



No. 03-018359- -00014-0000

Fecha: 2009-05-19 16:31:42 Dep. 2020 NUECREACION
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALI
Act. 444 RESPUESTAREQUE Folios: 6

Señores

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. _____ S. _____ D. _____

Ref: Expediente No. 03.018.359

Solicitud de patente de invención titulada: "17-METILEN ESTEROIDES 4-HALOGENADOS, MÉTODO PARA SU PREPARACIÓN Y COMPOSICIONES FARMACÉUTICAS QUE CONTIENEN ESTOS COMPUESTOS."

Solicitante: SCHERING AKTIENGESELLSCHAFT.

N. Ref.: P2003/000185

RESPUESTA AL OFICIO No. 2158 NOTIFICADO POR FIJACIÓN EN LISTA No. 40,
DE FECHA 20 DE FEBRERO DE 2009
(SEGUNDO EXAMEN DE FONDO)

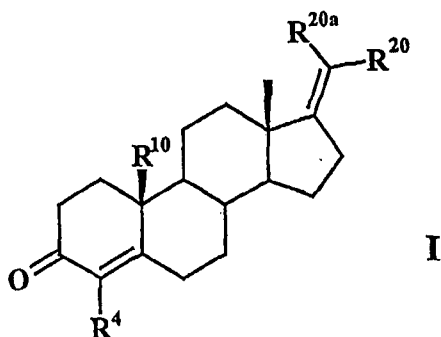
J. IAN RAISBECK, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal previsto en el artículo 45 de la Decisión 486:

1. a- El examinador sostiene en su informe saliente que frente a las enseñanzas de D4, los compuestos de las Reivindicaciones 1 a 5 no son inventivos y en consecuencia tampoco lo son las composiciones de la reivindicación 8 que comprenden dichos compuestos

b- En relación a lo anterior deseo señalar lo siguiente:

El documento D4: XP002187567, 6001 CHEMICAL ABST COLUMBOS, OHIO, US WITTIG REACTION OF STEROID KETONES J.PRAKT CHEM 27(1-2),18-33, 1965, describe la síntesis de compuestos 17-metilen-4-cloro-4-androsten-3-one que corresponden a compuestos de fórmula (I) de la presente invención cuando R20a =R20=H; R4= Cl; R10 =H

256
2



No obstante ello, tal como puede corroborarse de la lectura de reivindicación principal, dichos compuestos han sido específicamente excluidos del alcance de protección de dicha Reivindicación 1.

D4 no describe ni sugiere la substitución de R^{20a} o R^{20} por halógeno o pseudohalógeno o un grupo alquilo. Por lo tanto, basado en las enseñanzas de D4 y el conocimiento general en el campo técnico de la invención, una persona experta con conocimientos medios en la materia no encontraría ninguna motivación para modificar los compuestos de D4: 17-metilen-4-cloro-4-androsten-3-ona, en la dirección de los compuestos definidos en la Reivindicación 1, mediante la substitución de un átomo de H del grupo metileno en la posición 17 por halógeno, pseudohalógeno o un grupo alquilo C1-4 alquilo o un grupo hidroxialquilo.

Por otro lado, el experto en la materia reconoce claramente las diferencias de comportamiento fisicoquímico que existe entre un átomo de hidrógeno y un átomo de halógeno o pseudohalógeno o alquilo. Por ejemplo, un átomo de halógeno es mucho mas electronegativo que un átomo de hidrógeno, esto hace que su substitución tenga un notable influencia sobre la reactividad del resto de la molécula a la cual se une (ver libro de Química Orgánica de Robert Thorton Morrison and Robert Neisol Boyd). Por lo tanto, el comportamiento de una molécula compleja como la de los compuestos reivindicados aquí, y más aún su actividad farmacológica, no puede ser predicha a partir de D4, ya que la substitución de H del grupo metileno por Halógeno, pseudohalógeno o alquilo, no fue divulgada ni sugerida al momento de la presentación de la presente invención. Por lo tanto, la estructura de los compuestos de fórmula (I) de la presente invención no es obvia con respecto a la estructura de los compuestos divulgados por D4. Mas aún, la actividad híbrida que exhiben los compuestos reivindicados como inhibidor de 5-alfa reductasa y como gestágeno tampoco ha sido descrita o sugerida por D4.

Los datos experimentales que se muestran en la Tabla 1 y Tabla 2 demuestran este comportamiento híbrido de los compuestos de la presente invención.

TABLA 1

Actividad tipo 2 5[alfa]-Reductasa en un homogenato dérmico genital,
bajo condiciones óptimas a pH 5.5 (IC50 (nM))
Receptor de Progesterona unido a citosol de utero de conejo, cebado
Trazador: 3H-ORG 2058/ condiciones de incubación 0-4°C.; 18 horas
Sustancia de referencia: progesterona= 100%

Compuesto	IC50 (nM)	PR (Progesterona = 100%)
(I) E-17-Clorometileno-4-chloro-estr-4-en-3-one	250	60

TABLA 2

Datos in-vivo de la actividad gestagénica
Test de mantenimiento de la preñez de ratón después de la administración s.c.(subcutanea)

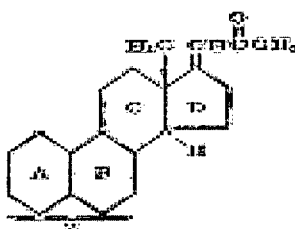
compuesto	dosage (mg/animal/dia) (s.c)	velocidad de preñez alcanzada
(I) E-17-Clorometilen-4-chloro-estr-4-en-3-ona	1.0 0.1	5/5 0/5

Los datos presentados en la Tabla 1 y Tabla 2 confirman el perfil de acción híbrida de los compuestos de acuerdo con la presente invención que actúan como inhibidores de 5[alfa]-reductasa y gestageno.

2. a- El Examinador a cargo cita los documentos D3 y D2 como afectando la actividad inventiva de las Reivindicaciones de procedimiento 6 y 7.

b- Al respecto deseo señalar lo siguiente:

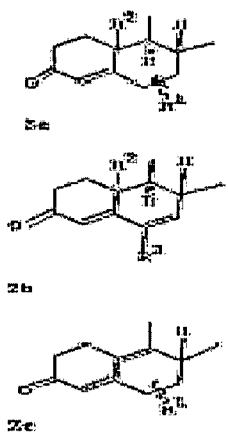
D3: US 3661940 describe un compuesto esteroide, mas particularmente, derivados de 21-metil-19-norpregnano. De acuerdo con esta invención, los compuestos de fórmula (I) poseen la siguiente formula:



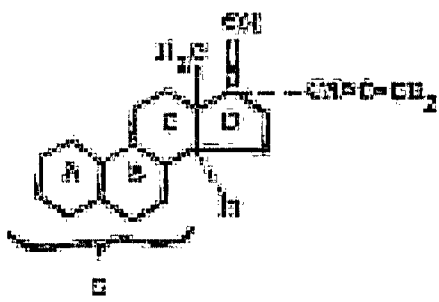
258

V

donde Z involucra los anillos A y B y los sustituyentes sobre el mismo representan:

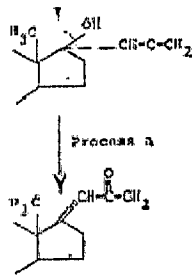


Los compuestos de Fórmula I se obtienen por medio de un tratamiento ácido (el proceso para un compuesto II, por ejemplo, corresponde a un compuesto 17alfa-propadienil-17beta-hidroxi esteroide)



Dicho tratamiento ácido (proceso (a)) puede ser llevado a cabo empleando como fuente ácida un agente de protonación fuerte en presencia de un agente contribuyente de anion hidroxi, aciloxi inferior o alcoxi inferior en un medio adecuado, a temperatura moderada, por ejemplo 10 a 100 °C preferiblemente a temperaturas de entre 15 a 35 °C. El agente contribuyente es líquido bajo las condiciones del proceso, y podría ser usado en exceso para servir como medio.

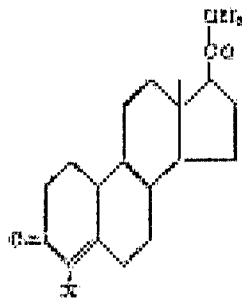
El agente de protonación fuerte incluye un ácido mineral, tal como HCl, o ácido sulfúrico. El proceso (a) se describe en la columna 6 del documento D3 como sigue:



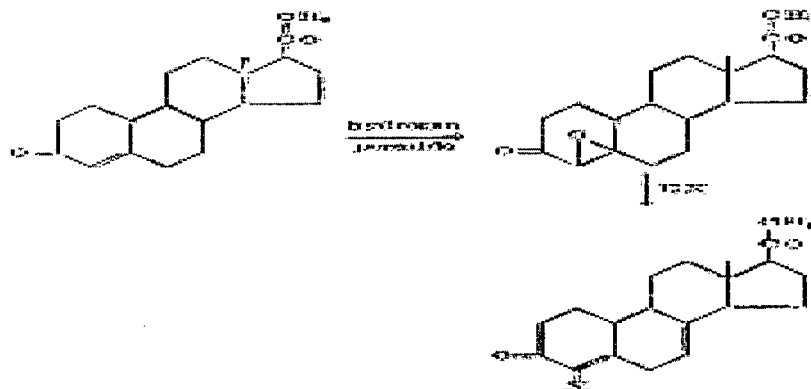
Estos compuestos de Fórmula (I) son útiles como agentes de control de la fertilidad en animales.

Por lo tanto, D3 describe sólo ciertos parámetros operativos del primer paso del procedimiento definido en las reivindicaciones de procedimiento 6 y 7, sin embargo este procedimiento no describe ni sugiere los compuestos de partida ni los compuestos finales obtenidos por medio de los procesos reivindicados en la presente invención.

El documento D2: US 3.146.239, describe compuestos de fórmula (I)



donde X representa cloro o bromo. Dichos compuestos son obtenidos por medio del siguiente proceso:



El compuesto 19- nor-progesterona, se hace reaccionar con peróxido de hidrógeno en presencia de una base tal como hidróxido de sodio. El 4,5-epóxido obtenido es disuelto en acetona (solvente aprótico dipolar), la solución es enfriada y se adiciona un agente nucleofílico tal como ácido clorhídrico concentrado. La mezcla es mantenida a temperatura ambiente y luego esparcida dentro

de una solución acuosa saturada de cloruro de sodio, luego es enfriada y posteriormente se recogen los cristales precipitados.

Por lo tanto, D2 describe la etapa de epoxidación y la apertura del anillo epoxídico con un agente nucleofílico, la cual es empleada como la segunda etapa del proceso definido en las reivindicaciones 6 y 7. Sin embargo, los materiales de partida así como los compuestos finales obtenidos son diferentes a los compuestos de partida y finales del proceso descrito por D2.

Por lo tanto, teniendo en cuenta que la estructura de los compuestos de fórmula (I) definidos en las Reivindicaciones 1 a 5, es nueva e inventiva, tal como se demuestra precedentemente, el proceso para su producción de acuerdo con las Reivindicaciones 6 y 7 también debe ser considerado nuevo e inventivo, toda vez que una reivindicación de proceso debe ser definido por el conjunto de etapas y parámetros operativos que obviamente incluyen los materiales de partida así como los productos finales obtenidos.

Por lo tanto, considero que con los argumentos aquí expuestos ha quedado demostrado el nivel inventivo de la presente invención por sobre las enseñanzas del arte previo citado.

3. Sin perjuicio de lo antes mencionado, deseamos señalar que la invención que aquí se reivindica fue concedida por la Oficina de Patentes de los Estados Unidos como patente número US 6.855.836 B2 (15/02/2005).

4. Por todo lo anterior, considero que las objeciones presentadas por el Examinador en su examen de fondo No.02457 han sido resueltas y por consiguiente solicito respetuosamente que el Despacho tengan por cumplidos los requisitos de patentabilidad, en tanto no se logró desvirtuar ninguno de ellos por parte del Despacho, y se proceda con la concesión de la patente.

Atentamente,



J. IAN RAISBECK

C.C. 79'376.996 de Bogotá D.C.

T.P. 135.799 del C. S. de la J.

Bioisosterism: A Rational Approach in Drug Design

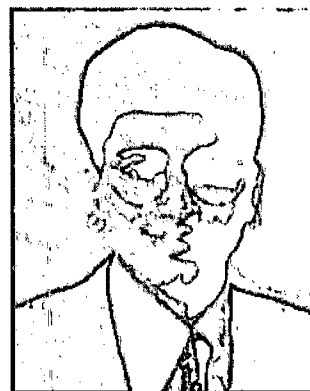
George A. Patani and Edmond J. LaVoie*

Department of Pharmaceutical Chemistry, College of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08855-0789

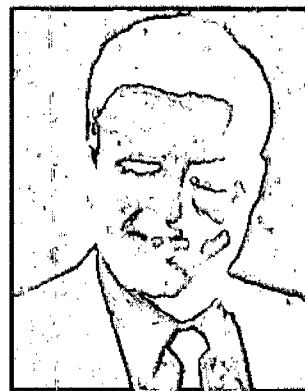
Received May 15, 1996 (Revised Manuscript Received July 25, 1996)

Contents

I. Introduction	3147
II. Classical Bioisosteres	3149
A. Monovalent Atoms or Groups	3149
1. Fluorine vs Hydrogen Replacements	3149
2. Interchange of Hydroxyl and Amino Groups	3150
3. Interchange of Hydroxyl and Thiol Groups	3151
4. Fluorine and Hydroxyl, Amino, or Methyl Groups as Replacements for Hydrogen (Grimm's Hydride Displacement Law)	3152
5. Monovalent Substitutions Involving Chloro, Bromo, Thiol, and Hydroxyl Groups (Erlenmeyer's Broadened Classification of Grimm's Displacement Law)	3154
B. Divalent Isosteres	3155
1. Divalent Replacements Involving Double Bonds	3155
2. Divalent Replacements Involving Two Single Bonds	3155
C. Trivalent Atoms or Groups	3156
D. Tetrasubstituted Atoms	3157
E. Ring Equivalents	3158
1. Divalent Ring Equivalents	3158
2. Trivalent Ring Equivalents	3159
III. Nonclassical Bioisosteres	3160
A. Cyclic vs Noncyclic Nonclassical Bioisosteric Replacements	3160
B. Nonclassical Bioisosteric Replacements of Functional Groups	3165
1. Hydroxyl Group Bioisosteres	3165
2. Carbonyl Group Bioisosteres	3166
3. Carboxylate Group Bioisosteres	3168
4. Amide Group Bioisosteres	3170
5. Thiourea Bioisosteres	3171
6. Halogen Bioisosteres	3172
IV. Conclusion	3172
V. Acknowledgments	3172
VI. References	3172



George Patani graduated with a B.Pharm. in 1992 from the College of Pharmaceutical Sciences, Mangalore University at Manipal, India. In 1996, he received his M.S. in Pharmaceutical Science at Rutgers University under the direction of Professor Edmond J. LaVoie. He is presently pursuing graduate studies in pharmaceuticals. His current research interests are focused on drug design and controlled drug delivery.



Edmond J. LaVoie received his B.S. in Chemistry from Fordham University in 1971 and his Ph.D. in Medicinal Chemistry from S.U.N.Y. at Buffalo under the direction of Dr. Wayne K. Anderson. After postdoctoral study with Dr. S. Morris Kupchan at the University of Virginia, he joined the American Health Foundation in Valhalla, NY. In 1988, he was appointed Professor of Medicinal Chemistry in the College of Pharmacy at Rutgers University. His current research interests are in the design and synthesis of cancer chemotherapeutics and in the elucidation of mechanism(s) of carcinogenesis.

I. Introduction

Years of cumulative research can result in the development of a clinically useful drug, providing either a cure for a particular disease or symptomatic relief from a physiological disorder. A lead compound with a desired pharmacological activity may have associated with it undesirable side effects, characteristics that limit its bioavailability, or structural features which adversely influence its metabolism and excretion from the body. Bioisosterism represents one approach used by the medicinal chemist

for the rational modification of lead compounds into safer and more clinically effective agents. The concept of bioisosterism is often considered to be qualitative and intuitive.¹

The prevalence of the use of bioisosteric replacements in drug design need not be emphasized. This topic has been reviewed in previous years.²⁻⁵ The objective of this review is to provide an overview of bioisosteres that incorporates sufficient detail to enable the reader to understand the concepts being delineated. While a few popular examples of the successful use of bioisosteres have been included, the

trienes produced by the 5-lipoxygenase enzyme pathway may also contribute to both inflammation and NSAID-induced effects. Table 8 summarizes the concentrations of test compounds required to cause a 50% inhibition of 5-LO and CO formation.

Replacement of the hydroxyl with an amino group resulted in more potent activity toward 5-LO while the potency toward CO remained the same. However, replacement with a thiol resulted in enhanced potency toward both 5-LO and CO. Comparison of the electronegativity values of oxygen, nitrogen and sulphur (Table 8) suggests that this could be a factor that modulates the degree of inhibition of 5-LO. Size, however, may play a significant role with regard to inhibition of CO (Table 7). Thus, the thiol group may be a suitable and informative bioisostere for the amino and hydroxyl groups in several different series of medicinal agents by the virtue of its size, lower electronegativity, and ability to hydrogen bond.

4. Fluorine and Hydroxyl, Amino, or Methyl Groups as Replacements for Hydrogen (Grimm's Hydride Displacement Law)

This monovalent group of isosteres is a result of the direct adaptation of Grimm's Hydride Displacement Law. The basis for the fluorine-hydrogen interchange and the hydroxyl-amino interchange was discussed previously. The existence of this larger group of isosteres might be attributable to a greater tolerance of the different physicochemical parameters of these functionalities within a particular series of agents. However, in the studies outlined in this section, an attempt was made to correlate a physicochemical parameter of this group of bioisosteres with the observed effect on biological activity.

In designing agents for the treatment of cardiovascular diseases, it may be beneficial to associate the hypotensive effects resulting from the inhibition of angiotensin II formation with the diuretic and natriuretic responses. Diuretic and natriuretic effects can be mediated by protection of the endogenous atrial natriuretic peptide (ANP) from inactivation by inhibition of epithelial neutral endopeptidase (NEP). Inhibition of angiotensin II formation may be brought about by inhibition of endothelial angiotensin-converting enzyme (ACE). A series of dual metalloproteinase inhibitors have been designed on the basis of the characteristics of the active sites of both enzymes. Monovalent substitution by fluorine, hydroxyl, and amino in place of hydrogen has recently been used in the design of these metalloproteinase inhibitors (Figure 11, Table 9).³⁰

In this study optically pure *N*-[2-(mercaptomethyl)-3-phenylbutanoyl] amino acids (**17**) were evaluated as dual inhibitors of NEP and ACE. Substitution with isosteres (-F, -OH, -NH₂) as described by Grimm's Hydride Displacement Law (Table 2) resulted in retention of activity. It was observed within this series, however, that the increase in the effective van der Waal's radii of the isosteric substituents resulted in a decrease in activity (Table 9). In this instance, no significant alteration in preferential activity with either of the peptidases, ACE or NEP, was observed for these bioisosteres.

The empirical approach used to advance the structure-activity relationships with these peptidase

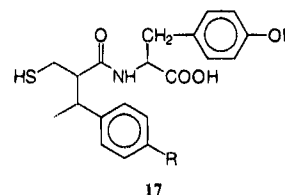


Figure 11.

Table 9. *In Vitro* Inhibition of NEP and ACE by *N*-[2-(Mercaptomethyl)-3-phenylbutanoyl] Amino Acids

compound	R	effective van der Waal's radii (Å) ³¹	IC ₅₀ (nM)	
			angiotensin converting enzyme	neutral endopeptidase
17a	H	1.20	4.3	2.5
17b	F	1.47	6.9	5.9
17c	OH	1.53	7.9	9.0
17d	NH ₂	1.79	12.0	16.0

inhibitors is useful despite the fact that a more selective ACE inhibitor was not developed. Retention of activity within this series of bioisosteres permits an assessment of the validity of a possible correlation with one or more specific physicochemical parameters. This study, for example, did provide insight into structural features which were critical to their activity as inhibitors of these peptidases.

Recently, several 8-substituted *O*⁶-benzyl guanines (**18**, Figure 12) were evaluated for their ability to inactivate the human DNA repair protein, *O*⁶-alkylguanine-DNA alkyltransferase (AGT) (Table 10).³² Inactivation of the human DNA repair protein *O*⁶-alkylguanine-DNA alkyltransferase by exposure to compounds such as *O*⁶-benzylguanine leads to a dramatic enhancement in the cytotoxic response of human tumor cells and tumor xenografts to chemotherapeutic drugs. This effect is principally observed for chemotherapeutic agents whose mechanism of action involves modification of DNA guanine residues at the *O*⁶-position. In this study the effect of the interchange of NH₂, OH, as well as CF₃ (a bioisostere for a methyl group based on the replacement of hydrogen with fluorine) on activity was assessed. Analogues possessing electronegative groups at the 8-position were more effective as inactivators of AGT in human HT29 colon tumor cell extracts. The relative activities of these bioisosteres based on the dose required for 50% inhibition (ED₅₀) along with their electronegativities are outlined in Table 10.

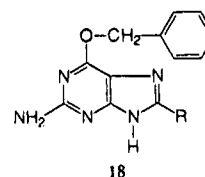


Figure 12.

Table 10. Alkyl Guanine Transferase Inactivating Activity of 6-(Benzoyloxy)purine Derivatives

compound	R	electronegativity ²⁹	ED ₅₀ (μM) ^a
18a	NH ₂	2.61	2.0
18b	CF ₃	3.46	0.25
18c	OH	3.51	0.15

^a Effective dose required to produce 50% inactivation in HT-29 cells upon incubation for 4 h.

Again, the retention of activity within a series of bioisosteres provides the basis for the discovery of a possible correlation between pharmacological activity and the physicochemical properties of specific agents.

As an extension to the above group defined by Grimm's Hydride Displacement Law, the widespread use of the chlorine atom as a bioisostere has been observed in several different series of biologically active compounds. This could be attributed to the similarity in size between these atoms, a comparison of which is made in Table 11. Further, there exists similarity in the lipophilicity of the methyl group with that of chlorine which may be responsible for its suitability as a monovalent bioisosteric replacement.

N-(Substituted-3-pyridyl)-*N'*-alkylthioureas (**19**, Figure 13), which have been evaluated as novel potassium channel openers,³³ are among the more recent illustrations of the replacement of chlorine with isosteres from Grimm's Hydride Displacement Law (Table 11). Potassium channel openers cause vasorelaxation in vascular smooth muscle through hyperpolarization of the cell membrane. There is an increased interest in these compounds based on their therapeutic potential in the treatment of cardiovascular diseases. Substitution at the 6-position with monovalent isosteres ($-\text{NH}_2$, $-\text{CH}_3$, $-\text{Cl}$) results in analogues with similar biological activity. It was observed that substituents with similar biological activity had comparable effective van der Waal's radii (Table 11). The methyl group, which has a lower electronegativity, elicited a weaker pharmacological response, suggesting an additional correlation between activity and a physicochemical property.

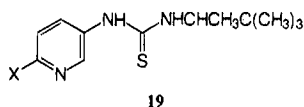


Figure 13.

Table 11. Inhibition of Spontaneous Mechanical Activity in Rat Portal Vein (*in vitro*)

compound	X	maximum fall in SBP ^a (%)	electro-negativity ²⁹	effective van der Waal's radii ³¹ (Å)
19a	NH ₂	29	2.61	1.79
19b	CH ₃	18	2.27	1.80
19c	Cl	27	3.0	1.73

^a Antihypertensive activity measured as maximum % fall in systolic blood pressure in anesthetized normotensive rat by iv injection.

Table 12 lists the relative potency of a group of bioisosteres which act as inhibitors of thymidylate synthase. Each of these benzo[*f*]quinazolin-1(2*H*)-ones (**20**, Figure 14), inhibit thymidylate synthase by virtue of their structural relation to its cofactor, 5,10-methylenetetrahydrofolic acid.³⁴ They are, therefore, referred to as folate-based thymidylate synthase inhibitors. These analogues differ from other folate-based thymidylate synthase inhibitors as the absence of a glutamate residue suggests that they are not dependent upon active folate transport and polyglutamylation for activity, the two mechanisms of resistance that have been observed with agents such

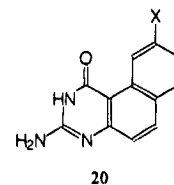


Figure 14.

Table 12. Thymidylate Synthase Enzyme Inhibition Data for Benzo[*f*]quinazolin-1(2*H*)-ones

compound	X	thymidylate synthase inhibitory activity IC ₅₀ (μM) ^a
20a	Cl	0.025
20b	CH ₃	0.178
20c	OH	0.48
20d	NH ₂	0.63
20e	H	1.08

^a Inhibitor concentration producing 50% inhibition and determined by the tritium release assay of Roberts³⁵ as modified by Dev et al.³⁶

as methotrexate. Within this series, it was observed that hydrogen bond donors were more potent than the unsubstituted parent compound. These analogues, however, were less active than compact lipophilic groups in elevating thymidylate synthase inhibition. Thus, at the 9-position, optimal size and lipophilicity appear to be critical factors associated with their ability to inhibit thymidylate synthase.

In another study aimed at designing cholinergic agents which would be capable of penetrating the central nervous system and displaying high efficacy at the cortical muscarinic receptors, a series of oxadiazole-based tertiary amines **21** (Figure 15, Table 13) were tested. The assay used was designed to measure affinity and predict cortical efficacy from the antagonist-agonist (i.e. NMS/OXO-M) binding ratio in rat cortical membranes.³⁷ The log of this ratio has been shown to correlate with the ability of the ligand to stimulate the hydrolysis of cortical phosphatidylinositol. It is known that at least three muscarinic receptor subtypes m1, m3, and m5 may be capable of positively stimulating phosphatidylinositol hydrolysis. In this series it was observed that replace-

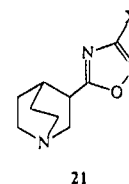


Figure 15.

Table 13. *In Vitro* Binding Data for Substituted Oxadiazoles

compound	X	<i>K_a</i> (app) (μM)		ratio ^c
		[³ H]NMS ^a	[³ H]OXO-M ^b	
21a	CH ₃	0.44	0.0009	490
21b	Cl	0.19	0.00048	400
21c	NH ₂	0.60	0.0005	1200

^a Displacement of [³H]-*N*-methylscopolamine. ^b Displacement of [³H]oxotremorine-M. ^c Ratio of *K_a*(app) for NMS to *K_a*(app) for OXO-M.

ment of the methyl with the less lipophilic amino group resulted in an increase in this antagonist-agonist binding ratio. However, replacement with a chloro substituent which is isosteric and isolipophilic to the methyl group resulted in a compound which had lower binding affinity, but a very similar antagonist-agonist binding compared to the methyl-substituted analogue.

While the chlorine atom is often viewed to be isosteric and isolipophilic with the methyl group, it is very often selected as a bioisosteric replacement because of its ability to alter the metabolism. Previous research has very effectively illustrated the feasibility of altering the metabolism of an agent by the interchange of chlorine with one of the bioisosteres from Grimm's Hydride Displacement Law. Replacement of a chloro atom with a methyl substituent can facilitate metabolism of a xenobiotic. Lipid-soluble chemicals tend to be distributed into adipose tissue where, unless they are metabolized, they tend to accumulate for long periods of time, e.g. DDT (22, Figure 16). The replacement of the trichloromethyl moiety with a *tert*-butyl group (23, Figure 16) results in diminished persistence of this pesticide.³⁸ The methyl substituents provide a site which is susceptible to metabolic degradation.

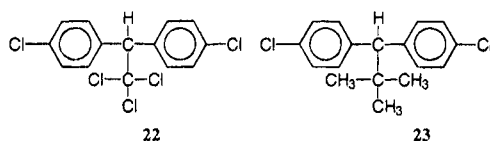


Figure 16.

Alternatively, the addition of a chloro substituent can be effective in inhibiting metabolic oxidation. Phenobarbital (24) is metabolized by aromatic hydroxylation at the *para* position to produce 25 (Figure 17), which then forms a glucuronide conjugate. Replacement of the hydrogen at the *para* position with chlorine (26) prevents this route of metabolism. Thus, in this instance the chlorine atom blocks the metabolism of phenobarbital and thereby increases its duration of action.

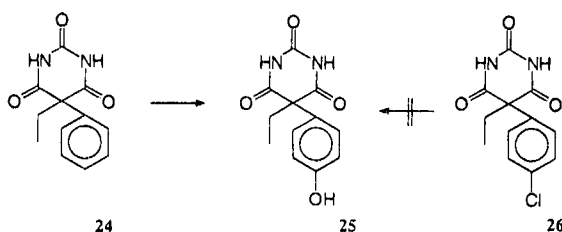


Figure 17.

2-(Diethylamino)ethyl benzoates (27, Figure 18), which are used as local anesthetics provide yet another example where classical bioisosteric replacement affects the observed duration of action by altering metabolism.³⁹ On the basis of the results outlined in Table 14, it appears that the decreased half-lives ($t_{1/2}$) associated with those analogues having electron-withdrawing groups is likely associated with their increased susceptibility to hydrolysis.

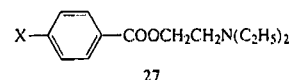


Figure 18.

Table 14. Biological Half-Lives of *Para*-Substituted 2-(Diethylamino)ethyl Benzoates

compound	X	σ_p	$t_{1/2}$
27a	NH ₂	-0.66	96.29
27b	OCH ₃	-0.26	26.75
27c	Cl	0.23	4.94
27d	CN	0.63	1.28

5. Monovalent Substitutions Involving Chloro, Bromo, Thiol, and Hydroxyl Groups (Erlenmeyer's Broadened Classification of Grimm's Displacement Law)

The last class of monovalent isosteres involves chloro, bromo, thiol, and hydroxyl group interchanges. While we have already rationalized the hydroxyl-thiol interchanges, the classification of the chloro, bromo and thiol group together is based on their similarity in their number of peripheral electrons as defined by Erlenmeyer. Use of these monovalent isosteric replacements is illustrated for certain C8-substituted guanosine analogues (28, Figure 19). To evaluate the efficacy of C8-substituted guanosine analogues in the enhancement of primary antibody response *in vitro*, these analogues were cultured with antigen and adjuvant.⁴⁰ B lymphocytes were incubated with 1.0 mM of the guanosine analogues in presence or absence of type 2 antigen TNP-Ficoll and after 3 days the cells were processed, collected, and then evaluated for the number of antibody-forming cells. The results of this study demonstrated that the bromo-, hydroxyl-, and thio-substituted analogues could stimulate polyclonal immunoglobulin secretion and in the presence of antigen enhanced the magnitude of the anti-TNP plaque-forming cell (Anti-TNP PFC) antibody response to antigen TNP-Ficoll as summarized in Table 15. The thiol bioisostere was more potent as compared to the hydroxyl and the bromo isosteres.

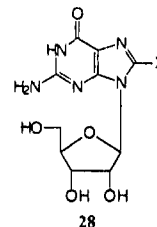


Figure 19.

Table 15. Enhancement of Antibody Responses of DBA/2 B Cells by Guanosine Analogs

compound	X	antigens: no. of anti TNP plaque-forming cells/culture	
		medium	TNP-Ficoll
28a	medium	14	8
28b	medium + Br analogue	78	198
28c	medium + OH analogue	118	200
28d	medium + SH analogue	80	296

Dihydrotestosterone is a potent androgen known to be essential for male differentiation, growth, and function of androgen-sensitive sex organs and is involved in the pathophysiology of many diseases