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4WD to Travel Inside the 5-HT$_{1A}$ Receptor World

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Additional information is available at the end of the chapter

Abstract

5-HT$_{1A}$ receptor is one of the most important members of the numerous families of serotoninergic receptors. Though it was the first 5-HT receptor to be identified and cloned, the knowledge of its activation/transduction mechanisms, mediated effects, and connection with other systems is still uncompleted. For this reason, relevant is the study of the four Ws of the title: first of all "who" this receptor is, then "why" it continues to be a so attractive target after several years after its identification, then "where" is 5-HT$_{1A}$ receptor expressed within the body, and, finally, "what" effects this receptor can elicit under physiological and pathological conditions. Obviously, more and more potent, safe, and selective "drugs" might be discovered once the responses to these questions are given.

Keywords: 5-HT$_{1A}$ receptor, 5-HT$_{1A}$ transduction mechanisms, central nervous system diseases, 5-HT$_{1A}$ ligands, structure-activity relationship studies

1. Introduction

The rational research of novel efficacious and safe drugs is mainly based on the knowledge of biological systems, whose dysfunctions cause several pathological conditions. Receptors and enzymes are the most common targets to which the so-called charmed bullets by Paul Ehrlich (1854–1915), Nobel Prize in Physiology and Medicine in 1908, should be addressed to mean the selectivity of interaction and, therefore, the reduced occurrence of unwanted side effects. Serotonin receptors (5-HTRs) are the most widespread targets of drugs because of the numerous

Figure 1. Chemical structure of 5-HT.

biological effects of the endogenous ligand serotonin (5-HT; **Figure 1**) and the wide presence of different 5-HTR subtypes in both the central and peripheral nervous systems (CNS and PNS) [1].

5-HT is biosynthesized at the periphery into the gut by intestinal enterochromaffin cells and in the CNS in the raphe nucleus from the essential amino acid L-tryptophan. A 5-HT reuptake protein (SERT) is responsible for carrying the neurotransmitter from the synaptic cleft to its target nerve and acts as a regulator of 5-HT levels. In the CNS, SERT is a key target for various antidepressant drugs such as tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), and serotonin-norepinephrine reuptake inhibitors (SNRIs). 5-HT is mainly deaminated by monoamine oxidase A (MAO A) to the corresponding aldehyde in the liver. The physiological effects of 5-HT are mediated by several 5-HTRs, whose heterogeneity was hypothesized from pharmacological characterization in the 1950s. From radioligand experiments, the first evidences of 5-HT subtypes were reported in 1979 [2]. To date molecular cloning techniques, amino acid sequence determination, evaluation of its pharmacological properties, second messenger coupling, and signal transduction characterization have allowed the identification of at least seven subfamilies (5-HT$_{1-7}$), some of which are further subdivided into different subtypes (**Figure 2**).
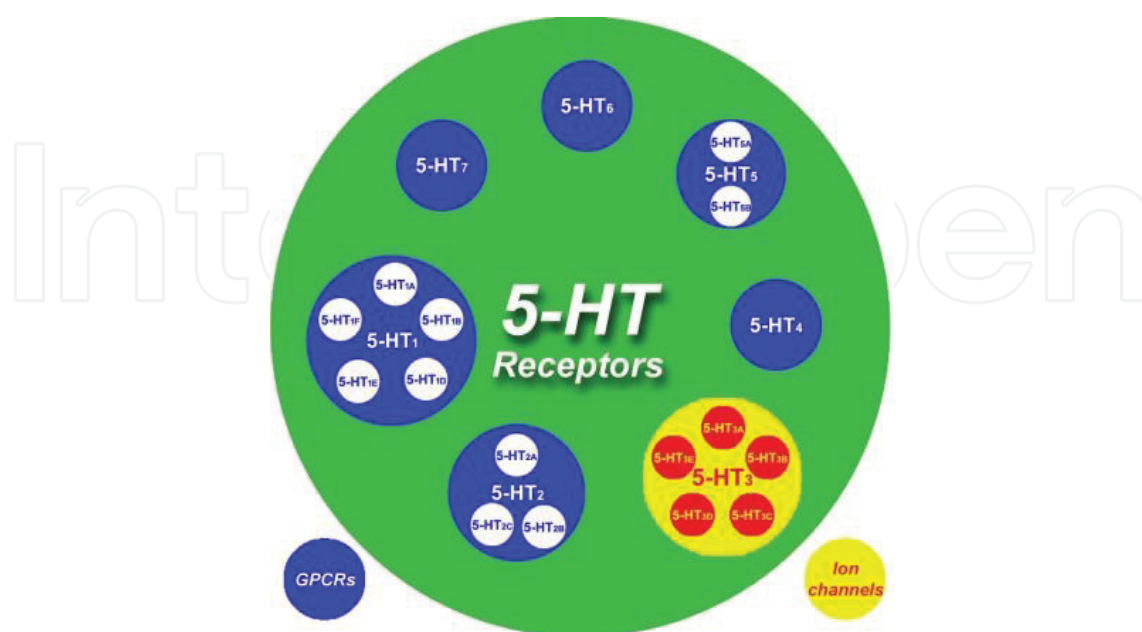

Figure 2. Classification of 5-HT$_{1A}$Rs.

While 5-HT$_3$Rs are cation-permeable ion channels, all the others are G-protein-coupled recep-tors (GPCRs) and are classified as rhodopsine-like receptors (class A). Among the 5-HTRs, the 5-HT$_{1A}$ subtype was the first to be cloned [3] and pharmacologically characterized, and it is one of the most studied. For this reason, it is often ironically called "old target" [4]. The human 5-HT$_{1A}$R consists of 422 amino acid residues with a molecular weight of about 46,000 Da. Though its structure is still unknown, mutagenesis studies have allowed the identification of amino acid residues responsible for ligand binding and G-protein coupling [1].

2. Localization

5-HT$_{1A}$Rs are widely expressed in the brain of mammals, including humans [5]. The main expressions are in limbic areas, such as the hippocampus, lateral septum, cortical brain regions, as well as dorsal and medial raphe nuclei (DRN and MRN) (**Figure 3**).

5-HT$_{1A}$Rs are located within the brain both pre- and postsynaptically. Presynaptic 5-HT$_{1A}$Rs are expressed in all 5-HT neurons (autoreceptors) and in a lot of non-5-HT neurons (hetero-receptors). The latter modulate the release of several neurotransmitters, including glutamate and dopamine, and hormones (adrenocorticotropin (ACHT), oxytocin, prolactin, growth hor-mone, β-endorphin). In the brainstem, presynaptic autoreceptors are expressed in serotoner-gic neurons in DRN and MRN, where their activation inhibits cell firing rate. These neurons send ascending 5-HT fibers to the forebrain attenuating 5-HT synthesis, turnover, and release in projection areas from axon terminals, working on a basis of a negative feedback. Presynaptic
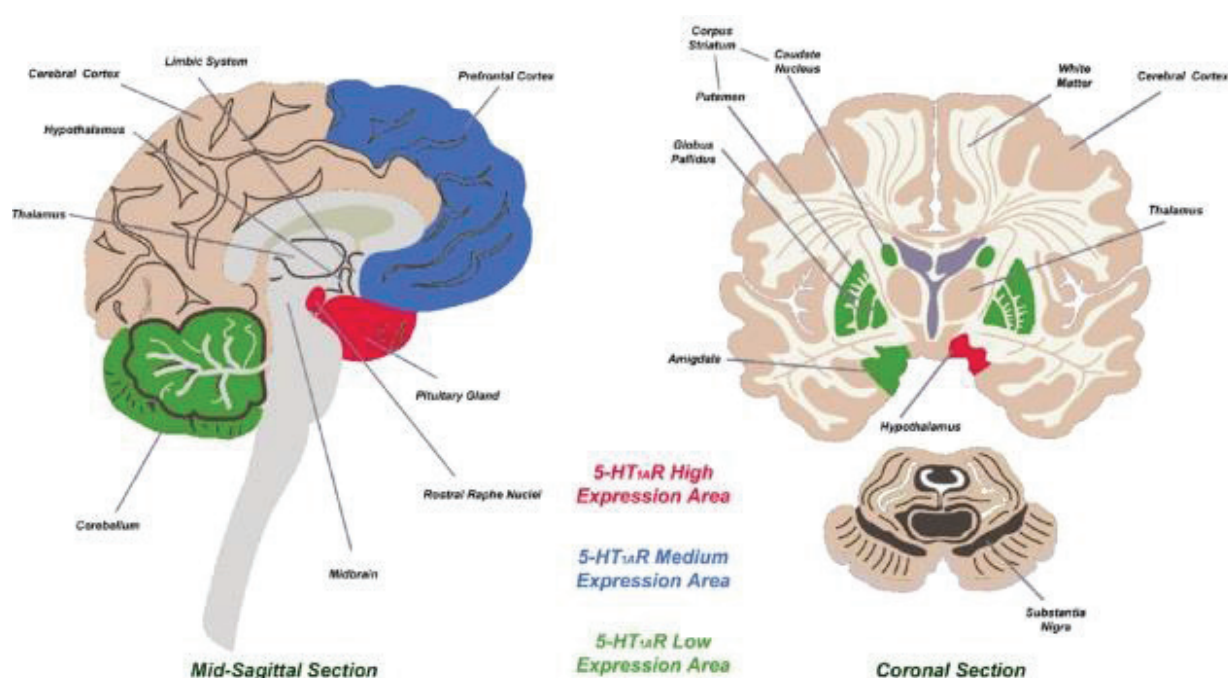

Figure 3. Central localization of 5-HT$_{1A}$Rs (Adapted from CNSforum image bank, Lundbeck Institute "Distribution of 5-HT$_{1A}$ receptors" http://www.cnsforum.com/imagebank/item/hrl_Rcpt_sys_SN1A_dist/default.aspx).

5-HT$_{1A}$Rs expressed in DRN, through coupling to G$\alpha_{i/o}$ proteins, decrease rate of cell firing by the activation of inwardly rectifying potassium channels. Postsynaptic 5-HT$_{1A}$Rs are found at high density in limbic regions, such as the hippocampus and septum, and in the frontal and entorhinal cortices. Lower 5-HT$_{1A}$R levels are observed in the amygdala. As in the case of presynaptic receptors, the activation of postsynaptic 5-HT$_{1A}$Rs generally decreases the firing rate of postsynaptic cells. Electrophysiological, pharmacological, and biochemical evidences have demonstrated that 5-HT$_{1A}$Rs are localized in primary afferent neurons [4]. They are also present in the gut, in the enteric nervous system, as well as in smooth muscle, where their activation inhibits relaxation or contraction.

3. Signal transduction pathways of 5-HT$_{1A}$Rs

The primary transduction pathway of 5-HT$_{1A}$Rs is the inhibition of adenylate cyclase (AC). Nevertheless, various other pathways are coupled to this receptor depending on the target cell. Indeed, 5-HT$_{1A}$R stimulation activates or inhibits different enzymes, channels, and kinases, as well as modulates the production of several second messengers (**Figure 4**) [6, 7].

Whatever is the activated second messenger, the signals initiated by the stimulation of 5-HT$_{1A}$Rs implicate the involvement of G$_{i/o}$ protein. Moreover, a G-protein-independent pathway of 5-HT$_{1A}$R coupling to a smooth inward current has also been suggested.

3.1. AC inhibition

The activation of 5-HT$_{1A}$Rs inhibits AC and reduces the production of cAMP with a consequent decrease of protein kinase A (PKA) activity. The Gα_i-induced inhibition of AC is coupled to 5-HT$_{1A}$ heteroreceptors, whereas the situation is still unclear for 5-HT$_{1A}$ autoreceptors. Indeed, some results reveal that 5-HT$_{1A}$R partial agonists negatively regulate presynaptic AC activity in raphe nuclei. On the other hand, a lot of evidences highlight that 5-HT$_{1A}$R agonists do not inhibit forskolin-stimulated AC activity in homogenates of the raphe region, suggesting that these autoreceptors do not couple to AC. 5-HT$_{1A}$R agonists also reduce PKA activity in the hippocampus, determining increased protein phosphatase-1 activity and reduction of Calcium/calmodulin-dependent protein kinase II phosphorylation. This signaling effect is joined to cognitive deficits. Therefore, cognitive behaviors can be mediated by the inhibition of AC/PKA activity induced by 5-HT$_{1A}$Rs.

3.2. GIRK and Ca^{2+} channel opening

Through coupling to G$\alpha_{i/o}$ proteins, 5-HT$_{1A}$Rs activate inwardly rectifying potassium channels (GIRKs) in the hippocampus and DRN. Such an action hyperpolarizes neurons and decreases firing. Moreover, Ca^{2+} entry is reduced by the inhibition of voltage-gated Ca^{2+} channel following 5-HT$_{1A}$R activation.

3.3. ERK/MAPK pathway activation

The stimulation of 5-HT$_{1A}$Rs induces the release of $\beta\gamma$-complex that participates in the activation of phosphatidylinositol-3 kinase (PI3K). It triggers the activation of extracellular signal-regulated
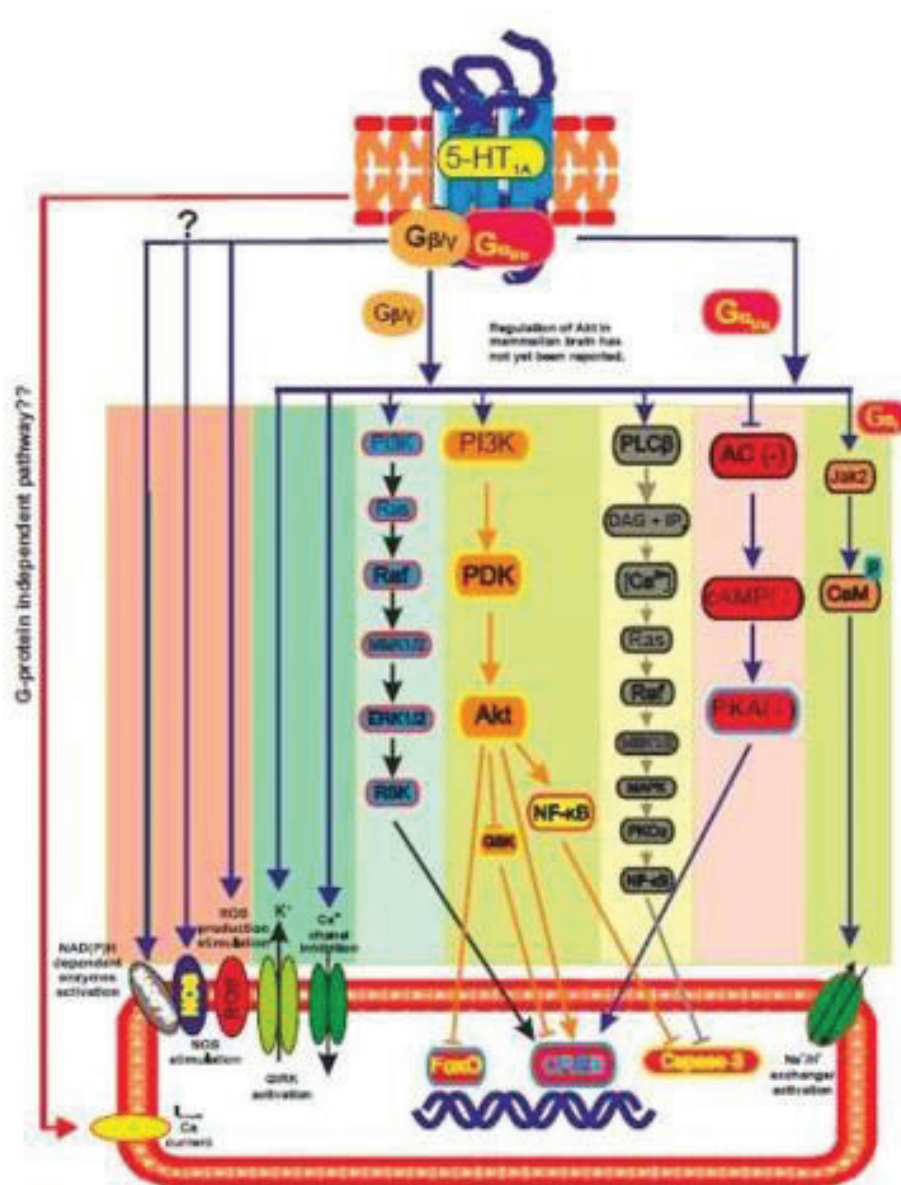
Figure 4. Main transduction pathways of 5-HT$_{1A}$Rs (Reprinted with permission from Ref. [6]).

protein kinase (ERK) (or MAPK), implicated in cell proliferation and differentiation through two pathways involving Ras-Raf-MEK proteins. In addition, 5-HT$_{1A}$-induced ERK activation in nonneuronal cells can be mediated by phosphatidylcholine-specific phospholipase C (PC-PLC) in a G-protein-dependent manner. In neuronal cells, the effects on ERK activity produced by 5-HT$_{1A}$Rs can be different. Indeed, in the hypothalamus a rapid but transient increase of ERK phosphorylation is observed, and this effect might be an intermediate step for the 5-HT$_{1A}$R-mediated increase of oxytocin, ACTH, and prolactin. In HN2-5 hippocampal-derived cell lines, 5-HT$_{1A}$R activation favors ERK phosphorylation and activity. This effect does not occur in the primary culture of hippocampal or fetal rhombencephalic neurons. On the contrary, in the rat hippocampus, 5-HT$_{1A}$R activation decreases ERK phosphorylation. Analogously it reduces MEK activity and ERK phosphorylation in differentiated raphe neurons. Different ERK-related effectors can be modulated by 5-HT$_{1A}$Rs: activation of the ribosomal S6 kinase (RSK), stimulation of

nuclear factor κB (NF-κB), and inhibition of caspase 3. This pathway seems to be involved in neuroprotective mechanisms. ERK also activates cAMP response element binding (CREB), a transcription factor that plays fundamental roles in stress, anxiety, and depression. Finally, the activation of MAPK/ERK transduction pathway may inhibit apoptosis by phosphorylation of the proapoptotic protein Bad and by increasing the expression of antiapoptotic Bcl-2.

3.4. PI3K and Akt pathway activation

5-HT$_{1A}$R stimulation can also regulate the activation of the PI3K/Akt signaling pathway through βγ-complex. The Akt protein kinase plays a key role in several cellular processes, such as glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration. In the mammalian brain, the PI3K/Akt pathway is also implicated in synaptic plasticity, learning, and memory. Consequently, Akt dysfunction can be associated with metabolic diseases (e.g., diabetes and obesity), central disorders (e.g., depression, schizophrenia, and drug abuse), and the most frequent alterations observed in human cancer and tumor cells. Akt phosphorylates and inactivates the protein glycogen synthase kinase 3 (GSK3), whose inhibition produces antidepressant and antimanic effects. Active Akt also phosphorylates and inactivates Forkhead box O (FoxO) transcription factors, whose deficiency in mice develops antidepressive and anxiolytic behavioral phenotypes.

3.5. Na$^+$/H$^+$ exchanger activation

Another complex pathway following 5-HT$_{1A}$R stimulation and involving G(i2)α and/or G(i3)α induces Janus kinase 2 (Jak2) activation, which leads to tyrosine phosphorylation of calmodulin (CaM). The consequent increase of CaM binding to Na$^+$/H$^+$ exchangers (NHEs) induces a conformational modification that activates NHEs, unmasking an obscured proton-sensing and/or proton-transporting region. NHEs, expressed on the surface of all mammalian cells, regulate cell volume, intracellular pH, and transepithelial transport of Na$^+$ and acid-base equivalents.

3.6. NO production

5-HT$_{1A}$Rs can also regulate the production of nitric oxide (NO) that plays an important role in the brain. In rat ventral prostate cells, 5-HT$_{1A}$Rs can stimulate NO synthase (NOS) activity, whereas in the adult rat hippocampus and in human neocortical slices, they inhibit NMDA-induced NO production. Therefore, the regulation of NO synthesis by 5-HT$_{1A}$Rs is complex and appears to be cell specific.

4. Biological interest of 5-HT$_{1A}$Rs

5-HT$_{1A}$R is one of the most important among the 5-HTRs because of its high affinity for 5-HT and involvement in nearly all 5-HT-mediated effects. The main behavioral and physiological functions mediated by this receptor are summarized in **Figure 5**.
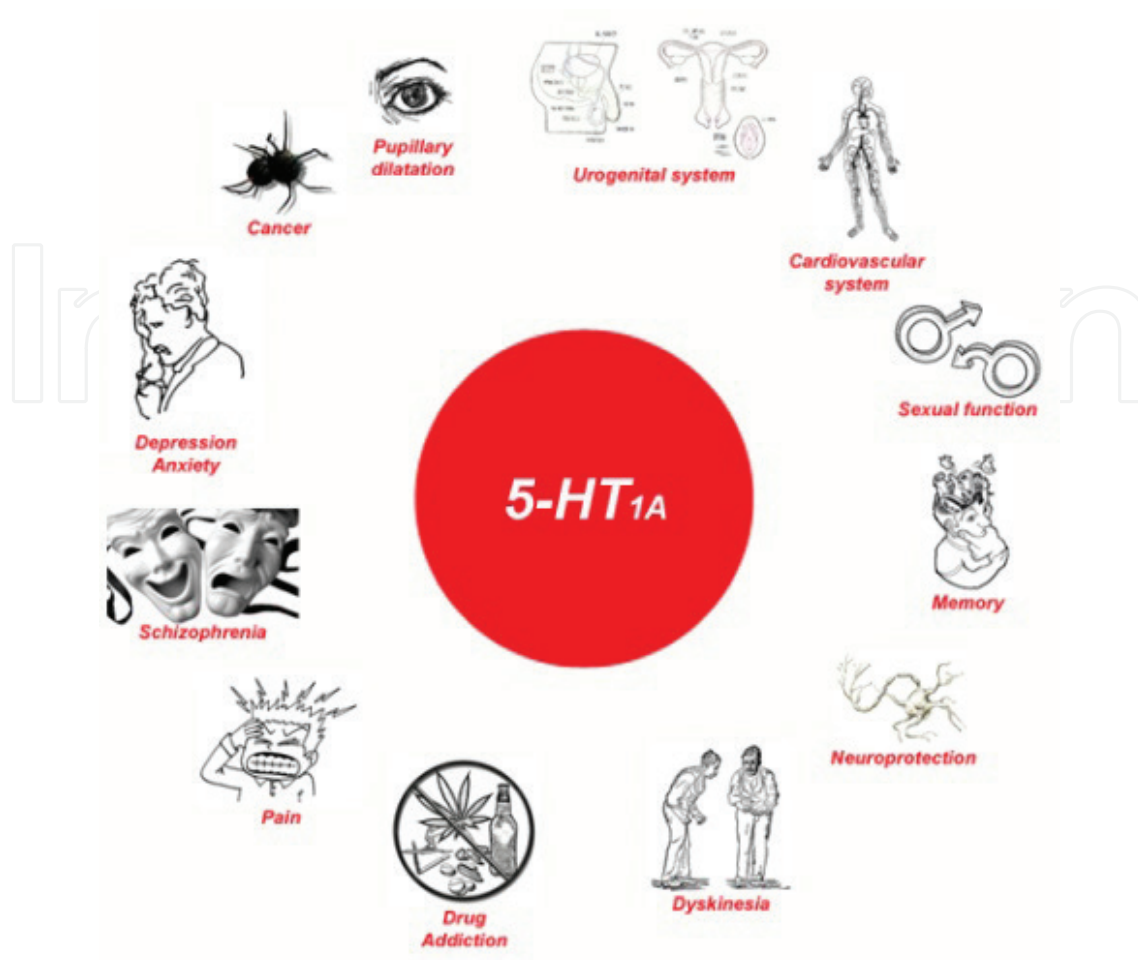
Figure 5. Main behavioral and physiological functions mediated by 5-HT$_{1A}$Rs.

4.1. Depression

The dysfunction of 5-HT$_{1A}$ autoreceptors has been proven to be associated with the major depressive disorders. This correlation is confirmed by the observation that significant antidepressant activity is elicited by 5-HT$_{1A}$R agonists [4]. Though the mechanism responsible for their antidepressant action is still unclear, desensitization or downregulation of presynaptic 5-HT$_{1A}$Rs appears to be implicated in this pharmacological effect. Indeed, in DRN and MRN, prolonged treatment with 5-HT$_{1A}$R agonists desensitizes presynaptic 5-HT$_{1A}$Rs inducing a reduction of autoreceptor-mediated inhibition of 5-HT release.

SSRIs represent the first-line treatment of depression. However, the inhibition of the reuptake of 5-HT increases 5-HT concentration in the synaptic cleft and simultaneously activates 5-HT$_{1A}$ autoreceptors, with a consequent suppression of 5-HT release from presynaptic terminals [8]. Therefore, only prolonged treatment with SSRIs allows the desensitization of 5-HT$_{1A}$ autoreceptors, leading to the recovery of neurotransmission in 5-HT neurons. Beneficial effects on depression are also produced by the combination of SSRIs with 5-HT$_{1A}$R agonists or antagonists, leading to faster onset of antidepressant action and greater antidepressant efficacy.

In particular, $5\text{-}HT_{1A}R$ antagonists can improve the efficacy of SSRIs by blocking inhibitory $5\text{-}HT_{1A}$ autoreceptors, while $5\text{-}HT_{1A}R$ agonists exert antidepressant-like effect through the activation of postsynaptic $5\text{-}HT_{1A}Rs$ and/or faster desensitization of $5\text{-}HT_{1A}$ autoreceptors. Finally, antidepressant-like effect can also be produced by $5\text{-}HT_{1A}$ partial agonism combined with 5-HT reuptake inhibition [4].

4.2. Anxiety

Several studies have been performed to demonstrate the possible role of $5\text{-}HT_{1A}Rs$ in anxiety [1]. Interestingly, mice with genetically inactivated $5\text{-}HT_{1A}R$ gene develop an anxiety-like phenotype, probably resulting from impaired autoinhibitory control of midbrain 5-HT neurons. On the contrary, mice with overexpressed $5\text{-}HT_{1A}Rs$ display diminished anxiety when compared to wild-type animals. These findings support the crucial role of the stimulation of $5\text{-}HT_{1A}Rs$ in the control of anxiety-like behavior. Therefore, $5\text{-}HT_{1A}R$ agonists and partial agonists have been developed as novel anxiolytic agents, devoid of dependence and side effect profile of other anxiolytics and antipsychotics.

4.3. Schizophrenia

Several studies performed in postmortem schizophrenia patients report an overexpression of $5\text{-}HT_{1A}Rs$ in the prefrontal cortex, indicating that these receptors are not adequately stimulated by 5-HT [1]. Therefore, $5\text{-}HT_{1A}R$ agonists might be useful to contrast this apparent deficit. Two mechanisms are advantageously activated by $5\text{-}HT_{1A}R$ stimulation in the treatment of schizophrenia. The first one involves the attenuation of parkinsonian symptoms, such as catalepsy, caused by the antagonism at dopamine D_2 receptor (D_2R) produced by antipsychotics. Since atypical antipsychotic drugs, such as clozapine, quetiapine, and ziprasidone, also behave as potent $5\text{-}HT_{1A}R$ agonists, it has been suggested that the reduced incidence of motor side effects observed with these drugs might be due to their inherent $5\text{-}HT_{1A}R$ agonism. The second mechanism involves the ability of $5\text{-}HT_{1A}R$ agonists to increase dopamine release in the prefrontal cortex, consequently reducing the negative symptoms of schizophrenia. Based on these observations, a novel approach in the treatment of schizophrenia concerns the development of novel atypical antipsychotic agents characterized by a mixed D_2R antagonist/$5\text{-}HT_{1A}R$ agonist profile.

4.4. Pain

Full and partial $5\text{-}HT_{1A}R$ agonists are beneficial in pain treatments, including efficacy in neuropathic pain models, arousing great interest as future therapeutic agents. In knockout mice, $5\text{-}HT_{1A}Rs$ have also been demonstrated to mediate an endogenous inhibitory control of nociception evoked by thermal noxious stimuli [4].

4.5. Drug addiction

A critical role in the effects of psychostimulants, including addiction, is played by $5\text{-}HT_{1A}Rs$. Some psychostimulant drugs, including cocaine, amphetamine, methamphetamine,

and 3,4-methylenedioxymethamphetamine (MDMA), increase not only dopamine but also 5-HT that can hyperactivate 5-HT$_{1A}$Rs. Interestingly, the contribution of pre- and postsynaptic 5-HT$_{1A}$Rs can be dissociated and frequently is responsible for opposite effects. In fact, 5-HT$_{1A}$ autoreceptors indirectly facilitate psychostimulant addiction-related behaviors by reducing 5-HT response in projection terminal areas, while postsynaptic 5-HT$_{1A}$Rs directly contrast the expression of various addiction-related behaviors [9]. Several studies have also demonstrated that 5-HT$_{1A}$R agonists alleviate opioid-induced respiratory depression in rodent models. The mechanisms involved in this effect are still unclear. However, concomitant decreases in opioid-induced analgesia, as well as altered baseline ventilation and behavior, have also been observed.

4.6. Dyskinesia

5-HT$_{1A}$Rs are involved in the regulation of locomotor activity. In particular, the stimulation of 5-HT$_{1A}$Rs facilitates the establishment of locomotor sensitization [10]. Parkinsonian patients in therapy with L-3,4-dihydroxyphenylalanine (L-DOPA) may develop motor complications, such as dyskinesia. The development of this side effect involves several pathways, including an abnormal 5-HT-mediated neurotransmission [4]. It has been highlighted that parkinsonian animals chronically treated with L-DOPA have increased levels of 5-HT$_{1A}$Rs in the striatal matrix. Accordingly, treatment with 5-HT$_{1A}$R agonists attenuates dyskinesia but, in some cases, also reduces the antiparkinsonian benefit of L-DOPA. Some evidences suggest that a lot of 5-HT$_{1A}$R agonists are also endowed with D$_2$R antagonism, which alleviates dyskinesia, though at the expense of worsening parkinsonism. The challenge is to obtain compounds able to selectively stimulate 5-HT$_{1A}$Rs in striatum and/or in middle layers of the cortex, avoiding the involvement of 5-HT$_{1A}$Rs in external cortical layers.

4.7. Neuroprotection

The activation of 5-HT$_{1A}$Rs exerts a neuroprotective effect in different animal models of ischemia, interfering with excitotoxic and apoptotic cell death processes in the postischemic brain [1]. Though the cellular mechanisms underlying such a neuroprotective effect are still unclear, the hyperpolarization of pyramidal neurons inhibits the glutamate-induced excitotoxicity consequent to cerebral ischemia. 5-HT$_{1A}$Rs may mediate brain protective mechanisms, by contrasting the effects of glutamatergic NMDA receptor overstimulation and the consequent NMDA-induced Ca^{2+} influx. Moreover, the inhibition of 5-HT$_{1A}$R-induced cyclases might produce neuroprotective effects due to the reduction of adenylyl cyclase excess following reperfusion after ischemic attack. 5-HT$_{1A}$R agonists can also be useful for the treatment of traumatic brain injury (TBI) [11].

4.8. Memory

Several experimental evidences highlight that the activation of postsynaptic 5-HT$_{1A}$Rs, attenuating the neuronal activity, impairs emotional memory. On the contrary, presynaptic 5-HT$_{1A}$R activation reduces 5-HT release and exerts pro-cognitive effects. 5-HT$_{1A}$R antagonism facilitates memory retention, probably by the activation of 5-HT$_7$Rs, and evidence is provided that 5-HT$_7$Rs can facilitate emotional memory upon reduced 5-HT$_{1A}$R

transmission [12]. Moreover, tonic and phasic 5-HT release can exert different and potentially opposite effects on emotional memory, depending on the states of 5-HT$_{1A}$Rs and 5-HT$_7$Rs and their interaction. Consequently, individual differences due to genetic and/or epigenetic mechanisms play an essential role in the responsiveness to drug treatment [13].

4.9. Sexual function

5-HT$_{1A}$Rs and 5-HT$_{2C}$Rs produce two distinct and opposite effects on sexual function: the activation of 5-HT$_{1A}$Rs decreases ejaculatory latency and erection, directly promoting the sympathetic emission, while the activation of 5-HT$_{2C}$Rs increases them, directly favoring parasympathetic expulsion and erection [4]. Therefore, 5-HT$_{1A}$R antagonists are under investigation for the treatment of primary premature ejaculation.

4.10. Cardiovascular system

Several studies have demonstrated that 5-HT$_{1A}$Rs in the medullary raphe mediate protective responses to stress [4]. Indeed, the activation of 5-HT$_{1A}$Rs induces bradycardia and blood pressure decrease, suggesting that 5-HT$_{1A}$Rs can reduce the sympathetic outflow. Moreover, 5-HT$_{1A}$R agonists reduce the cutaneous vasoconstriction evoked by physical and psychological stressors. 5-HT$_{1A}$Rs located in limbic regions can also reduce stress-evoked cardiovascular responses. However, this action does not occur via a direct effect on brainstem cardiovascular neurons, but is consequent to the anxiolytic effect. Psychological stress, cold exposure, or fever might elicit cardiovascular responses also mediated by neurons within the dorsomedial hypothalamus. Therefore, 5-HT$_{1A}$R agonists might be useful therapeutic agents to reduce the sympathetic responses occurring in some forms of hypertension and heart failure. The cardiovascular responses of 5-HT$_{1A}$R agonists could also be useful to reduce side effects in the treatment of hyperphagia and obesity with noradrenaline (NA) uptake inhibitors. Such inhibitors are able to reduce food intake due to increased noradrenergic activity that also causes an increased cardiovascular activity. When 5-HT$_{1A}$R agonists are combined with NA uptake inhibitors, side effects, such as hypertension and tachycardia, are mitigated. Postsynaptic 5-HT$_{1A}$R activation may contribute to hypophagia efficacy. Moreover, presynaptic 5-HT$_{1A}$Rs may reduce food intake by inhibiting spontaneous noradrenergic cell firing.

4.11. Urogenital system

5-HT$_{1A}$Rs mediate effects in the lower urinary tract function [4]. Indeed, their stimulation activates the micturition reflex, inducing an increase in the frequency of isovolumic bladder contractions. Conversely, 5-HT$_{1A}$R agonists elicit periodic external urethral sphincter relaxation, inducing an increase in micturition volume, a decrease in bladder capacity, and an increase in voiding efficiency.

4.12. Pupillary dilation

Pupillary response to 5-HT$_{1A}$R agonists is species dependent [14]. Indeed, 5-HT$_{1A}$R activation produces miosis in humans and rabbits and mydriasis in mice. In humans, 5-HT$_{1A}$Rs induce

miosis solely by inhibiting sympathetic mechanisms. However, evidences suggest that the parasympathetic nerve is also involved. Indeed, the activation of central 5-HT$_{1A}$Rs induces NA release, which in turn reduces parasympathetic neuronal tone to the iris sphincter muscle by the stimulation of postsynaptic α_2-adrenoceptors (α_2-ARs) within the Edinger-Westphal nucleus.

4.13. Cancer

5-HT$_{1A}$Rs are known to be involved in the proliferation of human tumor cells, but their function still remains poorly understood [4]. 5-HT$_{1A}$R antagonists inhibit the growth of different prostatic tumor cell lines, such as PC-3, DU-145, and LNCaP, as well as the proliferation of PC-3 xenografted subcutaneously in athymic nude mice. Multitarget ligands, acting as α_{1A}/α_{1D}-AR and 5-HT$_{1A}$R antagonists, in which a synergic effect occurs, have proved to be useful in the management of benign prostatic hyperplasia. 5-HT$_{1A}$Rs are also reported to be involved in the mitogenic effect of 5-HT in human small cell lung carcinoma cells.

5. Ligands

Several structurally different ligands, such as aryloxyalkylamines, arylpiperazines, amino-tetralins, indolyl-alkylamines, ergolines, and aporphines, are known to bind 5-HT$_{1A}$Rs [15]. Recently, new classes of ligands, including 2-imidazoline and 1,4-dioxane derivatives, have also shown high 5-HT$_{1A}$R affinity. Due to the high homology among 5-HT$_{1A}$Rs and other receptor systems, in binding studies several molecules show nanomolar and subnanomolar affinity not only for 5-HT$_{1A}$Rs but also for other receptors (5-HT$_{2A}$Rs, 5-HT$_{2C}$Rs, 5-HT$_7$Rs, α_1- and α_2-ARs, as well as D$_1$Rs and D$_2$Rs).

5.1. Aryloxyalkylamines

The sequence analysis of the 5-HT$_{1A}$R genomic clone indicates 43% amino acid homology with the β_2-AR in the transmembrane domain. Therefore, some compounds show good affinity for both systems. The first examples of dualistic interaction are offered by pindolol (**1**) and propranolol (**2**) (**Figure 6**) [16].

In several studies, an Asn amino acid residue in the putative helix VII of 5-HT$_{1A}$Rs has been demonstrated to play a crucial role in the binding of aryloxypropanolamines. Indeed, for example, propranolol **2** shows significantly reduced affinity for human 5-HT$_{1A}$Rs, in which
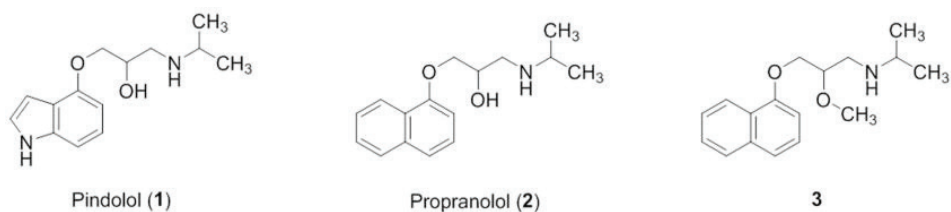

Figure 6. Chemical structures of **1–3**.

the Asn386 is replaced by valine, while the affinity of the neurotransmitter 5-HT is hardly affected. It was initially hypothesized that the formation of two hydrogen bonds occurs between the oxypropanol moiety and the amide group of Asn386. Moreover, since the (S)-enantiomer of propranolol is 13-fold more potent than the (R)-enantiomer at wild type (pK_i 5-HT$_{1A}$R = 6.8 and 5.7, respectively) and the enantioselectivity is significantly reduced (three-fold) in Asn386Val mutant human 5-HT$_{1A}$Rs (pK_i 5-HT$_{1A}$R = 5.4 and 5.0, respectively), Asn386 proves to behave as a chiral discriminator. Moreover, the observation that the replacement of the hydroxyl substituent of **2** with a methoxy group does not affect the high affinity for the wild-type receptor suggests that one or both ether oxygen atoms of (S)-**3** may act as hydrogen bond acceptors. (S)-**3** (pK_i 5-HT$_{1A}$R = 6.8) also shows high affinity for the Asn386Val mutant receptor because of a favorable lipophilic contact of its methoxy group with Val386.

5.2. Arylpiperazines

Arylpiperazines are one of the most important classes of 5-HT$_{1A}$R ligands from which a second generation of anxiolytics, including buspirone (**4**), the antipsychotics ziprasidone (**5**), perospirone (**6**), and aripiprazole (**7**), and several pharmacological tools originated (**Figure 7**) [8].

These ligands bind with high affinity to different GPCRs; the two multitarget drugs **5** and **6**, for example, acting as D$_2$R antagonists and 5-HT$_{1A}$R agonists, were marketed in 2001 and 2002, respectively, for the management of schizophrenia [4]. Compound **4** is the most known member of long-chain arylpiperazines (LCPAs) [17]. It was initially investigated as a putative antipsychotic agent devoid of the typical side effects of this class of drugs but was launched in the market as an anxiolytic in the USA in the 1980s. It behaves as a
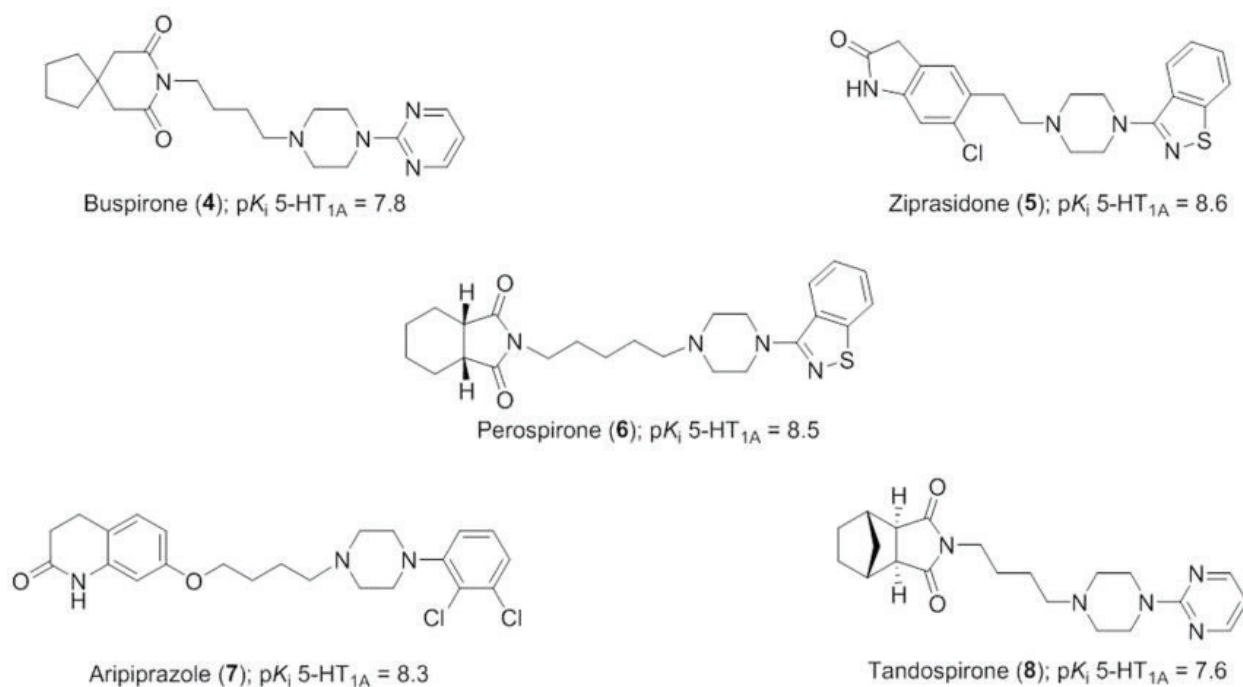

Buspirone (**4**); pK_i 5-HT$_{1A}$ = 7.8

Ziprasidone (**5**); pK_i 5-HT$_{1A}$ = 8.6

Perospirone (**6**); pK_i 5-HT$_{1A}$ = 8.5

Aripiprazole (**7**); pK_i 5-HT$_{1A}$ = 8.3

Tandospirone (**8**); pK_i 5-HT$_{1A}$ = 7.6

Figure 7. Chemical structures of **4–8**.

potent but nonselective partial 5-HT$_{1A}$R agonist and D$_2$R antagonist. Since its launch, several *N*4-(2-pyrimidinyl)piperazines containing an *N*1-imidobutyl substituent have originated as the third generation of anxiolytic agents, including the partial agonist tandospirone (**8**) (**Figure 7**).

The general structure of arylpiperazines consists of a terminal fragment containing an amide, imide, alkyl, arylalkyl, heteroarylalkyl, or tetralin function linked through a flexible aliphatic chain of variable length to the *N*1-arylpiperazine moiety [8]. The search for new derivatives has been focused on the modification of one or more portions of such a pharmacophore. Some of the main changes are schematically reported in **Figure 8**.

5.2.1. Modification of the aryl group

The replacement of the 2-pyrimidinyl moiety of **4** with a 2-methoxyphenyl group leads to the antidepressant BMY 8227 (**9**), from which BMY 7378 (**10**) originates by shortening its butyl
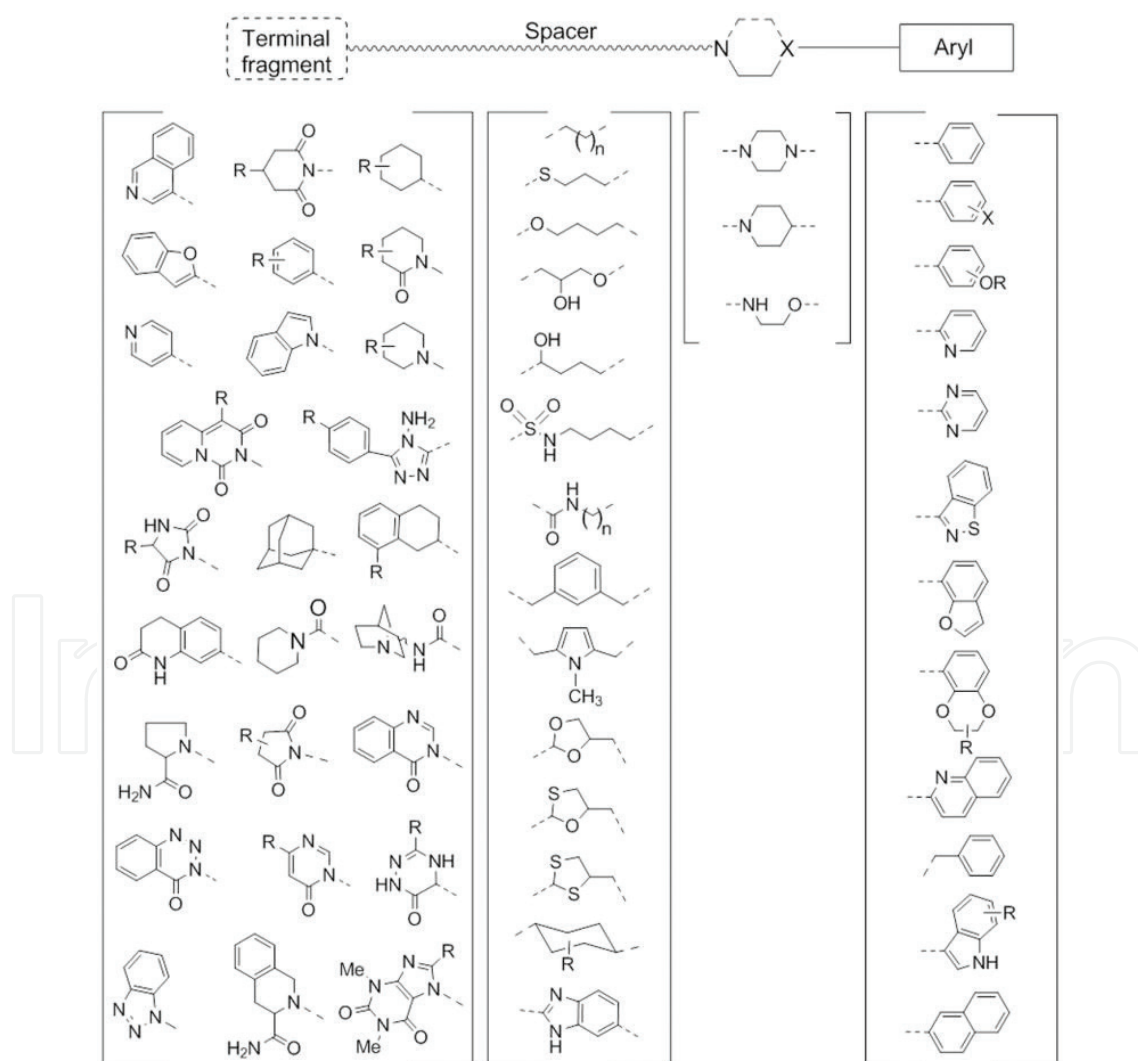

Figure 8. Pharmacophore of arylpiperazines.

to ethyl chain (**Figure 9**) [15]. Compounds **9** and **10** belong to a generation of postsynaptic 5-HT$_{1A}$R antagonists, which also behave as low efficacy partial agonists [4].

The 2-methoxyphenyl group is also present in the WAY series, including WAY 100135 (**11**) and WAY 100635 (**12**) (**Figure 9**). These compounds, also called "silent" 5-HT$_{1A}$R antagonists, behave as antagonists at both pre- and postsynaptic 5-HT$_{1A}$Rs. In the case of **11**, the (*S*)-enantiomer is 28-fold more potent than its (*R*)-antipode.

The incorporation of the *o*-methoxy group into an annulated benzodioxane or benzofurane ring, affording two series of heterobicyclic arylpiperazines, is consistent with the maintenance of high 5-HT$_{1A}$R affinity [15]. The benzodioxane fragment is present in the structure of flesinoxan (**13**) (**Figure 10**), a potent agonist at both pre- and postsynaptic 5-HT$_{1A}$Rs [15]. An example of benzofuran derivative showing high 5-HT$_{1A}$R affinity is compound **14** (**Figure 10**).

Moderate to high affinity for 5-HT$_{1A}$Rs and SERT and low affinity for 5-HT$_{2A}$R are recorded by ligands, whose four-carbon chain bears a quinoline moiety (**Figure 11**) [8].
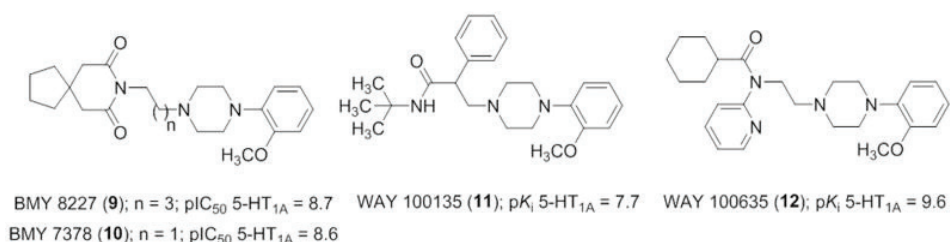

BMY 8227 (**9**); n = 3; pIC$_{50}$ 5-HT$_{1A}$ = 8.7   WAY 100135 (**11**); pK_i 5-HT$_{1A}$ = 7.7   WAY 100635 (**12**); pK_i 5-HT$_{1A}$ = 9.6
BMY 7378 (**10**); n = 1; pIC$_{50}$ 5-HT$_{1A}$ = 8.6

Figure 9. Chemical structures of **9–12**.

Flesinoxan (**13**); pK_i 5-HT$_{1A}$ = 8.8   **14**; pK_i 5-HT$_{1A}$ = 8.1
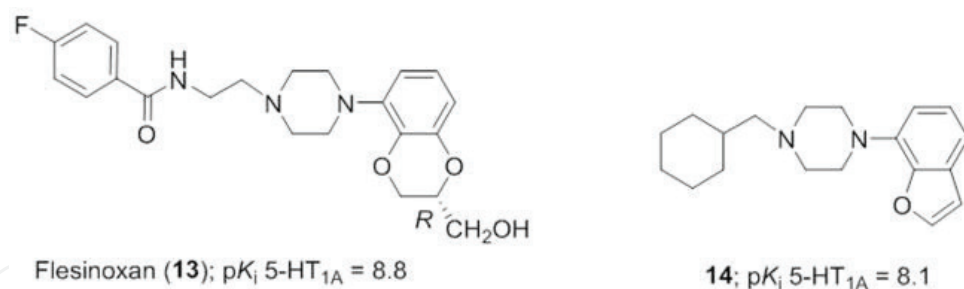
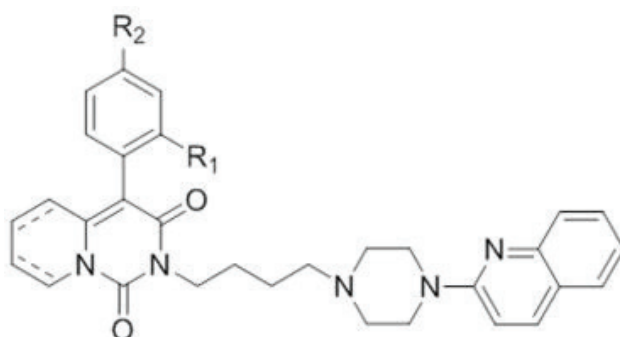**Figure 10.** Chemical structures of **13–14**.

Figure 11. General structure of quinoline derivatives.

5.2.2. Modification of the piperazine ring

*N*1-Arylpiperazine moiety plays an important role in the affinity for 5-HT$_{1A}$Rs. This template has been duplicated to successfully obtain selective homo- and heterobivalent ligands [18]. Indeed, compound **15** shows high affinity for 5-HT$_{1A}$Rs and selectivity over 5-HT$_7$Rs, whereas compound **16** selectively targets 5-HT$_7$Rs (pK_i = 7.4) (**Figure 12**).

The piperazine ring can be replaced by a piperidine one. The most representative example is befiradol (**17**), a very potent and highly selective 5-HT$_{1A}$R full agonist (**Figure 13**), that also shows efficacy in a rodent model of neuropathic, inflammatory, and surgical pain. It is endowed with potent analgesic and antiallodynic effects that are comparable to those of high doses of opioids. However, lower and fewer side effects are triggered, and little or no development of tolerance is manifested by **17**. In 2013, **17** was marketed by Neurolixis with indication for the treatment of L-DOPA-induced dyskinesia in Parkinson's disease [19]. The 3-chloro-4-fluorophenyl moiety of **17** can be bioisosterically replaced by both unsaturated and saturated lipophilic moieties [20]. Among the investigated compounds, the highly selective 5-HT$_{1A}$R superagonist benzothiophene-3-carboxamide **18** almost exclusively recognizes 5-HT$_{1A}$Rs (**Figure 13**).

A series of 2*H*-pyrido[1,2-c]pyrimidine derivatives, bearing a piperidinyl-indole residue in their pharmacophore (**Figure 13**), shows very high-affinity values for both 5-HT$_{1A}$Rs and SERTs. Compound **19** is a representative example [21]. The presence of a tetrahydropyridinyl-indole moiety reduces binding to 5-HT$_{1A}$Rs, while a Cl substituent in R$_3$ reduces binding to both 5-HT$_{1A}$Rs and SERTs.
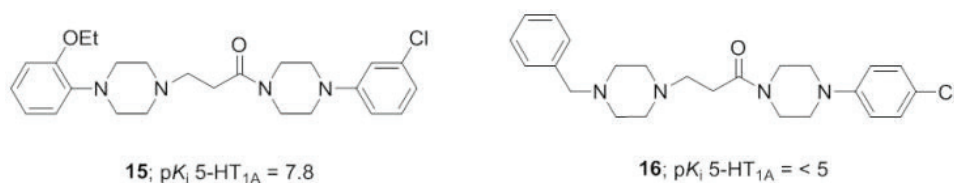

15; pK_i 5-HT$_{1A}$ = 7.8   **16**; pK_i 5-HT$_{1A}$ = < 5

Figure 12. Chemical structures of **15** and **16**.

Befiradol (**17**); R = ...   pK_i 5-HT$_{1A}$ = 9.5   **19**; R$_1$ = R$_2$ = H, R$_3$ =F; pK_i 5-HT$_{1A}$ = 7.9

18; R = ...   pK_i 5-HT$_{1A}$ = 8.6   **20**; R$_1$ = CH$_3$, R$_2$ = H, R$_3$ =F; pK_i 5-HT$_{1A}$ = 8.3
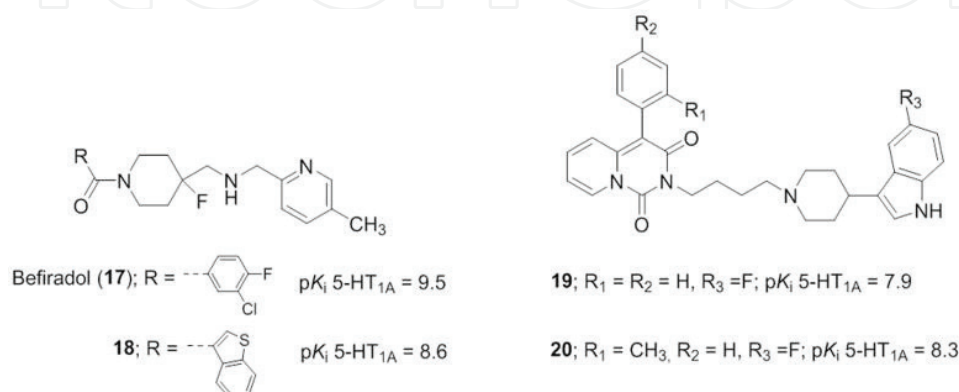
Figure 13. Chemical structures of **17–20**.

Finally, the presence of a 3β-aminotropane moiety instead of the piperazine or piperidine ring is unfavorable for the development of High affinity $5\text{-HT}_{1A}R$ ligands (**Figure 14**) [22].

5.2.3. Modification of the spacer

In LCPAs, the four-carbon alkyl chain seems to be the most favorable for high $5\text{-HT}_{1A}R$ affinity. Indeed, its shortening reduces affinity, according to the rank order of potency C-4 > C-2 > C-3 [4].

However, the butyl chain can be substituted by a propylthio bridge, as confirmed by the high $5\text{-HT}_{1A}R$ affinity of compound **21** (**Figure 15**). The NH_2 function is responsible for its selectivity over α_1-ARs ($5\text{-HT}_{1A}R/\alpha_1$-AR = 55) [15].

The oxybutynin chain of aripiprazole (**7**) is also favorable for high $5\text{-HT}_{1A}R$ affinity. Besides its main use in the treatment of schizophrenia and bipolar disorder, **7** is also employed as an add-on treatment in major depressive disorder, tic disorders, and irritability associated with autism. In addition, its systemic or local administration induces antinociceptive effects. Unlike other atypical antipsychotics approved by FDA (e.g., clozapine, olanzapine, quetiapine, ziprasidone, and risperidone), which are D_2R antagonists, **7** behaves as a D_2R and D_3R partial agonist. Moreover, it shows partial agonism at $5\text{-HT}_{1A}Rs$ and, similarly to the other atypical antipsychotics, is an antagonist at $5\text{-HT}_{2A}Rs$ and 5-HT_7Rs as well as a partial agonist at $5\text{-HT}_{2C}Rs$ [23].

The presence of a hydroxyl group in the butyl chain is well tolerated. BMY 14802 (**22**) (**Figure 16**), for example, is a $5\text{-HT}_{1A}R$ agonist that also attenuates dyskinesia produced by L-DOPA.
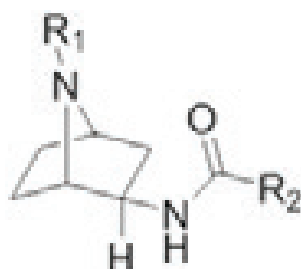

Figure 14. General structure of 3β-aminotropane derivatives.

A hydroxyalkyl chain also characterizes a series of molecules (**23–26**) (**Figure 17**), in which the combination of structural elements favoring the affinity for $5\text{-HT}_{1A}Rs$ (heterocyclic nucleus, hydroxyalkyl chain, and 4-substituted piperazine) was used to obtain ligands with high

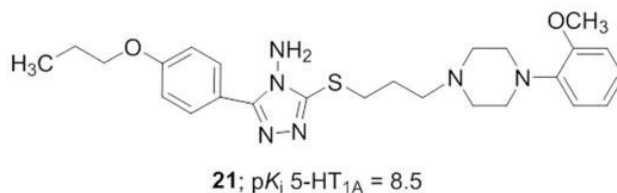**21**; pK_i 5-HT_{1A} = 8.5

Figure 15. Chemical structure of **21**.

5-HT$_{1A}$ affinity and selectivity over other 5-HT subtypes [24]. In particular, while compounds **23–25** show an outstanding 5-HT$_{1A}$R affinity, compound **26** is selective for 5-HT$_{2C}$Rs (pK_i = 8.3).

In a series of compounds prepared to discover mixed 5-HT/dopamine receptor agents as novel antipsychotics, amide **27** (**Figure 18**) emerges for its high affinity for D$_3$Rs, 5-HT$_{1A}$Rs, and 5-HT$_{2A}$Rs. Its low affinity for D$_2$Rs, 5-HT$_{2C}$Rs, and hERG channels reduces extrapyramidal side effects, risk of obesity under chronic treatment, and incidence of torsade des pointes, respectively [25]. The replacement of the ether/amide bridge with a sulfonamide function affords a series of quinoline or isoquinoline derivatives endowed with multireceptor 5-HT$_{1A}$R/5-HT$_{2A}$R/5-HT$_7$R/D$_2$R/D$_3$R profile and behaving as 5-HT$_{1A}$R agonists, D$_2$R partial agonists, and 5-HT$_{2A}$R/5-HT$_7$R antagonists (**Figure 18**). They produce significant antidepressant activity in mice [26]. In particular, **28** also displays remarkable antipsychotic effects in MK-801-induced hyperlocomotor activity in mice.

The inclusion of the alkyl chain of LCAPs in a cyclohexyl ring leads to more conformationally constrained analogues (e.g., **29**) (**Figure 19**) [15]. Trans derivatives show 5-HT$_{1A}$R affinity significantly higher than that of their corresponding cis isomers (e.g., trans **29** and cis **29**). The insertion of a hydroxyl substituent in the cyclohexyl moiety is also well tolerated (**30**). Interestingly, compared to flexible 4-carbon alkyl chain analogues, 1e,4e-disubstituted cyclohexane derivatives maintain very high 5-HT$_{1A}$R affinity, but in some cases, the functional profile is modulated from partial agonism to antagonism [27].
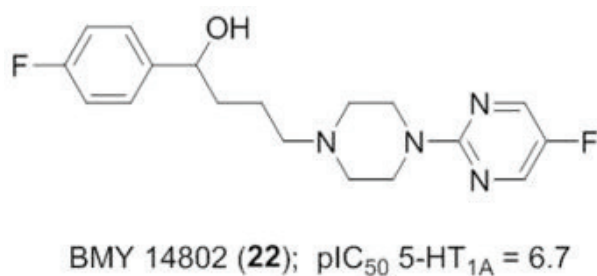

BMY 14802 (**22**); pIC$_{50}$ 5-HT$_{1A}$ = 6.7

Figure 16. Chemical structure of BMY 14802 (**22**).

23; X = CH$_2$, R = --〈 〉CF$_3$; pK_i 5-HT$_{1A}$ = 8.7   **24**; X = CH$_2$, R = --〈 〉H$_3$C CH$_3$; pK_i 5-HT$_{1A}$ = 8.4

25 X = O, R = --〈 〉CF$_3$; pK_i 5-HT$_{1A}$ = 8.5   **26**; X = CH$_2$, R = --〈 〉O ; pK_i 5-HT$_{1A}$ = 6.0
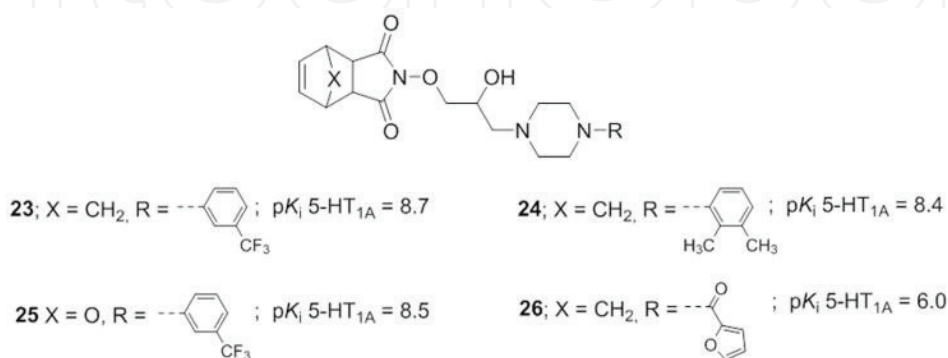
Figure 17. Chemical structures of **23–26**.

The alkyl chain can be partially included in aromatic functions, including pyrrole (RWJ 25730, **31**), phenyl (mazapertine, **32**), or benzimidazole (**33**) (**Figure 20**) [15]. The multireceptor affinity of **32** can be ascribed to its ability to adopt a variety of low-energy conformations. Indeed, constraining its 2-isopropoxyphenyl and piperazine moieties, affording compound **34**, significantly reduces affinities for α_1-ARs and D$_2$Rs, but not that for 5-HT$_{1A}$Rs.

The insertion of the 1,3-dioxolane nucleus in the chain is also well tolerated. Compound **35**, for example, is a potent partial agonist and shows moderate selectivity over α_1-ARs (**Figure 21**) [28]. Substitutions at C-8 position of the 1,4-dioxaspiro[4, 5]decane moiety reduce 5-HT$_{1A}$R/α_1-AR selectivity ratio because of the significant decrease of binding affinity and intrinsic activity for 5-HT$_{1A}$Rs with respect to α_1-ARs. The isosteric replacement of one (oxathiolane derivative **36**) and especially of two (dithiolane derivative **37**) oxygen atoms with sulfur atoms proves to be tolerated (**Figure 21**). The replacement of the piperazine ring with a more flexible basic chain affords compound **38**, which behaves as a potent and selective 5-HT$_{1A}$R partial agonist endowed with neuroprotective activity in vitro and potent antinociceptive activity in an in vivo model [28]. A similar profile is shown by the unsubstituted analogue **39** characterized by good 5-HT$_{1A}$/ α_1-AR selectivity (**Figure 21**).

Similar structure-activity relationships (SARs) can be observed when the spiro-cyclohexyl terminal fragment in both piperazine and open-chain series is replaced by a 2,2-diphenyl moiety.
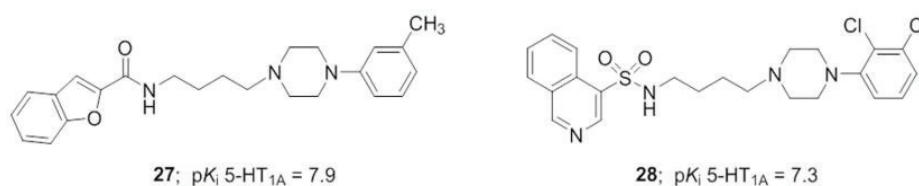

27; pK_i 5-HT$_{1A}$ = 7.9

28; pK_i 5-HT$_{1A}$ = 7.3

Figure 18. Chemical structures of **27** and **28**.

trans-**29**; pK_i 5-HT$_{1A}$ = 10.7

cis-**29**; pK_i 5-HT$_{1A}$ = 7.1

30; pIC$_{50}$ 5-HT$_{1A}$ = 8.7
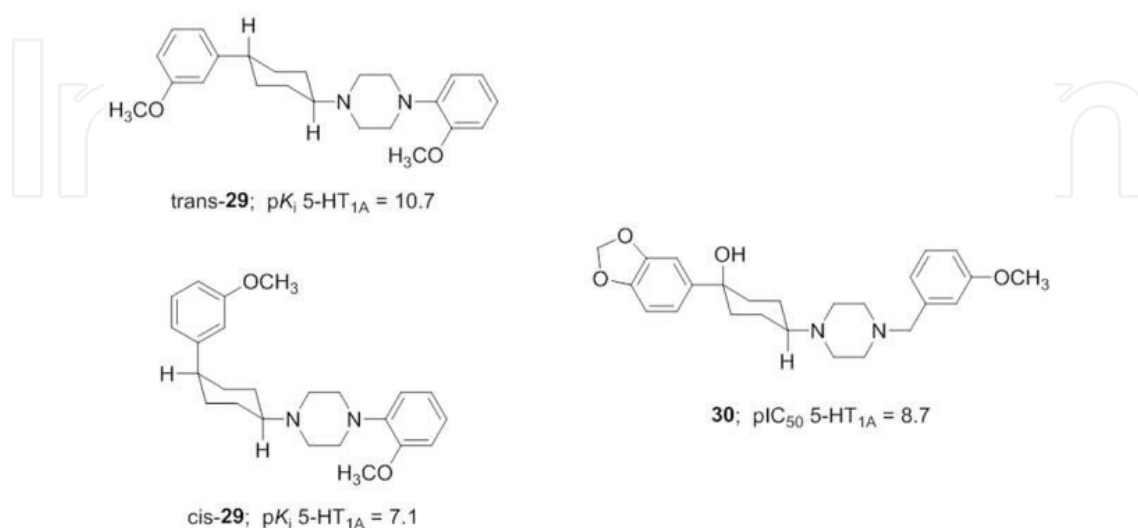
Figure 19. Chemical structures of **29** and **30**.
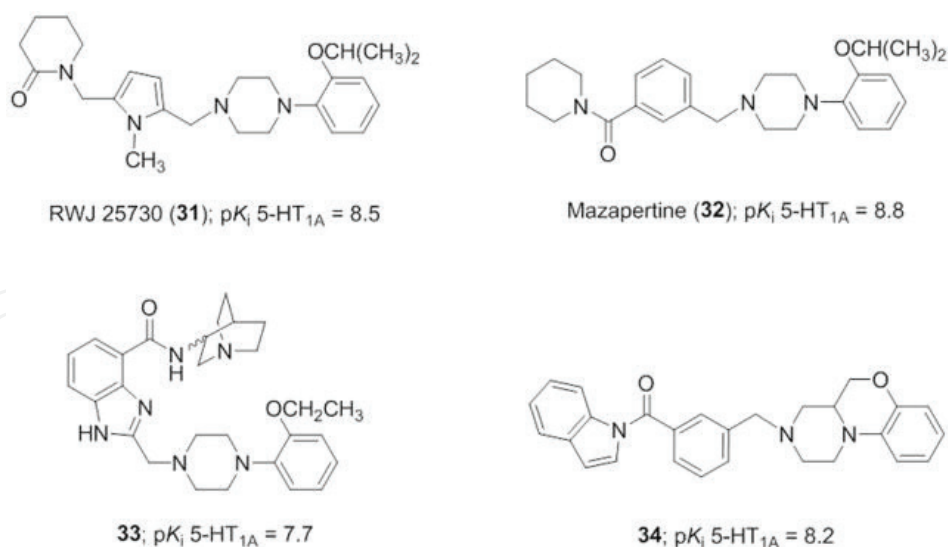
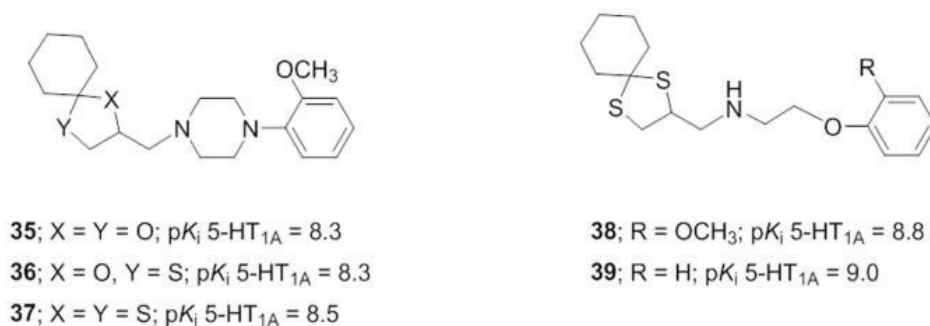**Figure 20.** Chemical structures of **31–34**.

Figure 21. Chemical structures of **35–39**.

The replacement of the 1,3-dioxolane nucleus with other pentatomic rings bearing H-bond acceptor groups (tetrahydrofuran or cyclopentanone) or an H-bond acceptor and donor group (cyclopentanol) (**Figure 22**) causes an overall reduction of affinity at α_1-ARs, while both potency and efficacy are increased at 5-HT$_{1A}$Rs.

5.2.4. Modification of the terminal fragment

The numerous structurally different terminal fragments, as already seen for ligands reported above, demonstrate that this moiety is less critical for 5-HT$_{1A}$R interaction [8]. The dual SSRI and 5-HT$_{1A}$R agonist vortioxetine (**40**), approved by FDA for the treatment of major depressive disorders in adult in 2013, even lacks this function (**Figure 23**).

The replacement of the azaspirodecanedione moiety of **9** with an *N*-phthalimido group affords the nonselective ligand **41** (**Figure 24**) [15]. Shortening the length of its butyl chain to three or two units significantly decreases the affinity. The presence of an isosteric sulfonyl function instead of a carbonyl group of the phthalimide moiety, as in ipsapirone (**42**), is compatible with
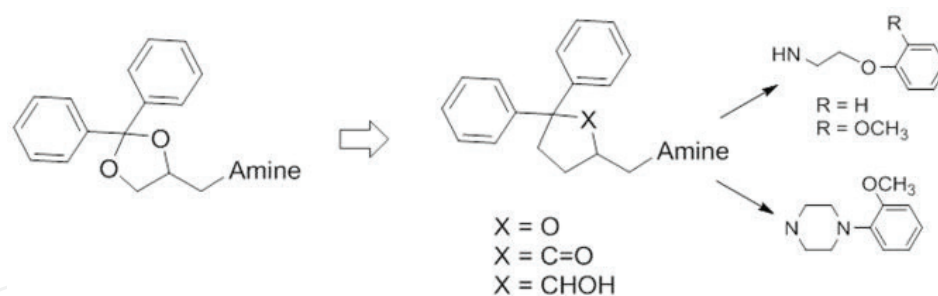
Figure 22. Bioisosteric replacement of oxygen atoms of 5-HT$_{1A}$R 1,3-dioxolane ligands.

the maintenance of similar 5-HT$_{1A}$R affinity and improved selectivity over α_1-ARs (**Figure 24**) [15]. The replacement of the phthalimide moiety of **41** with an adamantyl amide group, leading to **43**, also increases the selectivity for 5-HT$_{1A}$Rs over α_1-ARs (**Figure 24**) [15]. As in the case of the prototypical 5-HT$_{1A}$R antagonist **12**, substituents can be present at amidic NH [15]. The replacement of the pyridine ring of **12** with a pyrimidine substituent leads to the similarly potent 5-HT$_{1A}$R antagonist **44**. The isosteric inversion of the amide function and the presence of a phenyl group in the bridge, affording **45**, are tolerated (**Figure 24**). Considering both affinity and selectivity for 5-HT$_{1A}$Rs, among some 5-methoxy-1,2,3,4-tetrahydronaphthalen-1-yl derivatives obtained by inserting an alkyl chain of variable length (preferably a three-membered alkyl chain) in the α, β, or ω position, the best derivatives are **46** and **47** (**Figure 24**) [15].

Several molecules, bearing an isonicotinic moiety as the terminal fragment of LCAPs, show nanomolar and subnanomolar affinities for 5-HT$_{1A}$Rs, 5-HT$_{2A}$Rs, and 5-HT$_{2C}$Rs and moderate or no affinity for other relevant receptors (D$_1$Rs, D$_2$Rs, α_1- and α_2-ARs) [29]. In particular, derivative **48**, bearing a propyl chain as a spacer, shows the highest affinity for 5-HT$_{1A}$Rs and selectivity over dopaminergic, adrenergic, and other serotoninergic receptors (**Figure 25**). LCAPs bearing a 1,2,3,4-tetrahydroisoquinoline-3-carboxamide in the terminal fragment can show affinity for 5-HT$_{1A}$Rs and/or 5-HT$_7$Rs [30]. Indeed, while compounds **49** and **50**, with a methylthio substituent in the ortho-position show high
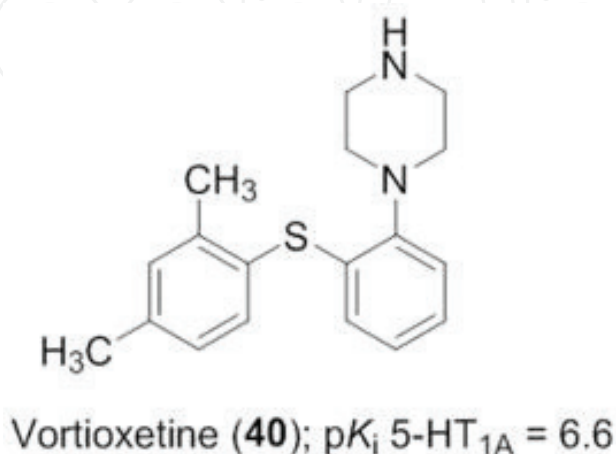

Vortioxetine (**40**); pK_i 5-HT$_{1A}$ = 6.6

Figure 23. Chemical structure of vortioxetine (**40**).

41; pK_i 5-HT$_{1A}$ = 9.2

Ipsapirone (**42**); pK_i 5-HT$_{1A}$ = 8.1

43; pK_i 5-HT$_{1A}$ = 9.4

44; pK_i 5-HT$_{1A}$ = 9.0

45; pK_i 5-HT$_{1A}$ = 8.0

46; R = 2-pyridyl; pK_i 5-HT$_{1A}$ = 9.3
47; R = 3-benzisothiazolyl; pK_i 5-HT$_{1A}$ = 9.3
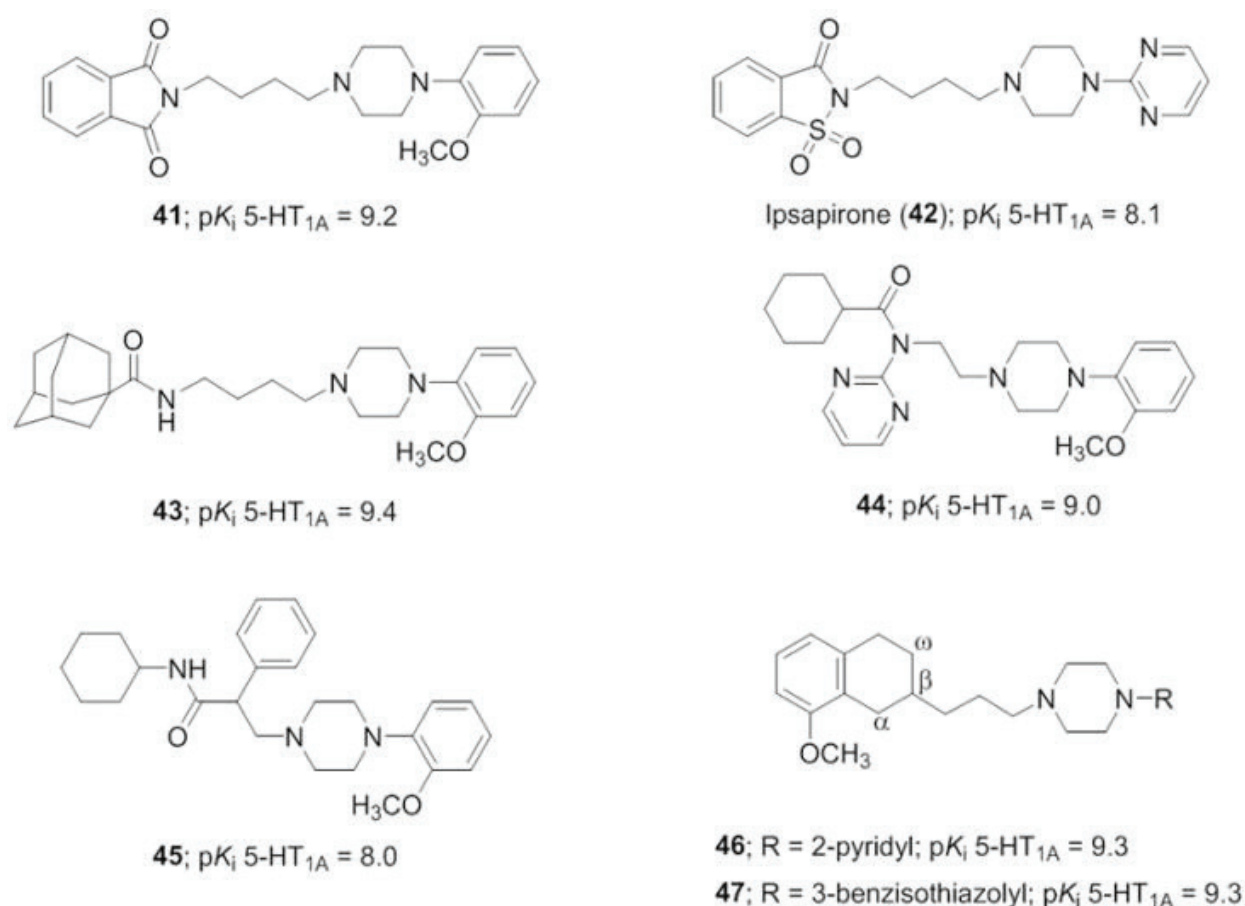
Figure 24. Chemical structures of **41**–**47**.

5-HT$_{1A}$R affinity, the replacement of the phenyl ring in the arylpiperazine moiety with a benzisoxazole system, affording, for example, **51** and **52**, significantly increases the affinity for 5-HT$_7$R (pK_i = 7.7 and 7.6, respectively) (**Figure 25**). The insertion of a spiro-cyclopentane or cyclohexane in position 3 of pyrrolidin-2,5-dione leads to a series of arylpiperazines, among which derivatives **53** and **54** with an ethylene spacer and a CF$_3$ substituent in meta position of the phenyl ring show both anticonvulsant activity and high 5-HT$_{1A}$R and 5-HT$_{2A}$R affinity (**Figure 25**) [31].

A β-tetralonohydantoin as terminal fragment characterizes a series of compounds, which show high 5-HT$_{1A}$R affinity (pK_i = 7.3–8.2) combined with moderate to high 5-HT$_{2A}$R affinity (pK_i = 6.7–7.3). Among them, compound **55** (**Figure 26**) is a postsynaptic 5-HT$_{1A}$R antagonist and produces the characteristic effect of presynaptic 5-HT$_{1A}$R agonists [32]. Moreover, it behaves as a 5-HT$_{2A}$R antagonist. Due to its interesting 5-HT$_{1A}$/5-HT$_{2A}$ functional profile, **55**, tested for its potential psychotropic activity, shows diazepam-like anxiolytic activity and behaves as a weak antidepressant.

Among new LNCPs with structural modifications in the terminal fragment, in the alkyl chain length and in the substituents of the piperazine fragment, the 2-ethoxy quinazolinone derivatives **56** and **57** are the most interesting ligands, showing high affinity for 5-HT$_{1A}$Rs and 5-HT$_7$Rs (**Figure 26**) [33].
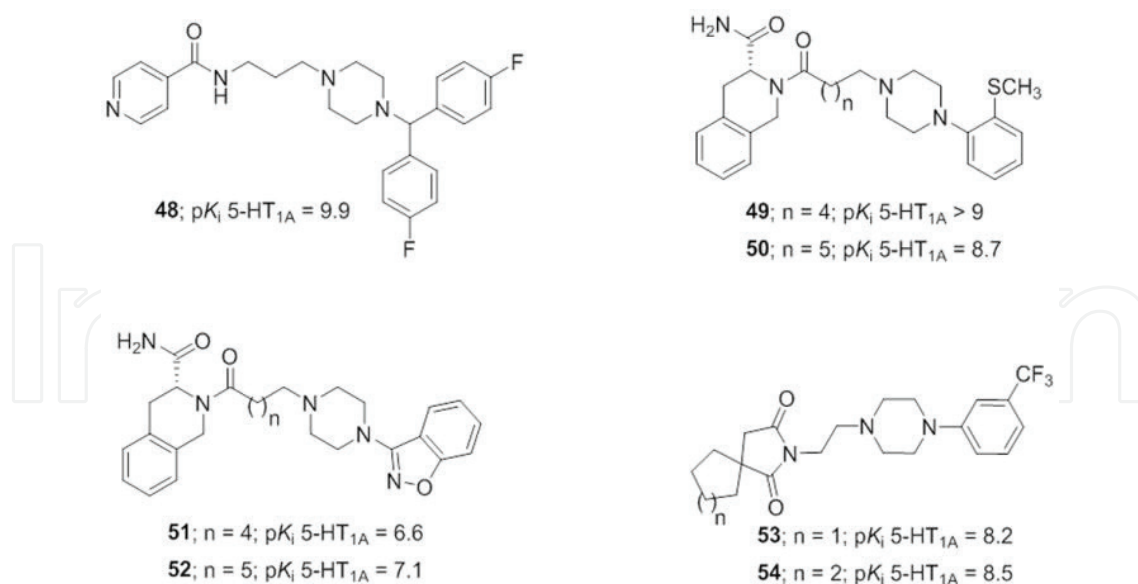
Figure 25. Chemical structures of **48–54**.

In a more recent work, the quinazolinone system has been replaced by 6-phenyl-4(3*H*)-pyrimidinone as a result of splitting bicyclic quinazolinone system [34]. The benzo-cracking strategy (compounds **58–62**) causes a decrease in affinity for both receptors. In functional assays, these derivatives behave as weak 5-HT$_{1A}$R and 5-HT$_7$R antagonists (**Figure 26**).

1,2,4-Triazine-6(1*H*)-one derivatives also display dual affinity for 5-HT$_{1A}$Rs and 5-HT$_7$Rs. SAR studies have revealed that receptor affinity and selectivity depend on the nature of the substituent in position 3 of the triazinone fragment as well as on the substitution pattern of the phenylpiperazine moiety [35]. The best 5-HT$_{1A}$R affinity values and selectivity over 5-HT$_7$Rs are displayed by compounds **63** and **64** (**Figure 26**).

The 3,5-dioxo-(2*H*,4*H*)-1,2,4-triazine-tethered arylpiperazines have been identified as agonists with high affinity for 5-HT$_{1A}$Rs. Several members of this series such as **65** show nanomolar affinity for 5-HT$_{1A}$Rs, high selectivity over α_1-AR, and potent agonist activity (**Figure 26**) [36]. The 1,2,3-benzotriazin-4-one terminal fragment characterizes some 5-HT$_{1A}$R antagonists prepared as potential antiproliferative agents in cancer cell lines [37]. These compounds are endowed with high 5-HT$_{1A}$R affinity and moderate or no affinity for other receptors (5-HT$_{2A}$Rs, 5-HT$_{2C}$Rs, D$_1$Rs, D$_2$Rs, α_1- and α_2-ARs). In particular, derivative **66** shows picomolar affinity for 5-HT$_{1A}$Rs (**Figure 26**).

MP 3022 (**67**), the lead compound of a large series of 4-alkyl-1-(*o*-methoxyphenyl)-piperazines containing a benzotriazole terminal fragment, behaves as a potent pre- and postsynaptic 5-HT$_{1A}$R antagonist, but it is not selective for 5-HT$_{1A}$Rs over α_1-ARs (**Figure 26**) [15]. 4-Benzoyl-1,2,3-triazole derivatives (e.g., **68**), open-chain analogues of their benzotriazole bioisosteres, bind to 5-HT$_{1A}$Rs in a nanomolar range and are highly selective over 5-HT$_{2A}$Rs and 5-HT$_{2C}$Rs (**Figure 26**) [15].

Purine 2,6-dione core has also been used as a terminal fragment to combine the 5-HT$_{1A}$R activity with the phosphodiesterase (PDE) inhibition [38]. Both effects might be advantageous in
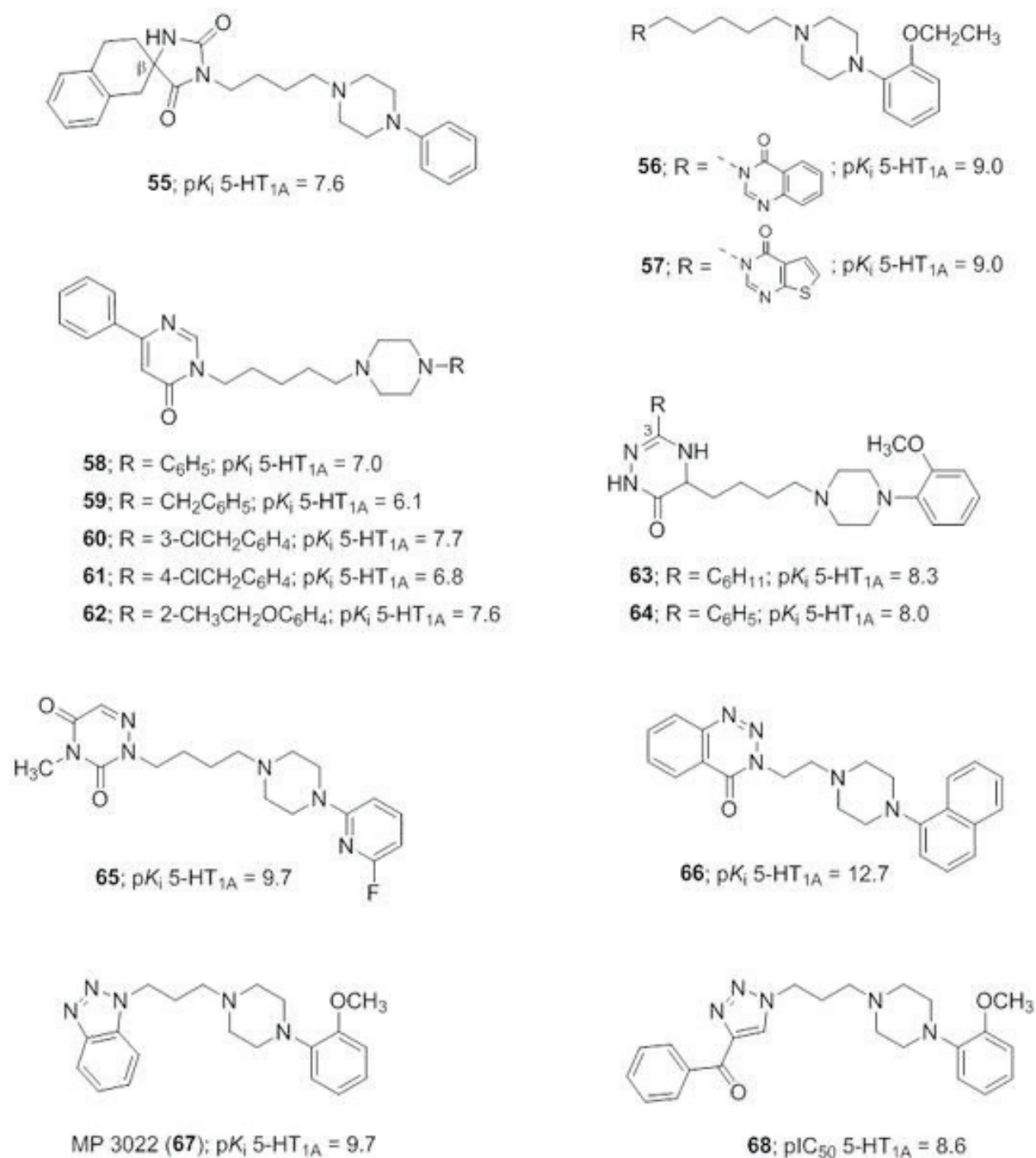
Figure 26. Chemical structures of **55–68**.

the treatment of neuropsychiatric disorders. Among the compounds bearing this core, **69–72** show high affinity for 5-HT$_{1A}$Rs and, in the case of **69** and **70**, also for 5-HT$_7$R. At the same time, compounds **69–72** show a moderate to very low D$_2$R affinity. From functional assays, **69–71** behave as 5-HT$_{1A}$R antagonists, whereas **72** is an agonist (**Figure 27**) [38, 39]. The anti-depressant activity of **69** and **70** at a dose of 1.25 mg/kg is similar to that of citalopram given at the same dose [38]. The annulation of the purine system at 7,8-positions with an imidazole moiety affords ligands with a wide spectrum of activities (high 5-HT$_{1A}$R or 5-HT$_7$R affinity,

mixed 5-HT$_{1A}$R/5-HT$_7$R affinity, and additional affinity for D$_2$R) [40]. The tested compounds are in the ranges defined by the "rule of five" (logP < 5), which indicates good intestinal permeability and metabolic stability. In preliminary pharmacological in vivo studies, the selected compound **73** behaves as a potential antidepressant in mice and, at the dose of 2.5 mg/kg, shows anxiolytic effect (**Figure 27**). Finally, purine 2,4,8-trione derivatives show affinity values lower than those of the corresponding purine 2,4-dione analogues (**Figure 27**) [41].

5.2.5. Main interactions of arylpiperazines with 5-HT$_{1A}$Rs

Two main interactions prove to be important for the affinity of arylpiperazines for 5-HT$_{1A}$Rs: (a) an ionic bond between the protonated nitrogen atom of the piperazine ring and the carboxyl oxygen of the side chain of Asp3.32 and (b) an edge-to-face CH-π interaction between the aromatic ring and the Phe6.52 residue, which stabilizes the ligand binding. The basic pharmacophore of the 5-HT$_{1A}$R is the same for agonists and antagonists and consists of an aromatic nucleus and a basic nitrogen atom, whose optimal distance is 5.2 Å, while the nitrogen lies at 0.2 Å above the plane defined by the reference ring (**Figure 28**) [4].

Due to the highly flexible linker (usually 2-4 methylene units), using different experimental and modeling techniques, various attempts have been conducted to determine the bioactive conformation of LCAPs [42]. Assuming that active conformations of LCAPs are closely related to those in solutions or in solid state, two-dimensional (2D) NMR and crystallographic methods were often applied. The 2D NMR studies indicated that compounds with tetramethylene spacer can adopt extended, bent, or folded conformations. On the other hand, analysis of Cambridge Structural Database showed that linear geometries predominated. Molecular
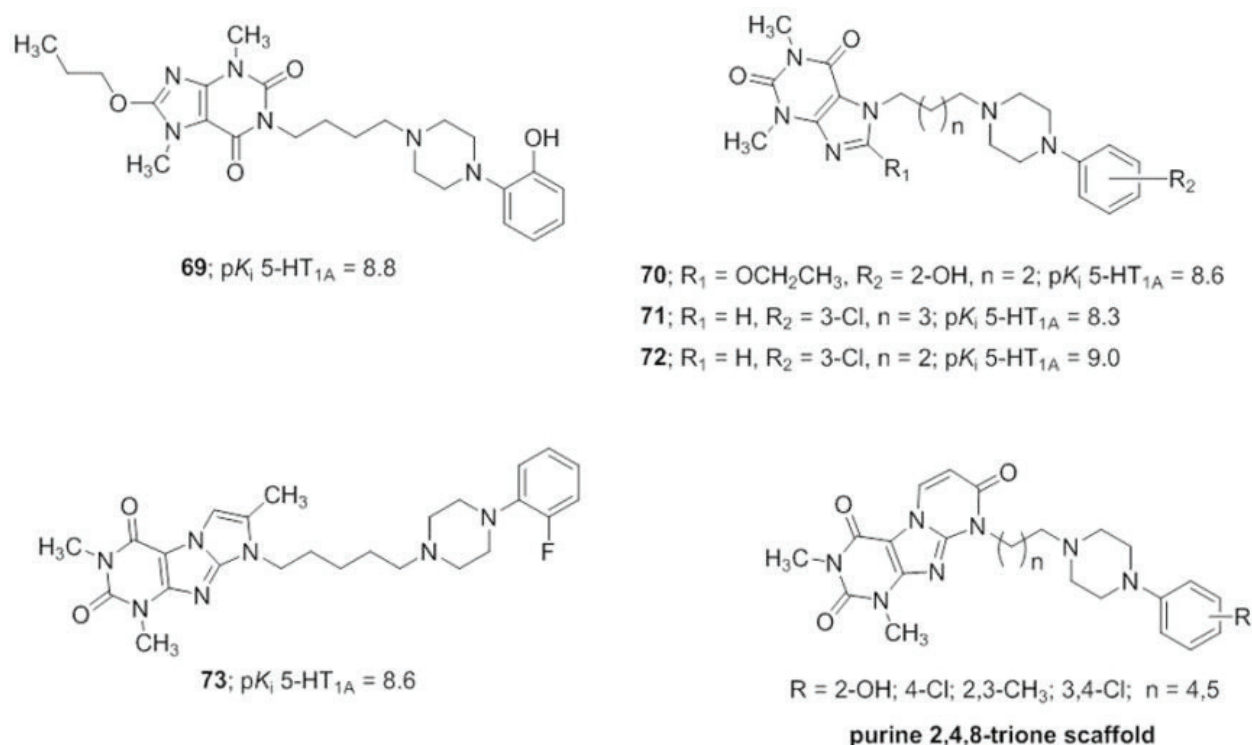

69; pK_i 5-HT$_{1A}$ = 8.8

70: R$_1$ = OCH$_2$CH$_3$, R$_2$ = 2-OH, n = 2; pK_i 5-HT$_{1A}$ = 8.6
71: R$_1$ = H, R$_2$ = 3-Cl, n = 3; pK_i 5-HT$_{1A}$ = 8.3
72: R$_1$ = H, R$_2$ = 3-Cl, n = 2; pK_i 5-HT$_{1A}$ = 9.0

73; pK_i 5-HT$_{1A}$ = 8.6

R = 2-OH; 4-Cl; 2,3-CH$_3$; 3,4-Cl; n = 4,5

purine 2,4,8-trione scaffold

Figure 27. Chemical structures of **69–73** and general structure of purine 2,4,8-trione derivatives.
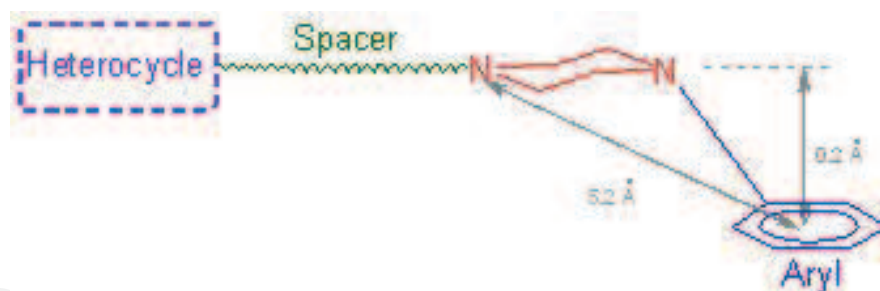
Figure 28. General structure of LCAPs and pharmacophoric model of 5-HT$_{1A}$R (Adapted with permission from Ref. [4]. Copyright (2014) American Chemical Society).

modeling studies (conformational analysis, docking, dynamics), provided with structural investigations or conducted separately, also gave equivocal results suggesting the possibility of different bioactive conformations of LCAPs.

5.3. Aminotetralins

For a long time, 2-aminotetralin structure has been known to be pharmacologically important. Initially, aminotetralins were characterized by their sympathomimetic action, i.e., the induction of mydriasis, contraction of the uterus, changes in blood pressure, and respiration, as well as increased intestinal motility in in vivo experiments. During the late 1960s, the discovery of their activity at central dopamine receptor led to active synthesis programs all over the world. The 2-aminotetralin structure has proven to be a valuable scaffold not only for the development of 5-HTR ligands, but it also characterizes dopamine and adrenergic receptor ligands, as well as compounds interacting with melatonin receptors [15]. The main SARs of aminotetralins are summarized in **Figure 29**.

The position of the hydroxyl group in the aromatic ring of the tetralin scaffold is crucial to address ligands toward 5-HT or dopamine receptors. Indeed, 8-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin (8-OH-DPAT, **74**) (**Figure 30**) is a very potent and selective 5-HT receptor ligand, while its 5- and 7-hydroxy regioisomers (5- and 7-OH-DPAT) are potent dopamine receptor ligands. [³H]8-OH-DPAT is frequently used to label 5-HT$_{1A}$Rs. Both its enantiomers show high affinity for 5-HT$_{1A}$Rs. However, in functional experiments, the (*R*) enantiomer behaves as a full agonist while its antipode as a partial agonist.

Compounds obtained by replacing the 8-hydroxy substituent with 8-methoxy (8-MeO-DPAT, **75**), 8-acetyl (**76**), and 8-methoxycarbonyl (**77**) or 8-carboxamide (**78**) groups are about as potent as the parent compound, indicating that the proton of the 8-hydroxy group is not essential for drug-receptor interaction (**Figure 30**). A carboxylic group in the same position (**79**) is not favorable. Aryl and heteroaryl groups, such as phenyl, fluorophenyl, methoxyphenyl, acetylphenyl, 2-furyl, and benzylthio, are well tolerated. For most derivatives, the (*R*)-enantiomers are more potent than the (*S*)-enantiomers. The introduction of a fluorine atom at position C-5 of **74**, affording **80**, slightly decreases 5-HT$_{1A}$R affinity. In functional studies, the (*R*)-enantiomer behaves as a partial agonist, while the (*S*)-enantiomer is a pure antagonist at both pre- and postsynaptic receptors. An antagonist is also obtained by introducing a methyl group in 5-position of **74** (compound **81**) (**Figure 30**). The replacement of the *N*,*N*-di-*n*-propyl groups of **74** or **75** with smaller or larger di-*n*-alkyl substituents results in a significant
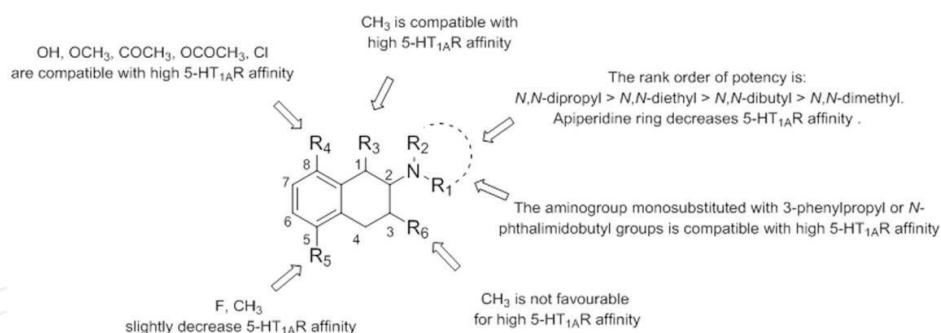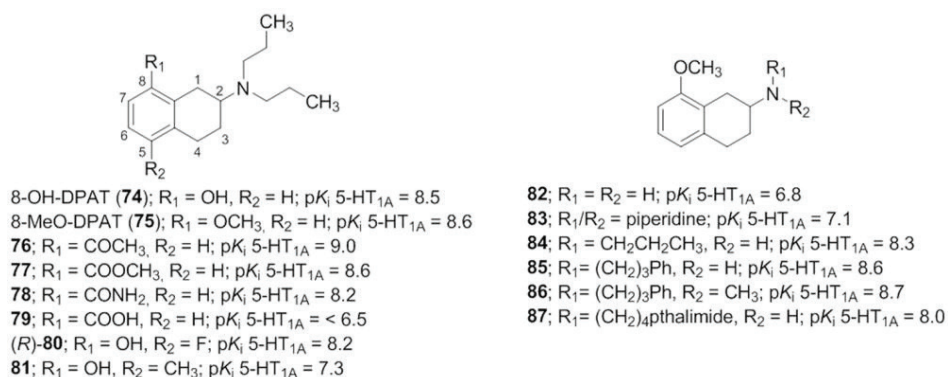
Figure 29. Main SARs of aminotetralins.

8-OH-DPAT (**74**); R_1 = OH, R_2 = H; pK_i 5-HT$_{1A}$ = 8.5
8-MeO-DPAT (**75**); R_1 = OCH$_3$, R_2 = H; pK_i 5-HT$_{1A}$ = 8.6
76; R_1 = COCH$_3$, R_2 = H; pK_i 5-HT$_{1A}$ = 9.0
77; R_1 = COOCH$_3$, R_2 = H; pK_i 5-HT$_{1A}$ = 8.6
78; R_1 = CONH$_2$, R_2 = H; pK_i 5-HT$_{1A}$ = 8.2
79; R_1 = COOH, R_2 = H; pK_i 5-HT$_{1A}$ = < 6.5
(*R*)-**80**; R_1 = OH, R_2 = F; pK_i 5-HT$_{1A}$ = 8.2
81; R_1 = OH, R_2 = CH$_3$; pK_i 5-HT$_{1A}$ = 7.3

82; R_1 = R_2 = H; pK_i 5-HT$_{1A}$ = 6.8
83; R_1/R_2 = piperidine; pK_i 5-HT$_{1A}$ = 7.1
84; R_1 = CH$_2$CH$_2$CH$_3$, R_2 = H; pK_i 5-HT$_{1A}$ = 8.3
85; R_1= (CH$_2$)$_3$Ph, R_2 = H; pK_i 5-HT$_{1A}$ = 8.6
86; R_1= (CH$_2$)$_3$Ph, R_2 = CH$_3$; pK_i 5-HT$_{1A}$ = 8.7
87; R_1= (CH$_2$)$_4$pthalimide, R_2 = H; pK_i 5-HT$_{1A}$ = 8.0

Figure 30. Chemical structures of **74–87**.

decrease in affinity. The rank order of potency is *N,N*-dipropyl > *N,N*-diethyl > *N,N*-dibutyl > *N,N*-dimethyl group.

Compared to the *N,N*-dialkylated 8-MeO-DPAT (**75**), the monoalkylated N-propyl derivative **84** shows slightly lower affinity, whereas the non-substituted 8-methoxy-2-aminotetralin (**82**) is almost inactive (**Figure 30**). The piperidine analogue **83** (**Figure 30**) is 16–29-fold less active than the *N*-mono (**84**) or *N,N*-dipropyl derivative (**75**). Compounds with high-affinity values are obtained if the amino group is monosubstituted with relatively large substituents as a phenylalkyl moiety, with the 3-phenylpropyl-8-methoxy group being optimal (**85**). Even an extra *N*-methyl group (**86**) or bulky substituents such as an *N*-(phthalimidobutyl) group are also well tolerated (**87**).

The incorporation of the nitrogen atom in the tetralin nucleus furnishes the series of 1,2,3,4-tetrahydroisoquinoline (THIQ) derivatives, which bind to 5-HT$_{1A}$Rs and 5-HT$_{2A}$Rs. SAR studies performed on the THIQ class lead to the synthesis of 1-adamantoyloaminoalkyl derivatives endowed with high affinity for 5-HT$_{1A}$Rs (pK_i = 7.3–8.3) and behaving as postsynaptic 5-HT$_{1A}$R partial agonists (**Figure 31**).

Ring contraction (indamines) or ring expansion (benzocycloheptamines) of the cycloexyl ring of 2-aminotetralins decreases 5-HT$_{1A}$R affinity. The replacement of the tetralin scaffold with the chroman nucleus does not influence affinity and selectivity.

Among the four enantiomers obtained by the introduction of a methyl group in position 1 of **75**, only (*S,R*)-**88** displays high affinity for 5-HT$_{1A}$Rs (**Figure 32**). In functional tests, it behaves as a mixed partial 5-HT$_{1A}$R agonist/D$_2$R antagonist.

The restriction of the conformation of **88** by the incorporation of the C-1 methyl and the C-2 nitrogen into an azetidine (**89**) or pyrrolidine (**90**) ring significantly enhances 5-HT$_{1A}$R affinity (**Figure 32**). These more rigid four/six and five/six fused angular tricyclic 2-aminotetralins are *N*-substituted with either *n*-propyl or its bioequivalent 2-propenyl group. The cis racemates of both series are more potent than cis-**88**. The hydroxy derivatives display selective 5-HT$_{1A}$R agonist activity, whereas the methoxy analogues show mixed 5-HT$_{1A}$R agonist and dopamine antagonist activities. In general, the cis analogues are more potent than the corresponding trans analogues, and in the cis series, the (*S,R*)-enantiomers display higher potency (**Figure 32**). Nitrogen substitution with either an *n*-propyl or an allyl group leads to ligands with similar activities, whereas their replacement with a bulky α-methylbenzyl group produces a decrease in activity. The incorporation of the C-1 methyl and the C-2 nitrogen into a more flexible six-membered piperidine ring (**91**) is less favorable for 5-HT$_{1A}$R affinity. In contrast to the pyrrolidine series, in these six/six fused angular tricyclic 2-aminotetralins, the trans enantiomers are more potent than the cis antipodes (**Figure 32**).

The introduction of a methyl group in position 3 of **75** is not favorable for high 5-HT$_{1A}$R affinity. Consequently, the incorporation of the C-2 nitrogen and C-3 methyl into a five-membered
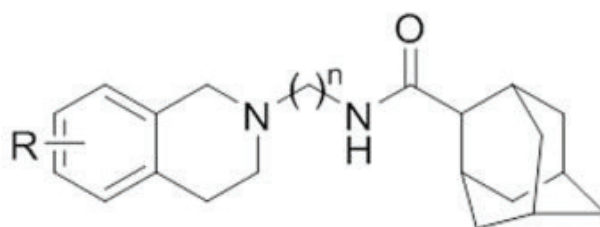

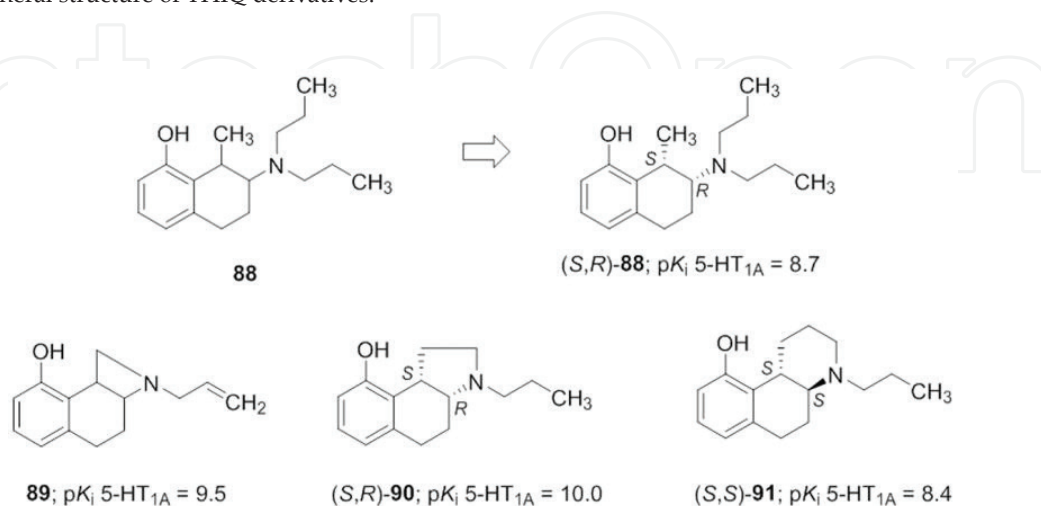**Figure 31.** General structure of THIQ derivatives.

Figure 32. Chemical structures of **88–91**.

pyrrolidine ring also leads to five/six fused linear tricyclic 2-aminotetralins, which are only moderately active.

A different six/six fused angular tricyclic of 2-aminotetralin is obtained by incorporating the 8-oxygen atom and C-7 into a six-membered ring, obtaining **92** and **93**, respectively. However, these modifications reduce affinity. The (R) configuration is more favorable than the (S) one (**Figure 33**).

A further decrease in affinity is shown by compounds bearing an annulated pyrrole ring in which the NH moiety is in the same position as the hydroxy group of **74**. On the contrary, the annulation in which the indole NH is in C-7 of the tetralin nucleus affords potent 5-HT$_{1A}$R ligands (**94**) (**Figure 34**).

The introduction of a formyl group at C-1 of **94**, affording **95** (**Figure 34**), modulates the pharmacological profile from a mixed D$_2$/5-HT$_{1A}$R agonist to a selective 5-HT$_{1A}$R agonist. The enantiomers of **95** are full agonists with affinities comparable to that of **74**. Both affinity and selectivity for 5-HT$_{1A}$Rs are improved by the substitution at C-1 of the pyrrole ring with a cyano group. In fact, the enantiomers of the 1-cyano derivative **96** are almost equipotent to the corresponding formyl derivative **95**, while 1-chloro (**97**) and 1-(1,1,1-trifluoroethyl) (**98**) substituents lead to less potent derivatives. The substitution at the C-2 of the pyrrole with a carboxamide (**99**) or cyano function (**100**) is also well tolerated, compound **100** being a potent 5-HT$_{1A}$R agonist. In the C-1 and C-2 substituted series, the (R)-enantiomers display high and moderate affinity for 5-HT$_{1A}$Rs and D$_2$Rs, respectively. The (S)-enantiomers are somewhat less potent but even more selective 5-HT$_{1A}$R ligands. An unsubstituted indole-NH moiety is crucial for the interaction with 5-HT$_{1A}$Rs. Indeed, the N-methyl compounds are significantly less potent. Without loss in 5-HT$_{1A}$R affinity, one of the propyl groups can be replaced by a variety of large substituents such as the glutarimide-butyl one (**101–103**) (**Figure 34**). In functional tests, most of the (R)-enantiomers behave as full agonists, whereas the corresponding (S)-enantiomers are partial agonists.

5.4. Indolylalkylamines

The prototype of this class of compounds is the endogenous ligand 5-HT (**Figure 1**), which behaves as a potent 5-HT$_{1A}$R agonist (pK_i = 8.4). The alkylation at α or β positions of tryptamine moiety, as well as the incorporation of its alkylamine side chain into a 4-substituted tetrahydropyridine ring, strongly decreases 5-HT$_{1A}$R affinity [15]. The removal of the hydroxyl group at position C-5 also reduces 5-HT$_{1A}$R affinity, the unsubstituted tryptamine analogue
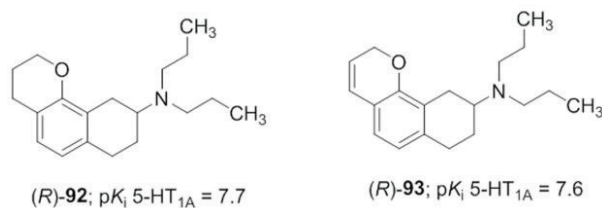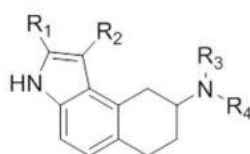

(R)-**92**; pK_i 5-HT$_{1A}$ = 7.7   (R)-**93**; pK_i 5-HT$_{1A}$ = 7.6

Figure 33. Chemical structures of **92** and **93**.

94; R$_1$ = R$_2$ = H, R$_3$ = R$_4$ = CH$_2$CH$_2$CH$_3$; pK_i 5-HT$_{1A}$ = 7.9

95; R$_1$ = H, R$_2$ = CHO, R$_3$ = R$_4$ = CH$_2$CH$_2$CH$_3$; pK_i 5-HT$_{1A}$ = 8.9

96; R$_1$ = H, R$_2$ = CN, R$_3$ = R$_4$ = CH$_2$CH$_2$CH$_3$; pK_i 5-HT$_{1A}$ = 8.7

97; R$_1$ = H, R$_2$ = Cl, R$_3$ = R$_4$ = CH$_2$CH$_2$CH$_3$; pK_i 5-HT$_{1A}$ = 7.9

98; R$_1$ = H, R$_2$ = CH$_2$CF$_3$, R$_3$ = R$_4$ = CH$_2$CH$_2$CH$_3$; pK_i 5-HT$_{1A}$ = 6.7

(*R*)-**99**; R$_1$ = CONH$_2$, R$_2$ = H, R$_3$ = R$_4$ = CH$_2$CH$_2$CH$_3$; pK_i 5-HT$_{1A}$ = 8.8

(*R*)-**100**; R$_1$ = CN, R$_2$ = H, R$_3$ = R$_4$ = CH$_2$CH$_2$CH$_3$; pK_i 5-HT$_{1A}$ = 10.0

(*R*)-**101**; R$_1$ = CN, R$_2$ = H, R$_3$ = CH$_2$CH$_2$CH$_3$, R$_4$ = (CH$_2$)$_2$-glutarimide; pK_i 5-HT$_{1A}$ = 9.2

(*R*)-**102**; R$_1$ = CONH$_2$, R$_2$ = H, R$_3$ = CH$_2$CH$_2$CH$_3$, R$_4$ = (CH$_2$)$_2$-glutarimide; pK_i 5-HT$_{1A}$ = 9.7

(*R*)-**103**; R$_1$ = H, R$_2$ = CHO, R$_3$ = CH$_2$CH$_2$CH$_3$, R$_4$ = (CH$_2$)$_2$-glutarimide; pK_i 5-HT$_{1A}$ = 9.2

Figure 34. Chemical structures of **94–103**.

being 30-fold less potent than 5-HT. However, the 5-hydroxyl group can be replaced by a 5-methoxy or 5-carboxamide function, leading to 5-MeOT (**104**) and 5-CT (**105**), respectively, which show high 5-HT$_{1A}$R affinities (**Figure 35**).

The 4-substituted tetrahydropyridine analogue of **104** (RU 24969, **106**) and the *N,N*-di-*n*-propyl analogue of **105** (DP-5-CT, **107**) also show high 5-HT$_{1A}$R affinities and behave as potent and selective 5-HT$_{1A}$R agonists (**Figure 35**). The incorporation of the side chain of **105** into a 3-substituted tetrahydropyridine, affording **108**, slightly decreases 5-HT$_{1A}$R affinity, which is further reduced by the removal of the 5-carboxyamido function or its replacement with substituents such as a methoxy or cyano group. Linking the indolyl moiety to an *N*-substituted piperazine ring through a proper alkyl spacer (LCAPs) also proves to be compatible with high 5-HT$_{1A}$R affinity and selectivity [43]. In particular, hydroxy, methoxy, or carboxamide groups in position 5 of the indole moiety yield ligands with high 5-HT$_{1A}$R affinity. Such ligands tolerate several substituents in the piperazine ring. Though the optimal linker to connect the indolyl moiety to the *N*-substituted piperazine is the *n*-butyl chain, an *n*-propyl spacer is also suitable, as demonstrated by the good 5-HT$_{1A}$R affinity showed by compounds **109** and **110** (**Figure 36**) [44].

A compound with an *n*-butyl chain is the potent and selective 5-HT$_{1A}$R ligand **111** (**Figure 36**). Within this series of derivatives, the introduction of a residue in the para position of the phenyl ring reduces dopaminergic activity and, consequently, improves 5-HT$_{1A}$R selectivity [45].

The indolylalkylamine moiety is also present in multitarget compounds simultaneously acting as SSRIs and 5-HT$_{1A}$R antagonists and potentially useful for the treatment of depression. Among these, the benzoxazine derivative **112** shows high affinity for both 5-HT$_{1A}$Rs and SERTs (pK_i SERT = 8.5), but no selectivity over α_1-ARs. It behaves as a 5-HT$_{1A}$R partial agonist [46]. On the contrary, the aryloxyalkylamine derivative **113** (pK_i SERT = 9.3) behaves as a full 5-HT$_{1A}$R antagonist (**Figure 37**) [47].

The hybridation between the chromane-based structure, present in 5-HT$_{1A}$R antagonists, and the 3-indolyl-alkylamine moiety, embedded in numerous SSRIs, leads to compounds with mixed profiles. 5-Carboxamide-8-fluoro derivatives as well as 5-carboxamide-8-des-fluoro analogues with proper *N*-alkyl chains display good affinities for both 5-HT$_{1A}$Rs and 5-HT reuptake site [48]. In particular, **114** (**Figure 37**) behaves as a very potent 5-HT$_{1A}$R antagonist and SSRI. The constrained amide conformation inherent in the lactam group results in less potent 5-HT$_{1A}$R antagonist activity [49]. Another LCAP, obtained by combining 3-indolyl-alkylamine and arylpiperazine through a butyl chain (vilazodone, **115**), proves to be suitable for the interaction with both SERTs and 5-HT$_{1A}$Rs. Indeed **115**, showing subnanomolar 5-HT reuptake inhibitor activity and subnanomolar 5-HT$_{1A}$R affinity, behaves as a 5-HT$_{1A}$R agonist high selective over other GPCRs [43]. 5-Substituted *bis*-3-propylindole derivatives connected to *N*1 and *N*4 atoms of the piperazine ring also bind both SERTs and 5-HT$_{1A}$Rs, as suggested by compounds **116** and **117** (**Figure 37**), which show good affinities for both targets [50].

5.5. Ergolines

The tetracyclic ergoline skeleton is a common structural element contained in all ergot alkaloids. Such compounds are used in the treatment of several pathophysiological conditions, because of their wide spectrum of central and peripheral pharmacological activities. They can be considered as rigid analogues of both indolylalkylamines and catecholamines. Therefore, it is not surprising that they are able to nonselectively bind to adrenergic, dopaminergic, and serotoninergic receptors. Potent and selective 5-HT$_{1A}$R ligands have been developed by combining the structural elements of the indolylethylamines and the 2-aminotetralins into a
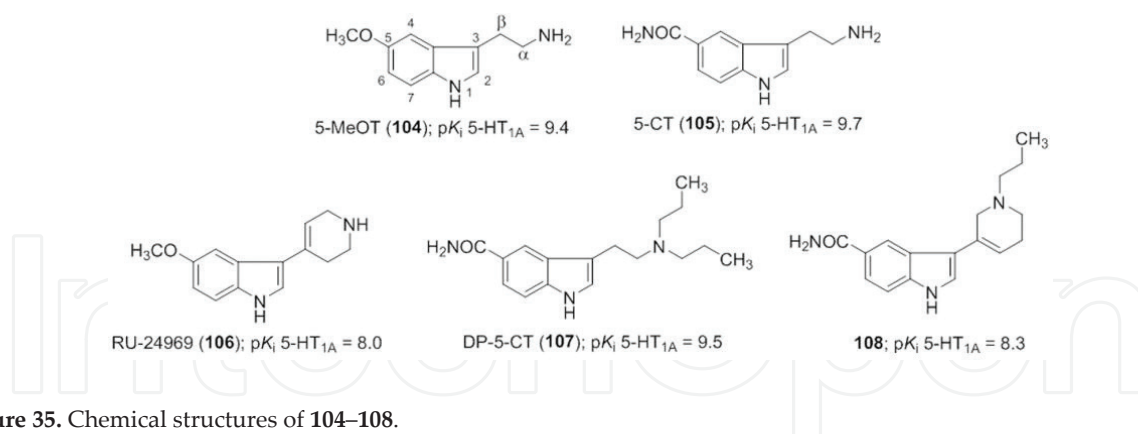
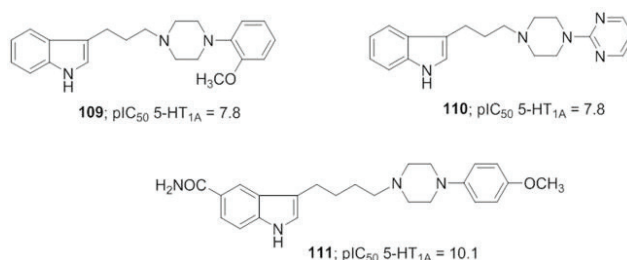

Figure 35. Chemical structures of **104–108**.

Figure 36. Chemical structures of **109–111**.

112; pK$_i$ 5-HT$_{1A}$ = 8.0

113; pK$_i$ 5-HT$_{1A}$ = 7.6

114; pK$_i$ 5-HT$_{1A}$ = 8.0

Vilazodone (**115**); pIC$_{50}$ 5-HT$_{1A}$ = 9.5

116; R = F; pIC$_{50}$ 5-HT$_{1A}$ = 7.8
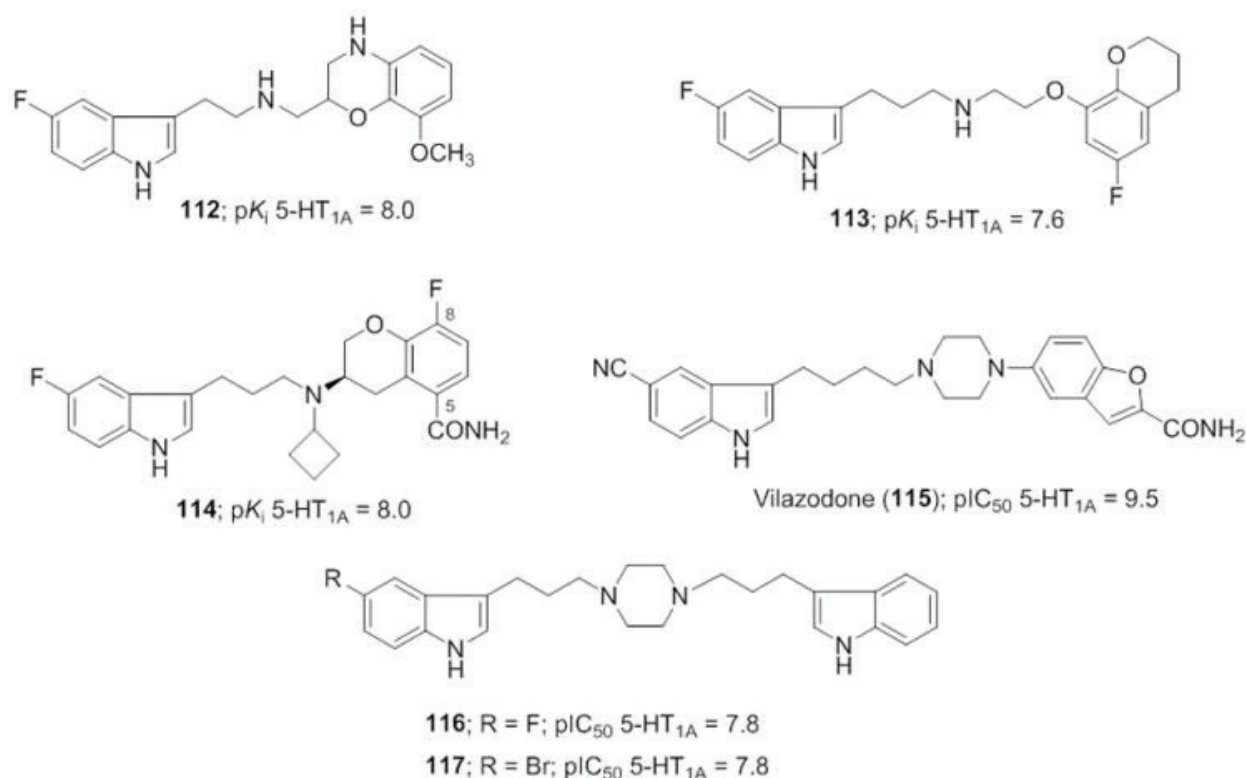**117**; R = Br; pIC$_{50}$ 5-HT$_{1A}$ = 7.8

Figure 37. Chemical structures of **112–117**.

partial ergoline skeleton [15]. Among the compounds belonging to this series, LY228729 (**118**; **Figure 38**) displays the highest affinity for 5-HT$_{1A}$Rs and good selectivity over a lot of other monoaminergic receptors. In functional assays, **118** behaves as a both pre- and postsynaptic 5-HT$_{1A}$R agonists.

Though several tetracyclic ergolines, such as LSD (**119**), lisuride (**120**), or pergolide (**121**), show high affinities for 5-HT$_{1A}$Rs, they lack of selectivity over the other monoaminergic receptors. The improvement of the selectivity for 5-HT$_{1A}$Rs over 5-HT$_2$Rs as well as D$_1$Rs, D$_2$Rs, and α-ARs can be obtained by introducing the bulky and metabolically stable *tert*-butyl group in the phenyl ring at C-13 of the ergoline skeleton. Some derivatives (**122–124**; **Figure 38**), bearing a heteroaryl substituent at C-9, display nM affinity for 5-HT$_{1A}$Rs and at least 100-fold selectivity over the other tested receptors. In contrast, the presence of a *tert*-butyl group at C-14 favors the selectivity for 5-HT$_2$R.

Among the 5(10→9)*abeo*-ergoline derivatives, compound **125** displays good 5-HT$_{1A}$R affinity and selectivity over 5-HT$_2$Rs, D$_1$Rs, D$_2$Rs, and α-ARs. In this class of compounds, 5-HT$_{1A}$R affinity is enhanced by the conversion of the 8β-hydroxymethyl group into a methyl group. Indeed, the transformation of **125** into the deoxy derivative **126** leads to appreciable increase of 5-HT$_{1A}$R affinity. An improvement of 5-HT$_{1A}$R selectivity can be obtained by the reduction of the 2,3-double bond of **126**, leading to the indolines **127** and **128** (**Figure 39**).

The stereochemistry at C-3 is very important for the 5-HT$_{1A}$R profile. In particular, compound **128** displays an outstanding selectivity for 5-HT$_{1A}$Rs over 5-HT$_2$Rs, D$_1$Rs, D$_2$Rs, and α$_1$- and α$_2$-ARs.
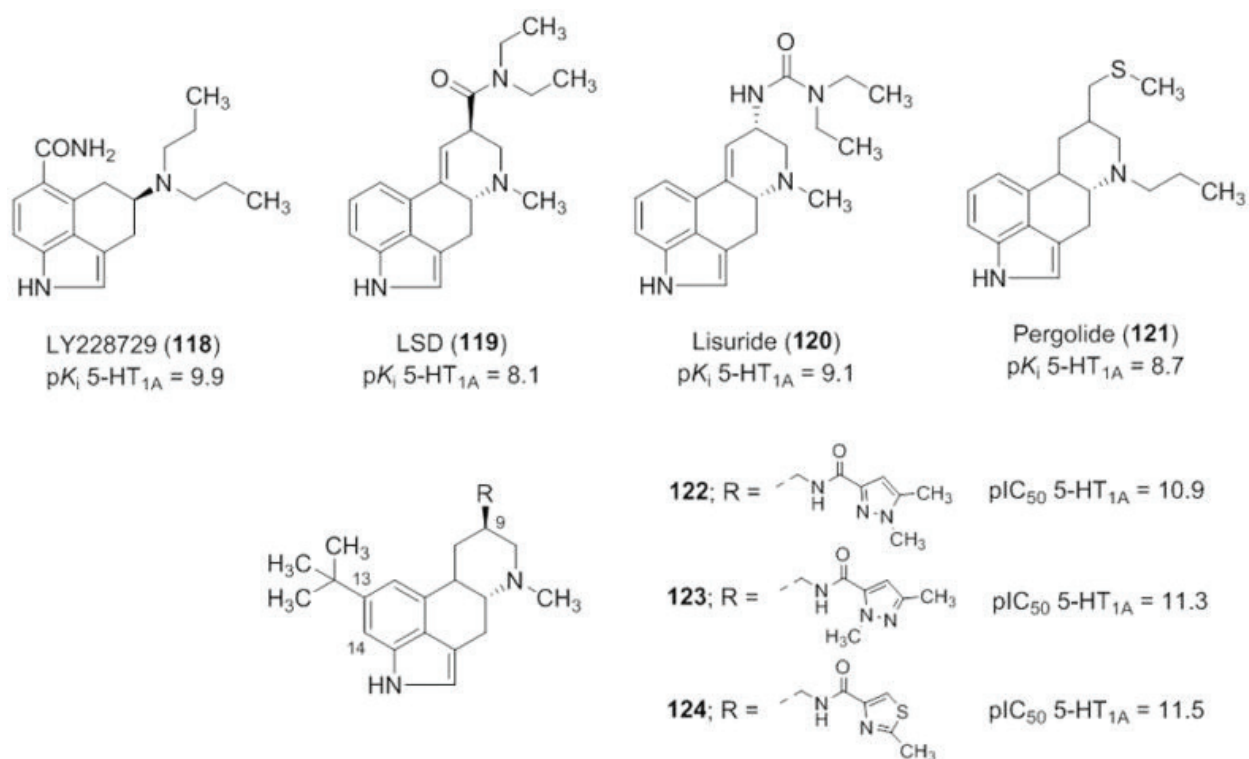
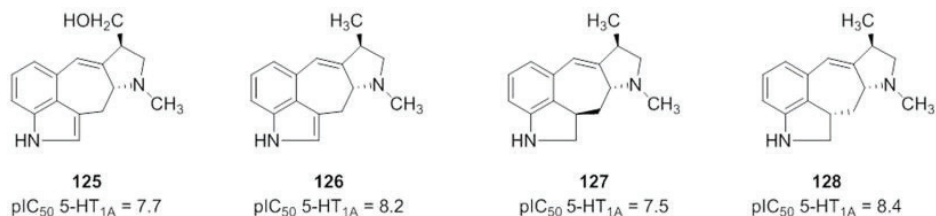**Figure 38.** Chemical structures of **118–124**.

Figure 39. Chemical structures of **125–128**.

5.6. Aporphines

These compounds, whose prototype is (*R*)-apomorphine (**129**), have extensively been studied for their interaction with dopamine receptors in the CNS. In the effort to extend SAR studies of (*R*)-aporphines at dopamine receptors, (*R*)-(−)-10-methyl-11-hydroxyaporphine **130** (**Figure 40**), the 10-methyl substituted derivative of **129**, was surprisingly discovered [15] as a potent and selective 5-HT$_{1A}$R agonist devoid of dopaminergic activity. The corresponding (*S*)-enantiomer behaves as an antagonist at postsynaptic 5-HT$_{1A}$Rs and is tenfold less potent than its antipode. Changes in steric bulk and/or electronic properties of the C10-substituent as compared to a C10-methyl group produce a decrease in 5-HT$_{1A}$R affinity. For example, the substitution of the methyl at C-10 with an ethyl group (**131**) reduces the 5-HT$_{1A}$R affinity of about 20-fold. Compound **132**, the *N*-desmethyl derivative of **130**, shows about 7-fold lower than 5-HT$_{1A}$R affinity (**Figure 40**). However, such a modification mostly reduces the affinities

for D$_1$Rs (62-fold) and D$_2$Rs (>9.3-fold) and, consequently, improves 5-HT$_{1A}$R selectivity. The removal of the substituent at position C-10 is compatible with 5-HT$_{1A}$R interaction. In particular, among the C-11-monosubstituted aporphines, ethyl (**133**) and phenyl (**134**) derivatives show the highest affinities for 5-HT$_{1A}$Rs and good selectivity over both D$_1$Rs and D$_2$Rs (**Figure 40**).

Rigidifying (*R*)-aporphines derivatives by linking C-1 and C-11 into a fused pentacyclic or hexacyclic ring strongly reduces 5-HT$_{1A}$R affinity. However, among the compounds within this series, the imino derivative **135** displays poor selectivity for 5-HT$_{1A}$Rs over both 5-HT$_7$Rs and D$_2$Rs, whereas the regioisomer **136** is selective for 5-HT$_7$Rs.

5.7. Imidazolines

The observation that the beneficial properties of the α_{2C}-AR agonists and α_{2A}-AR antagonists allyphenyline (**137**) and cyclomethyline (**138**) on morphine dependence proved to be associated to a significant antidepressant effect led to the hypothesis that ligands bearing the 2-substituted imidazoline nucleus as a structural motif can also be suitable to interact with 5-HT$_{1A}$Rs (**Figure 41**).

Experiments carried out in the presence of the 5-HT$_{1A}$R antagonist WAY100135 confirmed that 5-HT$_{1A}$R activation is involved in the observed antidepressant-like activity [51]. The investigation of a wide series of 2-substituted imidazolines linked to an aromatic moiety by
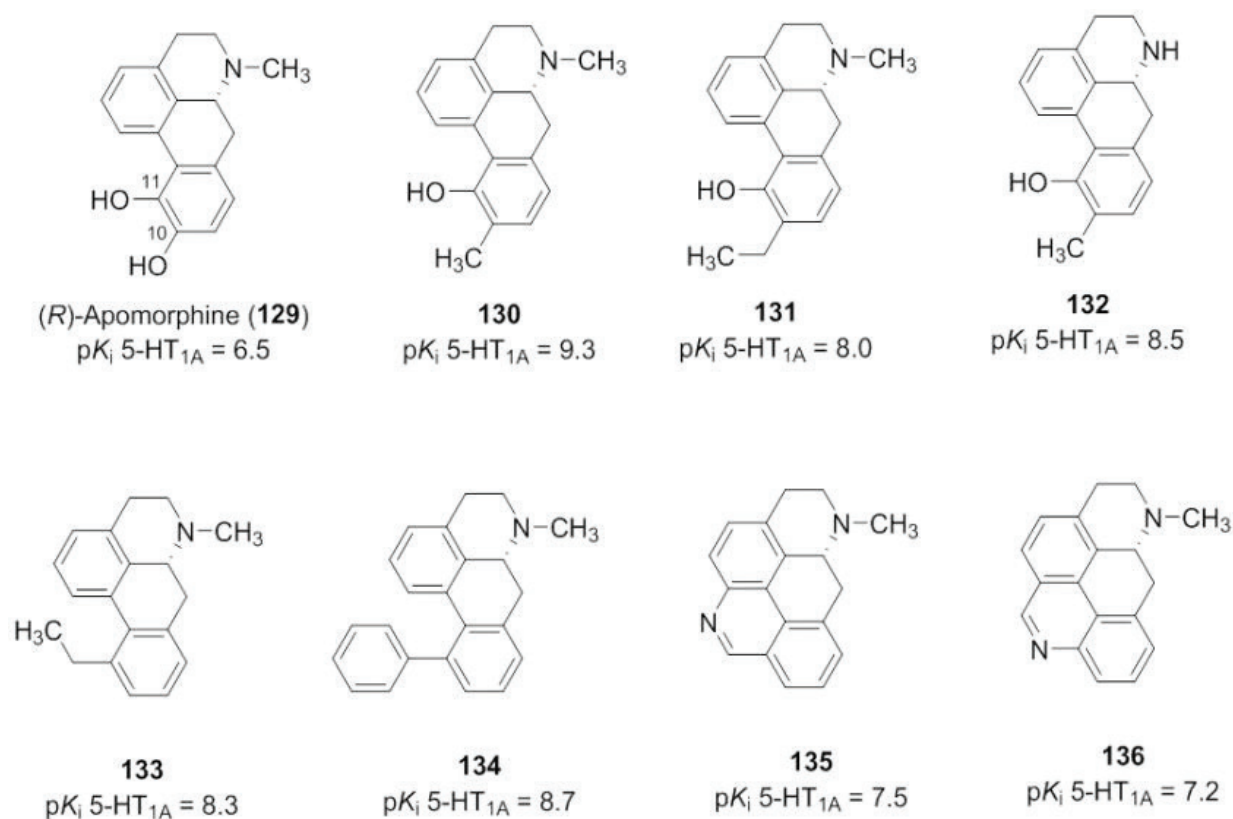

Figure 40. Chemical structures of **129–136**.

a biatomic bridge highlighted that a polar function (-O- or –NH- group) and a methyl group in the bridge as well as the suitable chirality and a proper steric hindrance in the aromatic area favor 5-HT$_{1A}$R recognition and activation. In particular, (S)-naphthaline (**139**) shows the highest 5-HT$_{1A}$R affinity within the series (**Figure 41**). In mice it displays antidepressant-like effect at a very low dose (0.01 mg/Kg) and proves to be more efficacious and potent than amitriptyline (15 mg/kg), a tricyclic antidepressant commonly used in human therapy [52].

5.8. 1,4-Dioxanes

The design and synthesis of 5-HT$_{1A}$R ligands bearing the 1,4-dioxane nucleus were inspired by the observation that the potent α_1-AR antagonist WB4101 (**140**) also shows high 5-HT$_{1A}$R affinity [53]. In the effort to discriminate between 5-HT$_{1A}$R and α_1-ARs, the quite planar 1,4-benzodioxane structure of **140** was replaced by the less conformationally constrained 6-aryl-1,4-dioxane ring, maintaining the 2,6-dimethoxy substitution or removing one or both methoxy groups of the phenoxy terminal. The most interesting results are shown by the 6,6-diphenyl substituted compounds **141–143**, which display nanomolar 5-HT$_{1A}$R affinities (**Figure 42**).

In particular, **143** behaves as a potent full 5-HT$_{1A}$R agonist with a pD$_2$ value significantly higher than those of the reference compounds 5-HT and 8-OH-DPAT. This derivative also shows a good selectivity for 5-HT$_{1A}$Rs over α_{1A}–, α_{1B}–, and α_{1D}–AR subtypes [54]. The stereogenic center in position 2 of the 1,4-dioxane nucleus appears to play a critical role in the
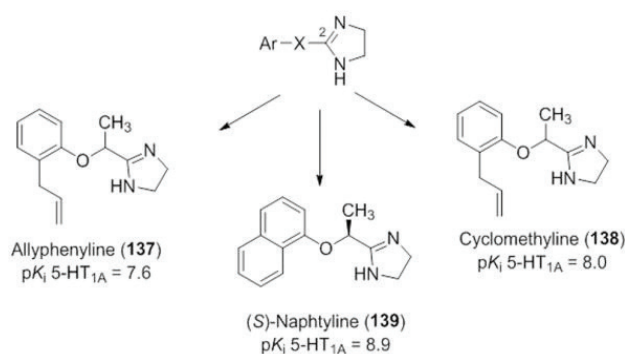

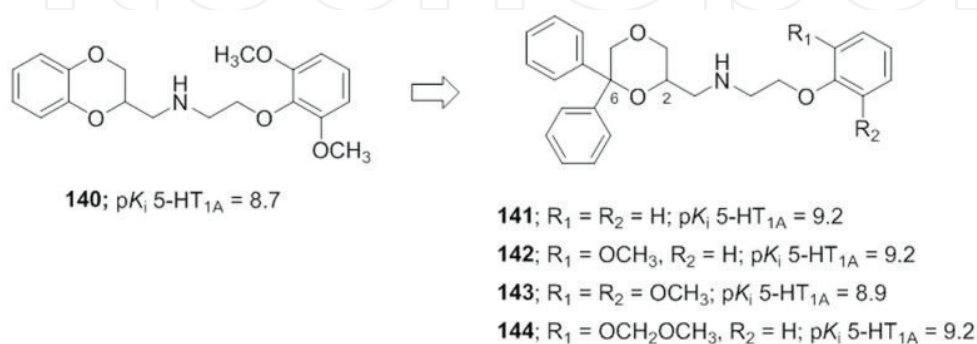**Figure 41.** Chemical structures of **137–139**.

Figure 42. Chemical structures of **140–144**.

interaction with α_1-AR and 5-HT$_{1A}$ R systems, a reversal enantioselectivity governing the 5-HT$_{1A}$R or α_1-AR recognition. Indeed, concerning 5-HT$_{1A}$Rs, the optimal affinity resides in the 2-(*S*) configuration, which, on the contrary, is less favorable for the interaction with α_1-AR subtypes. This result is particularly interesting because, as the eutomers for 5-HT$_{1A}$Rs behave as distomers for α_1-AR, the 5-HT$_{1A}$R/α_1-AR selectivity ratio significantly increases compared to the corresponding racemate [55].

A good selectivity for 5-HT$_{1A}$Rs over α_1-ARs and dopamine D$_2$-like receptors is also obtained by inserting a –OCH$_2$OCH$_3$ group in 2-position of the phenoxy terminal (compound **144**; **Figure 42**). The pharmacological profile of **144** and docking studies suggest that 5-HT$_{1A}$Rs also accommodate substituents bulkier than the methoxy group. Instead, both α_1-ARs and D$_2$-like receptors have more stringent steric requirements being intolerant to the increase of steric bulk itself. Due to its 5-HT$_{1A}$R activation, **144** significantly reduces anxiety-linked behaviors in mice [56].

6. Conclusion

In summary, the currently main knowledges of the four-wheel drive (4WD: who, why, where, what, and drugs) vehicle by which to travel inside the 5-HT$_{1A}$R world, have been presented. Such a travel, begun 30 years ago with the identification of 5-HT$_{1A}$R coding gene, is far from the conclusion. Indeed, despite no X-ray structure is deposited to date, it is possible to answer quite exhaustively the question "who" this receptor is. However, the most intriguing question is "why" it continues to be a so attractive target several years after its identification. Several evidences are available about "where" 5-HT$_{1A}$R is expressed throughout the body, at both central and peripheral levels. Between presynaptic (auto- and heteroreceptors) and postsynaptic receptors, are there differences which could allow us to target them selectively? Wider and wider is the field of "what" effects this receptor can elicit under physiological and pathological conditions directly or through the modulation of several other receptor systems or the stimulation of the secretion of various hormones. Well known is its involvement in anxiety, depression, epilepsy, mood disorders, learning, and memory. Consequently, growing is its importance in the treatment of such pathologies. Moreover, the interest for 5-HT$_{1A}$R as an attractive target of drugs is increased by further physiologically governed functions, including feeding/satiety, temperature regulation, sleep, pain perception, and sexual activity. The stimulation of 5-HT$_{1A}$Rs has been demonstrated to activate several different biochemical pathways and signals through both G-protein-dependent and G-protein-independent pathways. However, it cannot be ruled out that underlying mechanisms are far from being completely understood, making more and more complex the net of pathways through which the primary impulses unwind themselves. Finally, the discovery of "drugs" able to selectively activate or inhibit 5-HT$_{1A}$R might help to better characterize such a receptor and the physiological functions in which it is involved. Despite the numerous published papers and synthesized and tested molecules, the results are not completely satisfactory yet. The reasons can be ascribed partly to the great similarity of the ligand recognition transmembrane region of 5-HT$_{1A}$Rs with other members of the family or other GPCRs, partly to bimodal effect of 5-HT$_{1A}$R activation dependent on the neuroanatomical location of the receptors and the concentration of the ligand.

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