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Review article

Phthalazin-1(2H)-one as a remarkable scaffold in drug discovery

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ABSTRACT

Phthalazinones are an important kind of nitrogen atom containing heterocyclic compounds due to their synthetic and pharmacological versatility. This fused heterocycle system represents a common structural feature for many bioactive compounds showing a variety of pharmacological activities such as anticancer, anti-diabetic, anti-asthmatic, antihistaminic, antihypertensive, antithrombotic, anti-inflammatory, analgesic, antidepressant or antimicrobial agents, which makes it an attractive scaffold for the design and development of new drugs. This review summarizes detailed and updated information, described in recent non-patent literature, about the most relevant pharmacological properties of phthalazinone derivatives, highlighting the application of this potent pharmacophore in drug discovery.

1. Introduction

Heterocyclic compounds constitute a huge group of organic compounds playing a key role in drug discovery because of their biological properties. Therefore, several heterocycles are fundamental for life of plants and animals, such as the heme group of chlorophylls and haemoglobin or the nitrogen bases and sugars of nucleic acids, and many of them are nitrogen containing heterocycles [1].

Phthalazin-1(2H)-one (**1**), also called 2H-benzo[d]pyridazin-1-one, is a benzo-fused heterocycle that presents a 1,2-diazine ring with two adjacent nitrogen atoms exhibiting a tautomeric equilibrium in solution which has been the subject of many studies. This heterobicyclic system can exist either in the lactam form (**1A**) or in the lactime structure (**1B**) (Scheme 1), with the first one (**1A**) being the predominant one despite its minor aromaticity [2]. In this regard, recent theoretical studies in the gas phase of different heterocyclic lactams and their benzo-fused derivatives show that benzo-fusion stabilizes the lactam form reducing the aromaticity difference between the two tautomeric forms [3]. Thus, in the crystal structure of phthalazin-1(2H)-one the benzene and pyridazine rings are almost coplanar [4].

Natural products are a traditional source of nitrogen-rich heterocycles as lead structures for drug development like indole, quinoline or quinazoline derivatives [5]. However, the presence of a 1,2-diazine ring is not very common in compounds isolated from living organisms and the biosynthesis of this structural unit is poorly understood. Pyridazomycin (**2**), antrimycin (**3**), cirratiomycin (**4**), L 365209 (**5**) and azamerone (**6**), all of them isolated from *Streptomyces* culture, are representative examples of natural products containing a 1,2-diazine ring with different pharmacological activities [6], with azamerone being the only one with a pyridazino-fused ring structure like phthalazinone (Fig. 1).

Despite its small presence in natural products phthalazinone nucleus and especifically phthalazin-1(2H)-one system is a versatile scaffold in Medicinal Chemistry providing derivatives able to interact with different kinds of biological targets. They were developed as enzyme inhibitors, such as aldose reductase (AR) inhibitors [7], poly-[ADP-ribose] polymerase (PARP) inhibitors [8] or phosphodiesterase (PDE) inhibitors [9], as ligands acting at G protein-coupled receptors (GPCRs), in particular histamine receptors [10], adrenoceptors [11], dopamine/serotonin receptors [12], or adenosine receptors [13], or even as modulators of ion channel-coupled receptors [14] or ligands for nuclear receptors [15]. Thus, phthalazinone derivatives have a wide variety of biological properties like antidiabetic [16], anticancer [17], anti-asthmatic [18], anti-inflammatory and analgesic [19], antihistaminic [20], antihypertensive and antithrombotic [21],

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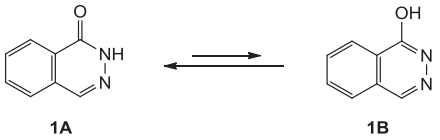


Scheme 1. Tautomeric equilibrium of phthalazin-1(2H)-one.

anticonvulsant [22], antimicrobial [23], antiviral [24], antiparasitic [25], as well as antidepressant and antipsychotic activities [26,12]. Their use as diagnostic agents [27] or even as herbicides [28] was also described. Moreover, a number of bioactive compounds are accessible using the phthalazinone nucleus as synthetic intermediates [29]. Some important established drugs which are related to the phthalazinone scaffold are detailed in Fig. 2.

A compilation of reports on the different biological activities associated with the phthalazine nucleus has been recently reported [30] and the therapeutic potential of phthalazinone derivatives for some activities was also mentioned in this review. However, a comprehensive report on different activities of phthalazinone based compounds is not available in literature till now. The present review summarizes the current information about the relevant pharmacological applications of phthalazinone derivatives in different therapeutic fields and described in non-patent literature, highlighting the application of this potent pharmacophore in drug discovery.

2. Chemistry

Synthesis of phthalazin-1(2H)-one derivatives is a research field of continuing interest not only because of their diverse pharmacological properties but also because of their potential as synthetic intermediates. A number of methods have been developed for obtaining phthazin-1(2H)-ones, especially referred to analogues substituted at C4 because this position along with N2 represent two key positions to modulate and optimize the biological activity of phthalazinone derivatives [31].

The chemistry of phthalazin-1(2H)-one was reviewed by Haider and Holzer in an interesting paper about phthalazines describing the different methodologies followed for phthalazine derivatives synthesis, in which most of analyzed strategies involve obtaining different precursors of phthalazinone structure [2]. Other review papers about chemistry of diazines and their benzo-fused systems were also recently published [32].

The most habitual methods for phthalazinone nucleus synthesis consist of cyclocondensation reactions of phthalic acid derivatives, such as phthalic anhydrides and phthalimides, or 2-formyl and 2-acylbenzoic acids with hydrazine or substituted hydrazines (Scheme 2), in a polar solvent and in the presence of an acid or basic catalyst [2]. Functionalization at N2 with several acyl, aryl or alkyl groups can also be achieved by substitution of the hydrogen atom and using different and specific conditions [2].

In addition, phthalimides are suitable to react under Friedel–-Crafts conditions [33] or with organometallic compounds [33,34] giving 2-keto benzoic acids hydrazides or 3-substituted 3-hydroxy indolinones, good building blocks to synthesize phthalazin-1(2H)-ones substituted at C4 with aryl, heteroaryl, aralkyl or alkyl groups (Scheme 3).

A frequent approach to 4-substituted phthalazin-1(2H)-ones involves 3-substituted benzofuran-1-ones or their tautomeric 2-acylbenzoic acids as synthetic intermediates [7,31a-b]. The synthesis of these key intermediates starts from the corresponding phthalic anhydrides and follows different pathways, such as condensation with active methylene compounds [35], Wittig-type alkenylation [7], a Friedel–Crafts reaction [31a,36] or through a metallation process [31a,37], depending on the selected substituents (Scheme 4).

Moreover, the reaction of 2-substituted 1,3-indanediones with hydrazine or hydrazine derivatives represents another useful method, also based on ring transformations, for the preparation of 4-diarylmethyl phthalazin-1(2H)-one core [38]. Likewise, an interesting approach based on hydrazine induced ring contraction of β-dicarbonyl functionalized tropolones was recently described for the synthesis of 2,4 disubstituted phthalazin-1(2H)-one derivatives [39].

Finally, microwave-assisted synthesis [40], palladium-catalyzed intramolecular cross-coupling reactions [41], one-pot synthesis [42] and other multicomponent syntheses, such as palladium-catalyzed carbonylation or isocyanide insertion on haloarenes [43], provide significant progress in phthalazinone derivatives chemistry. Green chemistry procedures have also been recently described. For example, reactions performed using ionic liquids

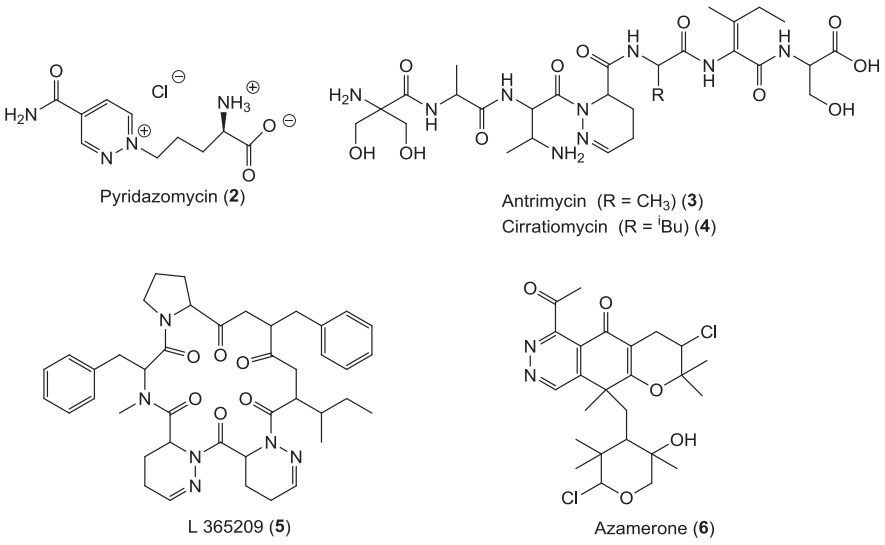


Fig. 1. Several natural products containing a 1,2-diazine ring in their structure.

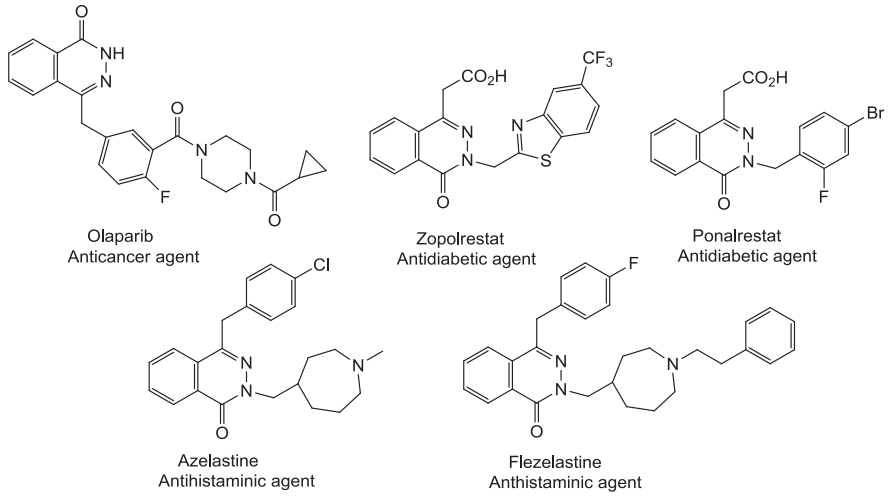
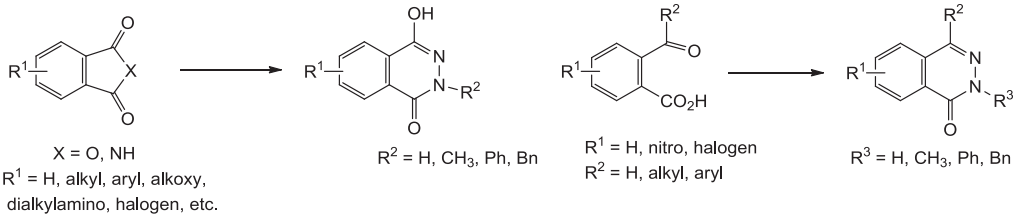
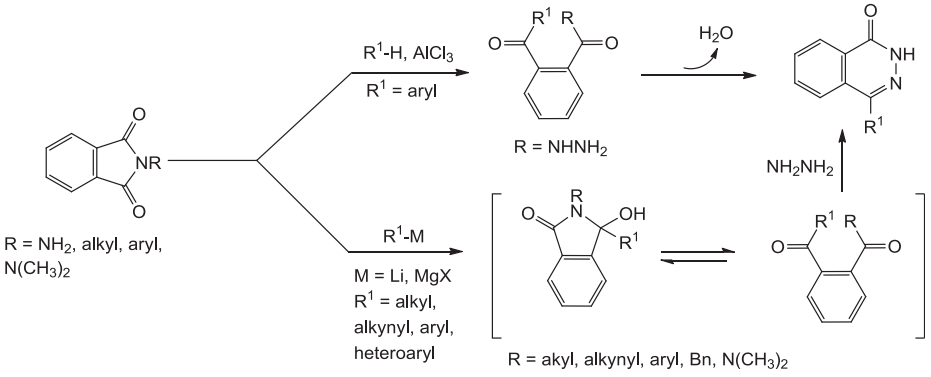


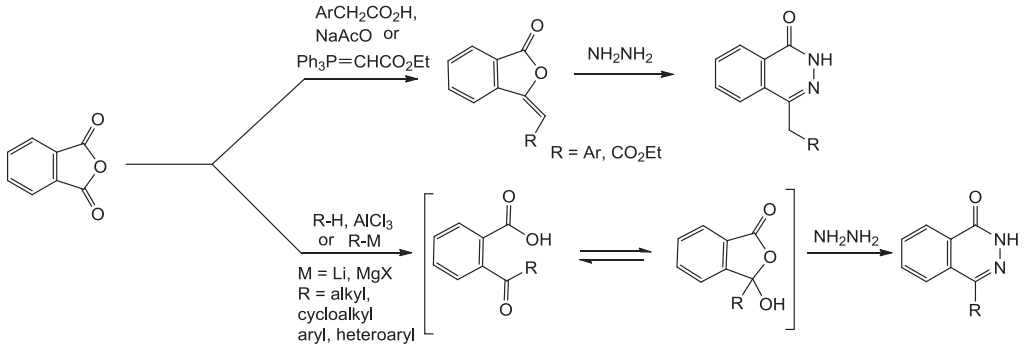
Fig. 2. Some commercial drugs of phthalazinone based structure.



Scheme 2. Construction of phthalazin-1(2H)-one core from phthalic anhydrides, phthalimides or 2-acylbenzoic acids.



Scheme 3. Synthesis of phthalazinone core from phthalimides via Friedel–Crafts conditions or with organometallic reactions.



Scheme 4. Construction of phthalazinone core from phthalic anhydride and following different pathways, among them Wittig-type alkenylation and metallation processes.

under ultrasound irradiation [44], reusable unsupported or heterogeneous catalyst [45], or palladium-catalyzed carbonylation under microwave irradiation in which the toxic CO gas was replaced by molybdenum hexacarbonyl ($Mo(CO)_6$) [46].

3. Pharmacological activities of phthalazin-1(2H)-ones

Phthalazin-1(2H)-one nucleus is a remarkable scaffold for drug discovery, so a literature survey reveals that phthalazinone derivatives have widespread applications in Medicinal Chemistry (Fig. 3). Taking this into account and in order to analyse the most relevant pharmacological activities of phthalazinone analogues they were organized into the following groups: anticancer, antidiabetic, antiasthmatic, antihistaminic, antihypertensive and antiplatelet, anti-inflammatory and analgesic, antidepressant and antimicrobial agents.

3.1. Anticancer agents

Cancer consists in an uncontrolled proliferation of abnormal cells. These mutated cells invade surrounding tissues and sometimes migrate through the blood or lymph spreading to other body organs causing metastases, which are the major cause of death from cancer. Although a significant proportion of cancers can be cured by surgery combined with radiotherapy or chemotherapy, especially if they are detected early, this disease is currently a leading cause of death in developed countries [47].

Cells of living organisms are equipped with different mechanisms to repair DNA damages caused by endogenous and environmental factors, known as DNA Damage Response (DDR) pathways. Disturbances in DDR pathways can contribute to the genomic instability and cancer development, and inhibition of the DDR represents a novel strategy in cancer therapy [48].

Currently, there is an increasing interest in the anticancer potential of DDR inhibitors. Poly[ADP-ribose] polymerases, PARP-1 and PARP-2, are nuclear enzymes activated by DNA damage playing a key role in single-strand break repair (SSBR) [48]. Both enzymes have a great structural and functional similarity. Taking into account that a number of anticancer therapies (radiotherapy and chemotherapy) are based on inducing irreversible breaks in DNA chain which may lead to cell death by apoptosis, PARP inhibition was considered a suitable target to improve the efficacy of these cytotoxic treatments [49].

More recent studies have demonstrated that PARP inhibitors (PARPi) may selectively kill cancer cells with defects in some DNA repair mechanisms. For instance, breast cancer, ovarian cancer and other solid tumours, particularly breast cancer showing *BRCA1/2* mutations [50].

Nicotinamide (**2**) was used as a starting point in development of PARP-1 inhibitors to mimic the interaction of NAD^+ with the enzyme (substrate–protein interaction) and most of PARP inhibitors contain nicotinamide-based pharmacophore fragments [48], such as 3-aminobenzamide (**3**), 5-substituted dihydroisoquinolin-1-ones (**4**), 2,8-disubstituted quinazolin-4-ones (**5**), benzo-condensed heteroaryl carboxamide derivatives (**6**) and 4-substituted phthalazinones (**7**) [51] (Fig. 4).

The PARP-1 inhibitory activity detected for phthalazin-1(2H)-one and 4-aryl phthalazin-1(2H)-ones [52,51d] was the starting point in discovery of a number of potent PARP-1 antagonists that bear the phthalazinone core [8,17,31b,53,54]. A hit exploratory study conducted by KuDOS pharmaceuticals led to 4-benzylphthalazin-1(2H)-one (**8**) with an IC_{50} against human PARP-1 of 0.77 μM. Further structural modifications supported by docking studies led to potent PARP-1 inhibitors, via inclusion of carbonyl-containing substituents at C3 position of the benzyl fragment (Fig. 5). Large series of analogues with different kind of carbonyl substituents at C3, such as anilide, amide, lactam and

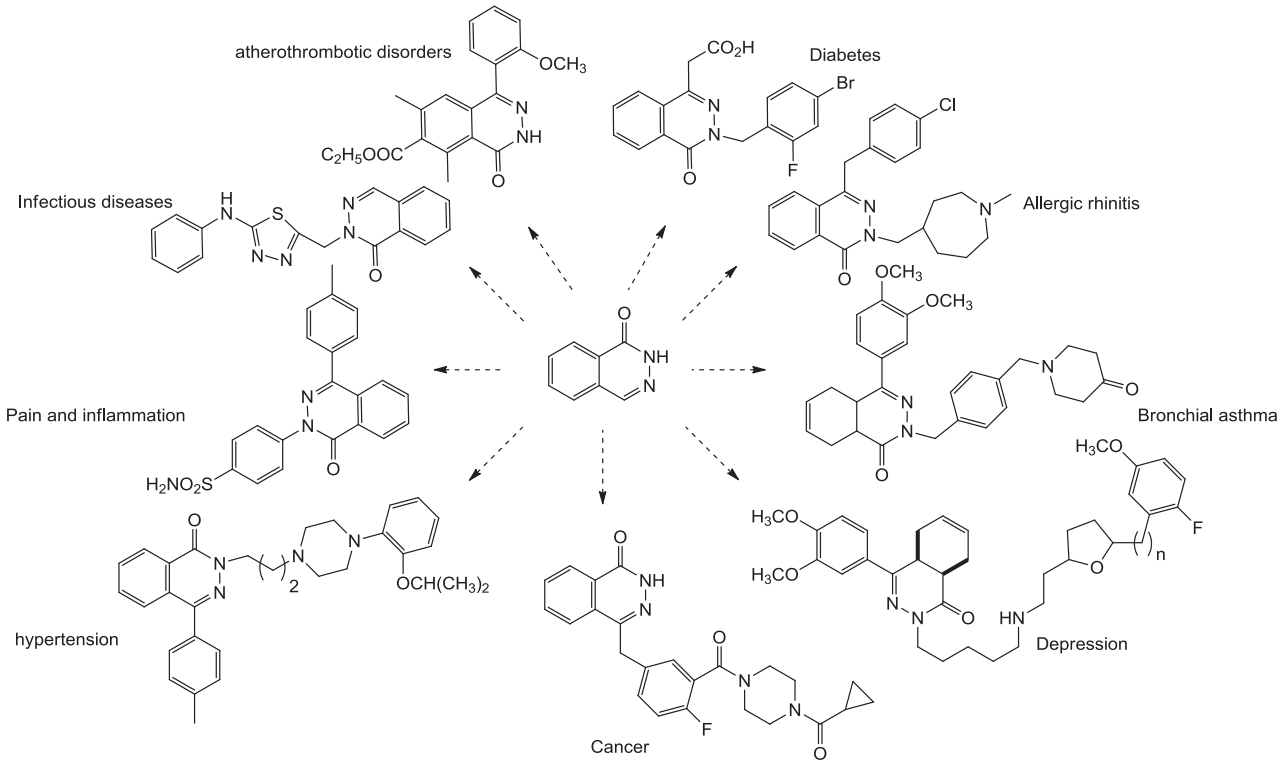


Fig. 3. Several phthalazinone derivatives with relevant therapeutic applications.

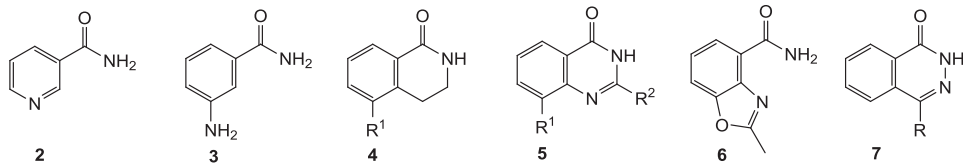


Fig. 4. Several prototypes of PARP-1 inhibitors including the phthalazinone scaffold.

imide derivatives, were obtained using parallel synthesis methodologies [8,31b], most of them with IC_{50} values in the nM range but showing significant differences in cellular efficacy and pharmacokinetics. Thus, the anilide analogues (compound **9**) exhibited poor metabolic stability in preliminary *in vitro* studies (mouse hepatic microsomes) [8]; however, a small change in this functional group to give amide (compound **10**) or imide derivatives (compound **11**) substantially enhanced the metabolic stability retaining the PARP-1 inhibitory potency [8]. A further optimization of both prototypes (amides and imides) was required to increase their activity in the cellular medium whilst maintaining *in vitro* potency and metabolic stability. The cellular efficacy was measured by the ability of target compounds to sensitize HeLa B cells to the killing effect of the alkylating agent methyl methanesulfonate (MMS) and was expressed by the potentiation factor value (PF_{50}, ratio of the IC_{50} growth curve for the MMS divided by the IC_{50} of the curve of MMS + PARP inhibitor). Better cellular efficacy was achieved for both series by introduction of a fluorine atom at C4 of the phenyl ring (compound **12**, Fig. 5) [8,31b]. In addition, the pharmacokinetic properties of amides series were improved to obtain compounds

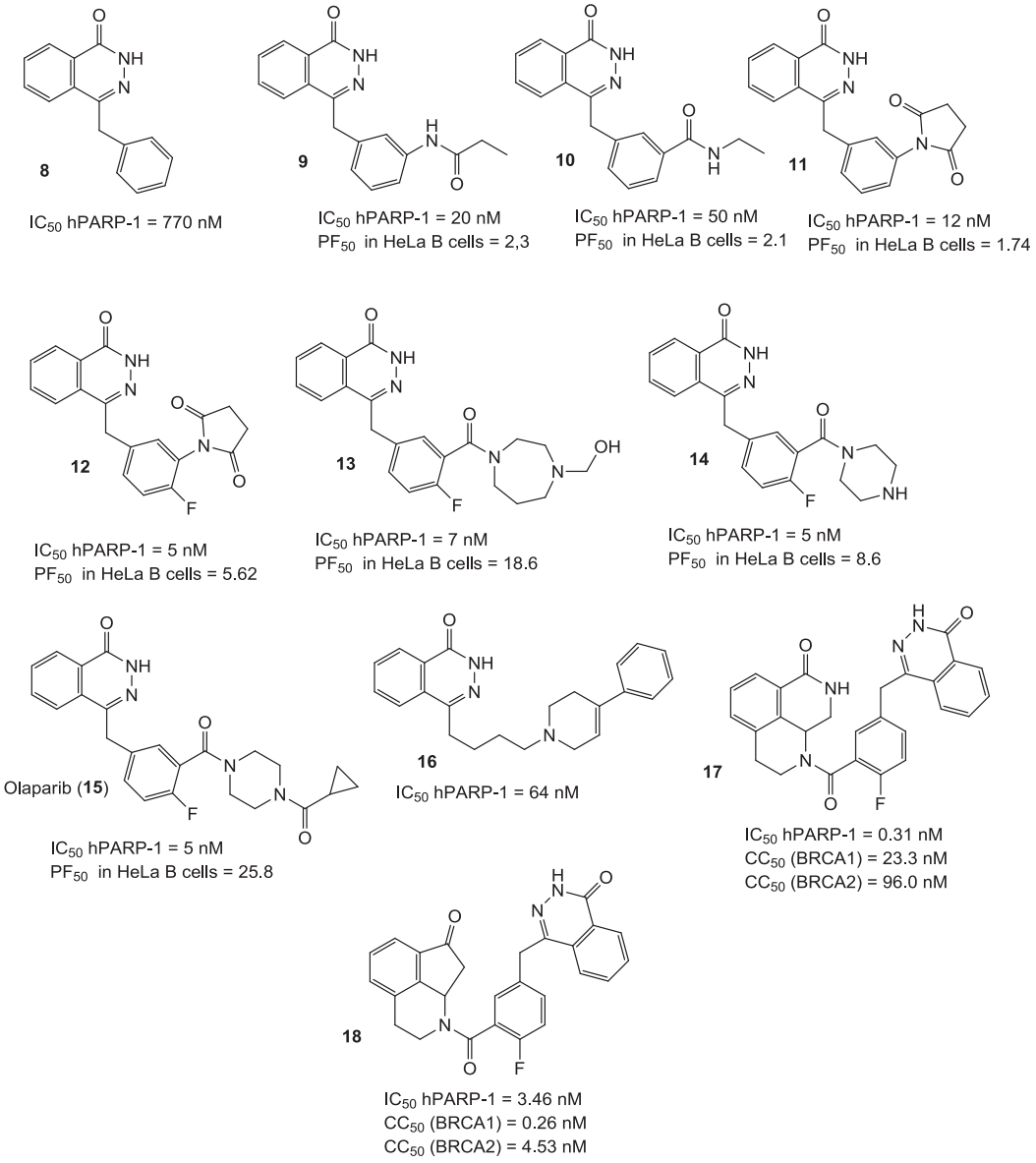


Fig. 5. Phthalazinone-derived inhibitors of PARP-1 as anticancer agents.

showing good *in vitro* and *in vivo* half lives, such as piperazine and homopiperazine analogues (compounds **13**–**14**) [17]. Subtle changes in the side chain of piperazine and homopiperazine led to the identification of olaparib (KU-0059436, AZD-2281, compound **15**), an equipotent inhibitor of both PARP-1 ($IC_{50} = 5$ nM) and PARP-2 ($IC_{50} = 1$ nM) enzymes with good cellular potency, pharmacokinetic profiles and oral bioavailability. Olaparib displayed *in vivo*, in a mouse xenograft model of SW620 colorectal cancer, the ability to potentiate the antitumour activity of the alkylating agent temozolomide [17,55]; it also shows standalone activity against BRCA1/2-deficient breast and ovarian cancer cell lines and is currently undergoing clinical trials as a single agent or in combination with other chemotherapeutic agents [17,55].

A small series of phthalazinone analogues were also designed by combining this moiety, essential to mimic the NAD^+ in the catalytic domain of PARP-1, with the 4-phenyl-1,2,3,4-tetrahydropyridine, a fragment which plays an important role in the enzyme-inhibitor binding, in order to improve the PARP-1 inhibitory potency of phthalazin-1(2H)-one scaffold [53]. A preliminary screening of this series revealed that its activity, with IC_{50} values in the nM range (compound **16**, Fig. 5), was comparable to that detected for some above-mentioned 4-benzylphthalazinone hits.

The recent discovery of benzo[de][1,7]naphthyridin-7(8H)-one scaffold as a new pharmacophore capable of interacting with the NAD^+ binding site [56] has promoted the development of new PARP-1 inhibitors bearing this lactam framework combined with key structural elements of olaparib, in particular a long-chain functionalized with amide groups or a bulky 4-benzylphthalazinone core [54]. Some of these compounds of hybrid structure benzonaphthyridinone phthalazinone displayed a high *in vitro* PARP-1 inhibitory activity, being more potent against the enzyme than olaparib, but only showing moderate potency for BRCA-deficient cells (compound **17**, Fig. 5). Further Structure–Activity Relationship (SAR) studies were performed in order to increase the potency in whole cells and validate the benzo[de][1,7]naphtyridin-7(8H)-one core as an effective scaffold for PARP inhibition. These studies conducted to a new lead (compound **18**, Fig. 5) in which the benzo[de][1,7]naphtyridin-7(8H)-one was replaced by the cyclopenta[ij]isoquinolin-7(1H)-one, showing high potency against both the PARP-1 enzyme and BRCA-deficient cells.

Aurora kinases and a wide variety of protein kinases are involved in protein phosphorylation events in cells playing an important role in the regulation of several cellular processes. They also constitute important therapeutic targets for cancer disease and most of kinase inhibitor scaffolds consist of planar heterocycles bearing both hydrogen bond donors and acceptor groups [57], a set of structural features also present on phthalazinone core.

Aurora kinases (consisting of auroras A, B and C) have a crucial role in cellular division being over-expressed in diverse solid tumours, in particular aurora A [57b,58]. Therefore, inhibition of aurora kinases has opened a new front in the search for antitumour drugs. Several aurora inhibitors were designed and synthesized, some of which are currently undergoing clinical development. The majority of these drugs display selective inhibition against auroras B and C and there are few examples of selective aurora A inhibitors [59]. Recently a series of compounds with hybrid structure phthalazinone pyrazole were proposed as a novel class of aurora A inhibitors with over 1000-fold selectivity against aurora B, a selectivity index much higher than that of previously described aurora A inhibitors. Some of these compounds (**19**, **20**, Fig. 6) showed good *in vitro*/*in vivo* profiles, with good biochemical potency, moderate activity against the three studied cell lines (MCF7, HTC116, Colo205), and good oral bioavailability and plasma concentration [60].

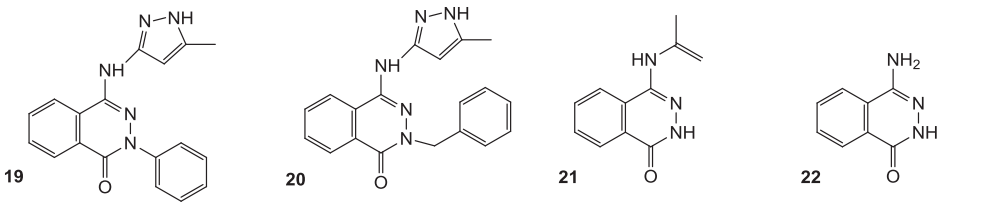
Phthalazinone scaffold was also described as starting hit (compounds **21**, **22**, Fig. 6) for designing selective inhibitors of other protein kinases, such as cyclin-dependent kinases (CDKs), glycogen synthase kinase-3 (GSK-3), casein kinase (CK) or dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) [61], most of them involved in signalling pathways regulating cell proliferation and apoptosis.

3.2. Antidiabetic agents

Diabetes mellitus is a chronic multifactorial disease characterized by a high blood glucose concentration (hyperglycemia). This complex disorder resulting from insulin deficiency (type I diabetes) or insulin resistance (type II diabetes), disturbs the metabolism of carbohydrates, fats and proteins giving rise to not only acute but also long-term complications. More than 90% of diabetic patients suffer from type II diabetes, i.e. non-exogenous insulin dependent diabetes [62].

The long-term vascular complications, microangiopathies, such as retinopathy, neuropathy or nephropathy, and macroangiopathies, such as coronary artery disease, peripheral vascular disease and cerebrovascular disease, are responsible for the morbidity and mortality detected in diabetic patients [63].

Diabetes disease results in the intracellular production of excess sorbitol and fructose through the polyol pathway, due to an increased glucose flux across the cell membrane. The polyol pathway is regulated by two enzymes, aldose reductase (AR) and sorbitol dehydrogenase. AR is present in all tissues susceptible to diabetic complications, where insulin is not necessary for glucose transport into the cell and in which the intracellular concentration of glucose could be equal to the plasma. The polyol pathway was proposed as one of the main molecular mechanisms involved in the pathogenesis of the diabetic complications [64]. Several preclinical studies and clinical trials indicate that inhibition of AR may prevent



19: IC_{50} Aur-A = 0.031 μM; IC_{50} Aur-B > 100 μM; CC_{50} (HTC116) = 7.8 μM; CC_{50} (Colo205) = 2.9 μM; CC_{50} (MCF7) = 1.6 μM

20: IC_{50} Aur-A = 0.065 μM; IC_{50} Aur-B > 100 μM; CC_{50} (HTC116) = 1.5 μM; CC_{50} (Colo205) = 4.4 μM; CC_{50} (MCF7) = 12.6 μM

21: IC_{50} DYRK1A > 10 μM; IC_{50} CK1 > 10 μM; IC_{50} CDK5 > 10 μM; IC_{50} GSK3 > 10 μM

22: IC_{50} DYRK1A = 4.1 μM; IC_{50} CK1 > 10 μM; IC_{50} CDK5 > 10 μM; IC_{50} GSK3 > 10 μM

Fig. 6. Phthalazinone-derived inhibitors of kinases as anticancer agents.

the appearance of long-term complications, such as retinopathy [65], neuropathy [63], nephropathy [66] and other diabetic vascular complications [67] and a number of aldose reductase inhibitors (ARIs) have been described in the last decades [68].

Currently, available ARIs can be divided into three main classes: carboxylic acids, hydantoins and structurally diverse ARIs [68].

Most of ARIs have in common two structural features, an acidic group, which is responsible for hydrogen bond interactions with the enzyme active site, and one or more aromatic groups in order to interact, via van der Waals and hydrophobic forces, with the lipophilic region in the active site [68].

Ponalrestat (ICI 128 436, **23**, Fig. 7), developed by Imperial Chemical Industries (ICI) in the 1980s, with an IC_{50} value against AR from human lens of 20 nM, was the first oxophthalazineacetic acid derivative described as clinical candidate for treatment of diabetic complications [69]. The interesting properties shown by ponalrestat along with the relative lack of carboxylic acid structures with potent activity *in vivo* promoted the development of new potential ARIs based on phthalazinone scaffold. A variety of oxophthalazineacetic acid derivatives were described as potent ARIs (Fig. 7), of which zopolrestat (**24**), a compound that combines the oxophthalazineacetic acid backbone with the benzothiazole group linked by a methylene spacer, is one of the most interesting. Zopolrestat was developed by Pfizer in 1990s under the hypothesis that there is an additional binding site on the AR enzyme with strong affinity for the benzothiazole scaffold [7]. The lead compound of this series of 3,4-dihydro-4-oxo-3-(benzothiazolylmethyl)-1-phthalazine acetic acids was the compound **25** (Fig. 7), which is a potent inhibitor of AR from human placenta (IC_{50} = 19 nM) and orally active in some animal models of diabetic complications (ED_{50} for inhibition of sorbitol accumulation in rat sciatic nerve, in an acute test of diabetic complications, of 18.5 mg/kg). Optimization of this lead in order to increase potency ($ED_{50} \leq 5$ mg/kg) and duration of action (potential for once-a-day treatment in the clinic study) provided different congeners with lipophilic substituents in the different benzene ring positions of the benzothiazole scaffold. The most potent compounds of this series were the 5-subtituted analogues, such as the 5-Br derivative **26** (hAR IC_{50} = 3.1 nM, sciatic nerve ED_{50} = 5.6 mg/kg) and 5-CF_3 analogue **24** (zopolrestat hAR IC_{50} = 3.1 nM, sciatic nerve ED_{50} = 3.6 mg/kg). In addition, zopolrestat showed good oral absorption, high blood level and favourably long plasma half-life and has reached the stage of clinical trials [7] but so far has not been marketed.

Searching for new zopolrestat congeners, and in order to analyze the importance for the activity of benzothiazole side chain and oxophthalazineacetic acid scaffold, both fragments were alternatively replaced by other heterocycles [16,70]. On the one

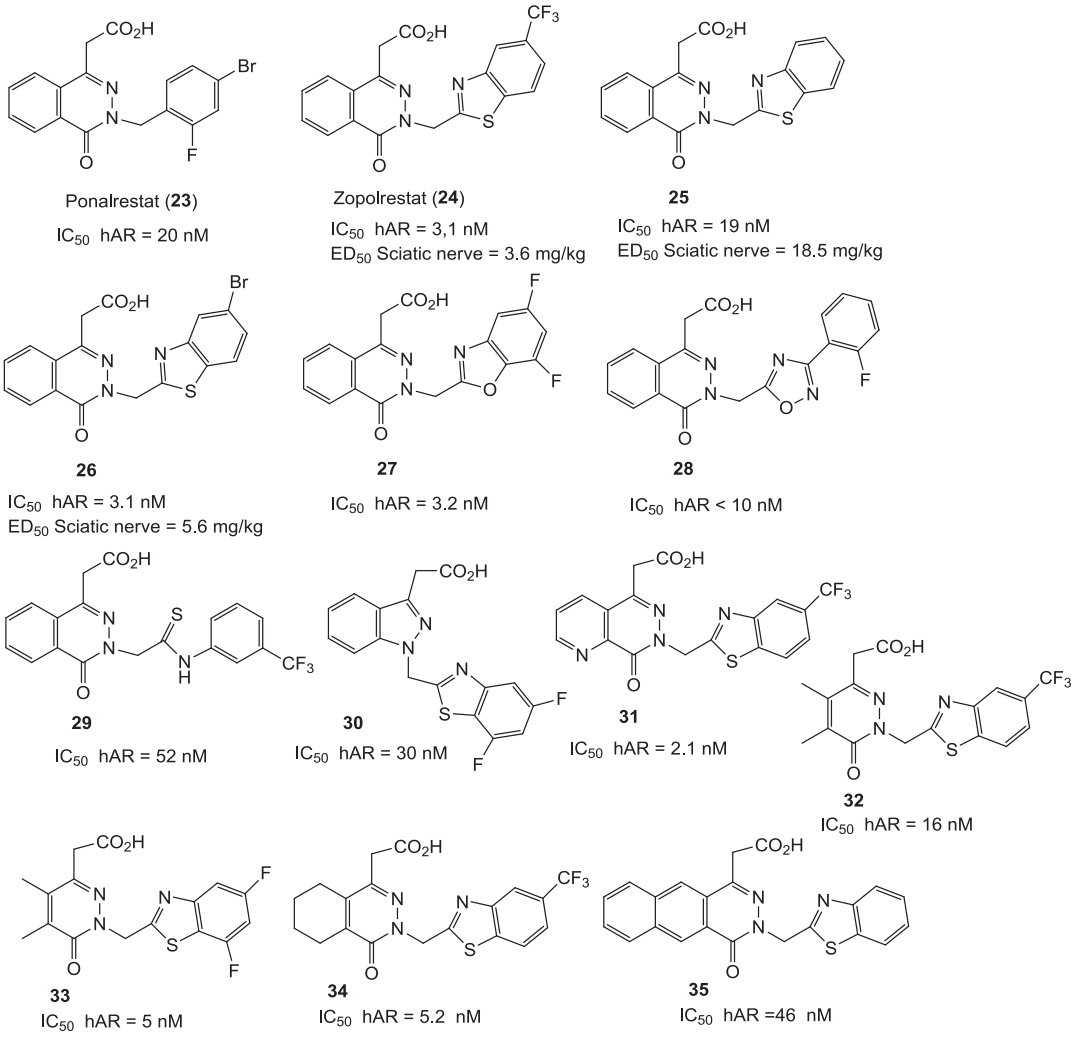


Fig. 7. Phthalazinone-derived AR inhibitors as antidiabetic agents.

hand, the results of these studies indicated that benzoxazole, 3-aryl-1,2,4-oxadiazole and thioanilide moiety are effective surrogates of key benzothiazole side chain giving rise to potent *in vitro* ARIs, such as compounds **27** (hAR IC_{50} = 3.2 nM), **28** (hAR IC_{50} < 10 nM) and **29** (hAR IC_{50} = 52 nM) (Fig. 7), and only the analogue **27** showing significant oral activity when administered at 10 mg/kg [70]. On the other hand, replacement of oxophthalazineacetic acid pattern by different heterocycles with a pendant acetic acid moiety, including indazole, pyridopyridazinone, pyridazinone and benzophthalazinone resulted also in potent *in vitro* ARIs (compound **30**–**35**, Fig. 7). The obtained results indicated that the phthalazinone moiety upsizing had a similar effect on *in vitro* activity as its downsizing when a benzothiazole side chain was incorporated. The best compounds of this series were the oxopyridazineacetic acid analogues **33** and **34** (Fig. 7), which also inhibit sorbitol accumulation in the above mentioned animal models when administered orally at 10 mg/kg [70].

In addition, regarding the known pharmacokinetic properties of zopolrestat, which in recent years was reported as a potent inhibitor of glyoxilase I (GLOI) with an *in vitro* K_i value of 1.2 μM [71], it is noteworthy that this phthalazinone analogue could serve as a molecular template for designing novel GLOI inhibitors as potential antineoplastic agents.

The discovery of 2,4-thiazolidinediones or glitazones as effective insulin resistance reducers was a major breakthrough in treating type II diabetes and its long-term complications [72]. These compounds reported as agonists of Peroxisome Proliferator Activated Receptor gamma (PPARγ) [73], a nuclear receptor which regulates the gene expression evolved in carbohydrate and lipid metabolism [74], enhance insulin action without stimulating its release from pancreatic β-cells. Some phthalazinones containing the thiazolidine-2,4-dione pharmacophore (compound **36**, Fig. 8) were described as potent *in vitro* PPARγ activators, showing both plasma glucose and triglycerides lowering activity *in vivo*, in insulin resistant db/db mice [15]. In addition, the N-substituted phthalazinone acetamide scaffold was recently identified as a potential glucose uptake activator by using a high throughput screening (HTS) assay [75] and several derivatives were synthesized. The *in vitro* preliminary screening results indicated that the active compounds increased glucose uptake in skeletal muscle cells (L6 rat myotubes) without inducing PPARγ agonist activity. Most of these active analogues contain a thiazole moiety linked to phthalazinone acatamide fragment (compounds **37**–**39**, Fig. 8).

3.3. Antiasthmatic agents

Bronchial asthma is a lung disease characterized by airway obstruction, inflammation and hyperresponsiveness [76]. In the traditional treatment for bronchial asthma two different types of drugs, bronchodilators, such as β_2-agonists and xanthine derivatives, to inhibit the airway obstruction process and steroidal anti-inflammatory drugs to control the airway inflammation and prevent their hyperresponsiveness are involved [77].

The discovery that thromboxane A_2 (TXA_2) is involved in the progressive airway inflammation [78] has promoted the development of TXA_2 synthetase inhibitors as potential antiasthmatic agents [79]. Thus, some TXA_2 synthetase inhibitors were marketed in Japan for treatment of bronchial asthma in adults reducing the need for concomitant steroid therapy [76].

A number of compounds of varied structure were reported as potent TXA_2 synthetase inhibitors, the common structural features for these compounds are a 1-imidazolyl or a 3-pyridyl group linked to a carboxylic acid function by an alkenyl chain [80]. In addition, several 1-imidazolylalkyl derivatives without the carboxylic group were also reported as very potent inhibitors of TXA_2 synthetase [81]. Taking this into account, the 1-imidazolyl group was initially selected to combine with the phthalazinone scaffold present in azelastine (**40**, Fig. 9), a known antiallergic compound having also bronchodilatory properties, in order to develop antiasthmatic agents with both TXA_2 synthetasa inhibitory and bronchodilatory activities. A number of 4-subtituted 2-[ω-(1-imidazolyl)alkyl]-1-(2H)-phthalazinones synthesized were effective for TXA_2 synthetase inhibition and bronchodilation [31a]. SAR studies indicated that both activities were more affected by the nature of the substituent at C4 than by the length of alkyl chain at N2. Compounds with phenyl or thienyl groups, such as **41** and **42**, exhibited good and well-rounded activities *in vitro* and *in vivo* (Fig. 9). The introduction of a carboxyl group reduced both TXA_2 synthetase inhibition and bronchodilation activities (compound **43**) and the 4-(3-pyridyl)phthalazinone **44** was particularly interesting because of its high activities *in vivo* in spite of lacking both activities *in vitro*. The results obtained for compound **44** led to consider its metabolites (the deimidazolyl and deimidazolylethyl analogues) as the

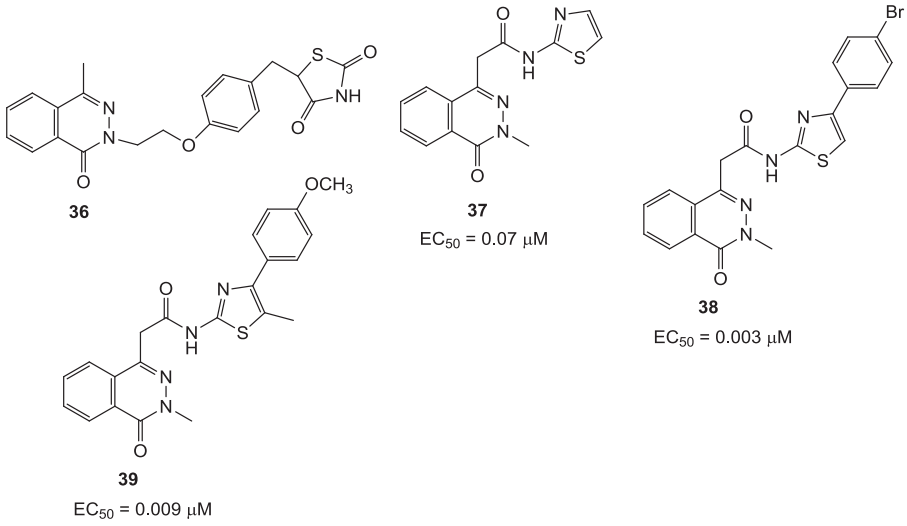


Fig. 8. Phthalazinone derivatives as antidiabetic agents.

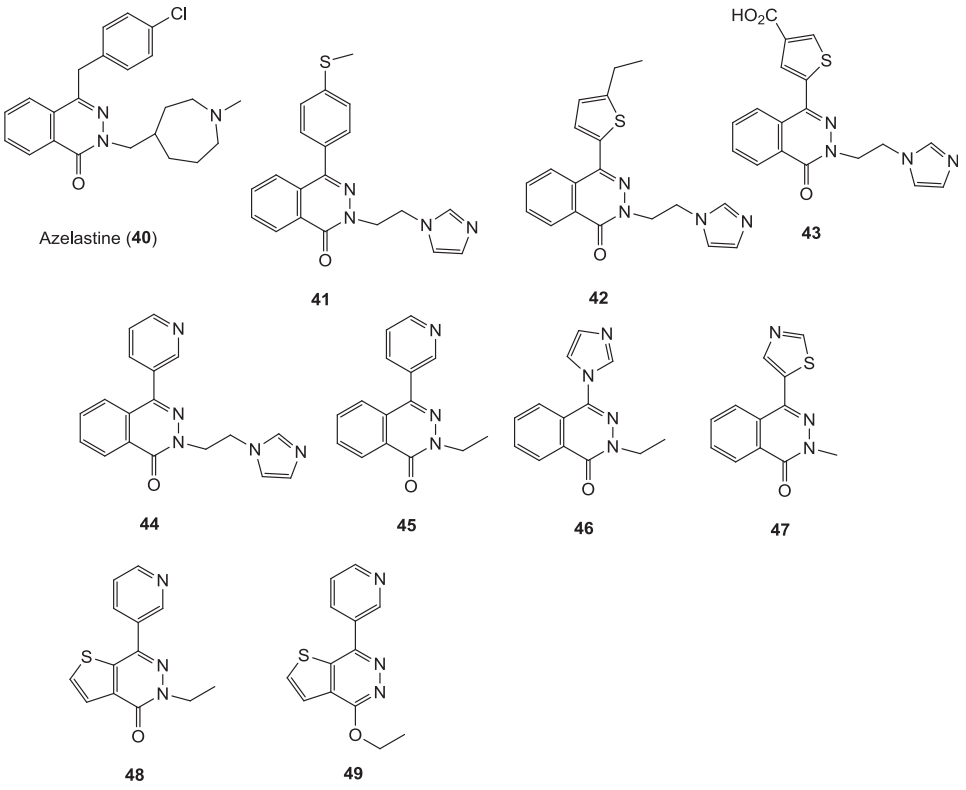


Fig. 9. Phthalazinone derivatives with TXA2 synthetase inhibition and bronchodilatory activities as antiasthmatic agents.

possible active species. A further study about the relationship between activities and N2 and C4-substituents structure of 3-pyridyl derivative **44** indicated that the removal of imidazolyl group causes an increase of bronchodilatory activity due to an increase of hydrophobicity [81b], and alkyl groups, especially the ethyl group, resulted the most effective for both activities (compound **45**). Furthermore, the heteroaromatic nucleus at C4 position of phthalazinone moiety and specifically the 3-pyridyl group, plays a critical role in TXA_2 synthetase inhibition, even though the 1-imidazolyl and 5-thiazolyl groups are well tolerated (compounds **46** and **47**, Fig. 9) [79a].

In addition, the role of benzene ring of the phthalazinone scaffold was also studied for compounds **41** and **45** finding that this group results in a key moiety for both TXA_2 synthetase inhibition and bronchodilatory activity, because benzene removal or substituent inclusion into the benzene ring led to a significant reduction of both activities [79b–c]. Further SAR studies showed that thiophene (bioisotere of benzene) was able to replace the benzene ring of phthalazinone without making the structure to lose its biological activities (compound **48**) [79d], supporting the hypothesis, also corroborated years later by other researchers [82], that benzene ring plays an important role for both TXA_2 synthetase inhibition and bronchodilatory activities [79b-c]. Finally, it was demonstrated that the carbonyl moiety of phthalazinone is not essential for these activities (compound **49**, Fig. 9) [79d].

Phosphodiesterases (PDEs) are enzymes responsible for the hydrolysis of purine cyclic nucleotides, cAMP and cGMP, two important second messengers which are involved in the regulation of a number of cell functions. Among the different PDE isoenzymes, PDE4 is characterized by a cAMP high-affinity and its inhibition results in cAMP increased cellular levels [83], giving rise both to the relaxation of airway smooth muscle (bronchodilation) and to the prevention of proinflammatory cell activation (anti-inflammatory effect), without the cardiovascular effects associated with PDE3 inhibition, and therefore PDE4 inhibitors (PDE4Is) have emerged in the last decades as promising antiasthmatic agents [77,84].

PDE4Is can be organized into three structural categories, catechol ethers (rolipram-related compounds), heterobicyclic compounds, such as nitraquazone and xanthine derivatives, exemplified by theophylline derivatives, which are considered structural prototypes to get new analogues more potent and selective, without cardiovascular and gastrointestinal side effects [77]; emesis and nausea are common gastrointestinal side effects of PDE4Is that have traditionally been associated either to binding at an allosteric center of PDE4, also called high-affinity rolipram binding site [85], or to activation of emetic centres in the Central Nervous System (CNS) [86].

Nitraquazone, an N1-benzyl N3-ethyl disubstituted quinazoline-2,4-dione, and their congeners were a starting point for developing several pyridopyridazinone derivatives and other heterocyclic fused pyridazinones [9,87] and a number of phthalazinone analogues [88–90].

Thus, a series of 4-(3,4-dimethoxyphenyl)-2H-phthalazin-1-ones resulting from combining structural elements present in known PDE4Is, such as the pyridazinone nucleus and the catechol ether group, and showing variations in the saturation level as well as in the stereochemistry of phthalazinone scaffold (*cis*/*trans* ring fusion) were synthesized and tested against PDE3 and PDE4 enzymes [88a]. The results of this study indicated that all designed phthalazinone derivatives selectively inhibited the PDE4 and that N-substitution is beneficial for activity, which is in agreement with previously described SAR for nitraquazone and related compounds. The N-benzyl analogue with a benzene fused pyridazinone (phthalazinone **50**, Fig. 10) and in particular its (±) *cis*-annulated cyclohexane and cyclohex-3-ene analogues (hexahydrophthalazinone **51** and tetrahydrophthalazinone **52**) presented high

PDE4 inhibitory activity (potency expressed as $pIC_{50} = -\log IC_{50}$), whereas the corresponding *trans*-racemic mixtures exhibited only weak to moderate inhibitory activity [88a].

Molecular modelling studies were conducted in order to analyze the detected differences in PDE4 inhibition upon changes in the phthalazinone moiety structure of 4-(3,4-dimethoxyphenyl)-2H-phthalazin-1-one series [89a]. These studies indicated that *cis*-fused cyclohexane and cyclohexene rings occupy a different region in the enzyme from that occupied by the fused rings of the other studied phthalazinones. Both chiral compounds, the (±)-*cis*-hexahydrophthalazinone **51** and the (±)-*cis*-tetrahydrophthalazinone **52**, were used as lead structures for the development of novel PDE4Is [88b].

In order to analyze the structural requirements of the 4-aryl substituent for the PDE4 inhibition, and taking previous data about the importance of the catechol ether group for the activity into account, several substituents were placed at the 2, 3 and 4 position of the 4-phenyl ring finding that the 4 position of this phenyl ring was restricted to small lipophilic groups, preferably a methoxy group, larger groups could result in unfavourable steric interactions with the enzyme. However, different alkoxy groups were permitted at the 3 position, including various ring systems and functional groups. In this study it was also indicated that in general the (±) *cis*-4a,5,8,8a-tetrahydro-2H-phthalazin-1-ones (compounds **53** and **54**, Fig. 10) were more potent than their hexahydrophthalazinone analogues (compounds **55** and **56**) [88b].

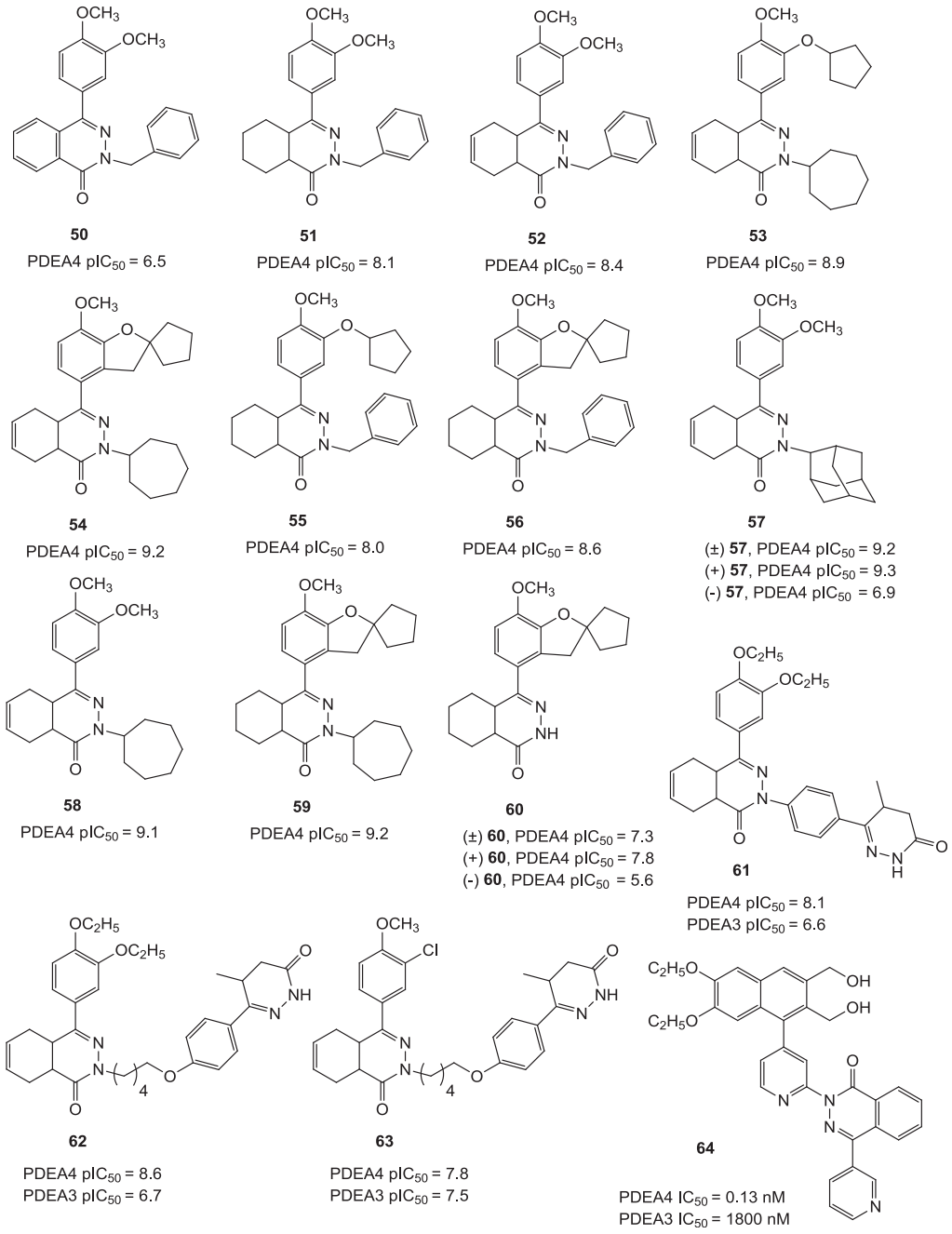


Fig. 10. Phthalazinone-derived PDE4 inhibitors as antiasthmatic agents.

In a subsequent study the influence of N-substitution in the (4a,8a)-*cis*-racemates of these hexahydro and tetrahydrophthazinones was discussed describing that the N-cycloalkyl substituted compounds exhibit the highest PDE4 inhibitory activity (nanomolar range, compounds **54**, **57**, **58** and **59**, Fig. 10) [89a]. The *in vitro* and *in vivo* anti-inflammatory efficacy was evaluated for selected compounds in order to know their possible applicability in the treatment of chronic inflammatory conditions. Some of the studied compounds inhibited *in vitro* the release of Tumour Necrosis Factor α (TNFα) and production of Reactive Oxygen Species (ROS) (compounds **57** and **58**), two processes of particular importance in chronic inflammation; compounds **54**, **57** and **58** also displayed high *in vivo* anti-inflammatory activity in the mouse ear oedema assay, being considered as promising anti-inflammatory agents for the treatment of asthma, rheumatoid arthritis and other inflammatory diseases [89a].

A number of optically active *cis*-hexahydro and tetrahydrophthalazinones were synthesized starting from adequate enantiomers of various γ-keto acids in order to determine the absolute configuration and PDE4 inhibitory activity of individual enantiomers. The authors concluded that (+)-enantiomers of *cis*-hexahydro and tetrahydrophthalazinones display high PDE4 inhibitory activity, whereas the corresponding (−)-enantiomers exhibit only weak or moderate activity (compounds **60** and **57**, Fig. 10). The N-substituted 4a,5,8,8a-tetrahydrophthalazinones included in this study also posses *in vivo* anti-inflammatory activity [89b].

The same authors in a subsequent study combine two pharmacophores, the tetrahydrophthalazinone (PDE4 inhibition) and 6-arylpyridazin-3(2H)-one (PDE3 inhibition) scaffolds with the idea of getting dual PDE3/PDE4 inhibitory agents because of their possible synergistic bronchorelaxant effect [90]. In the designed hybrid compounds the aromatic ring of phenylpyridazinone moiety was connected directly or through different linkers to N2 position of tetrahydrophthalazinone scaffold. Most of studied compounds are dual PDE3/PDE4 inhibitors. The SAR data regarding PDE inhibition are in agreement with those previously observed for pyridazinone and phthalazinone analogues. Compounds with a 5-methyl-4,5-dihydro-pyridazin-3(2H)-one fragment linked to N2 through the *para* position of the aryl ring are the most interesting ones. The data of *in vivo* study suggested compounds **61**, **62** and **63** as the most effective as anti-inflammatory agents (Fig. 10). However, the authors indicate that there is no correlation between the *in vivo* anti-inflammatory activity and the *in vitro* PDE3/PDE4 inhibitory activity.

In addition, a series of 1-pyridylnaphthalene derivatives substituted with a heterocyclic moiety containing a carbonyl group was reported as a new class of potent and selective PDE4 inhibitors [18]. The best compound of this series was the phthalazinone analogue **64** (Fig. 10). The compound **64** inhibits the PDE4 activity in the subnanomolar range (IC_{50} = 0.13 nM), exhibiting very high selectivity for PDE4 over PDE3 (selectivity ratio = 14 000), and showing potent antispasmogenic activities in guinea pigs, with little cardiovascular and emetic effects in both ferrets and dogs, which is consistent with its lower affinity by the high-affinity rolipram binding site (K_i = 2.6 nM) and therefore with the broad margin between both the K_i and the IC_{50} values.

More recently, with the aim to develop topically active PDE4Is and in order to minimize gastrointestinal side effects associated with oral administration of classical PDE4Is, novel *cis*-tetrahydro and hexahydrophthalazinone related compounds showing substituents with increased lipophilicity at N2 were synthesized and biologically evaluated as both PDE4 and TNF-α inhibitors [91]. SAR studies confirmed that there is a strong dependence between the substituent at N2 of the phthalazinone system and these inhibitory activities. Thus, the presence at this position of a benzyl moiety linked to a six-membered heterocyclic ring, such as an aminobenzyl fragment, provided potent activity. The authors also corroborated the important effect of the *cis*-fused cyclohexene ring for the activity (compounds **65** and **66**, Fig. 11). Compound **65** and its optically pure enantiomers were selected for further studies; in the PDE4 and TNF-α inhibition assays the *cis* (+)-**65** compound displayed 29 and 20 times more potency, respectively, than the corresponding (−)-enantiomer. The compounds more potent of this series, such as (+) **65**, **67** and **68**, were topically effective in a mouse dermatitis model.

The pharmacophore 4-(3,4-dimethoxyphenyl)-4a,5,6,7,8,8a-hexahydrophthalazinone, was also recently combined with different phenylethanolamine fragments present in the salmeterol and formoterol, two representative long-acting β_2-agonists very effective as bronchodilators, in a multivalent approach to obtain dual pharmacology bronchodilators that target both the β_2-adrenoceptor and PDE4 [92]. The different kind of hybrid compounds designed displayed moderate to high β_2-agonist activity on isolated guinea pig tracheal rings precontracted with histamine, among them compounds (R)-**69**, **70** and (R,R)-**71** (Fig. 12) exhibited higher β_2-agonist potency than salmeterol (pEC_{50} = 8.3) [92a–b] their potency being comparable to that presented by (R,R)-formoterol (pEC_{50} = 9.9) [92c]. Additionally, the obtained results for PDE4 inhibitory activity also indicated that N-substitution of phthalazinone resulted in a significant increase in the PDE4 inhibitory potency being (R,R)-**71** (IC_{50} = 0.092 μM) the most active compound of these series [92].

3.4. Antihistaminic agents

Allergic rhinitis and allergic processes in general are mediated by histamine, an intracellular chemical messenger which is released from several cells and specifically by mast cells [93]. Four G-protein coupled receptor subtypes (H_1, H_2, H_3 and H_4) are currently recognized to modulate the histamine physiological effects, showing differences in their expression, mechanism of signal transduction and histamine-binding mode [94]. The H_1R is widely distributed throughout the CNS and peripheral tissues and performs a key role in regulating the inflammatory processes and CNS activity, such as wakefulness. Drugs that block H_1R subtype are considered first-line medication for allergic rhinitis [95]. The H_2R regulates gastric acid secretion and H_2 antagonists have been used to treat gastric ulceration. The histamine H_3R subtype is a presynaptic autoreceptor mainly located in distinct regions of CNS, where it modulates synthesis and release of histamine as well as release of several important neurotransmitters (serotonin, GABA, dopamine, acetylcholine, glutamate and noradrenalin etc.), and to a lesser extent in the peripheral nerve endings. Currently, the H_3R is recognized as a new target for the treatment of CNS disorders and H_3R antagonists may be considered as novel therapeutic agents for epilepsy, Parkinson and Alzheimer disease, attention deficit hyperactivity disorder etc [96]. The H_4R, more recently identified, seems to be restricted to various cells of immune system and mast cells [95] and H_4R antagonists may be effective candidates to treat inflammatory diseases. The potential clinical usefulness of H_4R antagonists in autoimmune diseases, like rheumatoid arthritis, has also been proposed [97].

It is noteworthy that antagonists for H_1R and H_2R subtype have been for many years blockbusters in therapy of allergic conditions and gastric ulcer, respectively, and that several H_3R and H_4R antagonists are currently in different stages of preclinical or clinical development [93].

A frequent side effect of classical H_1R antagonists is sedation, attributed to their ability to block this receptor subtype in the CNS,

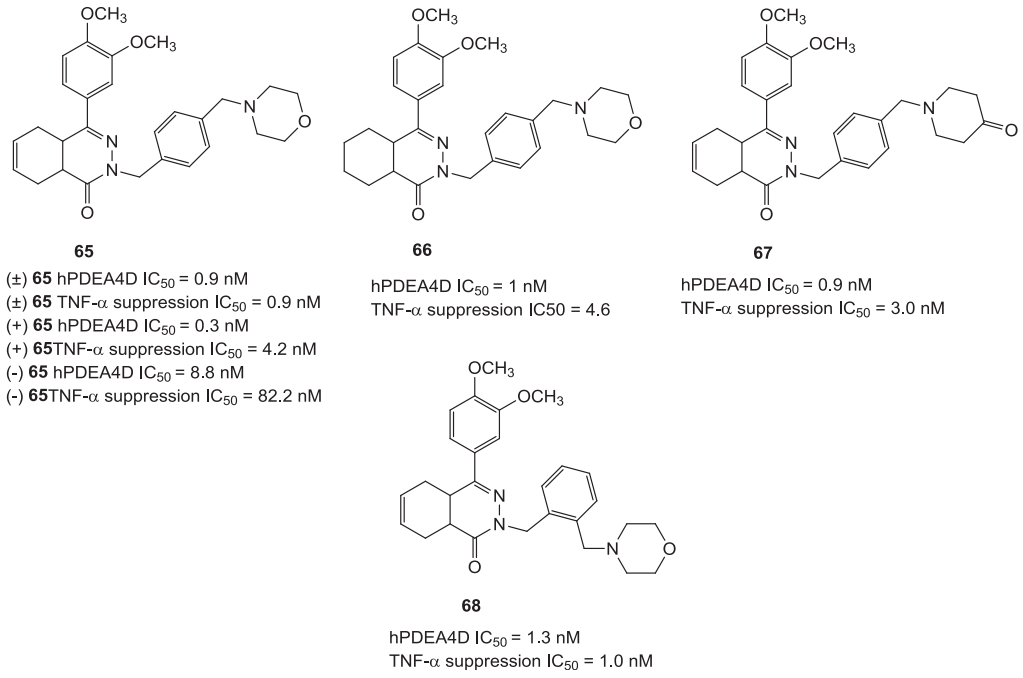


Fig. 11. Phthalazinone-derived both PDE4 and TNF-α inhibitors as antiasthmatic agents.

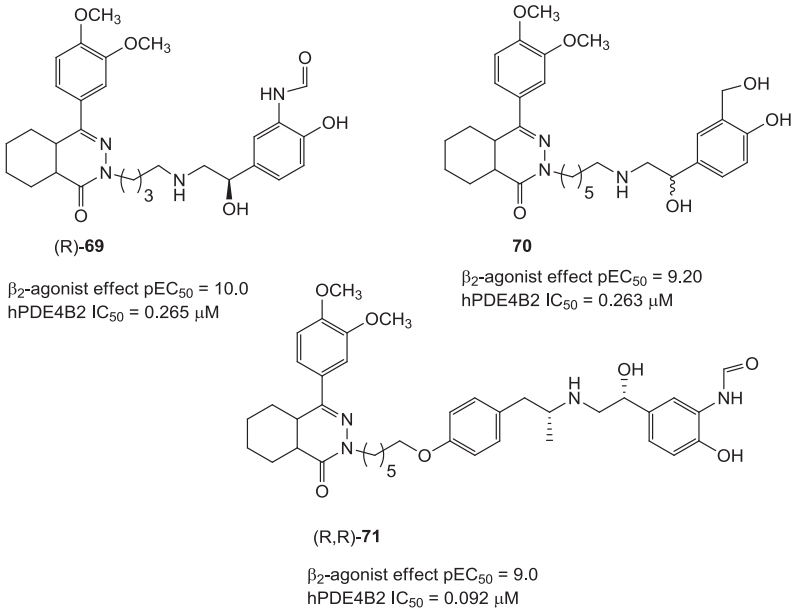


Fig. 12. Phthalazinone derivatives that target both β2-adrenoceptor and PDE4 as antiasthmatic agents.

which has encouraged the development of new orally or topically active drugs with limited access to CNS.

Most of the H_1R antagonist used clinically are diarylmethyl derivatives substituted with nitrogen-containing heterocyclic groups, including some phthalazinone analogues. Thus, the 4-(*p*-chlorobenzyl)-2-(hexahydro-1-methyl-1H-azepin-4-yl)-phthalazin-1(2H)-one [10] or azelastine (**40**, Figs. 9 and 13), a second generation of selective H_1R antagonist developed by ASTA-medica, is the recommended topical antihistamine therapy for allergic rhinitis [98], and the flezelastine (**72**, Fig. 13), which has also been developed for the same indication. The two phthalazinone analogues, azelastine and flezelastine, are chiral compounds and are resolved in their corresponding enantiomers. However, both compounds were developed as racemates because of the similar preclinical activity exhibited by their corresponding enantiomers [99].

The discovery that H_3R may play an important role in the modulation of sympathetic nasal vascular tone and nasal patency [100] and that a combined H_1R and H_3R blockade caused nasal decongestion [101] has promoted the development of dual antagonists. The first dual H_1/H_3 receptor antagonists were described in literature as the result of combining the chlorpheniramine, a known first generation H_1 antihistamine, with H_3 ligands of aza-heterocyclic structure like 4-imidazolylalkylamine pattern [102]. Searching for new H_1/H_3 dual antagonists suitable for intranasal

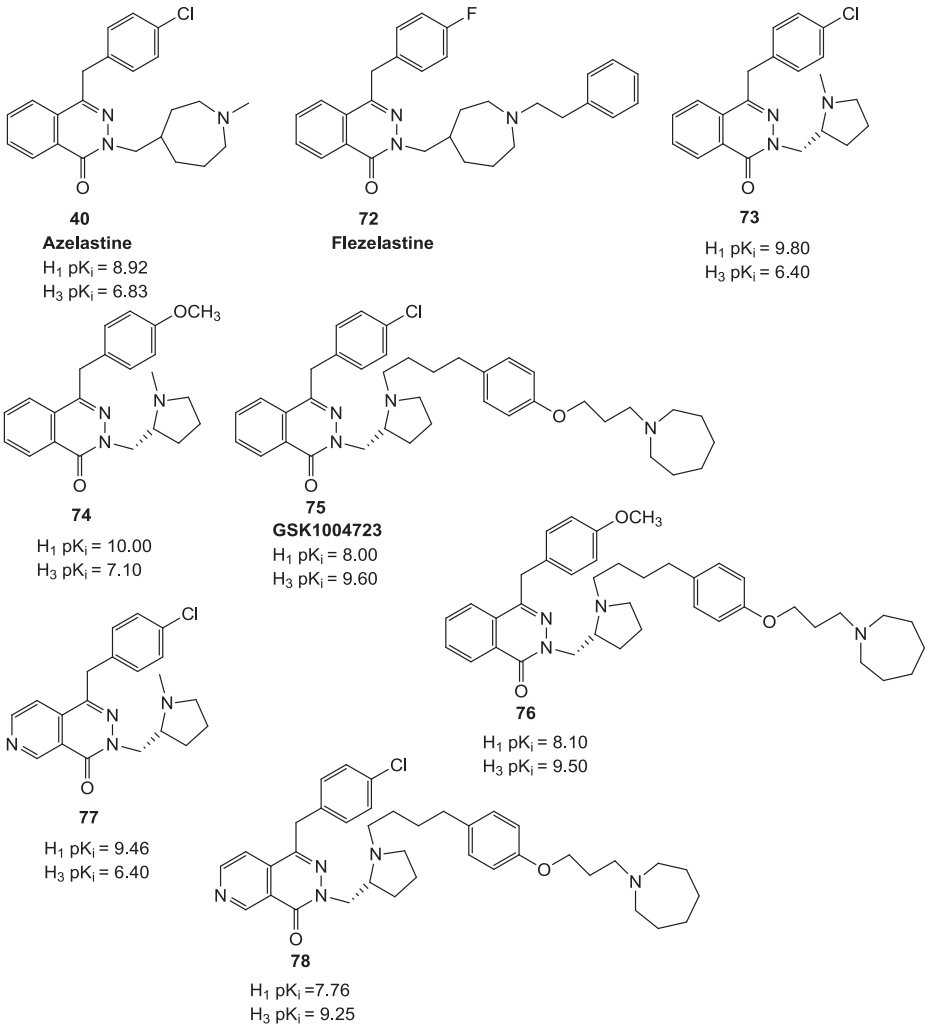


Fig. 13. Phthalazinone derivatives as antihistaminic agents.

administration as potential drugs for treatment of allergic rhinitis, a series of homochiral phthalazinone derivatives were recently described by researchers of the pharmaceutical company GlaxoSmithKline (GSK) [20]. The reported analogues were designed starting from azelastine structure and using homology modelling. Firstly and in order to maximize the H_1 potency the optimal N2 and C4 phthalazinone substitution was established, being the 2-pyrrolidinomethyl group at N2 and a *p*-substituted benzyl group at C4, like *p*-chlorobenzyl or *p*-methoxybenzyl, the preferred (compounds **73**, **74**, Fig. 13). Then, a phenoxypropylamino fragment associated with H_3 antagonism, such as the homopiperidinopropyloxyphenyl group [103], was linked to the basic nitrogen of azelastine type structure through an alkyl chain of variable length, ranged between one and four carbons. Compounds **75** and **76** (Fig. 13) were the best compounds of this series of H_1/H_3 bivalent ligands, both homochiral phthalazinone analogues showed a H_1 potency slightly lower than azelastine but greater H_3 potency than that of clinical standard, azelastine. On the basis of pharmacokinetic study results compound **75** (GSK1004723) was proposed as a candidate for further progression about intranasal treatment for allergic rhinitis.

The same researchers describe later different regioisomeric azaphthalazinone and diazaphthalazinone analogues of compound **73** as potent histamine H_1 antagonists [104]. As in previous work the combination of the most potent and selective core (6-azaphthalazinone analogue **77**) was used to append the homopiperidinopropyloxyphenyl group in order to develop H_1/H_3 dual ligands like compound **78** (Fig. 13) whose pharmacological profile was very similar to the GSK1004723 above-mentioned intranasal clinical candidate.

3.5. Antihypertensive agents

Hypertension is a leading risk factor for cardiovascular disease development like coronary disease, myocardial infarction or stroke being also a recognized contributor to sudden death as well as to congestive cardiac failure and renal insufficiency. Therefore, antihypertensive agents are of critical importance in order to achieve a better control of blood pressure. The actual antihypertensive therapy relies on the use of three main categories of agents, drugs inhibiting the renin-angiotensin system, such as angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers, calcium channel blockers, and diuretics [105]. In addition, drugs such as peripheral vasodilators, nitrates, α-adrenoceptor antagonists or β-adrenoceptor blockers are also useful in some clinical conditions [106].

Hydralazine (1-hydrazinophthalazine, **79**, Fig. 14), developed in the fifties, is a direct vasodilator useful to treat severe hypertension

in pregnant women [107]. This commercially available drug has been widely used as a lead for developing new antihypertensive compounds. In many cases, the new analogues contain the 6-arylpyridazin-3(2H)-one residue, which is considered as a pharmacophoric group for the cardiovascular activity [30]. However, some of them, such as compounds **80**, **81**, **82** and **83** (Fig. 14) are based on phthalazin-1(2H)-one scaffold [21a,35,108,109], which is described some decades ago as a hydralazine metabolite [110].

As mentioned above, compounds acting as selective α_1-adrenoceptor (α_1-AR) antagonists are useful to control blood pressure because of their vasodilator and negative inotropic effects. Many of α_1-AR antagonists contain in their structure an arylpiperazinyl group, and it is described that the link of this pharmacophore to different heterocycles, such as quinazolinone, pyrrolidinone, as well as pyridazinone or phthalazinone, through a polymethylene spacer provides selective α_1-AR antagonists [111,31c,112]. Compounds **84**, **85** and **86** (Fig. 15) are representative examples of 4-arylphthalazin-1(2H)-one analogues in which the phenylpiperazinealkyl was a key moiety for determining both affinity and selectivity toward α_1-AR [31c]. The phthalazinone scaffold was also successfully used in combination with the phenylpiperazine group to obtain β-adrenoceptor antagonists, blood pressure-lowering drugs recommended in patients with coronary heart disease and heart failure [105]. In this β-adrenoceptor antagonist, phthalazinone and phenylpiperazine groups, were connected through the 2-propanol spacer resulting in the 2-aminoethanol skeleton a typical structure of β-adrenoceptor blockers [31d].

3.6. Antiplatelet agents

Blood platelet aggregation is recognized as a primary event in the development of thrombotic events. Thrombus formation is a dynamic process involving a wide range of platelet receptors, signalling pathways and release of platelet proteins and inflammatory substances acting in a coordinated way with the vascular endothelium and other blood cells and coagulation factors. This process is the major pathological mechanism underlying atherothrombotic disorders, such as coronary artery disease, cerebrovascular disease or peripheral arterial disease [113]. Accordingly, platelet aggregation inhibitors (antiplatelets) are of great importance in prophylaxis and treatment of arterial thrombosis.

Among the compounds reported as antiplatelet agents a number of phthalazinone derivatives are included [21b,114,115,116], such as compound **87** and their congeners (Fig. 16). This compound provided to be an *in vitro* inhibitor of platelet aggregation induced by ADP and arachidonic acid (AA) [114]. Subsequent studies showed that the inclusion of phenyl groups at N2 of **87** resulted in derivatives which inhibited platelet aggregation induced by AA without affecting aggregation induced by ADP (compound **88**) [115]

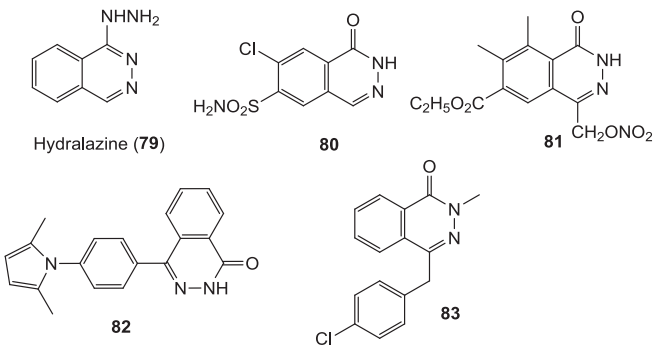


Fig. 14. Hydralazine and phthalazinone derivatives as antihypertensive agents.

and that this activity was markedly increased when the hydroxymethyl group at C4 was replaced by a hydrogen atom (compound **89**, Fig. 16) or by an ortho-substituted phenyl ring (compound **90**) [21b,116a]. However, the activity decreased when the carbonyl moiety was substituted by other stable moieties, like an ethoxy, ethylthio or alkylamino group [116a]. In addition, hydrogenation of 3,4-double bond of phthalazinone nucleus resulted in a complete loss of activity [21b].

Furthermore, in the 2-phenylphthalazinone series, 4-hydroxymethyl derivatives as well as analogues lacking this group at C4, the prototype compounds, 2-phenyl derivatives (compounds **88** and **89**), and the *ortho*-substituted 2-phenyl derivatives (compounds **91** and **92**, Fig. 16) showed the highest inhibitory activity. It was suggested that all these selective antiplatelet phthalazinones are in general inhibitors of cyclooxygenase enzyme like nonsteroidal antiinflammatory agents [21b].

3.7. Analgesic and anti-inflammatory agents

Pain is an unpleasant sensory and emotional experience associated with potential or actual tissue damage, or described in terms of such damage [117]. Pain represents a major symptom of many pathological conditions and millions of people worldwide must use analgesic agents in order to treat different types of pain. Non-steroidal anti-inflammatory drugs (NSAIDs) and opioids are the two main classes of analgesic drugs. The effects of NSAIDs are mediated by cyclooxygenase (COX) inhibition, an enzyme responsible for prostaglandin synthesis and for which two isoforms are described, COX-1 and COX-2 [118], while opioid effects are associated to activation of central G-protein coupled receptors μ, δ and κ [119].

The NSAIDs are generally used to treat mild or moderate pain whereas opioid drugs are used in the treatment of severe pain. Both classes of analgesic agents have important side effects that include gastrointestinal irritation and renal toxicity (NSAIDs), as well as respiratory depression, tachyphylaxis and physical dependence (opioids). In this regard, the search for new analgesic agents safer than traditional ones has attracted the attention of many researchers. Thus, the identification and characterization of COX-2, the inducible cyclooxygenase isoform involved in production of inflammatory prostanoids, led to the discovery of NSAIDs devoid of gastrointestinal problems [120]. The common structural feature for these selective NSAIDs was a diarylheterocyclic fragment. Many heterocyclic cores were investigated in order to develop safer analgesic agents [121], among them the phthalazinone scaffold [19,122].

Thus, several phthalazinone derivatives substituted at N2 with an acetamide or a propanamide side chain were described as potent antinociceptive (reducing sensitivity to painful stimuli) and anti-inflammatory agents [122]. The results indicated that in general propanamides were more potent than acetamides analogues, with compounds **93**, **94** and **95** (Fig. 17) showing the highest antinociceptive (p-benzoquinone-induced writhing test) and anti-inflammatory (carrageenan-induced hind paw oedema model) activities, which were comparable to aspirin and indomethacin, respectively, without any gastric lesion and bleeding and whose anti-inflammatory activity may be related to COX inhibition. A similar antinociceptive activity was described for some N2-dimethylaminoethyl derivatives of phthalazinone as well as for their pyridopyridazinone isosteres (compounds **96**, **97** and **98**) using hot-plate, tail-flick and writhing-like tests [19]. Compound **96** containing a phenyl attached to the 4-position of phthalazinone ring showed the highest activity; the removal of the phenyl group or its replacing by a methyl or a pyridyl group resulted in a reduced antinociceptive activity.

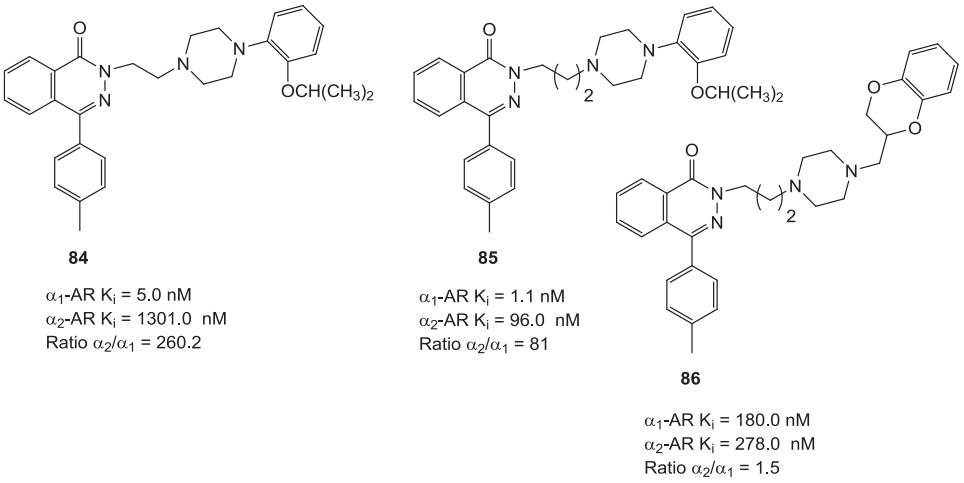


Fig. 15. Phthalazinone-derived α1-adrenoceptor antagonists as antihypertensive agents.

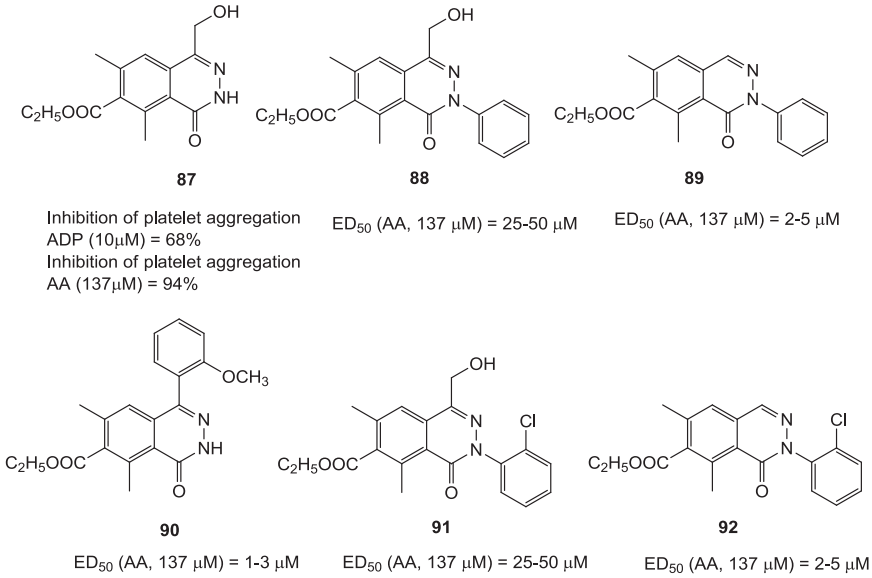


Fig. 16. Phthalazinone derivatives as antiplatelet agents.

Considering that the presence of an aryl substituted group at C4 of the phthalazinone nucleus is very important for both anti-inflammatory and antinociceptive activities, novel series of 4-arylphthalazin-1(2H)-one analogues showing at N2 different pharmacophoric groups, such as several azaheterocyclic groups, among them the oxadiazole and pyrazolone rings (compounds **99** and **100**, Fig. 17), different functional groups containing nitrogen, among them thiosemicarbazide, ethoxy imide, hydrazine hydrazide and phenylsulfonamide functions (compounds **101**, **102**, **103** and **104**, Fig. 17), were synthesized and their anti-inflammatory properties studied *in vivo* [123]. The compounds **99**–**103** exhibited an anti-inflammatory activity comparable to standard drug indomethacin in a sponge implantation test [123a]. Furthermore, some of the N2 phenylsulfonamide derivatives, such as compound **104**, showed significant anti-inflammatory activity which was comparable to celecoxib (selective inhibitor of COX-2) in a carrageenan-induced rat paw oedema model [123b]. The compound **104** was also a selective inhibitor of COX-2 with an IC_{50} value (6.0 μM) very similar to the celecoxib (IC_{50} = 6.5 μM) showing better selectivity index COX-1/COX-2 than the standard drug (333.33 vs. 325.00) [123b].

In addition, there are many examples of analgesic compounds bearing an aryl piperazinylalkyl moiety linked through alkyl chains to a cyclic hydrazide-like system whose antinociceptive effect is not related to acting on prostaglandins or opioid receptors [124], some of them being phthalazinone derivatives or isosteric analogues of bicyclic structure like isoxazolopyridazinone, pyrazolopyridazinone or pyridopyridazinone, such as compounds **105**, **106**, **107**, **108** and **109** (Fig. 17) [125], which showed an interesting antinociceptive activity in two different experimental models, writhing test and hot plate test. The biological results obtained indicated that compounds **105**–**109** were able to reduce by more than 50% the number of abdominal constrictions in writhing test, showing an efficacy comparable to that of morphine in the hot plate test. Furthermore, the structure–activity relationship data suggested that when the lactam-like nitrogen is substituted with the 3-chlorophenylpiperazinylpropyl group, both the fused ring (benzene, isoxazole, pyrazole and pyridine) and the group bonded to the pyridazine nucleus (H, methyl, phenyl and 2-thienyl) can be

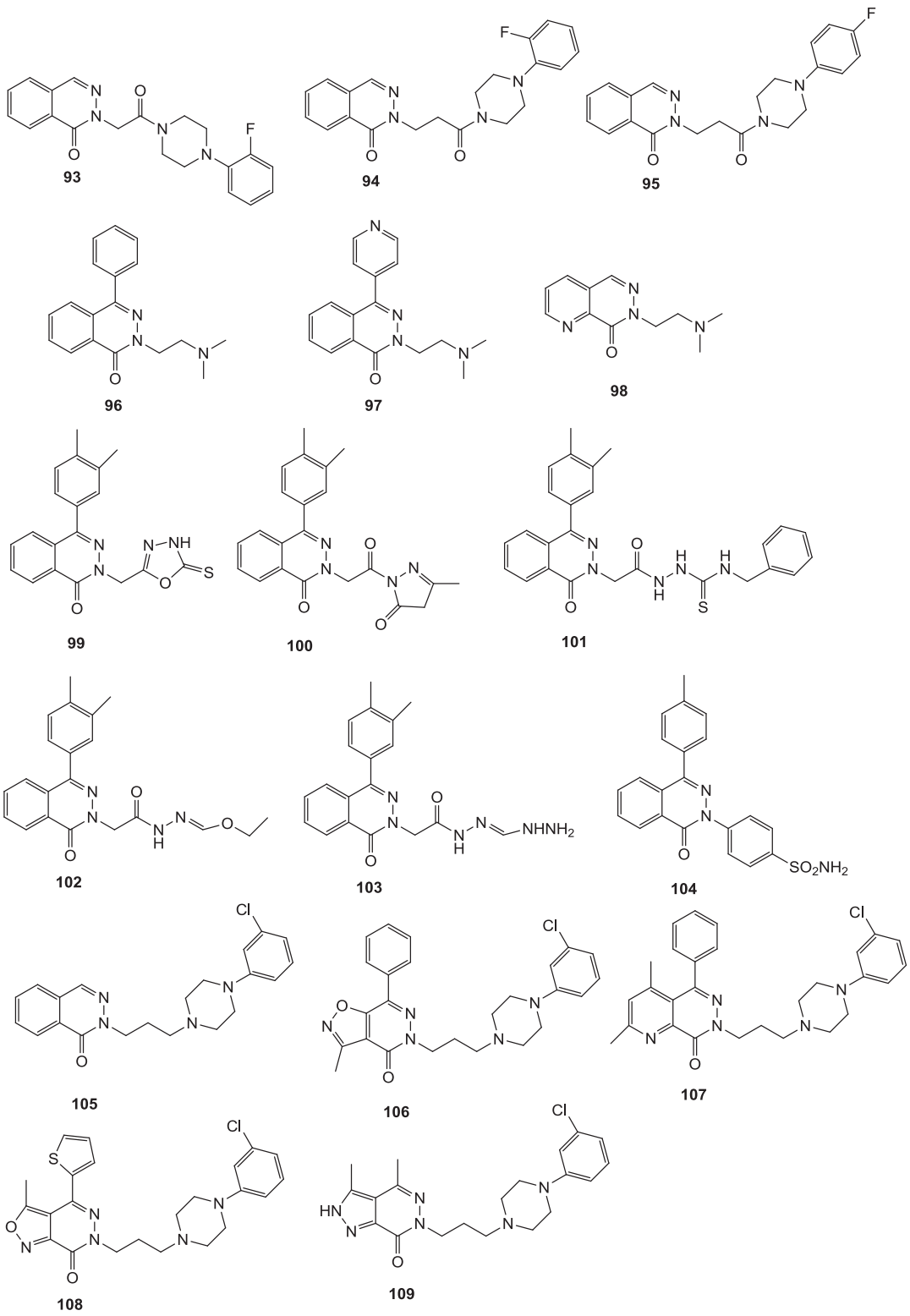


Fig. 17. Phthalazinone derivatives as analgesic and anti-inflammatory agents.

modified without significant loss of activity. The pharmacological study about these antinociceptive compounds indicates that their site of action is within the CNS and that their mechanism of action entail an amplifying adrenergic neurotransmission via noradrenaline reuptake inhibition, in which seems to be involved the α_2-adrenoceptor [125b], and therefore these compounds could be suitable to treat the neuropathic pain, a particular form of pain which is refractory to most of conventional painkillers.

Likewise, in more recent years antagonists of bradykinin receptors have been proposed as very promising agents for the treatment of pain [126]. Kinins are a family of natural peptides, including bradykinin, kallidin and some metabolites, which are

important biological mediators in cardiovascular homoeostasis, inflammation and nociception. They exert their biological effects through the interaction with two G-protein-coupled receptors, termed B1R and B2R, which show differences in their expression function and regulation. For instance, the B2R is ubiquitously expressed, whereas the B1R is expressed at very low level in healthy tissues but is rapidly induced by tissue injury or inflammation. These differences may explain their distinct functions in pain and inflammation. The B2R seems to play a significant role in early phases or acute state of inflammatory pain. In contrast B1R can be involved in the establishment and maintenance of chronic pain [126]. Accordingly, the B1R is considered an interesting therapeutic target for the treatment of chronic inflammatory and neuropathic pain.

Several peptides as well as a large number of small molecules were reported as selective B1R antagonist. The majority of small molecules can be classified as arylsulfonamides or amides [126]. The pharmacophore defined for the B1R antagonism suggests the presence of a hydrophobic unit on the left of the molecule, a hydrogen bond acceptor, a linker group and finally a basic domain on the right, frequently a bicyclic amine [127].

Taking these molecular features into account and searching for novel B1R antagonists a chemotype with improved pharmacokinetic (PK) properties and based on phthalazinone scaffold was recently discovered [128]. The phthalazinone analogues, that can be included in the amide group, emerged as a consequence of replacement of hydrogen bond acceptor sulfonamide with a cyclic carbonyl moiety. The new chemotype resulted in potent B1R antagonists, like compounds **110**, **111** and **112** (Fig. 18) with subnanomolar binding affinity to the human B1R and good cross-species PK properties.

3.8. Antidepressant agents

Depression represents a big health challenge in developed countries being a leading contributor to the global burden of disease. Currently, the recommended clinical guidelines to treat moderate to severe depression consist of psychological intervention combined with pharmacotherapy [129], in particular a selective serotonin reuptake inhibitor (SSRI) or a serotonin (5-HT) norepinephrine (NE) reuptake inhibitor (SNRI). It is recognized that 5-HT may be involved in depression and that increasing of 5-HT levels at post-synaptic sites in the brain by reuptake inhibition could be clinically useful, and SSRIs, showing high affinity towards the 5-HT transporter, are widely prescribed as antidepressant agents. *In vivo* pharmacological studies revealed that when animals are treated with SSRIs an increased 5-HT receptor-mediated cAMP signalling was initially observed and that upon repeated treatment this effect was reduced, since increased cAMP raises the expression of a number of PDE4 variants in neurons [130]. In addition, it is described that the combination of a PDEA4 selective inhibitor with an SSRI may be very effective for depression therapy because of the enhanced cAMP signal transduction pathway [131].

Under this hypothesis several SSRIs, such as (R)- and (S)-norfluoxetine as well as some furylalkylamines, were linked to the (±)-*cis*-tetrahydrophthalazinone scaffold, key pharmacophore for selective PDE4 inhibition [88,89], via a five carbon bridge in order to develop dual SSRI/PDE4 inhibitors [26,132]. The described compounds ((**R**)-**113**, (**S**)-**113**, **114** and **115**, Fig. 19) showed moderately potent but highly selective 5-HT re-uptake inhibition and significant inhibition of some recombinant PDE4 isoforms *in vitro*. In addition, the *in vivo* studies for acute and sub-chronic antidepressant-like effects using forced-swim test in mice indicated that compound **114** was more effective than fluoxetine, used as a standard drug [132].

3.9. Antimicrobial agents

Infectious diseases caused by microorganisms, bacteria, fungi and viruses have registered significant growth in recent years. The objective of microorganisms is to survive and many of them have determinants of resistance which can be expressed phenotypically as needed [133]. In spite of remarkable advances in antimicrobial therapy resistance of pathogenic bacteria towards available antimicrobial agents is becoming a serious threat to public health world-wide. In addition it is known the lack of selectivity for antifungal drugs because of the biochemical similarity between human and fungi cells. Therefore, there are many research works focused on antibacterial and antifungal compounds [134], some of them referred to the phthalazin-1(2H)-one scaffold.

Thus, the phthalazin-1(2H)-one nucleus was combined with different azole heterocycles recognized pharmacophores for antibacterial and antifungal activities, such as 1,2,4-triazole, 1,3,4-thiadiazole, 1,3,4-oxadiazole, thiazole, isoxazole or pyrazole, looking for compounds with increased antimicrobial activity [23,135–137]. Most of aforementioned compounds were

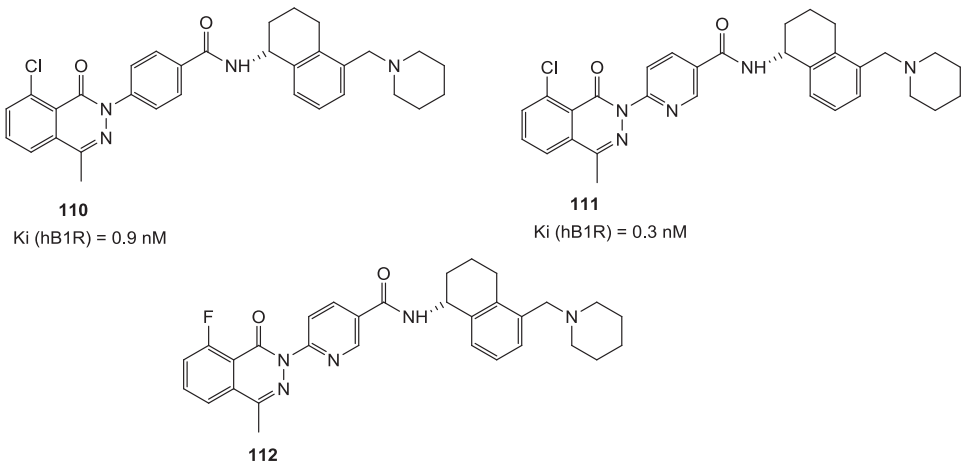


Fig. 18. Phthalazinone-derived B1R antagonists as potential analgesic and anti-inflammatory agents.

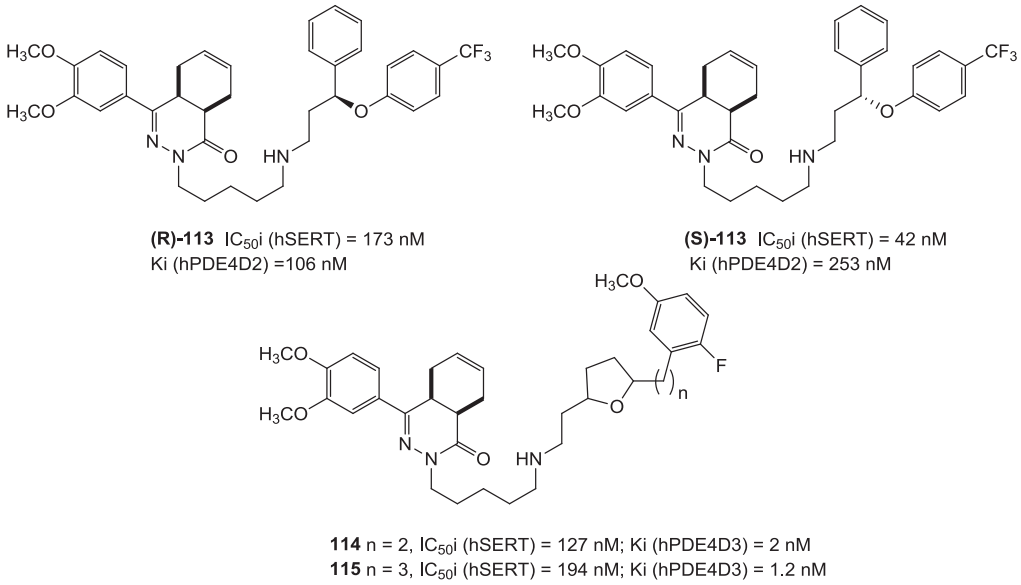


Fig. 19. Phthalazinone-derived dual SSRI/PDE4 inhibitors as potential antidepressant agents.

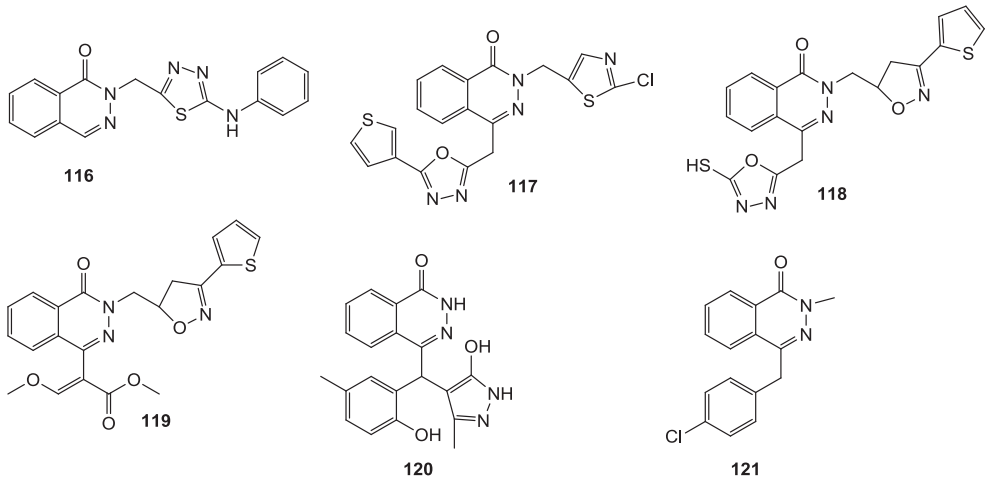


Fig. 20. Phthalazinone derivatives as antimicrobial agents.

investigated *in vitro* against several Gram-positive and Gram-negative bacteria as well as yeast and dermathophyte-like fungi.

Some analogues carrying a 5-arylamino-1,2,4-thiadiazole group linked at N2 of phthalazinone nucleus by a methyl or ethyl chain showed activity against *Bacillus subtilis* (Gram-positive bacteria) and yeast-like fungi as *Candida albicans* and *Candida parapsilosis*, with compound **116** (Fig. 20) being the most active of this series [135].

In the phthalazine-oxadiazole series the azole ring was linked at C4 of phthalazinone nucleus through a methylene group and an additional heterocyclic moiety was included at N2. The data of their antimicrobial activity revealed the phthalazinone analogue **117**, with a 2-chloro-1,3-thiazol-5-ylmethyl substitution at N2 [23], and the analogue **118**, substituted at N2 with a 3-(2-thienylisoxazolin-5-yl) group [136a], as the most interesting of these series, showing activity against all studied microorganisms, bacteria and fungi, although they were significantly less potent than the positive controls used. Similar results were obtained when the 3-(2-thienylisoxazolin-5-yl) group was incorporated into a phthalazinone core containing a β-methoxyacrylate fragment at C4 (compound **119**) [136b].

More series of polysubstituted phthalazinone derivatives with significant antibacterial or antifungal activities were recently reported [38b,137], a substituted benzyl group located at C4 of phthalazinone scaffold is the common structural feature of these new antimicrobial (compounds **120** and **121**, Fig. 20).

4. Conclusion

Phthalazinone because of its capability to interact with different kinds of biological targets represents a very attractive scaffold in Medicinal Chemistry. Many bioactive compounds exhibiting a variety of pharmacological effects, such as anticancer, antidiabetic, antiasthmatic, antihistaminic, antihypertensive, antithrombotic, anti-inflammatory, analgesic, antidepressant or antimicrobial activities show the phthalazinone core in their structure and therefore synthesis of phthalazinone derivatives can be a promising way for new lead compounds.

This review summarizes detailed and updated information described in recent non-patent literature, about the most relevant pharmacological properties of phthalazinone derivatives, highlighting the structural requirement of phthalazinone derivatives for their interaction with several targets or for their pharmacological activities in order to give useful information for designing new analogues.

Acknowledgements

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