Industria y Comercio
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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
RAD: 13-41983- -5	**FECHA**: 2014-02-20 17:53:27
DEP: 2020 DIRECCION DE NUEVAS CREACIONES	**EVE:** 378 FASE NACIONAL INCLUIDO CAPITULO I
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	**FOLIOS**: 8
ACT: 519 PRESENTACION	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
JAIME EDUARDO DELGADO VILLEGAS

Asunto: Radicación: 13-41983- -5
Trámite: 11
Evento: 378
Actuación: 519
Oficio: 2150
Folios: 8

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I	X	Capítulo II	
No. Solicitud Internacional	PCT/EP2011/064831				
Fecha de Presentación Internacional	29/08/2011				

1. Documentos estudiados

Descripción:	Radicado N° 13-041983-00000-0000	01/03/2013
Reivindicaciones:	Radicado N° 13-041983-00000-0000	01/03/2013
Prioridad:	EP 10175181.6	03/09/2010
	US 61/381,163	09/09/2010
	EP 11158802.6	18/03/2011
	US 61/454,753	18/03/2011

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. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del Capítulo Primero del Título X de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa pagada por el solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada.

Título de la solicitud: “COMPUESTOS DE DITIÍNATETRA (TIO) CARBOXIMIDAS Y PROCEDIMIENTOS PARA LA PREPARACIÓN DE ESTOS COMPUESTOS” (modificado Radicado N° 13-041983-00002-0000).

El problema planteado en esta solicitud consiste en la necesidad de desarrollar composiciones fungicidas novedosas que tengan ventajas sobre las composiciones conocidas

Para resolver este problema técnico la solicitud en estudio proporciona un compuesto de fórmula (I), composiciones que lo contienen y procedimientos para su obtención (Ver radicado N° 13-041983-00000-0000).

El objeto de la presente Solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): C07D495/14; A01N43/90

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4. **Reivindicaciones relativas a un uso o a un segundo uso** (Art 14 y 21 D 486)

El uso de la reivindicación 9 no es patentable, de acuerdo con el Art 14.

El objeto de la reivindicación 7 hace referencia a: “*un procedimiento para controlar hongos fitopatógenos dañinos caracterizados porque los compuestos de fórmula (I)….* ***se aplican a los hongos*** *….”*

Como la única etapa definida en el método corresponde a la aplicación, esta corresponde a un uso y por lo tanto no es patentable.

5. De la Solicitud de Patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

El capítulo reivindicatorio no es conciso porque las definiciones de los sustituyentes R^1 y R^2 conllevan a un número indefinido de compuestos que no encuentran soporte en la descripción. Al revisar en la descripción se observa según los ejemplos que dichos sustituyentes corresponden en todos los casos a un sustituyente metilo. Por lo tanto, se sugiere restringir la solicitud a una razonable generalización de tales ejemplos.

La reivindicación 9 indica el uso que puede darse al compuesto el cual no es una característica técnica que defina la invención, la materia objeto de la reivindicación 9 ya se encuentra contemplada en las reivindicaciones 1 a 6. Se requiere eliminar esta reivindicación del pliego reivindicatorio.

6. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de la cual se invoca prioridad, es decir, el 03 de septiembre de 2010.

Consultadas las fuentes de información con que se cuenta, se encontró (encontraron) el (los) siguiente(s) documento(s) que anticipa(n) el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1[1]	US20100120884	"USE OF DITHIINE-TETRACARBOXIMIDES FOR CONTROLLING PHYTOPATHOGENIC FUNGI"	13/Mayo/2010
D2[2]	F. Zentz et al. / Il Farmaco 60 (2005) 944–947	"SYNTHESES, IN VITRO ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF A SERIES OF N-ALKYL, 1,4-DITHIINES"	18/Octubre/2005
D3[3]	US3364229	"1, 4 DITHIIN-2, 3, 5, 6-TETRACARBOXIMIDES AND PROCESS FOR THEIR PREPARATION "	16/Enero/1968
D4	Current Medicinal Chemistry, 2005, 12, 23-49	"BIOISOSTERISM: A USEFUL STRATEGY FOR MOLECULAR MODIFICATION AND DRUG DESIGN"	2005

El documento D1 divulga un compuesto ditiína-tetracarboximida de la fórmula (I) útil como fungicida (Pág. 1, Párr. [0004] a [0021])

El documento D2 divulga un compuesto ditiína-tetracarboximida de la fórmula (I) útil como fungicida (Págs. 944 y 945)

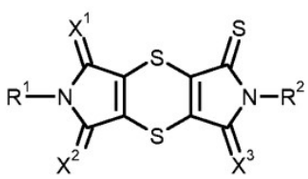
El documento D3 divulga un compuesto ditiína-tetracarboximida de la fórmula (I) útil como insecticida (Col.1)

El documento D4 divulga la relación bioisostérica entre los grupos =O y =S (Pág. 40)

7. **Examen de Patentabilidad** (Art 14 D486)

Nivel Inventivo (Art18 D486)

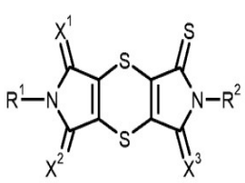
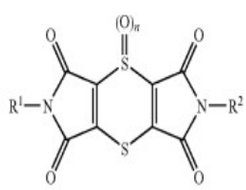
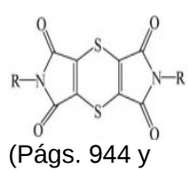
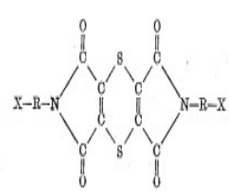
La reivindicación 1 consiste en compuestos ditiínatetra(tio) carboximidas de fórmula (I)



(I)

(Ver radicado 13-041983-00000-0000)

Comparación de las características técnicas esenciales, con las del estado de la técnica:

SOLICITUD	D1	D2	D3	D4
 X^1 es O o S X^2 es O o S X^3 es O o S	 (Pág. 1, Párr. [0004] a [0021]) R^1 y R^2 son iguales o diferentes y representan hidrógeno, alquilo C_1-C_8 el	 (Págs. 944 y 945) R es bencilo, ciclohexilo, isopropilo, propilo o butilo (Págs. 944 y 945, Figs. 1 y 2)	 (Col.1) R es un radical hidrocarbileno de hasta 20 átomos de carbono y X es un radical monovalente seleccionado del	Relación bioisostérica entre los grupos =O y =S (Pág. 40)

1[]URL:http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=2010120884A1&KC=A1&FT=D&ND=3&date=20100513&DB=worldwide.espacenet.com&locale=en_EP
2[]URL: http://www.sciencedirect.com/science/article/pii/S0014827X05001503
3[]URL:http://worldwide.espacenet.com/publicationDetails/originalDocument?CC=US&NR=3364229A&KC=A&FT=D&ND=3&date=19680116&DB=worldwide.espacenet.com&locale=en_EP

R^1 y R^2 son iguales o diferentes y son cada uno hidrógeno, alquilo C_1-C_6 sustituido opcionalmente con mono- o poli-halógeno, OR^3 o COR^4, cicloalquilo C_3-C_7 sustituido opcionalmente con mono- o poli-halógeno, alquilo C_1-C_4 o haloalquilo C_1-C_4, en cada caso arilo sustituido opcionalmente con mono o poli-halógeno, - alquilo C_1-C_4, -haloalquilo C_1-C_4, COR^4 o –sulfonilamino, o aril-(alquilo C_1-C_4), R^3 es hidrógeno, alquilo C_1-C_4, alquilcarbonilo C_1-C_4 o arilo sustituido opcionalmente con mono o poli-halógeno, alquilo C_1-C_4 o haloalquilo C_1-C_4, R^4 es hidroxilo, alquilo C_1-C_4 o alcoxi C_1-C_4 Y las sales agroquímicamente activas de las mismas	cual es opcionalmente mono sustituido o polisustituido por halógeno, OR^3 o COR^4, o representa cicloalquilo C_3-C_7 el cual es opcionalmente mono sustituido o polisustituido por halógeno, alquilo C_1-C_4 o haloalquilo C_1-C_4, o representa arilo o aril-(alquilo C_1-C_4) sustituido el cual es opcionalmente mono sustituido o polisustituido por halógeno, - alquilo C_1-C_4, -haloalquilo C_1-C_4, COR^4 o sulfonilamino (Pág. 1, Párr. [0005] R^3 representa hidrógeno, alquilo C_1-C_4, alquilcarbonilo C_1-C_4 o representa arilo el cual es opcionalmente mono sustituido o polisustituido por halógeno, alquilo C_1-C_4 o haloalquilo C_1-C_4 (Pág. 1, Párr. [0006] R^4 representa hidroxilo, alquilo C_1-C_4 o alcoxi C_1-C_4 (Pág. 1, Párr. [0007] n representa 0 o 1 (Pág. 1, Párr. [0008]		grupo consistente de hidrógeno, cloro, bromo, hidroxi y acetoxi (Col.1, líneas 56 a 62)	

El documento D1 divulga un compuesto ditiína-tetracarboximida de la fórmula (I) útil como fungicida (Pág. 1, Párr. [0004] a [0021])

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D1 consiste en la presencia de un compuesto carboximida en lugar del compuesto tiocarboximida reclamado

El documento D2 divulga un compuesto ditiína-tetracarboximida útil como fungicida (Págs. 944 y 945)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D2 consiste en la presencia de un compuesto carboximida en lugar del compuesto tiocarboximida reclamado

El documento D3 divulga un compuesto ditiína-tetracarboximida útil como insecticida (Col.1)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D3 consiste en la presencia de un compuesto carboximida en lugar del compuesto tiocarboximida reclamado

El documento D4 divulga la relación bioisostérica entre los grupos =O y =S (Pág. 40)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D4 consiste en que no se divulga un compuesto ditiína-tetratiocarboximida

El efecto que logra el incluir el grupo =S para obtener un compuesto tiocarboximida es que proporciona nuevos compuestos alternativos como fungicidas, útiles en el control de crecimiento de plantas.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el compuesto conocido en los documentos D1, D2, y D3 para proporcionar nuevos compuestos alternativos como fungicidas, útiles en el control de crecimiento de plantas ”.

Sin embargo, el documento D4 divulga la relación bioisostérica existente entre los grupos =S y =O y la posibilidad de que dichos grupos puedan ser intercambiables (Pág. 40)

En consecuencia, la persona del oficio normalmente versada en la materia, reemplazaría uno o más grupos =O por grupos =S en los compuestos conocidos en el estado de la técnica, obteniendo compuestos tiocarboximidas y esperando se mantuviera el efecto fungicida y de control de crecimiento de plantas presentados por estos compuestos para llegar así al objeto de la reivindicación 1 de la solicitud. Por lo cual se considera obvia a la luz de los documentos D1 con D4, D2 con D4 y D3 con D4. Adicionalmente la solicitante no presenta evidencia del efecto técnico sorprendente presentado por los compuestos ditiínatiocarboximidas con respecto a los compuestos ditiínacarboximidas conocidos en el estado de la técnica.

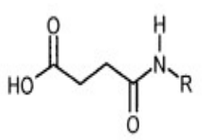
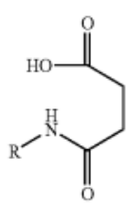
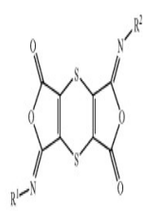
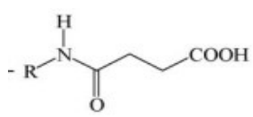
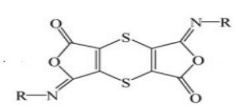
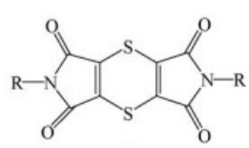
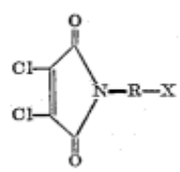
En conclusión la reivindicación 1 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

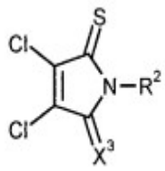
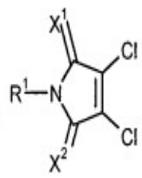
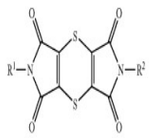
Puesto que las reivindicaciones dependientes 2 – 6, 8 y 10 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

La reivindicación 11 consiste en un procedimiento para preparar ditiínatetra(tio) carboximidas de fórmula (I) de acuerdo con la reivindicación 1, 2, 3, 4, 5 ó 6

(Ver radicado 13-041983-00000-0000)

Comparación de las características técnicas esenciales, con las del estado de la técnica:

SOLICITUD	**D1**	**D2**	**D3**	**D4**
Procedimiento para preparar ditiínatetra(tio) carboximidas caracterizado porque A) en una primera etapa, monoamidas succínicas de fórmula (VI)  (VI) En la que R es R^1 o R^2, cada uno de los cuales es como se define en la reivindicación 1, 2 ó 3, se hacen reaccionar con un exceso de cloruro de tionilo, opcionalmente en presencia de un diluyente, despues, se retira el exceso de cloruro de tionilo y se convierte la mezcla de producto asi obtenida, en una segunda etapa en una mezcla de un disolvente	Reacción del compuesto  Con $SOCl_2$ para obtener el compuesto  y posterior conversión al compuesto	Reacción del compuesto  Con $SOCl_2$ para obtener el compuesto  Y posterior conversión hacia el compuesto  (Pág. 945, Fig. 2)	Reacción de una dicloromaleimida  Con $Na_2S_2O_3.5H_2O$ Para obtener una imida del ácido 1,4-ditíin-2,3,5,6-tetracarboxilico (Col. 7, Ej. 9)	Relación bioisostérica entre los grupos =O y =S (Pág. 40)

organico y agua, opcionalmente en presencia de un catalizador de transferencia de fase, y finalmente se aislan las ditiínatetra(tio) carboximidas de fórmula (I) por procedimientos de cromatografía, o B) dicloromaleimidas de fórmula (VII)  (VII) en la que R^2 y X^3 son cada uno como se define en la reivindicación 1, 2 ó 3, se hacen reaccionar con dicloromaleimidas de fórmula (VIII)  (VIII) en la que R^1, X^1 y X^2 son cada uno como se define en la reivindicación 1, 2 ó 3, y con un tiosulfato inorgánico en un disolvente o mezcla de disolventes	 (Pág. 3, Párr. [0089])			

El documento D1 divulga un proceso para obtener una ditiína-tetracarboximida (Pág. 3, Párr. [0089])

La diferencia entre la invención, definida en la reivindicación 11 y el compuesto del documento D1 consiste en que el proceso divulgado produce una ditiína-tetracarboximida en lugar de la ditiína-tetratiocarboximida de la solicitud

El documento D2 divulga un proceso para obtener una ditiína-tetracarboximida (Pág. 945, Fig. 2)

La diferencia entre la invención, definida en la reivindicación 11 y el compuesto del documento D2 consiste en que el proceso divulgado produce una ditiína-tetracarboximida en lugar de la ditiína-tetratiocarboximida de la solicitud

El documento D3 divulga un proceso para obtener una ditiína-tetracarboximida (Col. 7, Ej. 9)

La diferencia entre la invención, definida en la reivindicación 11 y el compuesto del documento D2 consiste en que el proceso divulgado produce una ditiína-tetracarboximida en lugar de la ditiína-tetratiocarboximida de la solicitud

El documento D4 divulga la relación bioisostérica entre los grupos =O y =S (Pág. 40)

La diferencia entre la invención, definida en la reivindicación 1 y el compuesto del documento D4 consiste en que no se divulga un proceso para producir una ditiína-tetratiocarboximida

El efecto que logra el incluir el grupo =S en los compuestos intermedios del proceso es que proporciona un proceso para obtener nuevos compuestos alternativos como fungicidas, útiles en el control de crecimiento de plantas.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: “cómo modificar el proceso conocido en los documentos D1, D2, y D3 para proporcionar un proceso para obtener nuevos compuestos alternativos como fungicidas, útiles en el control de crecimiento de plantas.”.

Sin embargo, el documento D4 divulga la relación bioisostérica existente entre los grupos =S y =O y la posibilidad de que dichos grupos puedan ser intercambiables (Pág. 40)

En consecuencia, la persona del oficio normalmente versada en la materia, reemplazaría uno o más grupos =O por grupos =S en los compuestos intermediarios de los procesos conocidos en el estado de la técnica; ya sea la reacción a partir de las monoamidas succínicas conocidas en los documentos D1 y D2 o la reacción a partir de las dicloromaleimidas conocida en el documento D3 para obtener procesos alternativos que permitan la preparación de los compuestos ditiína-tetra(tio)carboximidas y llegar así al objeto de la reivindicación 11 de la solicitud. Por lo cual se considera obvia a la luz de los documentos D1 con D4, D2 con D4 y D3 con D4.

En conclusión la reivindicación 11 cumple el requisito de novedad (Art. 16), pero no cumple con el requisito de nivel inventivo (Art. 18).

En consecuencia las reivindicaciones 1 – 11 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

8. Conclusión

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

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	US20100120884	1 – 6, 8, 10 y 11	Nivel Inventivo
D2	SYNTHESES, IN VITRO ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF A SERIES OF N-ALKYL, 1,4-DITHIINES	1 – 6, 8, 10 y 11	Nivel Inventivo
D3	US3364229	1 – 6, 8, 10 y 11	Nivel Inventivo
D4	BIOISOSTERISM: A USEFUL STRATEGY FOR MOLECULAR MODIFICATION AND DRUG DESIGN	1 – 6, 8, 10 y 11	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 del día siguiente 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 de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

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)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2014-02-25

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Original article

Syntheses, in vitro antibacterial and antifungal activities of a series of *N*-alkyl, 1,4-dithiines

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Abstract

A series of dithiines were synthesized by cyclization of 4-(alkylamino)-4-oxobutanoic acids under the action of $SOCl_2$. Their in vitro antibacterial and antifungal activities have been evaluated against reference strains and versus reference compounds. The so-called 'isoimides' **2a**, **2b** were totally inactive whereas some imides had low MICs for few bacteria and for few fungal microorganisms.
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Keywords: Dithiine; Imide; Isoimide; Antimicrobial agents

1. Introduction

In previous works [1–3], we have investigated the syntheses and the biological properties of a series of maleimides and succinimides. In order to obtain other succinimides derivatives possessing antimicrobial and/or antifouling activities, we have studied the cyclization of 4-(alkylamino)-4-oxobutanoic acids **1**, easily obtained from aliphatic amines and succininic anhydride (Fig. 1). Surprisingly a very few papers are dealing with the synthesis and properties of dithiines.

2. Chemistry

In these experimental conditions, the corresponding succinimides were not obtained and it could be shown that, when the reaction was carried out in polar aprotic solvents (e.g. THF, dioxane), the 4,8-dithiine-indacene-1,3,5,7-tetraones **3** (diimides **3**) were produced via 3,7-bis-isoimino-4,8-dithia-indacene-1,5-diones **2** (diisoimides **2**). The diisomides **2** could be considered as the kinetically controlled compounds and may be isolated (as rose crystals), only when they had a low solubility in the reaction medium. Otherwise, an isomerization led to the green diimides **3**, which were always poorly soluble (Fig. 2).

This methodology has been reported (without experimental details) by Michaïlidis et al. [4], and three of these 1,4-dithiin-2,3,5,6-tetracarboxydiimides were used as synthons for Diels–Alder cycloadditions, by Hayakawa et al. [5]. Only one patent described a biological use of some 1,4-dithiin-2,3,5,6-tetracarboxydiimides as anthelmintics [6]. These poor data prompt us to synthesize a series of these compounds and evaluate their biological properties. Hence, we report herein, next to the synthesis, the in vitro antibacterial and antifungal properties of a series of dithiines **2** and **3**. One will observed that dithiin diimide **3b** was not obtained.

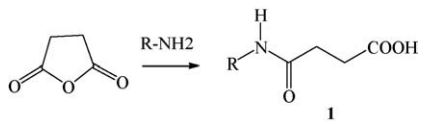

Fig. 1. Syntheses of succinamic acids. Substituents: a: R = benzyl; b: R = cyclohexyl; c: R = isopropyl; d: R = propyl; e: R = butyl.

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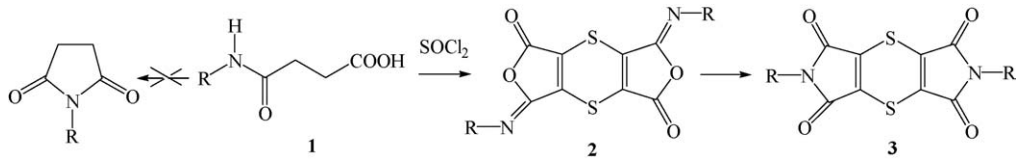

Fig. 2. Syntheses and structures of dithiin diisoimides (**2**) and diimides (**3**).

3. Microbiological evaluation

3.1. Antibacterial activities

The results of the screening tests of *N*-alkyl, 1,4-dithiines isoimides **2a,b** and *N*-alkyl, 1,4-dithiines **3a–e** were reported in Table 1 which showed the minimum inhibitory concentrations (MICs) of dithiines against two Gram-positive bacteria (*S. aureus* ATCC 25923, *E. faecalis* ATCC 29212) and two Gram negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853). *D*imethyl sulfoxide has no antibacterial effect at a concentration up to 512 μg ml^{-1}. The isoimides **2a** and **2b** are totally inactive versus the four strains. Conversely, the imides **3a** to **3e** have some activities against *S. aureus* and *E. faecalis* with MICs ranging from 2 to 16 μg ml^{-1}. Concerning *E. coli* and *P. aeruginosa* MICs of 128 μg ml^{-1} are considered as inactive. Nevertheless reference compounds (ampicillin, kanamycin, erythromycin and ofloxacin) are much more efficient with the exception of *P. aeruginosa* usually resistant to many antibiotics.

Table 1
In vitro antibacterial activities of *N*-alkyl, 1,4-dithiines isoimides **2** and *N*-alkyl, 1,4-dithiines **3** compounds (MICs, μg ml^{-1})

Compound	*S. aureus* ATCC 25923	*E. faecalis* ATCC 29212	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
2a	256	256	256	256
2b	256	256	256	256
3a	16	2	128	128
3c	4	4	128	128
3d	8	4	128	128
3e	8	4	128	128
Reference compounds				
Ampicillin	0.5	1	4	> 16
Kanamycin	2	32	2	> 16
Erythromycin	0.25	2	> 4	> 4
Ofloxacin	0.5	2	0.03	2

a: R = benzyl; b: R = cyclohexyl; c: R = isopropyl; d: R = propyl; e: R = butyl.

3.2. Antifungal activities

The antifungal activities of the compounds **2a,b** and **3a–e** (see Table 2) were determined according to the guidelines of the Clinical and Laboratory Standards Institute (formerly NCCLS) for yeasts and for filamentous fungi [7,8]. Fungal strains such as *Candida albicans*, *Candida parapsilosis*, *Aspergillus fumigatus* were used in the aim to evaluate interest of the investigational compounds against the species currently responsible for the majority of human mycoses. The test strains included also some yeast species of which a characteristic feature is the frequent resistance to major antifungals: *Candida krusei* has an intrinsic resistance to fluconazole, while majority of *Candida lusitaniae* strains presents high MIC to amphotericin B.

As observed for bacteria, the isoimides **2a** and **2b** are relatively inactive, whereas the isomide **3d** has some antifungal activity especially against *C. albicans* (< 0.1 μg ml^{-1}), and **3e** against *C. parapsilosis and C. krusei* (0.4 μg ml^{-1}). The product **3c** shows a low MIC particularly against *A. fumigatus* (0.1 μg ml^{-1}), and also against *C. albicans* (0.8 μg ml^{-1}). The compound **3a** shows a poorest spectrum of activity of *N*-alkyl, 1,4-dithiines molecules.

If we compare these results to the in vitro susceptibility of these fungal species to the reference antifungals mainly to

Table 2
In vitro antifungal activities of *N*-alkyl, 1,4-dithiines isoimides **2** and *N*-alkyl, 1,4-dithiines **3** compounds (MICs 50, μg ml^{-1})

Compound	*C. parapsilosis* ATCC 22019	*C. krusei* ATCC 26258	*C. albicans* ATCC 90028	*C. lusitaniae* ATCC 410086	*A. niger* ATCC 980483435	*A. fumigatus* wild strain [a]
	MIC50	MIC50	MIC50	MIC50	MIC50	MIC50
2a	> 51.2	> 12.8	> 51.2	> 51.2	> 51.2	> 25.6
2b	> 51.2	> 51.2	> 51.2	> 51.2	> 51.2	> 51.2
3a	6.4	3.2	3.2	3.2	> 51.2	> 51.2
3c	> 51.2	51.2	0.8	12.8	> 51.2	0.1
3d	3.2	1.6	< 0.1	6.4	3.2	1.6
3e	0.4	0.4	1.6	6.4	> 51.2	0.8
Reference compounds (MIC range)						
Amphotericin B	0.25–1	0.25–2	0.5–2	0.12–1	0.25–1	0.5–2
Fluconazole	2–8	16–64	0.25–1	0.25–1	–	–
Flucytosine	0.12–0.5	4–16	0.5–2	0.12–4	–	–
Itraconazole	–	–	–	–	0.5–12	0.12–1

a: R = benzyl; b: R = cyclohexyl; c: R = isopropyl; d: R = propyl; e: R = butyl.
[a] Fungal collection of the 'Centre Hospitalier Universitaire de Grenoble'; Service de Parasitologie–Mycologie B.P.217, Grenoble cedex 9 France.

amphotericin B, which remains the "gold standard" in mycology because of its wide spectrum of activity, all the other compounds can be considered as inactive.

However, we must note that the screening of antifungal activity only on the basis of in vitro techniques can be uncertain, as demonstrated these last years during the development of the new class of echinocandins. Indeed, the fungal cell is particularly complex, and according to the target of antifungal classes and their site of activity, MIC results can show wide variations between strains and species.

4. Conclusions

The reaction of thionyl chloride with succinamic acids appeared to be a useful method for the synthesis of *S*-containing heterocyclic compounds such as 1,4-dithiins, which were usually obtained with yields close to 50%. In the high majority of examples, the products crystallized spontaneously and the products did not require further purification.

Some dithiin diimides (compounds **3a**, **3c**, **3d** and **3e**) have a moderate antibacterial activity against Gram-positive organisms (*S. aureus* and *E. faecalis*), but *E. coli* and *P. aeruginosa* were resistant.

The antifungal activity of the new dithiins compounds **3c**, **3d** and **3e** against some yeasts and moulds of medical interest could open a new way of investigation for future. To confirm the potential value of these new compounds, a large number of strains should be tested for the species which appear sensitive to the products, especially *C. albicans*, *C. krusei* and *A. fumigatus*. Indeed there is a need for research of new antifungals to address infections due to an expanding number of opportunistic fungi.

Conversely, dithin diisoimides (compounds **2a** and **2b**) are totally inactive despites of their close structure similarities with the dithin diimides. The present study adds new data in the relationships of imides and their antimicrobial activities [1–3].

5. Experimental part

Melting points were taken on a Leitz 350 heated stage microscope and are not corrected. ^{1}H NMR spectra were recorded on a Bruker Avance DPX 400 and a WP80 DS instruments and were reported in ppm downfield from internal tetramethylsilane. Chemical shifts were reported in ppm (δ) relative to TMS. IR spectra were run on a Bruker IF 55 spectrometer. Elemental analyses were within ± 0.35 of the theoretical values. Analytical thin layer chromatography was performed on a plastic sheet (0.2 mm, silica gel 60 F_{254}, Merck). Silica gel 60 (70–230 mesh, Merck) was used for column chromatography.

*5.1. General procedure for the preparation of compounds **1***

Fifteen millimols of succinic anhydride were dissolved into 10 ml of dioxane and 15 mmol of the correspondent amine in 10 ml of dioxane was slowly added. The solution was warmed at 80 °C for 30 min. and the succinamic acid **1** crystallize by cooling. The white crystals were filtered off, dried and recrystallized from dioxane.

1a: m.p. 144 °C (85%). IR (KBr): *ν* CO 1691; 1642.
1b: m.p. 171 °C (90%). IR (KBr): *ν* CO 1697; 1642.
1C: m.p. 108 °C (90%). IR (KBr): *ν* CO 1731; 1641.
1d: m.p. 101 °C (85%). IR (KBr): *ν* CO 1414; 1642.
1e: m.p. 96 °C (85%). IR (KBr): ν CO 1695; 1650.

*5.2. General procedure for the preparation of compounds **2***

One gram of the succinamic acid was dissolved into 10 ml of ether and 13 ml of thionyl chloride was slowly added. The solution was allowed to r.t. for 6 h and the diisoimide was filtered off, washed with 2 × 5 ml of ether and recrystallized.

2a: Rose crystals, m.p.: 210 °C. IR: 1791, 1696 cm^{-1}. ^{1}H NMR ($CDCl_3$): 4.78 (s, 4H, CH_2); 7.31–7.38 (m, 10H, Ar).

2b: Rose crystals, m.p.: 228 °C. IR: 1789, 1774, 1701 cm^{-1}. ^{1}H NMR ($CDCl_3$): 1.23–2.13 (m, 20H, CH_2); 3.75–3.82 (m, 2H, CH).

*5.3. General procedure for the preparation of compounds **3***

By the same method used for the preparation of the compounds **2**, using dioxane as solvent.

3a: Green needles, m.p.: 224 °C. IR: 1712, 1697 cm^{-1}. ^{1}H NMR ($CDCl_3$): 4.64 (s, 4H, CH_2); 7.31–7.35 (m, 10H, Ar).

3c: Green needles, m.p.: 220 °C. IR: 1713, 1696 cm^{-1}. ^{1}H NMR (DMSO d_6): 1.35 (d, 12H, CH_3, J = 6.9 Hz); 4.23–4.30 (m, 2H, CH).

3d: Green needles, m.p.: 211 °C. IR: 1714, 1693 cm^{-1}. ^{1}H NMR (DMSO d_6): 0.88 (t, 6H, CH_3, J = 7.38 Hz); 1.48–1.61 (m, 4H, CH_2); 3.45 (t, 4H, CH_2, J = 7.09 Hz).

3e: Green needles, m.p.: 214 °C. IR: 1713, 1693 cm^{-1}. ^{1}H NMR (DMSO d_6): 0.91 (t, 6H, CH_3, J = 7.35 Hz); 1.26–1.35 (m, 4H, CH_2); 1.51–1.58 (m, 4H, CH_2);.3.49 (t, 4H, CH_2, J = 7.05 Hz).

6. Bacteriological assays

In vitro antimicrobial activities of the compounds were determined by the twofold broth dilution method in Mueller Hinton nutrient broth. The concentration of mother solutions was 1024 μg ml^{-1} (50/50 water–methyl sulfoxide). Minimal Inhibitory Concentration (MIC), was defined as the lowest drug concentration resulting in complete inhibition of growth after 18 h of incubation at 37 °C. Methyl sulfoxide has no antibacterial activity at a concentration up to 20% in water. The tested organisms were *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853.

7. Antifungal assays

Antifungal susceptibility testing was determined by the reference methods of the Clinical and Laboratory Standards Insti-

tute (formerly NCCLS). Broth microdilution methodologies: M27-A2 for yeasts and M38-A for filamentous fungi (7, 8). Briefly the test isolates included two quality control (QC) strains (*C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 26258), three yeast and mould reference strains (*C. albicans* ATCC 90028, *C. lusitaniae* ATCC 410086, *Aspergillus niger* ATCC 9804.83435) and one *A. fumigatus* clinical isolate, obtained from a proven invasive aspergillosis case at the University Hospital of Grenoble (France). Stock solutions of dithiines were prepared in *d*imethyl sulfoxide (water–methyl sulfoxide: 50/50), then they were diluted by the twofold broth dilution method in RPMI 1640 medium buffered to pH 7.0 with MOPS, as recommended by the NCCLS. The final solvent concentration was reduced to 1%, and at this concentration it had no antifungal effect. Regarding susceptibility testing, isolates were grown on Sabouraud medium at 35 °C for a period of 24–48 h for yeasts and 7 days for moulds. Final inoculum of yeast suspensions was 5×10^3 CFU ml^{-1} and suspensions of conidial spores of *Aspergillus* were adjusted to obtain an inoculum of 1×10^4 CFU ml^{-1}. The final ranges of drug concentrations tested were 0.1–51.2 μg ml^{-1} for all the dithiines compounds. Minimal inhibitory concentrations (MICs) were determined after 24 h of incubation of microdilution trays at 35 °C for *Candida* strains and 48 h at 30 °C for *Aspergillus* strains. MICs were determined spectrophotometrically at 405 nm, and defined as the lowest concentration of drug that produced 50% inhibition of growth relative to the drug-free growth control.

Acknowledgements

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Bioisosterism: A Useful Strategy for Molecular Modification and Drug Design

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Abstract: This review aim to demonstrate the role of bioisosterism in rational drug design as well as in the molecular modification and optimization process aiming to improve pharmacodynamic and pharmacokinetic properties of 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 pharmacophoric 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 amply 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 innumerous 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, Langmuir [8] studying the chemical behavior and reactivity of determined substances possessing atoms or groups with the same number of valence electrons, *i.e.* isoelectronic, created the concept of isosterism to define atoms or organic or inorganic molecules which possess the same number and/or arrangement of electrons examples:

$$O^{-2} \text{ x } F^{-} \text{ x } Ne \text{ x } Na^{+} \text{ x } Mg^{+2}$$

$$ClO_4^{-} \text{ x } SO_4^{-2} \text{ x } PO_4^{-3}$$

$$N{=}N \text{ x } C{=}O$$

$$CO_2 \text{ x } NO_2$$

$$N{=}N{=}N \text{ x } N{=}C{=}O^{-}$$

In 1925, Grimm [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 pseudoatom presenting the same physical properties as those 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 pseudoatoms.

In 1932, Erlenmeyer [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 bioisosters as compounds which fit the definitions of isosteres and which exercise their biological activity of bioreceptor, 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 antagonistic biological properties [11]. Later, Thornber [3] proposed a broadening of the term bioisosteres, defining them as sub-units or groups or molecules which possess physicochemical properties of similar biological effects.

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

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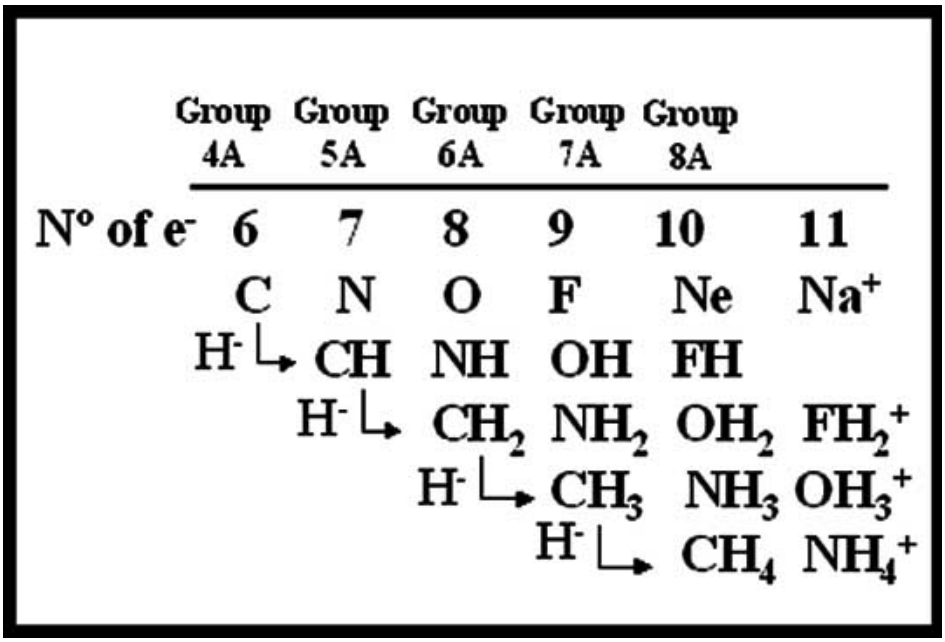
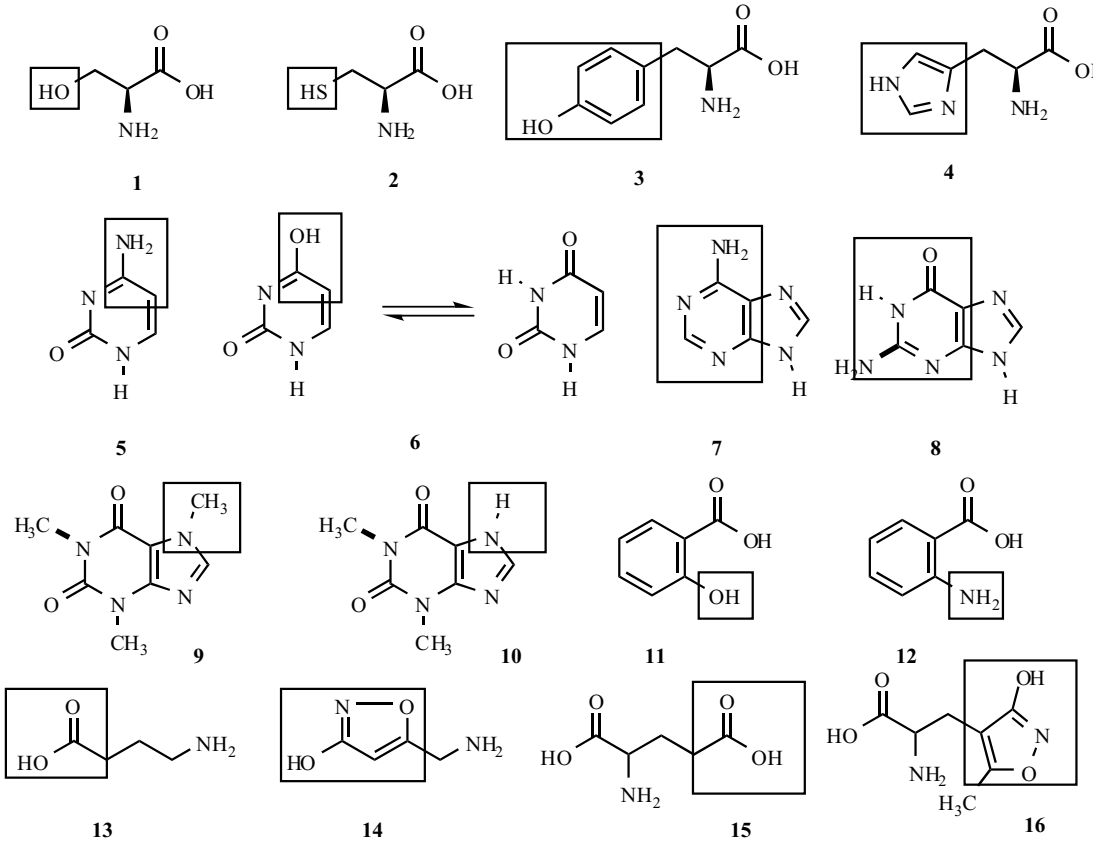


Fig. (1). Grimm's hydride displacement law.

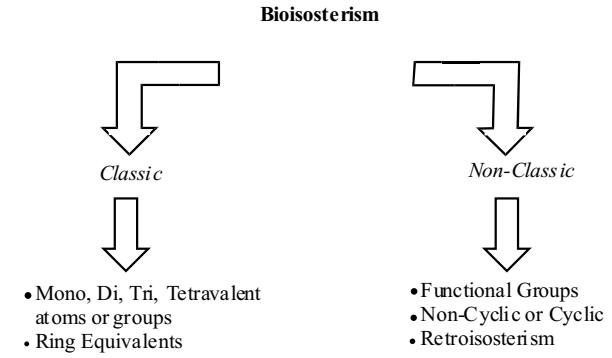
form of broadening chemodiversity (Scheme **1**), striking among which are the classic bioisosteric relation existing between the essential amino acids serine (1) and cysteine (2), tyrosine (3) and histidine (4) among the pyrimidine and purine bases cytosine (5) and uracil (6), adenine (7) and guanine (8); among the xanthines caffeine (9) and theophyline (10); and among the salicylic (11) and anthranilic (12) acids, which originated two important classes of non-steroid anti-inflammatory drugs, *e.g.* acetylsalicylic acid and mefenamic acids, respectively. Furthermore, examples of the application of non-classic bioisosterism are also found in nature - such as the bioisosteric relationship existing between γ-aminobutyric acid (GABA) (13) and muscimol (14), between the neurotransmittors glutamate (15) and AMPA (16).



Scheme 1.

3. CLASSIFICATION OF BIOISOSTERISM: CLASSIC AND NON-CLASSIC

In 1970, Alfred Burger classified and subdivided bioisoteres into two broad categories: Classic and Non-Classic [12] (Scheme **2**).



Scheme 2.

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

1 Classic 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

2 Non-Classic Bioisosteres (Table **2**)

- 2.1 Cyclic *vs* Noncyclic
- 2.2 Functional groups
- 2.3 Retroisosterism

Table 1 Classic Bioisostere Groups and Atoms

Monovalent	*Divalent*	*Trivalent*	*Tetravalent*
, -OH, $-NH_2$, $-CH_3$, -OR	$-CH_2-$	=CH-	=C=
-F -CI, -Br, - I, -SH, $-PH_2$,	-O-	=N-	=Si=
$-Si_3$, -SR	-S-	=P-	$=N^+=$
	-Se-	=As-	$=P^+=$
	-Te-	=Sb-	$=As^+=$
			$=Sb^+=$

Many authors have contributed to updating the tables of functional bioisostere groups, which explains why, in that which we refer to as non-classic bioisosterism, we have observed a growing number of bioisosteres described in the literature [6-7].

4. BIOISOSTERISM AS A STRATEGY OF MOLECULAR MODIFICATION

Among the most recent numerous examples used in the strategy of bioisosterism for designing new pharmacotherapeutically attractive substances [6-7,13], there is a significant predominance on non-classic bioisosterism, distributed in distinct therapeutic categories, be they selective receptor antagonist or agonist drugs, enzymatic inhibitors or anti-metabolites. The use of classic bioisosterism for the structural design of new drugs, while less numerous, has also been carried out successfully [6].

The correct use of bioisosterism demands physical, chemical, electronic and conformational parameters involved in the planned bioisosteric substitution, carefully analyzed so as to predict, although theoretically, any eventual alterations in terms of the pharmacodynamic and pharmacokinetic properties which the new bioisosteric substance presents.

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

a) 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;

b) degree of lipidic and aqueous solubility, so as to allow prediction of alteration of the physicochemical properties such as logP and pKa;

c) chemical reactivity of the functional groups or bioisosteric 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;

d) conformational factors, including the differential capacity formation of inter- or intramolecular hydrogen bonds.

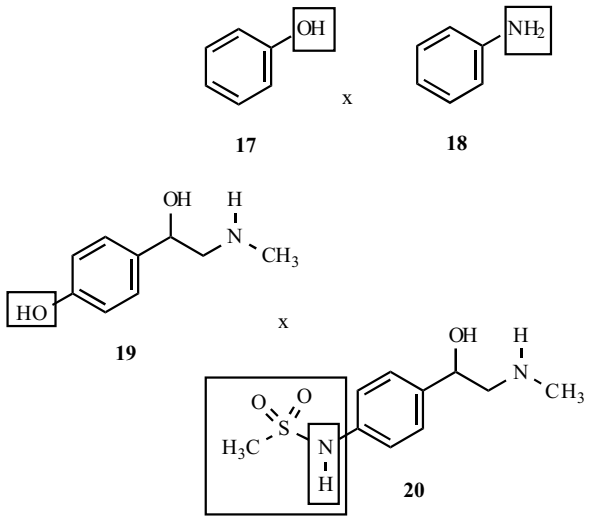
4.1. BIOISOSTERISM AND ALTERATIONS OF PHYSICOCHEMICAL PROPERTIES

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 classic bioisosterism of monovalent groups according to Grimm's 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 pKa 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 change form one positively charged function ($-NH_3^+$), originating from basic aromatic amine function (pKb = 9,30) by another acid (pKa = 10,0) present in phenol, which may, quite probably, abolish the original activity [14]. Thus, in

Table 2 Non-Classic Bioisosteres

-CO-	**-COOH**	**-SO_2NH_2**	**-H**	**-CONH-**	**-COOR**	**-$CONH_2$**
-CO_2-	**-SO_3H**	**-$PO(OH)NH_2$**	**-F**	**-NHCO-**	**-ROCO-**	**-$CSNH_2$**
-SO_2-	**-tetrazole**					
-SO_2NR-	**-SO_2NHR** **-SO_2NH_2**		**-OH** -CH_2OH		-catechol	
-CON-	**-3-hydroxyisoxazole**				**-benzimidazole**	
-CH(CN)-	**-2-hydroxychromones**		**-$NHCONH_2$**			**C_4H_4S**
R-S-R			**-NH-CS-NH_2**			**-C_5H_4N**
(R-O-R')	**=N-**					**-C_6H_5**
R-N(CN)-	**C(CN)=R'**		**-NH-C(=$CHNO_2$)-NH_2** **-NH-C(=CHCN)-NH_2**			**-C_4H_4NH**
-halides						
	-CF_3					
	-CN					
	-$N(CN)_2$					
	-$C(CN)_3$					

this example, we may predict that the use of bioisosterism, even the classic type, can promote severe alterations of molecular properties, as much in terms of lipidic-aqueous solubility as well as chemical reactivity, among others, which, broadly speaking, is not observed in the same homologue carbonic series. Otherwise, the system's enzymatic capacity for hepatic detoxification of xenobiotics, involving the microsomal mixed function oxidase also called cytochrome P-450 system [14], is distinct in the presence of these functional isosteric groups, which does not allow a simplistic comparison between the lead compound aniline (18) and the hydroxylated isostere (17) in terms of metabolism, altering, therefore, the pharmacokinetic phase as well as the pharmacodynamics of the isosteres.

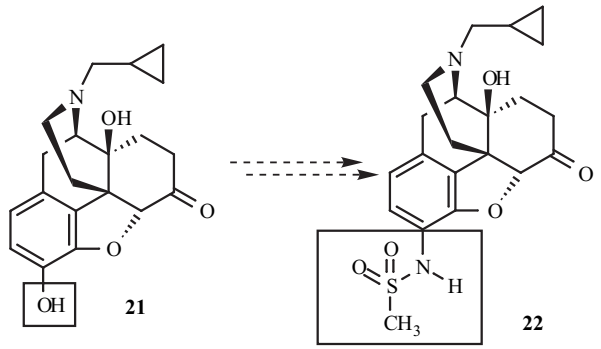


Scheme 3.

Another classic example of isosteric replacement involving phenol (PhOH), can be found in the search for adrenergic derivatives, structurally related to catecholamines (Scheme **3**) [15]. This example illustrates the exchange of phenolic hydroxyl group present in compound 19 with the arilsulfonamide 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 bioisosteres. This bioisosteric relationship was experimentally evidenced by determining the degree of acidity of these substances. Both compounds are of comparable acidity (pK_A = 9.1 and 9.6 respectively), explaining the similarity of biological profile in function of equivalent interactions of both molecules with site receptor, possibly through ionic bonding, in the presence of a similar acidity or even through hydrogen bonding [15]. Furthermore, both acidic groups , *i.e.* R-PhO<u>H</u> and R-Ph<u>N</u>HSO_2CH_3 are, in this case, monovalent groups. However, the identified bioisosteric relationship between the Ph<u>OH</u> and Ph<u>NHSO$_2$CH$_3$</u> groups, confirmed for adrenergic derivatives 19 and 20, is not extensive to other bioreceptors in which the process of molecular recognition is distinct. An example of how isostere groups can, in certain systems, not maintain a bioisosteric relationship, previously defined in another bioreceptor, is well illustrated with the analyses of 21 and 22, described by McCurdy and coworkers (Scheme **4**) [16]. The structural design of 3-sulfonamido compound 22, targeting the attainment of new bonds for opioid receptors, was based on the structural data of lead compound 21, which point to the relevance of phenolic hydroxyl as site of molecular recognition. This way, the replacement OH by the $NHSO_2CH_3$ group would respect the acidic properties and the characteristics donor and/or acceptor H-bonds as evidenced by the previous work of Larsen and Lish (1964). Nonetheless, the determination of the pharmacological properties of arilsulfonamide derivative 22, evidenced the absence of affinity by the opioid receptors, *in vitro* models,

indicating that in this system the groups PhOH and $PhNHSO_2CH_3$ are not bioisosteres, despite their great structural similarity.



Scheme 4.

Thus, when bioisosteric 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, bioisosteric 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.*

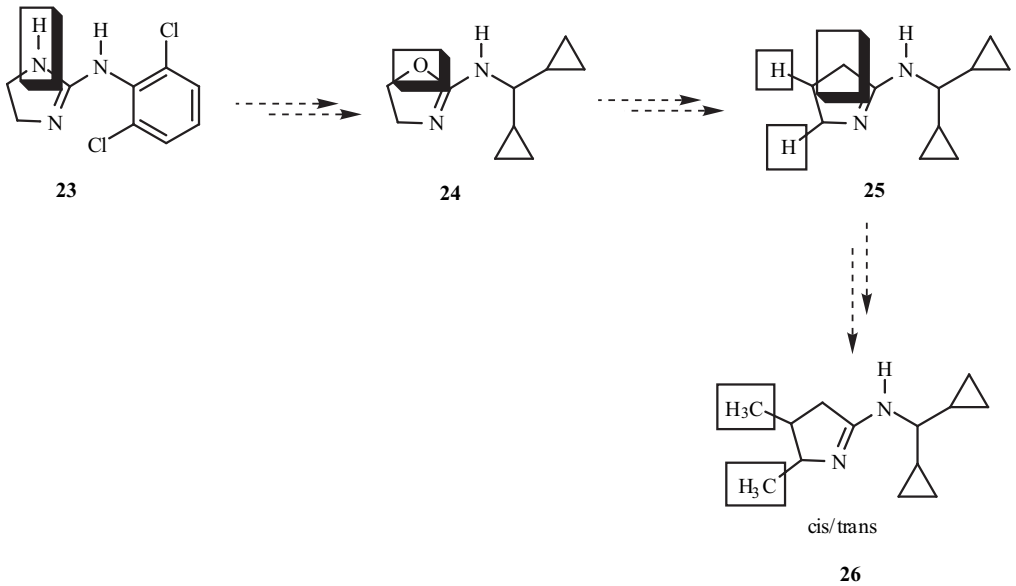
5. CLASSIC BIOISOSTERISM

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

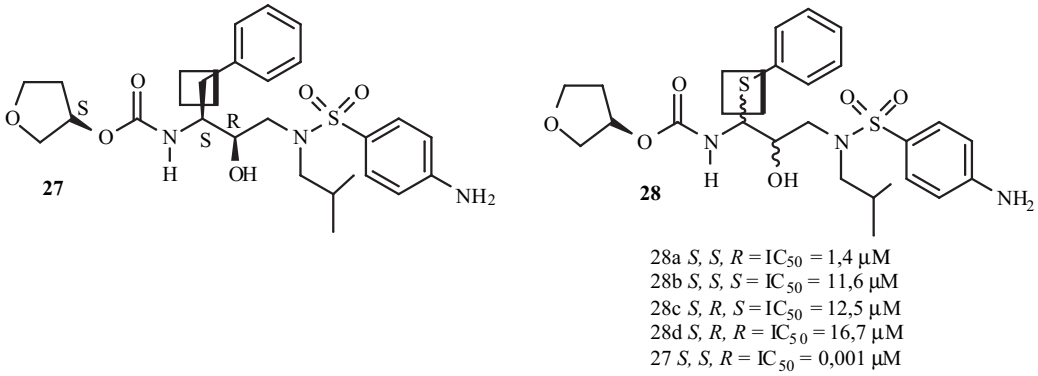
In the search for new anti-hypertensive drugs analogous to clonidine (23), with a greater selectivity by I_1 imidazoline receptors (I_1R) and reduced action on α_2-adrenoceptors, Schann and coworkers described the attainment of new candidates for anti-hypertensive drugs designed by molecular modifications in the structure of the lead compound rilmenidine (24) (Scheme **5**) [17]. These modifications were based on classic bioisosterism of bivalent groups, exemplified by replacement of the bivalent oxygen atom (O) present in the oxazoline ring, by the methylene group (CH_2), in the structure of the new pyrroline derivative (25); and between replacement of monovalent groups illustrated by the substitution of hydrogen atoms (H) in C-4 and C-5 of derivative (25) by the methyl group (CH_3), originating the *cis/trans*-4,5-dimethyl homologue 26 [17]. The *binding* tests with I_1R and α_2-adrenoceptors evidence that the modifications occurring in the structure of rilmenidine (24), allow the attainment of derivatives with affinities comparable to lead compound (24), although with a superior selectivity for I_1R , and illustrated an example of the success of classic bioisosterism.

To develop new HIV-protease inhibitors, Rocheblave and coworkers described the bioisosteric exchange between bivalent groups (Scheme **6**), realizing the exchange of the methylene group (CH_2), present in the structure of the lead compound amprenavir (27), for the sulfur atom (S) in 28 [18]. Knowing, *a priori,* that this isosteric replacement could induce several modifications in terms of size, shape, electronic distribution, chemical reactivity, lipophilicity and hydrogen bonding capacity, this new isoster was synthesized, and its stereoisomeres were duely 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 *ca.* 1400 less potent than 27. These results could be explained by the high sensitivity to hydrolysis of thio-isoster 28a-d. In fact, the half-life value found for thiophenoxy derivative 28a-d was 10 min, while amprenavir (27) was recovered unchanged after 1440 min.

Another illustration of the application of classic bioisosterism can be found in the works of Penning and coworkers (Scheme **7**) [19]. Continuing 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

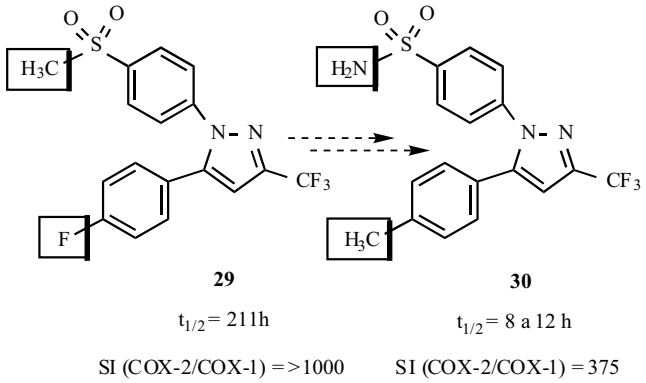


Scheme 5.



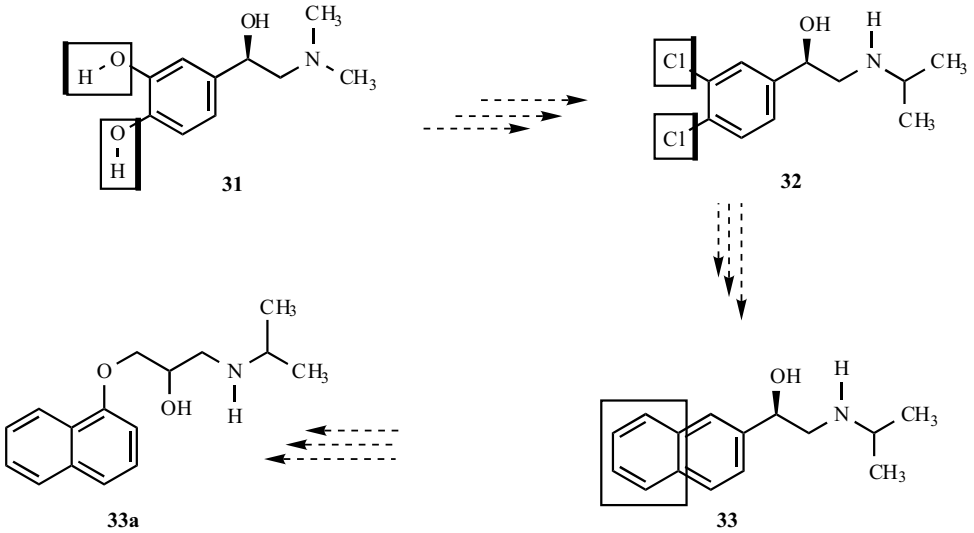
Scheme 6.

effect of isosteric modifications on the structure of lead compound SC-58125 (29), to improve its pharmocokinetic properties. In spite of the high rate of selectivity by PGHS-2 (SI> 1000) 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 bioisosteric monovalent group replacements, represented by replacing the methyl group, (CH_3) by the NH_2 group and exchanging the fluorine atom (F) with the CH_3 group (Scheme **7**). Both isosteric replacements suggested allow introducing into the structure of the new bioisostere of SC-58125, vulnerable soft 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 bioisostere 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/Searle 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].

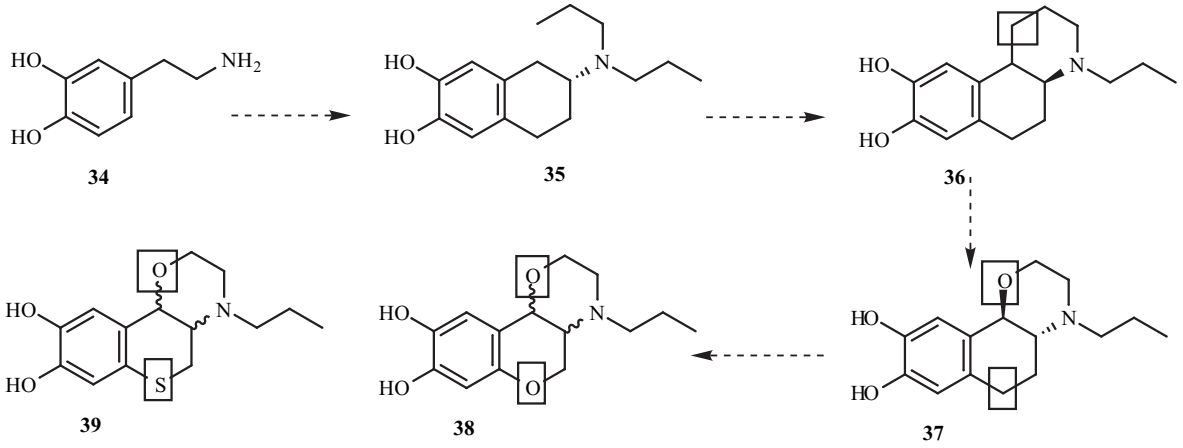


Scheme 7.

The design of dichloroisoproterenol (32) from isoproterenol (31) is, without a doubt, an unique example of the contribution of the application of bioisosterism 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 bioisosteric replacement allows blocking one of the main sites of metabolism of this catecholamine derivative.

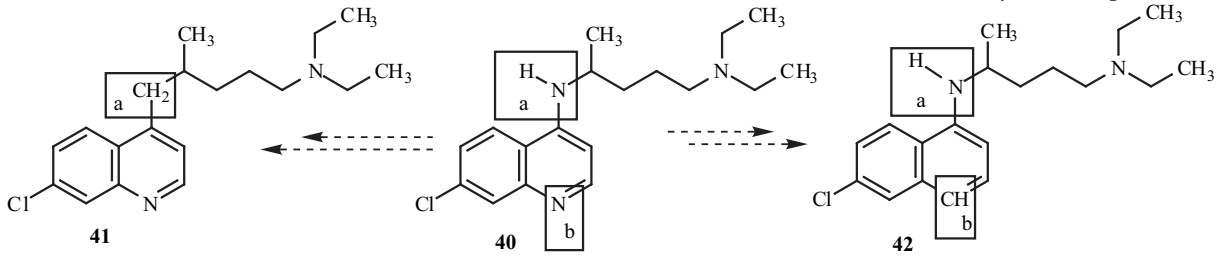


Scheme 8.



Scheme 9.

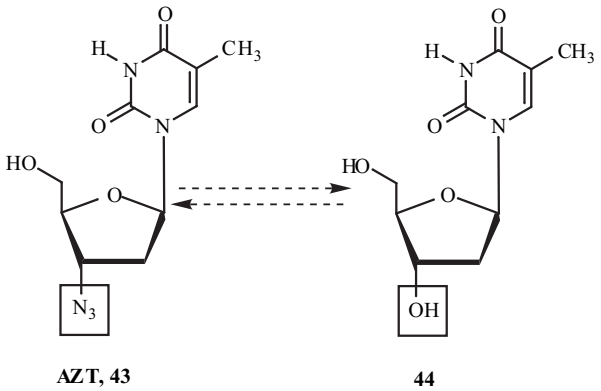
Furthermore, the discovery of dichloroisoproterenol (32), made feasible the comprehension of the relevance of structural characteristics of the catecholic subunit for adrenergic activity, allowing *a posteriori* the discovery of pronethalol (33), the first selective antagonist for adrenergic receptors for subtype β. Pronethalol(33), discontinued during the 1980s, was the precursor of propranolol (33a), a therapeutic innovation 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 innumerous other more selective adrenergic β_1 antagonists.



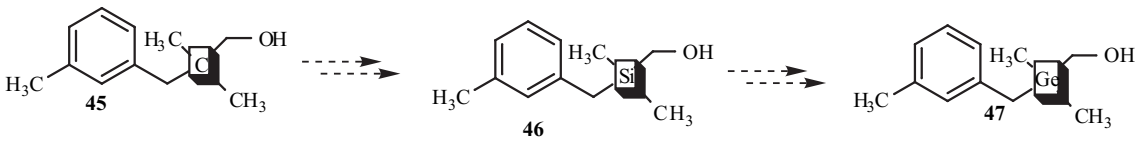
Scheme 10.

The bioisosteric replacement of the bivalent oxygen (O) atom 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 Vliete and coworkers (Scheme **9**), in their search for selective agonists of dopaminergic receptors, subtype 3 (D_3), molecular target for the design of anti-psychotic drugs, realized modifications in the hexahydronaphthoxazine system 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 (CH_2) 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 thio-isostere (39, log D= 1,62) compared to oxa-isostere (38, log D= 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 test*, leads us to believes in an inverse relationship between the increase in lipophilicity and the selectivity desired by the D_3 receptors.

To understand the contributions of 4-aminoquinoline subunit present in the structure of the first generation antimalarial chloroquine (CLQ, 40), Cheruku and coworkers described the attainment and determination of the pharmacological profile of modified analogs of CLQ (Scheme **10**) [22]. In this study, the authors carried out the classic bioisosteric exchange of NH (a; *NH-anilinic*) and N (b, *N-quinolinic*) with the CH_2 and CH groups in the structure of compounds 41 and 42, respectively, observing the loss of antimalarial activity in the presence of the modifications realized. Carbo-isosteres 41 and 42 were inactive due to the inhibition of *Plasmodium falciparum* NK 54 or K1 strains, even in concentrations over 3000 nM (CLQ, IC_{50}= 8,5 and 150 nM, respectively), and also proved to be unable to inhibit the formation of hemozoine



Scheme 11.



Scheme 12.

(CLQ IC_{50}= 80 μM; 41 IC_{50}= 1500 μM; 42 IC_{50}= >2500 μM). Hence, the isosteric modifications realized reinforced the hypothesis of the importance of *N*-quinolinic interaction process with hematine, the molecular target of antimalarial action of 4-aminoquinoline drugs.

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

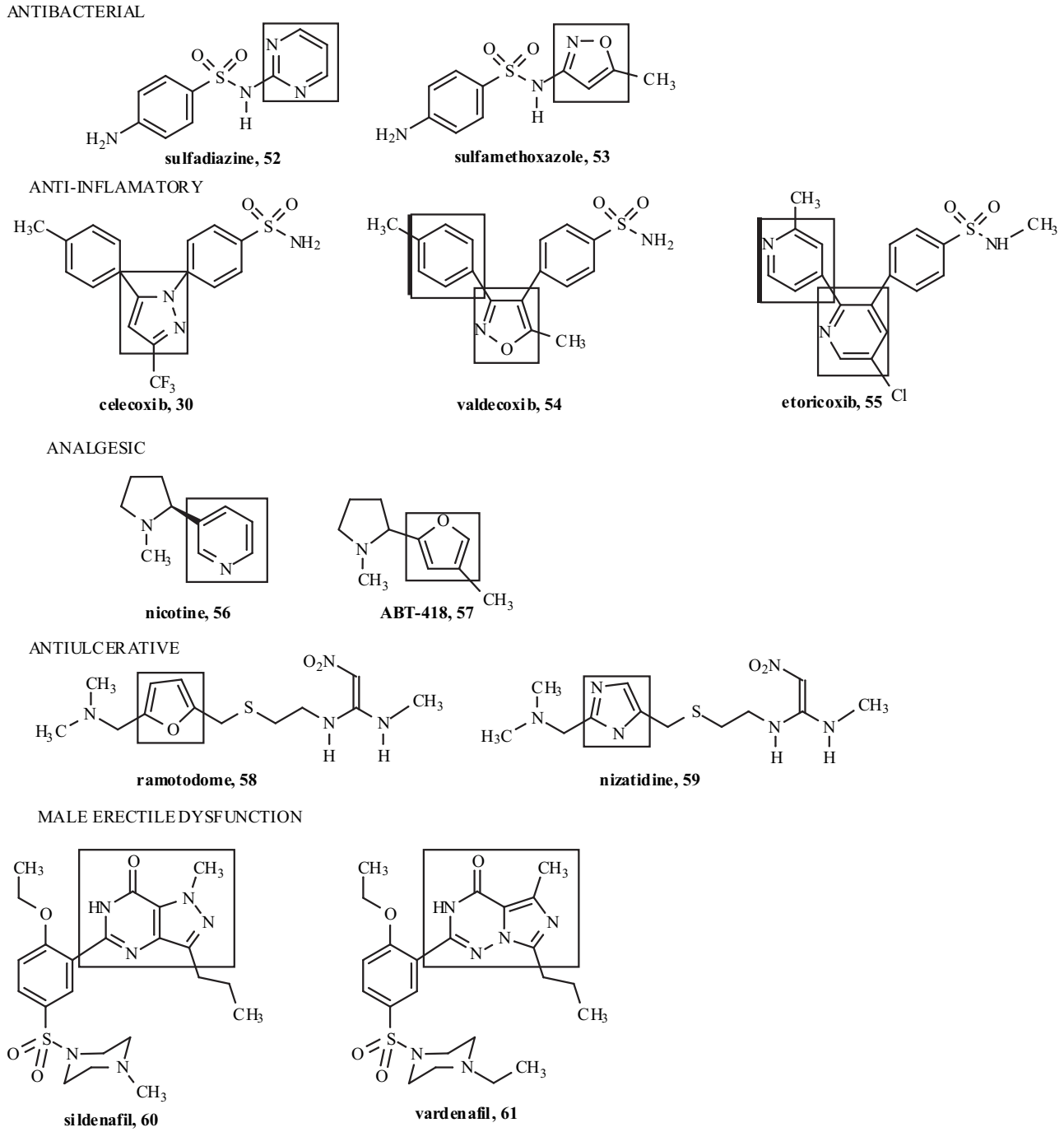


Fig. (2). Examples of ring bioisosterism between drugs belonging to different therapeutic classes.

transcriptase reverse enzyme, enables us to ascertain the existence of a classic bioisosteric relationship of monovalent groups between the nucleoside thymine (44) (endogenous substrate for the synthesis of DNA and RNA) and AZT, exemplified by the presence of the hydroxyl (OH) unit in 44 and azido (N_3) group present in 43 (Scheme **11**) [23]. Furthermore, although classic monovalent isosteres, the OH and N_3 groups possess dramatic electronic differences, easily demonstrated by simple functional analysis.

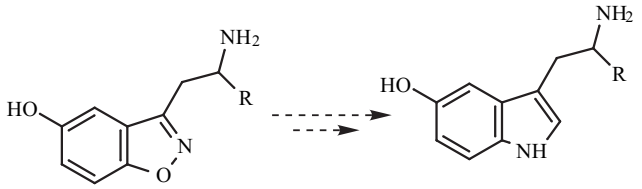
Although applicability of the strategy of molecular modification of a given lead compound through bioisosterism is well consolidated in the pharmaceutical industry, recent studies by Tacke and coworkers (Scheme **12**) shows that this approach may be used efficiently to optimize the organoleptic properties of compounds used in fragrances of industrial interest [24]. In this study, the application of classic bioisosterism of tetrasubstituted atoms, illustrated by exchange of the carbon atom (C), present in the structure of majantol (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 bioisosteres 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 phosphorous, 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].

5.2. Ring Bioisosteres

Ring bioisosterism, is undoubtedly the most frequent relationship in drugs of different therapeutic classes [8], as can be seen in (Fig. **2**), and certain specific examples will be commented upon.

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

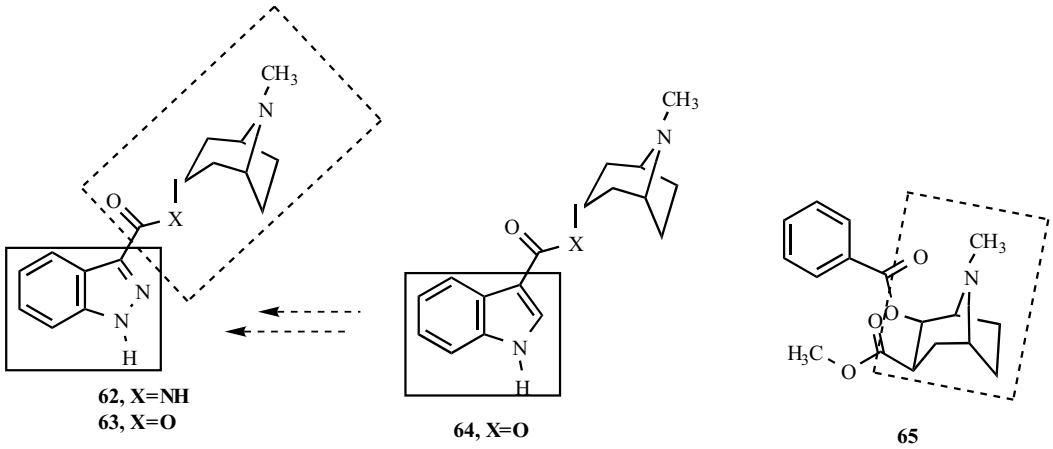


48, R=H
50, R=CO_2H

49, R=H
51, R=CO_2H

Scheme 13.

Applying classic bioisosterism in the design of new serotonin receptor (5-HT_3) antagonists Fludzinski and coworkers (Scheme **14**) described the obtention of new indazolyl compounds 62 and 63 with important antagonistic properties of receptor 5-HT_3 [28]. These two substances were developed as bioisosteres of 64, a compound which possesses as main structural characteristic the hybrid

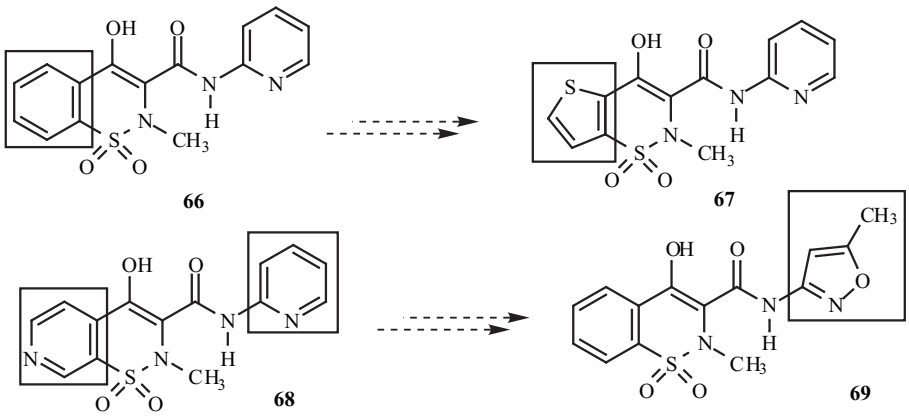


62, X=NH
63, X=O

64, X=O

65

Scheme 14.



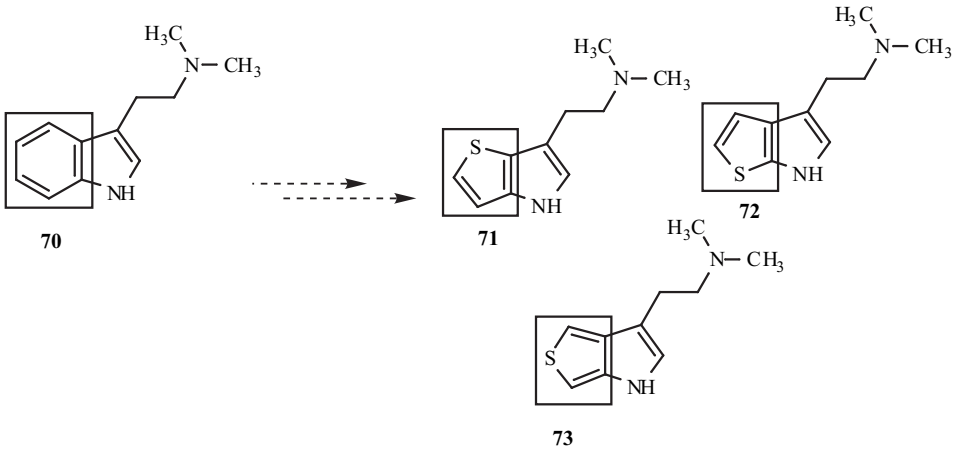
Scheme 15.

character between serotonin (49), represented by the indole ring substituted in C-3, and cocaine (65), represented by the nature of the substituent in C-3, a known antagonist of receptors 5-HT_3 [29]. The bioisosteric relationship of these two nuclei was proven. In this case, by the results obtained in bioassays which showed for 62 a rate of selectivity by the receptors 5-HT_3 superior to that of 64, 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 amidic bond present in chain 62 is responsible for a more favorable oral bioavailability .

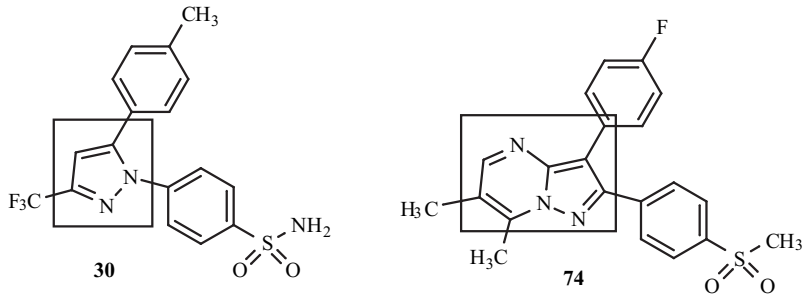
The application of ring bioisosterism was also successfully explored by Binder and coworkers (Scheme **15**) [30] in developing new non-steroid anti-inflammatory agents of the oxican group [31]. This class of NSAID was discovered by Lombardino and collaborators [32], at Pfizer Laboratories in England,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

Applying the strategy of ring bioisosterism, Binder proposed tenoxican 67, the newest member of the class of arylthiazine-1,1-dioxides, where the benzothiazinic nucleus of 66 was replaced by the thienothiazinic moiety (Scheme **15**) [30]. This example represents the bioisosteric relationship existing between aromatic heterocyclic rings and the phenyl group. The profile of pharmacotherapeutic activity of 67 proved to be comparable to that of 66, being able to be administered in single daily doses of 20 mg, because of its long plasmatic half-life, a desirable quality for cases of arthritis as well as osteoarthritis. Both derivatives act by the same mechanism of action, at the same receptor level, *i.e.* cyclooxygenase, an enzyme involved in arachidonic acid metabolism. It is noteworthy that other ring bioisosteres at the structural subunit level represented by the BTA nucleus may possess the same phramacotherapeutic profile as 66 and 67 (*cf.*68) [33], being differentiated from other bioisosteres developed by structural alterations in the heteroaromatic ring, present in the carboxamide unit, whose representative is isoxican 69, which possesses 5-methylisoxazole ring as equivalent of the pyridine ring present in compounds 66 and 67 (Scheme **15**). This derivative was recently taken off the market due to the dermatologic reactions it provoked.

The similarity between the physicochemical properties of the benzene (PE = 80 °C) and thiophene (PE= 84 °C) rings is well fundamented and was used to create the concept of ring bioisosterism or ring equivalents. However, the exchange of the phenyl ring by the thiophene ring in the structure of bicyclic derivatives, should be carried out



Scheme 16.



IC_{50}=0, 6 μM (COX-2)
IC_{50}=13 μM (COX-1)
SI>21

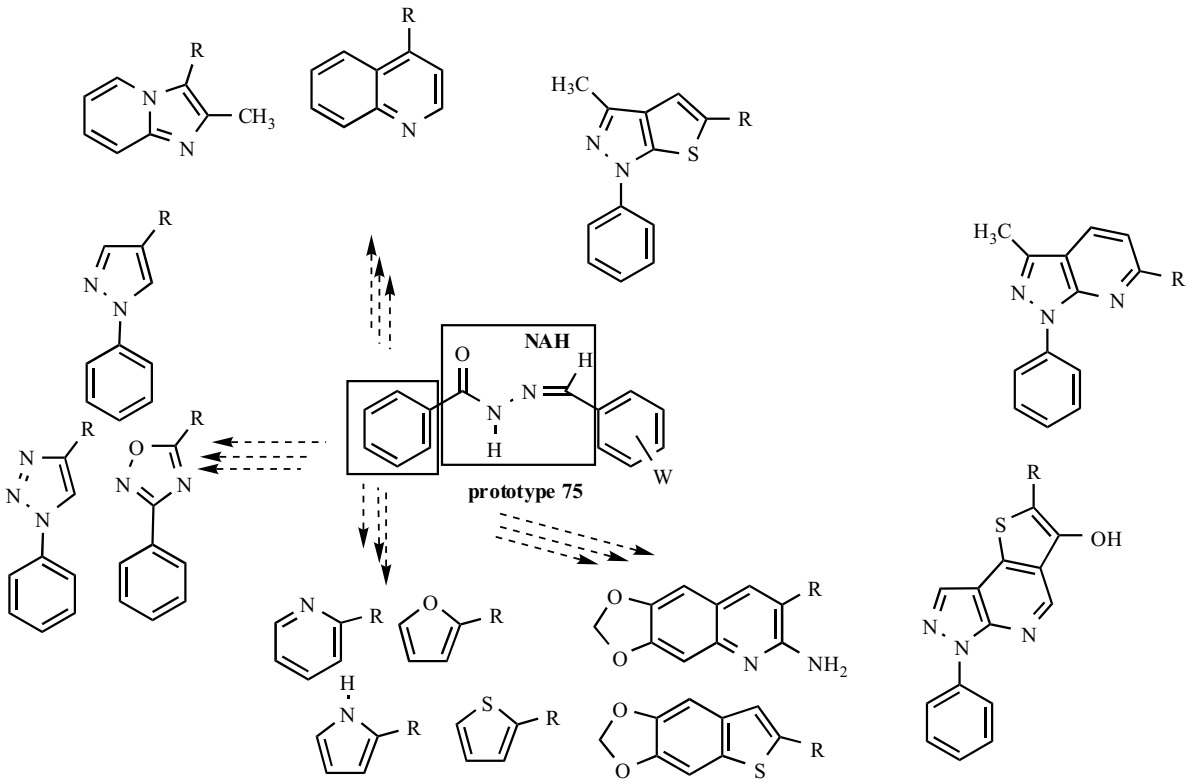
IC_{50}=0, 08 μM (COX-2)
IC_{50}=10 μM (COX-1)
SI>125

Scheme 17.

considering the possible regioisomeres involved in this replacement. Studies by Blair 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*-dimethyltryptamine (**70**) was replaced by the thiophene ring, giving rise to the isomeric systems thieno[3,2-*b*]pyrrole (**71**), thieno[2,3-*b*]pyrrole (**72**), thieno[4,3-*b*]pyrrole (**73**) (Scheme **16**) [34]. However, the results described by these authors show that with the exception of the thieno[4,3-*b*]pyrrole system (**73**), of unstable and difficult preparation, the other heterocyclic isomeric systems are authentic bioisosteres of the indole ring, presenting affinity and selectivity by the serotonin receptors similar to lead compound **70**.

Concerning the non-steroid anti-inflammatory drugs (NSAIDs), Almansa and coworkers (Scheme **17**) [35] described the existing bioisosteric relationship between the pyrazolo and pyrazolo[1,5-*a*]pyrimidine rings present in compounds **30** and **74**, respectively. The exchange of the pyrazolic ring, present in celecoxib (**30**), by the pyrazolo[1,5-*a*]pyrimidine system in **74**, resulted in an optimization of the pharmacodynamic properties of **30**, although it compromised its oral bioavailability .

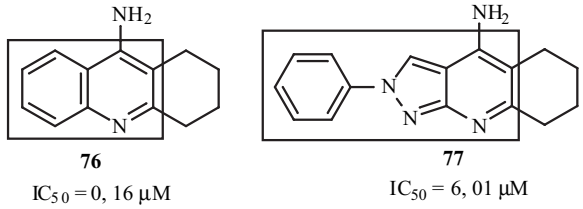
The use of ring bioisosterism may serve for designing congeneric series of lead compound candidates for new drugs, in view of the detailed study of the distinct hydrophobic contributions resulting from rings equivalents. This strategy has revealed itself as a fundamental tool in designing new *me-too* drugs, *i.e.* therapeutic copy (*vide* Figure 2). In a recent study, Barreiro and coworkers described the application of ring bioisosterism for designing functionalized *N*-acylhydrazone (NAH) derivatives (Scheme



Scheme 18.

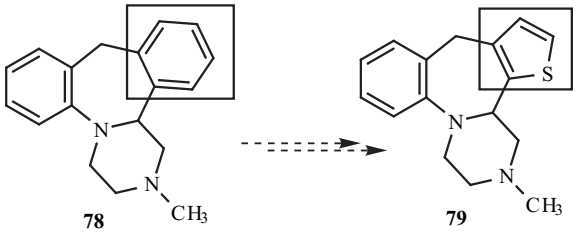
18) [36], in an effort to correlate the alterations found in the profile of anti-platelet, analgesic and anti-inflammatory activities, of each new hetero-aromatic system studied, with its respective electronic characteristics.

More recently, Barreiro and coworkers (Scheme **19**) described the attainment of new selective acetylcholinesterase (AChE) inhibitors designed by modifications in the structure of the lead compound tacrine (76) [37]. These modifications allow for identification of a new bioisosteric relationship existing between the quinoline nuclei in 76 and pyrazolo-pyridine in 77, which have shown a similar inhibitory capacity for rat brain cholinesterases.



Scheme 19.

In the field of antidepressives, bioisosteric ring replacement of a determined lead compound also permits the discovery of new agents of therapeutic interest. In 1983, Watthey and coworkers developed, at Ciba-Geigy Laboratories, compound 79 as a bioisoster of mianserine 78 (Scheme **20**) [38]. As principal structural characteristic, this substance possesses the thiophenic ring integrated to the cyclic unit of this representative of atypical antidepressive agents, which act as serotonin receptors (5-HT) antagonists [39]. Once again, this example illustrates the equivalency of the benzene and thiophene rings, in distinct pharmacological activities, allowing for a generalization on the bioisosteric relationship between heterocyclic nuclei of five atoms and benzene ring [40].



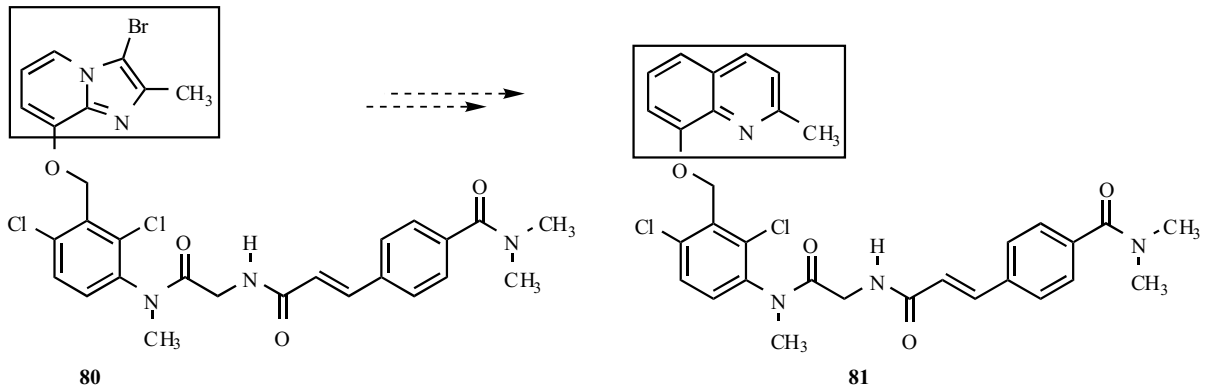
Scheme 20.

The recognition of the importance of bradykinin in the processes of pain, inflammation, rhinitis and hypertension, has shown the relevance of bradykinin receptors as a new target for therapeutic intervention. Although many bradykinin receptors antagonists have been described in the literature, its peptidic characteristics have limited its therapeutic applications. Seeking non-peptidic bradykinin receptors antagonists, Abe and coworkers described the attainment of 2-methylimidazo[1,2-*a*]pyridine derivatives, highlighting the derivative FR167344 as lead compound (80) (Scheme **21**) [41]. Later, these authors proposed the bioisosteric exchange of 2-methylimidazo[1,2-*a*]pyridine ring, present in compound 80, by the 2-methylquinoline subunit, identifying the new derivative FR175657 (81), as a potent bradykinin receptors antagonists of non-peptidic structure.

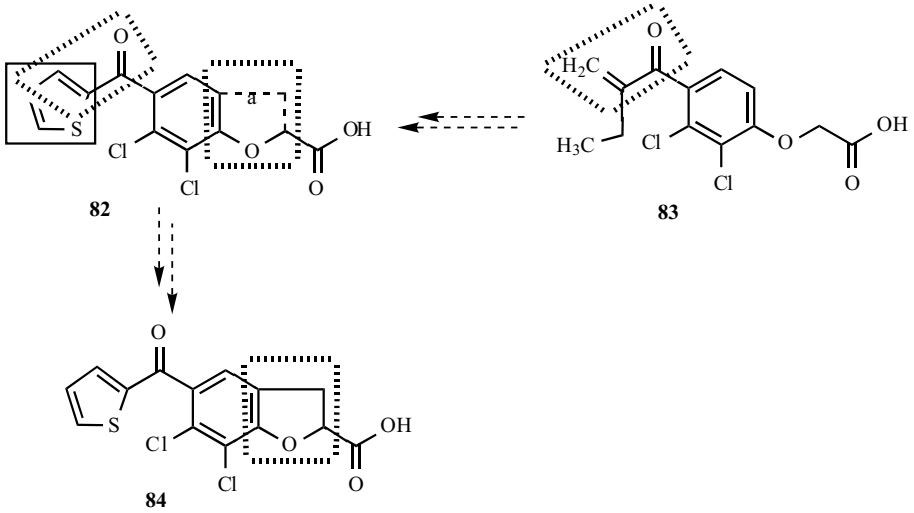
6 – NON-CLASSIC BIOISOSTERISM

6.1. Cyclic *vs* Non-Cyclic

In the category of diuretic substances of the phenoxyacetic class, in which etacrinic acid 82 is the main representative, we have found innumerous examples of the use of non-classic bioisosterism in the discovery of new drugs. To develop new diuretic substances, with uric activity superior to 82, Hoffman and coworkers applied non-classic bioisosterism represented by *ring-closing*, or anelation, as a strategy to choose definitions of new lead compounds for diuretic drugs [42]. These authors working at Merck, Sharp and Dohme Laboratories, proposed compound 84 as a bioisostere of 83 (Scheme **22**). The structure of 84 was defined based on the anelation of the phenoxyacetic chain (a) of 83, which, in turn, arose by the replacement of the ethylenone function present in 82 by the thiophene nucleus of 83, including all four carbon atoms of the replacement of 82, there being a clear correspondence between the carbons with sp^2 hybridization in 82 and 84. This type of anelation in the ethylenone chain of 82 had been proposed previously by Thuillier [43-44], who described the synthesis of 83 as being an acid equivalent to etacrinic acid (82) in terms of its diuretic properties, while with superior uric properties. In fact, this last compound represents one of the first examples of bioisosteres of 82, obtained by application of non-classic bioisosteric strategies. Compound 84, proposed and synthesized by Hoffman, presented a profile comparable to



Scheme 21.



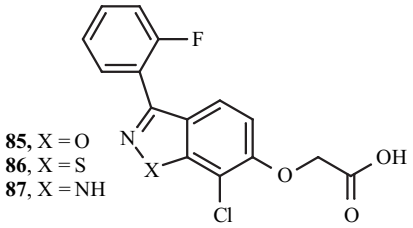
Scheme 22.

83, its uric potential being even greater. Furthermore, the introduction of the thiophene ring in 83 to replace the ethylenone subunit of 82, eliminates Michael's acceptor site present in the structure of etacrinic acid (82), responsible for the hepatotoxic effects of this drug.

Still on the subject of diuretics related to etacrinic acid (82), Shutske and coworkers later developed the synthesis of new aryl-benzisoxazolyloxyacetic acids (*e.g.*, 85) [45], based on the probable bioisosteric relationship existing between the aryl-benzisoxazole unit and the 2-acyl-thiophenyl moiety present in acid 83 [46] (Scheme **22**). This illustrative example is sufficient to show the potential of the strategy of non-classic bioisosterism for designing molecular modifications in substances of pharmacological interest.

Shutske and coworkers successfully explored the possibility of integrating the thiophenacyl 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 bioisosterism of the *closing ring* type. Later, the same authors [47] used classic bioisosteric ring to design structurally new heterocyclic diuretic compounds from 85, possessing, now, the indazole ring in 87 and benzisothiazole in 86 (Scheme **23**).

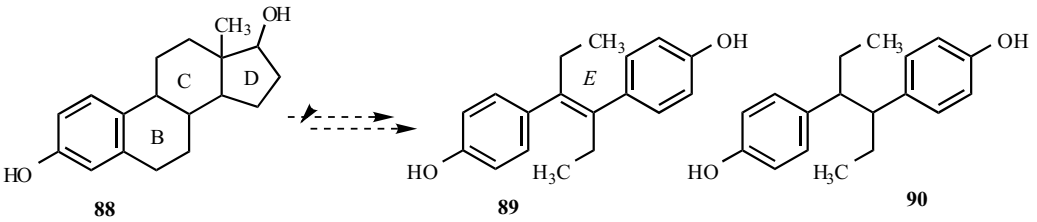
Innumerous other examples can illustrate the validity of the use of this strategy in discovering new bioactive agents which are more therapeutically attractive. A classic example is represented by the discovery of the estrogenic properties of *trans*-diethylstilbestrol 88 [48], illustrating the application of non-classic ring opening bioisosterism. This example shows that the molecular design of 89 could be carried out from the opening of rings B and C of the steroidal skeleton of estradiol 88 (Scheme **24**). However, in analogy to what was observed for estradiol (88), the activity of 89 is dependent on the configurational aspects, such that, the diastereoisomer *E* presents an estrogen profile significantly superior to the diastereoisomer *Z*, with reduced estrogen activity also being observed for the dihydrogenated compound 90 (Scheme **24**).



Scheme 23.

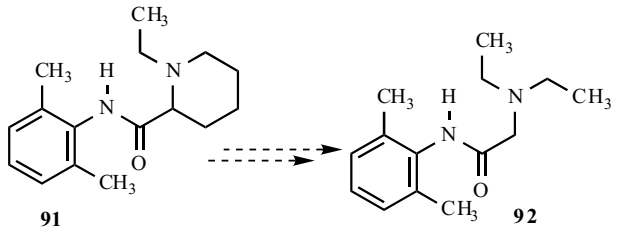
Another example of the application of non-classic bioisosterism can be illustrated by the discovery in 1957 of lidocaine 92 from mepivacaine 91 (Scheme **25**), contributing to the design of the important anesthetic agent with predominant antiarrhythmic properties, identified *a posteriori*.

Exploring the bioisosteric relationship between the benzoyl and benzisoxazole groups, Strupczewski and coworkers of Hoechst-Roussell Laboratories [49], developed



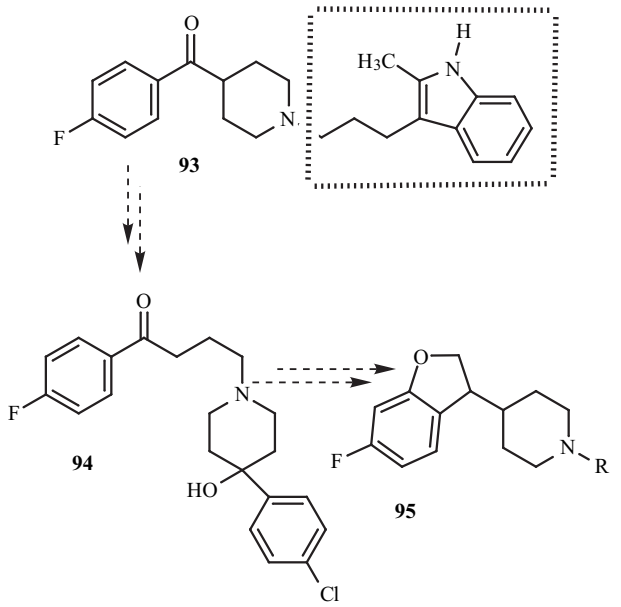
Scheme 24.

a new series of neuroleptic compounds of series 4-benzoylpiperidine, in which 93 (HP-291) is one of the main representatives. This substance is structurally related to haloperidol 94, the main representative of the butirophenones, a class of neuroleptics discovered by Janssen [50], in which, conceptually, the C-2, C-3 and C-4 carbon atoms of the butirophenone chain of 94 are contained in the piperidine moiety of 93, thus representing, a non-classic bioisostere of 94 (Scheme **26**). These authors further developed compound 95, in which a benzisoxazole nucleus bioisosterically replaces the 4-benzoyl moiety, represented by the 4-fluorobenzoyl unit in 93.



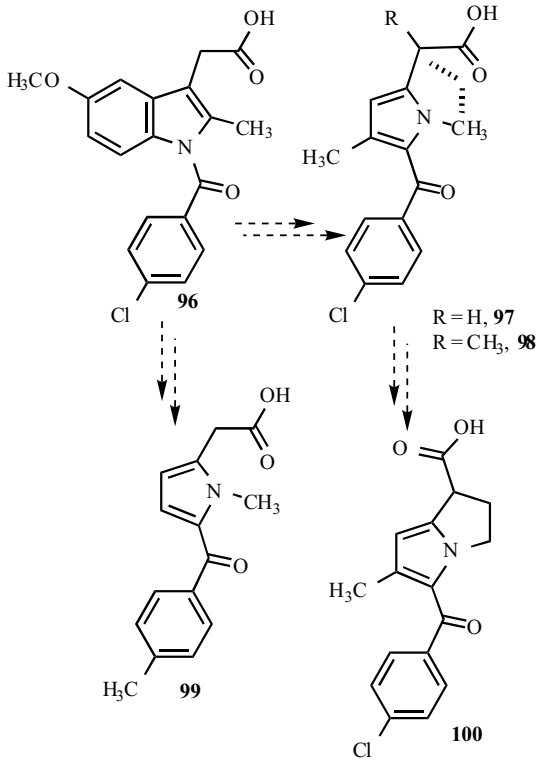
Scheme 25.

In the class of non-steroid anti-inflammatory drugs (NSAIDs) other examples were found of the use of the strategy of cyclic non-classical bioisosterism. In 1987, Muchowsky and coworkers [51], developed the systhesis of a new NSAID called cetroplac (100), designed from the cyclic non-classical bioisosterism strategy, exploring the ring closing between the acetic acid unit and the *N*-CH_3 moiety of lead compound tolmetin (99) (Scheme **27**) [52], a well known NSAID of the heteroarylacetic 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 Dolhme Laboratories in 1962 [54], applying the inverse principle, *i.e.* noncyclic nonclassical bioisosteric replacement.



Scheme 26.

In tolmetin (99) the indole subunit of indomethacin was replaced by the pyrrole ring carrying the benzoyl unit in C-2, as the bioequivalent of the *para*-chlorobenzoyl subunit of 96. This same approach was taken by Carlson developing zomepirac (97) (Scheme **27**) [53], another NSAI agent of the pyrrole-2-acetic acid class. This compound is an analog of 99, presenting as the only structural difference the presence of the methyl group in C-3, and may, therefore, be considered a homolog of 99.

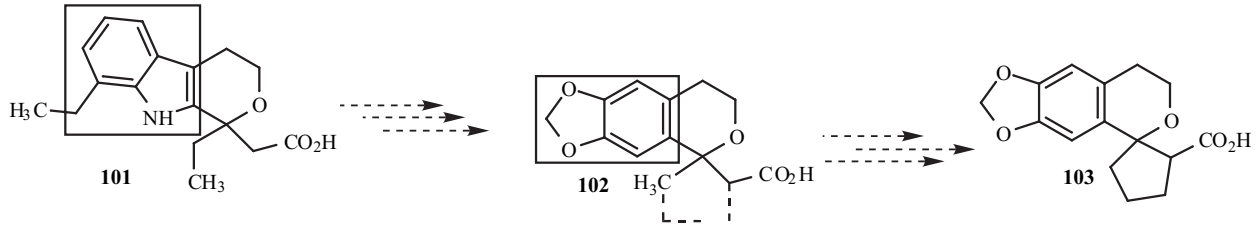


Scheme 27.

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 heteroarylacetic derivatives, as presenting a profile of anti-inflammatory properties greater then those of the acetic series. Substance 98, in fact, was seen to possess anti-inflammatory properties greater than 99 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-acyl-1, 2-dihydro-3*H*-pyrrol-(1,2-*a*)-pyrrolyl carboxylic acid family, represents, at the same time, a bioisostere of 99 and 97 (Scheme **27**), by *anelation* of the main pharmacophore unit of this family and is a new lead compound of the NSAIDs.

In 1995, Cabral and Barreiro (Scheme **28**) applied non-classic ring bioisosterism 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 *spiro*-isochromanyl acid derivative (103) when compared to lead compounds (101) and (102).

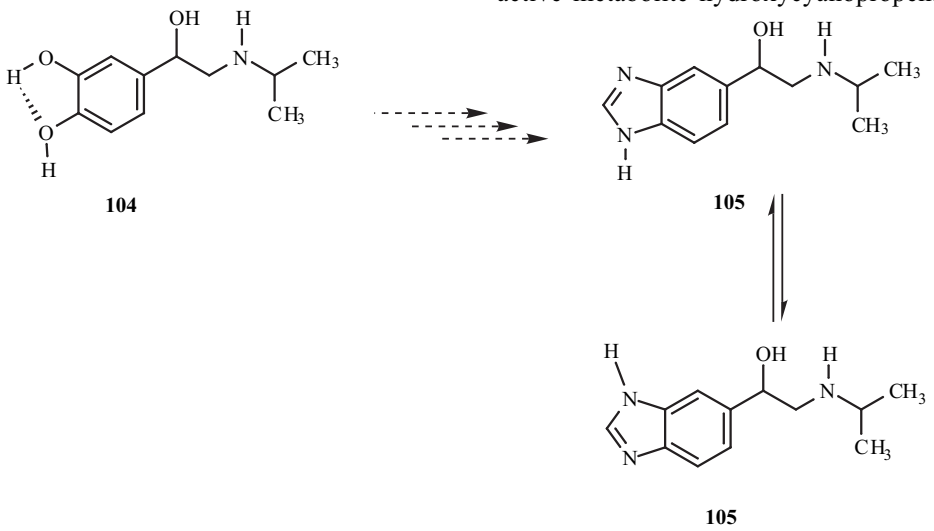


Scheme 28.

A supplementary example of bioisosterism, may be found in the work by Arnett and coworkers [57]. These authors developed the synthesis of compound 105, exploring the bioisosteric relationship existing between the benzimidazole rings and catechol subunit (Scheme **29**), based on isoproterenol 104, a compound with β-adrenergic activity. Compound 105 proved to be a bioisostere of 104, 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 catecholic group present in 104.

synthase-2 (PGHS-2) inhibitors, as well as to demonstrate the electronic similarities existing between the indanone and benzodioxole rings, suggesting similar profiles of supramolecular interaction with the enzyme involved in the pharmacological response of the flusolide and the derivative LASSBio 341 (107), possessing the benzodioxole moiety from safrole, an abundant natural alilbenzene present in *Piper spp* and *Ocotea spp.*

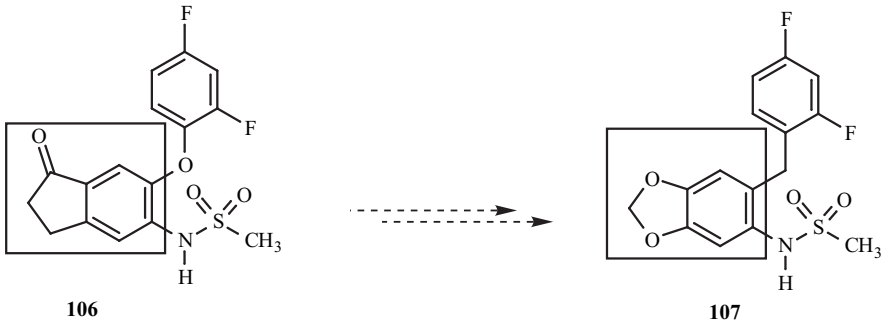
Leflunomide (108), an isoxazole immunosuppressor drug that can prevent antibody-mediates rejection in heart xenotransplantation, is a pro-drug metabolized *in vivo* in its active metabolite hydroxycyanopropenamide (109). In this



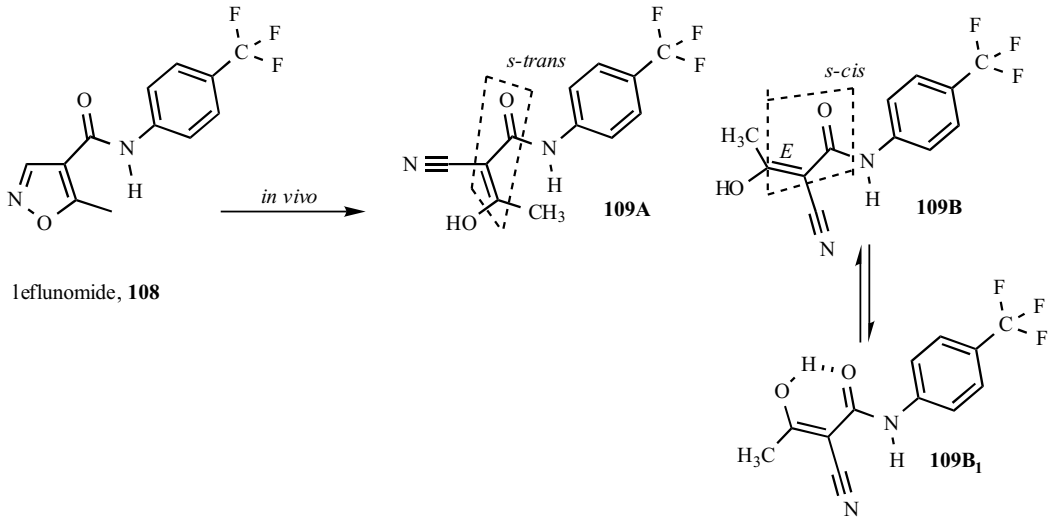
Scheme 29.

In a similar context, Barreiro and coworkers described the non-classic bioisosteric relationship between the indanone ring, present in the structure of the prototype flusolide (106) and benzodioxole unit present in compound 107 (Scheme **30**) [58,88]. The results obtained from this study allow us to identify a new molecular standard of prostaglandin H-

way, knowledge of the physical, chemical, conformational and configurational characteristics of this metabolite are essential to lead to the design of new immunosuppressive drugs, acting through mechanisms of action common to leflunomide (108). Among the conformational and configurational possibilities, relative to the α,β-unsaturated



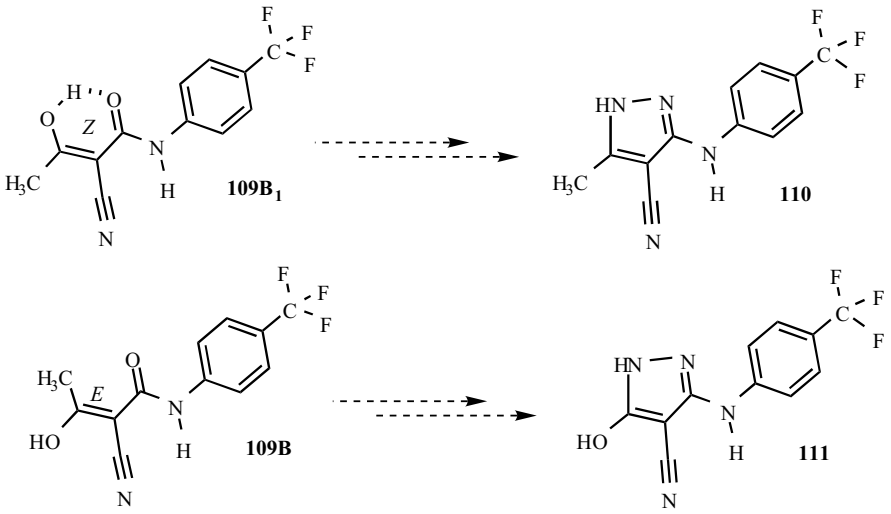
Scheme 30.



Scheme 31.

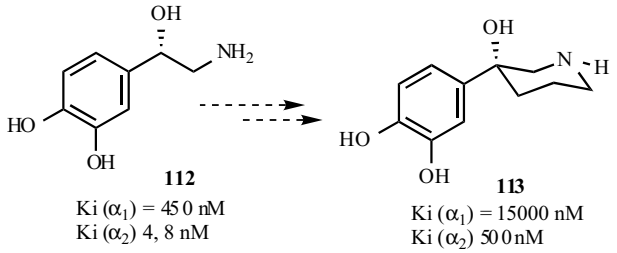
carbonyl system, referring to the double bond geometry, possible for the functionalized hydroxycyanopropenamide (109), the *S-cis* conformer (109B) and *Z*-configuration derivative (109B1), were found in X-ray crystallography studies (Scheme **31**). The presence of this single diastereoisomer might be due to the stabililization from the formation of intramolecular hydrogen bonds involving hydrogen in the enolic system with the oxigen of amide function [59]. In an effort to corroborate with this experimental evidence, and to corelate it with the eventual bioactive conformation of hydroxycyanopropenamide (109), Papageorgiou and coworkers proposed modifications in the structure of metabolite 109B and 109B$_1$, applying non-classic strategies of ring closing bioisosterism (Scheme **32**). In this study it was possible to design two novel pyrazoles analogs (110 and 111) conformationally restricted which mimic the diastereoisomeres *E* and *Z* of hydroxycyanopropenamide (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 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 (B1) and 110. In summary, this study allowed for identification of a new bioisosteric relationship between the pyrazole ring and the hydroxycyanopropenamide subunit, as well as validating the elucidation of the bioactive conformation of the active metabolite of leflunomide (108).

It is important to point out that the greatest conformational restriction ever reached by way of a anelation process may annul 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 acyclic lead compound. This concept may be well illustrated by the works of Macchia and coworkers who described the attainment of piperidinol (DDP3, 113) from noradrenaline (112) (Scheme **33**), applying the strategy of anelation or ring closing [60]. In this study, the authors observed an expressive loss of affinity by the α_1 and α_2-adrenoceptors observed for the DDP3 derivative (113), attributed to the



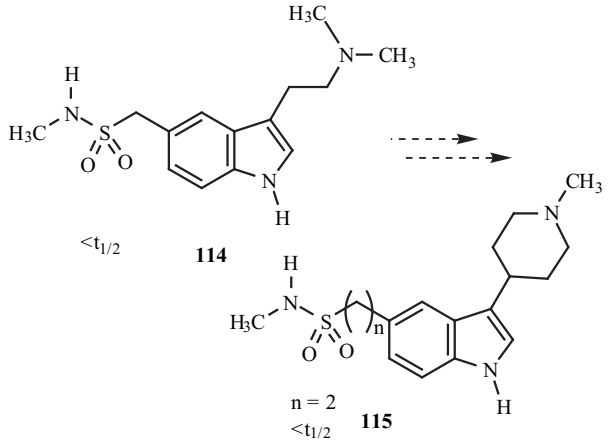
Scheme 32.

process of anelation of 2-hydroxyethylamine subunit, common to noradrenaline (112).



Scheme 33.

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

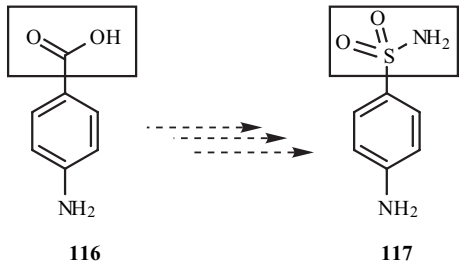


Scheme 34.

6.2. Non-Classic Bioisosterism of Functional Groups

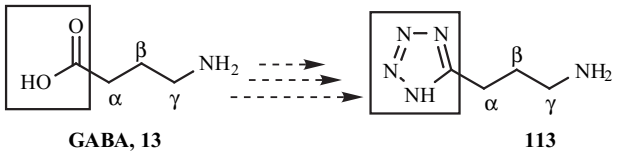
The landmark of the recognition of the importance of the bioisosterism of functional groups is the discovery of the antibacterial properties of sulfanilamide (117). Sulfanilamide (117), an active metabolite of Prontosil® [62], revolutionized chemotherapy during the 30s and the later elucidation of its mechanism of molecular action allowed evidence of the similarity of its structure with *para*-aminobenzoic acid (PABA, 116). These 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 sulfonamide (SO_2NH_2) and carboxylic acid functionalities (CO_2H) [63] (Scheme **35**).

Diverse functional groups are known for their bioisosteric relationships with the carboxylate group. Some examples of the bioisosteric replacement of –COOH by a tetrazole group are known [64]. An illustrative example is described in the synthesis of 118, a tetrazole bioisostere of γ-aminobutyric acid (GABA, 13), which presents important selective inhibitory properties of GABA-transaminase (GABA-T) (Scheme **36**) [65] presenting a potential pharmacotherapeutic application as an anticonvulsant agent.



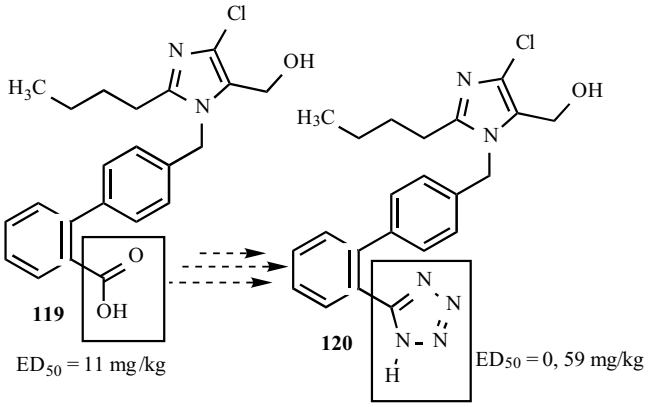
Scheme 35.

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



Scheme 36.

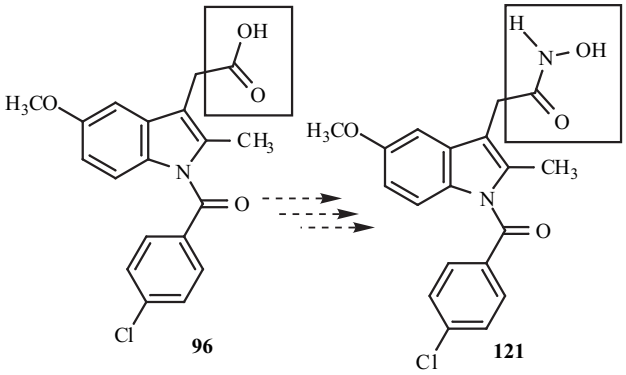
Another more recent example of the bioisosteric relationship existing between tetrazole and carboxylic acid groups may be seen by comparing the structures of losartan (120), angiotensin receptors antagonist approved in 1995 to treat hypertension with the lead compound EXP 7711 (119) (Scheme **37**) [66].



Scheme 37.

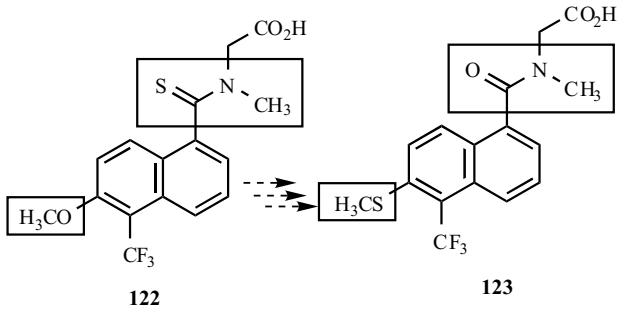
Derivatives possessing a hydroxamic-acid instead of a carboxylic acid function have been developed as carboxylate bioisosteres. Among other examples, compounds with anti-allergic properties presenting this function have been synthesized [67-68]. While in certain cases the metabolic lability of -CONHOH function, has been demonstrated, which would merely characterize a pro-drug effect [69], hydroxamic derivative 121 (Scheme **38**), designed by

modifications in indomethacin (96), has proved to be metabolically stable, evidencing the non-classic bioisosteric relationship existing between these two groups (CO_2H x CONHOH).



Scheme 38.

The discovery of oxatolrestat 123 [70], designed as a classic bioisostere of tolrestat 122 (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-).



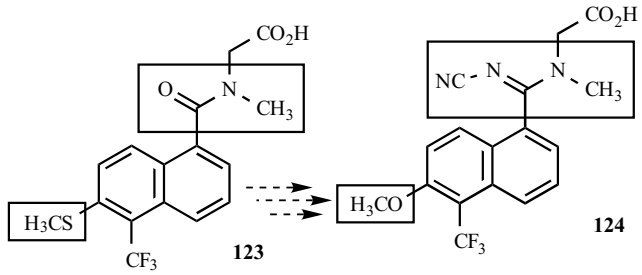
Scheme 39.

Tolrestat 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 -OMe (122) and –SMe (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, *i.e.*: A-CONH-B ⇒ A-NHCO-B and alkylydene (-CH=CH_2) [4,72].

Wrobel and coworkers used the principle of non-classic bioisosterism of functional groups to propose the synthesis of analog 124, from modifications in the lead compound oxatolrestat 123 (Scheme **40**) [70], identifying the bioisosteric relationship between the thioamide and cyanoamidine functions.

This same type of bioisosteric relationship between the thiocarbonyl (-C=S) and cyanoimine (-C=N-CN) groups was reported during the development of cimetidine by Ganellin and coworkers [71].



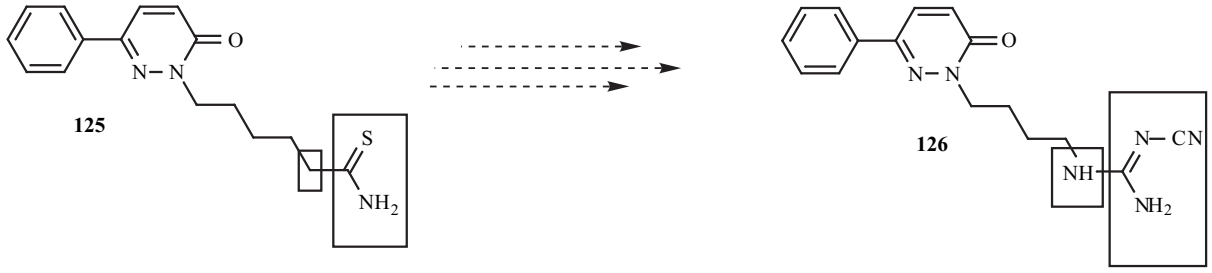
Scheme 40.

The same type of bioisosterism was further used by Yamanada and coworkers to develop pyridazinone derivatives 125 and 126 (Scheme **41**), which present a similar spectrum of antiulcerative activity, while also presenting a classic bioisosteric relationship between the substituents of the alkyl chain represented by the replacement of -CH_2- with–NH- group [73].

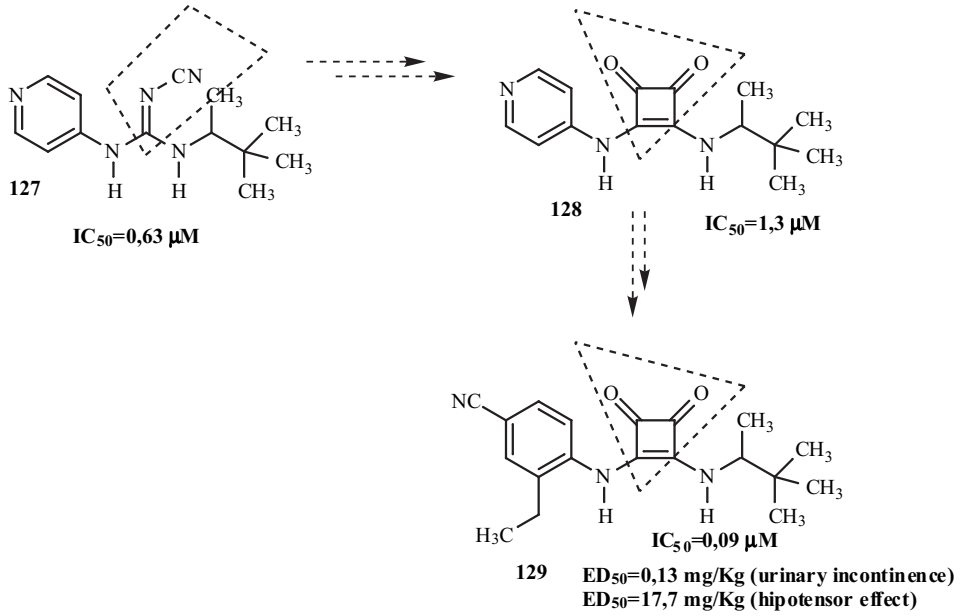
In the quest for new selective agonists of potassium channels Butera and coworkers carried out modifications in the structure of anti-hypertensive lead compound pinacidil (127) (Scheme **42**) [74]. These authors described the replacement of the *N*-cyanoguanidine template, present in pinacidil (127), with a 1,2-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 biososterism of functional groups may be exemplified by the isosteric relationship existing between the sulfonylhydrazone and acylhydrazone functions, identified in studies by Lima and coworkers (Scheme **43**) [75]. In this work, the authors demonstrated that the acylhydrazone function (CONHN=CH) may be efficiently replaced by the sulfonylhydrazone function (SO_2NHN=CH) 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 torasemide (132), Wounters and coworkers demonstrated the bioisosteric relationship existing between the sulfonylureia



Scheme 41.

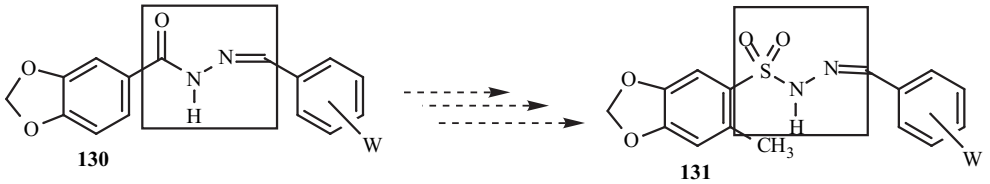


Scheme 42.

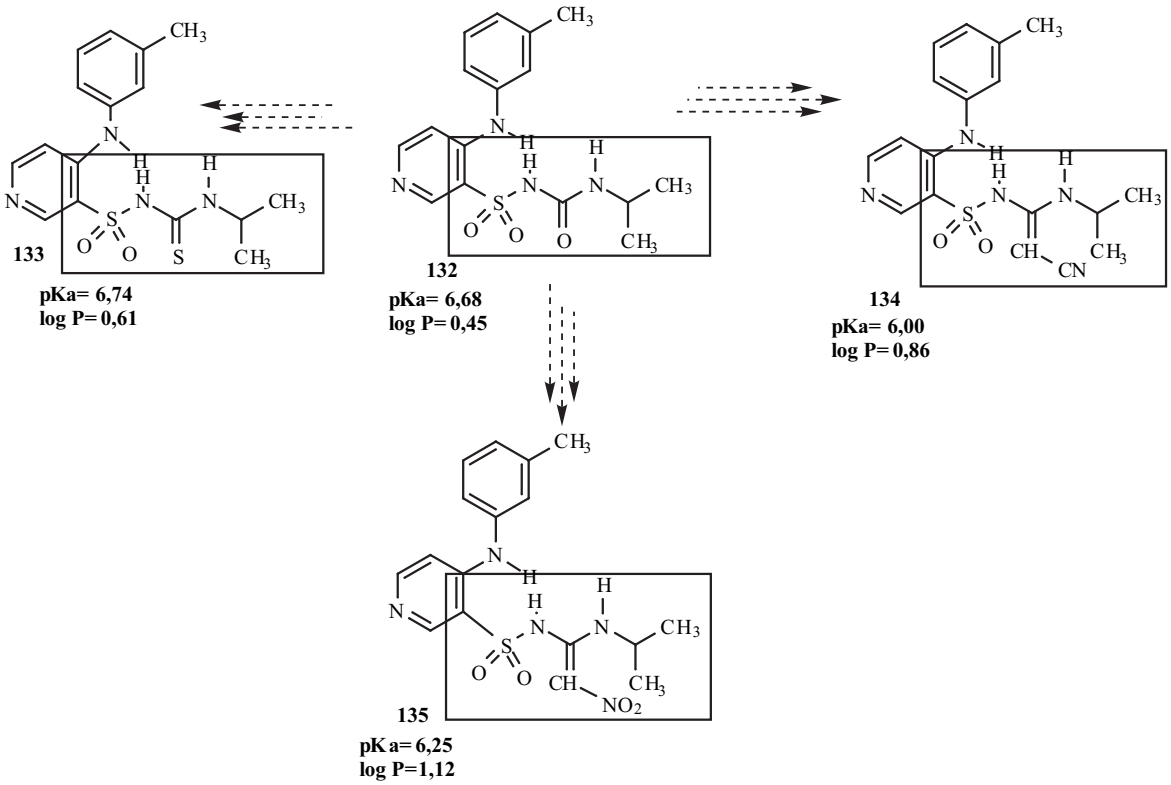
(132), sulfonylthioureia (133), sulfonylcyanoguanidine (134) and sulfonylaminonitroethylene (135) functionalities (Scheme **44**) [76]. These authors demonstrated that while these groups present similar electronic and geometric properties, determined by X-ray crystallography and similar acidity (pKa 6.0 to 6.7), compounds 132, 133, 134 and 135 each possess a distinct lipophilicity, expressed in logP 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 Na^+ K^+ $2Cl^-$ 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 hydrophylic compounds, while tubular reabsorption is increased for lipophilic compounds.

The importance of ionotropic receptors of glutamate (iGlu), the principal excitatory neurotransmittor 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 pharmokinetic 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 (15) with 3-hydroxy-1,2,5-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-hydroxyimidazole ring 138 may be explained by the basicity of this compound. Actually, the experimental determination of pKa value of the *N*-hydroxy group of 138 was found to be at least three pKa units higher than those found for the 3-hydroxy-1,2,5-thiadiazole, 1-hydroxy-1,2,3-triazole and 1-hydroxypyrazole subunits.

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, Jimonet and coworkers described the attainment of imidazo[1,2-*a*]indeno-[1,2-*e*]pyrazin- 2-substituted derivatives (Scheme **46**) [78]. These authors proposed the exchange of the carboxylic acid function in C-2 (140) by the phosphonic acid (141), tetrazole (142) and *N*-methylsulfonylcarboxamide groups (143) (Scheme **46**). The results obtained show that all of the isosteres 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.

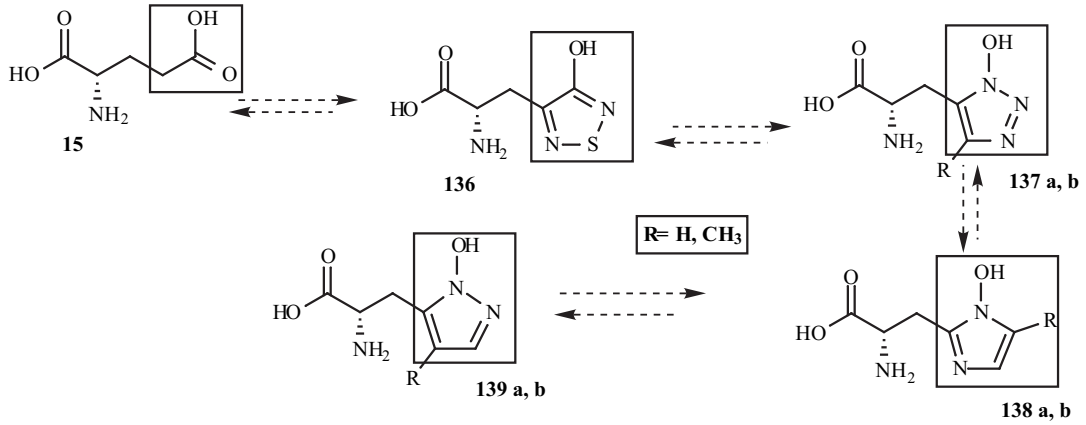


Scheme 44.

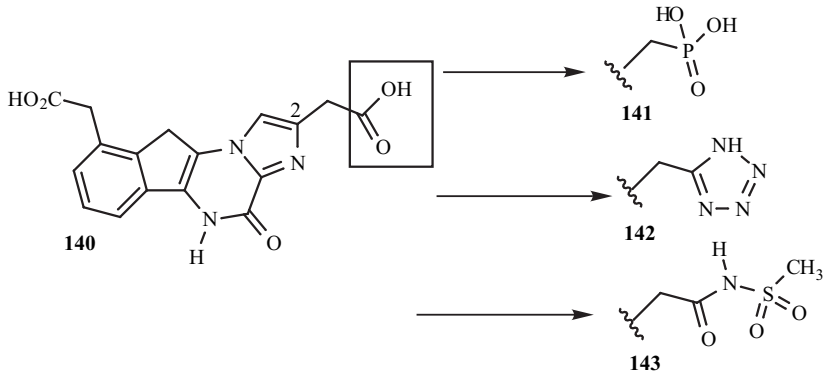
proves to be approximately 10 times more selective than the corresponding carboxylic acid derivative (140) .

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 bioisosteric 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 bioisosteric strategies. The bioisosterism of methylsulfonamide (CH_3SO_2NH-), sulfonylamine (-SO_2NHCH_3) 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 (ion-ion) with amino acid residues from the active site of PGHS, (the example of Arg_{513} residue), being, however, an authentic bioisostere of methylsulfonamide and methylsulfone functions, presenting comparable molecular volume and electrostatic potential.

Quinuclidine (148), designed by modifications in the natural alkaloid structure arecoline (147), is an important non-selective muscarinics (M1 and M2) receptors agonist with marginal actions on nicotinic ganglionar 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



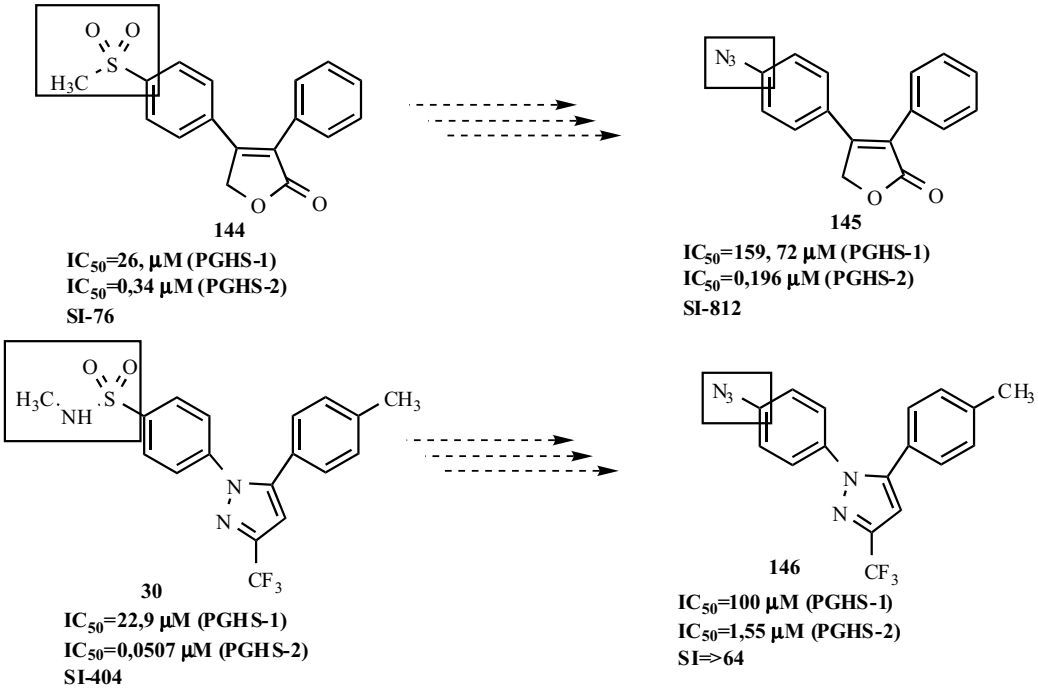
Scheme 45.



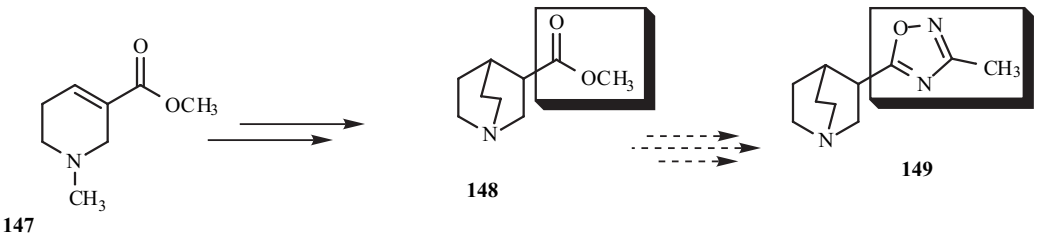
Scheme 46.

pharmacotherapeutics profile of quinuclidine (148), Orlek and coworkers proposed the exchange of the methyl ester group, present in 148, with the 3-methyl-1,2,4-oxadiazole group in the structure of compound 149 (Scheme **48**) [80], being able to identify electrostatic similarities between the methyl-oxadiazole and methyl ester groups, as well as in the profile of muscarinic agonist activity of these derivatives. However, the metabolic stability of methyl-oxadiazole bioisostere (149) was greater than to that found for the quinuclidine lead compound (148), conferring to compound 149 a better oral bioavailability.

Another successful example of a bioisosteric replacement of an ester functionality with an oxadiazole moiety, for improving the metabolic stability, can be illustrated in the development of cyclin-dependent kinase 2 (CDK2) inhibitors as anticancer agents. Despite the potent CDK2 inhibitory activity found for compound 2-amino 5-thio-substituted thiazole (150), 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 a attempts to increase the cellular stability of 150, Kim and coworkers realized the

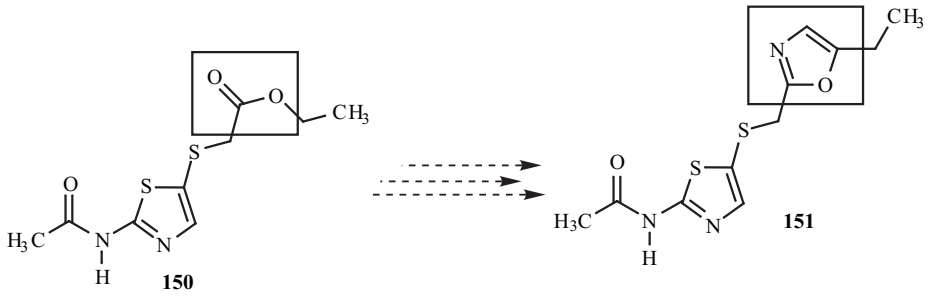


Scheme 47.



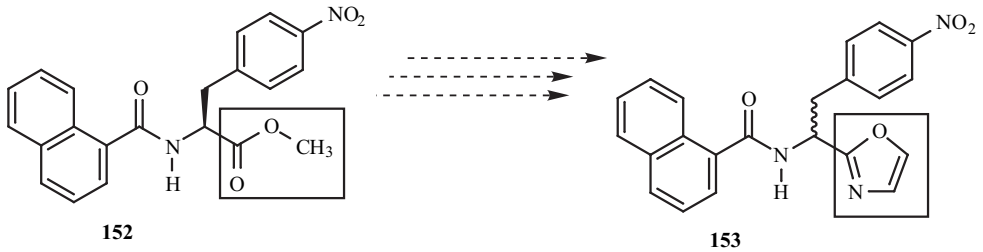
Scheme 48.

replacement of the ethyl ester with a bioisostere oxazole moiety led to compound 151 (Scheme **49**), which is metabolically stable against esterases, maintains the selectivity and shows increased CDK2 inhibitory potency as well as potent antiproliferative activity in cancer cell lines [7].



Scheme 49.

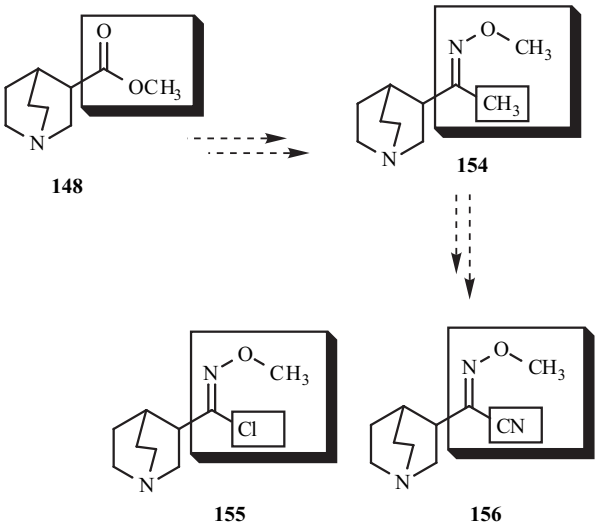
Nonetheless, bioisosteric replacement of the ester with oxazole and other heteroaromatics may not be universally effective, as demonstrated by Dhanak and coworkers in the design and synthesis of a series of highly selective and potent phenylalanine derived CCR2 antagonists as anti-inflammatory agents (Scheme **50**). While compound 152 was highly effective in blocking both the binding and the functional activity of a number of CCR3 agonists, compound 153 lacks all CCR affinity which suggests a more subtle role for the ester moiety that the heterocycles were unable to mimic [7].



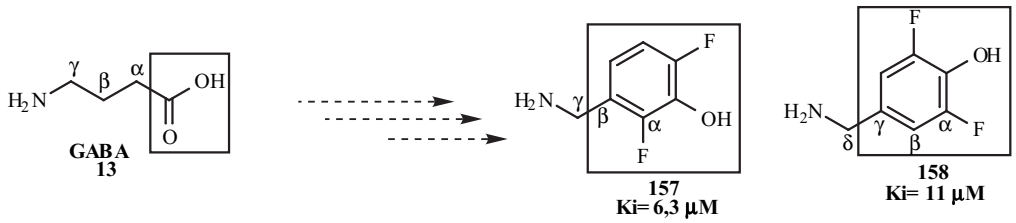
Scheme 50.

To identify non-aromatic bioisosteres, alternatives of carboxylic esters, Bromidge and coworkers described the synthesis and determination of the affinity of ketoxime ethers derivatives (154-156) for muscarinic receptors in rat cerebral cortex (Scheme **51**) [81]. In this study it was possible to confirm the bioisosteric relationship existing between the methyl ester (148) and (*E*)-ketoxime ethers (154) functions, and the later use of additional classic bioisosteric exchanges of monovalent 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 bioisosterism in drug designing can be well illustrated by planning 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 its use impossible for treating epilepsy, in view of its rapid inactivation and excessively low logP. In their search for GABA aminotransferase inhibitors, the enzyme responsible for GABA catabolism, Qiu and coworkers proposed structural modifications in the enzymatic substrate, *i.e.* GABA based on the application of non-classic bioisosterism of functional groups [82]. In this study, the authors carried out the exchange of the carboxylic acid group (pKa = *ca.* 4,50) present in GABA with 2,4-difluorophenol (pKa= 7,1) subunit present in compounds 157 and 158 (Scheme **52**), observing that both compounds were competitive inhibitors of GABA aminotransferase having Ki 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-difluorophenol group may be an authentic lipophilic bioisostere of carboxylic acids.



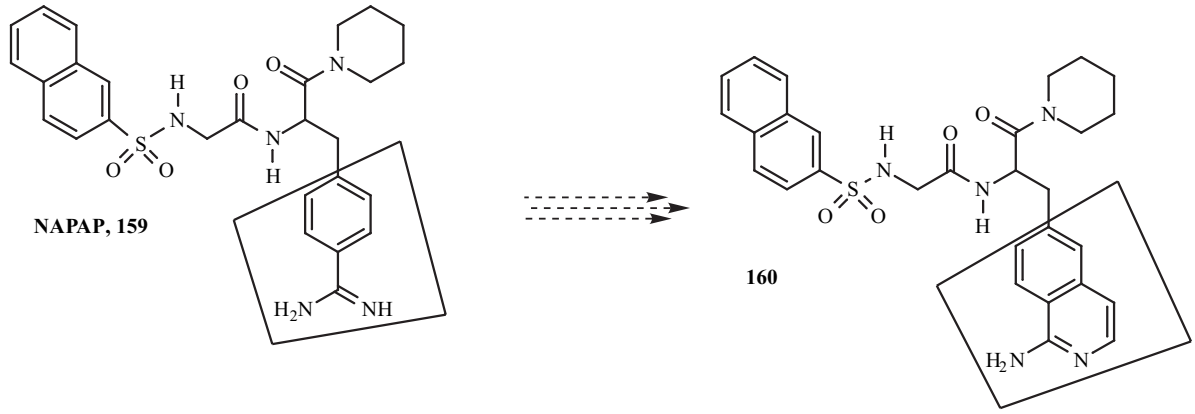
Scheme 51.



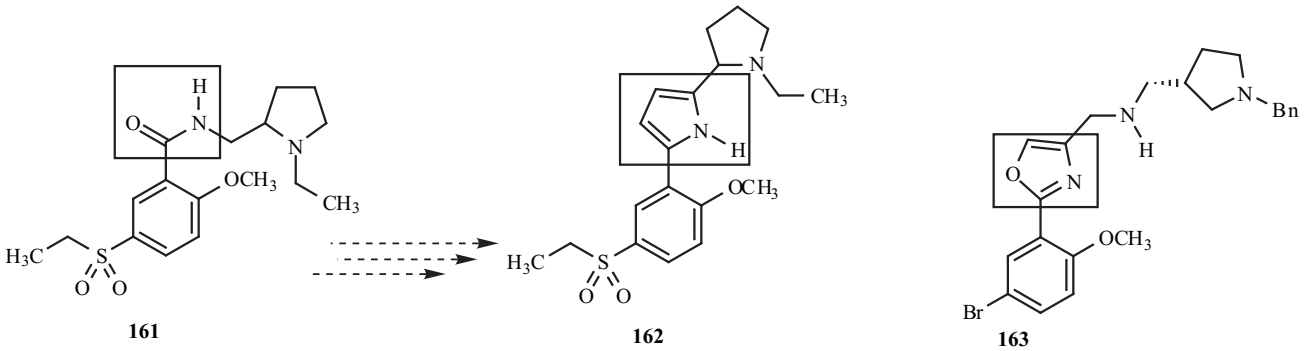
Scheme 52.

The anticoagulant activity reached by way of thrombine inhibition has proved to be of landmark therapeutic relevance, in view of the recent FDA approval of argatroban approved to treat thromboembolic disorders. [83]. However, the great challenge of this class of therapeutic drugs is in obtaining lead compounds of adequate oral bioavailability. NAPAP (159), (Scheme **53**) is a high affinity reversible inhibitor of thrombine (Ki= 6 nM) and low oral bioavailability. Many studies have suggested that the inadequate pharmacokinetic properties of NAPAP (159) and its structurally related derivatives is due to the elevated basicity of the benzamidine function (160, pKa=12). To deal with these pharmacokinetic limitations, Rewinkel and coworkers proposed the bioisosteric exchange of the benzamidine group by the 1-aminoisoquinoline function (Scheme **53**) [84]. In this study the authors demonstrated that the lower basicity of the 1-aminoisoquinoline group (pKa=7,5) conferred a greater absorption to compound (160) determined using cell monolayers as a model for intestinal absorption (*Caco-2*) [85]. Nonetheless, the inherent basicity of this function (160, pKa= 7,5) is sufficient to guarranty ionic interactions with Asp_{189} amino acid residue, present at the thrombine catalytic site and facilitate membrane permeability. Thus, replacement of the highly basic benzamidine moiety in NAPAP (159) with 1-aminoquinoline yielded new thrombin inhibitor and confirming a new existing bioisosteric relationship between these functions.

In the course of searching for high affinity dopamine D3 receptor antagonists, conformationally restricted benzamide isosteres, such as pyrroles, oxazoles, and thiazoles were investigated by Einsiedel and coworkers (Scheme **54**). Replacement of the amide sultopride (161) by a pyrrole ring led to compound 162 which maintained affinity for the dopamine D3 receptor and introduced modest selectivity over the dopamine D2 receptor. Replacement of the benzamide with oxazole subunit resulted in compound 163,



Scheme 53.

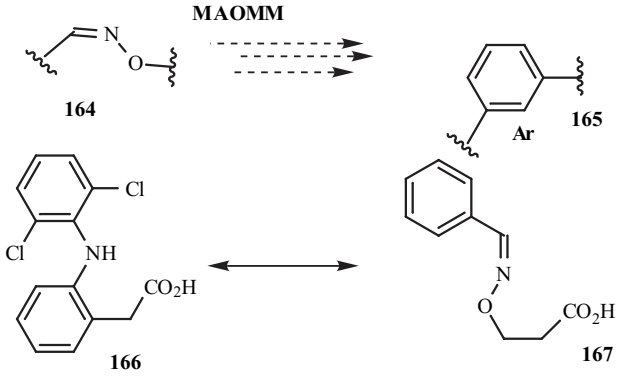


Scheme 54..

which exhibits dopamine D3 and dopamine D4 binding affinities comparable to those of the atypical neuroleptics sultopride and clozapine, respectively [7]

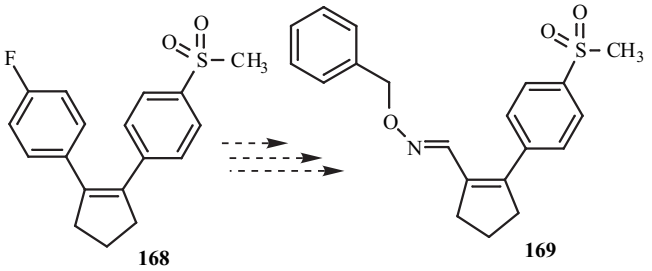
6.3. The Versatility of Non-Classic Bioisostersism

A spectacular example of non-classic bioisosterism may be illustrated by the work of Macchia and coworkers, involving the bioisosteric relationship between the benzene ring (165) and the methyleneaminoxy methyl moiety (MAOMM, 164) (Scheme **55**) [86]. 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 (Ph). The results observed in the anti-inflammatory activity of 167 and derivatives, in comparison with standard diclofenac (166), an aryl-acetic acid anti-inflammatory drug, prove this amazing bioisosteric relationship [6].



Scheme 55.

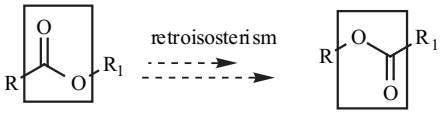
More recently, Balsamo and coworkers described the attainment of new selective inhibitors of PGHS-2 isoform, applying bioisosterism as a strategy of molecular modification [87]. This study also illustrates the non-classic bioisosteric relationship between aromatic rings and the methyleneaminoxy methyl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



Scheme 56.

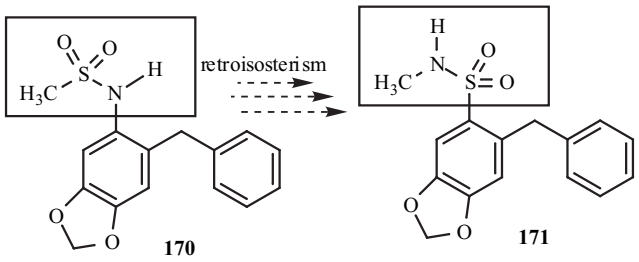
6.4. Retroisosterism

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 commented on in this paper, aims to optimize the pharmacotherapeutic properties of the original lead compound, thus aiding in optimizing the profile of interaction with the bioreceptor in designing drugs with half-lives more adequate for therapeutic use and may even be used in the attempt to avoid the formation of potentially toxic metabolic intermediates.



Scheme 57.

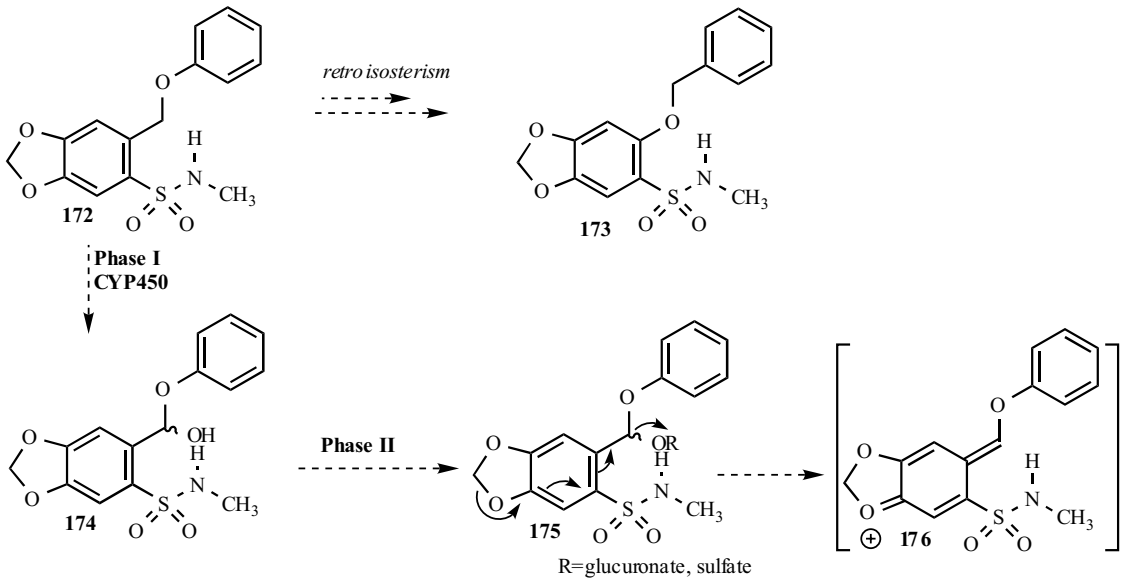
In a recent study, Lages and coworkers described the existing retroisosteric relationship between the methylsulfonylamine and methylsulfonamide functions (Scheme **58**) present in the structures of new selective COX-2 inhibitor lead compounds 170 and 171 [88]. This type of retroisosteric relationship 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 Arg_{513} and Ser_{353} 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 retroisosterism [14] and aimed to minimize the possibility of eletrophylic species formation (176) (Scheme **59**), potentially toxic, obtained by the action of enzymatic pool involved in the metabolism of xenobiotics. Furthermore, the use of retroisosterism 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.

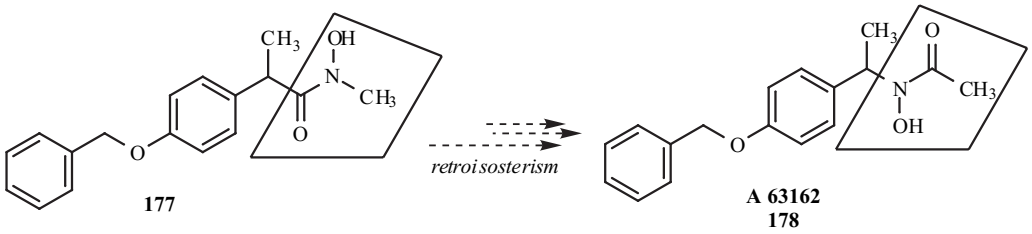
Among the different categories of 5-lipoxygenase inhibitors (5-LOX), the hydroxamic acids stand out acting through the formation of complexes with the Fe^{3+} ion present at the catalytic site of 5-LOX. It has been shown that although many hydroxamic acids are potent inhibitors of the enzyme *in vitro*, 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 hydroxamic



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 derivatives, which are unable to block leukotriene synthesis. Taken together these results suggest the pharmacophoric profile of hydroxamic acid subunit ($CON(OH)CH_3$).

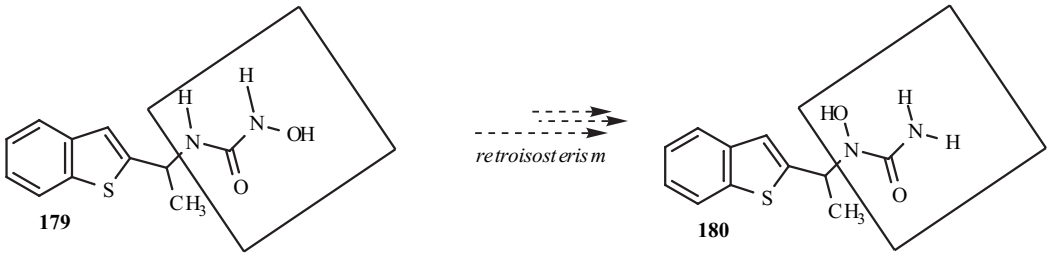
Later, Summers and coworkers (1998) [90] demonstrated that hydroxamic acids with 5-LOX inhibitor activity have small substituents (hydrogen or methyl) attached to the hydroxamic acid nitrogen and relatively large groups (aryl ring system) appended to the carbonyl group. Based on this observation, the authors proposed the attainment of the retroisostere 178 (A 63162), through the application of retroisosterism 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 administered orally to rats, this retroisostere produced higher plasma concentrations and longer duration. For example, compound 178 produced peak plasma concentrations in the rats of 140 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.



Scheme 60.

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 (ED_{50}= 8mg/Kg) was 5-fold more potent than 177 (ED_{50}= 40mg/Kg).

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



Scheme 61.

zileuton (180), the first selective 5-LOX inhibitor to receive approval by the FDA to treat asthma, discovered at Abbott Laboratories [91]. In analogy to the results obtained with hydroxamic acid 178 (A 63162), the application of the retroisosterism strategy in the structure of lead compound 179 allowed the attainment of *N*-hydroxyurea derivative 180 (zileuton) with a half life, oral bioavailability and inhibitory 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 bioisosterism represents in rational drug design a successful strategy, useful in the molecular modification and design of new therapeutically attractive substances of different pharmacological classes including the design of *me-too* drugs.

The correct use of the strategy of molecular modification also allows the identification of new classes of lead compound with attractive pharmacotherapeutic activity, minimizing 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 "*ad-hoc*", when determined theoretic principles of Medicinal Chemistry are carefully applied, thus allowing a prediction of the structural effects which govern phramacokinetic 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.

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