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SOLICITANTE: BAYER CORPORATION
FECHA DE SOLICITUD: ENERO 13 DE 2000

DILIA RODRÍGUEZ D´ALEMAN, en mi condición de apoderada del solicitante, por el presente escrito atentamente doy respuesta a su auto notificado por estado de Enero 27 de 2005 que Vence Abril 27 de 2005, y me permito presentar los siguientes documentos:

- Database HCAPUS, DN 127:293717, Abstract, Vysocomol. Soedin. 1996, Vol. 38, Nr. 12, Páginas 2038 a 2041. (La traducción de este documento será presentada posteriormente).
- Database HCAPUS; DN 127: 34137, Escrito por KUBO, publicado el 15, 1997
- Database HCAPUS, DN 126: 166148, Journal of Pharmacol. 1996, Vol 279, Nr. 2, Pag. 956 a 966;
- Database HCAPUS, DN 98:78152, CHUGAY PHARMACEUTICAL CO. LTD., publicado en Noviembre 15, 1982 (La traducción de este documento será presentada posteriormente).
- Database HCAPUS, DN 127: 273945, 1996, Vol 23, Pag 93 a 104.
- Database HCAPUS, DN 125:245169, Jornal of Immonulogical Methods, 1996, Vol 196, Nr. 2, Pag 163 a 173. Por favor tenga en cuenta que al parecer hubo un error mecanográfico en el auto emitido por esa Superintendencia y se colocó el número 241569 cuando el número del documento citado en el informe de búsqueda es 245169.

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Señor Jefe,

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Title

Optical properties of segmented oligourethane with azomethine terminal fragments

Author

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Organization

Natl. Acad. of Sci. of Ukraine, Inst. of Phys., Kiev, 252028, Ukraine

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Abstract

Segmented oligourethane contg. polar fragments based on 2-hydroxybenzylidene deriv. of sulfanilamide at the ends of a flexible nonpolar chain and model azomethine compd. were synthesized. Electronic absorption spectra of luminescence spectra of the synthesized compds. were studied. The state of the sample (soln., solid layer) was demonstrated to affect the optical properties. It was found that the absorption and luminescence spectra of oligourethane are controlled by its mol. structure. The synthesized compd. is an org. luminophore with moderate quantum efficiency.

Document Type

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1997:624629 CAPLUS

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ВЫСОКОМОЛЕКУЛЯРНЫЕ СОЕДИНЕНИЯ, Серия Б, 1996, том 38, № 12, с. 2038–2041

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УДК 541.64:535.5

ОПТИЧЕСКИЕ СВОЙСТВА СЕГМЕНТИРОВАННОГО ОЛИГОУРЕТАНА С КОНЦЕВЫМИ АЗОМЕТИНСОДЕРЖАЩИМИ ФРАГМЕНТАМИ

© 1996 г. **М. В. Курик*, В. В. Шевченко**, В. А. Шрубович**, Л. И. Цикора***

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252028 Киев, пр. Науки, 46

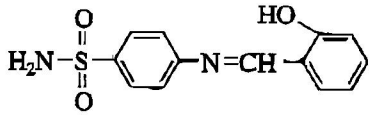
** *Институт химии высокомолекулярных соединений Национальной академии наук Украины*
253160 Киев, Харьковское ш., 48

Поступила в редакцию 24.12.95 г.

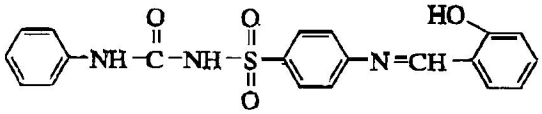
Синтезированы сегментированный олигоуретан, содержащий на концах гибкой неполярной цепи полярные фрагменты на основе 2-гидроксибензилиденового производного 4-сульфамидоанилина, а также модельные азометинсодержание соединения. Исследованы электронные спектры поглощения и люминесценции синтезированных соединений; установлено влияние структуры соединений типа образца (раствор, твердый слой) на оптические свойства. Показано, что спектры поглощения и люминесценции олигоуретана определяются его молекулярной структурой; данное соединение является органическим люминофором со средней квантовой эффективностью излучения.

В связи с развитием исследований по молекулярной и биомолекулярной электронике существенно возрастает интерес к изучению физических свойств амфифильных соединений, нахождению корреляции между молекулярной структурой и свойствами этих соединений, созданию на их основе элементов молекулярной электроники [1–3]. Это стимулирует развитие исследований в области синтеза и изучения свойств функционализированных амфифильных соединений, среди которых большой интерес представляют разрабатываемые нами бианкерные ПАВ олигомерного типа [4, 5]. В частности, большие возможности для введения функциональных групп различной химической природы представляют бианкерные ПАВ на основе сегментированных олигоуретанов (**ОУ**). Эти соединения содержат в своем составе гибкие неполярные цепи и жесткие полярные группы на концах цепей, что обусловливает различия в межмолекулярных взаимодействиях и молекулярных колебаниях [6]. Поэтому при введении в состав сегментированных ОУ хромофоров можно ожидать проявления этими соединениями характерных оптических свойств.

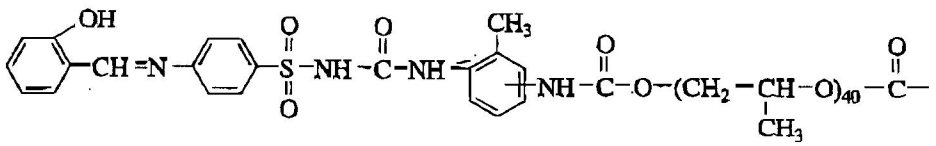
В настоящей работе синтезированы соединения, содержащие в качестве хромофора азометиновые группы: соединение I, сегментированный ОУ на его основе, и соединение II, моделирующее концевой фрагмент ОУ, общие формулы которых имеют вид

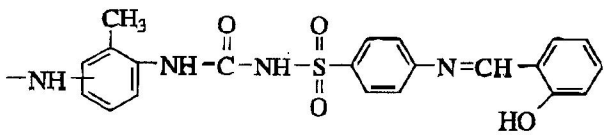


I



II





ОУ

Кроме того, нами проведено сравнительное исследование спектров поглощения и люминесценции полученных соединений.

ЭКСПЕРИМЕНТАЛЬНАЯ ЧАСТЬ

Исходный 4-салицилиденаминобензолсульфамид (I) получали по следующей методике. К 23.7 г 4-аминобензолсульфамида, растворенного при нагревании в 160 мл спирта, прибавляли 16.8 г салицилового альдегида. Раствор выдерживали при 50–60°С в течение 1 ч. При охлаждении до комнатной температуры выпадали кристаллы желтого цвета, которые перекристаллизовывали из спирта. Выход 97%. $T_{пл}$ = 211–212°С.

Найдено, %:	N 19.27;	C 11.41.
Для $C_{13}H_{12}N_2O_3S$		
вычислено, %:	N 10.14;	C 11.60.

N-(салицилиденаминофениленсульфо)-N'-фенилмочевину (II) синтезировали следующим образом. 1.3 г соединения I смешивали с 1.3 г триэтиламина и полученную смесь растворяли в 10 мл воды при небольшом нагревании. К полученному оранжевому раствору соли соединения I после охлаждения прибавляли при перемешивании 0.6 г раствора фенилизоцианата в 2 мл ацетона, при этом раствор приобретал желтую окраску. После непродолжительного выдерживания при комнатной температуре раствор упаривали досуха, и сухой остаток перекристаллизовывали несколько раз из спирта. Выход 76%. $T_{пл}$ > 230°С.

Найдено, %:	N 10.51;	C 7.90.
Для $C_{20}H_{17}N_3O_4S$		
вычислено, %:	N 10.63;	C 8.10.

ОУ синтезировали по методике [7] в две стадии. Сначала взаимодействием олигооксипропиленгликоля (M = 2300) с двукратным мольным избытком толуилендиизоцианата (смесь изомеров 2,4- и 2,6- в соотношении 65 : 35) при 100°С получали изоцианатный преполимер с M = 2700. На второй стадии 11.5 г изоцианатного преполимера, растворенного в 10 мл диоксана, обрабатывали раствором 2.4 г соединения I в смеси 5 мл ДМФА и 2.5 мл триэтиламина. После окончания экзотермической реакции образовавшийся желтый раствор упаривали досуха и полученное вещество переосаждали несколько раз из спиртового раствора водой. Выход ОУ составил 94%, его ММ по данным ИТЭК равна 3470.

Для спектральных исследований синтезированные вещества растворяли в спирте и из полученных растворов формировали твердые слои на кварцевой подложке; толщина пленок находилась в пределах 10–50 мкм. Пленки соединений I и II имели кристаллическую структуру, а ОУ – аморфную. Спектры поглощения измеряли на двухлучевом спектрометре типа 356 ("Hitachi", Япония). Спектры люминесценции регистрировали с помощью фотоэлектрической установки, созданной на базе монохроматора SPM-2 ("Carl Zeiss", Jena, ГДР). Возбуждение люминесценции проводили под углом 45° к образцу, используя ртутную лампу и фильтр, пропускающий излучение с длиной волны 365 нм. Интенсивность люминесценции определяли с учетом спектральной чувствительности установки. При этом реабсорбцию и рассеяние света не учитывали, так как исследование проводили на тонких пленках. Все измерения спектров поглощения и люминесценции выполняли на воздухе. За это время в спектрах люминесценции не обнаружено химических изменений образцов под действием УФ-излучения. Люминесценцию наблюдали при 300 и 77 К.

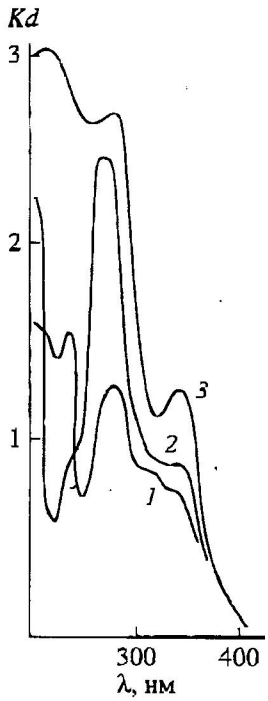


Рис. 1. Спектр поглощения растворов соединений I (*1*), II (*2*) и ОУ (*3*) в спирте. Kd – оптическая плотность (K – коэффициент поглощения, d – толщина образца).

РЕЗУЛЬТАТЫ И ИХ ОБСУЖДЕНИЕ

Как видно из приведенной формулы, ОУ имеет в своем составе гибкую неполярную часть, образованную олигооксипропиленгликолем, на обоих концах которой расположены жесткие полярные сегменты, содержащие азометиновые группы.

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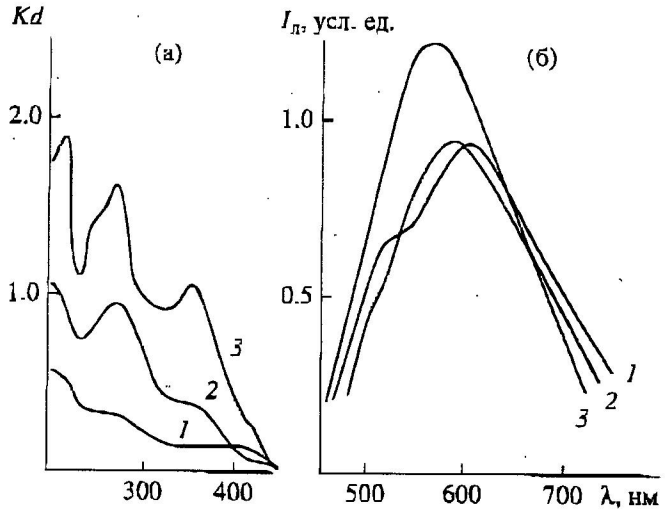


Рис. 2. Спектры поглощения (а) и люминесценции (б) пленок соединений I (*1*), II (*2*) и ОУ (*3*). $I_л$ – интенсивность люминесценции.

Спектры поглощения растворов в спирте (концентрация 10^{-2} моль/л) и твердых пленок синтезированных соединений приведены на рис. 1 и 2а. Структура спектра поглощения практически одинакова во всех случаях. Спектр состоит из трех полос при 235, 260–265 и 340 нм соответственно, интенсивность которых падает с ростом длины волны. Наблюдается только усиление поглощения в области 235–340 нм при переходе от соединений I и II к ОУ и незначительное смещение полос в длинноволновую область. Аналогичные изменения характерны для твердых пленок (рис. 2а), только при переходе от растворов к пленкам интенсивность полос понижается в ~3 раза и спектр смещается в длинноволновую область на 10 нм. В спектре поглощения пленки ОУ появляется новая полоса с максимумом 240 нм. Отметим также, что ОУ, нанесенный механическим способом на кварцевую подложку, имеет полосы поглощения, совпадающие по положению с полосами соединения II.

Более существенные изменения наблюдаются в спектрах люминесценции (рис. 2б). При переходе от соединений I и II к ОУ изменяется структура спектров и в 4 раза увеличивается интенсивность полос. Полосы люминесценции значительно более широкие по сравнению с полосами поглощения. В спектре люминесценции соединения I наблюдаются две полосы с максимумами 590 и 570 нм соответственно. Понижение температуры до 77 K приводит к смещению полос люминесценции в длинноволновую область на 50 нм и к увеличению интенсивности люминесценции почти в 4 раза. Большие значения полуширины полос люминесценции, стоксового сдвига спектра поглощения и люминесценции, по-видимому, могут быть доказательством эксимерной природы полосы излучения. Эксимеры в исследованных соединениях имеют смешанную природу, так как являются результатом взаимодействия возбужденных и невозбужденных молекул. Полоса люминесценции у ОУ шире, чем у соединения II. Это свидетельствует о том, что данная полоса обусловлена межмолекулярными и внутримолекулярными колебаниями, набор которых в ОУ существенно шире. Таким образом, оптические спектры поглощения и люминесценции определяются в первую очередь молекулярной структурой ОУ.

Полученные результаты позволяют заключить, что синтезированный амфифильный ОУ с концевыми азометиновыми фрагментами является органическим люминофором со средней квантовой эффективностью излучения. Такое амфифильное соединение, вероятно, наряду с возможностью использования в качестве модельного соединения для молекулярной электроники может представлять интерес и в качестве люминесцентной метки при изучении различных биологических структур в молекулярной биологии и медицине.

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Optical Properties of Segmented Oligourethane with Azomethine Terminal Fragments

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Abstract—Segmented oligourethane containing polar fragments based on 2-hydroxybenzylidene derivative of sulfanilamide at the ends of a flexible nonpolar chain and model azomethine compound were synthesized. Electronic absorption spectra and luminescence spectra of the synthesized compounds were studied. The state of the sample (solution, solid layer) was demonstrated to affect the optical properties. It was found that the absorption and luminescence spectra of oligourethane are controlled by its molecular structure. The synthesized compound is an organic luminophore with moderate quantum efficiency.

US006143764A

United States Patent [19]

Kubo et al.

[11] **Patent Number: 6,143,764**

[45] **Date of Patent: Nov. 7, 2000**

[54] **QUINOLINE AND QUINAZOLINE DERIVATIVES INHIBITING PLATELET-DERIVED GROWTH FACTOR RECEPTOR AUTOPHOSPHORYLATION AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME**

[75] Inventors: **Kazuo Kubo**; **Shinichi Ohyama**; **Toshiyuki Shimizu**; **Tsuyoshi Nishitoba**; **Shinichiro Kato**; **Hideko Murooka**; **Yoshiko Kobayashi**, all of Takasaki, Japan

[73] Assignee: **Kirin Beer Kabushiki Kaisha**, Tokyo-to, Japan

[21] Appl. No.: **09/068,660**

[22] PCT Filed: **Nov. 5, 1996**

[86] PCT No.: **PCT/JP96/03229**

§ 371 Date: **May 6, 1998**

§ 102(e) Date: **May 6, 1998**

[87] PCT Pub. No.: **WO97/17329**

PCT Pub. Date: **May 15, 1997**

[30] **Foreign Application Priority Data**

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Feb. 23, 1996 [JP] Japan 8-62121

[51] **Int. Cl.**[7] **A61K 31/47**; A61K 31/535; C07D 413/00; C07D 215/16; C07D 215/20

[52] **U.S. Cl.** **514/312**; 514/235.2; 514/253; 544/128; 544/235; 544/238; 544/363; 544/405; 154/155; 154/154

[58] **Field of Search** 546/154, 155; 544/128, 238, 363, 405, 235; 514/253, 312, 235.2

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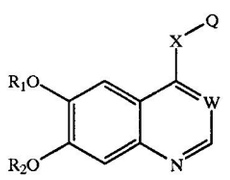
CA 76:42038, Wright, 1971.

Primary Examiner—D. Margaret Seaman
Attorney, Agent, or Firm—Foley & Lardner

[57] **ABSTRACT**

The present invention relates to novel quinoline derivatives and quinazoline derivatives represented by the following formula (I):

(1)



[wherein R_1 and R_2 are each independently H or C_1–C_4-alkyl, or R_1 and R_2 together form C_1–C_3-alkylene, X is O, S or CH_2, W is CH or N, and Q is a substituted aryl group or substituted heteroaryl group] and their pharmaceutically acceptable salts, having platelet-derived growth factor receptor autophosphorylation inhibitory activity, to pharmaceutical compositions containing these compounds, and to methods for the treatment of diseases associated with abnormal cell growth such as tumors.

52 Claims, No Drawings

QUINOLINE AND QUINAZOLINE DERIVATIVES INHIBITING PLATELET-DERIVED GROWTH FACTOR RECEPTOR AUTOPHOSPHORYLATION AND PHARMACEUTICAL COMPOSITIONS CONTAINING THE SAME

This application is a 371 of PCT/JP96/03229, filed Nov. 5, 1996.

FIELD OF THE INVENTION

The present invention relates to novel quinoline derivatives and quinazoline derivatives having an inhibitory action against abnormal cell growth and to their pharmaceutically acceptable salts. More specifically, the present invention relates to novel quinoline derivatives and quinazoline derivatives having an inhibitory action against autophosphorylation of the platelet-derived growth factor receptor and to their pharmaceutically acceptable salts.

BACKGROUND OF THE INVENTION

Growth factors such as insulin, the epidermal growth factor and the platelet-derived growth factor (hereinafter referred to as PDGF) have an important role in cell growth. In particular, PDGF is known to be a powerful cell growth factor related to the control of cell growth and division (Cell 46, 155 (1986)). However, in diseases or pathophysiological situations such as leukemia, cancers, psoriasis, glomerular nephritis, organofibrosis, atherosclerosis, restenosis after percutaneous coronary angioplasty or bypass surgery and rheumatoid arthritis, abnormal production of PDGF or PDGF receptor occurs at the pathophysiological sites, and abnormal cell growth is observed at the pathophysiological sites in such diseases. Namely, this is an excess of cell growth signals associated with overproduction of the PDGF or PDGF receptors. Thus, it is necessary to suppress cell growth signal transduction to improve these pathophysiological situations.

In one trial, it was reported that abnormal cell growth in pathophysiological situations was suppressed by administration of an anti-PDGF antibody (J. Exp. Med. 175, 1413 (1992)); however, there are problems such as in vivo stability and methods of administration as a therapeutic agent because the anti-PDGF antibody is a protein molecule.

On the other hand, in cells, PDGF is known to bind to the PDGF receptor to activate tyrosine kinase present in this receptor. This receptor tyrosine kinase relates to intracellular signal transduction via autophosphorylation of the receptor itself and plays an important role in cell growth. In pathophysiological situations, this intracellular signal transduction is considered to be excessive. Therefore, inhibition of PDGF receptor autophosphorylation can be considered as another possible strategy to suppress cell growth signal transduction.

Examples of suppressing cell growth using the PDGF receptor autophosphorylation inhibitors, which are low molecular weight compounds, are described in the literature (Cancer Research 54, 6106 (1994); Proc. Natl. Acad. Sci. USA. 92, 2558 (1995)). Therefore, PDGF receptor autophosphorylation inhibitors could be useful in many diseases such as leukemia, cancers, psoriasis, glomerular nephritis, organofibrosis, atherosclerosis, restenosis after percutaneous coronary angioplasty or bypass surgery and rheumatoid arthritis.

Examples of previously known PDGF receptor autophosphorylation inhibitors include 3-arylquinolines (J. Med. Chem. 37, 2627 (1994); J. Med. Chem. 37, 2129 (1994) and Publication of Japanese Patent Laid-open No. 94/507643), 3-arylquinoxalines (Cancer Research 54, 6106 (1994)), and 4-pyridyl-2-arylpyrimidines (Proc. Natl. Acad. Sci. USA. 92, 2558 (1995)).

However, as far as the present inventors know, compounds which have a structure in which an aryl group or heteroaryl group is bonded to position 4 of the quinoline backbone or quinazoline backbone via one oxygen atom, sulfur atom or carbon atom and which have a PDGF receptor autophosphorylation inhibitory activity are not known.

Furthermore, as to previously known quinoline derivatives, {4-[(7-chloro-4-quinolinyl)oxy]phenyl}(4-fluorophenyl)methanone is described in Publication of Examined Japanese Patent Application No. 89/246263. This known compound is used as a fungicide, and there are no reports as to its PDGF receptor autophosphorylation inhibitory activity.

Furthermore, 4-arylquinoline derivatives are described in J. Med. Chem. 14, 1060 (1971), Acta Chim. Hung 112, 241 (1983), Publication of Examined Japanese Patent Application No. 88/51375, German Patent No. 3101544, Publication of Examined Japanese Patent Application No. 89/246263, and U.S. Pat. No. 3,936,461. These known compounds are each reported as an antihypertensive agent or intermediate for its production, agent to prevent harmful organisms (preservative), plant growth control agent, fungicide or anti-angina agent, respectively, and there are no reports as to their PDGF receptor autophosphorylation inhibitory activity.

Consequently, the development of novel compounds having the PDGF receptor autophosphorylation inhibitory activity would be of great industrial benefit.

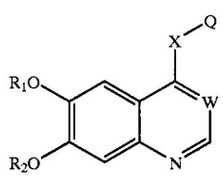
A major objective of the present invention is to provide novel compounds having the PDGF receptor autophosphorylation inhibitory activity.

DISCLOSURE OF THE INVENTION

As a result of intensive studies to attain the abovementioned objective, the present inventors found that compounds having a structure in which an aryl group or heteroaryl group is bound to position 4 of the quinoline backbone or quinazoline backbone via one oxygen atom, sulfur atom or carbon atom best serve the objective, and thus came to complete the present invention based on this finding.

Namely, the present invention is to provide quinoline derivatives and quinazoline derivatives represented by the following formula (I), and pharmaceutically acceptable salts thereof:

(1)



{wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl, or R_1 and R_2 together form C_1–C_3-alkylene, and W is CH or N, 1. when W is CH,

(a) X is O or S, and Q is a phenyl group represented by formula (II):

NO_2, OH, NH_2, halogens, C_1–C_4-alkyl, cyclic (C_3–C_{10}) alkyl, C_1–C_4-alkoxy, C_1–C_4-alkoxycarbonyl, C_1–C_3-alkylenedioxy, C_1–C_4-alkylamino, di-(C_1–C_4-alkyl)amino, C_2–C_4-alkylamido, trifluoromethyl, C_1–C_4-alkylthio, phenyl, phenoxy, phenylthio, phenyl(C_1–C_4-alkyl), benzoyl and C_2–C_4-acyl;

substituted benzoyl: a benzoyl group substituted by 1 to 3 groups selected from the group consisting of CN, NO_2, OH, NH_2, halogens, C_1–C_4-alkyl, cyclic (C_3–C_{10})alkyl, C_1–C_4-alkoxy, C_1–C_4-alkoxycarbonyl, C_1–C_3-alkylenedioxy, C_1–C_4-alkylamino, di-(C_1–C_4-alkyl)amino, C_2–C_4-alkylamido, trifluoromethyl, C_1–C_4-alkylthio, phenyl, phenoxy, phenylthio, phenyl(C_1–C_4-alkyl), benzoyl and C_2–C_4-acyl.

The compounds represented by formula (I) may be acid addition salts and base addition salts thereof. The compounds of the present invention include these addition salts. Further, the compounds of the present invention, including these addition salts, include hydrates thereof.

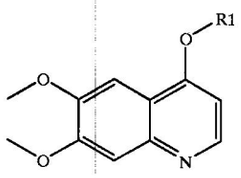
Examples of the acid addition salts include salts with inorganic acids such as hydrochloric acid, sulfuric acid, phosphoric acid, hydrobromic acid and nitric acid, and salts with organic acid such as maleic acid, fumaric acid, malic acid, oxalic acid, tartaric acid, succinic acid, citric acid, acetic acid, lactic acid, methanesulfonic acid and p-toluenesulfonic acid.

Further, examples of the base addition salts include salts with alkaline metal compounds (e.g., sodium hydroxide and potassium hydroxide), salts with alkaline earth metal compounds (e.g., calcium hydroxide and magnesium hydroxide), ammonium salts and salts with organic bases (e.g., triethylamine, ethanolamine).

Further, when these addition salts are used as drugs, the acids and bases naturally have to be pharmaceutically acceptable.

Representative examples of the compounds of formula (I) of the present invention are shown in Table 1. They are preferred embodiments of compound groups.

TABLE 1



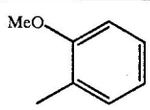
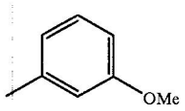
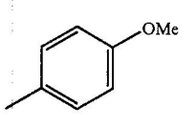
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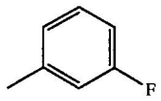
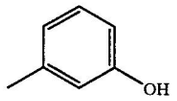
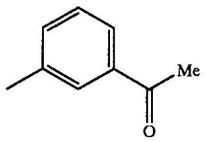
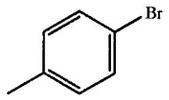
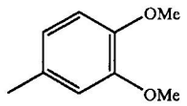
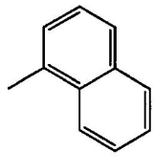
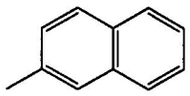
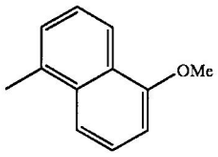
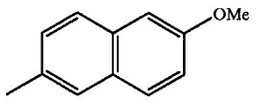
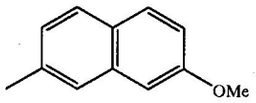
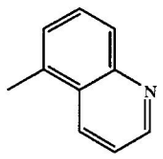
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TABLE 1-continued

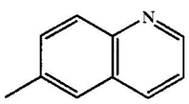
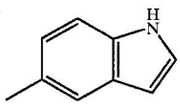
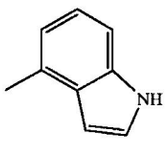
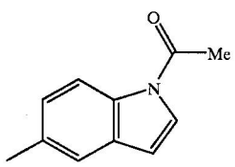
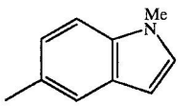
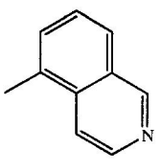
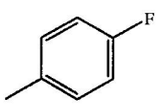
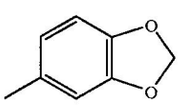
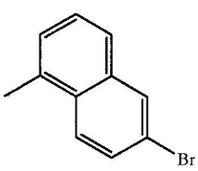
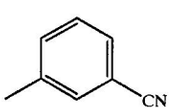
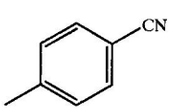
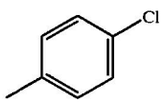
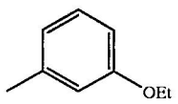
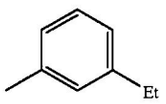
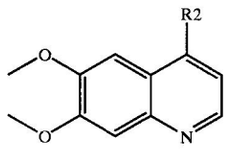
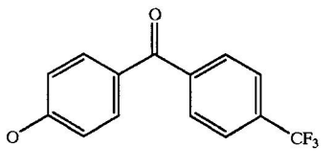
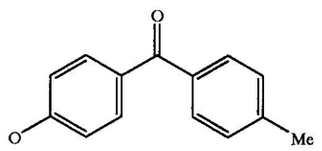
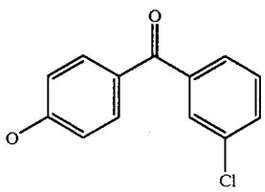
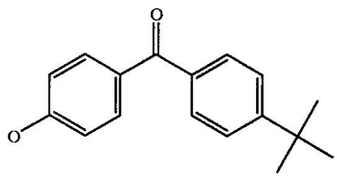
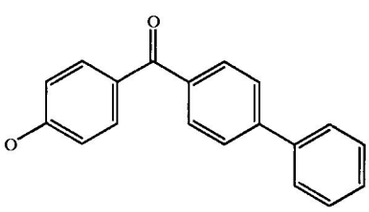
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TABLE 1-continued

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TABLE 1-continued

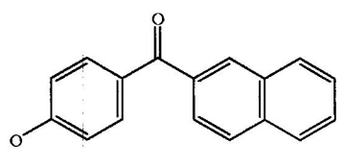
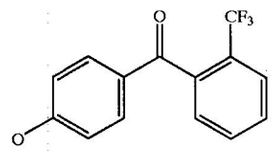
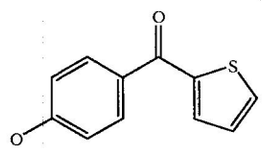
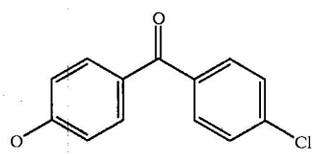
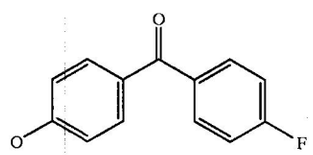
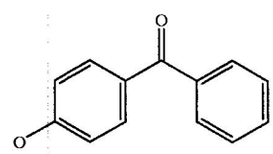
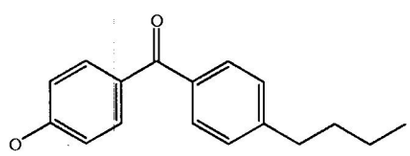
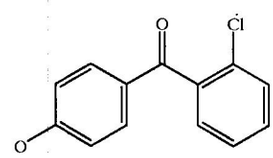
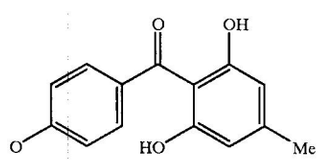
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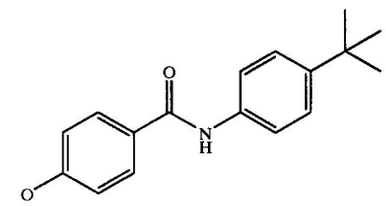
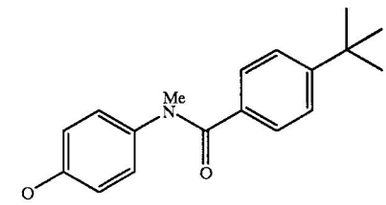
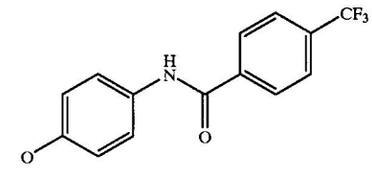
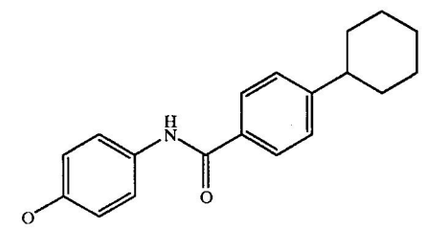
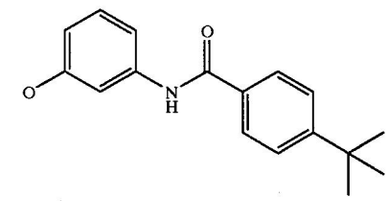
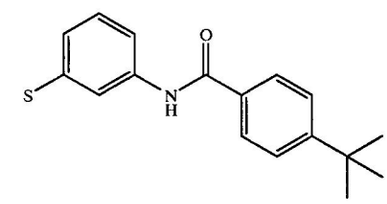
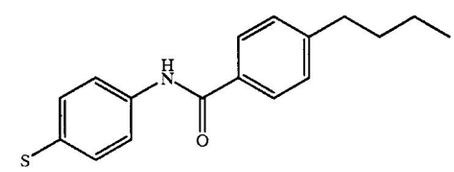
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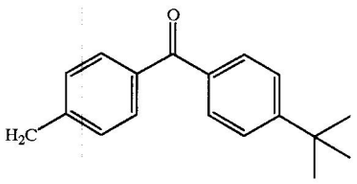
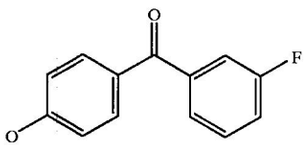
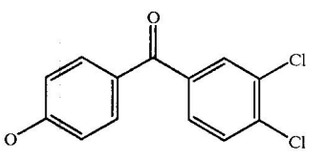
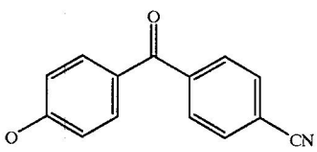
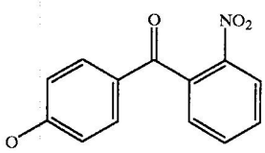
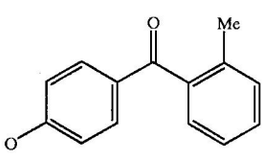
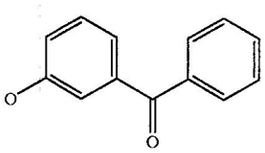
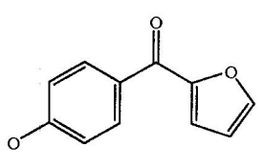
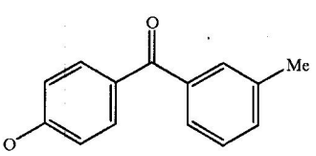
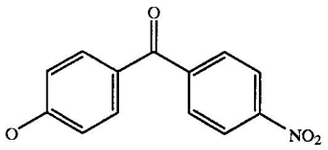
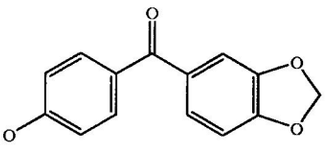
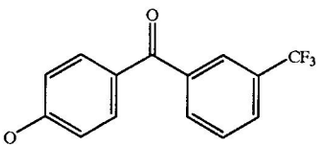
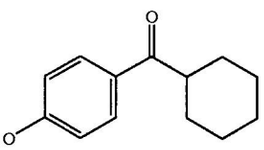
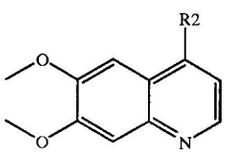
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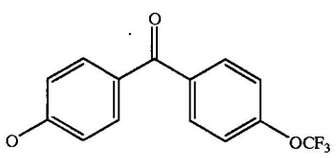
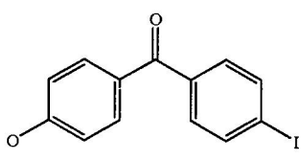
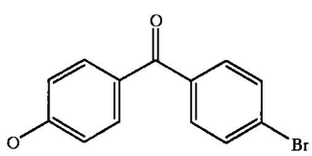
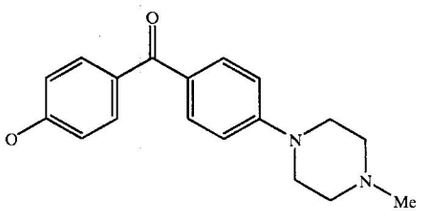
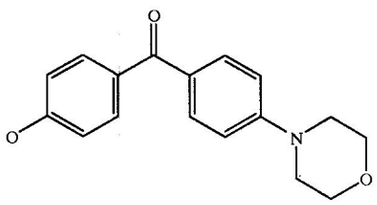
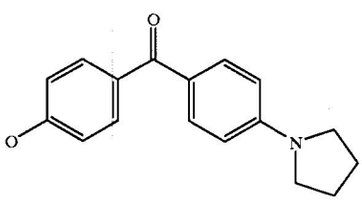
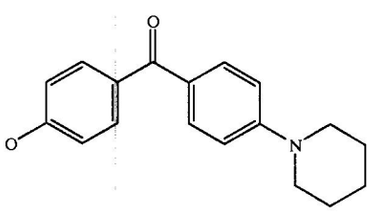
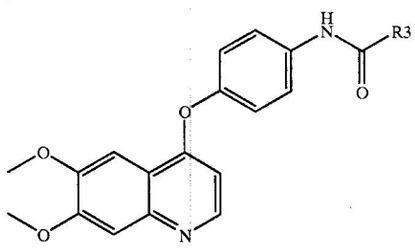
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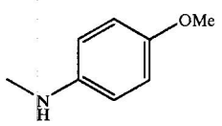
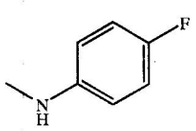
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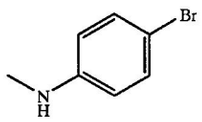
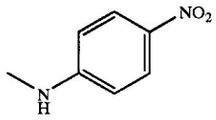
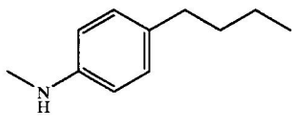
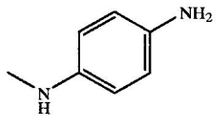
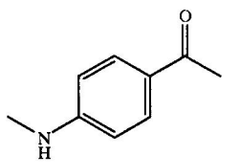
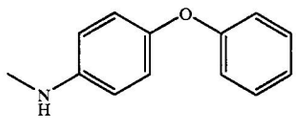
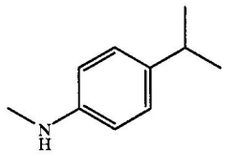
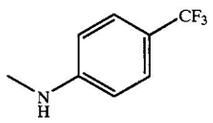
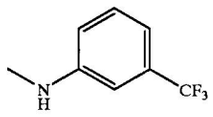
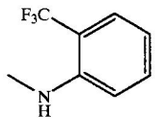
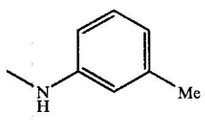
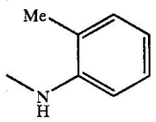
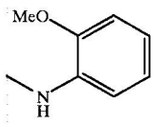
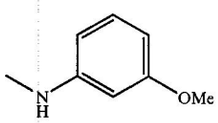
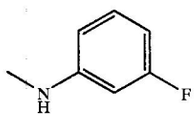
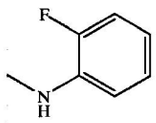
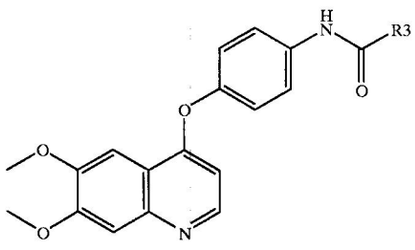
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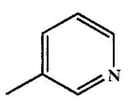
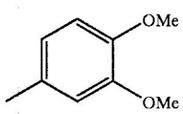
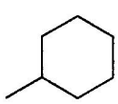
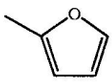
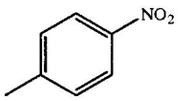
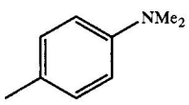
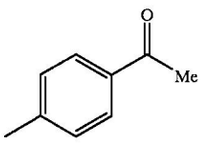
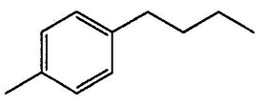
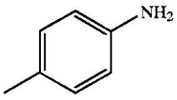
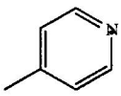
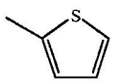
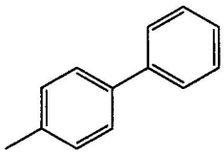
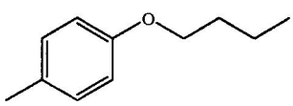
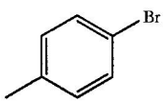
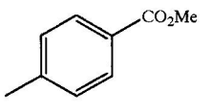
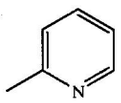
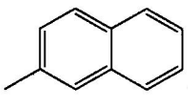
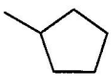
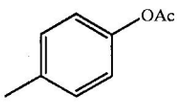
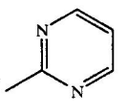
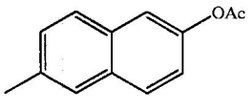
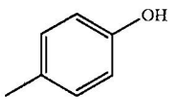
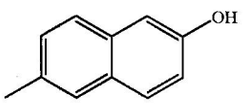
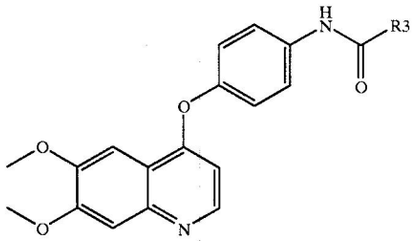
Compound No.	R3
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TABLE 1-continued

Compound No.	R3
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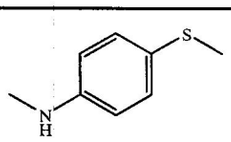
Compound No.	R3
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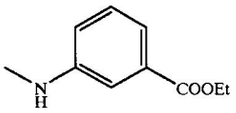
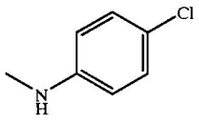
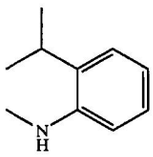
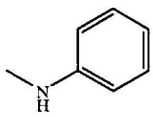
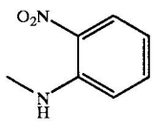
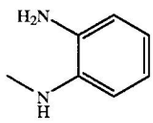
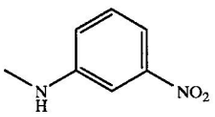
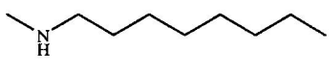
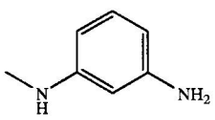
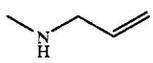
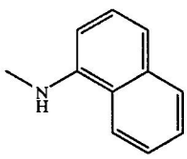
Compound No.	R3
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TABLE 1-continued

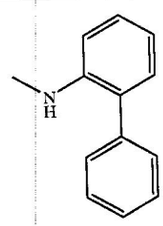
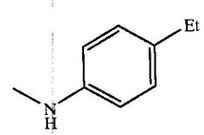
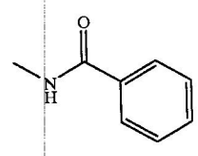
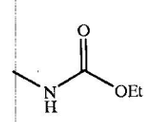
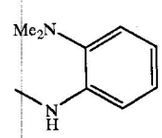
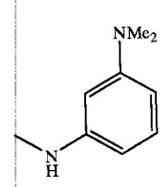
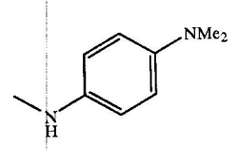
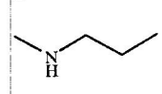
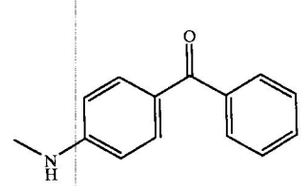
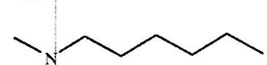
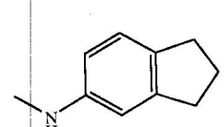
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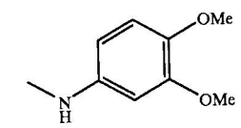
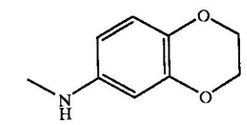
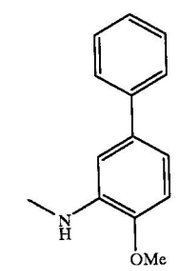
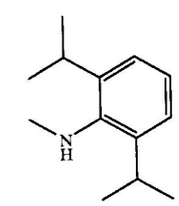
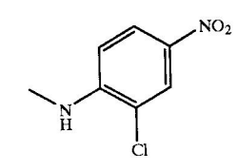
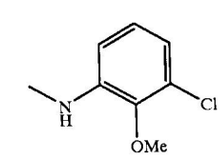
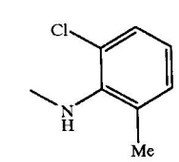
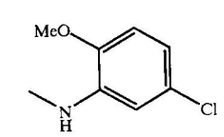
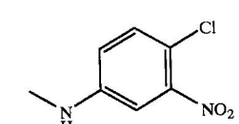
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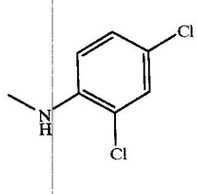
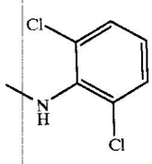
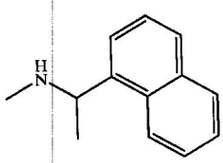
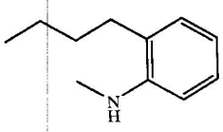
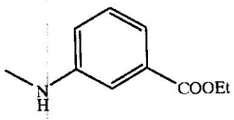
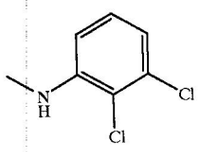
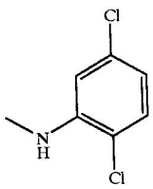
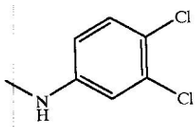
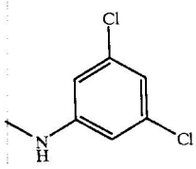
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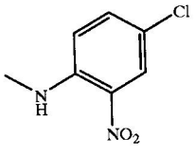
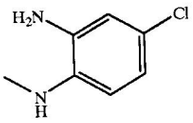
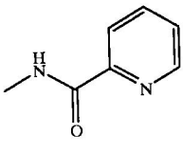
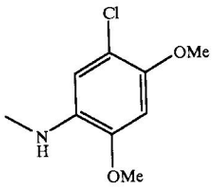
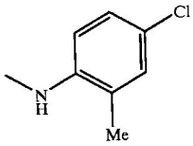
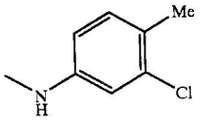
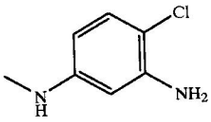
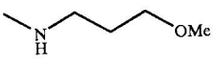
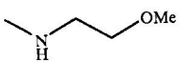
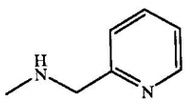
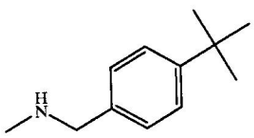
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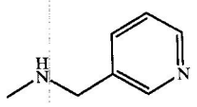
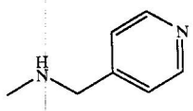
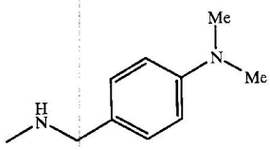
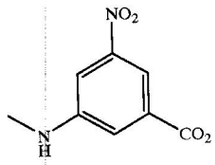
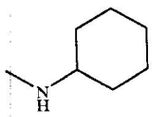
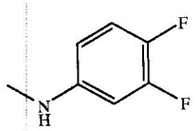
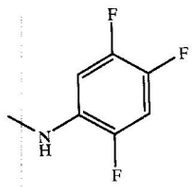
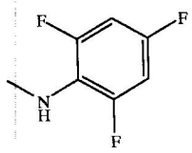
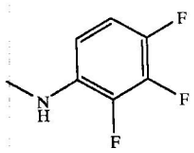
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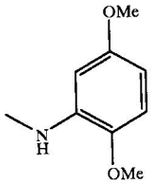
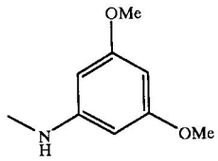
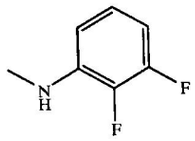
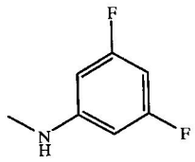
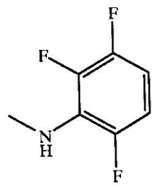
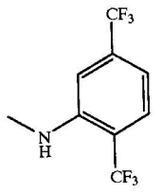
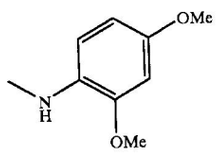
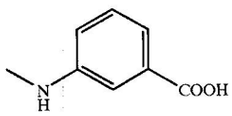
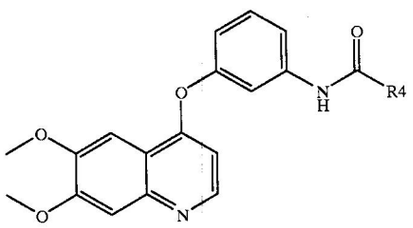
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TABLE 1-continued

Compound No.	
283	



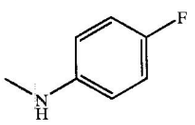
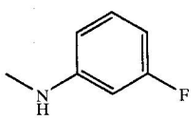
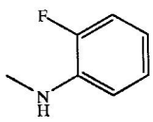
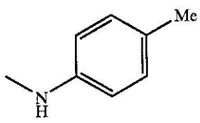
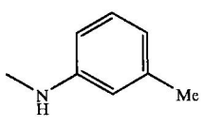
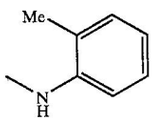
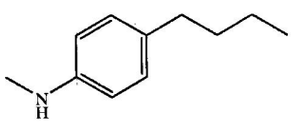
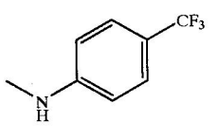
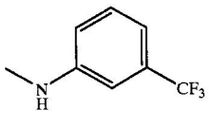
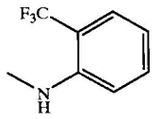
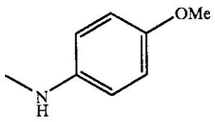
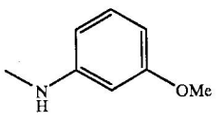
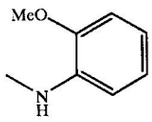
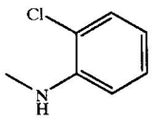
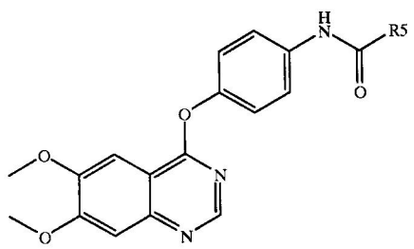
Compound No.	R4
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TABLE 1-continued

Compound No.	
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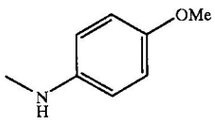
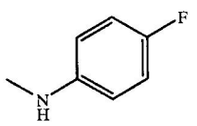
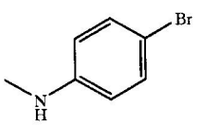
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100	
101	

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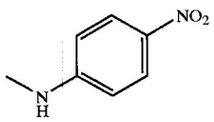
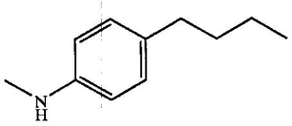
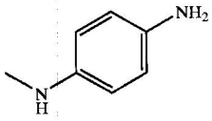
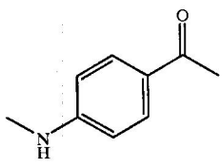
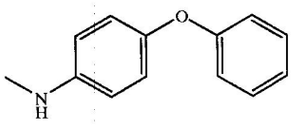
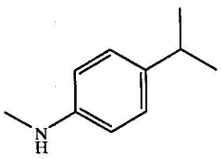
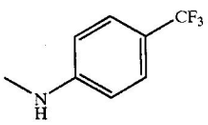
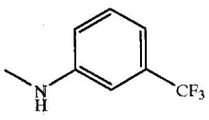
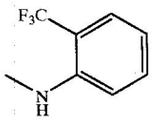
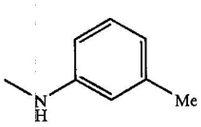
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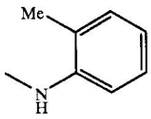
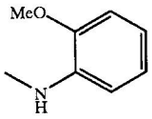
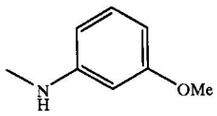
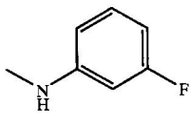
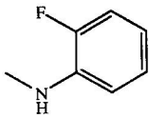
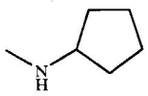
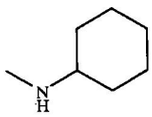
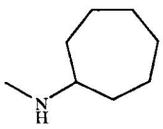
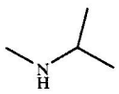
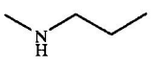
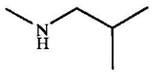
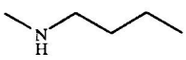
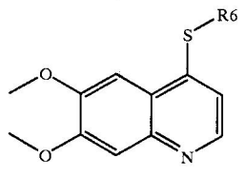
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TABLE 1-continued



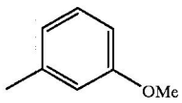
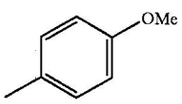
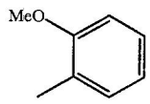
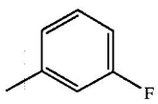
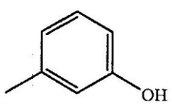
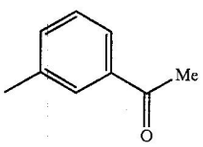
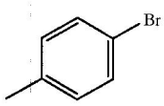
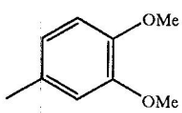
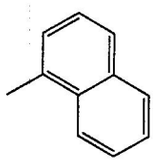
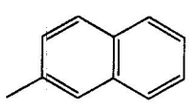
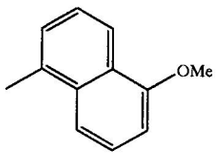
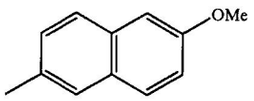
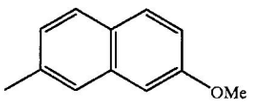
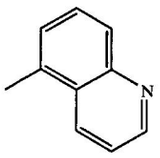
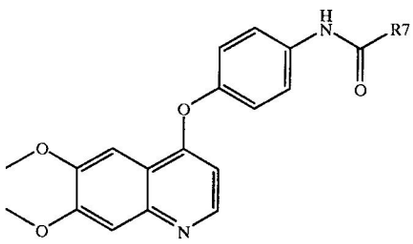
Compound No.	R6
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118	
119	
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126	

TABLE 1-continued

Compound No.	
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128	
129	
130	



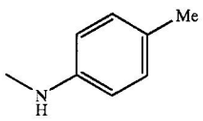
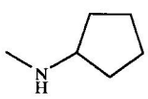
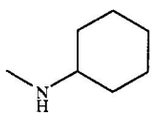
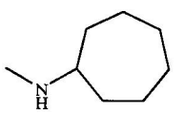
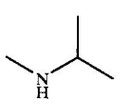
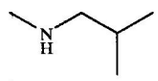
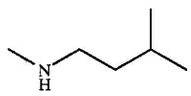
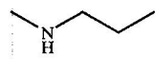
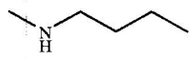
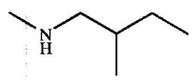
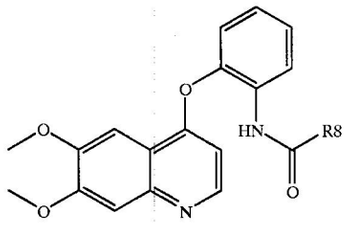
Compound No.	R7
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132	
133	
134	
135	

TABLE 1-continued

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137	
138	
139	
140	



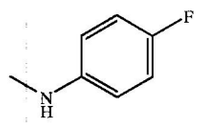
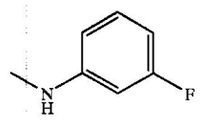
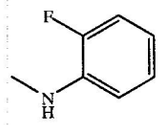
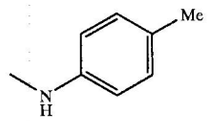
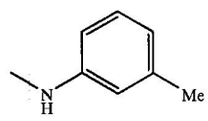
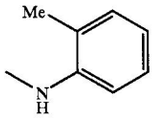
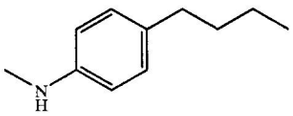
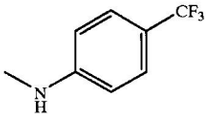
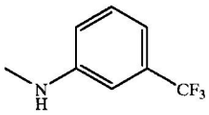
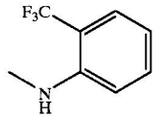
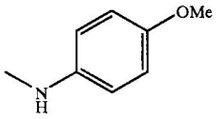
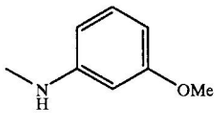
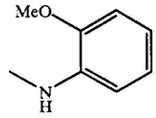
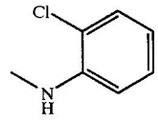
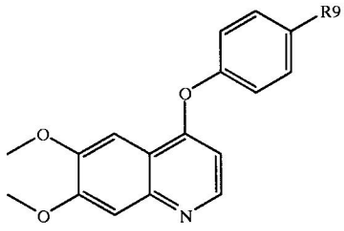
Compound No.	R8
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142	
143	
144	
145	

TABLE 1-continued

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147	
148	
149	
150	
151	
152	
153	
154	



Compound No.	R9

TABLE 1-continued

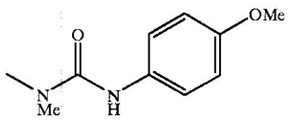
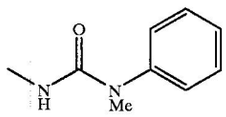
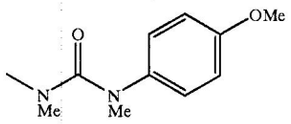
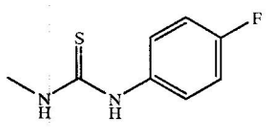
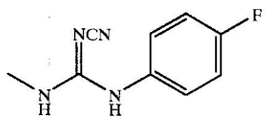
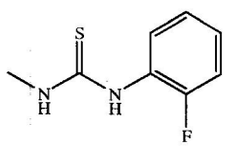
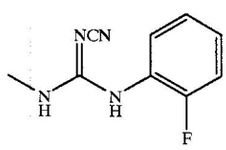
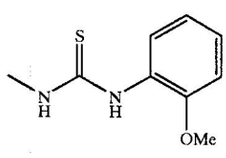
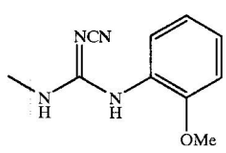
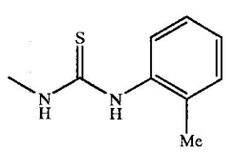
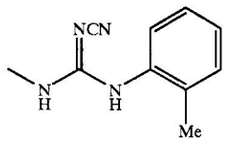
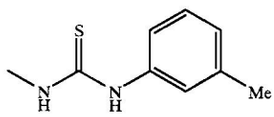
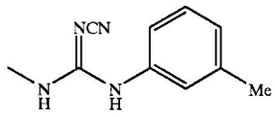
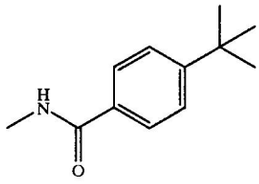
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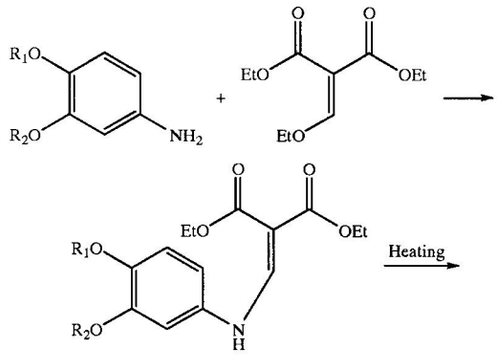
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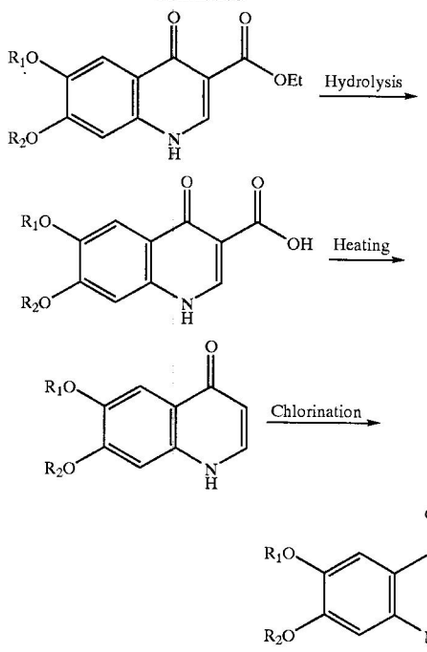
II. Methods of producing compounds of the present invention

Compounds of the present invention can be synthesized, for example, according to the methods described below. However, it should be understood that methods of producing compounds of the present invention are not limited to these methods.

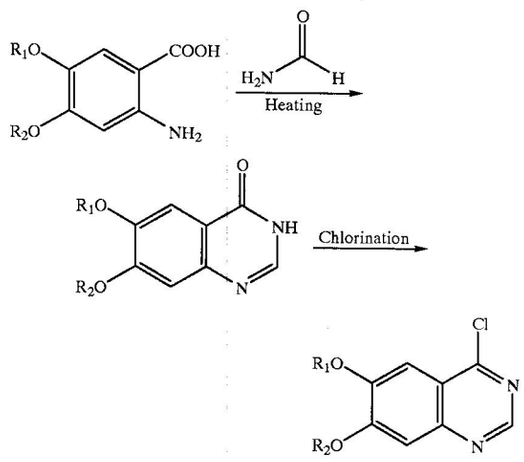
Although all of the compounds of the present invention are novel compounds which are not described in the literature, they can be produced using known chemical techniques. Further, the raw materials used for the production may be commercially available or synthesized by customary methods, if necessary. For example, 4-chloroquinoline derivatives can be synthesized by various known methods. For example, the method described in Org. Synth. Col. Vol. 3, 272 (1955) and Acta Chim. Hung. 112, 241 (1983) can be used. Many quinoline derivatives used in Examples hereinafter can be produced according to the following reaction scheme:



-continued



4-Chloroquinazoline derivatives can also be synthesized by various known methods. For example, the methods described in Dai Yuki' Kagaku (Comprehensive Organic Chemistry), Vol. 17, 150, edited by Kotake, Asakura Shoten (1967) can be used. Many quinazoline material compounds used in Examples hereinafter can be produced according to the following reaction scheme:



General methods of producing compounds of the present invention will be explained as follows:

1. Compounds of formula (I) wherein Q is represented by formula (II), formula (III) or formula (IV)

a. Synthesis of a compound wherein X is O:

A compound of formula (I) wherein X is O is produced by condensation of an aryl compound or heteroaryl compound having a hydroxyl group with a 4-chloroquinoline derivative.

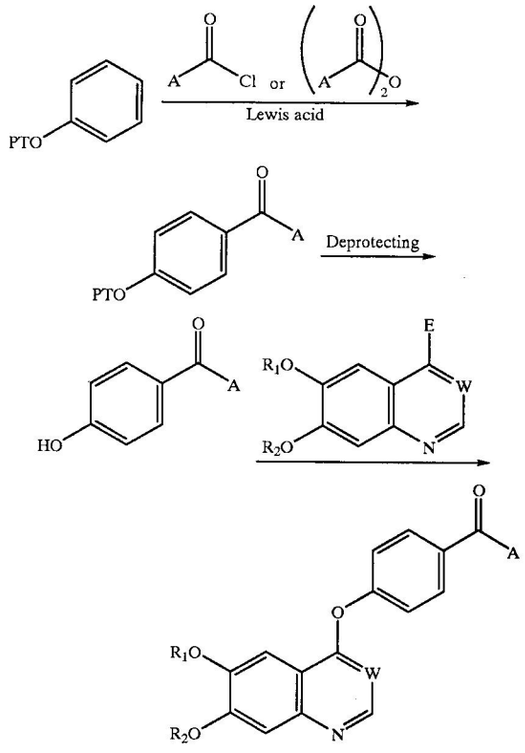
b. Synthesis of compounds wherein X is S:

A compound of formula (I) wherein X is S is produced in the same manner as described for a compound wherein X is O, by condensation of an aryl compound or heteroaryl compound having a thiol group with a 4-chloroquinoline derivative.

2. Compounds of formula (I) wherein Q is represented by formula (V) wherein both j and k are 0

a. Synthesis of a compound wherein X is O:

A Friedel-Crafts acylation reaction is carried out with phenol with a protected hydroxyl group by an appropriate protecting group in the presence of a Lewis acid (e.g., rare earth trifluoromethanesulfonate, in particular, scandium (III) trifluoromethanesulfonate or ytterbium (III) trifluoromethanesulfonate) described in literatures (e.g., J. Chem. Soc., Chem. Commun., 1157 (1993); Synlett, 1157 (1994)) to obtain an acylated compound. Next, the protecting group of the hydroxyl group of the acylated compound is removed, and the resultant compound is reacted with a 4-chloroquinoline derivative or 4-chloroquinazoline derivative at a temperature between 80 and 200° C., preferably between 130 and 180° C. in no solvent or in the presence of an appropriate polar solvent for 1 minute to 5 hours, preferably for 10 minutes to 1 hour to obtain the target compound.

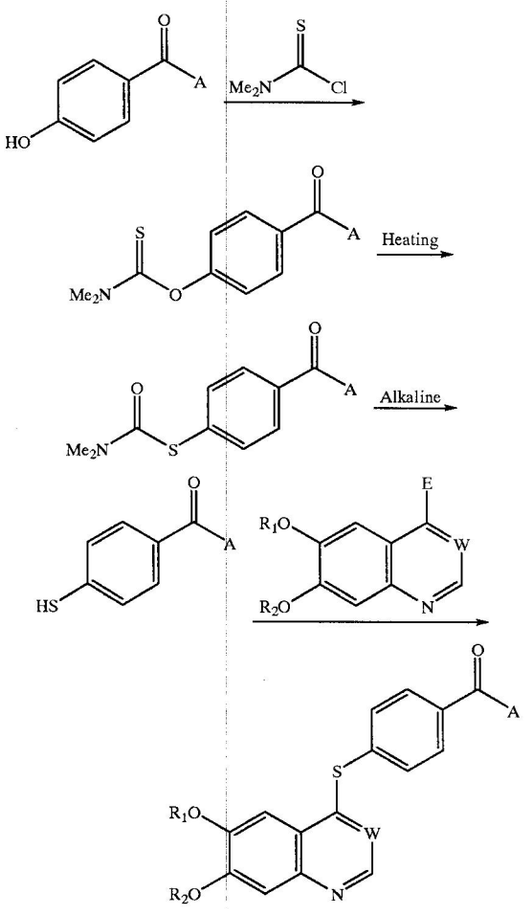


[In the formulas, A, R_1, R_2 and W are defined as described above, E is a leaving group (e.g., halogen, methanesulfonate, p-toluenesulfonate or trifluoromethanesulfonate, preferably chlorine), and PT is a protecting group (e.g., methyl or methoxymethyl)].

b. Synthesis of a compound wherein X is S:

For example, an acylthiophenol derivative can be synthesized by the following method described in the literature (Org. Syn. 51, 139 (1971)). Namely, an acylphenol derivative which can be synthesized as described in a. above is reacted with N,N-dimethylthiocarbamoylchloride to synthesize an O-aryl-N,N-dimethylthiocarbamate derivative. This derivative is heated (for example, at 200–300° C.) so that an S-aryl-N,N-dimethylthiocarbamate derivative with rear-

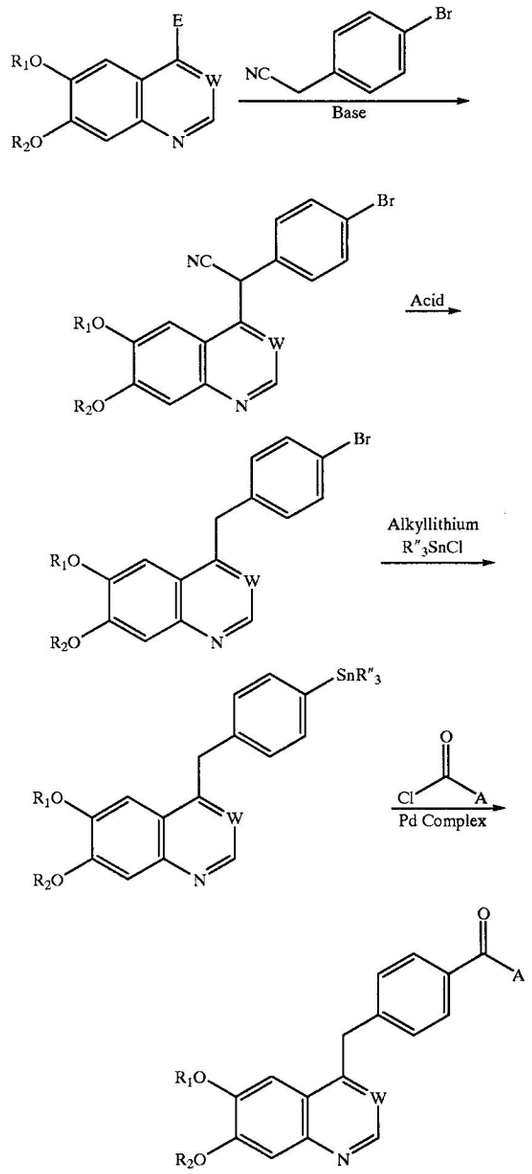
ranged oxygen and sulfur is formed in situ, which is then treated under alkaline conditions to yield the corresponding acylthiophenol derivative. Next, in the same manner as described for producing a compound wherein X is O, or in a polar solvent (e.g., N,N-dimethylformamide), the acylthiophenol derivative is reacted with a 4-chloroquinoline derivative or 4-chloroquinazoline derivative with sodium hydroxide at a temperature between 80 and 150° C. to obtain the target compound.



[In the formulas, A, R_1, R_2, W and E are defined as described above].

c. Synthesis of a compound wherein X is CH_2:

A compound represented by formula (I) wherein X is CH_2 can be synthesized using the method described by R. Cutler et al. (J. Am. Chem. Soc. 71, 3375 (1949)). In the present method, a 4-chloroquinoline derivative or 4-chloroquinazoline derivative is added to 4-bromophenylacetonitrile sodium salt in an appropriate organic solvent (e.g., toluene), then refluxed, and the diaryl-substituted acetonitrile so obtained is isolated and purified. The purified product is dissolved in an acidic solvent (e.g., aqueous sulfuric acid)and refluxed for 1–20 hours to obtain a 4-(4-bromobenzyl)quinoline derivative or said quinazoline derivative. Next, using the method of J. K. Stille et al. (J. Org. Chem. 48, 4634 (1983)), the 4-(4-bromobenzyl) quinoline derivative or said quinazoline derivative is reacted with alkyllithium in an appropriate solvent (e.g., tetrahydrofuran) to form an organic lithium compound in situ. Trialkyltin chloride is then added to yield an organic tin compound, and the resulting compound and an acid chloride are refluxed with heat in an appropriate organic solvent (e.g., chloroform) in the presence of a catalytic amount of a palladium complex (e.g., bis(triphenylphosphine)palladium (II) chloride) to produce the target compound.



[In the formulas, A, R_1, R_2, W and E are defined as described above, and R" is methyl or butyl].

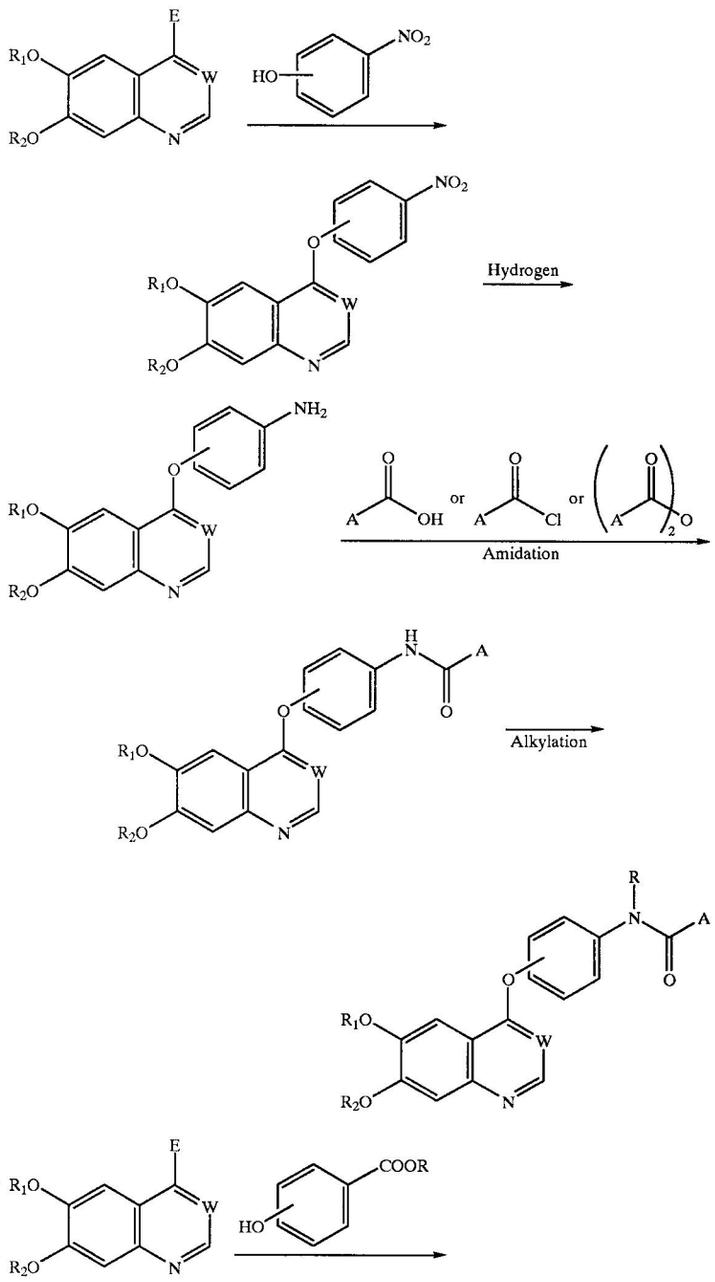
3. Compounds of formula (I) wherein Q is represented by formula (V) wherein B is O, and j is 1 and k is 0, or j is 0 and k is 1

a. Synthesis of a compound wherein X is O:

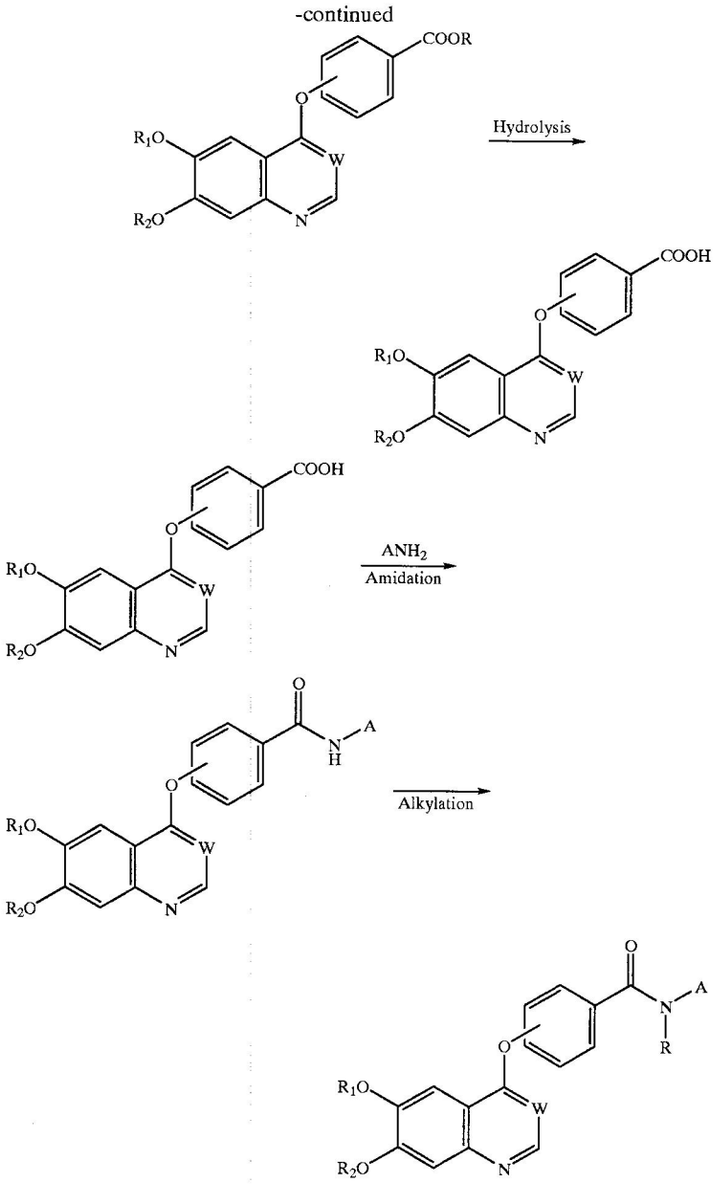
A 4-chloroquinoline derivative or 4-chloroquinazoline derivative is reacted with nitrophenol in an appropriate solvent or no solvent to synthesize a 4-(nitrophenoxy) quinoline derivative or said quinazoline derivative. The resulting derivative is then stirred in an appropriate solvent (e.g., N,N-dimethylformamide) in the presence of a catalyst (e.g., palladium hydroxide/carbon) under a hydrogen atmosphere to obtain the 4-(aminophenoxy)guinoline derivative or said quinazoline derivative. The resulting derivative can

be amidated using carboxylic acids or their derivatives to produce the target compound according to known methods. Further, its N-alkylamides can be produced by reaction with an alkyl halide in an appropriate solvent (e.g., N,N-dimethylformamide) in the presence of a base (e.g., sodium hydride).

Further, analogously, a 4-(carboxyphenoxy)quinoline derivative or said quinazoline derivative can be obtained by reacting a hydroxybenzoic acid ester with a 4-chloroquinoline derivative or 4-chloroquinazoline derivative, followed by acid or base hydrolysis. The resultant derivative can be amidated using an alkylamine or arylamine to produce the target compound according to known methods. Further, its N-alkylamides can be produced by the abovementioned method.



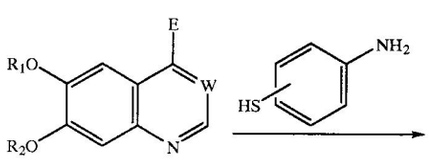
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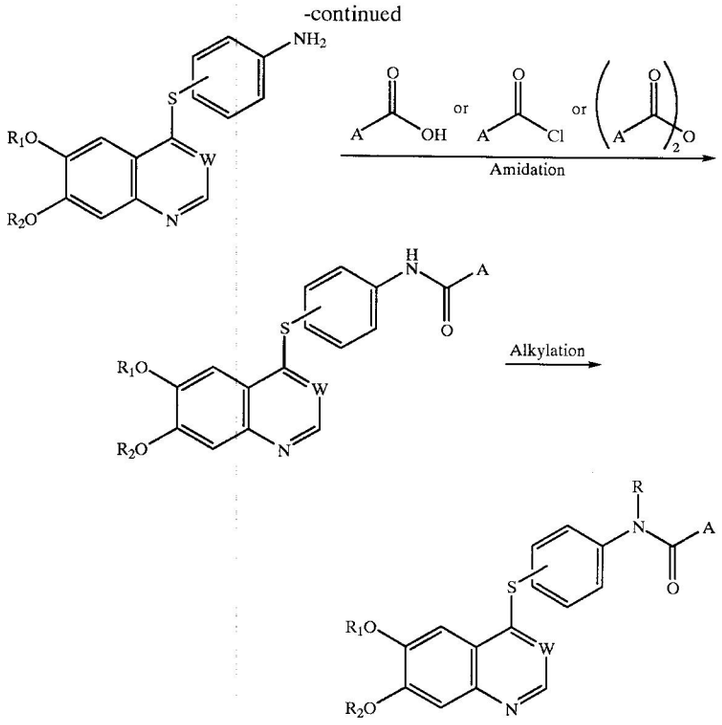
[In the formulas, A, R_1, R_2, W and E are defined as described above, and R is methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl or t-butyl.]

b. Synthesis of a compound wherein X is S:

A 4-(aminophenylthio)quinoline derivative or said quinazoline derivative is obtained by reacting a 4-chloroquinoline derivative or 4-chloroquinazoline derivative with aminothiophenol in an appropriate solvent or no solvent. The resulting derivative can be amidated using carboxylic acids or their derivative to produce the target compound according to known methods. Further, its N-alkylamides can be produced by reaction with an alkyl halide in an appropriate solvent (e.g., N,N-dimethylformamide) in the presence of a base (e.g., sodium hydride).



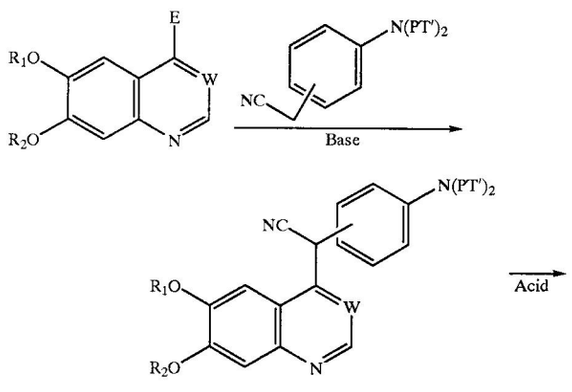
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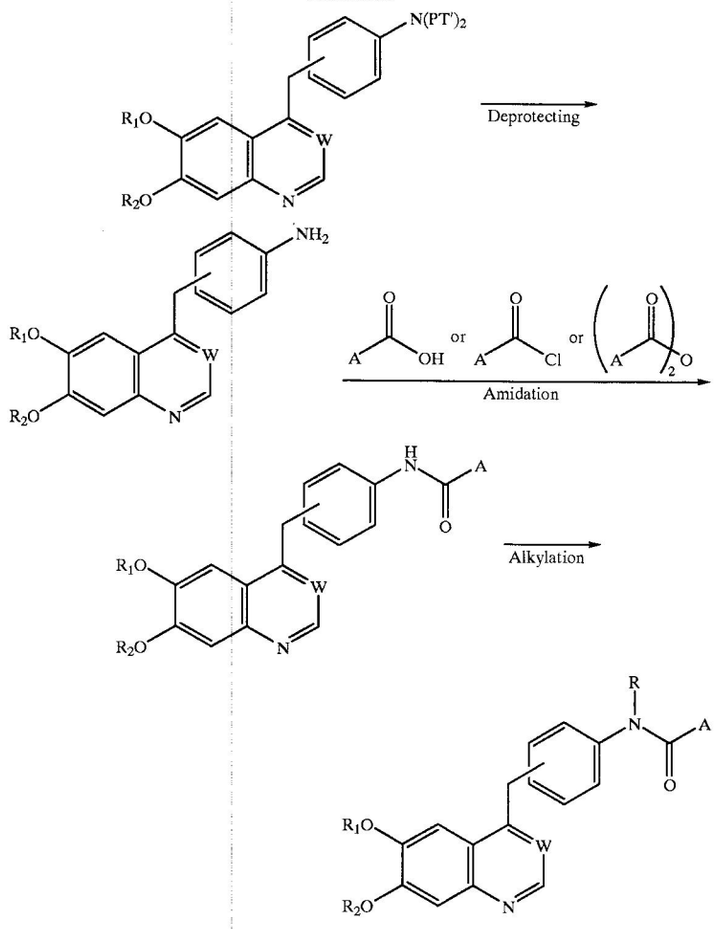
[In the formulas, A, R_1, R_2, W, E and R are defined as described above.]

c. Synthesis of a compound wherein X is CH_2:

A compound represented by formula (I) wherein X is CH_2 can be synthesized by the method described by R. Cutler et al. (J. Am. Chem. Soc. 71, 3375 (1949)). In this method, a base (e.g., sodium hydride) is added to aminophenylacetonitrile with a protected amino group (e.g., by a benzyl group) in an appropriate organic solvent (e.g., toluene), a 4-chloroquinoline derivative or 4-chloroquinazoline derivative is added, and the mixture is refluxed. The diarylsubstituted acetonitrile so obtained is isolated and purified, then dissolved in an acidic solvent (e.g., aqueous sulfuric acid) and refluxed for 1–20 hours, and then the protecting group is removed to obtain a 4-(aminobenzyl)quinoline derivative or 4-(aminobenzyl)quinazoline derivative. The resulting derivative can be amidated using carboxylic acids or their derivative to produce the target compound according to known methods. Further, its N-alkylamides can be produced by reaction with an alkyl halide in an appropriate solvent (e.g., N,N-dimethylformamide) in the presence of a base (e.g., sodium hydride).



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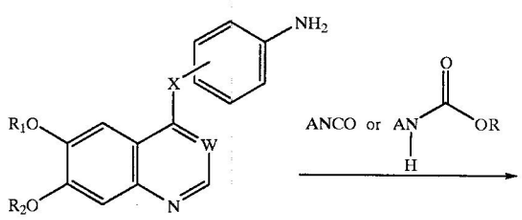


[In the formulas, A, R_1, R_2, W, E and R are defined as described above, and PT' is a protecting group (e.g., benzyl group.]

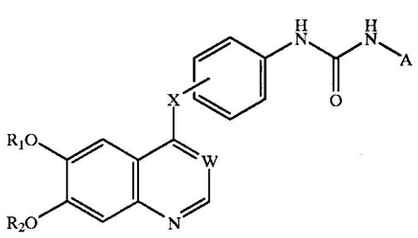
4. Compounds of formula (I) in which Q is represented by formula (V) wherein both j and k are 1

a. Synthesis of a compound wherein B is O:

A compound wherein B is O can be produced by reacting one of the abovementioned intermediates with an isocyanate derivative or carbamate derivative according to known methods.



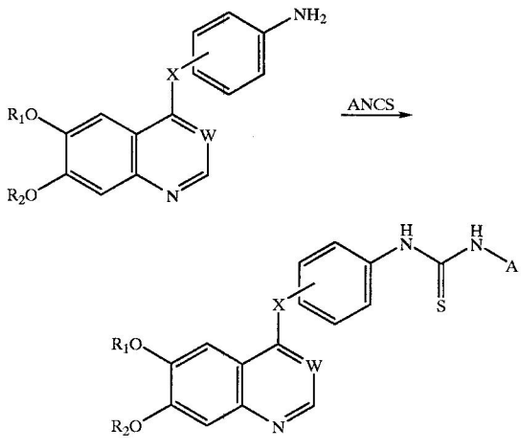
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[In the formulas, A, R_1, R_2, W and R are defined as described above, and X is O, S or CH_2.]

b. Synthesis of a compound wherein B is S:

A compound wherein B is S can be produced by reacting one of the abovementioned intermediates with an isocyanate derivative or thiocarbamate derivative according to known methods.

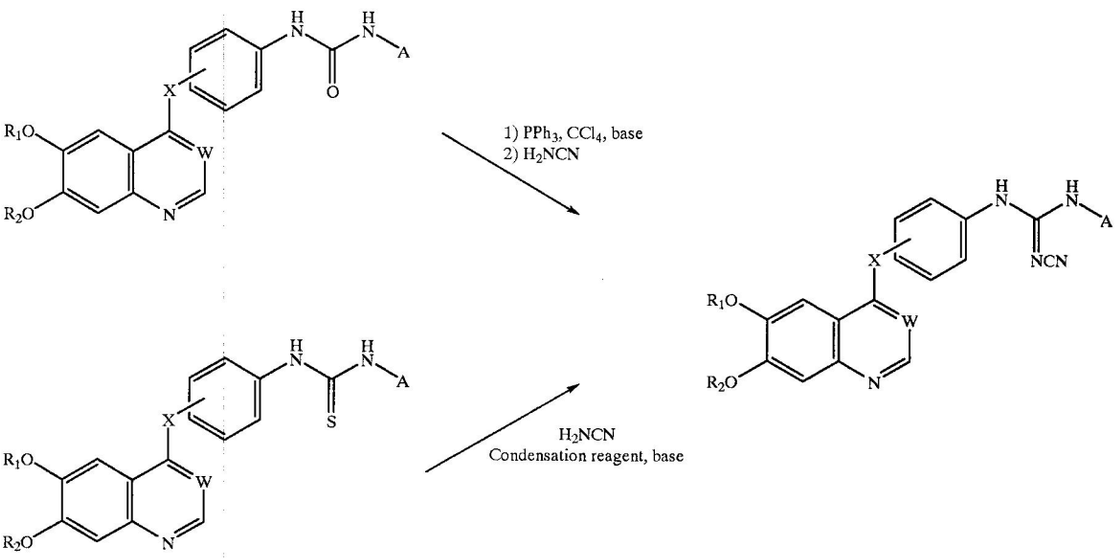


[In the formulas, A, R_1, R_2 and W are defined as described above, and X is O, S or CH_2.]

c. Synthesis of a compound wherein B is NCN:

A compound wherein B is NCN can be produced according to the method described by H. J. Petersen et al. (J. Med. Chem. 21, 773 (1978)).

In this method, the compound obtained in the abovementioned a. is refluxed in the presence of a triphosphorus compound (e.g., triphenylphosphine), a base (e.g., triethylamine) and carbon tetrachloride in an appropriate organic solvent (e.g., methylene chloride) to produce the corresponding carbodiimide, and then the carbodiimide is reacted with cyanamide to obtain the target compound. Alternatively, the target compound can be produced by reacting the compound obtained in the abovementioned b. with cyanamide in the presence of a condensation reagent (e.g., dicyclohexylcarbodiimide) and a base (e.g., ethyldiisopropylamine) in an appropriate organic solvent (e.g., ether).



[In the formulas, A, R_1, R_2 and W are defined as described above, and X is O, S or CH_2.]

Salts of the quinoline derivatives or quinazoline derivatives obtained by the methods of the abovementioned 1–4 can be produced by general methods which are conventionally used to produce salts.

III. Use of compounds of the present invention

Intracellular signal transduction mediated by growth factor receptor autophosphorylation is involved in various pathophysiological situations including neoplastic or other abnormal cell growth. Since quinoline derivatives and quinazoline derivatives of the present invention and their pharmaceutically acceptable salts have an inhibitory activity on platelet-derived growth factor (PDGF) receptor autophophorylation, they are expected to be useful as a therapeutic agent to treat various diseases which are caused by abnormal cell growth generated by PDGF receptor autophosphorylation due to an excessive amount of PDGF (e.g., leukemia, cancers, psoriasis, glomerular nephritis, organofibrosis, atherosclerosis, restenosis after percutaneous coronary angioplasty or bypass surgery and rheumatoid arthritis).

Pharmaceutical compositions which contain compounds of the present invention as an effective component, namely medicinal compositions, can be administered to human and other animals either orally or non-orally (e.g., intravenous, intramuscular, subcutaneous, rectal or endermic administration). Accordingly, medicinal compositions which contain compounds of the present invention as an effective component are prepared into a suitable dosage form depending on the method of administration.

Examples of oral preparations include tablets, capsules, powders, granules and syrups, and examples of non-oral preparations include injections, rectal agents, oily suppositories and aqueous suppositories.

These various pharmaceutical preparations can be produced using ordinally excipients, disintegrating agents, binding agents, lubricating agents, coloring agents, diluents or release controlling agents.

Examples of excipients are lactose, glucose, corn starch, sorbitol, and crystalline cellulose; examples of disintegrating agents are starch, sodium alginate, gelatine powder, calcium carbonate, calcium citrate and dextrine; examples of

bonding agents are dimethylcellulose, polyvinyl alcohol, polyvinyl ether, methycellulose, ethylcellulose, gum arabic, gelatine, hydroxypropylcellulose and polyvinylpyrrolidone; examples of lubricating agents are talc, magnesium stearate, polyethylene glycol and hydrogenated vegetable oils.

Further, the abovementioned injectable agents can be produced by adding buffering agents, pH controlling agents, stabilizing agents or the like, if necessary.

Contents of the compounds of the present invention in the medicinal formulations vary depending on their dosage form, but they are generally between about 0.5 and 50% by wight, preferably between about 1 and 20% by weight of the total.

The particular dose for each individual patient is determined as a function of age, body weight and sex of the patient, type or severity of the disease to be treated. For example, a daily dose of between 1 and 100 mg/kg of body weight, preferably between 1 and 50 mg/kg of body weight, is administered one or more times.

EXAMPLES

The present invention is illustrated in greater detail by the following Examples and Test Examples. The numbers in parentheses at the end of title compound in the Examples correspond to the compound numbers given in Table 1.

Example 1 (Reference Example)

6-Methoxy-2-naphthol

Potassium carbonate (1.244 g) was added to a solution of commercially available 2,6-dihydroxynaphthalene (961 mg) in N,N-dimethylformamide (100 ml), and the admixture was stirred at room temperature for 30 minutes. Dimethyl sulfate (1.14 ml) was slowly added dropwise to the mixture and the resulting mixture was further stirred at room temperature overnight. The reaction mixture thus prepared was neutralized with a 2N HCl solution and then partitioned between water and ethyl acetate, and the ethyl acetate layer was dried with anhydrous magnesium sulfate. After removing the solvent by distillation under reduced pressure, the resulting residue was purified by column chromatography on silica gel (eluting with chloroform/ethyl acetate) to obtain 307 mg of the title compound (yield: 29%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.89 (s, 3H), 7.00~7.18 (m, 4H), 7.53~7.70 (m, 2H)

Mass spectrometry data (FD-MS, m/z): 174 (M^+)

Example 2

6,7-Dimethoxy-4-(6-methoxy-2-naphthyloxy) quinoline [12]

4-Chloro-6,7-dimethoxyquinoline (75 mg) and 6-methoxy-2-naphthol (174 mg) obtained in Example 1 were mixed and stirred at 180° C. for 30 minutes, and the reaction mixture was then purified by thin layer chromatography on silica gel (eluting with hexane/acetone (2/1)) to obtain 71 mg of the title compound (yield: 59%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.96 (s, 3H), 4.07 (s, 3H), 4.08 (s, 3H), 6.53 (d, J=5.71 Hz, 1H), 7.18~7.36 (m, 3H), 7.56~7.91 (m, 5H), 8.50 (br, 1H)

Mass spectrometry data (FD-MS, m/z): 361 (M^+)

Example 3

6,7-Dimethoxy-4-(2-naphthyloxy)quinoline [10]

4-Chloro-6,7-dimethoxyquinoline (45 mg) and commercially available β-naphthol (144 mg) were mixed and stirred at 180° C. for 2 hours, and the reaction mixture was then purified in the same manner as described in Example 2 to obtain 45 mg of the title compound (yield: 68%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.05 (s, 3H), 4.06 (s, 3H), 6.52 (d, J=5.28 Hz, 1H), 7.27~7.61 (m, 6H), 7.75~8.00 (m, 3H), 8.50 (br, 1H)

Mass spectrometry data (FD-MS, m/z): 331 (M^+)

Example 4

6,7-Dimethoxy-4-(7-methoxy-2-naphthyloxy) quinoline [13]

4-Chloro-6,7-dimethoxyquinoline (89 mg) and commercially available 7-methoxy-2-naphthol (209 mg) were mixed and stirred at 180° C. for 30 minutes, and the reaction mixture was then purified in the same manner as described in Example 2 to obtain 59 mg of the title compound (yield: 41%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.92 (s, 3H), 4.05 (s, 3H), 4.07 (s, 3H), 6.54 (d, J=5.49 Hz, 1H), 7.10~7.24 (m, 3H), 7.49~7.91 (m, 5H), 8.49 (d, J=5.49, 1H)

Mass spectrometry data (FD-MS, m/z): 361 (M^+)

Example 5 (Reference Example)

5-Methoxy-1-naphthol

Using commercially available 1,5-dihydroxynaphthalene, the synthesis and purification were carried out in the same manner as described in Example 1 to obtain the title compound (yield: 28%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.00 (s, 3H), 6.79~6.89 (m, 2H), 7.30~7.48 (m, 2H), 7.69~7.90 (m, 2H)

Mass spectrometry data (FD-MS, m/z): 174 (M^+)

Example 6

6,7-Dimethoxy-4-(5-methoxy-1-naphthyloxy) quinoline [11]

4-Chloro-6,7-dimethoxyquinoline (85 mg) and 5-methoxy-1-naphthol (67 mg) obtained in Example 5 were mixed and stirred at 180° C. for 20 minutes, and the reaction mixture was then purified in the same manner as described in Example 2 to obtain 19 mg of the title compound (yield: 14%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.04 (s, 3H), 4.08 (s, 6H), 6.31 (d, J=5.27 Hz, 1H), 6.83~6.92 (m, 1H), 7.34~7.61 (m, 4H), 7.75 (s, 1H), 8.20~8.29 (s, 1H), 8.43 (brs, 1H)

Mass spectrometry data (FD-MS, m/z): 361 (M^+)

Example 7

6,7-Dimethoxy-4-(4-indolyloxy)quinoline [17]

4-Chloro-6,7-dimethoxyquinoline (112 mg) and commercially available 4-hydroxyindole (200 mg) were mixed and stirred at 180° C. for 30 minutes. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and chloroform, and the chloroform layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (3/1) to obtain 51 mg of the title compound (yield: 32%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.95 (s, 3H), 3.96 (s, 3H), 6.08~6.13 (m, 1H), 6.35 (d, J=5.28 Hz, 1H), 6.83~6.93 (m, 1H), 7. 29~7.45 (m, 5H), 7.62 (s, 1H), 8.40 (d, J=5.05 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 320 (M^+)

Example 8

6,7-Dimethoxy-4-(3,4-dimethoxyphenoxy)quinoline [8]

4-Chloro-6,7-dimethoxyquinoline (90 mg) and commercially available 3,4-dimethoxyphenol (187 mg) were mixed and stirred at 180° C. for 30 minutes, and the mixture was then purified by column chromatography on silica gel eluting with hexane/acetone to obtain 26 mg of the title compound (yield: 19%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.87 (s, 3H), 3.93 (s, 3H), 4.05 (s, 3H), 4.06 (s, 3H), 6.45 (d, J=4.9 Hz, 1H), 6.75 (dd, J=2.4 Hz, 9.2 Hz, 1H), 6.75 (d, J=2.4 Hz, 1H), 6.93 (d, J=9.2 Hz, 1H), 7.42 (s, 1H), 7.57 (s, 1H), 8.48 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 341 (M^+)

Example 9

6,7-Dimethoxy-4-(6-quinolyloxy)quinoline [15]

Using 4-chloro-6,7-dimethoxyquinoline (90 mg) and commercially available 6-hydroxyquinoline (176 mg), reaction and purification were carried out in the same manner as described in Example 8 to obtain 62 mg of the title compound (yield: 46%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.05 (s, 3H), 4.06 (s, 3H), 6.56 (d, J=5.5 Hz, 1H), 7.44~7.62 (m, 5H), 8.13 (d, J=8.5 Hz, 1H), 8.22 (d, J=9.2 Hz, 1H), 8.53 (d, J=4.9 Hz, 1H), 8.94 (d, J=4.3 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 332 (M^+)

Example 10

6,7-Dimethoxy-4-(3-fluorophenoxy)quinoline [4]

4-Chloro-6,7-dimethoxyquinoline (100 mg) and commercially available 3-fluorophenol (0.20 ml) were mixed and stirred at 180° C. for 30 minutes. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate and then with chloroform to obtain 125 mg of the title compound (yield: 94%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.04 (s, 3H), 4.06 (s, 3H), 6.56 (d, J=4.9 Hz, 1H), 6.93 (d, J=9.2 Hz, 1H), 6.95~7.05 (m, 2H), 7.39~7.45 (m, 1H), 7.47 (s, 1H), 7.50 (s, 1H), 8.53 (d, J=4.3 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 299 (M^+)

Example 11

6,7-Dimethoxy-4-(3-hydroxyphenoxy)quinoline [5]

4-Chloro-6,7-dimethoxyquinoline (300 mg) and commercially available resorcinol monoacetate (0.835 ml) were mixed and stirred at 180° C. for 30 minutes. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was crystallized with chloroform to obtain 37 mg of the title compound (yield: 9%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.01 (s, 3H), 4.02 (s, 3H), 6.48 (t, J=2.4 Hz, 1H), 6.59 (d, J=5.5 Hz, 1H), 6.77 (dd, J=2.4 Hz, 7.3 Hz, 1H), 6.82 (dd, J=2.4 Hz, 8.6 Hz, 1H), 7.33 (t, J=7.9 Hz, 1H), 7.39 (s, 1H), 7.47 (s, 1H), 8.40 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 297 (M^+)

Example 12

6,7-Dimethoxy-4-(4-bromophenoxy)quinoline [7]

4-Chloro-6,7-dimethoxyquinoline (1.00 g) and commercially available 4-bromophenol (115 mg) were mixed and stirred at 180° C. for 40 minutes. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and chloroform, and the chloroform layer was washed with brine and dried with sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel, eluting in sequence with hexane/acetone, chloroform and chloroform/methanol, to obtain 1.20 g of the title compound (yield: 76%).

^{1}H-NMR ($CDCl_3$, 500 MHz): 4.04 (s, 3H), 4.05 (s, 3H), 6.48 (d, J=4.9 Hz, 1H), 7.08 (d, J=8.5 Hz, 2H), 7.43 (s, 1H), 7.51 (s, 1H), 7.58 (d, J=8.5 Hz, 2H), 8.51 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 359 (M^+), 361 (M^++2)

Example 13

6,7-Dimethoxy-4-(2-methoxyphenoxy)quinoline [1]

4-Chloro-6,7-dimethoxyquinoline (102 mg) and commercially available 2-methoxyphenol (0.5 ml) were mixed and stirred at 150° C. for 8 hours. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting first with hexane/ethyl acetate and then with chloroform/methanol to obtain 76 mg of the title compound (yield: 54%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.78 (s, 3H), 4.05 (s, 3H), 4.05 (s, 3H), 6.31 (d, J=5.3 Hz, 1H), 6.9~7.4 (m, 4H), 7.42 (s, 1H), 7.63 (s, 1H), 8.45 (d, J=5.3 Hz, 1H)

Mass spectrometry data (FAB-MS, m/z): 312 (M^++1)

Example 14

6,7-Dimethoxy-4-(3-methoxyphenoxy)quinoline [2]

4-Chloro-6,7-dimethoxyquinoline (103 mg) and commercially available 3-methoxyphenol (158 mg) were mixed and stirred at 160° C. for 9 hours. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate to obtain 29 mg of the title compound (yield: 52%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.82 (s, 3H), 4.04 (s, 3H), 4.05 (s, 3H), 6.53 (d, J=5.3 Hz, 1H), 6.7~6.9 (m, 3H), 7.35 (t, J=7.0 Hz, 1H), 7.43 (s, 1H), 7.54 (s, 1H), 8.50 (d, J=5.3 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 311 (M^+)

Example 15

6,7-Dimethoxy-4-(4-methoxyphenoxy)quinoline [3]

4-Chloro-6,7-dimethoxyquinoline (103 mg) and commercially available 4-methoxyphenol (286 mg) were mixed and stirred at 150° C. for 6 hours. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and dried with anhydrous sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting first with hexane/ethyl acetate and then with chloroform/ethyl acetate to obtain 2.21 g of the title compound (yield: 88%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.85 (s, 3H), 4.05, (s, 3H), 4.05 (s, 3H), 6.41 (d, J=5.3 Hz, 1H), 6.97 (d, J=9.5 Hz, 2H), 7.14 (d, J=9.5 Hz, 2H), 7.43 (s, 1H), 7.58 (s, 1H), 8.46 (d, J=5.3 Hz, 1H)

Mass spectrometry data (FAB-MS, m/z): 312 (M^++1)

Example 16

6,7-Dimethoxy-4-(5-quinolyloxy)quinoline [14]

4-Chloro-6,7-dimethoxyquinoline (50 mg) and commercially available 5-hydroxyquinoline (50 mg) were mixed and stirred at 170° C. for 10 minutes. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by thin layer chromatography on silica gel eluting first with hexane/ethyl acetate (1/1) and then with chloroform/methanol (50/1) to obtain 47 mg of the title compound (yield: 64%).

^{1} H-NMR ($CDCl_3$, 90 MHz): δ 4.06 (s, 3H), 4.07 (s, 3H), 6.35 (d, J=5.3 Hz, 1H), 7.2~7.5 (m, 3H), 7.68 (s, 1H), 7.7~8.4 (m, 3H), 8.46 (d, J=5.3 Hz, 1H), 8.49 (dd, J=1.76 Hz, 4.17 Hz, 1 H)

Mass spectrometry data (FD-MS, m/z): 332 (M^+)

Example 17

6,7-Dimethoxy-4-(1-naphthyloxy)quinoline [9]

4-Chloro-6,7-dimethoxyquinoline (97 mg) and commercially available 1-naphthol (340 mg) were mixed and stirred at 150° C. for 8 hours. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting first with hexane/ethyl acetate (3/1) and then with chloroform/methanol (100/1), and further purified by thin layer chromatography on silica gel eluting with hexane/ethyl acetate (1/1) to obtain 57 mg of the title compound (yield: 40%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.05 (s, 3H), 4.05 (s, 3H), 6.32 (d, J=5.3 Hz, 1H), 7.2~7.6 (m, 5H), 7.74 (s, 1H), 7.8~8.0 (m, 3H), 8.35 (d, J=5.3 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 331 (M^+)

Example 18

6,7-Dimethoxy-4-(5-indolyloxy)quinoline [16]

4-Chloro-6,7-dimethoxyquinoline (1.0 g) and commercially available 5-hydroxyindole (1.19 g) were mixed and stirred at 150° C. for 1.5 hours. The reaction mixture was neutralized with saturated aqueous sodium hydrogen carbonate and then partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting in sequence with hexane/ethyl acetate, chloroform and chloroform/methanol, to obtain 898 mg of the title compound (yield: 63%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.05 (s, 3H), 4.07 (s, 3H), 6.42 (d, J=5.3 Hz, 1H), 6.5~6.7 (m, 1H), 6.9~7.6 (m, 5H), 7.67 (s, 1H), 8.44 (d, J=5.3 Hz, 1H), 8.55 (br, 1H)

Mass spectrometry data (FD-MS, m/z): 320 (M^+)

Example 19

6,7-Dimethoxy-4-(3-methoxyphenylthio)quinoline [117]

4-Chloro-6,7-dimethoxyquinoline (50 mg) and commercially available 3-methoxybenzenethiol (77 mg) were mixed and stirred at 180° C. for 30 seconds. Purification was carried out in the same manner as described in Example 18 to obtain 72 mg of the title compound (yield: 100%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.79 (s, 3H), 4.01 (s, 3H), 4.03 (s, 3H), 6.84 (d, J=4.8 Hz, 1H), 6.9~7.5 (m, 6H), 8.45 (d, J=5.1 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 327 (M^+)

Example 20

6,7-Dimethoxy-4-(4-methoxyphenylthio)quinoline [118]

4-Chloro-6,7-dimethoxyquinoline (50 mg) and commercially available 4-methoxybenzenethiol (63 mg) were mixed and stirred at 150° C. for 5 minutes. Purification was carried out in the same manner as described in Example 18, and further crystallization with chloroform gave 80 mg of the title compound (yield: 100%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.88 (s, 3H), 4.03 (s, 3H), 4.05 (s, 3H), 6.60 (d, J=4.8 Hz, 1H), 7.15 (d, J=8.7 Hz, 2H), 7.38 (s, 1H), 7.39 (s, 1H), 7.52 (d, J=8.8 Hz, 2H), 8.39 (d, J=5.1 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 327 (M^+)

Example 21 (Reference Example)

4-Bromo-1-methoxymethylphenol

Commercially available 4-bromophenol (17.3 g) was dissolved in N,N-dimethylformamide (90 ml), to which sodium hydride (2.64 g) was added while cooled in ice. After stirring at room temperature overnight, chloromethyl methyl ether (8.35 ml) was added, and the admixture was stirred for a further 1 hour. The reaction mixture was partitioned between

water and ethyl acetate, and the ethyl acetate layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/acetone to obtain 18.25 g of the title compound (yield: 84%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.46 (s, 3H), 5.13 (s, 2H), 6.91 (d, J=9.2 Hz, 2H), 7.38 (d, J=9.2 Hz, 2 H)

Mass spectrometry data (FD-MS, m/z): 216 (M^+), 218 (M^++2)

Example 22 (Reference Example)

4-Tri-n-butyltin-1-methoxymethylphenol

4-Bromo-1-methoxymethylphenol (15.99 g) obtained in Example 21 was dissolved in anhydrous tetrahydrofuran (20 ml), the solution was added to commercially available magnesium powder (1.97 g) under argon, and a drop of an iodine solution in anhydrous tetrahydrofuran was added to the solution to start the reaction. When magnesium disappeared and the temperature of the reaction mixture returned to room temperature, commercially available tri-n-butyltin chloride (23.99 g) dissolved in tetrahydrofuran (10 ml) was added slowly dropwise. The reaction mixture was stirred at room temperature for 4 hours and then partitioned between 5% aqueous ammonium chloride and chloroform. The chloroform layer was dried with anhydrous magnesium sulfate and the solvent was then removed by reduced-pressure distillation to obtain 31.39 g of the title compound (yield: 100%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 0.80–1.65 (m, 27H), 3.48 (s, 3H), 5.17 (s, 2H), 6.91~7.42 (m, 4H)

Mass spectrometry data (FD-MS, m/z): 428 (M^++1)

Example 23 (Reference Example)

4-Hydroxyphenyl 4-trifluoromethylphenyl ketone

4-Tri-n-butyltin-1-methoxymethylphenol (1.282 g) obtained in Example 22 and commercially available 4-(trifluoromethyl)benzoyl chloride (626 mg) were dissolved in chloroform (5 ml), commercially available bis (triphenylphosphine)palladium (II) chloride (8 mg) was added, and the admixture was refluxed for 5 hours. The reaction mixture was partitioned between water and ether, the ether layer was washed with saturated aqueous potassium fluoride, and the ether layer obtained by partitioning with brine was dried with anhydrous magnesium sulfate. The residue (1.028 g) obtained by removing the solvent by reduced-pressure distillation was dissolved in tetrahydrofuran (2 ml), water (5 ml) and 6 N aqueous hydrochloric acid (12 ml) were added, and the admixture was refluxed for 4.5 hours. The resulting reaction mixture was partitioned between brine and ether, and the ether layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/acetone to obtain 348 mg of the title compound (yield: 30%).

Mass spectrometry data (FD-MS, m/z): 266 (M^+)

Example 24

(4-Trifluoromethylphenyl){4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}methanone [29]

4-Chloro-6,7-dimethoxyquinoline (81 mg) and 4-hydroxyphenyl 4-trifluoromethylphenyl ketone (288 mg) obtained in Example 23 were mixed and stirred at 180° C. for 20 minutes, and the reaction mixture was then purified by thin layer chromatography on silica gel eluting with hexane/acetone (2/1) to obtain 98 mg of the title compound (yield: 60%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.03 (s, 3H), 4.06 (s, 3H), 6.68 (d, J=4.9 Hz, 1H), 7.29 (d, J=8.5 Hz, 2H), 7.46 (s, 1H), 7.47 (s, 1H), 7.78 (d, J=7.9 Hz, 2H), 7.91 (d, J=7.9 Hz, 2H), 7.94 (d, J=9.2 Hz, 2H), 8.59 (d, J=4.9 Hz, 1H),

Mass spectrometry data (FD-MS, m/z): 453 (M^+)

Example 25 (Reference Example)

4-Hydroxyphenyl 4-methylphenyl ketone

4-Tri-n-butyltin-1-methoxymethylphenol (1.282 g) obtained in Example 22 and commercially available 4-toluoyl chloride (464 mg) were dissolved in chloroform (5 ml), commercially available bis(triphenylphosphine) palladium(II) chloride (8 mg) was added, and the admixture was refluxed for 8 hours. The reaction mixture was partitioned in the same manner as described in Example 23 and the resulting ether layer was dried with anhydrous magnesium sulfate. The residue (967 mg) obtained by removing the solvent by reduced-pressure distillation was dissolved in tetrahydrofuran (0.5 ml), water (4 ml) and 6 N aqueous hydrochloric acid (10 ml) were added, and the admixture was refluxed for 7 hours. The reaction mixture was treated in the same manner as described in Example 23 to obtain 271 mg of the title compound (yield: 34%).

Mass spectrometry data (FD-MS, m/z): 212 (M^+)

Example 26

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(4-methylphenyl)methanone [30]

4-Chloro-6,7-dimethoxyquinoline (89 mg) and 4-hydroxyphenyl 4-methylphenyl ketone (248 mg) obtained in Example 25 were mixed and stirred at 180° C. for 10 minutes, and the reaction mixture was then purified in the same manner as described in Example 24 to obtain 118 mg of the title compound (yield: 74%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.45 (s, 3H), 4.03 (s, 3H), 4.06 (s, 3H), 6.65 (d, J=5.5 Hz, 1H), 7.27 (d, J=8.6 Hz, 2H), 7.30 (d, J=7.9 Hz, 2H), 7.46 (s, 1H), 7.49 (s, 1H), 7.74 (d, J=7.9 Hz, 2H), 7.91 (d, J=8.5 Hz, 2H), 8.57 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 399 (M^+)

Example 27 (Reference Example)

3-Chlorophenyl 4-hydroxyphenyl ketone

4-Tri-n-butyltin-1-methoxymethylphenol (1.282 g) obtained in Example 22 and commercially available 3-chlorobenzoyl chloride (525 mg) were dissolved in chloroform (5 ml), commercially available bis (triphenylphosphine)palladium(II) chloride (8 mg) was added, and the admixture was refluxed for 7 hours. The reaction mixture was partitioned in the same manner as described in Example 23, and the resulting ether layer was dried with anhydrous magnesium sulfate. A portion (1.563 g) of the residue (1.914 g) obtained by removing the solvent by reduced-pressure distillation was dissolved in tetrahydrofuran (1 ml), water (7 ml) and 6 N aqueous hydrochloric acid (17 ml) were added, and the admixture was refluxed for 8 hours. The reaction mixture was treated in the same

manner as described in Example 23 to obtain 313 mg of the title compound (yield: 22%).

Mass spectrometry data (FD-MS, m/z): 232 (M^+)

Example 28

(3-Chlorophenyl){4-[(6,7-dimethoxy-4-quinolyl) oxy]phenyl}methanone [31]

4-Chloro-6,7-dimethoxyquinoline (89 mg) and 3-chlorophenyl 4-hydroxyphenyl ketone (279 mg) obtained in Example 27 were mixed and stirred at 180° C. for 20 minutes. The reaction mixture was then purified in the same manner as described in Example 24 to obtain 67 mg of the title compound (yield: 40%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.05 (s, 3H), 4.01 (s, 3H), 6.71 (d, J=5.5 Hz, 1H), 7.31 (d, J=8.5 Hz, 2H), 7.51 (s, 1H), 7.69 (s, 1H), 7.94 (d, J=8.5 Hz, 2H), 8.12 (s, 1H), 7.37~8.03 (m, 4H)

Mass spectrometry data (FD-MS, m/z): 419 (M^+)

Example 29 (Reference Example)

4-t-Butylphenyl 4-methoxyphenyl ketone

To commercially available nitromethane (5 ml) were added commercially available anisole (541 mg), commercially available 4-t-butylbenzoyl chloride (983 mg) and commercially available scandium(III) trifluoromethanesulfonate (492 mg), and the admixture was stirred at 60° C. for 21 hours. The reaction mixture was partitioned between water and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/acetone to obtain 862 mg of the title compound (yield: 64%).

Mass spectrometry data (FD-MS, m/z): 268 (M^+)

Example 30 (Reference Example)

4-t-Butylphenyl 4-hydroxyphenyl ketone

4-t-Butylphenyl 4-methoxyphenyl ketone (862 mg) obtained in Example 29 was dissolved in N,N-dimethylformamide (35 ml), sodium thiomethoxide (562 mg) was added, and the admixture was refluxed for 3 hours under argon. The reaction mixture was partitioned between 10% aqueous phosphoric acid and ethyl acetate. The ethyl acetate layer was then dried with anhydrous magnesium sulfate and the solvent was removed by reduced-pressure distillation to obtain 794 mg of the title compound (yield: 97%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.36 (s, 9H), 6.92 (d, J=8.5 Hz, 2H), 7.49 (d, J=8.6 Hz, 2H), 7.72 (d, J=7.9 Hz, 2H), 7.79 (d, J=8.5 Hz, 2H)

Mass spectrometry data (FD-MS, m/z): 254 (M^+)

Example 31

(4-t-Butylphenyl){4-[(6,7-dimethoxy-4-quinolyl) oxy]phenyl}methanone [32]

4-Chloro-6,7-dimethoxyquinoline (341 mg) and 4-t-butylphenyl 4-hydroxyphenyl ketone (775 mg) obtained in Example 30 were mixed and stirred at 150° C. for 15 minutes. The reaction mixture was then purified by column chromatography on silica gel eluting with hexane/acetone to obtain 72 mg of the title compound (yield: 11%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.38 (s, 9H), 4.04 (s, 3H), 4.06 (s, 3H), 6.65 (d, J=5.5 Hz, 1H), 7.27 (d, J=8.6 Hz, 2H), 7.45 (s, 1H), 7.50 (s, 1H), 7.52 (d, J=8.5 Hz, 2H), 7.78 (d, J=8.6 Hz, 2H), 7.94 (d, J=8.6 Hz, 2H), 8.57 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FAB-MS, m/z): 442 (M^++1)

Example 32 (Reference Example)

4-Biphenyl 4-methoxyphenyl ketone

To commercially available nitromethane (50 ml) were added commercially available anisole (3.244 g), commercially available 4-phenylbenzoyl chloride (6.500 g) and commercially available ytterbium(III) trifluoromethanesulfonate (1.861 g), and the admixture was stirred at 60° C. for 24 hours. The reaction mixture was partitioned between water and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, a portion (1.00 g) of the resulting residue (9.842 g) was purified by column chromatography on silica gel eluting with hexane/acetone to obtain 300 mg of the title compound (yield: 34%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.90 (s, 3H), 6.98 (d, J=8.5 Hz, 2H), 7.40 (t, J=7.3 Hz, 1H), 7.48 (t, J=7.3 Hz, 2H), 7.65 (d, J=7.3 Hz, 2H), 7.76 (d, J=8.6 Hz, 2H), 7.85 (d, J=8.5 Hz, 2H), 7.87 (d, J=9.2 Hz, 2H)

Mass spectrometry data (FD-MS, m/z): 288 (M^+)

Example 33 (Reference Example)

4-Biphenyl 4-hydroxyphenyl ketone

4-Biphenyl 4-methoxyphenyl ketone (267 mg) obtained in Example 32 was dissolved in N,N-dimethylformamide (20 ml), sodium thiomethoxide (162 mg) was added, and the admixture was refluxed under argon for 3 hours. The reaction mixture was partitioned between 10% aqueous phosphoric acid and ethyl acetate. The ethyl acetate layer was washed with 0.5 N aqueous silver nitrate and then dried with anhydrous magnesium sulfate, the solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate to obtain 207 mg of the title compound (yield: 82%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 6.91 (d, J=8.5 Hz, 2H), 7.43 (t, J=7.3 Hz, 1H), 7.52 (dd, J=7.3 Hz, 7.9 Hz, 2H), 7.70 (d, J=8.5 Hz, 2H), 7.75 (d, J=7.9 Hz, 2H) 7.77 (d, J=7.9 Hz, 2H), 7.83 (d, J=7.9 Hz, 2H)

Mass spectrometry data (FD-MS, m/z): 274 (M^+)

Example 34

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(4-biphenyl)methanone [33]

4-Chloro-6,7-dimethoxyquinoline (65 mg) and 4-biphenyl 4-hydroxyphenyl ketone (79 mg) obtained in Example 33 were mixed and stirred at 180° C. for 30 minutes. The reaction mixture was purified by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (10/1) to obtain 50 mg of the title compound (yield: 37%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.11 (s, 3H), 4.14 (s, 3H), 6.74 (d, J=5.3 Hz, 1H), 7.30~8.09 (m, 15H), 8.65 (d, J=5.3 Hz, 1H)

Mass spectrometry data (FAB-MS, m/z): 462 (M^+)

Example 35 (Reference Example)

4-Methoxyphenyl 2-naphthyl ketone

To commercially available nitromethane (10 ml) were added commercially available anisole (1.081 g), commer-

Hz, 1H), 7.27 (s, 1H), 7.28 (s, 1H), 7.47 (d, J=8.5 Hz, 2H), 7.86 (d, J=8.5 Hz, 1H), 7.88 (d, J=8.5 Hz, 1H), 7.90 (d, J=8.5 Hz, 2H), 8.58 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 403 (M^+)

Example 43

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(phenyl) methanone [39]

4-Chloro-6,7-dimethoxyquinoline (91 mg) and commercially available 4-hydroxybenzophenone (243 mg) were mixed and stirred at 180° C. for 30 minutes. The reaction mixture was purified in the same manner as described in Example 31 to obtain 38 mg of the title compound (yield: 24%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.03 (s, 3H), 4.05 (s, 3H), 6.66 (d, J=5.3 Hz, 1H), 7.31~7.99 (m, 11H), 8.57 (d, J=5.3 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 385 (M^+)

Example 44 (Reference Example)

3,5-dimethoxy toluene

Commercially available orcinol (5.77 g) was dissolved in acetonitrile (200 ml), dimethyl sulfate (8.47 ml) and potassium carbonate (12.3 g) were added, and the admixture was refluxed for 2.5 hours. The reaction mixture was poured into ice water and partitioned with chloroform, and the chloroform layer was dried with anhydrous magnesium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate (5/1) to obtain 5.04 g of the title compound (yield: 82%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.30 (s, 3H), 3.76 (s, 6H), 6.30 (s, 1H), 6.37 (s, 2H)

Example 45 (Reference Example)

2,6-Dimethoxy-4-methylphenyl 4-methoxyphenyl ketone

3,5-Dimethoxy toluene (1.00 g) obtained in Example 44 was dissolved in anhydrous tetrahydrofuran (10 ml), commercially available 2.5 M n-butyllithium-hexane solution (2.90 ml) was added at −45° C. under nitrogen, and the admixture was stirred for 3 hours. Commercially available 4-methoxybenzoyl chloride (1.23 g) was dissolved in tetrahydrofuran (10 ml), and the resulting solution was added slowly to the admixture at −45° C. The reaction mixture was stirred at room temperature for 1 hour and then partitioned between water and chloroform. The chloroform layer was dried with anhydrous magnesium sulfate, the solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate (5/1) to obtain 384 mg of the title compound (yield: 20%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.40 (s, 3H), 3.69 (s, 6H), 3.85 (s, 3H), 6.43 (s, 2H), 6.89 (d, J=9.2 Hz, 2H), 7.82 (d, J=9.2 Hz, 2H)

Example 46 (Reference Example)

2,6-Dihydroxy-4-methylphenyl 4-hydroxyphenyl ketone

2,6-Dimethoxy-4-methylphenyl 4-methoxyphenyl ketone (321 mg) obtained in Example 45 was dissolved in anhydrous methylene chloride (10 ml), commercially available boron tribromide (349 ml) was added slowly while cooled in ice, and the admixture was stirred at room temperature overnight. The reaction mixture was poured into ice water and then partitioned with ethyl acetate. The ethyl acetate layer was washed with brine and then dried with anhydrous magnesium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate to obtain 87 mg of the title compound (yield: 68%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.16 (s, 3H), 6.16 (s, 2H), 6.79 (d, J=8.6 Hz, 2H), 7.56 (d, J=8.5 Hz, 2H)

Example 47

(2,6-Dihydroxy-4-methylphenyl){4-[6,7-dimethoxy-4-quinolyl)oxy]phenyl}methanone [42]

4-Chloro-6,7-dimethoxyquinoline (61 mg) and 2,6-dihydroxy-4-methylphenyl 4-hydroxyphenyl ketone (100 mg) obtained in Example 46 were dissolved in diethylene glycol dimethyl ether (0.5 ml), and the solution was stirred at 170° C. for 20 minutes. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was washed with brine and then dried with anhydrous magnesium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 32 mg of the title compound (yield: 27%).

^{1}H-NMR (DMSO, 500 MHz): δ 2.18 (s, 3H), 3.90 (s, 3H), 3.95 (s, 3H), 6.20 (s, 2H) 6.68 (d, J=4.9 Hz, 1H), 7.32 (d, J=9.2 Hz, 2H), 7.42 (s, 1H), 7.43 (s, 1H), 7.82 (d, J=8.6 Hz, 2H), 8.54 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 432 (M^++1)

Example 48 (Reference Examples)

6,7-Dimethoxy-4-(4-nitrophenoxy)quinoline

4-Chloro-6,7-dimethoxyquinoline (1.84 g) and commercially available 4-nitrophenol (3.42 g) was mixed and stirred at 170° C. for 50 minutes. After cooling to room temperature in air, aqueous sodium hydrogen carbonate was added to the reaction mixture, and the admixture was extracted 3 times with ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol to obtain 4.54 g of the title compound (yield: 89%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.01 (s, 3H), 4.06 (s, 3H), 6.69 (d, J=4.9 Hz, 1H), 7.27 (d, J=9.1 Hz, 2H), 7.37 (s, 1H), 7.47 (s, 1H), 8.32 (d, J=9.1 Hz, 2H), 8.62 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 326 (M^+)

Example 49 (Reference Example)

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline

6,7-Dimethoxy-4-(4-nitrophenoxy)quinoline (1.00 g) was dissolved in N,N-dimethylformamide/ethyl acetate (30 ml/15 ml), 10% palladium hydroxide-carbon (69 mg) was added, and the admixture was stirred at room temperature under hydrogen for 17 hours. The reaction mixture was filtered using Celite. The filtrate was washed with brine and

then dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation to obtain 799 mg of the title compound (yield: 88%).

^{1}H-NMR (CD_3OD, 500 MHz): δ 4.00 (s, 3H), 4.00 (s, 3H), 6.47 (d, J=5.5 Hz, 1H), 6.82 (d, J=8.6 Hz, 2H), 6.96 (d, J=9.2 Hz, 2H), 7.32 (s, 1H), 7.62 (s, 1H), 8.36 (d, J=5.5 Hz, 1H),

Mass spectrometry data (FD-MS, m/z): 296 (M^+)

Example 50

N-{4-[6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-3-pyridinecarboxamide [61]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (57 mg) and commercially available nicotinic acid (37 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (79 mg) was added, and the admixture was stirred at room temperature for 5 hours. The reaction mixture was partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting crystallized product was washed with ether to obtain 47 mg of the title compound (yield: 61%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.05 (s, 3H), 4.06 (s, 3H), 6.49 (d, J=4.9 Hz, 1H), 7.22 (d, J=8.6 Hz, 2H) 7.42 (s, 1H), 7.47 (dd, J=4.9, 7.9 Hz, 1H), 7.56 (s, 1H), 7.77 (d, J=9.2 Hz, 1H), 8.26 (d, J=8.5 Hz, 2H), 8.49 (d, J=4.9 Hz, 1H), 8.79 (d, J=3.7 Hz, 1H), 9.13 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 401 (M^+)

Example 51

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(3,4-dimethoxyphenyl)carboxamide [62]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (56 mg) and commercially available 3,4-dimethoxybenzoic acid (60 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (81 mg) was added, and the admixture was stirred at room temperature for 22 hours. The reaction mixture was partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting chloroform/acetone (10/1) to obtain 7 mg of the title compound (yield: 8%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.87 (s, 3H), 3.87 (s, 3H), 4.06 (s, 3H), 4.07 (s, 3H), 6.48 (d, J=5.5 Hz, 1H), 6.63 (d, J=8.5 Hz, 2H), 7.18 (d, J=9.2 Hz, 2H), 7.34 (m, 2H), 7.43 (s, 1H), 7.55 (s, 1H), 7.57 (s, 1H), 7.76 (d, J=8.6 Hz, 2H), 8.48 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 460 (M^+)

Example 52

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-cyclohexanecarboxamide [63]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) and commercially available cyclohexanecarboxylic acid (46 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (84 mg) was added, and the admixture was stirred at room temperature for 22 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 45 mg of the title compound (yield: 64%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.30 (m, 2H), 1.56 (m, 2H), 1.73 (m, 2H), 1.85 (m, 2H), 1.97 (m, 2H), 2.26 (m, 1H), 4.05 (s, 3H), 4.05 (s, 3H), 6.43 (d, J=5.5 Hz, 1H), 7.14 (d, J=8.5 Hz, 2H), 7.36 (s, 1H), 7.42 (s, 1H), 7.55 (s, 1H), 7.63 (d, J=8.5 Hz, 2H), 8.47 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 406 (M^+)

Example 53

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-2-furancarboxamide [64]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (55 mg) and commercially available 2-furancarboxylic acid (32 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (91 mg) was added, and the admixture was stirred at room temperature for 22 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 35 mg of the title compound (yield: 48%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.04 (s, 3H), 4.05 (s, 3H), 6.49 (d, J=4.9 Hz, 1H), 6.57 (dd, J=1.8, 8.7 Hz, 1H), 7.20 (d, J=8.6 Hz, 2H), 7.28 (d, J=7.9 Hz, 1H), 7.42 (s, 1H), 7.51 (s, 1H), 7.56 (s, 1H), 7.77 (d, J=9.2 Hz, 2H), 8.35 (s, 1H), 8.49 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 390 (M^+)

Example 54

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-3-thiophenecarboxamide [65]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) and commercially available 3-thiophenecarboxylic acid (44 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (82 mg) was added, and the admixture was stirred at room temperature for 31 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 41 mg of the title compound (yield: 55%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.02 (s, 3H), 4.04 (s, 3H), 6.46 (d, J=5.5 Hz, 1H), 7.17 (d, J=8.6 Hz, 2H), 7.38 (m, 1H), 7.55 (d, J=1.2 Hz, 1H), 7.56 (s, 1H), 7.73 (d, J=8.6 Hz, 2H), 8.05 (dd, J=1.2, 3.1 Hz, 1H), 8.37 (s, 1H), 8.47 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 406 (M^+)

Example 55

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-nitrophenyl)carboxamide [66]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (110 mg) and commercially available 4-nitrobenzoic acid (100 mg) were dissolved in N,N-dimethylformamide (4 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (176 mg) was added, and the admixture was stirred at room temperature for 22 hours. The reaction mixture was then purified in the same manner as described in Example 50 to obtain 126 mg of the title compound (yield: 77%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.49 (d, J=5.5 Hz, 1H), 7.29 (d, J=9.2 Hz, 2H), 7.39 (s, 1H), 7.52 (s, 1H), 7.93 (d, J=9.2 Hz, 2H), 8.21 (d, J=8.6 Hz, 2H), 8.39 (d, J=9.2 Hz, 2H), 8.48 (d, J=5.5 Hz, 1H), 10.7 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 445 (M^+)

Example 56

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(N,N-dimethylaminophenyl)carboxamide [67]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) and commercially available 4-(dimethylamino)benzoic acid (44 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (83 mg) was added, and the admixture was stirred at room temperature for 16 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 7 mg of the title compound (yield: 10%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.06 (s, 6H), 4.05 (s, 3H), 4.06 (s 3H), 6.48 (d, J=4.9 Hz, 1H), 6.71 (d, J=8.6 Hz, 2H), 7.17 (d, J=8.5 Hz, 2H), 7.44 (s, 1H), 7.57 (s, 1H), 7.74 (d, J=8.5 Hz, 2H), 7.81 (d, J=9.2 Hz, 2H), 7.94 (s, 1H), 8.49 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 443 (M^+)

Example 57

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-acetylphenyl)carboxamide [68]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) and commercially available 4-acetylbenzoic acid (46 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (87 mg) was added, and the admixture was stirred at room temperature for 22 hours. The reaction mixture was then purified in the same manner as described in Example 50 to obtain 43 mg of the title compound (yield: 53%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 2.66 (s, 3H), 3.94 (s, 3H), 3.95 (s, 3H), 6.48 (d, J=4.9 Hz, 1H), 7.28 (d, J=9.2 Hz, 2H), 7.39 (s, 1H), 7.52 (s, 1H), 7.94 (d, J=9.2 Hz, 2H), 8.10 (m, 4H), 8.48 (d, J=5.5 Hz, 1H), 10.6 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 442 (M^+)

Example 58

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-butylphenyl)carboxamide [69]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) and commercially available 4-butyl benzoate (54 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (85 mg) was added, and the admixture was stirred at room temperature for 22 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 65 mg of the title compound (yield: 78%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.95 (t, J=7.3 Hz, 3H), 1.38 (m, 2H), 1.64 (m, 2H), 2.69 (t, J=8.0 Hz, 2H), 4.05 (s, 3H), 4.06 (s, 3H), 6.48 (d, J=5.5 Hz, 1H), 7.19 (d, J=9.2 Hz, 2H), 7.30 (d, J=8.6 Hz, 2H), 7.42 (s, 1H), 7.57 (s, 1H), 7.75 (d, J=8.6 Hz, 2H), 7.82 (d, J=8.6 Hz, 2H), 8.03 (s, 1H), 8.49 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 456 (M^+)

Example 59

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-aminophenyl)carboxamide [70]

{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-nitrophenyl)carboxamide (100 mg) was dissolved in N,N-dimethylformamide/ethyl acetate (9 ml/5 ml), 10% palladium-carbon (69 mg) was added, and the admixture was stirred at room temperature under hydrogen for 22 hours. The reaction mixture was filtered using Celite. The filtrate was then distilled under reduced pressure to remove the solvents, and the resultant residue was purified by column chromatography on silica gel eluting chloroform/acetone to obtain 85 mg of the title compound (yield: 91%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 5.75 (brs, 2H), 6.46 (d, J=4.9 Hz, 1H), 6.61 (d, J=8.6 Hz, 2H), 7.22 (d, J=9.2 Hz, 2H), 7.39 (s, 1H), 7.52 (s, 1H), 7.73 (d, J=8.5 Hz, 2H), 7.89 (d, J=9.2 Hz, 2H), 8.46 (d, J=5.5 Hz, 1H), 9.90 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 415 (M^+)

Example 60

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-4-pyridinecarboxamide [71]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) and commercially available isonicotinic acid (45 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (98 mg) was added, and the admixture was stirred at room temperature for 62 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 22 mg of the title compound (yield: 31%).

^{1}H-NMR(DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.96 (s, 3H), 6.48 (d, J=5.5 Hz, 1H), 7.29 (d, J=9.2 Hz, 2H), 7.39 (s, 1H), 7.52 (s, 1H), 7.88 (d, J=4.3 Hz, 2H), 7.92 (d, J=8.5 Hz, 2H), 8.48 (d, J=4.9 Hz, 1H), 8.80 (d, J=4.3 Hz, 2H), 10.6 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 401 (M^+)

Example 61

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-2-thiophenecarboxamide [72]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) and commercially available 2-thiophenecarboxylic acid (46 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (99 mg) was added, and the admixture was stirred at room temperature for 62 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 37 mg of the title compound (yield: 54%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.02 (s, 3H), 4.04 (s, 3H), 6.46 (d, J=5.5 Hz, 1H), 7.11 (m, 1H), 7.17 (d, J=8.6 Hz, 2H), 7.40 (s, 1H), 7.55 (m, 1H), 7.56 (s, 1H), 7.73 (d, J=8.5 Hz, 2H), 7.73 (m, 1H), 8.38 (s, 1H), 8.48 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 406 (M^+)

Example 62

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-4-biphenylcarboxamide [73]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was suspended in triethylamine/methylene chloride (3 ml/2 ml), commercially available biphenylcarbonyl chloride (80 mg) was added, and the admixture was stirred at room temperature for 25 hours. Aqueous sodium hydrogen carbonate was added to the reaction mixture, and the resulting admixture was extracted with ethyl acetate. The ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. The solvent was removed by reduced-

pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone and further by washing resulting crystals with acetoneto obtain 9 mg of the title compound (yield: 10%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.95 (s, 3H), 3.95 (s, 3H), 6.48 (d, J=5.5 Hz, 1H), 7.28 (d, J=9.2 Hz, 2H), 7.40 (s, 1H), 7.43 (t, J=7.3 Hz, 1H), 7.50~7.53 (m, 3H), 7.77 (d, J=7.3 Hz, 2H), 7.85 (d, J=8.6 Hz, 2H), 7.96 (m, 2H), 8.08 (d, J=8.5 Hz, 2H), 8.48 (d, J=4.9 Hz, 1H), 10.4 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 476 (M^+)

Example 63

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-butoxyphenyl)carboxamide [74]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was suspended in triethylamine/methylene chloride (3 ml/2 ml), commercially available 4-butoxybenzoyl chloride (0.07 ml) was added, and the admixture was stirred at room temperature for 30 hours. The reaction mixture was then purified in the same manner as described in Example 62 to obtain 34 mg of the title compound (yield: 41%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.99 (t, J=7.3 Hz, 3H), 1.52 (m, 2H), 1.80 (m, 2H), 4.03 (m, 2H), 4.04 (s, 3H), 4.05 (s, 3H), 6.47 (d, J=4.9 Hz, 1H), 6.95 (d, J=9.2 Hz, 2H), 7.18 (d, J=9.2 Hz, 2H), 7.46 (s, 1H), 7.57 (s, 1H), 7.74 (d, J=9.2 Hz, 2H), 7.86 (d, J=9.2 Hz, 2H), 8.10 (s, 1H), 8.49 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 472 (M^+)

Example 64

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-bromophenyl)carboxamide [75]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) and commercially available 4-bromobenzoic acid (78 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (102 mg) was added, and the admixture was stirred at room temperature for 17 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 43 mg of the title compound (yield: 52%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.07 (s, 3H), 4.07 (s, 3H), 6.53 (d, J=5.5 Hz, 1H), 7.22 (d, J=9.2 Hz, 2H), 7.37 (s, 1H), 7.50 (s, 1H), 7.62 (s, 1H), 7.65 (d, J=8.6 Hz, 2H), 7.84 (d, J=8.6 Hz, 2H), 7.86 (d, J=8.6 Hz, 2H), 8.41 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 478 (M^+), 480 (M^++2)

Example 65

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-methoxycarbonylphenyl)carboxamide [76]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (100 mg) and commercially available monomethylterephthalic acid (130 mg) were dissolved in N,N-dimethylformamide (4 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (227 mg) was added, and the admixture was stirred at room temperature for 18 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 99 mg of the title compound (yield: 64%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.98 (s, 3H), 4.06 (s, 3H), 4.06 (s, 3H), 6.49 (d, J=4.9 Hz, 1H), 7.22 (d, J=9.2 Hz, 2H), 7.43 (s, 1H), 7.56 (s, 1H), 7.76 (d, J=9.2 Hz, 2H), 7.97 (d, J=8.6 Hz, 2H), 8.18 (d, J=8.6 Hz, 2H), 8.49 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 458 (M^+)

Example 66

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-2-pyridinecarboxamide [77]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) and commercially available picolinic acid (42 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (123 mg) was added, and the admixture was stirred at room temperature for 19 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 38 mg of the title compound (yield: 56%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 4.06 (s, 3H), 4.06 (s, 3H), 6.49 (d, J=5.5 Hz, 1H), 7.23 (d, J=8.6 Hz, 2H), 7.43 (s, 1H), 7.51 (m, 1H), 7.58 (s, 1H), 7.89 (d, J=9.2 Hz, 2H), 7.94 (m, 1H), 8.33 (d, J=8.0 Hz, 1H), 8.50 (d, J=5.5 Hz, 1H), 8.64 (dd, J=1.8, 4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 401 (M^+)

Example 67

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-hydroxycarbonylphenyl)carboxamide [78]

{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-methoxycarbonylphenyl)carboxamide was dissolved in methanol (3 ml), 33% aqueous potassium hydroxide (1.5 ml) was added, and the admixture was stirred at room temperature for 2 hours. The reaction mixture was washed 2 times with ethyl acetate, and the water layer was neutralized with dilute hydrocholoric acid and extracted 2 times with chloroform. The chloroform layer was dried with sodium sulfate, and the solvent was then removed by reduced-pressure distillation to obtain 34 mg of the title compound (yield: 45%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.93 (s, 3H), 3.94 (s, 3H), 6.47 (d, J=5.5 Hz, 1H), 7.28 (d, J=9.2 Hz, 2H), 7.39 (s, 1H), 7.51 (s, 1H), 7.92 (d, J=9.2 Hz, 2H), 8.07 (s, 4H), 8.47 (d, J=5.5 Hz, 1H), 10.5 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 444 (M^+)

Example 68

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}cyclopentanecarboxamide [79]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) and commercially available cyclopentanecarboxylic acid (66 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (110 mg) was added, and the admixture was stirred at room temperature for 17 hours. The reaction mixture was purified in the same manner as described in Example 51 to obtain 35 mg of the title compound (yield: 50%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.64 (m, 2H), 1.81 (m, 2H), 1.91 (m, 4H), 2.75 (m, 1H), 4.04 (s, 3H), 4.04 (s, 3H), 6.44 (d, J=4.9 Hz, 1H), 7.13 (d, J=9.2 Hz, 2H), 7.45 (s, 1H), 7.55 (m, 1H), 7.65 (d, J=8.6 Hz, 2H), 8.47 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 392 (M^+)

Example 69

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}adamantanecarboxamide [81]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) and commercially available adamantanecarboxylic acid

(110 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (107 mg) was added, and the admixture was stirred at room temperature for 15 hours. The reaction mixture was purified in the same manner as described in Example 51 to obtain 13 mg of the title compound (yield: 17%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.82 (m, 6H), 2.00 (m, 6H), 2.13 (m, 3H), 4.05 (s, 3H), 4.05 (s, 3H), 6.44 (d, J=4.9 Hz, 1H), 7.15 (d, J=9.2 Hz, 2H), 7.42 (s, 1H), 7.55 (m, 1H), 7.64 (d, J=9.2 Hz, 2H), 8.46 (d, J=4.9 Hz, 1H),

Mass spectrometry data (FD-MS, m/z): 458 (M^+)

Example 70

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-acetoxyphenyl)carboxamide [80]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (110 mg) and commercially available 4-acetoxybenzoic acid (207 mg) were dissolved in N,N-dimethylformamide (6 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (223 mg) was added, and the admixture was stirred at room temperature for 7 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 123 mg of the title compound (yield: 72%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.35 (s, 3H),4.06 (s, 3H), 4.06 (s, 3H), 6.49 (d, J=4.9 Hz, 1H), 7.21 (d, J=9.2 Hz, 2H), 7.25 (d, J=9.1 Hz, 2H), 7.43 (s, 1H), 7.56 (s, 1H), 7.73 (d, J=9.2 Hz, 2H), 7.87 (s, 1H), 7.93 (d, J=8.6 Hz, 2H), 8.50 (d, J=5.5 Hz, 1H),

Mass spectrometry data (FD-MS, m/z): 458 (M^+)

Example 71

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-2-(6-acetoxynaphthyl)carboxamide [82]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) and commercially available 6-acetoxy-2-naphthoic acid (121 mg) were dissolved in N,N-dimethylformamide (2 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (124 mg) was added, and the admixture was stirred at room temperature for 13 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 66 mg of the title compound (yield: 75%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 2.38 (s, 3 H), 4.03 (s, 3H), 4.05 (s, 3H), 6.49 (d, J=5.5 Hz, 1H), 7.20 (d, J=9.2 Hz, 2H), 7.30 (dd, J=2.4, 9.2 Hz, 1H), 7.42 (s, 1H), 7.57 (m, 1H), 7.61 (d, J=1.8 Hz, 1H), 7.81 (d, J=9.2 Hz, 2H), 7.86 (d, J=8.6 Hz, 1H), 7.89 (d, J=9.2 Hz, 1H), 7.94 (d, J=8.6 Hz, 1H), 8.38 (s, 1H), 8.49 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 508 (M^+)

Example 72

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-hydroxyphenyl)carboxamide [83]

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-(4-acetoxyphenyl)carboxamide (103 mg) was dissolved in methanol (4 ml), 35% aqueous potassium hydroxide (2 ml) was added, and the admixture was stirred at room temperature for 5 hours. The reaction mixture was partitioned with ethyl acetate/water, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting crystals were washed with ether to obtain 77 mg of the title compound (yield: 82%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.46 (d, J=4.9 Hz, 1H), 6.87 (d, J=8.5 Hz, 2H), 7.24 (d, J=9.2 Hz, 2H), 7.39 (s, 1H), 7.52 (s, 1H), 7.87 (d, J=8.6 Hz, 2H), 7.90 (d, J=9.2 Hz, 2H), 8.47 (d, J=5.5 Hz, 1H), 10.1 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 417 (M^++1)

Example 73

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-2-(6-hydroxynaphthyl)carboxamide [84]

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-2-(6-acetoxynaphthyl)carboxamide (47 mg) was dissolved in methanol (4 ml), 35% aqueous potassium hydroxide (2 ml) was added, and the admixture was stirred at room temperature for 5 hours. The reaction mixture was then purified in the same matter as described in the Example 72 to obtain 37 mg of the title compound (yield: 86%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.95 (s, 3H), 3.95 (s, 3H), 6.49 (d, J=4.9 Hz, 1H), 7.18 (m, 1H), 7.20 (s, 1H), 7.27 (d, J=8.6 Hz, 2H), 7.39 (s, 1H), 7.53 (s, 1H), 7.80 (d, J=8.5 Hz, 1H), 7.92~7.98 (m, 4H), 8.47~8.48 (m, 2H), 10.0 (brs, 1H), 10.4 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 467 (M^++1)

Example 74

N-(4-Methoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolinyl)oxy]phenyl}urea [43]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) was dissolved in toluene (3 ml) with heat, 4-methoxyphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 30 minutes. The separated crystals were filtered and washed with toluene to obtain 54 mg of the title compound (yield: 68%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.72 (s, 3H), 3.94 (s, 3H), 3.95 (s, 3H), 6.44 (d, J=4.9 Hz, 1H), 6.87 (d, J=8.5 Hz, 2H), 7.19 (d, J=9.2 Hz, 2H), 7.37 (d, J=8.5 Hz, 2H), 7.38 (s, 1H), 7.52 (s, 1H), 7.58 (d, J=8.5 Hz, 2H), 8.46 (d, J=5.5 Hz, 1H), 8.50 (s, 1H), 8.73 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 445 (M^+)

Example 75

N-(4-Fluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolinyl)oxy]phenyl}urea [44]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) was dissolved in toluene (3 ml) with heat, 4-fluorophenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 70 minutes. The separated crystals were filtered and washed with toluene to obtain 38 mg of the title compound (yield: 48%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.44 (d, J=4.9 Hz, 1H), 7.12 (m, 2H), 7.19 (m, 2H), 7.38 (s, 1H), 7.47 (m, 2H), 7.51 (s, 1H), 7.59 (d, J=9.2 Hz, 2H), 8.45 (d, J=5.5 Hz, 1H), 8.72 (s, 1H), 8.79 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 433 (M^+)

Example 76

N-(4-Bromophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolinyl)oxy]phenyl}urea [45]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (3 ml) with heat, 4-bromophenyl

isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 50 minutes. The separated crystals were filtered and washed with toluene to obtain 56 mg of the title compound (yield: 64%).

^{1}H-NMR (DMSO-d_6, 500 MHz): 3.94 (s, 3H), 3.95 (s, 3H), 6.44 (d, J=5.5 Hz, 1H), 7.19 (d, J=9.2 Hz, 2H), 7.39 (s, 1H), 7.45 (m, 4H), 7.51 (s, 1H), 7.59 (d, J=9.2 Hz, 2H), 8.46 (d, J=5.5 Hz, 1H), 8.86 (s, 1H), 8.86 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 493 (M^+), 495 (M^++2)

Example 77

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-N'-(4-nitrophenyl)urea [46]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (103 mg) was dissolved in toluene (10 ml) with heat, 4-nitrophenyl isocyanate (366 mg) was added, and the admixture was refluxed with heat for 60 minutes. The separated crystals were filtered and washed with toluene to obtain 141 mg of the title compound (yield: 88%).

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 3.94 (s, 3H), 3.95 (s, 3H), 6.45 (d, J=5.5 Hz, 1H), 7.23 (d, J=8.6 Hz, 2H), 7.39 (s, 1H), 7.51 (s, 1H), 7.62 (d, J=9.2 Hz, 2H), 7.71 (d, J=9.2 Hz, 2H), 8.20 (d, J=9.2 Hz, 2H), 8.46 (d, J=5.5 Hz, 1H), 9.08 (s, 1H), 9.49 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 460 (M^+)

Example 78

N-(4-Butylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolinyl)oxy]phenyl}urea [47]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (3 ml) with heat, 4-butylphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 26 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 36 mg of the title compound (yield: 45%).

^{1}H-NMR ($CDCl_3$/CD_3OD, 500 MHz): δ 0.93 (m, 3H), 1.35 (m, 2H), 1.57 (m, 2H), 2.57 (m, 2H), 4.06 (s, 3H), 4.06 (s, 3H), 6.49 (m, 1H), 7.14 (m, 4H), 7.33~7.62 (m, 6H), 8.39 (m, 1H)

Mass spectrometry data (FD-MS, m/z): 471 (M^+)

Example 79

N-(4-aminophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolinyl)oxy]phenyl}urea [48]

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-N'-(4-nitro)phenylurea (111 mg) was dissolved in N,N-dimethylformamide (7 ml), 10% palladium hydroxide-carbon (100 mg) was added, and the admixture was stirred at room temperature under hydrogen for 22 hours. The reaction mixture was filtered using Celite. The filtrate was washed with brine and then dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation to obtain 66 mg of the title compound (yield: 63%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.94 (s, 3H), 4.74 (brs, 2H), 6.43 (d, J=4.9 Hz, 1H), 6.52 (d, J=8.6 Hz, 2H), 7.08 (d, J=8.6 Hz, 2H), 7.16 (d, J=9.2 Hz, 2H), 7.38 (s, 1H), 7.51 (s, 1H), 7.55 (d, J=9.2 Hz, 2H), 8.14 (s, 1H), 8.45 (d, J=4.9 Hz, 1H), 8.61 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 430 (M^+)

Example 80

N-(4-Acetylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolinyl)oxy]phenyl}urea [49]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (3 ml) with heat, 4-acetylphenyl isocyanate (210 mg) was added, and the admixture was refluxed with heat for 20 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 50 mg of the title compound (yield: 64%).

^{1}H-NMR ($CDCl_3$/CD_3OD, 500 MHz): δ 2.59 (s, 3H), 4.07 (s, 3H), 4.07 (s, 3H), 6.50 (d, J=5.5 Hz, 1H), 7.17 (d, J=8.5 Hz, 2H), 7.36 (s, 1H), 7.58 (s, 1H), 7.58 (d, J=8.5 Hz, 2H), 7.60 (d, J=8.5 Hz, 2H), 7.94 (d, J=8.5 Hz, 2H), 8.40 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 457 (M^+)

Example 81

N-{4-[(6,7-Dimethoxy-4-quinolinyl)oxy]phenyl}-N'-(4-phenoxyphenyl)urea [50]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) was dissolved in toluene (3 ml) with heat, 4-phenoxyphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 30 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 55 mg of the title compound (yield: 60%).

^{1}H-NMR ($CDCl_3$/CD_3OD, 500 MHz): δ 4.46 (s, 3H), 4.46 (s, 3H), 6.51 (m, 1H), 6.98~7.62 (m, 15H), 8.40 (m, 1H)

Mass spectrometry data (FD-MS, m/z): 507 (M^+)

Example 82

N-(4-Isopropylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolinyl)oxy]phenyl}urea [51]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (3 ml) with heat, 4-isopropylphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 26 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 70 mg of the title compound (yield: 90%).

^{1}H-NMR (CD_3OD, 500 MHz): δ 1.18 (d, J=6.7 Hz, 6H), 2.82 (m, 1H), 3.97 (s, 3H), 4.01 (s, 3H), 6.37 (d, J=5.5 Hz, 1H), 7.04 (d, J=8.5 Hz, 2H), 7.11 (d, J=8.5 Hz, 2H), 7.25 (d, J=8.5 Hz, 2H), 7.26 (s, 1H), 7.38 (s, 1H), 7.41 (d, J=9.2 Hz, 2H), 7.54 (s,1H), 7.82 (s, 1H), 8.03 (s, 1H), 8.42 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 457 (M^+)

Example 83

N-(4-Trifluoromethylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolinyl)oxy]phenyl}urea [52]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) was dissolved in toluene (3 ml) with heat, 4-trifluoromethylphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 10 minutes. The separated crystals were filtered and washed with toluene to obtain 77 mg of the title compound (yield: 88%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.05 (s, 3H), 4.05 (s, 3H), 6.52 (d, J=5.5 Hz, 1H), 7.18 (d, J=9.2 Hz, 2H), 7.36 (s, 1H), 7.56 (s, 1H), 7.59 (d, J=8.6 Hz, 2H), 7.62 (d, J=8.6 Hz, 2H), 7.63 (s, 1H), 8.40 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 483 (M^+)

Example 84

N-(4-n-Butylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [147]

6,7-Dimethoxy-4-(2-aminophenoxy)quinoline (56 mg), which was obtained, analogously to Example 49, by reduc-

ing 6,7-dimethoxy-4-(2-nitrophenoxy)quinoline obtained in the same manner as described in Example 48, except that 2-nitrophenol was used in place of 4-nitrophenol, was dissolved in toluene (3 ml) with heat, 4-n-butylphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 30 minutes. The resulting residue was purified by chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 45 mg of the title compound (yield: 50%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.89 (t, J=7.3 Hz, 3H), 1.30 (m, 2H), 1.52 (m, 2H), 2.50 (m, 2H), 3.90 (s, 3H), 3.93 (s, 3H), 6.22 (d, J=4.9 Hz, 1H), 6.87 (d, J=7.3 Hz, 1H), 6.96~7.01 (m, 4H), 7.15 (brs, 1H), 7.28~7.31 (m, 4H), 8.12 (brs, 1H), 8.26 (d, J=4.9 Hz, 1H), 8.58 (d, J=7.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 471 (M^+)

Example 85

N-(4-n-Butylphenyl)-N'-{3-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [91]

6,7-Dimethoxy-4-(3-aminophenoxy)quinoline (51 mg), which was obtained, analogously to Example 49, by reducing 6,7-dimethoxy-4-(3-nitrophenoxy)quinoline obtained in the same manner as described in Example 48, except that 3-nitrophenol was used in place of 4-nitrophenol, was dissolved in toluene (3 ml) with heat, 4-n-butylphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 50 minutes. The resulting residue was purified by chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 26 mg of the title compound (yield: 32%).

^{1}H-NMR ($CDCl_3$, 500 MHz): 60.89 (t, J=7.3 Hz, 3H), 1.30 (m, 2H), 1.52 (m, 2H), 2.51 (m, 2H), 3.95 (s, 1H), 4.00 (s, 1H), 6.49 (d, J=5.5 Hz, 1H), 6.81 (d, J=8.0 Hz, 1H), 7.04 (d, J=7.9 Hz, 2H), 7.2~7.30 (m, 5H), 7.37 (s, 1H), 7.51 (s, 1H) 7.84 (brs, 1H), 8.24 (brs, 1H), 8.43 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 471 (M^+)

Example 86

N-n-Butyl-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [139]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (3 ml) with heat, n-butyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 14 hours. The resulting residue was purified by chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 31 mg of the title compound (yield: 46%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.93 (t, J=7.8 Hz, 3H), 1.37 (m, 2H), 1.53 (m, 2H), 3.28 (m, 2H), 4.04 (s, 3H), 4.04 (s, 3H), 5.04 (brs, 1H), 6.44 (d, J=5.5 Hz, 1H), 6.98 (brs, 1H), 7.11 (d, J=9.2 Hz, 2H), 7.41 (s, 1H), 7.43 (d, J=9.2 Hz, 2H), 7.55 (s, 1H), 8.46 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 395 (M^+)

Example 87

N-{4-[6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-(4-trifluoromethylphenyl)carboxamide [157]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) and commercially available 4-trifluoromethanebenzoic acid (102 mg) were dissolved in N,N-dimethylformamide (3 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (102 mg) was added, and the admixture was stirred at room temperature for 14 hours. The reaction mixture was partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting crystallized product was washed with ethyl acetate to obtain 32 mg of the title compound (yield: 38%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.04 (s, 3H), 4.05 (s, 3H), 6.48 (d, J=4.9 Hz, 1H), 7.22 (d, J=8.5 Hz, 2H), 7.41 (s, 1H), 7.55 (s, 1H), 7.76 (d, J=8.5 Hz, 2H), 7.76 (d, J=8.6 Hz, 2H), 8.02 (d, J=7.9 Hz, 2H), 8.13 (brs, 1H), 8.49 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 468 (M^+)

Example 88

N-{4-[6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-(4-cyclohexylphenyl)carboxamide [158]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) and commercially available 4-cyclohexylbenzoic acid (109 mg) were dissolved in N,N-dimethylformamide (3 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (110 mg) was added, and the admixture was stirred at room temperature for 14 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 54 mg of the title compound (yield: 64%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.25~1.86 (m, 10H), 2.55 (m, 1H), 4.01 (s, 3H), 4.03 (s, 3H), 6.46 (d, J=5.5 Hz, 1H), 7.16 (d, J=8.5 Hz, 2H), 7.27 (d, J=7.9 Hz, 2H), 7.39 (s, 1H), 7.55 (s, 1H), 7.75 (d, J=9.2 Hz, 2H), 7.83 (d, J=8.6 Hz, 2H), 8.46 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 482 (M^+)

Example 89

N-{3-[6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-(4-t-butylphenyl)carboxamide [159]

6,7-Dimethoxy-4-(3-aminophenoxy)quinoline (54 mg) and commercially available 4-t-butyl benzoic acid (102 mg) were dissolved in N,N-dimethylformamide (3 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (106 mg) was added, and the admixture was stirred at room temperature for 6 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 29 mg of the title compound (yield: 35%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.34 (s, 9H), 4.01 (s, 3H), 4.04 (s, 3H), 6.55 (d, J=4.9 Hz, 1H), 6.97 (d, J=7.3 Hz, 1H), 7.29~7.68 (m, 7H), 7.8 5 (d, J=7.9 Hz, 2H), 8.48 (d, J=4.9 Hz, 1H), 8.59 (brs, 1H)

Mass spectrometry data (FD-MS, m/z): 456 (M^+)

Example 90

N-{3-[6,7-Dimethoxy-4-quinolyl)thio]phenyl}-(4-t-butylphenyl)carboxamide [160]

6,7-Dimethoxy-4-(3-aminophenylthio)quinoline (62 mg) and commercially available 4-t-butyl benzoic acid (109 mg) were dissolved in N,N-dimethylformamide (3 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (112 mg) was added, and the admixture was stirred at room temperature for 25 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 54 mg of the title compound (yield: 57%).

^{1}H-NMR ($CDCl_3$, 500 MHz): 61.31 (s, 9H), 4.00 (s, 3H), 4.01 (s, 3H), 6.83 (d, J=4.9 Hz, 1H), 7.20~7.9 9 (m, 9H), 8.06 (m, 1H), 8.39 (d, J=4.9 Hz, 1H), 8.60 (brs, 1H)

Mass spectrometry data (FD-MS, m/z): 472 (M^+)

Example 91

N-{4-[6,7-Dimethoxy-4-quinolyl)thio]phenyl}-(4-butylphenyl)carboxamide [161]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (62 mg) and commercially available 4-butyl benzoic acid (104 mg) were dissolved in N,N-dimethylformamide (3 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (117 mg) was added, and the admixture was stirred at room temperature for 21 hours. The reaction mixture was then purified in the same manner as described in Example 51 to obtain 21 mg of the title compound (yield: 22%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.94 (t, J=7.3 Hz, 3H), 1.38 (m, 2H), 1.63 (m, 2H), 2.69 (t, J=7.9 Hz, 2H), 4.03 (s, 3H) 4.05 (s, 3H), 6.72 (d, J=4.9 Hz, 1H), 7.30 (d, J=7.9 Hz, 2H), 7.39 (s, 1H), 7.42 (s, 1H), 7.57 (d, J=8.5 Hz, 2H), 7.78 (d, J=8.5 Hz, 2H), 7.81 (d, J=7.9 Hz, 2H), 8.08 (s, 1H), 8.42 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 472 (M^+)

Example 92

N-{4-[6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(4-methoxythiophenyl)urea [163]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) was dissolved in toluene (3 ml) with heat, 4-methylthiophenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 8 minutes. The separated crystals were filtered and washed with toluene to obtain 62 mg of the title compound (yield: 76%).

^{1}H-NMR (DMSO-d_6, 500 MHz): 2.44 (s, 3H), 3.94 (s, 3H), 3.95 (s, 3H), 6.44 (d, J=4.9 Hz, 1H), 7.19 (d, J=9.2 Hz, 2H), 7.23 (d, J=8.5 Hz, 2H), 7.38 (s, 1H), 7.44 (d, J=9.2 Hz, 2H), 7.52 (s, 1H), 7.58 (d, J=8.6 Hz, 2H), 8.46 (d, L=4.9 Hz, 1H), 8.70 (s, 1H), 8.79 (s, 1H) Mass spectrometry data (FD-MS, m/z): 461 (M^+)

Example 93

N-(3-Ethoxycarbonylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [164]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) was dissolved in toluene (5 ml) with heat, 3-ethoxycarbonylphenyl isocyanate (284 mg) was added, and the admixture was refluxed with heat for 1 hour. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 18 mg of the title compound (yield: 20%).

^{1}H-NMR ($CDCl_3$, 500 MHz): 1.38 (m, 3H), 3.98 (s, 3 H), 4.05 (s, 3H), 4.3 5 (m, 2 H), 6.45 (d, J=5.5 Hz, 1H), 7.12 (d, J=9.2 Hz, 2H), 7.40 (s, 1H), 7.47 (d, J=9.2 Hz, 2H), 7.47 (d, J=8.6 Hz, 2H), 7.58 (s, 1H), 7.96 (d, J=9.2 Hz, 2H), 8.05 (s, 1H), 8.17 (s, 1H), 8.47 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 487 (M^+)

Example 94

N-(4-Chlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [165]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 4-chlorophenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 1 hour. The separated crystals were filtered and washed with toluene to obtain 43 mg of the title compound (yield: 55%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.44 (d, J=5.5 Hz, 1H), 7.20 (d, J=9.2 Hz, 2H), 7.32 (d, J=8.5 Hz, 2H), 7.38 (s, 1H), 7.49 (d, J=9.2 Hz, 2H), 7.51 (s, 1H), 7.58 (d, J=9.2 Hz, 2H), 8.46 (d, J=4.9 Hz, 1H), 8.83 (brs, 1H), 8.83 (brs, 1H)

Mass spectrometry data (FD-MS, m/z): 449 (M^+), 451 (M^++2)

Example 95

N-(2-Isopropylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [166]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (44 mg) was dissolved in toluene (5 ml) with heat, 2-isopropylphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 80 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 14 mg of the title compound (yield: 20%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.21 (m, 6H), 3.21 (m, 1H), 4.04 (s, 3H), 4.04 (s, 3H), 6.42 (m, 1H), 6.63 (m, 1H), 7.09–7.55 (m, 11H), 8.46 (m, 1H)

Mass spectrometry data (FD-MS, m/z): 457 (M^+)

Example 96

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-phenylurea [167]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, phenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 50 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 21 mg of the title compound (yield: 41%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.04 (s, 3H), 4.04 (s, 3H), 6.61 (m, 1H), 7.05 (m, 1H), 7.36~7.44 (m, 10H), 7.99 (s, 1H), 8.31 (s, 1H), 8.36 (m, 1H)

Mass spectrometry data (FD-MS, m/z): 431 (M^+)

Example 97

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(2-nitrophenyl)urea [168]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (83 mg) was dissolved in toluene (8 ml) with heat, 4-nitrophenyl isocyanate (370 mg) was added, and the admixture was refluxed with heat for 40 minutes. The deposited crystals were filtered and washed with toluene to obtain 58 mg of the title compound (yield: 45%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.46 (d, J=5.5 Hz, 1H), 7.22 (m, 1H), 7.23 (d, J=9.2 Hz, 2H), 7.38 (s, 1H), 7.51 (s, 1H), 7.62 (d, J=9.2 Hz, 2H), 7.71 (m, 1H), 8.11 (m, 1H), 8.31 (m, 1H), 8.46 (d, J=5.5 Hz, 1H), 9.62 (s, 1H), 9.96 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 460 (M^+)

Example 98

N-(2-Aminophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [169]

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl)-}-(2-nitrophenyl)urea (42 mg) was dissolved in N,N-

dimethylformamide/ethyl acetate (12 ml/6 ml), 20% palladium hydroxide-carbon (70 mg) was added, and the admixture was stirred at room temperature under hydrogen for 3 hours. The reaction mixture was filtered using Celite, after which the filtrate was washed with brine and then dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation to obtain 39 mg of the title compound (yield: 100%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 4.77 (br s, 2H), 6.44 (d, J=5.5 Hz, 1H), 6.58 (m, 1H), 6.75 (m, 1H), 6.85 (m, 1H), 7.18 (d, J=8.5 Hz, 2H), 7.34 (m, 1H), 7.38 (s, 1H), 7.52 (s, 1H), 7.58 (d, J=9.2 Hz, 2H), 8.29 (s, 1H), 8.46 (d, J=4.9 Hz, 1H), 8.92 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 430 (M^+)

Example 99

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(3-nitrophenyl)urea [170]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (83 mg) was dissolved in toluene (6 ml) with heat, 3-nitrophenyl isocyanate (384 mg) was added, and the admixture was refluxed with heat for 40 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 44 mg of the title compound (yield: 34%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.99 (s, 3H), 4.04 (s, 3H), 6.43 (d, J=4.9 Hz, 1H), 7.10 (d, J=9.2 Hz, 2H), 7.38 (m, 2H), 7.47 (d, J=8.6 Hz, 2H), 7.57 (s, 1H), 7.81 (m, 2H), 8.19 (s, 1H), 8.31 (s, 1H), 8.46 (d, J=4.9 Hz, 1H), 8.59 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 460 (M^+)

Example 100

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-octylurea [171]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, octyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 2 hours. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 32 mg of the title compound (yield: 41%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.87 (m, 3H), 1.26~1.30 (m, 10H), 1.53 (m, 2H), 3.27 (m, 2H), 4.03 (s, 3H), 4.03 (s, 3H), 5.04 (m, 1H), 6.44 (d, J=5.5 Hz, 1H), 6.97 (s, 1H), 7.10 (d, J=8.6 Hz, 2H), 7.41 (s, 1H), 7.42 (d, J=8.6 Hz, 2H), 7.55 (s, 1H), 8.46 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 451 (M^+)

Example 101

N-(3-Aminophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [172]

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(3-nitrophenyl)urea (28 mg) was dissolved in N,N-dimethylformamide/ethyl acetate (5 ml/2.5 ml), 20% palladium hydroxide-carbon (97 mg) was added, and the admixture was stirred at room temperature under hydrogen for 3 hours. The reaction mixture was filtered using Celite, the filtrate was washed with brine and then dried with anhydrous sodium sulfate. The solvent was then removed by reduced-pressure distillation to obtain 13 mg of the title compound (yield: 51%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.02 (s, 3H), 4.03 (s, 3H), 6.41 (m, 1H), 6.41 (d, J=5.5 Hz, 1H), 6.61 (m, 1H), 6.90 (s, 1H), 7.05 (m, 1H), 7.09 (d, J=8.5 Hz, 2H), 7.40 (s, 1H), 7.43 (m, 1H), 7.47 (d, J=8.5 Hz, 2H), 7.55 (s, 1H), 8.03 (s, 1H), 8.44 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS m/z): 430 (M^+)

Example 102

N-Allyl-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [173]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in N,N-dimethylformamide (5 ml), allyl isocyanate (0.2 ml) was added, and the admixture was stirred at 80° C. for 15 hours with heat. Water was added to the reaction mixture, the admixture was extracted 2 times with ethyl acetate, and the organic layer was washed with brine and then dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 61 mg of the title compound (yield: 96%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.90 (m, 2H), 4.03 (s, 3H), 4.03 (s, 3H), 5.12 (d, J=10.4 Hz, 1H), 5.23 (d, J=1.77 Hz, 1H), 5.62 (s, 1H), 5.88 (m, 1H), 6.41 (d, J=4.9 Hz, 1H), 7.08 (d, J=8.5, 2H), 7.39 (s, 1H), 7.44 (d, J=8.5 Hz, 2H), 7.54 (s, 1H), 7.75 (s, 1H), 8.44 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 379 (M^+)

Example 103

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(1-naphthyl)urea [174]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, 1-naphthyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 20 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 41 mg of the title compound (yield: 52%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.95 (s, 3H), 3.95 (s, 3H), 6.45 (d, J=5.5 Hz, 1H), 7.21 (d, J=9.2 Hz, 2H), 7.38 (s, 1H), 7.48–7.65 (m, 7H), 7.92~8.15 (m, 3H), 8.46 (d, J=4.9 Hz, 1H), 8.80 (s, 1H), 9.20 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 465 (M^+)

Example 104

N-(2-Biphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [175]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 2-biphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 30 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 56 mg of the title compound (yield: 64%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.97 (s, 3H), 4.04 (s, 3H), 6.35 (d, J=5.5 Hz, 1H), 6.96 (s, 1H), 7.40 (d, J=9.2 Hz, 2H), 7.14~7.39 (m, 11H), 7.54 (s, 1H), 7.89 (brs, 1H), 8.08 (d, J=8.6 Hz, 1H), 8.33 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 491 (M^+)

Example 105

N-(4-Ethylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [188]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, 4-ethylphenyl

isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 90 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 42 mg of the title compound (yield: 56%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.18 (m, 3H), 2.58 (m, 2H), 3.99 (s, 3H), 4.02 (s, 3H), 6.39 (d, J=5.5 Hz, 1H), 7.07 (d, J=9.2 Hz, 2H), 7.11 (d, J=7.9 Hz, 2H), 7.25 (d, J=8.5 Hz, 2H), 7.40 (s, 1H), 7.42 (d, J=8.5 Hz, 2H), 7.55 (s, 1H), 7.60 (s, 1H), 7.81 (s, 1H), 8.43 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 443 (M^+)

Example 106

N-Benzoyl-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [189]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, benzoyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 50 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 11 mg of the title compound (yield: 15%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.06 (s, 3H), 4.07 (s, 3H), 6.49 (d, J=4.9 Hz, 1H), 7.20 (d, J=9.2 Hz, 2H), 7.43 (s, 1H), 7.54 (m, 2H) 7.58 (s, 1H), 7.65 (m, 1H), 7.71 (d, J=9.2 Hz, 2H), 8.04 (d, J=7.9 Hz, 2H), 8.50 (d, J=5.5 Hz, 1H), 9.53 (s, 1H), 11.04 (s, 1 H)

Mass spectrometry data (FD-MS, m/z): 443 (M^+)

Example 107

N-Ethoxycarbonyl-N'-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}urea [190]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, ethoxycarbonyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 15 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 20 mg of the title compound (yield: 28%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.37 (t, J=7.3 Hz, 3H), 4.10 (s, 3H), 4.17 (s, 3H), 4.31 (q, J=7.3 Hz, 2H), 6.70 (d, J=6.1 Hz, 1H), 7.20 (d, J=8.6 Hz, 2 H), 7.64 (s, 1H), 7.72 (d, J=9.2 Hz, 2 H), 8.12 (s, 1H), 8.50 (d, J=6.7 Hz, 1H), 10.08 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 411 (M^+)

Example 108

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(2-methylphenyl)urea [56]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, 2-methylphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 15 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 61 mg of the title compound (yield: 82%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.20 (s, 3H), 4.00 (s, 3H), 4.03 (s, 3H), 4.31 (q, J=7.3 Hz, 2H), 6.40 (d, J=5.5 Hz, 1H), 7.05–7.20 (m, 6H), 7.39 (s, 1H), 7.42 (d, J=8.5 Hz, 2H), 7.55, (s, 1H), 7.60 (d, J=7.9 Hz, 2H), 7.80 (s, 1H), 8.45 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 429 (M^+)

Example 109

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(3-methylphenyl)urea [55]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, 3-methylphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 15 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 41 mg of the title compound (yield: 56%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.32 (s, 3H), 4.02 (s, 3H), 4.04 (s, 3H), 6.43 (d, J=5.5 Hz, 1H), 6.93 (d, J=7.3 Hz, 1H) 7.10~7.22 (m, 6H), 7.41 (s, 1H), 7.44~7.47 (m, 3H), 7.56 (s, 1H), 8.46 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS m/z): 429 (M^+)

Example 110

N-(2-Fluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [60]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, 2-fluorophenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 15 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 54 mg of the title compound (yield: 73%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.01 (s, 3H), 4.04 (s, 3H), 6.44 (d, J=5.5 Hz, 1H), 6.97~7.04 (m, 2H), 7.10~7.12 (m, 3H), 7.42 (s, 1H), 7.49 (d, J=9.2 Hz, 2H), 7.57 (s, 1H), 7.73 (s, 1H), 8.17 (m, 1H), 8.35 (s, 1H), 8.48 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 433 (M^+)

Example 111

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-propylurea [194]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) was dissolved in N,N-dimethylformamide (3 ml), propyl isocyanate (0.2 ml) was added, and the admixture was stirred at 80° C. for 24 hours. Water was added to the reaction mixture, the admixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 59 mg of the title compound (yield: 84%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.93 (m, 3H), 1.55 (m, 2H), 3.24 (m, 2H), 4.02 (s, 3H), 4.02 (s, 3H), 5.47 (t, J=5.5 Hz, 1H), 6.41 (d, J=4.9 Hz, 1H), 7.08 (d, J=8.5 Hz, 2H), 7.39 (s, 1H), 7.43 (d, J=9.2 Hz, 2H), 7.55 (s, 1H), 7.60 (brs, 1H), 8.44 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 381 (M^+)

Example 112

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(4-phenylcarbonylphenyl)urea [195]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) was suspended in toluene (5 ml), triphosgene (53 mg) was added, and the admixture was refluxed with heat for 20 minutes. 4-Aminobenzophenone (85 mg) was added to the reaction mixture, and the admixture was refluxed for 1 hour

with heat. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 19 mg of the title compound (yield: 21%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.01 (s, 3H), 4.04 (s, 3H), 6.44 (d, J=4.9 Hz, 1H), 7.12 (d, J=9.2 Hz, 2H), 7.41 (s, 1H), 7.46~7.80 (m, 12H), 8.14 (s, 1H), 8.30 (s, 1H), 8.47 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 519 (M^+)

Example 113

N-Hexyl-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy] phenyl}urea [196]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in N,N-dimethylformamide (3 ml), hexyl isocyanate (0.2 ml) was added, and the admixture was stirred at 60° C. for 15 hours. After the addition of water, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 63 mg of the title compound (yield: 84%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.85 (m, 3H), 1.25~1.47 (m, 6H), 1.50 (m, 2H), 3.26 (q, d=6.7 Hz, 2H), 4.02 (s, 3H), 4.02 (s, 3H), 5.68 (brs, 1H), 6.40 (d, J=5.5 Hz, 1H) 7.07 (d, J=9.2 Hz, 2H), 7.39 (s, 1H), 7.44 (d, J=9.2 Hz, 2H), 7.55 (s, 1H), 7.94 (s, 1H), 8.43 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 423 (M^+)

Example 114

N-(5-Indanyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl) oxy]phenyl}urea [197]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was suspended in toluene (5 ml), triphosgene (54 mg) was added, and the admixture was refluxed with heat for 12 minutes. 4-Aminoindan (36 mg) was added, and the admixture was refluxed with heat for 36 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 13 mg of the title compound (yield: 16%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.04 (m, 2H), 2.82~2.86 (m, 4H), 4.01 (s, 3H), 4.03 (s, 3H), 6.40 (d, J=5.5 Hz, 1H), 7.03 (m, 1H), 7.08 (d, J=8.5 Hz, 2H), 7.13 (m, 1H), 7.32 (s, 1H), 7.40~7.57 (m, 6H), 8.44 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 455 (M^+)

Example 115

N-(3,4-Dimethoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [198]

3,4-Dimethoxyaniline (42 mg) was dissolved in toluene (5 ml), triphosgene (24 mg) was added, and the admixture was refluxed with heat for 22 minutes. 6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was added, and the admixture was refluxed with heat for 18 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 15 mg of the title compound (yield: 18%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.84 (s, 3H), 3.85 (s, 3H), 4.01 (s, 3H), 4.04 (s, 3H), 6.43 (d, J=4.9 Hz, 1H), 6.75~6.80 (m, 2H), 7.10 (d, J=9.2 Hz, 2H), 7.16 (s, 1H), 7.38 (s, 1H), 7.41 (s, 1H), 7.45 (d, J=8.5 Hz, 2H), 7.56 (s, 1H), 7.59 (s, 1H), 8.46 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 475 (M^+)

Example 116

N-(3,4-Ethylenedioxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [199]

3,4-Ethylenedioxyaniline (80 mg) was dissolved in toluene (5 ml), triethylamine (0.5 ml) and then triphosgene (50 mg) were added, and the admixture was refluxed with heat for 1 hour. 6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was added, and the admixture was refluxed with heat for 100 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 62 mg of the title compound (yield: 74%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.94 (s, 3H), 4.19~4.21 (m, 4H), 6.43 (d, J=4.9 Hz, 1H), 6.74~6.78 (m, 2H), 7.09 (s, 1H), 7.17 (d, J=9.2 Hz, 2H), 7.37 (s, 1H), 7.51 (s, 1H), 7.56 (d, J=8.5 Hz, 2H), 8.45 (d, J=4.9 Hz, 1H), 8.47 (s, 1H), 8.69 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 473 (M^+)

Example 117

N-Methyl-N-{4-[(6,7-dimethoxy-4-quinolyl)oxy] phenyl}-N'-(4-methoxyphenyl)urea [200]

Formic acid (0.13 ml) was added to acetic anhydride (0.27 ml), and the admixture was stirred at 60° C. for 2 hours. A solution of 6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (303 mg) in tetrahydrofuran (8 ml) was added to the reaction mixture, and the admixture was stirred at room temperature for 45 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 327 mg of 6,7-dimethoxy-4-(4-formylaminophenoxy)quinoline.

6,7-Dimethoxy-4-(4-formylaminophenoxy)quinoline (317 mg) thus obtained was dissolved in tetrahydrofuran (9 ml), lithium aluminum hydride (181 mg) was added, and the admixture was stirred at room temperature for 2 hours. After the addition of aqueous ammonium chloride, the reaction mixture was extracted 2 times with ethyl acetate, and the

organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 239 mg of 6,7-dimethoxy-4-(4-methylaminophenoxy)quinoline.

6,7-Dimethoxy-4-(4-methylaminophenoxy)quinoline (49 mg) thus obtained was dissolved in toluene (5 ml) with heat, 4-methoxyphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 12 hours. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 47 mg of the title compound (yield: 51%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.38 (s, 3H), 3.77 (s, 3H), 4.05 (s, 3H), 4.06 (s, 3H), 6.14 (brs, 1H), 6.59 (d, J=5.5 Hz, 1H), 6.82 (d, J=9.2 Hz, 2H), 7.21 (d, J=9.2 Hz, 2H), 7.29 (d, J=9.2 Hz, 2H), 7.44 (d, J=8.6 Hz, 2H), 7.45 (s, 1H), 7.52 (s, 1H), 8.55 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 459 (M^+)

Example 118

N-Methyl-N-phenyl-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [201]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (72 mg) was suspended in toluene (7 ml), triphosgene (52 mg) was added, and the admixture was refluxed with heat for 3 hours. N-Methylaniline (0.05 ml) was added to the reaction mixture, and the admixture was refluxed for 1 hour with heat. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 48 mg of the title compound (yield: 46%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.37 (s, 3H), 4.04 (s, 3H), 4.04 (s, 3H), 6.32 (brs, 1H), 6.41 (d, J=4.9 Hz, 1H), 7.07 (d, J=9.2 Hz, 2H), 7.37~7.53 (m, 8H), 7.54 (s, 1H), 8.45 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 429 (M^+)

Example 119

N-Methyl-N-(4-methoxyphenyl)-N'-methyl-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [202]

N-(4-Methoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea (99 mg) was dissolved in N,N-dimethylformamide (3 ml), sodium hydride (42 mg) was added, and the admixture was stirred at room temperature for 20 minutes, methyl iodide (0.03 ml) was added, and the admixture was stirred at the same temperature for 1 hour. After the addition of aqueous ammonium chloride, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 91 mg of the title compound (yield: 87%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.23 (s, 3H), 3.24 (s, 3H), 3.75 (s, 3H), 4.05 (s, 3H), 4.06 (s, 3H), 6.27 (d, J=5.5 Hz, 1H), 6.69 (d, J=8.5 Hz, 2H), 6.81 (d, J=9.2 Hz, 2H), 6.89 (d, J=9.2 Hz, 2H), 6.91 (d, J=9.2 Hz, 2H), 7.42 (s, 1H), 7.51 (s, 1H), 8.53 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS m/z): 473 (M^+)

Example 120 (Reference Example)

3,4-Dichlorophenyl 4-methoxyphenyl ketone

To commercially available nitromethane (10 ml) were added commercially available anisole (1.081 g), commercially available 3,4-dichlorobenzoyl chloride (2.095 g) and commercially available ytterbium(III) trifluoromethanesulfonate (620 mg), and the admixture was stirred at 60° C. for 8 hours. The reaction mixture was partitioned between water and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate to obtain 270 mg of the title compound (yield: 10%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.90 (s, 3H), 6.98 (d, J=9.2 Hz, 2H), 7.56 (s, 1H), 7.58 (d, J=1.8 Hz, 1H), 7.79 (d, J=9.2 Hz, 2H), 7.84 (d, J=1.8 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 280 (M^+) 282 (M^++2)

Example 121 (Reference Example)

3,4-Dichlorophenyl 4-hydroxyphenyl ketone

3,4-Dichlorophenyl 4-methoxyphenyl ketone (235 mg) obtained in Example 120 was dissolved in dichloromethane (2 ml), a solution of 1.0 M boron tribromide in dichloromethane (7 ml) was added while cooled in ice, and the admixture was stirred at room temperature for two days. The reaction mixture was then poured into ice water and partitioned between water and chloroform. The chloroform layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate to obtain 173 mg of the title compound (yield: 77%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 6.91 (d, J=8.5 Hz, 2H), 7.62 (dd, J=1.8, 8.6 Hz, 1H), 7.68 (d, J=8.5 Hz, 2H), 7.80 (d, J=7.9 Hz, 1H), 7.85 (d, J=1.8 Hz, 1H), 10.55 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 266 (M^+), 268 (M^++2)

Example 122

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(3,4-dichlorophenyl)methanone [177]

Under argon, 3,4-dichlorophenyl 4-hydroxyphenyl ketone (154 mg) obtained in Example 121 and 4-dimethylaminopyridine (77 mg) were added to xylene (5 ml), and the admixture was stirred at room temperature for 3 hours. 4-Chloro-6,7-dimethoxyquinoline (129 mg) was added, and the admixture was refluxed with heat for 21 hours. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by thin layer chromatography on silica gel eluting with hexane/acetone (2/1) to obtain 81 mg of the title compound (yield: 31%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.03 (s, 3H), 4.07 (s, 3H), 6.68 (d, J=5.5 Hz, 1H), 7.28 (d, J=8.5 Hz, 2H), 7.46 (s, 1H), 7.47 (s, 1H), 7.60 (d, J=8.5 Hz, 1H), 7.66 (dd, J=1.8, 8.6 Hz, 1H), 7.90 (d, J=9.2 Hz, 2H), 7.91 (d, J=1.8 Hz, 1H), 8.60 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 453 (M^+), 455 (M^++2)

Example 123 (Reference Example)

4-(4-Hydroxybenzoyl)benzonitrile

4-Tri-n-butyltin-1-methoxymethylphenol (1.282 g) obtained in Example 22 and commercially available 4-cyanobenzoyl chloride (497 mg) were dissolved in chloroform (5 ml), commercially available bis (triphenylphosphine)palladium(II) chloride (8 mg) was added, and the admixture was refluxed with heat for 11 hours. The reaction mixture was partitioned in the same manner as described in Example 23, and the resulting ether layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue (859 mg) was dissolved in tetrahydrofuran (2 ml), water (5 ml) and 6 N aqueous hydrochloric acid (12 ml) were added, and the admixture was refluxed with heat for 4 hours. The reaction mixture was partitioned between brine and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate to obtain 250 mg of the title compound (yield: 37%).

^{1}H-NMR (DMSO-d_6, 500 MHz): 66.91 (d, J=9.2 Hz, 2H), 7.67 (d, J=8.5 Hz, 2H), 7.80 (d, J=7.9 Hz, 2H), 8.01 (d, J=8.6 Hz, 2H), 10.57 (s, 1H)

Mass spectrometry data (FD-MS m/z): 223 (M^+)

Example 124

4-{4-[(6,7-Dimethoxy-4-quinolyl)oxy] benzoyl}benzonitrile [178]

Under argon, 4-(4-hydroxybenzoyl)benzonitrile (230 mg) obtained in Example 123 and 4-dimethylaminopyridine (138 mg) were added to xylene (5 ml), and the admixture was stirred at room temperature for 1 hour. 4-Chloro-6,7-dimethoxyquinoline (230 mg) was added, and the admixture was refluxed with heat for 20 hours. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate and then by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1) to obtain 199 mg of the title compound (yield: 47%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.03 (s, 3H), 4.06 (s, 3H), 6.68 (d, J=5.5 Hz, 1H), 7.29 (d, J=8.5 Hz, 2H), 7.45 (s, 1H), 7.46 (s, 1H), 7.82 (d, J=7.9 Hz, 2H), 7.90 (d, J=8.6 Hz, 2H), 7.91 (d, J=8.5 Hz, 2H), 8.60 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 410 (M^+)

Example 125 (Reference Example)

4-Methoxyphenyl 2-methylphenyl ketone

To commercially available nitromethane (10 ml) were added commercially available anisole (1.081 g), commercially available 2-methylbenzoyl chloride (1.546 g) and commercially available ytterbium(III) trifluoromethanesulfonate (620 mg), and the admixture was stirred at 60° C. for 7 hours. The reaction mixture was treated in the same manner as described in Example 120 to obtain 1.679 g of the title compound (yield: 74%).

Mass spectrometry data (FD-MS, m/z): 226 (M^+)

Example 126 (Reference Example)

4-Hydroxyphenyl 2-methylphenyl ketone

4-Methoxyphenyl 2-methylphenyl ketone (1.660 g) obtained in Example 125 was dissolved in chloroform (10 ml), a solution of 1.0 M boron tribromide in dichloromethane (29 ml) was added while cooled in ice, and the admixture was stirred at room temperature overnight. A solution of 1.0 M boron tribromide in dichloromethane (15 ml) was further added, and the admixture was stirred at room temperature for 2 days. The reaction mixture was then treated in the same manner as described in Example 121 to obtain 1.501 g of the title compound (yield: 96%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.30 (s, 3H), 6.87 (d, J=8.5 Hz, 2H), 7.22~7.29 (m, 3H), 7.37 (td, J=1.2, 7.3 Hz, 1H), 7.73 (d, J=8.5 Hz, 2H)

Mass spectrometry data (FD-MS, m/z): 212 (M^+)

Example 127

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(2-methylphenyl)methanone [180]

Under argon, 4-hydroxyphenyl 2-methylphenyl ketone (1.334 g) obtained in Example 126 and 4-dimethylaminopyridine (845 mg) were added to xylene (15 ml), and the admixture was stirred at room temperature for 1 hour. 4-Chloro-6,7-dimethoxyquinoline (1.406 g) was added, and the admixture was refluxed with heat for 23 hours. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate and then with chloroform, followed by thin layer chromatography on silica gel eluting with hexane/acetone (2/1), to obtain 1.258 g of the title compound (yield: 50%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.37 (s, 3H), 4.02 (s, 3H), 4.06 (s, 3H), 6.65 (d, J=4.9 Hz, 1H), 7.23 (d, J=8.6 Hz, 2H), 7.25~7.35 (m, 3H), 7.39~7.42 (m, 1H), 7.45 (s, 1H), 7.46 (s, 1H), 7.91 (d, J=8.5 Hz, 2H), 8.57 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 399 (M^+)

Example 128

{3-[(6,7-Dimethoxy-4-quinolyl)oxy] phenyl}phenylmethanone [181]

A mixture of 4-chloro-6,7-dimethoxyquinoline (112 mg) and commercially available 3-hydroxybenzophenone (297 mg) was stirred at 170° C. for 10 minutes, the reaction mixture was then purified by thin layer chromatography on silica gel eluting with hexane/acetone (2/1) to obtain 126 mg of the title compound (yield: 65%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.4 (s, 3H), 4.05 (s, 3H), 6.53 (d, J=5.3 Hz, 1H), 7.34~7.88 (m, 11H), 8.53 (d, J=5.3 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 385 (M^+)

Example 129 (Reference Example)

2-Furoyl 4-hydroxyphenyl ketone

4-Tri-n-butyltin-1-methoxymethylphenol (1.282 g) obtained in Example 22 and commercially available 2-furoyl

chloride (392 mg) were dissolved in chloroform (5 ml), commercially available bis(triphenylphosphine)palladium (II) chloride (8 mg) was added, and the admixture was refluxed for 11 hours. The reaction mixture was partitioned in the same manner as described in Example 23, and the resulting ether layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue (662 mg) was dissolved in tetrahydrofuran (2 ml), water (5 ml) and 6 N aqueous hydrochloric acid (12 ml) were added, and the admixture was refluxed for 3 hours with heat. The reaction mixture was treated in the same manner as described in Example 123 to obtain 218 mg of the title compound (yield: 39%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 6.76 (dd, J=1.2, 3.1 Hz, 1H), 6.91 (d, J=8.5 Hz, 2H), 7.33 (d, J=3.1 Hz, 1H), 7.87 (d, J=8.5 Hz, 2H), 8.06 (d, J=1.2 Hz, 1H), 10.39 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 188 (M^+)

Example 130

2-Furoyl-{4-[(6,7-dimethoxy-4-quinolyl)oxy] phenyl}methanone [182]

Under argon, 2-furoyl 4-hydroxyphenyl ketone (205 mg) obtained in Example 129 and 4-dimethylaminopyridine (146 mg) were added to xylene (5 ml), and the admixture was stirred at room temperature for 1 hour. 4-Chloro-6,7-dimethoxyquinoline (244 mg) was added, and the admixture was then refluxed with heat for 20 hours. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate and then with chloroform, followed by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1), to obtain 192 mg of the title compound (yield: 47%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.03 (s, 3H), 4.07 (s, 3H), 6.63 (dd, J=1.8, 3.7 Hz, 1H), 6.65 (d, J=5.5 Hz, 1H), 7.28 (d, J=8.5 Hz, 2H), 7.32 (d, J=3.7 Hz, 1H), 7.47 (s, 1H), 7.48 (s, 1H), 7.73 (d, J=1.8 Hz, 1H), 8.14 (d, J=9.2 Hz, 2H), 8.58 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 375 (M^+)

Example 131 (Reference Example)

4-Methoxyphenyl 3-methylphenyl ketone

To commercially available nitromethane (5 ml) were added commercially available anisole (541 mg), commercially available 3-methylbenzoyl chloride (773 mg) and commercially available scandium(III) trifluoromethanesulfonate (49 mg), and the admixture was stirred at 60° C. overnight. The reaction mixture was partitioned between water and chloroform, and the chloroform layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/acetone to obtain 635 mg of the title compound (yield: 56%).

Mass spectrometry (FD-MS, m/z): 226 (M^+)

Example 132 (Reference Example)

4-Hydroxyphenyl 3-methylphenyl ketone

4-Methoxyphenyl 3-methylphenyl ketone (603 mg) obtained in Example 131 was dissolved in dichloromethane (3 ml), a 1.0 M boron tribromide-dichloromethane solution (11 ml) was added while cooled in ice, and the admixture was stirred at room temperature for 22 hours, after which the reaction mixture was treated in the same manner as described in Example 121 to obtain 316 mg of the title compound (yield: 56%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 2.38 (s, 3H), 6.89 (d, J=8.6 Hz, 2H), 7.37~7.47 (m, 4H), 7.65 (d, J=8.6 Hz, 2H), 10.42 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 212 (M^+)

Example 133

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(3-methylphenyl)methanone [183]

Under argon, 4-hydroxyphenyl 3-methylphenyl ketone (307 mg) obtained in Example 132 and 4-dimethylaminopyridine (194 mg) were added to xylene (5 ml), and the admixture was stirred at room temperature for 1 hour. 4-Chloro-6,7-dimethoxyquinoline (324 mg) was added, and the admixture was then refluxed with heat for 23 hours. The reaction mixture was treated in the same manner as described in Example 122 to obtain 262 mg of the title compound (yield: 45%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.44 (s, 3H), 4.03 (s, 3H), 4.06 (s, 3H), 6.66 (d, J=4.9 Hz, 1H), 7.27 (d, J=8.6 Hz, 2H), 7.38 (t, J=7.3 Hz, 1H), 7.41 (d, J=7.3 Hz, 1H), 7.46 (s, 1H), 7.49 (s, 1H), 7.59 (d, J=7.3 Hz, 1H), 7.64 (s, 1H), 7.93 (d, J=8.6 Hz, 2H), 8.57 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 399 (M^+)

Example 134 (Reference Example)

4-Hydroxyphenyl 4-nitrophenyl ketone

4-Tri-n-butyltin-1-methoxymethylphenol (1.282 g) obtained in Example 22 and commercially available 4-nitrobenzoyl chloride (557 mg) were dissolved in chloroform (5 ml), commercially available bis(triphenylphosphine)palladium(II) chloride (8 mg) was added, and the admixture was refluxed with heat for 23 hours. The reaction mixture was partitioned in the same manner as described in Example 23, and the resulting ether layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue (1.047 g) was dissolved in tetrahydrofuran (4 ml), water (5 ml) and 6 N aqueous hydrochloric acid (15 ml) were added, and the admixture was refluxed with heat overnight. The reaction mixture was treated in the same manner as described in Example 123 to obtain 398 mg of the title compound (yield: 55%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 6.92 (d, J=8.6 Hz, 2H), 7.69 (d, J=8.6 Hz, 2H), 7.89 (d, J=8.5 Hz, 2H), 8.36 (d, J=8.5 Hz, 2H), 10.62 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 243 (M^+)

Example 135

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(4-nitrophenyl)methanone [184]

Under argon, 4-hydroxyphenyl 4-nitrophenyl ketone (370 mg) obtained in Example 134 and 4-dimethylaminopyridine (204 mg) were added to xylene (5 ml), and the admixture was stirred at room temperature for 1 hour. 4-Chloro-6,7-dimethoxyquinoline (340 mg) was added, and the mixture was then refluxed with heat for 23 hours. The reaction

mixture was treated in the same manner as described in Example 130 to obtain 238 mg of the title compound (yield: 36%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.63 (s, 3H), 4.07 (s, 3H), 6.69 (d, J=4.9 Hz, 1H), 7.29 (d, J=8.5 Hz, 2H), 7.44 (s, 1H), 7.47 (s, 1H), 7.93 (d, J=9.2 Hz, 2H), 7.96 (d, J=8.5 Hz, 2H), 8.37 (d, J=8.5 Hz, 2H), 8.60 (d, J=5.5 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 430 (M^+)

Example 136 (Reference Example)

4-Hydroxyphenyl 3,4-methylenedioxyphenyl ketone

4-Tri-n-butyltin-1-methoxymethylphenol (1.282 g) obtained in Example 22 and commercially available piperonyloyl chloride (664 mg) were dissolved in chloroform (5 ml), commercially available bis(triphenylphosphine) palladium(II) chloride (8 mg) was added, and the admixture was refluxed with heat overnight. The reaction mixture was partitioned in the same manner as described in Example 23, and the resulting ether layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue (1.030 g) was dissolved in tetrahydrofuran (2 ml), water (5 ml) and 6 N aqueous hydrochloric acid (12 ml) were added, and the admixture was refluxed with heat 10.5 hours. The reaction mixture was treated in the same manner as described in Example 123 to obtain 212 mg of the title compound (yield: 29%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 6.15 (s, 2H), 6.88 (d, J=8.6 Hz, 2H), 7.04 (d, J=7.9 Hz, 1H), 7.21 (d, J=1.2 Hz, 1H), 7.24 (dd, J=1.8, 7.9 Hz, 1H), 7.62 (d, J=9.2 Hz, 2H), 10.34 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 242 (M^+)

Example 137

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(3,4-methylenedioxyphenyl)methanone [185]

Under argon, 4-hydroxyphenyl 3,4-methylenedioxyphenyl ketone (202 mg) obtained in Example 136 and 4-dimethylaminopyridine (112 mg) were added to xylene (2 ml), and the admixture was stirred at room temperature for 1 hour. 4-Chloro-6,7-dimethoxyquinoline (187 mg) was added, and the admixture was refluxed with heat for 20 hours. The reaction mixture was treated in the same manner as described in Example 124 to obtain 135 mg of the title compound (yield: 38%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.04 (s, 3H), 4.06 (s, 3H), 6.09 (s, 2H), 6.64 (d, J=4.9 Hz, 1H), 6.89 (d, J=7.9 Hz, 1H), 7.26 (d, J=8.5 Hz, 2H), 7.38 (d, J=1.8 Hz, 1H), 7.41 (dd, J=1.8, 7.9 Hz, 1H), 7.46 (s, 1H), 7.49 (s, 1H), 7.88 (d, J=8.5 Hz, 2H), 8.57 (d, J=4.9 Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 429 (M^+)

Example 138 (Reference Example)

3-Trifluoromethylphenyl 4-methoxyphenyl ketone

To commercially available nitromethane (10 ml) were added commercially available anisole (1.081 g), commercially available 3-(trifluoromethyl)benzoyl chloride (2.086 g) and commercially available ytterbium(III) trifluoromethanesulfonate (620 mg), and the admixture was stirred at 60° C. overnight. The reaction mixture was treated in the same manner as described in Example 120 to obtain 719 mg of the title compound (yield: 26%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.91 (s, 3H), 6.99 (d, J=9.2 Hz, 2H), 7.60~7.64 (m, 1H), 7.80~7.83 (m, 1H), 7.82 (d, J=8.5 Hz, 2H), 7.93~7.94 (m, 1H), 8.01 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 280 (M^+)

Example 139 (Reference Example)

3-Trifluoromethylphenyl 4-hydroxyphenyl ketone

3-Trifluoromethylphenyl 4-methoxyphenyl ketone (657 mg) obtained in Example 138 was dissolved in N,N-dimethylformamide (35 ml), sodium thiomethoxide (411 ml) was added, and the admixture was refluxed with heat under argon for 7 hours. The reaction mixture was treated in the same manner as described in Example 33 to obtain 454 mg of the title compound (yield: 73%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.85 (s, 1H), 6.94 (d, J=9.2 Hz, 2H), 7.62 (t, J=7.3 Hz, 1H), 7.78 (d, J=9.2 Hz, 2H), 7.83 (d, J=7.3 Hz, 1H), 7.93 (d, J=7.3 Hz, 1H), 8.01 (s, 1H)

Example 140

(3-Trifluoromethylphenyl){4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}methanone [186]

Under argon, 3-trifluoromethylphenyl 4-hydroxyphenyl ketone (432 mg) obtained in Example 139 and 4-chloro-6,7-dimethoxyquinoline (363 mg) were dissolved in diethylene glycol dimethyl ether (10 ml), and the solution was then refluxed at 160° C. for 11 hours. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate and then by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1) to obtain 204 mg of the title compound (yield: 28%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.03 (s, 3H) 4.07 (s, 3H), 6,69 (d, J=4.9Hz, 1H), 7.30 (d, J=9.2Hz, 2H), 7.47 (s, 2H), 7.66 (t, J=7.9Hz, 1H), 7.87 (d, J=7.9Hz, 1H), 7.92 (d, J =9.2Hz, 2H), 8.00 (d, J=7.9Hz, 1H), 8.08 (s, 1H), 8.60 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 453 (M^+)

Example 141 (Reference Example)

4-Cyclohexylcarbonyl-1-methoxybenzene

To commercially available nitromethane (5 ml) were added commercially available anisole (0.5 ml), commercially available cyclohexanecarbonyl chloride (0.63 ml) and commercially available ytterbium(III) trifluoromethanesulfonate (288 mg), and the admixture was stirred at 60° C. for 3 hours. The reaction mixture was partitioned between water and chloroform, and the chloroform layer was washed with saturated aqueous sodium hydrogen carbonate and brine and then dried with anhydrous sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with chloroform to obtain 517 mg of the title compound (yield: 51%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.21~1.88 (m, 10H), 3.19~3.25 (m, 1H), 3.87 (s, 3H), 6.93 (d, J=9.2Hz, 2H), 7.94 (d, J=9.2Hz, 2H)

Mass spectrometry data (FAB-MS, m/z): 219 (M^++1)

Example 142 (Reference Example)

4-Cyclohexylcarbonyl-1-hydroxybenzene

4-Cyclohexylcarbonyl-1-methoxybenzene (517 mg) obtained in Example 141 was dissolved in N,N-

dimethylformamide (20 ml), sodium thiomethoxide (538 mg) was added, and the admixture was refluxed with heat for 1 hour under argon. The reaction mixture was partitioned between water and ethyl acetate, and the ethyl acetate layer was washed with brine and then dried with anhydrous sodium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/acetone to obtain 487 mg of the title compound (yield: 93%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.23~1.88 (m, 10H), 3.19~3.25 (m, 1H), 6.06 (brs, 1H) 6.89 (d, J=8.6Hz, 2H), 7.90 (d, J=8.6Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 204 (M^+)

Example 143

4-Cyclohexylcarbonyl-1-[(6,7-dimethoxy-4-quinolyl)oxy]benzene [187]

Under argon, 4-cyclohexylcarbonyl-1-hydroxybenzene (273 mg) obtained in Example 142 and 4-chloro-6,7-dimethoxyquinoline (100 mg) were dissolved in diethylene glycol dimethyl ether (0.2 ml), and the solution was refluxed at 160° C. for 30 minutes. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was washed with brine and then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/acetone and then with chloroform/methanol to obtain 58 mg of the title compound (yield: 33%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.21~1.93 (m, 10H), 3.24~3.29 (m, 1H), 4.03 (s, 3H), 4.06 (s, 3H), 6.60 (d, J=5.5Hz, 1H), 7.24 (d, J=8.5Hz, 2H), 7.45 (s, 1H), 7.47 (s, 1H), 8.05 (d, J=8.5Hz, 2H), 8.55 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 392 (M^++1)

Example 144 (Reference Example)

2-Trifluoromethylphenyl 4-methoxyphenyl ketone

To commercially available nitromethane (10 ml) were added commercially available anisole (1.081 g), commercially available 2-(trifluoromethyl)benzoyl chloride (2.086 g) and commercially available ytterbium(III) trifluoromethanesulfonate (620 mg), and the admixture was stirred at 60° C. for 6 hours. The reaction mixture was partitioned between water and chloroform, abd the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate to obtain 1.402 g of the title compound (yield: 50%).

^{1}H-NMR ($CDCl_3$, 500 MHz): 3.88 (s, 3H) 6.93 (d, J=9.2Hz, 2H), 7.37~7.39 (m, 1H), 7.60~7.63 (m, 2H), 7.75 (d, J=9.2Hz, 2H), 7.73~7.78 (m, 1H)

Mass spectrometry data (FD-MS, m/z): 280 (M^+)

Example 145 (Reference Example)

2-Trifluoromethylphenyl 4-hydroxyphenyl ketone

2-Trifluoromethylphenyl 4-methoxyphenyl ketone (1.402 g) obtained in Example 144 was dissolved in N,N-dimethylformamide (15 ml), sodium thiomethoxide (877 mg) was added, and the admixture was refluxed with heat for 4 hours under argon. The reaction mixture was treated in the same manner as described in Example 33 to obtain 1.050 g of the title compound (yield: 79%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 5.88 (s, 1H), 6.87 (d, J=8.5Hz, 2H), 7.37~7.38 (m, 1H), 7.58~7.63 (m, 2H), 7.71 (d, J 9.2Hz, 2H), 7.76~7.78 (m, 1H)

Mass spectrometry data (FD-MS, m/z) 266 (M^+)

Example 146

(2-Trifluoromethylphenyl){4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}methanone [35]

Under argon, 2-trifluoromethylphenyl 4-hydroxyphenyl ketone (959 mg) obtained in Example 145 and 4-dimethylaminopyridine (484 mg) were added to xylene (7 ml), and the admixture was stirred at room temperature for 1 hour. 4-Chloro-6,7-dimethoxyquinoline (805 mg) was added, and the admixture was then refluxed with heat overnight. The reaction mixture was treated in the same manner as described in Example 130 to obtain 899 mg of the title compound (yield: 55%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.01 (s, 3H), 4.06 (s, 3H), 6,67 (d, J=5.5Hz, 1H), 7.22 (d, J=9.2Hz, 2H), 7.42 (s, 1H), 7.42~7.44 (m, 1H), 7.45 (s, 1H), 7.62~7.68 (m, 2H), 7.80~7.81 (m, 1H), 7.87 (d, J=8.6Hz, 2H), 8.58 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/Z): 453 (M^+)

Example 147 (Reference Example)

4-n-Butylphenyl 4-methoxyphenyl ketone

To commercially available nitromethane (10 ml) were added commercially available anisole (1.081 g), commercially available 4-(n-butyl)benzoyl chloride (1.967 g) and commercially available ytterbium(III) trifluoromethanesulfonate (620 mg), and the admixture was stirred at 60° C. for 6 hours. The reaction mixture was treated in the same manner as described in Example 120 to obtain 863 mg of the title compound (yield: 32%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.95 (t, J=7.3Hz, 3H), 1.38 (tq, J=7.3, 7.3Hz, 2H), 1.64 (quint, J=7.3Hz, 2H), 2.69 (t, J=7.3Hz, 2H), 3.89 (s, 3H), 6.96 (d, J=9.2Hz, 2H), 7.27 (d, J=8.6Hz, 2H), 7.69 (d, J=8.6Hz, 2H), 7.82 (d, J=9.2Hz, 2H)

Mass spectrometry data (FD-MS, m/z): 268 (M^+)

Example 148 (Reference Example)

4-n-Butylphenyl 4-hydroxyphenyl ketone

4-n-Butylphenyl 4-methoxyphenyl ketone (863 mg) obtained in Example 147 was dissolved in N,N-dimethylformamide (50 ml), sodium thiomethoxide (563 mg) was added, and the admixture was refluxed with heat for 7 hours under argon. The reaction mixture was treated in the same manner as described in Example 33 to obtain 787 mg of the title compound (yield: 96%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 0.95 (t, J=7.3Hz, 3H), 1.38 (tq, J=7.3, 7.3Hz, 2H), 1.64 (septet, J=7.3Hz, 2H), 2.69 (t, J=7.9Hz, 2H), 6.35 (s, 1H), 6.92 (d, J=8.5, 2H), 7.28 (d, J=7.9Hz, 2H), 7.69 (d, J=8.6Hz, 2H), 7.77 (d, J=8.5Hz, 2H)

Mass spectrometry data (FD-MS, m/z): 254 (M^+)

Example 149

(4-n-Butylphenyl){4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}methanone [40]

Under argon, 4-n-butylphenyl 4-hydroxyphenyl ketone (727 mg) obtained in Example 148 and 4-chloro-6,7-

dimethoxyquinoline (639 mg) were dissolved in diethylene glycol dimethyl ether (10 ml), and the solution was then refluxed with heat at 180° C. for 10 hours. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate and then with chloroform, followed by chromatography on silica gel eluting with hexane/acetone to obtain 400 mg of the title compound (yield: 32%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.95 (t, J=7.3Hz, 3H), 1.38 (tq, J=7.3, 7.3Hz, 2H), 1.62~1.68 (m, 2H), 2.71 (t, J=7.3Hz, 2H), 4.04 (s, 3H), 4.07 (s, 3H), 6.65 (d, J=4.9Hz, 1H), 7.26 (d, J=8.5Hz, 2H), 7.31 (d, J=7.9Hz, 2H), 7.46 (s, 1H), 7.49 (s, 1H), 7.76 (d, J=7.9Hz, 2H), 7.92 (d, J=8.5Hz, 2H), 8.58 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 440 (M^+−1)

Example 150 (Reference Example)

3-Fluorophenyl 4-methoxyphenyl ketone

To commercially available nitromethane (5 ml) were added commercially available anisole (541 mg), commercially available 3-fluorobenzoyl chloride (793 mg) and commercially available scandium(III) trifluoromethanesulfonate (49 mg), and the admixture was stirred at 60° C. for 3 days. The reaction mixture was partitioned between water and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/acetone to obtain 585 mg of the title compound (yield: 51%).

Mass spectrometry data (FAB-MS, m/z): 231 (M^++1)

Example 151 (Reference Example)

4-Hydroxyphenyl 3-fluorophenyl ketone

3-Fluorophenyl 4-methoxyphenyl ketone (580 mg) obtained in Example 150 was dissolved in dichloromethane (5 ml), a solution of 1.0 M boron tribromide in dichloromethane (23 ml) was added while cooled in ice, and the admixture was stirred at room temperature for 3 days. The reaction mixture was treated in the same manner as described in 121 to obtain 402 mg of the title compound (yield: 74%).

^{1}H-NMR (DMSO-d_6, 500 MHz) 6.91 (d, J=8.5Hz, 2H), 7.44~7.49 (m, 3H), 7.57~7.61 (m, 1H), 7.68 (d, J=8.5Hz, 2H), 10.51 (brs, 1H)

Mass spectrometry data (FAB-M S, m/z): 217 (M^++1)

Example 152

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl})(3-fluorophenyl)methanone [176]

Under argon, 4-hydroxyphenyl 3-fluorophenyl ketone (373 mg) obtained in Example 151 and 4-dimethylaminopyridine (232 mg) were added to xylene (5 ml), and the admixture was stirred at room temperature for 3 hours. 4-Chloro-6,7-dimethoxyquinoline (386 mg) was added, and the mixture was then refluxed with heat for 21 hours. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/acetone and then with chloroform, followed by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1), to obtain 484 mg of the title compound (yield: 70%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.04 (s, 3H), 4.07 (s, 3H), 6.67 (d, J=4.9Hz, 1H), 7.28 (d, J=7.9Hz, 2H), 7.31~7.33 (m, 1H), 1.46 (s, 1H), 7.47 (s, 1H), 7.48~7.53 (m, 2H), 7.59~7.61 (m, 1H), 7.93 (d, J=8.6Hz. 2H), 8.59 (d, J-5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 403 (M^+)

Example 153

N-{4-[(6,7-Dimethoxy-4-quinazolinyl)oxy]phenyl}-N'-(2-methoxyphenyl)urea [113]

6,7-Dimethoxy-4-(4-aminophenoxy)quinazoline (100 mg) was dissolved in toluene (10 ml) with heat, 2-methoxyphenyl isocyanate (0.36 ml) was added, and the admixture was refluxed with heat for 40 minutes. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) and then with chloroform/acetone (5/1) to obtain 112 mg of the title compound (yield: 75%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.90 (s, 3H), 3.98 (s, 3H), 3.99 (s, 3H), 6.90 (t, J=7.9Hz, 1H), 6.96 (t, J=7.9Hz, 1H), 7.03 (t, J=7.9Hz, 1H), 7.23 (d, J=9.2Hz, 2H), 7.39 (s, 1H), 7.55 (d, J=9.2Hz, 2H), 7.57 (s, 1H), 8.15 (d, J=7.9Hz, 1H), 8.25 (s, 1H), 8.55 (s, 1H) 9.44 (s, 1H)

Mass spectrometry data (FD-MS m/z): 446 (M^+)

Example 154

N-{4-[(6,7-Dimethoxy-4-quinazolinyl)oxy]phenyl}-N'-(3-methoxyphenyl)urea [114]

6,7-Dimethoxy-4-(4-aminophenoxy)quinazoline (100 mg) was dissolved in toluene (10 ml) with heat, 3-methoxyphenyl isocyanate (0.36 ml) was added, and the admixture was refluxed with heat for 40 minutes. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) and then with chloroform/acetone (5/1) to obtain 31 mg of the title compound (yield: 21%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.74 (s, 3H), 3.98 (s, 3H), 3.99 (s, 3H), 6.56 (d, J=7.9Hz, 1H), 6.96 (d, J=7.9Hz, 1H), 7.15~7.25 (m, 4H), 7.38 (s, 1H), 7.53~7.60 (m, 3H), 8.55 (s, 1H), 8.76 (s, 1H) 8.81 (s, 1H)

Mass spectrometry data (FD-MS, m/z) 446 (M^+)

Example 155

N-{4-[(6,7-Dimethoxy-4-quinazolinyl)oxy]phenyl}-N'-(4-methoxyphenyl)urea [99]

6,7-Dimethoxy-4-(4-aminophenoxy)quinazoline (81 mg) was dissolved in toluene (5 ml) with heat, 4-methoxyphenyl isocyanate (0.29 ml) was added, and the admixture was refluxed with heat for 40 minutes. The separated solid was filtered and washed with toluene to obtain 60 mg of the title compound (yield: 49%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.79 (s, 3H), 4.06 (s, 3H), 4.07 (s, 3H), 6.48 (s, 1H), 6.64 (s, 1H), 6.91 (d, J=9.2Hz,

2H), 7.20 (d, J=8.6Hz, 2H), 7.27 (d, J=8.6Hz, 2H), 7.32 (s, 1H), 7.47 (d, J=9.2Hz, 2H), 7.55 (s, 1H), 8.60 (s, 1H)

Mass spectrometry data (FAB-MS, m/z): 447 (M^++1)

Example 156

N-{4-[(6,7-Dimethoxy-4-quinazolinyl)oxy]phenyl}-N'-(2-fluorophenyl)urea [116]

6,7-Dimethoxy-4-(4-aminophenoxy)quinazoline (100 mg) was dissolved in toluene (10 ml) with heat, 2-fluorophenyl isocyanate (0.30 ml) was added, and the admixture was refluxed with heat for 40 minutes. The separated solid was filtered and washed with toluene to obtain 96 mg of the title compound (yield: 66%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.98 (s, 3H), 3.99 (s, 3H), 7.00~7.04 (m, 1H), 7.13~7.17 (m, 1H), 7.23~7.27 (m, 3H), 7.39 (s, 1H), 7.54~7.57 (m, 3H), 8.10~8.20 (m, 1H), 8.55 (s, 1H), 8.65 (s, 1H), 9.26 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 434 (M^+)

Example 157

N-(4-[(6,7-Dimethoxy-4-quinazolinyl)oxy]phenyl)-N'-n-butylurea [220]

6,7-Dimethoxy-4-(4-aminophenoxy)quinazoline (100 mg) was dissolved in toluene (10 ml) with heat, n-butyl isocyanate (0.29 ml) was added, and the admixture was refluxed with heat for 40 minutes. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (5/1) to obtain 75 mg of the title compound (yield: 56%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 0.90 (t, J=7.3Hz, 3H), 1.28–1.46 (m, 4H), 3.09 (q, J=6.7Hz, 2H), 3.97 (s, 3H), 3.99 (s, 3H), 6.23 (brs, 1H), 7.14 (d, J=9.2Hz, 2H), 7.37 (s, 1 H), 7.47 (d, J=9.2Hz, 2H), 7.55 (s, 1H), 8.53 (s, 1H), 8.58 (brs, 1H)

Mass spectrometry data (FD-MS, m/z): 396 (M^+)

Example 158

N-{4-[6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-(4-tert-butylphenyl)carboxamide [213]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) and commercially available 4-tert-butylbenzoic acid (102 mg) were dissolved in N,N-dimethylformamide (3 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (106 mg) was added, and the admixture was stirred at room temperature for 6 hours, after which the reaction mixture was purified in the same manner as described in Example 51 to obtain 29 mg of the title compound (yield: 35%).

^{1}H-NMR ($CDCl_3$, 90 MHz): (1.36 (s, 9H), 4.05 (s, 3H), 4.08 (s, 3H), 6.48 (d, J=5.3Hz, 1H), 7.1~8.1 (m, 11H) 8.49 (d, J=5.3Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 456 (M^+)

Example 159

N-(4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl)-N-methyl-(4-t-butylphenyl)carboxamide [156]

N-(4-[6,7-Dimethoxy-4-quinolyl)oxy]phenyl)-(4-t-butylphenyl)carboxamide (100 mg) obtained in Example 158 was dissolved in N,N-dimethylformamide (3 ml), sodium hydride (10 mg) was added, and the admixture was stirred at 0° C. for 1 hour, methyl iodide (31 mg) was added, and the admixture was stirred for a further 3 hours. The reaction mixture was purified in the same manner as described in Example 51 to obtain 48 mg of the title compound (yield: 46%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 1.28 (s, 9H), 3.54 (s, 3H), 4.04 (s, 3H), 4.05 (s, 3H), 6.32 (d, J=5.3Hz, 1H), 7.0~7.3 (m, 8H), 7.42 (s, 1H), 7.50 (s, 1H), 8.47 (d, J=5, 3Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 470 (M^+)

Example 160

N-(4-t-Butylphenyl)-{4-[(6,7-dimethoxy-4-quinolyl) oxy]phenyl}carboxamide [155]

4-[6,7-Dimethoxy-4-quinolyl)oxybenzoic acid (54 mg) and commercially available 4-tert-butylaniline (102 mg) were dissolved in N,N-dimethylformamide (3 ml), 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (106 mg) was added, and the admixture was stirred at room temperature for 6 hours, after which the reaction mixture was purified in the same manner as described in Example 51 to obtain 29 mg of the title compound (yield: 35%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 1.34 (s, 9H), 4.03 (s, 3H), 4.06 (s, 3H), 6.57 (d, J=5.3Hz, 1H), 7.2~8.1 (m, 11H), 8.55 (d, J=5.3Hz, 1H)

Mass spectrometry dada (FD-MS, m/z): 456 (M^+)

Example 161 (Reference Example)

2- (6, 7-Dimethoxy-4-quinolyl)-2-(4-bromophenyl) acetonitrile

Commercially available 4-bromophenylacetonitrile (4.3 g) was dissolved in toluene (10 ml) and the temperature was lowered to 0° C., after which sodium hydride (1.0 g) was added, the temperature was raised to 50° C., and the admixture was then stirred for 1 hour. Next, 6,7-dimethoxy-4-chloroquinoline (2.2 g) was added, and the admixture was stirred for 15 hours, after which water was poured and the mixture was extracted with ethyl acetate. After drying with anhydrous sodium sulfate and removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate (2/1) to obtain 850 mg of the title compound (yield: 22%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.66 (s, 3H), 3.80 (s, 3H), 5.40 (s, 1H), 6.74 (s, 1H), 6.9~7.4 (m, 6H), 8.54 (d, J=4.6Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 382 (M^+), 384 (M^++2)

Example 162 (Reference Example)

6,7-Dimethoxy-4-(4-bromophenylmethyl)quinoline

2-(6,7-Dimethoxy-4-quinolyl)-2-(4-bromophenyl) acetonitrile (850 mg) obtained in Example 161 was dissolved in 60% aqueous sulfuric acid (5 ml), and the solution was refluxed with heat for 1 hour and then poured into a container with ice and aqueous ammonia. The solution was neutralized with hydrochloric acid and extracted with methylene chloride. After drying with anhydrous sodium sulfate and removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 260 mg of the title compound (yield: 33%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.90 (s, 3H), 4.02 (s, 3H), 4.31 (s, 2H), 6.9~7.5 (m, 7H), 8.63 (d, J=4.4Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 357 (M^+), 359 (M^++2)

Example 163 (Reference Example)

6,7-Dimethoxy-4-(4-tri-n-butylstanniophenylmethyl) quinoline

Under argon, 6,7-dimethoxy-4-(4-bromophenylmethyl) quinoline (250 mg) obtained in Example 162 was dissolved in tetrahydrofuran (4 ml), and the solution was cooled to −78° C., a solution of 2.5 M n-butyllithium in hexane (0.3 ml) was added dropwise, and the admixture was stirred for 1 hour. An solution of tri-n-butyltin chloride in tetrahydrofuran (1 ml) was then added dropwise, and the admixture was stirred at −78° C. for 2 hours. After adding water, extracting with ethyl acetate and drying with anhydrous sodium sulfate, the solvent was removed by distillation, and the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate (3/1) to obtain 85 mg of the title compound (yield: 22%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 0.7~1.6 (m, 27H), 3.88 (s, 3H), 4.02 (s, 3H), 4.34 (s, 2H), 7.0~7.5 (m, 7H), 8.63 (d, J=4.6Hz, 1H)

Example 164

(4-[(6,7-Dimethoxy-4-quinolyl)methyl]phenyl](4-t-butylphenyl)methanone [162]

6,7-Dimethoxy-4-tri-n-butylstanniophenylmethyl) quinoline (82 mg) obtained in Example 162, commercially available 4-t-butylbenzoyl chloride (31 mg) and a catalytic amount of commercially available bis(triphenylphosphine) palladium(II) chloride were dissolved in chloroform (3 ml), and the admixture was stirred for 15 hours under reflux with heat. After the addition of water, the admixture was extracted with methylene chloride, and the organic layer was washed with brine and saturated aqueous potassium fluoride and then dried with anhydrous sodium sulfate. After removing the solvent by distillation, the resulting residue was purified by thin layer chromatography on silica gel eluting with hexane/ethyl acetate (2/1) to obtain 6 mg of the title compound (yield: 10%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 1.36 (s, 9H), 3.91 (s, 3H), 4.03 (s, 3H), 4.45 (s, 2H), 7.0~7.9 (m, 11H), 8.66 (d, J=4.4Hz, 1H)

Mass spectrometry data (FAB-MS, m/z): 440$^+$+1)

Example 165

N-(4-Fluorophenyl)-N'-(4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl)thiourea [203]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (100 mg) was dissolved in toluene (10 ml) with heat, 4-fluorophenylthio isocyanate (52 mg) was added, and the admixture was refluxed with heat for 24 hours. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 32 mg of the title compound (yield: 21%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.93 (s, 3H), 3.95 (s, 3H), 6.50 (d, J=4.9Hz, 1H), 7.15~7.25 (m, 4H), 7.40 (S, 1H), 7.47~7.50 (m, 3H), 7.59 (d,. J=8.6Hz, 2H), 8.50 (d, J=5.5Hz, 1H), 9.80 (brs, 1H), 9.84 (brs, 1H)

Mass spectrometry data (FAB-MS, m/z 450 (M^++1)

Example 166

1-(4-Fluorophenyl)-2-cyano-3-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}guanidine [204]

N-(4-Fluorophenyl)-N'-(4-[(6,7-dimethoxy-4-quinolyl) oxy]phenyl)thiourea (33 mg) obtained in Example 165, dicyclohexylcarbodiimide (31 mg) and a catalytic amount of diisopropylethyl amine were dissolved in methylene chloride (10 ml). To this solution, a solution of cyanamide (16 mg) in THF (1 ml) was added, and the admixture was stirred at room temperature overnight. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 34 mg of the title compound (yield: 99%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.03 (s, 3H) 4.05 (s, 3H), 6.50 (d, J=5.1Hz, 1H), 6.8~7.6 (m, 10H), 8.52 (d, J=5.3Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 457 (M^+)

Example 167

N-(2-Fluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}thiourea [205]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (100 mg) was dissolved in toluene (10 ml) with heat, 2-fluorophenylthio isocyanate (0.05 ml) was added, and the admixture was refluxed with heat for 24 hours. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 66 mg of the title compound (yield: 42%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.03 (s, 3H) 4.05 (s, 3H), 6.53 (d, J=5.3Hz, 1H), 7.1~8.0 (m, 10H), 8.53 (d, J=5.1Hz, 1H)

Mass spectrometry data (FAB-MS, m/z): 450 (M^++1)

Example 168

1-(2-Fluorophenyl)-2-cyano-3-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}guanidine [206]

N-(2-Fluorophenyl)-N'-(4-[(6,7-dimethoxy-4-quinolyl) oxy]phenyl}thiourea (42 mg) obtained in Example 167, dicyclohexylcarbodiimide (37 mg) and a catalytic amount of diisopropylethyl amine were dissolved in methylene chloride (10 ml). To this solution, a solution of cyanamide (20 mg) in THF (1 ml) was added, and the admixture was stirred at room temperature overnight. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 41 mg of the title compound (yield: 99%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 4.04 (s, 3H), 4.06 (s, 3H) 6.53 (d, J=5.3Hz, 1H), 7.1~7.6 (m, 10H), 8.47 (d, J 5.3Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 457 (M^++1)

Example 169

N-(2-Methoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}thiourea [207]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (100 mg) was dissolved in toluene (10 ml) with heat,

2-methoxyphenylthio isocyanate (0.05 ml) was added, and the admixture was refluxed with heat for 24 hours. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 78 mg of the title compound (yield: 50%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 3.86 (s, 3H), 4.04 (s, 3H), 4.05 (s, 3H), 6.52 (d, J=5.3Hz, 1H), 6.9~8.1 (m, 10H), 8.52 (d, J=5.1Hz, 1H)

Mass spectrometry data (FAB-MS, m/z): 462 (M^++1)

Example 170

N-(2-Methylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}thiourea [209]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (80 mg) was dissolved in toluene (10 ml) with heat, 2-methylphenylthio isocyanate (0.04 ml) was added, and the admixture was refluxed with heat for 24 hours. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 31 mg of the title compound (yield: 26%).

^{1}H-NMR ($CDCl_3$, 90 MHz): 2.38 (s, 3H), 4.03 (s, 3H), 4.04 (s, 3H), 6.50 (d, J=5.3Hz, 1H), 7.1~7.8 (m, 10H), 8.50 (d, J=5.3Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 445 (M^+)

Example 171

N-(3-Methylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl)thiourea [211]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (81 mg) was dissolved in toluene (10 ml) with heat, 3-methylphenylthio isocyanate (0.04 ml) was added, and the admixture was refluxed with heat for 15 minutes. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 66 mg of the title compound (yield: 55%).

^{1}H-NMR ($CDCl_3$, 90 MHz): δ 2.40 (s, 3H), 4.03 (s, 3H), 4.05 (s, 3H), 6.52 (d, J=5.1Hz, 1H), 7.1~7.8 (m, 10H), 8.51 (d, J=5.3Hz, 1H)

Mass spectrometry data (FD-MS m/z): 445 (M^+)

Example 172

1-(2-Methylphenyl)-2-cyano-3-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}guanidine [210]

N-(2-Methylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl) oxy]phenyl}thiourea (24 mg) obtained in Example 170, dicyclohexylcarbodiimide (45 mg) and a catalytic amount of diisopropylethyl amine were dissolved in methylene chloride (7 ml), to which a solution of cyanamide (28 mg) in THF (1 ml) was added, and the admixture was stirred at room temperature overnight. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 18 mg of the title compound (yield: 76%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.36 (s, 3H), 4.03 (s, 3H), 4.04 (s, 3H), 6.46 (d, J=5.5Hz, 1H), 7.30~7.45 (m, 9H), 7.50 (s, 1H), 8.49 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 453 (M^+)

Example 173

1-(3-Methylphenyl)-2-cyano-3-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}guanidine [212]

N-(3-Methylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl) oxy]phenyl}thiourea (18 mg) obtained in Example 171, dicyclohexylcarbodiimide (35 mg) and a catalytic amount of diisopropylethyl amine were dissolved in methylene chloride (6 ml), to which a solution of cyanamide (23 mg) in THF (1 ml) was added, and the admixture was stirred at room temperature overnight. After removing the solvent by distillation, the resulting residue was purified by column chromatography on silica gel eluting with chloroform/methanol (100/1) to obtain 19 mg of the title compound (yield: 95%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.38 (s, 3H), 4.03 (s, 3H), 4.04 (s, 3H), 6.48 (d, J=5.5Hz, 1H), 7.10~7.45 (m, 9H), 7.51 (s, 1H), 8.48 (d, J=5.5Hz, 1H)

Mass spectrometry mass (FD-MS, m/z): 453 (M^+)

Example 174

4-Hydroxyphenyl 3-trifluoroxyphenyl ketone (Reference Example)

4-Tri-n-butyltin-1-methoxymethylphenol (1.3 g) obtained in Example 22 and commercially available 4-(trifluoromethoxy)benzoyl chlorobenzoyl chloride (674 mg) were dissolved in chloroform (5 ml), commercially available bis(triphenylphosphine)palladium(II) chloride (8 mg) was added, and the admixture was refluxed overnight. The reaction mixture was partitioned in the same manner as described in Example 23, and the ether layer was dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue (2.0 g) was dissolved in tetrahydrofuran (4 ml), water (5 ml) and 6 N aqueous hydrochloric acid (15 ml) were added, and the admixture was refluxed for 8 hours. The reaction mixture was partitioned between water and chloroform, and the chloroform layer was dried with magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate to obtain 2.1 g of the title compound.

Mass spectrometry data (FD-M S, m/z): 282 (M^+)

Example 175

(4-Trifluoromethoxyphenyl){4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}methanone [284]

4-Hydroxyphenyl 3-trifluoroxyphenyl ketone (581 mg) obtained in Example 174 and commercially available 4-dimethylaminopyridine (277 mg) were added to xylene (20 ml), and the admixture was stirred at room temperature under argon. After 1 hour, 4-chloro-6,7-dimethoxyquinoline (460 mg) was added, and the admixture was refluxed with heat for 24 hours. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate and then with chloroform, followed by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1), to obtain 470 mg of the title compound (yield: 49%).

^{1}H-NMR ($CDCl_3$, 500 MHz): (4.03 (s, 3H) 4.07 (s, 3H), 6.66 (d, J=4.5Hz, 1H), 7.28 (d, J=8.5Hz, 2H), 7.35 (d, J=8.7Hz, 2H), 7.46 (s, 1H), 7.47 (s, 1H), 7.89 (d, J=8.5Hz, 2H), 7.92 (d, J=9.2Hz, 2H), 8.59 (d, J=4.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 469 (M^+)

Example 176

4-Methoxyphenyl 4-iodophenyl ketone (Reference Example)

To commercially available nitromethane (10 ml) were added commercially available anisole (1.08 g), commer-

cially available 4-iodobenzoyl chloride (2.67 g) and commercially available ytterbium(III) trifluoromethanesulfonate (620 mg), and the admixture was stirred at 60° C. overnight. The reaction mixture was partitioned between water and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate to obtain 722 mg of the title compound (yield: 21%).

Mass spectrometry data (FD-MS, m/z): 338 (M^+)

Example 177

4-Hydroxyphenyl 4-iodophenyl ketone (Reference Example)

4-Methoxyphenyl 4-iodophenyl ketone (722 mg) obtained in Example 176 was dissolved in dichloromethane (20 ml), a solution of 1.0 M boron tribromide in dichloromethane (9 ml) was added while cooled in ice, and the admixture was stirred at room temperature overnight. The reaction mixture was then poured into ice water and partitioned between water and chloroform. The chloroform layer was dried with anhydrous magnesium sulfate and the solvent was removed by reduced-pressure distillation. The resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate to obtain 380 mg of the title compound (yield: 55%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 6.89 (d, J=8.6Hz, 2H), 7.44 (d, J=8.5Hz, 2H), 7.65 (d, J=8.6Hz, 2H), 7.92 (d, J=8.5Hz, 2H), 10.47 (s, H)

Mass spectrometry data (FAB-MS, m/z): 325 (M^++1)

Example 178

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(4-iodophenyl)methanone [285]

Under argon, 4-hydroxyphenyl 4-iodophenyl ketone (380 mg) obtained in Example 177 and 4-dimethylaminopyridine (158 mg) were added to xylene (11 ml), and the admixture was stirred at room temperature. After 2 hours, 4-chloro-6,7-dimethoxyquinoline (262 mg) was added, and the admixture was refluxed with heat overnight. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by chromatography on silica gel eluting with hexane/ethyl acetate and then with chloroform/ethyl acetate, followed by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1), to obtain 459 mg of the title compound (yield: 77%).

^{1}H-NMR ($CDCl_3$, 50 MHz): δ 4.03 (s, 3H), 4.07 (s, 3H), 6.66 (d, J=4.9Hz, 1H), 7.27 (d, J=7.6Hz, 2H), 7.46 (s, 1H), 7.4 7 (s, 1H), 7.5 4 (d, J 8.6Hz, 2H), 7.88 (d, J=7.9Hz, 2H), 7.90 (d, J=8.6Hz, 2H), 8.58 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS m/z): 511 (M^+)

Example 179

4-Methoxyphenyl 4-bromophenyl ketone (Reference Example)

To commercially available nitromethane (10 ml) were added commercially available anisole (1.08 g), commercially available 4-bromobenzoyl chloride (2.20 g) and commercially available ytterbium(III) trifluoromethanesulfonate (620 mg), and the admixture was stirred at 60° C. overnight. The reaction mixture was partitioned between water and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate to obtain 1.65 g of the title compound (yield: 57%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.89 (s, 3H), 6.97 (d, J=8.6Hz, 2H), 7.62 (d, J=1.8Hz, 2H), 7.62 (d, J=1.8Hz, 2H), 7.80 (d, J=9.2Hz, 2H)

Mass spectrometry data (FD-MS, m/z: 290 (M^+), 292 (M^++2)

Example 180

4-Hydroxyphenyl 4-bromophenyl ketone (Reference Example)

4-Methoxyphenyl 4-bromophenyl ketone (1.65 g) obtained in Example 122 was dissolved in dichloromethane (20 ml), a solution of 1.0 M boron tribromide in dichloromethane (23 ml) was added while cooled in ice, and the admixture was stirred at room temperature overnight. The reaction mixture was then poured into ice water and partitioned between water and chloroform. The chloroform layer was dried with anhydrous magnesium sulfate and the solvent was removed by reduced-pressure distillation. The resulting residue was purified by column chromatography on silica gel eluting with hexane/ethyl acetate to obtain 1.34 g of the title compound (yield: 85%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 6.90 (d, J=8.5Hz, 2H), 7.61 (d, J=8.5Hz, 2H), 7.66 (d, J=8.6Hz, 2H), 7.74 (d, J=8.5Hz, 2H), 10.46 (brs, 1H)

Mass spectrometry data (FD-MS, m/z): 276 (M^+), 278 (M^++2)

Example 181

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}(4-bromophenyl)methanone [286]

Under argon, 4-hydroxyphenyl 4-bromophenyl ketone (1.182 g) obtained in Example 123 and 4-dimethylaminopyridine (573 mg) were added to xylene (37 ml), and the admixture was stirred at room temperature. After 30 minutes, 4-chloro-6,7-dimethoxyquinoline (954 mg) was added, and the admixture was refluxed with heat overnight. The reaction mixture was partitioned between saturated aqueous sodium hydrogen carbonate and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified first by chromatography on silica gel eluting with chloroform/ethyl acetate and then by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1) to obtain 1.574 g of the title compound (yield: 79%).

^{1}H-NMR ($CDCl_3$, 50 MHz): δ 4.05 (s, 3H), 4.66 (s, 3H), 6.66 (d, J=4.9Hz, 1H), 7.27 (d, J=8.6Hz, 2H), 7.46 (s, 1H), 7.47 (s, 1H), 7.65 (d, J=7.9Hz, 2H), 7.70 (d, J=8.5Hz, 2H), 7.90 (d, J=8.6Hz, 2H), 8.58 (d, J=5.5Hz, 1 H)

Mass spectrometry data FD-MS, m/z): 463 (M^+), 465 (M++2)

Example 182

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}[4-(N-methylpiperazino)phenyl]methanone [287]

Under argon, 4-hydroxyphenyl 4-bromophenyl ketone (232 mg) obtained in Example 180 was added to toluene (5

ml), and commercially available N-methylpiperazine (61 mg), sodium t-butoxide (67 mg), bis (triphenylphosphine) palladium(II) chloride (7 mg) were further added, and the admixture was stirred at 100° C. for 17 hours. The reaction mixture was partitioned between brine and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by thin layer chromatography on silica gel eluting with chloroform/methanol (10/1) to obtain 53 mg of the title compound (yield: 22%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.37 (s, 3H), 2.58 (t, J=5.5Hz, 4H), 3.41 (t, J=5.5Hz, 4H) 4.04 (s, 3H), 4.06 (s, 3H), 6.63 (d, J=4.9Hz, 1H), 6.9 2 (d, J=9.2Hz, 2H), 7.2 5 (d, J=8.5Hz, 2H), 7.45 (s, 1H), 7.51 (s, 1H), 7.81 (d, J=8.5Hz, 2H), 7.87 (d, J=8.6Hz, 2H), 8.56 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 483 (M^+)

Example 183

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}[4-morpholinophenyl]methanone [288]

Under argon, 4-hydroxyphenyl 4-bromophenyl ketone (232 mg) obtained in Example 180 was added to toluene (5 ml), and commercially available morpholine (52 mg), sodium t-butoxide (67 mg), bis (triphenylphosphine) palladium(II) chloride (7 mg) were further added, and the admixture was stirred at 100° C. overnight. The reaction mixture was partitioned between brine and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1) to obtain 32 mg of the title compound (yield: 14%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.34 (t, J=4.9Hz, 4H), 3.88 (t, K=4.9Hz, 4H), 4.04 (s, 3H), 4.07 (s, 3H), 6.64 (d, J=5.5Hz, 1H), 6.92 (d, J=9.2Hz, 2H), 7.26 (d, J=7.3Hz, 2H), 7.83 (d, J=9.2Hz, 2H), 7.87 (d, J=8.5Hz, 2H), 8.56 (d, J=5.5Hz, 1H), 7.49 (s, 1H), 7.51 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 470 (M^+)

Example 184

(4-[{6,7-Dimethoxy-4-quinolyl)oxy]phenyl)[4-pyrrolidinophenyl]methanone [289]

Under argon, 4-hydroxyphenyl 4-bromophenyl ketone (232 mg) obtained in Example 180 was added to toluene (5 ml), and commercially available pyrrolidine (43 mg), sodium t-butoxide (67 mg), bis (triphenylphosphine) palladium(II) chloride (7 mg) were further added, and the admixture was stirred at 100° C. overnight. The reaction mixture was partitioned between brine and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1) to obtain 45 mg of the title compound (yield: 20%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.05~2.08 (m, 4 H), 3.38~3.41 (m, 4H), 4.04 (s, 3H), 4.06 (s, 3H), 6.57 (d, J=9.2Hz, 2H), 6.62 (d, J=4.9Hz, 1H), 7.24 (d, J=8.6Hz, 2H), 7.45 (s, 1H), 7.52 (s, 1H), 7.82 (d, J=9.2Hz, 2H), 7.84 (d, J=8.5Hz, 2H), 8.55 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 454 (M^+)

Example 185

{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}[4-piperidinophenyl]methanone [290]

Under argon, 4-hydroxyphenyl 4-bromophenyl ketone (232 mg) obtained in Example 180 was added to toluene (5 ml), and commercially available piperidine (51 mg), sodium t-butoxide (67 mg), bis (triphenylphosphine)palladium(II) chloride (7 mg) were further added, and the admixture was stirred at 100° C. overnight. The reaction mixture was partitioned between brine and chloroform, and the chloroform layer was then dried with anhydrous magnesium sulfate. After removing the solvent by reduced-pressure distillation, the resulting residue was purified by thin layer chromatography on silica gel eluting with chloroform/ethyl acetate (5/1) to obtain 75 mg of the title compound (yield: 32%)).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.68 (brs, 6H), 3.38~3.39 (m, 4H), 4.04 (s, 3H), 4.06 (s, 3H), 6.63 (d, J=4.9Hz, 1H), 6.89 (d, J=8.6Hz, 2H), 7.25 (d, J=8.5Hz, 2H), 7.45 (s, 1H), 7.52 (s, 1H), 7.80 (d, J=9.2Hz, 2H), 7.86 (d, J=8.6Hz, 2H), 8.56 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 468 (M^+)

Example 186

N-(2-Methoxy-4-biphenyl)-N'-(4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl urea [222]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was suspended in toluene (5 ml), after the addition of triethylamine (1 ml), triphosgene (52 mg) was added, and the admixture was refluxed with heat for 2 minutes 2-Methoxy-4-biphenylamine (103 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 13 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 89 mg of the title compound (yield: 98%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.67 (s, 3H), 4.00 (s, 3H), 4.04 (s, 3H) 6.44 (d, J=5.5Hz, 1H), 6.86 (d, J=8.5Hz, 1H), 7.10 (d, J=8.6Hz, 2H), 7.21~7.37 (m, 4H), 7.43 (s, 1H) , 7.53~7.59 (m, 5H), 7.79 (s, H), 8.41 (s, 1H), 8.48 (d, J=4.9Hz, 1H), 8.53 (d, J=2.4Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 521 (M^+)

Example 187

N-(2,6-Diisopropylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [223]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) was suspended in toluene (5 ml), after the addition of triethylamine (1 ml), triphosgene (53 mg) was added, and the admixture was refluxed with heat for 8 minutes. 2,6-Diisopropylaniline (0.05 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 15 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on

silica gel eluting with chloroform/acetone (10/1) to obtain 69 mg of the title compound (yield: 76%).

^{1}H-NMR ($CDCl_3$, 500 MHz): (1.26 (m, 12H), 3.39 (m, 2H), 4.03 (s, 3H), 4.04 (s, 3H), 6.09 (brs, 1H), 6.28 (brs, 1H), 6.42 (d, J=5.5Hz, 1H), 7.08 (d, J=8.5Hz, 2H), 7.2 6 (m, 1H), 7.29~7.40 (m, 5H), 7.53 (s, 1H), 8.46 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 499 (M^+)

Example 188

N-(2-Chloro-4-nitrophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [224]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (104 mg) was dissolved in toluene (10 ml) with heat, 2-chloro-4-nitrophenyl isocyanate (150 mg) was added, and the admixture was refluxed with heat for 10 minutes. The separated crystals were filtered and then washed with toluene to obtain 172 mg of the title compound (yield: 100%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.46 (d, J=5.5Hz, 1H), 7.24 (d, J=9.2Hz, 2H), 7.38 (s, 1H), 7.51 (s, 1H), 7.62 (d, J=8.6Hz, 2H), 8.23 (, 1H), 8.35 (m, 1H), 8.46 (d, J=5.5Hz, 1H), 8.57 (m, 1H), 8.84 (brs, 1H), 9.85 (brs, 1H)

Mass spectrometry data (FD-MS, m/z): 494 (M^+), 496 (M^++2)

Example 189

N-(3-Chloro-2-methoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [225]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (58 mg) was dissolved in toluene (5 ml) with heat, 3-chloro-2-methoxyphenyl isocyanate (0.1 ml) was added, and the admixture was refluxed with heat for 22 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 75 mg of the title compound (yield: 80%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.73 (s, 3H), 4.02 (s, 3H), 4.05 (s, 3H), 6.47 (d, J=5.5Hz, 1H), 7.01~7.06 (m, 2H), 7.15 (d, J=8.5Hz, 2H), 7.4 3 (s, 1H), 7.52 (d, J=9.2Hz, 2H), 7.57 (s, 1H), 7.81 (s, 1H), 8.16~8.19 (m, 2H), 8.50 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 479 (M^+) 481 (M^++2)

Example 190

N-(2-Chloro-6-methylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [226]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 2-chloro-6-methylphenyl isocyanate (0.1 ml) was added, and the admixture was refluxed with heat for 17 minutes. The separated crystals were filtered and then washed with toluene to obtain 61 mg of the title compound (yield: 74%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 2.2 8 (s, 3H), 3.94 (s, 3H), 3.94 (s, 3H), 6.43 (d, J=4.9Hz, 1H), 7.18 (d, J=9.2Hz, 2H), 7.20~7.36 (m, 3 H), 7.38 (s, 1H), 7.52 (s, 1H), 7.59 (d, J=9.2Hz, 2H), 7.98 (s, 1H), 8.45 (d, J=5.5Hz, 1H), 9.03 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 463 (M^+) 465 (M^++2)

Example 191

N-(3-Chloro-6-methoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [227]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 3-chloro-6-methoxyphenyl isocyanate (111 mg) was added, and the admixture was refluxed with heat for 29 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 84 mg of the title compound (yield: 100%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.60 (s, 3H), 3.99 (s, 3H), 4.05 (s, 3H), 6.46 (d, J=4.9Hz, 1H), 6.69 (d, J=8.5Hz, 1H), 6.90 (m, 1H), 7.11 (d, J=9.2Hz, 2H), 7.4 2 (s, 1H), 7.5 3 (d, J=8.5Hz, 2H), 7.58 (s, 1H), 7.82 (s, 1H), 8.32 (m, 1H), 8.48 (d, J=5.5Hz, 1H), 8.52 (s, 1H)

Mass spectrometry data (FED-MS, m/z): 479 (M^+), 481 (M^++2)

Example 192

N-(4-Chloro-3-nitrophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [228]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 4-chloro-3-nitrophenyl isocyanate (224 mg) was added, and the admixture was refluxed with heat for 8 minutes. The separated crystals were filtered and then washed with toluene to obtain 125 mg of the title compound (yield: 75%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.93 (s, 3H), 3.95 (s, 3H), 6.46 (d, J=4.9Hz, 1H), 7.21 (d, J=8.5Hz, 2H), 7.38 (s, 1H), 7.51 (s, 1H), 7.60 (d, J=8.5Hz, 2H), 7.65 (s, 1H), 7.67 (s, 1H), 8.31 (s, 1H), 8.46 (d, J=4.9Hz, 1H), 9.01 (s, 1H), 9.27 (s, 1H)

Mass spectrometry data (FD-MS m/z): 463 (M^+) 465 (M^++2)

Example 193

N-(2,4-Dichlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [229]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) was dissolved in toluene (5 ml) with heat, 2,4-dichlorophenyl isocyanate (115 mg) was added, and the admixture was refluxed with heat for 20 minutes. The separated crystals were filtered and then washed with toluene to obtain 74 mg of the title compound (yield: 85%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.45 (d, J=4.9Hz, 1H), 7.22 (d, J=8.6Hz, 2H), 7.39 (s, 1H), 7.40 (m, 1H), 7.51 (s, 1H), 7.59 (d, J=9.2Hz, 2H), 7.62 (m, 1 H), 8.21 (d, J=9.2Hz, 1H), 8.40 (s, 1H), 8.46 (d, J=5.5Hz, 1H), 9.56 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 483 (M^+)

Example 194

N-(2,6-Dichlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [230]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 2,6-dichlorophenyl isocyanate (125 mg) was added, and the admixture was refluxed with heat for 12 minutes. The separated crystals were filtered and then washed with toluene to obtain 81 mg of the title compound (yield: 96%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H) 3.94 (s, 3H), 6.43 (d, J=5.5Hz, 1H), 7.14~7.20 (m, 2H), 7.32 (dd, J=7.9, 7.9Hz, 1H), 7.38 (s, 1H) 7.51~7.54 (m, 3H), 7.60 (d, J=9.2Hz, 2H), 8.23 (s, 1H), 8.45 (d, J=5.5Hz, 1H), 9.09 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 483 (M^+)

Example 195

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-N'-[1-(1-naphthyl)ethyl]urea [231]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) was dissolved in toluene (5 ml) with heat, 1-(1-naphthyl)

ethyl isocyanate (0.05 ml) was added, and the admixture was refluxed with heat for 100 minutes. The resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 65 mg of the title compound (yield: 72%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 1.55 (d, J=6.7Hz, 3H), 3.93 (s, 3H), 3.94 (s, 3H), 4.62 (m, 1H), 6.39 (d, J=4.9Hz, 1H), 6.79 (s, 1H), 7.11 (d, J=9.2H z, 2H), 7.36 (s, 1H), 7.49~7.58 (m, 7H), 7.83 (m, 1H), 7.94 (m, 1H), 8.17 (m, 1H), 8.42 (d, J=5.5Hz, 1H), 8.52 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 493 (M^+)

Example 196

N-(2-n-Butylphenyl)-N'-(4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [232]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (119 mg) was suspended in toluene (12 ml), after the addition of triethylamine (2.4 ml), triphosgene (133 mg) was added, and the admixture was refluxed with heat for 2 minutes. 2-n-Butylphenylamine (0.13 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 10 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with chloroform, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (4/1) to obtain 130 mg of the title compound (yield: 69%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 0.92 (t, J=7.5Hz, 3H), 1.32~1.40 (m, 2H), 1.50~1.57 (m, 2H), 2.59 (t, J=8.9Hz, 2H), 3.93 (s, 3H), 3.94 (s, 3H), 6.44 (d, J=4.9Hz, 1H), 6.98~7.02 (m, 1H), 7.12∫7.17 (m, 2H), 7.19 (d, J=8.9Hz, 2H), 7.51 (s, 1H), 7.58 (s, 1H), 7.59 (d, J=8.5Hz, 2H), 7.71~7.75 (m, 1H), 7.89 (s, 1H), 8.46 (d, J=4.9Hz, 1H), 9.11 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 471 (M^+)

Example 197

N-(3-Ethoxycarbonylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [233]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (131 mg) was dissolved in toluene (10 ml) with heat, after the addition of triethylamine (2 ml), triphosgene (213 mg) was added, and the admixture was refluxed with heat for 2 minutes. Ethyl 3-aminobenzoate (0.1 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 7 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 214 mg of the title compound (yield: 100%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.36 (t, J=7.3Hz, 3H), 4.02 (s, 3H), 4.05 (s, 3H), 4.36 (q, J=7.3Hz, 2H), 6.45 (d, J=5.5Hz, 1H), 7.13 (d, J=9.2Hz, 2H), 7. 8~7.45 (m, 4H), 7.48 (d, J=9.2Hz, 2H), 7.5 6 (s, 1H), 7.75 (d, J=7.3Hz, 1H), 7.80 (d, J=7.9Hz, 1H), 7.90 (s, 1H), 8.48 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 487 (M^+)

Example 198

N-(2,3-Dichlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [234]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 2,3-dichlorophenyl isocyanate (0.05 ml) was added, and the admixture was refluxed with heat for 20 minutes. The reaction solution was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 84 mg of the title compound (yield: 100%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.00 (s, 3H), 4.05 (s, 3H), 6.45 (d, J=5.5Hz, 1H), 7.11~7.17 (m, 4H), 7.41 (s, 1H), 7.53 (d, J=9.2Hz, 2H), 7.57 (s, 1H), 7.91 (s, 1H), 8.22 (d, J=8.5Hz, 1H), 8.48 (d, J=5.5Hz, 1H), 9.12 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 483 (M^+)

Example 199

N-(2,4-Dichlorophenyl)-N'-(4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [235]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (58 mg) was dissolved in toluene (5 ml) with heat, 2,4-dichlorophenyl isocyanate (93 mg) was added, and the admixture was refluxed with heat for 20 minutes. The reaction solution was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 69 mg of the title compound (yield: 73%).

^{1}H-NMR ($CDCl_3$, 500 MHz): (4.04 (s, 3H), 4.06 (s, 3H), 6.47 (d, J=4.9Hz, 1H), 6.98 (d d, J=2.4, 8.5Hz, 1H), 7.19 (d, J=8.5Hz, 2H), 7.25 (s, 1H), 7.29 (s, 1H), 7.39 (s, 1H), 7.43 (s, 1H), 7.52 (d, J=8.5Hz, 2H), 7.56 (s, 1H), 8.40 (d, J=2.4Hz, 1H), 8.50 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 483 (M^+)

Example 200

N-(3,4-Dichlorophenyl)-N'-[4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [236]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, 3,4-dichlorophenyl isocyanate (102 mg) was added, and the admixture was refluxed with heat for 8 minutes. The separated crystals were filtered and then washed with toluene to obtain 71 mg of the title compound (yield: 84%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.44 (d, J=4.9Hz, 1H), 7.14~7.24 (m, 2H), 7.34 (m, 1H), 7.38 (s, 1H), 7.50~7.52 (m, 2H), 7.59 (d, J=9.2Hz, 2H) 7.88 (s, 1H), 8.46 (d, J=4.9Hz, 1H), 8.92 (s, 1H), 9.00 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 483 (M^+)

Example 201

N-(3,5-Dichlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [237]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) was dissolved in toluene (5 ml) with heat, 3,5-dichlorophenyl isocyanate (76 mg) was added, and the admixture was refluxed with heat for 8 minutes. The separated crystals were filtered and then washed with toluene to obtain 75 mg of the title compound (yield: 87%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.93 (s, 3H), 3.94 (s, 3H), 6.44 (d, J=4.9Hz, 1H), 7.15 (s, 1H), 7.20 (d, J=9.2Hz, 2H), 7.38 (s, 1H), 7.51 (s, 1H), 7.54 (s, 1H), 7.54 (s, 1H), 7.59 (d, J=9.2Hz, 2H), 8.46 (d, J=4.9Hz, 1H) 9.00 (brs, 1H), 9.07 (brs, 1H)

Mass spectrometry data (FD-MS, m/z): 483 (M^+)

Example 202

N-(4-Chloro-2-nitrophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [238]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (123 mg) was dissolved in toluene (12 ml) with heat, 4-chloro-2-

nitrophenyl isocyanate (172 mg) was added, and the admixture was refluxed with heat for 14 minutes. The separated crystals were filtered and then washed with toluene to obtain 174 mg of the title compound (yield: 85%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.46 (d, J=4.9Hz, 1H), 7.23 (d, J=7.3Hz, 2H), 7.39 (s, 1H), 7.51 (s, 1H), 7.62 (d, J=7.3Hz, 2H), 7.79 (d, J=9.2Hz, 1H), 8.14 (s, 1H), 8.35 (d, J=9.2Hz, 1H), 8.46 (d, J=5.5Hz, 1H), 9.62 (s, 1H), 10.0 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 494 (M^+), 496 (M^++2)

Example 203

N-(2-Amino-4-chlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [239]

N-(4-Chloro-2-nitrophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea (158 mg) was dissolved in chloroform/methanol/water (10 ml/70 ml/5 ml), sodium thiosulfate (1.01 g) was added, and the admixture was stirred at 60° C. for 37 minutes. The reaction mixture was extracted 2 times with chloroform, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 33 mg of the title compound (yield: 22%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 5.09 (brs, 2H), 6.43 (d, J=4.9 Hz, 1H), 6.57 (m, 1H), 6.78 (d, J=2.4Hz, 1H), 7.17 (d, J=9.2Hz, 2H), 7.34 (d, J=8.6Hz, 1H), 7.38 (s, 1H), 7.51 (s, 1H), 7.57 (d, J=8.5Hz, 2H), 7.77 (s, 1H), 8.45 (d, J=4.9Hz, 1H), 8.89 (s, 1H)

Example 204

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-N'-(2-pyridinecarbonyl)urea [240]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (75 mg) was dissolved in toluene (7 ml) with heat, after the addition of triethylamine (1.4 ml), triphosgene (201 mg) was added, and the admixture was refluxed with heat for 1 minute. 2-Pyridinecarboxamide (102 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 3 hours. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 86 mg of the title compound (yield: 77%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.06 (s, 3H), 4.06 (s, 3H), 6.49 (d, J=4.9Hz, 1H), 7.20 (d, J=9.2Hz, 2H), 7.4 3 (m, 1H), 7.57 (s, 1H), 7.59 (m, 1H), 7.71 (d, J=9.2Hz, 2H), 7.97 (m, 1H), 8.28 (d, J=7.9Hz, 1H), 8.50 (brs, 1H), 8.68 (d, J=4.9Hz, 1H), 10.1 (brs, 1H), 10.7 (brs, 1H)

Mass spectrometry data (FD-MS, m/z): 444 (M^+)

Example 205

N-(3-Chloro-4,6-dimethoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [241]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) was dissolved in toluene (5 ml) with heat, 3-chloro-4,6-dimethoxyphenyl isocyanate (76 mg) was added, and the admixture was refluxed with heat for 18 minutes. The reaction solution was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 93 mg of the title compound (yield: 100%)

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.76 (s, 3H), 3.87 (s, 3H), 4.02 (s, 3H), 4.05 (s, 3H), 6.46 (d, J=5.5Hz, 1H), 6.49 (s, 1H), 7.13 (d, J=8.5Hz, 2H), 7.20 (s, 1H), 7.42 (s, 1H), 7.50 (d, J=8.5Hz, 2H), 7.57 (s, 1H), 7.59 (s, 1H), 8.12 (s, 1H), 8.48 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 509 (M^+), 511 (M^++2)

Example 206

N-(4-Chloro-2-methylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [242]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (55 mg) was dissolved in toluene (5 ml) with heat, 4-chloro-2-methylphenyl isocyanate (90 mg) was added, and the admixture was refluxed with heat for 26 minutes. The separated crystals were filtered and then washed with toluene to obtain 69 mg of the title compound (yield: 80%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 2.26 (s, 3H), 3.94 (s, 3H), 3.94 (s, 3H), 6.44 (m, 1H), 7.18~7.20 (m, 3H), 7.25 (s, H), 7.38 (s, 1H), 7.51 (s, 1H), 7.59 (d, J=9.2Hz, 2H), 7.87 (m, 1H), 7.99 (s, 1H), 8.45 (m, 1H), 9.15 (s, 1H)

Mass spectrometry data (FD-MS; m/z): 463 (M^+), 465 (M^++2)

Example 207

N-(2-Chloro-3-methylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [243]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 2-chloro-3-methylphenyl isocyanate (0.2 ml) was added, and the admixture was refluxed with heat for 12 minutes. The reaction solution was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 30 mg of the title compound (yield: 37%).

^{1}H-NMR ($CDCl_3$, 500 MHz): 62.28 (s, 3H), 4.00 (s, 3H), 4.03 (s, 3H), 6.41 (d, J=4.9Hz, 1H), 7.09~7.15 (m, 4H), 7.37 (s, 1H), 7.39 (s, 1H), 7.42 (d, J=8.5Hz, 2H), 7.55 (s, 1H), 7.61 (s, 1H), 7.72 (s, 1H), 8.44 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z) 463 (M^+), 465 (M^++2)

Example 208

N-(3-Amino-4-chlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [244]

N-(4-Chloro-3-nitrophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea (100 mg) was dissolved in chloroform/methanol/water (5ml/15ml/3ml), sodium thiosulfate (358 mg) was added, and the admixture was stirred at 60° C. for 20 minutes. The reaction mixture was extracted 2 times with chloroform, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 39 mg of the title compound (yield: 41%).

^{1}H-NMR (DMSO-$d_6$500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 5.30 (brs, 1H), 6.45 (d, J=5.5Hz, 1H), 6.64 (m, 1H), 7.02 (m, 1H), 7.06 (d, J=8.6Hz, 1H), 7.20 (d, J=9.2Hz, 2H),

7.39 (s, 1H), 7.52 (s, 1H), 7.57 (d, J=8.6Hz, 2H), 8.47 (d, J=4.9Hz, 1H), 8.56 (s, 1H), 8.73 (s, 1H)

Mass spectrometry (FD-MS, m/z): 465 (M^++1)

Example 209

N-(3-Methoxypropyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [245]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (54 mg.) was added, and the admixture was refluxed with heat for 2 minutes. 3-Methoxypropylamine (0.05 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 12 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 51 mg of the title compound (yield: 72%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.82 (m, 2H), 3.31 (s, 3H), 3.39 (m, 2H), 3.50 (t, J=4.5Hz, 2H), 4.03 (s, 3H), 4.04 (s, 3H), 5.65 (brs, 1H), 6.44 (d, J=4.9Hz, 1H), 7.10 (d, J=8.7Hz, 2H), 7.4 1~7.55 (m, 4H), 7.55 (s, 1H), 8.46 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z) 411 (M^+)

Example 210

N-(2-Methoxyethyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [246]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (54 mg) was added, and the admixture was refluxed with heat for 3 minutes. 3-Methoxyethylamine (0.05 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 8 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 63 mg of the title compound (yield: 91%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.36 (s, 3H), 3.36~3.53 (m, 4H), 4.03 (s, 3H), 4.03 (s, 3H), 5.81 (brs, 1H), 6.43 (d, J=5.5Hz, 1H), 7.87 (d, J=8.5Hz, 2H), 7.40 (s, 1H), 7.45 (d, J=9.2Hz, 2H), 7.55 (s, 1H), 7.92 (s, 1H), 8.45 (d, J=4.9Hz, 1H)

Mass spectrometry data (FAB-MS, m/z): 398 (M^++1)

Example 211

N-(4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl)-N'-(2-pyridylmethyl)urea [247]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (54 mg) was added, and the admixture was refluxed with heat for 2 minutes. 2-Pyridylmethylamine (0.05 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 12 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 76 mg of the title compound (yield: 100%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 4.00 (s, 3H), 4.01 (s, 3H), 4.58 (d, J=5.5Hz, 2H), 6.39 (d, J=5.5Hz, 1H), 6.66 (m, 1H), 7.05 (d, J=9.2Hz, 2H), 7.18 (m, 1H), 7.34 (d, J=7.9Hz, 1H), 7.39 (s, 1H), 7.45 (d, J=9.2Hz, 2H), 7.54 (s, 1H), 7.66 (m, 1H), 8.40 (brs, 1H), 8.43 (d, J=5.5Hz, 1H), 8.47 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z) 430 (M^+)

Example 212

N-[(4-tert-Butylphenyl)methyl]-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [248]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 2 minutes. (4-tert-Butylphenyl)methylamine (0.05 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 12 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 23 mg of the title compound (yield: 28%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.99 (s, 3H), 4.01 (s, 3H), 4.38 (d, J=5.5Hz, 1H), 5.81 (brs, 1H), 6.38 (d, J=4.9Hz, 1H), 7.02 (d, J=9.2Hz, 2H), 7.20~7.40 (m, 7H), 7.52 (s, 1H), 7.64 (s, 1H), 8.40 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 485 (M^+)

Example 213

N-(4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl)-N'-(3-pyridylmethyl) urea [249]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (56 mg) was added, and the admixture was refluxed with heat for 2 minutes. 3-Pyridylmethylamine (0.05 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 12 minutes After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 71 mg of the title compound (yield: 93%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.93 (s, 3H) 3.94 (s, 3H), 4.34 (d, J=5.5Hz, 2H), 6.40 (d, J=4.9Hz, 1H), 6.72 (t, J=6.1Hz, 1H), 7.12 (d, J=9.2Hz, 2H), 7.3 5 (m, 1H), 7.37 (s, 1H), 7.50 (s, 1H), 7.53 (d, J=9.2Hz, 2H), 7.72 (d, J=7.9Hz, 1H), 8.43~8.46 (m, 2H), 8.53 (s, 1 H), 8.7 3 (s, 1H)

Mass spectrometry data (FD-MS, m/z) 430 (M^+)

Example 214

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-4-pyridylmethyl) urea [250]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, after the addition

of triethylamine (1 ml), triphosgene (53 mg) was added, and the admixture was refluxed with heat for 2 minutes. 4-Pyridylmethylamine (0.05 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 12 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 40 mg of the title compound (yield: 54%).

^{1}H-NMR ($CDCl_3$, 500 MHz): 4.01 (s, 3H), 4.03 (s, 3H), 4.43 (d, J=5.5Hz, 2H), 5.97 (m, 1H), 6.40 (d, J=5.5Hz, 1H), 7.08 (d, J=9.2Hz, 2H), 7.20 (d, J=5.5Hz, 2H), 7.38 (s, 1H), 7.42 (d, J38.6Hz, 2H), 7.54 (s, H), 7.81 (s, 1H), 8.42 (d, J=5.5Hz, 1H), 8.49 (d, J=6.1Hz, 2H)

Mass spectrometry data (FD-MS, m/z): 430 (M^+)

Example 215

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-[(4-dimethylaminophenyl)methyl]urea [251]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 2 minutes. (4-Dimethylaminophenyl)methylamine (104 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 12 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 12 mg of the title compound (yield: 15%).

^{1}H-NMR (DMSO-d_6, 500 MHz): (2.87 (s, 6H), 3.93 (s, 3H), 3.94 (s, 3H), 4.18 (d, J=5.5Hz, 2H), 6.40~6.43 (m, 2H), 6.70 (d, J=9.2Hz, 2 H), 7.1 3 (d, J=8.6Hz, 2H), 7.14 (d, J=8.6Hz, 2H), 7.37 (s, H), 7.51 (s, 1H), 7.52 (d, J=9.2Hz, 2H), 8.44 (d, J=4.9Hz, 1H), 8.57 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 472 (M^+)

Example 216

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(3,5-dinitrophenyl) urea [252]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (54 mg) was dissolved in toluene (5 ml) with heat, 3,5-dinitrophenyl isocyanate (81 mg) was added, and the admixture was refluxed with heat for 20 minutes. The reaction solution was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 53 mg of the title compound (yield: 57%).

^{1}H-NMR (DMSO-d_6, 500 MHz): 3.94 (s, 3H), 3.95 (s, 3H), 6.46 (d, J=4.9Hz, 1H), 7.24 (d, J=9.2Hz, 2H), 7.39 (s, 1H), 7.51 (s, 1H), 7.64 (d, J=8.5Hz, 2H), 8.42 (s, 1H) 8.47 (d, J=4.9Hz, 1H), 8.77 (s, 1H), 8.77 (s, 1H), 9.21 (s, 1H), 9.73 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 506 (M^++1)

Example 217

N-(Cyclohexyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl) oxy]phenyl}urea [253]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (200 mg) was dissolved in toluene (5 ml) with heat, cyclohexyl isocyanate (340 mg) was added, and the admixture was stirred with heat at 80–90° C. for 30 minutes. After the addition of water, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 210 mg of the title compound (yield: 74%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 1.11~1.22 (m, 2 H), 1.34~1.46 (m, 2H), 1.56~1.66 (m, 2H), 1.68~1.78 (m, 2H), 1.96~2.05 (m, 2H), 3.64~3.73 (m, 1H), 4.05 (s, 6H), 4.58 (d, J=7.9Hz, 1H), 6.36 (s, 1H), 6.46 (d, J=4.9Hz, 1H), 7.13 (d, J=8.6Hz, 2H), 7.42 (d, J=8.6Hz, 2H), 7.42 (s, 1H), 7.56 (s, 1H), 8.48 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 421 (M^+)

Example 218

N-(3, 4-Difluorophenyl)-N'-(4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl)urea [254]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 3,4-Difluoroaniline (66 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 63 mg of the title compound (yield: 83%).

^{1}H-NMR ($CDCl_3$, 500 MHz): 64.03 (s, 3H), 4.06 (s, 3H), 6.45 (d, J=5.5Hz, 1H), 6.96~7.02 (m, 1H), 7.02~7.10 (m, 1H), 7.11 (d, J=8.5Hz, 2H), 7.41~7.45 (m, 2H), 7.48 (d, J=8.6Hz, 2H), 7.5 8 (s, 1H), 7.72 (s, 1H), 7.74 (s, 1H), 8.44 (d, J=5.5H z, 1H)

Mass spectrometry data (FD-MS, m/z) 451 (M^+)

Example 219

N-(2,4,5-Trifluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [255]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 2,4,5-Trifluoroaniline (75 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 58 mg of the title compound (yield: 73%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.45 (d, J=5.5Hz, 1H), 7.24 (d, J=8.6Hz, 2H), 7.40 (s, 1H), 7.52 (s, 1H), 7.59 (d, J=8.6Hz, 2H), 7.62~7.70 (m, 1H), 8.17~8.25 (m, 1H), 8.48 (d, J=4.9Hz, 1H), 8.76 (s, 1H), 9.23 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 469 (M^+)

Example 220

N-(2,4,6-Trifluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [256]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 2,4,6-Trifluoroaniline. (75 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 37 mg of the title compound (yield: 47%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.4 3 (d, J=5.5Hz, 1H), 7.2 0 (d, J=8.6Hz, 2H), 7.29 (t, J=8.6Hz, 2H), 7.39 (s, 1H), 7.52 (s, 1H), 7.58 (d, J=8.6Hz, 2H), 8.07 (s, 1H), 8.46 (d, J=5.5Hz, 1H), 9.14 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 469 (M^+)

Example 221

N-(2,3,4-Trifluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [257]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 2,3,4-Trifluoroaniline (75 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 18 mg of the title compound (yield: 23%).

^{1}H-NMR (DMSO-d_6, 500 MHz) δ 3.94 (s, 3H), 3.95 (s, 3H), 6.44 (d, J=4.9Hz, 1H), 7.23 (d, J=8.6Hz, 2H), 7.25~7.33 (m, 1H), 7.39 (s, 1 H), 7.52 (s, 1H), 7.59 (d, J=8.6Hz, 2H), 7.85~7.92 (m, 1H), 8.47 (d, J=5.5Hz, 1H), 8.73 (s, 1H), 9.21 (s, 1H)

Mass spectrometry data (FD-MS, m/z) 469 (M^+)

Example 222

N-(2,5-Dimethoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [258]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (56 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (66 mg) was added, and the admixture was refluxed with heat for 3 minutes. 2,5-Dimethoxyaniline (102 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 10 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 81 mg of the title compound (yield: 90%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.68 (s, 3H), 3.78 (s, 3H), 4.02 (s, 3H), 4.05 (s, 3H), 6.46 (d, J=5.5Hz, 1H), 6.53 (dd, J=3.1, 8.5 Hz, 1H), 6.76 (d, J=9.2Hz, 1H), 7.13 (d, J=9.2Hz, 2H), 7.4 3 (s, 1H) 7.52 (d, J=9.2Hz, 2H), 7.55 (s, 1H), 7.5 7 (s, 1H) 7.78 (s, 1H), 7.91 (d, J=3.1Hz, 1H), 8.49 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 475 (M^+)

Example 223

N-(3,5-Dimethoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [259]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (101 mg) was added, and the admixture was ref luxed with heat for 4 minutes. 3,5-Dimethoxyaniline (62 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 13 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 85 mg of the title compound (yield: 100%)).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.73 (s, 6H), 4.00 (s, 3H), 4.03 (s, 3H), 6.18 (s, 1H), 6.42 (d, J=5.5Hz, 1H), 6.60 (s, 1H), 6.60 (s, 1H), 7.09 (d, J=8.6Hz, 2H), 7.40 (s, 1H), 7.45 (d, J=8.6Hz, 2H), 7.56 (s, 1H), 7.68 (s, 1H), 7.85 (s, 1H), 8.45 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 47 5 (M^+)

Example 224

N-(2,3-Difluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [260]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 2,3-Difluoroaniline (51 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 60 mg of the title compound (yield: 79%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.45 (d, J=4.9Hz, 1H), 7.02~7.08 (m, 1H), 7.11~7.19 (m, 1H), 7.23 (d, J=9.2Hz, 2H), 7.39 (s, 1H), 7.52 (s, 1H), 7.59 (d, J=9.2Hz, 2H), 7.94~7.99 (m, 1H), 8.47 (d, J=4.9Hz, 1H), 8.78 (s, 1H), 9.25 (s, 1H)

Mass spectrometry data (FD-M S, m/z): 451 (M^+)

Example 225

N-(3,5-Difluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [261]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition

of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 3,5-Difluoroaniline (66 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 49 mg of the title compound (yield: 64%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.45 (d, J=4.9Hz, 1H), 6.75~6.83 (m, 1H), 7.16~7.26 (m, 4H), 7.39 (s, 1H), 7.52 (s, 1H), 7.59 (d, J=8.6Hz, 2H), 8.47 (d, J=4.9Hz, 1H), 8.99 (s, 1H), 9.12 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 451 (M^+)

Example 226

N-(2,3,6-Trifluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [262]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 2,3,6-trifluoroaniline (53 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 30 mg of the title compound (yield: 38%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.93 (s, 3H), 3.94 (s, 3H), 6.44 (d, J=5.5Hz, 1H), 7.15~7.25 (m, 3H), 7.33~7.44 (m, 2H), 7.52 (s, 1H), 7.59 (d, J=8.6Hz, 2H), 8.41 (s, 1H), 8.46 (d, J=4.9Hz, 1H), 9.18 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 469 (M^+)

Example 227

N-(2,6-Difluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [263]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, 2,6-difluorophenyl isocyanate (104 mg) was added, and the admixture was refluxed with heat for 30 minutes. The separated crystals were filtered and then washed with toluene to obtain 57 mg of the title compound (yield: 74%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.44 (d, J=4.9Hz, 1H), 7.13~7.25 (m, 4H), 7.28~7.38 (m, 1H), 7.39 (s, 1H), 7.52 (s, 1H), 7.59 (d, J=9.2Hz, 2H), 8.15 (s, 1H), 8.46 (d, J=5.5Hz, 1H), 9.09 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 451 (M^+)

Example 228

N-(2,5-Bis(trifluoromethyl)phenyl]-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [264]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 2,5-Bis(trifluoromethyl)aniline (79 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 30 mg of the title compound (yield: 32%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.45 (d, J=4.9Hz, 1H), 7.24 (d, J=9.2Hz, 2H), 7.40 (s, 1H), 7.52 (s, 1H), 7.60~7.66 (m, 3H), 7.95 (d, J=7.9Hz, 1H), 8.38 (s, 1H), 8.46~8.52 (m, 2H), 9.70 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 551 (M^+)

Example 229

N-(2,4-Dimethoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [265]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (51 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (72 mg) was added, and the admixture was refluxed with heat for 3 minutes. 2,4-Dimethoxyaniline (60 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 11 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 44 mg of the title compound (yield: 53%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.74 (s, 3H), 3.80 (s, 3H), 4.03 (s, 3H), 4.05 (s, 3H), 6.44~6.51 (m, 3H), 7.06 (s, 1H), 7.12 (d, J=9.2Hz, 2H), 7.43 (s, 1H), 7.49~7.51 (m, 3H), 7.57 (s, 1H), 7.86 (d, J=8.6Hz, 1H), 8.48 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 475 (M^+)

Example 230

N-(2-Chlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [266]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 2-chlorophenyl isocyanate (0.05 ml) was added, and the admixture was refluxed with heat for 30 minutes. The reaction solution was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 64 mg of the title compound (yield: 81%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 4.01 (s, 3H), 4.05 (s, 3H), 6.46 (d, J=4.9Hz, 1H), 6.98 (d d, J=7.3, 7.9Hz, 1H), 7.14 (d, J=8.6Hz, 2H), 7.26 (dd, J=7.3, 7.9Hz, 1H), 7.31 (d, J=7.9 Hz, 1H), 7.43 (s, 1H), 7.52 (d, J=9.2Hz, 2H), 7.57 (s, 1H), 7.57 (s, 1H), 8.24 (d, J=7.9Hz, 1H), 8.37 (s, 1H), 8.50 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 449 (M^+) 451 (M^++2)

Example 231

N-(3-Chlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [267]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, 2-chlorophenyl

isocyanate (0.05 ml) was added, and the admixture was refluxed with heat for 30 minutes. The reaction solution was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 72 mg of the title compound (yield: 93%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 3.98 (s, 3H), 4.03 (s, 3H), 6.41 (d, J=4.9Hz, 1H), 6.99 (d, J=7.9Hz, 1H), 7.09 (d, J=8.5Hz, 2H), 7.16 (dd, J=7.9Hz, 7.9Hz, 1H), 7.23 (d, J=8.6Hz, 1H), 7.38 (s, 1H), 7.42~7.44 (m, 3H), 7.56 (s, 1H), 8.13 (s, 1H), 8.14 (s, 1H), 8.44 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 449 (M^+) 451 (M^++2)

Example 232

N-(2,3-Dimethoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [268]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (74 mg) was added, and the admixture was refluxed with heat for 3 minutes. 2,3-Dimethoxyaniline (0.05 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 11 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 83 mg of the title compound (yield: 100%).

^{1}H-NMR ($CDCl_3$, 500 MHz): (3.72 (s, 3H), 3.84 (s, 3H), 4.02 (s, 3H), 4.05 (s, 3H), 6.46 (d, J=5.5Hz, 1H), 6.62 (d, J=7.9Hz, 1H), 7.05 (dd, J=8.5, 8.5Hz, 1H), 7.13 (d, J=9.2Hz, 2H), 7.43 (s, 1H), 7.54 (d, J=8.6Hz, 2H), 7.58 (s, 1H), 7.85 (d, J=8.6Hz, 1H), 7.88 (s, 1H), 8.27 (s, 1H), 8.50 (d, J=5.5Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 475 (M^+)

Example 233

N-(4-Hydroxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [269]

N-(4-Benzyloxyphenyl)-N'-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}urea (228 mg) was dissolved in N,N-dimethylformamide/ethyl acetate (15 ml/20 ml), after the addition of triethylamine (4 ml), 20% palladium hydroxide (1.18 g) was added, and the admixture was stirred at room temperature under hydrogen for 15 hours. The reaction mixture was filtered using Celite, after which the filtrate was concentrated, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 188 mg of the title compound (yield: 100%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.98 (s, 3H), 3.9 9 (s, 3H), 6.60 (d, J=5.5Hz, 1H), 6.68 (d, J=8.6Hz, 2H), 7.21 (d, J=8.5Hz, 2H), 7.22 (d, J=8.5Hz, 2H), 7.47 (s, 1H), 7.60 (d, J=9.2Hz, 2H), 7.61 (s, 1H), 8.58 (s, 1H), 8.59 (s, 1H), 8.98 (s, 1H), 9.03 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 431 (M^++1)

Example 234

N-(4-Acetoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [270]

N-(4-Hydroxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea (47 mg) was dissolved in methylene chloride (6 ml), after the addition of triethylamine (2 ml), acetic anhydride (0.5 ml) was added, and the admixture was stirred at room temperature for 15 hours. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 35 mg of the title compound (yield: 68%).

^{1}H-NMR ($CDCl_3$, 500 MHz): δ 2.31 (s, 3H), 4.01 (s, 3H), 4.04 (s, 3H), 6.43 (d, J=5.5Hz, 1H), 7.01 (d, J=8.6Hz, 2H), 7.11 (d, J=9.2Hz, 2H), 7.26 (brs, 1H), 7.32 (d, J=9.2Hz, 2H), 7.40 (s, 1H), 7.45 (d, J=9.2Hz, 1H), 7.56 (s, 1H), 7.5 6 (brs, 1H), 8.46 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-M S, m/z) 473 (M^+)

Example 235

N-(3,4,5-Trimethoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [271]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (66 mg) was added, and the admixture was refluxed with heat for 2 minutes. 3,4,5-Trimethoxyaniline (76 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 19 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 76 mg of the title compound (yield: 87%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 3.81 (s, 6H), 3.83 (s, 3H), 4.02 (s, 3H), 4.05 (s, 3H), 6.44 (d, J=4.9Hz, 1H), 6.69 (s, 2H), 7.12 (d, J=9.2Hz, 2H), 7.41 (s, 1H), 7.41 (brs, 1H), 7.48 (d, J=8.6Hz, 2H), 7.57 (s, 1H), 7.58 (s, 1H), 8.47 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 505 (M^+)

Example 236

N-(2,4-Difluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [272]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (100 mg) was dissolved in toluene (10 ml) with heat, 2,4-difluorophenyl isocyanate (120 μl) was added, and the admixture was refluxed with heat for 30 minutes. The separated crystals were filtered and then washed with toluene to obtain 128 mg of the title compound (yield: 84%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.46 (d, J=4.9Hz, 1H), 7.03~7.10 (m, 1H), 7.22 (d, J=9.2Hz, 2H), 7.28~7.35 (m, 1H), 7.39 (s, 1H), 7.52 (s, 1H), 7.59 (d, J=9.2Hz, 2H) 8.05~8.12 (m, 1H) 8.47 (d, J=5.5Hz, 1H), 8.52 (s, 1H), 9.15 (s, 1H)

Mass spectrometry data (FD-MS, m/z) 451 (M^+)

Example 237

N-(2,5-Difluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [273]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (100 mg) was dissolved in toluene (10 ml) with heat, 2,5-

difluorophenyl isocyanate (120 μl) was added, and the admixture was refluxed with heat for 30 minutes. The separated crystals were filtered and then washed with toluene to obtain 132 mg of the title compound (yield: 87%).

^{1}H-NMR (DMSO-d_6, **500**MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.46 (d, J=5.5Hz, 1H), 6.80~6.87 (m, 1H), 7.23 (d, J=9.2Hz, 2H), 7.2 7~7.33 (m, 1H), 7.40 (s, 1H), 7.52 (s, 1H), 7.59 (d, J=8.6Hz, 2H), 8.03~8.08 (m, 1H), 8.47 (d, J=5.5Hz, 1H), 8.78 (s, 1H), 9.27 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 45 1 (M^+)

Example 238

N-(4-Amino-2-chlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [274]

N-(4-Chloro-3-nitrophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea (150 mg) was dissolved in chloroform/methanol/water (10 ml/13 ml/3 ml), sodium thiosulfate (501 mg) was added, and the admixture was stirred at 60° C. for 50 minutes. The reaction mixture was extracted 2 times with chloroform, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 48 mg of the title compound (yield: 34%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.94 (s, 3H), 5.14 (brs, 2H), 6.43 (d, J=4.9 Hz, 1H), 6.51 (m, 1H), 6.65 (d, J=2.5Hz, 1H), 7.17 (d, J=9.2Hz, 2H), 7.38 (s, 1H), 7.48 (d, J=8.6Hz, 1H), 7.51 (s, 1H), 7.56 (d, J=8.5Hz, 2H), 7.83 (s, 1H), 8.45 (d, J=5.5Hz, 1H), 9.10 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 464 (M.), 466 (M^++2)

Example 239

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-[2-ethoxycarbonylphenyl]urea [275]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (100 mg) was dissolved in toluene (10 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (100 mg) was added, and the admixture was refluxed with heat for 2 minutes. Ethyl-2-aminobenzoate (84 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 2 hours. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 86 mg of the title compound (yield: 52%).

^{1}H-NMR (DMSO-d_6, 90 MHz): δ 1.37 (t, J=7Hz, 3H), 3.94 (s, 3H), 3.94 (s, 3H), 4.38 (q, J=7 Hz, 2H), 6.4 7 (d, J=5Hz, 1H), 7.1~8.1 (m, 9H), 8.38 (d, J=9Hz, 1H), 8.47 (d, J=5Hz, 1H), 9.92 (s, 1H), 10.10 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 487 (M^+)

Example 240

N-(2-Methylthiophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [276]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (131 mg) was dissolved in toluene (13 ml) with heat, after the addition of triethylamine (2.6 ml), triphosgene (152 mg) was added, and the admixture was refluxed with heat for 2 minutes. 2-Methylthioaniline (0.11 ml) was added to the reaction mixture, and the admixture was refluxed with heat for 10 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with chloroform, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (4/1) to obtain 29 mg of the title compound (yield: 14%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 2.50 (s, 3H), 4.00 (s, 3H), 4.00 (s, 3H), 6.51 (d, J=5.5Hz, 1H), 7.08~7.15 (m, 1H), 7.25~7.35 (m, 3H), 7.45 (s, 1H), 7.48 (d, J=7.9Hz, 1H), 7.58 (s, 1H), 7.67 (d, J=8.5Hz, 2H), 7.99 (d, J=7.9Hz, 1H), 8.24 (s, 1H), 8.53 (d, J=5.5Hz, 1H), 9.61 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 461 (M^+)

Example 241

N-[3,5-Bis(trifluoromethyl)phenyl]-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [277]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 3,5-Bis (trifluoromethyl)aniline (116 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 54 mg of the title compound (yield: 58%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.93 (s, 3H), 3.94 (s, 3H), 6.45 (d, J=5.5Hz, 1H), 7.23 (d, J=9.2Hz, 2H), 7.39 (s, 1H), 7.51 (d, J=9.2Hz, 2H), 7.62 (d, J=9.2Hz, 2H), 7.64 (s, 1H), 8.15 (s, J5.5Hz, 2H), 9.13 (s, 1H), 9.43 (s, 1H)

Mass spectrometry data (FD-M S, m/z): 551 (M^+)

Example 242

N-(3-Chloropropyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [278]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (50 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 3-Chloropropylaniline (66 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 20 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 26 mg of the title compound (yield: 37%).

^{1}H-NMR (DMSO-$d_6$500 MHz): δ 1.87~1.94 (m, 2H), 3.20~3.27 (m, 2H), 3.69 (t, J=6.1Hz, 2 H), 3.93 (s, 3H), 3.94 (s, 3H), 6.29 (t, J=6.1Hz, 1H), 6.41 (d, J=5.5Hz, 1H), 7.14 (d, J=9.2Hz, 2H), 7.38 (s, 1H), 7.50~7.5 5 (m, 3 H), 8.46 (d, J=4.9H z, 1H), 8.61 (s, 1H)

Mass spectrometry data (FD-MS, m/z 415 (M^+)

Example 243

N-{5-[2-(4-fluoro)phenoxy]-pyridyl}-N'-(4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [279]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (53 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (58 mg) was added, and the admixture was refluxed with heat for 4 minutes. 5-Amino-2-(4-fluoro)phenoxypyridine (95 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 19 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 74 mg of the title compound (yield: 79%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.44 (d, J=5.5Hz, 1H), 7.0 (d, J=8.5Hz, 1H), 7.12~7.24 (m, 6H), 7.38 (s, 1 H), 7.51 (s, 1H), 7.59 (d, J=9.2Hz, 2H), 8.01 (d, J=8.6Hz, 1H), 8.19 (d, J=2.4Hz, 1H), 8.46 (d, J=4.9Hz, 1H), 8.77 (s, 1H), 8.89 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 526 (M^+)

Example 244

N-{4-[(6,7-Dimethoxy-4-quinolyl)oxy]phenyl}-N'-(3-dimethylaminophenyl)urea [280]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (52 mg) was dissolved in toluene (5 ml) with heat, after the addition of triethylamine (1 ml), triphosgene (55 mg) was added, and the admixture was refluxed with heat for 3 minutes. 3-Dimethylaminoaniline (69 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 12 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted 2 times with ethyl acetate, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was purified by column chromatography on silica gel eluting with chloroform/acetone (10/1) to obtain 72 mg of the title compound (yield: 89%).

^{1}H-NMR ($CDCl_3$, 500 MHz) δ 2.92 (s, 6H), 4.0 (s, 3H), 4.03 (s, 3H), 6.41 (d, J=4.9Hz, 1H), 6.48 (d, J=8.6Hz, 1H), 6.56 (d, J=9.2Hz, 1H), 6.88 (s, 1H), 7.08 (d, J=9.2Hz, 2H), 7.14 (dd, J=7.9, 8.5Hz, 1H), 7.35 (s, 1H), 7.41 (s, 1H), 7.45 (d, J=9.2Hz, 2H), 7.55 (s, 1H), 7.67 (s, H), 8.45 (d, J=4.9Hz, 1H)

Mass spectrometry data (FD-MS, m/z): 458 (M^+)

Example 245

N-(2-Hydroxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [281]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (105 mg) was dissolved in toluene (10 ml) with heat, after the addition of triethylamine (2 ml), triphosgene (118 mg) was added, and the admixture was refluxed with heat for 2 minutes. 2-Aminophenol (77 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 10 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted with chloroform, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was washed with chloroform and filtered to obtain 86 mg of the title compound (yield: 56%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.45 (d, J=4.9Hz, 1H), 6.73~6.88 (m, 3H), 7.20 (d, J=8.6Hz, 2H), 7.39 (s, 1H), 7.52 (s, 1H), 7.59 (d, J=8.6Hz, 2H), 8.03~8.08 (m, 1H), 8.19 (s, 1H), 8.47 (d, J=5.5Hz, 1H), 9.46 (s, 1H), 9.95 (s, 1H)

Mass spectrometry data (FD-MS, m/z) 431 (M^+)

Example 246

N-(3-Hydroxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [282]

6,7-Dimethoxy-4-(4-aminophenoxy)quinoline (115 mg) was dissolved in toluene (12 ml) with heat, after the addition of triethylamine (2 ml), triphosgene (113 mg) was added, and the admixture was refluxed with heat for 2 minutes. 3-Aminophenol (73 mg) was added to the reaction mixture, and the admixture was refluxed with heat for 10 minutes. After the addition of aqueous sodium hydrogen carbonate, the reaction mixture was extracted with chloroform, and the organic layer was then washed with brine and dried with anhydrous sodium sulfate. The solvent was removed by reduced-pressure distillation, and the resulting residue was washed with chloroform and filtered to obtain 95 mg of the title compound (yield: 60%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H), 3.95 (s, 3H), 6.35~6.42 (m, 1H), 6.44 (d, J=4.9Hz, 1H), 6.77~6.85 (m, 1H), 6.98~7.08 (m, 2H), 7.20 (d, J=8.6Hz, 2H), 7.39 (s, 1H), 7.52 (s, 1H), 7.58 (d, J=8.6Hz, 2H), 8.47 (d, J=4.9Hz, 1H), 8.57 (s, 1H), 8.73 (s, 1H), 9.31 (s, 1H)

Mass spectrometry data (FD-MS, m/z): 431 (M^+)

Example 247

N-(3-Hydroxycarbonylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea [283]

N-(3-Ethoxycarbonylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea (190 mg) obtained in Example 197 was dissolved in methanol (3 ml), 35% aqueous potassium hydroxide (5 ml) was added, and the admixture was stirred at room temperature for 30 minutes. After removing methanol by reduced-pressure distillation and neutralizing using dilute hydrochloric acid, the separated crystals were filtered to obtain 67 mg of the title compound (yield: 38%).

^{1}H-NMR (DMSO-d_6, 500 MHz): δ 3.94 (s, 3H) 3.95 (s, 3H), 6.45 (d, J=5.5Hz, 1H), 7.20 (d, J=8.6Hz, 2H), 7.38~7.65 (m, 7H), 8.13 (s, 1H), 8.46 (d, J=4.9H z, 1H), 8.88 (s, 1H), 8.97 (s, 1H)

Mass spectrometry data (FD-MS m/z 459 (M^+)

Test Example 1

As for pharmacological action of the compounds of the present invention, inhibition of PDGF receptor autophosphorylation was studied using cultured rat mesangial cells which are known to have the PDGF receptor.

1. Cultivation of rat mesangial cells

Kidneys were taken out from Wistar-Kyoto rats (purchased from Charles River Japan, Inc.), and the glomeruli were isolated by the sieving method (Nephron, 22, 454 (1978)). The glomeruli were cultured in RPMI 1640 medium (hereinafter referred to as the medium) containing 10% fetal calf serum (hereinafter abbreviated to FCS) at 37° C. in an

incubator in an atmosphere containing 5% carbon dioxide. The glomerulus-derived adhesive cells were subcultured several times to obtain mesangial cells. The mesangial cells subcultured more than 5 passages were used for the assay.

2.Evaluation of PDGF receptor autophosphorylation inhibition

The mesangial cells were seeded in 24-well flat-bottom microtiter plates and then cultured in the medium containing 10% FCS for 2 days. When the cells were grown to about 3×10^4 cells per well, the medium was changed to the medium containing 0.5% FCS, the cultivation was continued for another 3 days, and then cell growth was arrested. The culture medium was removed, the adhered cells were washed with 500 μl of the medium, and 250 μl of the medium containing 0.1% bovine serum albumin (hereinafter abbreviated to BSA) was then added. A test drug dissolved in 1.38 μl of dimethyl sulfoxide (hereinafter abbreviated to DMSO) was added to this culture medium, and the admixture was incubated at 37° C. for 1 hour. Recombinant human platelet-derived growth factor BB chain (hereinafter abbreviated to PDGF-BB) was dissolved in the medium containing 0.1% BSA, 25 μl per well of the solution was added at final 50 ng/ml, and the incubation was continued at 37° C. for 10 minutes. The medium was removed, the cells were washed with 500 μl of phosphate-buffered physiological saline (pH 7.4) (hereinafter abbreviated to PBS), and 50 μl of lysis buffer (Tris-buffered physiological saline, pH 7.4, containing 1% Triton X100, 2 mM sodium orthobanadate, and 1 mM ethylene-diamine-tetraacetic acid disodium (hereinafter abbreviated to EDTA) were added. The cells were allowed to stand at 4° C. for 30 minutes for lysis, and the resulting solution was thoroughly admixed with an equal amount of Tris-buffered physiological saline containing 1% sodium dodecyl sulfate (hereinafter abbreviated to SDS). An aliquot (20 μl) of the solution was subjected to SDS electrophoresis on 7.5% polyacrylamide gel. After the electrophoresis, proteins in the gel were electrically transferred to a PVDF filter, which was then subjected to Western blotting using monoclonal antibodies against phosphotyrosine. Bands of PDGF receptor (molecular weight: about 180 Kda) which were autophosphorylated due to the addition of PDGF-BB were quantitated by a densitometer. Autophosphorylation rates for individual wells with drugs were calculated by setting measurements for PDGF receptor autophosphorylation in the wells without drugs in the presence and absence of PDGF-BB as 100% and 0%, respectively.

PDGF receptor autophosphorylation inhibition rates were obtained at a series of different concentrations of individual test drugs, from which regression line formulas were determined to calculate PDGF receptor autophosphorylation 50% inhibition concentrations (IC_{50}s) for the test drugs.

The compounds of the present invention showed the PDGF receptor autophosphorylation 50% inhibition concentrations (IC_{50}s) of less than 100 μM.

As for representative examples of preferred compound groups of the present invention, test results for PDGF receptor autophosphorylation 50% inhibition concentrations (IC_{50}s) in mesangial cells are shown in Table 2.

TABLE 2

Compound No.	IC_{50} (μM) for PDGF receptor autophosphorylation inhibition
(2)	0.44
(6)	0.45
(8)	0.10
(16)	0.05
(29)	0.80
(32)	0.30
(43)	0.005
(44)	0.006
(45)	0.014
(46)	0.021
(47)	0.043
(49)	0.018
(50)	0.067
(51)	0.009
(52)	0.03
(53)	0.010
(54)	0.003
(55)	0.003
(56)	0.004
(57)	0.006
(58)	0.002
(59)	0.004
(60)	0.004
(63)	0.40
(66)	0.13
(67)	0.11
(68)	0.44
(69)	0.05
(74)	0.34
(75)	0.18
(79)	0.34
(94)	0.023
(113)	0.004
(114)	0.004
(131)	0.003
(133)	0.007
(135)	0.043
(138)	0.014
(139)	0.005
(163)	0.005
(165)	0.010
(166)	0.011
(167)	0.004
(168)	0.006
(169)	0.004
(170)	0.018
(172)	0.010
(173)	0.016
(174)	0.002
(175)	0.006
(188)	0.006
(189)	0.011
(190)	0.021
(192)	0.004
(194)	0.014
(195)	0.079
(196)	0.031
(197)	0.010
(198)	0.011
(199)	0.003
(200)	0.004
(220)	0.009
(224)	0.087
(225)	0.047
(226)	0.011
(227)	0.073
(228)	0.055
(229)	0.037
(230)	0.034
(231)	0.019
(232)	0.018
(233)	0.013
(234)	0.024
(235)	0.043

TABLE 2-continued

Compound No.	IC_{50} (μM) for PDGF receptor autophosphorylation inhibition
(236)	0.055
(237)	0.075
(238)	0.016
(239)	0.005
(240)	0.15
(241)	0.018
(242)	0.018
(243)	0.014
(244)	0.009
(245)	0.006
(246)	0.013
(247)	0.009
(248)	0.063
(249)	0.041
(250)	0.017
(251)	0.036
(253)	0.007
(254)	0.007
(255)	0.015
(256)	0.004
(257)	0.007
(258)	0.007
(259)	0.005
(260)	0.002
(261)	0.010
(262)	0.002
(263)	0.004
(265)	0.005
(266)	0.001
(267)	0.15
(268)	0.007
(269)	0.008
(270)	0.006
(271)	0.004
(272)	0.003
(273)	0.005
(274)	0.036
(276)	0.005
(278)	0.003
(279)	0.029
(281)	0.012
(282)	0.008
(283)	0.002
(288)	0.024
(289)	0.032
(290)	0.055

Test Example 2

Effect on nephritis associated with mesangial cell growth in rats

Groups of 6 Wistar-Kyoto male rats (7 weeks old, purchased from Charles River Japan, Inc.) were used. OX-7 (prepared according to Pathol. Int., 45, 409 (1995)), a monoclonal antibody against anti-rat Thy1.1, was injected intravenously into the tails of rats at a dose of 1.2 mg/kg to induce glomerulonephritis associated with mesangial cell growth and extracellular matrix accumulation. A test drug suspended in a vehicle, a 1% aqueous cremophor solution, was administered orally 2 times a day starting one day after an OX-7 injection for 8 consecutive days. On day 9, when pathological changes in glomeruli became markedly apparent, the rats were sacrificed and anatomized under anesthesia with ether, and left kidneys were taken out and fixed in formalin. The kidneys were embedded in paraffin and then sectioned thinly, and the sections were stained with a periodic acid-Schiff's reagent. The 20 glomeruli were arbitrarily selected for individual specimens thus prepared, and pathological sclerotic changes were scored as follows:

0: No pathological sclerotic changes were observed in glomeruli.

1: Pathological sclerotic changes were observed in less than 25% of glomeruli. 2: Pathological sclerotic changes were observed in 25–50% of glomeruli.

3: Pathological sclerotic changes were observed in 50–75% of glomeruli.

4: Pathological sclerotic changes were observed in 75–100% of glomeruli.

Average scores for sclerotic change of individual specimens were calculated to quantify the degree of glomerular sclerosis. Average scores of glomerular sclerosis for individual specimens of rat groups administered with the drug were compared with those for control groups administered with the vehicle only to calculate glomerular sclerosis inhibition rates (%). The significance of differences was determined by the Dunnet method after testing homoscedasticity according to the Bartlett method.

Glomerular sclerosis inhibition rates (%) for the compound number 32 are shown as follows:

Dose	Glomerular sclerosis inhibition rate (%)	Significance of difference
10 mg/kg	11.7	$p < 0.05$
30 mg/kg	10.2	$p < 0.05$
100 mg/kg	13.7	$p < 0.01$

From the results above, it was shown that the compound number 32 has an effect in inhibiting pathological sclerotic changes of glomeruli in nephrosis associated with mesangial cell growth.

Test Example 3

Antitumor activity

1. Antitumor effect of the compound number 43 against mouse leukemia cells (P388)

The compound number 43 exhibited the following effect on prolonging survival of mice injected with tumor cells.

P388 cells (1×10^5 cells), mouse leukemia cells obtained from ATCC, were inoculated intraperitoneally into CDF_1 mice (obtained from Japan SLC, Inc.), and then the test compound was administered intraperitoneally for 9 consecutive days at the dose of 100 mg/kg. The drug-treated animals survived longer than control animals by 130%.

2.Antitumor effect of the compound number 43 against human glioma cells (GLO7)

The compound number 43 exhibited an antitumor effect in the nude mice human tumor xenograft model, a model which reflects clinical effect, as follows.

Human glioma, GLO7 (obtained from the Central Institute for Experimental Animals), was transplanted into nude mice. When the tumor had grown to a volume of about 100 mm^3, the nude mice were divided into several groups of 4 animals so as to equalize the average tumor volumes of each group. The test drug was administered orally to animals in experimental groups 2 times a day for 14 consecutive days at 100 mg/kg, and the vehicle was administered to control animals. The tumor growth inhibition rate (TGIR, %) was calculated from the equation: $TGIR=(1-Tx/Cx)\times100$, in which Cx is the tumor volume in the control mice and Tx is the tumor volume in the drug-treated mice, on day X, when the initial tumor volume at the time treatment was commenced is set 1. During the test period of about 4 weeks, the maximum TGIR value was 76%, and thus an excellent antitumor effect was observed.

From the results above, the compound number 43 was revealed to have an antitumor activity.

3.Antitumor effect of the compounds of the present invention against human glioma (GLO7)

Each compounds exhibited an antitumor effect in the nude mice human tumor xenogroft model, a model which reflects clinical effect, as follows.

Analogously to 2, human glioma cells, GLO7 (obtained from the Central Institute for Experimental Animals), was transplanted into nude mice. When the tumor had grown to a volume of about 100 mm^3, the nude mice were divided into several groups of 4 animals so as to equalize the average tumor volumes of each group. The test drug was administered orally to animals in experimental groups once a day for 9 consecutive days at 50 mg/kg, and the vehicle was administered to control animals. The tumor growth inhibition rate (TGIR, %) was calculated from the equation: TGIR=(1-Tx/Cx)×100, in which Cx is the tumor volume in the control mice and Tx is the tumor volume in the drug-treated mice, on day X, when the initial tumor volume at the time treatment was commenced is set 1. Results are shown in Table 3.

TABLE 3

Compound No.	Tumor growth inhibition rate (TGIR, %)
(44)	78
(45)	68
(52)	86
(56)	52
(57)	64
(58)	57
(59)	82
(165)	70
(168)	56
(173)	54
(190)	50
(191)	50
(194)	59
(225)	58
(227)	78
(229)	81
(235)	78
(236)	84
(237)	74
(242)	62
(244)	73
(254)	85
(255)	86
(260)	52
(262)	87
(264)	63
(267)	65
(272)	84

4.Antitumor effect of compounds numbers 43 and 58 against various tumor cells

In the same manner as described in 2, various types of tumor cells (obtained from the Central Institute for Experimental Animals) were transplanted into nude mice. When the tumor had grown to a volume of about 100 mm^3, the animals were allocated to several groups each consisting of 4 animals so as to equalize the average tumor volume of each group. Test drugs were administered intraperitoneally to animals in experimental groups once a day for 9 consecutive days at 100 mg/kg, and the vehicle was administered to control animals. The resulting tumor growth inhibition rates (TGIR) are shown in Table 4.

TABLE 4

Cells	Tumors	Tumor growth inhibition rate (TGIR, %) (43)	(58)
COL-1	colon	68	74
St-4	stomach	78	86
L-27	lung	83	96

The results above revealed that the compounds of the present invention had antitumor effect on various types of tumors.

Test Example 4

Effect on collagen-induced arthritis in mice

9–10 male DBA/lJNCrj mice (9 weeks old, purchased from Charles River Japan, Inc.) were used. An emulsion consisting of 5 ml of 0.3% bovine collagen type II (K-41, Collagen Gijyutsu-kenshukai, Japan), 15 mg of *Mycobacterium tuberculosis* H37Ra (obtained from Difco Labs.), 2.5 ml of physiological saline and 7.5 ml of Freund's incomplete adjuvant (Difco Labs.) was prepared, and then injected subcutaneously at the base of the tail (0.1 ml/animal) 2 times at 3 week intervals to induce arthritis. A test drug suspended in a vehicle, physiological saline containing 10% each of cremophor and DMSO, was administered intraperitoneally for 16 consecutive days, beginning one day before the second injection of the emulsion. Swelling of four legs, characteristic of the onset of arthritis, was investigated every day.

Effect of the compound number 43 on the incidence (%) of collagen-induced arthritis is shown as follows:

Dose	Number of mice with arthritis/total mice	Incidence (%)
Vehicle only	7/10	70
10 mg/kg	1/9	11
100 mg/kg	0/10	0

The results shown above revealed that the compound number 43 suppressed the incidence of collagen-induced arthritis.

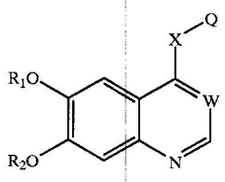
Possible Industrial Use

Since the compounds of the present invention have inhibitory activity on abnormal cell growth, more specifically PDGF receptor autophosphorylation inhibitory activity, they are useful for treating numerous diseases such as leukemia, cancers, psoriasis, glomerulonephritis, organofibrosis, atherosclerosis, restenosis after percutaneous coronary angioplasty or bypass surgery and articular rheumatism. Therefore, the compounds can benefit greatly in treating humans and other animals which need these treatments.

What is claimed is:

1. A quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (I):

(1)



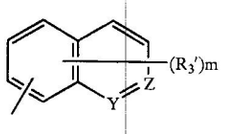
wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl or together form C_1–C_3-alkylene,

wherein W is CH,

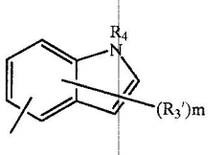
wherein X is O, S or CH_2,

wherein Q is a group represented by the following formula (III), (IV) or (V):

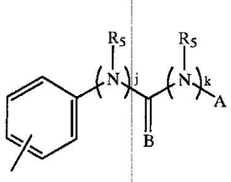
(III)



(IV)



(V)



wherein m is 1, 2 or 3,

wherein each R_3' is independently OH, C_1–C_5-alkyl or C_1–C_4-alkoxy,

wherein Y and Z are each independently N or CH,

wherein R_4 is H, C_1–C_5-alkyl or C_2–C_4-acyl,

wherein j and k are each independently 0 or 1,

wherein each R_5 is independently H or C_1–C_4-alkyl,

wherein A is C_1–C_8-alkyl, C_1–C_5-alkenyl, cyclic (C_3–C_{10})-alkyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, substituted benzoyl, C_2–C_4-acyl, or a 5- or 6-membered monocyclic or 9- or 10-membered bicyclic heteroaryl group,

wherein the heteroaryl group has 1 or 2 nitrogen atoms,

wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

wherein R_6 is C_1–C_5-alkyl,

with the proviso that if X is CH_2, then Q is not a group represented by the formula (III) or (IV).

2. The derivative, or pharmaceutically acceptable salt thereof, according to claim **1**, wherein the heteroaryl group has another heteroatom selected from the group consisting of N, O and S.

3. The derivative, or pharmaceutically acceptable salt thereof, according to claim **1**, wherein, if A is alkyl, aryl or heteroaryl, then A has 1 to 5 substituents selected from the group consisting of CN, NO_2, OH, NH_2, halogen C_1–C_5-alkyl, cyclic (C_3–C_{10}) alkyl, C_1–C_4-alkoxy, C_1–C_4-alkoxycarbonyl, C_1–C_5-acyl, C_1–C_5-acyloxy, C_1–C_3-alkylenedioxy, C_1–C_4-alkylamino, di-(C_1–C_4-alkyl)amino, CO_2H, $CONH_2$, N-(C_1–C_4-alkyl)amido, N,N-di-(C_1–C_4-alkyl)amido, C_2–C_4-alkylamido, trifluoromethyl, C_1–C_4-alkylthio, phenyl, substituted phenyl, phenoxy, substituted phenoxy, phenylthio, substituted phenylthio, phenyl(C_1–C_4-alkyl), substituted phenyl(C_1–C_4-alkyl), pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolidinyl, piperidinyl, piperazinyl, homopyperazinyl, morpholinyl, quinolyl, quinazolinyl, benzoyl, substituted benzoyl and C_2–C_4-acyl.

4. The derivative, or pharmaceutically acceptable salt thereof, according to claim **1**, wherein X is O or S, and Q is a group represented by the formula (III) or (IV).

5. The derivative, or pharmaceutically acceptable salt thereof, according to claim **4**, wherein X is O.

6. The derivative, or pharmaceutically acceptable salt thereof, according to claim **1**, wherein Q is a group represented by the formula (V).

7. The derivative, or pharmaceutically acceptable salt thereof, according to claim **1**, wherein R_1 and R_2 are each independently C_1–C_5-alkyl, Q is a group represented by the formula (V), j and k are each O, and B is O, S or NOR_6.

8. The derivative, or pharmaceutically acceptable salt thereof, according to claim **2**, wherein R_1 and R_2 are each independently C_1–C_5-alkyl, Q is a group represented by the formula (V), j is 0, k is 1, and R_5 is H or methyl.

9. The derivative, or pharmaceutically acceptable salt thereof, according to claim **1**, wherein R_1 and R_2 are each independently C_1–C_5-alkyl, Q is a group represented by the formula (V), j is 1, k is 0, and R_5 is H or methyl.

10. The derivative, or pharmaceutically acceptable salt thereof, according to claim **1**, wherein R_1 and R_2 are each independently C_1–C_5-alkyl, Q is a group represented by the formula (V), j and k are 1, and each R_5 is independently H or methyl.

11. The derivative, or pharmaceutically acceptable salt thereof, according to claim **1**, wherein X is 0; R_1 and R_2 are methyl; Q is a group represented by the formula (V); j and k are each independently 0 or 1; R_5 is H; A is C_1–C_5-alkyl, cyclopentyl, cyclohexyl, cycloheptyl, allyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl or benzoyl; and R_6 is methyl.

12. The derivative, or pharmaceutically acceptable salt thereof, according to claim **11**, wherein, if A is alkyl, aryl or heteroaryl, then A has 1–5 substituents selected from the group consisting of OH, CO_2H, fluoro, chloro, bromo, iodo, nitro, amino, di-(C_1–C_4-alkyl)amino, ethylenedioxy, acetoxy, methylthio, C_1–C_4-alkoxycarbonyl, trifluoromethyl, C_1–C_4-alkyl, C_1–C_4-alkoxy, pyridyl and phenyl.

13. The derivative, or pharmaceutically acceptable salt thereof, according to claim **1**, wherein the derivative is:

6,7-dimethoxy-4-(1-naphthyloxy) quinoline,

6,7-dimethoxy-4-(2-naphthyloxy)quinoline,

6,7-dimethoxy-4-(5-methoxy-1-naphthyloxy)quinoline,

6,7-dimethoxy-4-(6-methoxy-2-naphthyloxy)quinoline,

6,7-dimethoxy-4-(7-methoxy-2-naphthyloxy)quinoline,

6,7-dimethoxy-4-(5-quinolyloxy)quinoline,

6,7-dimethoxy-4-(6-quinolyloxy)quinoline,

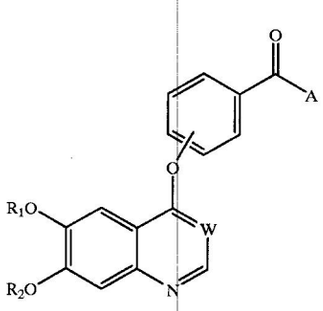
4-(4-indolyloxy)-6,7-dimethoxyquinoline, or

4-(5-indolyloxy)-6,7-dimethoxyquinoline.

14. A quinoline derivative, or a pharmaceutically acceptable salt thereof,

wherein the derivative is represented by the following formula (VI):

(VI)



wherein W is CH,

wherein R_1 and R_2 are each independently C_1–C_5-alkyl, and

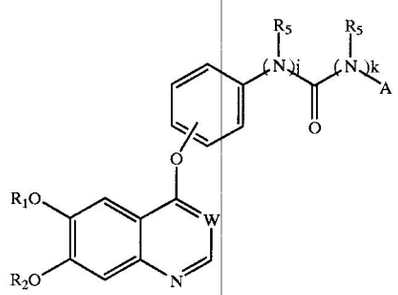
wherein A is methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, t-butyl, pentyl, isopentyl, cyclopentyl, cyclohexyl, cycloheptyl, phenyl, naphthyl, furyl, thienyl, pyridyl or pyrimidinyl.

15. A quinoline derivative, or a pharmaceutically acceptable salt thereof, according to claim **14**, wherein, if A is alkyl, aryl, or heteroaryl, then A has 1–5 substituents selected from the group consisting of fluoro, chloro, bromo, iodo, cyano, hydroxy, nitro, amino, methylamino, dimethylamino, diethylamino, dipropylamino, dibutylamino, trifluoromethyl, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, t-butyl, methoxy, ethoxy, propoxy, isopropoxy, morpholino, pyrrolidino, piperidino and butoxy.

16. A quinoline derivative, or a pharmaceutically acceptable salt thereof,

wherein the derivative is represented by the following formula (VII):

(VII)



wherein W is CH,

wherein j is O and K is 1, or j is 1 and k is 0,

wherein R_1 and R_2 are each independently C_1–C_5-alkyl,

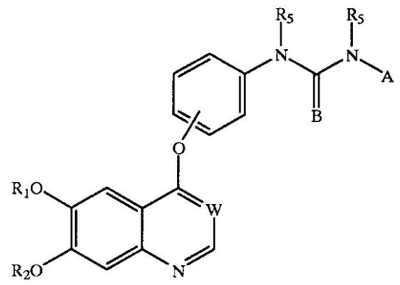
wherein R_5 is H or methyl, and

wherein A is methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, t-butyl, pentyl, isopentyl, cyclopentyl, cyclohexyl, cycloheptyl, phenyl, naphthyl, furyl, thienyl, pyridyl or pyrimidinyl.

17. A quinoline derivative, or a pharmaceutically acceptable salt thereof, according to claim **16**, wherein, if A is alkyl, aryl, or heteroaryl, then A has 1–5 substituents selected from the group consisting of fluoro, chloro, bromo, iodo, cyano, hydroxy, nitro, amino, methylamino, dimethylamino, diethylamino, dipropylamino, dibutylamino, trifluoromethyl, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, s-butyl, t-butyl, methoxy, ethoxy, propoxy, isopropoxy and butoxy.

18. A quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (VIII):

(VIII)



wherein W is CH,

wherein R_1 and R_2 are each independently C_1–C_5-alkyl, each R_5 is independently H or methyl,

wherein A is C_1–C_6-alkyl, C_1–C_4-alkenyl, cyclopentyl, cyclohexyl, cycloheptyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, acetyl, pyridyl, pyrimidinyl, pyrrolidinyl, piperidinyl, piperazinyl, homopiperazinyl or morpholino,

wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

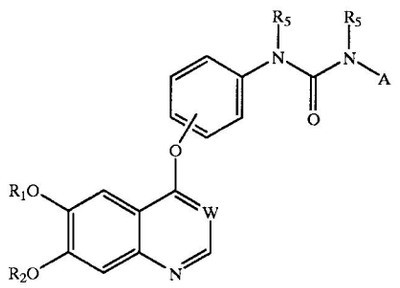
wherein R_6 is methyl.

19. A quinoline derivative, or a pharmaceutically acceptable salt thereof, according to claim **18**, wherein, if A is alkyl, aryl or heteroaryl, then A has 1–5 substituents selected from the group consisting of halogen, cyano, CO_2H, $CONH_2$, hydroxy, nitro, amino, C_1–C_4-alkylamino, di-(C_1–C_4-alkyl)amino, C_1–C_5-acyloxy, C_1–C_5-acyl, C_1–C_4-alkylthio, trifluoromethyl, C_1–C_5-alkyl, C_1–C_4-alkoxyl, C_1–C_4-alkoxycarbonyl, N-(C_1–C_4-alkyl)amido, N,N-di-(C_1–C_4-alkyl)amido, C_2–C_4-alkylamido, ethylenedioxy, phenyl, phenoxy, substituted phenyl, benzoyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinolyl and quinazolinyl.

20. A quinoline derivative, or a pharmaceutically acceptable salt thereof,

wherein the derivative is represented by the following formula (IX):

(IX)



wherein W is CH,

wherein R_1 and R_2 are each independently C_1–C_5-alkyl,

wherein each R_5 is independently H or methyl, and

wherein A is C_1–C_6-alkyl, C_1–C_4-alkenyl, cyclopentyl, cyclohexyl, cycloheptyl, C_1–C_4-alkoxycarbonyl,

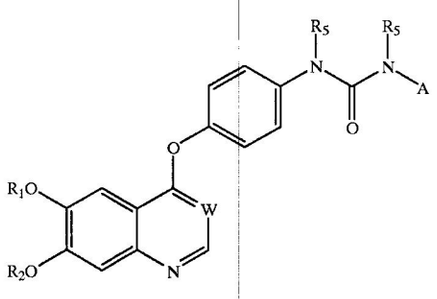
phenyl, naphthyl, furyl, thienyl, benzoyl, acetyl, pyridyl, pyrimidinyl, pyrrolidinyl, piperidinyl, piperazinyl, homopiperazinyl or morpholino.

21. A quinoline derivative, or a pharmaceutically acceptable salt thereof, according to claim **20**, wherein, if A is alkyl, aryl or heteroaryl, then A has 1–5 substituents selected from the group consisting of halogen, cyano, CO_2H, $CONH_2$, hydroxy, nitro, amino, C_1–C_4-alkylamino, di-(C_1–C_4-alkyl)amino, C_1–C_5-acyloxy, C_1–C_5-acyl, C_1–C_4-alkylthio, trifluoromethyl, C_1–C_5-alkyl, C_1–C_4-alkoxyl, C_1–C_4-alkoxycarbonyl, N-(C_1–C_4-alkyl)amido, N,N-di-(C_1–C_4-alkyl)amido, C_2–C_4-alkylamido, ethylenedioxy, phenyl, phenoxy, substituted phenyl, benzoyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, quinolyl and quinazolinyl.

22. A quinoline derivative, or a pharmaceutically acceptable salt thereof,

wherein the derivative is represented by the following formula (X):

(X)



wherein R_1 and R_2 are each independently C_1–C_5-alkyl,

wherein R_5 is each independently hydrogen or methyl, and

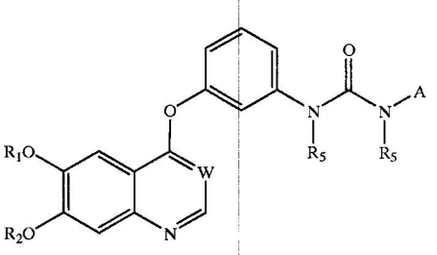
wherein A is C_1–C_5-alkyl, cyclopentyl, cyclohexyl, cycloheptyl, allyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl or benzoyl.

23. A quinoline derivative, or a pharmaceutically acceptable salt thereof, according to claim **22**, wherein, if A is alkyl, aryl or heteroaryl, then A has 1–5 substituents selected from the group consisting of OH, CO2H, fluoro, chloro, bromo, iodo, nitro, amino, di-(C_1–C_4-alkyl)amino, ethylenedioxy, acetoxy, methylthio, C_1–C_4-alkoxycarbonyl, trifluoromethyl, C_1–C_4-alkyl, pyridyl and phenyl.

24. The derivative, or pharmaceutically acceptable salt thereof, according to claim **22**, wherein R_1 and R_2 are methyl, and each R_5 is H.

25. A quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (XI):

(XI)



wherein R_1 and R_2 are each independently C_1–C_5-alkyl,

wherein each R_5 is independently H or methyl, and

wherein A is C_1–C_5-alkyl, cyclopentyl, cyclohexyl, cycloheptyl, allyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl or benzoyl.

26. A quinoline derivative, or a pharmaceutically acceptable salt thereof, according to claim **25**, wherein, if A is alkyl or aryl, then A has 1–5 substituents selected from the group consisting of OH, CO_2H, fluoro, chloro, bromo, iodo, nitro, amino, di-(C_1–C_4-alkyl)amino, ethylenedioxy, acetoxy, methylthio, C_1–C_4-alkoxycarbonyl, trifluoromethyl, C_1–C_4-alkyl, C_1–C_4-alkoxy, pyridyl and phenyl.

27. The derivative, or pharmaceutically acceptable salt thereof, according to any one of claims **1**, **11**, **13**, **14**, **16**, **18**, and **20**, wherein the derivative is:

(4-n-butylphenyl){4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}methanone,

(4-t-butylphenyl){4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}methanone,

(4-trifluoromethylphenyl){4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}methanone,

(4-t-butylphenyl){4-[(6,7-dimethoxy-4-quinolyl)methyl]phenyl}methanone,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-cyclohexanecarboxamide,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-(4-nitrophenyl)carboxamide,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-(N,N-dimethylaminophenyl)carboxamide,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-(4-acetylphenyl)carboxamide,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-(4-n-butylphenyl)carboxamide,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-(4-butoxyphenyl)carboxamide,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-(4-bromophenyl)carboxamide,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-cyclopentanecarboxamide,

N-(4-n-butylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(4-t-butylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(2-trifluoromethylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(3-trifluoromethylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(4-trifluoromethylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(2-methoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(3-methoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(4-methoxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(2-fluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(3-fluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(4-fluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(4-acetylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-N'-n-propylurea,

N-n-butyl-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}phenylurea,

{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}[4-morpholinophenyl]methanone,

{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}[4-pyrrolidinophenyl]methanone,

{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}[4-piperidinophenyl]methanone,

N-(2,4-dichlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(3,4-dichlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(3,5-dichlorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(4-chloro-2-methylphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(3-amino-4-chlorophenyl)-N -{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}-N'-(2-pyridylmethyl)urea,

N-(3,4-difluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(2,4,5-trifluorophenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea,

N-(3-chlorophenyl)-N'-{4-[(6,7-dimethoxy-4quinolyl)oxy]phenyl}urea, or

N-(4-hydroxyphenyl)-N'-{4-[(6,7-dimethoxy-4-quinolyl)oxy]phenyl}urea.

28. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to claim **1**.

29. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **1, 14, 16, 18, 20, 22**, and **25** in an amount effective in inhibiting platelet-derived growth factor receptor autophosphorylation.

30. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **1, 14, 16, 18, 20, 22** and **25** in an amount effective in treating tumors.

31. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **1, 14, 16, 18, 20, 22** and **25** in an amount effective in treating psoriasis.

32. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **1, 14, 16, 18, 20, 22** and **25** in an amount effective in treating atherosclerosis.

33. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **1, 14, 16, 18, 20, 22** and **25** in an amount effective in treating restenosis after percutaneous coronary angioplasty or bypass surgery.

34. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **1, 14, 16, 18, 20, 22** and **25** in an amount effective in treating glomerulonephritis.

35. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **1, 14, 16, 18, 20, 22** and **25** in an amount effective in treating organofibrosis.

36. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **1, 14, 16, 18, 20, 22** and **25** in an amount effective in treating leukemia.

37. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **1, 14, 16, 18, 20, 22** and **25** in an amount effective in treating articular rheumatism.

38. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **7** and **14** in an amount effective in inhibiting platelet-derived growth factor receptor autophosphorylation.

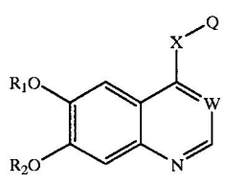
39. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **10, 18, 20, 22, 24**, and **25** in an amount effective in treating tumors.

40. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **10, 18, 20, 22, 24** and **25** in an amount effective in treating leukemia.

41. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and the derivative, or a pharmaceutically acceptable salt thereof, according to any of claims **10, 18, 20, 22, 24** and **25** in an amount effective in treating articular rheumatism.

42. A method for treating a tumor comprising administering to a patient suffering from a tumor an effective amount of a quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (I):

(1)



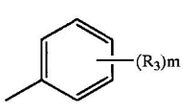
wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl or together form C_1–C_3-alkylene,

wherein W is CH,

wherein X is O, S or CH_2,

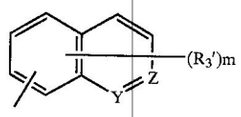
wherein Q is a group represented by the following formula (II), (III), (IV) or (V):

(II)

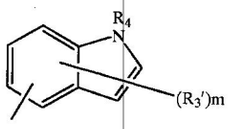


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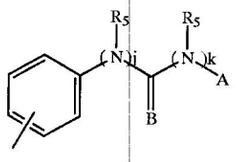
(III)



(IV)



(V)



wherein m is 1, 2 or 3,

wherein each R_3 is independently CN, OH, halogen, C_1–C_5-alkyl, C_1–C_4-alkoxy or C_2–C_4-acyl,

wherein each R_3' is independently OH, C_1–C_5-alkyl or C_1–C_4-alkoxy,

wherein Y and Z are each independently N or CH,

wherein R_4 is H, C_1–C_5-alkyl or C_2–C_4-acyl,

wherein j and k are each independently 0 or 1,

wherein each R_5 is independently H or C_1–C_4-alkyl,

wherein A is C_1–C_8-alkyl, C_1–C_5-alkenyl, cyclic (C_3–C_{10})-alkyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, substituted benzoyl, C_2–C_4-acyl, or a 5- or 6-membered monocyclic or 9- or 10-membered bicyclic heteroaryl group,

wherein the heteroaryl group has 1 or 2 nitrogen atoms,

wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

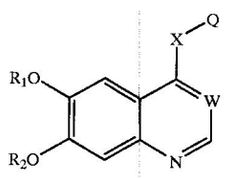
wherein R_6 is C_1–C_5-alkyl,

with the proviso that if X is CH_2, then Q is not a group represented by the formula (II), (III) or (IV).

43. The method of claim **42**, wherein the tumor is neoplastic.

44. A method for treating psoriasis comprising administering to a patient suffering from psoriasis an effective amount of a quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (I):

(1)



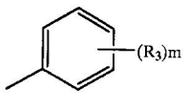
wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl or together form C_1–C_3-alkylene,

wherein W is CH,

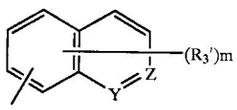
wherein X is O, S or CH_2,

wherein Q is a group represented by the following formula (II), (III), (IV) or (V):

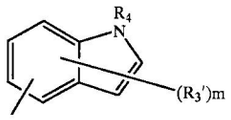
(II)



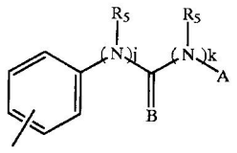
(III)



(IV)



(V)



wherein m is 1, 2 or 3,

wherein each R_3 is independently CN, OH, halogen, C_1–C_5-alkyl, C_1–C_4-alkoxy or C_2–C_4-acyl,

wherein each R_3' is independently OH, C_1–C_5-alkyl or C_1–C_4-alkoxy,

wherein Y and Z are each independently N or CH,

wherein R_4 is H, C_1–C_5-alkyl or C_2–C_4-acyl,

wherein j and k are each independently 0 or 1,

wherein each R_5 is independently H or C_1–C_4-alkyl,

wherein A is C_1–C_8-alkyl, C_1–C_5-alkenyl, cyclic (C_3–C_{10})-alkyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, substituted benzoyl, C_2–C_4-acyl, or a 5- or 6-membered monocyclic or 9- or 10-membered bicyclic heteroaryl group,

wherein the heteroaryl group has 1 or 2 nitrogen atoms,

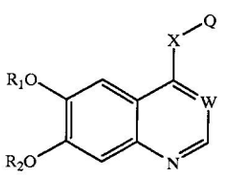
wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

wherein R_6 is C_1–C_5-alkyl,

with the proviso that if X is CH_2, then Q is not a group represented by the formula (II), (III) or (IV).

45. A method for treating atherosclerosis comprising administering to a patient suffering from atherosclerosis an effective amount of a quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (I):

(1)



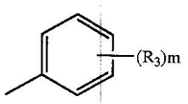
wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl or together form C_1–C_3-alkylene,

wherein W is CH,

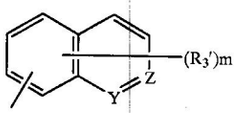
wherein X is O, S or CH_2,

wherein Q is a group represented by the following formula (II), (III), (IV) or (V):

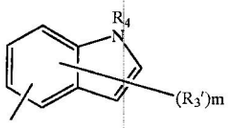
(II)



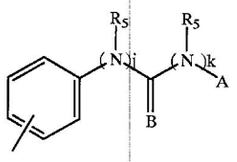
(III)



(IV)



(V)



wherein m is 1, 2 or 3,

wherein each R_3 is independently CN, OH, halogen, C_1–C_5-alkyl, C_1–C_4-alkoxy or C_2–C_4-acyl,

wherein each R_3' is independently OH, C_1–C_5-alkyl or C_1–C_4-alkoxy,

wherein Y and Z are each independently N or CH,

wherein R_4 is H, C_1–C_5-alkyl or C_2–C_4-acyl,

wherein j and k are each independently 0 or 1,

wherein each R_5 is independently H or C_1–C_4-alkyl,

wherein A is C_1–C_8-alkyl, C_1–C_5-alkenyl, cyclic (C_3–C_{10})-alkyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, substituted benzoyl, C_2–C_4-acyl, or a 5- or 6-membered monocyclic or 9- or 10-membered bicyclic heteroaryl group,

wherein the heteroaryl group has 1 or 2 nitrogen atoms,

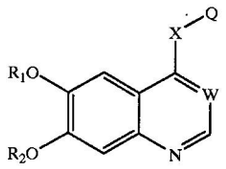
wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

wherein R_6 is C_1–C_5-alkyl,

with the proviso that if X is CH_2, then Q is not a group represented by the formula (II), (III) or (IV).

46. A method for treating restenosis after percutaneous coronary angioplasty or bypass surgery comprising administering to a patient suffering from restenosis after percutaneous coronary angioplasty or bypass surgery an effective amount of a quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (I):

(1)



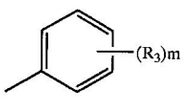
wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl or together form C_1–C_3-alkylene,

wherein W is CH,

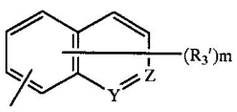
wherein X is O, S or CH_2,

wherein Q is a group represented by the following formula (II), (III), (IV) or (V):

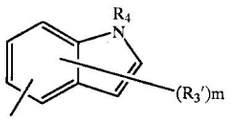
(II)



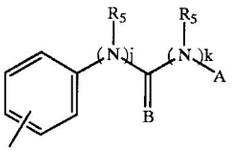
(III)



(IV)



(V)



wherein m is 1, 2 or 3,

wherein each R_3 is independently CN, OH, halogen, C_1–C_5-alkyl, C_1–C_4-alkoxy or C_2–C_4-acyl,

wherein each R_3' is independently OH, C_1–C_5-alkyl or C_1–C_4-alkoxy,

wherein Y and Z are each independently N or CH,

wherein R_4 is H, C_1–C_5-alkyl or C_2–C_4-acyl,

wherein j and k are each independently 0 or 1,

wherein each R_5 is independently H or C_1–C_4-alkyl,

wherein A is C_1–C_5-alkyl, C_1–C_5-alkenyl, cyclic (C_3–C_{10})-alkyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, substituted benzoyl, C_2–C_4-acyl, or a 5- or 6-membered monocyclic or 9- or 10-membered bicyclic heteroaryl group,

wherein the heteroaryl group has 1 or 2 nitrogen atoms,

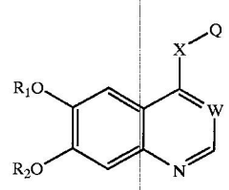
wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

wherein R_6 is C_1–C_5-alkyl,

with the proviso that if X is CH_2, then Q is not a group represented by the formula (II), (III) or (IV).

47. A method for treating glomerulonephritis comprising administering to a patient suffering from glomerulonephritis an effective amount of a quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (I):

(1)



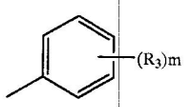
wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl or together form C_1–C_3-alkylene,

wherein W is CH,

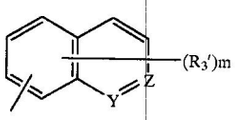
wherein X is O, S or CH_2,

wherein Q is a group represented by the following formula (II), (III), (IV) or (V):

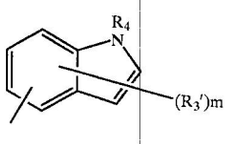
(II)



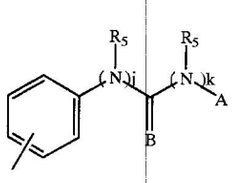
(III)



(IV)



(V)



wherein m is 1, 2 or 3,

wherein each R_3 is independently CN, OH, halogen, C_1–C_5-alkyl, C_1–C_4-alkoxy or C_2–C_4-acyl,

wherein each R_3' is independently OH, C_1–C_5-alkyl or C_1–C_4-alkoxy,

wherein Y and Z are each independently N or CH,

wherein R_4 is H, C_1–C_5-alkyl or C_2–C_4-acyl,

wherein j and k are each independently 0 or 1,

wherein each R_5 is independently H or C_1–C_4-alkyl,

wherein A is C_1–C_5-alkyl, C_1–C_5-alkenyl, cyclic (C_3–C_{10})-alkyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, substituted benzoyl, C_2–C_4-acyl, or a 5- or 6-membered monocyclic or 9- or 10-membered bicyclic heteroaryl group,

wherein the heteroaryl group has 1 or 2 nitrogen atoms,

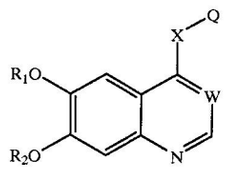
wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

wherein R_6 is C_1–C_5-alkyl,

with the proviso that if X is CH_2, then Q is not a group represented by the formula (II), (III) or (IV).

48. A method for treating organofibrosis comprising administering to a patient suffering from organofibrosis an effective amount of a quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (I):

(1)



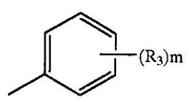
wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl or together form C_1–C_3-alkylene,

wherein W is CH,

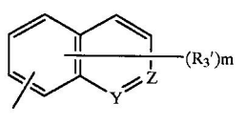
wherein X is O, S or CH_2,

wherein Q is a group represented by the following formula (II), (III), (IV) or (V):

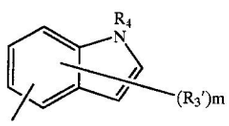
(II)



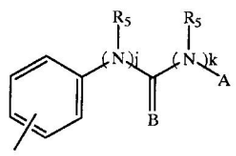
(III)



(IV)



(V)



wherein m is 1, 2 or 3,

wherein each R_3 is independently CN, OH, halogen, C_1–C_5-alkyl, C_1–C_4-alkoxy or C_2–C_4-acyl,

wherein each R_3' is independently OH, C_1–C_5-alkyl or C_1–C_4-alkoxy,

wherein Y and Z are each independently N or CH,

wherein R_4 is H, C_1–C_5-alkyl or C_2–C_4-acyl,

wherein j and k are each independently 0 or 1,

wherein each R_5 is independently H or C_1–C_4-alkyl,

wherein A is C_1–C_8-alkyl, C_1–C_5-alkenyl, cyclic (C_3–C_{10})-alkyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, substituted benzoyl, C_2–C_4-acyl, or a 5- or 6-membered monocyclic or 9- or 10-membered bicyclic heteroaryl group,

wherein the heteroaryl group has 1 or 2 nitrogen atoms,

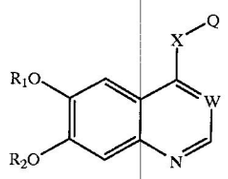
wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

wherein R_6 is C_1–C_5-alkyl,

with the proviso that if X is CH_2, then Q is not a group represented by the formula (II), (III) or (IV).

49. A method for treating leukemia comprising administering to a patient suffering from leukemia an effective amount of a quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (I):

(1)



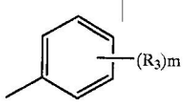
wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl or together form C_1–C_3-alkylene,

wherein W is CH,

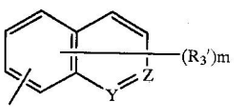
wherein X is O, S or CH_2,

wherein Q is a group represented by the following formula (II), (III), (IV) or (V):

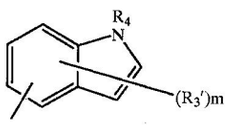
(II)



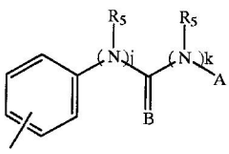
(III)



(IV)



(V)



wherein m is 1, 2 or 3,

wherein each R_3 is independently CN, OH, halogen, C_1–C_5-alkyl, C_1–C_4-alkoxy or C_2–C_4-acyl,

wherein each R_3' is independently OH, C_1–C_5-alkyl or C_1–C_4-alkoxy,

wherein Y and Z are each independently N or CH,

wherein R_4 is H, C_1–C_5-alkyl or C_2–C_4-acyl,

wherein j and k are each independently 0 or 1,

wherein each R_5 is independently H or C_1–C_4-alkyl,

wherein A is C_1–C_8-alkyl, C_1–C_5-alkenyl, cyclic (C_3–C_{10})-alkyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, substituted benzoyl, C_2–C_4-acyl, or a 5- or 6-membered monocyclic or 9- or 10-membered bicyclic heteroaryl group,

wherein the heteroaryl group has 1 or 2 nitrogen atoms,

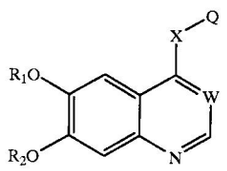
wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

wherein R_6 is C_1–C_5-alkyl,

with the proviso that if X is CH_2, then Q is not a group represented by the formula (II), (III) or (IV).

50. A method for treating articular rheumatism comprising administering to a patient suffering from articular rheumatism an effective amount of a quinoline derivative, or a pharmaceutically acceptable salt thereof, wherein the derivative is represented by the following formula (I):

(1)



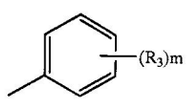
wherein R_1 and R_2 are each independently H, C_1–C_5-alkyl or together form C_1–C_3-alkylene,

wherein W is CH,

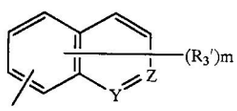
wherein X is O, S or CH_2,

wherein Q is a group represented by the following formula (II), (III), (IV) or (V):

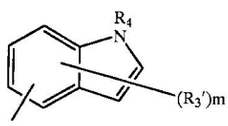
(II)



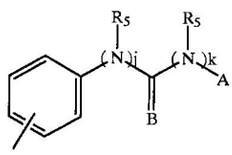
(III)



(IV)



(V)



wherein m is 1, 2 or 3,

wherein each R_3 is independently CN, OH, halogen, C_1–C_5-alkyl, C_1–C_4-alkoxy or C_2–C_4-acyl,

wherein each R_3' is independently OH, C_1–C_5-alkyl or C_1–C_4-alkoxy,

wherein Y and Z are each independently N or CH,

wherein R_4 is H, C_1–C_5-alkyl or C_2–C_4-acyl,

wherein j and k are each independently 0 or 1,

wherein each R_5 is independently H or C_1–C_4-alkyl,

wherein A is C_1–C_5-alkyl, C_1–C_5-alkenyl, cyclic (C_3–C_{10})-alkyl, C_1–C_4-alkoxycarbonyl, phenyl, naphthyl, furyl, thienyl, benzoyl, substituted benzoyl, C_2–C_4-acyl, or a 5- or 6-membered monocyclic or 9- or 10-membered bicyclic heteroaryl group,

819 830

wherein the heteroaryl group has 1 or 2 nitrogen atoms,

wherein B is O, S, NH, NCN, NR_6 or NOR_6, and

wherein R_6 is C_1–C_5-alkyl,

with the proviso that if X is CH_2, then Q is not a group represented by the formula (II), (III) or (IV).

51. The method according to any one of claims **42–50**, wherein the heteroaryl group has another heteroatom selected from the group consisting of N, O and S.

52. The method according to any one of claims **42–40**, wherein, if A is alkyl, aryl or heteroaryl, then A has 1 to 5 substituents selected from the group consisting of CN, NO_2, OH, NH_2, halogen C_1–C_5-alkyl, cyclic (C_3–C_{10}) alkyl, C_1–C_4-alkoxy, C_1–C_4-alkoxycarbonyl, C_1–C_5-acyl, C_1–C_5-acyloxy, C_1–C_3-alkylenedioxy, C_1–C_4-alkylamino, di-(C_1–C_4-alkyl)amino, CO_2H, $CONH_2$, N-(C_1–C_4-alkyl) amido, N,N-di-(C_1–C_4-alkyl)amido, C_2–C_4-alkylamido, trifluoromethyl, C_1–C_4-alkylthio, phenyl, substituted phenyl, phenoxy, substituted phenoxy, phenylthio, substituted phenylthio, phenyl(C_1–C_4-alkyl), substituted phenyl (C_1–C_4-alkyl), pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolidinyl, piperidinyl, piperazinyl, homopyperazinyl, morpholinyl, quinolyl, quinazolinyl, benzoyl, substituted benzoyl and C_2–C_4-acyl.

* * * * *

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Title

Inhibitors of coenzyme A-independent transacylase induce apoptosis in human HL-60 cells

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Abstract

ET-18-O-CH3 (1-O-octadecyl-2-O-methyl-sn-glycero-3-phosphocholine) is an antiproliferative agent, blocking the growth of cancer cells both in vitro and in vivo. However, there is controversy regarding the mechanism leading to its antiproliferative effects. CoA-independent transacylase (CoA-IT) is an enzyme that remodels arachidonate between specific phospholipid donor and acceptor mols. in a variety of mammalian cells. ET-18-O-CH3 was a potent inhibitor of CoA-IT (IC50, 0.5 .mu.M), and kinetic anal. revealed that its inhibition was competitive with the lyso-phospholipid substrate. The goal of the current study was to explore the connection between inhibition of CoA-IT and antiproliferative effects using several structurally distinct inhibitors of CoA-IT. ET-18-O-CH3 and other inhibitors of CoA-IT were found to inhibit cell proliferation and thymidine incorporation into the DNA, as well as to induce apoptosis in human HL-60 monocytic leukemia cells. The mechanism of apoptosis induced by ET-18-O-CH3 appeared to be different from that induced by tumor necrosis factor; the former failed to activate NF-.kappa.B, whereas tumor necrosis factor did. Closer examn. of the pharmacol. of apoptosis in this model revealed that compds. that were structurally related to CoA-IT inhibitors, but lacked CoA-IT inhibitory activity, also failed to induce apoptosis. In addn., compds. that inhibited other enzymes that participate in arachidonic acid metab., cyclooxygenase, 5-lipoxygenase and phospholipase A2, did not induce apoptosis. Taken together, these results demonstrate that inhibition of CoA-IT can be linked to blockade of proliferation and the induction of apoptosis in HL-60 cells.

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Inhibitors of Coenzyme A-Independent Transacylase Induce Apoptosis in Human HL-60 Cells[1]

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Accepted for publication July 3, 1996

ABSTRACT

ET-18-O-CH_3 (1-*O*-octadecyl-2-O-methyl-*sn*-glycero-3-phosphocholine) is an antiproliferative agent, blocking the growth of cancer cells both *in vitro* and *in vivo*. However, there is controversy regarding the mechanism leading to its antiproliferative effects. CoA-independent transacylase (CoA-IT) is an enzyme that remodels arachidonate between specific phospholipid donor and acceptor molecules in a variety of mammalian cells. ET-18-O-CH_3 was found to be a potent inhibitor of CoA-IT (IC_{50}, 0.5 μM), and kinetic analysis revealed that its inhibition was competitive with the lyso-phospholipid substrate. The goal of the current study was to explore the connection between inhibition of CoA-IT and antiproliferative effects using several structurally distinct inhibitors of CoA-IT. ET-18-O-CH_3 and other inhibitors of CoA-IT were found to inhibit cell proliferation and thymidine incorporation into the DNA, as well as to induce apoptosis in human HL-60 monocytic leukemia cells. The mechanism of apoptosis induced by ET-18-O-CH_3 appeared to be different from that induced by tumor necrosis factor; the former failed to activate NF-κB, whereas tumor necrosis factor did. Closer examination of the pharmacology of apoptosis in this model revealed that compounds that were structurally related to CoA-IT inhibitors, but lacked CoA-IT inhibitory activity, also failed to induce apoptosis. In addition, compounds that inhibited other enzymes that participate in arachidonic acid metabolism, cyclooxygenase, 5-lipoxygenase and phospholipase A_2, did not induce apoptosis. Taken together, these results demonstrate that inhibition of CoA-IT can be linked to blockade of proliferation and the induction of apoptosis in HL-60 cells.

There is extensive literature on the effects of alkyl-lyso-phospholipids on cell proliferation and cancer. One of the most studied compound is ET-18-O-CH_3. This compound, along with other alkyl-lyso-phospholipids, has been shown to decrease cell proliferation of leukemia, carcinoma and sarcoma cell lines (Andreesen *et al.*, 1979; Modolell *et al.*, 1979; Berdel *et al.*, 1981; Hoffman *et al.*, 1984; Morris-Natschke *et al.*, 1986; Brachwitz *et al.*, 1987; Lu and Arthur, 1992a). This effect on proliferation appears to be selective, as these compounds do not block proliferation of noncancerous cells, such as normal bone marrow cells, neutrophils and skin fibroblasts (Hoffman *et al.*, 1984; Verdonck *et al.*, 1990; Langen *et al.*, 1992). The mechanism of action of these antiproliferative alkyl-lyso-phospholipids remains elusive. It is thought that the actions ascribed to ET-18-O-CH_3 are due to the parent compound itself, as little cellular metabolism of ET-18-O-CH_3 is observed (Wilcox *et al.*, 1987; Lu *et al.*, 1993). Although structurally similar to platelet-activating factor, these compounds do not appear to act through the receptor for platelet-activating factor, because antagonists of this receptor do not block the antiproliferative effects of alkyl-lyso-phospholipids (Workman *et al.*, 1991). Other potential mechanisms for the antiproliferative effects of alkyl-lyso-phospholipids have been described: inhibition of phosphatidylinositol-phospholipase *C* with decreases in inositol-phosphate formation (IC_{50}, ~40 μM) (Powis and Phil, 1994); alteration of membrane-associated enzymes such as protein kinase *C* and Na,K-adenosine triphosphatase (Zheng *et al.*, 1990; Hoffman *et al.*, 1992); decreases in acyl-CoA acyltransferase activity (IC_{50}, ~80 μM) (Herrmann and Neumann, 1986); and decreases in phospholipid metabolism (Modolell *et al.*, 1979; Herrmann, 1985; Boggs *et al.*, 1995b). However, there are reports of a lack of correlation between decreases in

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ABBREVIATIONS: ET-18-O-CH_3, 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycero-3-phosphocholine; CoA, coenzyme A; CoA-IT, coenzyme A-independent transacylase; GPC, *sn*-glycero-3-phosphocholine; BSA, bovine serum albumin; 1-alkyl, 1-*O*-alkyl; 1-alkenyl, 1-*O*-alk-1′-enyl; DMSO, dimethylsulfoxide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; AA, arachidonic acid, 5-8-11-14 eicosatetraenoic acid; 5-LO, 5-lipoxygenase; PLA_2, phospholipase A_2; TNF, tumor necrosis factor α.

phospholipid metabolism and the sensitivity of different cell lines to the blockade of proliferation (Lu and Arthur, 1992a,b). Also, the concentrations of alkyl-lyso-phospholipids necessary to inhibit the above-mentioned cellular events are higher than the antiproliferative effects (3–10 μM). In addition, other compounds which inhibit these enzymes are not themselves antiproliferative (Daniel *et al.*, 1987).

CoA-IT is an enzyme responsible for the movement of arachidonate between phospholipid molecular species of inflammatory cells. CoA-IT removes arachidonate from the *sn*-2 position of 1-acyl-containing phospholipids, such as 1-acyl-2-arachidonoyl-GPC. It then transfers that arachidonate to a suitable lyso-phospholipid acceptor, such as 1-alkyl-2-lyso-GPC and 1-alkenyl-2-lyso-*sn*-glycero-3-phosphoethanolamine (Kramer and Deykin, 1983; Chilton *et al.*, 1983; Sugiura *et al.*, 1987). This activity is selective for 20 carbon fatty acyl groups and is thought to be the mechanism by which inflammatory cells move arachidonate into specific phospholipid pools before its release (Winkler and Chilton, 1993, 1995; Snyder *et al.*, 1994; Chilton *et al.*, 1996).

Because ET-18-O-CH_3 is structurally similar to one of the substrates of CoA-IT, 1-alkyl-2-lyso-GPC, we examined the ability of ET-18-O-CH_3 to block CoA-IT activity. This compound was found to be a potent and competitive inhibitor of CoA-IT activity. As expected, ET-18-O-CH_3 also induced apoptosis in HL-60 cells, but not in K562 cells, by a mechanism distinct from that of TNF. Other structurally distinct inhibitors of CoA-IT shared antiproliferative and apoptosis properties with ET-18-O-CH_3. Taken together, these studies reveal that inhibition of the enzyme CoA-IT by compounds such as ET-18-OCH_3 may represent an important mechanism leading to apoptosis.

Materials and Methods

Materials. 1-[^{3}H]alkyl-2-lyso-GPC (30–60 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Common laboratory chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). 1-alkyl-2-lyso-GPC was purchased from Biomol (Plymouth Meeting, PA). Silica gel G plates were acquired from Analtech Inc. (Newark, DE). Essentially, fatty acid-free BSA was obtained from Calbiochem (San Diego, CA).

Compounds. ET-18-O-CH_3 was obtained from Biomol and was made as a 100 mM stock in $CHCl_3$, then diluted in RPMI-1640 (>16,000) before addition to cell suspensions. The following compounds were made by the Department of Medicinal Chemistry, SmithKline Beecham (King of Prussia, PA): SK&F 98625 {diethyl 7-(3,4,5-triphenyl-2-oxo-2,3-dihydro-imidazol-1-yl)heptane-phosphonate}, SK&F 98628 {diethyl 7-(1,4,5-triphenyl imidazol-2-yloxy)heptane phosphonate}, SK&F 98414 {5-(3,4,5-triphenyl-2-oxo-2,3-dihydroimidazol-1-yl)valeric acid}, SK&F 98416 {ethyl 6-(1,4,5-triphenyl imidazol-1-yloxy)hexanoate}, SB 202718 {2-[2-[3,5-bis(trifluoromethyl)phenyl]ureidol-4-trifluoromethylphenoxy]-5-(1,1-dimethylpropyl)benzenesulfonamide}, SB 203347 {2-[2-[3,5-bis(trifluoromethyl) phenylsulfonamidol]-4-trifluoromethylphenoxy]benzoic acid sodium salt}, zileuton and SK&F 45905 {2-[2-[3-(4-chloro-3-trifluoromethylphenyl)ureido]-4-trifluoromethyl phenoxy]-4,5-dichlorobenzene sulfonic acid}. Indomethacin was from Sigma. Arachidonyl-trifluoromethyl ketone was purchased from Cayman (Ann Arbor, MI). These compounds were made as stocks (5–100 mM) in DMSO and were diluted in DMSO to provide final concentrations, with DMSO concentrations ranging from 0.1 to 1%. TNF (Sigma) was made as stock in distilled water and diluted to provide the desired concentrations.

Preparation of cells. HL-60, U937 and K562 cells were obtained from the American Type Culture Collection and were grown in RPMI-1640 media supplemented with 10% BSA at 37°C, 5% CO_2. Cells were seeded in T-flasks at 0.03 to 0.08 $\times$ 10^6 cells/ml and were used for experiments at 0.5 to 0.6 $\times$ 10^6 cells/ml. The cell concentration at the time of drug exposure was found to be an important parameter (Fujiwara *et al.*, 1994).

Proliferation and apoptosis. The functional status of cells was measured in several ways. First, cell viability was assessed by the ability of the cells to exclude trypan blue stain. Proliferation was determined in one set of experiments as the number of viable cells per flask over time.

A second, more sensitive and quantitative method was to measure DNA synthesis, by using the incorporation of [^{3}H]thymidine into newly formed DNA as the index of synthesis. In these assays, HL-60 cells were treated with various concentrations of compounds or vehicle and were kept in cell culture for different lengths of time (1–3 days). Cells were then given a pulse of [^{3}H]thymidine (1 μCi), then were removed from the culture 24 hr later, washed 2 times with PBS and then were treated with 0.2 N NaOH for 30 min, followed by the addition of 15% trichloroacetic acid for 2 hr. The total sample from each well was filtered through GF/C Watman glass microfilters under vacuum and was washed 3 times with 5% trichloroacetic acid. The incorporation of [^{3}H]thymidine into DNA bound to the filter was determined using liquid scintillation spectroscopy.

Another method, measurement of DNA fragmentation, determines not only the amount of cell death, but also provides insight into the mechanism of death. When cells undergo programmed cell death or apoptosis, but not necrosis, cellular DNA is broken down into oligo-nucleosomal fragments, which can be discerned on gels (Kerr *et al.*, 1994; Majno and Joris, 1995; Squier *et al.*, 1995). Cells were treated for various times (3–24 hr) with different concentrations of compounds or vehicle. The washed cells were lysed by resuspending them in 200 μl of cold, sterile detergent buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA and 0.2% Triton X-100) and were incubated on ice 30 min. Cellular protein and RNA were enzymatically degraded by incubation with 75 μg/ml of RNAse A for 1 hr at 37°C, then 200 μg/ml of Proteinase K and 0.5% sodium dodecyl sulfate (final) for 1 hr at 37°C. An internal standard of DNA was added during the cell lysis and its recovery was used as a confirmation of efficient extraction of cellular DNA fragments. The samples were extracted twice with an equal volume of cold, buffer-saturated phenol, once with phenol-chloroform-isoamyl alcohol (25:24:1, v/v) and once with chloroform, then NaCl (300 mM final) and cold ethanol were added, the solution was vortexed and let stand overnight. The ethanol was removed and the samples and DNA bp standards (Sigma) were run on a 1% agarose gel with TBE buffer with ethidium bromide (in millmolar: Tris, 45, pH 8.0; boric acid, 45; and EDTA, 1). The DNA was visualized under UV light (UVP Gel Documentation system) and the size of the DNA fragments were noted by comparison to MW standards.

A final method for measuring apoptosis is to quantitate the amount of broken DNA fragments using a fluorescent end-labeling method (Gorczyca *et al.*, 1993; Chapman *et al.*, 1995), a system used in the ApopTag kit from Oncor (Gaithersburg, MD). In brief, the enzyme terminal deoxynucleotidyl transferase extends the DNA fragments with digoxigenin-containing nucleotides, which are then detected with a antidigoxigenin antibody carrying fluorescein to allow detection by fluorescence (494 nm excitation and 523 nm emission). Propidium iodide is used as a counter stain to measure total DNA content. Flow cytometric analysis was done on a Becton-Dickinson (Rutherford, NJ) FACScan instrument using CellQuest software.

CoA-IT activity. CoA-IT activity was measured as described previously (Winkler *et al.*, 1991) in a total volume of 100 μl. Microsomes from HL-60 cells (5–12 μg of protein) from a 100,000 $\times$ *g* 60 min centrifugation (Winkler *et al.*, 1991) were diluted in PBS with 1 mM ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid to

the desired protein concentration. The reaction was initiated by the addition of 1-[^{3}H]alkyl-2-lyso-GPC (0.1 μCi/tube) and 1 μM final cold 1-alkyl-2-lyso-GPC in assay buffer with 0.25 mg/ml of fatty acid-poor BSA. The reaction was allowed to proceed for 10 min at 37°C. The reaction was stopped, the lipids were extracted (Bligh and Dyer, 1959) and materials from an aliquot of the chloroform extract were separated by TLC in chloroform-methanol-acetic acid-water (50:25: 8:4, v/v) and were visualized by radioscanning (Bioscan, Washington, D.C.). The product, 1-[^{3}H]alkyl-2-acyl-GPC, was then scraped and quantified by liquid scintillation spectroscopy.

The effects of ET-18-O-CH_3 on the kinetics of the CoA-IT reaction were also examined. Various concentrations of ET-18-O-CH_3 (0–15 μM) were mixed with various concentrations of substrate 1-alkyl-2-lyso-GPC (0.01–3 μM). Microsomes from U937 cells containing CoA-IT activity were added and the reaction was allowed to proceed for 3 min at 37°C. The amount of product formed was determined as described above and was not more than 5% of added substrate at any concentration. Analysis of the results was performed using the method of Cleland (1979). It is important to note that these mechanistic studies with ET-18-O-CH_3 were performed by using CoA-IT activity from microsomes of U937 cells, whereas the proliferation studies were done in HL-60 cells. ET-18-O-CH_3 is presumed to block CoA-IT activity by the same mechanism in the two different cell lines.

NF-κB electrophoretic mobility shift assay. Cells were treated with compounds as described previously and extracts of nuclear proteins were prepared as follows: cells were lysed (10 mM HEPES, pH 7.9; 1.5 mM $MgCl_2$; 10 mM KCl; 0.5 mM dithiothreitol; and 0.1% nonidet P40, on ice for 10 min), vortexed and microfuged for 10 min. The nuclear pellet was resuspended (20 mM HEPES, pH 7.9, 23% glycerol, 1.5 mM $MgCl_2$, 0.42 M NaCl, 0.2 mM EDTA, 0.5 mM dithiothreitol and 0.5 mM Phenylmethylsulfonyl fluoride), incubated at 4°C for 15 min and microfuged for 10 min. The supernatant fluid was collected and used for the assays. The electrophoretic mobility shift assay was performed using an oligonucleotide probe containing the NF-κB consensus sequence (5′-AGTTGAGGGGACTTTC-CCAGGC-3′) (Santa Cruz Biotechnology, Santa Cruz, CA). The DNA binding reactions were performed at 27°C for 30 min in a volume of 25 μl, which contained 10 μg of nuclear protein, 0.5 ng of ^{32}P-radiolabeled probe (2.5–10 × 10^4 cpm, T4 Kinase method, GIBCO, Grand Island, NY) and 5 μl of binding buffer (final concentrations: 10% glycerol, 10 mM HEPES-NaOH, pH 7.9), 4 mM Tris · Cl, pH 7.9, 60 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 5 μg of poly (dI-dC) · poly(dI-dC) and 300 μg/ml of BSA). After the binding reaction, the samples were subjected to electrophoresis in buffer (100 mM Tris, pH 8.0, 90 mM borate and 1 mM EDTA) using 4% native polyacrylamide gels. The results were visualized by auto radiographic analysis, with films exposed for 1 to 3 days.

Protein analysis. Protein concentrations were determined using the method of Bradford (1976), with reagents purchased from Bio-Rad (Hercules, CA).

Statistical analysis. Differences between treatments were analyzed by two-tailed Student's *t* test (figs. 3 and 4) or by analysis of variance with Scheffe *F* test (figs. 7 and 8), with statistical significance taken to be $P < .05$.

Results

Effect of ET-18-O-CH_3 on CoA-IT activity. ET-18-O-CH_3 is structurally related to the 1-ether-2-lyso-phospholipids used as arachidonate acceptors by the enzyme CoA-IT. Because ET-18-OCH_3 contains a nonhydrolyzable *O*-methyl group at the *sn-2* position, we postulated that this compound would act as a "dead end" inhibitor of CoA-IT activity. To test this hypothesis, the effects of ET-18-O-CH_3 on CoA-IT activity were determined in HL-60 microsomes by measuring the acylation of 1-[^{3}H]alkyl-2-lyso-GPC to form the product,

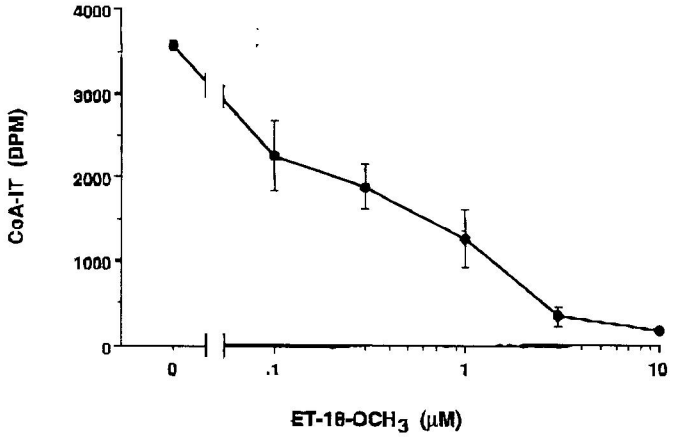


Fig. 1. Effect of ET-18-O-CH_3 on CoA-IT activity. Microsomes from HL-60 cells were incubated with the indicated concentrations of ET-18-O-CH_3 for 10 min. CoA-IT activity was then measured as described under "Materials and Methods." These data are the means ± S.D. of triplicate determinations and are representative of three experiments.

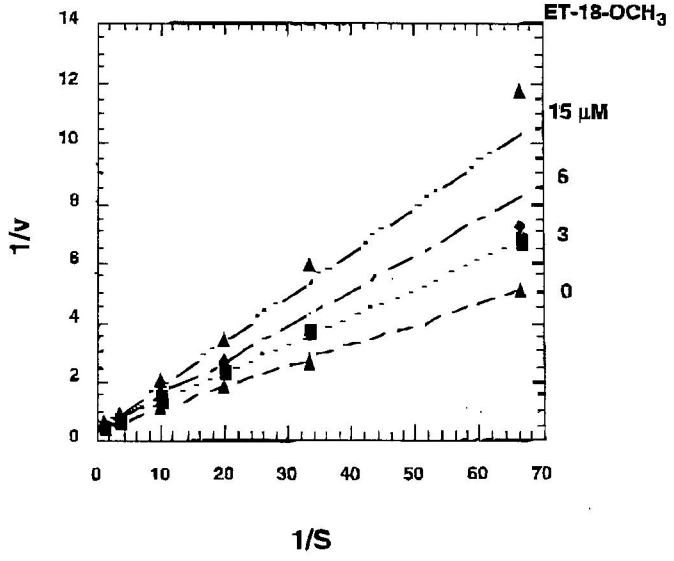


Fig. 2. Kinetics of ET-18-O-CH_3 Inhibition of CoA-IT activity. Microsomes from U937 cells were incubated with the indicated concentrations of ET-18-O-CH_3 and 1-alkyl-2-lyso-GPC as described under "Materials and Methods." The quantities of product produced in the reactions were determined. A double-reciprocal plot of 1/velocity (1/V) *vs.* 1/[substrate] (1/S) is shown.

[^{3}H]1-alkyl-2-arachidonoyl-GPC. The results (fig. 1) showed that ET-18-O-CH_3 was a potent inhibitor of CoA-IT activity with an IC_{50} of 0.5 μM and complete inhibition was observed at 3 μM. To explore further the interaction of ET-18-O-CH_3 with CoA-IT, kinetic analysis experiments were performed using CoA-IT enzyme in U937 cell microsomes. These data (fig. 2) revealed that ET-18-O-CH_3 inhibited CoA-IT activity in a manner that was best fit by a model of competitive inhibition. The apparent K_m of the substrate was 0.19 ± 0.01 μM and the K_{is} of ET-18-O-CH_3 was 1.9 ± 0.9 μM. These results show that ET-18-O-CH_3 is interacting with CoA-IT in a specific and predictable manner and that the inhibition of CoA-IT was not caused by a nonspecific effect, such as a detergent effect on the microsomal membranes.

Effect of ET-18-O-CH_3 and other CoA-IT inhibitors on cell proliferation. As described in the Introductory sec-

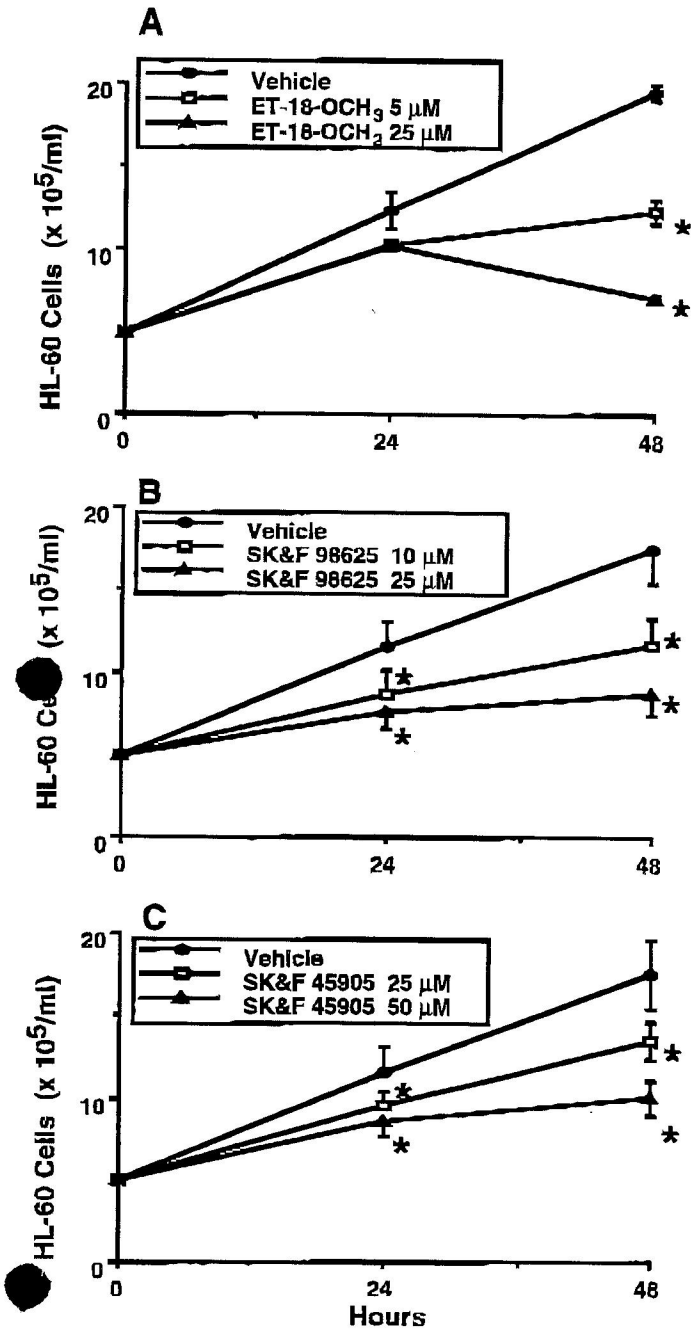


Fig. 3. Effect of ET-18-O-CH_3, SK&F 45905 and SK&F 98625 on HL-60 cell proliferation. HL-60 cells were treated at time 0 with the indicated concentrations of compounds and the number of viable cells was determined by trypan blue exclusion after 24 and 48 hr. These results are the means ± S.D. of triplicate determinations and are representative of three experiments. *P < .05 compared to vehicle.

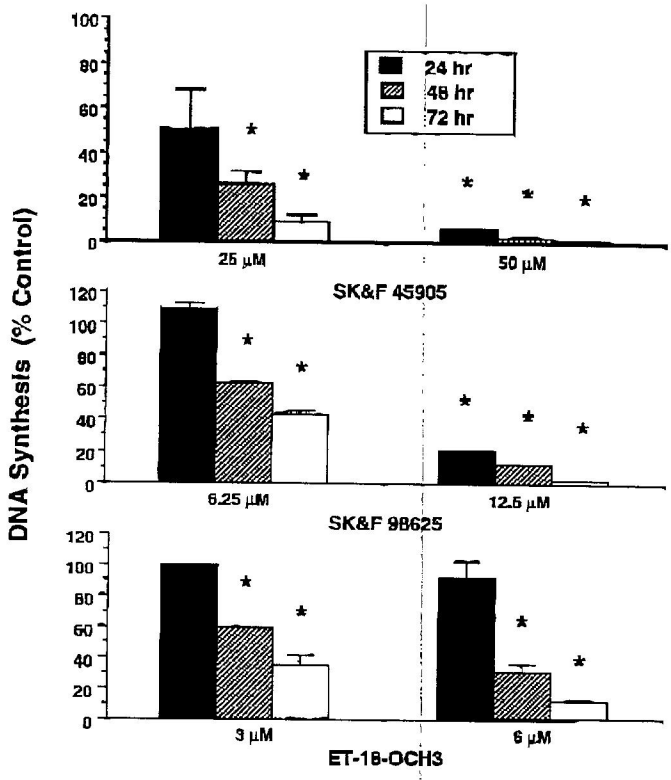


Fig. 4. Effect of ET-18-O-CH_3, SK&F 45905 and SK&F 98625 on DNA synthesis in HL-60 cells. HL-60 cells were treated with various concentrations of compounds or vehicle. Twenty-four hours before harvesting the cells, they were given a pulse of [^{3}H]thymidine. At the indicated time points, the cells were harvested, washed, lysed and the amount of ^{3}H incorporated into DNA was measured as described under "Materials and Methods." These results are the means ± S.D. of triplicate determinations and are representative of three experiments. *P < .05 compared to vehicle.

tion, ET-18-O-CH_3 has been shown to block the proliferation of cancer cells. The findings that ET-18-O-CH_3 is a potent inhibitor of CoA-IT raised the question of whether other structurally different CoA-IT inhibitors would influence proliferation in a manner comparable to ET-18-O-CH_3. We have developed recently two structurally distinct classes of CoA-IT inhibitors, SK&F 98625 and SK&F 45905 (Chilton *et al.*, 1995). Figure 3 illustrates the effects of two concentrations of ET-18-O-CH_3, SK&F 98625 and SK&F 45905, on the proliferation of HL-60 cells at 24 and 48 hr. These experiments were done with cells in culture in the presence of 10% fetal bovine serum. Because of critical differences between the *in vitro* CoA-IT assay and the cellular assays, in terms of protein binding of compounds and cell penetration, higher concentrations of compounds were needed to produce cellular effects, and direct comparison between the potency of compounds under these two conditions is problematic. Consistent with previous studies, ET-18-O-CH_3 reduced the number of HL-60 cells in culture in a dose- and time-dependent manner (fig. 3A). In addition, two other structurally different CoA-IT inhibitors, SK&F 98625 and SK&F 45905, blocked the increases in cell numbers in a comparable fashion to ET-18-O-CH_3 (figs. 3, B and C). All three inhibitors caused a delayed loss of cell viability that was observed between 24 and 48 hr (data not shown). K562 cells are reported to be much less sensitive to alkyl-lyso-phospholipids (Tidwell *et al.*, 1981). Similar to HL-60 cells, ET-18-O-CH_3 caused a concentration-dependent inhibition of K562 cell proliferation, with decreases of 43, 71 and 96% seen at 5, 10 and 20 μM, respectively. However, in contrast to HL-60 cells, K562 cells did not die in response to ET-18-OCH_3, even at high concentrations (96 μM).

To confirm the antiproliferative effects of the CoA-IT inhibitors, the synthesis of new DNA was monitored by [^{3}H]thymidine incorporation. The results depicted in figure 4 show that all three inhibitors of CoA-IT, ET-18-O-CH_3, SK&F 98625 and SK&F 45905, caused a time- and concen-

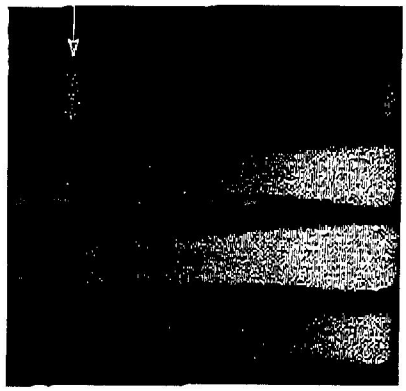


Fig. 5. Effect of ET-18-O-CH_3, SK&F 45905 and SK&F 98625 on DNA fragmentation in HL-60 cells. HL-60 cells were treated with the indicated concentration of compounds and DNA fragmentation was determined after 24 hr as described under "Materials and Methods." Control is vehicle treatment; →, internal standard fragment.

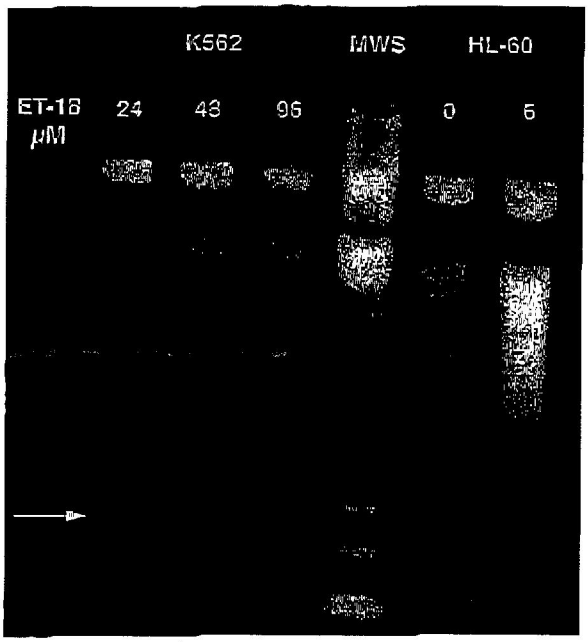


Fig. 6. Effect of ET-18-OCH_3 on DNA fragmentation in HL-60 and K562 cells. HL-60 and K562 cells were treated with the indicated concentration of ET-18-OCH_3 and DNA fragmentation was determined after 24 hr as described under "Materials and Methods." Control is vehicle treatment; →, internal standard fragment.

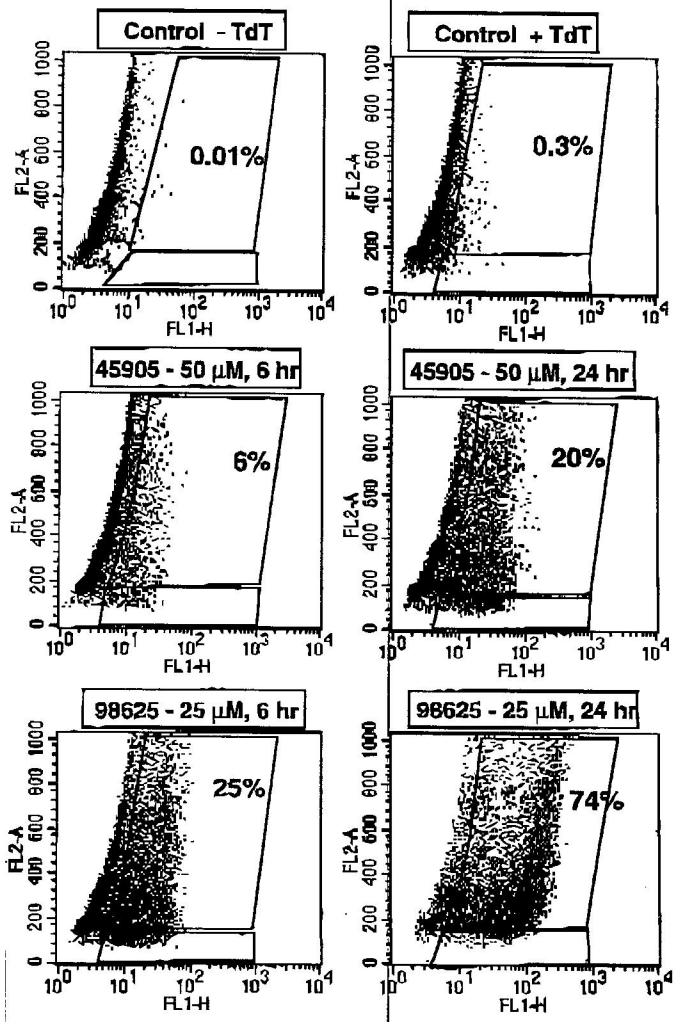


Fig. 7. Analysis of SK&F 45905 (45905) and SK&F 98625 (98625) DNA fragmentation by flow cytometry. HL-60 cells were treated with vehicle, 50 μM 45905 or 25 μM 98625 for 24 hr. DNA fragmentation was measured by flow cytometry as described under "Materials and Methods." The control cells were treated with and without terminal deoxynucleotidyl transferase (TdT) to assess nonspecific fluorescence. Areas were set on the control cells to exclude > 99% of the cells from the apoptotic windows, and then the drug-treated cells were analyzed. The number shown is the percentage of cells falling into the apoptotic window. The results are representative from three experiments. The means ± S.E. were 1.1 + 0.69, 4.2 + 0.88, 12.8 + 3.1*, 23.4 + 7.9* and 54.9 + 16.5* for Control: 459056, 24 hr; and 98625, 6 hr and 24 hr, respectively. *P < .05 compared to vehicle. FL, fluorescent.

tration-dependent reduction in DNA synthesis. Together, these data reveal that, in addition to sharing the capacity to block the enzyme CoA-IT, ET-18-O-CH_3, SK&F 98625 and SK&F 45905 all attenuate the growth and viability of HL-60 cells.

Effect of ET-18-O-CH_3 and other CoA-IT inhibitors on cellular apoptosis. A major mechanism that has been demonstrated to reduce cell numbers is the induction of programmed cell death or apoptosis (Bergamaschi *et al.*, 1994). The induction of apoptosis has been proposed to be responsible for much of the inhibition in HL-60 cell numbers observed after treatment with ET-18-O-CH_3 (Mollinedo *et al.*, 1993). The next group of experiments were performed to determine if other CoA-IT inhibitors would also instigate apoptosis. Apoptosis was determined in these studies by examining internucleosomal DNA fragmentation patterns before and after treatment with CoA-IT inhibitors. HL-60 cells treated with ET-18-O-CH_3 (6 μM), SK&F 98625 (50 μM) and SK&F 45905 (50 μM) for 24 hr all showed a pattern of DNA "ladder" fragmentation (fig. 5). DNA ladder formation in HL-60 cells depended on the dose of the CoA-IT inhibitor, occurred within 3 hr and was maximal 24 hr after treatment with CoA-IT inhibitors. In contrast to HL-60 cells, DNA fragmentation from the leukemia cell line K562 was not affected by ET-18-O-CH_3 treatment, even at concentrations as high as 96 μM for 24 hr (fig. 6). Again, K562 cells did not die in response to ET-18-OCH_3, confirming the selectivity of ET-18-OCH_3 for sensitive cells.

Another method to measure apoptosis is the use of flow cytometry to quantitate DNA fragmentation in individual

TABLE 1

Ability of compounds to induce apoptosis in HL-60 cells

For *in vitro* assays, CoA-IT activity has performed as described under "Materials and Methods" and remaining data are from References: Zileuton (Carter *et al.*, 1991); Indomethacin (Shen, 1985); SB 203347 (Marshall *et al.*, 1995); AA-CF_3-ketone (Street *et al.*, 1993); and CoA-IT inhibitors (Haidar *et al.*, 1994; Winkler *et al.*, 1995). For cellular assays, HL-60 cells were treated with the indicated concentration of compound for 24 hr and DNA fragmentation was measured as described under "Materials and Methods." Higher concentrations of compounds were used for the cellular assays because of the presence of serum proteins.

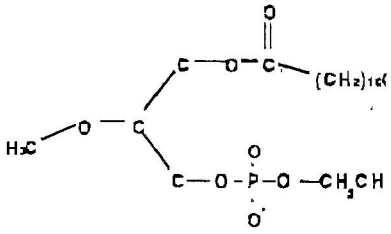
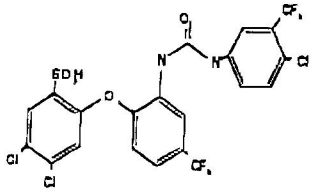
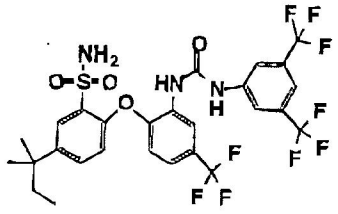
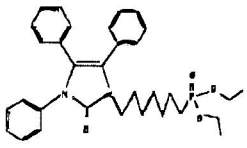
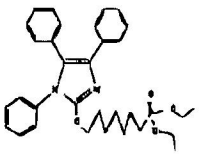
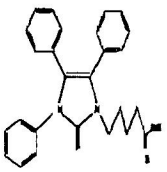
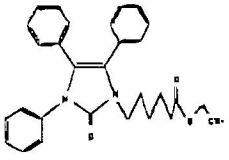
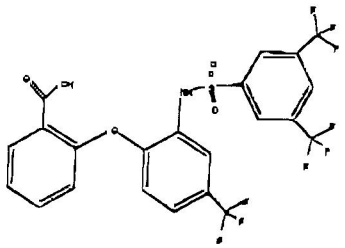
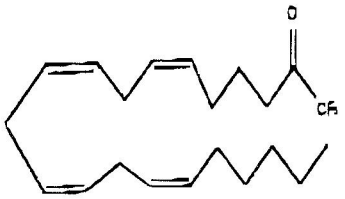
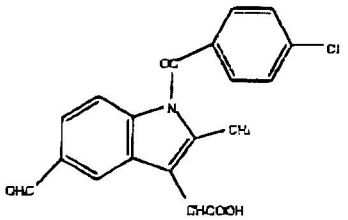
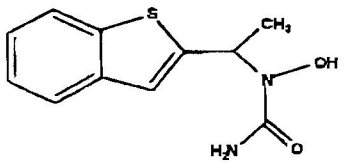
Compound	Structure	*In vitro* IC_{50} for CoA-IT:	*In vitro* IC_{50} of:	Cellular Study Conc.	Induction of Apoptosis
				μM	
ET-18-O-CH3		0.5 μM		6	Yes
SK&F 45905		6 μM		50	Yes
SB 202718		Inactive at 50 μM		50	No
SK&F 98625		9 μM		50	Yes
SK&F 98628		12 μM		50	Yes
SK&F 98414		Inactive at 50 μM		50	No
SK&F 98416		Inactive at 50 μM		50	No

TABLE 1
Continued

Compound	Structure	*In vitro* IC_{50} for CoA-IT:	*In vitro* IC_{50} of:	Cellular Study Conc.	Induction of Apoptosis
SB 203347		Weakly active (23 μM)	14 kDa PLA_2: 0.5 μM	50	No
AA-CF_3-ketone		Inactive at 50 μM	85 kDa PLA_2: 1 μM	10	No
Indomethacin		Inactive at 10 μM	COX: 0.5 μM	1	No
Zileuton		Inactive at 50 μM	5-LO: 2 μM	10	No

cells, measuring the incorporation of detectable nucleotides into the fragments with fluorescein-labeled antibody. Such analysis confirms that both SK&F 45905 and SK&F 98625 induce DNA fragmentation in a time-dependent fashion and that SK&F 98625 is more efficacious in this regard, producing a higher percentage of cells with increased DNA fragmentation than SK&F 45905 (fig. 7). The reason that SK&F 98625 is more efficacious than SK&F 45905 is not known. In the *in vitro* assay against CoA-IT, they have similar potency. Because the compounds have different structures, it is difficult to compare an *in vitro* effect to one that occurs inside the cell, due to differences in the ability of compounds to partition into lipids from the aqueous phase and to enter intracellular compartments.

Effect of other inhibitors of lipid metabolism on apoptosis. Previous studies in our laboratory have demonstrated that treatment of neutrophils with CoA-IT inhibitors can indirectly block the mobilization of AA and formation of eicosanoids (Winkler *et al.*, 1995). The next group of experiments in this study examined whether other more direct inhibitors of AA metabolism would induce apoptosis in HL-60 cells. Inhibitors of two phospholipase A_2 isotypes as well as 5-LO and cyclooxygenase were examined and apoptosis was determined by the presence of DNA "ladders." The results of these experiments are shown in table 1. SB 203347 is an interesting compound, as it inhibits 14 kDa PLA_2 at 0.5 μM and has effects on CoA-IT only at much higher concentrations (23 μM). Because of a rightward shift in the concentration-response curve due to increased protein concentration, this compound does not inhibit CoA-IT activity in intact cells (Marshall *et al.*, 1995). If the compound was used at concentrations high enough to block CoA-IT, it may then induce apoptosis. However, due to limitations of compound solubility and exposure to DMSO, higher concentrations could not be used.

Three important findings derived from these experiments are: 1) apoptosis is not caused by inhibitors of 5-LO, cyclooxygenase, 14 kDa PLA_2 or 85 kDa PLA_2; 2) CoA-IT inhibitors with widely divergent structures (ET-18-O-CH_3, SK&F 45905, SK&F 98625 and SK&F 98628) induce apoptosis; and 3) close structural analogs of CoA-IT inhibitors (SK&F 202718, SK&F 98414 and SK&F 98416), which lack the ability to inhibit CoA-IT, also lack the capacity to cause apoptosis.

NF-κB activation by CoA-IT inhibitors. Recent investigations have implied that NF-κB activation can have a role in mediating the effects of TNF-induced apoptosis (Machleidt *et al.*, 1994; Singh and Aggarwal, 1995; Majno and Joris, 1995). To determine if CoA-IT inhibitors can utilize this pathway, the capacity of ET-18-O-CH_3 and TNF to activate NF-κB was examined by an electrophoretic mobility shift assay. Nuclear extracts from TNF-stimulated HL-60 and K562 cells showed the presence of proteins that recognize and bind to the consensus sequence for NF-κB proteins (fig. 8A). This process was blocked completely by the addition of excess unlabeled competitors. In contrast, ET-18-O-CH_3 failed to activate NF-κB proteins in HL-60 or K562 cells (fig. 8B). These data suggest that the mechanisms which lead to apoptosis may be different for CoA-IT inhibitors and TNF.

Discussion

There is growing evidence that treatment of animals with ET-18-O-CH_3 and other alkyl-lyso-phospholipids can reduce the size of tumors (Berger and Schmahl, 1987; Scherf *et al.*, 1987; Hilgard *et al.*, 1988). In addition, ET-18-O-CH_3 has shown some efficacy in the treatment of patients suffering from leukemias and carcinomas (Berdel *et al.*, 1982; Andreesen and Munder, 1983; Andreesen, 1988). At the cellular level, ET-18-O-CH_3 has been shown to have selective toxic effects against transformed cells, like HL-60 cells, but not other "normal" cell such as neutrophils, fibroblasts and K562 cells (Tidwell *et al.*, 1981; Hoffman *et al.*, 1984; Chabot *et al.*, 1989; Mollinedo *et al.*, 1993; Diomede *et al.*, 1994). It has been proposed that ET-18-O-CH_3 influences two processes to block increases in cell numbers; the first is to attenuate the rate of cell proliferation and the second is to induce cellular apoptosis. As outlined in the Introductory section, ET-18-O-CH_3 provided to cells *in vitro* is known to inhibit several signal transduction pathways. However, because the signal transduction pathways that control cell proliferation and apoptosis are not well understood, the mode of action of ET-18-O-CH_3 is equivocal.

In the last 10 years, several laboratories have characterized an enzyme, CoA-IT, that moves arachidonate in a CoA-independent manner from specific arachidonate-containing phospholipids to specific lyso-phospholipid acceptors within inflammatory cells. Based on the structural similarities between ET-18-O-CH_3 and the "natural" lyso-phospholipid acceptors for CoA-IT, the capacity of this compound to block CoA-IT activity was tested in cell microsomes. ET-18-O-CH_3 was a potent inhibitor of CoA-IT with an IC_{50} of 500 nM. Our laboratory has reported recently two distinct structural classes of CoA-IT inhibitors; these classes are typified by SK&F 98625 and SK&F 45905 and have an IC_{50} in a microsomal assay of 9 and 6 μM, respectively (Chilton *et al.*, 1995). ET-18-O-CH_3 is therefore a more potent inhibitor of CoA-IT than the above-mentioned compounds and represents a third structurally distinct class of CoA-IT inhibitor.

The finding of ET-18-O-CH_3 as a CoA-IT inhibitor raised the interesting question of whether blockage of CoA-IT represented a major step that affects the growth of HL-60 cells. To test this hypothesis, the three structurally distinct inhibitors of CoA-IT were provided to HL-60 cells and cell growth as well as DNA synthesis were measured. It was reasoned

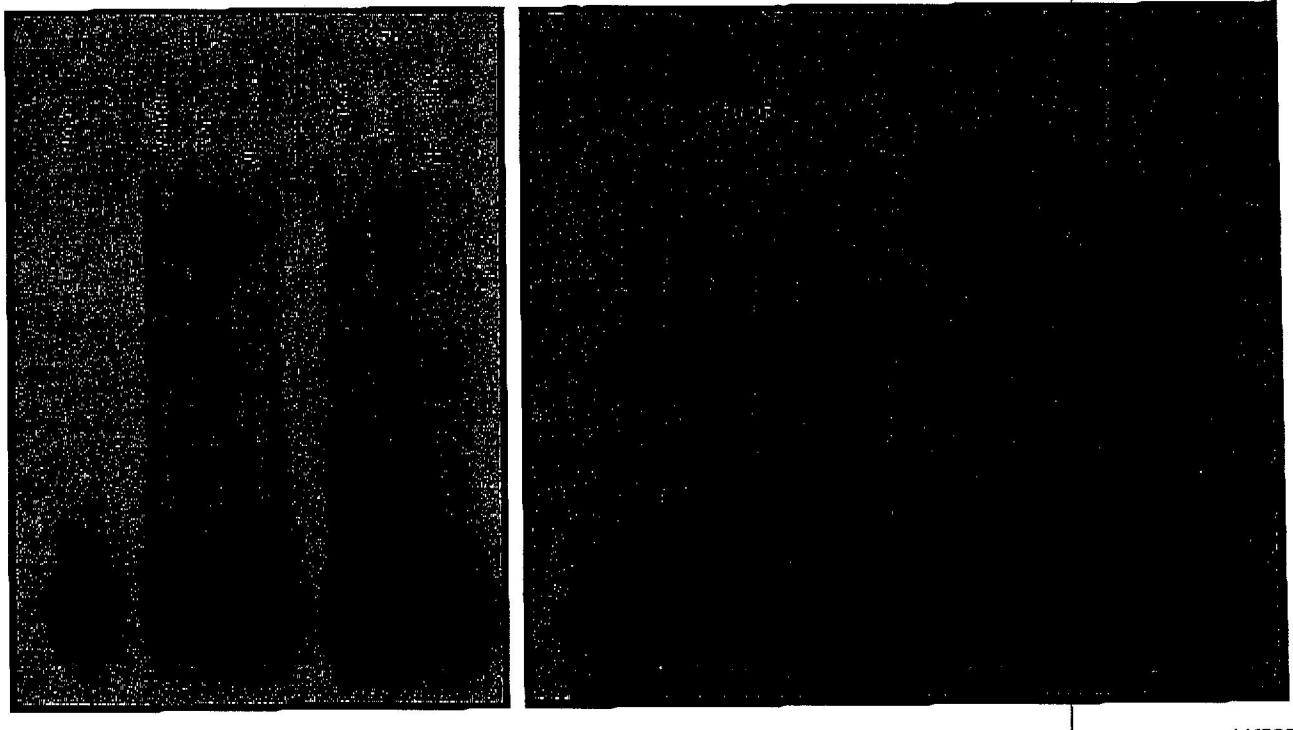


Fig. 8. Effect of ET-18-OCH_3 and TNF on activation of NF-κB in HL-60 and K562 cells. Extracts of nuclear proteins from HL-60 and K562 cells were examined in a electrophoretic mobility shift assay, as detailed under "Materials and Methods," in which specific binding of protein to the sequence is indicated by the retardation of the movement of the radioactive material through the gel. A, lane 1 is probe alone. HL-60 cells and K562 cells were vehicle- or TNF-treated (100 U/8 min) and extracts run in the absence or presence (+cold) of excess unlabeled probe (200 ×). B, the time (minutes) after treatment with ET-18-O-CH_3 (6 μM) is indicated. P, probe alone with no cell protein; +, in the presence of excess unlabeled probe (200×) added to determine selectivity. The results are representative of two experiments.

that, although each of these inhibitors are not total selective for CoA-IT (each have distinct profiles for the inhibition of other enzymes), they share inhibition of CoA-IT as their unifying feature. All three inhibitors of CoA-IT blocked HL-60 cell numbers and new DNA synthesis. These data suggest that some cellular event that is regulated by CoA-IT activity in HL-60 cells is critical to controlling cell numbers.

Recently, ET-18-O-CH_3 has been revealed to induce apoptosis of HL-60 cells (Mollinedo *et al.*, 1993). If inhibition of CoA-IT by ET-18-O-CH_3 has a role in apoptosis, then other CoA-IT inhibitors would be predicted to also instigate apoptosis. The current study showed that both SK&F 98625 and SK&F 45905 also caused apoptosis of HL-60 cells, as determined by DNA ladder formation and by flow cytometry analysis. To assure that something about the chemical structures of SK&F 98625 and SK&F 45905 other than their capacity to inhibit CoA-IT was not causing apoptosis, close structural analogs (not CoA-IT inhibitors) were tested. These latter compounds did not induce apoptosis, suggesting that one of the key processes regulated by CoA-IT is cellular apoptosis.

ET-18-O-CH_3 appears to have two separable effects on cell proliferation and induction of apoptosis. HL-60 cells treated with ET-18-O-CH_3 appear to undergo both processes, whereas in K562 cells only proliferation is blocked by ET-18-O-CH_3. A recent paper by Boggs *et al.* (1995a) lends support to this notion. They showed that ET-18-O-CH_3 could block both proliferation and cause cell death in a mouse macrophage cell line. However, addition of exogenous lyso-phosphatidylcholine attenuated cell death, but had no effect on inhibition of cell proliferation, suggesting that two mechanism may be involved. Additionally, the relative resistance of K562 cells to undergo apoptosis has also been seen after treatment with the anticancer agent etoposide (Ritke *et al.*, 1994).

There is strong evidence for a relationship between levels of free AA and proliferation in some cell systems. For example, tumor-promoting phorbol esters stimulate the release of AA from cellular phospholipids, leading to increased eicosanoid synthesis (Daniel *et al.*, 1987). In addition, there is a striking increase in eicosanoid concentrations found in human mammary tumors, compared with control tissue or benign tumors (Nigam *et al.*, 1985; Vergote *et al.*, 1985), and AA addition to the media is required for the growth of some mastocytoma cell lines (Butcher *et al.*, 1993). Finally, blockage of eicosanoid production with enzyme inhibitors of cyclooxygenase and/or 5-LO has been shown to stop proliferation of gastric tumor cells, mammary tumor cell lines and HL-60 leukemia cells (Lee and Ip, 1992; Najid *et al.*, 1992; Denizot *et al.*, 1993). Very recent studies in our laboratory suggest that CoA-IT inhibitors can indirectly block AA release by preventing arachidonate "loading" into critical phospholipid pools (Winkler *et al.*, 1995). Therefore, it was possible that CoA-IT inhibitors were inducing apoptosis by blocking AA release and subsequent eicosanoid generation. To address this question, selective inhibitors of AA release or metabolism were provided to HL-60 cells. Inhibitors of AA metabolism did not cause apoptosis of HL-60 cells, suggesting that levels of free AA acid or eicosanoids are not controlling this event.

It may be that the arachidonate remodeling itself is influencing apoptosis. It has long been recognized that many "cancer" cells contain high levels of 1-ether-linked phospholipids (Snyder and Wood, 1968, 1969). Moreover, Chabot and colleagues (1989) have shown that susceptibility to ET-18-O-CH_3 correlates with the ether lipid content of leukemia cell lines. Extending these studies to human leukemic patients, they showed that there is a clinical correlation between response to treatment and the size of 1-alkyl-linked phospholipid pools in leukemia cells (Chabot *et al.*, 1990). It may be that blocking CoA-IT prevents the loading of AA into 1-ether-linked phospholipid pools. Evidence to support this comes from experiments showing that SK&F 45905 and SK&F 98625 block the movement of arachidonate into 1-alkyl- and 1-alkenyl-containing phospholipids (Chilton *et al.*, 1995). In addition, there is some data to suggest that ET-18-O-CH_3 can reduce the movement of arachidonate into specific phospholipid pools (Lu and Arthur, 1992b). Because these pools are larger in cancer cells, CoA-IT inhibitors may influence more dramatically cancer cell growth.

A major question that remains is: How does blocking CoA-IT and perhaps AA remodeling translate into the induction of apoptosis? Several studies have shown that NF-κB activation can be achieved by numerous agents, such as TNF in HL-60 and U937 cells (Machleidt *et al.*, 1994). The current study confirms that TNF activates NF-κB. By contrast, ET-18-O-CH_3 does not affect NF-κB activation. Although the exact role of NF-κB activation in apoptosis has not been determined, one study found a lack of correlation between NF-κB activation and cell proliferation (Chaturvedi *et al.*, 1994), and the current studies suggest that ET-18-O-CH_3 may influence apoptosis by an NF-κB-independent mechanism.

In summary, ET-18-O-CH_3 has long been recognized to block cell growth of cancer cells by an unknown mechanism. We have found ET-18-O-CH_3 to be a potent inhibitor of the enzyme CoA-IT. Two other structurally distinct CoA-IT inhibitors also attenuated cell growth, new DNA synthesis and induced apoptosis, whereas other inhibitors of AA metabolism had no effect on apoptosis. Therefore, the remodeling of AA orchestrated by CoA-IT may be a crucial event that regulates cancer cell growth and particularly apoptosis and represents a new therapeutic target in proliferative disorders.

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Title

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Patent Assignee

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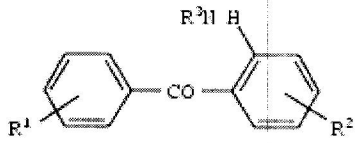
Abstract

Antitumor formulations contg. I (R1 and R2 = H, alkoxy, alkyl, or halogen; R3 = H, alkyl, or acyl) have relatively less side effects than com. available antitumor drugs like purine and pyrimidine derivs. Thus, 2-amino-5-chlorobenzophenone [719-59-5] 100, KH_2PO_4 58.5, cryst. cellulose 50, corn starch 40, and Ca stearate 1.5 parts were mixed and made into tablets (250 mg/tablet). The dosage for cancer patients ranges between 10 mg and 3.0 g/day. Twenty-eight I were synthesized by reaction of benzoyl chloride derivs. with aniline derivs and the antitumor activities in mice were demonstrated.

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(74) Representative:

(54) REMEDY FOR CANCER

(57) Abstract:

PURPOSE: To provide the titled remedy containing a benzophenone derivative as an active component, and capable of eliminating the proliferation activity of cancer cell or eliminating the cancer cell itself by acting exclusively to cancer cells.

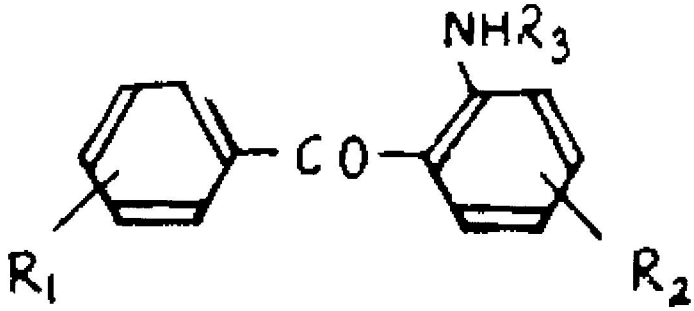
CONSTITUTION: The titled remedy contains the compound of formula (R1 and R2 are H, lower alkoxy, lower alkyl, or halogen; R3 is H, lower alkyl, or acyl) as an active component. Since the anticarcinomatous activity of an alkylation agent such as nitrogen mustard or an antimetabolite such as pyrine antagonist is non- selective, the agent attacks not only the cancer cells but also normal cells, and accordingly has high toxicity. On the

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contrary, the above benzophenone derivative attacks only the cancer cells, and eliminates its proliferation activity or eliminates the cancer cell itself. The activity of the benzophenone derivative is supposed as the redifferentiation of cancer cells produced by the abnormal differentiation of normal cells, i.e. elimination of cancer cells. It is administered orally or parenterally at a dose of usually 10mgW3g daily.

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（全 6 頁）

⑤4脱癌剤

⑳特　　願　昭56—70226
㉒出　　願　昭56(1981) 5 月12日
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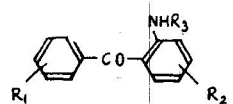
明　細　書

1. 発明の名称

脱　癌　剤

2. 特許請求の範囲

一般式

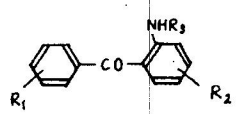


（式中 R_1 及び R_2 は同一又は異って、水素原子，低級アルコキシ基，低級アルキル基又はハロゲン原子を示し、R_3 は水素原子，低級アルキル基又はアシル基を示す）

で表わされる化合物を有効成分とする脱癌剤。

3. 発明の詳細な説明

本発明は次の一般式

 　〔I〕

（式中 R_1 及び R_2 は同一又は異って、水素原子，低級アルコキシ基，低級アルキル基又はハロゲン原子を示し、R_3 は水素原子，低級アルキル基又はアシル基を示す）

で表わされるベンゾフェノン誘導体を有効成分とする脱癌剤の発明である。

従来、癌の化学療法剤としては、ナイトロジェンマスタード類，エチレンイミン類などのアルキル化剤、葉酸拮抗剤，プリン拮抗剤，ピリミジン拮抗剤などの代謝拮抗物質、アクチノマイシンC，D，マイトマイシンCなどの抗生物質、下垂体副腎系ホルモン及びアルカロイド等が用いられている。

これらの化合物は、DNAから蛋白合成に至るいずれかの段階を阻害することにより癌細胞の増殖を抑え、直接的な癌細胞に対する致死作用をねらいとして用いられている。しかしながら、これらの作用は非選択的である為、癌細胞への攻撃に留まらず正常細胞にも作用するので、期待される制癌効果に比べて、あまりにも毒性が強い。

本発明者らは、これらの事情に鑑み、癌細胞に特異的に作用する薬物を求めて研究したところ、

前記一般式〔I〕で表わされるベンゾフェノン誘導体が、癌細胞にのみ作用して、その増殖性を失わしめ、或は癌細胞を消失せしめるという意外な事実を見出した。

本発明はこれらの知見に基づいて完成されたものである。

このベンゾフェノン誘導体の有する作用は、必ずしも明確ではないが、正常細胞の分化異常によって発生した癌細胞に対する再分化作用、いわゆる脱癌作用であると推定される。

上式(1)で表わされる化合物の中には、公知の化合物が含まれるが、それらの記載されている先行文献には脱癌作用ないしそれを示唆する薬理作用は全く記載されていない。

上式(1)(式中R_3=H又はアルキル基)で表わされる本発明の化合物はJ. O. E. Simpsonら〔J. Chem. Soc. 646(1945)〕の方法を応用して合成した。一方上式(1)においてR_3=アシルで表わされる化合物は、常法により式(1)においてR_3=Hの化合物をアシル化して容易に得ることができる。

参考例1.

O-フルオロ安息香酸クロライド11.3gを100℃に加熱し、P-クロロアニリン4.0gを撹拌下徐々に加える次いで外温を190℃にし、塩化亜鉛4.0gを加える。温度を徐々に上げ210℃で3時間加熱する。

次に温度を110℃に下げて、15%塩酸300mlを加え1時間還流し、塩酸をデカントして除く。本操作を3回行った後残渣を75%硫酸200mlに溶解し、1時間還流し、反応物を氷中に注ぎ析出する油状物をクロロホルムにて抽出する。クロロホルム層を希塩酸,希水酸化ナトリウム液および水で洗浄後無水硫酸ナトリウムで乾燥し、減圧下クロロホルムを留去し、残渣をメタノールより再結晶して黄色針状晶の2-アミノ-5-クロロ-2'-フルオロベンゾフェノン4.8gを得る。

収率62%　融点93～95℃

元素分析値　分子式$C_{13}H_9ClFNO$として

	C	H	N
理論値(%)	62.54	3.63	5.61
実測値(%)	62.49	3.66	5.58

参考例2.

2-アミノ-5-クロロベンゾフェノン2.3g,トリエチルアミン1.1gおよびベンゼン30mlの混合物にO-トルオイルクロライド1.6gを氷冷撹拌下徐々に加える。室温にて1時間撹拌後、反応液を希塩酸,希水酸化ナトリウム液および水で洗浄し、無水硫酸ナトリウムで乾燥し、減圧下ベンゼンを留去し、残渣をエタノールより再結晶し、淡黄色針状晶の5-クロロ-2-(O-トルオイルアミノ)ベンゾフェノン2.7gを得る。

収率77.3%　融点135～136℃

元素分析値　分子式$C_{21}H_{16}ClNO_2$として

	C	H	N
理論値(%)	72.10	4.61	4.00
実測値(%)	72.14	4.73	4.11

参考例3.

2-アミノ-5-クロロベンゾフェノン2.3g,シクロヘキシルイソシアネート1.3gおよびベンゼン30mlの混合物に室温にて撹拌下トリエチルアミン0.2mlを加える。同温度で6時間撹拌後、析出する結晶を戸取し、水洗しエタノールより再結晶して無色針状晶の5-クロロ-2-シクロヘキシルウレイドベンゾフェノン2.1gを得る。

収率59%　融点199～200℃

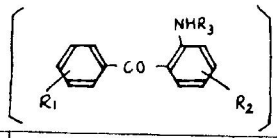
元素分析値　分子式$C_{20}H_{21}ClN_2O_2$として

	C	H	N
理論値(%)	67.32	5.93	7.85
実測値(%)	67.18	6.01	7.79

参考例4～28

上記参考例と同様にして表1の化合物を得た。

第 1 表



参考例	置換基及び置換位置			分子式	融点 (℃)	収率 (%)	元素分析値 (%)		
	R_1	R_2	R_3						
							理論値 C	H	N
							実測値		
4	H	5-Cl	H	$C_{13}H_{16}ClNO$	98～99	76	67.40 67.28	4.35 4.41	6.05 6.12
5	H	H	H	$C_{13}H_{11}NO$	105～106	68	79.16 79.07	5.62 5.65	7.10 7.17
6	H	5-CH_3	H	$C_{14}H_{13}NO$	61～62	63	79.59 79.47	6.20 6.23	6.63 6.59
7	H	5-Cl	-CH_3	$C_{14}H_{12}ClNO$	94～95	73	68.44 68.24	4.93 4.85	5.70 5.69
8	2-Cl	5-Cl	H	$C_{13}H_9Cl_2NO$	88～89	74	58.67 58.54	3.41 3.27	5.26 5.30
9	4-Cl	H	H	$C_{13}H_{10}ClNO$	103～104	77	67.40 67.31	4.35 4.40	6.05 5.97
10	H	5-Br	H	$C_{13}H_{10}BrNO$	114～115	74	56.54 56.48	3.65 3.67	5.07 5.14
11	4-Cl	5-Cl	H	$C_{13}H_9Cl_2NO$	117～118	70	58.67 56.44	3.41 3.52	5.26 5.17
12	4-Cl	5-Br	H	$C_{13}H_9BrClNO$	106～107	68	50.27 50.14	2.92 2.83	4.51 4.36
13	H	5-F	H	$C_{13}H_{10}FNO$	117～118	55	72.55 72.38	4.68 4.70	6.51 6.45
14	3-Cl	5-Cl	H	$C_{13}H_9Cl_2NO$	112～113	67	58.67 58.54	3.41 3.46	5.26 5.22
15	2,4-$DiCl_2$	5-Cl	H	$C_{13}H_8Cl_3NO$	63～64	61	51.95 52.03	2.68 2.71	4.66 4.58
16	2-CH_3	5-Cl	H	$C_{14}H_{12}ClNO$	52～53	45	68.44 68.51	4.93 4.88	5.70 5.77
17	4-Bu(t)	5-Cl	H	$C_{17}H_{18}ClNO$	50～51	55	70.95 70.78	6.30 6.18	4.87 4.85
18	4-CH_3	5-Cl	H	$C_{14}H_{12}ClNO$	87～88	62	68.44 68.41	4.93 5.02	5.70 5.66
19	2-Cl	3,5-$DiCl_2$	H	$C_{13}H_8Cl_3NO$	102～103	53	51.95 51.88	2.68 2.70	4.66 4.55
20	2-Cl	4,5-$DiCl_2$	H	$C_{13}H_8Cl_3NO$	58～59	57	51.95 51.79	2.68 2.73	4.66 4.75

21	H	5-OCH_3		$C_{21}H_{17}NO$	101～102	82	84.25 84.17	5.72 5.84	4.68 4.59
22	H	5-Cl		$C_{21}H_{16}ClNO_2$	137～138	87	72.10 72.13	4.61 4.58	4.00 3.97
23	H	5-Cl	C_2H_5CO-	$C_{16}H_{14}ClNO_2$	48～49	85	66.79 66.65	4.90 4.78	4.87 4.75
24	H	5-Cl		$C_{20}H_{14}ClNO_2$	107～108	82	71.54 71.37	4.20 4.16	4.17 4.21
25	H	5-Cl	CH_3CO-	$C_{15}H_{12}ClNO_2$	114～115	84	65.82 65.77	4.42 4.51	5.12 5.08
26	H	5-Cl	m-C_6H_7NHCO-	$C_{17}H_{17}ClN_2O_2$	96～97	77	64.46 64.39	5.41 5.47	8.84 8.77
27	H	5-Cl		$C_{21}H_{17}ClN_2O_2$	119～120	74	69.14 68.97	4.70 4.81	7.68 7.73
28	4-OCH_3	5-Cl	H	$C_{14}H_{12}ClNO_2$	100～101	71	64.25 64.11	4.62 4.73	5.35 5.32

実施例1

(1) 15%の馬血清及び2.5%の牛胎児血清を含むHam・sF-12培養液に、マウスのCloudman 891メラノーマ由来の培養株M-3細胞をけん濁し、プラスチック製培養びんに入れ、炭酸ガス培養器中（炭酸ガス5%，空気95%）、37℃で10日間培養した。増殖した細胞はびんの底面に付着しているので、上清の培養液を除き0.05%トリプシン溶液を加えて、2～3分処理した後トリプシン溶液を除き、更に培養液を加えて付着している細胞を洗いおとして細胞浮遊液とする。

(2) (1)で調製した1ml当り10^5個のM-3細胞を含む細胞浮遊液を2mlずつ直径3.5cmのプラスチック製シャーレに植え込み、炭酸ガス培養器中37℃で48時間培養した後上清の培養液を除き、予め被検化合物を添加、溶解した培養液2mlを加え、更に培養を続ける。48時間毎に同様の培地交換を行なった。また細胞増殖の状態は、倒立顕微鏡により連日観察した。

(3) 細胞増殖の測定

測定は1日おきにおこなった。培養器よりプラスチックシャーレをとり出し、上清の培養液をデカンテーションによって除いた。次に0.05%トリプシン溶液1mlを加え、駒込ピペットにより液の撹拌をおこなって充分に細胞をバラバラにしたのち、血球計算板を用いて細胞数を測定した。被検化合物の効力は次の4段階に分けて表わした。検体添加翌日から細胞数の増加が殆んどない(+++)，検体添加翌日から細胞増殖はあるが明らかに増殖速度が抑制されているもの(++)，検体添加後数日は対照との間に差はないが、その後、対照群と比べて増殖速度が明らかに低下したもの(+)，対照群と比べて増殖の抑制がいずれの時点においてもみられないもの(−)。

(4) 形態的変化

被検化合物添加後4日目に顕微鏡下で細胞を観察した。形態変化の度合を表わす指標としては、細胞からの突起の数，核と細胞の大きさの比を用い、それぞれ対照との比が2～3倍のものを(+)，

4倍以上のものを(++)として表示した。

(5) メラニン産生

培養10日目に細胞をとり出し、Whittaker の方法でメラニンを測定した。

メラニン含有量は、蛋白(mg)当りの重量として求め、次式によりメラニン産生増加率を算出した。

$$\text{メラニン産生増加率}(\%) = \left(\frac{\text{被検化合物添加群のメラニン含有量}}{\text{対照のメラニン含有量}} - 1\right) \times 100$$

なお、蛋白量はLowryらの方法に依った。

以上の測定結果を第2表に例示する。なお、表中の化合物番号は第1表の番号に対応している。

第 2 表

化合物番号	メラニン産生増加率(%)	形態変化	増殖抑制
1	249	++	+
2	43	+	+
3	76	+	+++
4	834	+++	++
5	48	++	−
6	38	++	+++
7	126	++	++
8	660	+++	+++
9	51	+	±
10	703	+++	++
11	295	+	+
12	220	+	+
13	129	++	++
14	544	+++	++
15	583	+++	++
16	321	++	++
17	447	+++	++
18	63	++	+
19	365	++	++
20	84	++	+
21	38	++	+
22	43	++	+
23	275	++	+
24	52	++	+
25	217	++	+
26	228	++	+++
27	142	++	+++
28	178	++	+

実施例2

生後5週令のCDF1雄性マウスのそけい部にCloudman S-91メラノーマ細胞を1匹当り10^6個皮下移植し、次の実験を行なった。

A) 移植後24時間目より、1日1回，19日間，0.2%カルボキシメチルセルロース水溶液に溶解又はけん濁した検体を200mg/kgずつ経口投与した。最終投与の24時間後に腫瘍組織を摘出し重量を測定した。測定結果は、各群10匹の平均値として表すと第3表に示すとおりである。

第 3 表

化合物No.	腫瘍重量(mg)（平均±標準誤差）	抑制率(%)
対照	1923±395	—
4	865±122	55.0
8	1102±193	42.7
17	1326±211	31.0
23	937±109	51.3
26	1066±204	44.6

B) 移植後13日目に触診により腫瘍の大きさを測定し、各群の腫瘍の大きさが同等になるよう公平に群分けした後1日1回，7日間検体をA)と同様に経口投与し、最終投与の24時間後に腫瘍組織を摘出し重量を測定した。測定結果は各群10匹の平均値として表すと第4表のとおりである。

第 4 表

化合物No.	腫瘍重量(mg)（平均±標準誤差）	抑制率(%)
対照	2584±602	—
4	1898±239	26.6
8	1773±386	31.4
17	1652±270	36.1
23	2114±403	18.2
26	2037±391	21.2

実施例3.

人由来神経芽細胞腫IMR-32細胞株を、実施例1，(1)移植細胞の調製，(2)細胞培養と被検化合物の添加，(3)細胞増殖の測定の項に従って実験を行なった。但し、培地については90%Ham's F-12-10%牛胎児血清（含ペニシリンGカリウム100単位／ml，ストレプトマイシン100μg力価／ml）を用いた。

被検化合物の効果は、対照の細胞密度が飽和に達した植込み10日後に判定し、飽和密度が対照の80～50%に減少したものを＋とし、その際顕微鏡観察において細胞どうしの重りがほとんど認められず、増殖の接触阻止様の像を示すものを卄とした。結果は第5表のとおりである。

第5表

化合物No.	効果
4	卄
8	卄
17	＋
23	卄
26	＋

実施例4.

人胃癌細胞HG-II株を用いて実施例3と同様にして行なった実験結果は第6表のとおりである。但し、判定は植込み後14日目に行なった。

第6表

化合物No.	効果
4	卄
8	卄
17	卄
23	卄
26	＋

実施例5.

2-アミノ-5-クロロベンソ~~セチドロール~~	100部
リン酸水素カリウム	585部
結晶セルロース	50部
コーンスターチ	40部
ステアリン酸カルシウム	1.5部

これらをよく混合し、常法により1錠250mgに打錠（有効成分100mg含有）し、脱ガン剤用錠剤として用いる。

本発明を実施するに当っては、上記製剤例の他日本薬局方に表示される通常の製剤基材を用いて所望のカプセル剤，液剤，けん濁剤として経口的に又は非経口的に、患者の症状に応じて通常1日10mg～3gの範囲で投与される。

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Title

Quantitative structure-biodegradability studies: an investigation of the MITI aromatic compound database

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Abstract

Multiple linear regression anal. of 79 arom. compds. found to be biodegradable in the MITI test did not produce satisfactory correlations. However, discriminant anal. based on 3 calcd. parameters (one from MOPAC 7 and two from MOLCONN-X) gave good discrimination between readily and non-readily biodegradable compds., with an overall correct cross-validated prediction rate of 78.6%.

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QUANTITATIVE STRUCTURE-BIODEGRADABILITY STUDIES: AN INVESTIGATION OF THE MITI AROMATIC COMPOUND DATABASE

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1. Introduction

Risk is a function of both intrinsic hazard and exposure. Knowledge of the persistence of compounds in the environment is thus of prime importance in the assessment of risk; clearly, therefore, ability to predict persistence (or lack of persistence, i.e. biodegradability) can be of great help in this respect.

Some years ago we obtained a 240 compound data-set of biodegradability data, in the form of 5-day BOD (biological oxygen demand) values (as % ultimate degradation), measured using acclimated sludge; the data were supplied by the U.S. Environmental Protection Agency Laboratory in Duluth, MN. They had not been determined there, but had been carefully screened for consistency of determination.

We broke the data down into chemical classes, and searched for quantitative structure-biodegradability relationships (QSBRs) with a range of relevant physico-chemical and structural parameters. One parameter that appeared to be significant was the modulus of the difference of atomic charge across one of the bonds in a molecule; this parameter was selected because of its potential to model the strength of a bond. For example, we found [1] the following QSBR for a series of 11 phenols:

$$BOD = 0.998 \times 10^3 \, \Delta|\delta|_{C\text{-}O} + 2.108 \qquad (1)$$

$$n = 11 \quad r = 0.991 \quad s = 4.04$$

However, it should be pointed out that the CINMIN program used to calculate atomic charges was not a standard program, and it has not been possible to reproduce these results using programs such as MOPAC. This must cast some doubt on the universal applicability of the results. We remain convinced, however, that a parameter that reflects bond strength should be able to contribute to the modelling of biodegradability.

We also found [1] that 5-day BOD values correlated well with electrophilic super-delocalisability (S_E). For example, for alcohols we found a good correlation with S_E on the carbon to which the hydroxyl group was attached:

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$$BOD = 0.093\ S_E - 3.163 \qquad (2)$$

$$n = 19 \quad r = 0.981 \quad s = 4.30$$

The superdelocalisabilities were calculated for energy-minimised molecules using MOPAC.

We have also carried out work [2] on the correlation of biodegradability with molecular connectivity indices, as have Boethling and co-workers [3,4]. For a series of 20 alcohols, we found a good correlation with the difference of third-order simple and valence-corrected molecular connectivities:

$$BOD = 12.40\ (^3\chi - {}^3\chi^v) + 0.52 \qquad (3)$$

$$n = 20 \quad r = 0.952 \quad s = 7.67$$

Clearly the size and shape of molecules can play an important role in their biodegradability, and several workers have reported QSBRs involving steric parameters [5]. We observed [6] a correlation between BOD values and the Sterimol substituent length L for halogenated hydrocarbons:

$$BOD = 8.29\ L - 1.187 \qquad (4)$$

$$n = 9 \quad r = 0.976 \quad s = 4.12$$

We further observed a good correlation of BOD values of alkanes with accessible surface area (ASA):

$$BOD = 0.0996\ ASA + 0.055 \qquad (5)$$

$$n = 12 \quad r = 1.000 \quad s = 0.27$$

These parameters did not correlate well with the biodegradability of polar compounds, and indeed such correlations would not be expected.

More recent work from our laboratory [7] has investigated the application of Comparative Molecular Field Analysis (CoMFA) to the correlation of biodegradability. This technique investigates the steric and electrostatic fields sensed by a probe at different points within a three-dimensional grid within which the molecules are aligned. No multiple linear regression-type QSAR equations are produced, but correlation coefficients indicate the degree of fit. We examined a number of different data-sets, and reasonable to good fits were obtained for all except a series of phenols. For example, for a series of 20 alcohols, an r^2 of 0.980 (and cross-validated (CV) r^2 of 0.724) was obtained, with 74.7 % steric and 25.3 % electrostatic contribution to biodegradation. For 20 linear alkylbenzene sulphonates, r^2 = 0.913 and CVr^2 = 0.761, with the

degradation being 100 % controlled by steric factors. This technique is generally applicable only to congeneric series, otherwise the alignment problems are very difficult.

2. The MITI Aromatic Compound Biodegradation Data-Base

We have recently obtained, courtesy of Dr. W.J.G.M. Peijnenburg, the biodegradation data for 240 aromatic compounds determined by MITI. The data-set comprised mostly compounds with a single aromatic ring, but there were 17 compounds with two aromatic rings and 10 compounds with three aromatic rings. There were no fused-ring compounds in the data-set, with the exception of two anhydrides. Substitution patterns were extremely varied, with numbers of substituents up to six per ring: there was a considerable number of chlorine-containing compounds. Molecular weights ranged from 78 (benzene) to 959 (decabromodiphenyl ether). Four compounds were sodium salts; these were treated as the corresponding acids. A few compounds were indicated as being mixtures of isomers; in such cases the most appropriate isomer was used. Three compounds could not be handled by the software that we used; they contained respectively iodine, tin and silicon.

For each compound, the data supplied were: ID number, CAS number, molecular structure, molecular formula, molecular weight, % BOD (biological oxygen demand) at 7 days, % BOD at 14 days, induction time to 5 % BOD, % BOD at 10 days after 5 % BOD, maximum biodegradation rate, test period, and % BOD at the end of the test. 122 of the compounds showed some biodegradation, whilst 138 did not (i.e. % BOD at end of test was zero). In a few cases replicate results were given; in such cases the first result was used, for consistency with the other data.

2.1 METHODS

For each compound a large number of descriptors was calculated. Log P values were calculated using CLOGP for Windows; for the few compounds for which CLOGP was unable to calculate a value because of missing fragments, values were calculated using ProLogP or, in one case, MicroQSAR. Molar refractivities were calculated using MEDCHEM ver. 3.55. For the calculation of molecular orbital properties, molecules were constructed and energy-minimised in COSMIC, and then MOPAC ver. 7.0 was used to calculate heat of formation, E_{HOMO} (energy of the highest occupied molecular orbital), E_{LUMO} (energy of the lowest unoccupied molecular orbital) and dipole moment. MOLCONN-X was used to calculate a large number of topological indices, including molecular connectivities up to tenth order, electrotopological indices and information contents. The method of Fujita et al. [8] was used to determine the hydrogen bond donor and acceptor abilities of each molecule, and the method of Yang et al. [9] to determine the number of donor and acceptor hydrogen bonds that each molecule was capable of forming. Statistical analysis was carried out using MINITAB ver. 10.1.

2.2 RESULTS AND DISCUSSION

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Virtually all of the QSAR work carried out to date on biodegradability has been done on congeneric series of compounds, in which mechanisms of biodegradation might be expected to be reasonably consistent. The present data set is extremely diverse, and *a priori* one would not expect to find good QSAR correlations. This was indeed found to be the case with the MITI data-set. There were 79 compounds for which all (or most) of the measured biodegradability end-points were given. Step-wise regression showed that the best correlation that could be obtained with final BOD values was:

$$BOD = - 28.2\ {}^{3}\chi^{v} - 0.00193\ I_{Bon} - 0.184\ HeatForm + 71.0 \qquad (6)$$

$$n = 79 \quad r^2(adj.) = 0.180 \quad s = 23.3 \quad F = 6.72$$

where ${}^{3}\chi^{v}$ = third order valence-corrected path molecular connectivity, I_{Bon} = Bonchev index, and HeatForm = heat of formation.

It is, of course, probable that considerably better correlations could be obtained if single chemical classes were examined, but we were concerned here to determine whether the data would allow of a reasonable prediction of biodegradability across a wide range of chemical classes, using a single regression equation. Clearly that was not the case. Similarly poor correlations were obtained with all the other biodegradability end-points: 7-day BOD, r^2 = 0.122; 14-day BOD, r^2 = 0.255; time to 5 % biodegradation, r^2 = 0.232; % BOD at 10 days after 5 % BOD, r^2 = 0.153; maximum biodegradation rate, r^2 = 0.229.

It may be noted here that in general there is not a particularly high correlation between the biodegradability end-points, as Table 1 shows. The high correlation between 14-day BOD and final BOD is because many of the compounds had achieved their final BOD by 14 days.

TABLE 1. Correlation matrix (r) between biodegradability end-points

	7-d BOD	14-d BOD	T5 %	10-day	Rmax
14-d BOD	0.771				
T5 %	-0.751	-0.719			
10-day	0.792	0.873	-0.618		
Rmax	0.546	0.497	-0.459	0.491	
Final BOD	0.634	0.878	-0.610	0.868	0.372

At this point in our analysis we observed a number of errors in the MITI data-base. One example will serve to illustrate these; for 3-nitrophenol, the 7-day BOD (63 %) and the 14-day BOD (65 %) values were both considerably higher than the final BOD value of 46 %. It may also be noted that in some instances where replicate BOD values were given for a compound, the replicate value differed considerably from the original

determination; for example, for 3-nitrobenzoic acid the two reported final BOD values were 0 % and 12 %.

In view of this, and because of the lack of quantitative correlation of the biodegradability end-points, we decided to investigate whether any of the parameters that we had generated could distinguish between readily and non-readily biodegradable compounds. The former we defined as those compounds with a final BOD greater than 50 %, and the latter as those with a final BOD of less than 20 %. The number of compounds with final BOD values between 20 and 50 % was only 15, although one compound (CAS no. 60628-17-3) had replicate final BOD values of 14 and 25 %; we included it as a non-readily biodegradable compound. Step-wise regression indicated that three parameters, namely heat of formation, sixth order chain molecular connectivity and the total topological index based on electrotopological state indices (the last two being obtained from MOLCONN-X), were important in modelling final BOD. Discriminant analysis on the 222 compounds, using these three parameters, yielded 172 correct predictions, i.e. a 77.5 % correct prediction rate. Of these, there were 117/160 (73.1 %) correct predictions for non-readily biodegradable compounds, and 55/62 (88.7 %) correct for readily biodegradable compounds. Whilst these prediction rates are reasonable, they nevertheless leave something to be desired, and so we examined which groups of compounds were outliers. Phosphorus-containing compounds were the most obvious in this respect, and a highly substituted fluorine compound (CAS no. 346-10-1) was observed to have an extremely influential effect; these compounds (which included that mentioned earlier with CAS no. 60628-17-3) were therefore removed from the data-set to give some improvement in the prediction rate. For the 210 compounds remaining, there were 114/150 (76.0 %)correct predictions for non-readily biodegradable compounds, and 53/60 (88.3 %) correct predictions for readily biodegradable compounds; the overall correct prediction rate was therefore 167/210 (79.5 %). Cross-validation yielded 113/150 (75.3 %) correct predictions for non-readily biodegradable compounds and 52/60 (86.7 %) correct for readily biodegradable compounds, with an overall correct prediction rate of 165/210 (78.6 %). Figure 1 shows the discrimination observed for the 210 compound data-set.

The heat of formation of a compound is a measure of its stability, and hence would be expected to reflect the ability of the compound to biodegrade. TETS2 is a topological index which is the sum of all path terms computed with E-state values [10]; it contains both electronic and topological information, but, being a newly-derived index, has not yet been fully evaluated. It is believed to relate to, *inter alia*, the compactness of a molecule. Sixth order chain molecular connectivity represents essentially the number of substituents on an aromatic ring, with high values of ChainS6 indicating fewer substituents. Figure 1 clearly shows that readily biodegradable compounds generally have high ChainS6 values, and one would expect that the more substituents on an aromatic ring, the less readily should the compound biodegrade. It is pleasing that as few as three readily calculated parameters are able to give good discrimination between readily and non-readily biodegradable aromatic compounds with widely diverse substituents.

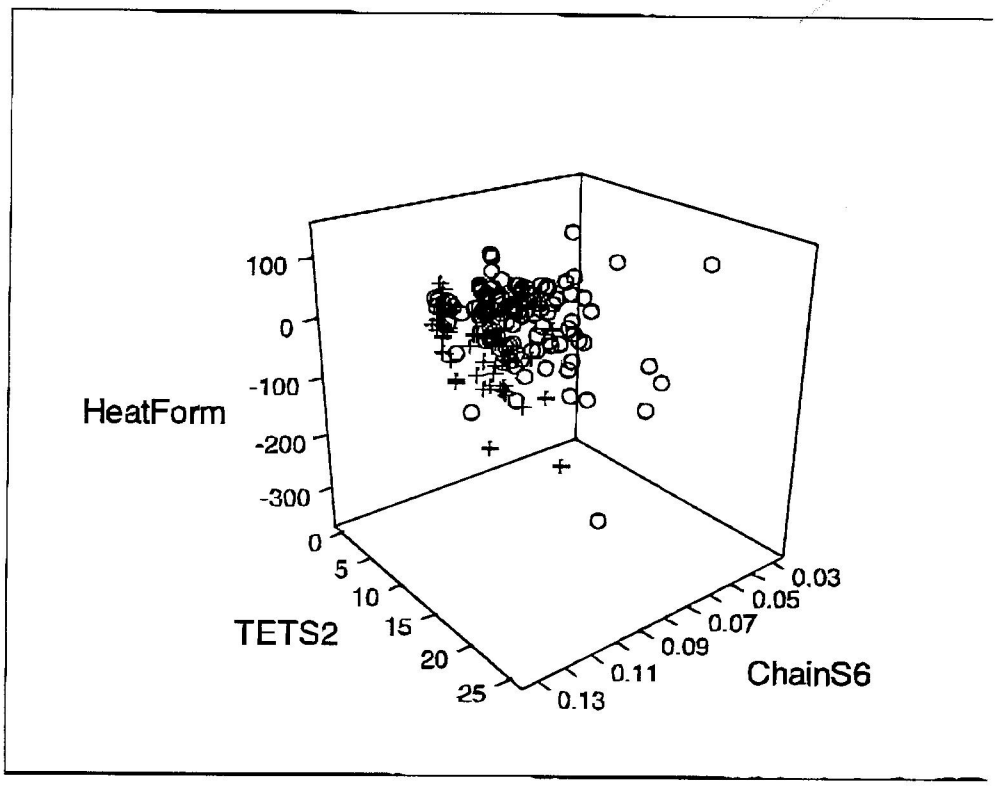


Figure 1. Discrimination between readily (+) and non-readily (o) biodegradable compounds (HeatForm = heat of formation, TETS2 = total topological index based on electrotopological state indices, ChainS6 = sixth order chain molecular connectivity)

Table 2. CAS numbers, final BOD values and physico-chemical properties of compounds used in this analysis

CAS number	Final BOD	Heat of Formation (kcal mol^{-1})	Chain 6	TETS2
71-43-2	40.0	22.028	0.1250	2.8967
108-88-3	100.0	14.423	0.1021	3.7304
100-42-5	53.0	38.719	0.1021	4.4407
99-87-6	88.0	-2.600	0.0833	6.0944
98-51-1	52.0	-3.978	0.0833	6.5193
100-41-4	39.0	8.678	0.1021	4.4299
98-87-3	90.0	3.182	0.1021	4.5682
100-44-7	71.0	6.919	0.1021	4.0774
103-84-4	69.0	-13.785	0.1021	4.4210
104-42-7	62.0	-56.560	0.0833	10.2502
95-53-4	34.0	17.713	0.0833	4.2441

2415-85-2	74.0	-61.046	0.0833	6.1738
64-04-0	90.0	13.499	0.1021	4.7743
100-46-9	64.0	19.557	0.1021	4.1024
588-46-5	77.0	-20.976	0.1021	4.6712
108-95-2	85.0	-22.237	0.1021	2.8405
1319-77-3	50.0	-28.853	0.0833	3.5102
1300-71-6	44.0	-36.366	0.0680	4.3271
108-46-3	67.0	-66.582	0.0833	1.9427
120-80-9	83.0	-64.368	0.0833	1.9070
123-31-9	70.0	-65.678	0.0833	2.3202
100-66-3	56.0	-15.814	0.1021	4.4085
103-71-1	63.0	-21.290	0.1021	5.0795
122-60-1	24.0	-18.849	0.1021	6.8248
90-05-1	90.0	-59.913	0.0833	3.6106
150-76-5	86.0	-59.195	0.0833	4.0848
91-16-7	96.0	-50.196	0.0833	5.8127
93-15-2	89.0	-37.736	0.0680	8.2197
614-80-2	60.0	-58.074	0.0833	3.8270
90-04-0	55.0	-16.254	0.0833	4.7196
104-94-9	65.0	-16.506	0.0833	4.9145
554-84-7	46.0	-17.420	0.0833	2.8290
25167-82-2	83.0	-38.288	0.0556	2.5716
118-79-6	49.0	-2.181	0.0556	4.3044
100-51-6	94.0	-28.248	0.1021	3.4687
140-11-4	95.0	-67.595	0.1021	5.3352
60-12-8	87.0	-35.265	0.1021	4.2937
96-09-3	81.0	18.269	0.1021	5.8385
104-51-1	91.0	-15.034	0.1021	5.1292
536-60-7	85.0	-47.736	0.0833	5.6787
100-52-7	66.0	-8.916	0.1021	3.7031
98-86-2	67.0	-14.988	0.1021	3.7503
118-90-1	94.0	-69.521	0.0833	4.8199
131-11-3	93.0	-137.333	0.0833	5.9474
84-61-7	69.0	-178.537	0.1021	10.9007
85-68-7	81.0	-110.660	0.0833	12.2276
1459-93-4	99.0	-128.823	0.0833	6.3923
131-17-9	82.0	-83.961	0.0833	6.8795
120-61-6	84.0	-143.006	0.0833	6.5230
121-91-5	78.0	-156.798	0.0833	3.2081
100-21-0	75.0	-144.634	0.0833	4.2001
536-66-3	89.0	-85.482	0.0833	6.1882
85-44-9	85.0	-68.834	0.1021	9.1486
94-36-0	84.0	-31.435	0.1021	11.9686
552-30-7	96.0	-148.639	0.0833	9.1099
1726-23-4	88.0	-277.264	0.0680	9.5852
495-69-2	81.0	-100.676	0.1021	6.1007
65-85-0	85.0	-61.976	0.1021	4.1355
119-68-6	85.0	-59.805	0.0833	5.3683
62-23-7	62.0	-55.332	0.0833	3.7476
552-16-9	100.0	-57.503	0.0833	5.7653
87-20-7	83.0	-118.109	0.0833	4.9636

69-72-7	88.0	-106.842	0.0833	3.2946
100-47-0	63.0	53.383	0.1021	3.9994
140-29-4	87.0	46.694	0.1021	4.5141
98-11-3	87.0	-97.075	0.1021	6.1908
1300-51-2#	86.0	-139.089	0.0833	6.3828
115-86-6*	90.0	-140.218	0.1021	27.3580
15205-57-9*	79.0	-104.387	0.1021	22.1709
1330-78-5*+	28.0	-158.872	0.0833	31.5726
1330-78-5*+	37.0	-163.251	0.0833	31.4299
122-40-7	51.0	-25.253	0.1021	8.2887
618-41-7#	22.0	-27.515	0.1021	4.7563
98-83-9	0.0	32.463	0.1021	5.2439
95-63-6	18.0	-0.098	0.0680	5.6159
108-67-8	0.0	-0.677	0.0680	5.6244
526-73-8	0.0	1.220	0.0680	5.6144
28106-30-1	0.0	25.390	0.0833	6.1971
535-77-3	0.0	-2.834	0.0833	6.1004
105-05-5	0.0	-4.709	0.0833	6.2203
141-93-5	0.0	-4.661	0.0833	6.2331
1321-74-0	0.0	55.452	0.0833	6.1497
25321-09-9	0.0	-12.480	0.0833	7.7169
108-90-7	0.0	14.810	0.1021	3.4256
108-86-1	0.0	26.758	0.1021	3.6653
95-49-8	0.0	7.918	0.0833	4.2127
106-43-4	0.0	7.121	0.0833	4.2663
95-50-1	0.0	9.220	0.0833	3.6624
541-73-1	0.0	8.199	0.0833	3.8133
1712-70-5	0.0	25.247	0.0833	5.7634
106-37-6	0.0	32.026	0.0833	4.4633
98-15-7	0.0	-140.453	0.0833	8.1972
87-83-2	7.0	47.058	0.0370	7.6061
87-61-6	0.0	4.197	0.0680	3.6587
108-70-3	0.0	2.228	0.0680	4.0119
120-82-1	0.0	2.930	0.0680	3.9030
95-94-3	0.0	-1.874	0.0556	3.7376
118-74-1	0.0	-7.822	0.0370	1.7108
608-93-5	0.0	-4.940	0.0454	2.9044
634-66-2	0.0	-0.600	0.0556	3.4390
98-08-8	0.0	-134.366	0.1021	8.2994
102-47-6	0.0	-5.300	0.0680	4.6483
100-61-8	1.4	26.702	0.1021	4.5374
579-10-2	1.0	-1.076	0.1021	5.2858
91-66-7	0.0	21.541	0.1021	7.1427
121-69-7	1.9	32.363	0.1021	5.5464
103-69-5	0.0	17.994	0.1021	5.2343
95-80-7	0.0	15.398	0.0680	4.6623
95-64-7	7.1	6.129	0.0680	5.1915
95-78-3	1.0	10.178	0.0680	5.1386
122-39-4	0.0	58.488	0.1021	11.3147
26603-23-6	0.0	-43.856	0.0833	23.9307
74-31-7	0.2	91.657	0.1021	21.2089

108-45-2	2.0	19.135	0.0833	3.8791
108-44-1	1.0	16.851	0.0833	4.2850
611-21-2	1.0	20.706	0.0833	5.4894
622-57-1	2.0	10.427	0.0833	6.2443
696-44-6	0.0	16.825	0.0833	5.4977
95-51-2	2.7	13.129	0.0833	3.7024
95-76-1	0.0	11.800	0.0680	3.9434
95-82-9	0.0	6.288	0.0680	3.9542
554-00-7	0.0	6.293	0.0680	3.9512
95-69-2	0.0	5.843	0.0680	4.6650
634-93-5	0.0	0.072	0.0556	3.7195
1477-55-0	22.0	14.953	0.0833	5.3587
103-83-3	2.0	26.509	0.1021	5.9811
88-74-4	0.0	26.125	0.0833	2.8185
99-09-2	0.0	27.909	0.0833	2.9270
100-01-6	0.0	21.694	0.0833	3.2963
89-63-4	0.0	16.331	0.0680	3.4248
98-95-3	3.3	26.105	0.1021	3.2178
88-72-2	0.5	18.939	0.0833	3.8216
99-08-1	2.0	17.805	0.0833	3.8949
99-99-0	0.8	17.292	0.0833	3.9259
88-73-3	8.2	23.863	0.0833	2.8682
100-00-5	0.0	19.284	0.0833	3.2425
25154-54-5	0.0	33.141	0.0833	4.9541
610-39-9	0.0	30.538	0.0680	6.5450
118-83-2	0.0	-128.507	0.0680	12.8858
99-54-7	0.0	15.112	0.0680	3.0826
102-06-7	0.0	114.907	0.1021	11.6025
98-54-4	0.0	-40.457	0.0833	5.0281
99-71-8	0.0	-45.396	0.0833	5.4647
1806-26-4	0.0	-76.637	0.0833	7.5271
25154-52-3	0.0	-83.486	0.0833	7.9916
5510-99-6	3.0	-64.103	0.0680	8.0938
96-76-4	0.0	-56.296	0.0680	6.2135
128-37-0	4.5	-59.138	0.0556	6.4651
732-26-3	0.0	-69.606	0.0556	8.7923
4130-42-1	0.0	-63.397	0.0556	7.3681
101-53-1	0.0	-2.060	0.1021	10.4433
67557-76-0	0.0	-39.365	0.0833	10.4016
120-71-8	0.7	-24.084	0.0680	5.6133
101-84-8	6.3	23.593	0.1021	10.9806
591-27-5	0.0	-24.030	0.0833	3.0575
156-43-4	0.0	-22.076	0.0833	5.5998
62-44-2	8.4	-56.976	0.0833	6.8836
346-10-1*	1.0	-476.510	0.0680	59.0480
88-75-5	0.0	-20.163	0.0833	3.8454
100-02-7	4.3	-19.549	0.0833	1.9265
91-23-6	0.0	-9.585	0.0833	2.8586
100-17-4	0.0	-13.449	0.0833	4.1996
555-03-3	1.0	-11.381	0.0833	3.8770
119-33-5	0.0	-27.609	0.0680	4.3638

2042-14-0	0.0	-23.572	0.0680	2.8913
2581-34-2	0.0	-25.889	0.0680	2.4465
5428-54-6	0.0	-24.768	0.0680	2.9709
51-28-5	0.0	-8.779	0.0680	6.6198
97-52-9	0.0	-14.933	0.0680	3.6874
96-96-8	0.0	-10.940	0.0680	4.2078
95-57-8	0.0	-26.627	0.0833	2.6693
106-48-9	2.0	-29.302	0.0833	3.1398
108-43-0	0.0	-29.208	0.0833	3.0165
95-56-7	0.0	-14.595	0.0833	3.2925
106-41-2	0.0	-17.545	0.0833	3.4832
591-20-8	0.0	-16.933	0.0833	3.4334
59-50-7	0.0	-36.210	0.0680	3.8056
615-74-7	0.0	-34.308	0.0680	3.3721
1570-64-5	0.0	-36.525	0.0680	3.7843
87-65-0	0.0	-32.186	0.0680	2.3766
95-77-2	0.0	-34.640	0.0680	2.9063
120-83-2	0.0	-33.137	0.0680	2.6095
576-24-9	0.0	-33.931	0.0680	2.0873
583-78-8	5.0	-33.242	0.0680	2.3302
591-35-5	0.0	-35.381	0.0680	2.8982
95-95-4	0.0	-38.237	0.0556	2.4830
609-89-2	0.0	-27.482	0.0556	4.3718
30171-80-3	0.0	-10.958	0.0556	9.4844
58-90-2	7.0	-41.677	0.0454	1.8011
1836-77-7	0.0	12.559	0.0833	8.6537
120-52-5	0.0	50.504	0.1021	19.7024
103-50-4	0.0	4.290	0.1021	11.1262
80-43-3	0.0	25.153	0.1021	17.1826
96-69-5	1.9	-75.994	0.0556	18.5044
117-81-7	43.0	-215.809	0.0833	12.0389
3648-21-3	36.0	-182.559	0.0833	10.2913
26761-40-0	42.0	-246.382	0.0833	13.7313
134-62-3	0.0	-13.900	0.0833	6.7786
98-73-7	0.0	-86.580	0.0833	6.1189
118-91-2	5.6	-66.762	0.0833	3.7789
121-92-6	0.0	-56.141	0.0833	4.4563
603-11-2	0.0	-136.242	0.0680	10.6115
91-15-6	3.3	87.699	0.0833	4.4901
623-26-7	0.0	86.143	0.0833	4.9026
626-17-5	0.0	86.162	0.0833	4.7756
1897-45-6	0.0	72.086	0.0370	1.4318
88-19-7	0.0	-50.818	0.0833	7.2016
88-44-8	0.0	-109.907	0.0680	7.2467
127-68-4#	0.0	-90.405	0.0833	9.4963
88-53-9	0.0	-115.916	0.0556	6.9987
946-30-5#	0.0	-92.167	0.0680	10.3243
330-55-2	0.0	-11.030	0.0680	7.5466
330-54-1	0.0	-9.652	0.0680	6.5388
24019-05-4	0.0	-21.302	0.0680	20.1781
2655-14-3	1.0	-60.739	0.0680	7.8530

3766-81-2	1.0	-65.010	0.0833	8.8373
2631-40-5	0.0	-58.812	0.0833	8.1476
644-97-3*	0.0	-28.527	0.1021	5.1768
1241-94-7*	2.0	-216.584	0.1021	24.0259
60628-17-3*	14.0	-188.863	0.1021	22.5740
26967-76-0*	0.0	-193.571	0.0833	37.1672
2597-03-7*	0.0	-144.500	0.1021	13.7624
122-14-5*	0.0	-132.895	0.0680	11.4238
2104-64-5*	3.0	67.154	0.0833	17.0437
80-54-6	8.0	-44.034	0.0833	7.8748
3319-31-1	4.2	-335.422	0.0680	14.7004
3886-69-9	6.0	15.621	0.1021	4.3026
782-74-1	0.0	75.952	0.0833	12.4101
1163-19-5	0.0	99.015	0.0370	18.8227
87-86-5	1.0	-44.715	0.0370	1.1598
36065-30-2	2.9	8.700	0.0556	8.7279
50-31-7	0.0	-75.048	0.0556	2.9728
10541-83-0	14.0	-67.225	0.0833	5.6621
98-10-2	0.0	-46.954	0.1021	6.3724
961-11-5*	0.0	-220.597	0.0556	10.7600
14816-18-3*	3.0	-64.576	0.1021	14.6704
1460-02-2	0.0	-32.100	0.0680	11.7982

*indicates phosphorus compound or highly influential compound which was removed
sodium salt; treated as undissociated acid
+o- and p-tricresyl phosphates respectively, although same CAS number given

3. Conclusions

Multiple linear regression analysis of 79 aromatic compounds found to be biodegradable in the MITI test did not produce satisfactory correlations. However, discriminant analysis based on three calculated parameters (one from MOPAC 7 and two from MOLCONN-X) gave good discrimination between readily and non-readily biodegradable compounds, with an overall correct cross-validated prediction rate of 78.6 %.

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Production of murine monoclonal antibodies against sulcofuron and flucofuron by in vitro immunisation

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Abstract

Monoclonal murine anti-pesticide antibodies were produced by in vitro immunisation (IVI) of cultured splenocytes with the pesticides sulcofuron and flucofuron. The majority of both anti-flucofuron and anti-sulcofuron antibodies obtained were of the IgM isotype, rather than IgG. When used in an indirect enzyme-linked immunosorbent assay (ELISA), the antibodies bound to plate coating antigens which incorporated haptens that mimicked moieties present within the immunising pesticide. The antibodies exhibited a high degree of specificity, with the degree of cross-reactivity related to the structural similarity between the hapten present in the plate coating antigen and the moieties present within the immunising pesticide. These results indicated that antibodies specific to both sulcofuron and flucofuron had been produced by IVI. Synthesis of both hapten analogues and immunogens as required for methods based on in vivo immunisation was avoided, whilst antibody production was also comparatively more rapid than traditional methods and minimised animal discomfort.

Keywords: In vitro immunization; Pesticide; Monoclonal antibody

1. Introduction

Immunoassays such as the enzyme-linked immunosorbent assay (ELISA) have been reported for many pesticides (Wratten and Feng, 1990). When developed, ELISAs are cheaper, more rapid and less technically demanding than methods such as high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). In addition, ELISAs which have used monoclonal antibodies (MAbs) have often shown both a high degree of specificity and an ability to detect sub-part per billion ($\mu g\ l^{-1}$) concentrations of the target analyte (Hammock et al., 1986; OTA, 1988). Consequently, ELISA methods have proved suitable for monitoring both environmental and food matrices for pesticide

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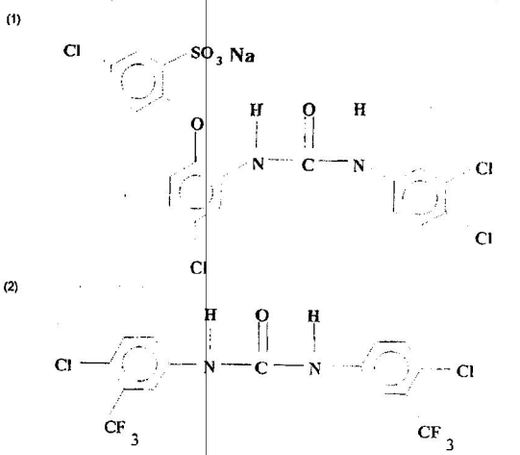


Fig. 1. Structures of the pesticides (1) sulcofuron and (2) flucofuron.

residues (Van Emon et al., 1989; Morgan et al., 1992).

Sulcofuron and flucofuron (Fig. 1) are the active ingredients of the commercial mothproofing formulations Mitin FF HC and Mitin N (Ciba-Geigy, Basle, Switzerland), respectively. Entry of these compounds into aquatic ecosystems occurs as a result of either direct discharge, or indirectly within the effluents of sewage treatment works (Zabel et al., 1988). Within the European Community, monitoring of freshwaters in areas where wool or wool-based textiles are processed is necessary in order to ensure that the relevant Environmental Quality Standards (EQS) are maintained (Directive 76/464/EEC). For sulcofuron and flucofuron the current EQS values are 25 $\mu g\ l^{-1}$ and 1 $\mu g\ l^{-1}$, respectively. Although reversed-phase high performance liquid chromatography (HPLC) can detect sulcofuron and flucofuron in freshwaters when present at concentrations of 0.1 $\mu g\ l^{-1}$ (Standing Committee of Analysts, 1991), the method is not specific for the target analytes and is prone to interference, particularly when matrices more complex than water are analysed (Antoine, 1994). The development of ELISA methods for screening both environmental and food samples for these analytes was therefore considered desirable.

Low molecular weight compounds such as the pesticides sulcofuron and flucofuron are generally regarded as being non-immunogenic but capable of acting as haptens when coupled to larger, immunogenic carrier molecules (Leskowitz, 1972). Anti-pesticide antibodies for use in ELISAs are produced by the immunisation of animals with synthetic antigens (immunogens) which consist of the pesticide molecule covalently linked (conjugated) to a larger immunogenic carrier molecule such as protein. An additional small molecule might also be incorporated into the immunogen between the hapten and carrier to act as a spacing group and thus improve immune recognition of the hapten (Harrison et al., 1990).

Inspection of the structures of sulcofuron and flucofuron suggested that both compounds were not only too small to be immunogenic, but also unsuitable for covalent bonding to carrier proteins. In order to obtain antibodies against these compounds by in vivo immunisation, it would prove necessary to synthesise suitable hapten analogues and then conjugate these to immunogenic carrier molecules. This strategy has been demonstrated successfully for other pesticides such as permethrin (Stanker et al., 1989).

Bonwick et al. (1994a) demonstrated that for some haptens it may be possible to avoid the complex and time consuming synthesis of hapten analogues. Instead of an analogue, an easily conjugated hapten molecule which mimics a similar moiety within the target molecule is used (hapten mimic). For example, anti-hapten mimic antibodies were raised against 3-phenoxybenzoic acid (PBA) and dichlorovinyl cyclopropane carboxylate (CPA) which subsequently exhibited binding to the corresponding phenoxybenzyl alcohol or dichlorovinyl cyclopropane carboxylate moieties within the pesticide permethrin. Through use of the anti-hapten mimic antibodies an ELISA was successfully developed for environmental monitoring (Bonwick et al., 1994b).

The compounds 3,4-dichloroaniline (DCA) and 5-amino-2-chlorobenzotrifluoride (ACBT) are both easily conjugated to carrier proteins and also mimic moieties present within the pesticides sulcofuron and flucofuron, respectively (Fig. 2). Consequently, immunisation of rabbits or mice with conjugates that contained either DCA or ACBT would be expected to result in the production of anti-DCA or anti-ACBT antibodies. Use of these anti-hapten mimic antibodies in an appropriate ELISA format should then permit quantification of sulcofuron and flucofuron.

This strategy for the raising of antibodies against sulcofuron and flucofuron was, however, not

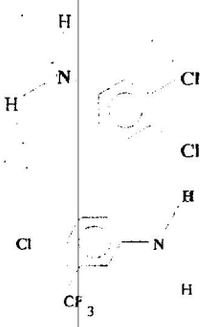


Fig. 2. Structures of the hapten mimics (1) DCA and (2) ACBT.

favoured by us following observations made during the earlier permethrin ELISA development study. In this study, in which the hapten mimics PBA and CPA were used to produce polyclonal rabbit antisera, it was found that serum antibody titres suitable for use in the permethrin ELISA were only obtained between 6 and 9 months after immunisation had commenced. A similar period of immunisation was also required for mice, in order to obtain antibody titres significantly greater than those of the pre-immune sera and thus signal that hybridoma production could commence (unpublished data). A more serious problem which was encountered, however, was that the administration of immunogens which contained CPA frequently resulted in the death of the immunised animals (unpublished observations). Therefore, methods based on the in vitro immunisation (IVI) of cultured splenocytes were considered rather than conventional in vivo immunisation procedures. Furthermore, any problems associated with immunogen toxicity and consequent animal suffering would be avoided.

Since the first demonstration of IVI of mouse splenocytes and the subsequent production of mouse monoclonal antibodies (Hentgartner et al., 1978), this technique has also been used for the production of antibodies against haptens such as dinitrophenol (DNP), trinitrophenol (TNP), benzo[*a*]pyrene and digoxin (Borrebaeck, 1983, 1986; Schilizzi et al., 1992). When compared to in vivo immunisation procedures, IVI has the advantages that smaller amounts of immunogen are required (nanogram as opposed to milligram quantities) and that the production of antibodies is more rapid (4 or 5 days as opposed to several months). Furthermore, antibodies may be obtained in circumstances where in vivo immunisation cannot be safely undertaken such as in the production of human antibodies (Borrebaeck, 1986). The main disadvantage is that the antibodies produced are predominantly of the IgM isotype, rather than those of the more desirable IgG isotype. This is because the length of time for which the splenocytes are cultured following immunisation is not sufficient for an immunoglobulin class switch (IgM to IgG) to occur. Although extended culture time would allow this switch to occur, this is offset by the poor survival of the splenocytes (De Boer et al., 1987; Moller and Borrebaeck, 1988). Reviews of the IVI method have been presented by Reading (1982) and Borrebaeck (1986, 1988, 1989).

The aims of this study were first, to determine whether antibodies could be obtained against the pesticides sulcofuron and flucofuron using IVI methods; second, to examine the nature of any antibodies obtained and third, to observe the performance of the antibodies when incorporated into indirect competitive ELISAs for the quantitative analysis of sulcofuron and flucofuron. A modified IVI method (Dougherty et al., 1987) was performed in which the unconjugated pesticides sulcofuron and flucofuron were introduced into splenocyte cultures stimulated by the addition of a mitogen. The splenocytes were subsequently fused with mouse myeloma cells to give hybridoma cultures which were then cloned using standard methods to produce monoclonal anti-pesticide antibodies.

2. Materials and methods

2.1. Reagents

The pesticides sulcofuron (5-chloro-2-(4-chloro-2-(3,4-chlorophenyl)) phenoxyureidobenzene sulphonate) and flucofuron (*N*,*N*′-bis(4-chloro-3-trifluoromethyl)phenyl urea) were provided by the National Rivers Authority (Yorkshire and Northumbria region). The hapten mimics 5-amino-2-chlorobenzotrifluoride (ACBT) and 3,4-dichloroaniline (DCA) used for production of ELISA plate coating antigens were obtained from the Aldrich Chemical Co. (Gillingham, Dorset, UK). The structures of DCA and

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ACBT are shown in Fig. 2. Dichlorovinyl dimethylcyclopropane carboxylate (CPA) was synthesized by B. Lygo, University of Salford. All other reagents were obtained from Sigma Chemical Co. (Poole, Dorset, UK) unless specified otherwise.

Stock solutions of the pesticides were made up in dimethyl sulphoxide (1 mg ml^{-1}) whilst stock solutions of ACBT and DCA were made up in dimethyl formamide (20 mg ml^{-1}). All stock solutions were stored in the dark at 4°C. Aqueous solutions of ACBT and DCA (0.1% w/v) were prepared by the dropwise addition of stock solution (5 ml) to distilled water (95 ml) immediately prior to use.

2.2. Cells and media

Murine myeloma cells (P3 × 63-Ag8.653) for fusion with in vitro immunised splenocytes were raised in Dulbecco's modified Eagle medium (DMEM) buffered with 20 mM Hepes (Hyclone Europe, Cramlington, Northumbria, UK) and supplemented with 2 mM glutamine (Integra Bioscience, Cramlington, Northumbria, UK) and 1 mM sodium pyruvate (Integra Bioscience) (DMEM1 medium). The DMEM1 medium was also supplemented with fetal calf serum (FCS, Hyclone Europe) to give a serum content of 10% (v/v) (DMEM1/FCS10). DMEM2 medium for hybridoma growth was produced by the addition of oxaloacetic acid (1 mM), pyruvic acid (0.45 mM) and insulin (0.2 U ml^{-1}) to DMEM medium, as directed by the manufacturer (OPI media supplement, Sigma). Ewing Sarcoma Growth medium (ESG, Integra Bioscience) (1% v/v) and glutamine (2 mM) were also added.

Splenocytes for use in an in vitro immunisation were prepared immediately prior to commencement of the procedure. Six-week-old, non-immune, BALB/c mice spleens were removed and disrupted by passage through a stainless steel screen into DMEM1 medium (10 ml). The splenocytes were washed three times by centrifugation (500 × g, 5 min) with fresh DMEM1 medium and finally resuspended in DMEM1/FCS10 medium.

Splenocyte feeder cells for hybridoma production were prepared 24 h before use. A BALB/c mouse spleen was disrupted by passage through a stainless steel screen into DMEM1 (10 ml). A crude removal of large debris from the splenocyte preparation was performed by sedimentation (2 min) within a conical tube. Splenocytes and media were carefully removed, resuspended in DMEM1/FCS10 medium (100 ml), dispensed (50 μl $well^{-1}$) into 96-well culture plates (Costar) and incubated (37°C, air) until required for use.

A thymocyte conditioned medium (TC medium) was prepared from the thymus of 6-week-old, non-immune, BALB/c mouse. The thymus was disrupted by passage through a stainless steel screen into DMEM1 medium (10 ml). The thymocytes were washed three times by centrifugation (500 × g, 5 min) with DMEM1 medium, resuspended in DMEM1 supplemented with 20% (v/v) FCS (DMEM1/FCS20) to give a cell concentration of 1×10^6 cells ml^{-1} and then incubated (37°C, air). After 48 h, the thymocyte culture was centrifuged (500 × g, 5 min) and the supernatant carefully removed, filter sterilised (0.22 μm filter), then stored at −20°C.

2.3. In vitro immunisation

Bacterial lipopolysaccharide (1.0 mg) dissolved in DMEM1/FCS10 (1 ml) was added to freshly prepared splenocytes in DMEM1/FCS10 (10 ml, 1×10^7 cells ml^{-1}) in a 75 cm^3 flask (Costar). Immediately prior to use, stock solutions of sulcofuron and flucofuron were diluted with dimethyl sulphoxide to give working solutions with final concentrations of 10 ng ml^{-1}. An aliquot (20 μl) of a working pesticide solution was added to a splenocyte culture. TC medium (10 ml) was added to the splenocyte culture and the mixture incubated (37°C, air). After 4 days the splenocytes were recovered and washed three times by centrifugation (500 × g, 5 min) with DMEM1 medium.

2.4. Hybridoma generation

Hybridomas were produced by mixing in polethylene glycol (PEG 1500) using standard methods (Harlow and Lane, 1988). The ratio of in vitro immunised splenocytes to 653 myeloma cells was approx. 2:1 as recommended by Borrebaeck (1983). The resulting hybridomas were resuspended in DMEM2/FCS10 medium, dispensed in two 96-well plates (100 μl $well^{-1}$) and incubated for 24 h (37°C,

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air). DMEM2/FCS10 supplemented with hypoxanthine (2×10^{-4} M), aminopterin (8×10^{-7} M) and thymidine (3.2×10^{-5} M) was used to replace 50% of the media within the wells. After a further 96 h incubation (37°C, air) another 50% media change was performed with DMEM2/FCS10 medium supplemented with hypoxanthine (1×10^{-4} M), aminopterin (4×10^{-7} M) and thymidine (1.6×10^{-5} M).

2.5. Hybridoma screening

Hybridoma supernatants were screened for the presence of antibodies using an indirect ELISA. Supernatants (50 μl well^{-1}) were added to an Immulon I microtitre plate (Dynatech Corp., Chantilly, VA, USA) and incubated overnight at 4°C. Supernatants were removed by aspiration and the plates washed three times with PBST (0.05% (v/v) Tween 20 in phosphate buffered saline (PBS, pH 7.2)), then blocked (1 h, 37°C) with blocking buffer (3% (w/v) dried non-fat milk (Marvel, Premier Brands UK Ltd., Knighton Adbaston, Stafford, UK) in PBS; 100 μl well^{-1}). After blocking, goat anti-rabbit peroxidase (1/5000 dilution in PBST; 50 μl well^{-1}) was added. After incubation (1 h, 256°C) the plate was washed (five times, PBST), substrate solution was added (2′,2-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS); 100 μl well^{-1}) and the product absorbance measured at a wavelength of 405 nm.

Hybridoma supernatants were also screened for anti-pesticide antibodies through use of an indirect ELISA. Microtitre plates were coated with a bovine serum albumin (BSA)-hapten conjugate, in which the hapten mimicked a moiety present within the original immunising pesticide. Anti-sulcofuron antibodies were detected using a DCA-BSA conjugate, whilst ACBT-BSA was used for anti-flucofuron antibody detection. The microtitre plate surfaces were initially coated with BSA and then either ACBT and DCA were coupled to the immobilised BSA with glutaraldehyde, using a modified method of Fujiwara and Kitagawa (1993). In all ELISA screening of hybridoma supernatants, a positive result was indicated by an absorbance value at 405 nm which exceeded the mean absorbance of the negative control by 2 SDs.

Two methods of coating with BSA were examined; either wet coating in which BSA adsorbed onto the microtitre plate surface from a buffer solution, or dry coating in which the buffer solution was evaporated to leave a BSA residue on the microtitre plate surface. Dry coating was performed by the addition of BSA solution to each well (1 mg ml^{-1} in PBS; 50 μl well^{-1}) of a 96-well microtitre plate (Immulon II) which was then dried onto the plate by incubation at 37°C. When dry, an aqueous solution of glutaraldehyde (0.25% w/v) was added (50 μl well^{-1}) and the plate incubated (37°C, 2 h). The glutaraldehyde solution was then discarded and replaced by an aqueous hapten solution (0.1% w/v, 50 μl well^{-1}). After overnight incubation at 4°C, the hapten solution was discarded and the plate blocked (1 h, 37°C) with blocking solution (100 μl well^{-1}). The blocking solution was discarded and the plate washed five times with PBST. Wash buffer was then discarded and the microtitre plate stored at 4°C until required.

Wet coating was performed in a similar fashion and involved addition of BSA (2 mg ml^{-1} in carbonate buffer 50 mM, pH 9.0; 50 μl well^{-1}) and subsequent overnight incubation (4°C). Excess solution was removed by aspiration and the haptens were coupled using glutaraldehyde as described for the dry coating procedure, above.

The uniformity of both the wet and dry coating methods was examined by performing an indirect ELISA on each type of plate and determining the percentage coefficient of variation (CV). The percentage CV value for a plate was determined thus:

$$\mathrm{CV}\,(\%) = \frac{\text{standard deviation of plate}}{\text{mean absorbance of plate}} \times 100$$

The specificity of the antibodies within the hybridoma culture supernatants was examined by an evaluation of their binding to additional conjugates, also immobilised on the microtitre plates by drying. The two conjugates selected for use as plate coating antigens consisted of either the hapten CPA coupled to BSA (CPA-BSA) or the hapten 3′-phenoxybenzoic acid (PBA) coupled to BSA (PBA-BSA). Both conjugates incorporated a six carbon spacing group (6-amino hexanoic acid, 6AHA) between the hapten and carrier protein molecules; the synthesis of these compounds, as well as the plate coating protocol, are

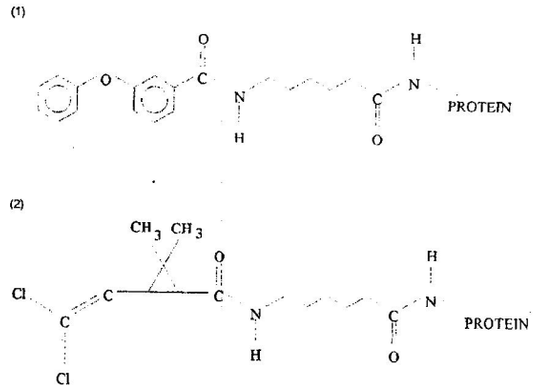


Fig. 3. Structures of the hapten-spacer conjugates (1) PBA-6AHA and (2) CPA-6AHA.

described by Bonwick et al. (1994a). The structures of the CPA-6AHA and PBA-6AHA hapten-spacer conjugates are illustrated in Fig. 3.

Hybridoma culture supernatants (50 μl well^{-1}) were incubated for 1 h at 37°C. In addition, DMEM2/FCS10 medium (50 μl well^{-1}) was included on the microtitre plates as a negative control, whilst non-specific binding was assessed using both pre-immune mouse sera and a hybridoma culture supernatant containing murine monoclonal anti-*Taenia* antibody (50 μl well^{-1}, antibody raised against *Taenia saginata* coproantigens and provided by J.C. Allen, Dept. of Biological Sciences, University of Salford, UK). The remainder of the indirect ELISA protocol was performed as described by Bonwick et al. (1994a). ABTS was used as the enzyme substrate and product absorbance was measured at a wavelength of 405 nm.

2.6. Cloning

After a further 7 days a 50% media replacement was performed with DMEM2/FCS10 medium supplemented with hypoxanthine (1×10^{-4} M) and thymidine (1.6×10^{-5} M) (DMEM2-HT). Additional 50% media replacements with DMEM2-HT were performed at least every 7 days, the frequency dependent on colony growth. Supernatant media from visible colonies were tested for the anti-pesticide antibodies by an indirect ELISA. Cells in ELISA positive wells were cloned by limiting dilution using 96-well plates with splenocyte feeder layers as described by Harlow and Lane (1988). ELISA positive clones were isolated and expanded to 24-well plates then 75 cm^3 flasks with DMEM2/FCS10 medium.

2.7. Hybridoma antibody isotyping

Murine monoclonal antibodies produced by the hybridomas were isotyped by Ouchterlony double diffusion (Ouchterlony, 1949). The procedure was performed as directed by the manufacturer (The Binding Site, Birmingham, UK).

2.8. Indirect competitive ELISA

The inhibition of MAb binding to immobilised conjugate by free pesticide in solution was conducted as described by Bonwick et al. (1994a) for the assay of permethrin. Both sulcofuron and flucofuron were first dissolved in methanol and the solutions were introduced into the ELISA to give a final methanol concentration of 10% (v/v).

3. Results

3.1. Antibody detection

Splenocytes immunised in vitro with flucofuron were successfully fused and a total of 192 culture plate wells were seeded with hybridomas. After 15 days, hybridoma colonies were observed in 37 wells of which 36 (97.3%) contained supernatants that tested positive for mouse immunoglobulin and 20 (54.1%) contained polyclonal antibodies that bound to ACBT-BSA. As ACBT mimicked a moiety within the flucofuron molecule, those supernatants that bound to ACBT-BSA were considered to contain anti-flucofuron antibodies.

Hybridomas produced from splenocytes immunised in vitro with sulcofuron were also seeded into 192 culture plate wells. After 15 days, hybridoma colonies were observed in 48 wells of which 47 (97.9%) contained supernatants that tested positive for mouse immunoglobulin and 41 (85.4%) contained polyclonal antibodies that bound to DCA-BSA. As DCA mimicked a moiety within the sulcofuron molecule, those hybridoma supernatants which tested

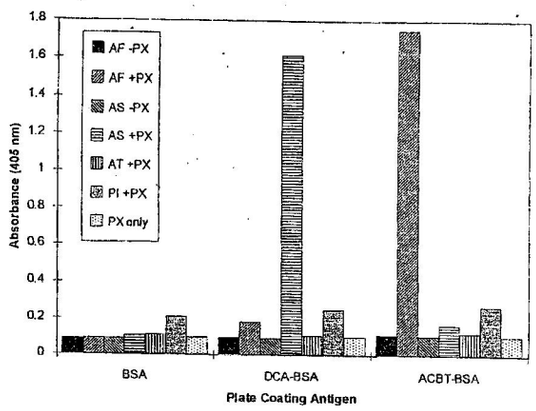


Fig. 4. Non-specific binding during the indirect ELISA of hybridoma supernatants. Anti-flucofuron (AF) and anti-sulcofuron (AS) both diluted 1/32, mouse anti-*Taenia* (AT) and mouse pre-immune serum (PI) undiluted. Omission or inclusion of goat anti-mouse peroxide indicated by −PX or +PX, respectively. Values presented are means of five observations.

positive were considered to contain anti-sulcofuron antibodies.

Controls included in the ELISA of the hybridoma supernatants showed that non-specific binding by the goat anti-mouse peroxidase contributed little to the final absorbance values obtained. Similarly, there was little evidence of binding to either ACBT-BSA or DCA-BSA by the mouse anti-*Taenia* antibody, whilst hybridoma supernatants from both the flucofuron and sulcofuron immunisations also failed to bind to plates coated with BSA alone. These observations helped confirm that anti-pesticide antibodies were present within the hybridoma supernatants and that the positive ELISA results were due to binding to the haptens within the plate coating antigens, rather than the BSA carrier protein. Despite the structural similarity of the haptens, little cross-reactivity in binding to the plate coating antigens was observed at the dilution used (Fig. 4).

Hybridoma supernatants from both the flucofuron and sulcofuron IVIs which had tested positive for the presence of antibody were used to examine the uniformity with which the microtitre plates were coated with antigen. Wet coated microtitre plates gave CV values of 10.9% and 11.5% for ACBT-BSA and DCA-BSA, respectively, whilst CV values of 5.6% and 4.9% were obtained for microtitre plates dry coated with ACBT-BSA and DCA-BSA, respectively. The lower CV values recorded for the dry coated plates indicated that a more consistent and uniform coverage with antigen had been achieved in comparison to the wet coated plates. On the basis of these observations it was decided that all subsequent ELISA tests would make use of dry coated microtitre plates.

Hybridoma cultures which tested positive by ELISA were frozen, with the exception of F2A8 (anti-flucofuron) and S2B5 (anti-sulcofuron). Cloning of F2A8 by limiting dilution resulted in 23 colonies of which 19 (82.6%) tested positive by ELISA. Isotyping of the immunoglobulins showed that antibodies of the the IgM isotype had been produced by 18 clones and antibodies of the IgG2A isotype had been produced by a single clone. Although cloning

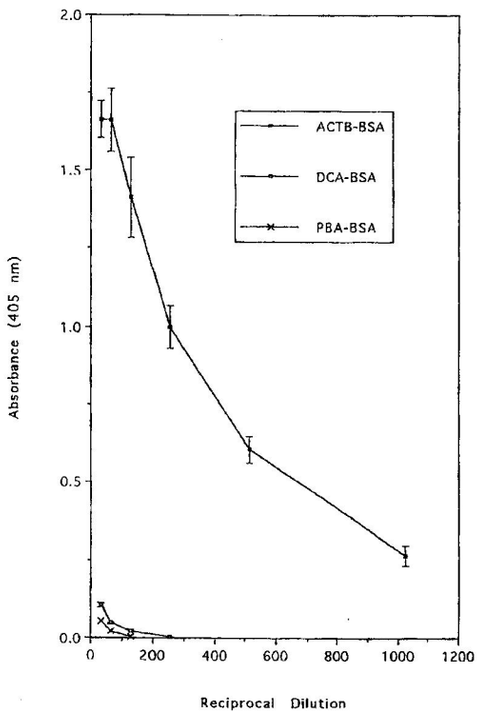


Fig. 5. Binding of monoclonal anti-flucofuron (F2A8/1/A4-B3) to hapten-BSA conjugates. Values presented are means of six observations ± SD.

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of S2B5 gave a total of 31 colonies, only 17 (54.8%) tested positive by ELISA. Isotyping of the immunoglobulins showed that all the clones had produced antibodies of the IgM isotype.

The binding of monoclonal anti-flucofuron (F2A8/1/A4-B3) and monoclonal anti-sulcofuron (S2B5/1/C3) antibodies to a range of conjugates was examined using an indirect ELISA. Both the F2A8/1/A4-B3 and S2B5/1/C3 antibodies showed poor binding to the CPA-BSA conjugate over the range of hybridoma supernatant dilutions examined. In contrast, both MAbs showed good binding with conjugates which possessed a hapten that most closely mimicked a moiety present within the original immunising pesticide (Fig. 5 and Fig. 6).

The performance of both MAbs when incorporated in an indirect competitive ELISA was subsequently examined. In these assays, the original immunising pesticide was included as the inhibitor. Binding of the monoclonal F2A8/1/A4-B3 to the ACBT-BSA conjugate was not inhibited by flucofuron, even when present at concentrations of 1 g l^{-1}. Similarly, the binding of S2B5/1/C3 to the DCA-BSA conjugate could not be inhibited by sulcofuron at the same concentration (Fig. 7).

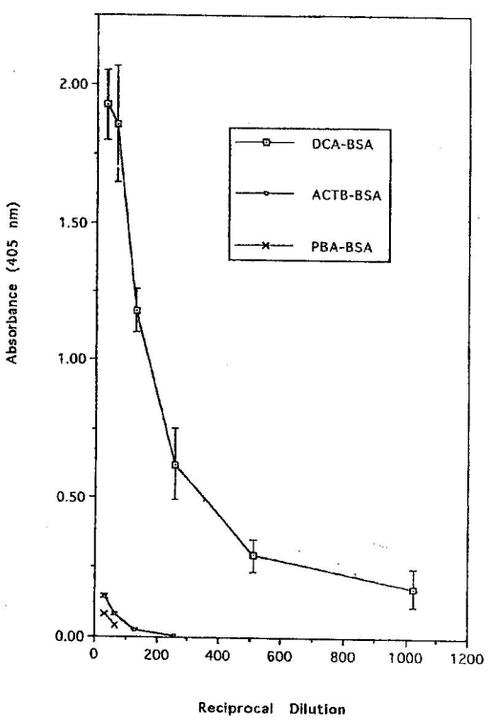


Fig. 6. Binding of monoclonal anti-sulcofuron (S2B5/1/G3) to hapten-BSA conjugates. Values presented are means of six observations ± SD.

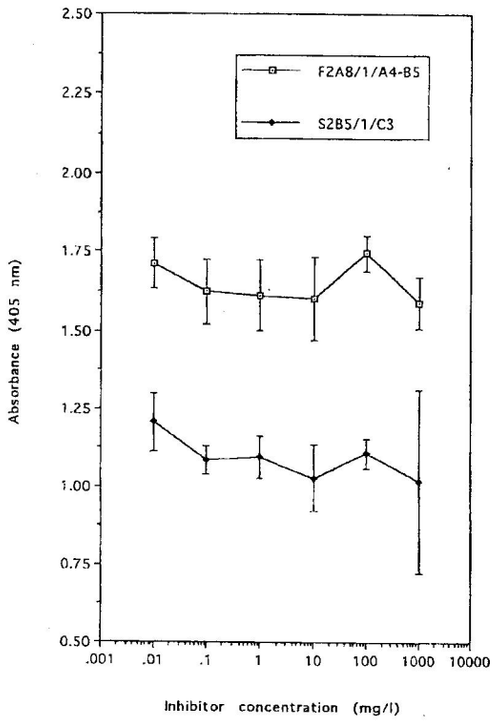


Fig. 7. Indirect competitive ELISA of flucofuron with monoclonal antibody F2A8/1/A4-B3 and of sulcofuron with monoclonal antibody S2B5/1/C3. Values presented are the means of six observations ± SD.

4. Discussion

The fusion of mouse myeloma cells with splenocytes immunised in vitro with the pesticides flucofuron or sulcofuron resulted in hybridomas which secreted polyclonal anti-flucofuron and anti-sulcofuron antibodies, respectively. This was confirmed by the binding of these antibodies to conju-

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gates that contained haptens which mimicked moieties present within the original immunising pesticides. Monoclonal antibodies (MAbs) were subsequently obtained from these cell lines and, as expected from the short initial splenocyte culture period, the majority were of the IgM isotype.

The monoclonal anti-flucofuron (F2A8/1/A4-B3) and anti-sulcofuron (S2B5/1/C3) antibodies showed very specific binding with conjugates in which the haptens mimicked moieties within the original immunising pesticide. The limited cross-reactivity which was observed reflected the structural differences between the haptens within the conjugates and the moieties present within the immunising pesticides. Thus, the least cross-reactivity was observed with conjugates that contained CPA, the hapten most structurally dissimilar from the moieties present within either flucofuron or sulcofuron. These results reflect those obtained from a previous study in which the performance of anti-hapten mimic antibodies have been examined (Bonwick et al., 1994a). It is considered that these latter observations further confirm that both anti-sulcofuron- and anti-flucofuron-specific MAbs were successfully produced.

Although both MAbs showed a high degree of specificity when binding to various immobilised conjugates was examined, a similar target specificity was not observed when these antibodies were used in an indirect competitive ELISA. Neither the F2A8/1/A4-B3, nor the S2B5/1/C3 antibodies were inhibited from binding by the original immunising pesticide, even when present at 1 mg ml^{-1}. It is thought that this was due to the combined effects of a high solid phase epitope density (due to the dry coating of the antigen onto the microtitre plate) and the properties of antibodies of the IgM isotype. Multi-specific, low affinity interactions would be favoured and would negate the inhibitory effects of the pesticides (Borrebaeck, 1989).

The objective of developing imunoassays for the pesticides sulcofuron and flucofuron has not yet been achieved. However, this is primarily considered to be the product of incomplete immunoassay method development rather than a problem associated with the in vitro immunisation procedure. It is thought that a functional quantitative assay based on antibody capture can be obtained if the microtitre plate coating method is modified to give a lower epitope density whilst retaining coating uniformity. New conjugates in which the haptens ACBT and DCA are bound to BSA via an adipic hydrazide spacing group are under development, and should permit microtitre plates to be coated using more traditional methods known to provide acceptable plate coating uniformity. Further screening of the other MAbs generated during this study is in progress to assess whether any of them have characteristics more desirable than those already observed. The incorporation of the antibodies obtained so far into alternative assay formats will also be examined, in order to obtain functional immunoassays for flucofuron and sulcofuron.

The IVI method conducted during this present study has since been repeated using a different unconjugated pesticide, to further examine the properties of the antibodies obtained in relation to hapten structure, and the prospects of obtaining greater proportions of IgG isotype antibodies. Initial results have shown that this method is also capable of generating anti-permethrin antibodies, many of which have exhibited differing specificities for the two moieties (PBA and CPA) within the permethrin molecule (in preparation). An extension of this IVI method to the generation of antibodies against some common food additives is also in progress. Overall, this method appears suitable for the rapid generation of antibodies against pesticides such as sulcofuron and flucofuron and other toxic low molecular weight compounds, and as a means of minimising laboratory animal suffering.

The mechanism by which the splenocytes were immunised and stimulated into producing the anti-pesticide antibodies was not revealed by these results. It is not clear whether the immune response elicited in vitro was due to the presence of the free pesticides alone, or whether interaction with the other media components was involved. During the IVI procedure both sulcofuron and flucofuron were introduced into the growth medium as free pesticides and it is likely that under such conditions these lipophilic molecules became associated with the proteins present within the growth medium, possibly through hydrophobic interactions. Although covalent bonding had not occurred, the proteins would now act as carrier molecules. This situation would then have similarities with previous observations in which

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the addition of antigenic proteins (scheleppers) to purified Forsmann material permitted an immune response in vivo without covalent bonding (Landsteiner and Forstmann (1928), cited in Leskowitz (1972)).

The majority of the anti-pesticide antibodies secreted by the hybridomas were of the IgM isotype. Increased proportions of hybridomas secreting IgG have been reported where splenocytes have been obtained from pre-immunised mice (in vitro boost, IVB; De Boer et al., 1989), or where carrier-specific T helper hybridomas have been introduced into the splenocyte culture (Schilizzi et al., 1992). In these studies, however, immunisation made use of hapten-carrier conjugates and would therefore not be of use for this IVI method, in which conjugates were not used. Additional work is considered desirable in order to improve the proportions of IgG secretors obtained by this method. This might be obtained simply by an increase in the immunisation period prior to fusion with the myeloma cells, in order to maximise the number of splenocytes in which a class switch to IgG production has occurred.

The advantages of this method are primarily related to the speed and number of hybridomas which can be generated, and the ability to dispense with hapten analogue synthesis and immunogen production. Furthermore, only small (nanogram) quantities of antigen are required which is advantageous where only small amounts have been purified. An additional potential use of this technique is as a rapid source of mRNA for the preparation of recombinant antibodies. This latter approach has been recently examined by us and has provided immunoglobulin heavy and light chains within 3 days of the initial splenocyte immunisation (unpublished observations). It is considered that under ideal conditions, recombinant antibodies could be prepared in under 2 weeks from the initial IVI.

Acknowledgements

This study was conducted as part of United Kingdom National Rivers Authority Research Contract 319, Mothproofing Agents and Water Quality Management. The authors would like to thank the NRA for permission to reproduce these data. Part of the research was supported by a DevR grant to J.H.H. Williams from the Higher Education Funding Council for Wales.

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2006-03-375

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES

2020
Bogotá, DC, 14 de febrero de 2006

Apoderado: Dilia D´Aleman

Oficio: 2338

ASUNTO

Radicación: 0-1451
Trámite 002
Evento 001
Actuación 430
Folios

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

Documentos estudiados:

◆capitulo descriptivo, folios 10 a 118;
◆capitulo reivindicatorio, folios 705 a 746;
◆1° examen de forma, folios 161 a 163;
◆respuesta al 1° examen de forma, folios 167 y 168;
◆2° examen de forma, folio 699;
◆solicitudes de las cuales se reivindica prioridad y la traducción exigida, folios 338 a 698;
◆ solicitud del examen de patentabilidad, folios 701 y 702.
◆Examen Art-46, folios 747 a 749;
◆Respuesta al examen Art-46, folios

Objeto de la invención:

El título de la invención "UNA SAL FARMACEUTICAMENTE ACEPTABLE, DE ACUERDO CON DIFENILUREAS SUSBSTITUIDAS CON ω-CARBOXILO COMO INHIBIDORES DE RAF QUINASA"
Tienen como objeto los compuestos derivados representados con una formula Markush con significados indefinidos.

El campo de la invención, según la Clasificación Internacional de Patentes: A61K 31/40/ 31/17

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Claridad de la solicitud:

En el artículo 30 de la Decisión 486 de la Comisión de la Comunidad Andina se establece: *"Las reivindicaciones definirán la materia que se desea proteger mediante la patente. Deben ser claras y concisas y estar enteramente sustentadas por la descripción".*

Las reivindicaciones 1,con sus relacionadas, 37, 38 con sus relacionadas, no son claras, y no se puede realizar ninguna búsqueda, porque su alcance es ilimitado; las reivindicaciones son excesivamente amplias y especulativas, porque se coloca una formula Markush general y luego a esa formula general, se coloca para cada sustituyentes otra formula Markush, y luego se da a cada porción un significado mediante una característica deseada, basado en un modalismo molecular que cubre cualquier molécula que tenga un núcleo urea sustituido con "cíclicos", porque dicho medelismo no comprenden ninguna porción fija como sustituyente, y además cuando se nombran sustituyentes se hacen de una manera ambigua, designándolos solo por una propiedad que se desea que tenga el sustituyente, tal como por ejemplo "donde L es una estructura cíclica de 5 o $_6$ miembros…", "M es un grupo enlazante que tiene al menos un átomo", "L^1-contiene 0-4miembros del grupo que consiste de nitrógeno, oxígeno y azufre, …", "B una unidad estructural sustituida o no sustituda hasta arilo tricíclico o heteroarilo con al menos una estructura cíclica de $_6$ miembros..", "arilo", "heteroarilo" estas definiciones como propiedades deseables, son términos ambiguos e inaceptables en las reivindicaciones, porque solo con cada sustituyente designado de esa manera ambigua, comprende un número indefinido de estructuras, y si se sigue con las posibles combinaciones por cada uno de los otros sustituyentes, podremos ver cuan especulativo es, y por lo tanto inaceptable en las reivindicaciones. Realmente no hay una estructura representativa de los compuestos reclamados, que se pueda considerar que delimita inequívocamente el alcance de las reivindicaciones, por lo cual no puede ser considerado que satisface el requerimiento de claridad y que este perfectamente definida la materia para la cual se solicita la protección mediante patente, tal como lo establece el artículo 30 de la Decisión 486 de la comisión de la Comunidad Andina.

-Evaluación de la Unidad de Invención:

Esta solicitud de patente comprende más de una invención o un grupo de invenciones no relacionadas entre sí, de manera que se integra un único concepto inventivo conforme al artículo 25 de la Decisión 486 de la Comisión de la Comunidad Andina.

Para el presente caso se conforman múltiples invenciones, de acuerdo al significado concreto que se le de a los grupos sustituyentes de A y B respectivamente, porque el núcleo de urea no es nuevo, y se conforma la unidad inventiva con la o las características técnicas especiales, o sea, las características nuevas e inventivas que se aportan al estado de la técnica y que además se deben conservar en todas las reivindicaciones esas mismas características especiales. Por ejemplo se deben agrupar toda las que B tiene un significado único, concreto e inequívoco, con A variable pero con significados concretos y que estén basados en la descripción. En otro grupo donde el significados de A es único, concreto e inequívoco y el significado de B variable pero con significados concretos y basados en la descripción.

Excepciones de Patentabilidad:

El artículo 20 de la Decisión 486 de la Comisión de la Comunidad Andina establece en su inciso d) que no serán patentables: "*los métodos terapéuticos o quirúrgicos para el tratamiento humano o animal, así como los métodos de diagnóstico aplicados a los seres humanos o a animales*".

Las reivindicaciones 58 a 62 reclaman un método para tratamiento del cuerpo humano o animal, el cual incluye la administración de una composición farmacéutica que contenga algún compuesto de la solicitud, por lo tanto están inmersas bajo el alcance del artículo 20 de la Decisión 486.

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2,literal e), del capítulo quinto del título primero de la circular única No. 10 de 2001.

ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de División de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista, de fecha 02 MAR. 2006

CONCEPTO TÉCNICO No. 240	**EXAMINADOR TÉCNICO**Código No.202012