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

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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 (IC50=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 (Panjrathe and Messerli, 2006). Also, much attention is being paid to α-adrenoceptor blockers, Ca entry blockade and α1-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 anti hypertensive 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 longer term 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-ami noethanol 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
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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 \( \alpha \)-, \( \beta \)-, and \( \gamma \)-classes and the more recently discovered \( \delta \)- and \( \zeta \)-classes (Zimmerman et al., 2007). The \( \alpha \)-CAs are found in vertebrates, algae, eubacteria, and cytoplasm of green plants whereas the \( \gamma \)-CAs are present mainly in Archaea and few eubacteria. The \( \beta \)-CAs are predominantly available in chloroplasts of mono- and dicotyledonous plants along with some algae and eubacteria. The \( \delta \)-CAs are primarily found in marine diatoms. In humans, 16 isoforms of \( \alpha \)-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 alkyllating 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-difluorophenylsulfanyl)methyl)-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).
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 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 high-affinity 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 led to a more rational approach in designing new drug combinations involving this 2-hydroxypropyl spacer. In this regard, keeping the basic 2-hydroxypropyl spacer for significant β-adrenoceptor 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 β-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.
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 phthalaldehydezide was reduced with tin (II) chloride in ethanol (Ono et al., 2007). The amino phthalaldehydezide was reacted with isocyanates or thioisocyanates to get the final products (5a–p) (Ogita et al., 2002).

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

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

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 b-blocking activity. In contrast, the p-methoxyphenyl derivative 1f did not show promising b-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 β-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-Bromobenzoyl)benzoic acids (Yamaguchi et al., 1993) (Abouzid et al., 2013).
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Table 2: IC50 (µM) values of the phthalazine substituted urea and thiourea derivatives

<table>
<thead>
<tr>
<th>Compd</th>
<th>X</th>
<th>R</th>
<th>Compd</th>
<th>X</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>S</td>
<td>Ph–</td>
<td>5i</td>
<td>O</td>
<td>4-N02–Ph</td>
</tr>
<tr>
<td>5b</td>
<td>S</td>
<td>3-MeO–Ph–</td>
<td>5j</td>
<td>O</td>
<td>3-MeO–Ph–</td>
</tr>
<tr>
<td>5c</td>
<td>S</td>
<td>4-Me–Ph–</td>
<td>5k</td>
<td>O</td>
<td>4-Me–Ph–</td>
</tr>
<tr>
<td>5d</td>
<td>S</td>
<td>4-Cl–Ph–</td>
<td>5l</td>
<td>O</td>
<td>4-F–Ph–</td>
</tr>
<tr>
<td>5e</td>
<td>S</td>
<td>4-I–Ph–</td>
<td>5m</td>
<td>O</td>
<td>CH3(CH2)5–</td>
</tr>
<tr>
<td>5f</td>
<td>S</td>
<td>4-Br–Ph–</td>
<td>5n</td>
<td>O</td>
<td>CH3(CH2)2-</td>
</tr>
<tr>
<td>5g</td>
<td>S</td>
<td>4-F–Ph–</td>
<td>5o</td>
<td>O</td>
<td>(CH 3)2CH-</td>
</tr>
<tr>
<td>5h</td>
<td>O</td>
<td>Ph–</td>
<td>5p</td>
<td>O</td>
<td>CH3CH2-</td>
</tr>
</tbody>
</table>

Phthalazine substituted urea and thiourea derivatives.

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 to an entire range of side effects, the most 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 hCA I and hCA II inhibitors among the synthesized and investigated compounds were 5a and 5i. For urea derivatives of aryphthalazine 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 hCAs I and II. For the aryl-aryl thiourea derivatives, electron donating groups as mesomorphic 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. Fluoro substituted urea derivatives (5i 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 bronchodilation, 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 phthalazines. In view of the fact the continuation of research interests for the synthesis of biologically active heterocycles.

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