CHEMICAL REVIEWS

REVIEW

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The Methylation Effect in Medicinal Chemistry

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CONTENTS

1. INTRODUCTION: THE METHYL GROUP AND ITS BIOLOGICAL INTERACTIONS

The monovalent methyl group is derived from methane through the removal of a hydrogen atom,[1] and its etymology is directly related to the discovery of methanol.[2] The first reports of the use of methanol were from ancient Egyptians. In their embalming process, they used a mixture of substances obtained from the pyrolysis of wood shards that contained a significant amount of methyl alcohol.[2] Pure methanol was first isolated and described in 1661 by Robert Boyle, who called it "*spirit of box*," as it is the product of the distillation of "*Boxwood*," the generic name given to ca. 70 types of trees from the *Buxaceae* family. In 1834, the French chemists Jean-Baptiste Dumas and Eugene Peligot determined its elementary composition as CH_4O through combustion analysis. They introduced the term methyl to organic chemistry through a mistranslation from the Greek, *methy* = "wine" + *hȳlē* = wood (wood bark), when the intention was to define "wood alcohol" or methanol.[2]

The methyl group is very important in the molecular recognition of endogenous and exogenous organic compounds by bioreceptors. Although it only participates in London dispersion interactions, which are the weakest of all intermolecular interactions,[3] methyl groups have stereoelectronic effects[4] on micromolecules and biomacromolecules, thereby leading to diverse biological effects, including selectivity among bioreceptors, increased potency, and protection against enzyme metabolism.[5] Cognizant of the methyl group's importance in molecular recognition, Wermuth wrote:[5]

"The methyl group, so often considered as chemically inert, is able to alter deeply the pharmacological properties of a molecule."

Received: February 18, 2011
Published: June 01, 2011

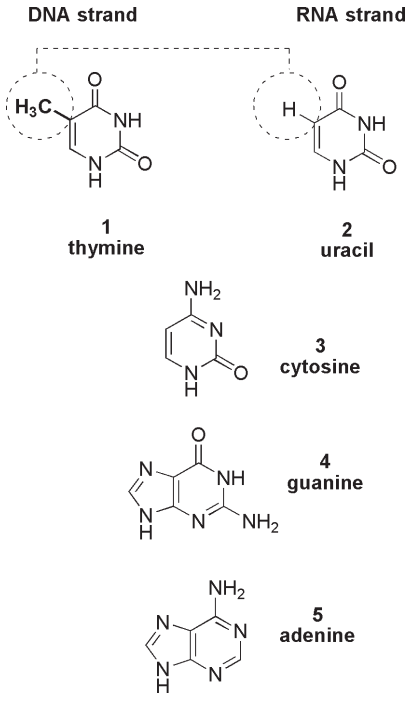


Figure 1. Nucleic bases of DNA and RNA and the structural difference between thymine (**1**) and uracil (**2**).

The stereoelectronic changes promoted by methyl groups are directly involved in many biological process.[6] For example, in pyrimidine bases (**1**, **2**, **3**), one difference between DNA and RNA is the exchange of a thymine (**1**) for a uracil (**2**), two pyrimidine bases that are differentiated by a methyl group at position 5 of the pyrimidine ring of (**1**) (Figure 1). Multiple biochemical events, including replication, transcription, and nucleosome recognition, are dependent on DNA conformation, which might be directly related to the CH/π type interactions of the thymine methyl group with purine nucleotides in the DNA helix.[6−8]

Although the purine nucleotides present in DNA and RNA, i.e., guanine (**4**) and adenine (**5**), do not contain methyls, the purine alkaloids theophylline (**6**), theobromine (**7**), and caffeine (**8**) are excellent examples of the influence of methyl groups in natural products. These alkaloids are methyl derivatives of xanthine (**9**), and they are subsequently methylated by *S*-adenosylmethionine (SAM) (see section 4.1).[9] They are biosynthesized similarly to purine nucleotides and are found in large quantities in cocoa extract, coffee, and cola drinks (Scheme 1).[10,11]

The stimulating properties of drinks such as mate, tea, and coffee derive from hydrosoluble alkaloids that act as adenosine receptor antagonists and phosphodiesterase (PDE) inhibitors, resulting in increased cAMP and subsequent adrenaline release.[12] Depending on the target tissue, this action leads to central nervous system (CNS) stimulation, bronchial smooth muscle relaxation, and diuresis.[10,12−14] The activity profiles of these substances are not common and depend upon their methylation pattern. Caffeine (**8**) is the only trimethylated representative and the most lipophilic; it is the best CNS stimulant and has little influence on diuresis.[12,13] Theobromine (**7**) has little stimulatory action and acts more on diuresis and pulmonary muscle relaxation.[10] Finally, theophylline (**6**) is the strongest bronchodilator of the three and also acts as a diuretic, but it is practically devoid of excitatory activity.[10,14]

The differences between these pharmacological profiles occur because PDE has 11 distinct isoforms (PDE1−11) and the adenosine receptor has four (A_1, A_{2A}, A_{2B}, and A_3).[15−19] These isoforms display different distribution at the biophase, and the methylation pattern of these alkaloids might lend it stereoelectronic characteristics such as in lipophilicity, which promote different effects on different isoforms and target tissues.

2. EFFECTS OF METHYL GROUPS ON THE SOLUBILITY OF BIOACTIVE COMPOUNDS: INCREASE LIPOPHILICITY AND PROMOTE THE HYDROPHOBIC INTERACTIONS

The insertion of one or more methyl groups into a bioactive molecule makes it more lipophilic and theoretically less water-soluble. However, in some cases, the insertion of methyl groups into a molecule leads to increased solubility through mechanisms such as reduced H intramolecular interactions, increased hydrophobic interactions, changes in the ionization state of functional groups, and lower energy of the crystalline network.[5]

The increased lipophilicity of a compound increases its solubility in biomembranes.[20] Biomembranes are composed of phospholipids, which are determining factors for the passage of compounds from the gastrointestinal tract to target tissues and for their transport through the bloodstream.[21,22] The lipophilic effect may help in interactions with hydrophobic sites, as described for some HMG-CoA reductase inhibitors (see section 5).[23]

The introduction of methyl groups is expected to increase the lipophilicity of a molecule known as Log *P*, e.g., logarithm of the partition coefficient between *n*-octanol and water. For example, the addition of a methyl group to benzene to generate toluene raises its Log *P* from 2.13 to 2.69.[24] The methylation of the generic aromatic compound (R)-H to generate (R)-CH_3 leads to a positive increment of 0.52 (π_X) based on values of the Hansch constant (π), which are calculated through the following equation:[25−28]

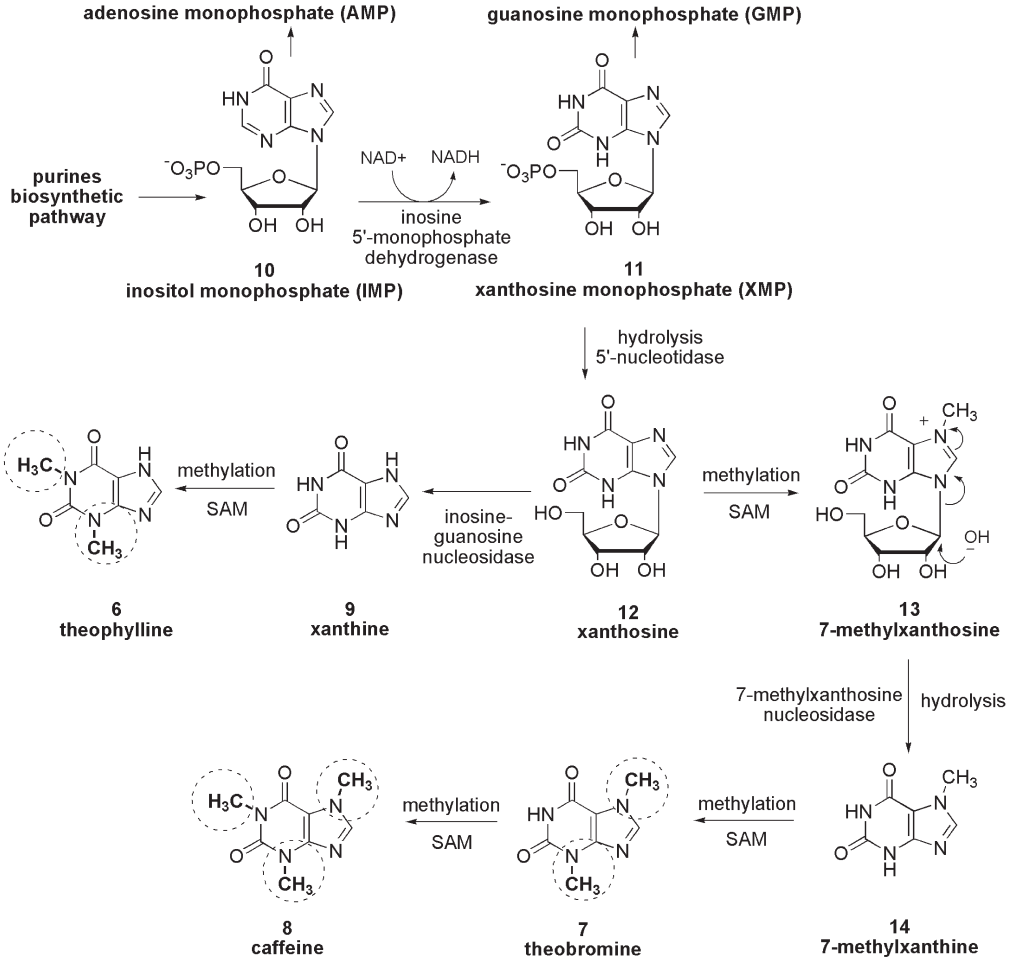
$$\pi_X = \mathrm{Log}(P_{\text{R-CH3}}/P_{\text{R-H}})$$

Increased lipophilicity due to methylation can drastically change the bioavailability and thus the efficacy of a bioactive molecule as well as its mode of interaction with bioreceptors. Dimethylation at C-2′ and C-6′ of the tyrosine unit of the synthetic opioid agent (2-D-penicillamine, 5-D-penicillamine]-enkephalin (DPDPE) (**15**) (Table 1)[29] to produce 2,6-dimethyl-L-tyrosine (DMT)-DPDPE (**16**) leads to increased affinity in vitro for both the δ-opioid receptor (10-fold, DPDPE K_i δ-receptor = 17.7 nM and DMT-DPDPE K_i δ-receptor = 1.8 nM) and the μ-opioid receptor (35-fold, DPDPE K_i μ-receptor = 2 018 nM and DMT-DPDPE K_i μ-receptor = 58.3 nM). Compared to nonmethylated DPDPE (**15**), DMT-DPDPE (**16**) has stronger analgesic activity in vivo as a result of its greater bioavailability (Table 2).[30,31]

A correlation between lipophilicity and activity can also be seen in a series of imidazolinediones (**17**−**20**) (Table 3).[32] The introduction of a methyl group to compounds **17** and **19** to generate **18** and **20**, respectively, increases their lipophilicity and their ability to dislocate rimonabant (SR-141716A) (**21**),[33,34] a specific antagonist for CB_1 cannabinoid receptors.

The addition of a methyl group to a specific molecule is usually done to increase its lipophilicity. However, there are exceptions, especially when this insertion causes the molecule to become

Scheme 1. Biosynthesis of Purine Nucleotides and Methylxanthines



more compact (more "globular") such as with aliphatic alcohols. As expected, the homologation of a methyl group to *n*-butanol (**22**) to generate *n*-pentanol (**23**) leads to an increase in lipophilicity and a decrease in solubility (Figure 2).[5] However, 2-pentanol (**25**) and (especially) neopentanol (**27**), in spite of having the homologation of one methyl in relation to *n*-butanol, are less lipophilic, which translates into higher solubility (Figure 2).[35]

This behavior may be explained by an entropic effect.[36,37] In aqueous solution, a molecule is trapped in a network of water molecules. Fewer water molecules are needed to cluster around a more compact molecule than an extended one (Figure 2). Therefore, compact structural arrangements are more favorable energetically.[5]

3. METHYLATED NATURAL PRODUCTS

Several examples of natural methylated products with recognized pharmacological activity are provided in Figure 3. The endogenous sex steroid hormones testosterone (**28**)[38] and progesterone (**29**)[39] play a key role in the development of male and female reproductive tissues. The hormone and neurotransmitter adrenaline (**30**) is a catecholamine produced by the adrenal glands from the amino acids phenylalanine and tyrosine,[40] and it increases heart rate,[41] regulates blood pressure,[42] and is involved in the fight-or-flight response of the sympathetic nervous system.[43] Like adrenaline (**30**), ephedrine (**31**) and pseudoephedrine (**32**), which are natural products obtained from plants, act at the adrenergic receptor level as bronchodilators,[44] thermogenic compounds,[45] and vasoconstrictors.[44,46]

Other plant alkaloids contain methyl groups that present distinct mechanisms of action. For example, nicotine (**33**) is a pyridine alkaloid that induces stimulation and pleasure and reduces stress.[47] The tyrosine alkaloids morphine (**34**, Figure 3) and tubocurarine (**35**) act as an analgesic[48] and a neuromuscular blocker, respectively.[49] The tropanic alkaloid cocaine (**36**) has a stimulatory effect on the central nervous system.[50] The more structurally complex natural compound paclitaxel (**37**) is used in cancer chemotherapy as a mitotic inhibitor; it was the first drug sold that acts by stabilizing microtubules.[51]

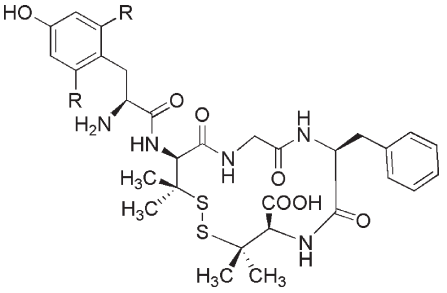
Vitamins B_1 (thiamine) (**38**) and B_2 (riboflavin) (**39**) are also examples of natural methylated products that play important roles in cell metabolism.[52,53] Their absence leads to diseases such as beriberi[52] (vitamin B_1 (**38**)) and iron absorption deficiency[54] (vitamin B_2 (**39**)).

3.1. Morphine and Methylated Analogues

Opium, which is obtained from latex extracted from *Papaver somniferum*, is composed of a mixture of over 20 different

dx.doi.org/10.1021/cr200060g |*Chem. Rev.* 2011, 111, 5215–5246

Table 1. Affinity for Opioid Receptors of DPDPE (15) and DMT-DPDPE (16)



15 R=H (DPDPE)
16 R=CH_3 (DMT-DPDPE)

compd	receptor binding K_i, nM: μ[a]	δ[b]	$K_i(\mu)/K_i(\delta)$
15	2018	17.7	114.0
16	58.3	1.8	39.4

[a] Based on displacement of [^{3}H]DAMGO. [b] Based on displacement of [^{3}H]DSLET.

Table 2. In Vivo Antinociceptive Activity of DMT-DPDPE (16) as Determined in Hot Plate and Phenylbenzoquinone (PBQ)-Induced Writhing Assays

compd	hot plate[a]: sc (mg/kg)	icv (mg)	PBQ writhing:[a] sc (mg/kg)
15	NT[b]	1.4	inactive
16	33	0.2	2.6

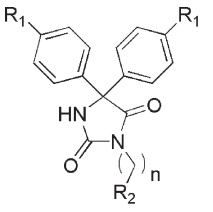
[a] ED_{50} value and 95% confidence limits. [b] Not tested due to the lack of effect of **16** in the less stringent writhing test.

alkaloids, with morphine being the main component and responsible for its analgesic activity.[55] Morphine (**34**) was first described in 1805 by the German pharmacist Sertürner,[56,57] although it was originally isolated by Seguin and Courtois in 1804.[58] Sertürner described the isolation of a white alkaline solid with hypnotic properties, which he called morphine (**34**) after the Greek god of sleep Morpheus.[56,57] The complex chemical structure of morphine was fully elucidated by Sir Robert Robinson in 1925,[59] who was awarded a Nobel Prize in Chemistry in 1947.

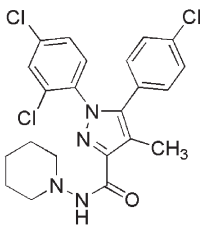
Morphine (**34**) is an analgesic that acts as an antagonist of the μ, κ, and δ opioid receptors (G-protein coupled receptors) at the central nervous system level.[48] Morphine provides a good illustration of the importance of methyl groups and how their simple introduction or removal may significantly alter the pharmacological activity of a bioactive compound, leading to different pharmacokinetic and pharmacodynamic profiles.

Studies of the structure−activity relationships of morphine (**34**) revealed that removal of the methyl group attached to the sp^3 nitrogen of its benzylisoquinoline ring to generate normorphine (**40**, Figure 4)[60] causes ca. 6-fold reduction in in vivo analgesic activity (morphine ED_{50} = 4.8 mg/kg and normorphine ED_{50} = 31.5 mg/kg in mice).[61] Analysis of the binding mode of morphine (**34**) to opioid receptors

Table 3. Structure and Affinity for CB_1 Cannabinoid Receptors of 3-Alkyl-5,5′-diphenylimidazolidinediones (17−20)



17-20



21
rimonabant

compd	R_1	n	R_2	percent displacement[a]
17	H	2	*N*-morpholine	<5
18	CH_3	2	*N*-morpholine	24
19	H	5	CH_3	36
20	CH_3	5	CH_3	47

[a] Results were obtained at 10 μM and are expressed as the percentage of the displaced specific binding of [^{3}H]SR-141716A (mean ± SEM, n = 3−5).

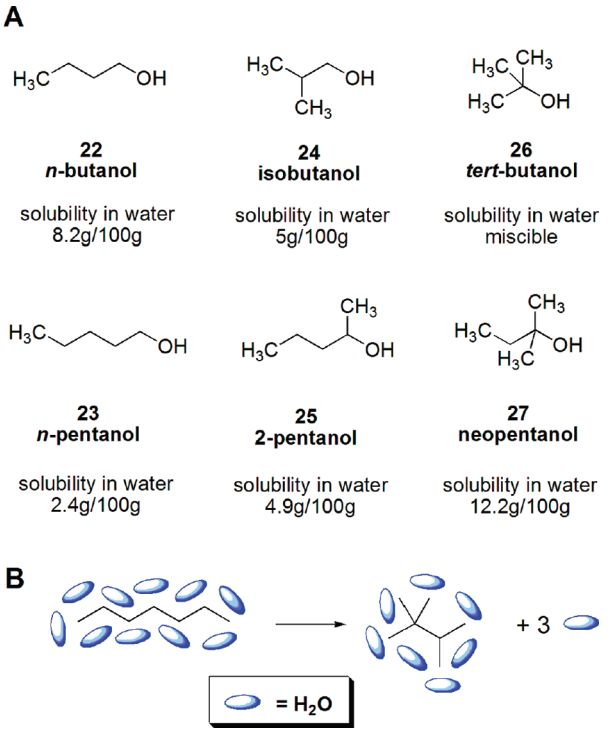


Figure 2. Solubility of different alcohols in water at 20 °C (A) and the representation of the entropic gain in solubilization of more folded compounds (B).

revealed that this reduction of activity is not due to pharmacodynamic factors, i.e., interactions with the target bioreceptor, because the main interactions with the catalytic site such as ionic ligation are maintained (Figure 4).[55,62,63] The pK_a^Hs of the nitrogens of the two compounds differ by just 0.48 (pK_a^H of morphine = 8.18 and pK_a^H of normorphine = 8.66);[64] they thus have similar ionization states in the biophase.

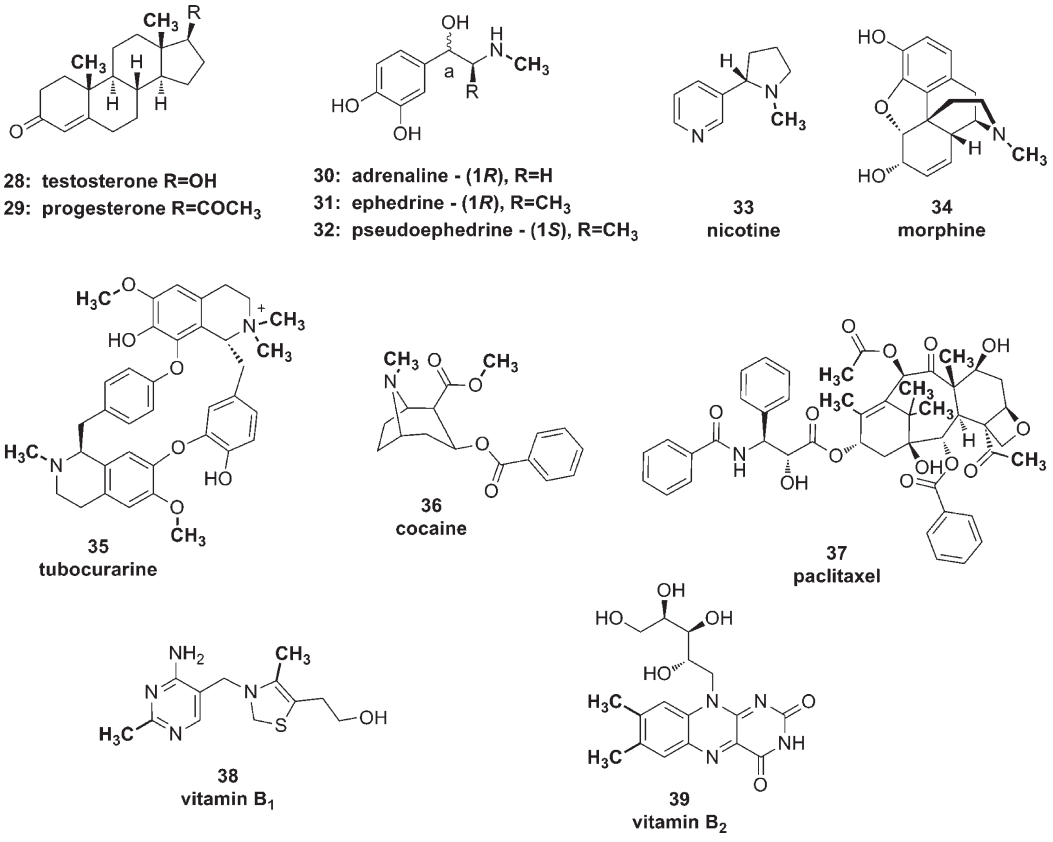


Figure 3. Example of methylated natural products.

The reduced activity of normorphine (**40**) is due to its more polar secondary nitrogen, which gives it physical-chemical activities different from those of morphine (**34**). Consequently, normorphine (**40**) has greater difficulty passing through the blood−brain barrier, where its target receptors are located. This theory is supported by the equipotent analgesic profile displayed by morphine (**34**) and normorphine (**40**), before electrostimulatory assays in mice.[65]

The simple *O*-methylation of morphine's phenolic hydroxyl at C-3 to generate codeine (**41**, Figure 5)[66] reduces its receptor affinity by 200-fold (**34**) (morphine K_i μ-receptor = 0.0018 μM and codeine K_i μ-receptor = 0.35 μM),[67] demonstrating that, in addition to being beneficial for an intended activity, methyl groups may also have a negative influence on activity. In this case, the introduction of a methyl group in the genesis of codeine (**41**) reduces its interaction with opioid receptors because the phenolic hydroxyl group of morphine is responsible for the hydrogen bond interactions with the receptor site (Figure 5).[62,63] Despite being 200-fold less potent in vitro, codeine (**41**) is only 3-fold less potent than morphine in vivo (**34**) (codeine ED_{50} = 14.5 mg/kg in mice).[61] This behavior is due to the demethylation of codeine (**41**) by a hepatic enzyme (CYP2D6), which converts a portion of it into morphine (**34**).[68,69] Codeine (**41**) is used for the treatment of moderate pain, cough, and diarrhea (Figure 5).[70]

Finally, heterocodeine (**42**, Figure 5), an *O*-methylated derivate at C-6, exhibits 2-fold more activity in vivo than morphine (**21**) (morphine ED_{50} = 0.75 mg/kg and normorphine ED_{50} = 0.48 mg/kg in cats).[71] Considering that heterocodeine's in vitro potency is roughly equivalent, methylation must effect its pharmacokinetic parameters, leading to increased lipophilicity and facilitating its passage into the central nervous system (Figure 5).

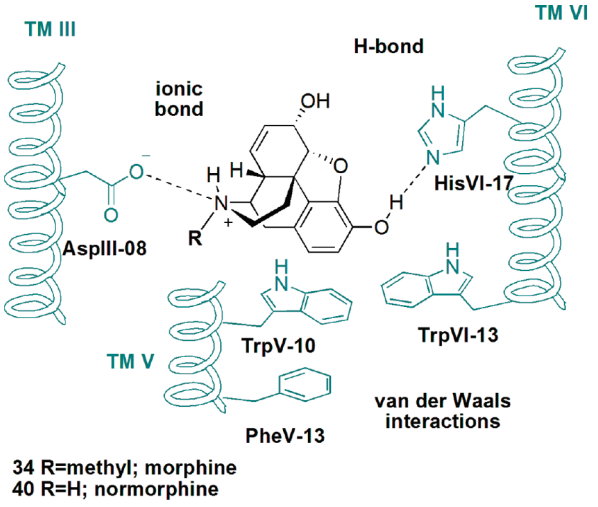


Figure 4. Representation of the interaction of morphine compounds and the opioid site. TM = transmembrane domain.

3.2. METHYL EFFECTS IN NATURAL AMINO ACIDS

Many of the nonpolar aliphatic amino acids, such as glycine (**43**), alanine (**44**), valine (**45**), isoleucine (**46**), leucine (**47**), and methionine (**48**), differ solely through homologation or inversion of the positions of methyl groups (Figure 6). For

dx.doi.org/10.1021/cr200060g |*Chem. Rev.* 2011, 111, 5215–5246

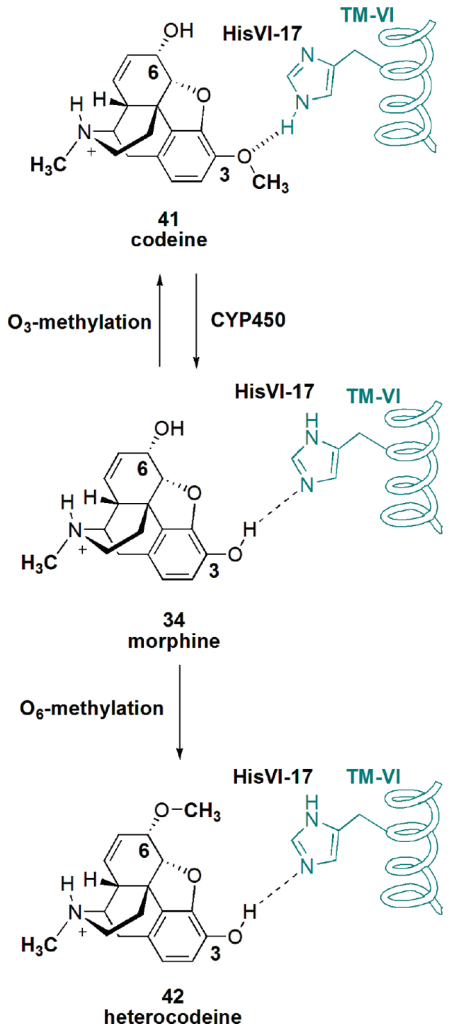


Figure 5. Representative illustration of the metabolism and receptor interactions of morphine derivatives. TM = transmembrane domain.

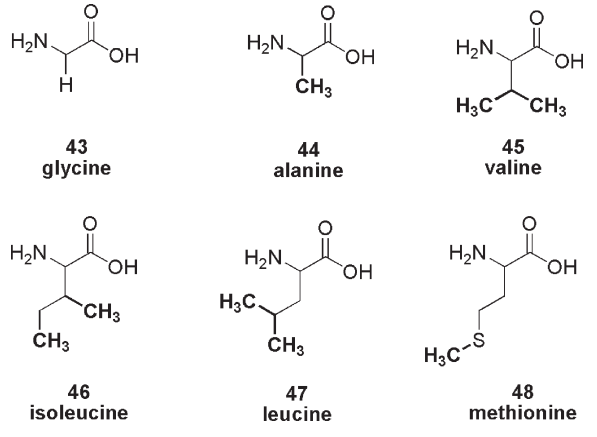


Figure 6. Aliphatic nonpolar amino acids and differences in their methylation patterns.

example, isoleucine (**46**) has one homologated methyl in comparison to valine (**45**). Moreover, alanine (**44**) is the methyl-homologue of glycine (**43**).

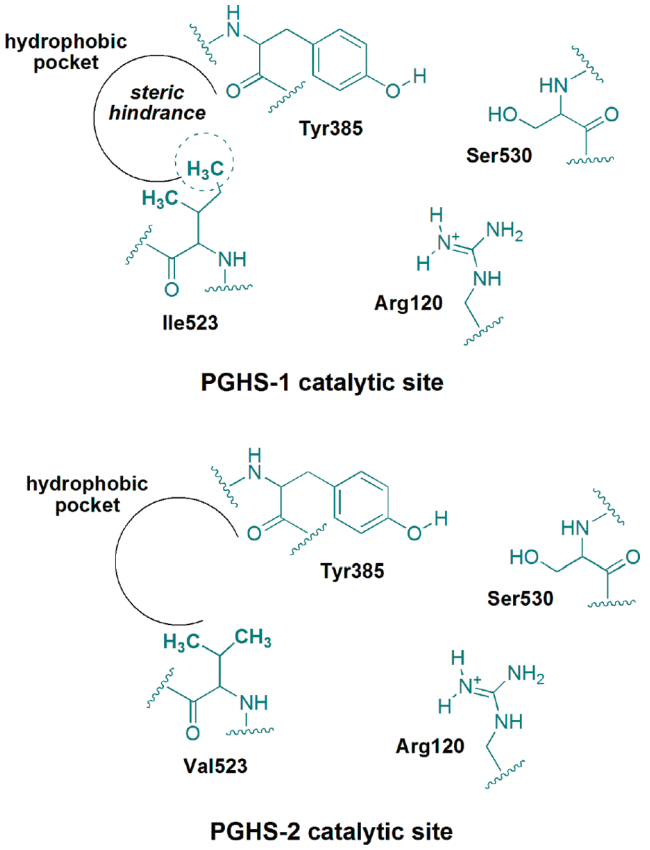


Figure 7. Representation showing the accessibility of the side hydrophobic pocket in the catalytic site of PGHS-1 and -2.

The presence or absence of a methyl group constitutes the key difference between the catalytic sites of prostaglandin endoperoxide synthases 1 and 2 (PGHS-1 and 2),[72,73] the enzymes responsible for the conversion of arachidonic acid (AA) to prostaglandins (PG) and the therapeutic target of nonsteroidal anti-inflammatory drugs (NSAIDs).[74] PGHS-1 has an isoleucine residue (MR = 34.48 cm^3/mol) (**46**) in position 523, whereas PGHS-2 has a valine (**45**) (Figure 6).[72] The smaller volume of valine (MR = 29.89 cm^3/mol) (**45**) allows access to a hydrophobic pocket in PGHS-2,[75] which was exploited by several research groups in the construction of selective PGHS-2 inhibitors such as SC-558[76] (Figure 7).

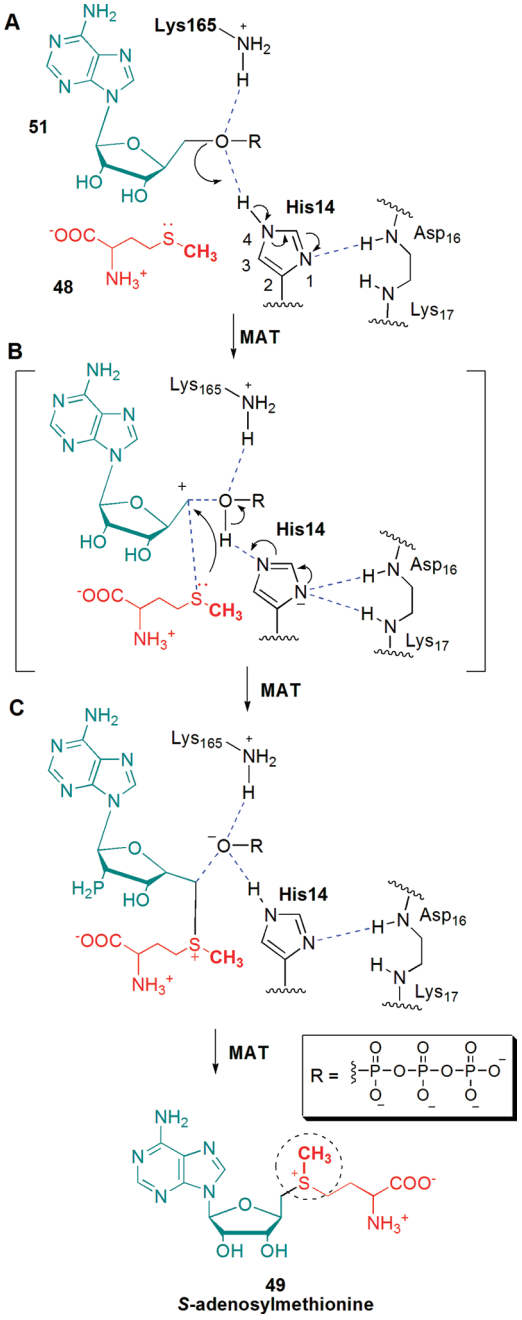
4. SPECIALIZED COFACTORS IN THE BIOMETHYLATION PROCESS

In biological systems, multiple reaction processes introduce a methyl group to a wide variety of natural compounds. However, few cofactors are capable of acting as methyl donors in these reactions.[78] Among these cofactors, we will highlight *S*-adenosylmethionine (SAM) (**49**) and 5,10-methylenetetrahydrofolic acid (**50**), a product derived from the folic acid pathway, as the main methylating agents in nature (Schemes 2 and 5).[78]

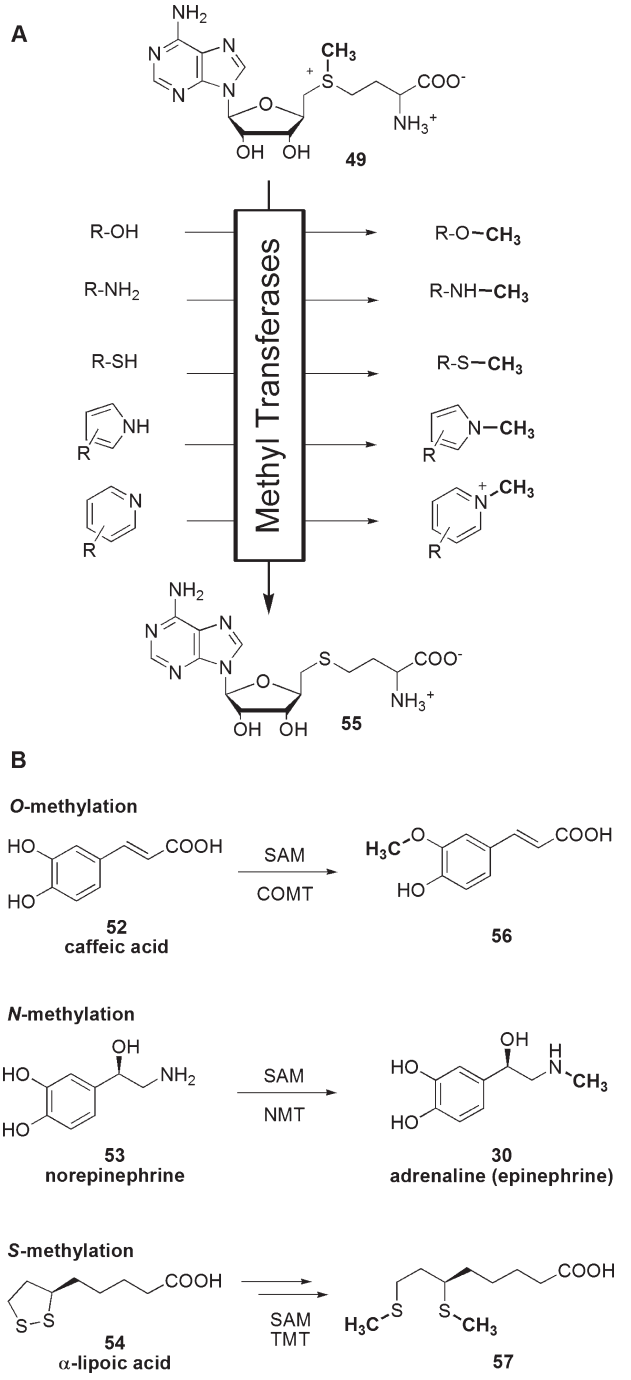
4.1. *S*-Adenosylmethionine (SAM)

Originally described by Cantoni in 1953, *S*-adenosylmethionine (SAM) (**49**) is a coenzyme involved in most methylation

Scheme 2. Biosynthesis of *S*-Adenosylmethionine (49)



Scheme 3. Reactions Catalyzed by Methyltransferases, Using *S*-Adenosylmethionine as Cofactor



processes in nature.[9] The formation of SAM (**49**) from methionine (**48**) and adenosine triphosphate (ATP) (**51**) is catalyzed solely by *S*-adenosylmethionine synthetase through a reaction that mainly involves three amino acids: His14, Asp16, and Lys165 (Figure 8, Scheme 2).[79]

The anchoring of ATP (**51**) to the catalytic site of *S*-adenosylmethionine synthetase (MAT) initially takes place through two hydrogen interactions between the oxygen of the triphosphate chain and His14 and Lys165 (Scheme 2A).[79] The imidazole ring of His14 may be polarized to allow the proton at *N*-4 to be promptly transferred to the oxygen of the triphosphate chain of ATP (**51**).[79] The negative charge generated in the imidazole ring is stabilized by interactions with the amides of Asp16 and Lys17; in this tautomeric form, the charge is located under *N*-1 (Scheme 2B). As the protonation of the triphosphoric oxygen takes place, the C−O bond of ATP (**51**) is cleaved, generating a positive charge on the ribose carbon (Scheme 2B); once polarized, this carbon moves toward the methionine (**48**) and is then subject to nucleophilic attack by sulfur to form SAM (**49**) and release pyrophosphate (PPPi) (Scheme 2B,C). Although this process is described here step-by-step, it takes place in a concerted manner.[79]

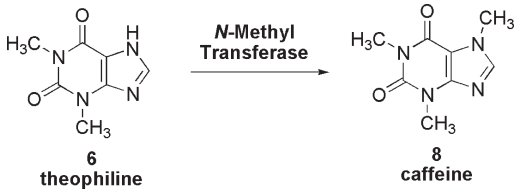
A methyl group connected to the methionine residue of SAM (**49**) has been described in the literature as a "natural methyl

dx.doi.org/10.1021/cr200060g |*Chem. Rev.* 2011, 111, 5215–5246

iodide."[80] Its chemical reactivity as an electrophile from the generation of the positive charge in the sulfonium center of the methionine facilitates S_N2 reactions involving this group.[80,81] As such, SAM (**49**) conducts *O*-methylation, *N*-methylation, and *S*-methylation reactions with natural products containing amine, hydroxyl, or sulfhydryl groups (Scheme 3A,B).[81] These methylations transfer a methyl group from the cofactor SAM to these groups, such as in the *O*-methylation of phenolic groups (mainly catechols) (**52**),[82] the *N*-methylation of endocyclic or exocyclic amino groups (**53**),[83] and the *S*-methylation of thiols (**54**)[84] (Scheme 3A,B). During these reactions, SAM loses a methyl group and a positive charge to become *S*-adenosylhomocysteine (**55**) (Scheme 3A). These processes are mediated by a number of specific enzymes of the methyltransferase class that target the *ortho*-diphenol moiety (catechol *O*-methyltransferase, COMT),[85] *N*-atoms in aromatic aza-heterocycles such as pyridines (nicotinamide *N*-methyltransferase, NNMT),[86] primary arylamines (indolethylamine *N*-methyltransferase, INMT),[87] endocyclic secondary amines (histamine *N*-methyltransferase, HNMT)[88] and a variety of thiols including heterocyclic ones (thiol methyltransferase, TMP; thiopurine methyltransferase, TPMT)[89,90] (Table 1, Scheme 3A,B).[91−93] A complete list of the major methyltransferases involved with the cofactor SAM is presented in Table 4.

Through the process of methyl conjugation, SAM (**49**) is the main agent involved in the metabolic processing of xenobiotics in humans.[95−97] The reaction of theophylline (**6**) to yield caffeine (**8**) is a toxicologically significant *N*-methylation reaction (Scheme 4).[98] This reaction can distinguish between individuals of different ages and counteracts the well-known oxidative *N*-demethylation reactions of methylxanthines.[99] This reaction is not seen in adult humans, but it is effective in neonates (about 5−10% of administered theophylline is converted to caffeine) and may cause unwanted side effects such as tachycardia and gastrointestinal intolerance.[100]

Scheme 4. *N*-Methylation of Theophylline (6) in Neonates



4.2. Folic Acid Pathway

Folic acid (**58**, Scheme 5) is a soluble B vitamin (vitamin B_9) with important roles in DNA synthesis, stability, integrity, and cellular repair in all living cells.[101] Folic acid (**58**) acts as an essential cofactor in the de novo biosynthesis of purines and especially thymidylate (**59**) by mediating the transfer of a methyl group (Scheme 5).[102] This transfer is

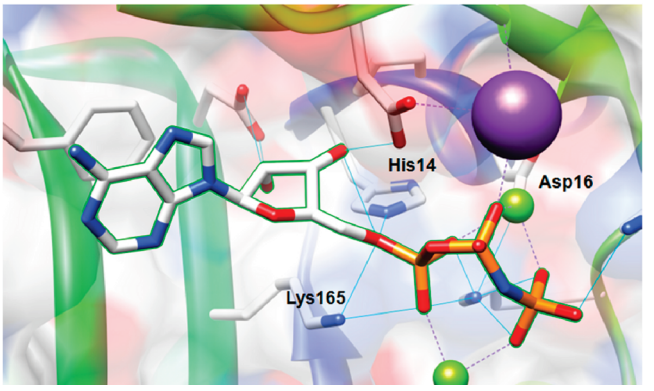
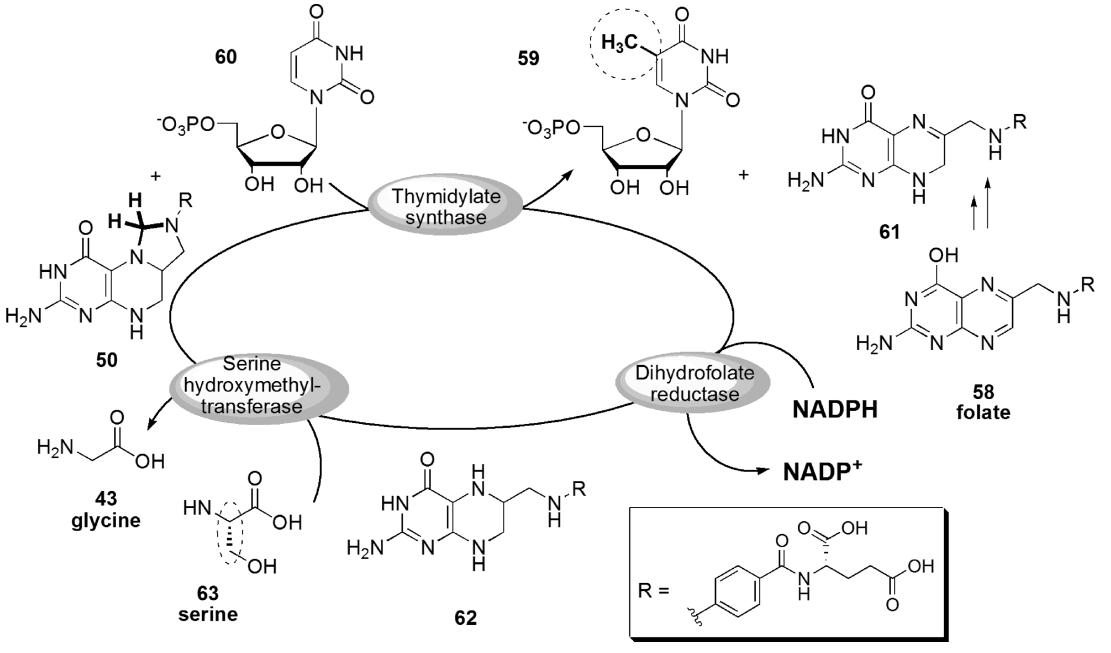


Figure 8. Catalytic site of *S*-adenosylmethionine synthetase (MAT). Picture made with the program Chimera[77] and the PDB ID structure is 1RG9.

Scheme 5. Biosynthesis of Thymidine Monophosphate (59), Using 5,10-Methylenetetrahydrofolate (50) as Cofactor



dx.doi.org/10.1021/cr200060g |*Chem. Rev.* 2011, 111, 5215–5246

Table 4. List of Major Methyltransferases Involved with SAM

name	EC number	type of reaction	substrates	ref
catechol *O*-methyltransferase (COMT)	EC 2.1.1.6	*O*-methylation	catecholamines and catechols	85
phenol *O*-methyltransferase (PMT)	EC 2.1.1.25	*O*-methylation	a variety of simple phenols	94
nicotinamide *N*-methyltransferase (NNMT)	EC 2.1.1.1	*N*-methylation	nicotinamide, pyridine, many drugs and other xenobiotics	86
histamine *N*-methyltransferase (HNMT)	EC 2.1.1.8	*N*-methylation	histamine, related heterocycles	88
phenylethanolamine *N*-methyltransferase (PNMT)	EC 2.1.1.28	*N*-methylation	noradrenaline and analogues	83
indolethylamine *N*-methyltransferase (INMT)	EC 2.1.1.49	*N*-methylation	very broad specificity; many primary, secondary, and tertiary amines	87
thiol methyltransferase (TMT)	EC 2.1.1.9	*S*-methylation	H_2S and a variety of alkyl, aryl, and heterocyclic thiols	89
thiopurine methyltransferase (TPMT)	EC 2.1.1.67	*S*-methylation	aromatic and heteroaromatic thiols	90

made specifically by 5,10-methylenetetrahydrofolate (**50**), a reduced form of folate generated by the consecutive actions of dihydrofolate reductase and serine hydroxymethyltransferase. A reductive methylation reaction catalyzed by thymidylate synthase (TS) yields thymidine monophosphate (dTMP) (**59**) from deoxyuridine monophosphate (dUMP) (**60**) (Scheme 5).[103−105] The presence of TS in most living organisms coupled with its important biochemical function in the synthesis of thymine (**1**) by the methylation of uridine (**2**) has made this enzyme an attractive target for multiple research groups interested in anticancer[106−108] and antiviral activities.[109,110]

5. FROM METHYLATED NATURAL PRODUCTS TO DRUGS: THE DISCOVERY OF STATINS

The importance of naturally methylated products as prototype substances for the development of new drugs can be observed in the history of the discovery of compactin (**64**, Figure 9).[111−113] This hexahydronaphthalene derivative is the source for antilipemic agents known as statins; in fact, the statin atorvastatin (Lipitor)[114−116] (**65**, Figure 10) is currently the best-selling drug worldwide.[117]

The pathogenic relationship between blood cholesterol (**66**) level and the risk of coronary incidents is well established, and cholesterol biosynthesis inhibitors are therapeutically useful.[118] Cholesterol biosynthesis is a complex process that involves more than 30 enzymes,[117] and it can be blocked by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG CoA_R).[111,119] This enzyme biocatalyzes the reduction of its substrate (HMG-CoA, K_m = 4 μM)[120] (**67**) to mevalonic acid (**68**) along with the reducing agent NADPH (Figure 9).[121]

During a screen of natural products conducted by a team of Japanese researchers led by Akira Endo, approximately 8000 microorganism lineages were tested for their steroid biosynthesis-inhibiting properties. The compound mevastatin (**64**) was discovered and isolated from cultures of the fungus *Penicillium citrinum* and was found to be a powerful inhibitor of HMG-CoA_R (IC_{50} = 5.6 nM).[111,113] Mevastatin (**64**) was identified and isolated from strains of the fungus *Penicillium brevicompactum* at nearly the same time by English researchers working for Beecham pharmaceuticals, who named it compactin (**64**).[112]

Research on these inhibitors from fungi led other research groups to follow their lead. In 1980, Merck industries discovered lovastatin (Mevacor) (**69**, Figure 9), which was initially called mevinolin, in *Aspergillus terreus*.[122] The structural difference between lovastatin (**69**) and compactin (**64**) is a methyl group

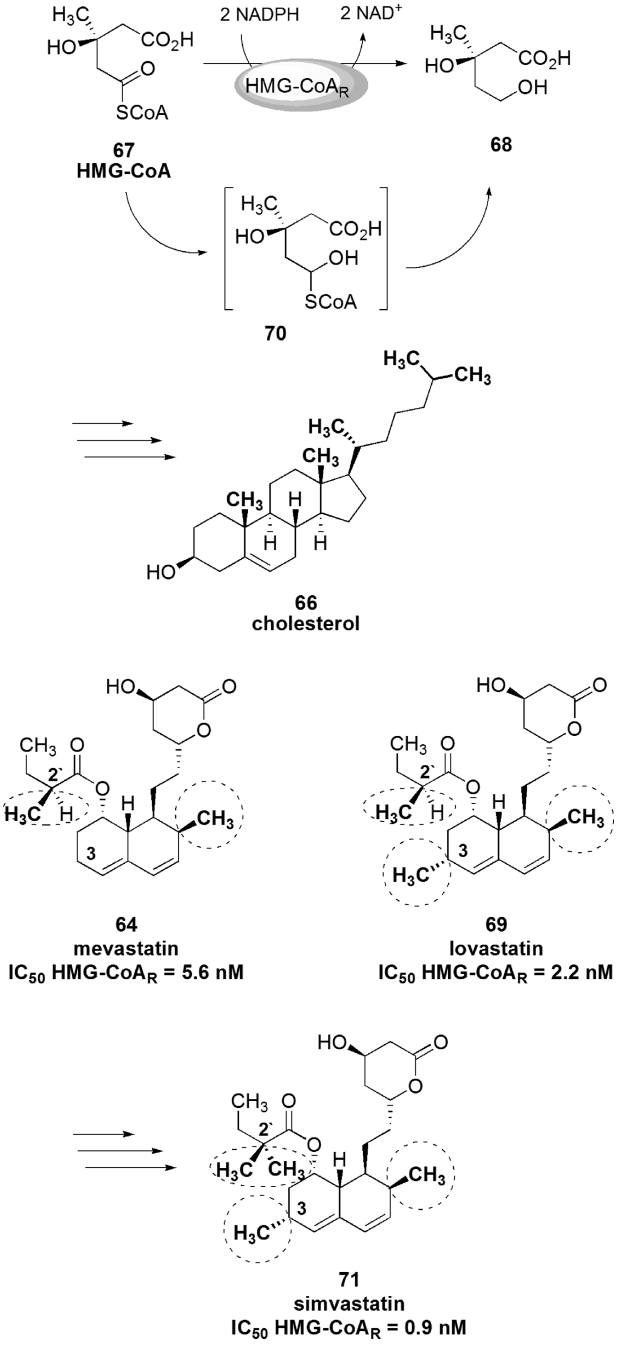


Figure 9. Discovery and evolution of statins from the methylated natural product mevastatin (**64**).

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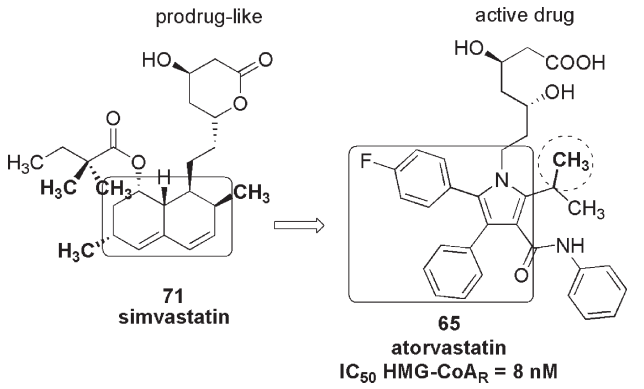

Figure 10. The genesis of atorvastatin (**65**) from first-generation HMG-CoA$_R$ inhibitors.

at the C-3 position of the hexahydronaphthalene ring in (**69**), which increases its inhibition of HMG-CoA$_R$ ~2.5-fold (IC$_{50}$ = 2.2 nM).[123] This compound was launched in 1987 by Merck Pharmaceuticals as the first safe and effective pro-drug of the statin class.[124−126]

Countless structural changes in lovastatin (**69**) and compactin (**64**) have been introduced with the goal of understanding the pharmacophoric contributions of each functional group to the overall pharmacological activity (structure−activity relationships).[123,127−130] From these studies, it became clear that the stereochemistry of the δ-lactonic subunit (after being hydrolyzed) is extremely important for the inhibitory activity (reversible competitive inhibition), as it mimics the intermediate (**70**) (Figure 9) in the reduction of HMG-CoA (**67**) by HMG-CoA$_R$.[127] In addition, the methyl groups at position 3 of the hexahydronaphthalene ring and position 2′ of the butanoate side chain are responsible for other important interactions with the target enzyme.[123] Removal of the methyl from the hexahydronaphthalene ring, as seen in compactin (**64**),[112] leads to a 2.5-fold reduction in inhibitory potency; however, removal of the methyl group α to the ester carbonyl unit (C-2′) reduces its potency toward HMG-CoA$_R$ by 4-fold (Figure 9).[123]

Hoffman[123] realized that the stereochemistry of the methyl group α to the carbonyl group does not influence the activity of these compounds. Led by A. Patchett, Merck developed simvastatin (Zocor) (**71**) (IC$_{50}$ HMG-CoA$_R$ = 0.9 nM),[123] a 2′-methyl derivative of lovastatin (**69**). Simvastatin (**71**) is 2.5 times more potent than lovastatin (**69**); in addition, it has one less chiral center and a longer half-life because the additional methyl group blocks ester hydrolysis (Figure 9).[123] Simvastatin (**71**) was launched in the North American pharmaceutical market in 1992.

Bruce Roth and associates at Parke-Davis laboratories designed a new class of HMG-CoA$_R$ inhibitors (second-generation inhibitors) that have a 1,2-diphenyl pyrrole system (Figure 10).[131] The molecular design of this new class was based on the hexahydronaphthalene system of lovastatin (**69**). This hydrophobic pharmacophoric unit was replaced with a 1,2-diphenyl pyrrole scaffold to achieve the desired hydrophobicity, thereby creating atorvastatin (**65**). This drug was launched in the pharmaceutical market in 1998 (Figure 10).[114−116,131−133]

Although the hexahydronaphthalene ring and side ester group are replaced by other groups in these second-generation inhibitors, the methyl groups are still present. Some examples of second-generation inhibitors are fluvastatin (**72**),[134−136] rosuvastatin (**73**),[137,138] cerivastatin (**74**)[139,140] (withdrawn from U.S. market

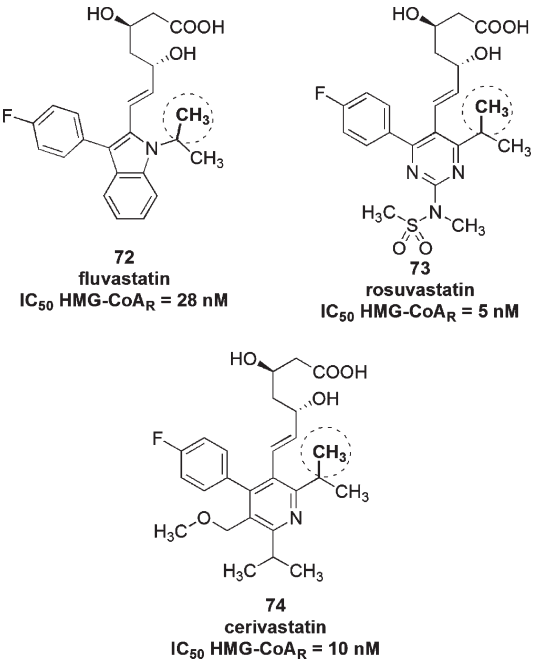

Figure 11. Second-generation HMG-CoA$_R$ inhibitors.

in 2001), and atorvastatin (**65**). All of these structures contain two methyl groups in an isopropyl group connected to the central heterocyclic system (Figure 11). These methyl groups are important for activity due to their involvement in hydrophobic interactions with Leu562, Cys561, and His752, which create an entropic gain in the interaction with the target receptor. The removal of the methyl groups leads to an 18-fold reduction in potency, as shown in initial structure−activity relationship studies with certain pyrrole derivatives (**75**−**77**) (Figure 12).[23,132]

6. STEREOELECTRONIC EFFECTS OF METHYL GROUPS IN DRUG DESIGN AND DISCOVERY

As seen with the discovery and evolution of statins from natural methylated products, the methyl group has been widely used in molecular modification strategies employed by numerous research groups dedicated to the invention of new drugs. The introduction of methyl groups can radically alter the potency, duration, and nature of the pharmacological effect.[5] As such, the medicinal chemist uses this group to modify aspects related to solubility, conformation, electronic factors, bioavailability, and pharmacokinetics. We must bear in mind that methyl effects are not limited to the simple transformation of functional groups, as in the alkylation of alcohols and carboxylic acid to form ethers and esters, respectively. Similarly, the process of extending alkyl chains by adding a linear methyl group, which is known as homologation, should not be considered a methyl effect. Conversely, if this addition is made through a branch to achieve steric effects or new hydrophobic interactions with a bioreceptor, an influence of the stereoelectronic effects of this group is clearly present.

6.1. The Inductive Effect of Methyl in the Discovery of Anti-Ulcer Drugs: Cimetidine and H^+-K^+-ATPase Inhibitors

Cimetidine (**78**)[141−143] is the first selective antagonist of subtype 2 histamine receptors,[144] and it is used in the treatment and prevention of gastric ulcers. This drug was discovered almost 40 years ago at a time when the structure of the chosen

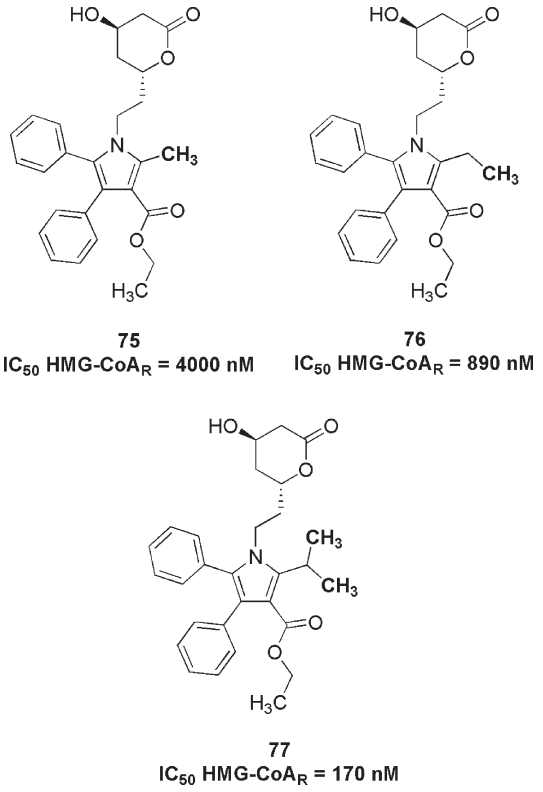

Figure 12. Methyl homologation increases the inhibitory potency of HMG-CoA$_R$ inhibitors.

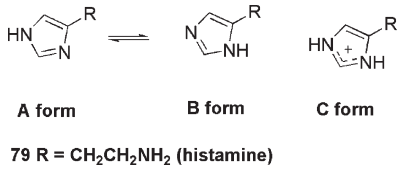

Figure 13. Tautomerism of the imidazole ring of histamine (**79**).

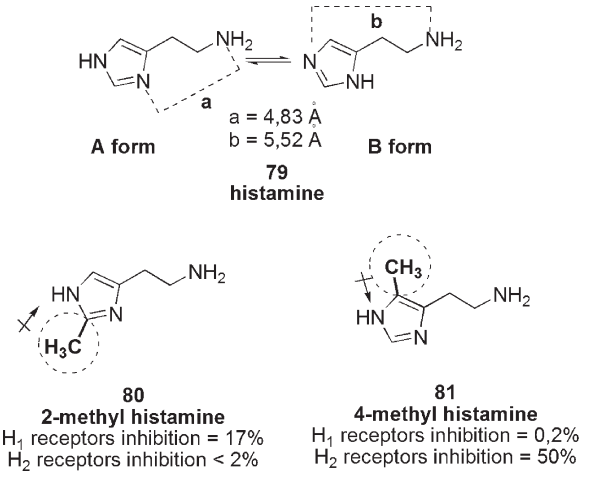

Figure 14. Structural design of inhibitors of H_2 histamine receptors based on the inductive effect of the methyl group.

therapeutic target was not known. Its discovery was an important and remarkable therapeutic innovation for the treatment and prevention of gastric ulcers, and it was later used as a prototype for the development of other drugs in its class.[145,146]

The main challenge faced by C. Robin Ganellin and his associates[145] with a molecular design based on cimetidine (**78**) was to introduce selectivity between the two subtypes of histamine (**79**) receptors known at the time: subtypes 1 (H_1) and 2 (H_2).[147] Subtype 2 is responsible for the action of this autacoid (**79**) in the gastric tract and was chosen as the therapeutic target.[144]

As the topography of these receptors was not known, Ganellin and his associates[144] adopted histamine (**79**), the natural agonist, as a prototype. Like other compounds of the imidazolyl class, histamine presents tautomerism (A−B forms, Figure 13) in aqueous solution at physiological pH.[148]

Because of the nature of the substituents, the population of tautomers changes because the ethylamine side chain alters the electron densities at the ring nitrogen atoms and therefore affects proton acidity. This effect is more pronounced at the nitrogen closest to the substituent. Therefore, electron-releasing groups make the adjacent nitrogen more basic, thus favoring the **B** tautomeric form of imidazole.[149] Moreover, if R is an electron-withdrawing group, the adjacent nitrogen atom becomes less acidic, favoring the **A** form. The percentage of the ionized form **C** (Figure 13) can be determined from the pK_a of imidazole and the pH. The electronic effect exerted by R can be calculated from the imidazole pK_a using the Hammett equation[150,151] as follows:

$$pK_a^R = pK_a^H + \rho\sigma m$$

where pK_a^R is the pK_a of the substituted imidazole, pK_a^H is the pK_a of imidazole, σm is the substituent electronic constant, and ρ is the reaction constant.[152] At physiological pH and temperature, the imidazole ring is 20% in its cationic form **C**, as it has a pK_a value of 6.80. Under the same conditions, histamine has a pK_a value of 5.90, indicating that its side chain has an electron-withdrawing effect that favors the tautomeric **A** form (to the extent of 80%); only 3% of the molecules are in the cationic **C** form and 17% are in the **B** form.[149,153]

Depending on the predominant tautomeric form, the distance between the protonated terminal amine group and the non-hydrogenated nitrogen atom of the imidazolyl ring (A and B) varies. This distance may be necessary for molecular recognition by the different subtypes of histamine receptors (Figure 14).[148]

Among several structural modifications of the imidazolyl system of the histamine prototype (**79**), Ganellin observed that methylation of the imidazolyl ring changed the tautomeric balance to favor the **A** shape. Moreover, methylation took place equally at the imidazolyl carbon *meta* to the ethylamine chain, leading to 2-methyl-histamine (**80**), and *ortho* to the ethylamine chain, leading to 4-methyl-histamine (**81**). However, 4-methyl-histamine (**81**) has greater selectivity for the subtypes of H_2 receptors of interest (Figure 14).[144,154,155] This selectivity results from the electron-releasing effect (σ Hammett −0.17) of the methyl group at C-5 over the imidazolyl ring, leading to an increase in the electronic density over N-1 and consequently a reduced pK_a, favoring its NH form (Figure 14).[154]

Cimetidine (**78**) contains an imidazolyl nucleus (**a**) and a methyl group (**b**) at C-5, thus favoring the tautomeric form necessary for H_2 receptor selectivity.[144,145] The thioether (**c**) in the side chain of cimetidine ensured adequate hydrophobic properties and led to increased selective antagonist activity. In addition, it also shifted the tautomeric balance of the heterocyclic ring toward considerable amounts of the cationic **B** form.[156] In turn, the cyanoguanidine function (**d**) of cimetidine

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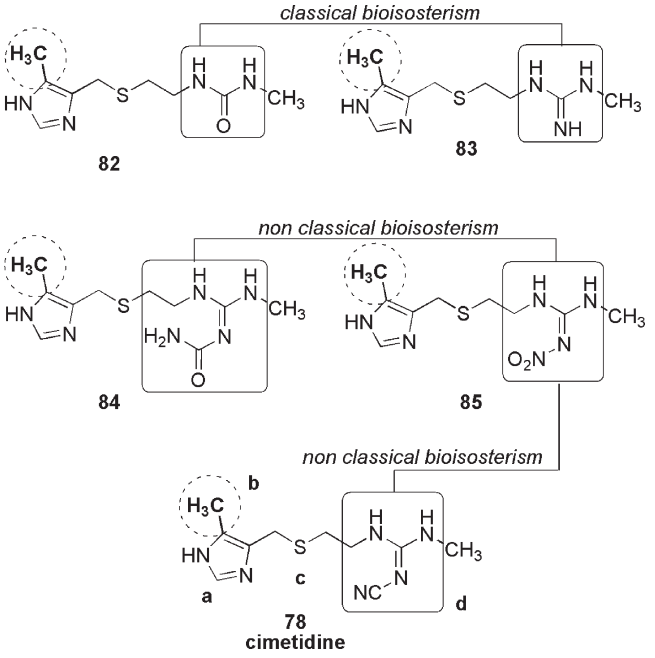

Figure 15. Structural design of cimetidine (**78**).

was included in subsequent changes to the *N*-alkyl-urea unit of **82** (Figure 15).[145,149]

Cimetidine (**78**) was the first drug to bring in more than $1 billion a year, and it thus became the first *blockbuster drug*.[149] It was first marketed in the United Kingdom in 1976, after only 12 years of the H_2-receptor antagonist program. Subsequently, two other H_2-antagonists, ranitidine (**86**)[157,158] and famotidine (**87**),[159−161] were launched after their discovery by analogue-based drug design from cimetidine (**78**) (Figure 16).[146] Nizatidine (**88**)[162] and roxatidine (**89**)[163] are two other H_2-antagonists currently marketed worldwide as medicines.

Omeprazole (Losec) (**90**), a pyrimidinyl-benzoimidazol derivative with important antiulcer properties, was the first selective gastric H^+-K^+-ATPase inhibitor, which are also referred to as proton pump inhibitors (PPIs).[164−166] This drug, which is actually a pro-drug,[164] was introduced for the treatment of gastric ulcers in 1989. It undergoes pH-dependent bioactivation to become a cyclic sulfenamide (**91**), which is the species reactive with gastric H^+-K^+-ATPase. Sulfenamide (**91**) forms an irreversible covalent complex with the enzyme via a disulfide bond (Figures 17 and 19).[167−169]

At the end of the 1960s, the Swiss pharmaceutical company Hässle started searching for compounds capable of blocking gastric acid secretion.[170] In 1971, Servier Pharmaceuticals discovered the thiobenzamide prototype compound (**92**) through random trials.[171] Hepatic toxicity caused by **92**, which was attributed to thioamide function, led researchers to conduct structural modifications. Thiohomologation and nonclassic annelation isosterism of the thioamide in **92**,[172,173] which presented good antisecretory activity, culminated in the discovery of **93**. The construction of a benzologue series followed by oxidation of the sulfur atom to the corresponding sulfoxide generated timoprazole (**94**),[174] a very potent derivative against gastric H^+-K^+-ATPase but with side effects on iodine recapture in the thyroid gland (Figure 17).[170,175]

The synthesis of timoprazole (**94**) analogues led to the identification of picoprazole (**95**), a dimethyl derivative of **94**

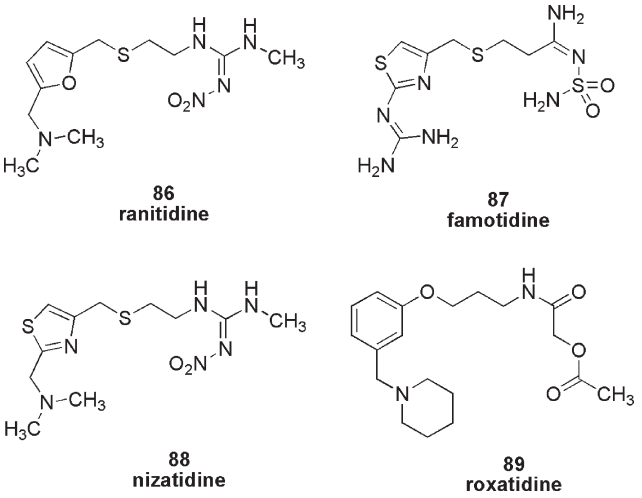

Figure 16. Current H_2-antagonists on the market.

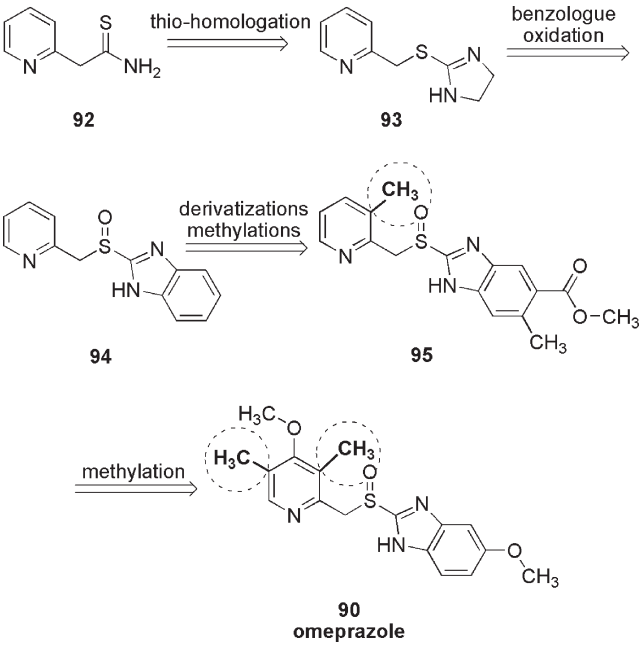

Figure 17. The influence of the inductive effect of methyl groups in the discovery of H^+-K^+-ATPase inhibitors.

that acts by inhibiting H^+-K^+-ATPase without the problem of blocking iodine recapture.[176] Structure−activity relationship studies of **95** showed that the introduction of methyl groups into the pyridine ring, due to their electron-donating effects, is beneficial for activity and increases its potency against H^+-K^+-ATPase. The best analogue was found to be omeprazole (Losec) (**90**) (Figure 17).[175]

More than 40 pharmaceutical companies have invested in the development of new PPI analogues of omeprazole (**90**).[177] These compounds include esomeprazole (**96**)[178−180] (Nexium , an *S*-enantiomer of omeprazole obtained by the chiral switch approach[181,182]), rabeprazole (**97**);[183,184] lansoprazole (**98**)[185,186] (Aciphex), pantoprazole (**99**)[187−189] (Prevacid), and tenatoprazole (**100**)[190,191] (Somac) (Chart 5). A common feature of these compounds is that they are composed of two heteroaromatic moieties: one is a pyridine moiety, usually replaced by one or more

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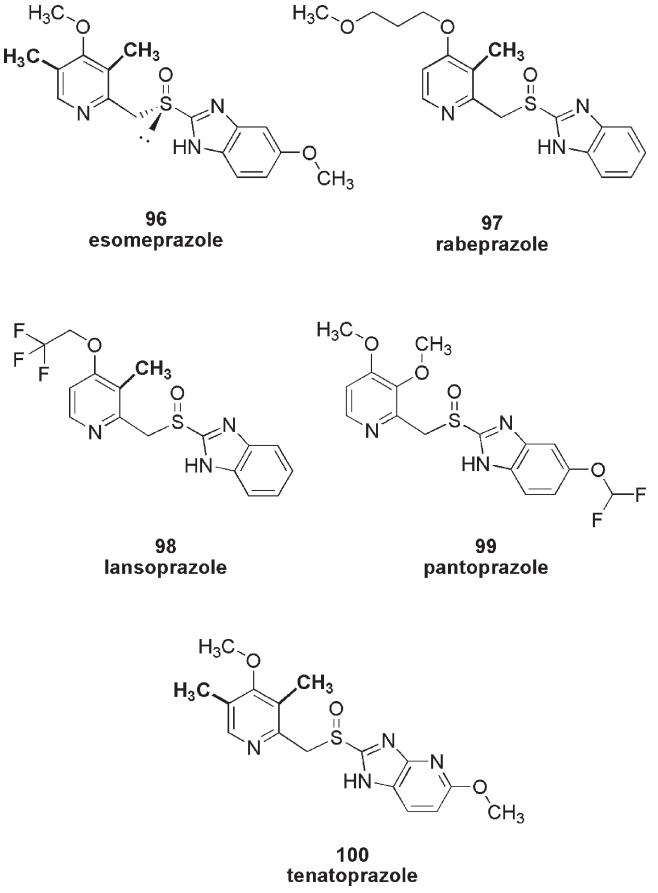

Figure 18. PPI analogues of omeprazole (**90**).

methyl groups, and the other is a benzimidazole or an imidazopyridine ring (Figure 18).

The importance of these methyl groups and the role of cysteines in the catalytic site were verified by several studies elucidating the chemical mechanism of gastric ATPase inhibition.[167−169,192] The introduction of methyl groups into these inhibitors allows fine-tuning of their pK_a and increases the nucleophilicity of the pyridine nitrogen atom, which is a lipophilic weak base (pK_a 4.0). By remaining in their nonionized form at physiological pH, these molecules can diffuse through plasma membranes to the target tissue.[167−169,192] However, a pH of around 1.0 inside of parietal cells quickly protonates the pyridine, preventing these compounds from leaving the canaliculus and immediately initiating the inactivation of H^+-K^+-ATPase through covalent bonds to the cysteine residues in the catalytic site (Figure 19).[167−169,192]

The first stage in the "activation" of these PPIs involves their transformation into a spiro dihydrobenzimidazole (**101**) intermediate through the nucleophilic addition of the acid-catalyzed pyridine nitrogen of **90a** on the benzimidazole ring, which is then rearomatized to generate sulfenic acid (**102**). This step is followed by dehydration to form tetracyclic sulphenamide (**91**).[167−169,192] This nucleophilic intermediate (**91**), which is a permanent cation, attacks the thiol groups of cysteines located in the catalytic site (Cys813 and Cys822) to produce chemically stable disulfides (**103**) (Figure 19).[167−169,192]

Aside from the importance of the compartmentalization of the drug in parietal cells, fine-tuning of the pyridine's pK_a by methyl

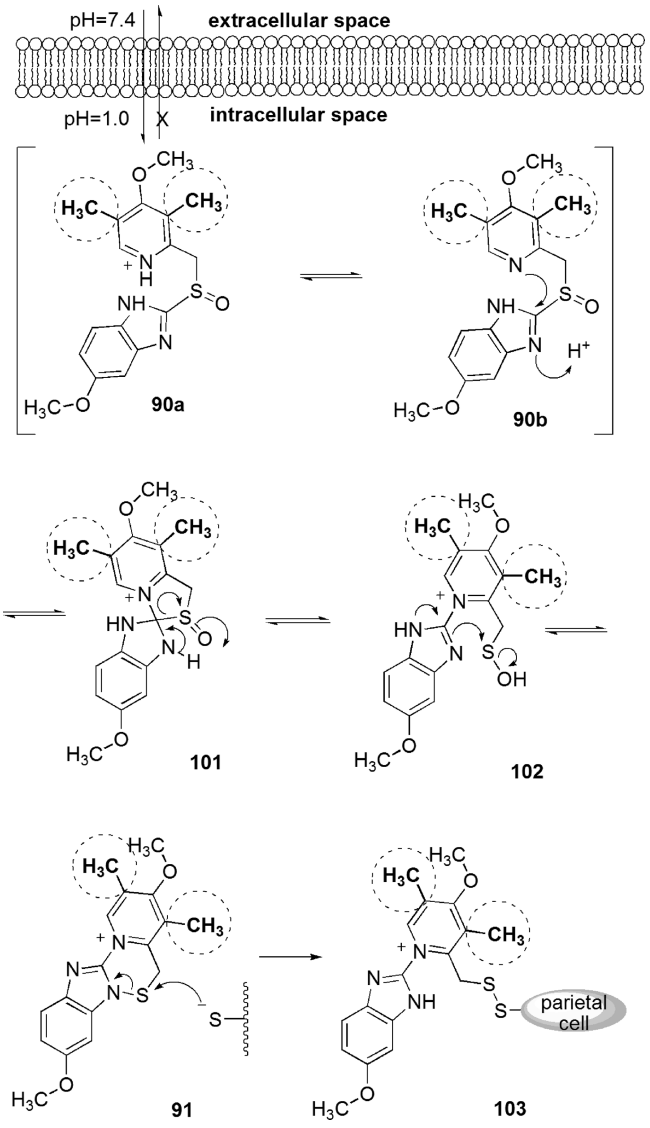

Figure 19. Proposed mechanism for the bioactivation of antiulcer drug omeprazole (**90**).

group introduction (pK_a at 3−4) is involved in the stability of these drugs at physiological pH (pH 7.4). In addition, the methyl group affects the speed of conversion into the active form (**91**) in microscopic channels (pH around 1.0). The $t_{1/2}$ values of different PPIs at pH 5.1 reveal the chemical stability of these compounds at this pH: pantoprazole (**99**) 4.7 h, lansoprazole (**98**) 1.5 h, omeprazole (**90**) 1.4 h, and rabeprazole (**97**) 0.12 h.[193] However, at pH 1.2, which is close to that of parietal cells, these PPIs are rapidly activated and their $t_{1/2}$ changes from 4.6 to 1.3 min.[193]

The activation mechanism of PPIs is directly related to the nitrogen nucleophilicity of the pyridine moiety, which depends on the electronic effect of substituents on the pK_a of the pyridine ring.[169] When electron-donating substituents, such as methyl groups, are attached to the pyridine ring, its pK_a increases, thus increasing its protonation rate at any given pH. Protonation of the pyridine decreases its nucleophilic reactivity due to the occupation of the lone pair of electrons of the pyridine nitrogen, but it also increases the nucleophilic reactivity of the

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minimum amount of the unprotonated form (**90b**) responsible for activation.[169]

6.2. The Conformational Effect Induced by Methyl: The Discovery of Imatinib

Until the beginning of the 1980s, programs for the discovery of compounds for cancer treatment focused almost exclusively on targets such as DNA synthesis and cell division, resulting in the birth of antimetabolic, alkyl, and microtubule-destabilizing drugs.[194] These drugs were effective, but at the cost of high toxicity due to their lack of selectivity between normal and cancer cells.[194] Therefore, the search for more selective drugs for cancer treatment led to the discovery of imatinib (Glivec) (**104**),[195−197] the first FDA-approved drug (in 2001) for the treatment of chronic myeloid leukemia (CML).[198,199] Imatinib acts on tyrosine kinase (TK), the main constituent of the *Bcr-Abl* oncogene responsible for this disease.[200]

Imatinib (**104**) is an authentic therapeutic innovation capable of treating leukemia.[198] This discovery revolutionized the treatment of this type of cancer, demonstrating the use of the modern hyphenated *combichem-HTS* tactic in drug discovery.[201,202]

The use of high-throughput screening (HTS) with a collection of nitrogenated heteroaromatic substances that mimic the ATP (**51**) purinic fragment, a natural TK substrate, led to the identification of the diaryl-amine scaffold (**105**) and began the process of the discovery of imatinib (**104**) (Figure 17).[194] Through the use of combinatorial chemistry, the construction of 300 analogues of **105** led to compound **106**, which was obtained by introduction of a 3-pyridinyl group at the C-4 position of the pyrimidine ring and presented strong inhibition of protein kinase C (PKC) in cellular assays.[194] During the optimization of this class of pyridinyl-pyrimidines (**106**), medicinal chemists observed that the introduction of an amide group linked to the phenyl ring led to a gain in activity against TKs as well as against *Bcr-Abl* kinase (*Bcr-AblK*) (Figure 20).[203]

Once the amide derivative **107** presented selectivity over other PKs, such as the serine/threonine kinases PKC-α and PKC-δ, structural optimization of this class of compounds was necessary for *Bcr-AblK* selectivity.[203] The Zimmermann research group realized that the addition of a simple methyl at C-6 of the diphenylamine ring to generate **108** led to a complete loss of activity for PKCs but maintained or even increased its activity for the target *Bcr-AblK* (Figure 20).[203−205] This gain in selectivity could be explained by a conformational change forced by the introduction of this "flag-methyl" group, leading to a steric *ortho* effect on the amine that induces a phenyl turn, as supported by X-ray diffraction analysis (Figure 20).[203,206]

Structural modifications were made to the phenyl ring connected to the amide of **108** in order to modulate its physicochemical properties and optimize the pharmacokinetic profile of the diphenylamines. Imatinib (**104**) was discovered through the introduction of a methylpiperazine group, which is protonatable at the biophase (Figure 20).[194,196,197,203] Schindler and colleagues described the structural mechanism for imatinib's (**104**) inhibition of *Bcr-AblK* by determining the crystal structure of **104** bound to the catalytic domain of the *Abl* TK at 2.4 Å resolution. These results again highlight the importance of the "flag-methyl" for **108**, which causes the pyridinyl-pyrimidine ring to turn, thus favoring interactions with Met318 and Lys271 and van der Waals interactions with Thr315 and Ala269.[207]

Imatinib (**104**) was the first TK inhibiting drug ("*tinib*") used in cancer treatment. This class of compounds now includes the *Bcr-AblK* inhibitors nilotinib (Tasigna) (**109**)[208−210] and dasatinib (Sprycel) (**110**),[211−213] the multiple vascular endothelial growth factor (VEGF) TK inhibitor sorafenib (Nexavar) (**111**),[214−217] the platelet-derived growth factor (PDGF-Rs) inhibitor sunitinib (Sutent) (**112**),[218,219] the epidermal growth factor receptor (EGFR) inhibitor erlotinib (Tarceva) (**113**),[220,221] the human EGFR type 1 (HER1) inhibitor gefitinib (Iressa) (**114**),[222−224] and lapatinib (Tykerb) (**115**),[225] a dual TK inhibitor associated with the oncogenes EGFR and HER2 (Figure 21).

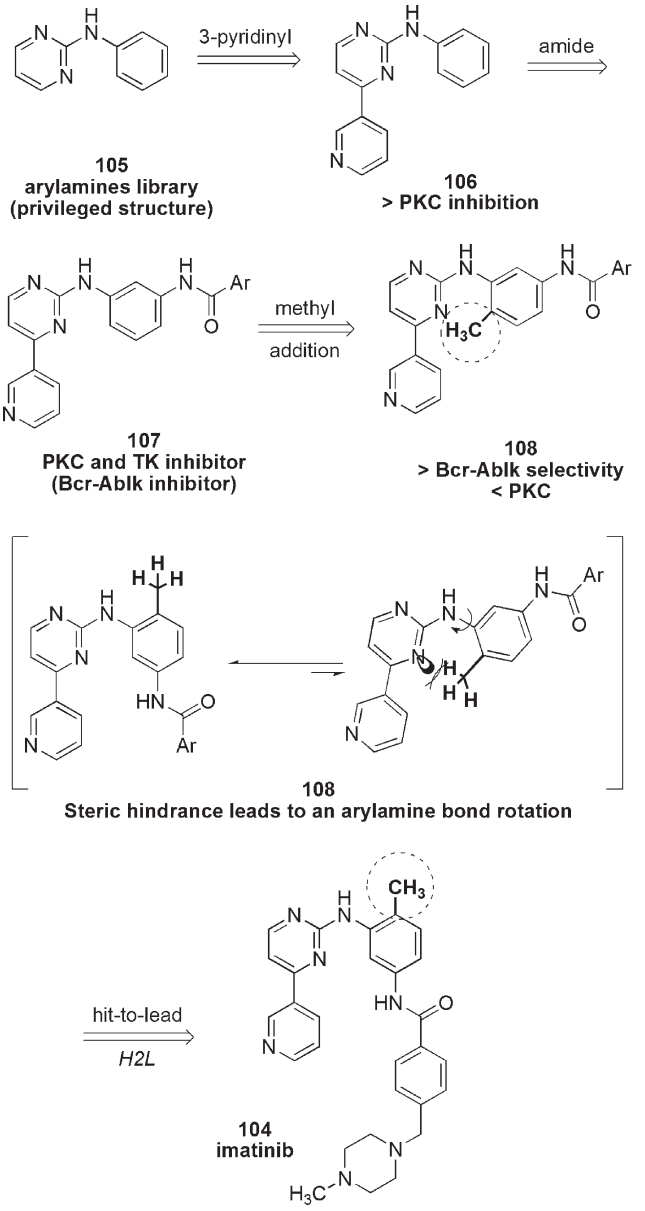

Figure 20. Structural modifications of diphenylamines and the influence of methyl group steric effects in the discovery of imatinib (**104**).

6.3. Conformational Changes Induced by *N*-Methylation of Bioactive *N*-Acylhydrazones (*NAH*)

In an attempt to optimize the cardiovascular properties of the cardioactive lead compound LASSBio-294 (**116**),[226] a series of structural modifications were introduced into the structure of *N*-acylhydrazone prototype **116**. Notably, *N*-methylation of the amide in the *N*-acylhydrazone group generated LASSBio-785

(**117**), a compound 7 times more potent as a vasodilator than prototype **116**; in addition, it exhibited selectivity toward vascular smooth muscle.[227]

Evaluation of the pharmacologic mechanism of action of these two compounds (**116** and **117**) showed that the vasodilator activity of LASSBio-294 (**116**) is totally dependent on the vascular endothelium, whereas LASSBio-785 (**117**) promotes vasodilation in a manner that is independent of the endothelium.[227] These results indicate different mechanisms of action and reveal that methylation of LASSBio-294 (**116**) led to more relevant structural differences than simply the loss of a hydrogen bond donor and increased lipophilicity. Studies using X-ray crystallography, molecular modeling, and ultraviolet spectroscopy have elucidated the bioactive conformations of these compounds (**116** and **117**), revealing that the methylation of the *N*-acylhydrazone leads to a conformational change that might be responsible for the different bioactivities of these two derivatives (Figure 22).[228]

X-ray diffractometry analysis showed that the *N*-acylhydrazone LASSBio-294 (**116**) in its solid state presents itself in a planar form (amide hydrogen *antiperiplanar* to the carbonyl oxygen); by contrast, the *N*-methyl-*N*-acylhydrazone LASSBio-785 (**117**) tends to turn 180° to the amide bond, generating a folded conformation (amide hydrogen *sinperiplanar* to the carbonyl oxygen). The folded conformation of **117** is due to the sterical effect generated by the additional methyl (Figure 22), which was confirmed by semiempirical molecular modeling studies indicating a high rotational barrier (ΔH_f = 17 kcal/mol) between this form and the planar form.[228]

These conformational differences observed in the solid stage have also been confirmed in solution through the use of ultraviolet spectroscopy.[228] In vivo, *N*-acylhydrazones and *N*-methyl-*N*-acylhydrazones are likely to present different conformations and therefore different pharmacological profiles.

6.4. The Steric Effect of the Methyl in the Selectivity of Lumiracoxib for PGHS-1/PGHS-2

Prostaglandins (PGs) induce a series of important beneficial and harmful biological responses, including pain, fever, and symptoms associated with the inflammatory response.[229] Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the PGHS enzyme, thus blocking the formation of prostaglandins and leading to analgesic, antipyretic, and anti-inflammatory effects.[230]

Selective inhibitors of the inducible PGHS isoform 2 (PGHS-2) represented a new generation of NSAIDs that lacked the gastro-irritating action of traditional PGHS-1 (a constitutive isoform) inhibitors[75,231,232] such as diclofenac (**118**).[233,234] Although it is a classic PGHS inhibitor, diclofenac (**118**) is nonselective,[235] with the same affinity for isoforms 1 and 2 (K_i values of 0.01 μM for both) (Figure 23).[235]

On the basis of the James W. Black (the discoverer of propranolol) paradigm, which says that "the most fruitful basis for the discovery of a new drug is to start with an old drug,"[236] researchers at Pfizer decided to use diclofenac (**118**), an NSAID that presented inhibitory activity for both isoforms (PGHS-1 IC_{50} = 0.075 μM and PGHS-2 IC_{50} = 0.038 μM).[232] In addition, because diclofenac (**118**) had well-established pharmacokinetic and toxicological properties,[237,238] they aimed to modify its structure in a way that would reduce its potency for PGHS-1 and thus increase its selectivity for PGHS-2.[235,239]

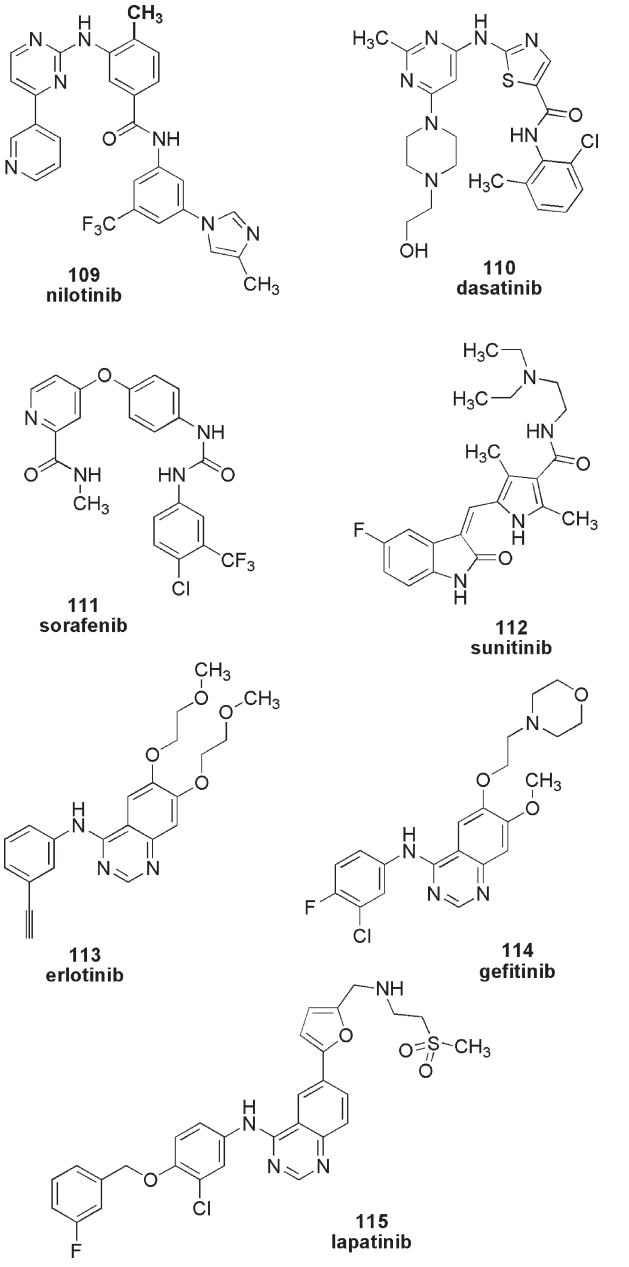

Figure 21. TK inhibitor drugs currently used in cancer treatment.

Using the classic monovalent bioisosteric exchange strategy,[172,173] the chlorine atom present in diclofenac was replaced by fluoride, and a methyl group was added at the C-4 position of the phenylacetic unit. This led Pfizer to discover lumiracoxib (**119**), a selective PGHS-2 inhibitor (Figure 23).[235,239] The gain in selectivity for PGHS-2 is related to the additional volume of the methyl group (MR = 5.65 cm^3/mol) on the phenylacetic ring in comparison to that of hydrogen (MR = 1.05 cm^3/mol). The methyl group generates steric repulsion with Ser353 and Tyr355, preventing the formation of a hydrogen bond between the carboxylate group and the side chain of Arg120 in PGHS-1, which occurs with diclofenac (**118**). Therefore, the potency for PGHS-1 is reduced (Figure 23).[240] By contrast, the methyl group does not generate this steric repulsion with PGHS-2; instead, it is inserted into a small pocket around Leu384, which is made

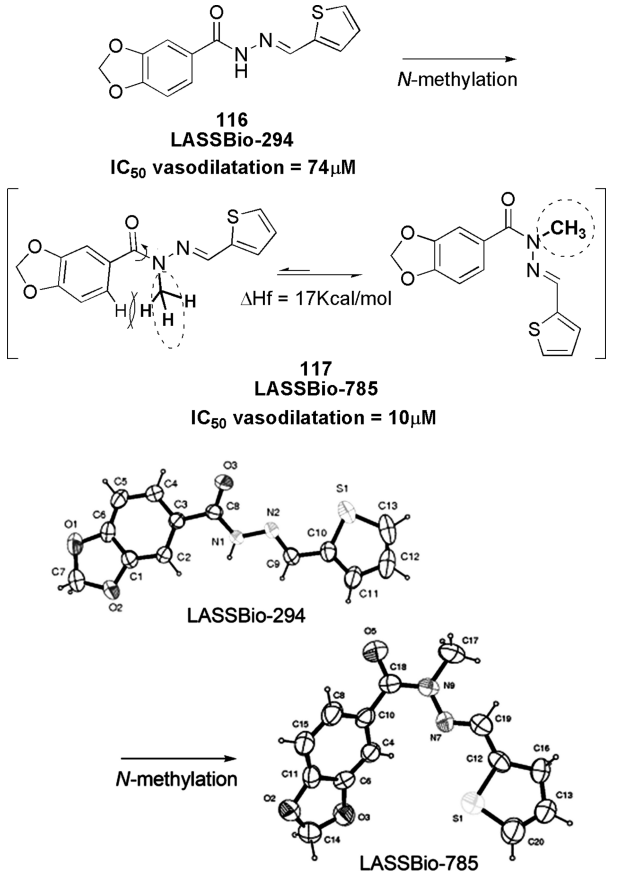

Figure 22. Conformational changes induced by *N*-methylation of *N*-acylhydrazones.

accessible by small secondary shell residues that exist only in PGHS-2 due to its higher volume catalytic site. Lumiracoxib (**119**) may interact in a way similar to that of diclofenac (**118**), and thus its potency against this enzyme subtype is not significantly reduced (Figure 23).[240−242]

The synthesis of 2-anilinophenylacetic acid derivates related to lumiracoxib (**119**) demonstrated that the steric effect of the methyl group on the PGHS-1/PGHS-2 selectivity of this compound is not an isolated case.[241,243] As demonstrated in Table 5, methylation at C-5 of the phenylacetic ring has systematically led to compounds selective for PGHS-2, without a significant reduction in their potency or the extent of their inhibition of this isoform.[241]

6.5. Methyl-Induced Effects in Chloroquine Analogues

The beginning of World War II and the interruption in the supply of quinine (**125**) initiated a search for synthetic compounds as alternatives to **125**.[244,245] With the basic understanding of the structure−activity relationships of quinine (**125**), new antimalarial agents were synthesized, culminating in the discovery of 4-aminoquinoline derivatives such as chloroquine (**126**).[244,245] Chloroquine (**126**) has a methyl group in its 4-diethylamine-1-methylbutylamine chain, which derives from a rearrangement in its synthetic process and generates a chiral center in this antimalarial agent. This methyl group has proven to be important for the desired activity, and it is also present in other antimalarial drugs such as hydroxychloroquine (**127**) and primaquine (**128**) (Figure 24).[244]

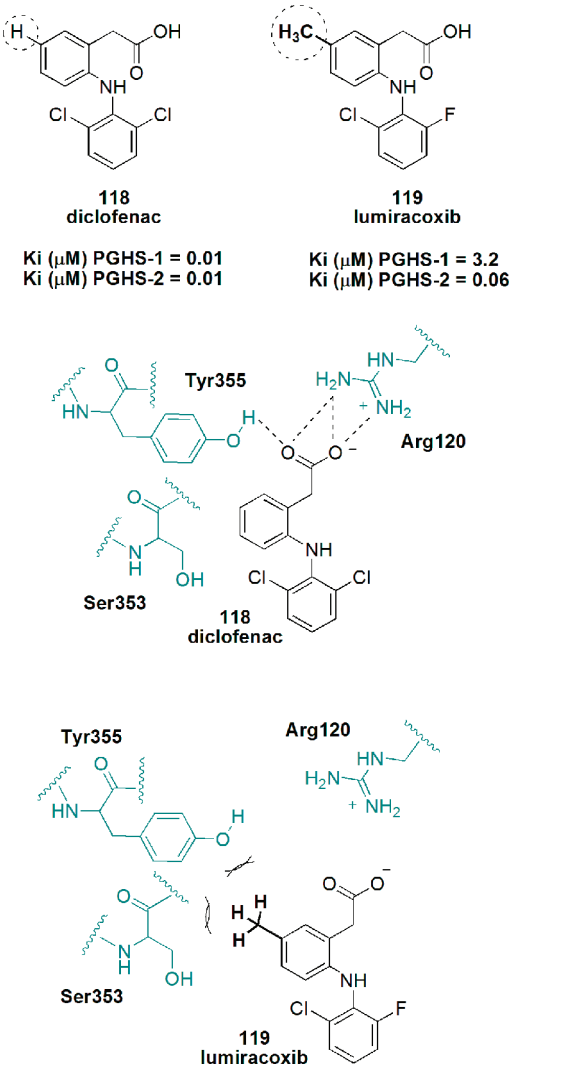

Figure 23. Molecular basis for PGHS-2/PGHS-1 selectivity induced by the methyl group in lumiracoxib (**119**).

The introduction of a methyl group in prototropic aromatic rings might favor one tautomeric form over others. As described earlier, this strategy was used in the discovery of cimetidine by Ganellin and associates,[144,154,156] and it was also used to obtain new antimalarial agent candidates structurally related to chloroquine (**126**). The main toxic characteristic that limits the usage of **126** lies in the formation of the reactive prototropic species **129** because of the 4-aminoquinolinic nature of **133** (Figure 25).[246]

Replacement of the quinoline system of this classic antimalarial agent with a pyrazolo[3,4-*b*]pyridine ring produced the new malarial agent candidate **130**. Because of the difference in pK_a between the two nitrogenated systems when faced with chloroquine-resistant *Plasmodium falciparum* strains, the pharmacophoric unit of the antimalarial drug **126** was maintained in the proposed isostere **130** (Figure 25).[246] Introduction of a methyl group to C-6 of the pyrazolo[3,4-*b*]pyridine nucleus was hypothesized to favor the **130** tautomer over the **131** tautomer, which would reduce the potential for toxicity when compared to chloroquine (**126**) (Figure 25).[3,246]

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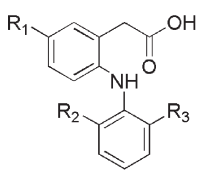
Table 5. IC_{50} and Inhibition Values of Lumiracoxib (119) Analogues for PGHS-1 and PGHS-2[a]



Compd	R_1	R_2	R_2
119	CH_3	F	Cl
120	H	F	Cl
121	CH_3	Cl	Cl
122	H	Cl	Cl
123	CH_3	Cl	CH_3
124	H	Cl	CH_3

	PGHS-2		PGHS-1	
compd	IC_{50} (nM)	max inhib (%)	IC_{50} (nM)	max inhib (%)
119	107	50	nd	–
120	nd	40	nd	30
121	47	90	nd	40
122	28	100	67	90
123	60	95	nd	–
124	20	100	58	100

[a] The line symbol indicates less than 20% inhibition up to inhibitor concentrations of 4 μM. nd = not determined.

6.6. Methyl-Induced Atropoisomerism in Psychoactive Drugs and Male Contraceptive Drugs

Atropoisomerism, a name derived from the Greek word atropos (i.e., without rotation), refers to a type of stereoisomerism that is characteristic of systems where free rotation around a simple ligation is prevented. An elevated energetic barrier exists, and this allows different rotamers or conformers, called atropoisomers, to be isolated or simply detected.[247,248]

Methaqualone (**132**) is a neuroactive drug with hypnotic and anticonvulsant properties and is a member of the class of psychoactive quinazolinone derivatives.[249] This drug presents an atropoisomeric effect due to the *ortho*-methyl (a) substitution pattern in the *N*-phenyl ring, which has a *pseudoperiplanar* relationship with the methyl (b) and carbonyl (c) groups of the heterocyclic system. This system is characterized by a steric barrier to the free rotation of this bond of approximately 132 kJ/mol (Figure 26).[250]

This atropoisomerism was confirmed by nuclear magnetic resonance (NMR) experiments in the presence of lanthanide displacement-inducing salts (LIS). In these experiments, signs corresponding to magnetically differentiated methyl groups were observed.[250] Atropoisomer resolution through high-resolution liquid chromatography using a chiral support allowed optical isomers to be obtained in enantiomeric excess of 70%.[250]

Pharmacological evaluation of these enantiopure compounds showed that the (−)-enantiomer (DE_{50} = 26 mg/kg) (**132a**) has more anticonvulsant activity than the (+)-enantiomer (DE_{50} = 36 mg/kg) (**132b**), showing the importance of conformational studies of pharmacologically active compounds with atropoisomerism induced by the presence of methyl groups (Figure 26).[250]

Diazepam (**133**) is a member of the class of 1,4-benzodiazepine derivatives, an important therapeutic class of drugs with antianxiety, hypnotic, anticonvulsant, and muscle relaxant actions.[251]

According to a report by Gilman et al.,[252] *N*-methylated 1,4-benzodiazepines may also present atropoisomerism in their structure with two possible conformations, **133a** and **133b**, and a conversion barrier of 17.6 kcal/mol between them. The NMR spectrum of diazepam (**133**) revealed the presence of an

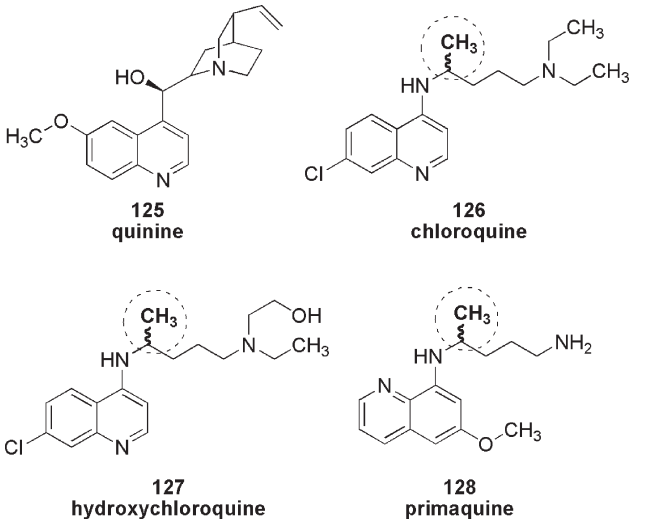


Figure 24. Quinoline antimalarial drugs.

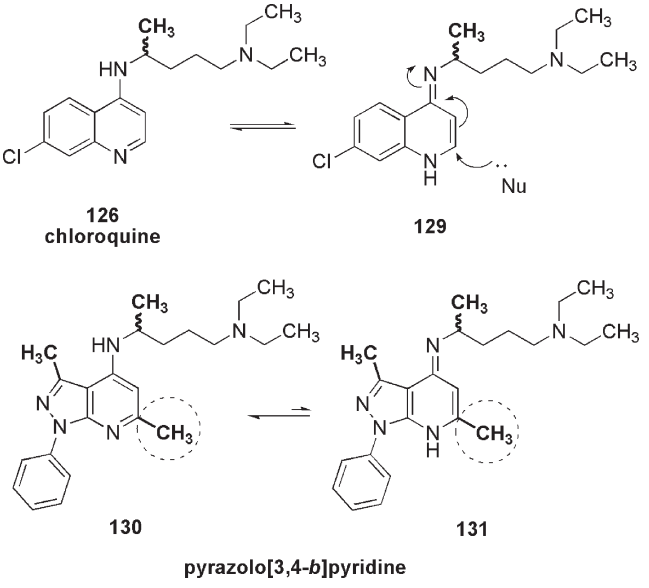


Figure 25. Design of a new pyrazole[3,4-*b*]pyridine derivative (**130**) analogue of chloroquine (**126**) and the inductive influence of the methyl group.

AB pattern with large coupling constants (J = 14.0 Hz) typical of germinal hydrogens. These data support the existence of conformational restriction capable of differentiating diastereotopic methylene hydrogens (C-3).[252] In fact, the methyl group is responsible for this energy barrier, as the methylene (C-3) of N^1-desmethyldiazepam (**134**) exhibited only a singlet in its NMR spectrum, indicative of a quick interconversion between the two atropisomeric forms (Figure 27).[252]

Biological assays with diazepam enantiomeric pairs (**133a**, **133b**) indicated that the enantiomer **133a** is stereoselectively recognized by benzodiazepine receptors. This observation was confirmed after separation of the atropoisomers through the use of resolution methodology for 1,4-benzodiazepine compounds, which was also developed by Gilman.[253]

Gossypol (**135**) is an important natural compound that also presents atropoisomerism due to the *ortho* effect of its methyls in

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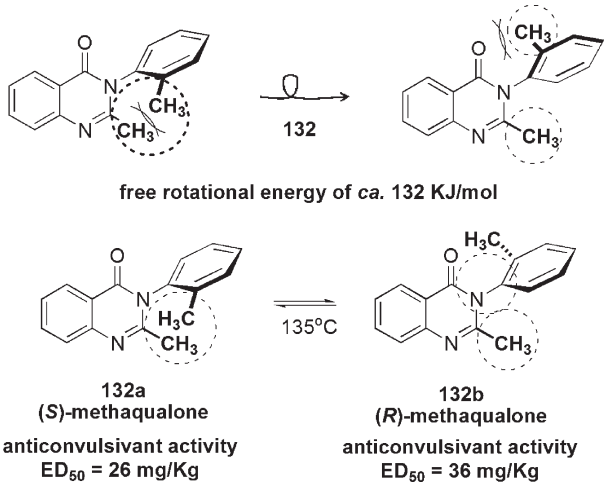

Figure 26. Atropoisomeric effect induced by methyl groups in methaqualone (**132**).

the biphenyl-like system. This compound is a polyphenol dialdehyde isolated from the seeds, trunks, and roots of the cotton plant (*Gossypium* sp.), and it has been widely studied since it was discovered in the late 1960s for its contraceptive activity.[254]

Aside from its contraceptive properties, gossypol (**135**) also displays antiviral and antiparasite activities in vitro at micromolar concentrations and it also displays marked antitumor activity.[255]

Gossypol (**135**) exhibits atropoisomerism as a result of rotational restriction around the C−C bond within the binaphthyl system, resulting in two optically active forms. In general, the (*aR*)-(−)-gossypol (**135a**) enantiomer has a more marked bioactivity profile when compared to the (*aS*)-(+)-gossypol (**135b**) optical antipode and the corresponding racemate (Figure 28). Shelley and associates[256] reported that the L isomer of **135** is significantly more potent as an antitumor agent than the D isomer. These results suggest that low concentrations of the levorotatory enantiomer of **135** can affect tumoral cells in a stereospecific manner. Nonspecific interactions are found when (D)-gossypol is used or with high concentrations of (L)-gossypol.[255]

7. STEREOELECTRONIC EFFECTS OF METHYL GROUPS AND DRUG METABOLISM

Although the effects of methyl groups on the metabolic processes are not directly related to the pharmacodynamic stage, i.e., interaction with bioreceptors, they can be of great importance in the pharmacokinetic stage. Introduction of methyl groups is a tool used in medicinal chemistry for controlling the plasmatic half-life of bioactive compounds and for bioactivating pro-drugs.

From a metabolic point of view, the methyl group has an important role. It can be used to accelerate the metabolism of bioactive compounds through its oxidation to an alcohol, aldehyde, or acid; alternatively, in can slow metabolism it due to its stereoelectronic effects.[3,257]

7.1. The *ortho*-Effect Due to Methyl Groups: The Metabolism of Lidocaine

Lidocaine (**136**) is a 50-year old antiarrhythmic drug originally developed as an anesthetic that likely acts against cellular receptors associated with sodium channels. Studies on the conformation of lidocaine allowed for the identification of structural factors responsible for its metabolic stability.[3]

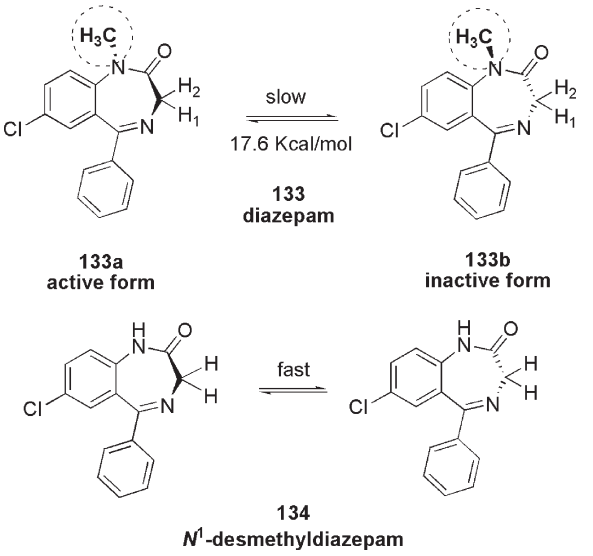

Figure 27. Atropoisomeric effect induced by the methyl groups in diazepam (**133**).

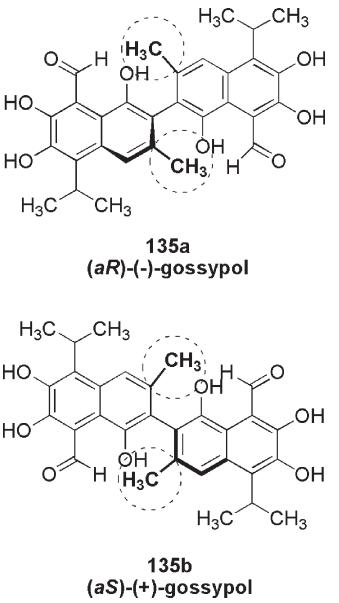

Figure 28. Representation of gossypol (**135**) atropoisomers.

Structure-based design of lidocaine began with repeated molecular modifications of cocaine (**36**), leading to the production of eucaine (**137**). Further molecular simplification[226] yielded the anesthetic procaine (**138**). Next, classic isosteric exchange[172,173] of the ester unit led to the genesis of a series of benzamide derivatives, such as procainamide (**139**). Its retroisostere finally led to lidocaine (**136**) (Figure 29).[3,258]

In addition to an inductive effect that reduces the reactivity of the amide groups, the presence of bis-*ortho*-methyl groups in this drug (**136**) introduces a conformational "twist" in the benzene ring plane in relation to the side chain. This conformational change is enough to provide steric "protection" against plasmatic amidases, enzymes capable of hydrolyzing the amide bonds of the drug in vivo (Figure 30).[259] As a result of this steric "protection," lidocaine (**136**), which is absorbed by the dermis when used as a

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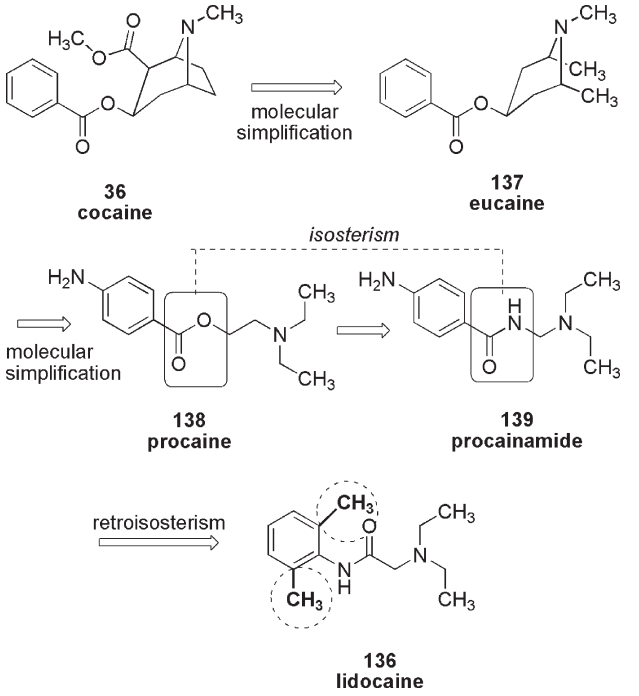


Figure 29. Structural design of lidocaine (**136**) from cocaine (**36**).

topical anesthetic, exhibits antiarrhythmic properties.[260] Lidocaine (**136**) also suffers from predominant hepatic metabolization, leading to the biotransformation of the major metabolite monoethylglycinexylidide (**140a**) by direct dealkylation of one ethyl chain in **141** and 3-(2,6-dimethylphenyl)-1-ethyl-2-methylimidazolidin-4-one (**140b**) by the production of a protonated imine (**142**), as shown in Figure 30.[3,261]

The methyl group has been widely used to protect newly discovered analgesic compounds against plasmatic amidases, and it is thus present in other drugs of this class such as bupivacaine (Marcain) (**143**), etidocaine (Duranest) (**144**), mepivacaine (Carbocaine) (**145**), and prilocaine (Citanest) (**146**) (Figure 31).

7.2. The Methyl Group As a Soft Metabolic Point: The Discovery of Celecoxib

The design of a selective PGHS-2 inhibitor presented a new and attractive therapeutic strategy that was more effective at fighting inflammatory conditions without inducing the characteristic gastroirritant effects that result from the use of first-generation NSAIDs.[262,263]

Celecoxib (Celebra) (**147**) was the first selective PGHS-2 anti-inflammatory drug launched, and its structural design was based on SC-236 (**148**), a promising lead compound with an extremely long plasmatic half-life (117 h in rats).[264] Celecoxib was designed through classic isosteric exchanges starting from SC-58125 (**149**) (Figure 32).[172,265] Although rapid metabolism of drugs is a big problem due to the reduced bioavailability and short duration of action, a very long half-life leads to the accumulation of the drug in tissues and thus to possible side effects.[3,266]

Compound SC-236 (**148**) has a chlorine in the *para* position of the aromatic ring, protecting this position from possible metabolic oxidation by cytochromes P450, mainly CYP2C9, CYP2D6, and CYP3A4.[257,267,268] The knowledge that lipophilic groups on the C-4 position of the aromatic ring are favorable for selectivity against PGHS-2, led researchers to find a group with this physicochemical behavior, but one that could be quickly

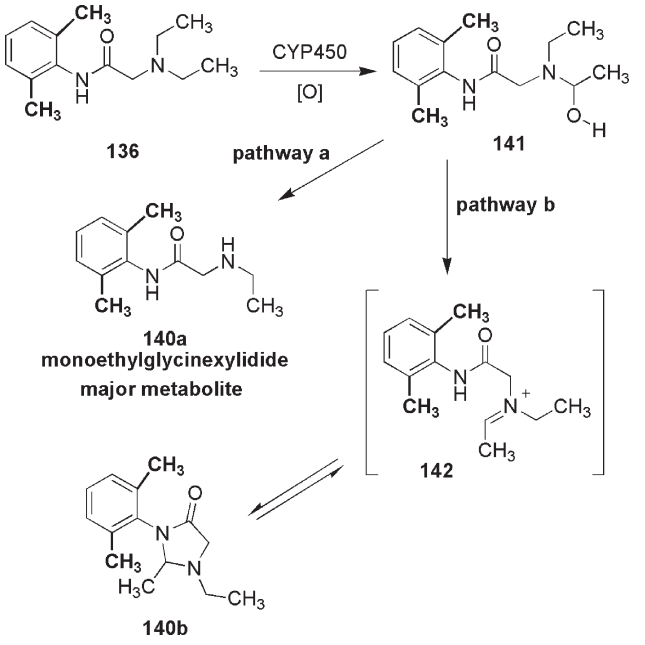


Figure 30. The importance of the *ortho* effect generated by methyl group in metabolic protection against plasmatic amidases.

metabolized. Aiming to reduce the plasmatic half-life of SC-236 (**148**), its structure was modified by making a classic monovalent isosteric exchange of the chlorine atom ($\pi_{p\text{-benzene}} = 0.73$) with a methyl group ($\pi_{p\text{-benzene}} = 0.60$) (Figure 32).[172] This group is converted into the corresponding hydroxymethylene (**150**) and carboxyl (**151**) derivatives mainly by human liver microsomal CYP2C9 (Scheme 6);[269,270] they present no activity against PGHS-1 but still act as weak PGHS-2 inhibitors.[264]

For this reason, the use of the *para*-methyl phenyl ring in this series of pyrazole compounds reduced its plasmatic half-life to 12 h in humans, giving rise to celecoxib (**147**), which was launched in 1999 due to its adequate bioavailability.[264]

The search for celecoxib analogues, notably with the diaryl-heterocyclic framework, has led to the release of other pharmaceuticals such as rofecoxib (Vioxx) (**152**) and valdecoxib (Bextra) (**153**) (Figure 33). However, due to cardiovascular problems associated with the chronic use of these PGHS-2 inhibitors, they have been banned.

7.3. Methyl Improves the Metabolic Stability of Thiazole- And Isoxazole-Containing Drugs

The thiazole and isoxazole rings are important heteroaromatic frameworks present in multiple classes of bioactive substances. Thiazole (**154**) is metabolized through CYP450 (CYP2C9)-dependent oxidation, resulting in ring-opening and formation of thioamide or thiourea metabolites (**155**) (Scheme 7), which are responsible for the toxicity displayed by drugs presenting this heterocyclic ring after bioactivation.[271] The production of species **155** results from the initial epoxidation of a C−C double bond, followed by hydrolysis of an unstable epoxide intermediate (**156**) (Scheme 7).

Isoxazole rings (**158**) are biotransformed by reductive cleavage of the O−N bond, resulting in the initial formation of an α-imine-enol intermediate (**159**), which can undergo further reactions in two different ways. The intermediate can be hydrolyzed and oxidized to produce the corresponding α-carboxy-enol metabolite (**160**)[271] (pathway A, Scheme 8); alternatively, it can

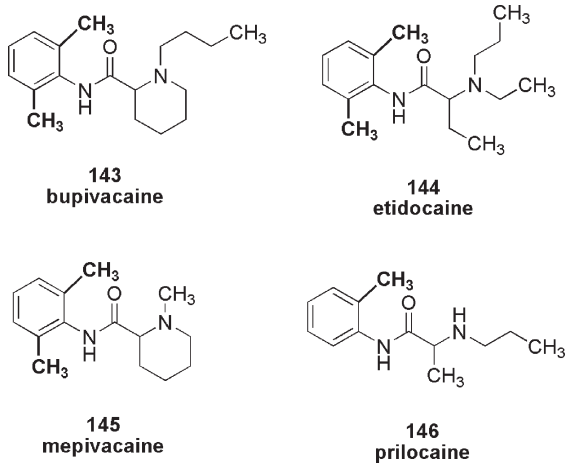


Figure 31. Analgesic compounds presenting the methyl *ortho* effect.

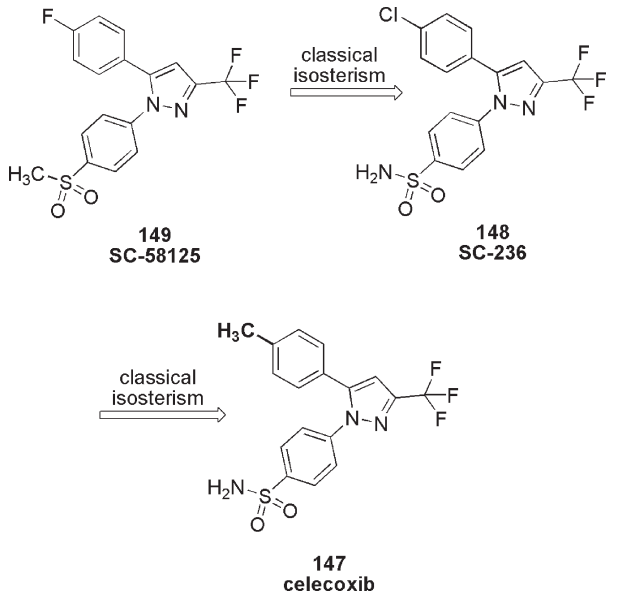
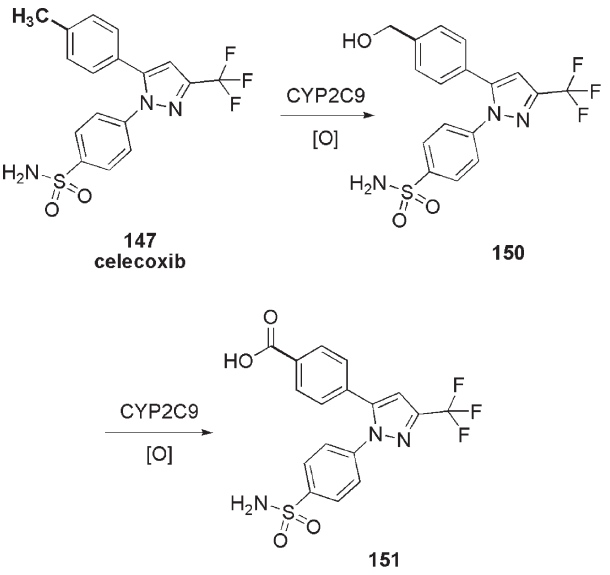


Figure 32. The discovery of celecoxib (**147**).

be *N*-oxidized to form an oxime intermediate (**161**), which is converted into the respective α-cyano-enol metabolite (**162**) through CYP450-dependent dehydration[272] (pathway B, Scheme 8). Moreover, **162** can also be produced after enzymatic or nonenzymatic removal of the hydrogen vicinal to the nitrogen atom of the isoxazole ring (**158**).[273]

Enolic anti-inflammatory drugs, which belong to the oxicam class, were described in the 1970s by the pioneering work of Lombardino and co-workers as a therapeutic alternative with improved efficacy and plasmatic half-life in comparison to drugs with carboxylate units as the pharmacophoric group.[274] Sudoxicam (**164**) was the first compound of this class to demonstrate efficacy in various animal models of inflammation.[275] However, despite the potential therapeutic application of the 1,2-benzothiazine derivative sudoxicam (**164**), its clinical development was discontinued due to its induction of severe hepatotoxicity. In addition, depletion of glutathione was promoted by its acylthiourea metabolite (**165**), which forms after CYP2C9 oxidation of the thiazole ring attached to carboxamide unit.[276] For these reasons,

Scheme 6. Major Metabolic Pathway of Celecoxib (147)



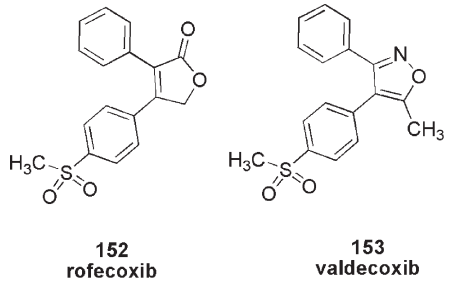
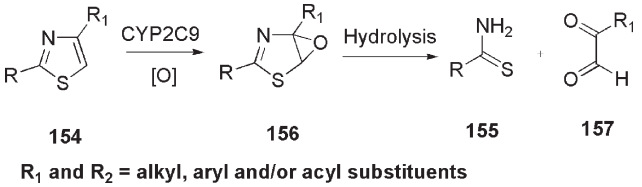


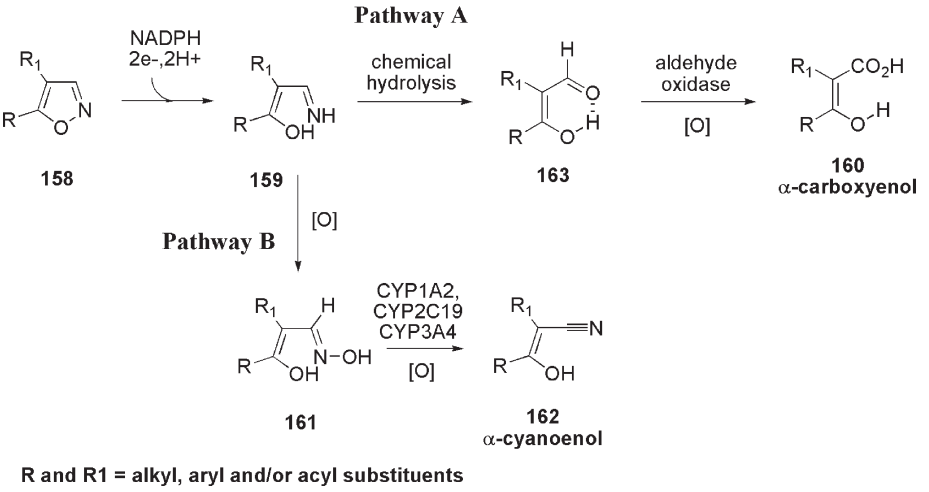
Figure 33. Selective PGHS-2 inhibitors.

Scheme 7. Metabolization of Thiazole Derivatives (154)



2-pyridine isostere piroxicam[277] (**166**) was developed and launched as the first marketed enol-carboxamide drug, and this was followed by thieno[2,3-*e*]-1,2-thiazine derivative tenoxicam[278] (**167**) a few years later (Figure 34). All of these oxicam derivatives provide their anti-inflammatory action by inhibiting PGHS and thereby blocking the production of prostaglandins.[279] The discovery of a second and inducible isoform of PGHS (named PGHS-2) responsible for the biosynthesis of prostaglandins during inflammatory processes changed the paradigm for the discovery of novel anti-inflammatory drugs.[280,281] The methylated analogue of sudoxicam (**164**) meloxicam (**168**) was characterized as a weakly selective PGHS-2 inhibitor.[282] The selectivity displayed by this compound results at least in part from the increased molecular volume due to the introduction of a methyl group. The methylation dramatically affects the metabolic

Scheme 8. Metabolization of Isoxazole Derivatives (158)



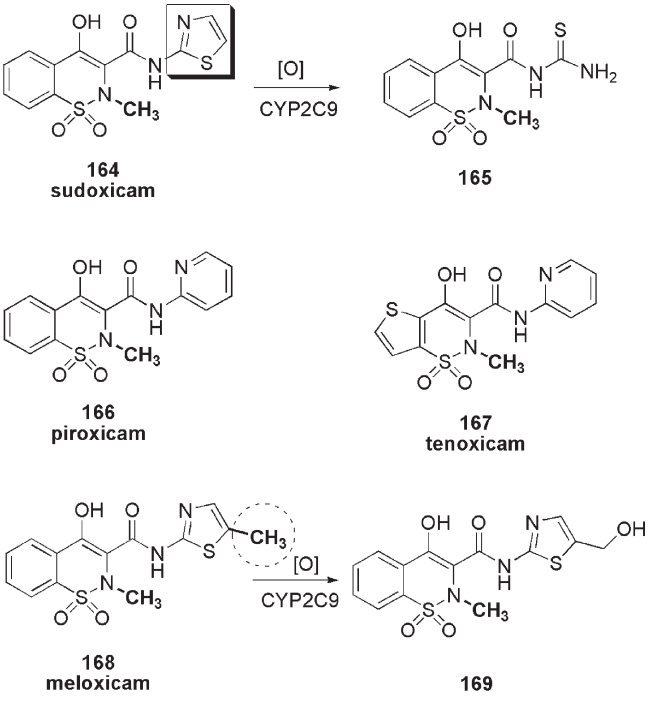


Figure 34. Metabolic protection by methyl groups in oxicams.

profile[276] by offering an additional labile group for CYP oxidation and avoiding the formation of a hepatotoxic species (**165**) (Figure 34).

Several other anti-inflammatory agents contain isoxazole units, e.g., the oxicam me-too drug isoxicam[283] (**170**), which was launched in 1983 by Warner-Lambert laboratories under the name of Pacyl, and the PGHS-2 selective inhibitor valdecoxib[284] (**153**) and its prodrug parecoxib[285] (**171**) (Dynastat). All of these drugs contain a metabolically soft methyl group at C-5 to improve the stability of the five-membered heteroaromatic ring (Figure 35).

The disease-modifying antiarthritic drug leflunomide[286] (**172**) (Arava) is another example of a bioactive compound presenting a 5-methyl-isoxazole unit. This immunosuppressor agent

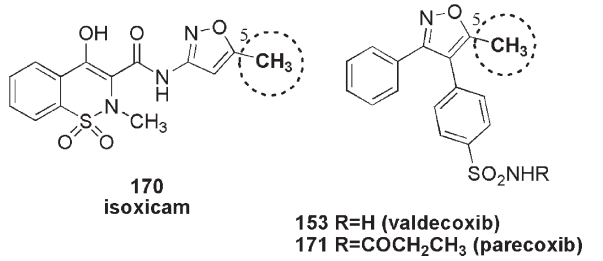


Figure 35. Anti-inflammatory agents presenting an isoxazole unit.

acts as a potent inhibitor of human dihydroorotate dehydrogenase after bioactivation to produce an α-cyanoenol metabolite A771726[287] (**173**) (Scheme 9). C3−H deprotonation during the first step of the formation of (**173**) by an enzymatic or nonenzymatic process was confirmed after comparison with the metabolic behavior of the corresponding C3-methyl derivative (**174**). This derivative (**174**) produces a mixture of hydroxylation products at C3-methyl (**175**) and C5-methyl (**176**) groups, whereas the active metabolite results from cleavage of the heterocyclic ring.[288]

7.4. The Methyl Effect in the Design of Orally Active Synthetic Prostaglandins

Prostaglandins became important in medicinal chemistry in the 1960s when these substances were "rediscovered" after a quiescent time after World War II. Industrial and academic laboratories attempted to exploit the therapeutic potential of prostaglandins for a diverse array of pathologies and therapeutic categories such as birth control, labor induction, asthma, arthritis, and peptic ulcers.[289,290]

In 1967, Robert and his team[291] discovered that prostaglandins of series E, PGE_1 (**177**) and PGE_2 (**178**), inhibit gastric secretion, ultimately leading to the use of these autacoids in vivo. Their rapid metabolism and short-lived pharmacological action result from the oxidation of the hydroxyl group at C-15 to the corresponding ketone by prostaglandin dehydrogenases (PGDH) (Figure 36).[289,291]

The biggest advance in the discovery of synthetic prostaglandin analogues was achieved by researchers at the no longer

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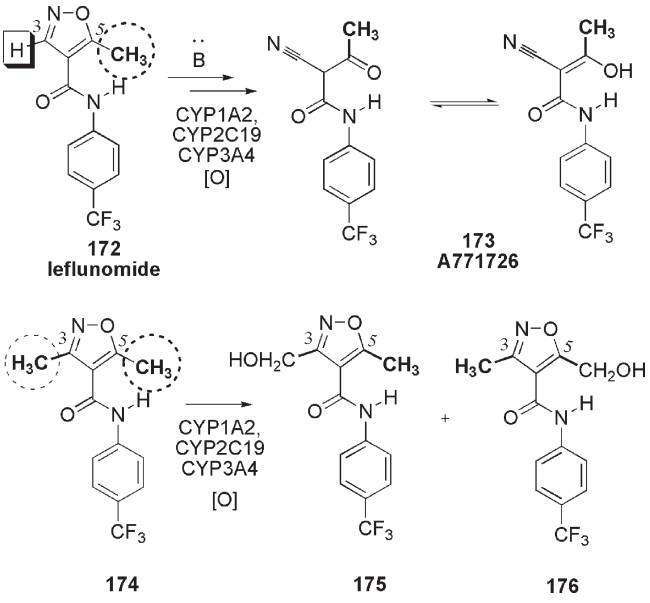
Scheme 9. The Bioactivation of Leflunomide (172)



existing pharmaceutical company Upjohn[292,293] through the addition of methyl groups to the 15 and 16 carbons of PGE_2 (**178**). The resulting molecule provides steric protection from the oxidative metabolism of the hydroxyl group at C-15.[292,293] The additional molecular volume resulting from the addition of these groups blocks the action of PGDH, which is dependent on the interaction of the substrate with the NAD^+ coenzyme and complexing of the zinc atom present in the catalytic site (Figure 37).[294]

(15*S*)-15-Methyl- and 16,16-dimethyl-PGE derivatives generated by the introduction of methyl groups were found to be active when administered orally. They are potent and have a relatively long half-life for the inhibition of gastric secretion.[295] A similar approach was successfully applied to the design of new prostaglandin analogues. Through methylation at C-15, other analogues were discovered such as arbaprostil ((15*R*)-15-methyl-PGE_2) (**181**),[296] prostalene (**182**),[297] and tiprostanide (13-thia-PGE_1) (**183**)[298] (Figure 38). Methylation at C-16 generates trimoprostil (isostere of 16,16-dimethyl-PGE_2) (**184**),[299,300] nocloprost (9-β-chloro analogue of 16,16-dimethyl-PGE_2) (**185**),[301] dimoxaprost (16,16-dimethyl-oxa-alkyl-PGE_2) (**186**),[302] gemeprost (C-2 (*E*)-analogue of 16,16-dimethyl-PGE_1) (**187**),[303] and meteneprost (9-methylene isostere of 16,16-dimethyl-PGE_2) (**188**)[304,305] (Figure 38).

However, these compounds still presented adverse effects such as diarrhea and emesis.[306] Several changes were made to reduce the side effects of these prostaglandin analogues.[289] The transposition of the hydroxyl group at PGE_1 (**177**) from C-15 to C-16 significantly reduces these effects while maintaining the antisecretory activity (Figure 39).[307]

The hydroxyl group without the steric protection of the methyl group in this new series of PG analogues was the target of the same dehydrogenases that oxidize the hydroxyl group at C-15 and inactivate PGE_1 (**177**) and PGE_2 (**178**). The introduction of methyl groups into this series of PG analogues as a form of metabolic protection led to the identification of misoprostol (**189**),[308,309] a C-16 methylated derivative that presents increased antisecretory potency when administered orally.[308] Misoprostol

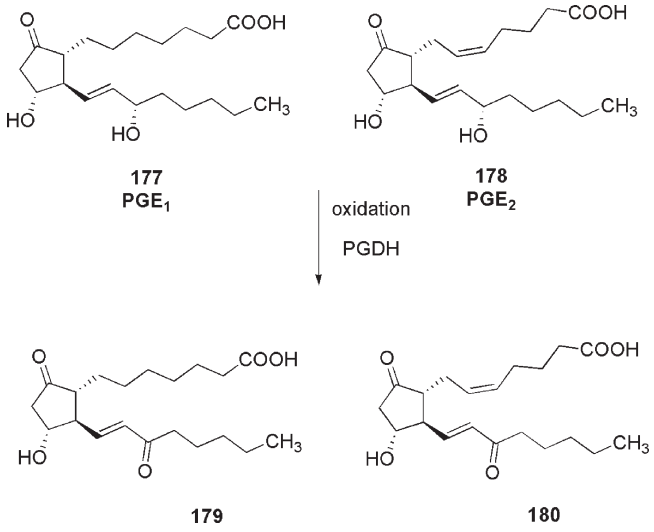


Figure 36. C-15 oxidation in prostaglandins.

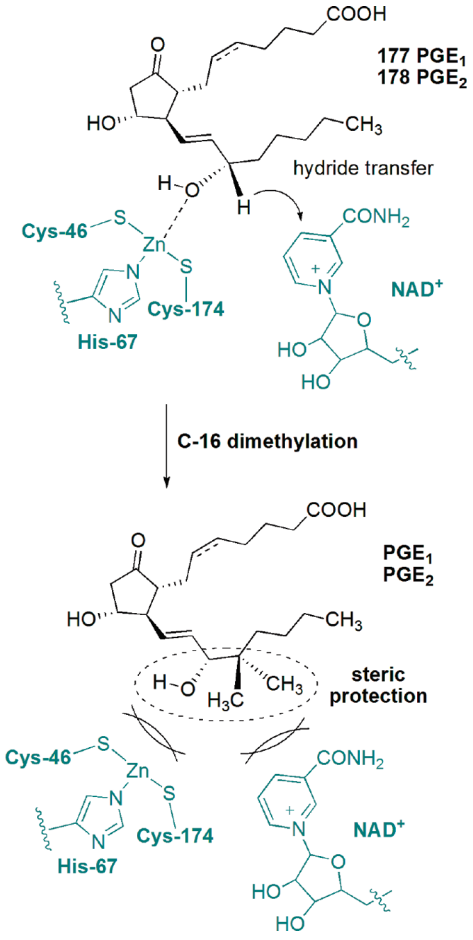


Figure 37. Steric protection from PGDH action promoted by methyl groups at ω-chain of PGE_1 (**177**) and PGE_2 (**178**).

(**189**) has been licensed for the treatment and prevention of gastric and duodenal ulcers, and side effects (such as diarrhea) that are characteristic of the 15-hydroxy prostaglandin analogues are reduced.[309] Metabolic protection through the use of a methyl group on C-16 of prostaglandin analogues has been widely used,

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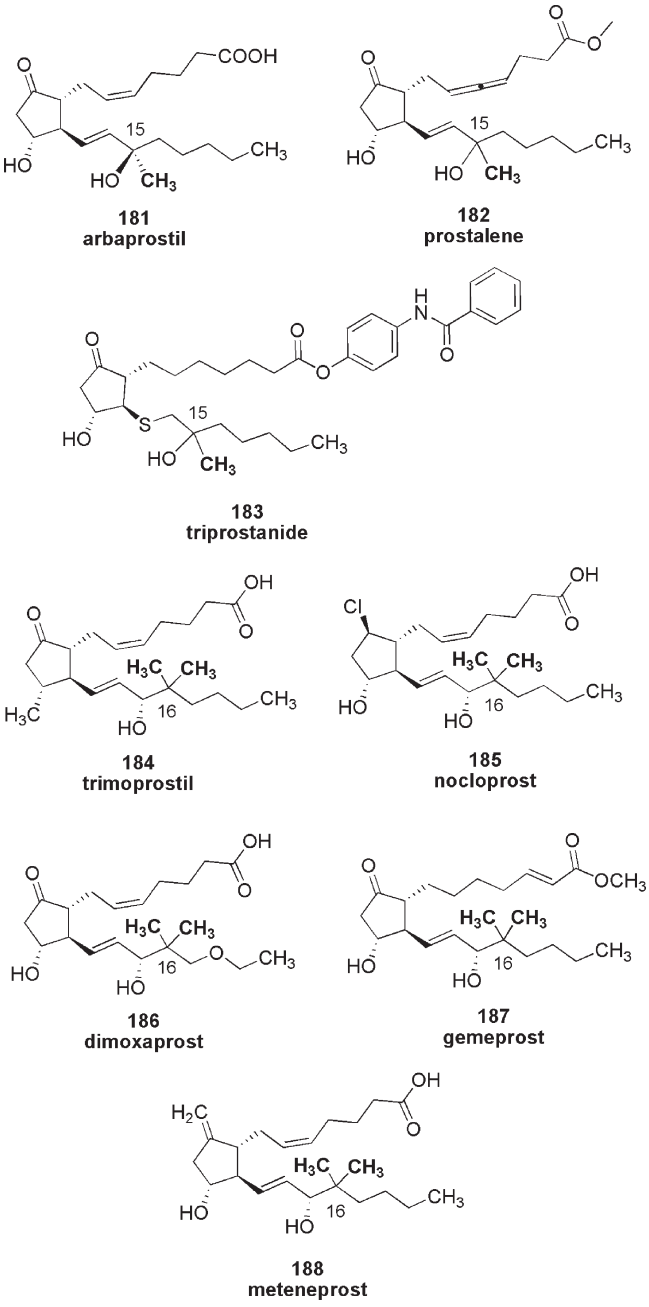

Figure 38. C-15 and C-16-modified PGE derivatives.

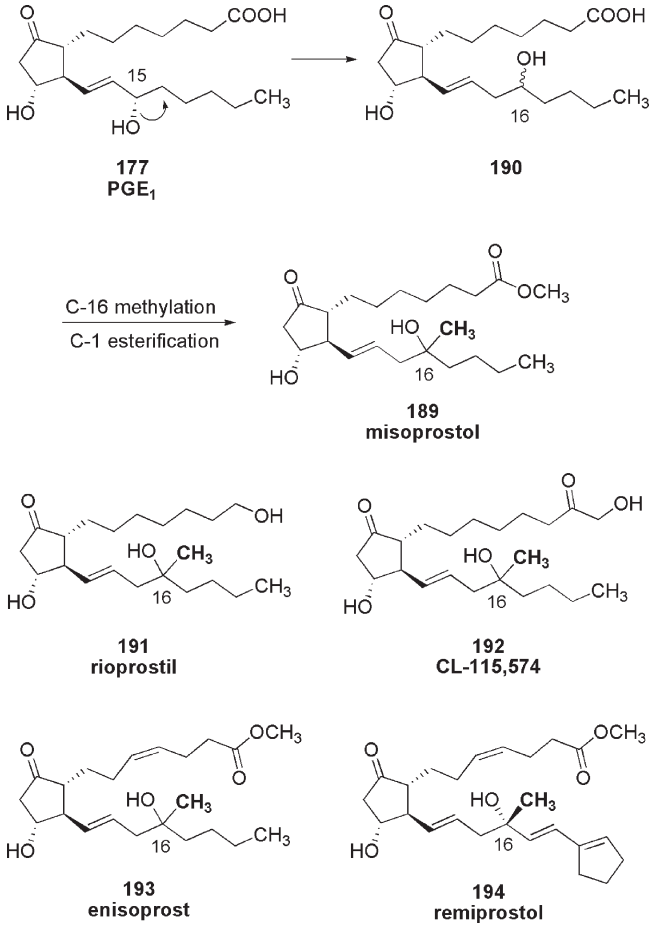

Figure 39. Methylation on C-16 and the discovery of orally bioavailable prostaglandins.

generating other synthetic prostaglandins such as the PGE_1 derivatives rioprostil (1-hydroxy analogue of misoprostol) (**191**)[310,311] and CL-115,574 (**192**),[312,313] as well as enisoprost (Δ^4-*Z*-analogue of misoprostol) (**193**)[309] and remiprostol (ω-chain cyclopentenyl analogue of enisoprost) (**194**),[314,315] an analogue of PGE_2 (Figure 39).

7.5. Methyl in Metabolic Activation of Prodrugs

The first-choice schistosomicide drug oxaminiquine[316,317] (**195**) (Mansil) was launched in 1974 by Pfizer after molecular simplifications of the thioxanthone derivative hycantone (**196**) (Etrenol), which was characterized by Archer et al.[318] as the active metabolite of lucanthone (**197**) (Mirasil A) (Figure 40). The superior antihelmintic profile of hycanthone[319] (**196**), in addition to its physiochemical properties, particularly its higher water solubility and consequently lower partition coefficient, explains the reduced central effects evidenced for (**196**) in comparison to the prodrug (**197**). Further studies of the molecular mechanism of action indicated that the benzyl alcohol of (**196**) is a selective substrate for parasitic enzymes, which can turn it into a better leaving group after phosphorylation or sulfation, thus favoring the formation of reactive azo-type quinones that are responsible for its lethal effects on *Schistosoma mansoni*.[320]

8. METHYLATION IN THE SOSA STRATEGY OF DRUG DESIGN

Selective Optimization of Side Activities (SOSA), which was coined by Wermuth,[321,322] is a medicinal chemistry approach that represents a valid alternative to HTS. It involves trying old drugs on new pharmacological targets with the goal of testing a limited amount of compounds with great structural and therapeutic diversity and known biodistribution and security profiles.[321,322]

A typical case in the use of methyls in the SOSA approach was the discovery of endothelin receptor antagonists (ET_A) by Bristol-Myers-Squibb (BMS) scientists.[321,323] From sulfathiazole (**198**) trials, a drug from BMS itself, they discovered that this antibiotic was a weak ligand for ET_A receptors.[323] By testing isosteric modifications on the thiazole ring and mainly through

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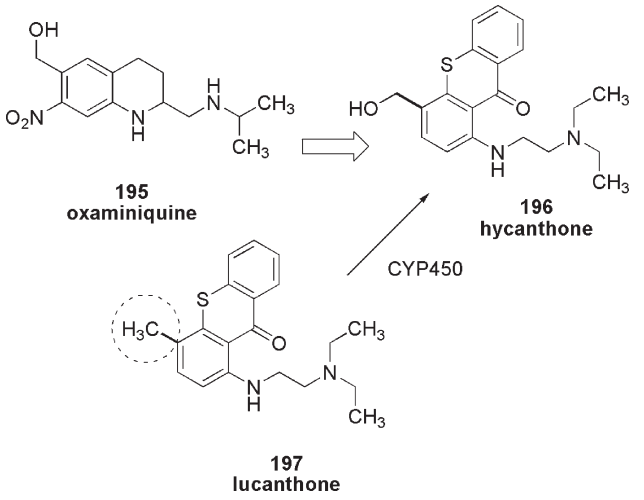


Figure 40. Metabolic activation of lucanthone (**197**).

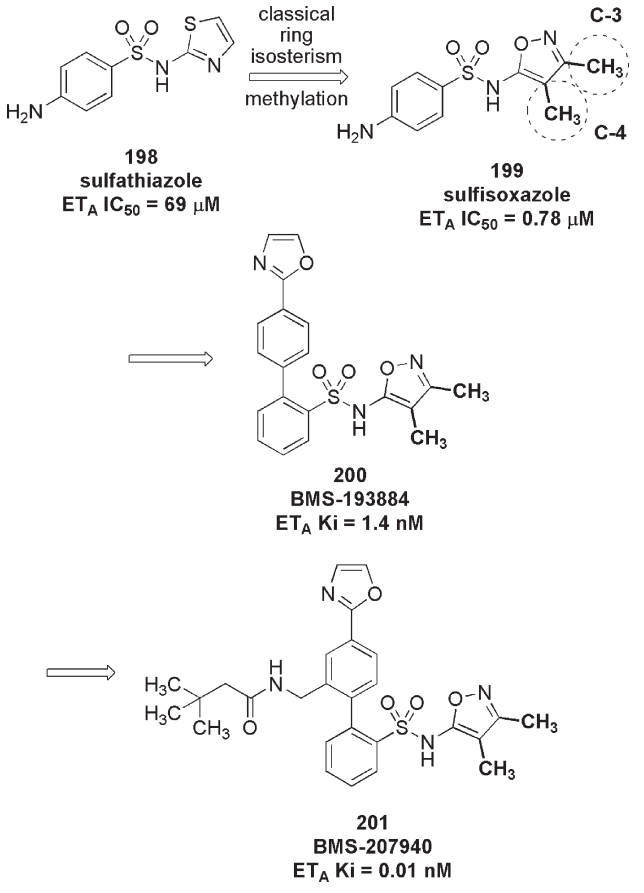


Figure 41. Methylation in the SOSA strategy applied to the discovery of new ET$_A$ receptor antagonists.

the introduction of two methyls (at C-3 and C-4) to the isoxazolic compound sulfisoxazole (**199**), researchers discovered compound BMS-193884 (**200**). This molecule exhibits optimized affinity for the ET$_A$ receptor, demonstrating that the methyl at C-4 is essential for potency, whereas the methyl at C-3 is responsible for selectivity (Figure 41).[323] Additional modifications on the *para*-aminobenzenesulfonamide ring to

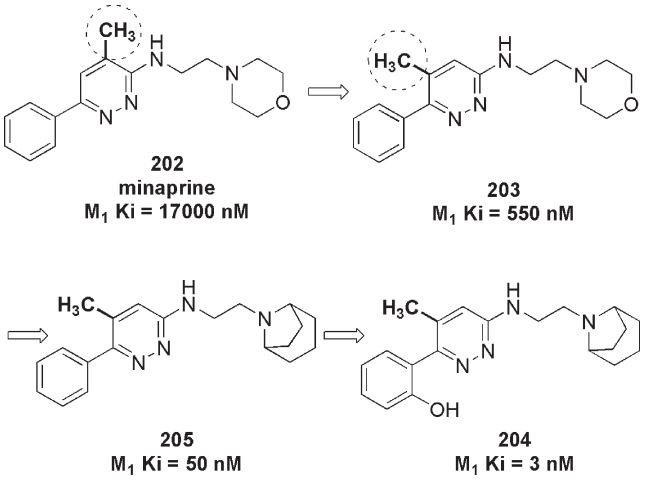
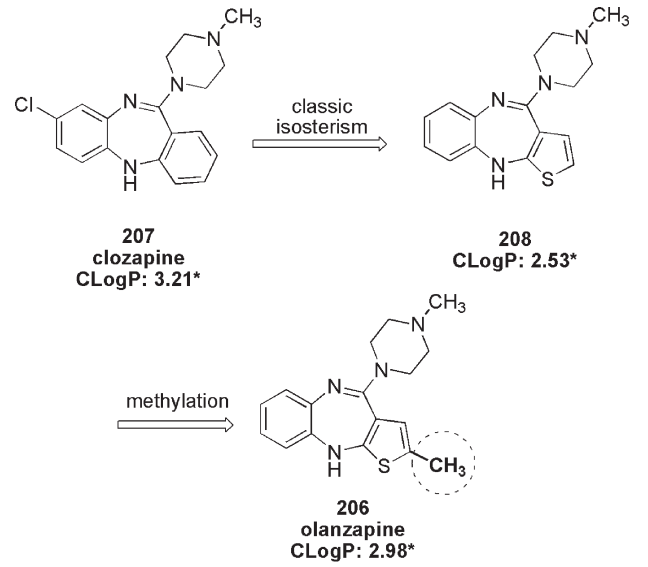


Figure 42. Methylation using the SOSA strategy in the discovery of new selective antagonists of M$_1$ receptors.



*calculated by using OSIRIS Property Explorer (ref 342)

Figure 43. The use of methyl groups in the discovery of me-too drugs.

increase its potency and bioavailability led to the discovery of BMS-207940 (**201**), a powerful and selective ET$_A$ receptor antagonist with in vivo hypotensive activity when taken orally (Figure 41).

In addition to the introduction of a methyl group to a bioactive compound, its regioisomers can also be important for optimizing its pharmacological activity.[321] For example, Wermuth and associates moved the methyl from C-4 to C-5 in minaprine (**202**), a drug that acts on dopaminergic and serotoninergic transmission and also has a weak affinity for muscarinic M$_1$ (K_i = 17 μM) receptors. This generated **203**, which shows a 35-fold increase in affinity for M$_1$ receptors (Figure 42). Additional modifications to the morpholine and the addition of an *ortho*-hydroxyl group to the phenyl group led to **204**, a potent partial agonist of M$_1$ devoid of activity on dopaminergic or serotonergic transmission (Figure 42).[324,325]

9. THE METHYL IN GENERATING ME-TOO DRUGS

Some examples of the use of methyls in the isosteric exchange of a six-membered ring for a heteroaromatic five-membered ring can be found in the literature, such as in the design of me-too drugs. This strategy aims to adjust the lipophilicity of molecules because the contraction of a six-membered ring into a five-membered one decreases its lipophilicity. In addition, this modification provides protection from oxidative metabolism, which might generate toxic species from these five-membered heterocycles.[5]

The drug olanzapine (Zyprexa) (**206**), an atypical antipsychotic approved by the FDA in 1996 for the treatment of schizophrenia,[326] was designed based on the benzodiazepine structure of clozapine (**207**) through the removal of the chlorine atom at C-8 and a classic isosteric exchange of phenyl in (**207**) by thiophene in (**208**).[327] However, these modifications led to a decrease of over one logarithmic unit in the calculated ClogP by OSIRIS Property Explorer.[328] Moreover, because this class of compounds acts on the central nervous system (CNS), this reduction could also reflect pharmacological activity. The methyl introduced into C-2 of (**208**) aimed to increase lipophilicity and to provide metabolic protection for the thiophene ring of olanzapine (**206**) (Figure 43).[327,329,330]

10. CONCLUSIONS

In this review, we aimed to highlight the importance of the simple methyl group as a very useful structural modification in the rational design of bioactive compounds and drugs. We selected different examples from distinct classes of organic compounds with several pharmacological properties in order to illustrate that an apparent subtle molecular modification can strongly alter the biological profile of a drug. The methyl effect alter both biological phases of a drug, represented by its pharmacodynamic and pharmacokinetc profile, due to the modifications introduced in the stereoeletronic properties. In fact, it was illustrated that distinct metabolic behavior or receptor affinity could be modified by the methyl effect. Among the examples included many other important ones could be used, such as the importance of the methyl in modified the bioprofile of phenyl ethanolamines with differentiation in the molecular recognition pattern among different adrenergic receptor subtypes. The introduction of an simple extra methyl connected to the *N*-alkylpiperidine moiety present in PDE5 inhibitor raisng the discovery of me-too drug vardenafil for erectile dysfunction. In fact, the correct use of this strategy for molecular modification and design of drugs or drug-candidate compounds may be useful from both a pharmacokinetic and a pharmacodynamic point of view, representing an important tool for the medicinal chemist.

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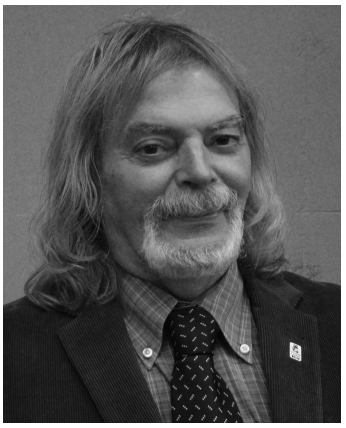
BIOGRAPHIES



Eliezer J. Barreiro concluded his scientific education (Docteur-ès-Sciences d'État) in Medicinal Chemistry at the University of Grenoble, France, in 1978, under the direction of Professor Pierre Crabbé and Dr Andrew E. Greene. He spent four years as Associate Professor of Organic Chemistry at Federal University of São Carlos, S.P. from 1979 to 1983, when he joined the Federal University of Rio de Janeiro where he got a permanent position. He works in the medicinal chemistry field and created the Laboratório de Avaliação e Síntese de Substâncias Bioativas (LASSBio) at the School of Pharmacy of Federal University of Rio de Janeiro. Professor Barreiro has published over 200 journal scientific articles and book chapters and a book concerning the field of medicinal chemistry and he is the inventor of 14 patents of bioactive compounds, drug candidates acting as cardioactive, CNS-active, anti-inflammatory, and antileishmania diseases.

Arthur Eugen Kümmerle was born in Rio de Janeiro, Brazil, in 1979. He received his degree in Pharmacy (2003), his M.Sc. in Organic Chemistry (2005), and his Ph.D. in Chemistry in 2009 from Federal University of Rio de Janeiro (UFRJ), Brazil, where he studied the effects of the methyl group on the analgesic and antiinflamatory properties of a chemical library of *N*-acylhyadrazones under the guidance of Prof. Eliezer J. Barreiro and Prof. Carlos A. M. Fraga. In 2009, he was assistant professor of Chemical Pharmaceutical Technology at the same university, and since 2010 he is professor of Organic and Medicinal Chemistry at Rural Federal University of Rio de Janeiro (UFRRJ), Brazil. His research interests focus on heterocyclic and microwave chemistry in the scope of medicinal chemistry, aiming at bioactive compounds for the treatment of anti-inflammatory, cardiovascular, tropical, and degenerative diseases.

Carlos Alberto Manssour Fraga was born in Rio de Janeiro (Brazil) in 1964. He obtained a B.Sc. degree in Pharmacy in 1988 and his M.Sc. degree in Sciences (Medicinal Chemistry) from Federal University of Rio de Janeiro (UFRJ). After obtaining his Ph.D. degree from Chemistry Institute of UFRJ in 1994, working with the synthesis of novel stable prostacyclin mimetics under the supervision of Professor Eliezer J. Barreiro, Carlos Alberto Manssour Fraga joined the Faculty of Pharmacy of UFRJ (Rio de Janeiro) as Associate Professor in 1996 and was promoted to Professor in May 2006. He was Head of the Drug Department of the Faculty of Pharmacy (UFRJ) from 2008 to 2010, and currently he is Coordinator of the Post Graduate Program in Pharmacology and Medicinal Chemistry of the Institute of Biomedical Sciences of UFRJ. Dr. Fraga is an effective member of Brazilian Chemical Society since 1991, where he was Director of the Medicinal Chemistry Division from 2002 to 2004. Apart from teaching, Professor Fraga develops his research activities in LASSBio (Laboratório de Avaliação e Síntese de Substâncias Bioativas, UFRJ), focusing the design, synthesis, and pharmacological evaluation of novel drug candidates able to act in multifactorial diseases, with particular emphasis in the use of *N*-acylhydrazone framework as a privileged structure to discover novel therapeutically valuable compounds.

ACKNOWLEDGMENT

We thank the CNPq (BR) and FAPERJ (BR) for fellowships and grants (CNPq nos. 302.918/07-8, 303.977/09-4, 142613/05-3; FAPERJ nos. E-26/102.695/08, E-26/102.350/09, E-26/100.329/08) and to Professor Angelo C. Pinto (UFRJ, BR) for helpful discussions and suggestions.

LIST OF ABBREVIATIONS

A_1	adenosine A_1 receptor
A_{2A}	adenosine A_{2A} receptor
A_{2B}	adenosine A_{2B} receptor
A_3	adenosine A_3 receptor
Ala	alanine
AMP	adenosine monophosphate
AMPc	cyclic adenosine monophosphate
Arg	arginine
Asp	aspartic acid
ATP	adenosine triphosphate
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
Bcr-Abl	Bcr (*breakpoint cluster region*)-abl (*gene*) fusion protein
Bcr-AblK	Bcr-Abl kinase
CB1	CB1 cannabinoid receptor
ClogP	Ccalculated Log *P*
CML	chronic myeloid leukemia
CNS	central nervous system
compd	compound
COMT	catechol *O*-methyl transferase
CYP1A2	cytochrome P450 1A2
CYP2C19	cytochrome P450 2C19
CYP2C9	cytochrome P450 2C9
CYP2D6	cytochrome P450 2D6
CYP3A4	cytochrome P450 3A4
CYP450	cytochrome P450
Cys	cysteine
DNA	deoxyribonucleic acid
dTMP	thymidine monophosphate
dUMP	deoxyuridine monophosphate
ED_{50}	effective dose
EGFR	epidermal growth factor receptor
ET_A	endothelin receptor antagonists
FDA	Food and Drug Administration
GMP	guanosine monophosphate
H^+-K^+-ATPase	hydrogen potassium adenosine triphosphatase
H_1	histamine receptors of subtype 1
H_2	histamine receptors of subtype 2
H-bond	hydrogen bond
HER1	human EGFR type 1
HER2	human EGFR type 2
His	histidine
HMG CoA_R	3-hydroxy-3-methylglutaryl-coenzyme A reductase
HMG-CoA	3-hydroxy-3-methylglutaryl-coenzyme A
HNMT	histamine *N*-methyl transferase
HTS	high-throughput screening
IC_{50}	half maximal inhibitory concentration
icv	intracerebroventricular administration
Ile	isoleucine
IMP	inositol monophosphate
INMT	indolethylamine *N*-methyl transferase
K_i	affinity constant
K_M	Michaelis Menten constant
LASSBio	Laboratório de Avaliação e Síntese de Substâncias Bioativas
Leu	leucine
LIS	lanthanide dislocation inducing salts
Log *P*	logarithm of partition coefficient
Lys	lysine
M_1	muscarinic receptor M_1
MAT	*S*-adenosylmethionine synthetase
max inhib	maximal inhibition
Met	methionine
MR	molar refractivity
NAD^+	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide
$NADP^+$	nicotinamide adenine dinucleotide phosphate
NADPH	nicotinamide adenine dinucleotide phosphate
nM	nanomolar
NMR	nuclear magnetic resonance experiments
NNMT	nicotinamide *N*-methyl transferase
NSAIDs	nonsteroidal anti-inflammatory drugs
PBQ writhing	phenylbenzoquinone writhing test
PDB ID	Protein Data Bank identification

PDE	phosphodiesterase
PDGF-Rs	platelet-derived growth factor
PG	prostaglandin
PGDH	15-hydroxyprostaglandin dehydrogenase
PGE_1	prostaglandina E1
PGE_2	prostaglandina E2
PGHS-1	prostaglandin endoperoxide synthase 1
PGHS-2	prostaglandin endoperoxide synthase 2
Phe	phenylalanine
PKC	protein kinase C
PKs	protein kinases
PMT	phenol *O*-methyl-transferase
PNMT	phenylethanolamine *N*-methyl-transferase
PPi	pyrophosphate
PPIs	proton pump inhibitors
RNA	ribonucleic acid
SAM	*S*-adenosylmethionine
sc	subcutaneous injection
SEM	standard error of the mean
Ser	serine
S_N2	bimolecular nucleophilic substitution
SOSA	selective optimization of side activities
$t_{1/2}$	half-life
Thr	threonine
TK	tyrosine kinase
TM	trans membrane helix
TMP	thiol methyltransferase

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