1,4-Benzodioxane, an evergreen, versatile scaffold in medicinal chemistry: a review of its recent applications in drug design

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Abstract

1,4-Benzodioxane has long been a versatile template widely employed to design molecules endowed with diverse bioactivities. Its use spans the last decades of medicinal chemistry until today concerning many strategies of drug discovery, not excluding the most advanced ones. Here, more than fifty benzodioxane-related lead compounds, selected from recent literature, are presented showing the different approaches with which they have been developed. Agonists and antagonists at neuronal nicotinic, α_1 adrenergic and serotoninergic receptor subtypes and antitumor and antibacterial agents form the most representative classes, but a variety of other biological targets are addressed by benzodioxane-containing compounds.

Contents

1. Introduction

1,4-Benzodioxane, whose synthesis was accomplished for the first time at the end of 1800s [1] and then improved during the first half of the successive century, has gained great popularity as a versatile scaffold for the achievement of biologically active compounds in the last five decades. Its molecular architecture continues to attract attention of organic, phyto and medicinal chemists as documented by the yearly number of scientific papers, constantly considerable since 2000 to date, the period here reviewed. Attractiveness of this bicyclic scaffold, in which planar six-carbon aromatic ring is fused with non planar six-membered 1,4-dioxa-alicycle, is due to its intrinsic interactions capabilities with amino acids residues of enzymes and proteic receptors and to multiple decoration options at both rings, which greatly improve portfolio for derivatization. In particular, substitution at one or both dioxane carbons generates chiral 1,4-benzodioxanes. In the last two decades, many efforts have been devoted to efficient preparations and analytical characterization of pure stereoisomers of these

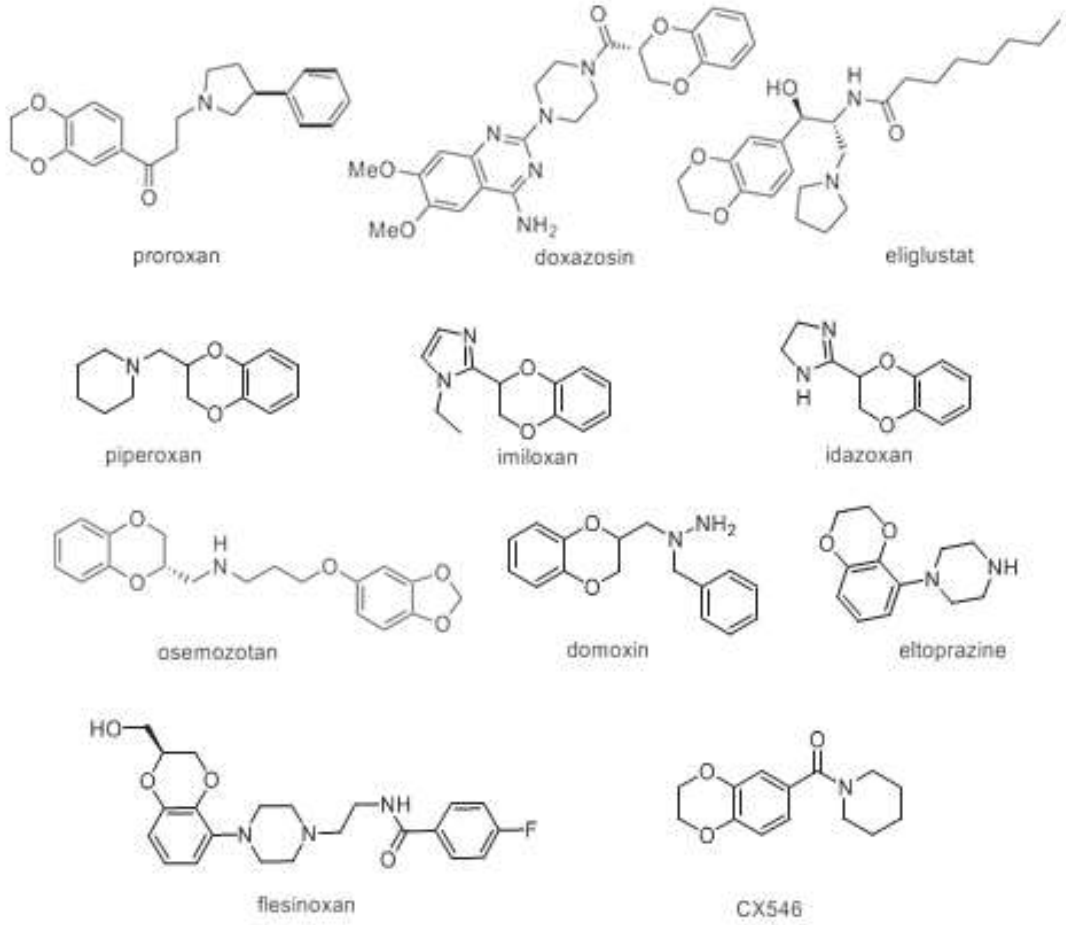
molecules by different approaches (asymmetric synthesis,[2-9] racemates resolution [10-18], 'chiral pool'[19-26] and chiral HPLC[27]), to their isolation from natural sources, structural elucidation and pharmacological characterization.

1,4-Benzodioxane scaffold is found in numerous drugs (Figure 1). Some are currently marketed, such as the glucosylceramide synthase inhibitor eliglustat (Cerdelga), used to treat Gaucher's disease, [28] and the antihypertensive α-adrenoblockers doxazosin (Cardura) [29] and proroxan (Pirroksan), [30] some have been marketed or have been discontinued in an advanced development phase for different reasons or are used as pharmacological tools and investigational drugs. This is the case for the sympatholytic prosympal, marketed until the 1950's, and for the α_2 adrenergic receptor antagonists piperoxan, [31] discontinued for incidence and severity of side effects, idazoxan, [32] antideprexant and antipsychotic investigational drug, and imiloxan, [33] whose development was terminated in clinical trials due to hypersensitivity. The 5-HT_{1A} agonist flesinoxan, [34] developed as a antihypertensive drug and repurposed as an antidepressive agent, was abandoned after clinical phase III by Solvay due to "management decisions". Osemozotan. [35] and eltoprazine, [36] other 5-HT_{1A} agonists, are, respectively, an investigational drug widely used to study the role of 5-HT_{1A} receptors in brain and a drug in advanced clinical development for the treatment of Parkinson's disease, levodopa-induces dyskinesia (PD-LID), adult attention hyperactivity disorder (ADHD) and Alzheimer's aggression. The monoamine oxidase inhibitor domoxin [37] was never marketed while the use of AMPA receptor positive allosteric modulator CX546, [38] investigated as a respiratory stimulant against barbiturates and opioids depression, was discouraged by limited oral bioavailability.

Despite wide and successful use of 1,4-benzodioxane skeleton in designing new bioactive molecules and continuous isolation of natural substances based on this heterocyclic ring system, literature concerning the use of 1,4-benzodioxane nucleus as a template for drug candidates has been seldom reviewed and never, to our knowledge, in an exclusive and comprehensive manner during the last decades. A review dating from 2008 mainly focuses on preparative methods and reports bioactive synthetic benzodioxanes developed before 2000. [39] Recently, bioactivity, biosynthesis and synthetic

procedures of natural 1,4-benzodioxanes have been exhaustively reviewed. [40] Therefore, our effort is to provide here a survey of the number of the bioactive benzodioxane-based molecules of synthetic origin developed since 2000, to show how the range of their biological targets has widened and diversified in the last twenty years and to discuss their structure activity relationships.

Figure 1. Selected examples of 1,4-benzodioxane based drugs and pharmacological tools



2. Natural chiral benzodioxanes

Natural chiral 1,4-benzodioxanes, widely distributed in the plant kingdom, but isolated also from animal sources, show a broad structural diversity and a variety of pharmacological properties. They would be biosynthesized by the oxidative dimerization of two phenylpropanoid units (benzodioxane lignans) or by the oxidative coupling of a phenylpropanoid with a flavonoid (flavonolignans) or with

a coumarin (coumarinolignans) or with a stilbenoid (stilbenolignans).[41] Figure 2 shows some of the most important terms of natural benzodioxane lignans, the first of the four classes of natural 1,4-benzodioxanes studied for their interesting biological activities in the last twenty years.

Here we find neurotrophic agents such as the methyl esters of americanoic and isoamericanoic acid A and the corresponding alcohols americanol and isoamericanol A. [42] The 3-*O*-methyl ether of isoamericanoic acid A exerts antitumor activity inhibiting the activation of the NO donor (±)-(*E*)-methyl-2-[(*E*)-hydroxyimino]-5-nitro-6-methoxy-3-hexemide, [43] while isoamericanoic acid B, isolated from the bark of *Acer tegmentosum*, behaves as a promising phytoestrogen for the development of natural estrogen supplements potentially beneficial in relieving menopausal symptoms. [44] Antiestrogenic activity resultant in lactation promotion is instead shown by princepin,[19, 45] which was characterized as one of the most potent inhibitors of estrogen action isolated from *Vitex glabrata*, a plant traditionally used in Thai medicine to support lactation. The other furofuran derivative, haedoxan A, is well known for its insecticidal properties in East Asia. [46] Among the several lignans isolated from *Phryma leptostachya* L., a plant widely distributed in temperate Asia, it has the highest insecticidal activity, whose mechanism of action has been recently investigated. [47] Nitidanin has been recognized as an antimalarial agent [48] with weak activity but also with minimal cytotoxicity unlike previously reported highly cytotoxic neolignas. [49]

Rodgersinines are an example of benzodioxane lignans with hepatoprotective anti-HCV activity. This is important because rodgersinines lack the chromanone core of hepatoprotective flavolignans, such as silybin, which has been suggested to be responsible for unspecific activity at several biological target [PAINS (pan-assay interference compounds) activity].[50] Natural *trans*-rodgersinines A and B have *R* configuration at the two stereocenters, while natural *cis*-rodgersinines A and B have *S* and *R* configuration at the phenyl substituted and the methyl substituted dioxane carbons respectively. All the natural stereoisomers show anti-HCV activity, with considerably lower toxicity than silybin. The maximum activity, which is due to reduction in expression of critical proteins in the lifecycle of HCV, is displayed by *trans*-rodgersinine B.[22]

The depside salvianolic acid P, isolated together with other fourteen secondary metabolites from *Origanum dictamnus* as the only benzodioxane based species, has shown the highest activity against Gram-negative clinical strains such as *Acinetobacter hemolyticus*, *Empedobacter brevis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*.[51]

Natural benzodioxanes have been isolated also from animal organisms. Orthidines, isolated from the New Zealand ascidian *Aplidium orthium*, inhibit the *in vitro* production of superoxide by PMA-stimulated human neutrophils and this is associated with inhibition of superoxide production by neutrophils *in vivo* in a murine model of gouty inflammation.[52] *N*-acetyldopamine dimers, isolated from *Periostracum cicadae*, exhibit a number of antioxidant and anti-inflammatory activities, more pronounced in the unsaturated dimer.[53]

Figure 3 shows three important exemplars of the other three classes of natural benzodioxanes (stilbenolignans, flavolignans and coumarinolignans). (-)-Aiphanol, a potent inhibitor of the cyclooxygenase enzymes COX-1 and COX-2 with IC_{50} values of 1.9 and 9.9 μM, respectively, possesses a stilbenolignan skeleton in which a hydroxylated stilbene unit is connected to a phenylpropane moiety via a 1,4-dioxane bridge.[54] Silybin, extracted from the seeds of blessed milkthistle, is an approximately equimolar mixture of two diastereoisomeric flavolignans differing in benzodioxane stereocenters configurations, *R* and *R* in silybin A and *S* and *S* in silybin B.[55] It is a well-documented hepatoprotective agent whose activity results from several non-mutually exclusive biological activities including antiviral (anti-HCV), antioxidant, anti-inflammatory and immunomodulatory functions.[56] Cleomiscosin-A, from *Aesculus turbinata*, is a coumarinolignans endowed with anti-cancer and anti-inflammatory activity.[57, 58]

Figure 2. Lignan natural products containing the 1,4-benzodioxane substructure

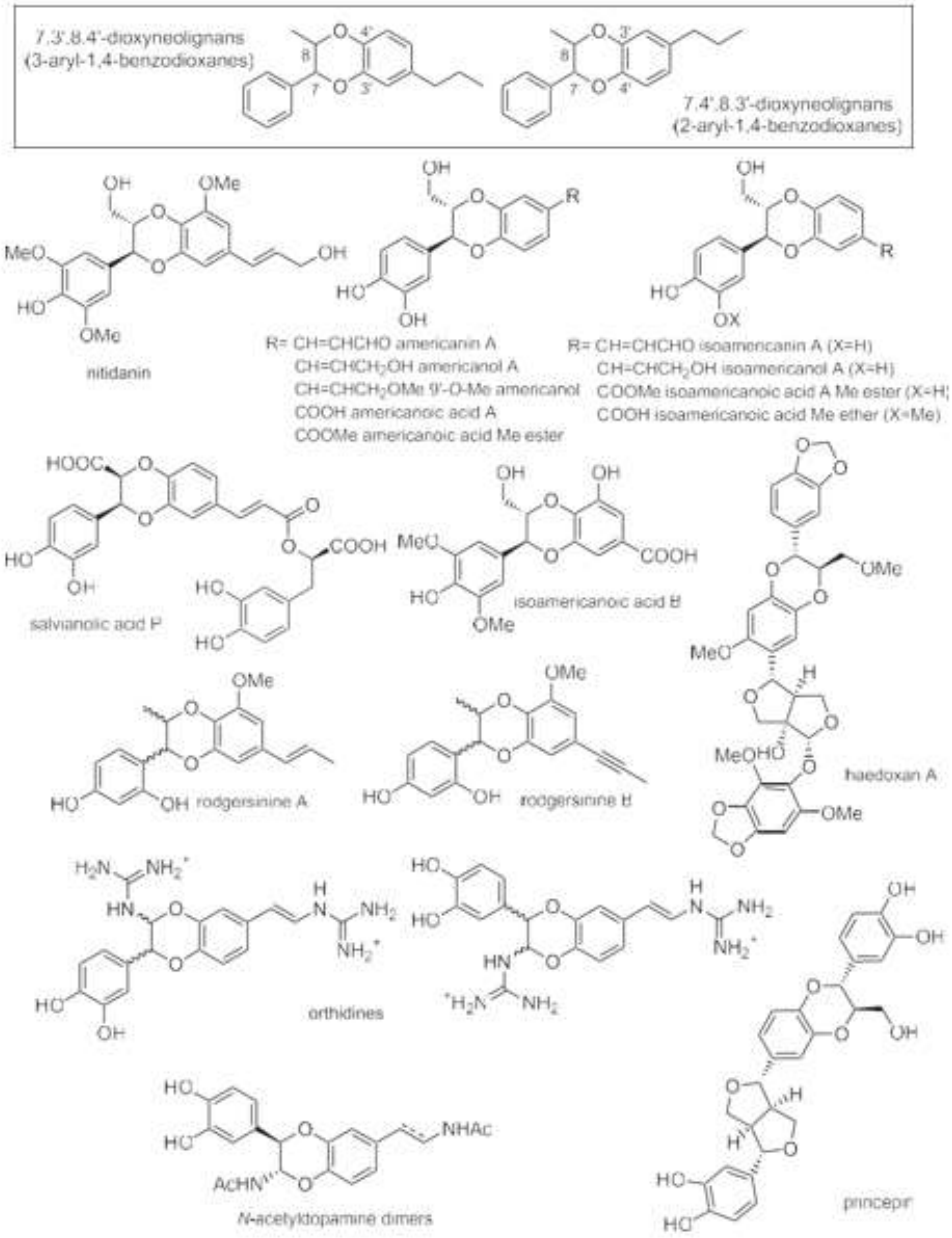
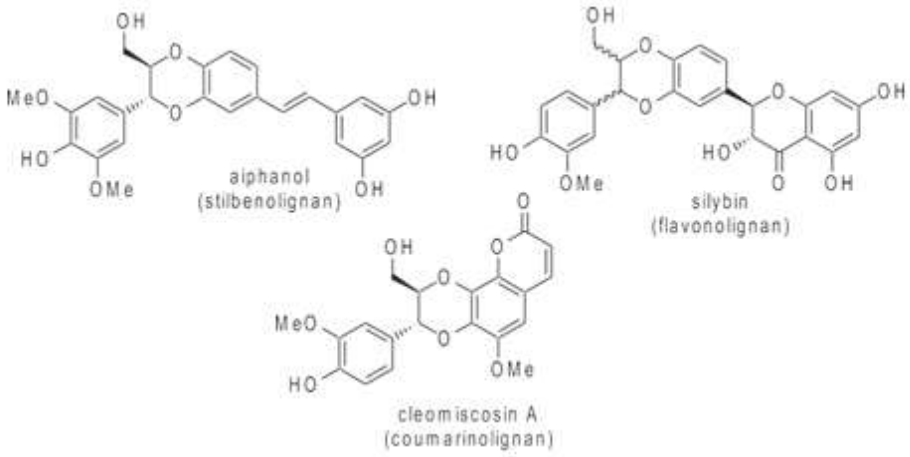


Figure 3. Natural stilbenolignans, flavolignans and coumarinolignans containing the 1,4-benzodioxane substructure.



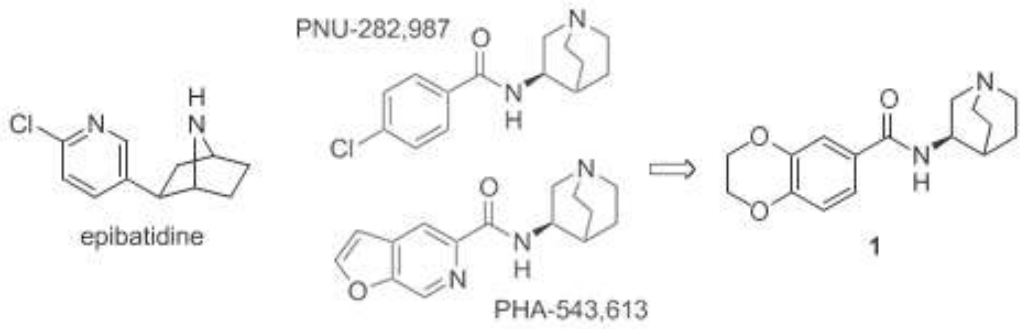
3. Pharmacological profile and SAR of benzodioxane derivatives

3.1. Benzodioxanes as neuronal nicotinic acethylcholine receptors (nAChRs) agonists and antagonists

Neuronal nicotinic acetylcholine receptors (nAChRs) are ligand-gated pentameric ion channels playing a pivotal role in neurotransmission in the central nervous system (CNS) and in the peripheral nervous system (PNS). Recently they have been also found in non-neuronal tissues.[59,60] In the CNS, the most abundant nAChR subtypes are the homopentameric α7 and the heteropentameric α4β2. The former, upon agonist binding, undergoes rapid activation followed by quick desensitization. [61] It is located on both presynaptic and postsynaptic membranes exerting different functions: control of neurotransmitter release (e.g. glutamate or noradrenaline) and cooperation to the fast synaptic transmission, respectively. α7 nAChRs have been proposed to modulate a variety of attention and cognitive processes and great attention has been paid to α7 nicotinic agonists as potential therapeutic agents for the treatment of the dysfunction of such processes.[62] Five identical orthosteric binding sites are located at each α7/α7 interface. Their features are highlighted by the X-ray crystal structure of the receptor accommodating the α7 agonist epibatidine, a 7-azabicyclo[2.2.1] ring system 6-chloro-3-pyridyl substituted at position 2.[63] In the early 2000s, many efforts have focused on another

azabicycle, the 1-azabicyclo [2.2.2]octane (quinuclidine) scaffold, bearing the aromatic system, through an amide linker, at position 3 in order to identify new potent and selective α7 agonists, exemplified by PNU-282,987 (Figure 4).[64] Optimization of this compound and of its congener PHA-543,613,[65] in particular of the acid portion by substitution of a set of 6,5- and 6,6-fused aryl rings led to the benzodioxane derivative **1** with very high α7 affinity (K_i 44 nM), optimal selectivity over the other nAChR subtypes (α3β4, α1β1γδ, α4β2) and the 5-HT_3 receptor, good rat liver microsomes (RLM) stability and oral bioavailability, significantly reduced inhibition of hERG compared to PNU-282,987, good CNS penetration and high full α7 agonist activity, similar to that of PNU-282,987 both *in vitro* (activation of rat hippocampal α7 nAChRs at 0.3-30 μM concentration) and *in vivo* (90% reversal of amphetamine-induced auditory gating deficits in rats at 1 mg/kg).[66]

Figure 4. Quinuclidine-based α7 nAChR agonists

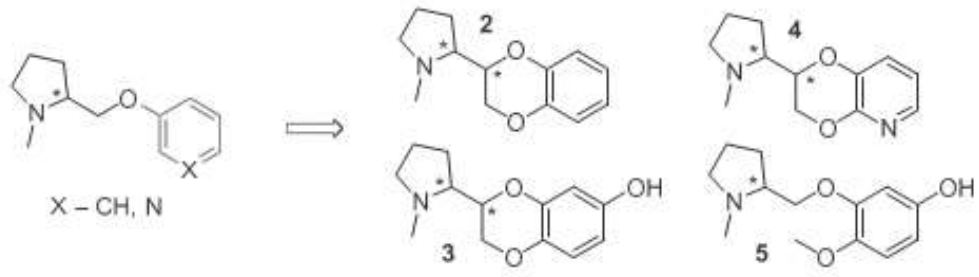


The benzodioxane scaffold has been applied also to the design of ligands of the α4β2 nAChR, the other major nicotinic receptor in CNS, assembled in two pentameric stoichiometries $(\alpha4)_3(\beta2)_2$ and $(\alpha4)_2(\beta2)_3$, both having two orthosteric agonist-binding sites at the two α4/β2 subunit interfaces.[67,68]

Interest in molecules selectively targeting this nicotinic receptor subtype has focused on full and partial agonists. Some of these have been proved beneficial in clinical and preclinical studies for CNS disorders such as Attention Deficit Hyperactivity Disorder (ADHD), Obsessive Compulsive Disorder

(OCD), Parkinson's and Alzheimer's disease, pain, autism and schizophrenia. [69,70] However, it is drug dependence, in particular the management of nicotine dependence, the therapeutic area where α4β2 nAChR partial agonists have achieved the most success, varenicline and cytisine being marketed as smoking cessation aids.[71] Linked to the azacycle, but through the dioxane portion, 1,4-benzodioxane allows to rigidify the aryloxymethyl portion typical of aryl ethers of prolinol endowed with high α4β2 nAChR affinity and potent full agonism at this receptor (Figure 5).[72,73] Such a rigidification, resulting in an additional stereocenter, has led to selective α4β2 nAChR ligands, whose affinity is highly conditioned by the stereochemistry of both the stereocenters and whose activity depends on decoration of the aromatic ring and on the structural constraint degree of the aromatic portion. Indeed, pyrrolidinyl benzodioxane **2**, with *S* and *R* configuration at the pyrrolidine and dioxane stereocenter, respectively, has moderate submicromolar α4β2 affinity and behaves as an α4β2 antagonist with 5.4 μM IC_{50}.[74-76] Decoration of **2** with OH at benzodioxane C(7) (**3**) confers α4β2/α3β4 selective partial agonism (0.3 and 17 μM EC_{50}, respectively), which becomes even more selective if associated to partial derigidification by C(2)-C(3) bond cleavage (**5**).[77-79] Analogous results are obtained when C(5) of the *S,R* diastereoisomer of **2** is replaced with N.[76] The pyridodioxane derivative **4** is a partial α4β2 agonist (10.1 μM EC_{50}) with no effect on the α3β4 subtype.

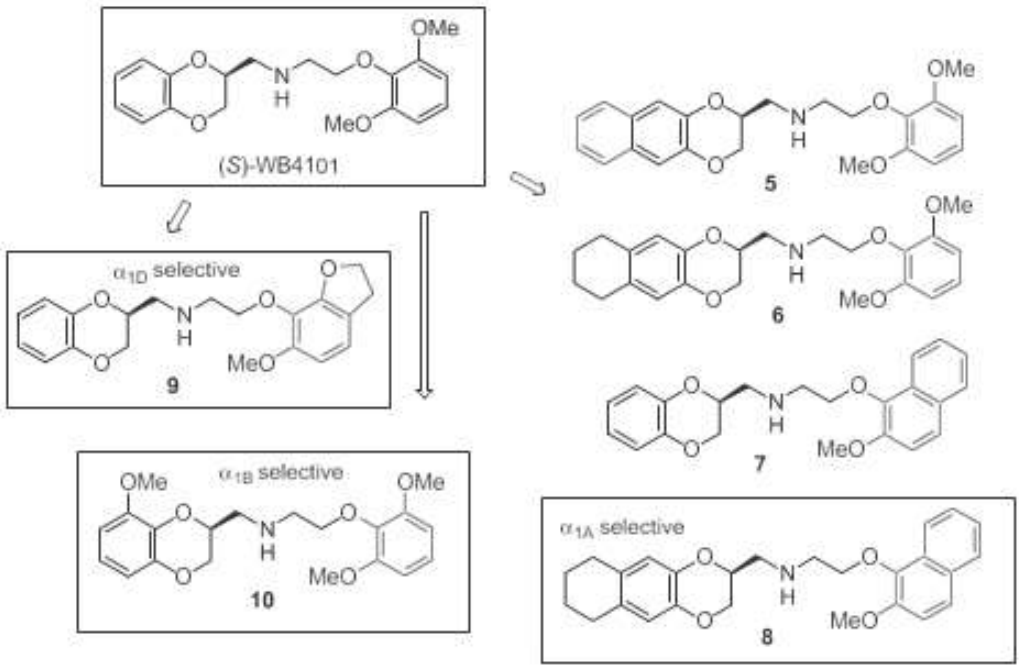
Figure 5. Design of benzodioxane based α4β2 nAChR antagonists and partial agonists



3.2. Benzodioxanes as α_1-adrenoceptors antagonists

The α_1 and α_2 adrenergic receptors (ARs) are families of G-protein coupled seven-transmembrane helix receptors involved in a number of cardiovascular and central nervous system functions. After intensive researches in the last decades of the past century, aimed at discovering α_1 versus α_2 selective adrenergic antagonists, medicinal chemists efforts have addressed, in more recent years, the development of subtype selective α_1 adrenergic antagonists with the support of molecular biology, binding and functional studies establishing the existence of three α_1-AR subtypes, α_{1A}, α_{1B} and α_{1D}.[80-82] Initially developed as antihypertensive drugs for their vascular smooth muscle relaxing properties, the alpha-1 adrenergic antagonists are, at present, recommended only as adjunctive therapy of hypertension and not as monotherapy or long term therapy, because improvement in survival has not been observed and increase in hearth failure, stroke and cardiovascular disease have been evidenced.[83] α_1-AR antagonists have successively aroused more interest as agents for the treatment of Lower Urinary Tract Symptoms (LUTS) secondary to Benign Prostatic Hypertrophy (BPH), the main indication for which they are currently prescribed. [84,85] Therefore, predominance of the α_{1A} and α_{1D} subtypes in prostatic stroma and bladder detrusor muscle and their role in mediating LUTS have made selective antagonism at these subtypes an attracting goal. It is within this context that WB-4101 (Figure 6), a benzodioxane related α_1-AR antagonist [86] endowed with high potency and very high α_1 versus α_2 selectivity and also proved effective as antihyperalgesic agent in multiple pain models,[87] has been considered as a lead compound to develop new unichiral subtype selective α_1-AR antagonists in more recent years. Indeed, WB-4101 itself and in particular its eutomer having *S* configuration displays a slight selectivity for α_{1A}- (9.39 pK_i) and, to a minor extent, for α_{1D}-ARs (9.29 pK_i) with respect α_{1B}-AR (8.24 pK_i) and 5-HT$_{1A}$ (8.61 pK_i) serotoninergic receptor.[88,89] Addition of a 6,7 fused benzene or cyclohexane to the benzodioxane scaffold of (*S*)-WB-4101 (compounds **5** and **6**) results in lower, but significantly more specific α_{1a} affinity (7.47 and 7.60 pK_i respectively), while

Figure 6. Benzodioxane based subtype-selective α_1-AR antagonists from (*S*)-WB4101



replacement of one of the two *ortho* methoxy groups with a 2,3 fused benzene maintains most of the very high α_{1a} affinity of (*S*)-WB-4101 (8.80 pK_i) but with less pronounced increase of α_{1a} selectivity (compound **7**) (Figure 6).[88] Indeed, the presence of two *ortho* substituents, at least one of which is methoxyl, at the phenoxy moiety is of great importance for the interaction with the α_1-ARs, especially with the α_{1a} subtype, while it is not essential to the interaction with the 5-HT_{1A} receptor.[90,91] Consistently with affinity data, functional studies of the antagonist activity at the α_{1A}-, α_{1B}-and α_{1D}-ARs show that the two combined modifications, fusion of cyclohexane at the benzodioxane and of benzene at the 2,6 dimethoxyphenyl, lead to a potent and selective α_{1A}-AR antagonist (compound **8**) (7.98 α_{1A} pA_2 vs <5 α_{1B} pA_2 and 5.59 α_{1D} pA_2) (Figure 6).[89] On the other hand, selective α_{1D}-AR antagonist activity is attained by maintaining the characteristic ortho hetero-disubstituted phenyl group of (*S*)-WB-4101, but rigidified in the 6-methoxy-2,3-dihydrobenzofuran substructure (9.58 α_{1D} pA_2 vs 8.49 α_{1B} pA_2 and 7.88 α_{1A} pA_2),[92,93] while 8-substitution at the benzodioxane, in particular with methoxyl, confers significantly selective α_{1B}-AR antagonism (9.58 α_{1B} pA_2 vs 8.55 α_{1A} pA_2 and 7.93 α_{1D} pA_2) (compounds **9** and **10**) (Figure 6).[94] (*S*)-WB4101, **7** and **9** show also intrinsic relaxant activity

on non-vascular smooth muscle and moderate negative inotropic effect, which parallel the calcium antagonist effects suggesting a direct interaction with L-type Ca^{2+} channels.[95]

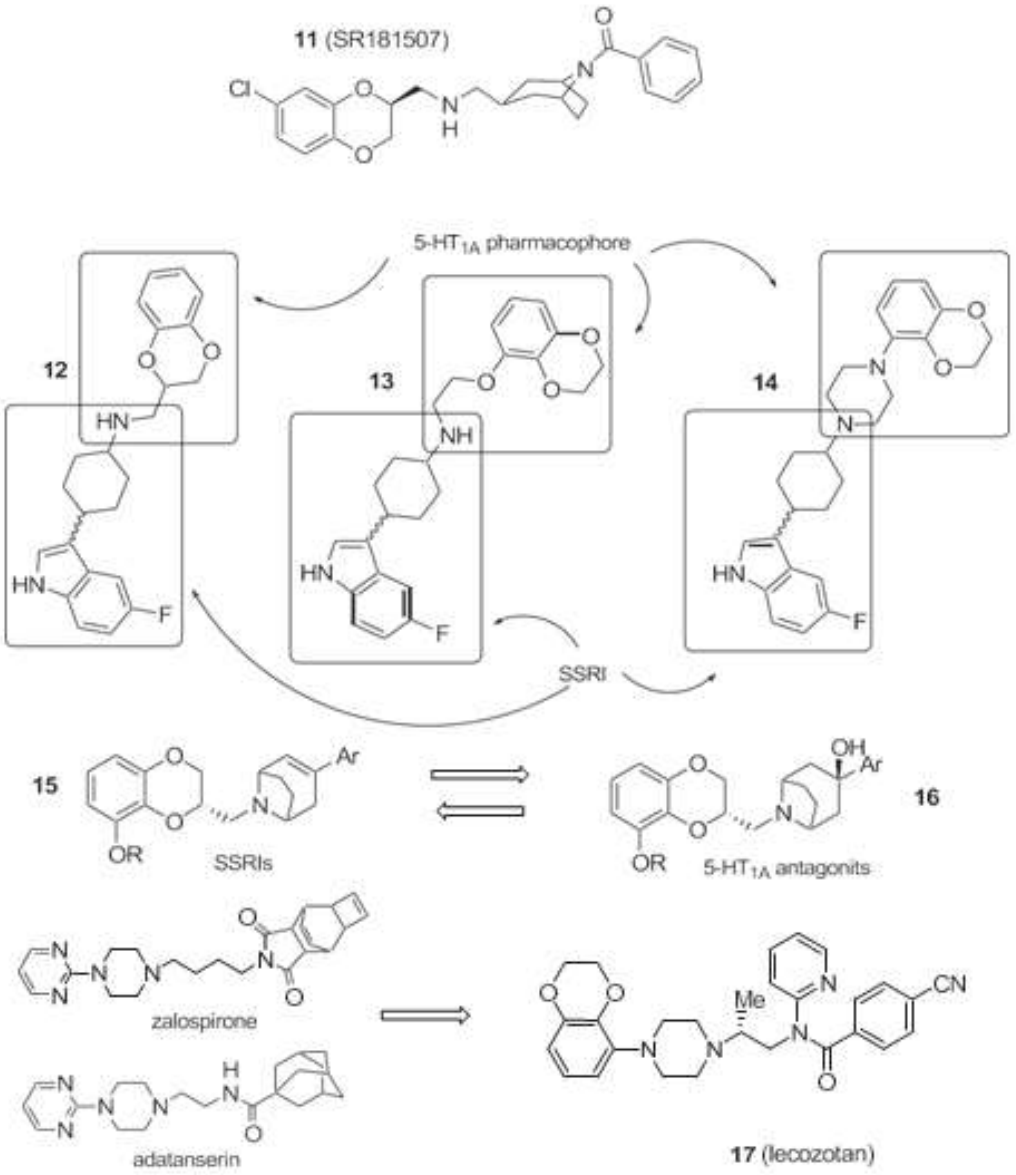
3.3. Benzodioxanes as 5-HT$_{1A}$ agonists and antagonists

The 5-HT$_{1A}$ receptor, the most thoroughly studied of all the serotonin (5-HT) receptors, is a 7-transmembrane G-protein-coupled receptor, found both presynaptically and postsynaptically in the central nervous system.[96] The presynaptic 5-HT$_{1A}$ receptors act as autoreceptors and their activation decreases 5-HT release, while the postsynaptic 5-HT$_{1A}$ receptors mediate the activity of 5-HT. Treatment of anxiety and, secondarily, of depression is the main indication of 5-HT$_{1A}$ partial and full agonists. As mentioned in the Introduction, 5-HT$_{1A}$ agonists have been actively investigated and developed in the eighties and in the nineties. Buspirone, currently marketed as an anxiolytic drug, exemplifies the success of such an approach, which continued after 2000 focusing on new therapeutic potentials. In 2003, the benzodioxane derivative SSR181507 (compound **11**) was developed as a 5-HT$_{1A}$ agonist and D_2 receptor antagonist and proposed, for its double-face profile, for the treatment of schizophrenia with the additional benefit of anxiolytic/antidepressant activities (Figure 7).[97,98]

In subsequent years, interest was addressed to 5-HT$_{1A}$ antagonism with the aim of developing a new generation of antidepressants by combining 5-HT$_{1A}$ antagonism with serotonin transporter (5-HT-T) inhibition in the same molecule. The rationale was that blocking 5-HT$_{1A}$ autoreceptors avoids their stimulation by the increase in 5-HT induced by 5-HT-T inhibition. In such a way, increase in synaptic 5-HT levels would not be counteracted by 5-HT$_{1A}$ stimulation exerted by 5-HT and consequent therapeutic antidepressant effects, even if not risk-free,[99] would be observed. Preclinical studies indicate that antidepressant activity of selective serotonin reuptake inhibitors (SSRIs) is enhanced by their association with a 5-HT$_{1A}$ antagonist. According to the 'overlapping type approach', the SSRI 4-(5-fluoro-1*H*-indol-3-yl)cyclohexylamine was combined with 5-HT$_{1A}$ pharmacophores, such as 2-aminomethyl-1,4-benzodioxane, by overlapping at the basic amine

(compounds **12**-**14**) with the aim of obtaining a SSRI endowed with 5-HT$_{1A}$ antagonist activity (Figure 7). [100] Among the three benzodioxane derivatives, which were each tested as *cis* and *trans* racemate, *cis*-**14** showed the most interesting profile: high affinities for both 5-HT transporter (K_i 5.3 nM) and 5-HT$_{1A}$ receptor (K_i 66 nM) and no 5-HT$_{1A}$ agonism.

Figure 7. Benzodioxane based 5-HT$_{1A}$ agonists and antagonists



Alternatively, potent 5-HT-T inhibitors, containing the 1,4-benzodioxane scaffold linked to the aryl 8-aza-bicyclo[3.2.1]oct-3-ene, are converted into potent 5-HT$_{1A}$ antagonists changing the aryl 8-aza-bicyclo[3.2.1]oct-3-ene moiety to 8-aza-bicyclo[3.2.1]octan-3-ol (general structural formulas **15** and

16, Figure 7). [101] Obtained according to this approach, the compound with structure **16** (R = Me, Ar = 3-benzothiophene) shows very high 5-HT_{1A} affinity (K_i 1.6 nM), with > 50 fold selectivity over α_1-AR, potent 5-HT_{1A} antagonism (IC_{50} 10-20 nM) and no 5-HT-T affinity.

Selective 5-HT_{1A} antagonists have been pursued also for their potential in the treatment of cognitive dysfunction such as that associated with Alzheimer's disease. A number of benzodioxan-5-ylpiperazine derivatives possessing a 4-substituted aryl amide moiety were designed building upon the structural features of the 5-HT_{1A} partial agonists zalospirone and adatanserin. [102] Among these, Lecozotan (compound **17**) is a potent (IC_{50} 4-23 nM), selective (over >55 receptors, transporters and ion channels), orally active 5-HT_{1A} antagonist (Figure 7).

2.4. Benzodioxanes as antibacterial agents

Development of new antibacterial agents has become one of the major challenges faced by medicinal chemistry. The increasing number of bacterial infections resistant to current antibiotic therapies is no longer just a serious menace to the health of human, it is an emergency requiring that novel mechanisms of antibacterial action are identified and tested for practicability. Interestingly, it is in this field that our versatile medicinal chemistry scaffold finds some of its most recent applications. A number of benzodioxane related compounds have been recently developed as agents active against drug resistant bacteria on the basis of new mechanisms, leaving aside all that literature where benzodioxane derivatives are described as having high antibacterial activity but without any information on toxicity and action mechanism. Novel targets addressed in this postantibiotic era are MmpL3 protein, essential for *Mycobacterium tuberculosis* viability, the golden pigment staphyloxanthin (STX) and biofilm-associated protein, determinant for *Staphylococcus aureus* virulence, fatty acid biosynthesis, essential for bacterial survival, and protein FtsZ, essential for bacterial cell division.

A benzodioxane based spirocyclic compound has been identified as a promising antitubercular hit, as it produces accumulation of intracellular trehalose monomycolate (TMM) by interfering with the ability of MmpL3 protein to act as a TMM transporter (compound **18**, Figure 8).[103] Optimization of this lead, which displays 0.30 μM MIC_{90} against *Mtb* H37Rv and 36 μM mammalian cell (HepG2) Tox_{50}, through fifty analogues has provided some compounds displaying striking potency, low toxicity and highly promising *in vivo* activity. Analogous approach has been applied to Naftifine hydrochloride (Figure 8), capable of blocking the STX biosynthesis pathway in *S. aureus* at nanomolar concentrations by targeting diapophytoene desaturase (CrtN).[104] Thirty-eight 1,4-benzodioxan-derived CrtN inhibitors were designed and synthesized and one of them (compound **19**, Figure 8) exhibited highly potent inhibitory activity against *S. aureus* Newman (2.2 nM IC_{50}), hundred-nanomolar CrtN enzymatic inhibitory activity (270 nM IC_{50}), single-nanomolar inhibitory activity against four methicillin-resistant *S. aureus* (MRSA) strains and low hERG inhibition (12.1 μM IC_{50}). Compound **19** was selected as a good candidate for combating vancomycin-intermediate and linezolid resistant *S. aureus* infections.

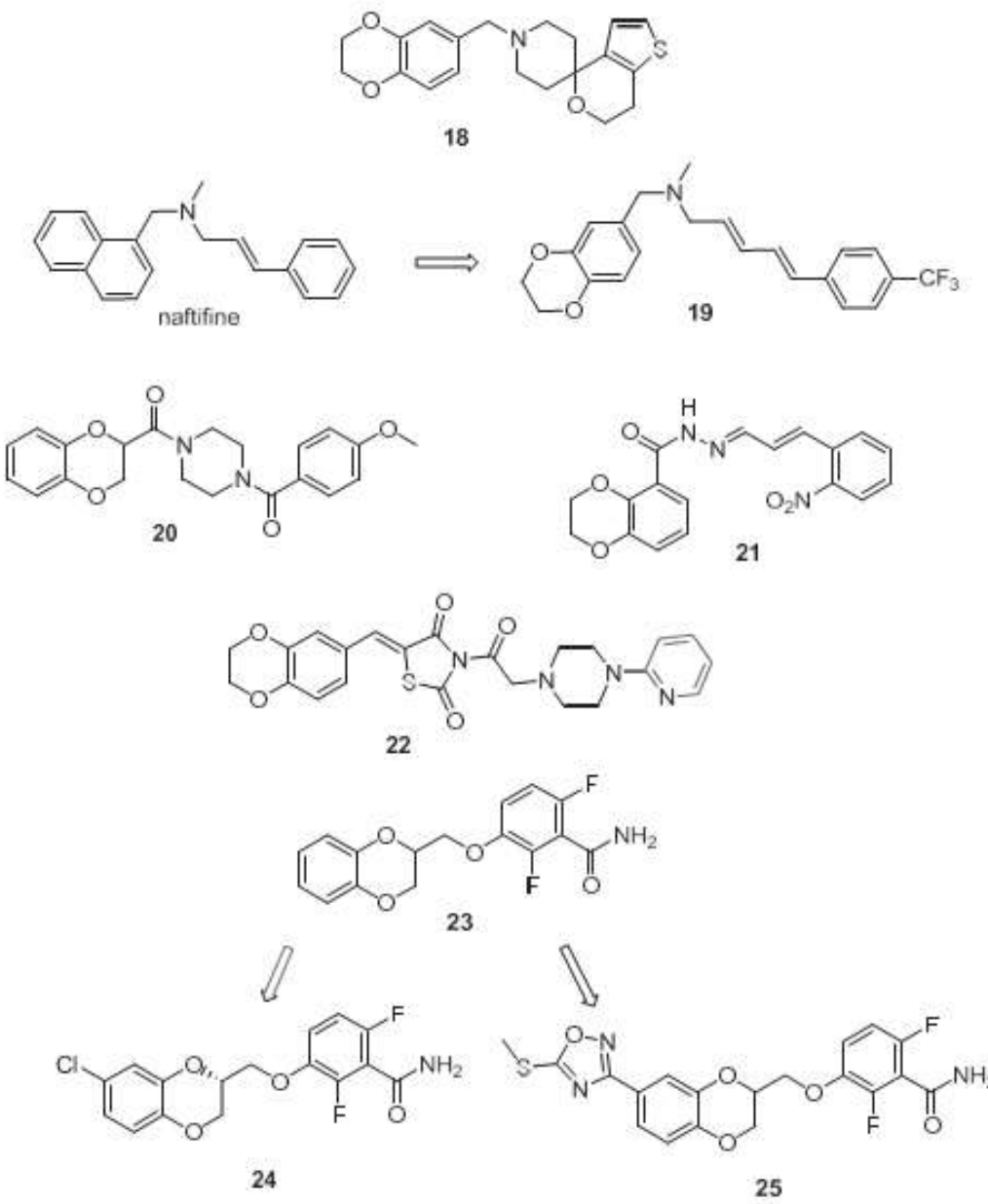
A benzodioxane-carbonyl-piperazine derivative (compound **20**, Figure 8) loaded into chitosan silver nanoparticles shows biocidal properties in controlling the MRSA biofilm formation, a process of bacterial defense against various environmental stresses facilitating surfaces colonization by *S. aureus*.[105,106]

Fatty acid biosynthesis (FAB) is essential for bacterial survival and differs from human FAB. The β-ketoacyl-acyl carrier protein (ACP) synthase III (FabH) is an essential enzyme in bacterial FAB and it has no homologs in human. Twenty-one cinnamaldehyde acylhydrazones, in which the acyl portion derives from 1,4-benzodioxane-2-carboxylic acid or from its 5- and 6-carboxylic regioisomers, were tested for activity against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*.[107] Within the series, the *o*-nitrocinnamaldehyde hydrazone derivative of 1,4-benzodioxane-5-carboxylic acid (compound **21**, Figure 8) showed the most potent antibacterial activity, with minimum inhibitory concentrations against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*S.*

aureus and *B. subtilis*) ranging between 1.5 and 6 μg/mL, and with the highest inhibitory activity of *E. coli* FabH (3.5 μM IC_{50}). More recently, a series of 1,4-benzodioxane thiazolidinedione piperazine derivatives have been developed as FabH inihibitors.[108] Their antibacterial activities strongly correlate with the inhibitory activity of the enzyme. The compound in which piperazine is *N*-substituted with 2-pyridyl is the most active one (compound **22**, Figure 8) showing minimum inhibitory concentrations against the same bacterial species tested with **21** ranging between 1.5 and 7 μM and sub-hundred nanomolar inhibitory activity of *E. coli* FabH (60 nM IC_{50}).

1,4-Benzodioxane scaffold was successfully used also to design inhibitors of FtsZ, the bacterial cell protein essential for cellular separation and division, recognized as an appealing target for antibacterial agents discovery. In 2007, English researchers described 2,6-difluoro-3-alkyloxynemzamides as FtsZ inhibitors, active against *S. aureus* and *B. subtilis* and, among these, the 3-(benzodioxan-2-yl)methyloxy derivative (compound **23**, Figure 8).[109] Some years after, this compound has been subjected to SAR optimization studies, identifying in the *S* enantiomer of the derivative 7-chloro substituted at the benzodioxane (compound **24**, Figure 8) a potent FtsZ inhibitor active against MRSA (0.39 μg/mL MIC), vancomycin resistant *E. faecalis* (VRE) (25 μg/mL MIC) and *Mtb* (8 μg/mL MIC) .[110,111] Successive studies have allowed FtsZ to be validated as the real target of **24** through specific biochemical assays.[112] The more recent identification of other 7-substituted benzodioxane based potent FtsZ inhibitors, such as compound **25** (Figure 8), consolidates benzodioxane-benzamides as one of the main class of these antibacterials.[113]

Figure 8. Benzodioxane related antibacterial agents



2.5. Benzodioxanes as cancer chemotherapics

The main objective of cancer chemotherapy research is the development of classes of therapeutics selectively acting on targets, which allow differences between tumor and normal cells to be fully exploited so as to reduce toxicity. This is well illustrated by the use of benzodioxane by medicinal chemists in designing new anticancer agents. We find the benzodioxane scaffold in a series of molecules proposed for different targets on the basis of new therapeutic approaches and strategies.

For instance, a number of benzodioxanes 6-substituted with different penta-atomic heterocycles have been reported as inhibitors of enzymes that are potential therapeutic targets in cancer because playing critical role in cell proliferation, survival and motility or because necessarily more active in tumor than in normal cells.

The arylpyrazolyl benzodioxane CCT018159 (compound **26**) (Figure 9), selected from the enzymatic screening of 50,000 compounds, and a number of close analogues have been proved to inhibit heat-shock protein 90 (Hsp90), a molecular chaperone responsible for folding, stability and function of 'client proteins' and critical to maintain the health of cells.[114] Its inhibition results in cell apoptosis and this effect, for reasons explained by different hypotheses, is more pronounced on tumor than on normal cells. Results of assays for activity of CCT018150 and its congeners against the yeast Hsp90 ATPase well correlate with their potency in inhibiting HCT116 human colon cancer cells. CCT018150 exerts the highest growth inhibition (4.1 μM GI_{50}) with a micromolar inhibitory potency against yeast Hsp90 (7.1 μM IC_{50}).

1,3,4-Thiadiazole derivatives containing 1,4-benzodioxane have been studied for their ability to inhibit Focal Adhesion Kinase (FAK), an enzyme involved in many cellular functions such as proliferation and motility and thus proposed to be a new potential therapeutic target in cancer.[115,116] Among nineteen derivatives, the compound **27** (Figure 9) showed the best antiproliferative activity against HEPG2 cancer cell line (10.28 μg/mL EC_{50} for HEPG2, 10.79 μM EC_{50} for FAK). The top 10 compounds inhibited FAK with a potency well correlated with their antitumor activities. A number of 2-styryl-5-nitroimidazoles, 1,4-benzodioxane-5-carbonyloxyethyl substituted at N(1), have been submitted to analogous investigations finding that the 2-fluoro-6-chlorostyryl derivative (compound **28**, Figure 9) is the most potent inhibitor of the growth of A549 and Hela cancer cells (3.11 and 2.54 μM IC_{50} respectively) and of FAK activity (0.45 μM IC_{50}).[117] The analogues 1,4-benzodioxane-6-carbonyloxyethyl substituted have been instead reported as Janus Kinase 3 (JAK-3) inhibitors with potent activity against A549, Hela, HepG-2 and U251 cancer cells *in vitro*. The 2,4-dichlorostyryl derivative (compound **29**, Figure 9) shows the highest antiproliferative ability against the above four

cultured cell lines (65 nM, 21 nM, 16 nM and 44 nM IC_{50} resectively) and JAK-3 inhibition (9 nM IC_{50}).[118] More recently, a series of 1,3,4-oxadiazole-2(3*H*)-thiones linked, through C(5), to 1,4-benzodioxane C(6) were assayed for the FAK inhibition and the anticancer activity against four cancer cells, HepG2, Hela, SW116 and BGC823, again finding the trends of the two activities consistent and identifying the compound **30** (Figure 9) as the most potent derivative (5.78 μM IC_{50} for HepG2 and 47.15 μM IC_{50} for SW116; 0. 78 μM IC_{50} anti-FAK activity).[119]

Among the kinases emerging in the last years as targets for cancer therapy, phosphatidylinositol 3-kinase (PI3K) has a prominent place.[116] PI3K is a family of intracellular enzymes converting PIP2 into PIP3, which is involved in AKT activation. Many PI3K inhibitors are in advanced clinical trials for the treatment of diverse types of cancer. [116] Starting from LY294002, an inhibitor of all PI3K isoforms (α, β, γ, δ), a benzodioxane based selective inhibitor of the PI3K-α isozyme (34 nM IC_{50} versus 158-960 nM IC_{50} for β, γ, δ isoforms) has been developed by isosteric replacement of benzene with thiophene in the chromenone core and of the phenyl substituent with a benzodioxan-6-yl residue (compound **31**, Figure 9).[120] Efficacy of this derivative was proved in mouse xenograft tumor models.

Recent studies have enforced evidence for the role of p38α mitogen-activated protein kinase (p38α MAPK) in cancer.[121] Using a funnel approach consisting of computer-aided drug discovery methods and biological experiments, four novel chemotypes of p38α MAPK inhibitors have been identified. Among these, an anilide of benzodioxane-6-carboxylic acid turned out to be a potent and efficient p38α MAPK inhibitor (compound **32**, Figure 9) (0.07 μM IC_{50}) with pronounced selectivity against a panel of other 15 human kinases.[122]

Another anticancer target addressed by benzodioxanes 6-substituted with a penta-atomic heterocycle is methionine aminopeptidase 2 (MetAP2), an enzyme involved in protein maturation, because removing the initiator methionine residue from nascent polypeptide chains, and believed to play a critical role in cell proliferation. The compounds **33** and **34** (Figure 9) are the most potent

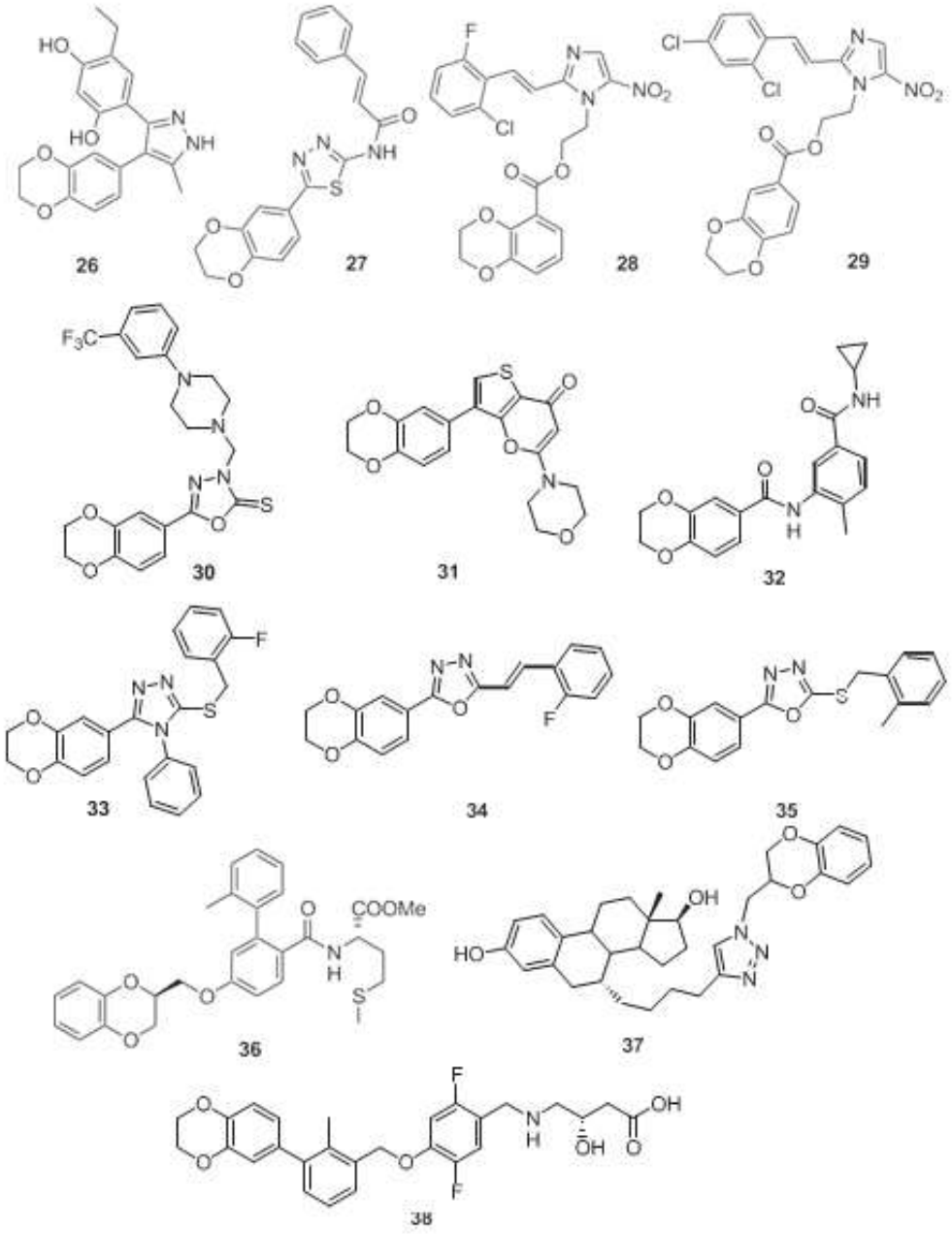
MetAP2 inhibitors (0.93 μM and 1.16 μM IC_{50}) discovered in two different researches and those showing the highest antiproliferative activity against HEPG2 cancer cells (0.81 μM IC_{50}) and Human Umbilical Vein Endothelial cells (2.08 μM IC_{50}).[123,124]

1,3,4-Oxadiazolyl benzodioxanes have been developed also as inhibitors of telomerase, an enzyme tirelessly working in maintaining telomere length and chromosomal integrity in rapidly dividing cells such as those of tumor.[125] The compound **35** (Figure 9), one of the most potent term in the series for antitumor activity against four cancer cell lines (HEPG2, HELA, SW1116 and BGC823), was also the most potent one in inhibiting telomerase activity (1.27 μM IC_{50}).

Prenylation of proteins, in particular farnesylation of Ras proteins, is essential for the role of these proteins in mitogenesis. Ras proteins are abnormally active in cancer cell and inhibition of their farnesylation by farnesyltransferase (FTase) is considered a suitable strategy for cancer therapy. Mimetics of the C-terminal tetrapeptide of Ras protein, in which benzodioxane replaces cysteine in fourth last position, were found to exert both antiproliferative effect and RAS prenylation inhibition.[126,127] In particular, compound **36** (Figure 9), tested in a cellular assay measuring inhibition of human aortic smooth muscle cells proliferation, showed 6.6 μM IC_{50} and directly interfered with Ras prenylation (2.8 μM IC_{50}).

Estrogen receptor (ER) antagonists are used in hormonal therapy for ERα-positive human breast cancer. Most of the pure ER antagonists contain structure of 17β-estradiol substituted at the 7α position with a long side chain. A study of nine bulky ring-based structures attached to this position of 17β-estradiol has demonstrated that such a modification can produce ER antagonists with ER affinity comparable to that of fulvestrant achieving the best results, in terms of inhibition of human breast cancer cell lines proliferation, with a triazolylbutyl chain bearing a terminal benzodioxan-2-ylmethyl moiety (compound **37**, Figure 9).[128] Compound **37** showed human ERα and ERβ affinities of 10 nM order with pure ER antagonistic activity.

Figure 9. Benzodioxane-related enzymatic inhibitors with anticancer activity

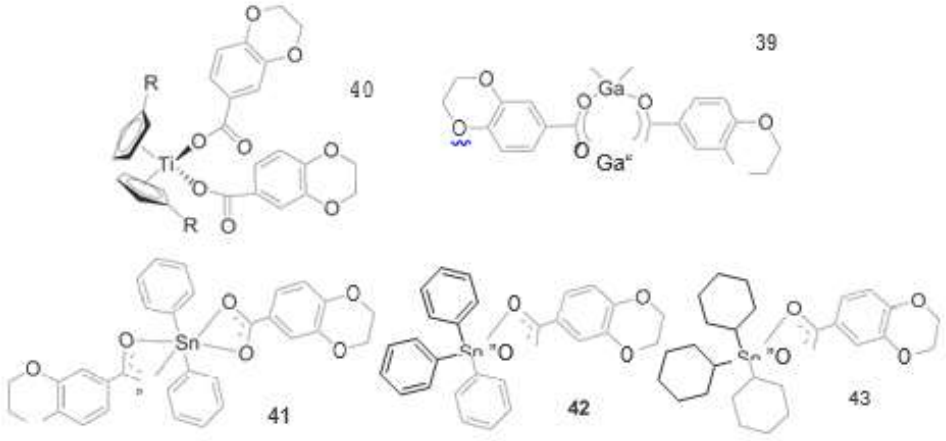


Recently, benzodioxane scaffold has been used also in the development of small molecules inhibiting programmed death protein 1 (PD-1)/programmed cell death ligand 1 (PD-L1), the immune checkpoint proteins blocked with monoclonal antibodies such as nivolumab and atezolizumab. The drawbacks of the antibody-based immunothepay of cancer are well known and traditional small molecules interfering with the PD-1/PD-L1 pathway have recently aroused interest. The compound **38** (Figure 9), resulting from studies based on biochemical data and on NMR and X-ray

characterization, binds to the dimeric PD-L1 inducing conformational changes. Investigation of these changes can create new possibilities for design of novel small molecules as PD-L1 inhibitors.[129]

Benzodioxane derivatives were also used as chelating donor ligands in different transition metal complexes with cytotoxic activity, when research efforts initially focused on the evaluation of platinum-based antitumor drugs were shifted to non-platinum metal-based agents with the aim of minimizing side effects. Gallium(III) salts present antitumor effects, probably affecting cellular acquisition of iron by a competitive binding to transferrin, but they have tendency toward hydrolysis and formation of non-soluble gallium oxides. Chelation with benzodioxane-6-carboxylic acid stabilizes gallium against hydrolysis and the resulting dimeric complex (compound **39**, Figure 10) is one of the best chelates among a number of other gallium(III) carboxylate complexes for activity (6.6 μM-22.8 IC_{50} values) against a series of human tumor cell lines such as 8505C analastic thyroid cancer, A253 head and neck tumor, A549 lung carcinoma, A2780 ovarian cancer and DLD-1 colon carcinoma.[130] In order to increase water solubility, different carboxylate ligands, among which benzodioxane-6-carboxylate (compound **40**, Figure 10), have been used to synthesize titanocene(IV) compounds with notable cytotoxic properties (44.5 μM IC_{50} against A2780 ovarian cancer cell line).[131] Diphenyltin(IV) complex containing two benzodioxane-6-carboxylate ligands (compound **41**, Figure 10) and triphenyl- and tricyclohexyltin(IV) complexes containing one benzodioxane-6-carboxylate ligand (compounds **42** and **43**, Figure 10) have been synthesized and sub-micromolar activities, higher than those of cis-platin, against a number of human tumor line cells have been found in *in vitro* tests.[132,133]

Figure 10. Cytotoxic complexes of benzodioxane carboxylate with transition metals



2.6. Benzodioxanes on other targets

As mentioned in the introduction, the hepatoprotective properties of the flavonolignan silybin, the major component of silymarin, are well known. They have inspired studies on synthetic benzodioxane derivatives endowed with antihepatotoxic activity. Benzodioxanes 2-substituted with a 2-phenyl-1,3,4-oxadiazol-5-yl residue or 6-substituted with a 3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl residue (compounds **44** and **45**, Figure 11) show hepatoprotective activity against CCl_4-induced hepatotoxicity in rats. The activity is comparable with that of silymarin when phenyl is 4-chloro or 4-methoxy substituted, in the case of the benzodioxane-oxadiazole, and 4-chloro or 2,4-dihydroxy substituted, in the case of the benzodioxane-dihydropyrazoles.[134,135] Compounds **44** and **45**, administered to CCl_4-treated rats at the same dose (10 mg/kg) as silymarin, similarly prevent the CCl_4-induced elevation of the activities of the liver enzymes serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate oxaloacetate transaminase (SGPT), alkaline phosphatase (ALP) and the decrease of serum total protein.

The anti-inflammatory activity of aryl acetic and aryl propionic acids has prompted the use of benzodioxane scaffold to design benzodioxane-based carboxylic acids as COX inhibitors. (*S*)-2-(benzodioxan-6-yl)propionic acid and 2-[*N*-(benzodioxan-6-yl)pyrrol-2-yl]acetic (compounds **46** and **47**, Figure 11) acid are inhibitors of both COX-1 and COX-2 showing higher anti-inflammatory activity *in vivo* than ibuprofen (carrageenan-induced oedema test).[136] In particular, **47** inhibited

mammalian 3α-hydroxysteroid dehydrogenase with 5.8 μM IC_{50}, COX-1 with 1.9 μM IC_{50} and COX-2 with 2.2 μM IC_{50}, while the respective values were 100 μM, 6.9 μM and 6.2 μM for ibuprofen. More recently, selective COX-2 inhibition has been demonstrated for phenylpiperazine derivatives of 1,4-benzodioxane, in particular for the derivative substituted at the piperazine distal nitrogen with 3-trifluoromethylphenyl (compound **48**, Figure 11).[137] This derivative inhibited COX-2 with a 70-fold higher potency (0.12 μM IC_{50}) than COX-1 (8.35 μM IC_{50}) and was significantly more potent than diclofenac in the carrageenan induced paw edema test.

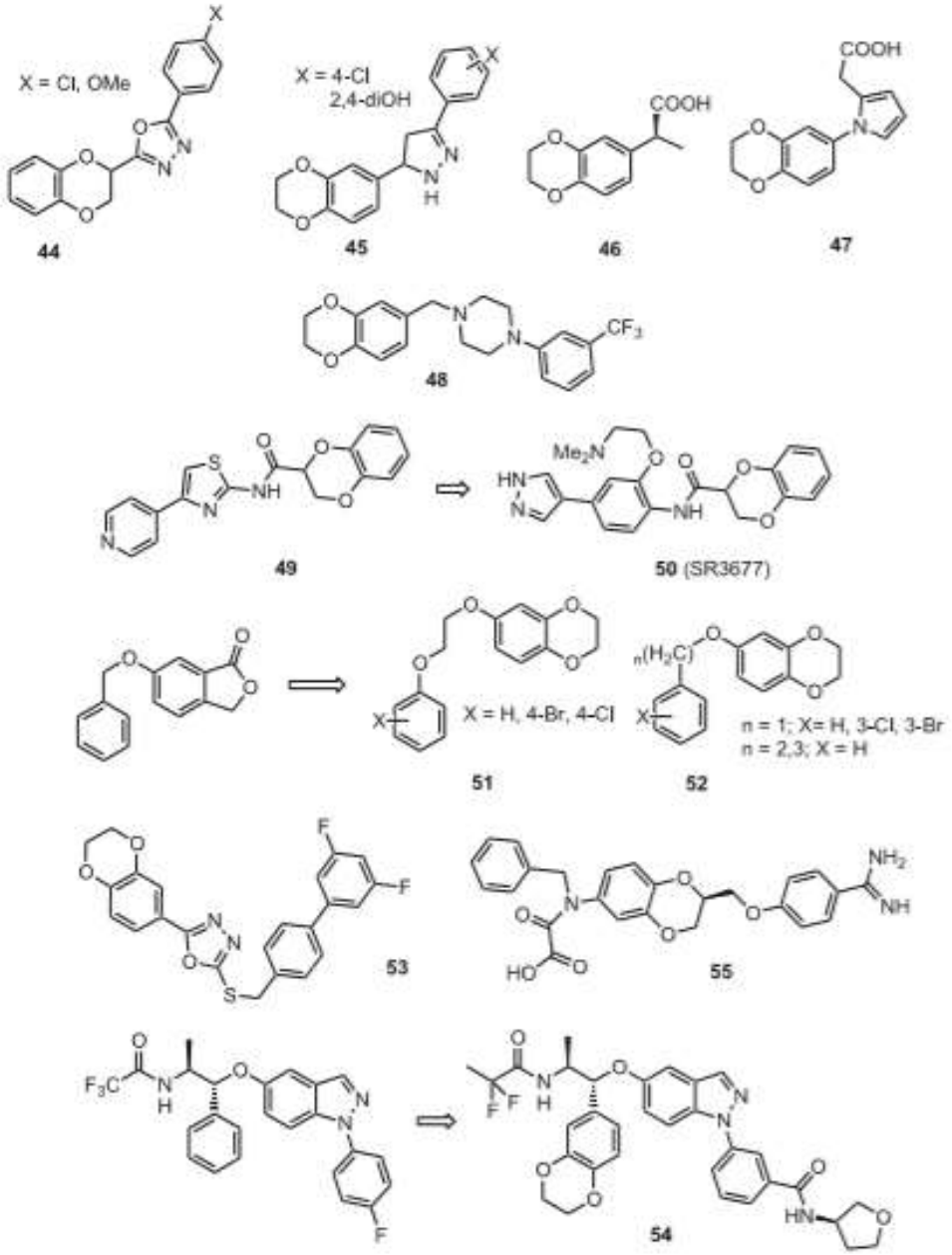
Rho kinase (ROCK) inhibitors are promising therapeutic agents;[138] they are currently used for corneal diseases and glaucoma. Inhibition of ROCK, in particular of ROCK-II isoform, promotes relaxation of vascular smooth muscle fibers decreasing intraocular pressure and improving aqueous humor outflow. Starting from the benzodioxane derivative **49** (Figure 11), a potent ROCK-II inhibitor (0.0072 μM IC_{50}) with a good selectivity against a few other kinases, an optimization process has been undertaken which has led to SR3677 (compound **50**, Figure 11), another benzodioxane related compound endowed with higher potency (0.0032 μM IC_{50}) and higher selectivity over other 353 kinases.[139]

MAO-B inhibitors are used for the treatment of Parkinson's disease. In the central nervous system, they contribute to conserve the depleted dopamine levels in the parkinsonian brain. Their safety profile is excellent and not associated with risks that limit therapeutic use of MAO-A inhibitors. Therefore, development of novel MAO inhibitors possessing selectivity for the MAO-B isoform is an important goal. Phthalide is a weak MAO-B inhibitor, while 6-benzyloxyphthalide inhibits human MAO-B at ten-nanomolar concentration and C-6 substituted phthalide derivatives have been considered suitable leads for the design of new MAO-B inhibitors by replacing the phthalide nucleus with benzodioxane.[140] Some benzodioxane 6-substituted with phenoxyethyloxy or phenylakyloxy residues (compounds **51** and **52**, Figure 11) have shown highly potent (<1 μM IC_{50}) and selective (IC_{50} MAO-A/IC_{50} MAO-B > 100) inhibition of recombinant human MAO-B.

Inhibition of glycogen synthase kinase-3 (GSK-3) has been demonstrated to decrease β-amiloid production and protein tau hyperphosphorylation, which are both associated with Alzheimer's disease. That's why inhibitors of GSK-3 have aroused interest, in particular if endowed with remarkably differentiated activity at the two GSK-3 isoforms, α and β, and selectivity versus other kinases. Starting from GSK inhibitors characterized by a central 1,3,4-oxadiazole 2-substituted with an arylmethylthio group and 5-substituted with a heterocyclic system, a series of analogues have been developed in which the heterocyclic system is benzodioxane-6-yl and the 2-substituent a biphenylmethylthio group with differently substituents at the terminal phenyl.[141] Some of these, in particular the 3,5-difluoro derivative **53** (Figure 11), show nanomolar inhibition activity, good selectivity for GSK-3α over GSK-3β and very high selectivity versus other kinases (**53**: 35 nM IC_{50} for GSK-3α and 27-fold selectivity over GSK-3β).

A series of selective nonsteroidal glucocorticoid receptor modulators (SGRMs) containing the benzodioxane scaffold was developed for the inhaled treatment of respiratory disease.[142] Starting from the structure of an orally available anti-inflammatory compound, the derivative **54** (Figure 11) was identified through a soft-drug strategy and a successive further optimization, involving replacement of a phenyl residue with 3-methoxyphenyl and then with benzodioxane-6-yl group. The compound **54**, compared to the benchmark inhaled corticosteroid fluticasone propionate, showed higher potency in lung edema inhibition (0.8 μg/kg vs 6.0 μg/kg in the rat model of Sephadex-induced airway inflammation), improved therapeutic ratio (50 vs 0.7) and more efficacious prolonged lung edema inhibition (76% lung edema reduction vs no significant effect when dosed 24 h prior to Sephadex challenge).

Figure 11. Benzodioxane related compounds with different activities



Joining anticoagulant and platelet antiaggregatory activities in the same molecule could be a successful strategy towards multitarget antithrombotic agents. A novel benzodioxane enantiomeric series act both as thrombin inhibitors and as fibrinogen GPIIb/IIIa binding inhibitors, the derivative **55** possessing the best balanced dual activity with 1.67 μM $K_{i\ (thrombin)}$ and 0.665 μM $IC_{50(GPIIb/IIIa)}$ (Figure 11).[143]

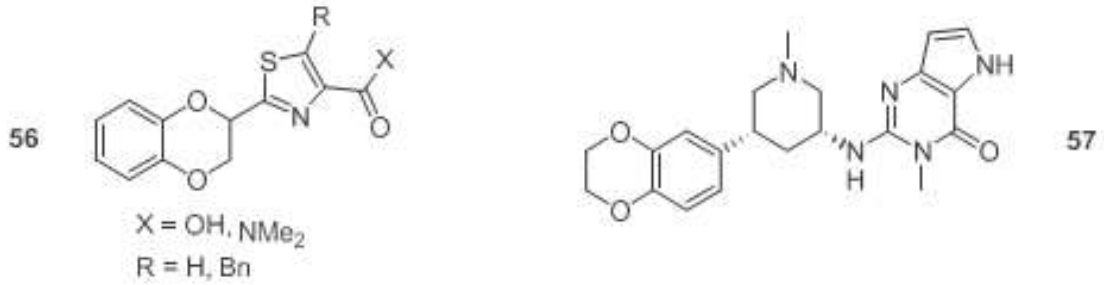
3. Conclusions

More than fifty benzodioxane-related compounds of synthetic origin exerting different biological activities are here reported as a result of last twenty years literature screening, in which scientific works have been selected if presenting sound chemical and biological characterization and, if possible, investigation of the mechanisms of action. We have found a high number of benzodioxane derivatives successfully designed as antitumor agents, antibacterial agents, α_1 adrenergic antagonists, neuronal nicotinic and 5-HT_{1A} agonists and antagonists. Therefore, we have divided them into these five groups bringing together benzodioxanes endowed with other less represented activities into an additional sixth group. The reported benzodioxane-containing compounds address different targets, including G-protein coupled receptors, ligand-gated ion channels and enzymes, benzodioxane substructure often acting as an essential pharmacophoric element. As here exemplified, different design strategies, both structure- and ligand-based, have inspired the use of the benzodioxane scaffold by medicinal chemists: replacement of other cyclic systems, such as naphthalene, tetrahydronaphthalene, chromane, phtalide, benzodioxole, aimed at improving druglikeness and selectivity, rigidification of oxy-substituted aromatic moieties, structural complication with the introduction of the dioxane stereocenter and the decoration of the aromatic ring, mimicking natural bioactive benzodioxanes, *in silico* screening based on funnel approach. Revealingly, benzodioxane template is found also in ligands optimization performed according the most advanced fragment-based approaches. Relying on biophysical and structural methods applied to protein constructs, benzodioxane-, benzoxazine-related small molecules (compounds **56**, Figure 12) interacting with anti-apoptotic proteins have been recently identified.[144] In 2019, a hit discovery process and subsequent structure-based optimization and SAR analyses led to the identification of a new lead benzodioxane-related derivative (compound **57**, Figure 12) for drug discovery targeting the bromodomain (BRD) containing protein PCAF (P300/CBP-associated factor). The developed compound, having *R* configuration at the two stereocenters, is a potent and selective PCAF inhibitor

(7 nM IC_{50} by homogeneous time-resolved fluorescence assay; 78 nM K_D by isothermal titration calorimetry assay) that could be very useful to unravel the functions of PCF BRD and the medicinal potential of its inhibition.[145]

The benzodioxane scaffold fortunes seem to not decline.

Figure 12. Lastly developed benzodioxane related compounds



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