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Review Article

A BRIEF REVIEW ON PHARMACOLOGICAL EFFECT OF SOME PHTHALAZINE DERIVATIVES ON CARDIOVASCULAR AND KIDNEY FUNCTIONS

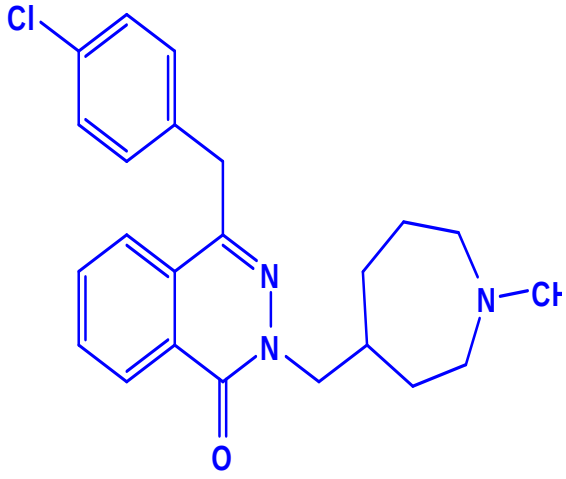
***Mohammad Asif**
Department of Pharmacy, GRD(PG) Institute of Management & Technology, 248009, Dehradun, (Uttarakhand), India
**Author for Correspondence*

ABSTRACT
Phthalazine derivatives are biological potential compounds having diverse biological activities. In this review, briefly discuss about the effect of phthalazine derivatives on cardiac and kidney functions. A series of 4-(4-bromophenyl) phthalazine and phthalazinone analogs connected through 2-propanol spacer to N-substituted piperazine residue were tested for their effect on β-adrenergic blocking activity. Most compounds exhibited appreciable β-adrenolytic activity compared to propranolol. Compounds **1a, 1d, 1e** and **2c** showed appreciable inhibition of norepinephrine induced aortic ring contraction. Another series of phthalazine substituted urea and thiourea derivatives **(5a–p)** were tested for their inhibitory actions on the activity of human carbonic anhydrase (hCAs I and II) enzymes. All these compounds inhibited the CA isoenzymes activity. Compound **5a** (IC_{50}=6.40μM for hCA I and 6.13 μMfor hCA II) has the most inhibitory activity. Phthalazine derivatives were showed both β-adrenergic blocking and carbonic anhydrase activities and useful for both cardiac and kidney functions.

Keywords: *Phthalazine Analogs; Adrenergic β-blockers, Carbonic Anhydrase Inhibitors*

INTRODUCTION
Despite the significant progress made in prevention and treatment, cardiovascular diseases are still the main cause of death worldwide (Lopez *et al.*, 2006). The use of β-adrenoceptor antagonists is well-established in the treatment of various cardiovascular disorders. Since development of this class of drugs in the late 1950s of twentieth century, they are administered in the therapy of hypertension, coronary artery disease, arrhythmia, myocardial infarction and heart failure (Panjrath and Messerli, 2006). Also, much attention is being paid to b-blockers that possess vasodilator action produced through different mechanisms, such as release of nitric oxide (NO), antioxidant action, β2-agonistic action, Ca entry blockade and a1-blockade (Toda, 2003). In the last decade, a new generation of β-blockers with additional a-adrenoceptor blocking activity was introduced to therapy. The α/β-blockers (bucindolol, carvedilol and labetalol) have vasodilating properties via relaxation of arterial smooth muscle, with no reflex tachycardia, as a result of β-adrenoceptor blockade (Matsuda *et al.*, 2000; Marona *et al.*, 2008). They have also beneficial effects on the regular circulation in contrast to classic b-blockers (Toda, 2003; Carella *et al.*, 2010). Pyridazinone and phthalazinone derivatives have been reported to possess a variety of pharmacological effects on the cardiovascular system (Demirayak *et al.*, 2004a; Del Olmo *et al.*, 2006; Bansal *et al.*, 2009). Within the drugs in the market, hydralazine, one of the first antihypertensive agents, is considered as a lead for developing new drugs, due to its direct vasodilator effect (Del Olmo *et al.*, 2006). Structural modification of hydralazine led to the discovery of new phthalazine candidates possessing antihypertensive effect (Demirayak *et al.*, 2004b). However, in the longterm treatment of hypertension, the use of vasodilators alone does not suffice and it is the concomitant use of b-blockers which has proved to be useful for achieving adequate control of blood pressure (Bisi *et al.*, 2003).
The β-Adrenergic blocking agents are very homogeneous in their chemical structures, which generally include the 2-aminoethanol basic skeleton to which the other groups of molecules are linked and which should be associated principally with the ability of these compounds to bind with the receptors (Saccomanni *et al.*, 2003). On the other hand, the nature of aromatic nucleus generally determines the blocking or stimulant properties of these compounds, (Macchia *et al.*, 1985). β-Adrenergic antagonists

containing phenylpiperazine moiety comprise a class of compounds with ancillary vasodilator properties and superior clinical efficacy compared to classical b-blockers (Toda, 2003; Gonec *et al.*, 2008; Racanska *et al.*, 2010). The carbonic anhydrases (CAs) are commonly characterized as zinc metalloenzymes whose primary physiological function is to rapidly catalyze the reversible hydration of carbon dioxide to form bicarbonate and a proton (Silverman and Lindskog, 1988). CAs are ubiquitous enzymes present in prokaryotes and eukaryotes which are encoded by five known CA structural families, the structurally characterized α-, β-, and γ-classes and the more recently discovered δ- and ζ-classes (Zimmerman *et al.*, 2007). The α-CAs are found in vertebrates, algae, eubacteria, and cytoplasm of green plantswhereas the γ-CAs are presentmainly in Archaea and few eubacteria. The β-CAs are predominantly available in chloroplasts of mono- and dicotyledonous plants along with some algae and eubacteria. The δ-CAs are primarily found in marine diatoms. In humans, 16 isoforms of α-CAs have been reported, of which three are CARP or CA-related proteins (Supuran and Scozzafava, 2007).There are sixteen isozymes which are characterized, and many of them are involved in critical physiological processes (Carta *et al.*, 2012). CAs are found in a variety of tissues such as kidneys, lungs, eyes, skins, the nervous systems, and the gastrointestinal tract in humans (Supuran, 2011). Biological activities of this metalloenzyme family have several medicinal applications which are commonly used as diuretics for the treatment of symptoms of hypertension (Supuran and Scozzafava, 2002), as antiglaucoma drugs (Supuran and Scozzafava, 2000), and for the treatment of high altitude sickness, gastric and duodenal ulcers, epilepsy, and osteoporosis (Richalet *et al.*, 2005). More recently CA inhibitors have been shown to have potential as antiobesity drugs (Supuran, 2008). Some alkylating agents bearing amino acid residues showed high cytotoxic activity against various cancer cell lines, such as melphalan (L-phenylalanine mustard hydrochloride). Furthermore, amino acids could also improve the cell uptake of antitumor agents. However, although new cytotoxic agents with unique mechanisms of action have been developed continuously, many of them have not been therapeutically useful due to low tumor selectivity and harsh side effects. These facts prompted us to design and develop novel potent and selective anti-breast cancer agents. 1,4-Disubstituted phthalazines have received a considerable attention as antitumor agents in the past few years. A successful example is N-(4-chlorophenyl)-4-(pyridin-4-ylmethyl) phthalazin-1-amine also known as Vatalanib (PTK-787) which is VEGFR (vascular endothelial growth factor receptor) inhibitor and is currently in Phase III clinical trials for metastatic colorectal cancer. (4-(3,4-difluoro-phenylsulfanylmethyl)-phthalazin-1-yl)-(3-fluoro-phenyl)-amine II displayed excellent selectivity against MDA-MB-231 cell line. Furthermore, N-(4-fluoro-phenyl)-2-(4-(4-pyridin-4-ylmethyl-phthalazin-1-yl)-piperazin-1-yl)-acetamide III has shown more potent cytotoxicity than cisplatin (El-Nezhawy *et al.*, 2009; Khalil *et al.*, 2011).



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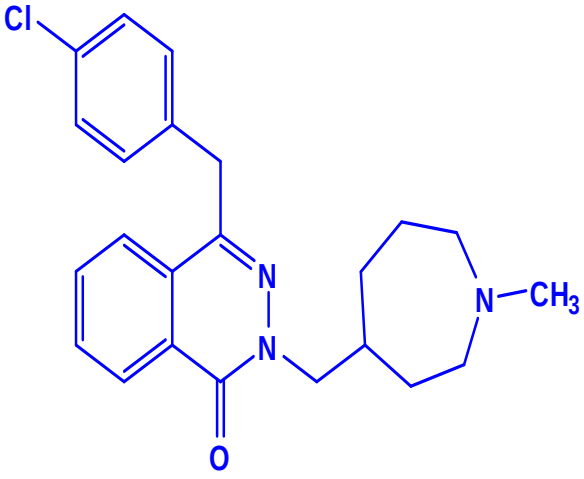

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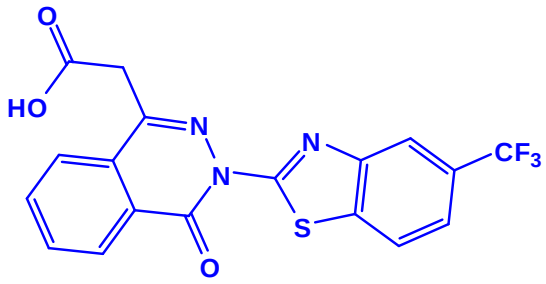

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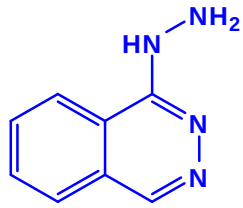

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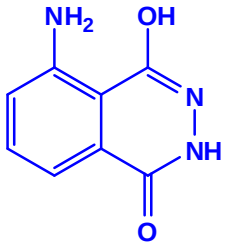
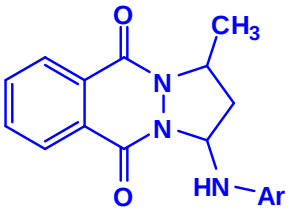
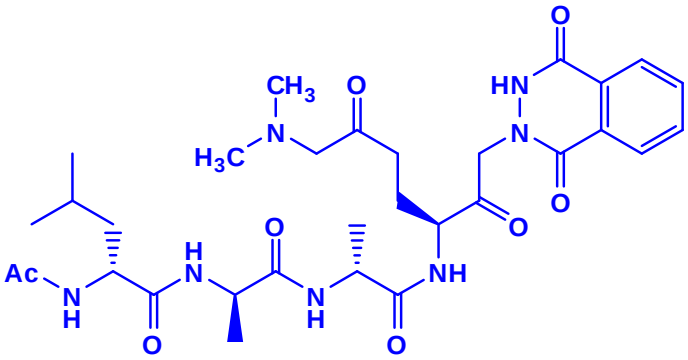


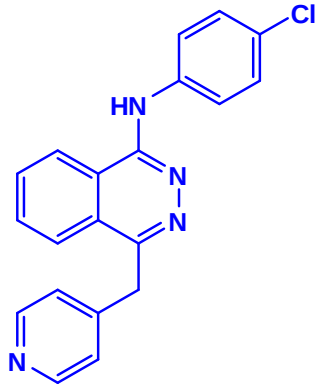
Luminol



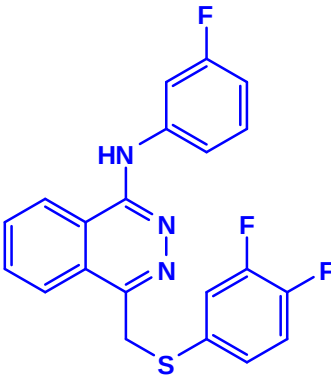
Antihypoxic and antipyretic agent



HAV 3C inhibitor



Vatalanib (PTK-787)



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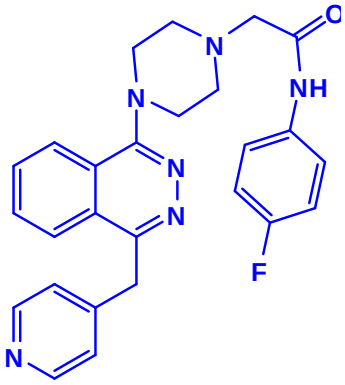


III

Figure 1: Some commercially used phthalazine derivatives

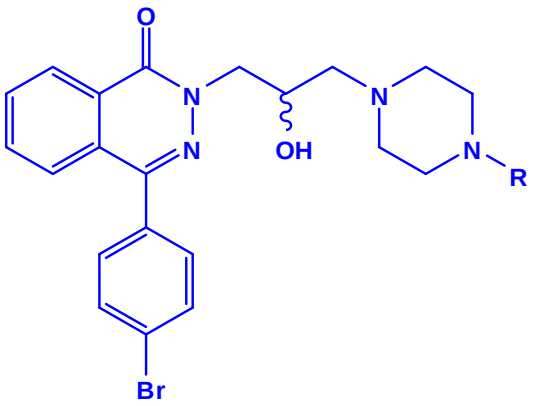
In particular, hydrazine containing heterocyclic compounds has been considered of great importance on account of pharmacological properties and clinical applications (Turk *et al.*, 2001). Moreover, these of combined phthalazines have biological properties such as inhibition of p38 MAP kinase (Mavel *et al.*, 2002) for selective binding of GABA receptor (Carling *et al.*, 2004), antianxiety drug (Imamura *et al.*, 2003), antitumor agent (Kim *et al.*, 2004), and highaffinity ligand to the a2d-1 subunit of calcium channel (Lebsack *et al.*, 2004). Phthalazine derivatives have been greatly used as therapeutic agents owing to their anticonvulsant, cardiotonic, vasorelaxant, anti-inflammatory properties (Tsoungas and Searcey, 2001; Sivakumar *et al.*, 2002; Coelho *et al.*, 2004; Demirayak *et al.*, 2004; Dogruer *et al.*, 2003), and antimicrobial activity (Sonmez *et al.*, 2006).

Like azelastine, the phthalazine derivatives have antihistaminic effects in the treatment of allergic rhinitis (Tanizaki *et al.*, 1992), and hydralazine is used as antihypertensive agent in the treatment of pulmonary hypertension (Groves *et al.*, 1985; Packer *et al.*, 1982; Keller *et al.*, 1984). Some commercially used phthalazine derivatives are shown in Figure 1.

In the past few decades, progress in understanding the biochemical pharmacology of β-blockers has lead to a more rational approach in designing new drug combinations involving this 2-hydroxylpropyl spacer. In this regard, keeping the basic 2-hydroxypropyl spacer for significant b-adrenoreceptor antagonistic activity in combination with the vasorelaxant-substituted phthalazine pharmacophore, two series of 4-(4-bromophenyl) phthalazine derivatives connected through 2-propanol spacer to N-substitutedpiperazine residue **1a–f** and **2a–f** were synthesized with the aim to elicit their β-adrenolytic activity.

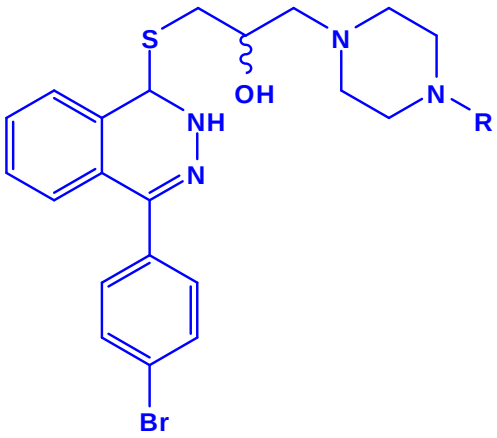
Ureidosubstituted benzenesulfonamides show very interesting profile for the inhibition of several human carbonic anhydrases (hCAs) such as hCAs I and II (cytosolic isoforms) and hCAs IX and XII (transmembrane, tumor-associated enzymes). It is mentioned that the compounds have excellent inhibitory effects for all these isoforms due to the ureamoiety (Pacchiano *et al.*, 2011). On the other hand, it has been reported that some urea derivatives have CA inhibitor activities (Nixha *et al.*, 2013; Celik *et al.*, 2013). Therefore, the investigation of clinically useful ureas/thioureas is a growing field of interest. A series of phthalazine substituted urea and thiourea derivatives were synthesized, and their inhibitory effects on the activity of purified human carbonic anhydrases (hCAs I and II) were evaluated.

β-Adrenergic antagonists activity: The pharmacological evaluation of the possible b-blocking activity of the test compounds **1a–f** and **2a–f** has been carried out on the norepinephrine (NE)-induced precontracted aortic rings module. Blunting isoprenaline-induced relaxation was quantified as described in methodology section. Isoprenaline relaxed the NE-induced precontracted aortic rings by 19.75 % of the contracted tension. The reference drug, propranolol not only blunted the isoprenaline-induced relaxation, but also induced further 1.16 % contraction in the aortic ring preparation (negative sign indicates further contraction). Compounds **1a, 1d, 1e** and **2c** showed the most potent β-blocking activity by complete blunting and even further contracting the aortic ring preparation by 0.71 to 6.18 % of its pre-contracted tension.



3a-f

3a : R=CH_3
3b : R =C_6H_5
3c : R = 4-ClC6H4
3d: R=2-$OCC_2H_5C_6H_4$
3e : R= 2-$OCH_3C_6H_4$
3f: R=4-$OCH_3C_6H_4$



7a-f

7a : R= CH
7b : R= C_6H_5
7c : R = 4-ClC_6H_4
7d: R= 2-$OCC_2H_5C_6H_4$
7e : R= 2-$OCH_3C_6H_4$
7f : R= 4-$OCH_3C_6H_4$

Table 1: Change in aortic muscle tension after NE and isoprenaline exposure

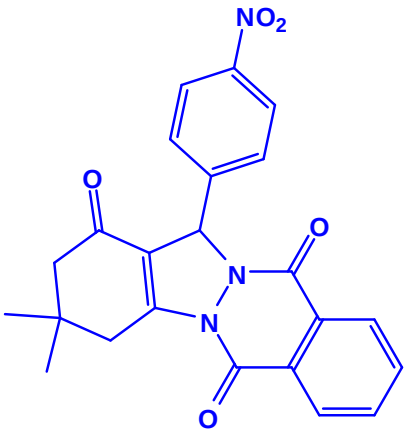
Compd	NE-induced change in aortic muscle tension (%)	Isoprenaline-induced change in precontracted aortic muscle (%)	compd	NE-induced change in aortic muscle tension (%)	Isoprenaline-induced change in precontracted aortic muscle (%)
1a	9.52	-6.18	**2a**	46.6	18.51
1b	42.03	2.2	**2b**	45.32	12.79
1c	31.05	3.2	**2c**	42.35	-1.83
1d	55.2	-3.87	**2d**	31.55	12.05
1e	54.57	-0.71	**2e**	17.4	17.4
1f	47.36	18.01	**2f**	15.43	15.43
Control	51.18	19.75	Propranolol	63.91	-1.16

Change in tension is expressed as average muscle tone over duration of 1–2 min of recording.

Compounds **1b** and **1c** displayed potentially strong b-blocking activity by decreasing the isoprenaline-induced relaxation to 2.2 and 3.2 %, respectively, of pre-contracted aortic tension compared to 19.75 % of control untreated aortic ring preparation. However, compounds **2b, 2d** and **2f** exhibited mild to moderate b-blocking activity by decreasing the isoprenaline-induced relaxation to 12.05–15.43 % of pre-contracted aortic tension. On the other hand, compounds **1a**, **2e** and **2f** did not show tangible blocking of isoprenaline-induced relaxation. It is worth to mention that, the compound **1a** also strongly inhibited the NE-induced contraction of the aortic ring preparation to 9.52 % compared to 51.18 % of control untreated preparation. However, the test compounds **1c**, **2d**, **2e** and **2f** showed similar but weaker inhibition effects to NE-induced aortic ring contraction (Table 1). The pharmacological screening revealed that the N-substituted derivatives **1a–f** displayed more potent b-adrenergic blocking activity than the S-substituted analogues **2a–f**. In the first series of compounds, it is obvious that the compounds with methyl or o-substituted phenyl groups on the piperazine nitrogen **1a, 1d, 1e** showed the highest b-blocking activity. Moreover, the unsubstituted and p-chloro substituted analogues **1b, 1c** were found to possess appreciable β-blocking activity. In contrast, the p-methoxyphenyl derivative **1f** did not show promising β-blocking effect. Within the series of S-substituted compounds **2a–f**, the p-chloro-substituted derivative **2c** was the only compound that displayed potent β-adrenergic blockade. Other derivatives showed from weak to moderate activities (Abouzid *et al.*, 2013).

Certain 4-(4-bromophenyl) phthalazine and phthalazinone derivatives connected through 2-propanol spacer to N-substituted piperazine residue were synthesized and screened for their β-adrenergic blocking activity on the norepinephrine-induced precontracted aortic ring module. All compounds were obtained and tested as racemates. The results revealed that N-substituted derivatives **1a–f** generally displayed more potent b-adrenergic blocking activity than the S-substituted analogues **2a–f**. The test compounds **1a, 1d, 1e** and **2c** showed the most potent β-blocking activity by complete blunting and even further contracting the aortic ring preparation by 0.71 to 6.18 % of its precontracted tension 2-(4-Bromodbenzoyl)benzoic acids (Yamaguchi *et al.*, 1993) (Abouzid *et al.*, 2013).

Carbonic anhydrases Inhibitory activity: For evaluation of the physiologically relevant human CA isozymes (hCAs I and II) inhibitory activity, several new urea and thiourea compounds were subjected to CA inhibition assay with CO2 as a substrate. The results showed that phthalazine substituted urea and thiourea derivatives **(3, 4** and **5a–p)** inhibited the CA enzyme activity. The inhibition constants of the synthesized compounds against CAs were given in Table 1. We have determined the IC50 values of 6.40–20.38 μM and 6.13–23.63 μM for hCA I and hCA II, respectively, and they are all competitive inhibitors (Sayyafi *et al.*, 2008). The nitro containing phthalhydrazide was reduced with tin (II) chloride in ethanol (Ono *et al.*, 2007). The amino phthalhydrazide was reacted with isocyanates or thioisocyanates to get the final products **(5a–p)** (Ogita *et al.*, 2002).



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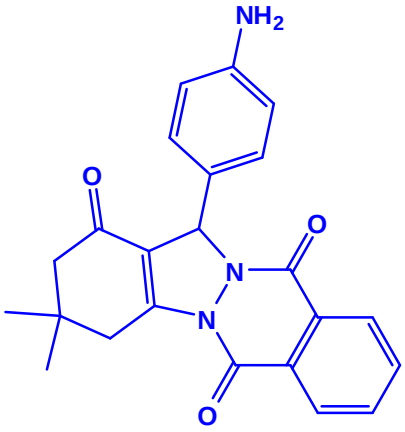

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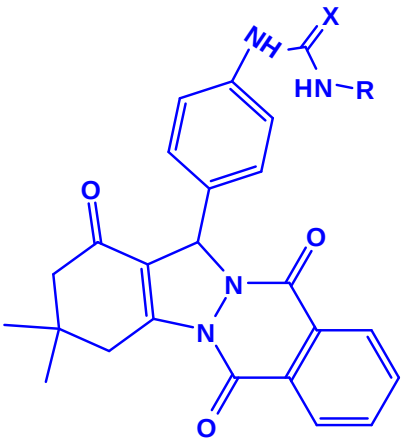

5a-p

Compd	X	R	Compd	X	R
5a	**S**	Ph–	**5i**	**O**	4-NO2–Ph
5b	**S**	3-MeO–Ph–	**5j**	**O**	3-MeO–Ph–
5c	**S**	4-Me–Ph–	**5k**	**O**	4-Me–Ph–
5d	**S**	4-Cl–Ph–	**5l**	**O**	4-F–Ph–
5e	**S**	4-I–Ph–	**5m**	**O**	CH3(CH2)5–
5f	**S**	4-Br–Ph–	**5n**	**O**	CH3(CH2)2-
5g	**S**	4-F–Ph–	**5o**	**O**	(CH 3)2CH-
5h	**O**	Ph–	**5p**	**O**	CH3CH2-

Phthalazine substituted urea and thiourea derivatives.

Table 2: IC_{50} (μM) values of the phthalazine substituted urea and thiourea derivatives

Compd	hCA I	hCA II	Compd	hCA I	hCA II
1	12,08	11,89	**5h**	14,04	17,78
2	10,18	10,25	**5i**	6,76	6,75
5a	6,40	6,13	**5j**	20,38	22,73
5b	13,97	15,35	**5k**	16,38	16,34
5c	18,20	23,63	**5l**	12,62	12,34
5d	19,59	18,21	**5m**	7,63	9,67
5e	11,02	8,50	**5n**	8,20	8,74
5f	12,20	19,12	**5o**	7,45	10,03
5g	8,72	8,25	**5p**	11,72	10,04

The CA inhibitors decrease intraocular pressure by reducing bicarbonate formation in the ciliary process, so lowering Na+ transport and flow of aqueous humour: this is the basis for their use in glaucoma treatment. Unfortunately, systemic therapy with parenteral sulphonamides leads to significant side effects, many of them being probably due to inhibition of CA isoforms in other tissues. Acetazolamide which is 20 times less active against hCA I than against hCA II in erythrocytes is the most widely used inhibitor. But the inhibition of various CA isoforms which are present in tissues other than eye leads toanentire range of side effects, themost prominent being numbness and tingling of extremities, metallic taste, depression, fatigue, malaise, weight loss, decreased libido, gastrointestinal irritation, metabolic acidosis, renal calculi, and transient myopia (Arslan *et al.*, 1997).

Sulfonamide compounds are coordinated to the zinc (II) ion within the hCAs active site, whereas its organic scaffold fills the entire enzyme cavity, making an extensive series of van der Waals and polar

interactions with amino acid residues both at the bottom, middle, and entrance of the active site cavity (Maresca *et al.,* 2010). Coumarins derivatives may possess various tautomeric forms which may bind within the CA active site similarly to phenols, that is, by anchoring to the zinc-bound water molecule/hydroxide ion (Ebbesen *et al.,* 2009). Coumarins cannot bind enzyme effectively in the restricted space near Zn^{2+} ion because they have bulky group and exhibit unusual binding mode not interacting with the metal ion of the enzyme (Maresca and Supuran, 2010). We assume that the synthesized compounds are very big pendant group to be able to bind near the zinc ion. Hence, they much more probably bind as the coumarin derivatives. The results showed that all the compounds **(5a–p)** inhibited the enzyme activity.The inhibition constants of the synthesized compounds against CAs were given in Table 1. The following structure-activity relationship (SAR) observations can be drawn from the data.The slow cytosolic isoform hCA I and the second off-target isoform hCA II were inhibited by the synthesized compounds with inhibition values in the range of 6.00–24.00 μM.

The best hCAI andhCAII inhibitors among the synthesized and investigated compounds were **5a** and **5i**. For urea derivatives of aryl-phthalazine substituted compounds, electron withdrawing groups (nitro and fluorine) bonded on phenyl ring (**5i** and **5l**) increased the hCAs I and II inhibitory activity. In contrast, electron donating groups (methoxy, methyl) on phenyl ring (**5j** and **5k**) have moderate inhibitory activity for the hCAIs and II. For the aryl-aryl thiourea derivatives, electron donating groups as mesomeric or inductive (methoxy, methyl, and halogens) on the phenyl ring (from **5b** to **5f**) have moderate inhibitory activity, but the compound **(5g)** with fluorine atom has good inhibition effect on hCAs I and II. Flouro substituted urea derivatives (**5l** and **5g**) showed inhibitorier effect than methoxy, methyl, chloro, and bromo substituted ureas. Fluorophenyl sulfamate adducts were reported where the sulfamates possess a rather variable binding pattern within the hCA II active site (Winum *et al.,* 2009; Kim *et al.,* 2000). Alkyl-phthalazine substituted ureas have different inhibition effects. When alkyl chain increases, inhibition effect increases with alkyl chain length due to their steric effect. It is obviously clear that bulky phthalazine group affects inhibition for the compounds. In summary, enzyme inhibition is a more important issue for drug design and biochemical applications (Gencer and Arslan, 2011; Demir *et al.,* 2012; Senturk *et al.,* 2012; Gencer *et al.,* 2012). The results showed that new phthalazine substituted urea and thiourea derivatives inhibited the hCAs I and II enzyme activity. Therefore, our results suggested that the compounds are likely to be adopted as candidates to treat glaucoma and may be taken for further evaluation in *in vivo* studies (Berber *et al.,* 2013).

CONCLUSION

Phthalazin-1(2H)-one is of considerable interest due to their antidiabetic, antiallergic, Vasorelaxant, PDE4 inhibitors, beta adrenergic antagonist, VEGF (vascular endothelial growth factor) receptor tyrosine kinases for the treatment of cancer, antiasthmatic agents with dual activities of thromboxane A2 (TXA2) synthetase inhibition and bronchodialation, herbicidal, carbonic anhydrase inhibitor like activities. A number of established drug molecules like (N-(4-methylpent-3-en-2-ylidene amino) phthalazin-1-amine) is known as Budralazine, ((RS)-4-((4-chlorophenyl)methyl)-2-(1-methylazepan-4-yl)-phthalazin-1-one) known as Azelastine are prepared from the corresponding phthalazinones. In view of the fact the continuation of research interests for the synthesis of biologically active heterocycles.

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