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EDIFICIO SISKI
CARRERA 4 No. 72 - 35
BOGOTÁ 8, COLOMBIA

Teléfono: (57-1) 347 3611
Telefax: (57-1) 211 8650
(57-1) 700 4814
Fax in U.S.A. + 1 205 675 7743

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-090848- -00007-0000

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Señor Director
DIRECCIÓN DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Trámite : 011
Evento : 378
Actuación : 444

Referencia : Expediente No. **07.090.848**
Asunto : Solicitud de patente para: **COMPUESTOS QUE
COMPRENDEN PORCIONES HETEROARILLO LIGADAS Y
SU USO COMO DEGUSTANTES, MODIFICADORES DE
SABOR UMAMI NOVEDOSOS Y MEJORADORES DE
GUSTO PARA COMPOSICIONES COMESTIBLES**
Solicitante : SENOMYX INCORPORATED
Fecha solicitud : 04 de septiembre de 2007

1. Yo, Luz Clemencia DE PÁEZ, en mi condición de apoderada de la sociedad solicitante de la patente de la referencia, de conformidad con la sustitución de poder otorgada a mí por el doctor Emilio FERRERO me refiero al oficio No. **7252** notificado por fijación en lista del 09 de MAYO de 2012.

2. Mediante oficio No. **7252** el Examinador a cargo del estudio de patentabilidad de la presente solicitud, en virtud del artículo 46 de la Decisión 486, solicitó la presentación de los siguientes documentos:

2.1.1 CLARK R. L et Al: "SYNTHESIS AND ANALGESIC ACTIVITY OF 1,3-DIHYDRO-3-(SUBSTITUTED PHENYL)IMIDAZOL 4,5-BUPYRIDIN-2-ONES AND 3-(SUBSTITUTED PHENYL)-1,2,3-TRIAZOL 4,5-BÜPYRIDINES". JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CUEMICAL SOCIETY, US, vol. 21, no. 9, 1978, pages 965-978, XP001191133 ISSN 0022-2623.

2.1.2 BIAGI G. et Al: "N⁶-Cycloalkyl-2-phriyl-3-deaza-8-azaadenines: a new class of Al adenosine receptor ligands. A comparison with the corresponding adenines and 8-

azaadenines". EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, EDITIONS SCIENTIFIQUE ELSEVIER, PARIS, FR, vol. 38, no. 11-12, November 2003 (2003-11), pages 983-990, XP004475843 ISSN: 0223-5234.

2.1.3 HUANG D. et Al: "2-(2-[3-(Pyridin-3-yloxy)phenyl]-2H-tetrazol-5-yl}pyridine: a highly potent, orally active, metabotropic glutamate subtype 5-(mGlu5) receptor antagonist". BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 14, no. 22, 15 November 2004 (2004-11-15), pages 5473- 5476, XP004598576, ISSN: 0960-894K.

2.1.4 PERNAK J. et Al: "ACTIVITY OF NEW QUATERNARY AMMONIUM COMPOUNDS ON STRAINS OF BACTERIA AND FUNGI. PART 5: SYNTHESIS OF 3-METHYL-N-ALKYLTHIOMETHYLPYRIDINE-, 1-METHYL-3-N-ALKYLTHIOMETHYLIMIDAZOLE- AND 1-ETHYL-3-N-ALKYLTHIOLIMIDAZOLINE CHLORIDES / WIRKUNG NEUER QUARTAERER AMMONIUMVERBINDUNGEN AUF BAKTERLEN AND PILZE. T" PHARMAZIE, DIE, GOVI VERLAG, ESCHBORN, DE, vol. 38, no. 11,1983, pages 752-754, XP008069005 , ISSN: 0031-7144.

2.1.5 EVANGELISTA 5. et Al: "SINTESI ED ATTIVITA ANTIULCERA DI ALCUNI NUOVI COMPOSTI A STRUTTURA ARILTOMETIL-PIRIDINICA/SYNTHESIS AND ANTI-ULCER ACTIVITY OF SOME NEW COMPOUNDS WITH ARYLTHIOMETHYL-PYRIDINE STRUCTURE" FARMACO, EDIZIONE SCIENTIFICA, SQCIETA CHIMICA ITALIANA, PAVIA, IT, vol. 43, no. 11, November 1988 (1988-11), pagas 901-908, XP008068997, ISSN: 0430-092.0.

3. Respuesta al requerimiento

Con el ánimo de dar respuesta al requerimiento formulado por el Examinador, se presenta junto con el presente memorial, copia de los cinco documentos arriba mencionados.

4. Puesto que se han atendido a cabalidad las observaciones hechas por su Despacho sobre la presente solicitud de patente, atentamente solicito que se continúe su tramitación.

5. Anexos

- Documento: CLARK R. L et Al: "synthesis and analgesic activity of 1,3-dihydro-3-(substituted phenyl)imidazol 4,5-bupyrudin-2-ones and 3-(substituted phenyl)-1,2,3-triazol 4,5-büpyridines"

- Documento: BIAGI G. et Al: "N⁶-Cycloalkyl-2-phriyl-3-deaza-8-azaadenines: a new class of Al adenosine receptor ligands. A comparison with the corresponding adenines and 8-azaadenines"
- Documento: HUANG D. et Al: "2-(2-[3-(Pyridin-3-yloxy)phenyl]-2H-tetrazol-5-yl}pyridine: a highly potent, orally active, metabotropic glutamate subtype 5-(mGlu5) receptor antagonist"
- Documento: PERNAK J. et Al: "Activity of new quaternary ammonium compounds on strains of bacteria and fungi. part 5: synthesis of 3-methyl-n-alkylthiomethylpyridine-, 1-methyl-3-n-alkylthiomethylimidazole- and 1-ethyl-3-n-alkylthiolimidazoline chlorides/wirkung neuer quartaerer ammoniumverbindungen auf bakterlen and pilze"
- Documento: EVANGELISTA 5. et Al: "Sintesi ed attivita antiulcera dl alcuni nuovi composti a struttura ariltiometil-piridinica/synthesis and anti-ulcer activity of some new compounds with arylthiomethyl-pyridine structure"
- Copia simple de la sustitución del poder otorgado por el Doctor EMILIO FERRERO, a mi favor, para lo cual solicito me sea reconocida personería y así continuar con el trámite de este asunto en favor de la sociedad solicitante.

Señor Director,


LUZ CLEMENCIA DE PÁEZ
C.C. 35.456.344 de Usaquén
T.P.A. No. 23.555

✓ COLO-15159-841-004-2
JMS\JMS\ID-222875\WPC15159
No. M-2012-222875\JMS

Synthesis and Analgesic Activity of 1,3-Dihydro-3-(substituted phenyl)imidazo[4,5-*b*]pyridin-2-ones and 3-(Substituted phenyl)-1,2,3-triazolo[4,5-*b*]pyridines

Robert L. Clark, Arsenio A. Pessolano, Tsung-Ying Shen,* David P. Jacobus, Howard Jones,

Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065

Victor J. Lotti, and Lars M. Flataker

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486. Received June 13, 1977

In a study of nonsteroidal antiinflammatory and analgesic agents, a series of 1,3-dihydro-3-(substituted phenyl)imidazo[4,5-*b*]pyridin-2-ones and 3-(substituted phenyl)triazolo[4,5-*b*]pyridines was prepared. Many of the imidazolones were alkylated on the free nitrogen. In a modified Randall-Selitto analgesic assay, the pain thresholds of both the inflamed and normal foot were elevated. This is not commonly observed with nonsteroidal antiinflammatory agents. The most active compounds were 1,3-dihydro-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-15) and its *N*-allyl (I-21) and *N*-isopropyl (I-121) derivatives. In the triazole series the 3-(2-fluoro- and 2,4-difluorophenyl)triazolo[4,5-*b*]pyridines (T-1 and T-8) were the best. The imidazole compounds were somewhat superior in analgesic activity to codeine and *d*-propoxyphene without showing any narcotic characteristics. Some of the compounds also possessed activity against carrageenan-induced foot edema in the rat, so these compounds represent a new class of nonnarcotic analgesic antiinflammatories, capable of producing a greater degree of analgesia than that obtainable with other nonsteroidal antiinflammatory agents.

In our continuing study of nonacidic antiinflammatory agents¹ it was found that several 1,3-dihydro-3-(substituted phenyl)imidazo[4,5-*b*]pyridin-2-ones and 3-(substituted phenyl)triazolo[4,5-*b*]pyridines possessed interesting analgesic activity in a modified Randall-Selitto assay. The pain thresholds of both inflamed and normal feet were elevated. The analgetic effect on the normal foot is not commonly observed with nonsteroidal antiinflammatory agents, whose peripheral analgesic actions are usually limited to the inflamed foot only.² Since nonacidic antiinflammatory agents are generally less irritating in the gastrointestinal tract,³ the potential utility of these two series of compounds was investigated.

Previously, a group of 1,3-dihydro-1-aminoalkyl-3-phenylimidazo[4,5-*b*]pyridin-2-ones was studied by Ciba Geigy⁴ as antidepressant agents. 1,3-Dihydroimidazo[4,5-*b*]pyridin-2-ones with substituents on the pyridine ring were reported by American Cyanamid,⁵ Shell,⁶ and Deutsche Gold and Silber Scheideanstalt⁷ to possess antiphlogistic activity or have postemergence applications on broad leaved plants. A number of chemical investigations⁸ have been made of the reaction of a 2,3-diaminopyridine and ethyl acetoacetate. One of the products is a 1,3-dihydroimidazo[4,5-*b*]pyridin-2-one. In the triazolopyridine series, 3-phenyl-5-chlorotriazolo[4,5-*b*]pyridine was used by Glaxo⁹ as an intermediate in the preparation of α -carbolines.

About 200 compounds in the two series were prepared and each compound was tested for its analgesic activity. Common intermediates can be used for both series. They are 2-(substituted phenylamino)-3-nitropyridines, 2. These were prepared from the appropriate aniline and 2-chloro-3-nitropyridine. These reactions were carried out under various conditions. A mixture of 2-fluoroaniline and 2-chloro-3-nitropyridine reacted exothermally after it was warmed on a steam bath. Other reactants, such as 4-aminoacetanilide and 2-chloro-3-nitropyridine, required heating to 190 °C to initiate the reaction. Some reactions, such as 3,4-(methylenedioxy)aniline with 2-chloro-3-nitropyridine, were controlled better if the reactants were heated in acetic acid in the presence of sodium acetate. The vigorous reaction between 3,4-(methylenedioxy)aniline and 2-chloro-3,5-dinitropyridine was controlled by first diluting the aniline with methanol and then adding the

2-chloro-3,5-dinitropyridine at room temperature. The product crystallized almost immediately. In other cases the reactants were refluxed in pyridine or dimethylformamide. Other variations involved the use of 2-chloronitrobenzene or 2,4-dichloro-5-nitropyrimidine instead of 2-chloro-3-nitropyridine. These reacted similarly to the pyridine. A few benzylamines were used instead of aniline; they were especially reactive and the reactants needed to be diluted with a solvent such as benzene to control the reaction (Tables I-III).

Most of the nitro compounds, 2, were catalytically hydrogenated to give the 2,3-diaminopyridines. In many cases this diamine, 3, was unstable, so it was not characterized but immediately used in the next step. With a 3,5-dinitropyridine the 3-nitro group was reduced with hydrogen sulfide in an ammoniacal solution. Hydrogenation of the 2-chloro-5-nitropyrimidine removed the chlorine atom as well as reducing the nitro group.

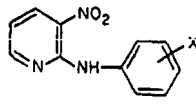
The most convenient method of converting the diamine, 3, to the imidazo[4,5-*b*]pyridin-2-one, 4, was through reaction with phosgene. In some cases this was not satisfactory and the diamine was heated with urea to give the desired product. Potassium ethyl xanthate was used to give the corresponding thio compound, 7.

The free nitrogen of the imidazo[4,5-*b*]pyridin-2-one was alkylated with alkyl halide in refluxing acetone solution in the presence of powdered potassium hydroxide according to the method of Pachter and Kloetzel.¹⁰ In the case of imidazo[4,5-*b*]pyridine-2-thione (I-24), the alkylation occurred on the sulfur and not on the nitrogen. Alkylation of I-15 with *tert*-butyl bromide gave low yields of a mixture of *O*- and *N*-alkylated products (I-130 and I-131). The chemical preparation of these compounds is depicted in Scheme I.

Discussion

The analgesic assay is essentially the one described by Winter and Flataker.^{11a} One foot of a Sprague-Dawley female rat is made sensitive by an injection of brewer's yeast, and the response threshold was determined by applying pressure to each foot and reading on a manometer the pressure at which an audible "squeak" was elicited. For the initial evaluation, three rats are given an aqueous suspension of the compound orally by stomach tube at 32

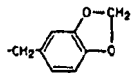
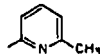
Table I. 2-Anilino-3-nitropyridines



compd	X	yield, %	method	mp, °C	formula
N-1	2-F	50	A-180 ^a	102-103	C ₁₁ H ₈ FN ₂ O ₂
N-2	H		^b		
N-3	4-MeO	80	A-180	78-80	C ₁₁ H ₁₁ N ₂ O ₃
N-4	4-F	85	A-200	130-131	C ₁₁ H ₈ FN ₂ O ₂
N-5	2- <i>i</i> -Pr	80	A-190	96-98	C ₁₃ H ₁₃ N ₂ O ₂
N-6	2,6-Me ₂	75	A-190	114-115	C ₁₃ H ₁₃ N ₂ O ₂
N-7	3-CF ₃	80	A-185	81-82	C ₁₁ H ₈ F ₃ N ₂ O ₂
N-8	2,4-F ₂	75	A-190	114-116	C ₁₁ H ₈ F ₂ N ₂ O ₂
N-9	2-Cl	50	A-190	128-129	C ₁₁ H ₈ ClN ₂ O ₂
N-10	2-Me ₂ N	40	A-165	^c	C ₁₁ H ₁₂ N ₄ O ₂
N-11	3-F	75	A-185	102-104	C ₁₁ H ₈ FN ₂ O ₂
N-12	4-Et	85	A-180	83-84	C ₁₂ H ₁₁ N ₂ O ₂
N-13	2-F,5-Me	75	A-200	114-117	C ₁₁ H ₁₀ FN ₂ O ₂
N-14	4-Me		^b		
N-15	3,4-OCH ₂ O-	92	^d	146-148	C ₁₁ H ₉ N ₂ O ₄
N-16	4-NHAc	45	A-190	176-177	C ₁₂ H ₁₁ N ₂ O ₃
N-17	2,4-Me ₂	70	A-175	121-125	C ₁₃ H ₁₃ N ₂ O ₂
N-18	2,5-F ₂	70	A-180	150-152	C ₁₁ H ₈ F ₂ N ₂ O ₂
N-22	2-Br	40	A-185	138-140	C ₁₁ H ₈ BrN ₂ O ₂
N-25	3,4-O(CH ₂) ₂ O-	90	B	126-127	C ₁₃ H ₁₁ N ₂ O ₄
N-26	2,4-Cl ₂	80	A-185	144-145	C ₁₁ H ₈ Cl ₂ N ₂ O ₂
N-27	2,6-F ₂	25	A-190	^c	C ₁₁ H ₈ F ₂ N ₂ O ₂
N-28	4-Cl	55	A-190	145-147	C ₁₁ H ₈ ClN ₂ O ₂
N-30	3,4-(MeO) ₂	85	B	97-98	C ₁₃ H ₁₃ N ₂ O ₄
N-43	2-Me,3-Cl	63	^d	134-135	C ₁₁ H ₁₀ ClN ₂ O ₂
N-47	3,4-Cl ₂	90	A-180	167-168	C ₁₁ H ₈ Cl ₂ N ₂ O ₂
N-49	3,4,5-(MeO) ₃	90	B	139-140	C ₁₄ H ₁₃ N ₂ O ₆
N-50	2,4-(MeO) ₂	80	B	139-140	C ₁₃ H ₁₃ N ₂ O ₄
N-59	4-C ₆ H ₅ O	75	A-100	105-107	C ₁₇ H ₁₅ N ₂ O ₂
N-65	3,4-CH ₂ OCH ₂ -	65	B	146-147	C ₁₃ H ₁₁ N ₂ O ₄
N-67	2-F ^e	31	^d	159-161	C ₁₁ H ₈ FN ₂ O ₂
N-68	3-MeO	65	A-175	98-100	C ₁₂ H ₁₁ N ₂ O ₃
N-74	2,5-(MeO) ₂	90	B	145-147	C ₁₃ H ₁₃ N ₂ O ₄
N-77	3,4-Me ₂	65	B	134-136	C ₁₃ H ₁₃ N ₂ O ₂
N-81	3-C ₆ H ₅ O	55	A-185	81-82	C ₁₇ H ₁₅ N ₂ O ₂
N-82	2-F ^f	50	A-190	114-115	C ₁₁ H ₈ FN ₂ O ₂
N-85	3-MeO,4-Me	85	B	101-102	C ₁₃ H ₁₃ N ₂ O ₃
N-88	3,4-OC(CH ₃) ₂ O-	65	B	oil ^g	C ₁₄ H ₁₃ N ₂ O ₄
N-90		75	A-155	114-116	C ₁₃ H ₁₃ N ₂ O ₂
N-91	3,4-OCH ₂ O-,6-Me	65	B	167-168	C ₁₃ H ₁₁ N ₂ O ₄
N-92	3,4-(CH ₂) ₂ -	55	B	103-104	C ₁₄ H ₁₃ N ₂ O ₂
N-95		90	B	117-118	C ₁₃ H ₁₇ N ₂ O ₄
N-96	3,4-OC(Et) ₂ O-	70	B	95-96	C ₁₆ H ₁₇ N ₂ O ₄
N-98	3,4-CH ₂ OCH ₂ O-	45	^g	130-131	C ₁₃ H ₁₁ N ₂ O ₄
N-100	3,4-(CH ₂) ₂ -	40	B	116-118	C ₁₄ H ₁₃ N ₂ O ₂
N-103	2,3-OCH ₂ O-	30	B	194	C ₁₂ H ₁₁ N ₂ O ₄
N-105	4-COOH	55	A-172	292	C ₁₂ H ₉ N ₂ O ₄
N-106	2-F ^h	45	^d	145-148	C ₁₁ H ₈ FN ₂ O ₂
N-107	3,4-OCH ₂ O-	50	A-100	196	C ₁₂ H ₁₁ N ₂ O ₄
N-110	3-COOEt	80	A-200	82-83	C ₁₄ H ₁₃ N ₂ O ₄
N-138	4-CH ₂ COOH	25	^j	169	C ₁₃ H ₁₁ N ₂ O ₄
N-139	4- <i>i</i> -Bu	50	B	46-48	C ₁₅ H ₁₇ N ₂ O ₂
N-140	2-MeO	55	B	151-152	C ₁₂ H ₁₁ N ₂ O ₃
N-143	2-F ^k	20	A-160	107-109	C ₁₁ H ₈ ClFN ₂ O ₂
N-144	3-CN	40	A-175	155-157	C ₁₁ H ₈ N ₄ O ₂
N-145	4-Me ₂ N	70	A-170	137	C ₁₁ H ₁₂ N ₄ O ₂
N-147	4-COCH ₃	25	A-185	155-157	C ₁₃ H ₁₁ N ₂ O ₃
N-149	4-Et	80	A-180	83-84	C ₁₃ H ₁₃ N ₂ O ₂

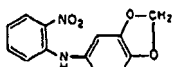
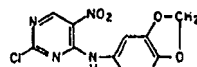
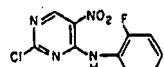
^a Refers to the maximum temperature (°C) attained in the preparation. ^b Reference 24. ^c Not purified. ^d Preparation given in the Experimental Section. ^e Also 5-nitro in pyridine ring. ^f Also 5-methyl in pyridine ring. ^g Reaction done in refluxing pyridine. ^h Also 5-carbomethoxy in pyridine ring. ⁱ C: calcd, 53.00; found, 52.54. ^j Reaction done in refluxing DMF. ^k Also 6-chloro in pyridine ring.

Table II. 2-Amino-3-nitropyridines

compd	R	yield, %	method	mp, °C	formula
N-23	-CH ₂ CH ₂ C ₆ H ₅	75	A-130 ^a	86-87	C ₁₃ H ₁₃ N ₃ O ₂
N-31		32	b	113-115	C ₁₃ H ₁₁ N ₃ O ₄
N-48	-CH ₂ C ₆ H ₄ -2-Fl	83	b	116-118	C ₁₃ H ₁₀ FN ₃ O ₂
N-72	c-C ₆ H ₁₁	65	A-80	oil ^c	C ₁₁ H ₁₁ N ₃ O ₂
N-76		50	A-175	153-154	C ₁₁ H ₁₀ N ₃ O ₂
N-141		30	A-185	165-167	C ₁₀ H ₉ N ₃ O ₂

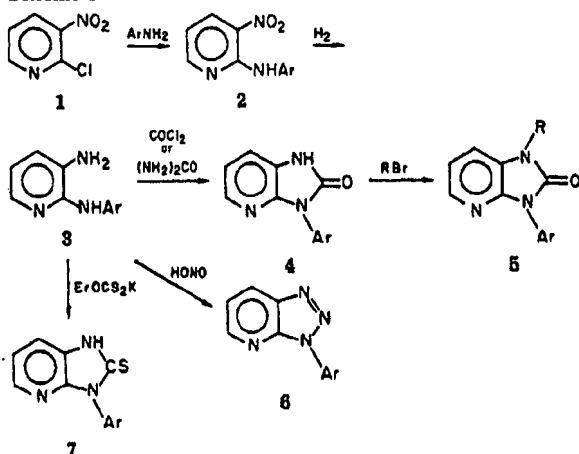
^a Refers to the maximum temperature (°C) attained in the preparation. ^b Preparation given in the Experimental Section. ^c Not purified.

Table III. Miscellaneous Nitro Compounds

compd	structure	method	yield, %	mp, °C	formula
N-84		a	17	109-111	C ₁₃ H ₁₀ N ₃ O ₄
N-120		a	73	143-145	C ₁₁ H ₇ ClN ₃ O ₄ ^b
N-133		c	40	147-148	C ₁₀ H ₈ ClFN ₃ O ₂

^a Preparation given in the Experimental Section. ^b C: calcd, 44.83; found, 45.47. ^c The two reagents were mixed in an ether solution at room temperature.

Scheme I



or 64 mg/kg. Tests with compounds that show activity are repeated using six rats per dose level. The results are shown in Tables IV-VIII.

In this assay 1,3-dihydro-3-phenylimidazo[4,5-b]pyridin-2-one (I-2) possessed activity of the order of codeine. The related 3-phenyltriazolopyridine (T-2) was less active, but in this triazole series the substitution of a 2-fluorophenyl for phenyl resulted in a compound (T-1) which also approached the activity of codeine. Many compounds in both series were prepared with electron-withdrawing and electron-donating substituents on the phenyl ring. Although many of these compounds showed

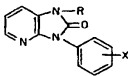
modest activity, the 3,4-(methylenedioxy)phenyl group on the imidazopyridine seemed to give the most active compound (I-15). Surprisingly, the same group did not enhance the activity in the triazolopyridine series (T-15). Seemingly minor changes in the (methylenedioxy)phenyl group lowered or destroyed the activity. An ethylenedioxy group (I-25), or the substitution of dimethyl (I-88), diethyl (I-96), or pentamethylene (I-95), on the methylene carbon gave compounds devoid of activity. Similar groups such as 2,3-(methylenedioxy)phenyl (I-103), 3,4-(methylenedioxy)-6-methylphenyl (I-91), 3,4-dimethoxyphenyl (I-30), indanyl (I-92), or 1,2-dimethylbenzofuryl (I-90) also produced inactive compounds.

A few examples of other related structures were also prepared and tested for analgesic activity. Instead of using substituted anilines to give the 3-substituted phenyl derivatives, compounds such as 2-amino-6-methylpyridine (I-76), phenylethylamine (I-23), 3,4-(methylenedioxy)-benzylamine (I-31), and cyclohexylamine (I-72) were used, but these resulted in inactive compounds. Substituents on the pyridine ring, or replacement of it by a benzene or pyrimidine ring, also resulted in inactive compounds. The sulfur analogue of an active compound (I-24) was also inactive as well as a 2-phenylimidazopyridine (I-75).

Alkylation of the unsubstituted nitrogen sometimes yielded compounds with increased activity. The alkyl groups conferring the best activity were allyl, *n*-propyl, and isopropyl. The cyclopropylmethyl group, an activity enhancing group in many analgesics, did not help in this series.

An attempted "Hansch analysis" of most of the 1,3-dihydro-3-(substituted phenyl)imidazo[4,5-b]pyridin-2-

Table IV. 3-(Substituted phenyl)imidazo[4,5-b]pyridin-2-ones



compd	X	R	method	yield, %	mp, °C	formula	analgesic act.	
							sensitive foot	normal foot
I-1	2-F	H	a	31	199-202	C ₁₂ H ₇ FN ₂ O	+ ^b	-
I-2	H	H	D	30	240-241	C ₁₂ H ₇ N ₂ O	+++	-
I-3	4-MeO	H	D	30	257-258	C ₁₃ H ₉ N ₂ O ₂	-	+
I-4	4-F	H	D	35	271-272	C ₁₂ H ₇ FN ₂ O	+	+
I-5	2- <i>i</i> -Pr	H	D	15	202-204	C ₁₄ H ₁₁ N ₂ O	-	-
I-6	2,6-Me ₂	H	E	60	236-239	C ₁₄ H ₁₁ N ₂ O	+	-
I-7	3-CF ₃	H	D	15	177-178	C ₁₃ H ₆ F ₃ N ₂ O	-	-
I-8	2,4-F ₂	H	D	20	240-242	C ₁₂ H ₆ F ₂ N ₂ O	+	-
I-9	2-Cl	H	D	30	242-244	C ₁₂ H ₇ ClN ₂ O	++	-
I-10	2-Me ₂ N	H	D	25	222-224	C ₁₄ H ₁₁ N ₃ O	-	-
I-11	3-F	H	D	40	224	C ₁₂ H ₇ FN ₂ O	-	-
I-12	4-Et	H	D	60	214-215	C ₁₄ H ₁₃ N ₂ O	-	-
I-13	2-F,5-Me	H	D	45	206-208	C ₁₃ H ₉ FN ₂ O	-	+
I-14	4-Me	H	D	33	222-224	C ₁₃ H ₁₁ N ₂ O	++	++
I-15	3,4-OCH ₂ O-	H	a	30	259-260	C ₁₄ H ₁₁ N ₂ O ₂	++++	++++
I-16	4-NH ₂	H	D ^c	65	301	C ₁₂ H ₇ N ₃ O	++	-
I-17	2,4-Me ₂	H	D	25	110-112	C ₁₄ H ₁₃ N ₂ O·0.5H ₂ O	++	-
I-18	2,5-F ₂	H	D	65	229	C ₁₂ H ₆ F ₂ N ₂ O	-	-
I-19	3,4-OCH ₂ O-	Ac	H	70	211-212	C ₁₄ H ₁₁ N ₂ O ₂	+	+
I-20	3,4-OCH ₂ O-	Me	F	85	238-239	C ₁₄ H ₁₃ N ₂ O ₂	++	++++
I-21	3,4-OCH ₂ O-	CH ₂ CH=CH ₂	a	69	130-131	C ₁₆ H ₁₃ N ₂ O ₂	++++	++++
I-22	2-Br	H	D	35	240-243	C ₁₂ H ₆ BrN ₂ O ^d	+	-
I-25	3,4-O(CH ₂) ₂ O-	H	D	35	267-269	C ₁₄ H ₁₃ N ₂ O ₂	-	-
I-26	2,4-Cl ₂	H	D	25	172-174	C ₁₂ H ₆ Cl ₂ N ₂ O	-	-
I-27	2,6-F ₂	H	D	10	252-255	C ₁₂ H ₆ F ₂ N ₂ O	-	-
I-28	4-Cl	H	D	85	267-269	C ₁₂ H ₇ ClN ₂ O	-	-
I-29	3,4-OCH ₂ O-	(CH ₂) ₃ NEt ₃	F	50	172-173 ^e	C ₁₆ H ₁₅ N ₂ O ₂ ·2C ₂ H ₅ SO ₃	-	-
I-30	3,4-(MeO) ₂	H	D	50	245-246	C ₁₄ H ₁₃ N ₂ O ₂	-	-
I-32	2-F	CH ₂ CH=CH ₂	F	65	92-94	C ₁₄ H ₁₁ FN ₂ O	+	-
I-33	3,4-OCH ₂ O-	CH ₂ C≡CH ₂	F	40	207-209	C ₁₄ H ₁₁ N ₂ O ₂	-	-
I-34	3,4-OCH ₂ O-	CH ₂ C(CH ₃)=CH ₂	F	75	119-121	C ₁₅ H ₁₃ N ₂ O ₂	-	-
I-35	3,4-OCH ₂ O-	SO ₂ CF ₃	F	80	155-156	C ₁₄ H ₉ F ₃ N ₂ O ₂ S	-	-
I-36	3,4-OCH ₂ O-	<i>i</i> -Am	F	45	91-93	C ₁₄ H ₁₃ N ₂ O ₂	-	-
I-37	3,4-OCH ₂ O-	CH ₂ C ₆ H ₄	F	75	111-113	C ₁₆ H ₁₃ N ₂ O ₂	-	-
I-38	3,4-OCH ₂ O-	CH ₂ COOC ₂ H ₅	F	18	144-146	C ₁₆ H ₁₅ N ₂ O ₄	-	-
I-39	3,4-OCH ₂ O-	CH ₂ Cl=CH ₂	a	8	122-123	C ₁₄ H ₁₁ ClN ₂ O ₂	++	++
I-40	3,4-OCH ₂ O-	CH=CC=CH ₂	a	15	195-196	C ₁₄ H ₁₁ N ₂ O ₂	-	-
I-41	3,4-OCH ₂ O-	C(=O)-c-C ₆ H ₄	F	45	192-193	C ₁₆ H ₁₃ N ₂ O ₂	-	-
I-42	3,4-OCH ₂ O-	CH ₂ CH=C(CH ₃) ₂	F	75	109-110	C ₁₆ H ₁₅ N ₂ O ₂	-	-
I-43	2-Me,3-Cl	H	D	15	224-225	C ₁₂ H ₇ ClN ₂ O	-	-
I-44	3,4-OCH ₂ O-	CH ₂ -c-C ₆ H ₄	F	38	141-142	C ₁₆ H ₁₃ N ₂ O ₂	++	++
I-45	3,4-OCH ₂ O-	<i>n</i> -Pr	a	80	112-114	C ₁₆ H ₁₅ N ₂ O ₂	+++	++++

I-46	3,4-OCH ₃ O-	(CH ₃) ₂ C ₆ H ₄	F	40	123-124	C ₁₁ H ₁₂ N ₂ O ₃	-	-
I-47	3,4-Cl ₂	H	D	18	282	C ₁₂ H ₈ Cl ₂ N ₂ O	-	-
I-49	3,4,5-(MeO) ₃	H	D	23	265-266	C ₁₂ H ₁₄ N ₂ O ₄	-	-
I-50	2,4-(MeO) ₂	H	D	72	204-206	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-51	3,4-OCH ₃ O-	CH ₃ COOH	a	18	283	C ₁₃ H ₁₄ N ₂ O ₅ ·H ₂ O	-	-
I-52	3,4-OCH ₃ O-	-CH ₂ CH=CH ₂	F	55	171-173	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-53	3,4-OCH ₃ O-	(CH ₃) ₂ N(CH ₃) ₂	F	13	97-99	C ₁₂ H ₁₆ N ₂ O ₃	-	-
I-54	3,4-OCH ₃ O-		a	30	224-226	C ₁₂ H ₁₂ N ₂ O ₃ S	-	-
I-55	3,4-OCH ₃ O-	CH ₃ CH=CHC ₆ H ₄	F	75	160-161	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-56	3,4-OCH ₃ O-	COOC ₆ H ₄	F	75	191-192	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-57	3,4-OCH ₃ O-	CH ₃ COC ₆ H ₄	F	80	79-81	C ₁₂ H ₁₂ N ₂ O ₄	-	+
I-58	3,4-OCH ₃ O-	CH ₃ OH	a	72	268	C ₁₂ H ₁₂ N ₂ O ₄	++	+
I-59	4-C ₆ H ₄ O	H	D	30	236-237	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-60	3,4-OCH ₃ O-	(CH ₃) ₂ OH	F	25	194-196	C ₁₂ H ₁₂ N ₂ O ₄	+++	++
I-61	3,4-OCH ₃ O-	CH ₃ C(=O)C ₆ H ₄ N(CH ₂ CH ₃) ₂ O	a	14	212-213	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-63	3,4-OCH ₃ O-	S(=O) ₂ C ₆ H ₄ -4-F	F	70	144-145	C ₁₂ H ₁₂ FN ₂ O ₄ S	-	-
I-64	3,4-OCH ₃ O-	i-Bu	F	15	124-126	C ₁₂ H ₁₂ N ₂ O ₄	++	-
I-65	3,4-CH ₃ OCH ₃ -	H	D	15	248-250	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-66	2-F	H	D	85	220-221	C ₁₂ H ₁₂ FN ₂ O	-	-
I-67	2-F	H	E	40	234-236	C ₁₂ H ₁₂ FN ₂ O	-	-
I-68	3-MeO	H	D	50	196-198	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-70	3,4-OCH ₃ O-	(CH ₃) ₂ C ₆ H ₄	F	70	103-104	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-71	3,4-OCH ₃ O-	CONHC ₆ H ₄	a	24	129-130	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-74	2,5-(MeO) ₂	H	D	55	243-244	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-77	3,4-Me ₂	H	D	63	188-190	C ₁₂ H ₁₂ N ₂ O	-	-
I-78	3,4-OCH ₃ O-	(CH ₃) ₂ CH ₂	F	68	65-66	C ₁₂ H ₁₂ N ₂ O ₄	+	-
I-79	2-Me, 3-Cl	CH ₃ CH=CH ₂	F	80	95-96	C ₁₂ H ₁₂ CHN ₂ O	-	-
I-81	3-C ₆ H ₄ O	H	D	25	188-190	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-82	2-F	H	D	45	183-184	C ₁₂ H ₁₂ FN ₂ O	+	-
I-83	3,4-(MeO) ₂	CH ₃ CH=CH ₂	F	55	120-121	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-85	3-MeO, 4-Me	H	D	15	237-239	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-86	3-MeO, 4-Me	CH ₃ CH=CH ₂	F	75	104-105	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-88	3,4-OC(CH ₃) ₂ O-	H	D	10	223-224	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-90	3,4-Cl(CH ₃) ₂ O-	H	D	30	269-270	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-91	3,4-OCH ₃ O-, 6-Me	H	D	30	214-215	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-92	3,4-(CH ₃) ₂ -	H	D	50	179-180	C ₁₂ H ₁₂ N ₂ O	-	-
I-94	3,4-(CH ₃) ₂ -	CH ₃ CH=CH ₂	F	80	81-82	C ₁₂ H ₁₂ N ₂ O	-	-
I-95		H	D	10	276-278	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-96	3,4-OC(Et) ₂ O-	H	D	15	187-189	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-97	3,4-OCH ₃ O-	CH ₃ CH=CH ₂ , CH ₃ I	a	41	202-203	C ₁₂ H ₁₂ IN ₂ O ₄	-	-
I-98	3,4-CH ₃ OCH ₃ O-	H	D	25	277-279	C ₁₂ H ₁₂ N ₂ O ₄	-	-
I-99		CH ₃ CH=CH ₂	F	60	112-113	C ₁₂ H ₁₂ N ₂ O ₄	-	+
I-100	2,3-(CH ₃) ₂ -	H	D	32	265-267	C ₁₂ H ₁₂ N ₂ O	-	-
I-101	2,3-(CH ₃) ₂ -	CH ₃ CH=CH ₂	F	80	162-163	C ₁₂ H ₁₂ N ₂ O	-	-

Table IV (Continued)

compd	X	R	method	yield, %	mp, °C	formula	analgesic act.	
							sensitive foot	normal foot
I-102	3,4-CH ₂ OCH ₂ O-	CH ₂ CH=CH ₂	F	85	162-163	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-103	2,3-OCH ₂ O-	H	D	60	221-223	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-104	2,3-OCH ₂ O-	CH ₂ CH=CH ₂	F	90	143-144	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-105	4-COOH	H	D	45	290	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-106	2-F ^k	H	D	70	211-213	C ₁₂ H ₁₂ FN ₂ O ₂	-	-
I-107	3,4-OCH ₂ O- ^h	H	D	50	283-285	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-108	2-F ^l	H	G	80	316-318	C ₁₂ H ₁₂ FN ₂ O-CH ₂ OH	-	-
I-109	3-COOEt	CH ₂ CH=CH ₂	F	90	101-102	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-110	3-COOEt	H	D	35	193-194	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-112	3,4-OCH ₂ O- ^l	H	a	37	325-328	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-113	3,4-OCH ₂ O-6-NO ₂	H	a	68	249-250	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-121	3,4-OCH ₂ O-	i-Pr	F	40	175-176	C ₁₂ H ₁₂ N ₂ O ₂	++++	++++
I-122	3,4-OCH ₂ O-	s-Bu	F	55	129-131	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-123	3,4-OCH ₂ O-	c-C ₄ H ₉	F	80	156-158	C ₁₂ H ₁₂ N ₂ O ₂	+++	++
I-124	3,4-OCH ₂ O-	CF ₃ CHClF	a	18	86-88	C ₁₂ H ₁₂ ClF ₂ N ₂ O ₂	-	-
I-125	3,4-OCH ₂ O-	CH(CH ₃)CH=CH ₂	F	20	106-107	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-126	3,4-OCH ₂ O-	(CH ₃) ₂ OAc	a	42	111-113	C ₁₂ H ₁₂ N ₂ O ₂	+	-
I-127	3,4-OCH ₂ O-	CH ₂ CH ₂	F	72	145-146	C ₁₂ H ₁₂ N ₂ O ₂	++	-
I-128	3,4-OCH ₂ O-	CH ₂ COCH ₃	F	40	209-210	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-129	3,4-OCH ₂ O-	CH ₂ OAc	H	30	141-142	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-130	3,4-OCH ₂ O-	t-Bu	a	3	142	C ₁₂ H ₁₂ N ₂ O ₂	++	++
I-132	3,4-OCH ₂ O-	CH ₂ CH(OH)CH ₃	a	15	139-139	C ₁₂ H ₁₂ N ₂ O ₂	++	+
I-134	3,4-OCH ₂ O-	CH ₂ OC ₂ H ₅	F	90	137-138	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-136	H	n-Pr	D	70	78-80	C ₁₂ H ₁₂ N ₂ O ₂	++	+
I-137	3,4-OCH ₂ O-	CH ₂ SCH ₃	F	75	149-150	C ₁₂ H ₁₂ N ₂ O ₂ S	-	-
I-138	4-CH ₂ COOH	H	D	20	295	C ₁₂ H ₁₂ N ₂ O ₂	-	-
I-139	4-t-Bu	H	D	55	193-194	C ₁₂ H ₁₂ N ₂ O ₂	-	-
aspirin							++	+
d-propoxyphene							+++	++
codeine							++++	++++
morphine							++++	++++

^a Preparation given in the Experimental Section. ^b The results are recorded as follows. In the sensitive or hyperesthetic foot: - = <25 mm at 32 mg/kg; + = 25-34 mm at 32 mg/kg; ++ = >34 mm at 32 mg/kg or 25-34 mm at 16 mg/kg; +++ = >34 mm at 16 mg/kg or 25-34 mm at 8 mg/kg; ++++ = >34 mm at 8 mg/kg. In the normal foot: - = <40 mm at 32 mg/kg; + = 40-49 mm at 32 mg/kg; ++ = >50 mm at 32 mg/kg or 40-49 mm at 16 mg/kg; +++ = 50-59 mm at 16 mg/kg or 40-49 mm at 8 mg/kg; ++++ = >60 mm at 16 mg/kg or >50 mm at 8 mg/kg. ^c Resulted from the 4-acetylamino compound when COCl₂ was added. ^d C: calcd, 49.67; found, 50.53. ^e Melting point of ditosyl acid salt. ^f Also 6-amino in pyridine ring. ^g From I-67 by hydrogenation using Pd/C. ^h Also 6-nitro in pyridine ring. ⁱ Also 6-methyl in pyridine ring. ^j Contains 1.5% water. ^k Also 6-carbomethoxy in pyridine ring. ^l Also 6-carboxy in pyridine ring.

Table V. Miscellaneous Imidazo[4,5-*b*]pyridin-2-ones

compd	R	R'	method	yield, %	mp, °C	formula	analgesic act.	
							sensi- tive foot	normal foot
I-23	-(CH ₂) ₃ C ₆ H ₅	H	D	25	163-164	C ₁₄ H ₁₃ N ₂ O	- ^a	-
I-31		H	D	45	236-238	C ₁₄ H ₁₁ N ₂ O ₃	-	-
I-48	-CH ₂ C ₆ H ₄ -4-F	H	D	15	191-192	C ₁₃ H ₁₀ FN ₂ O	-	-
I-69		CH ₂ CH=CH ₂	F	30	111-112	C ₁₁ H ₁₀ N ₂ O	-	-
I-72	<i>c</i> -C ₆ H ₁₁	H	D	25	225-226	C ₁₇ H ₁₇ N ₂ O	-	-
I-76		H	D	60	217-219	C ₁₁ H ₁₀ N ₂ O	-	-

^a See footnote b, Table IV.

ones showed that there was no correlation of activity with lipophilicity, electronic effect, or steric bulk. The enhancing effect of a 3,4-methylenedioxy substituent appeared to be a specific effect not correlated with any of the above parameters. Compounds I-21, I-121, and I-45, as well as the unsubstituted compound I-15, were the most interesting and were studied more extensively. They were tested in rats in a modification of the Eddy and Leimbach^{11b} hot-plate assay. Compounds I-15 and I-21 at 20 mg/kg were several times as potent as *d*-propoxyphene and without showing any overt side effects as shown in Table IX. Despite this analgesic activity the compounds have no propensity to induce physical dependence in mice. This is shown by a lack of withdrawal symptoms after the acute administration of naloxone. Under these experimental conditions compounds such as morphine, codeine, pentazocine, and *d*-propoxyphene all produce physical dependence as revealed by the mice which show excitement and make many "escape jumps". In mice, these naloxone precipitated withdrawal effects provide a valid measure of physical dependence. Absence of these effects indicates a lack of physical dependence capacity. None of the compounds tested induced withdrawal signs. However, they did show significant analgesic activity in mice using the Haffner^{11c} tail clamp procedure (see Table X). Naloxone, when given to rats simultaneously with the compound (I-21), did not affect its analgesic activity in the hyperesthesia assay as shown in Table XI.

Two of the compounds (I-15 and I-21) possess activity approaching that of phenylbutazone in both the carrageenan-induced foot edema and adjuvant arthritis assays in the rat.^{12a,b} These data are given in Tables XII and XIII. In comparison, *d*-propoxyphene is inactive in the carrageenan edema assay, although it is moderately active in the adjuvant arthritis model.

Experimental Section

All melting points are corrected and were taken on a Thomas-Hoover capillary melting point apparatus. Infrared spectra were obtained from a Nujol mull with a Perkin-Elmer 137 IR spectrometer. A Varian T-60 NMR spectrometer and an LKB

Model 9000 mass spectrometer were also used. All compounds were analyzed for C, H, and N and were within 0.4% of calculated theoretical values unless designated otherwise.

The following chloronitropyridines that could not be purchased were prepared according to the cited literature references: 2,6-dichloro-3-nitropyridine,¹³ 2-chloro-3,5-dinitropyridine,¹⁴ 2,5-dichloro-3-nitropyridine,¹⁵ 2-chloro-3-nitro-5-methylpyridine,¹⁶ ethyl 6-chloro-5-nitronicotinate,¹⁷ and 2,4-dichloro-5-nitropyrimidine.¹⁸

The following anilines that could not be purchased were prepared according to the cited literature references or as designated: 2,3-(methylenedioxy)aniline,¹⁹ 3,4-(ethylenedioxy)aniline,²⁰ 3,4-(isopropylendioxy)aniline,²¹ 2,3-dimethyl-6-aminobenzofuran,²² 3,4-(methylenedioxy)-6-methylaniline²³ (aniline prepared from the known nitro compound), 6-amino-1,3-benzodioxan (aniline prepared from the commercial nitro compound), 5-aminophthalan (experimental given), 5-amino-spiro[1,3-benzodioxole-2,1'-cyclohexane] and 3,4-[(1,1-diethyl)-methylenedioxy]aniline²¹ (anilines prepared from known nitro compounds), and 4-isobutylaniline (experimental given).

Many of the compounds are prepared by the same general method. These general methods are described by the synthesis of specific compounds. Designation of the method used and the properties of the compounds are given in Tables I-VIII. The preparation of all other new compounds is given specifically for the compounds desired.

Many of the reactions were run only once, and purity rather than yield was the dominating factor.

Reactions of 2-Chloro-3-nitropyridine and Related Compounds with Substituted Anilines. Method A. 2-(3-Chloro-2-methylanilino)-3-nitropyridine (N-43). A mixture of 6.3 g (0.04 mol) of 2-chloro-3-nitropyridine and 16.9 g (0.12 mol) of 3-chloro-2-methylaniline was heated in an oil bath to 170 °C, when the temperature spontaneously rose to 185 °C. After an additional 10 min at 180 °C the mixture was cooled to 50 °C and extracted with a solution of 75 mL of water and 24 mL of HOAc. The insolubles were collected, air-dried, and dissolved in methylene chloride, and the solution was dried, concentrated to a small volume, and diluted with 50 mL of Et₂O which caused crystallization of 6.6 g (63%), mp 134-135 °C.

Method B. 2-[3,4-(Methylenedioxy)anilino]-3-nitropyridine (N-15). A mixture of 6.3 g (0.04 mol) of 2-chloro-3-nitropyridine, 6.8 g (0.05 mol) of 3,4-(methylenedioxy)aniline, and 4.1 g (0.05 mol) of NaOAc in 125 mL of HOAc was stirred and refluxed for 5 h. The reaction mixture was concentrated to about one-fourth of the original volume and diluted with 100 mL of

Table VI. Miscellaneous Structures

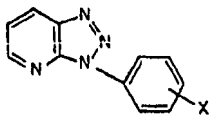
compd	structure	method	yield, %	mp, °C	formula	analgesic act.	
						sensitive foot	normal foot
I-24		a	13	285	C ₁₁ H ₉ N ₃ O ₂ S	- ^b	-
I-75		a	6	154-155	C ₁₉ H ₁₃ N ₃ O ₂	-	-
I-84		a	24	230-231	C ₁₄ H ₁₀ N ₃ O ₂	-	-
I-87		F	65	107-109	C ₁₇ H ₁₄ N ₃ O ₂	-	-
I-89		a	58	123-124	C ₁₆ H ₁₃ N ₃ O ₂ S	-	-
I-119		F	65	177-178	C ₁₁ H ₁₃ N ₄ O ₂	-	-
I-120		a	28	274-275	C ₁₁ H ₈ N ₄ O ₂	-	-
I-131		a	61	160-162	C ₁₇ H ₁₇ N ₃ O ₂	+++	-
I-133		c	75	242	C ₁₁ H ₇ FN ₄ O	-	-
I-135		F	72	131-132	C ₁₆ H ₁₁ FN ₄ O	-	-

^a Preparation given in the Experimental Section. ^b See footnote b, Table IV. ^c Prepared like I-120.

water. The precipitate was collected, 9.5 g (92%), and a small sample was recrystallized from EtOH to give a product melting at 146-148 °C.

2-[3,4-(Methylenedioxy)phenylmethylamino]-3-nitro-

pyridine (N-31). To 18 g (0.12 mol) of piperonylamine was added portionwise 6.3 g (0.04 mol) of 2-chloro-3-nitropyridine. The reaction is exothermic and so it was cooled occasionally. After all had been added, the reactants had solidified. It was heated

Table VII. 3-(Substituted phenyl)triazolopyridines^a


compd	X	yield, %	mp, °C	formula	analgesic act.	
					sensitive foot	normal foot
T-1	2-F	60	118-119	C ₁₁ H ₇ FN ₄	+++ ^b	-
T-2	H	25	76-77	C ₁₁ H ₈ N ₄	-	-
T-3	4-MeO	35	129-130	C ₁₃ H ₉ N ₄ O	-	-
T-4	4-F	50	182-184	C ₁₁ H ₇ FN ₄	-	-
T-5	2-i-Pr	20	oil	C ₁₄ H ₁₁ N ₄	+	-
T-6	2,6-Me ₂	60	155-156	C ₁₃ H ₁₁ N ₄ ^c	+	-
T-7	3-CF ₃	30	100	C ₁₁ H ₇ F ₃ N ₄	+	-
T-8	2,4-F ₂	30	138-139	C ₁₁ H ₆ F ₂ N ₄	+++	++
T-9	2-Cl	25	100-101	C ₁₁ H ₇ ClN ₄	+	-
T-10	2-Me ₂ N	40	78-79	C ₁₃ H ₁₃ N ₄	-	-
T-11	3-F	40	144-145	C ₁₁ H ₇ FN ₄	-	-
T-13	2-F,5-Me	25	107-108	C ₁₂ H ₈ FN ₄	-	-
T-14	4-Me	20	114-115	C ₁₃ H ₁₀ N ₄	-	-
T-15	3,4-OCH ₂ O-	16	169-170	C ₁₃ H ₁₀ N ₄ O ₂	+	+
T-16	4-NHAc	15	187-188	C ₁₃ H ₁₁ N ₄ O	-	-
T-17	2,4-Me ₂	35	124-125	C ₁₃ H ₁₂ N ₄	-	-
T-18	2,5-F ₂	70	144-145	C ₁₁ H ₆ F ₂ N ₄	++	-
T-22	2-Br	70	116-118	C ₁₁ H ₇ BrN ₄	+	-
T-25	3,4-O(CH ₂) ₂ O-	65	162-164	C ₁₃ H ₁₀ N ₄ O ₂	++	-
T-26	2,4-Cl ₂	35	150-151	C ₁₁ H ₆ Cl ₂ N ₄	-	+
T-27	2,6-F ₂	15	173-174	C ₁₁ H ₆ F ₂ N ₄	+	-
T-28	4-Cl	35	162-163	C ₁₁ H ₇ ClN ₄	-	-
T-30	3,4-(MeO) ₂	60	137-138	C ₁₃ H ₁₂ N ₄ O ₂	-	-
T-43	2-Me,3-Cl	20	152-153	C ₁₁ H ₇ ClN ₄	-	-
T-47	3,4-Cl ₂	60	175-176	C ₁₁ H ₆ Cl ₂ N ₄	-	-
T-49	3,4,5-(MeO) ₃	20	138-140	C ₁₃ H ₁₄ N ₄ O ₃	-	-
T-50	2,4-(MeO) ₂	45	163-164	C ₁₃ H ₁₂ N ₄ O ₂	-	-
T-59	4-C ₂ H ₅ O	50	60-61	C ₁₃ H ₁₂ N ₄ O ₂	-	-
T-65	3,4-CH ₂ OCH ₂ -	15	174-175	C ₁₃ H ₁₀ N ₄ O	-	-
T-68	3-MeO	45	78-80	C ₁₃ H ₁₀ N ₄ O	-	-
T-74	2,5-(MeO) ₂	70	140-142	C ₁₃ H ₁₂ N ₄ O ₂	-	-
T-77	3,4-Me ₂	70	110-111	C ₁₃ H ₁₂ N ₄	-	-
T-82	2-F ^d	75	122-123	C ₁₁ H ₇ FN ₄	-	-
T-85	3-MeO,4-Me	25	132-133	C ₁₃ H ₁₂ N ₄ O	-	-
T-88	3,4-OC(CH ₃) ₂ O-	10	122-123	C ₁₃ H ₁₂ N ₄ O ₂	-	-
T-90	3,4-C(CH ₃)=C(CH ₃)O-	10	152-153	C ₁₃ H ₁₂ N ₄ O	-	-
T-91	3,4-OCH ₂ O-,6-Me	80	147-148	C ₁₃ H ₁₀ N ₄ O ₂	-	-
T-92	3,4-(CH ₃) ₂ -	35	133-134	C ₁₃ H ₁₂ N ₄	-	-
T-96	3,4-OC(Et) ₂ O-	65	56-57	C ₁₅ H ₁₄ N ₄ O ₂	-	-
T-98	3,4-CH ₂ OCH ₂ O-	60	183-184	C ₁₃ H ₁₀ N ₄ O ₂	-	-
T-105	4-COOH	35	292	C ₁₁ H ₇ N ₄ O ₂ ^e	-	-
T-106	2-F ^f	40	112-113	C ₁₁ H ₇ FN ₄ O ₂	-	-
T-107	3,4-OCH ₂ O-, ^g	15	189-191	C ₁₃ H ₁₀ N ₄ O ₂	-	-
T-110	3-COOEt	70	114-115	C ₁₃ H ₁₂ N ₄ O ₂	-	-
T-139	4-i-Bu	65	40-42	C ₁₅ H ₁₆ N ₄	-	-
T-140	2-MeO	60	154-155	C ₁₃ H ₁₀ N ₄ O	+	-
T-142	H ^h	15		^h	-	-
T-143	2-F ⁱ	65	132-133	C ₁₁ H ₇ ClFN ₄	+	-
T-144	3-CN	20	165-166	C ₁₁ H ₇ N ₅	-	-
T-145	4-Me ₂ N	15	139-141	C ₁₃ H ₁₂ N ₄ ^{j,k}	-	-
T-146	3-CONH ₂	75	237-239	C ₁₁ H ₈ N ₄ O	+	-
T-147	4-COCH ₃	80	184-185	C ₁₃ H ₁₀ N ₄ O	-	-
T-148	2-OH	44	165-166	C ₁₁ H ₈ N ₄ O ^l	-	-
T-149	4-Et	65	61-62	C ₁₃ H ₁₂ N ₄	-	-
T-150	2-CN	75	177-179	C ₁₁ H ₇ N ₅ ^l	+	-
T-151	2-C(=O)NH ₂	45	>340	C ₁₁ H ₈ N ₄ O ^l	+	-

^a All the examples were prepared by method I unless designated otherwise. ^b See footnote b, Table IV. ^c C: calcd, 70.08; found, 69.63. ^d Also 6-methyl in pyridine ring. ^e Contains 1% water. ^f Also 6-carbomethoxy in pyridine ring. ^g Also 5-chloro in pyridine ring. ^h Reference 5. ⁱ Also 6-chloro in pyridine ring. ^j N: calcd, 29.27; found, 28.62. ^k Prepared by method K. ^l Preparation given in the Experimental Section.

on the steam bath 15 min. EtOH (25 mL) was then added and heating continued for 20 min. Water was slowly added to give a complete solution. Upon cooling a yellow solid crystallized: 10.1 g (32%); mp 113-115 °C.

2-(2-Fluorobenzylamino)-3-nitropyridine (N-48). To a solution of 6.3 g (0.04 mol) of 2-chloro-3-nitropyridine in 100 mL

of benzene was added 10.4 g (0.082 mol) of 2-fluorobenzylamine. The solution began to get cloudy after a few minutes. It was heated on the steam bath and a solid began to crystallize. After 1 h the HCl salt of the starting benzylamine was removed by filtration (4.1 g). The filtrate was heated for another hour and another 1.3 g of hydrochloride was removed. After another hour

Table VIII. 3-Substituted Triazolopyridines^a

compd	R	yield, %	mp, °C	formula	analgesic act.	
					sensitive foot	normal foot

T-31		55	107-108	C ₁₁ H ₁₀ N ₄ O ₂	— ^b	—
T-48	—CH ₂ C ₆ H ₄ —2-F	20	84-85	C ₁₁ H ₈ FN ₄	—	—
T-76		70	141-142	C ₁₁ H ₉ N ₄	—	—
T-141		45	195-197	C ₁₀ H ₈ N ₄	—	—
T-152	—(CH ₂) ₃ —C ₆ H ₅	35	69-70	C ₁₃ H ₁₂ N ₄	—	—

^a All compounds were prepared by method I. ^b See footnote b, Table IV.Table IX. Analgesic Activity in the Rat Hot-Plate Assay^a

compd	dose, mg/kg, oral aq susp	increase in mean reaction time (s) over control times				
		1 h	2 h	3 h	4 h	5 h

I-21	5	0.1	3.8	5.3	3.9	0
	10	8.8	7.3	11.6	7.1	6.2
	20	13.5	21.7	24.4	28.1	24.5
<i>d</i> -propoxyphene	30	-3.2	-2.6	-2.2	-0.8	2.4
	60	-3	-0.8	-2.4	-0.8	-1.0
	120	41.9	30	24.5	15	3.6
control times		7.7	6.6	6.6	5.7	6.2
I-15	5	1.5	2.1	-0.1	1.3	0.4
	10	2.0	3.9	2.8	2.3	-0.2
	20	6	15	20.4	20	18.3
<i>d</i> -propoxyphene	30	6.5	0.7	-4.4	0.2	0.1
	60	8.7	4.4	0.2	1.8	-1.1
	90	40.6 ^b	42 ^b	40.7 ^b	36.4 ^b	10.5 ^b
control times		7.9	9.3	16.3	13.6	13.5

^a The assay is described in ref 11b. The temperature of the hot plate was maintained at 58 °C. Instead of mice, young Sprague-Dawley rats (ten per dose level) were used. ^b Sedation was evident at all time intervals; six of ten animals succumbed prior to 5-h reading.

of heating the cloudy mixture was filtered through Super cel and concentrated to about 30 mL. Upon the addition of 50 mL of petroleum ether the product crystallized: yield 8.1 g (83%); mp 116-118 °C.

3,5-Dinitro-2-(2-fluoroanilino)pyridine (N-67). When 2.0 g (0.01 mol) of 2-chloro-3,5-dinitropyridine and 3 mL (0.03 mol) of 2-fluoroaniline were mixed the temperature of the mixture rose to 80 °C. The reaction was completed by heating the mixture on the steam bath for 15 min, and then the solid product was crystallized from MeOH to give 850 mg (31%), mp 159-161 °C.

1-[3,4-(Methylenedioxy)anilino]-2-nitrobenzene (N-84). A mixture of 12.6 g (0.08 mol) of 2-chloronitrobenzene and 21.9 g (0.17 mol) of 3,4-(methylenedioxy)aniline was stirred and heated in an oil bath to 220 °C when there was an exotherm to 245 °C. The dark reaction mixture was cooled and extracted with ether. The ether extract was washed once with 50 mL of 1 N HCl, dried, and finally evaporated to a small volume. Petroleum ether was added to precipitate a deep red solid which was crystallized from ethanol. There was obtained 3.5 g (17%) of deep red rods, mp 109-111 °C.

Methyl 6-(2-Fluoroanilino)-5-nitronicotinate (N-106). A mixture of 1 g (0.0046 mol) of methyl 6-chloro-5-nitronicotinate and 2 mL of 2-fluoroaniline was heated on the steam bath for 90 min. MeOH (15 mL) was added and the solid removed by filtration. Crystallization from MeOH gave 600 mg (45%), mp 145-148 °C.

2-Chloro-4-[3,4-(methylenedioxy)anilino]-5-nitropyrimi-

dine (N-120). To 15 g (0.078 mol) of 2,4-dichloro-6-nitropyrimidine in 100 mL of ether was added over 1 h a solution of 22 g (0.16 mol) of 3,4-(methylenedioxy)aniline in 100 mL of ether. A deep red solid formed during the addition and finally the mixture was so thick that more ether was added to facilitate stirring. After a further 2 h of stirring at room temperature the solid was removed and washed well with warm water. It was dissolved in 150 mL of hot CHCl₃ and clarified by filtration. After concentration to about 75 mL, ether was added to promote crystallization: yield 16.5 g (73%); mp 143-145 °C.

Hydrogenation Reactions. Method C. 3-Amino-2-[3,4-(methylenedioxy)anilino]pyridine. The crude nitro compound N-15 (9.5 g) was hydrogenated at 3 atm in 175 mL of MeOH using 0.5 g of 5% Pd/C as a catalyst, the theoretical amount of hydrogen being consumed in 16 h. The catalyst was removed, the filtrate was concentrated in vacuo, and the dark residue was extracted with 75 mL of 2.5 N HCl and 75 mL of H₂O. The crude acid solution was used in methods D and I.

1,3-Dihydro-3-[3,4-(methylenedioxy)phenyl]-1-propyl-imidazo[4,5-*b*]pyridin-2-one (I-45). I-21 (1 g) was hydrogenated at 3 atm in 30 mL of EtOH over 100 mg of PtO₂ as catalyst for 1.5 h. The catalyst was removed and the filtrate concentrated to a small volume. The product crystallized and was collected to give 800 mg (80%), mp 112-114 °C.

5-Aminophthalan. The 5-nitrophthalan (19 g) was hydrogenated at 3 atm in 200 mL of MeOH over 1 g of 5% Pd/C for 30 min. The catalyst was removed by filtration and the filtrate

Table X. Lack of Physical Dependence as Shown by Abrupt Withdrawal with Naloxone^a

compd	dose, mg/kg po, aq suspn	no. of mice with analgesia	no. of mice with escape jumps	total no. of jumps
I-15	75	8/10	0/10	0
	50	6/10	0/10	0
	25	5/10	2/10	21
d-propoxyphene	12.5	4/10	1/10	1
	75	7/10	8/10	159
	50	7/10	7/10	78
methocel (control)	25	7/10	7/10	86
	12.5	5/10	7/10	63
		0/10	2/10	3
I-21	75	5/10	0/10	0
	50	5/10	1/10	1
	25	5/10	1/10	21
d-propoxyphene	12.5	2/10	2/10	12
	75	7/10	9/10	78
	50	5/10	8/10	98
methocel (control)	25	3/10	3/10	15
	12.5	2/10	2/10	14
		0/10	2/10	10

^a The mice are given the test compound and tested 2 h later for analgesia (Haffner clamp). Three hours after administration of the test compound the mice are abruptly withdrawn with naloxone, 10 mg/kg ip.

Table XI. Lack of Physical Dependence as Shown by Simultaneous Treatment with Naloxone^a

pretreatment	treatment	dose, mg/kg po, aq suspn	mean pain reaction threshold in mmHg (N = 8)	in-flamed foot	normal foot
methocel	— (control)		18.3	35.3	
methocel	I-21	5	28.8	40.5	
methocel	I-21	10	44.4	52.3	
methocel	I-21	20	74.4	88.5	
naloxone	— (control)		19.4	34.5	
naloxone	I-21	5	33.4	38.4	
naloxone	I-21	10	47.8	71.1	
naloxone	I-21	20	66.6	79.3	

^a The standard hyperesthesia test protocol was used. Two groups of six Sprague-Dawley rats were randomly administered various dose levels of I-21 1 h before testing. Fifteen minutes prior to testing, one group was administered naloxone (5.0 mg/kg ip); the other group was given methocel.

Table XII. Rat Foot Carrageenan Edema Activity^a

compd	dose, mg/kg, oral suspn	% inhibn of edema
I-15	30	54, 38, 37
I-21	30	49, 57, 42
phenylbutazone	30	58, 49, 41, 39, 49
d-propoxyphene	30	23, 32

^a The standard assay, ref 12a, was used with Sprague-Dawley rats, six rats per test.

was evaporated to dryness to give 15.1 g (97%), mp 102–104 °C. Recrystallization from C₆H₆-petroleum ether raised the melting point to 104–105 °C. Anal. (C₉H₉NO) C, H, N.

5-Amino-4-[3,4-(methylenedioxy)anilino]pyrimidine. A solution of 7.2 g (0.025 mol) of N-120 in 250 mL of EtOH was hydrogenated at 3 atm in the presence of 1.5 g of 5% Pd/C and 2.94 g (0.03 mol) of KOAc. Only one-third of the calculated hydrogen pressure drop was recorded in 3.5 h, so another 1.5 g of Pd/C was added and the mixture heated to 75 °C. In another

Table XIII. Adjuvant Arthritis Activity^a

compd	dose, mg/kg po, agar suspn	% inhibn of swelling
I-15	50	68.3
I-21	50	78.2
phenylbutazone	50	78
d-propoxyphene	50	56.2

^a On day 0 female Lewis rats (ten per group) weighing 170–190 g are sensitized in the right hind foot pad sub-plantar with 0.1 mL of a suspension containing mycobacterium butyricum in light mineral oil. Compounds are viritized in 0.25% sterile agar and administered orally on days 1–13. Foot volume of the paws is measured on day 0 and 14. Percent inhibition of swelling is determined.

3 h another one-third of the theoretical hydrogen pressure drop was recorded and so a third sample of 1.5 g of Pd/C was added and the mixture again heated to 75 °C for 7 h. The theoretical hydrogen pressure drop was attained. The catalyst was removed and 50 mL of 2.5 N HCl was added to the filtrate. This was concentrated to a small volume and a brown solid separated which was collected. This was suspended in 50 mL of water, an excess of NaHCO₃ was added, and the mixture was stirred for 30 min. The brown solid was collected which weighed 1.7 g (33%) and melted at about 183 °C. The IR showed NH₂ bands and the Beilstein test for chlorine was negative. This was used in the next step.

4-Isobutylaniline. 4-Nitroisobutylbenzene (20 g) was hydrogenated at 3 atm in 250 mL of EtOH in the presence of 0.5 g of 5% Pd/C for 16 h. The catalyst was removed by filtration and the filtrate concentrated to a dark oil (15.5 g, 87%) which was completely soluble in 2.5 N HCl. This crude material was used in the reaction with 2-chloro-3-nitropyridine.

2,2-Diethyl-5-amino-1,3-benzodioxole. A solution of 14.1 g of 2,2-diethyl-5-nitro-1,3-benzodioxole in 200 mL of MeOH was hydrogenated at 3 atm in the presence of 0.5 g of 5% Pd/C for 1.5 h. The product was isolated and used in the reaction with 2-chloro-3-nitropyridine.

5-Aminospiro[1,3-benzodioxole-2,1'-cyclohexane]. A solution of 20 g of 5-nitrospiro[1,3-benzodioxole-2,1'-cyclohexane] in 250 mL of MeOH was hydrogenated at 3 atm in the presence of 1 g of 5% Pd/C. After 3 h the hydrogen uptake was completed, and the amine was then isolated and used directly in the next reaction.

2-[3,4-(Methylenedioxy)anilino]aniline. N-84 (11 g) was hydrogenated at 3 atm in 175 mL of MeOH using 0.5 g of 5% Pd/C. After 16 h the theoretical amount of hydrogen was absorbed, the catalyst was removed, and 75 mL of 2.5 N HCl was quickly added to the filtrate. Most of the MeOH was taken off in vacuo and the solution containing the o-diamine was used for the reaction with phosgene.

Formation of Imidazo[4,5-b]pyridin-2-ones and Related Compounds. Method D. 1,3-Dihydro-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-b]pyridin-2-one (I-15). About one-third of the acid solution from method C (i.e., ~3 g of the product) was treated with phosgene gas for 30 min and the dark solution was allowed to stand at room temperature overnight. NH₄OH was added dropwise with stirring to the ice-cold reaction mixture until alkaline. The precipitate was collected and extracted with 50 mL of 2.5 N NaOH. The alkaline solution was treated with decolorizing charcoal and the filtrate therefrom was acidified with HOAc. The precipitate (1.1 g) was collected and recrystallized from DMF-Et₂O to give 900 mg (30%), mp 259–260 °C.

Method E. 1,3-Dihydro-3-(2-fluorophenyl)imidazo[4,5-b]pyridin-2-one (I-1). A mixture of 2.7 g of 3-amino-2-(2-fluoroanilino)pyridine hydrochloride and 3 g of urea was heated at 180 °C for 25 min. After cooling, the residue was stirred with 50 mL of 2.5 N NaOH. The alkaline solution was treated with charcoal and filtered. The filtrate was made acidic with HOAc which caused crystallization of the product (1 g, mp 195 °C). This was recrystallized from EtOAc-petroleum ether to give 800 mg (31%), mp 199–202 °C.

1,3-Dihydro-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-b]pyridine-2-thione (I-24). A mixture of 2.6 g (0.01 mol) of

3-amino-2-[3,4-(methylenedioxy)anilino]pyridine hydrochloride, 1.8 g (0.011 mol) of potassium ethyl xanthate, and 840 mg (0.01 mol) of NaHCO_3 in 45 mL of EtOH and 10 mL of H_2O was heated at reflux for 3.5 h. There was added 3 mL of 2.5 N NaOH and the mixture was filtered. The filtrate was acidified with HOAc and the precipitated product was collected: 500 mg (13%); mp 278 °C. After recrystallization from dioxane the product melted at 285 °C.

1-[3,4-(Methylenedioxy)phenyl]-2-benzimidazolinone (I-84). The aqueous acid solution containing crude 2-[3,4-(methylenedioxy)anilino]aniline was reacted with an excess of phosgene for 30 min. The reaction was cooled and treated with an excess of NH_4OH . A gum formed which was crystallized twice from dioxane-petroleum ether to give 700 mg (24%) of product melting at 230–231 °C.

1,3-Dihydro-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*d*]pyrimidin-2-one (I-120). The above crude *o*-diamine (1.7 g) from the hydrogenation of N-120 was mixed with 10 g of urea and heated in an oil bath under a blanket of N_2 . The internal temperature was maintained at 190 °C for 15 min. After cooling to 90 °C, 50 mL of water was added and the solid collected. This crude solid was extracted with 25 mL of 2.5 N NaOH. The filtered extract was treated with Darco and HOAc added to precipitate the product, which weighed 1.1 g. It was crystallized from 125 mL of boiling dioxane to give 500 mg (28%), mp 274–275 °C. The mass spectrum gives a molecular ion of 256.

Alkylations. Method F. 1,3-Dihydro-1-allyl-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-21). To a suspension of 500 mg (0.002 mol) of I-15 in 50 mL of Me_2CO was added 220 mg (0.004 mol) of powdered KOH. After being stirred for 15 min 480 mg (0.004 mol) of allyl bromide was added. After 1 h of stirring at room temperature, the mixture was heated on a steam bath to evaporate about half the solution. Water was added to precipitate a solid (500 mg, mp ~100 °C). After recrystallization from C_6H_6 -petroleum ether there was obtained 400 mg (69%), mp 130–131 °C.

1,3-Dihydro-1-(ethoxycarbonylmethyl)-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-38) and 1,3-Dihydro-1-(carboxymethyl)-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-51). To a stirred suspension of 2 g (0.008 mol) of I-15 in 100 mL of Me_2CO was added 980 mg (0.016 mol) of powdered KOH. After being stirred at room temperature for 30 min 1.7 g (0.01 mol) of ethyl bromoacetate was added over 3 min. After 3 h at room temperature and 5 min on the steam bath, the Me_2CO was evaporated. The residue was extracted with H_2O leaving 700 mg of solid. Crystallization from C_6H_6 -petroleum ether gave 500 mg (18%) of I-38, mp 144–146 °C.

The H_2O extract from above was acidified with HOAc. The precipitated solid (1 g) was extracted with a solution of 50 mL of H_2O and 20 mL of NH_4OH . The extract was acidified with HOAc. The precipitate was recrystallized from 60 mL of EtOH to give 450 mg (18%) of I-51, mp 283 °C.

1-Allelyl-1,3-dihydro-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-40) and 1-(2-Chloroallyl)-1,3-dihydro-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-39). A mixture of 1.0 g (0.0039 mol) of I-15, 100 mL of Me_2CO , and 220 mg (0.039 mol) of powdered KOH was stirred at room temperature 5 min and to it was added 960 mg (0.0085 mol) of 2,3-dichloropropene. The mixture was refluxed for 20 h. H_2O (100 mL) was added and after standing overnight it was filtered and the product crystallized from 35 mL of EtOAc. There was obtained 170 mg (15%) of I-40, mp 195–196 °C. The Me_2CO - H_2O filtrate was concentrated to remove the Me_2CO . The remaining solution was extracted with 4 × 20 mL of CH_2Cl_2 . The combined extracts were dried and concentrated to an oil. Trituration with hexane caused crystallization. Crystallization from EtOAc-hexane and from MeOH gave 100 mg (7.8%) of I-39, mp 122–123 °C.

1,3-Dihydro-3-[3,4-(methylenedioxy)phenyl]-1-(2-thiazolyl)imidazo[4,5-*b*]pyridin-2-one (I-54). A suspension of 1.2 g (0.0047 mol) of I-15 in 100 mL of dry dimethoxyethane was stirred and treated with 336 mg (0.007 mol) of a 50% NaH emulsion. After 15 min at room temperature, there was added 850 mg (0.007 mol) of 2-chloroethyl isothiocyanate and the mixture was refluxed 3 h. Most of the solvent was evaporated and the

residue was diluted with 40 mL of 1 N NaOH. The precipitate was recrystallized from 5 mL of dioxane-Et₂O to give 450 mg (30%), mp 224–226 °C.

1,3-Dihydro-1-hydroxymethyl-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-58). A mixture of 500 mg of I-15 and 30 mL of 37% formaldehyde was heated on the steam bath for 1.5 h. The hot solution was treated with decolorizing carbon and filtered, and the filtrate was treated with 2 mL of 2.5 N NaOH and 30 mL of H_2O . The precipitate weighed 400 mg (72%), mp 268 °C.

2-Allylthio-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridine (I-89). A solution of 1.8 g of I-24 in 50 mL of 2.5 N NaOH and 30 mL of water was stirred vigorously as 1 mL of allyl bromide was added. A light tan solid formed after a short time and stirring was continued for 30 min. The crude product was collected and crystallized from C_6H_6 -petroleum ether giving 1.2 g (58%) of white needles melting at 123–124 °C. The NMR is in accord with *S*-allyl rather than *N*-allyl: NMR (CDCl_3) δ 4.02 (d, 2- SCH_2) whereas for - NCH_2 - δ would be ~4.55.

1-(2-Chloro-1,1,2-trifluoroethyl)-1,3-dihydro-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-124). A mixture of 1.3 g (0.005 mol) of I-15, 3 g of 1-chloro-1,2,2-trifluoroethylene, and 50 mg (0.009 mol) of NaOMe as catalyst in 40 mL of DMF was heated in a bomb at 80 °C for 5 h. The yellow solution was concentrated to a small volume and a mixture of 5 mL of 2.5 N NaOH and 75 mL of H_2O was added. The solid was collected and recrystallized twice from C_6H_6 -petroleum ether: 350 mg (18%); mp 86–88 °C.

1,3-Dihydro-1-*tert*-butyl-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-130) and 2-*tert*-Butoxy-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridine (I-131). To a stirred suspension of 2.5 g (0.01 mol) of I-15 in 150 mL of acetone was added 1.12 g (0.02 mol) of powdered KOH. This was stirred at room temperature for 10 min and 2.74 g (0.02 mol) of *tert*-butyl bromide was added. After refluxing for 36 h and then evaporation, the residue was extracted with 25 mL of 2.5 N NaOH and 100 mL of H_2O . There was a small amount of insoluble oil which solidified. This weighed 150 mg and was a mixture of the *N*-*tert*-butyl and *O*-*tert*-butyl derivatives. The solid was placed on a silica gel GF prep plate (1000 μ) and eluted with a solution of hexane-ether (2:1). The *O*-*tert*-butyl isomer moved the faster. The isomers were easily distinguished by their IR spectra. The *N*-*tert*-butyl isomer I-130, 90 mg (3%), melted at 142 °C and then solidified and melted again at 258–259 °C. The mass spectrum gave a molecular ion of 311. Anal. C: calcd, 65.58; found, 64.71. The *O*-*tert*-butyl isomer I-131, 35 mg (1.1%), melted at 150–152 °C.

Nitration Reactions. 5-Nitrophthalan. Phthalan (6 g, 0.05 mol) was dissolved in 75 mL of concentrated H_2SO_4 and cooled to 5 °C, and with stirring a solution of 5.1 g (0.05 mol) of KNO_3 in 25 mL of concentrated H_2SO_4 was added dropwise over 40 min while maintaining the temperature at <7 °C. After an additional 30 min at ice-bath temperature and 30 min at room temperature, the solution was poured onto ice. The precipitate was collected and recrystallized from C_6H_6 -petroleum ether to give 5.5 g (67%): mp 90–92 °C; NMR δ 8.15 (m, 1, J = 2 Hz, aromatic proton 6), 8.1 (s, 1, aromatic proton 4), 7.40 (d, 1, J = 8 Hz, aromatic proton 7), 5.17 (s, 4, - CH_2OCH_2 -). Anal. ($\text{C}_8\text{H}_7\text{NO}_3$) C, H, N.

5-Nitrospiro[1,3-benzodioxole-2,1'-cyclohexane]. While 200 mL of 70% HNO_3 was stirred and cooled to 0 °C, 19.5 g of spiro[1,3-benzodioxole-2,1'-cyclohexane]¹⁹ was added over 1 h. The nitro compound formed as a precipitate during the acid addition. This was stirred another 15 min at 0 °C and poured into ice water. The yellow nitro compound was collected and recrystallized from hexane giving 17.9 g (74%): mp 99–100 °C; NMR (CDCl_3) δ 7.83 (m, 1, aromatic proton 6), 7.55 (d, 1, J = 2 Hz, aromatic proton 4), 6.75 (d, 1, J = 8.9 Hz, aromatic proton 7), 1.75 [m, 10, -(CH_2)₅].

2,2-Diethyl-5-nitro-1,3-benzodioxole. 2,2-Diethyl-1,3-benzodioxole¹⁹ (14 g) was added with good stirring to 140 mL of 70% HNO_3 at 0 °C over 30 min. The reaction mixture was stirred another 20 min at 0 °C and then poured onto ice. The oil that separated was extracted with Et₂O which was washed with aqueous NaHCO_3 . The Et₂O was evaporated and the oil was taken up in petroleum ether and cooled in dry ice to crystallize the product: 14.5 g (82%); mp 30–31 °C. Anal. ($\text{C}_{11}\text{H}_{13}\text{NO}_4$) C, H, N.

1,3-Dihydro-3-[3,4-(methylenedioxy)-6-nitrophenyl]-imidazo[4,5-*b*]pyridin-2-one (I-113). To 7 mL of HNO_3 ($d = 1.5$) with stirring and ice cooling was added portions of 500 mg of I-15 over 10 min. The solution was kept another 5 min at ice temperature and then poured onto ice. A yellow solution formed which soon crystallized. The product was collected and washed with NaHCO_3 solution. This was recrystallized from DMF- Et_2O giving 400 mg (68%) of yellow crystals: mp 249–250 °C; NMR (DMF- d_7) δ 7.94 (dd, 1, $J = 1.5$ Hz, α -H on pyridine), 7.78 (s, 1, α to NO_2), 7.48 (dd, 1, γ -H on pyridine), 7.39 (s, 1, β to NO_2), 7.10 (dd, 1, $J = 5.0$ Hz for α - β , $J = 7.6$ Hz for β - γ , β proton on pyridine).

4-Nitroisobutylbenzene. To 200 g (1.5 mol) of rapidly stirred isobutylbenzene was added dropwise a solution of 95 mL of 70% HNO_3 and 95 mL of concentrated H_2SO_4 . Two layers were always present. The temperature was kept below 32 °C using occasional cooling. The addition took 5.5 h. The mixture was then stirred overnight. To the two layers were then added 200 mL of ice and 100 mL of hexane. The organic layer was separated and washed with water and with a NaHCO_3 solution until all the acid was neutralized. It was then dried, the hexane evaporated, and the yellow liquid distilled twice at 1 mm; the fraction, 69.5 g (26%), boiling at 105–107 °C was shown to be the correct isomer by a study of its NMR spectra [NMR (CDCl_3) δ 8.12 (d, 2, $J = 8.8$ Hz), 7.31 (d, 2, $J = 8.8$ Hz)]. This was used in the next experiment.

Miscellaneous Reactions. Method G. 1,3-Dihydro-3-[3,4-(methylenedioxy)phenyl]-2-oxoimidazo[4,5-*b*]pyridine-6-carboxylic Acid (I-112). Compound I-107 (500 mg) was warmed on the steam bath for 3 h with 5 mL of 2.5 N NaOH . The cooled solution was neutralized with 5 mL of 2.5 N HCl . A gelatinous precipitate was removed by filtration and dried. Repeated extraction with boiling MeOH and then concentration of the combined MeOH extract gave 200 mg (37%) of product, mp 325–328 °C. This contained 1 mol of MeOH which was lost by a thermal gravimetric analysis at 140 °C. Anal. C: calcd, 54.38; found, 53.81.

Method H. 1,3-Dihydro-1-(β -acetoxyethyl)-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-126). A mixture of I-60 (250 mg) and Ac_2O (10 mL) was heated under reflux for 2 h. The solvents were removed in vacuo and the resulting oil was dissolved in Et_2O and washed with NaHCO_3 solution. The Et_2O solution was dried and evaporated. The gum was crystallized from CH_2Cl_2 by the addition of hexane: 120 mg (42%); mp 111–113 °C.

Method I. 3-[3,4-(Methylenedioxy)phenyl]-3-*H*-1,2,3-triazolo[4,5-*b*]pyridine (T-15). About two-thirds of the solution described in method C was diluted with 25 mL of EtOH . This was stirred and cooled to 0 °C. Some of the hydrochloride precipitated, but a solution of 1.3 g of NaNO_2 in 30 mL of water was slowly added. The product began to precipitate and after 20 min at room temperature 3 g of crude brown solid was removed by filtration. This was dissolved in 150 mL of warm C_6H_6 and treated with charcoal and then with 10 g of Al_2O_3 . The filtrate was concentrated to a small volume and 900 mg (16%) of product crystallized by the addition of petroleum ether, mp 169–170 °C.

Method J. 3-(2-Cyanophenyl)-1,2,3-triazolo[4,5-*b*]pyridine (T-150). A mixture of 1 g (0.0036 mol) of T-22 and 1 g (0.01 mol) of CuCN in 10 mL of dry *N*-methylpyrrolidinone was purged with nitrogen and heated in an oil bath at 175 °C for 3 h. After cooling to 50 °C a mixture of 40 mL of NH_4OH and 40 mL of water was added. The precipitate was collected and washed with $\text{NH}_4\text{O}-\text{H}_2\text{O}$ (1:1) and H_2O and air-dried. The solid was extracted with 40 mL of hot C_6H_6 . The C_6H_6 extract was cooled and diluted slightly with petroleum ether to give 600 mg (75%), mp 177–179 °C.

Method K. 3-(2-Carbamoylphenyl)-1,2,3-triazolo[4,5-*b*]pyridine (T-151). T-150 (600 mg) was dissolved in 8 mL of concentrated H_2SO_4 with ice cooling. After standing at room temperature for 7 h it was poured onto ice. The cold solution was treated with an excess of NH_4OH . The precipitate was collected, air-dried, and recrystallized from DMF- H_2O to give 300 mg (45%), mp >340 °C.

1,3-Dihydro-1-(morpholinocarbonylmethyl)-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridin-2-one (I-61). A mixture of 600 mg (0.0019 mol) of I-51 and 10 mol of thionyl chloride was heated on a steam bath for 3 h. The mixture was

evaporated to dryness, the residue was dissolved in Et_2O , and a slight excess of morpholine was added. The mixture was diluted with H_2O and the precipitate was recrystallized from 10 mL of MeOH to give 100 mg (14%), mp 212–213 °C.

1,3-Dihydro-3-[3,4-(methylenedioxy)phenyl]-2-oxoimidazo[4,5-*b*]pyridine-1-*n*-butylcarboxamide (I-71). A suspension of 1.2 g (0.0047 mol) of I-15 in 75 mL of dry dimethoxyethane was treated with 1 g (0.01 mol) of *n*-butyl isocyanate, and the mixture was refluxed until solution was complete. After filtering and evaporating to near dryness it was diluted with petroleum ether to precipitate the product which was recrystallized from 30 mL of EtOH by addition of H_2O to give 400 mg (24%), mp 129–130 °C.

2-Phenyl-3-[3,4-(methylenedioxy)phenyl]imidazo[4,5-*b*]pyridine (I-75). A mixture of 1.15 g (0.005 mol) of 2-[3,4-(methylenedioxy)phenyl]-3-aminopyridine and 600 mg (0.005 mol) of benzoic acid was added to 20 mL of POCl_3 . The mixture was stirred and refluxed for 2 h. The excess of POCl_3 was removed in vacuo and the dark residue treated with ice and NaHCO_3 . The resulting solid was extracted with warm benzene. Petroleum ether was added to the cloud point and the mixture filtered through Super cel. The filtrate with more petroleum ether gave a gum which crystallized. It was recrystallized from 2 mL of hot benzene by the addition of 10 mL of ether. The yield was 100 mg (6%), mp 154–155 °C.

1-Allyl-1,3-dihydro-3-[3,4-(methylenedioxy)phenyl]-2-oxoimidazo[4,5-*b*]pyridinium Methiodide (I-97). A solution of 500 mg of I-21 and 3 mL of MeI in 50 mL of acetone was refluxed for 2 h when another 3 mL of MeI was added and refluxing was continued for another 2 h. The solution was concentrated to a small volume and ether was added to crystallize the quaternary salt. There was obtained 450 mg which was recrystallized from acetone-ether giving 300 mg (41%) melting at 202–203 °C.

3-(2-Hydroxyphenyl)-1,2,3-triazolo[4,5-*b*]pyridine (T-148). A mixture of 1.2 g of T-140, 12.5 g of AlCl_3 , and 175 mL of dry C_6H_6 was refluxed 16 h. The cooled mixture was stirred into ice-water containing 5 mL of concentrated HCl . The C_6H_6 layer was separated, washed with H_2O and NaHCO_3 solution, dried, and evaporated to dryness. The residue was recrystallized from C_6H_6 to give 500 mg (44%), mp 165–166 °C.

2-(2-Fluoroanilino)-3-amino-5-nitropyridine. A mixture of 2.4 g (0.0085 mol) of N-67, 45 mL of EtOH , and 15 mL of NH_4OH was heated to 70 °C with stirring and H_2S bubbled in for 15 min. Upon cooling, 1.4 g of solid separated. This was crystallized from 10 mL of EtOH to give 600 mg (28%), mp 171–172 °C. Anal. ($\text{C}_{11}\text{H}_9\text{FN}_3\text{O}_2$) C, H, N:

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Effects of Thiophene Analogues of Chloroamphetamines on Central Serotonergic Mechanisms

S. Conde, R. Madroñero,*

Department of Chemistry

M. P. Fernández-Tomé, and J. del Río

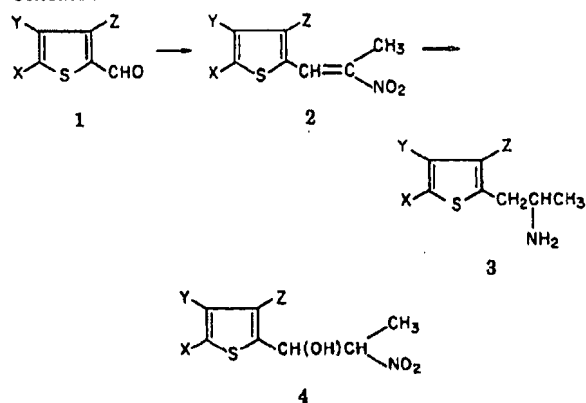
Department of Pharmacology, Instituto de Química Médica, Juan de la Cierva-3, Madrid-6, Spain. Received March 20, 1978

Ring-chlorinated thienylisopropylamines, thiophene analogues of chloroamphetamines, have been synthesized and their effects on serotonergic mechanisms in the rat brain have been evaluated. With 4,5-dichlorothiénylisopropylamine (3e), a pharmacological profile similar to that of *p*-chloroamphetamine, consisting in a marked and long-lasting serotonin depletion and a rather strong and prolonged inhibition of synaptosomal uptake of serotonin, was found. Chloro substitution in position C₃ of the thiophene ring did not determine brain serotonin depletion nor serotonin uptake inhibition but enhanced brain MAO inhibitory activity present in all these compounds. 3,5-Dichlorothiénylisopropylamine (3g) was the only compound of the series in which the inhibition of serotonin uptake was more marked than the serotonin depleting property.

Ring-halogenated amphetamines affect predominantly brain serotonin (5-HT) metabolism.¹ *p*-Chloroamphetamine (PCA) has been extensively studied in this regard, and it is known that this drug exerts multiple actions on 5-HT metabolism. PCA inhibits tryptophan hydroxylase,² releases serotonin from storage granules,^{3,4} blocks serotonin reuptake into the serotonergic neurons,⁴ and inhibits 5-HT metabolism by monoamine oxidase (MAO).⁵ After treatment of rats with a high enough dose of PCA, some of the effects of this drug become irreversible. Thus, brain levels of 5-HT are markedly reduced for several months and the 5-HT synaptosomal uptake system shows also a long-term inhibition.⁶ The selective neurotoxic actions of PCA on 5-HT neurons in the brain may result from the formation of a neurotoxic metabolite or from the prolonged permanence of the drug inside the neuron.⁷ In any case, the continual uptake of PCA into the 5-HT neurons seems necessary to obtain neuronal destruction.⁸ This continual uptake of PCA is possible due to the long half-life of this drug in the organism.⁹ Human studies have shown that PCA, like other blockers of 5-HT reuptake, is an effective antidepressant.¹⁰ However, the possibility of a toxic destruction of 5-HT neurons has perhaps prevented more extensive trials with this drug in psychiatry.

Fuller and Molloy¹¹ suggested that the multiple actions of PCA were potentially dissociable and, in fact, some PCA analogues which only retain some pharmacological features of the parent drug have already been developed.^{12,13} With

Scheme I



this aim, we have prepared some ring-chlorinated thienylisopropylamines, thiophene analogues of chloroamphetamines, and have studied their effects on some aspects of brain 5-HT dynamics. The bioisosteric equivalence of the benzene and thiophene rings is well known,¹⁴ and we have recently found in our laboratory new examples of this bioequivalence.¹⁵⁻¹⁷ On the other hand, the substitution of benzene by thiophene could perhaps lead in the present case to chloroamphetamines analogues more easily metabolized in the brain and, in consequence,



Original article

*N*⁶-Cycloalkyl-2-phenyl-3-deaza-8-azaadenines: a new class of A₁ adenosine receptor ligands. A comparison with the corresponding adenines and 8-azaadenines

Giuliana Biagi^a, Irene Giorgi^{a,*}, Oreste Livi^a, Antonio Nardi^a, Federica Pacchini^a,
Valerio Scartoni^a, Antonio Lucacchini^b

^a Dipartimento di Scienze Farmaceutiche, Università di Pisa, via Bonanno, 6-56126 Pisa, Italy

^b Dipartimento di Psichiatria, Neurobiologia, Farmacologia e Biotecnologie, Università di Pisa, via Bonanno, 6-56126 Pisa, Italy

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Abstract

Several 9-benzyl-*N*⁶-cycloalkyl-2-phenyladenines, 9-benzyl-*N*⁶-cycloalkyl-2-phenyl-8-azaadenines and 4-cycloalkylamino-1-benzyl-6-phenyl-1*H*-1,2,3-triazolo[4,5-*c*]pyridines were prepared and assayed as A₁ adenosine receptor ligands. The 1*H*-1,2,3-triazolo[4,5-*c*]pyridines were obtained starting from *N,N*-diethyl-1-benzyl-4-carboxyamido-5-methyl-1*H*-1,2,3-triazole by lithiation in anhydrous tetrahydrofuran in the presence of benzonitrile. The usual work up afforded the isolation of 1-benzyl-6-phenyl-1*H*-1,2,3-triazolo[4,5-*c*]pyridin-4-one which was treated with phosphorous oxychloride and cycloalkylamines. Some compounds showed high affinity and selectivity and the trend of *K_i* values corresponds to the series of 9-benzyl-*N*⁶-cycloalkyl-2-phenyladenines and 9-benzyl-*N*⁶-cycloalkyl-2-phenyl-8-azaadenines, therefore they can be considered bioisosteres. The affinity data permitted us to ascertain the role and the importance of the N(3) in the adenine or 8-azaadenine moiety in the receptor binding and to study the dimension of the receptor lipophilic pocket which is filled by the *N*⁶ substituent of adenosine derivatives.

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1. Introduction

Adenosine is an endogenous nucleoside which mediates a variety of important physiological effects by its specific receptors: A₁ receptors were the first subtype to be identified. They are widely distributed in the central nervous system and in peripheral tissues and mediate diverse biological effects. The role of adenosine as a neuroprotective agent during hypoxia and ischemic conditions seems to be mediated by the A₁ receptors; these receptors have been involved in sedative, anti-seizure and anxiolytic effects. A₁ receptors mediate cardiac depression through negative chronotropic, dromotropic and inotropic effects. In the kidney, activation of A₁ receptors causes vasoconstriction, inhibition of

renin secretion, diuresis, natriuresis and other effects [1]. A great number of physiological effects have been demonstrated for A₁ receptors. For example, hundreds of agonists and antagonists have been assayed to obtain some compounds useful in therapy. A number of A₁ antagonists have been studied as novel potassium sparing diuretics with kidney-protection properties, in dementia, Alzheimer's disease, cardiac therapy and kidney disease [2].

In the course of our studies directed toward synthesis of A₁ adenosine receptor antagonists, we had occasion to prepare and test a number of 8-azaadenines and adenines having three lipophilic substituents in the positions 2, 9 and *N*⁶ [3]. Initially, we synthesised 2-, *N*⁶- and 9-substituted-8-azaadenines by a facile two-stage method [4]. This procedure afforded a large number of compounds with good affinity towards A₁ adenosine receptors. These had high selectivity A₁/A₂

* Corresponding author.

E-mail address: igiorgi@farm.unipi.it (I. Giorgi).

and some were very effective having a K_i in the nanomolar range. The most active compounds were 9-benzyl-2-phenyl-8-azaadenines N^6 -cyclopentyl and N^6 -cyclohexyl substituted (general formula I, Fig. 1), having K_i 11 [3] and 1.6 nM [5], respectively. Thus, we have completed the series, by synthesising all the N^6 -cycloalkyl derivatives from three to eight carbons. We also prepared the corresponding adenine derivatives (general formula II, Fig. 1) in order to compare their affinity with the analogous 8-azaadenines (I), and to confirm the bioisosterism of the two nuclei, which we demonstrated recently [6].

In an effort to further explore other structural modifications of the purine nucleus, we sought to verify the importance of N(3) in the binding of ligands with A_1 adenosine receptors. In the past, some studies regarding the relative importance of nuclear nitrogen atoms in several deaza analogs of adenosine were synthesised [7]. More recently, N -cycloalkyl derivatives of adenosine and 1-deazaadenosine, were compared regarding the importance both of N-1 atom and of the dimension of the cycloalkyl inserted on N^6 [8]. Similar analogous studies on adenine derivatives have not been reported in the literature.

First we planned to prepare 9-benzyl-2-phenyl-3-deaza-8-azaadenines (general formula III, Fig. 1) and 9-benzyl-2-phenyl-3-deazaadenines (general formula IV, Fig. 1) by cyclisation of 3,4-diamino-2-chloro-6-phenylpyridine by classical methods, but this first idea was rejected because these compounds are not easy to obtain by this synthetic pathway. In fact in the literature the preparation of N^6 -substituted-2-chloro-3,4-diaminopyridine [9] or 2-hydroxy-3,4-diaminopyridine [10] is reported, but their 6-phenylsubstituted analogs are not. So we studied a new synthetic route which permitted us to prepare, starting from 1-benzyl-5-methyl-1H-1,2,3-triazole-4-carboxylic acid, 4-cycloalkyl derivatives of 7-phenyl-1-benzyl-1H-1,2,3-triazolo[4,5-c]pyridine (2-phenyl-9-benzyl-3-deaza-8-azaadenines) which, up to now, have not been reported. Comparing the capability of these compounds to bind A_1 adenosine receptors with the corresponding adenine and 8-azaadenine derivatives, we were able to study the importance of the purine N(3) atom in the binding with A_1 receptors. Synthesis of the

three series of compounds (2-phenyl-9-benzyl- N^6 -cycloalkyl substituted adenines, 8-azaadenines and 3-deaza-8-azaadenines) was also useful to explore the steric tolerance of the A_1 receptor lipophilic pocket in front of N^6 of adenosine which is able to hold a cyclopentyl or a cyclohexyl moiety in potent agonists (as cyclohexyladenosine or cyclopentyladenosine) or antagonists as N^6 -cyclohexyl- or cyclopentyl-2-phenyl-9-benzyl-8-azaadenines.

2. Chemistry

The 9-benzyl-2-phenyladenine (4) and its N^6 -cycloalkyl derivatives (5–11) were obtained from 9-benzyl-6-chloro-2-phenylpurine (3) according to described procedures [6] (Fig. 2); also the 9-benzyl- N^6 -cycloalkyl-2-phenyl-8-azaadenines (14–18) were prepared as previously reported [11] (Fig. 3).

Compounds 27–34 were obtained by a new method (Fig. 4). In fact, up to now, 1,2,3-triazolo[4,5-c]pyridines have been prepared from the corresponding 3,4-diaminopyridines by diazotation reaction [9,12]. In this paper we present a synthetic route starting from 1-benzyl-5-methyl-1H-1,2,3-triazole-4-carboxylic acid ethyl ester (19), obtained as described previously in the literature [14] by regioselective and base-catalysed cycloaddition of benzylazide and ethyl acetoacetate under mild conditions; 19 was hydrolysed with sodium hydroxide to obtain the corresponding acid (compound 20) [15] which, in turn, was converted into chloride 21 by treatment with thionyl chloride at reflux: this was converted in four stages into 1-benzyl-4-aminosubstituted-6-phenyl-1H-1,2,3-triazolo[4,5-c]pyridines. This synthetic method is based on a lithiation reaction: a first attempt was carried out on 1-benzyl-5-methyl-1H-1,2,3-triazole-4-(N -methyl)-carboxamide (22) in the presence of benzonitrile and lithium diisopropyl amide (LDA). This reaction was used by Poindexter [15] who transformed 1-methyl-2-(N -methyl)-carboxamido benzene into an isochinoline derivative. The chloride 21 was reacted with methyl amine to obtain the corresponding amide 22. A treatment of 22 with benzonitrile and a 2 M solution of LDA at -74°C , did not give the expected 1-

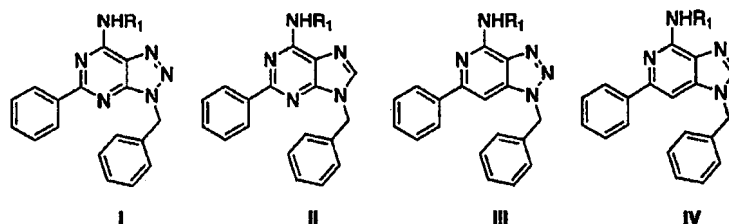


Fig. 1. General formulas of N^6 -substituted 9-benzyl-2-phenyl-8-azaadenines (I), N^6 -substituted 9-benzyl-2-phenyladenines (II), N^6 -substituted 9-benzyl-2-phenyl-3-deaza-8-azaadenines (III) N^6 -substituted 9-benzyl-2-phenyl-3-deazaadenines (IV).

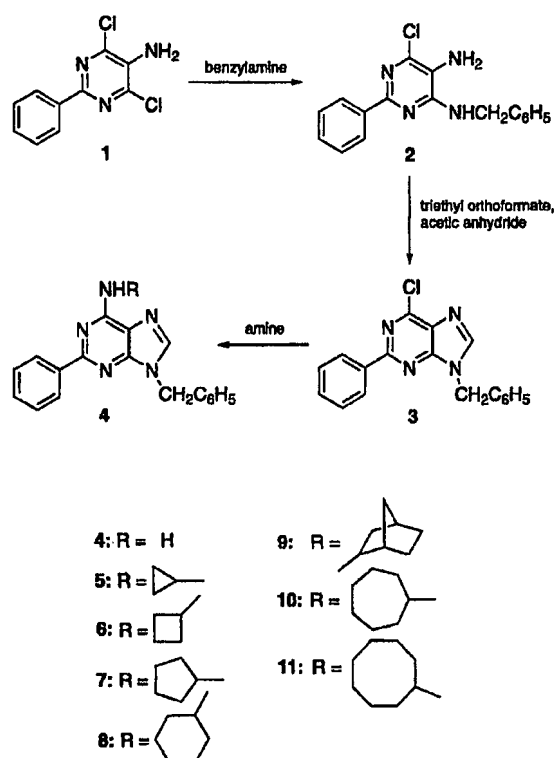
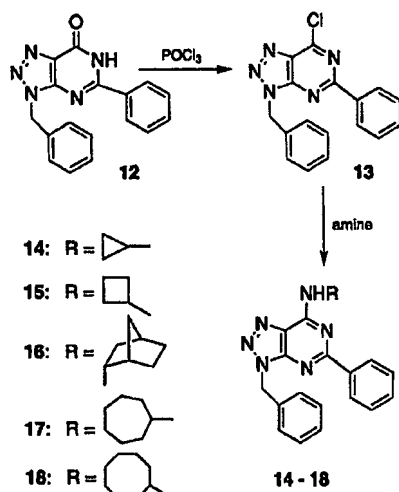
Fig. 2. Synthesis of N^6 -substituted 2-phenyl-9-benzyladenines (4–11).

Fig. 3. Synthesis of 9-benzyl-2-phenyl-6-cycloalkylamino-8-azaadenines (14–18).

benzyl-6-phenyl-1H-1,2,3-triazolo[4,5-c]pyridine-4-one (25). From the reaction mixture, only the open imino intermediate adduct 23, formed between lithiated triazole and benzonitrile, was isolated. All attempts to

obtain compound 25 by heating 23 in basic or acid conditions failed. Only the starting material, sometimes contaminated with decomposition products, was recovered. A good yield (60%) of 25 was obtained employing the lithiation reaction on 1-benzyl-5-methyl-1H-1,2,3-triazole-4-(*N,N*-diethyl)-carboxamide (24), prepared from 21 by reaction with diethylamine. Examples of cyclisation by lithiation reaction using diethylamides were described by Mitsuaki et al. in 1984 [16]. 1-Benzyl-6-phenyl-1H-1,2,3-triazolo[4,5-c]pyridine-4-one (25) was transformed into the corresponding chloride 26 by reaction with phosphorous oxychloride. Finally, the 4-aminosubstituted compounds 27–34 were obtained by treatment of the chloride with the appropriated amine at 140–150 °C in a sealed tube.

3. Biological evaluation

Compounds 4–11, 14–18 and 27–34 were tested in radioligand binding assays for affinity at A₁ and A_{2A} adenosine receptors in bovine brain cortical membranes and bovine striatum respectively. In Table 1 the results of A₁ adenosine receptor binding assays are reported; in A_{2A} adenosine receptor binding assays, all the compounds showed inhibition values <60% at 1 μM, and are considered inactive.

4. Results and discussion

All synthesised compounds, assayed as A₁ and A_{2A} adenosine receptor ligands, showed good affinity and selectivity for A₁ subtype. As can be seen in the histogram of Table 1, trends of K_i values in series I and II are very similar; by increasing the size of the N^6 -cycloalkyl substituent, affinity increased up to five or six carbon atoms, then affinity decreased for the compounds with N^6 -cycloheptyl or -cyclooctyl substituents. The biological results (Table 1) also indicate that the two series would bind to the receptor in the same site with the same orientation; the receptor pocket in front of N^6 of adenosine would be very large and capable of binding either little or large cycloalkyl substituents. The different size of the cycloalkyl substituent modulated the affinity, and cyclopentyl, cyclohexyl or norbornyl groups conferred the highest affinity to the molecules.

The comparison of the values of K_i of adenines (Table 1) with those of the corresponding 8-azaadenines (Table 1, newly developed compounds 14–18 and compounds A–C already reported in the literature [3,5]) showed that they are very close, either for N^6 -unsubstituted as for N^6 -cycloalkylsubstituted compounds. This strong similarity of affinity permits us to consider the two nuclei as bioisosteric: the N(8) did not contribute to the binding

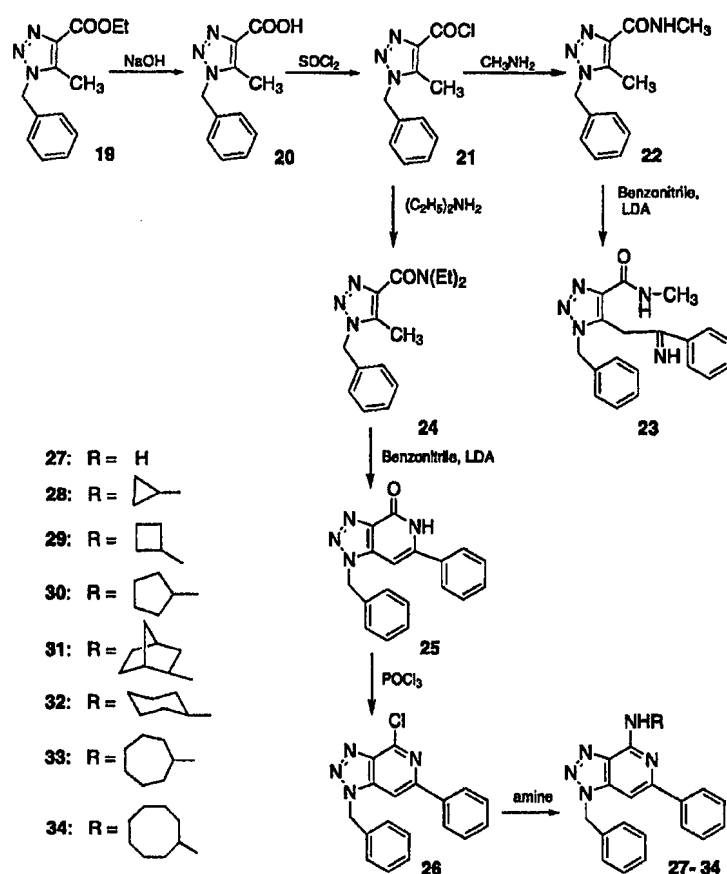


Fig. 4. Synthesis of 1-benzyl-4-amino-6-phenyl-1H-1,2,3-triazol[4,5-c]pyridine (27) and 1-benzyl-4-cycloalkylamino-6-phenyl-1H-1,2,3-triazol[4,5-c]pyridines (28–34).

of these molecules towards the receptor protein in a different manner from the C(8).

Regarding triazolopyridines (series III), biological results (Table 1) indicated a general lowering of A_1 affinity with respect to the analogous compounds having nitrogen in the position 3 (series I and II): most of the triazolopyridine derivatives (compounds 27, 28, 32, 33, 34) have a $K_i > 100$ nM, and only compounds 29, 30 and 31 are very potent A_1 ligands, having K_i values of 44, 14 and 11 nM, respectively. The trend of K_i values in series III is similar to that of series I and II for the first five compounds (compounds 27–31 vs. 4–7 and A, 14, 15, B, 16) but it is different for the other ones, in which we can observe an abrupt lowering of activity. So, in the triazolopyridines, the size of the cycloalkyl substituent was more critical, because affinity decreased quickly when the cycloalkyl moiety was different from cyclobutyl, cyclopentyl or norbornyl group. In conclusion, from the biological data we may presume that nitrogen in the position 3 of 2-phenyl-8-azaadenines (I) and their bioisoster 2-phenyladenines (II) was impor-

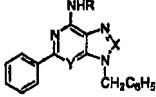
tant, but not crucial, to stabilise the complex between these ligands and the A_1 receptors.

5. Experimental

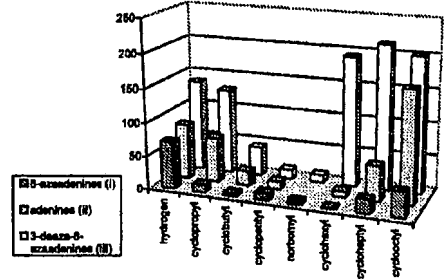
5.1. Chemistry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. $^1\text{H-NMR}$ spectra were recorded on a Varian Gemini 200 spectrometer in δ units using TMS as an internal standard; the compounds were dissolved in CDCl_3 . Mass spectra were performed on a Hewlett–Packard GC/MS System 5988A. TLC was performed on precoated silica gel F₂₅₄ plates (Merck). Flash-column chromatography was performed using Merck Kieselgel 60 (230–400 mesh). Microanalyses (C H N) were carried out on a Carlo Erba elemental analyser (model 1106) and were within $\pm 0.4\%$ of the theoretical values. Petroleum–ether corresponds to fraction boiling at 40–60 °C.

Table 1
A₁ adenosine receptor binding results and related histogram



R	8-azaadenines (I) X=N; Y=N	Ki (nM)	adenines (II) X=C; Y=N	Ki (nM)	3-deaza-8-azaadenines (III) X=N; Y=C	Ki (nM)
□	A ^a	71 ^a	4	83±7	27	137±10
△	14	11.3±1	5	68±7	28	128±11
◇	15	5±0.5	6	22±2	29	44±4
○	B ^a	11 ^a	7	12±2	30	14±1
⬢	16	5±0.4	8	*	31	11.1±1
⬢	C ^a	1.6 ^a	9	9±1	32	193±20
⬢	17	20.7±2	10	53±4	33	214±22
⬢	18	38±4	11	167±10	34	201±19



^a See Fig. 1.
* Compound 8 is not enough soluble at the assay conditions.
^a Ref. [3]; ^b Ref. [5].

5.1.1. General synthesis of N⁶-substituted 2-phenyl-9-benzyladenines (4–11)
9-Benzyl-6-chloro-2-phenylpurine 3 [6] (0.15 g, 0.47 mmol), the suitable amine (Table 2) (6.60 mmol) and some drops of *N,N*-diethylaniline were dissolved in absolute ethanol (3 mL), and refluxed for 12 h. After evaporation, the mixture was diluted with chloroform, washed with 10% HCl and 5% NaHCO₃ and H₂O, evaporated and crystallised from diethyl ether–hexane to give the title compounds (Tables 2 and 3).

5.1.2. General synthesis of 9-benzyl-2-phenyl-6-cycloalkylamino-8-azaadenines (14–18)
To a mixture of 2-phenyl-9-benzyl-8-azaxanthine (12) [17] (1.0 g, 3.31 mmol) and *N,N*-diethylaniline (0.8 g, 5.36 mmol), phosphorous oxychloride (6 mL, 65.1 mmol) was added and heated at 90 °C for 4 h. Then the mixture was evaporated at reduced pressure and the resulting 6-chloro-2-phenyl-9-benzyl-8-azaadenine (13) [18] was used without purification for the substitution reactions. In a well-stoppered flask a mixture of 13 (0.2 g, 0.62 mmol), ethanol (5 mL), the suitable amine (Table

Table 2
Reaction and physico-chemical data

Compound	Amine	Yield (%)	M.p. (°C)	Analysis (C, H, N)
4	NH ₃	85	210	C ₁₈ H ₁₅ N ₅
5	cyclopropylamine	62	173	C ₂₁ H ₁₉ N ₅
6	cyclobutylamine	58	225	C ₂₂ H ₂₁ N ₅
7	cyclopentylamine	80	165–167	C ₂₃ H ₂₃ N ₅
8	norbornylamine	47	150	C ₂₅ H ₂₅ N ₅
9	cyclohexylamine	80	170	C ₂₄ H ₂₅ N ₅
10	cycloheptylamine	39	140	C ₂₅ H ₂₇ N ₅
11	cyclooctylamine	79	137–139	C ₂₆ H ₂₉ N ₅
14	cyclopropylamine	63	193–195	C ₂₀ H ₁₈ N ₆
15	cyclobutylamine	70	170	C ₂₁ H ₂₀ N ₆
16	norbornylamine	58	185	C ₂₄ H ₂₄ N ₆
17	cycloheptylamine	58	144	C ₂₄ H ₂₆ N ₆
18	cyclooctylamine	62	176–178	C ₂₅ H ₂₈ N ₆
27	NH ₃	68	145–146	C ₁₈ H ₁₅ N ₅
28	cyclopropylamine	93	156–157	C ₂₁ H ₁₉ N ₅
29	cyclobutylamine	95	136	C ₂₂ H ₂₁ N ₅
30	cyclopentylamine	92	147	C ₂₃ H ₂₃ N ₅
31	norbornylamine	44	140–143	C ₂₅ H ₂₅ N ₅
32	cyclohexylamine	90	138–139	C ₂₄ H ₂₅ N ₅
33	cycloheptylamine	83	121	C ₂₅ H ₂₇ N ₅
34	cyclooctylamine	87	106–107	C ₂₆ H ₂₉ N ₅

2) (6 mmol) and *N,N*-diethylaniline (0.1 g, 0.67 mmol) was refluxed for 12 h. Then the solution was evaporated at reduced pressure, diluted with chloroform and washed with 10% HCl and water. After evaporation of the organic layer, the residuc was crystallised from isopropanol (Tables 2 and 3).

5.1.3. 1-Benzyl-5-methyl-1*H*-1,2,3-triazol-4-carboxylic acid ethyl ester (19)

Title compound was obtained as described in the literature [13]. M.p. 78–79 °C (lit. 76–78 °C).

5.1.4. 1-Benzyl-5-methyl-1*H*-1,2,3-triazol-4-carboxylic acid (20)

A mixture of 19 and 30 mL of 10% NaOH was refluxed for 2 h; after cooling, the title compound, precipitated by acidification to pH of 2, was filtered, washed with water and crystallised from chloroform–hexane. M.p. 168–169 °C (lit. 168–169 °C [15]). MS (*m/e*): 217 [M⁺], 188, 91, 65. ¹H-NMR (CDCl₃): 7.38–7.16 (m, 5H); 5.51 (s, 2H); 2.48 (s, 3H).

5.1.5. 1-Benzyl-5-methyl-1*H*-1,2,3-triazol-4-carbonyl chloride (21)

1-Benzyl-5-methyl-1*H*-1,2,3-triazol-4-carboxylic acid (20) (3.0 g, 13.8 mmol) was refluxed in SOCl₂ for 3 h. By evaporation under reduced pressure a solid was obtained (3.1 g, 13.3 mmol, 96% yield) which was crystallised from chloroform–hexane. M.p. 94–95 °C. MS (*m/e*): 235 [M⁺], 206, 200, 178, 91, 65. ¹H-NMR

Table 3
1H-NMR and MS spectral data of some synthesised compounds

Compound	1H-NMR				MS (m/z, %)
	Aromatic H	Benzylic H	Aliphatic H	Exchang. H	
4	7.52–7.32 (m 8H), 7.77 (s 1), 8.46–8.43 (m 2H)	5.45 (m 2H)	–	5.62 (2H)	301 ([M ⁺] 28), 91 (100)
5	7.56–7.36 (m 8H), 7.84 (s 1), 8.58–8.53 (m 2H)	5.45 (m 2H)	1.02–0.86 (m 4H), 3.45 (m 1H)	–	341 ([M ⁺] 3.5), 91 (100)
6	7.58–7.38 (m 8H), 7.89 (s 1), 8.56 (m 2H)	5.46 (m 2H)	2.51–1.21 (m 7H), 5.30 (m 1H)	5.30 (1H)	355 ([M ⁺] 2), 300 (2) 236 (11), 91 (100)
7	7.63–8.41 (s 1H), 7.60–7.43 (m 8H), 8.53–8.41 (m 2H)	5.39 (m 2H)	2.16–1.73 (m 8H), 4.95 (m 1H)	5.7 (1H)	369 ([M ⁺] 5), 300(1) 333 (13), 91(100)
8	7.53–7.32 (m 8H), 7.72(s 1H), 8.54–8.49 (m 2H)	5.44 (m 2H)	1.67–0.89 (m 10H), 5.30 (m 1H)	5.86 (1H)	395 ([M ⁺] 11), 301 (16), 91 (100)
9	7.83 (m 1H), 7.58–7.50(s 8H), 8.54–8.49 (m 2H)	5.44 (m 2H)	1.67–0.89 (m 10H), 5.86 (m 1H)	6.15 (1H)	395 ([M ⁺] 11), 301 (16), 91 (100)
10	7.51–7.37 (m 8H), 7.75 (s 1H), 8.56–8.52 (m 2H)	5.44 (m 2H)	1.65 (m 10H), 2.19–2.13 (m 2H), 4.60 (m 1H)	4.91 (1H)	397 ([M ⁺] 5), 301 (32), 91 (100)
11	8.55–8.50 (m 2H), 7.71 (s 1H), 7.46–7.36 (m 8H)	5.43 (m 2H)	1.66 (m 12H), 2.13–2.03 (m 2 H), 4.64 (m 1H)	5.75 (1H)	411([M ⁺] 3), 301(25), 91 (100)
14	8.59–8.54 (m 2H), 7.58–7.53 (m 5H), 7.31 (m 3H)	5.84 (s 2H)	1.16–0.80 (m 4H) 3.68 (m 1H)	6.30 (1H)	342 ([M ⁺] 17), 315 (2), 91 (100)
15	8.55–8.52 (m 2H), 7.53–7.50 (m 5H), 7.37–7.34 (m 3H)	5.81 (s 2H)	1.94 (m 2H), 2.20 (m 2H), 2.60 (m 2H), 4.97 (m 1H)	6.32 (1H)	328 ([M ⁺] – 28 49), 300 (5.6), 91 (100)
16	8.54–8–51 (m 2H), 7.51–7.30 (m 8H)	5.80 (s 2H)	2.80 (m 1H), 2.34 (m 2H), 1.65–1.00 (m 7H), 4.66 (m 1H)	6.25 (1H)	396 ([M ⁺] 10), 339 (4), 273 (15), 91 (100)
17	8.56–8.53 (m 2H), 7.52–7.49 (m 5H), 7.33–7.31 (m 3H)	5.81 (s 2H)	1.75–1.58 (m 10H), 2.20 (m 2H), 4.560 (m 1H)	6.21 (1H)	398 ([M ⁺] 2.8), 302 (18) 273 (36), 91 (100)
18	8.60–8.57 (m 2H), 7.64–7.37 (m 8H)	5.83 (s 2H)	1.70–1.55 (m 12H), 2.10–2.04 (m 2H), 5.10 (m 1H)	6.22 (1H)	412 ([M ⁺] 10), 302 (19) 273 (42), 91 (100)
27	7.91–7.85 (m 2H), 7.51–7.31 (m 8H), 6.93 (s 1H)	5.80 (s 2H)	–	5.69 (1H)	301 ([M ⁺] 10), 272 (5), 91(100)
28	8.03–7.99 (m 2H), 7.48–7.30 (m 8H), 6.91 (s 1H)	5.78 (s 2H)	3.22 (m 1H), 0.95 (m 2H), 0.75 (m 2H)	6.38 (1H)	341([M ⁺] 7), 91(100), 77(13)
29	7.99–7.95 (m 2H), 7.48–7.30 (m 8H), 6.87 (s 1H)	5.77 (s 2H)	4.97 (m 1H), 2.54 (m 2H), 2.06 (m 2H), 1.84 (m 2H)	6.01 (1H)	355 ([M ⁺] 2), 326 (23), 91(100)
30	8.01–7.96 (m 2H), 7.44–7.27 (m 8H), 6.87 (s 1H)	5.77 (s 2H)	4.78 (m 1H), 2.21 (m 2H), 1.79–1.67 (m 6H)	5.94 (1H)	369 ([M ⁺] 2), 301(10) 272(5), 91(100)
31	7.99–7.94 (m 2H), 7.49–7.30 (m 8H), 6.86 (s 1H)	5.77 (s 2H)	4.64 (m 1H), 2.32–0.85 (m 10H)	5.96 (1H)	395 ([M ⁺] 15.4), 366(12) 301(30), 91(100)
32	7.99–7.94 (m 2H), 7.50–7.30 (m 8H), 6.86 (s 1H)	5.77 (s 2H)	4.35 (m 1H), 2.21 (m 2H), 1.87–1.26 (m 8H)	5.81 (1H)	383 ([M ⁺] 6), 301(72) 272(28.8) 91(100)
33	8.00–7.97 (m 2H), 7.48–7.30 (m 8H), 6.86 (s 1H)	5.77 (s 2H)	4.57 (m 1H), 2.19 (m 2H), 1.67–1.60 (m 10H)	5.82 (1H)	397([M ⁺] 7), 301(81) 272(22), 91(100)
34	8.00–7.97 (m 2H), 7.47–7.30 (m 8H), 6.86 (s 1H)	5.77 (s 2H)	4.61 (m 1H), 2.18–1.57 (m 14H)	5.82 (1H)	411([M ⁺] 2), 301(14) 272(4), 91(100) 77(6)

(CDCl₃): 7.38–7.16 (m, 5H); 5.52 (s, 2H); 2.45 (s, 3H). Anal.: C,H,N.

5.1.6. 1-Benzyl-5-methyl-4-(N-methyl)-carboxamido-1H-1,2,3-triazole (22)

To an iced solution of 21 (2.9 g, 12.7 mmol) in THF (50 mL), 40% aqueous methylamine (25 mL) was added slowly. After stirring for a day at room temperature (r.t.), the mixture was concentrated at reduced pressure, then filtered and the solid washed with water and crystallised from chloroform–hexane. The pure title compound was obtained with high yield (2.7 g, 11.8

mmol, 92% yield). M.p. 134–135 °C. MS (m/e): 230 [M⁺], 201, 91, 41. 1H-NMR (CDCl₃): 7.30–7.12 (m, 5H+1H exchangeable); 5.47 (s, 2H); 2.97 (d, 3H); 2.49 (s, 3H). Anal.: C,H,N.

5.1.7. 1-Benzyl-4-(N-methyl)-carboxamido-5(1-phenyl-1-iminoethyl)-1H-1,2,3-triazole (23)

To a stirring solution of 22 (2.0 g, 8.68 mmol) and benzonitrile (1.1 g, 0.01 mol) in anhydrous THF (50 mL), under nitrogen and cooled to –74 °C, 10 mL of 2M LDA was added. Stirring was maintained for 30 min at –74 °C, then the mixture was allowed to reach r.t.

After evaporation at reduced pressure, the residue was dissolved in CH_2Cl_2 and the solution was washed with saturated NH_4Cl , H_2O and evaporated. The orange oil, treated at reflux with petroleum ether 40–60 to dissolve the unreacted benzonitrile, was repeatedly crystallised from toluene and finally from ethanol to give pure 23 (0.43 g, 1.35 mmol, 15% yield). M.p. 183–185 °C. MS (*m/e*): 375 [M^+], 234. $^1\text{H-NMR}$ (CDCl_3): 8.00 (s, 1H, exc.); 7.97 (s, 1H, exc.); 7.61–7.11 (m, 10H); 5.62 (s, 2H); 4.72 (s, 2H); 2.98 (d, 3H). Anal.: C, H, N.

5.1.8. 1-Benzyl-5-methyl-1H-4-(*N,N*-diethyl)-carboxamido-1,2,3-triazole (24)

To an iced solution of 21 (3.0 g, 12.7 mmol) in CH_2Cl_2 , *N,N*-diethylamine (5.0 g, 33.7 mmol) was slowly added. The mixture was refluxed for 2 h, then cooled and repeatedly washed with 5% HCl. The organic layer was evaporated to give an oil which was flash-chromatographed using as eluent CHCl_3 –MeOH 99:1. The pure title compound was obtained as an oil (2.96 g, 10.9 mmol, 85% yield). MS (*m/e*): 272 [M^+], 201, 91. $^1\text{H-NMR}$ (CDCl_3): 7.41–7.17 (m, 5H); 5.50 (s, 2H); 3.84 (q, 2H); 3.53 (q, 2H); 2.42 (s, 3H); 1.32 (t, 3H); 1.24 (t, 3H). Anal.: C, H, N.

5.1.9. 1-Benzyl-6-phenyl-1H-1,2,3-triazol[4,5-*c*]pyridin-4-oxo (25)

To a stirring solution of 24 (3.0 g, 11.0 mmol) and benzonitrile (1.25 g, 12.1 mmol) in anhydrous THF (20 mL), under nitrogen and cooled to –70 °C, 8 mL of 2 M LDA was added. Then the mixture was allowed to reach r.t. and stirring was maintained overnight. After evaporation at reduced pressure, the oily residue was dissolved in CH_2Cl_2 (200 mL) and the solution was washed with saturated NH_4Cl and concentrated to 15 mL, obtaining a solid which was filtered (2.05 g, 6.78 mol, 62% yield) and crystallised from ethanol. M.p. 240–242 °C. MS (*m/e*): 302 [M^+], 273, 91, 77, 65. $^1\text{H-NMR}$ (CDCl_3): 9.43 (s, 1H, exc.); 7.47–7.22 (m, 10H); 6.30 (s, 1H); 5.71 (s, 2H). Anal.: C, H, N.

5.1.10. 1-Benzyl-4-chloro-6-phenyl-1H-1,2,3-triazol[4,5-*c*]pyridine (26)

A solution of 25 (0.2 g, 0.66 mmol) and POCl_3 (1 mL) was refluxed for 30 min. To the mixture, iced and stirred, ethanol (15 mL) and then H_2O (30 mL) were slowly added. The solid obtained was filtered, washed with H_2O and crystallised from 1,2-dimethoxyethane– H_2O to give 0.19 g (0.61 mmol, 92% yield) of the title compound. M.p. 173–174 °C. MS (*m/e*): 320 [M^+], 291, 257, 91, 77, 65. $^1\text{H-NMR}$ (CDCl_3): 7.97 (d, 1H); 7.93 (d, 1H); 7.49–7.31 (m, 9H); 5.91 (s, 2H). Anal.: C, H, N.

5.1.11. 1-Benzyl-4-amino-6-phenyl-1H-1,2,3-triazol[4,5-*c*]pyridine (27)

An iced solution of 26 (0.1 g, 0.31 mmol) in a mixture of 1,2-dimethoxyethane– H_2O (1:1) was saturated with gaseous NH_3 , then heated in a steel bomb at 140 °C for 36 h. After cooling, 5% HCl was added dropwise to the mixture. The solid obtained was filtered, washed with H_2O and crystallised from 1,2-dimethoxyethane– H_2O to give 0.63 g (0.21 mmol, 67% yield) of the title compound (Tables 2 and 3).

5.1.12. General synthesis of 1-benzyl-4-cycloalkylamino-6-phenyl-1H-1,2,3-triazol[4,5-*c*]pyridine (28–34)

To a solution of 26 (0.31 mmol) in 1,2-dimethoxyethane, the suitable amine (Table 2) (2.0 mmol) was added, then heated in a steel bomb at 150 °C for 24 h. After cooling, 20 mL of 5% HCl was added dropwise to the mixture. The solid obtained was filtered, washed with H_2O and crystallised from 1,2-dimethoxyethane– H_2O (Tables 2 and 3).

5.2. Biochemistry

5.2.1. A_1 receptor binding

Bovine cerebral cortex was homogenised in ice-cold 0.32 M sucrose containing protease inhibitors, as previously described [19]. The homogenate was centrifuged at $1000 \times g$ for 10 min at 4 °C and the supernatant again centrifuged at $48\,000 \times g$ for 15 min at 4 °C. The final pellet was dispersed in 10 volumes of fresh buffer, incubated with adenosine deaminase (2 U mL^{-1}) to remove endogenous adenosine at 37 °C for 60 min and then recentrifuged at $48\,000 \times g$ for 15 min at 4 °C. The pellet was suspended in buffer and used in the binding assay.

The [^3H]CHA binding assay was performed in triplicate by incubating aliquots of the membrane fraction (0.2–0.3 mg of protein) at 25 °C for 45 min in 0.5 mL of Tris–HCl, pH 7.7, containing 2 mM MgCl_2 , with approximately 1.2 nM [^3H]CHA. Nonspecific binding was defined in the presence of 50 μM R-PIA. Binding reactions were terminated by filtration through Whatman GF/C filters under reduced pressure. Filters were washed three times with 5 mL of ice-cold buffer.

5.2.2. A_{2A} receptor binding

Bovine striatum was homogenised in 20 volumes of ice-cold 50 mM Tris–HCl, pH 7.5, containing 10 mM MgCl_2 and protease inhibitors. The membrane homogenate was centrifuged at $48\,000 \times g$ for 10 min at 4 °C. The resulting pellet was resuspended in buffer containing 2 U mL^{-1} of adenosine deaminase and incubated at 37 °C for 30 min. The membrane homogenate was centrifuged, and the final pellet frozen at –80 °C. Routine assays were performed in triplicate by incubation an aliquot of striatal membranes (0.2–0.3 mg of

protein) in cold 50 mM Tris–HCl, pH 7.5, containing 10 mM MgCl_2 with approximately 5 nM [^3H]CGS 21680 in a final volume of 0.5 mL. Incubation was carried out for 90 min at 25 °C. Non-specific binding was defined in the presence of 50 μM CGS 21680. Binding reactions were terminated by filtration through Whatman GF/C filters under reduced pressure. Filters were washed three times with 5 mL of ice-cold buffer and placed in scintillation vials. The radioactivity was counted in a 4 mL Beckman Ready-Protein scintillation cocktail in a scintillation counter. The compounds were dissolved in DMSO and added to the assay mixture to make a final volume of 0.5 mL. Blank experiments were carried out to determine the effect of the solvent (2%) on binding. The concentrations of the tested compounds to produce 50% inhibition of specific [^3H]CHA or [^3H]CGS 21680 binding (IC_{50}) were determined from semilog plots of data from experiments of binding inhibition. The K_i values were calculated from the IC_{50} values using the equation $\text{IC}_{50}/(L/K_d)$ [20] ([^3H]CHA $K_d = 10.5$ nM and $L = 1.2$ nM; [^3H]CGS 21680 $K_d = 1$ nM and $L = 5$ nM); protein estimation was based on the method reported [21] using bovine serum albumin as standard.

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2-{2-[3-(Pyridin-3-yloxy)phenyl]-2H-tetrazol-5-yl}pyridine: a highly potent, orally active, metabotropic glutamate subtype 5 (mGlu5) receptor antagonist

Dehua Huang,^{a,*} Steve F. Poon,^a Deborah F. Chapman,^c Janice Chung,^b Merryl Cramer,^a
Thomas S. Reger,^a Jeffrey R. Roppe,^a Lida Tehrani,^a Nicholas D. P. Cosford^a and
Nicholas D. Smith^a

^aDepartment of Medicinal Chemistry, Merck Research Laboratories, MRLSDB2, 3535 General Atomics Court,
San Diego, CA 92121, USA

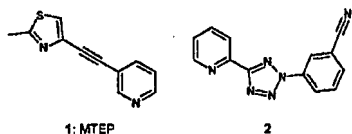
^bDepartment of Molecular Profiling, Merck Research Laboratories, MRLSDB2, 3535 General Atomics Court,
San Diego, CA 92121, USA

^cDepartment of Neuropharmacology, Merck Research Laboratories, MRLSDB1, 3535 General Atomics Court,
San Diego, CA 92121, USA

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Abstract—Structure–activity relationship studies on 3-(5-pyridin-2-yl-2H-tetrazol-2-yl)benzonitrile **2** led to the discovery of 2-{2-[3-(pyridin-3-yloxy)phenyl]-2H-tetrazol-5-yl}pyridine (**10**)—a highly potent and selective mGlu5 receptor antagonist with good brain penetration and in vivo receptor occupancy in rat and cross-species oral bioavailability.
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The major excitatory neurotransmitter in the central nervous system, glutamate, activates both ionotropic and metabotropic glutamate (mGlu) receptors.¹ The G protein-coupled mGlu receptor subtype 5 (mGlu5), which is predominantly localized post-synaptically couples via phospholipase C leading to an increase in intracellular Ca²⁺ levels.² A number of reports have indicated that selective antagonism of mGlu5 receptors may improve disease states such as anxiety and depression,^{3–8} pain,⁹ drug dependence¹⁰ and mental retardation.¹¹



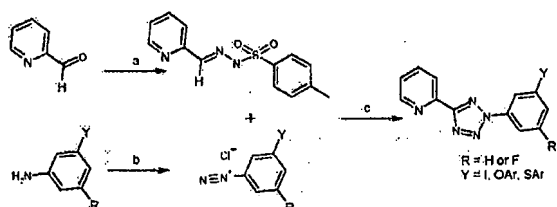
Recent publications from this laboratory have described the discovery of MTEP (**1**), a potent and selective mGlu5 receptor antagonist.¹² In our search for further structural classes, we examined the replacement of the alkyne linker in **1** with a series of heteroaromatic azoles.¹³ Of the 16 N-linked azoles examined, tetrazole **2** was found to be the most promising in terms of potency, selectivity,¹⁴ brain penetration, and rat pharmacokinetics. However, tetrazole **2** showed only moderate in vitro potency and binding affinity and suffered from relatively poor aqueous solubility.¹⁵ Due to these short comings we sought to replace the nitrile moiety of **2** with groups that would confer improved potency and physicochemical properties. Herein we describe our efforts towards these goals.

The tetrazole derivatives described herein were synthesized as outlined in Schemes 1–3. A 1,3-dipolar cycloaddition was employed between a diazonium salt and a tosyl hydrazone (derived from condensation of 2-pyridylaldehyde with tosyl hydrazide, Scheme 1).¹⁶

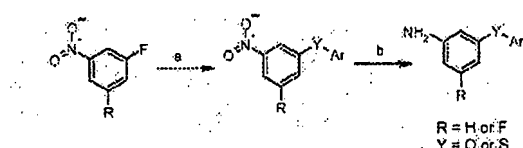
In the case of oxygen and sulfur linked biaryls **3–8**, **10**, and **11**, the required aniline derivative was prepared

Keywords: Metabotropic glutamate; Antagonist; Tetrazole.

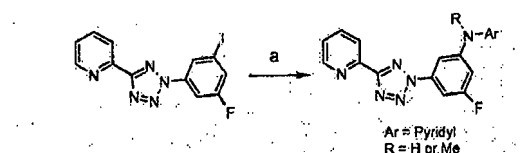
* Corresponding author. Tel.: +1 858 202 5248; fax: +1 858 202 5743; e-mail: nicholas_smith@merck.com



Scheme 1. Reagents and conditions: (a) tosyl hydrazide, EtOH, rt; (b) NaNO_2 , HCl, H_2O , EtOH, 0°C ; (c) NaOH, 0°C .



Scheme 2. Reagents and conditions: (a) K_2CO_3 , Ar-OH or ArSNa; DMF, $80\text{--}150^\circ\text{C}$; (b) H_2 , Pd/C.



Scheme 3. Reagents and conditions: (a) NaOtBu , biphenyl-2-yl-(dicyclohexyl)phosphine, $\text{Pd}(\text{dba})_3$, Ar-NH $_2$, dioxane, 110°C .

via an $\text{S}_{\text{N}}\text{Ar}$ reaction of a phenol or thiophenol with an appropriately substituted nitroarene (Scheme 2).

As shown in Scheme 3, for the N-linked biaryls **13** and **15**, the fourth ring was appended using palladium-catalyzed heteroatom cross-coupling chemistry between an aryl iodide and an appropriately substituted aniline (or for compound **15**, 7-azaindole).^{17,18}

The data in Table 1 illustrate the effect of replacing the nitrile moiety in **2** with oxygen-linked aryl groups. Thus O-phenyl derivative **3** and O-2-pyridyl derivative **4** both showed a significant loss in potency compared to **2**. However, moving the pyridyl nitrogen to the 3-position as in **5** gave an increase in the functional Ca^{2+} flux ($\text{IC}_{50} = 30\text{ nM}$) and binding ($K_i = 59\text{ nM}$) assays. Moving the pyridyl nitrogen to the 4-position as in **6** led to a loss of potency.¹⁹ Finally, if the O-3-pyridyl group of **5** is placed at the 2- or 4-positions of the phenyl ring as in **7** and **8**, again potency was lost relative to **2** (Table 1).

Previous research from this laboratory demonstrated the dramatic increase in potency obtained when benzonitrile **2** is substituted at the 5-position with fluorine to give **9** (Ca^{2+} flux $\text{IC}_{50} = 3.8\text{ nM}$ compared to 73 nM for **2**; Table 1).¹³

A key question was whether application of this SAR to 3-pyridyl derivative **5** would lead to a similar increase in

Table 1. In vitro potencies of tetrazole mGlu5 receptor antagonists

Compd	Structure	mGlu5 Ca^{2+} flux IC_{50} (nM) ^a	mGlu5 K_i (nM) ^b
2		73	186
3		1458	617
4		2859	633
5		30	59
6		NA ^c	NA ^c
7		1577	NT ^d
8		737	633
9		3.9	14
10		6.7	12
11		13	16
12		17	38
13		124	29
14		22	30
15		16	25

^a Ca^{2+} flux assay using glutamate ($10\text{ }\mu\text{M}$) as agonist ($n = 2\text{--}4$, SD $< +/ - 25\%$).²⁰

^b Displacement by test compounds of [^3H]-3-methoxy-5-(pyridin-2-ylethynyl)pyridine from rat cortical membranes ($n = 2\text{--}4$, SD $< +/ - 25\%$).²¹

^c Not active at $2\text{ }\mu\text{M}$.

^d Not tested.

potency. Gratifyingly compound **10**, containing a 5-fluorine substituent, demonstrated an increase in mGlu5

receptor functional ($IC_{50} = 6.7$ nM) and binding ($K_i = 12$ nM) potency compared to **5** ($IC_{50} = 30$ nM and $K_i = 59$ nM).

Encouraged by this result, we decided to also investigate the effect of the atom linker between the phenyl and 3-pyridyl rings. As seen from the data in Table 1, sulfur linked derivative **11** and carbon linked derivative **12** both maintain good potency in the Ca^{2+} flux assay at $IC_{50} = 13$ and 17 nM, respectively, while nitrogen linked derivative **13** lost functional potency ($IC_{50} = 124$ nM). Interestingly, functional potency can be regained with a nitrogen linkage by methylation of the secondary aniline as in **14** ($IC_{50} = 22$ nM) or by using the conformationally restricted 7-azaindole derivative **15** ($IC_{50} = 16$ nM).

Having identified compounds with good potency at the mGlu5 receptor, brain penetration and receptor occupancy was evaluated using receptor occupancy assay in rats (Table 2).²² As represented by **10**, **11**, **12**, and **14**, this class of compounds has good rat brain penetration with levels of 1.5 – 9.7 μ M at 1 h following a dose 10 mg/kg ip. In particular, compound **10** showed brain levels of 6.7 μ M, which translated into excellent rat receptor occupancy of 90%.

Since derivative **10** exhibited excellent mGlu5 receptor potency and in vivo receptor occupancy and the corresponding hydrochloride salt showed improved water solubility¹⁵ (compared to hydrochloride salts of **2** and **9**). It was selected for further in vivo profiling.

The pharmacokinetic data for **10** in rat, dog and monkey are summarized in Table 3. In all three species good to excellent bioavailability was observed (27–100%), with a similar half-life across species ($t_{1/2} = 2.3$ – 2.7 h). A rat receptor occupancy–dose titration for **10** was also carried out with the occupancy ED_{50} determined to be 10.7 mg/kg, p.o.

In conclusion, we have demonstrated that the 3-nitrile moiety of **2** and **9** may be replaced with an oxygen linked 3-pyridyl group leading to compounds **5** and **10**, respectively. Tetrazole **10** has excellent potency and selectivity against the mGlu5 receptor, has good rat

Table 2. Rat receptor occupancy, brain levels and plasma for selected compounds^{a,b}

Compd	Recep. occ (%)	Hippocampus levels (μ M)	Plasma levels (μ M)
2	96	12	13
5	60	ND ^c	ND ^c
9	97	9.7	8.7
10	90	6.7	7.4
11	83	1.5	3.4
12	43	2.7	0.9
14	58	4.9	3.5

^a Measured at 1 h following 10 mg/kg dose ip.¹⁹

^b All compounds were administered in solution, except **2**, **9** in the form of suspension, of PEG 400/water (V:V = 1:1).

^c Not determined.

Table 3. Pharmacokinetic data for compound **10**

	Rat ^a	Dog ^b	Monkey ^b
Cl_p (mL/min/kg)	21	14	7.7
Vd (L/kg)	22	1.5	0.7
$t_{1/2}$ (h)	2.7	2.3	2.5
%F	100	54	27
Cmax (μ M)	11	1.4	0.3

^a Iv dosing at 2 mg/kg, p.o. dosing at 10 mg/kg. In solution of PEG 400/water (V:V = 1:1).

^b Iv dosing at 1 mg/kg, p.o. dosing at 1 mg/kg. In solution of PEG 400/water (V:V = 1:1).

brain penetration and cross-species pharmacokinetics and demonstrates good rat receptor occupancy when dosed orally.

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- The selectivity of the prototypical tetrazole **2** was determined by extensive profiling against a battery of in vitro assays (MDS Pharma Services screen). In addition, **2** is highly selective for mGlu5 over mGlu1 (mGlu1 Ca^{2+} flux $IC_{50} > 10$ μ M).

15. Solubility of HCl salt of **2** is 1.6 mg/mL in PEG 400/water (V/V = 1:1). While the HCl salt of **9** is 36 mg/mL in PEG/400 water (V/V = 1:1).
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19. On a related system, when a methyl group was scanned at the *ortho*, *meta* and *para* positions of the terminal phenyl of **3**, the compounds exhibited activities >2 mM in the Ca²⁺ flux assay.
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Wirkung neuer quartärer Ammoniumverbindungen auf Bakterien und Pilze

Teil 5¹: Synthese von 3-Methyl-n-alkylthiomethylpyridinium-,
1-Methyl-3-n-alkylthiomethylimidazolinium- und 1-Ethyl-3-n-alkylthiomethylimidazoliniumchloride

J. PERNAK, S. KUCHARSKI und J. KRYSINSKI

Es wird die Synthese und antibakterielle Wirkung der im Untertitel genannten Chloride beschrieben. Die quartären Pyridinium- und Imidazoliniumchloride wurden durch Reaktion von Chlormethyl-n-alkylthioether mit 3-Methylpyridin oder 1-Methyl- und 1-Ethylimidazol synthetisiert. Die Eigenschaften der Verbindungen wurden gegenüber 13 Bakterien- und Pilzstämmen geprüft. Chloride mit dem Dodecylrest in der Alkylkette besitzen die höchste antibakterielle Wirkung.

Activity of New Quaternary Ammonium Compounds on Strains of Bacteria and Fungi

Part 5: Synthesis of 3-Methyl-n-alkylthiomethylpyridine-, 1-Methyl-3-n-alkylthiomethylimidazole- and 1-ethyl-3-n-alkylthiomethylimidazoline chlorides

Synthesis of some chlorides indicated in headline and their antibacterial properties was described. Synthesis of quaternary pyridine and imidazoline chlorides was performed by reaction of chlormethyl-n-alkylthioether with 3-methylpyridine or 1-methyl- and 1-ethylimidazole. Antibacterial properties of obtained compounds was tested on 13 strains of bacteria and fungi. Chlorides with dodecyl radical indicated the best antibacterial activity.

1. Einleitung

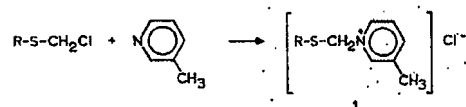
Quartäre Ammonium- und Pyridiniumsalze besitzen hohe antibakterielle Aktivität [2]. Die Struktur dieser Verbindungen hat einen großen Einfluß auf ihre Eigenschaften. Pyridiniumchloride mit einem Octyl- bis Hexadecylrest wirken auf Bakterien und Pilze am besten [7]. Im folgenden beschreiben wir die Synthese von 3-Methyl-n-alkylthiomethylpyridinium-, 1-Methyl-3-n-alkylthiomethylimidazolinium- und 1-Ethyl-3-n-alkylthiomethylimidazoliniumchloriden und ihre antibakterielle Wirkung gegenüber 13 Bakterien- und Pilzstämmen.

2. Untersuchungen und Ergebnisse

2.1. Synthesen

3-Methyl-n-alkylthiomethylpyridiniumchloride 1 wurden durch Reaktion von Chlormethyl-n-alkylthioether mit 3-Methylpyridin dargestellt (Schema 1).

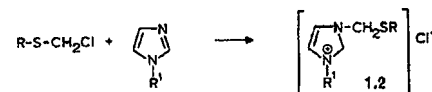
Schema 1



Ausgangsmaterial waren die aus dem entsprechenden Thioalkohol gewonnenen Thioether [1, 6]. Die Reaktion wurde mit äquivalenten Mengen der Reaktionspartner unter nahezu wasserfreien Bedingungen bei 353 K durchgeführt. 3-Methylpyridin war destilliert und über Molekularsieb Typ 4 A aufbewahrt. Die

in dem Reaktionsgemisch anwesende, geringe Wasser führte zur Chlormethyl-n-alkylthioetherhydrolyse. Der stehende Chlorwasserstoff gab mit 3-Methylpyridin das I-chlorid. Die Nebenprodukte wurden durch Hexenextrakt Soxhlet-Apparat und durch Kristallisation aus Aceton entfernt. Bei der Reaktion von Chlormethyl-n-alkylthioethern mit 1-Methylimidazol wurden die 1-Methyl-3-n-alkylthiomethylimidazoliniumchloride 2 erhalten. Die Reaktion von Chlormethyl-n-alkylthioethern mit 1-Ethylimidazol ergab die 1-Ethyl-3-n-alkylthiomethylimidazoliniumchloride 3 (Schema 2).

Schema 2



Reaktions- und Reinigungsbedingungen waren dieselben wie bei der Synthese von 1. Von allen Verbindungen wurden IR-Spektren aufgenommen. Die fehlende Absorptionsbande bei 2200 cm⁻¹ zeigt, daß keine 3-Methylpyridinium-, 1-Methylimidazolinium- und 1-Ethylimidazoliniumhydrochloride vorhanden waren. Die Deformationsschwingungen bei 1630 cm⁻¹ und Valenzschwingungen für die OH-Gruppe (breite Absorptionsbande bei 3500–3200 cm⁻¹) wiesen auf wasserhaltige Chloride hin. Sie kristallisieren mit 1 Molekül Wasser.

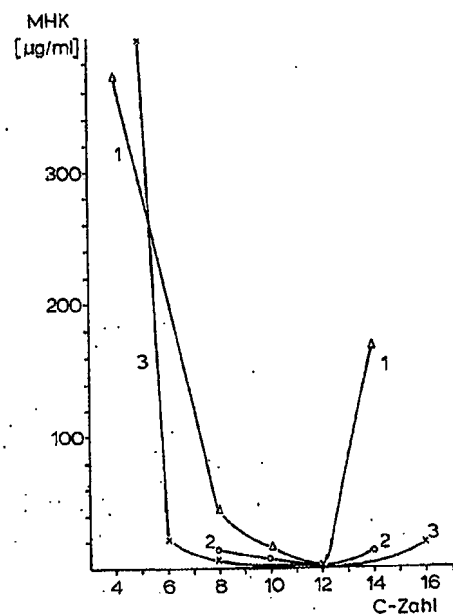


Abb. 1. MHK-Mittelwerte in Abhängigkeit von der Anzahl der C-Atome der / für Coccaceae, Ziffern entsprechen Substanzklassen

¹ Teil 4: Pharmazie 35, 644 (1980)

AP008069005										
Verbindung	Alkyl R	R ¹	Schmp. [K]	Analyse C ber.	gef.	H ber.	gef.	N ber.	gef.	
1	a	C ₄ H ₉	—	flüssig	52,9	52,7	8,0	7,7	5,6	5,5
	b	C ₆ H ₁₃	—	397—398	58,9	57,9	9,2	9,2	4,6	4,6
	c	C ₁₀ H ₂₁	—	399—400	61,2	61,0	9,6	9,5	4,1	4,1
	d	C ₁₂ H ₂₅	—	399,5—400	63,0	62,9	10,0	9,5	3,9	3,8
	e	C ₁₄ H ₂₉	—	402—403	64,7	64,6	10,3	10,2	3,6	3,4
2	a	C ₆ H ₁₃	CH ₃	flüssig	53,0	53,1	9,2	9,0	9,5	9,3
	b	C ₁₀ H ₂₁	CH ₃	314,5—316	55,8	55,3	9,7	10,0	8,7	8,6
	c	C ₁₂ H ₂₅	CH ₃	312—314	58,2	57,3	10,1	10,2	7,8	7,6
	d	C ₁₄ H ₂₉	CH ₃	326—327	60,2	59,8	10,3	10,0	7,4	7,3
3	a	C ₅ H ₁₁	C ₂ H ₅	flüssig	49,5	49,3	8,6	8,3	10,5	10,5
	b	C ₆ H ₁₃	C ₂ H ₅	flüssig	51,3	51,0	8,9	8,5	9,9	9,7
	c	C ₈ H ₁₇	C ₂ H ₅	flüssig	54,5	54,0	9,4	9,1	9,1	9,0
	d	C ₁₂ H ₂₅	C ₂ H ₅	flüssig	59,3	59,1	10,2	9,8	7,7	7,6
	e	C ₁₆ H ₃₃	C ₂ H ₅	353—354	62,8	62,4	10,7	10,3	6,7	6,6

Tabelle 2 Minimale Hemmkonzentration (MHK) nach 24 h [µg/cm³]

Stamm*	1a	1b	1c	1d	1e	2a	2b	2c	2d	3a	3b	3c	3d	3e	
Fungi, Bacteriaceae Coccaceae	Staphylococcus aureus 209 P	375	11,8	11,7	0,006	93,7	5,9	2,9	0,4	11,7	375,0	11,8	2,9	1,4	23,5
	Sarcina lutea ATTC 9341	375	11,8	11,7	0,4	375,0	11,8	5,8	0,003	5,8	500,0	46,9	5,9	1,4	11,7
	Gaffkya tetragenae PZH 2/49	375	1,4	23,5	0,008	93,7	2,9	5,8	0,003	11,7	375,0	11,8	2,9	0,7	11,7
	Staphylococcus albus 614	375	1,4	11,7	0,0004	93,7	1,4	11,7	0,01	23,5	375,0	11,8	5,9	2,9	23,5
	Streptococcus faecalis 8040	375	187,5	11,7	0,7	187,5	46,9	11,7	1,4	11,7	375,0	23,5	5,9	2,9	23,5
	Pseudomonas aeruginosa A-52	500	750,0	187,5	46,9	500,0	375,0	187,5	93,7	375,0	500,0	500,0	375,0	93,7	375,0
	Klebsiella pneumoniae 138	500	93,7	23,5	5,9	375,0	93,7	23,5	11,8	63,6	93,7	46,9	11,8	2,9	46,9
	Escherichia coli 503	500	46,9	63,6	5,9	500,0	93,7	11,7	5,9	23,5	500,0	46,9	11,8	2,9	46,9
	Serratia marcescens 6/46	500	375,0	63,6	46,9	500,0	375,0	46,9	93,7	46,9	500,0	375,0	46,9	46,9	46,9
	Serratia marcescens 76	500	0,4	46,9	0,4	93,7	0,4	5,8	0,003	11,7	187,5	23,5	2,9	1,4	11,7
	Candida albicans 505	500	93,7	23,5	0,4	187,5	93,7	5,8	2,9	11,7	187,5	23,5	2,9	2,9	11,7
	Rhodotorula glutinis 29	500	5,9	23,5	0,02	187,5	11,8	5,8	0,09	5,8	375,0	23,5	5,9	2,9	11,7
	Bacillus subtilis 32	500	23,5	23,5	0,4	187,5	46,9	23,5	0,02	23,5	187,5	23,5	23,5	11,8	11,7

* Inoculum für alle Stämme lag zwischen 2 · 10⁴ und 5 · 10⁵

2.2. Antibakterielle Eigenschaften

Die antibakteriellen Eigenschaften wurden im Reihenverdünnungstest auf Müller-Hinton-Bouillon und Sabouraud-Nährboden (Fungi) ermittelt [4, 5].

In Tab. 2 sind die minimale Hemmkonzentration (MHK) für 18 Bakterien- und Pilzstämmen nach 24 h und auf den Abb. 1—4

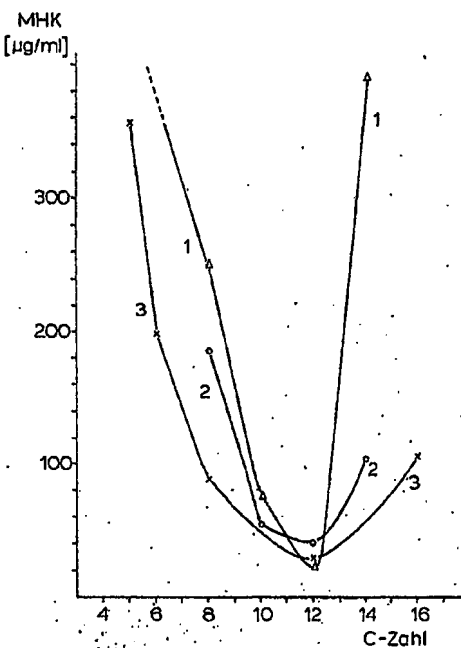


Abb. 2. MHK-Mittelwerte in Abhängigkeit von der Anzahl der C-Atome der Alkylkette für Bacteriaceae. Vgl. auch Abb. 1

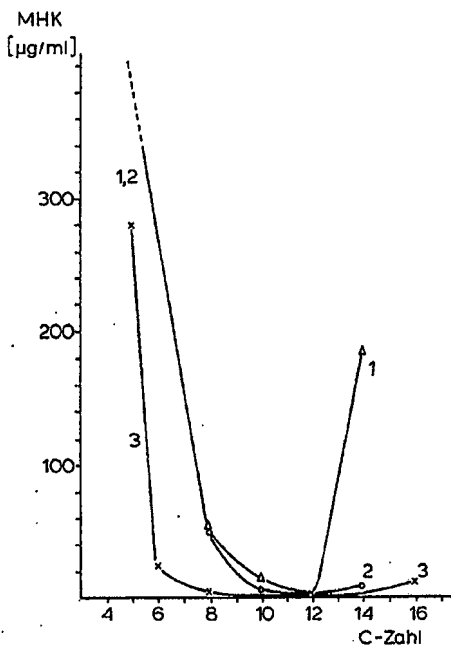


Abb. 3. MHK-Mittelwerte in Abhängigkeit von der Anzahl der C-Atome der Alkylkette für Fungi. Vgl. auch Abb. 1

die MHK-Mittelwerte in Abhängigkeit von der Anzahl der C-Atome in der Alkylkette dargestellt.

Die Ergebnisse zeigen, daß die Pyridinium- und Imidazoliumchloride mit dem Dodecylrest die höchste Wirksamkeit gegen Bakterien und Pilze besitzen und daß bei der Laurylgruppe für alle Verbindungen das Optimum der MHK-Werte liegt. Die Pyridiniumchloride mit Octyl- bis Dodecyl- und die Imidazol

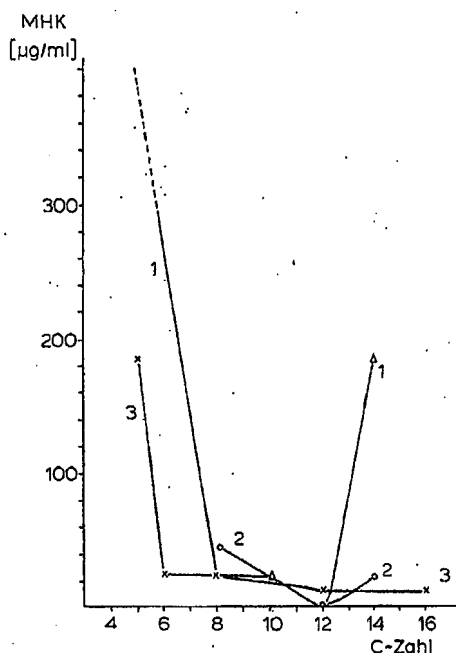


Abb. 4. MHK-Mittelwerte in Abhängigkeit von der Anzahl der C-Atome der Alkylkette für *Bacillus subtilis*. Vgl. auch Abb. 1

niumchloride mit Hexyl- bis Hexadecylrest ergeben ebenfalls eine hohe Wirkung. Die Anzahl der C-Atome in der Alkylkette verändert die Aktivität dieser Verbindungen jedoch deutlich. Wir beobachteten fast keine Unterschiede in der Aktivität der Imidazoliniumchloride mit Methyl- bzw. Ethylgruppe am Imidazolring. Unsere Untersuchungen bestätigen, daß diese

Verbindungen besonders stark gegen *Bacillus subtilis* und *I* wirken [3].

3. Experimenteller Teil

3.1. Synthese von 1—3

0,3 bzw. 0,2 mol Chlormethyl-n-alkylthioether werden mit 0,3 m Methylpyridin bzw. 0,2 mol 1-Methylimidazol versetzt und 1,5 auf 353 K bzw. 363 K (Methylimidazol) erhitzt. 3-Methylpyridin war (liert und über Molekularsieb Typ 4 A aufbewahrt. Die Nebenpro Mercaptal des Formaldehyds, HCHO und 3-Methylpyridiniumhydroc wurden durch Hexenextraktion im Soxhlet-Apparat und durch Kri sation aus Aceton entfernt.

Die Schmp. wurden auf dem Mikroheiztischgerät nach Boëtius best. Die IR-Spektren wurden mit Spektrometer Specord IR-72 (VEB Carl Jena) aufgenommen.

3.2. Prüfung der Wirkung

Die Wirkung der Pyridinium- und Imidazoliniumchloride wurde Reihenverdünnungstest gegenüber 13 Bakterien- und Pilzstämmen (Tab. 2) verfolgt. Der Müller-Hinton-Bouillon und dem Sabouraud-boden (Fungi) wurden abnehmende Konzentrationen der in H₂O ge Substanzen hinzugefügt. Die Untersuchungen wurden bei 310 K c geführt.

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Doz. Dr. hab. Sławój Kuc
ul. Grunwaldzka 6
PL — 60-780 Poznań/VR

SINTESI ED ATTIVITÀ ANTIULCERA DI ALCUNI NUOVI COMPOSTI A STRUTTURA ARILTIOMETIL-PIRIDINICA

S. EVANGELISTA - M. GHELARDONI - A. MELI - V. PESTELLINI - G. VITI
MENARINI RICERCHE - FIRENZE

RIASSUNTO. — *Si riferiscono i risultati dell'indagine sull'attività antiulcera di alcuni composti di struttura ariltiometilpiridinica. Alcuni derivati hanno mostrato attività gastroprotettiva nelle lesioni da etanolo di entità superiore all'Omeprazolo. Nelle ulcere da Indometacina, maggiormente dipendenti dalla secrezione gastrica, si sono rivelati inferiori al composto di riferimento.*

SUMMARY. — *We report the results of the antiulcer activity of some arylthiomethylpyridine compounds. Some of these showed gastroprotective activity against 96% ethanol-induced gastric lesions greater than Omeprazole. However they possess antiulcer properties lower than the reference compound against Indomethacin-induced gastric ulcers.*

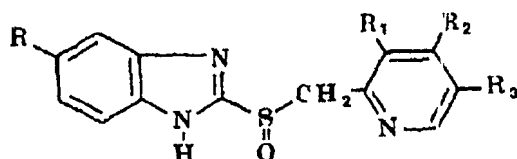
Introduzione

Sono noti in letteratura vari derivati benzoimidazolici che presentano una attività antisecretoria gastrica attraverso un meccanismo di inibizione selettiva della pompa $H^+/K^+-ATPasi$ delle cellule parietali della mucosa gastrica (1,2,3,4).

Gli studi di struttura-attività eseguiti su questi composti (5) hanno evidenziato l'importanza per l'attività antisecretoria dell'anello piridinico sostituito, del gruppo benzoimidazolico sostituito e del ponte metil sulfinilico o tiometilenico fra di essi. Inoltre è stato messo in evidenza che l'attività biologica risente della natura dei sostituenti sull'anello piridinico e, anche se in misura minore, di quelli sull'anello benzoimidazolico. Sono stati individuati diversi inibitori della pompa $H^+/K^+-ATPasi$, strettamente correlati fra loro, e che rispondono ai requisiti sopra citati (6-13). Recentemente sono stati sintetizzati nuovi composti dotati di attività antisecretoria in cui il gruppo benzoimidazolico è stato sostituito da un gruppo benzotiazolico

o benzossazolico (14, 15).

Al fine di meglio chiarire i rapporti struttura/attività, particolarmente per quanto concerne la presenza del gruppo benzoimidazolico, abbiamo sostituito questo nucleo con altri eterociclici. È stata determinata la loro potenziale attività antiulcera nel ratto nei confronti di ulcere gastriche indotte da Indometacina o da etanolo al 96% la cui formazione è rispettivamente dipendente ed indipendente dalla ipersecrezione gastrica (16,17,18).



	R	R ₁	R ₂	R ₃
OMEPRAZOLO	OCH ₃	CH ₃	OCH ₃	CH ₃
TIMOPRAZOLO	H	H	H	H

Chimica

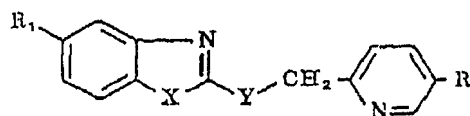
I prodotti sintetizzati (vedi Tabella I) sono stati ottenuti per reazione dei 2-tiolo derivati dei vari eterocicli in esame con 2-cloro-metil-piridinio cloruro o suoi derivati in presenza di una base.

I 2-piridil metil-tio-eteri così ottenuti sono stati poi ossidati con acido 3-cloro-perbenzoico (MCPB) come già precedentemente descritto per prodotti analoghi (1,6,20).

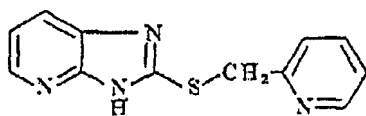
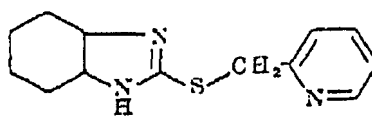
Il processo di ossidazione del tio etere al corrispondente solfossido risulta più difficile al diminuire della basicità degli eterocicli azoici (21) presenti nella molecola: per esempio, mentre il 2-[(2-piridil-metil)tio]-benzoimidazolo (Timoprazolo, I) dà il corrispondente solfossido a basse temperature in tempi brevi, al corrispondente derivato del benzotiazolo (IV) occorrono tempi molto più lunghi o temperature più elevate per passare completamente a solfossido (V) e quello del benzossazolo, pur in presenza di un gruppo metossile in posizione 5 (I), non viene praticamente ossidato dopo 24 ore a temperatura ambiente. L'introduzione sul derivato benzossazolico di un ulteriore gruppo metossilico nella posizione 4 dell'anello piridinico (II) ci permette di ottenere per ossidazione, anche se con basse rese, il sulfone derivato (III), ma non il solfossido.

Anche altri derivati con gruppi meno basici dell'imidazolo quali il pirido [4,5-b]imidazolo (VI) e il 1,2,4-triazolo (VII), sono risultati resistenti all'ossidazione nelle condizioni impiegate.

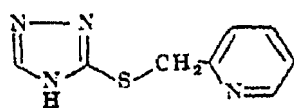
TABELLA I



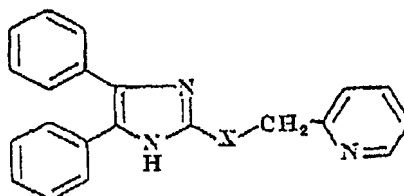
	R ₁	R	X	Y	
(I)	OCH ₃	H	O	S	
(II)	OCH ₃	OCH ₃	O	S	
(III)	OCH ₃	OCH ₃	O	SO ₂	
(IV)	H	H	S	S	a
(V)	H	H	S	SO	b

(VI)^c

(VII)



(VIII)

(IX) X = S
(X) X = SO

- a) rif. (14)
b) rif. (15)
c) rif. (19).

Nel caso del derivato dell'esaidrobenzimidazolo (VII), questo risulta molto sensibile all'ossidazione e dà facilmente, anche a temperature molto basse, una miscela complessa di prodotti di ossidazione da cui non è stato possibile isolare il solfossido.

Parte Sperimentale

I punti di fusione sono stati determinati con un Büchi Apparatus e non sono corretti; gli spettri ^1H -R.M.N. sono stati registrati con uno spettrometro Varian, modello EM-360 usando TMS come standard interno. Le analisi elementari del C, H, N sono comprese nell'intervallo $\pm 0,4\%$ intorno ai valori teorici per tutti i composti nuovi.

2-Mercapto-5-metossi-benzossazolo

5 g Di 2-ammino-4-metossi fenolo cloridrato (22) furono trattati in alcool etilico 96% con 9 g di potassio etil xantogenato e mantenuti a reflusso per 6 h. Il solvente fu evaporato e l'olio residuo versato in acqua. Il solido presente fu allontanato per filtrazione e la soluzione acidificata. Il precipitato fu raccolto ottenendo 5,1 g di prodotto. P.f. 223-225° (Toluene).

Anal. ($\text{C}_8\text{H}_7\text{NO}_2\text{S}$): C, H, N.

R.M.N. δ p.p.m. 3,8 (s, 3H, OCH_3), 6,6-7,3 (m, 3H, H arom), 13,7 (bs, 1H, NH).

(E)-2-Mercapto-3a,4,5,6,7,7a-esaidro-benzoimidazolo

Il composto è ottenuto in letteratura a partire dal diammino cicloesano (miscela *cis-trans*) come miscela di isomeri p.f. 151-160° (23). Partendo dal *trans*-diammino cicloesano (Aldrich) è possibile ottenere in modo analogo il solo isomero *E*, p.f. 198-199°.

R.M.N. δ p.p.m. miscela *E* + *Z*: 1,1-2 (m, 8H, CH_2), 3 + 3,6 (m, 2H, CH), 7,9 + 8,1 (s, 2H, NH tioureidici); isomero *E* 1,1-2 (m, 8H, CH_2), 3 (m, 2H, CH), 7,9 (s, 2H, NH tioureidici).

2-(2-Piridil-metil-tio)-5-metossi-benzossazolo (I)

10 g Di 2-mercapto-5-metossi-benzossazolo furono sciolti in 130 ml di acqua contenente 6 g di NaOH, trattati con 11 g di 2-clorometil-piridina cloridrato (24) e mantenuti sotto agitazione a temperatura ambiente per 4 h. La soluzione fu acidificata fino a solubilizzazione, lavata con etere, riportata a pH basico con carbonato di sodio ed estratta con etile acetato. Per allontanamento del solvente si ottiene un olio che cristallizza da cicloesano. P.f. 55,5-56° (Cicloesano). Resa 90%.

Anal. ($\text{C}_{14}\text{H}_{12}\text{N}_2\text{O}_2\text{S}$): C, H, N.

R.M.N. δ p.p.m. 3,8 (s, 3H, OCH_3), 4,7 (s, 2H, SCH_2), 6,7-7,7 (m, 6H, H arom), 8,6 (d, 1H, H piridinico in 6).

In condizioni analoghe furono ottenuti i seguenti composti:

2-[2-(4-Metossi-piridil)metil-tio]-5-metossi-benzossazolo (II)

[Da 2-mercapto-5-metossi-benzossazolo e 2-clorometil-4-metossipiridina cloridrato (25)] p.f. 62-64°, cicloesano, resa 35%.

Anal. ($\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}_3\text{S}$): C, H, N.

R.M.N. δ p.p.m. 3,8 (s, 6H, OCH_3), 4,6 (s, 2H, SCH_2), 6,6-7,6 (m, 5H, H arom), 8,3 (d, 1H, H piridinico in 6).

(E)-2(2-Piridilmetil-tio)-3a,4,5,6,7,7a-esaidro-benzoimidazolo (VII)

[Da *(E)*-2-mercapto-3a,4,5,6,7,7a-esaidro-benzoimidazolo e 2-clorometilpiridina cloridrato] p.f. 121-122°, cicloesano e alcool/acqua, resa 20%.

Anal. ($C_{13}H_{17}N_3S$), C, H, N.

R.M.N. δ p.p.m. 1,2-2,2 (m, 8H, CH_2), 3 (m, 2H, CH), 4,5 (s, 2H, SCH_2), 6,9 (s, 1H, NH), 7,2-8 (m, 3H, H piridinici in 3,4,5), 8,6 (d, 1H, H piridinico in 6).

3-(2-Piridil-metil-tio)-1,2,4-triazolo (VIII)

[Da 3-mercapto-1,2,4-triazolo (Aldrich) e 2-clorometil piridina cloridrato] p.f. 109-111°, toluene, resa 85%.

Anal. ($C_8H_8N_4S$), C, H, N.

R.M.N. δ p.p.m. 4,6 (s, 2H, SCH_2), 7,1-8 (m, 3H, H piridinici in 3,4,5), 8,4 (s, 1H, H triazolo), 8,5 (d, 1H, H piridinico in 6).

2-(2-Piridil-metil-tio)-4,5-difenil-imidazolo (IX)

[Da 2-mercapto-4,5-difenil-imidazolo (26) e 2-clorometil piridina cloridrato] p.f. 155-156°, toluene ed alcool/acqua, resa 35%.

Anal. ($C_{21}H_{17}N_3S$), C, H, N.

R.M.N. δ p.p.m. 4,3 (s, 2H, SCH_2), 7,1-7,9 (m, 13H, H arom), 8,5 (d, 1H, H piridinico in 6), 13,2 (bs, 1H, NH).

I composti 2-(2-piridilmetiltio)-benzotiazolo (IV) e il corrispondente solfossido (V) furono ottenuti come descritto in letteratura (14,15).

2-(2-Piridil-metil-sulfenil)-4,5-difenil-imidazolo (X)

Il composto (IX) (1 g) fu sciolto in cloroformio, la soluzione raffreddata a -10° e addizionata di MCPB (0,65 g). La miscela fu mantenuta sotto agitazione alla temperatura suddetta per 1 h e poi per un'altra ora a 0° . La soluzione fu lavata con bicarbonato di sodio al 5% ed acqua, il solvente evaporato e l'olio residuo cristallizzato da toluene. Resa 75%. P.f. 197-9° (dec). Anal. ($C_{21}H_{17}N_3OS$), C, H, N.

R.M.N. δ p.p.m. 4,8 (s, 2H, $SOCH_2$), 7,1-7,9 (m, 13H, H arom), 8,6 (d, 1H, H piridinico in 6), 13,8 (bs, 1H, NH).

Nelle stesse condizioni, variando la temperatura ed i tempi di reazione, furono eseguiti anche i tentativi di ossidazione dei prodotti (I), (VI), (VII), (VIII).

2-[2-(4-Metossi)-piridil-metil-sulfonil]-5-metossi-benzoxazolo (III)

Il cloridrato del composto (II) (3 g) fu sospeso in cloroformio e trattato con MCPB (2,5 g) per 16 h a temperatura ambiente sotto agitazione fino a scomparsa della macchia corrispondente al prodotto di partenza (TLC, cicloesano / etile acetato 2/1). La soluzione fu lavata con bicarbonato di sodio al 5%, il solvente fu allontanato per distillazione sotto vuoto e l'olio residuo cristallizzato con poco etile acetato. Resa 10%.

P.f. 118-120° (etile acetato). Anal. ($C_{15}H_{14}N_2O_5S$), C, H, N.

R.M.N. δ p.p.m. 3,7 (s, 3H, OCH_3), 3,8 (s, 3H, OCH_3), 4,5 (s, 2H, SO_2CH_2), 6,5-7,3 (m, 5H, H arom), 8,3 (d, 1H, H piridinico in 6).

Farmacologia

MATERIALI E METODI

Sono stati utilizzati ratti albin maschi Sprague-Dawley della ditta Nossan (Correzzana, Milano), del peso di 180-210 g, tenuti alla temperatura ambiente di $21 \pm 1^\circ$, umidità relativa del 60% ed ad un ciclo di luce-buio di 12 ore (luce alle 6 del mattino).

Le ulcere gastriche sono state indotte somministrando *per os* Indometacina (20 mg/kg) oppure etanolo al 96% (5 ml/kg) a ratti digiuni da 24 ore. I prodotti in studio sono stati somministrati *per os*, 30 min prima dell'agente ulcerogeno alle dosi di 20 o 50 mg/kg, sospesi in un veicolo acquoso contenente NaCl 0.9%, carbossimetilcellulosa 0.5% e Tween 80 0.5%. Ogni gruppo era costituito da almeno 8 ratti. L'Omeprazolo era invece sospeso in 1% carbossimetilcellulosa contenente 0.2% NaHCO_3 ed aggiustato a pH 9 con NaOH 1N (4).

Un'ora dopo l'etanolo e 4 ore dopo l'indometacina gli animali sono stati sacrificati con anidride carbonica e lo stomaco rimosso per valutarne l'ulcerazione con un punteggio da 0 a 3 da un operatore ignaro dei trattamenti (27).

Risultati e discussione

I dati nella Tabella II indicano che i composti in esame esplicano una attività inferiore a quella dell'Omeprazolo nell'antagonizzare le ulcere da

TABELLA II

Composto	Dose mg/kg <i>os</i>	Riduzione ulcere indotte da: (*)	
		Indometacina	96% Etanolo
Omeprazolo	20	75,2	23,6
(I)	20	12	10
(I)	50	56,8	34,6
(II)	20	0	20,6
(II)	50	34,4	38,4
(III)	20	0	0
(IV)	20	0	55,1
(IV)	50	38,9	46,6
(V)	20	20	96,5
(V)	50	49,6	100
(VI)	20	15,2	41,4
(VI)	50	98,2	70
(VII)	20	27,6	48,3
(VII)	50	88,6	50
(VIII)	20	0	20,6
(VIII)	50	23,9	43,3
(IX)	20	0	46,4
(IX)	50	74	40
(X)	20	0	20,6
(X)	50	0	11

(*) % riduzione del grado delle ulcere indotte da indometacina o etanolo rispetto ai controlli.

Indometacina e che per i composti (VI), (VII) e (IX) tale attività è dose dipendente.

Nelle ulcere da etanolo dove la componente ipersecretoria è di scarsa importanza (17,18), alcuni dei composti in esame [prodotti (IV), (V), (VI), (IX)] ed in particolare il composto (V), esplicano attività inibitoria superiore a quella dell'Omeprazolo (Tabella II). D'altra parte l'attività gastroprotettiva dell'Omeprazolo in modelli sperimentali di ulcere gastriche non strettamente dipendenti da un aumento della secrezione gastrica acida è messa in discussione da alcuni autori (28).

In base a questi risultati i composti studiati ed in particolare quelli citati, si sono dimostrati validi gastroprotettori in quanto pur mantenendo, seppure in parte, le caratteristiche dell'Omeprazolo, proteggono dalle ulcere di tipo necrotico prodotte da etanolo e possono essere utili nella terapia delle ulcere gastriche per categorie di pazienti che non rispondono ai farmaci antisecretorii (29). Ulteriori studi ne chiariranno il meccanismo di azione.

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EDIFICIO SISKI
CARRERA 4 No. 72 - 35
BOGOTA 8, COLOMBIA

WEB SITE: www.cavelier.com
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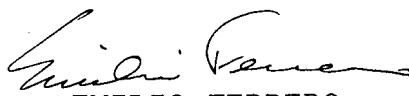
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Poder conferido el : 28/08/2007

Yo, EMILIO FERRERO, obrando como apoderado principal del poderdante arriba mencionado, mediante el presente escrito sustituyo el poder a mi otorgado a la doctora LUZ CLEMENCIA DE PAEZ, identificada con cédula de ciudadanía No. 35.456.344 y Tarjeta Profesional N°23.555, con todas las facultades que me fueron conferidas.

Hago la anterior sustitución conforme al artículo 66 del Código de Procedimiento Civil.


, EMILIO FERRERO

T.P.A. N° 31.929

C.C. N° 438.019 de Usaquén

Acepto:


LUZ CLEMENCIA DE PAEZ

T.P.A. N° 23.555

C.C. No. 35.456.344 de Usaquén

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