

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCIÓN DE NUEVAS CREACIONES**

2020
Bogotá, D.C.,

Abogado
CARLOS R. OLARTE.
E-mail:office@olarteraisbeck.com

ASUNTO	Radicación	10-1889
	Trámite	011
	Evento	378
	Actuación	519 115171
	Oficio	
	Folios	005

Patente de Invención	☐	Patente de Modelo de Utilidad	☐
Vía Nacional	☐		
Vía PCT (fase nacional)	☐	Cap. I ☐	Cap. II ☐

No. Solicitud Internacional PCT/GB2008/050431
Fecha de Presentación Internacional 11 de junio de 2008.

Como resultado del Examen de Fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Folios 199 a 359	08/enero/2010
Reivindicaciones:	Folios 360 a 378	08/enero/2010
Prioridad:	US 60943440	12/junio/2007

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque esta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Modificaciones no aceptadas (Art 34 D 486 y Art 19, 22 y 34 PCT)

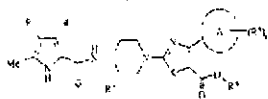
Las modificaciones de las Reivindicaciones 11-38, folios 363-376, no son aceptables porque no se pagó la tasa respectiva. Lo anterior, de acuerdo con requerimiento N° 01272 del 28 de enero de 2011

4. Objeto de la invención

Título de la solicitud: "Derivados carboxílicos y alcoxi de 4-sustituido-2-[4-((5-metil-1H-pirrol-2-il)carbonil)amino)-piperidin sustituido-1-il]-1,3-tiazol como inhibidores de la DNA girasa y/o topoisomerasa IV y composiciones farmacéuticas de los mismos" (modificado en el folio 387).

El problema planteado en esta solicitud consiste en suministrar compuestos que presentan actividad antibacteriana para tratar infecciones bacterianas en animales de sangre caliente

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de la fórmula (Ia) y composiciones que lo contienen



Compuesto Ia

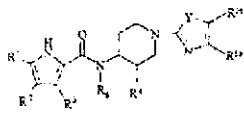
El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D 417/08

5. Determinación del Estado de la Técnica

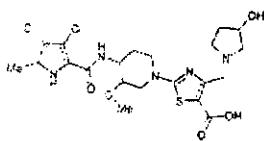
El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: junio 12 de 2007 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación*
D1	WO 2006/087543	Antibacterial piperidine derivatives	24/agosto/2006
D2	Current Medicinal Chemistry, 2005, Vol. 12, No. 1	Bioisosterism: A Useful Strategy for Molecular Modification and Drug Design	2005

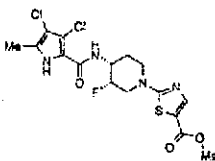
D1:<http://patentscope.wipo.int/search/en/WO2006087543> divulga una serie de compuesto con actividad antibacterial (Pág. 2, Ren. 1-10) derivados del compuesto le (Pág. 241) inhibidores de la DNA girasa (Pág 53, Ren. 3-10) y que da a conocer por medio realizaciones preferidas 380 (Pág. 166) y 426 (Pág. 177) los compuestos.



Compuesto Ia



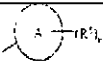
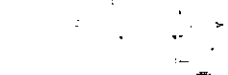
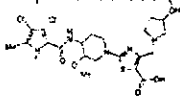
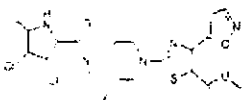
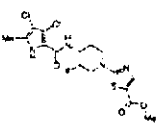
Realización preferida 380



Realización preferida 426

D2: divulga (Pág 1. Int.) que el bioisosterismo es una estrategia de la química medicinal para el diseño racional de nuevos fármacos, el cual a partir de un compuesto líder o base a través de un procesos especial de modificaciones moleculares. El objeto de este proceso es desarrollar nuevas sustancias que son terapéuticamente atractivas y se ha observado una un crecimiento significativo en distintas clases terapéuticas, siendo ampliamente utilizado por la industria farmacéutica para descubrir nuevos análogos de las innovaciones terapéuticas comercialmente atractivos y también como una herramienta útil en el nivel molecular modificación.

Adicionalmente, da a conocer (Pág. 8) algunos grupos heterocíclicos bioisósteros como la diazina y el oxazol para los antibacterianos del grupo de las sulfas

SOLICITUD	D1
R ³	R ⁴
Fluoro Metilo Metoxi Etoxi Propoxi Aliloxi benciloxi	Halo carboxi C ₁₋₄ alquilo C ₁₋₄ alcanoilo C ₁₋₄ alcanoiloxi
Azufre	Y
	S
Hidrógeno	R ⁴
	Hidrógeno
	R ^{5a}
os carbociclilo o heterociclilo; donde si dicho heterociclilo contiene nitrógeno un resto-NH- este puede ser sustituido por R ⁶	Carbociclil-R ¹⁰ o heterociclil-R ¹¹ donde R ¹⁰ o R ¹¹ puede ser sustituido por un grupo seleccionado
	R ^{5b}
Donde R ⁴ es hidrógeno o C ₁₋₄ alquilo	Carboxi opcionalmente sustituido C ₁₋₄ alquilo
REALIZACIONES PREFERIDAS Realización preferida 114 (Fol. 289)	REALIZACIONES PREFERIDAS Realización preferida 380 (Pág. 166)
	
Realización preferida 61 (Fol. 270)	Realización preferida 426 (Pág. 177)
	
Correspondiente al compuesto 2-((3S,4R)-4-(((3,4-dicloro-5-metil-1H-pirrol-2-il)carbonil]amino)3-fluoropiperidin-1-il)-4-isoxazol-5-il-1,3-tiazol-5-carboxilato	Correspondiente a la sal del compuesto 2-((3S,4R)-4-(((3,4-dicloro-5-metil-1H-pirrol-2-il)carbonil]amino)3-fluoropiperidin-1-il)-1,3-tiazol-5-carboxilato

El documento D1 se consideran el estado de la técnica cercano a la invención definida en la reivindicación 1 porque divulga compuestos derivados del ácido 2-((3S,4R)-4-(((3,4-sustituidos-5-metil-1H-pirrol-2-il)carbonil]amino)-3-sustituidos piperidin-1-il)-1,3-tiazol-4-carbociclil o heterociclil-5-carboxílico inhibidores de la DNA girasa y/o topoisomerasa IV y por lo tanto útiles para el tratamiento de infecciones bacterianas.

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto (Ie) del documento D1 corresponden a que 1.) La invención divulga explícitamente un sustituyente carbociclil o heterociclil en la posición 4 del anillo 1,3-tiazol y el documento I refiere estos sustituyentes como parte de un grupo seleccionado para la posición 4 o 5 del anillo 1,3-tiazol. Por lo cual se considera que esta invención es una selección.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo seleccionar los derivados del ácido 2-((3S,4R)-4-(((3,4-sustituido-5-metil-1H-pirrol-2-il)carbonil]amino)3-sustituidos-piperidin-1-il)-1,3-tiazol-4-carbociclil o heterociclil-5-carboxílico del compuesto (Ie) conocidos en D1 para obtener inhibidores de la DNA girasa y/o topoisomerasa IV y por lo tanto útiles para el tratamiento de infecciones bacterianas.

El efecto que se logra al seleccionar los derivados del ácido 2-((3S,4R)-4-(((3,4-sustituido-5-metil-1H-pirrol-2-il)carbonil]amino)3-sustituidos piperidin-1-il)-1,3-tiazol-4-

carbociclíl o heterociclíl-5-carboxílico del compuesto (Ie) divulgado en D1 es obtener nuevos inhibidores alternativos de la DNA girasa y/o topoisomerasa IV.

Sin embargo, dado que documento D1 refiere que 1.) Los compuestos 2-((3S,4R)-4-[[[(3,4-sustituido-5-metil-1H-pirrol-2-il)carbonil]amino]3-sustituidos piperidin-1-il)-1,3-tiazol como el compuesto 426 (Pág. 177) son inhibidores de la DNA girasa y/o topoisomerasa IV y por lo tanto útiles para el tratamiento de infecciones bacterianas y el documento D2 indica que el bioisosterismo es una estrategia de la química medicinal para el diseño racional de nuevos fármacos, dando a conocer una serie de grupos heterocíclicos bioisósteros como lo son 1.) La diazina y el oxazol y 2.) El pirazol, el oxazol y la piridina.

En consecuencia, una persona del oficio normalmente versada en la materia, estaría suficientemente motivada para seleccionar los derivados del ácido 2-((3S,4R)-4-[[[(3,4-sustituido-5-metil-1H-pirrol-2-il)carbonil]amino]3-sutituidos piperidin-1-il)-1,3-tiazol-4-carbociclíl o heterociclíl-5-carboxílico del compuesto (Ie) y obtener inhibidores alternativos de la DNA girasa y/o topoisomerasa IV como lo sugieren el documento D1 o sustituir en la posición 4 del anillo 1,3 tiazol de la realizaciones preferidas 380 y 426 del documento D1 o incluir en la posición 4 del anillo 1,3 tiazol del compuesto 426 (Pág. 177) de acuerdo con lo sugerido el documento D2 un grupo carbociclíl o heterociclíl bioisósteros alternativo al definido en el documento D1 para obtener nuevos inhibidores alternativos dado que esta sustitución o inclusión esta sugerida por los documentos D1 y D2. Por lo cual la reivindicación 1 se considera obvia.

Puesto que los documentos D1 y D2 mencionan todas las características técnicas esenciales de las reivindicaciones dependientes 2-3 y 6-10, estas tampoco se pueden considerar inventivas.

7. Conclusión

Los requisitos de Patentabilidad de la presente Solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	WO 2006/087543	1-3 y 6-10	Nivel Inventivo
D2	Current Medicinal Chemistry, 2005, Vol. 12, No. 1	1-3 y 6-10	Nivel Inventivo

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante (60 días) y presentarla por escrito.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.


JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (C)

28 AGO. 2012

El anterior requerimiento fue notificado por fijación en lista, de fecha _____

Bioisosterism: A Useful Strategy for Molecular Modification and Drug Design

Lidia Moreira Lima and Eliezer J. Burreiro*

Laboratório de Biofarmacologia, Universidade Federal do Rio de Janeiro, 20221-900 Rio de Janeiro, RJ, Brazil

Abstract: This review aims to demonstrate "the use" of bioisosterism, a rational drug design, as well as a the molecular modification and optimization process, as a tool to develop pharmacologically active and pharmaceutically acceptable lead compounds.

1. INTRODUCTION

Bioisosterism is a strategy of Medicinal Chemistry for the rational design of new drugs, applied with a lead compound (LC) as a special process of molecular modification [1]. The LC should be of a completely well known chemical structure and possess an equally well known mechanism of action, if possible at the level of topographic interaction with the receptor, including knowledge of all of its pharmacophore group. Furthermore, the pathways of metabolic inactivation [2], as well as the main determining structural factors of the physicochemical properties which regulate the bioavailability, and its side effects, whether directly or not, should be known so as to allow for a broad prediction of the definition of the bioisosteric relation to be used.

The success of this strategy in developing new substances which are therapeutically attractive has observed a significant growth in distinct therapeutic classes, being simply used by the pharmaceutical industry to discover new analogs of therapeutic innovations commercially attractive (*me-too*), and also as a tool useful in the molecular modification.

There may be innumerable reasons for the use of bioisosterism to design new drugs, including the necessity to improve pharmacological activity, gain selectivity for a determined receptor or enzymatic isoform subtype - with simultaneous reduction of certain adverse effects -, or even optimize the pharmacokinetics the LC might present.

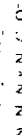
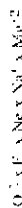
In this paper, we will discuss bioisosterism as a strategy of molecular modification, showing its importance in building a new series of congeners compounds designed as candidate of new drugs, giving examples of successful cases in distinct therapeutic classes [3-7].

2. BACKGROUND

In 1919, Longuir [8] studying the chemical behavior and reactivity of determined substances possessing atoms or groups with the same number of valence electrons, i.e.,

*Address correspondence to this author at the Laboratório de Farmacologia, Universidade Federal do Rio de Janeiro, 20221-900, Rio de Janeiro, RJ, Brazil. E-mail: eliezer@pharm.ufrj.br

works from, created the concept of isosterism to define atoms or organic or inorganic molecules which possess the same number and/or arrangement of electrons examples:



In 1923, Grinm [9] formulated the *Hydride Displacement Law*, an empiric rule which states that the addition of a hydrogen atom with a pair of electrons (i.e. hydride) to an atom, produces a pseudocation presenting the same physical properties as these present in the column immediately behind on the Periodic Table of the Elements for the initial atom (Fig. 1), showing that any atom belonging to groups 4A, 5A, 6A, 7A on the Periodic Table change their properties by adding a hydride, becoming isoelectronic pseudocations.

In 1933, Fehrmeyer [10] proposed a broadening of the term isosterism, defining isosteres as elements, molecules or ions which present the same number of electrons at the valence level. His contribution includes the proposition that elements of the same column on the Periodic Table are isosteres among themselves (e.g. C x Si x Ge) and the creation of a concept of rings electronically equivalent, later broadened to the term ring bioisosterism.

The coining of the term bioisosterism goes back to the pioneer work of Friedman and Thornber during the early 50s. Friedman [11], recognizing the usefulness of the concept *isosterism* to design bioactive molecules, defined isosteres as compounds which fit the definitions of isosteres and which exercise their biological activity of receptor, whether through agonist or antagonist actions. However, Friedman introduced the term *bioisosterism* to describe the phenomenon observed between substances structurally related which presented similar or analogous biological properties [11]. Later, Thornber [3] proposed a broadening of the term bioisosteres, defining them as subunits or groups or molecules which possess physicochemical properties of similar biological effects.

Over the years, innumerable bioisosteric relations have been identified in compounds both natural and synthetic. In nature, we have identified many examples of isosterism as a

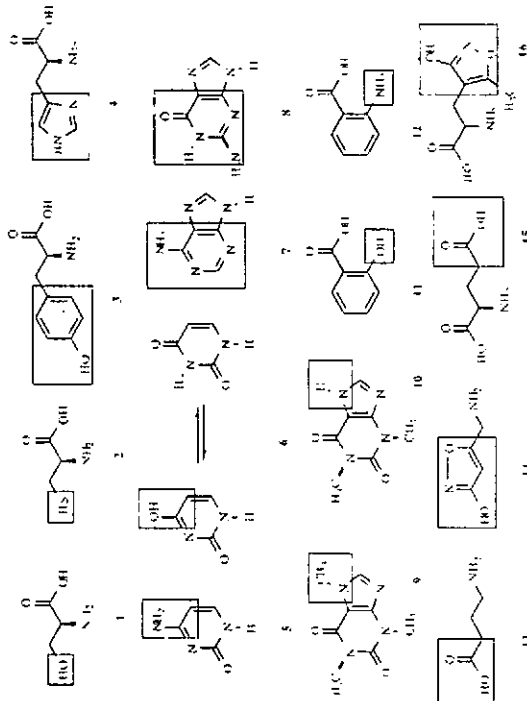
Group Group Group Group Group
4A 5A 6A 7A 8A

N° of e ⁻	6	7	8	9	10	11
C	N	O	F	Ne	Na ⁺	
H $\rightarrow$ CH	NH	OH	FH			
H $\rightarrow$ CH ₂	NH ₂	OH ₂	FH ₂ ⁺			
H $\rightarrow$ CH ₃	NH ₃ ⁺	OH ₃ ⁺				
H $\rightarrow$ CH ₄	NH ₄ ⁺					

Fig. (1). Grinm's hydride displacement law.

form of broadening chemodiversity (Scheme 1), striking among which are the classic bioisosteric relation existing between the essential amino acids serine (1) and cysteine (2), tyrosine (3) and histidine (4) among the pyrimidine and purine bases cytosine (5) and uracil (6), adenine (7) and guanine (8), among the xanthines caffeine (9) and theophylline (10); and among the xanthine (11) and anthranilic (12) acids, which originated two important

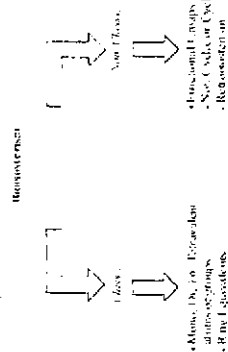
classes of non-steroid anti-inflammatory drugs, i.e. acetylsalicylic acid and metanilic acids, respectively. Furthermore, examples of the application of bioisosteric relations are also found in nature, such as the bioisosteric relationship existing between γ -aminobutyric acid (GABA) (13) and muscimol (14), between the neurotransmitters glutamate (15) and AMPA (16).



Scheme 1.

B. CLASSIFICATION OF RECONSTRUCTION:

In 1970, A few Budget classified and subdivided
parameters into two basic categories: "Assets and Non-
Classified" (Scheme 2)



Scheme 2.

Burger's definition significantly broadened this concept, now denoting these atoms or molecular subunits or functional groups of the same valence and rings equivalents as classic biosteroids (Table 1), while non-classic biosteroids were those which practically did not fit the definitions of the first class (Table 2).

- | Class I Bioisosteres (Table 1) | |
|----------------------------------|----------------------------|
| 1.1 | Monovalent atoms or groups |
| 1.2 | Divalent atoms or groups |
| 1.3 | Trivalent atoms or groups |
| 1.4 | Tetrasubstituted atoms |
| 1.5 | Ring equivalents |
| Non-Class Bioisosteres (Table 2) | |
| 2.1 | Cycle vs Noncycle |
| 2.2 | Functional groups |
| 2.3 | Retrosynthesis |

Table 1 Classic Ricinostere Groups and Atoms

Monofunctional	Divalent	Trivalent	Trifunctional
-OH, NH ₂ , CH ₃ , OR -F, Cl, Br, I, SH, PH ₂ -Si ₃ -SR	-CH ₂ - -Ar- -Ar- -Ar- -N ₂ - -Te-	-CH ₂ - -N- P- -N ₃ - Sb-	-C≡ -NH ₂ N' -P' As' SH ⁺ ≡

Many authors have contributed to updating the tables of functional biostere groups, which explains why, in that which we refer to as non-classic biostereotaxism, we have observed a growing number of biosteres described in the literature [2-7].

FOR INFORMATION OF THE DIRECTOR

Young *et al.* had recent anti-tuberculars, and the sensitivity of bacitracin for *M. fortuitus* [10]. Pharmacologically, bacitracin is active against *M. fortuitus* as a lipopeptide, but its apparent predominance in non-lipid bacitracin is distributed in distinct therapeutic categories by the selective receptor antagonist at almost 100% enzymatic inhibition of anti-metabolites. The use of classic bacitracin for the structural design of new drugs, while *M. fortuitus* has a structure that is necessary to

The correct use of biostatistical demands physical, chemical, economic and computational models involved in the planned biostatistical substitution, carefully analyzed so as to predict, although theoretically, any eventual alterations in terms of the pharmacodynamic and pharmacokinetic properties which the new biostatistical substance presents.

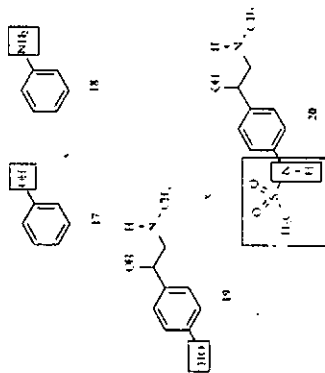
Thus being, any biostatistic replacement should be rigorously preceded by careful analysis of the following parameters [5]:

- size, volume and electronic distribution of the atoms or the considerations on the degree of hybridization, polarizability, bonding angles and inductive and mesomeric effects when fitting;
- degree of lipidic and aqueous solubility, so as to allow prediction of alteration of the physicochemical properties such as $\log P$ and pK_a ;
- chemical reactivity of the functional groups or biospecific structural subunits, mainly to predict significant alterations in the processes of biotransformation, including for the eventual alteration of the toxicity profile relative to the main metabolites;
- conformational factors, including the differential capacity formation of inter- or intramolecular hydrogen bonds

1.1. BIOSISTERISM AND ALTERATIONS OF PHYSICO-CHEMICAL PROPERTIES

Some bioisosteric groups dramatically alter the physicochemical properties of substances and, therefore, their activities. This can be easily understood by comparing classic isosteres resulting from bioisosteric replacement between hydroxyl (OH) and amine (NH_2), an example of a classic bioisosterism of monovalent groups according to the *Isomorphous Rule*. In this case, considering the bioisosteric replacement of aromatic amine present in aniline (18) by hydroxyl, we have phenol (17) (Scheme 3) resulting in a significant change in the acid-base properties of isosteres, with dramatic modification of the pK_a of the compounds, which is responsible for the distinct pharmacokinetic profiles among the isosteres in question. Furthermore, in terms of molecular recognition of a given receptor site, we have a chemical change form one positively charged function (NH_2^+) to another charged function (phenol pK_b 9, 30) or another acid (pK_a 10.0) present in phenol, which may, in turn, enable, although the present in phenol, which may, in

In this example, we may predict that the use of biosolvent, rather than the classic type, can promote severe alterations of the molecular properties, as such in terms of lipidic-aqueous solubility as well as chemical reactivity, among others. In fact, broadly speaking, is not observed in the same homogeneous carbonic series. Otherwise, the system's enzymatic capacity for hepatic detoxification of xenobiotics involving the microsomal mixed function oxidase also associated with cytochrome P-450 system [42], is distinct in the presence of these functional isomeric groups, which does not allow a simplistic comparison between the lead compound (moline 18) and the hydroxylated isostere (17) in terms of its pharmacokinetic behavior, its metabolism, its pharmacokinetic phase as well as the pharmacodynamics of the isomers.



Scheme 1.

Another classic example of isosteric replacement involving phenol (PhOH), can be found in the search for catecholic derivatives, structurally related to catecholamines.

26. Current Methods Chemistry, 1968, Vol. 12, No. 1

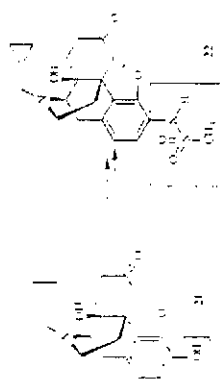
[illegible]

(Scheme 3).¹⁵ This example illustrates the exchange of phenolic hydroxyl group present in compound 19 with the arylsulfonamide unit in compound 20, through the use of non-classic bioisosterism of functional groups.

The results obtained, carrying out bioassays with these compounds, witness comparable biological activities through the mechanism of equivalent action, allowing us to conclude that both functional groups involved are authentic biosites. This biotensite relationship was experimentally evidenced by determining the degree of acidity of these substances. Both compounds are of comparable acidity ($pK_a = .91$ and $.76$, respectively), explaining the similarity of biological profile in function of their equivalent interactions of both molecules with site receptor, possibly through ionic bonding, in the presence of a similar acidity or even though hydrogen bonding [15]. Furthermore, both acidic groups - i.e., R-PHOH and R- $\text{P}(\text{NH}_2)_2\text{CH}_2\text{C}(=\text{O})_2-$ are, in this case, monovalent groups. However, the identified biosensitive relationship between R-PHOH and PHNISO-C(=O)-R groups, confirmed for adrenergic derivatives 19 and 20, is not extensive to other biosensors such as those which process molecular recognition is distinct. An example of how ionene groups can, in certain systems, not maintain a biosensitive relationship, previously defined in another acceptor, is well illustrated with the analysis of compound 22 and 22c described by McCarty and coworkers (Scheme 4) [21] and 22, described by McCarty and coworkers (Scheme 4) [21]. The structural design of 3-sulfonamide compound 22, targeting the attainment of new bonds for opiate receptors, was based on the structural data of lead compound 21, which brought attention to the relevance of phenolic hydroxyl as site of attachment to the relevancy of phenolic hydroxyl as site of attachment to the relevancy of phenolic hydroxyl as site of attachment to the relevancy of phenolic hydroxyl as site of attachment.

This way, the replacement OFF by the NPHNISO-C(=O)- group would respect the acidic properties and characteristics donor and/or acceptor H-bonds as evidenced by the previous work of Larsen and Lush (1964). Nonetheless, the determination of the pharmacological properties of arilfonamide derivative 22 showed the absence of affinity the opiate receptors, it also indicated the lack of ability to interact with the same sites.

from 2.0 to 0.0 in this system the groups PhCH₃ and p-PhSO₂CH₃ are not biososteric, despite their great chemical similarity.



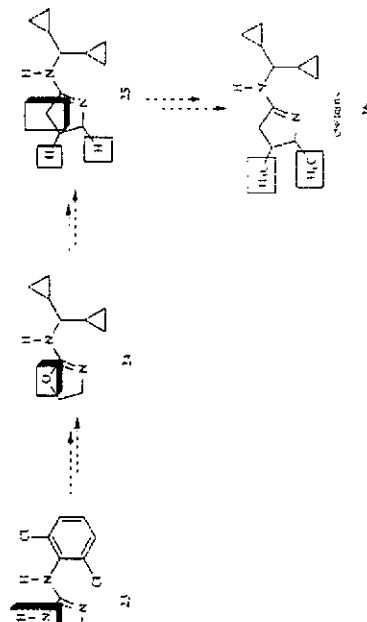
Scheme 3.

Thus, when biososteric replacement occurs in functional groups involved in the pharmacophore subunit of a certain bioactive substance, the relative activity of the resulting compounds may be dramatically modified. However, *biososteric replacement which successfully occurs in a series of compounds acting as a type of bioreceptor, will not necessarily be successful in another therapeutic series acting through other receptors.*

5. CLASSIC BIOSOSTERISM

5.1. Biosostericism of Mono-, Di-, Tri- and Tetravalent Atoms or Groups

In the search for new and hypertensive drugs analogous to clonidine (23), with a greater selectivity by H_1 imidazoline receptors (116), and reduced action on α_2 -adrenoceptors, Schöten and coworkers described the attainment of new candidates for anti-hypertensive drugs designed by nucleolar modifications in the structure of the lead compound rilmenidine (24) (Scheme 5) [17]. These modifications were based on classic biosostericism of bivalent groups,

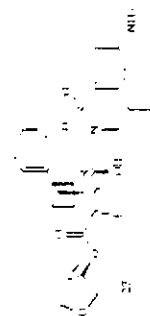


Scheme 5.

exemplified by replacement of the biocent oxygen atom (11) present in the oxazoline ring by the nitrogen atom (11a) at the structure of the new pyridine derivative (25), and between replacement of monovalent groups (fluorine) by the substitution of hydrogen atoms (H) in C-3 and C-5 of arisartan (25) by the methyl group (CH₃), originating the *ortho*-methyl analogues 26 [17]. The *bioing* modifications securing in the structure of rilmenidine (23), allow the attainment of derivatives with activities comparable to lead compound (24), although with a superior selectivity for H_1R , and illustrated an example of the success of classic biosostericism.

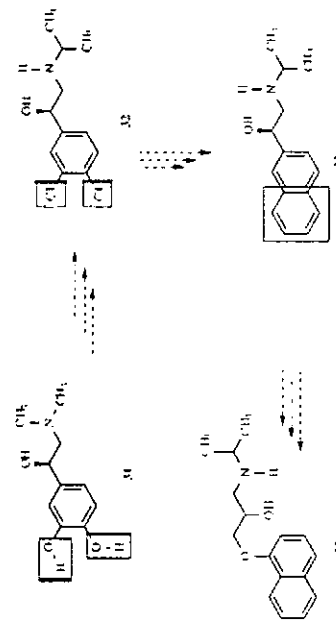
In develop new HIV-protease inhibitors, Rochehave and coworkers described the biososteric exchange between bivalent groups (Scheme 6), realizing the exchange of the methylene group (CH₂), present in the structure of the lead compound amprenavir (27), for the sulfur atom (S) in 28 [18]. Knowing, *a priori*, that this biososteric replacement could induce several modifications in terms of size, shape, electronic distribution, chemical reactivity, lipophilicity and hydrogen bonding capacity, this new isomer was synthesized, and its stereoisomers were duly separated by HPLC and tested as recombinant HIV protease inhibitors. The results obtained revealed that the four diastereoisomers tested were only weak inhibitors of recombinant HIV protease, while the diastereoisomer (28a), of the same absolute configuration as the lead compound amprenavir (27), being 10,000 less potent than 27. These results could be explained by the high sensitivity to hydrolysis of this isomer 28a d. In fact, the half-life value found for thiophenyl derivative 28a-d was 10 min, while amprenavir (27) was recovered unchanged after 1440 min.

Another illustration of the application of classic biosostericism can be found in the works of Penning and coworkers (Scheme 7) [19]. Confining the research to find new anti-inflammatory drugs acting through the selective inhibition of prostaglandin-H synthase-2 (PGHS-2) or cyclooxygenase-2 (COX-2), the authors investigated the



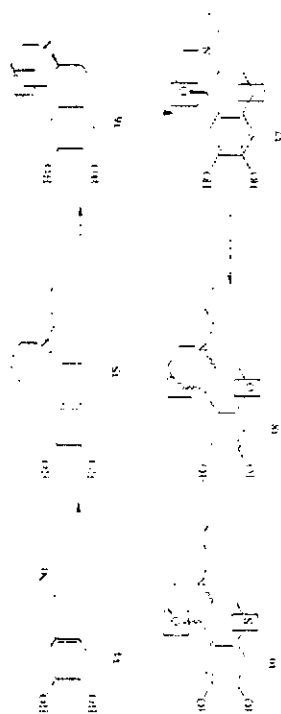
Scheme 6.

effect of steric modifications on the structure of lead compound SC-58125 (29), to improve its pharmacokinetic properties. In spite of the high rate of selectivity by PGHS-2 (815-1700) and good inhibitory potential, the derivative SC-58125 (29) exhibited a half-life of over 200 h, thus reflecting its low susceptibility in the presence of the complex enzyme involved in the hepatic metabolism of xenobiotics. Hence the authors suggested two classic biososteric monovalent group replacements, represented by replacing the methyl group (CH₃) by the NH₂ group and exchanging the fluorine atom (F) with the CH₃ group (Scheme 7). Both steric replacements suggested allow introducing into the structure of the new biososteric of SC-58125, vulnerable and metabolic sites taking advantage of the effect of the first passage through reactions of conjugation with glucuronic acid and the benzylic hydroxylation catalyzed by CYP450, respectively. This new biososteric optimized by SC-58125, *i.e.* celecoxib (30), of a half-life of from 8-12 h, was introduced on the Brazilian market in 1999 by Pfizer/Seale Laboratories to treat rheumatoid arthritis and other inflammatory conditions, being the first non-steroidal anti-inflammatory drug (NSAID) acting selectively upon the inducible isoform prostaglandin-H synthase (*i.e.*, PGHS-2) and, therefore, without the irritating gastric effects typical of the first generation NSAIDs [20].



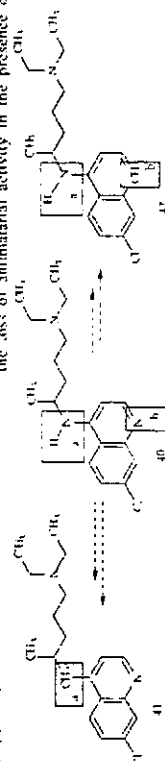
Scheme 7.

The design of dichloroisoproterenol (32) from isoproterenol (31) is, without a doubt, an unique example of the contribution of the application of biosostericism to optimize pharmacodynamic and pharmacokinetic properties of new drugs (Scheme 8). The replacement of catecholic hydroxyls, present in isoproterenol (31), by the monovalent chloro (Cl) group in the structure of dichloroisoproterenol (32), represented a useful strategy to obtain new analogs with half-lives greater than the lead compound 31, since this biososteric replacement allows blocking one of the main sites of metabolism of this catecholamine derivative.



Sicherung 7.

Furthermore, the discovery of diethylpropion (12), a virtually tasteless compound of the relevance of unusual characteristics of the catechololic substituent for anesthetic activity, allowing a *protonated* the discovery of amphetamine (13), the first selective antagonist for adrenergic receptors for salivary B₂ (proctolinol(33), discontinued during the 1980s, was the precursor of "propranolol" (34), a therapeutic innovator which revolutionized the treatment for hypertension. The discoverer, Sir James Black, received the Nobel Prize in Medicine for this feat and his discovery was the lead compound for innumerable other more selective adrenergic B₂ antagonists

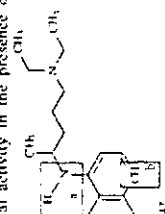


Schwarz 10.

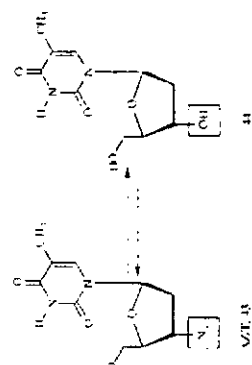
The bioisosteric replacement of the bivalent oxygen (O) by sulfur (S) results in a significant alteration in lipophilicity, and may be used in the design of new drugs to act upon the central nervous system (CNS). Van Vleet and coworkers (Scheme 9), in their search for selective agonists of dopaminergic receptors, subtype 3 (D3), molecular targets for the design of anti-psychotic drugs, realized modifications to the hexyldopamine derivative present in lead compound 36, an analog conformationally restricted to dopamine (34), of high potency and low selectivity [21]. These structural modifications, based on the bioisosteric exchange of the methylene (CH₂) group in 37, by oxygen (O) and sulfur (S) atoms in compounds 38 and 39, respectively, allow identification of two new dopamine analogs. The coefficient of partition of these isomers (log *P*, 1.62) compared to octanediene (38, log *P* 1.13), showed a significant increase in lipophilicity, a typical consequence of the bioisosteric replacement carried out. However, the determination of selectivity by the different subtypes of dopaminergic receptors, assayed by the *binding* tests, leads us to believe in an inverse relationship between

the increase in hydrophilicity and the selectivity desired by the D₂ receptors.

To authors and the contributors of 1-aminopropylamine substituent present in the structure of the 1st generation antimalarial chloroquine (C1Q, 40), Chereka and co-workers have described the attainment and determination of the pharmacological profile of modified analogs of C1Q (Scheme 10) [21]. In this study, the authors carried out the selective bi-ionic exchange of NH in *MAL-nitrile* (I) and N-methyl-*N*-nitrosamine with the CH₃ and Cl groups in the structure of compounds 41 and 42, respectively, observing the loss of antimalarial activity in the presence of the



modifications realized. Carbo-esters 41 and 42 were inactive due to the inhibition of *Plasmodium falciparum* NK-624 or K1 strains, even in concentrations over 3000 nM. Carbo-esters 43 and 44 (8.5 and 150 nM, respectively), and also 45, were able to inhibit the formation of benzoxime



Scheme 11.



Page 12.

ClO₂ (10–80 μM) (10, 1000 μM, 12 h, 2500 μM). Hence, the bacteria disaccharides cellulose reinforced the effectiveness of the treatment of a quaternary interaction process with hemagglutinin, the molecular target of antimicrobial action of 3-aminopropylate drugs.

Zidovudine (AZT, 43), an important chemotherapeutic resource available for the treatment of acquired human immunodeficiency syndrome, was discovered from the structures identified in nucleosides isolated from seaweed. The structural analysis of AZT (43), a powerful inhibitor of

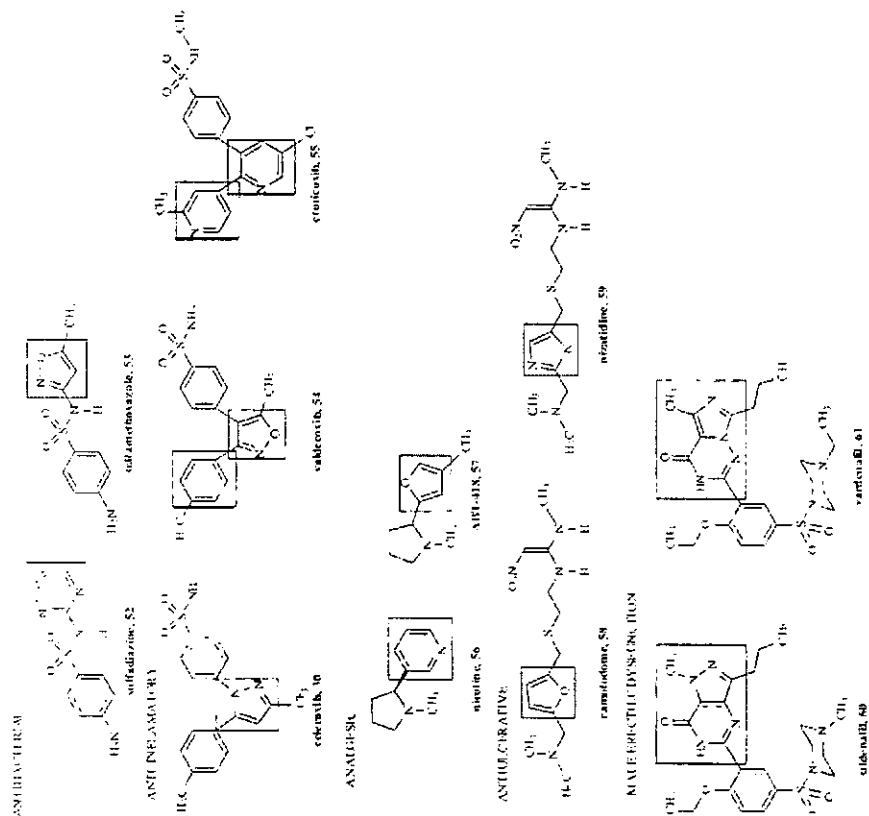
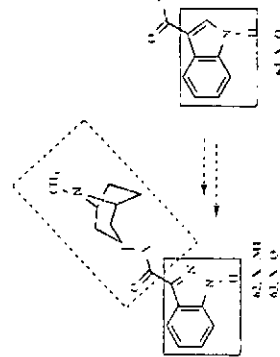


Fig. (2). Parameter of zinc homeostasis between jars belonging to different therapeutic classes.

transference reverse source, enables us to ascertain the existence of a class of bioisosteric relationship of intramolecular groups between the bicyclobutene (23) tetraeneous substrate for the synthesis of DNA and RNA and $\Delta^1,2$ exemplified by the systems of the indole, (60) and in 41 and azide (N₃) group present in 43 (Scheme 11) [73]. Furthermore, through a cyclic isomeric isosteres the OH and N₃ groups possess adequate electronic differences, easily demonstrated by simple functional analysis.

Although applicability of the strategy of molecular modification on a given lead compound through bioisosterism is well consolidated in the pharmaceutical industry, recent studies by Lack and coworkers (Scheme 12) shows that this approach may be used efficiently to optimize the regiospecific properties of compounds used in fragrances of industrial interest [74]. In this study, the application of classic bioisosterism of aryl-substituted moieties illustrated by exchange of the carbon atom (C), present in the structure of imatinib, (45), by silicon atoms (Si) and germanium (Ge) in 46 and 47, respectively, allows the identification of new standards of fragrances of synthetic origin. Although this isosteric exchange is rare in drugs, the modification of molecular volume by the substitution C x Si x Ge, producing bioisosters 45, 46, and 47, results in conformational and electronic similarities evidenced by diffraction and X-ray studies and the determination of electrostatic potential through the Program GAUSSIAN 98.

More recently Showell and Mills reported that one of the advantages of classic bioisosterism application of tetrasubstituted atoms involving the replacement of the carbon atom (C) by the silicon (Si) atom in the structure of existing drugs, based on the possibility of designing new drug-like candidates that have beneficial biological properties and a clear intellectual property position [25]. In fact, according to Steele, in 2001, less than 1% of patent applications related to compounds containing phosphorus, silicon or other less common elements of the periodic table [26]. However, although this may seem attractive, the differences in atomic size, electronegativity and lipophilicity between C and Si atoms, associated with the instability of Si-H bonds, in physiological conditions prove the applicability of isosteric exchange C x Si in the structure of new drugs to be quite limited [25].

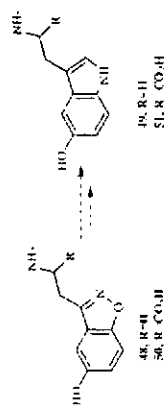


Scheme 14.

5.2. Ring Bioisosteres

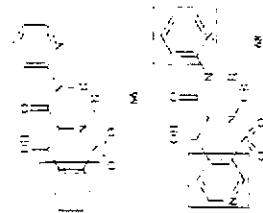
Ring bioisosterism is undoubtedly the most frequent relationship in terms of different therapeutic classes [8] as can be seen in Fig. 21 and certain specific examples will be commented upon.

In 1973, Campagne and coworkers synthesized compound 48 [27] as being a derivative structurally related to serotonin 49, an antagonist with the function of inhibitor in different physiological phenomena (Scheme 13). These authors based their findings on the probable classic bioisosteric relationship existing between the benzoxazole and indole rings present in 48 and 49, respectively. The results of these pharmacological assays showed that compound 48 presented no type of serotonergic activity, not even anti-serotonin, when tested on rat uterus preparations. In contrast, derivative 50, presenting the same benzoxazole nucleus in replacing the indole ring of 51, showed activity when tested with substrate for serotonin decarboxylase enzyme, dependent on 5-hydroxytryptophane 51 (Scheme 13), an indole compound which is the natural substrate of the enzyme involved in serotonin biosynthesis. This example highlights the bioisosteric relationship existing between the benzoxazole ring and the indole nucleus in 50 and 51, respectively, depending on the receptor site involved.



Scheme 13.

Applying classic bioisosterism in the design of new serotonin receptor (5-HT₁) antagonists Fludzinski and coworkers (Scheme 14) described the obtention of new indazole compounds 62 and 63 with important anagonistic properties of receptor 5-HT₁ [28]. These two substances were developed as bioisosters of 64, a compound which possesses as main structural characteristic the hybrid

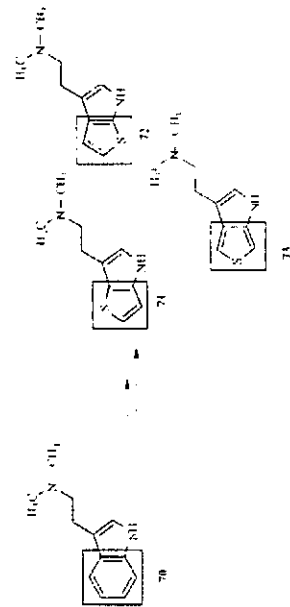


Scheme 15.

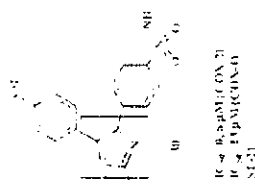
character between serotonin (49), represented by the indole ring substituted in C-2, and oxime (65), represented by the nature of the substituent in C-2, a known antagonist of receptors 5-HT₁ [29]. The bioisosteric relationship of these two nuclei was proven in this case, by the results obtained in bioassays which showed for 67 a rate of selectivity by the receptors 5-HT₁ superior to that of 61, when administered intravenously as well as orally, showing that the indazole and indole moieties, when substituted equivalently, possessing the same electronic properties and the same standard of aromaticity, may be considered bioequivalent at the level of interactions with the same receptor. However, compound 62 showed a profile of activity superior to 63, administered *p.o.*, indicating that the amide bond present in chain 62 is responsible for a more favorable oral bioavailability.

The application of ring bioisosterism was also successfully explored by Binder and coworkers (Scheme 15) [30] in developing new non-steroid anti-inflammatory agents of the oxazone group [31]. This class of NSAID was discovered by Lombardino and collaborators [32], at Pfizer laboratories in England, piroxicam 66 being the main representative of this class of 1,1-dioxybenzene-1,2-dithione (HFA) synthetics.

Applying the strategy of ring bioisosterism, Binder proposed tenoxicam 67, the newest member of the class of arylthiazine-1,1-dioxides, where the benzothiazine nucleus



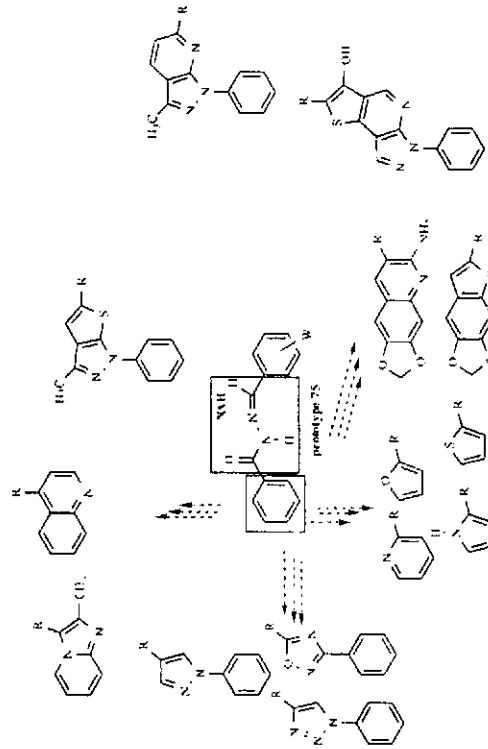
Scheme 16.



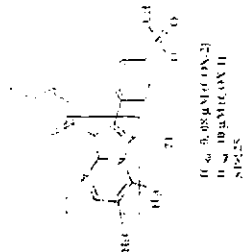
Schönemann 17.

considering the possible resonances involved in this replacement. Stokes et al. and coworkers have illustrated very well this type of replacement, in which the phenyl ring of the indolic nucleus, present in the structure of lead compound, *N,N*-dimethylpyromethene 170, was replaced by the thiophene ring, giving rise to the isomeric systems, thietherol 1,2,3-b-pyrole (71), dietherol 1,3,4-b-pyrole (72), thietherol 1,2,4-b-pyrole (73), dietherol 1,3,4-b-pyrole (74). However, the results described by these authors show that with the exception of the dietherol 1,3,4-b-pyrole system (73), of unobtainable and difficult preparation, the other heterocyclic isomeric systems are authentic bioisosteres of the indole nucleus, presenting affinity and selectivity by the serotonin receptors similar to lead compound 70.

Concerning the non-steroid anti-inflammatory drugs (NSAIDs), Almaraz and coworkers (Scheme 17) [35]



Scheme 18.

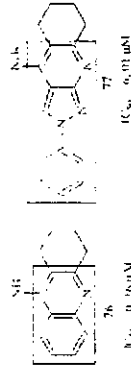


described the existing biostereic relationship between the pyrazolo and pyrazolyl 1,5-olpyridine rings present in compounds 30 and 74, respectively. The exchange of the pyrazole ring, present in celecoxib (30), by the pyrazolyl 1,5-olpyridine system in 74, resulted in an optimization of the pharmacodynamic properties of 30 although it compromised its oral bioavailability.

The use of ring bioisosterism may serve as a design strategy for identifying a series of related compounds for new drugs, in view of the detailed study of the distinct hydrophobic contributions resulting from rings equivalents. This strategy has revealed itself as a fundamental tool in the design of new *new*-mold drugs, i.e. therapeutic copy trade (Figure 2). In a recent study, Barreiro and coworkers¹² described the application of ring bioisosterism for designing functionalized *N*-acetylhydrazine (NAH) derivatives (Scheme 1).

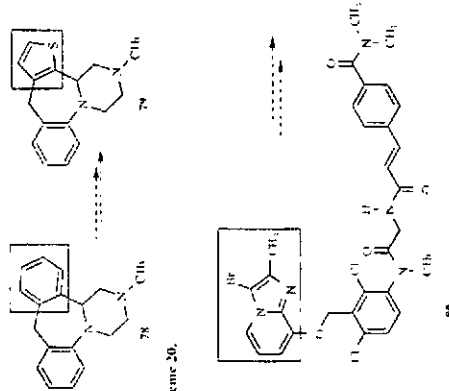
183) [26], in an effort to combine the advantages found in the world of computer, analogue and non-inflammatory activities of each new heterogeneous system studied, with its respective electronic characteristics.

More recently, Barone and coworkers (Scheme 19) described the attachment of new selective acetylcholinesterase (AChE) inhibitors designed by modifications in the structure of the lead compound tacrine (26) (37). These modifications allow for identification of a new biospecific relationship existing between the quinuclidine nuclei in 76 and pyrazolopyridine in 77, which have shown a similar inhibitory capacity on rat brain cholinergic enzymes.



Scheme 19.

In the field of antidepressives, bisoxazone ring replacement of a determined lead compound also permits the discovery of new agents of therapeutic interest. In 1983, Watkley and coworkers developed, at Ciba-Geigy Laboratories, compound 79 as a bisoxazone of miniseries 78 (Scheme 20) [28]. As principal structural characteristic, this substance possesses the thienophene ring integrated to the bicyclic unit of this representative of atypical antidepressive agents, which act as serotonin receptors (5-HT) antagonists [34]. Once again, this example illustrates the equivalency of the benzene and thiophene rings, in distinct pharmacological activities, allowing for a generalization on the bisoxazone relationship between heterocyclic nuclei of five atoms and benzene ring [40].



20

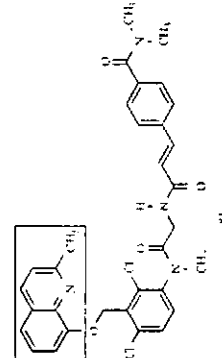
Sicherung 21

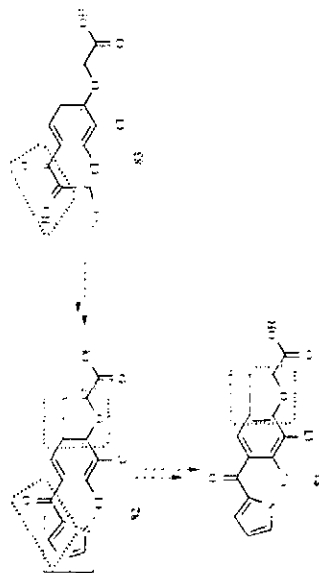
The recognition of the importance of bradykinin in the processes of pain, inflammation, fluids and hypertension, has drawn the attention of bradykinin receptors as a new target for therapeutic intervention. Although many bradykinin receptors antagonists have been described in the literature, few of them have been marketed. Among these, non-peptide antagonists seeking non-peptide bradykinin receptor antagonists. And coworkers described the development of 2-azetidinyl-1,2-dipyrrolone derivatives highlighting the derivative **1** (Fig. 1) as a lead compound (Schäfer 21). Later, these authors proposed the 2-azetidinyl-1,2-dipyrrolone **2** as a novel non-peptide antagonist, as a new structural analogue of **1**, 2-dipyrrolone present in contrast 80 by the 2-methyl-2-pyrrolone substituent, identifying the new derivative **1** (Fig. 1) as a structural analogue of non-peptide bradykinin receptor antagonists.

CLASSIC ROCK 9

6.1. Cyclic vs Non-Cyclic

In the category of aromatic substances, of the phenylacetylenic class, in which chlorine atom 82 is the main representative, we have found several interesting examples of the use of non-classic biosynthesis in the discovery of new drugs. To develop new diuretic substances, with ure activity superior to 82, Hollman and co-workers applied non-classic biosynthesis represented by *ring- β lactone*, or anelation, as a strategy to choose definitions of new lead compounds for the discovery of new diuretic drugs [42]. These authors working at Merck, Sharp and Dohme Laboratories, proposed compound 84, a chlorobiosisester of 83 (Scheme 22). The structure of 84 was based on the anelation of the phenylacetylenic chain (n) of 83, which, in turn, arose by the replacement of the phenylacetylenic function present in 82 by the thiophene nucleus of 83, including all four carbon atoms of the replacement of 83, there being a clear correspondence between the carbons of 82, 83, and 84, with sp^2 -hybridization in 82 and 84. This type of anelation in the cyclohexene chain of 82 had been proposed previously by Thullier [43-44], who described the synthesis of 83 as being an acid equivalent to electronic acid 82 in terms of its pharmacologic properties, while with superior ure properties. In fact, this last compound represents one of the first examples of biosynthesis of 82, obtained by application of non-classic biosynthetic strategies. Compound 84, proposed and synthesized by Hollman, presented a profile comparable to 82.





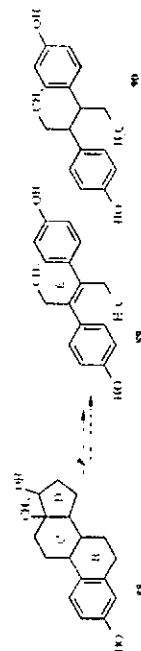
Scheme 22.

83, its ureic potential being even greater. Furthermore, the introduction of the thiophene ring in 83 to replace the ethylene substituent of 82, diminishes Michael's acceptor site present in the structure of enamine acid 82, responsible for the hepatotoxic effects of this drug.

Still on the subject of diuretics related to elacrine acid (82), Shustke and coworkers later developed the synthesis of new aryl-benzisoxazole acids (e.g., 85) [45], based on the probable biosynthetic relationship existing between the aryl-benzisoxazole unit and the 2-acyl-thiophenyl moiety present in acid 83 [46] (Scheme 23). This illustrative example is sufficient to show the potential of the strategy of non-classic biosynthesis for designing molecular modifications in substances of pharmacological interest.

Shustke and coworkers successfully explored the possibility of integrating the thiophenyl group of 83 into one aryl-benzisoxazole nucleus, respecting the basic pharmacophore of this class of agents, so as to assure, at least theoretically, an action through a similar mechanism, i.e. through molecular recognition by the same site receptor. In this example, the authors introduced a factor of conformational restriction in 85, typical of non-classic biosynthesis of the *clawing* ring type. Later, the same authors [47] used classic biosynthetic ring to design structurally new heterocyclic diuretic compounds from 85, possessing, now, the indazole ring in 87 and benzisothiazole in 86 (Scheme 23).

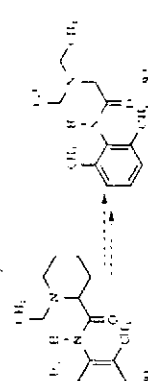
Innumerable other examples can illustrate the validity of the use of this strategy in discovering new bioactive agents which are more therapeutically attractive. A classic example



Scheme 23.

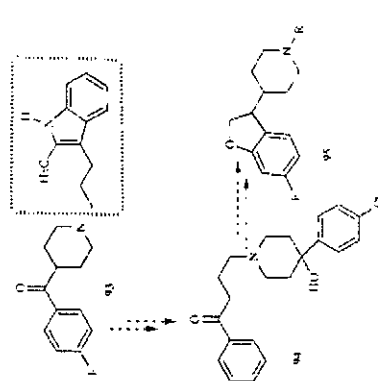
In compound (99) the indole substituent of indometacin was replaced by the pyrrole ring carrying the benzoyl unit in C-2,

a new series of non-steroid compounds of series I (aroylpyrrolidine, in which 91 (HP 201) is one of the main representatives. This substance is structurally related to haloperidol 91, the main representative of the antipsychotics, a class of neuroleptics associated by Loewen [50], in which, conceptually, the C-2, C-3 and C-4 carbon atoms of the butylenic chain of 91 are contained in the pyridine moiety of 93, thus representing a non-classic biosynthetic of 91 (Scheme 26). These authors further developed compound 95, in which a benzoxazole moiety was biosynthetically replaces the 4-benzoyl moiety, represented by the 4-fluorobenzoyl unit in 93.



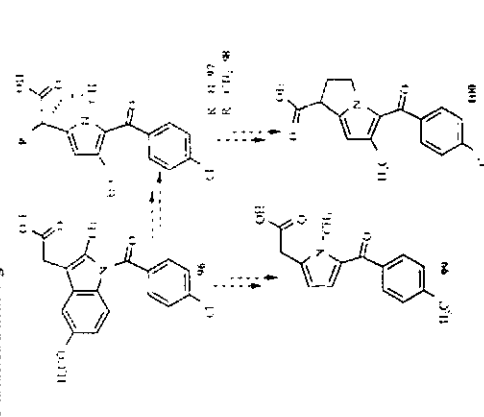
Scheme 25.

In the class of non-steroid anti-inflammatory drugs (NSAIDs) other examples were found of the use of the strategy of cyclic non-classic biosynthesis. In 1987, Muchosky and coworkers [51], developed the synthesis of a new NSAID called etopirone (100), designed from the cyclic non-classic biosynthesis strategy, exploring the ring closing between the acetic acid unit and the N-CH₃ moiety of lead compound tolmetin (99) (Scheme 27) [52], a well known NSAID of the heterocyclic acid class [53]. Tolmetin (99), in turn, was developed by molecular modification of the indomethacin structure 96, an important representative of the 3-indolylacetic acid class with anti-inflammatory properties developed by Shen and coworkers at Merck, Sharp and Dohme Laboratories, in 1962 [54], applying the inverse principle, i.e. noncyclic nonclassical biosynthetic replacement.



Scheme 26.

as the biosynthetic of the *pyrro* class observed, substituent of 96. This same approach was later used for the development of compound (97) (Scheme 27) [55], another NSAID of the pyridyl 3-acetic acid class. This compound is in study of being presenting as the only structure of active the presence of the methyl group at C-3, also may therefore be considered a form of 97.

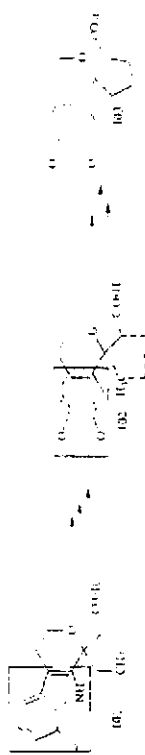


Scheme 27.

In 1973, Carlson and Wong demonstrated that homologation of the acetic chain of 97 leads to a new anti-inflammatory derivative, α -methylbenzene acetic acid 98 (Scheme 28), belonging to the propionic acid series, a class lauded by Shen [55], in the light of studies on the relationship between the chemical structure of aryl and heterocyclic derivatives, as presenting a profile of anti-inflammatory properties greater than those of the acetic series. Substance 98, in fact, was seen to possess anti-inflammatory properties greater than 90 and was even able to reduce adverse effects on the gastrointestinal tract, corroborating the previous observations made by Shen.

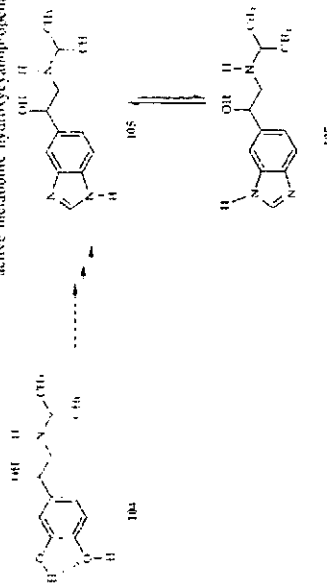
Considering this data, we may observe that compound (100), belonging to the 5-ethyl-2,2-dihydro-1H-pyridyl-1,2-*o*-pyridyl carboxylic acid family, represents, at the same time, a biosynthetic of 90 and 97 (Scheme 27), by *amidation* of the main pharmacophore unit of this family and is a new lead compound of the NSAID.

In 1995, Cabrel and Barrero (Scheme 28) applied non-classic ring biosynthesis as a strategy for molecular modification [56], to obtain analogs conformationally restricted to etodolac (101), an important drug with anti-inflammatory, analgesic and antipyretic properties. In this study it was possible to observe the optimization of the analgesic properties of the *pyrro*-indomethacin (101) derivative (103) when compared to lead compounds (101) and (102).



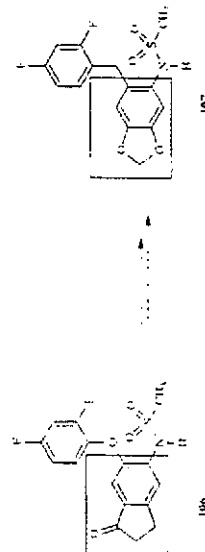
Scheme 28.

A supplementary example of biosynthesis may be found in the work by Avast and coworkers [37]. These authors developed the synthesis of compound 103, exploring the biosynthetic relationship between the benzimidazole ring and the side chain. The biosynthetic relationship between the benzimidazole ring and the side chain is shown in Scheme 29. Compound 103 proved to be a bioisostere of 101, presenting a pharmacological profile comparable to this level of β -adrenoceptors. In this case it is important to point out that the tautomerism of the benzimidazole ring present in 105, mimics, with a single proton the catechol group present in 104.



Scheme 29.

In a similar context, Barreiro and coworkers described the non-classic biosynthetic relationship between the indanone ring present in the structure of the prototype thioride (106) and benzimidazole unit present in compound 107 (Scheme 30) [58,59]. The results obtained from this study allow us to identify a new molecular standard of prostaglandin H-



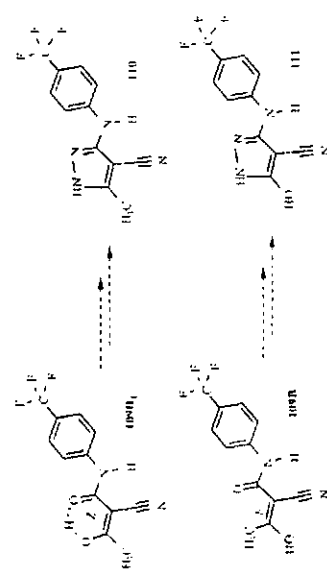
Scheme 30.

synthase (PHS 2) inhibitors, as well as to determine the electronic similarities existing between the indanone and benzimidazole rings, suggesting similar profiles of supra-molecular interaction with the enzyme involved in the pharmacological response of the thioride and the derivative (ANSB 34) (107). Possessing the benzimidazole moiety from salivary, an abundant natural alkaloid present in *Piper spp* and *Chelidonium*.

Leffluamide (108), an isozole aminomimetic drug that can prevent antibody-mediated rejection in heart xenotransplantation, is a pro-drug metabolized *in vivo* in its active metabolic hydroxyisopropenamide (109). In this

Scheme 31.

carbon system, referring to the double bond geometry, possible for the functionalized hydroxyisopropenamide (109), the *S*-*isomer* (109B) and *Z*-configuration derivative (109H), were found in X-ray crystallography studies (Scheme 31). The presence of this single diastereoisomer might be due to the stabilization from the formation of intramolecular hydrogen bonds involving hydrogen in the enolate system with the oxygen of amide function [50]. In an effort to corroborate with this experimental evidence, and to correlate it with the eventual bioactive conformation of hydroxyisopropenamide (109), Papageorgiou and coworkers proposed modifications in the structure of metabolite 109B and 109H, applying non-classic strategies of ring closing biotransformation (Scheme 32). In this study it was possible to design two novel pyrazoles analogs (110 and 111) conformationally restricted which mimic the diastereoisomers *E* and *Z* of hydroxyisopropenamide (109). The pharmacological results obtained with compounds 110 and 111 demonstrated that only derivative 110, whose structure was equivalent to diastereoisomer *Z* of 109 was active in the bioassays realized. Furthermore, X-ray studies carried out with the

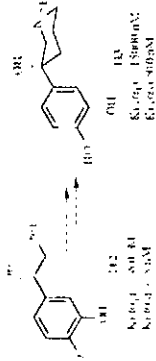


Scheme 32.

pyrazole derivative 110 demonstrated the existence of a single tautomer relative to the pyrazole ring, evidencing the similarity of the spatial arrangement between compounds 109 (101) and 110. In summary, this study allowed for identification of a new biosynthetic relationship between the pyrazole ring and the hydroxyisopropenamide substrate, as well as validating the elucidation of the bioactive conformation of the active metabolite of leffluamide (108).

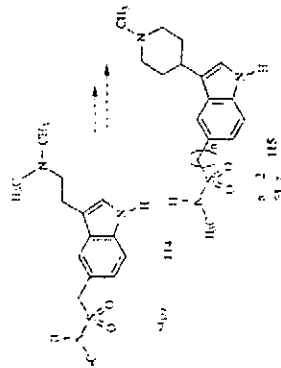
It is important to point out that the greatest conformational restriction ever reached by way of a anisotropic process may allow the possibility that the newly derived molecule adopt the bioactive conformation necessary for its molecular recognition by a determined bioreceptor, resulting in the loss of activity when compared to the active lead compound. This concept may be well illustrated by the works of Macchia and coworkers who described the attainment of piperidine (DDP3, 113) from noradrenaline (112) (Scheme 33), applying the strategy of anisotropic ring closing [60]. In this study, the authors, observed an expressive loss of affinity by the α - and α -adrenoceptors observed for the DDP3 derivative (113), attributed to the

process of introduction of 2-hydroxyethylamine substituent on compound 112 (2).



Scheme 33.

The molecular design of natriuretic (115) from the lead compound sumatriptan (114) (Scheme 34), the first agonist of serotonin receptors of subtype 5HT_{1D} commercialized for the treatment of migraine, illustrates how an increase in molecular volume from a ring process may be explored as a surrogate of metabolic protection, to obtain new lead compounds with more appropriate half-lives [61]. In fact, the introduction of the hexahydroindole ring in the natriuretic structure (115) resulted in protection, by steric blocking, of carbon α -amine from oxidative actions of hepatic metabolism, which act as an analog of monoamine oxidases (like MAO), producing the corresponding indole-3-acetic acid derivative.



Scheme 34.

6.2. Non-Classical Bioisosterism of Functional Groups

The landmark of the recognition of the importance of the bioisosterism of functional groups is the discovery of the antidiabetic properties of sulfamamide (117). Sulfamamide (117), an active metabolite of Promotril® [62], revolutionized chemodiscovery during the 70s and the later elucidation of its mechanism of molecular action allowed evidence of the similarity of its structure with *para*-aminobenzoic acid (PABA, 116). Those similarities based on electronic and conformational aspects, as well as the physicochemical properties such as pKa and log P, denote an authentic bioisosteric relationship existing between the sulfamamide (SO₂NH₂) and carboxylic acid functionalities (CO₂H) [63] (Scheme 35).

Diverse functional groups are known for their bioisosteric relationships with the carboxylate group. Some examples of the bioisosteric replacement of a COOH by a tetrazole group

are known [64]. An allyl-CH₂CO₂ example is described in the synthesis of 118, a tetrazole bioisostere of γ -aminobutyric acid (GABA, 119), which prevents its important selective inhibitory properties of GABA transaminase (GABA-T) (Scheme 36) [65, 66], an important pharmacokinetic application as an anticonvulsant agent.



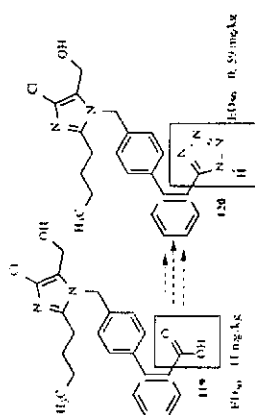
Scheme 35.

The tetrazole group mimics the carboxylate group, principally in terms of its physicochemical properties related to acidity, although the former is more stable and lipophilic. These differences allow this bioisostere to present a greater possibility of overcoming the blood brain barrier, with the type of topology favorable to the desired activity.



Scheme 36.

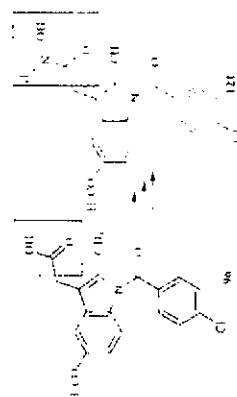
Another more recent example of the bioisosteric relationship existing between tetrazole and carboxylic acid groups may be seen by comparing the structures of losartan (120), angiotensin receptors antagonist approved in 1995 to treat hypertension with the lead compound EXP-7711 (119) (Scheme 37) [66].



Scheme 37.

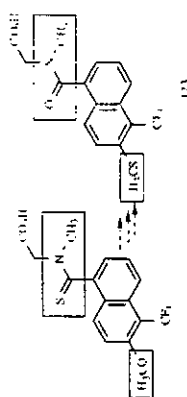
Derivatives possessing a hydroxamic-acid instead of a carboxylic acid function have been developed as carboxylic bioisosteres. Among other examples, compounds with anti-allergic properties presenting this function have been synthesized [67-68]. While in certain cases the metabolic stability of α -CONHOH function, has been demonstrated, which would merely characterize a pro-drug effect [69],

bioisosterism in sulfonylureas in the lead compound α -acyl-urea 123 (Scheme 40) [69]. Identifying the bioisosteric relationship between the thioamide and cycloamide functions.



Scheme 38.

The discovery of oxalofresat 122 [70], designed as a classic bioisostere of tolfesat 121 (Scheme 39), a potent aldose reductase inhibitor [71], illustrates the non-classic bioisosteric relationship between the functional groups thioamide (CS-NH-) and amide (CO-NH-).

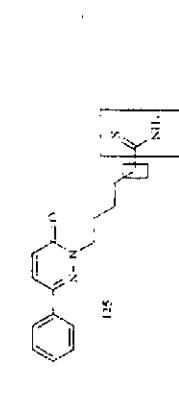


Scheme 39.

Tolfesat 122, currently undergoing clinical assays for its use to treat complications arising from diabetes, may also illustrate the classic bioisosteric relationship between the monovalent groups α -OMe (122) and α -Me (123). Significant differences as to the metabolism of these molecules may be expected, however, in view of the possibility that sulfur undergoes specific metabolic oxidation.

Other common examples of amide function bioisosteres include the "retro-amide" groups, α -C(CN)H₂ $\Rightarrow$ α -NH(CO)H and allylhydrazide α -CH₂CH₂NH₂ $\Rightarrow$ α -NH(CO)H [4, 72].

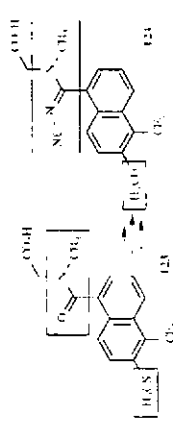
Wrobel and coworkers used the principle of non-classic bioisosterism of functional groups to propose the synthesis



Scheme 40.

of analog 124 from modifications in the lead compound α -acyl-urea 123 (Scheme 40) [69]. Identifying the bioisosteric relationship between the thioamide and cycloamide functions.

This same type of bioisosteric relationship between the thioamide (CS-NH-) and cycloamide (C-NH-) groups was reported during the development of cimetidine by Glaxo and coworkers [71].



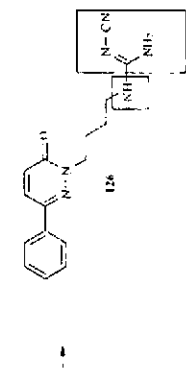
Scheme 41.

The same type of bioisosterism was further used by Yamashita and coworkers to develop pyridazine derivatives 125 and 126 (Scheme 41), which present a similar spectrum of antitumor activity, while also presenting a classic bioisosteric relationship between the substituents of the alkyl chain represented by the replacement of α -CH₂- with NH-group [73].

In the quest for new selective agonists of potassium channels Hutter and coworkers carried out modifications in the structure of anti-hypertensive lead compound pinacidil (127) (Scheme 42) [74]. These authors described the replacement of the *N*-cyanoguanidine template, present in pinacidil (127), with a 1,3-diaminocyclobutene-3,4-dione moiety afforded a novel series of potent bladder-selective agonists of the K_{ATP} channel 128 and 129, as a novel drug candidate to treat urge urinary incontinence.

Another important example of non-classic bioisosterism of functional groups may be exemplified by the isosteric relationship existing between the sulfonylhydrazide and acylhydrazide functions, identified in studies by Lima and coworkers (Scheme 43) [75]. In this work, the authors demonstrated that the acylhydrazide function (CONHNH-) may be efficiently replaced by the sulfonylhydrazide function (SO₂NHNH-) to design new anti-inflammatory and analgesic candidates, despite the subtle differences between the pKa of these groups.

During research to obtain new diuretics, analogs of furosemide (132). Wrobel and co-workers demonstrated the bioisosteric relationship existing between the sulfonylurea





Scheme 42.

(132), sulfamylthiourea (133), sulfamylthioacetamide (134) and sulfamylthioacetamide (135) functionalities (Scheme 43) [76]. These authors demonstrated that while these groups present similar electronic and geometric properties, determined by X-ray crystallography and similar acidity (pK_a 6.6 to 6.7), compounds 132, 133, 134 and 135 each possess a distinct lipophilicity, expressed in $\log P$ values (Scheme 44). The pharmacological results obtained for compounds 132, 133, 134 and 135 *in vivo* show that their diuretic potency is inversely proportional to their lipophilicity, a fact which may be explained considering that the diuretic activity of these molecules is the result of inhibition of the $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter located on the luminal membrane of the thick ascending limb of the loop of Henle, and that the glomerular filtration process is generally enhanced for hydrophilic compounds, while tubular reabsorption is increased for lipophilic compounds.

The importance of ionotropic receptors of glutamate (iGlu), the principal excitatory neurotransmitter in rapid synaptic signal pathways, as well as in processes of learning and memory are well characterized and the deficits within the glutamatergic signaling pathways in Alzheimer's patients may, in part, explain the loss of cognitive function of this disease. However, the use of glutamate to treat Alzheimer's disease is limited because of its inadequate pharmacokinetic properties in the biophase. Thus being, diverse research groups have sought to design new analogs of glutamate (Glu) with a half-life and lipophilicity superior to Glu. Recently, Stensbol and coworkers proposed replacement of

the carboxylic acid group, present in Glu (135) with 3-hydroxy-1,3,5-thiadiazole (136), 1-hydroxy-1,2,3-triazole (137), 1-hydroxyimidazole (138) and 1-hydroxypyrazole (139) groups (Scheme 45), observing that with the exception of the imidazole derivative (138), all the other compounds presented similar affinity for AMPA receptors, without, however, showing significant affinity for the other glutamate ionotropic receptors, i.e. NMDA and kainate receptors [77]. The absence of activity of the derivative containing the 1-hydroxypyrazole ring 139 may be explained by the basicity of the compound. Actually, the experimental determination of pK_a value of the 3-hydroxy group of 139 was found to be at least three pK_a units higher than those found for the 3-hydroxy-1,2,5-thiadiazole, 1-hydroxy-1,2,3-triazole and 1-hydroxypyrazole substituents.

In developing of new drug candidates to treat cerebrovascular ischemia designed as (*R,S*)-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptors antagonists, Jimenez and coworkers described the attachment of imidazol[1,2-*a*]indeno[1,2-*c*]pyrazin-2-substituted derivatives (Scheme 46) [78]. These authors proposed the exchange of the carboxylic acid function in C-2 (140) by the phosphine acid (141), tetrazole (142) and *N*-methylthiothiocarbamate groups (143) (Scheme 46). The results obtained show that all of the isomers studied present affinity for the AMPA receptors, in varying degrees. However the selectivity measured by way of AMPA and NMDA receptors is significantly affected by the replacements realized, such that the tetrazole derivative (142)



Scheme 43.

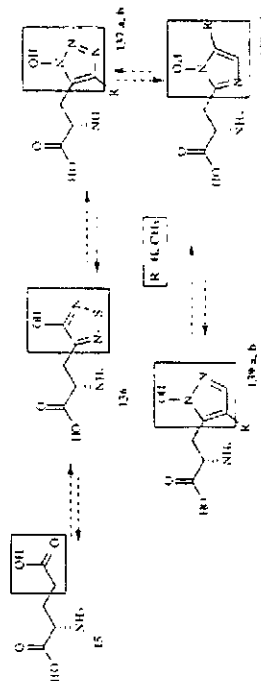
Scheme 44.

proves to be approximately 10 times more selective than the corresponding carboxylic acid derivative (140).

In 2001 Habeeb and coworkers described the design and synthesis of new analogs for celecoxib and rofecoxib, non-steroid anti-inflammatory drugs of second generation, reporting for the first time the biostereic relationship existing between methylsulfonamide, methylsulfone and azido functional groups (Scheme 47) [79]. Actually, many authors have reported obtaining new PGHS-2 inhibitors, using both classic as well as non-classic biostereic strategies. The biostereic relationship of methylsulfonamide ($\text{CH}_3\text{SO}_2\text{NH}_2$), sulfonamide ($\text{CH}_3\text{SO}_2\text{NH}_2$) and methylsulfone (CH_3SO_2) functions, for example, is well characterized within the class of selective PGHS-2 inhibitors, as are its contributions to other pharmacodynamic

and pharmacokinetic factors [58]. The results obtained from the studies by Habeeb and coworkers revealed that the azido group (N_3) is capable of realizing electrostatic interactions (non-ionic) with amino acid residues from the active site of PGHS, (the example of Arg513 residue), being, however, an authentic biostereic of methylsulfonamide and methylsulfone functions, presenting comparable molecular volume and electrostatic potential.

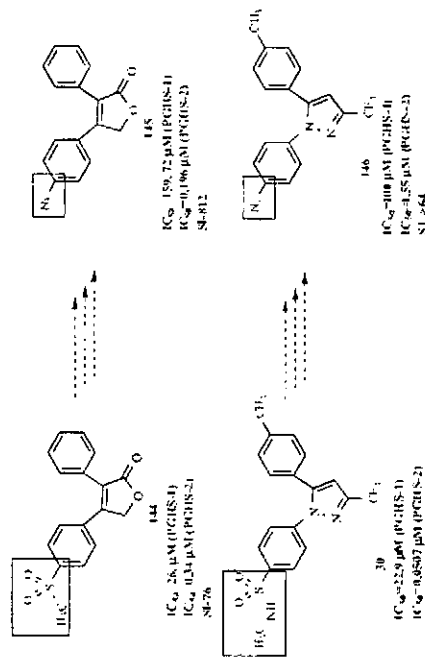
Chumacrine (118), designed by modifications in the natural alkaloid structure nicotine (147), is an important non-selective muscarinics (M1 and M2) receptors agonist with marginal actions on nicotinic ganglionic receptors. However, the low selectivity associated with low therapeutic index and inadequate bioavailability, have annulled its use for treating Alzheimer's disease. In an effort to optimize the



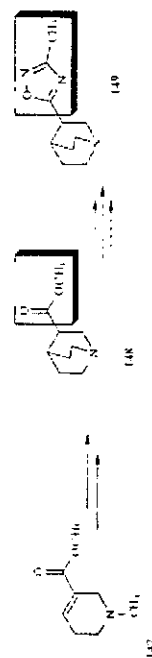
Scheme 45.



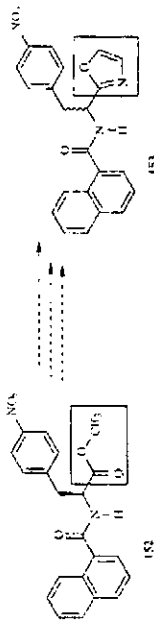
Section 46

[illegible]

Scheme 17



Another successful example of a transactivator, represson factor and an ester fibronectin with an exfoliative activity, for improving the metabolic stability, can be formulated in the development of a cyclin-dependent kinase (CDK2) inhibitors as anticancer agents. Despite the potent CDK2 inhibitory activity found for compound 2-amino-5-hydroxy-substituted benzimidazole (50), it lacks cellular activity in cell proliferation assays due to hydrolysis of the ethyl ester to the corresponding carboxylic acid, that is completely devoid of CDK2 inhibitory activity. In an attempt to increase the cellular stability of 50, Kim and coworkers realized the



Nichrome 50,

functional activity of a number of CCR3 agonists, compound 15 lacks all CCR affinity which suggests a more subtle role for the ester moiety than the heterocycles were unable to mimic [7].

To identify non-aromatic bisoesters, alternatives of carboxylic esters, Bromidge and coworkers described the synthesis and determination of the affinity of ketoxime esters derivatives (154-156) for muscarinic receptors in rat cerebral cortex (Scheme 51) [8]. In this study it was possible to confirm the bioisosteric relationship existing between the methyl ester (118) and 153-ketoxime ethers (154) (inset), and the later use of additional classic bioisosteric functions, such as the presence of hydrogen bond donors, exchanges of non-aromatic groups, led to the attainment of optimized compound 156 (Scheme 51), which present a pKa value inferior to that found for lead compound 154, resulting in a decrease in basicity, facilitating the passage through the blood-brain barrier.

The application of the strategy of functional group transposition in drug designing can be well illustrated by synthesizing new anti-convulsant agents structurally related to γ -aminobutyric acid (GABA), an inhibitory neurotransmitter of low molecular weight. GABA's polar and hydrophilic nature makes it easy impossible for treating epilepsy, in view of its rapid inactivation and excessively low half-life.

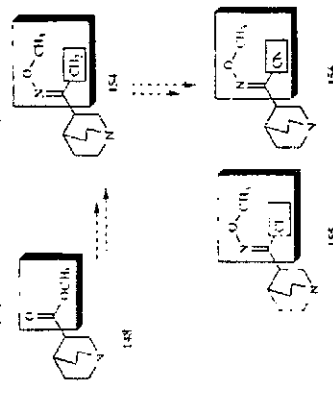
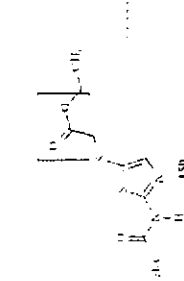


Figure 51.

replaced by the new ester with a bioisosteric analog, substituted to compound 18 (Scheme 4), which is more biologically stable against esterases, maintains the selectivity, and shows increased CDK2 inhibitory potency as well as potent, if proliferative activity in cancer cell lines.



1997-1998

Nonethless, butyrolactone replacement of the ester with oxazole and other heterocyclics may not be universally effective, as demonstrated by Dharuk and coworkers in the design and synthesis of a series of highly selective and potent phenylamine derived CCR_2 antagonists as anti-inflammatory agents (Scheme 50).¹⁵² While compound 152 was highly effective in blocking both the binding and the

their search for GABA aminotransferase inhibitory, the enzyme responsible for GABA catabolism [14] and the co-workers proposed structural modifications in the pyrazolopyrimidine substrate, *see* Table 1 based on the application of the following rules:

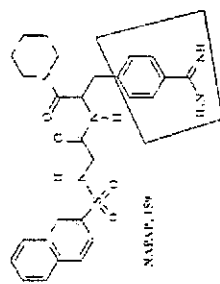
acid group (pK_a ca. 4.50) present in GABA with 2,4-dichlorophenol (pK_a 7.4) substituent present in compounds 57 and 58 (Scheme 52), observing that both compounds were competitive inhibitors of GABA aminotransferase with K_i values of 6.3 μM and 11 μM , respectively. These compounds were tested as substrates for GABA aminotransferase by the radiochemical procedure of

measuring the conversion of [^{14}C]- α -ketoglutarate to [^{14}C]-glutamate, and both were poor substrate. These results suggest that the 2,6-difluorophenyl group may be an authentic lipophilic bioisostere of carboxylic acids.



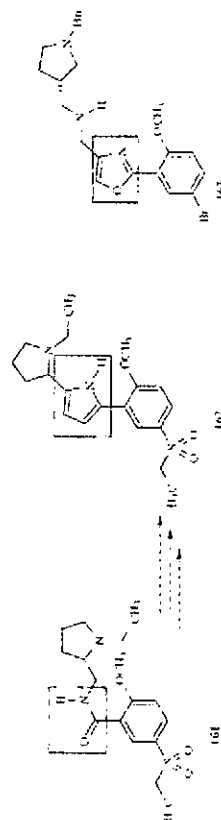
Scheme 52.

The activity and activity reached by way of therapeutic relevance, in view of the recent FDA approval of apizadone, a novel antipsychotic drug, 83). However, the great challenge of this class of therapeutic drugs is in obtaining low compounds of adequate oral bioavailability. NAPIAP (159) (Scheme 53) is a high affinity reversible inhibitor of thrombin (K_i 6 nM) and low oral bioavailability. Many studies have suggested that the inadequate pharmacokinetic properties of NAPIAP (159) and its structurally related derivatives is due to the elevated basicity of the benzimidazole function (160, pKa 12). To deal



Scheme 53.

with these pharmacokinetic limitations, Rewinkel and coworkers proposed the bioisosteric exchange of the benzimidazole group by the 1-aminoquinoline function (Scheme 54) [84]. In this study the authors demonstrated that the lower basicity of the 1-aminoquinoline group (pKa 7.5) conferred a greater absorption to compound (166) determined using cell monolayers as a model for intestinal

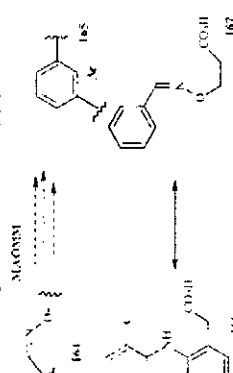


Scheme 54.

which exists dopamine D1 and dopamine D2 binding affinities comparable to those of the atypical neuroleptics sulpiride and clozapine respectively [7].

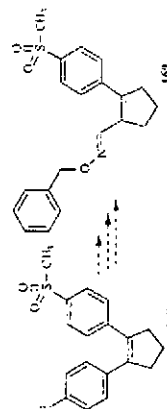
6.3. The Availability of Non-Classical Bioisosterism

A structural example of non-classical bioisosterism may be illustrated by the work of Macchia and coworkers, involving the bioisosteric relationship between the benzene ring (165) and the methylenecyclohexene methyl group (MAOMM, 164) (Scheme 55) [85]. These authors, studying the synthesis of new non-steroid anti-inflammatory agents of easy synthetic access, prepared substance 167 possessing the MAOMM function as bioequivalent to the benzene ring (165). The results observed in the anti-inflammatory activity of 167 and derivatives, in comparison with standard diclofenac (166), an arylacetic acid anti-inflammatory drug, prove this amazing bioisosteric relationship [6].



Scheme 55.

More recently, Balsamo and coworkers described the attainment of new selective inhibitors of PGHS-2 isoform, applying bioisosterism as a strategy of molecular modification [87]. This study also illustrates the non-classical bioisosteric relationship between aromatic rings and the methylenecyclohexene methyl moiety (MAOMM) (Scheme 56). However, it must be considered that introducing the oxime-ether (O-N, CH₃) substituent in 169 introduces inconsistent molecular characteristics in the structure of lead compound SC 57666 (168).

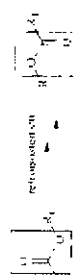


Scheme 56.

6.4. Retroisosterism

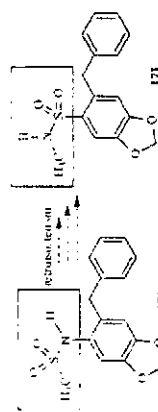
Retroisosterism is based on the inversion of a determined functional group present in the lead compound structure, producing an isostere with the same function (Scheme 57). This strategy, just as the other bioisosteric strategies

conducted on in this paper, aims to replicate the characteristic properties of the original lead compound thus aiding in optimizing the profile of interaction with the biological systems with full 3D, more adequate for therapeutic use and may even be used in the attempt to avoid the formation of potentially toxic metabolic intermediates.



Scheme 57.

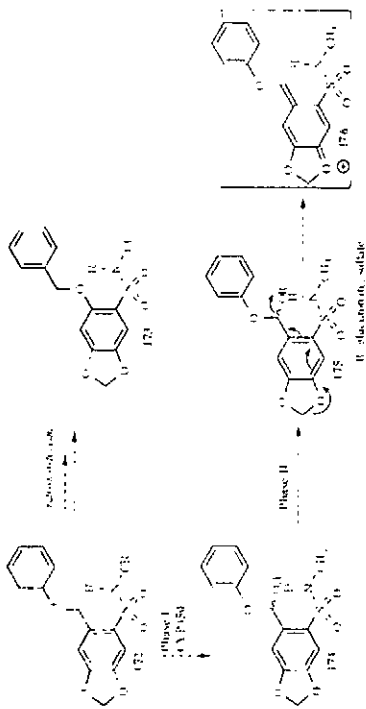
In a recent study, Lages and coworkers described the existing stereoisosteric relationship between the methyl sulfonamide and methylsulfonamide functions (Scheme 58) present in the structures of new selective COX-2 inhibitor lead compounds 170 and 171 [88]. This type of retroisosteric relationship confers metabolic susceptibility and distinct pKa values between compounds 170 and 171, while the greatest activity found for the methylsulfonamide derivative (170) may be explained considering the profile of interaction of this group with the Arg389 and Ser353 amino acid residues present at the catalytic site of COX-2, favored in 170 when compared to the 171 retroisostere.



Scheme 58.

Based on studies of molecular modeling, Barreiro and coworkers proposed two new molecular standards, exemplified by compounds 172 and 173 as new candidates for second generation anti-inflammatory drugs. The molecular design of 173 from the lead compound 172 (Scheme 59), was realized applying the strategy of retrosynthesis [14] and aimed to minimize the possibility of electrophilic species formation (176) (Scheme 59), potentially toxic, obtained by the action of enzymatic pool involved in the metabolism of xenobiotics. Furthermore, the use of retrosynthesis for derivative 173 enables the formation of intramolecular hydrogen bonds, which confer greater conformational restriction allowing the prediction that derivatives 172 and 173 will present pharmacokinetic concerning the process of metabolism and pharmacodynamics distinct profiles.

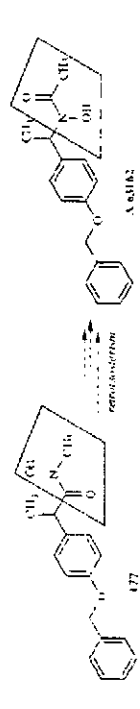
Among the different categories of 5-lipoxygenase inhibitors (5-LOX), the hydroxyacids stand out acting through the formation of complexes with the Fe³⁺ ion present at the catalytic site of 5-LOX. It has been shown that although many hydroxyacids are potent inhibitors of the enzyme *in vitro*, they fail to produce significant inhibition *in vivo*. This dichotomy between *in vitro* vs *in vivo* pharmacological results can be explained based on pharmacokinetic studies realized with different hydroxyacids



Scheme 59.

acids [89]. In these studies it was demonstrated that hydroxamic acid derivatives (e.g., 177) are rapidly metabolized to corresponding inactive carboxylic acid

administered orally to rats, this retroester produced higher plasma concentrations and longer duration. For example, compound 178 produced peak plasma concentrations in the



Scheme 60.

derivatives, which are unable to block leukotriene synthesis. Taken together these results suggest the pharmacophore profile of hydroxamic acid substituent (CON(OH)CH₃).

Later, Summers and co-workers (1998) [90] demonstrated that hydroxamic acids with 5-LCOX inhibitor activity have small substituents (hydrogen or methyl) attached to the ring system) appended to the carbonyl group. Based on this observation, the authors proposed the attainment of the retroisostere 178 (A 63162), through the application of retroisosteric strategy in the structure of lead compound 177 (Scheme 60) [90]. The study of the pharmacokinetic behavior of retroisostere 178 (A 63162) revealed that when

rats of 160 µM at 3 h after a 100 mg/kg oral dose, while its congener 177 reached a peak level of only 15 µM 30 min after dosing.

From the pharmacodynamic point of view, the pharmacological assays carried out with hydroxamic acid 177 and its retroisostere 178 (A 63162) reveal that 178 was significantly more potent than 177 in inhibiting *in vivo* leukotriene synthesis in the rat peritoneal anaphylaxis model. Following oral administration, 178 (160 µg/kg) was 5-fold more potent than 177 (ED₅₀ 40mg/kg).

The unmistakable demonstration of the benefits of the strategy of retroisostericism, applied to the class of 5-LCOX inhibitors, may be well illustrated by the attainment of



Scheme 61.

of compound 179, the first selective 5-LCOX inhibitor to receive approval by the FDA to treat asthma, discovered at Abbott Laboratories [91]. In analogy to the results obtained with hydroxamic acids (A 63162), the application of the retrosynthetic strategy in the structure of lead compound 179 allowed the attainment of hydroxamic derivative 180 (Scheme 61) with a half-life and bioavailability and multiplicity potency greater than lead compound 179 (Scheme 61) [92].

7. FINAL COMMENTS

As we have demonstrated in this paper, through comments made on selected examples, the bioisostericism represents an rational drug design a successful strategy used in the molecular modification and design of new therapeutically attractive substances of different pharmacological classes including the design of *de novo* drugs.

The correct use of the strategy of molecular modification also allows the identification of new classes of lead compound with attractive pharmacotherapeutic activity, maximizing the efforts of synthetic work and, consequently, maximizing the chances for success in discovering medications both more efficient and of safer use.

In this paper, we have also demonstrated that drug discovery may be planned "retro-hoc", when determined theoretic principles of Medicinal Chemistry are carefully applied, thus allowing a prediction of the structural effects which govern pharmacokinetic factors, such as absorption and biotransformation; consequently, in theoretical terms, we are able to predict the expected bioavailability for the new bioactive compound designed as new drug candidate.

8. REFERENCES

[1] Berger, A. *A Guide to the Chemical Basis of Drug Design*, Wiley, E.A., Wiley, 1983, p. 119.

[2] Suckling, J. B. *Fundamentals of Molecular Pharmacology*, 1978, Vol. 2, The Chemical Basis of Drug Action, London, Inglaterra, Addison Press, p. 213-290.

[3] Florschütz, C. W. *Chem Soc. Rev.*, 1979, 8, 363.

[4] Lipinski, C. A. *Bioisostericism in drug design*, *Int. Rev. Med. Chem.*, 1986, 27, 283.

[5] Barreira, P. *Rev. Bras. Farm.*, 1991, 72, 34.

[6] Pádua, G. A.; Lázaro, J. *J. Chem. Res.*, 1996, 36, 1047.

[7] Chen, X.; Wang, W. *Int. Rev. Med. Chem.*, 2003, 35, 31.

[8] Burger, A. *Prog. Drug Res.*, 1991, 37, 287.

[9] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[10] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[11] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[12] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[13] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[14] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[15] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[16] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[17] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[18] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[19] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[20] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[21] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[22] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[23] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[24] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[25] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[26] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[27] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[28] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[29] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[30] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[31] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[32] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[33] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[34] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[35] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[36] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[37] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[38] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[39] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[40] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[41] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[42] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[43] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[44] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[45] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[46] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[47] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[48] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[49] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[50] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[51] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[52] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[53] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[54] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[55] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[56] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[57] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[58] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[59] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[60] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[61] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[62] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[63] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[64] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[65] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[66] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[67] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[68] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[69] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[70] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[71] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[72] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[73] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[74] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[75] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[76] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[77] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[78] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[79] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[80] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[81] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[82] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[83] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[84] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[85] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[86] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[87] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[88] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[89] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[90] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[91] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

[92] Kunkel, A.; Buchhalter, J. G. *Quinn's Pharmacology*, 1982, 10, 100.

