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Review Article

Brief Overview on Biological Potential of Various Substituted Phthalazine and Phthalazinone Analogues

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Abstract

Various phthalazine derivatives have been used as therapeutic agents such as antimicrobial, anticonvulsant, cardiotonic, antihypertensive, anti-inflammatory, antitumor, anti-virus, antiallergic; antifungal; anti-proliferative, COX-2 and LOX-5 inhibitors; anti-obesity; anti-diabetes and other properties useful biological activities. Some phthalazine derivatives are used as marketed drug Like azelastine has antihistaminic activity in the treatment of allergic rhinitis, and hydralazine is used as antihypertensive drug etc. Various methods have been developed for synthesized different pthalazine derivatives in excellent yields and the compounds were tested for their different types of biological activities. This review is high light various investigated biological activities in chronically.

Keywords: Antitumor; antimicrobial, antivirus; antiallergic; anti-inflammatory; analgesic, pthalazines, phthalazinone.

Introduction

Phthalazine derivatives, like the other members of the isomeric benzodiazine series, have been widely applied as therapeutic agents due to their anticonvulsant, cardiotonic, vasorelaxant and antiinflammatory properties [1, 2]. Heterocyclic compounds containing nitrogen group have large area in nature, and their utilizationis becoming progressively important as biologically active pharmaceuticals, agrochemicals, and functional materials. In particular, hydrazines containing heterocyclic compounds have been considered of great importance on account of pharmacological activities and clinical applications. These of combined phthalazines have biological properties like inhibition of p38MAPkinase for selective binding of GABA receptor, antianxiety drug, and antitumor agents [3-5]. Phthalazine derivatives have been greatly used as therapeutic agents owing to their antimicrobial, anticonvulsant, cardiotonic, vasorelaxant, anti-inflammatory, antitumor; PARP-1 inhibitors; anti-dengue virus and poly-(phthalazinone ether sulfone ketone) (PPESK); Histamine H1 receptor antagonist; allergic rhinitis; antifungal; imaging agents; anti-proliferative activity; COX-2 and LOX-5 inhibitors; anti-obesity; anti-Type-2 diabetes properties [1, 6-9]. Like azelastine, the phthalazine derivatives have antihistaminic effects in the treatment of allergic rhinitis, and hydralazine is used as antihypertensive drug in the therapy of pulmonary hypertension [10, 11]. Pthalazines are synthetically versatile moiety and hence can be used for the synthesis of a large variety of heterocyclic compounds. Pthalazines occupy a distinct and unique place in our life. This hetero cyclic moiety has great biological and medicinal significance. Various synthetic aspects indicate that pthalazines derivatives are easy to synthesize which can produce a wide variety of activity. Encouraged by the diverse biological activities of pthalazine compounds, it was decided to prepare a new series of Pthalazines derivatives. Some commercially used phthalazine drugs are shown in Figure 1.

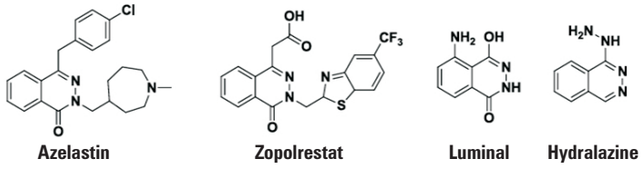
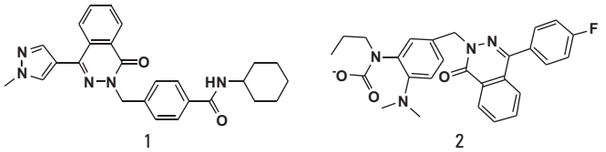


Figure 1: Some commercially used phthalazine derivatives & Structure of Pthalazine

Pharmacological potential of phalazinines

A series of 2,4-disubstituted phthalazinones were tested for their antiproliferation, inhibition against Aurora kinases and cell cycle effects. Among them, N-cyclohexyl-4-((4-(1-methyl-1H-pyrazol-4-yl)-1-oxophthalazin-2(1H)-yl)methyl) benzamide (1) was exhibited the most potent antiproliferative activity against five carcinoma cell lines (HeLa, A549, HepG2, LoVo and HCT116 cells) with IC50 values (2.2-4.6μM), as compare to standard drug VX-680 (8.5-15.3μM). Aurora kinase assays exhibited that this compound was potent inhibitor of AurA and AurB kinase with the IC50 values were 118±8.1 and 80±4.2nM, respectively. Molecular docking result showed that this compound forms better interaction with both AurA and AurB. This compound induced G2/M cell cycle arrest in HeLa cells by regulating protein levels of cyclinB1 and cdc2. The results suggested that this compound is a promising pan-Aurora kinase inhibitor for the potential treatment of cancer [12]. Using a dengue replicon cell line-based testing, 3-(dimethylamino) propyl(3-((4-(4-fluorophenyl)-1-oxophthalazin-2(1H)-yl) methyl)phenyl)carbamate (2) act as a potent DENV-2 inhibitor, with an IC50 value of 0.64μM. A series of phthalazinone derivatives based on hit 2 were tested for their in vitro anti-DENV activity and cytotoxicity. The subsequent structure activity relationship (SAR) study and optimization led to the discovery of the most promising compound, which exhibited potent anti-DENV-2 activity, with low IC50 value against DENV-2 RNA replication of 0.13μM and high selectivity (SI=89.2) with appropriate pharmacokinetics profile [13].



During liver development, nonpolarized hepatic progenitor cells differentiate into mature hepatocytes with different polarity. This polarity is vital for sustaining the intrinsic properties of hepatocytes. The balance between the epithelial-mesenchymal transition (EMT)

and mesenchymal-epithelial transition (MET) acts a decisive role in differentiation of polarized hepatocytes. The phthalazinone pyrazole (PP) is a selective inhibitor of Aurora-A kinase (Aurora-A), suppressed the EMT during the differentiation of hepatocyte-like cells (HLCs) from human embryonic stem cells. The differentiated HLCs treated with PP at the hepatoblast stage exhibited enhanced hepatic morphology and functions, generally with regard to the expression of drug metabolizing enzymes. The result provided insights into the regulatory role of the EMT on in vitro hepatic maturation, suggesting that inhibition of the EMT may drive transformation of hepatoblast cells into mature and polarized HLCs [14].

Two series of 4-phenylphthalazin-1-ones and 4-benzylphthalazin-1-ones compounds were tested as anti-lung adenocarcinoma agents with potential inhibitory activity against PARP-1. All the phthalazinones were tested for their anti-proliferative activity against A549 lung carcinoma cell line. Some phthalazinone compounds were showed significant cytotoxic activity against A549 cells at different concentrations (0.1, 1 and 10μM) for two time intervals (24h and 48h). These phthalazinones were also tested for their inhibitory activity towards PARP-1. One of the compound emerged as the most potent PARP-1 inhibitor with IC50 value of 97nM, compared to that of Olaparib (IC50＝139nM). These phthalazinones passed the filters of Lipinski and Veber rules, and predicted to have good pharmacokinetics profile. Western blotting in A549 cells revealed the enhanced expression of the cleaved PARP-1, alongside, with the reduced expression of pro-caspase-3 and phosphorylated AKT. The ELISA assay confirmed the up-regulation of active caspase-3 and caspase-9 levels, suggesting the activation of the apoptotic machinery in the A549 cells [15]. The amide-containing phthalazinone H1 histamine receptor antagonists are described. Some analogues were equipotent with azelastine and were longer-acting in vitro. Few analogues had low oral bioavailability, low brain-penetration, high metabolic clearance, and long duration of action in vivo, and it was suitable for once-daily dosing intranasally, with a predicted dose for humans of approximately 0.5 mg per day [16]. Four phthalazinones (22334057, 22333974, 22334032, 22334012) were exhibited a potent enhancers of antifungal activity of fluconazole against Candida albicans. Some even more potent analogues of these compounds were identified, some with EC50 as low as 1 nM, against C. albicans. These compounds were exhibited pharmacological synergy (FIC＜0.5) with fluconazole and also shown enhance antifungal activity of isavuconazole, an azole antifungal drug. Some Phthalazinone were shown to be active against several resistant clinical isolates of C. albicans [17].

Phthalazinone derivatives were designed as optical probes for one- and two-photon fluorescence microscopy imaging. The design strategy involves stepwise extension and change of pyridazinone by 1) expansion of pyridazinone to phthalazinone, a larger conjugated system, as the electron acceptor, 2) coupling of electron-donating aromatic groups such as N,N-diethylaminophenyl, thienyl, naphthyl, and quinolyl to the phthalazinone, and 3) anchoring of an alkyl chain to the phthalazinone with various terminal substituents such as triphenylphosphonio, morpholino, triethylammonio, N-methylimidazolio, pyrrolidino, and piperidino. The desired fluorescent probes were synthesized by two different routes in considerable yields. Twenty-two phthalazinone derivatives were for their photophysical properties were measured. Selected compounds were applied in cell imaging, and valuable information was obtained. The designed compounds showed excellent performance in two-photon microscopic imaging of mouse brain slices [18]. A series of proteasome inhibitors using pyridazinone as initial scaffold, and extended the structure with rational design by computer aided drug design (CADD). Two different synthetic routes were explored and the biological testing of the phthalazinone derivatives was tested. Importantly, electron positive triphenylphosphine group was first introduced in the structure of proteasome inhibitors and potent inhibition was achieved [19].

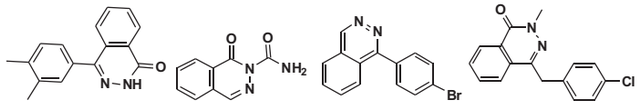
Phthalazinethione has been synthesized and its behavior towards hydrazine hydrate, oxidizing agent and ethyl chloroacetate has been tested. The synthesized compounds were characterized. The antimicrobial, the cytotoxic, and the antioxidant activities of some of the compounds were tested. Some of the compounds showed very strong cytotoxic activity as compare to the standard [20].

Synthesize a library of phthalazine derivatives and to test their anti-inflammatory and anti-proliferative activities. Sixteen phthalazinone derivatives were tested for their in-vitro antiproliferative and in-vivo anti-inflammatory activities. Two compounds, showed significant anti-inflammatory activity comparable to the standard drug etoricoxib in the carrageenan-induced rat paw edema. Three compounds were showed moderate sensitivity toward the renal cancer cell line UO-31[21]. Inflammation is a natural reaction of our body in response to infection or any other injury to renovate that damage. The majority of the available Non-steroidal anti-inflammatory drugs (NSAIDs) is nonselective and consequently, causes gastric irritation and ulceration. Therefore, it is design a series of NSAIDs with minimal gastric complications. A series of 4-(3,4-dimethylphenyl)-2(1H)-phthalazinone derivatives (3) were designed and tested for their in vivo anti-inflammatory activity. The compounds that showed powerful anti-inflammatory activities were assessed for their in vitro COX-1/COX-2 inhibitory activity and in vivo ulcerogenic profile. The interaction between the designated compounds and the binding pocket of the COX-2 enzyme was predicted by molecular docking stimulation. Some compounds were exhibited significant anti-inflammatory activities as compared to standard drug celecoxib. Few compounds were the most potent and selective COX-2 inhibitors. Moreover, all the tested compounds exhibited higher gastric safety profile compared to celecoxib. A series of phthalazinone derivatives were successfully tested for their in vivo anti-inflammatory activity. Some compounds presented powerful anti-inflammatory activity compared to celecoxib. Moreover, few compounds were the most potent inhibitors to COX-2 and were inactive to COX-1. The screened compounds showed better ulcer protection and less gastric lesion compared to celecoxib. Some Compounds were promising candidate with more gastric safety [22].

The androgen receptor (AR) plays important roles in multiple physiological functions, including differentiation, growth, and maintenance of male reproductive organs, and also has effects on hair and skin. The synthesis of nonsteroidal AR antagonists having a 4-benzyl-1-(2H)-phthalazinone skeleton, compounds, with two ortho-substituents on the phenyl group potently inhibited SC-3 cell proliferation (IC50: 0.18 μM) and showed high wt AR-binding affinity (IC50: 10.9 μM), comparable to that of hydroxyflutamide. This compound also inhibited proliferation of LNCaP cells containing T877A-mutated AR. Docking study of compound with the AR ligand-binding domain indicated that the benzyl group is vital for the antagonism. These phthalazinone derivatives may be useful for testing potential clinical applications of AR antagonists [23].

Discover orally active small molecules that stimulate glucose uptake, high throughput screening of a library of 5000 drug-like compounds was conducted in differentiated skeletal muscle cells in presence of insulin. N-Substituted phthalazinone acetamide (4) was identified as a potential glucose uptake modulator. Several derivatives were establishing structure activity relationships. Identified lead thiazolyl-phthalazinone acetamide (7114863) increased glucose uptake (EC50 of 0.07±0.02 μM) in differentiated skeletal muscle cells in presence of insulin. Furthermore, 7114863 was superior to rosiglitazone under similar experimental conditions without inducing PPAR-γ agonist activity thus making it a very interesting scaffold [24]. The 4-(4-bromophenyl)phthalazine derivatives (5) connected via an alkyl spacer to amine or N-substituted piperazine were designed and synthesized as promising α-adrenoceptor antagonists. Twelve of the tested compounds exhibited significant α-blocking activity.

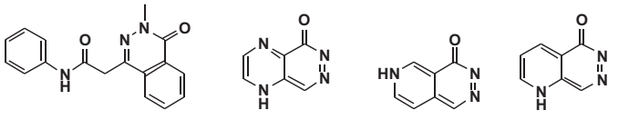
Molecular modeling studies were carried out to rationalize the biological results. Among the tested compounds, one compound displayed the best-fitting score and the highest in vitro activity [25]. Twenty-five poly substituted phthalazinone derivatives were tested for their antifungal activity against yeasts and filamentous fungi. Compound 4-(4-chlorobenzyl)-2-methylphthalazin-1(2H)-one (6) exhibited a significant antifungal activity against strains of dermatophytes and Cryptococcus neoformans, as well as against some clinical isolates. A physicochemical study on compound 6 is providing us with useful data for the future design of novel related antifungal analogues [26].



(3) (4) (5) (6)

The designs of pyridazinone and phthalazinone derivatives were tested on a panel of four kinases in order to test their activity and potential selectivity. In addition, the promising compounds were tested on four cancer cell lines to examine cytotoxic effects and inhibited DYRK1A and GSK3 with different activity. SAR analysis and docking calculations were carried out to aid in the interpretation of the results. The findings suggest that pyridazinone and phthalazinone scaffolds are interesting starting points for design of potent GSK3 and DYRK1A inhibitors [27]. Poly(phthalazinone-ether-sulfone) (PPES) polymer is a newly developed material with a bis(4-fluorodiphenyl) sulfone group. The effects of PPES concentration and two additives, polyvinylpyrrolidone (PVP) and oxalic acid (OA), on the apparent viscosity and gelation rate of PPESK/NMP solutions and membrane performance have also been investigated. It was found that the gelation rate is important to obtain a sponge-like membrane structure, however favored by a fast gelation rate. The membrane obtained by a fast gelation rate showed a high pure water flux and rejection of bovine serum albumin (BSA), contrary to previous findings. The actual membrane structure and pure water flux were related, and in agreement with the optical micrograph and gelation rate. The results provide a fundamental insight in this copolymer, useful in future applications, especially in the membrane formation process [28].

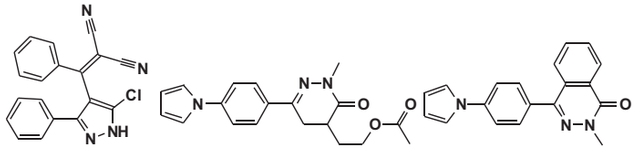
Cryptosporidium parvum (Cp) is a potential biowarfare agent and major cause of diarrhea and malnutrition. This protozoan parasite relies on inosine 5'-monophosphate dehydrogenase (IMPDH) for the production of guanine nucleotides. A CpIMPDH-selective N-aryl-3,4-dihydro-3-methyl-4-oxo-1-phthalazineacetamide (7) inhibitor was identified in a high throughput screening (HTS) campaign. In addition, the antiparasitic activity of select analogs in a Toxoplasma gondii model of C. parvum infection is also presented [29]. The 5-Aza, 6-aza, 7-aza and 8-aza-phthalazinone, and 5,8-diazaphthalazinone templates were formed by stereoselective routes. All four mono-azaphthalazinone series had higher affinity (pKi) for the human H-1 receptor than azelastine, but were not as potent as the parent non-aza phthalazinone. The 5,8-diazaphthalazinone (8) was equipotent with azelastine. The least potent series were the 7-azaphthalazinones (9), whereas the 5-azaphthalazinones (10) were the most lipophilic. The more hydrophilic series were the 8-aza series. Replacement of the N-methyl substituent on the pyrrolidine with the n-butyl group caused an increase in potency (pA2) and a corresponding increase in lipophilicity. Introduction of β-ether oxygen in the n-butyl analogues (2-methoxy-ethyl group) reduced the H-1 pA2 slightly, and increased the selectivity against hERG. The duration of action in vitro was longer in the 6-azaphthalazinone series. The more potent and selective 6-azaphthalazinone core was used to append an H3-receptor antagonist fragment, and to convert the series into the long acting single-ligand, dual H-1,H-3 receptor antagonist [30].



(7) (8) (9) (10)

The bradykinin B1 receptor is rapidly induced upon tissue injury and inflammation, stimulating the production of inflammatory mediators resulting in plasma extravasation, leukocyte trafficking, edema, and pain. Sulfonamide and sulfone-based B1 antagonists containing a privileged bicyclic amine moiety leading to potent series of 2-oxopiperazines. The oxopiperazine sulfonamides led us to seek B1 antagonists with improved drug like properties. Designed a series of amide antagonists with targeted physicochemical properties and led to a novel series of potent phthalazinone B1 antagonists, where we successfully replaced a sulfonamide acceptor with a cyclic carbonyl unit. Compounds with subnanomolar B1 binding affinity, demonstrate excellent cross-species PK properties with high oral bioavailability and potent activity in a rabbit biochemical challenge pharmacodynamic study [31]. A series of potent phthalazinone-based human H1 and H3 bivalent histamine receptor antagonists, suitable for intranasal administration for the potential treatment of allergic rhinitis, were identified. Blockade of H3 receptors is thought to improve efficacy on nasal congestion, a symptom of allergic rhinitis that is currently not treated by current antihistamines. Some analogues had slightly lower H-1 and H-3 potency than azelastine. One compound had longer duration of action than azelastine, low brain penetration, and low oral bioavailability, which coupled with the predicted low clinical dose, should limit the potential of engaging CNS-related side-effects associated with H1 or H3 antagonism [32]. Cultures of Aspergillus niger NRRL-599 in fluid Sabouraud medium were grown with phthalazine for 7 days. Phthalazine was oxidized to 1-phthalazinone [33]. The inhibition of Aurora kinases in order to arrest mitosis and subsequently inhibit tumor growth via apoptosis of proliferating cells. A class of Aurora kinase inhibitors based upon a phthalazinone pyrazole scaffold, resulted in a potent Aurora-A selective series of compounds (typically >1000-fold selectivity over Aurora-B) that display good pharmacological profiles with significantly bioavailability compared to the Aurora inhibitor VX-680 [34]. Inhibitors of phosphodiesterase-4 (PDE4) is an important class of anti-inflammatory drug that act by inhibiting the production of proinflammatory cytokines, including tumor necrosis factor-alpha (TNF-α) and tested a series of 2-substituted phthalazinone derivatives as PDE4 inhibitors. The SAR studies led to the identification of benzylamine-substituted phthalazinones as potent PDE-4 inhibitors that also suppressed TNF-α formation by whole rat blood cells [35]. 2-(5-Chloro-1,3-diphenyl-1H-pyrazol-4-ylmethylene)-malononitrile (11) reacts with the arylidenes of malononitrile to afford the triaryl-5-chloropyrazoles, respectively. Compound 11 reacts with the active methylene pyrazolinones to afford different products depending on the substitution in the pyrazole ring. Compound 11 reacts also with the pyridazinone derivative to afford the phthalazinone and with the thiazolinones afford the pyrano[2,3-d]thiazoles, respectively. It reacts also with the malononitrile dimer and with ethyl cyanoacetate dimer to yield the pyrazolyl pyridines respectively. The synthesized compounds showed a moderate molluscicidal activity towards Biomphalaria alexandrina snails [36]. Several phthalazinone derivatives were synthesized for their vasorelaxant activity and measured on isolated rat aorta rings pre-contracted with phenylephrine (10-5M). Some phthalazinones attained, the total relaxation of the organ at micromolar concentrations. For the most potent compound 9h (EC50=0.43μM) the affinities for α1A, α1B and α1D adrenergic sub-receptors were determined [37]. Discovery of poly(ADP-

ribose)polymerase-1 (PARP-1) inhibitors based on a phthalazinone scaffold, subsequent optimisation of inhibitory activity, metabolic stability and pharmacokinetic parameters has led to a series of meta-substituted 4-benzyl-2H-phthalazin-1-one PARP-1 inhibitors which retain low nM cellular activity and show good stability in vivo and efficacy in cell based models [38]. Some 2-nonsubstituted/2-methyl-/2-(2-acetyloxyethyl)-6-[4-(substitutedpyrrol-1-yl)phenyl]-4,5-dihydro-3(2H)-pyridazinone derivatives (12) and 2-nonsubstituted/2-methyl-4-[4-(substituted pyrrol-1-yl)phenyl]-1(2H)-phthalazinone derivatives (13) were synthesised by reacting hexan-2,5-dion or 1-aryl-3-carbethoxypent-1,4-diones with corresponding 2-substituted/nonsubstituted 6-(4'-aminophenyl)-4,5-dihydro-3(2H)-pyridazinone (14) or 2-substituted/nonsubstituted-4-(4'-aminophenyl)-(2H)-phthalazinone (15) under Paal-Knorr pyrrole synthesis conditions. The antihypertensive activities of the compounds were examined both in vitro and in vivo. Some pyridazinone derivatives showed appreciable activity. The 6-[(4-arylidene-2-phenyl-5-oxoimidazolin-1-yl)phenyl]-4,5-dihydro-3(2H)-pyridazinone (16) and 4-[(4-arylidene-2-phenyl-5-oxoimidazolin-1-yl)phenyl]-1(2H) phthalazinone derivatives were prepared by reacting 6-(4-aminophenyl)-4,5-dihydro-3(2H)-pyridazinone (17) or 4-(4-aminophenyl)-1(2H)-phthalazinone (18) compound with different 4-arylidene-2-phenyl-5(4H)-oxazolone derivatives. The vasodilator activities of the compounds were examined both in vitro and in vivo. Some pyridazinone derivatives showed appreciable activity [9, 39].



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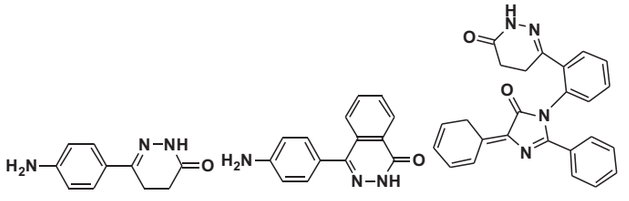

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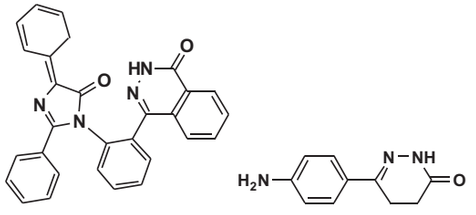
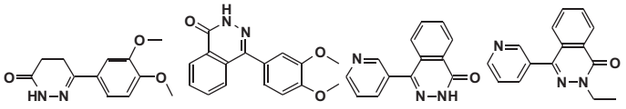


(17) (18)

A series of 5-[4-[2-[substituted phthalazinones-2(or 4)yl]ethoxy]phenylmethyl]thiazolidine-2,4-diones and 5-[4-[2-[2,3-benzoxazine-4-one-2-yl]ethoxy]phenylmethyl]thiazolidine-2,4-diones and their plasma glucose and plasma triglyceride lowering activity in db/db mice. In-vitro PPARγ transactivation assay was performed in HEK 293T cells. In vitro and in vivo pharmacological studies showed that the phthalazinone analogue has better activity. These compounds were showed better in vitro PPAR trans activation potential than troglitazone and pioglitazone. Subchronic toxicity study in Wistar rats did not show any treatment-related adverse effect [40]. Some 6-(3,4-dimethoxyphenyl)-4,5-dihydro-2H-pyridazin-3-ones (19) and 4-(3,4-dimethoxy-phenyl)-2H-phthalazin-1-ones (20) were ested on the cGMP-inhibited phosphodiesterase (PDE-3) and cAMP-specific PDE-4 enzymes. Most compounds were found to specifically inhibit PDE4, which showed moderate PDE4 (pIC50 = 6.5) as well as PDE3 (pIC50 = 6.6) inhibitory activity. In both the pyridazinone and phthlazinone series it was found that N-substitution is beneficial for PDE-4 inhibition, whereas in the pyridazinone series it also accounts for PDE-4 selectivity. In the phthalazinone series, the cis-4a,5,6,7,8,8a-hexahydrophthalazinones and their corresponding 4a,5,8,8a-tetrahydro analogues were showed potent PDE-4 inhibitory potency (10/11c,d: pIC50 =7.6-8.4) [41]. A series of 4-(3-pyridyl)-1(2H)-phthalazinone derivatives (21) possess dual activities of thromboxane A2 (TXA2) synthetase inhibition and bronchodilation. While the length and the bulk of 2-alkyl substituents had no influence on either activity, the 2-substituents with polar groups reduced bronchodilatory activity. Furthermore, introduced heteroaromatic nuclei into the 4-position of the phthalazinone and found that 1-imidazolyl and 5-thiazolyl derivatives were as active as the parent 3-pyridyl compound. The heteroaromatic nuclei at the 4-position of phthalazinones are play a vital role in TXA2 synthetase inhibition. Hydrophobicity of the compounds was found to exert a marked influence on bronchodilatory activity. These remarks led to the selection of 2-ethyl-4-(3-pyridyl)-1(2H)-phthalazinone (22) (KK-505) and 2-methyl-4-(5-thiazolyl)-1(2H)-phthalazinone (23) (KK-562) for further studies. Although their precise mechanism of action remains unclear, this series of novel phthalazinone derivatives represents a class of antiasthma agents with dual activities [42]. Various 4-substituted 2-[omega-(1-imidazolyl)alkyl]-1(2H)-phthalazinones (24) were possessing both thromboxane A2 synthetase inhibitory and bronchodilatory activities. These compounds disclosed that they have both activities to various extents. Both activities were slightly dependent on the length of the 2-substituents and largely affected by the nature of the 4-substituents. Compounds bearing phenyl and thienyl groups exhibited relatively high and well-rounded activities. Some compounds were found to be the most effective agents having well-rounded activities in vitro and in vivo. Introduction of a carboxyl group reduced both activities contrary to our expectation. 4-(3-Pyridyl)phthalazinone (25) was of particular interest because of unexpectedly high in vivo activities in spite of an absence of significant in vitro activities [43].



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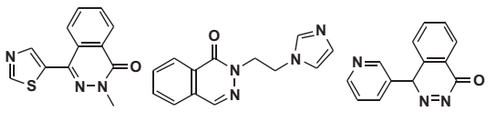
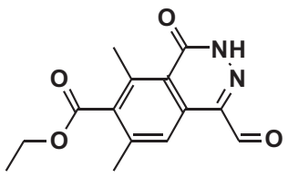


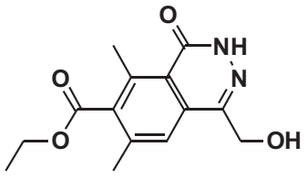
(23) (24) (25)

In female NMRI mice, the phthalazinone azelastine was used orally once daily over 7 days. The drug influenced the epidermal thymidine triphosphate and amino acid incorporation rates at doses between 1 and 5 mg/kg. In control mice, an epidermal hyperproliferation induced by abrasion of superficial epidermal layers was characterized by enhanced prostaglandins (PGs) and leukotriene (LKT) concentrations

in epidermal homogenate, an increase in thymidine triphosphate and amino acid incorporation and an increase in epidermal thickness. In mice treated with 1 mg/kg azelastine HCl, this epidermal reaction was changed. Compared to controls, the increase in leukotriene level was diminished, and that of PGs was enhanced. The incorporation of thymidine triphosphate and of amino acids as well as the epidermal thickness and the ratio cell count/epidermal thickness were increased in irritated skin of azelastine-treated mice. The azelastine influences the epidermal metabolism in irritated and unirritated skin. So, a beneficial role of this phthalazinone in the treatment of psoriasis and related skin disorders seems to be possible [44]. Derivatives of 7-ethoxycarbonyl-4-formyl-6,8-dimethyl-1(2H)-phthalazinone (26) derivatives were tested for their inhibitory effect on platelet aggregation, and their relaxing effect on blood vessels [45]. The 4-substituted phthalazinone derivatives with possible anti-bacterial activity [46] and 4-phenyl and 4-benzyl substituted phthalazinone and its derivatives [47]. The inhibitory effect exhibited on platelet aggregation of 2-phenyl-1(2H)-phthalazinone derivatives [48]. The 7-Ethoxycarbonyl-4-hydroxy-methyl-6,8-dimethyl-1(2H)-phthalazinone (27) (0.3-3 mg/kg i.v.), a cyclic AMP-PDE inhibitor, increased femoral, renal, coronary, carotid, vertebral and sagittal blood flows in anesthetized dogs. Systolic tension in left ventricular wall, heart rate and cardiac output also increased. These properties of 27 resembled those of papaverine (0.1-1 mg/kg i.v.). However, the heart rate increasing activity of 27 was less than that of papaverine at equipotent does on the vasodilator actions. In the isolated right atria of guinea pigs, 27 was more selective for increasing contractility than for increasing the sinus rate. The cardiovascular actions of 27 were also examined in perfused vascular beds and papillary muscle preparations of dogs. An i.v. infusion of 27 or papaverine (0.3 mg/kg/min) enhanced the increasing action of isoprenaline on left ventricular systolic pressure and coronary sinus outflow. The vasodilator effect of 27 was no suppressed by atropine, propranolol nor clemastine, and the inotropic action was not modified by pindolol. These results indicate that 27 may be a useful vasodilator with a cardiotonic activity but with less potency to cause tachycardia than papaverine [49]. Compound 27 and several analogues were synthesized and their inhibitory effects on platelet aggregation were evaluated. All synthesized compounds showed no appreciable effect on platelet aggregation induced by adenosine diphosphate, but most of them inhibited effectively the arachidonic acid induced platelet aggregation. The parent compound, 2-phenyl derivatives, and ortho-substituted 2-phenyl derivatives show the most potent inhibition of all compounds [50]. Effects of 27 on the spinal trigeminal nucleus (STN), ventral posteromedial nucleus (VPM), and sensory cortex were examined in cats anesthetized with α-chloralose in comparison with the effects of morphine. Compound 27 produced a dose-dependent inhibition of the polysynaptic components of the cortical field potentials upon VPM stimulation and either facilitatory or inhibitory effects on the polysynaptic components of the VPM field potential upon stimulation of the medial lemniscus, while the drug failed to affect the STN field potential with trigeminal nerve stimulation. Morphine inhibited the postsynaptic components of the STN field potentials and to a lesser extent, the polysynaptic components of the cortical field potential; and the effects of morphine on the VPM field potential were similar to those seen with 27. Pretreatment of the animal with naloxone antagonized the facilitatory effect on the VPM field potentials produced by morphine, but not those by 27. Morphine and 27 induced either a prolonged increase in the blood flow or transient increase followed by a decrease in the blood flow in the VPM. These results suggest that 27 may impair the polysynaptic transmission and/or neuron excitability in the sensory cortex and the VPM at least partly due to the change in blood flow there as does morphine. Unlike morphine, however, 27 did not produce any obvious effect on the STN [51] and compound 27 also act as antiatherosclerotic agents [52]. The 4-(p-chlorobenzyl)-2-[N-methyl-perhydroazepinyl-(4)]-1-(2H)-phthalazinone hydrochloride (azelastine) exhibited anti-hypersensitive activity [53].



(26)



(27)

Compound 27 was found to be a considerably potent cardiotonic agent. It produced both the positive chronotropic and inotropic actions in the guinea pig heart muscle. Positive inotropic action of isoproterenol was potentiated by 27 at the concentration which did not produce a substantial positive inotropic action by itself. Cardiac action potential was not modified by 27 at concentrations sufficient to produce positive inotropic actions. Compound 27 has a strong activity to produce slow responses in the depolarized myocardium, indicating that it can increase the density of the slow channels. The possibility was shown that the increase in the density of the slow channel may play an important role in the positive inotropic action of 27. The increase in the intracellular cyclic AMP due to the PDE inhibition is tentatively most likely to be the cause of the 27 induced in the density of the slow channels [54]. Anti-aggregating activity of 27 was tested using rabbit platelets in vitro. The compound 27 alone, when added before, prevented platelet aggregation induced by ADP, as did PGI2, papaverine and dipyridamole. Spontaneous disaggregation was also accelerated when 27 was added after the maximal aggregation induced by ADP. Compound 27 alone also inhibited platelet aggregation induced by collagen and arachidonic acid. ID50s of these agents in ADP-induced aggregation were 7-9 nM for PGI2, 223 μM for 27, 266 μM for papaverine and 957 μM for dipyridamole. When EG-626 was used in combination with PGI2, a threshold dose (50 μM) of EG-626 potentiated the antiaggregation effect of subthreshold dose (3 nM) of PGI2 upto 100% inhibition in collagen-induced platelet aggregation. The marked potentiating effect of EG-626 was accompanied by an accumulation of cyclic AMP in the platelets. These effects might be due to inhibition of PDE. Papaverine and dipyridamole, other PDE inhibitors, also potentiated the anti-aggregating activity of PGI2. The activity of papaverine, however, was one eighth of EG-626 and that of dipyridamole was much less. The most effective combination of PGI2 and EG-626 to induce 50% inhibition was obtained with 20% of ID50 of each agent, whereas that of PGI2 and papaverine or dipyridamole was 39 or 41%, respectively [55]. The metabolic fate of an antiallergic agent, azelastine (4-(p-chlorobenzyl)-2-[N-methyl-perhydroazepinyl-(4)]-1-(2H)-phthalazinonehydrochloride) in rats and guinea pigs was investigated using its 14C-labelled compound [56]. Compound 27 was reported as an antagonist of thromboxane (Tx) A2 in the contraction of rabbit aorta. Compound 27 did inhibit the contraction of superfused rabbit aorta, but also did inhibit that of rabbit coeliac artery, rat stomach strip and rat colon induced by TxA2, PG endoperoxides, angiotensin II and PGF2 alpha in non-specific manner. Compound 27 had no effect on the biosynthesis of PG endoperoxides as well as TxA2. These results indicate that EG-626 is not a TxA2 antagonist, but has a general inhibitory effect on the smooth muscles. This inhibitory effect of EG-626 may be explained by the inhibition of PDE [57]. Anti-allergic properties of azelastine (A-5610) were tested focusing the most attention on its decongestive effect. Intravenous injection of azelastine into anesthetized dogs with doses more than 0.1 mg/kg prevented the changes in nasal impedance provoked by histamine sprayed into the nasal cavity. When azelastine was given orally, the minimum effective dose to abolish the impedance reduction due to histamine was 2 mg/kg, in the case of cleamastine the same dose was required. Histamine release from the rat mesentery pieces by the condensation product of N-methyl-

homoanisylamine formaldehyde (compound 48/80) (0.005%) was inhibited almost completely by pretreatment with azelastine at the concentrations of 10-4 to 10-3 g/ml, and in those concentrations azelastine alone released histamine scarcely. When 5 mg/kg of azelastine was given i.v. to rabbits, the characteristic changes in EEG-a high-voltage low-frequency pattern-persisted more than 1 h, but not the least inhibition in arousal response was noted. With the dose of 0.5 mg/kg, diphenhydramine impaired arousal response and slow waves with high amplitude dominantly appeared in EEG [58].

Conclusion

In conclusion, the phthalazine derivatives have been of increasing interest since many of these compounds have found many pharmacological and chemotherapeutic applications. Various novel pthalazine derivatives were synthesized, characterized and tested for their diverse types of biological activities.

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Conflicts of interest

The authors report no conflict of interest.

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