From Nature to Drug Discovery: The Indole Scaffold as a '*Privileged Structure*'

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Abstract: The indole scaffold probably represents one of the most important structural subunits for the discovery of new drug candidates. The demonstration that many alkaloids contain the indole nucleus, the recognition of the importance of essential amino acid tryptophan in human nutrition and the discovery of plant hormones served to bring about a massive search on indole chemistry, giving rise to a vast number of biologically active natural and synthetic products, with a wide range of therapeutic targets, such as anti-inflammatories, phosphodiesterase inhibitors, 5-hydroxytryptamine receptor agonists and antagonists, cannabinoid receptors agonists and HMG-CoA reductase inhibitors. Many of these target-receptors belong to the class of GPCRs (integral membrane G-protein coupled receptors) and possess a conserved binding pocket that is recognized by the indole scaffold in a "common" complementary binding domain, explaining the great number of drugs that contain the indole substructure, such as indomethacin, ergotamine, frovatriptan, ondansetron, tadalafil, among many others.

Key Words: Indole scaffold, privileged structure, GPCR, bioisosterism, drug discovery.

INTRODUCTION

The term 'privileged structure' was introduced in medicinal chemistry by Evans and co-workers to define scaffolds which were capable of providing useful ligands for diverse receptors and judicious modification of such structures could be a viable alternative for design of new receptor agonists and antagonists [1]. Since then, the term has appeared in literature many times and some organic scaffolds were classified as privileged structures such as 1,4-benzodiazepin-2-one, biphenyl, 1,4-dihydropyridine, 4-substituted piperidine and indole, which were synthesized and screened against a variety of different receptors, yielding several active compounds [2].

In 2000, Patchett and Nargund published a review focusing primarily on the use of privileged structures in the design of G-protein coupled receptor (GPCR) agonists and antagonists, highlighting many examples of strategies used in the discovery of ligands such as privileged structure-based antagonists, peptidomimetic GPCR agonists and privileged structure libraries [3]. More recently, Duarte and co-workers have described structures pursuing the privileged status, not covered in other papers, emphasizing the use of the concept 'privileged structure' in the finding of new leads in medicinal chemistry [4].

The indole scaffold probably represents one of the most important structural subunits for the discovery of new drug candidates. The demonstration that many alkaloids contain the indole nucleus, the recognition of the importance of es-

sential amino acid tryptophan in human nutrition as structural constituent of many proteins and biosynthetic precursor of neurotransmitter serotonin, and the discovery of plant hormones served to bring about a massive search on indole chemistry, giving rise to a vast number of biologically active natural and synthetic derivatives containing the indole scaffold [5-6], which were pharmacologically evaluated as G-protein coupled receptors agonists and antagonists, ion channel blockers and enzyme inhibitors, as will be discussed below.

ENZYME INHIBITORS

Enzymes are natural biocatalysts involved in all catabolic and metabolic reactions at biophase, which in addition to present high efficiency, specificity and stereoselectivity, could be regulated through the binding of effector molecules, such as an enzyme-specific inhibitor, as a ubiquitous and general principle for the fine tuning and control of their metabolic activity [7].

Enzyme inhibition is one of the most used approaches in drug discovery and many diseases may be successfully resolved by interrupting an enzymatic pathway intrinsically associated with the expressed physiopathology [8]. In recent years, the discovery of enzyme inhibitors not only have provided an increased number of potent therapeutically useful agents for the treatment of diseases, but also have significantly contributed to the better comprehension of the role of a determined biochemical pathway and a target enzymatic transformation in the development of some physiopathological states [9].

HMG-CoA Reductase Inhibitors

Statins are a group of cholesterol-lowering agents that have become some of the largest selling drugs worldwide.

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They are used therapeutically to reduce risk of coronary heart disease by serum cholesterol levels and upregulating low-density lipoprotein receptors in the liver, through competitive inhibition of 3-hydroxy-3-methylgluta-ryl-coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis, which is responsible for conversion of HMG-CoA to mevalonate [10]. All statin drugs, *e.g.* atorvastatin (**1**, Fig. (**1**)), function similarly through binding to the active site of HMG-CoA reductase and thus inhibiting the enzyme. However, structural differences in statins may partially account for differences in inhibitory enzyme potencies [11].

In this context, fluvastatin (**2**, Fig. (**1**)), an indole-containing type II statin, was synthesized in 1983 as the first non-compactin HMG-CoA reductase inhibitor [12], which generated the corresponding bisphenyl-pyrrole analogue atorvastatin (**1**) by exploiting classical ring opening strategy [13]. Istvan and Deisenhofer showed how the specificity and tight binding of statins, such as fluvastatin (**2**) and atorvastatin (**1**), was achieved, using a crystal structure of the catalytic region of human HMG-CoA reductase receptor bound to the inhibitor, through van der Waals interactions with amino acid residues Leu^{562}, Val^{683}, Leu^{853}, Ala^{856} and Leu^{857} located at hydrophobic side chains of the enzyme. The bulky, hydrophobic statin derivatives occupy the HMG-binding pocket and part of the CoA binding surface, blocking the access of natural substrate HMG-CoA to HMG-CoA reductase active site [14] (Fig. (**1**)).
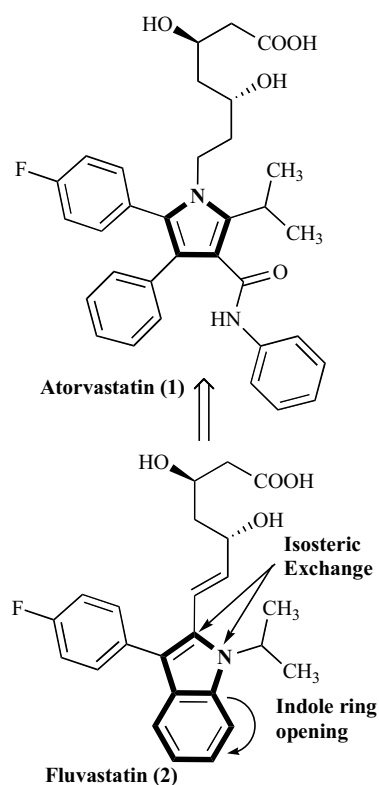
Cyclooxygenase Inhibitors

Non-steroidal anti-inflammatory drugs (NSAIDs) continue to be one of the most widely used groups of therapeutic agents for the treatment of pain, inflammation and fever associated to some diseases. They produce their pharmacological effects by the common ability to inhibit the cyclooxygenase (COX), a key enzyme that catalyzes the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), the immediate precursor to prostaglandins, thromboxane A_2 and prostacyclin [15].

As result of a systematic investigation of anti-inflammatory activity of 350 indole acetic acid derivatives structurally related to serotonin and its metabolites, Shen and co-workers reported in 1963 the synthesis and characterization of antipyretic and anti-inflammatory profiles of the most potent compound of this series, *i.e.* indomethacin (**3**, Fig. (**2**)), a member of the arylalkanoic acids class, which was launched in U.S. market in 1965 [16].

After the discovery of the second isoform of cyclooxygenase enzyme, whose production could be induced by inflammatogenic stimulus, Hu and co-workers showed that the indole ring proved to be an effective scaffold for the design of new COX-2 inhibitors, belonging to the terphenylic class. This study led to the discovery of two compounds, *i.e.* 2-phenyl-3-sulfonylphenyl-indole derivatives (**4**) and (**5**), which possess higher COX-2 inhibitory activity than celecoxib (**6**) on cellular assay [17] (Fig. (**2**)).
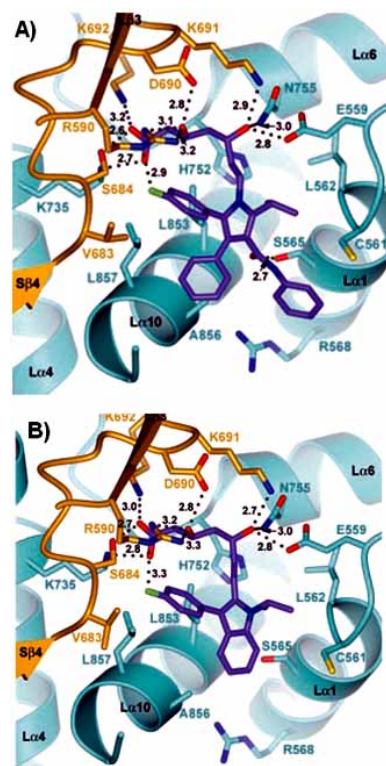
Fig. (1). Structural design of HMG-CoA reductase inhibitor atorvastatin (**1**) from molecular modifications on indole derivative fluvastatin (**2**), illustrating their respective binding modes (**A**) and (**B**) in the active site of target enzyme. Figure adapted from Istvan and Deisenhofer [14].
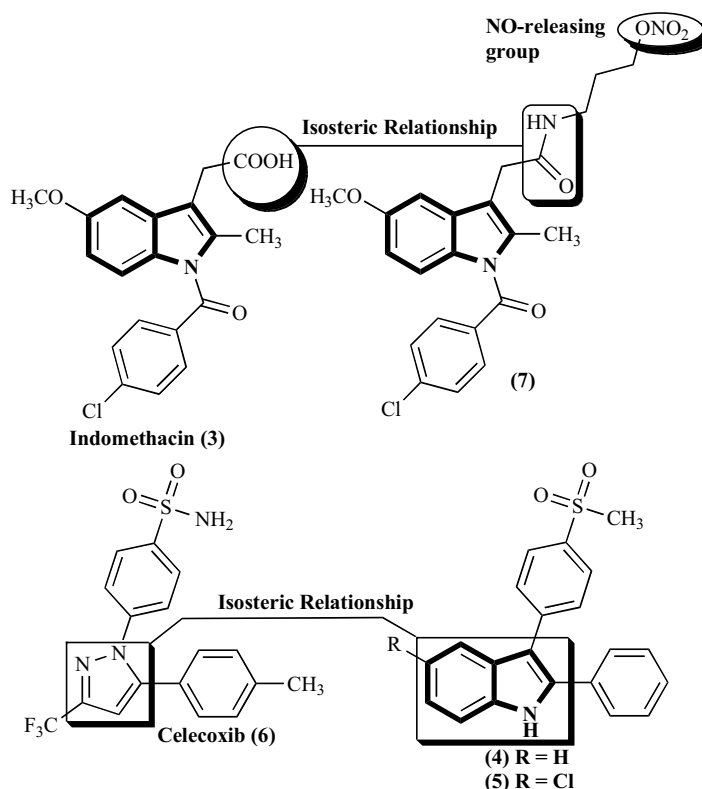
Fig. (2). Structure of some indole-containing anti-inflammatory drugs that act as COX inhibitors.

On the other hand, Wey and colleagues reported a series of nitric oxide-donating COX-2 selective inhibitors, using indomethacin (**3**) as a starting template structure exploited in an attempt both to increase cyclooxygenase-2 selectivity and to enhance drug safety, through adequate modifications in the acidic moiety. The compound (**7**, Fig. (**2**)) was successfully designed and synthesized as a NO-enhanced COX-2 selective inhibitor *in vivo*, while being exceptionally well tolerated without apparent gastrointestinal problems [18].

Phosphodiesterase (PDE) Inhibitors

The cyclic nucleotide phosphodiesterases (PDEs) are a family of related phosphohydrolases that selectively catalyze the hydrolysis of the 3'-cyclic phosphate bond of adenosine and/or guanosine 3',5'-cyclic monophosphate (cAMP or cGMP, respectively), modulating their ability to act as intracellular second messengers. PDEs are recognized as good drug targets due to the fact that there are so many different isoforms, with particular and prevalent tissue distributions. Currently, it is widely accepted that there are 11 different families of PDEs comprising 21 different gene products [19].

The physiological importance of PDE-5 in regulation of smooth muscle tone has been demonstrated most clearly by clinical use of its specific inhibitors for the treatment of erectile dysfunction and more recently pulmonary hypertension [20]. Tadalafil (**8**, Fig. (**3**)), a potent and selective indole-containing PDE-5 inhibitor, was selected as clinical development candidate for the treatment of hypertension and congestive heart failure and in 2003 was approved for the treatment of male erectile dysfunction in the U.S. [21].

On the other hand, in the search for an alternative anti-inflammatory treatment to corticosteroids, selective phosphodiesterase 4 (PDE-4) inhibitors have received so much attention because they are able to suppress the functions of several cell types involved in allergic and inflammatory dis-
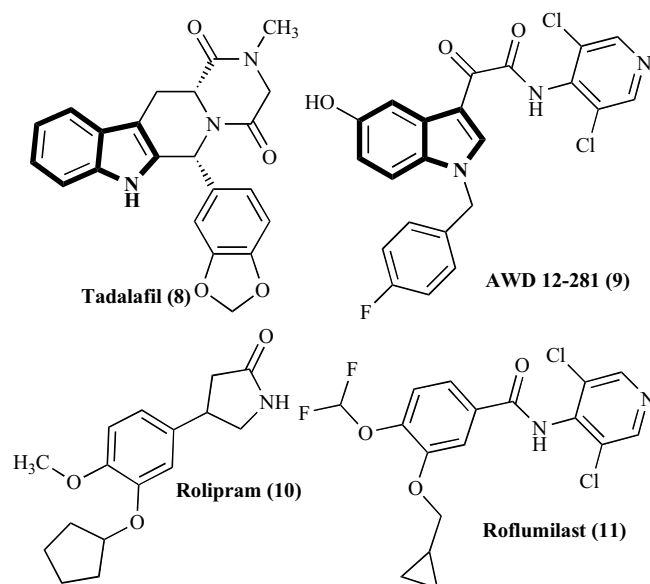

Fig. (3). Structure of some indolic PDE inhibitors (**8**) and (**9**), in comparison with PDE-4 inhibitors rolipram (**10**) and roflumilast (**11**).

orders such as asthma or chronic obstructive pulmonary disease (COPD) [22]. The *N*-benzylindole derivative AWD 12-281 (**9**, Fig. (**3**)) was described as a potent and selective PDE-4 inhibitor, which showed a strong anti-inflammatory effect in animal models of asthma and COPD and it has a considerably lower emetic potential than other PDE-4 inhibitors, such as rolipram (**10**) and roflumilast (**11**) [23] (Fig. (**3**)).

BIORECEPTOR MODULATORS

5-Hydroxytryptamine (5-HT) Receptors

Serotonin (5-HT) receptors can be classified into seven groups and sixteen subtypes. All of these receptors belong to the G-coupled metabotropic receptor family, with the only exception of 5-HT$_3$ receptors, *i.e.* 5-HT$_{3A}$, 5-HT$_{3B}$ and 5-HT$_{3C}$, which are part of the ionotropic receptor family [24].

Serotonin (5-hydroxytryptamine, **12**, Fig. (**4**)), the endogenous agonist of these receptors, plays a number of very important roles in normal brain function, which include modulation of mood states, hunger, sex, sleep, memory, emotion, anxiety and endocrine effects, among others. As one of the first discovered neurotransmitter systems, its functions have been conserved and even expanded up through the various branches of the evolutionary tree [25].

Serotonin receptors are widely expressed throughout the brain and in many key structures responsible for cognition and basic brain functions (Table **1**) [26]. The important roles of 5-HT receptors in various pathologies such as anxiety, memory impairment, depression, schizophrenia, migraine, drug abuse, gastric motility disorders, appetite control and cardiovascular disorders has led to a massive development of novel agonists and antagonists with greater subpopulation selectivity [27].

Drugs Acting as 5-HT$_{1A}$ Receptor Ligands

The 5-HT$_{1A}$ receptors are the best studied among the 5-HT receptors and it is generally accepted that they are involved in psychiatric disorders such as anxiety and depression. They are localized dendritically as inhibitory autoreceptors on serotonergic cell bodies of the median and dorsal raphe nuclei, which innervate several different CNS areas, *e.g.* hippocampus and frontal cortex, respectively [28].

During the last decade, the discovery of new ligands for 5-HT$_{1A}$ receptors has been an area of active neurobiological research and, among several classes that bind to these receptors, we highlight here the indolylalkylamines such as the potent and selective 5-HT$_{1A}$ agonists DP-5-CT (**13**) and RU-24969 (**14**), the full agonist TD-59 (**15**) and the partial agonist TD-60 (**16**) [28] (Fig. (**4**)).
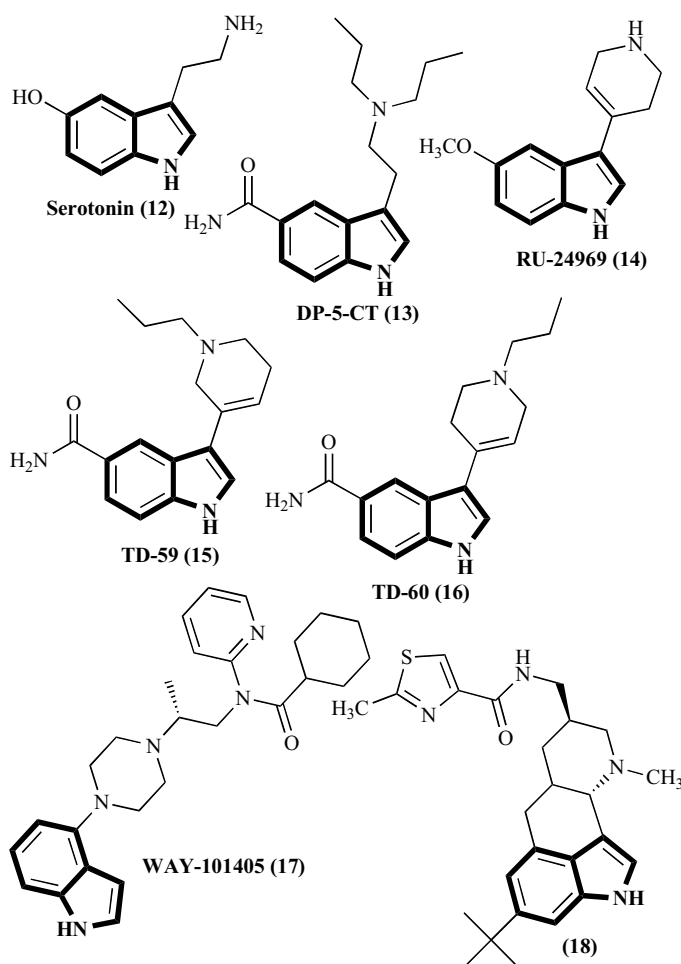

Fig. (4). Serotonin (**12**) and some indolic 5-HT$_{1A}$ receptor ligands (**13-18**).

Table 1.   Cellular and Biochemical Functions of Main Serotonin Receptors

5-HT Receptor	Signal Transduction	Function, Occurrence and Therapeutic Implications
5-HT_{1A}	Gi/o AC ↓	Inhibition of cellular function; somadendritic "autoreceptor" in raphe nuclei; also highly expressed in cingulate and entorhinal cortex, hippocampus, and lateral septum; involved in learning, memory, in anxiety and depression, and in ACTH release.
5-HT_{1B}	Gi/o AC ↓	Inhibition of cellular function; presynaptic autoreceptor; highly expressed in basal ganglia, hippocampus, and striatum; involved in anxiety and depression, drug abuse, and migraine therapy.
5-HT_{1D}	Gi/o AC ↓	Inhibition of cellular function; present in basal ganglia, periaqueductal grey, and spinal cord; involved in migraine therapy.
5-HT_{2A}	Gq/11 PLC ↑	Facilitation/stimulation of cellular function and neuronal depolarization; highly expressed in neocortex, pyriform and entorhinal cortex, caudatum, claustrum, nucleus accumbens, olfactory tubercle, and hippocampus; involved in learning and therapy of schizophrenia with atypical antipsychotics.
5-HT_{2C}	Gq/11 PLC ↑	Facilitation/stimulation of cellular function; strongly expressed in choroid plexus, less in pyriform cortex, cingulate, nucleus accumbens, hippocampus, amygdala, caudatum, and substantia nigra; involved in cerebrospinal fluid secretion; therapy of schizophrenia with atypical antipsychotics.
5-HT_3	$Na^+/K^+/Ca^{2+}$ channel	Facilitation/stimulation of cellular function; in hippocampus, striatum, and superficial neocortex layers; involved in emesis, anxiety, and reward.
5-HT_4	Gs AC ↑	Facilitation/stimulation of cellular function and excitatory function on cholinergic transmission; occurrence in nigrostriatal and mesolimbic system, and neocortex; involved in cognitive performance.

AC, adenylate cyclase; PLC, phospholipase C. Adapted from reference [26].

Hirst and co-workers reported the therapeutic potential of WAY-101405 (**17**, Fig. (**4**)), a potent and selective indole piperazine derivative, able to cross the blood-brain barrier, orally bioavailable 5-HT_{1A} receptor "silent" antagonist, designed to be used in the treatment of cognitive dysfunction associated with psychiatric and neurological conditions [29].

The ergolines are another class of indole derivatives which bind to 5-HT_{1A} receptors. The ergot alkaloids and their synthetic derivatives have a wide spectrum of central and peripheral pharmacological activity, being effective in certain neurological diseases. In this context, Mantegani and co-workers reported a series of 13-*tert*-butyl-ergoline derivatives as 5-HT_{1A} receptor ligands and among them they discovered the tetracyclic indole derivative (**18**, Fig. (**4**)), which presented nM affinity for 5-HT_{1A} receptor sites accompanied by more than a hundred fold selectivity over the α_1, α_2, D_1, D_2 and 5-HT_2 receptors [30].

Drugs Acting as 5-HT_{1D} Receptor Ligands

The GPCR-coupled 5-HT_{1D} receptors associated to inhibition of adenylate cyclase are widely distributed throughout the central nervous system. The clinical significance of these receptors still remains unknown but there is speculation that they might be involved in anxiety, depression, and other neuropsychiatric disorders. Some studies show that 5-HT_{1D} receptors are the dominant subtype in human cerebral blood vessels, which led to the development of 5-HT_{1D} receptor agonists for the treatment of migraine [31].

Sumatriptan (**19**) has been discovered among a hundred of serotonin analogs, all of them screened on isolated blood vessels. Various studies confirmed the high degree of selectivity of this compound for 5-HT_{1D} receptors, with little or

no effects on other 5-HT receptor types or other non-5-HT receptors. After toxicological assays and testing in volunteers, sumatriptan (**19**, Fig. (**5**)) was launched in 1991 for the pharmacological treatment of acute migraine [32].

Similarly to sumatriptan (**19**), other compounds were synthesized and launched on the market, including zolmitriptan (**20**), naratriptan (**21**) and rizatriptan (**22**), all of them containing the indole scaffold in their structures [33] (Fig. (**5**)).

Drugs Acting as 5-HT_2 Receptor Ligands

5-HT_{2A} receptors, originally referred to as 5-HT_2 and less commonly as $5\text{-HT}_{2\alpha}$, were among the first 5-HT receptors to be identified. They are widely distributed at varying densities throughout the brain, being their highest density evidenced in the neocortex. 5-HT_{2A} receptors are directly coupled to a phosphoinositol second messenger system. In certain brain regions, serotonin stimulates phospholipase A_2 through 5-HT_2 receptor modulation [34].

Besides the potential therapeutic roles of 5-HT_{2A} ligands and their possible involvement in regulating normal physiological functions, they have also received considerable attention from a neuropsychiatric standpoint, due to the fact that various antipsychotic and antidepressant agents are able to bind to these receptors with relatively high affinity [26].

Perregaard and co-workers reported a series of 3-substituted 1-(4-fluorophenyl)-1*H*-indoles as potent, centrally acting dopamine D_2 and serotonin 5-HT_2 receptor antagonists. Among them, the compound 1-[2-[4-[5-chloro-l-(4-fluorophenyl)-1*H*-indol-3-yl]-l-piperidinyl]ethyl]-2-imidazolidinone (**23**, Fig. (**6**)) was selected for further development as a result
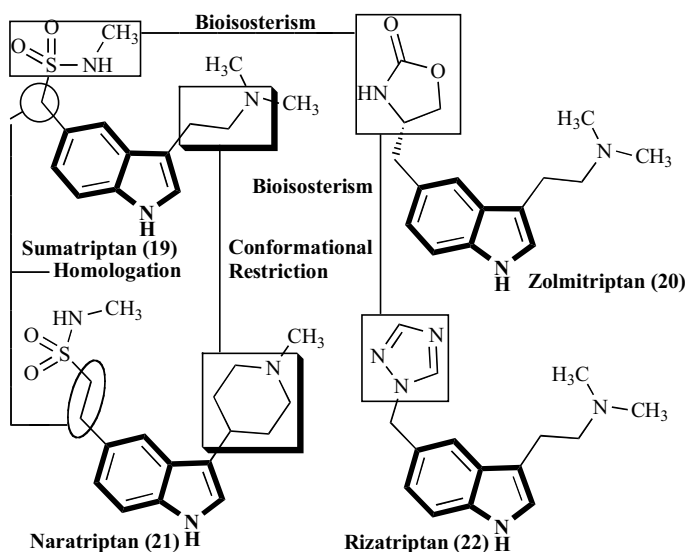
Fig. (5). Structural modifications in indole-containing 5-HT$_{1D}$ receptor agonists, useful as anti-migraine agents.

of these structure-activity relationship studies, being then launched as sertindole, an antipsychotic drug [35].

Moreover, Funakoshi and co-workers reported the *in vitro* and *in vivo* pharmacological results of compound NRA0562 (**24**, Fig. (**6**)) which showed an atypical antipsychotic profile in animal models, like other atypical antipsychotics, with little incidence of extrapyramidal side effects. Although the involvement of the dopamine D$_4$ receptor has not been elucidated, potent occupancies of both the 5-HT$_{2A}$ receptor and the α$_1$ adrenoceptor in the frontal cortex and moderate occupancy of the dopamine D$_2$ receptor in the striatum might be involved in atypical antipsychotic actions of NRA0562 [36].

On the other hand, Brea and co-workers characterized the pharmacological profile of QF2004B (**25**, Fig. (**6**)), a compound that mirrored the characteristics of clozapine in terms of its receptor binding profile and inverse agonist activity at 5-HT$_{2C}$ receptors. The results point to QF2004B (**25**) as a new lead compound with a relevant multi-receptor interaction profile for the discovery and development of new antipsychotics [37].

Drugs Acting as 5-HT$_3$ Receptor Ligands

In spite of being recognized as a unique receptor so far among the seven serotonin receptor families, research interest on basic and clinical pharmacology of 5-HT$_3$ receptors
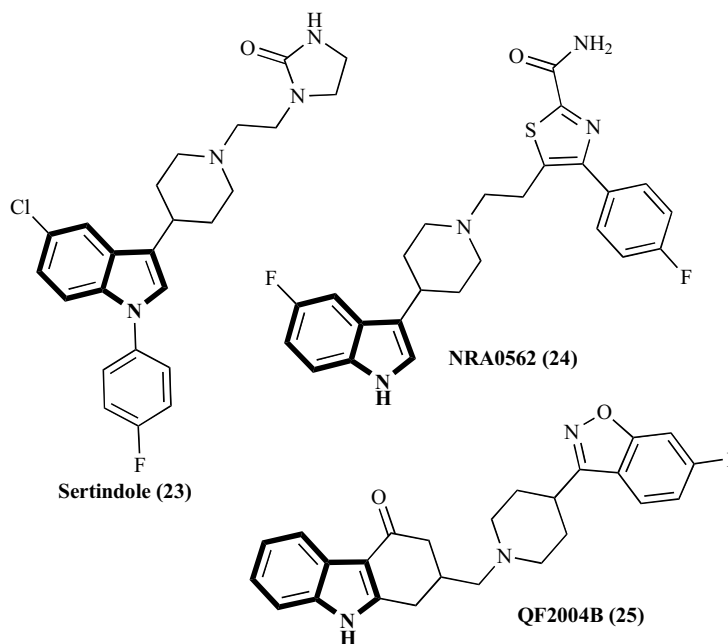

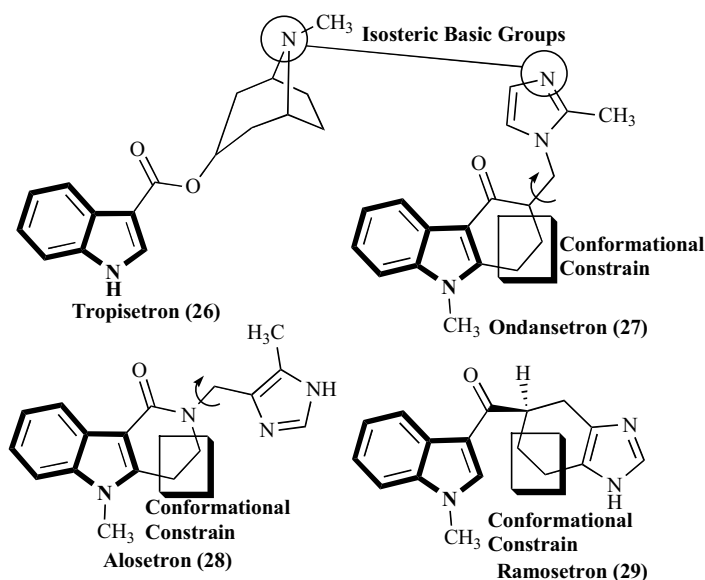**Fig. (6).** Sertindole (23) and other 5-HT$_{2A}$ receptor ligands.

Fig. (7). Clinically useful indole-containing 5-HT$_3$ receptor antagonists (**26-29**).

has grown in recent years, because these receptors are able to modulate several neural functions [38].

The 5-HT$_3$ receptor antagonists, *e.g.* tropisetron (**26**), ondansetron (**27**), alosetron (**28**) and ramosetron (**29**) (Fig. (**7**)), are used for the treatment of chemotherapy-induced or radiation-induced nausea and vomiting and there are indications that they may be effective in the treatment of migraine or the pain associated with it [39].

Cannabinoid Receptors

Cannabinoid receptors are membrane proteins of class A rhodopsin-like GPCRs and they are remarkably abundant and present in a wide variety of tissues and organs. They are among the most ubiquitous neurotransmitter receptors in the mammalian brain, where they are present in almost all regions and on many different types of neurons. The multiple consequences of cannabinoid receptor activation, *i.e.* a reduction in adenylate cyclase, modulation of ion channels and reduction in intracellular Ca^{2+} provide an important basis for control of multiple cellular signaling processes within the brain [40].

They are divided into two subtypes, termed cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). The CB1 receptor is one of the most abundant receptors in several brain regions and its modulation is related to most of behavioral effects. CB2 receptors are located in the spleen and other cells with immunochemical functions and thus are thought to have an immunomodulatory role [41]. Recently, van Sickle and co-workers have shown that CB2 receptors are found in the brainstem and also in the cortex and cerebellum. CB2 receptors represent an alternative site of endocannabinoids action that opens the possibility of nonpsychotropic therapeutic interventions using enhanced endocannabinoid levels in localized brain areas [42].

The compound WIN-55,212-2 (**30**, Fig. (**8**)) belongs to the class of aminoalkylindoles and acts as a potent CB1 and CB2 receptor agonist with slight preference for CB2. It has

played an important role in the identification and characterization of cannabinoid receptors and in their associated functions and is now in use as a CB1/CB2 radioligand. It is interesting to notice that the aminoalkylindoles bear no structural relationship to the cannabinoids and were developed as nonsteroidal anti-inflammatory drugs [41].

In 2008, Moloney and colleagues reported structure-activity relationship studies directed to the investigation of a series of 1-substituted-indole-3-oxadiazoles as potential CB1 agonists, which has led to the discovery of the compound (**31**, Fig. (**8**))[43]. Additionally, in the same year Frost and co-workers reported a series of potent indol-3-yl-tetramethyl-cyclopropyl ketones designed as CB$_2$ receptor ligands. Compound (**32**, Fig. (**8**)) was discovered as a novel, high affinity ligand for CB$_2$ receptors, exhibiting selectivity towards the CB$_1$ binding site. It displays full agonist efficacy in an *in vitro* functional assay and is active in a model of chronic inflammatory pain, an effect that is selectively blocked by
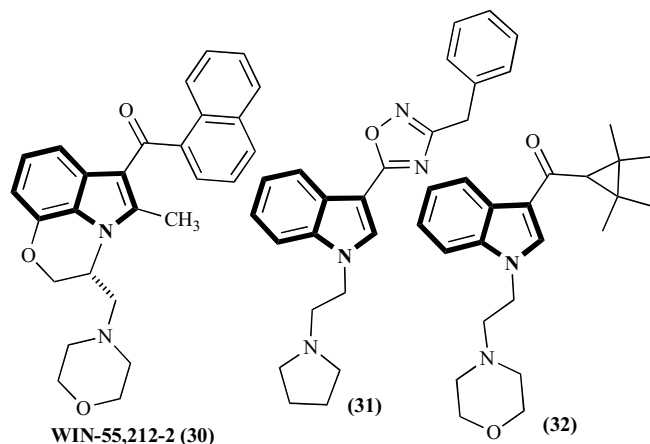

Fig. (8). Structure of some functionalized indole-containing cannabinoid CB1 and CB2 receptor ligands.

pretreatment with a CB2 antagonist and not by a CB1 antagonist [44].

DISCUSSION

The indole ring is an electron-rich aromatic subunit with characteristic properties due to the presence of an electron-rich pyrrole moiety. It is widely distributed in biological systems as an important constituent of biomolecules and natural products such as the essential amino acid tryptophan, ergot alkaloids and the neurotransmitter serotonin. Furthermore, the indole scaffold is present in many drugs and synthetic drug-candidate prototypes.

As a constituent of proteins, tryptophan has the highest hydrophobicity among the amino acids and forms a hydrophobic environment that could contribute to stabilize the protein structure [45]. Interestingly, the indole ring of the tryptophan residue near the active site of some metalloenzymes, *e.g.* cytochrome c peroxidase, has been reported to be involved in the electron-transfer pathways [46], indicating that the presence of these amino acid residues in proteins not only contribute to provide an adequate hydrophobic environment but also could take part in some enzymatic reactions, through the exploitation of the indole ring as an active component.

Considering all known protein sequences, tryptophan is the rarest among the twenty amino acids commonly found, occurring only a little over 1% of the time. Among these amino acids, only four possess aromatic side chains and only three of those present electron-rich rings. Imidazole at side chain of histidine is electron-poor. Phenylalanine and tyrosine occur about 4 and 3% of the time, respectively. As in histidine (~2.5% occurrence), the indole side chain of tryptophan is a nitrogen-containing heterocycle. Like tyrosine, indole presents a hydrogen bond donor subunit, *i.e.* N-H, in addition to the electron-rich behavior of heterocyclic ring, which can engage it in a variety of supramolecular interactions. These features make the tryptophan's indole ring a rare but very special chemical framework of our peptides and proteins [47].

Some researchers have highlighted the importance of the presence of indole N-H group in receptor interactions, as reported by Da Settimo and co-workers after the isosteric replacement of the indole nucleus in a series of glyoxylylamine derivatives, *e.g.* (**33**, Fig. (**9**)) designed as benzodiazepine receptor ligands, by benzothiophene and benzofuran [48]. This work illustrated that despite literature's indication that the presence of a hydrogen bond donor group like N-H

was not necessary to elicit a benzodiazepine agonist response, all the benzothiophene and benzofuran derivatives synthesized showed moderate to scarce affinity for the target bioreceptor, demonstrating that the indole N-H plays a decisive role in the interaction of this class of glyoxylylamine ligands [49].

Indole Properties

The nitrogen electron lone pair of indole ring is involved in maintenance of the aromatic system, so the N-H bond is acidic (pKa ~17) rather than nitrogen being basic. The indole ring is capable of various non covalent interactions with other molecules by hydrogen bonding through the N-H moiety and by $\pi-\pi$ stacking or cation–π interactions, through the aromatic moiety [45].

Additionally, Rekker and co-workers reported the calculated values of the hydrophobic fragmental constant (f_{oct}) of the indole scaffold and other aromatic isosteric subunits that are summarized in Table **2** [50]. The comparative analysis of these values evidentiated that indole ring is much less hydrophobic than classical isosteric benzothiophene and benzofuran rings, presenting hydrophobic contribution similar to a phenyl subunit.

Table 2.   Fragmental Constants in Octanol/Water (f_{oct}) of Indole and Other Isosteric Aromatic Rings

Fragment	$f_{(oct)}$
Phenyl	1.903
Naphthyl	3.191
Benzimidazolyl	1.241
Indolyl	1.902
Benzofuryl	2.374
Benzothienyl	2.901

Indole Ring Bioisosteres

Bioisosteres are groups or molecules that have chemical and physical similarities producing broadly similar biological properties [51]. The successful use of this kind of strategy in the development of new lead-prototypes and drugs, aiming at the improvement of pharmacological activity or even the optimization of pharmacokinetic profile, has ob-
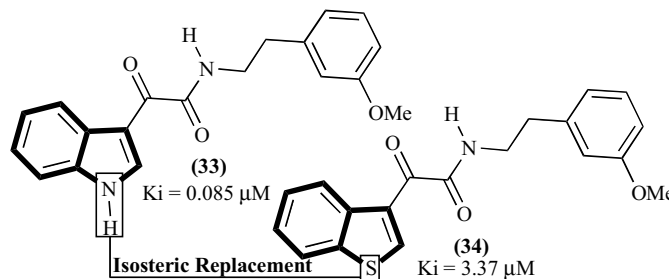
Fig. (9). Deleterious effect of indole N-H group replacement on benzodiazepine receptor affinity of some glyoxylylamine derivatives.
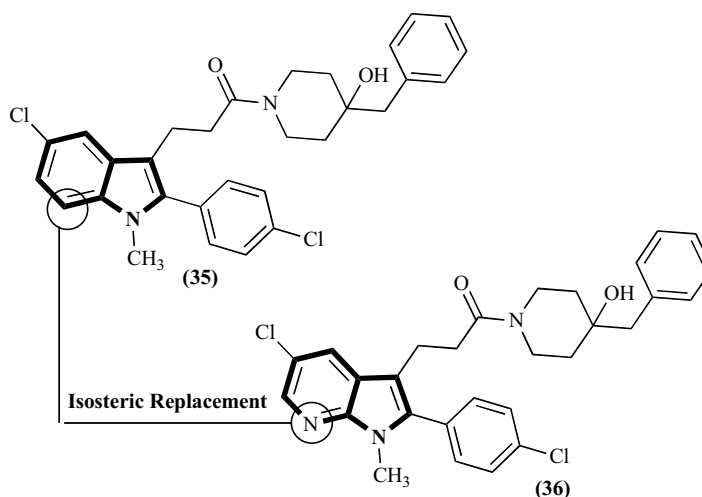
Fig. (10). Bioisosteric replacement in indole NK$_1$ receptor antagonists.

served a significant growth in a variety of therapeutic classes, being widely used to discover new analogs and also as a useful molecular modification tool [52].

In this context, Cooper and co-workers reported a series of 2-aryl indole NK$_1$ receptor antagonists, among them derivative (**35**, Fig. (**10**)) was considered the best ligand but it suffered from low oral bioavailability in rats. To solve this problem, a basic nitrogen was introduced to increase solubility and absorption, leading to an azaindole analogue (**36**, Fig. (**10**)), which showed the same NK$_1$ binding affinity of compound (**35**) [53].

On the other hand, Blair and colleagues reported the synthesis and pharmacological evaluation of thieno[3,2-*b*]- and thieno[2,3-*b*]pyrrole bioisosteric analogues of hallucinogen and serotonergic agonist *N,N*-dimethyltryptamine (**37**, Fig. (**11**)). The thienopyrrole ring in compounds (**38**) and (**39**) serves as an authentic bioisostere for the indole nucleus in the molecular recognition by serotonin 5-HT$_{1A}$ receptor [54] (Fig. (**11**)).
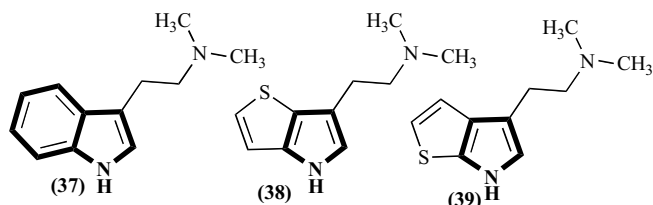
Fig. (11). Thienopyrrole bioisosteres of serotonin 5-HT$_{1A}$ agonist *N,N*-dimethyltryptamine (**37**).

Moreover, Fludzinski and co-workers, from Lilly Research Laboratories, reported a classical bioisosterism strategy between indole (**40**) and indazole (**41**) derivatives for the development of new 5-HT$_3$ receptor antagonists [55] (Fig. (**12**)).

The Indole Privileged Status

The privileged structure concept has been initially defined as a selected substructure that is able to provide high-affinity ligands for more than one type of receptor after the

adequate modulation of accessory structural subunits [4]. All the examples of many different compounds and receptors that we reported herein validate the indole scaffold as a privileged structure. No matter if the inspiration of synthesized compounds has been based on nature (indole alkaloids, tryptophan) or combinatorial chemistry, these approaches gave rise to a diverse number of biologically important structures, many of which have become drugs, others important probes for pharmacological assays and the great majority became lead-prototypes.

To explain this concept, Bondensgaard and co-workers reported an analysis of the class A family of GPCRs emphasizing ligand recognition [56]. Using privileged structure fragments selected from literature, like 2-phenyl-indole, they docked three pairs of ligands recognizing widely different receptor types into receptor models of their target receptors, *e.g.* serotonin 5-HT$_6$ receptor bound to compound (**42**) and melanocortin-4 (MC4) receptor bound to compound (**43**) (Fig. (**13**)). The authors showed that the comparison of ligand-receptor complexes for each pair of indole ligands revealed the conserved nature of the binding subpocket where the privileged scaffold can be accommodated and that additional interactions with non conserved parts of the binding pocket are responsible for important differences in the molecular recognition by the corresponding target receptor [56].

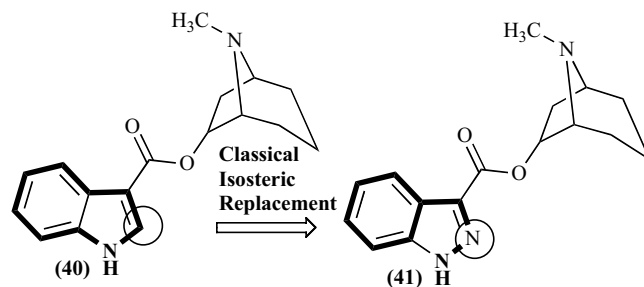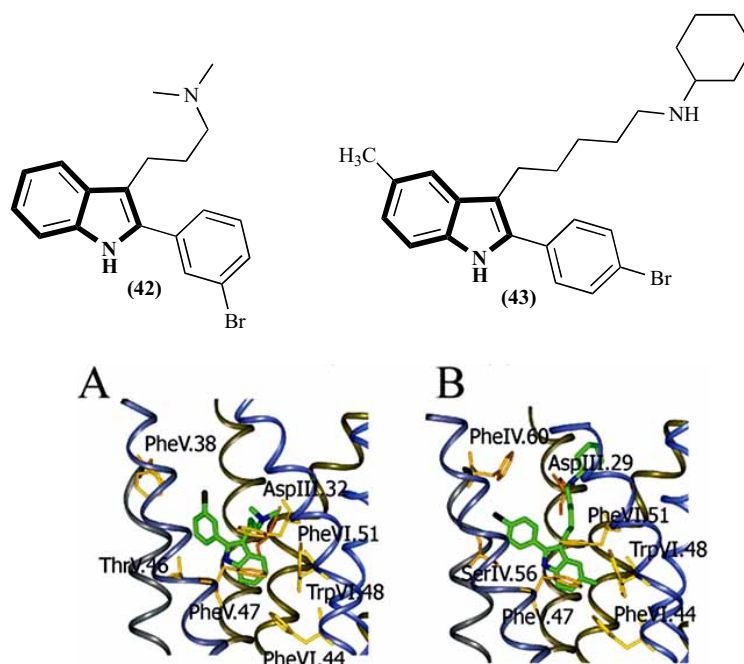Additionally, in a preliminary study based on privileged substructures, Rad and colleagues reported that the indole

Fig. (12). Indazole bioisostere (**41**) of tropanic serotonin 5-HT$_3$ antagonist (**40**).

Fig. (13). Illustration of representative interactions of indole ligands (**42**) and (**43**), respectively at serotonin 5-HT$_6$ (**A**) and melanocortin-4 (**B**) receptors. Figure adapted from Bondensgaard and co-workers [56].

substructure could be validated as a truly privileged scaffold for GPCR targets. Among 615 structures from medicinal chemistry literature, 41 substructures were classified as 'chemically privileged' and just 6 of them, including indole, were classified as biologically privileged. This concept of 'biological validation' was used to distinguish 'chemically privileged' substructures (*i.e.*, those that are recurring motifs in medicinal chemistry literature) from those privileged scaffolds that have passed the test of biological and perhaps clinical significance [57].

CONCLUSIONS

This mini-review focuses on the recurrence and importance of indole scaffold in many lead-prototypes and drug candidates as well as in nature. This substructure is found in a variety of receptor ligands useful as drugs or pharmacological probes, from enzyme inhibitors to bioreceptor modulators, validating this scaffold as a truly privileged structure.

In conclusion, we reported, based on scientific literature, that no matter what the drug discovery approach used such as starting from inspiration on natural products, combinatorial chemistry or virtual screening techniques, the indole scaffold became an interesting and valuable starting point for drug development or lead optimization in a variety of receptors until now.

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REFERENCES

[1]   Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Freidinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. Methods for drug discovery – development of potent, selective, orally effective cholecystokinin antagonists. *J. Med. Chem.*, **1988**, *31*, 2235-46.

[2]   Horton, D. A.; Bourne, G. T.; Smythe, M. L. The combinatorial synthesis of bicyclic privileged structures or privileged substructures. *Chem. Rev.*, **2003**, *103*, 893-930.

[3]   Patchett, A. A.; Nargund, R.P. Privileged structures - an update. *Annu. Rep. Med. Chem.*, **2000**, *35*, 289-98.

[4]   Duarte, C. D.; Barreiro, E. J.; Fraga, C. A. M. Privileged structures: a useful concept for the rational design of new lead drug candidates. *Mini-Rev. Med. Chem.*, **2007**, *7*, 1108-19.

[5]   Smith, A. L.; Stevenson, G. I.; Swain, C. J.; Castro, J. L. Traceless solid phase synthesis of 2,3-disubstituted indoles. *Tetrahedron Lett.*, **1998**, *39*, 8317-20.

[6]   Joule, J. A.; Mills, K.; Smith, G. F. In *Indoles: reactions and synthesis*, Stanley Thornes: Cheltenham, **1998**; pp. 305-06.

[7]   Krauss, G. In *The Regulation of Enzyme Activity*, Wiley-VCH Verlag: Weinheim, **2001**, pp. 89-117.

[8]   Dolle, R. E. Discovery of enzyme inhibitors through combinatorial chemistry. *Mol. Divers.*, **1997**, *2*, 223-36.

[9]   McLeish, M. J.; Kenyon, G. L. In *Approaches to the Rational Design of Enzyme Inhibitors*, Abraham, D. J., Ed.; John Wiley and Sons: New Jersey, **2003**; Vol. *1*, p. 716.

[10]   Blumenthal, R. S. Statins: effective antiatherosclerotic therapy. *Am. Heart J.*, **2000**, *139*, 577-83.

[11]   Istvan, E. Statin inhibition of HMG-CoA reductase: a 3-dimensional view. *Atheroscl. Suppl.*, **2003**, *4*, 3-8.

[12]   Deslypere, J. P. The role of HMG-CoA reductase inhibitors in the treatment of hyperlipidemia: a review of fluvastatin. *Curr. Ther. Res. Clin. Exp.*, **1995**, *56*, 111-28.

[13]   Roth, B. D. The discovery and development of atorvastatin, a potent novel hypolipidemic agent. *Prog. Med. Chem.*, **2002**, *40*, 1-22.

[14]   Istvan, E. S.; Deisenhofer, J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science*, **2001**, *292*, 1160-64.

[15]   Smith, W.L.; DeWitt, D. L.; Garavito, R. M. Cyclooxygenases: structural, cellular, and molecular biology. *Annu. Rev. Biochem.*, **2000**, *69*, 145-82.

[16]   Shen, T. Y.; Windholz, T. B.; Rosegay, A.; Witzel, B. E.; Wilson, A. N.; Willett, J. D.; Holtz, W. J.; Ellis, R. L.; Matzuk, A. R.; Lucas, S.; Stammer, C. H.; Holly, F. W.; Sarett, L. H.; Risley, E. A.;

Nuss, G. W.; Winter, C. A. Non-Steroid anti-inflammatory agents. *J. Am. Chem. Soc*., **1963**, *85*, 488-89.

[17]   Hu, W.; Guo, Z.; Yi, X.; Guo, C.; Chu, F.; Cheng, G. Discovery of 2-phenyl-3-sulfonylphenyl-indole derivatives as a new class of selective COX-2 inhibitors. *Bioorg. Med. Chem*., **2003**, *11*, 5539-44.

[18]   Wey, S.-J.; Augustyniak, M. E.; Cochran, E. D.; Ellis, J. L.; Fang, X.; Garvey, D. S.; Janero, D. R.; Letts, L. G.; Martino, A. M.; Melim, T. L.; Murty, M. G.; Richardson, S. K.; Schroeder, J. D.; Selig, W. M.; Trocha, A. M.; Wexler, R. S.; Young, D. V.; Zemtseva, I. S.; Zifcak, B. M. Structure-based design, synthesis, and biological evaluation of indomethacin derivatives as cyclooxygenase-2 inhibiting nitric oxide donors. *J. Med. Chem*., **2007**, *50*, 6367-82.

[19]   Bender, A. T.; Beavo, J. Cyclic nucleotide phosphodiesterases: molecular regulation to clinical use. *Pharmacol. Rev*., **2006**, *58*, 488-520.

[20]   Jeon, Y. H.; Heo,Y.-S.; Kim, C. M.; Hyun, Y.-L.; Lee, T. G.; Ro, S.; Cho, J. M. Phosphodiesterase: overview of protein structures, potential therapeutic applications and recent progress in drug development. *Cell. Mol. Life Sci*., **2005**, *62*, 1198-220.

[21]   Daugan, A.; Grondin, P.; Ruault, C.; Le Monnier de Gouville, A.-C.; Coste, H.; Linget, J. M.; Kirilovsky, J.; Hyafil, F.; Labaudiniere, R. The discovery of tadalafil: a novel and highly selective PDE5 inhibitor. 2: 2,3,6,7,12,12a-Hexahydropyrazino[1′,2′:1,6] pyrido[3,4-b]indole-1,4-dione analogues. *J. Med. Chem*., **2003**, *46*, 4533-42.

[22]   Schmidt, D.; Dent, G.; Rabe, K. F. Selective phosphodiesterase inhibitors for the treatment of bronchial asthma and chronic obstructive pulmonary disease. *Clin. Exp. Allergy*, **1999**, *29*, 99-109.

[23]   Kuss, H.; Hoefgen, N.; Johanssen, S.; Kronbach, T.; Rundfeldt, C. *In vivo* efficacy in airway disease models of N-(3,5-dichloro-pyrid-4-yl)-[1-(4-fluorobenzyl)-5-hydroxy-indole-3-yl]-glyoxylic acid amide (AWD 12-281), a selective phosphodiesterase 4 inhibitor for inhaled administration. *J. Pharmacol. Exp. Ther*., **2003**, *307*, 373-85.

[24]   Kitson, S.L. 5-hydroxytryptamine (5-HT) receptor ligands. *Curr. Pharm. Des*., **2007**, *13*, 2621-37.

[25]   Nichols, D. E.; Nichols, C. D. Serotonin receptors. *Chem. Rev*., **2008**, *108*, 1614-41.

[26]   Fink, K. B.; Göthert, M. 5-HT receptor regulation of neurotransmitter release. *Pharmacol. Rev*., **2007**, *59*, 360-417.

[27]   Saxena, P. R. Serotonin receptors: subtypes, functional responses and therapeutic relevance. *Pharmacol. Ther*., **1995**, *66*, 339-68.

[28]   Caliendo, G.; Santagada, V.; Perissutti, E.; Fiorino, F. Derivatives as 5HT1A receptor ligands - past and present. *Curr. Med. Chem*., **2005**, *12*, 1721-53.

[29]   Hirst, W.D.; Andree, T. H.; Aschmies, S.; Childers, W. E.; Comery, T. A.; Dawson, L. A.; Day, M.; Feingold, I. B.; Grauer, S. M.; Harrison, B. L.; Hughes, Z. A.; Kao, J.; Kelly, M. G.; Van der Lee, H.; Rosenzweig-Lipson, S.; Saab, A. L.; Smith, D. L.; Sullivan, K.; Rizzo, S. J. S.; Tio, C.; Zhang, M.-Y.; Schechter, L. E. Correlating efficacy in rodent cognition models with *in vivo* 5-hydroxytryptamine1A receptor occupancy by a novel antagonist, (R)-N-(2-methyl-(4-indolyl-1-piperazinyl)ethyl)-N-(2-pyridinyl)-cyclohexane carboxamide (WAY-101405). *J. Pharmacol. Exp. Ther*., **2008**, *325*, 134-45.

[30]   Mantegani, S.; Brambilla, E.; Caccia, C.; Fornaretto, M. G.; Mc Arthur, R.A.; Varasi, M. Synthesis and *in vitro* structure-activity relationship of 13-tert- butyl-ergoline derivatives as 5-HT1A receptor ligands. *Eur. J. Med. Chem*., **1997**, *32*, 795-804.

[31]   Tepper, S. J.; Rapoport, A. M.; Sheftell, F. D. Mechanisms of action of the 5-HT 1B/1D receptor agonists. *Arch. Neurol*., **2002**, *59*, 1084-88.

[32]   Humphrey, P. P. A. The discovery and development of the triptans, a major therapeutic breakthrough. *Headache*, **2008**, *48*, 685-87.

[33]   Pascual, J.; Mateos, V.; Roig, C.; Sanchez-Del-Rio, M.; Jiménez, D. Marketed oral triptans in the acute treatment of migraine: a systematic review on efficacy and tolerability. *Headache*, **2007**, *47*, 1152-68.

[34]   Baxter, G.; Kennett, G.; Blaney, F.; Blackburn, T. *Trends Pharmacol. Sci*., **1995**, *16*, 105-10.

[35]   Perregaard, J.; Arnt, J.; Bogeso, K. P.; Hyttel, J.; Sanchez, C. Non-cataleptogenic, centrally acting dopamine D-2 and serotonin 5-HT2 antagonists within a series of 3-substituted 1-(4-fluorophenyl)-1H-indoles. *J. Med. Chem*., **1992**, *35*, 1092-101.

[36]   Funakoshi, T.; Chaki, S.; Kawashima, N.; Suzuki, Y.; Yoshikawa, R.; Kumagai, T.; Nakazato, A.; Kameo, K.; Goto, M.; Okuyama, S. *In vitro* and *in vivo* pharmacological profile of 5-{2-[4-(6-fluoro-1H-indol-3-yl)piperidin-1-yl]ethyl}-4-(4-fluorophenyl) thiazole-2-carboxylic acid amide (NRA0562), a novel and putative atypical antipsychotic. *Life Sci.*, **2002**, *71*, 1371-84.

[37]   Brea, J.; Castro, M.; Loza, M. I.; Masaguer, C. F.; Raviña, E.; Dezi, C.; Pastor, M.; Sanz, F.; Cabrero-Castel, A.; Galán-Rodríguez, B.; Fernández-Espejo, E.; Maldonado, R.; Robledo, P. QF2004B, a potential antipsychotic butyrophenone derivative with similar pharmacological properties to clozapine. *Neuropharmacology*, **2006**, *51*, 251-62.

[38]   Gyermek, L. 5-HT3 receptors: pharmacologic and therapeutic aspects. *J. Clin. Pharmacol*., **1995**, *35*, 845-55.

[39]   Olivier, B.; Van Wijngaarden, I.; Soudijn, W. 5-HT3 receptor antagonists and anxiety; a preclinical and clinical review. *Eur. Neuropsychopharmacol*., **2000**, *10*, 77-95.

[40]   Howlett, A. C.; Breivogel, C. S.; Childers, S. R.; Deadwyler, S. A.; Hampson, R. E.; Porrino, L. J. Cannabinoid physiology and pharmacology: 30 years of progress. *Neuropharmacology*, **2004**, *47*, 345-58.

[41]   Pavlopoulos, S.; Thakur, G. A.; Nikas, S. P.; Makriyannis, A. Cannabinoid receptors as therapeutic targets. *Curr. Pharm. Des*., **2006**, *12*, 1751-69.

[42]   Van Sickle, M. D.; Duncan, M.; Kingsley, P. J.; Mouihate, A.; Urbani, P.; Mackie, K.; Stella, N.; Makriyannis, A.; Piomelli, D.; Davison, J. S.; Marnett, L. J.; Di Marzo, V.; Pittman, Q. J.; Patel, K. D., Sharkey, K. A. Neuroscience: identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*, **2005**, *310*, 329-32.

[43]   Moloney, G. P.; Angus, J. A.; Robertson, A. D.; Stoermer, M. J.; Robinson, M.; Wright, C. E.; McRae, K.; Christopoulos, A. Synthesis and cannabinoid activity of 1-substituted-indole-3-oxadiazole derivatives: novel agonists for the CB1 receptor. *Eur. J. Med. Chem*., **2008**, *43*, 513-39.

[44]   Frost, J. M.; Dart, M. J.; Tietje, K. R.; Garrison, T. R.; Grayson, G. K.; Daza, A. V.; El-Kouhen, O. F.; Miller, L. N.; Li, L.; Yao, B. B.; Hsieh, G. C.; Pai, M.; Zhu, C. Z.; Chandran, P.; Meyer, M. D. Indol-3-yl-tetramethylcyclopropyl ketones: effects of indole ring substitution on CB2 cannabinoid receptor activity. *J. Med. Chem*., **2008**, *51*, 1904-12.

[45]   Shimazaki, Y.; Yajima, T.; Takani, M.; Yamauchi, O. Metal complexes involving indole rings: structures and effects of metal-indole interactions. *Coord. Chem. Rev*., **2009**, *253*, 479-92.

[46]   Nocek, J. M.; Zhou, J. S.; De Forest, S.; Priyadarshy, S.; Beratan, D. N.; Onuchic, J. N.; Huffman, B. M. Theory and practice of electron transfer within protein-protein complexes: application to the multidomain binding of cytochrome c by cytochrome c peroxidase. *Chem. Rev*., **1996**, *96*, 2459-89.

[47]   Gokel, G. W. Indole, the aromatic element of tryptophan, as a pi-donor and amphiphilic headgroup. *Int. Congr. Ser.*, **2007**, *1304*, 1-14.

[48]   Da Settimo, F.; Lucacchini, A.; Marini, A. M.; Martini, C.; Primofiore, G.; Senatore, G.; Taliani, S. Isosteric replacement of the indole nucleus by benzothiophene and benzofuran in a series of indolylglyoxylylamine derivatives with partial agonist activity at the benzodiazepine receptor. *Eur. J. Med. Chem*, **1996**, *31*, 951-56.

[49]   Bianucci, A. M.; Da Settimo, A.; Da Settimo, F.; Primofiore, G.; Martini, C.; Giannaccini, G.; Lucacchini, A. Benzodiazepine receptor affinity and interaction of some N-(indol-3-ylglyoxylyl)amine derivatives. *J. Med. Chem*, **1992**, *35*, 2214-20.

[50]   Mannhold, R.; Rekker, R. F. The hydrophobic fragmental constant approach for calculating log P in octanol/water and aliphatic hydrocarbon/water systems. *Perspect. Drug Discov. Des.*, **2000**, *18*, 1-18.

[51]   Thornber, C. W. Isosterism and molecular modification in drug design. *Chem. Soc. Rev*., **1979**, *8*, 563-80.

[52]   Lima, L. M.; Barreiro, E. J. Bioisosterism: a useful strategy for molecular modification and drug design. *Curr. Med. Chem*., **2005**, *12*, 23-49.

[53]   Cooper, L. C.; Chicchi, G. G.; Dinnell, K.; Elliott, J. M.; Hollingworth, G. J.; Kurtz, M. M.; Locker, K. L.; Morrison, D.; Shaw, D. E.; Tsao, K.-L.; Watt, A. P.; Williams, A. R.; Swain, C. J. 2-Aryl indole NK1 receptor antagonists: optimisation of indole substitution. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 1233-36.

[54]	Blair, J. B.; Marona-Lewicka, D.; Kanthasamy, A.; Lucaites, V. L.; Nelson, D. L.; Nichols, D. E. Thieno[3,2-b]- and thieno[2,3-b]pyrrole bioisosteric analogues of the hallucinogen and serotonin agonist N,N-dimethyltryptamine. *J. Med. Chem.*, **1999**, *42*, 1106-11.

[55]	Fludzinski, P.; Evrard, D. A.; Bloomquist, W. E.; Lacefield, W. B.; Pfeifer, W.; Jones, N. D.; Deeter, J. B.; Cohen, M. L. Indazoles as indole bioisosteres: synthesis and evaluation of the tropanyl ester

and amide of indazole-3-carboxylate as antagonists at the serotonin 5HT3 receptor. *J. Med. Chem.*, **1987**, *30*, 1535-37.

[56]	Bondensgaard, K.; Ankersen, M.; Thøgersen, H.; Hansen, B. S.; Wulff, B. S.; Bywater, R. P. Recognition of privileged structures by G-protein coupled receptors. *J. Med. Chem.*, **2004**, *47*, 888-99.

[57]	Rad, R.; Mracec, M.; Mraceca, M.; Oprea, T. The privileged structures hypothesis for G protein-coupled receptors - some preliminary results. *Rev. Roum. Chim.*, **2007**, *52*, 853-58.