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Computer – based drug design, synthesis and biological evaluation of new pyrimidinone derivatives linked to arylpiperazine and 2′-carbethoxy-biphenylylmethyl moeities as α_1- adrenoceptor antagonists and angiotensin II AT_1 receptor antagonists

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Two new series of pyrimidinone derivatives linked to arylpiperazine moieties and 2′-carbethoxy-biphenylylmethyl moieties were designed, synthesized and biologically evaluated for their *in vivo* hypotensive activities. The design of arylpiperazine analogues (**IIa-f**, **IIIa-c**, **VIIa.b**, **IX**) was based upon structural modification of the newly discovered selective α_1-AR antagonist drug; Urapidil. Compare/fit studies of these molecules with the previously generated and validated α_1-AR antagonist hypothesis showed that these molecules have comparable affinities for the α_1-AR antagonist hypothesis while compound **IIIc** had the highest fitting value. The *in vivo* biological evaluation of these compounds for their effects on blood pressure of normotensive cats in comparison to the lead compound prazosin, was consistent with the results of molecular modeling fit values. As expected, compound **IIIc** exhibited the highest hypotensive activity among the test set compounds. Meanwhile, the design of 2′-carbethoxy-biphenylylmethyl analogues (**XIa,b**) was based upon the molecular modeling simulation fitting of their carboxylic acid bioprecursors with the previously generated and validated Ang II receptor antagonist hypothesis. Such compare/fit studies predicted that the designed compounds (**XIa,b**) showed comparable fitting affinities between their de-esterified analogues and the Ang II antagonist pharmacophore. *In vivo* biological evaluation of these compounds for their effects on blood pressure of normotensive cats showed that compound **XIa** exhibited hypotensive activity more or less similar to losartan.

1. Introduction

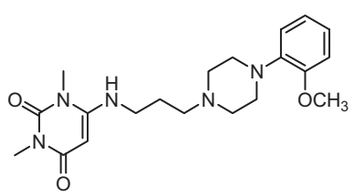
In a continuation of our previous research (Ismail et al. 2006a,b) we aimed to introduce new antihypertensive agents having various pyrimidinone-ring systems connected to the 4-arylpiperazino moiety or biphenyl-carboxylic acid scaffold that are needed to effectively bind to the α_1-ARs or the Ang II receptors, respectively.

The adrenoceptors (ARs) play a key role in the modulation of sympathetic nervous system activity and are a site of action for many important therapeutic agents. The ARs, members of the G-protein-coupled receptors superfamily, are divided into three principal groups: α_1, α_2, and β. The α_1-ARs are further subdivided into α_{1A}, α_{1B}, α_{1D} and α_{1L} subtypes (Barbaro et al. 2002), and they are mainly located in the cardiovascular system. They play a prominent role in regulating vascular tone and hypertrophic growth of smooth muscle and cardiac cells. Inhibition of these receptors decreases blood pressure (BP) and symptomatically treats begnin prostate hypertrophy by relaxation of the vascular smooth muscles. The first selective α_1-AR antagonist was prazosin.

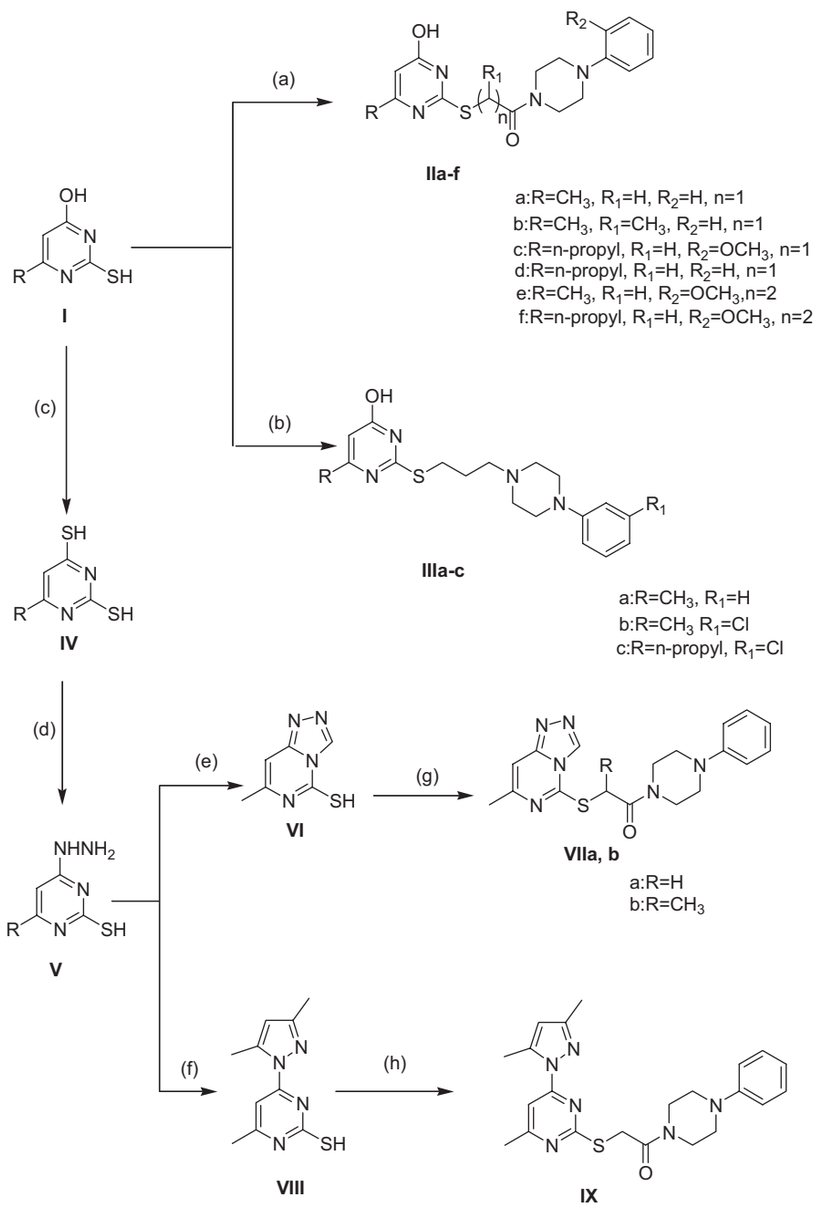
In 2001, Betti et al. (2001) generated a hypothesis that was further developed by our research team (Ismail et al. 2006a) comprising five features; a positive ionizable (PI), three hydrophobic pockets (HY1, HY2 and HY3), and a hydrogen bond acceptor (HBA) located at a suitable distance from the PI.

Unfortunately, the α_1-AR antagonists still lack selectivity as many of these agents possess dopaminergic and serotonergic activity. This may be due to the close similarity of the 3D features of these receptors.

In the past few years, the search for new selective α_1-AR antagonists revealed that substitution of the quinazoline ring of the α-blockers by pyrimidine may result in more potent agents. Accordingly, a new molecule, ŭrapidil (Corsano et al. 1999; Merieau et al. 2008; Bugnicourt et al. 2008) was among the developed molecules that contain a phenylpiperazine linked to a uracil ring. This drug was a safe and selective α_1-AR antagonist (Bugnicourt et al. 2008).



Urapidil

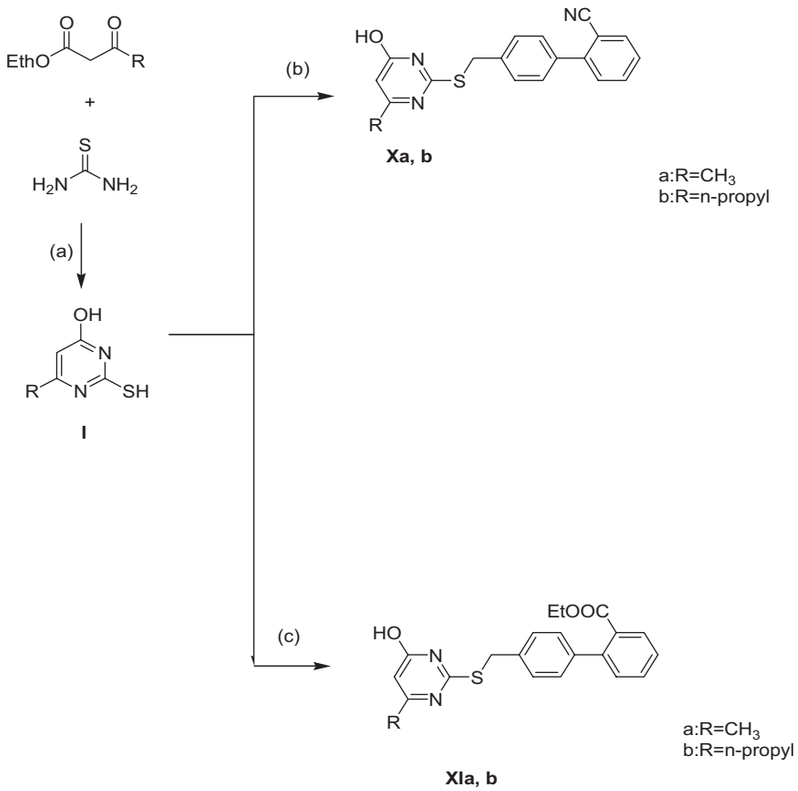


Scheme 1: (a) 4-aryl-1-chloroacylpiperazines, K_2CO_3, DMF, reflux, 24 h. (b) 1-(aryl)-4-(3-chloropropyl)piperazine, K_2CO_3, DMF, reflux, 18 h. (c) P_2S_5, xylene, reflux, 8 h. (d) Hydrazine hydrate 99%, ethanol, reflux, 1 h. (e) Formic acid, reflux, 24 h. (f) Acetyl acetone, reflux, 12 h. (g) 4-aryl-1-chloroacylpiperazines, K_2CO_3, DMF, reflux, 24 h. (h) 4-aryl-1-chloroacylpiperazine, K_2CO_3, DMF, reflux, 24 h.

In the current investigation, the design of new α_1-AR antagonists (viz; **IIa-f**, **IIIa-c**, **VIIa**, **b** & **IX**) was based upon modification in the structure of ŭrapidil by replacing its uracil ring by pyrimidinone ring systems, namely; pyrimidinones, triazinopyrimidines or pyrazolyl-pyrimidines. In addition, the substituents at the phenyl ring of ŭrapidil were changed by different isostearic moieties, the secondary amino group at its side chain was replaced by sulphur isostear, the arylpiperazine ring system was retained and the optimum length of the alkyl linker between the piperazine and pyrimidine ring system was changed to be 2-3 carbons. The similar structural features between ŭrapidil and our designed compounds **IIa-f**, **IIIa-c**, **VIIa**, **b** & **IX** may offer promising, more active molecules (Betti et al. 2002).

Furthermore, the design of these new α_1-AR antagonists was confirmed by molecular modeling simulation studies based upon compare/fit studies between our recently reported and approved α_1-AR antagonists hypothesis (Ismail et al. 2006a), and these test set targeted molecules. The virtual study revealed that most of the designed compounds have comparable prioritized fit values at the α_1-AR antagonists hypothesis with low conformation energy in comparison to the reference lead molecules. Accordingly, these hit molecules were planed to be synthesized according to the sequence outlined in Scheme 1.

Additionally, the renin-angiotensin system (RAS) plays an important role in the regulation of blood pressure through the actions of angiotensin II (Ang II) enzyme. Such enzymes have many activities involving; vasoconstriction, aldosterone secretion, renal sodium reabsorption, and norepinephrine release. Thus, this is an appropriate target for therapeutic intervention in hypertension. Inhibition and antagonism of the various components of the RAS has been the subject of extensive research, culminating in such drugs as angiotensin converting enzyme (ACE) inhibitors, rennin inhibitors, and more recently Ang II receptor antagonists (Javed et al. 2008). Ang II receptor antago-



Scheme 2: (a) Sodium metal, ethanol, steam bath, 7 h (b) 4′-(Bromomethyl)-2-cyanobiphenyl, K_2CO_3, DMF, reflux, 24 h (c) 4′-Bromomethyl-biphenyl-2-yl-carboxylic acid ethyl ester, K_2CO_3, DMF, reflux, 48 h.

nists have proved to lower blood pressure effectively and are better tolerated than other classes of drugs (Ellingboe et al. 1994). The development of non-peptide Ang II receptor antagonists was one of the most important achievements in this era. Optimization of the new molecules gave rise to the discovery of various lead compounds (Ellingboe et al. 1994).

The SAR of Ang II AT_1 receptor antagonists which was proposed by Carini et al. (1991), suggested the importance of the existence of an acidic function at the ortho position of the biphenyl moiety of the lead Ang II antagonists. In this research we included new molecules (**XIa**-**b**), that involved pyrimidinone nucleus linked to the 2′-carbethoxy-biphenylylmethyl scaffold, to have potential Ang II receptor antagonist activity (Ellingboe et al. 1994; Elokdah et al. 2002). The carbethoxy group has the ability to be deesterified *in vivo* into the active carboxylic acid analogues needed for effective binding with the receptor sites. The compare/fit virtual screening study between our recently generated and validated Ang II receptor antagonist hypothesis (Ismail et al. 2006b) and the de-esterified test molecules (**XIa**-**b**), showed comparable prioritized fit values with low conformation energy. Accordingly, these active hit molecules (**XIa**, **b**) were synthesized adopting the sequence outlined in Scheme 2.

2. Investigations and results

2.1. Synthesis of 2-(arylpiperazinyloxoalkylthio) pyrimidine derivatives IIa-f, VIIa, b & IX

The synthesis of 2-(arylpiperazinyloxoalkylthio)pyrimidine derivatives **IIa-f**, **VIIa**, **b** & **IX** (Scheme 1) was carried out starting from 6-alkyl uracil **I** via its thiation at position 4 to afford the key intermediate **IV**. The latter was reacted with hydrazine hydrate to introduce the hydrazino group at the 4-position to give **V**. Refluxing **V** with formic acid afforded **VI**, while refluxing it with acetyl acetone afforded the new intermediate **VIII**. S-alkylation of the key intermediates **I**, **VI** & **VIII** with the respective 4-aryl-1-chloroacylpiperazines generated the corresponding targeted compounds **IIa-f**, **VIIa**, **b** & **IX**. Structural confirmation of the new compounds was forthcoming from IR, NMR, MS, and elemental microanalysis data.

2.2. Synthesis of 6-alkyl-2{3′[(4″-arylpiperazin-1″-yl)propyl]thio}-4-hydroxy-pyrimidines IIIa-c

Compounds **IIIa-c** (Scheme 1) were synthesized by alkylating the key intermediate **I** with the respective 1-(aryl)-4-(3-chloropropyl)piperazine. IR, NMR, MS, and Elemental Microanalysis data confirmed the structures of the targeted compounds.

2.3. Synthesis of 4-hydroxy-6-alkyl-2-[(2″-cyanobiphenyl-4′-yl)methylthio]-pyrimidines Xa, b and 4-hydroxy-6-alkyl-2-[(2″-carboethoxybiphenyl-4′-yl)methylthio]pyrimidines XIa, b

The molecules **Xa, b** & **XIa, b** were constructed by the introduction of substituted biphenyl derivatives into the molecules of 6-alkyl-2-thio-(1H,3H)-pyrimidin-4-one (**I**) (Scheme 2). Thus, the key intermediates (**I**) were subjected to S-alkylation with 4′-(bromomethyl)-2-cyanobiphenyl and 4′-bromomethyl-biphenyl-2-yl-carboxylic acid ethyl ester in the presence of anhydrous potassium carbonate in DMF, following reported procedures (Le Bourdonnec et al. 2002). Products **Xa**, **b** & **XIa**, **b** were isolated in 20-27% yields. The structures of these compounds were confirmed by spectral and analytical data.

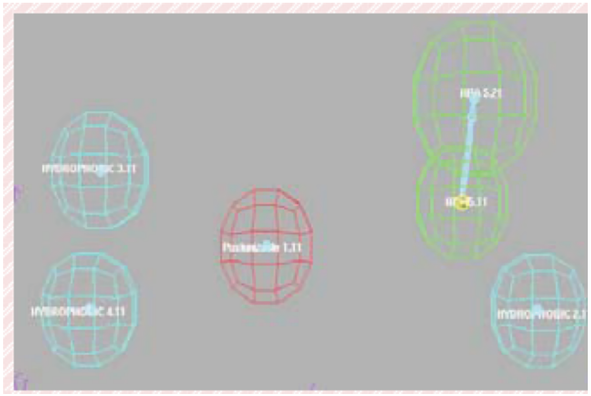


Fig. 1: Generated hypothesis for α_1-AR antagonist

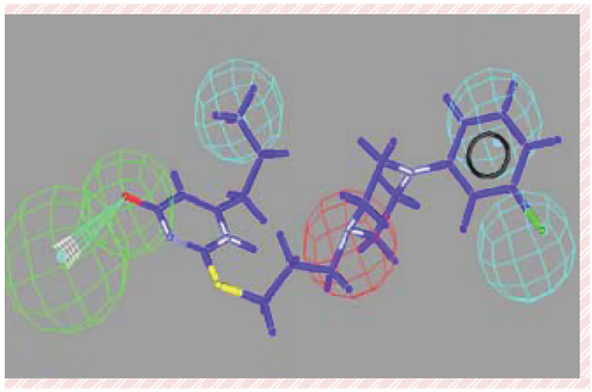


Fig. 2: mapping of α_1-AR antagonist hypothesis and **IIIc**

2.4. Molecular modeling

2.4.1. Molecular simulation fitting of the α_1-AR antagonist hypothesis and the test set of the target arylpiperazine derivatives

Molecular modeling studies were performed using ***Catalyst*** **s**oftware, ***HipHop*** module. The lead compounds, which were reported to have potent α_1-AR antagonistic activity (Ismail et al., 2006a), were chosen as training set to generate common-feature hypothesis according to the reported methods by our computer drug design lab. In this case, the conformational model for each compound was generated, and then used to construct the common-feature hypothesis (by default) for α_1-AR antagonists. The generated hypothesis (Fig. 1) was used as a template to predict and prioritize the biological activity of the test compounds (**IIa-f**, **IIIa-c**, **VIIa**, **b** & **IX**). The compare fit values of mapping of the test set compounds (**IIa-f**, **IIIa-c**, **VIIa**, **b**, and **IX**) with the generated hypothesis were collected together with the number of generated conformers and the conformational energy of the best fit conformer in Table 1. The molecular modeling study revealed that most of the test compounds showed prioritized comparable affinities to the generated α_1-AR antagonist hypothesis in comparison to prazosin. Thus, they could be considered as promising active α_1-AR antagonists hits. Compound **IIIc** showed the highest fitting affinity with the hypothesis among the test set compounds (Fig. 2), indicating that it is the most promising one. Accordingly, all these compounds were synthesized and the most promising hits (**IIa**,**e** & **f**, **IIIa-c**, **VIIb** and **IX**) were examined for their hypotensive activity while the molecules with low fit values (**IIb**, **IIc**, **IId** & **VIIa**), were not biologically examined.

2.5. Molecular simulation fitting of the AT_1 antagonist hypothesis and the test set of the target 2′-carbethoxy-biphenylylmethyl derivatives

In this search, the sets of conformational models of 6 leads, namely losartan (Cozaar®), irbesartan, olmesartan, candesartan, tasosartan, and telmisartan (Ismail et al. 2006b) were used to generate HipHop Ang II antagonists common features hypothesis. The mapping of the test compounds with the generated hypothesis (Fig. 3) was carried out using Best Fit algorithm, during the Compare/Fit process. The data collected included the fit value of the best fit conformer for each of the test set compounds (**XIa**, and **XIb**) with the generated hypothesis. The values of the compare fit were collected together with the number of generated conformers and the conformational energy of the best fit conformer in Table 1. Such compare/fit studies predicted that the designed compounds (**XIa**,**b**) showed prioritized comparable fitting affinities of their de-esterified molecules with the AT_1 antagonist pharmacophore in comparison to losartan (Fig. 4). Accordingly, these final compounds (**XIa**-**b**) were synthesized and examined for their hypotensive activity.

Table 1: Effect of test compounds (II∼IX) on systolic blood pressure of un-anaesthetized normotensive rats in comparison with fit values at the α_1-AR antagonist hypothesis

Compd	Conf. energy (kcal/mol)	Fit-values at the *α_1-AR antagonist* hypothesis	Systolic BP (mmHg) Mean ± SE	Mean change %
Control	–	–	105 ± 1.4	–
Prazosin	1.98	3.88	85 ± 2.15	−19.05%
IIe	16.33	3.64	97 ± 1.76	−7.62%
IIf	15.57	3.7	94 ± 1.96	−10.48%
IIIa	8.86	2.99	94 ± 2.04	−10.48%
IIIb	4.5	3.57	95 ± 2.87	−9.52%
IIIc	12.06	3.73	90 ± 0.79	−14.30%
IIa	8.1	2.22	105 ± 1.3	0%
VIIb	1.04	1.47	106 + 0.4	0%
IX	12.46	2.85	105 + 1.4	0%
IIb	19.66	1.41	–	–
IIc	12.99	1.39	–	–
IId	10.12	1.45	–	–
VIIa	19.01	1.49	–	–

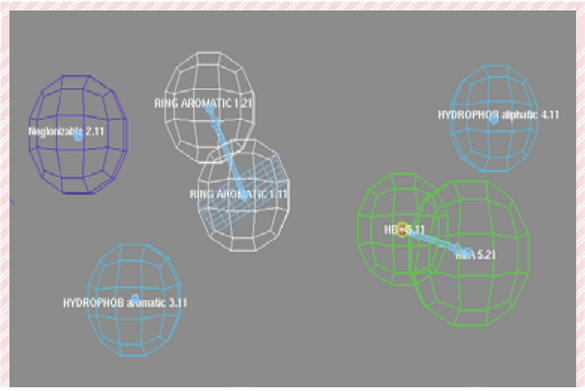


Fig. 3: Generated hypothesis for AT_1 receptor antagonists

2.6. Biological in vivo hypotensive activity testing

Preliminary *in vivo* hypotensive activity testing for the hit compounds which have good fit values with α_1-AR antagonist hypothesis (**IIa**,**e**,**f**, **IIIa-c**, **VIIb** & **IX**) (Table 1), as well as the promising hit compounds (**XIa** & **XIb**) which have high fit values with Ang II antagonist hypothesis were examined in comparison to the α_1-AR antagonist lead compound; prazosin, and the AT_1 antagonist lead compound; losartan as reference drugs, respectively. The experiments were carried out in unanaesthetized normotensive rats according to a reported method (Ismail et al. 2000). The effect of the tested compounds on systolic blood pressure of un-anaesthetized normotensive rats was demonstrated in Tables 1 and 2.

2.7. Conclusion

In this project we were able to synthesize a set of new molecules having pyrimidinone ring system linked to 4-arylpiperidino moieties or biphenyl-carboxylic acid scaffold that could be act as either α_1-AR antagonists or Ang II antagonists, respectively. *In vivo* biological evaluation revealed that compounds **IIe**,**f** and **IIIa-b** showed moderate antihypertensive activities than prazosin, where compound **IIIc** demonstrated the highest antihypertensive activity (Table 1). Also, compounds **XIa**,**b** showed comparable prioritized activity to their Ang II antagonist's analogue, losartan (Table 2). The virtual screening that have been performed in this research (Compare Fit with the corresponding hypothesis), appeared to be matched with the *in vivo* hypotensive activity indicating reliable designing strategy to develop new antihypertensive agents at either the α_1-AR or the Ang II receptors.

3. Experimental

Melting points were determined with Stuart Scientific apparatus and are uncorrected. FT-IR spectra were recorded on a Perkin-Elmer spectrophotometer and measured by ν' cm^{-1} scale using KBr cell. ^{1}H NMR spectra were measured in δ scale on a JEOL 300 MHz spectrometer. Unless otherwise stated, the spectra were obtained on solutions in DMSO and referred to TMS. The electron impact (EI) mass spectra were recorded on Finnigan Mat SSQ 7000 (70 ev) mass spectrometer. The peak intensities, in parentheses, are expressed as percentage abundance. Analytical thin layer chromatography (TLC) was performed on Merk Kieselgel 60PF_{254} silica on aluminum backed sheets. All R_f values were recorded from the center of the spots. All TLC solvent proportions were measured volume by volume. Column chromatography was performed using Merk Silica gel (mesh size = 0.032- 0.064 mm). all reagents and solvents were purified and dried by standard techniques. Solvents were removed under reduced pressure in a rotary evaporator. Elemental microanalysis was performed at the Microanalytical Center, Cairo University. The preparation of was performed according to the reported procedures.

3.1. Preparation of 6-alkyl-4-hydroxy-2[2′(4″-aryl-piperazin-1″-yl)-2′-oxoalkyl]-thiopyrimidines II a–f

To a mixture of the corresponding pyrimidine derivatives **Ia**, **b** (0.005 mol) and K_2CO_3 (1.38 g, 0.01 mol) in dry DMF (7 mL), the appropriate 4-aryl-1-chloroacyl-piperazine (0.005 mol of each) was added. The reaction mixture was refluxed for 24 h, cooled and filtered. The filtrate was evaporated under vacuum almost to dryness, ice cold water and brine were added where a white solid was precipitated. The formed solid was crystallized from acetone-ether to produce the titled compounds (**II a**–**f**).

3.1.1. Compound **IIa**

It was separated as white crystals (72% yield) m.p. >300 °C. MS (EI): *m/z* 344 (M^+, 24.29%), IR (FT): 3500 (OH), 1633 (CO). ^{1}H NMR; 2.08 (s, 3H, CH_3), 3.18–3.25 (m, 4H, $2CH_2$ piperazine), 3.68–3.74 (m, 4H, $2CH_2$

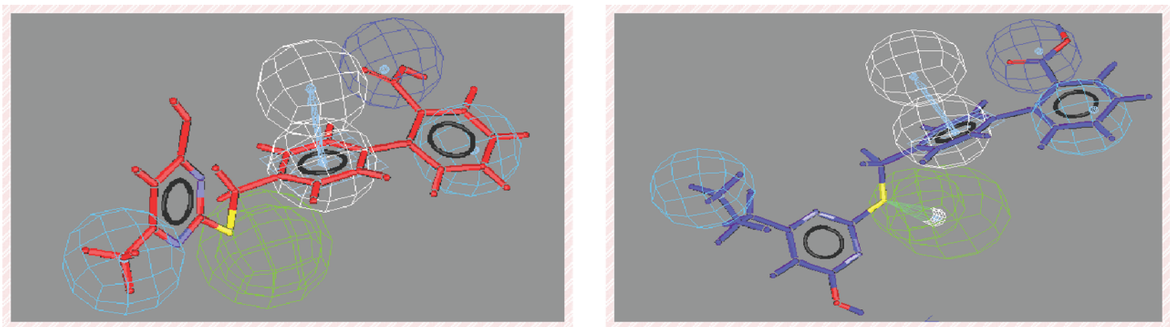


Fig. 4: Mapping of AT1 hypothesis with XIa and XIb

Table 2: Effect of test compounds (XIa-XIb) on systolic blood pressure of unanaesthetized normotensive rats in comparisone with fit values at the Ang II antagonist hypothesis

Compd	Conf. energy (kcal/mol)	Fit-values at the *Ang II antagonist* hypothesis	Systolic BP (mmHg) Mean ± SE	Mean change %
Control	–	–	105 ± 1.4	–
Losartan	3.2	5	81 ± 1.93	−20%
XIa	3.93	3.93	87 ± 1.86	−17.14%
XIb	2.58	3.95	89 ± 1.49	−15.24%

piperazine), 4.02 (s, 2H, -S-CH2-C=O), 5.65 (s, 1H, pyrimidyl-H), 6.80–7.23 (m, 5H, Ar-H).
$C_{17}H_{20}N_4O_2S$

3.1.2. Compound **IIb**

It was separated as yellow crystals (65% yield), m.p. 85 °C. MS (EI): *m/z* 358 (M^+, 9.1%), IR (FT): 3640 (OH), 1655 (CO). ^{1}H NMR; 1.45 (d, 3H, CH_3), 2.52 (s, 3H, CH_3), 3.06–3.17 (m, 4H, $2CH_2$ piperazine), 3.62–3.72 (m, 4H, $2CH_2$ piperazine), 4.83–4.92 (q, 1H, -S-CH-C=O), 6.60 (s, 1H, pyrimidyl-H), 6.81–7.19 (m, 5H, Ar-H).
$C_{18}H_{22}N_4O_2S$

3.1.3. Compound **IIc**

It was separated as white crystals (68% yield), m.p. 181 °C. MS (EI): *m/z* 402 (M^+, 8.1%), IR (FT): 3540 (OH), 1651 (CO). ^{1}H NMR; 0.75 (t, ***J***=7 Hz, 3H, CH_3-CH_2-CH_2-), 1.3 (sextet, ***J*** = 7 Hz, 2H, CH_3-CH_2-CH_2-), 2.22 (t, ***J*** = 7 Hz, 2H, CH_3-CH_2-CH_2-), 2.60–2.68 (m, 4H, $2CH_2$ piperazine), 3.26-3.40 (m, 4H, $2CH_2$ piperazine), 3.86 (s, 3H, -O-CH_3), 4.76 (s,2H, -S-CH2-C=O), 6.23 (s, 1H, pyrimidyl-H), 6.54–6.63 (m, 4H, Ar-H).
$C_{20}H_{26}N_4O_3S$

3.1.4. Compound **IId**

It was separated as faint yellowish white crystals (76% yield), m.p. 159 °C. MS (EI): *m/z* 372 (M^+, 26.4%), IR (FT): 3586 (OH), 1684 (CO). ^{1}H NMR; 0.80 (t, ***J*** = 7 Hz, 3H, CH_3-CH_2-CH_2-),1.56 (sextet, ***J*** = 7 Hz, 2H, CH_3-CH_2-CH_2-),2.32 (t, ***J*** = 7 Hz, 2H, CH_3-CH_2-CH_2-),3.08–3.17 (m, 4H, $2CH_2$ piperazine), 3.58–3.67 (m, 4H, $2CH_2$ piperazine), 4.16 (s,2H, -S-CH2-C=O), 5.89 (s, 1H, pyrimidyl-H), 6.78–7.23 (m, 5H, Ar-H).
$C_{19}H_{24}N_4O_2S$

3.1.5. Compound **IIe**

It was separated as yellow crystals (45% yield), m.p. 92 °C. MS (EI): *m/z* 388 (M^+, 8.9%), IR (FT): 3600 (OH), 1643 (CO). ^{1}H NMR; 2.16 (s, 3H, CH_3), 2.78 (t, ***J*** = 7 Hz, 2H, -CH2-C=O), 2.91–2.93 (m, 4H, $2CH_2$ piperazine), 3.30 (t, ***J*** = 7 Hz, 2H, -S-CH2-), 3.58–3.59 (m, 4H, $2CH_2$ piperazine), 3.78 (s, 3H, -O-CH_3), 5.93 (s, 1H, pyrimidyl-H), 6.83–6.98 (m, 4H, Ar-H).
$C_{19}H_{24}N_4O_3S$

3.1.6. Compound **IIf**

It was separated as yellow crystals (34% yield), m.p. 123 °C. MS (EI): *m/z* 416 (M^+, 14%), IR (FT): 3600 (OH), 1655 (CO). ^{1}H NMR; 0.88 (t, ***J*** = 7 Hz, 3H, CH_3-CH_2-CH_2-),1.61 (sextet, ***J*** = 7 Hz, 2H, CH_3-CH_2-CH_2-),2.39 (t, ***J*** = 7 Hz, 2H, CH_3-CH_2-CH_2-),2.79 (t, ***J*** = 7 Hz, 2H, -CH2-C=O), 2.88–2.91 (m, 4H, $2CH_2$ piperazine), 3.31 (s, 2H, -S-CH_2-),3.58–3.59 (m, 4H, $2CH_2$ piperazine), 3.78 (s, 3H, -O-CH_3), 5.93 (s, 1H, pyrimidyl-H), 6.87–6.95 (m, 4H, Ar-H).
$C_{21}H_{28}N_4O_3S$

3.2. Preparation of 4-hydroxy-6-substituted-2[3′(4″-aryl-piperazin-1″-yl)propyl-thio]pyrimidines (III)

To a mixture of the corresponding pyrimidine derivatives **Ia**, **b** (0.005 mol of each) and K_2CO_3 (1.38 g, 0.01 mol) in DMF (3 mL), the appropriate 1-(aryl)-4-(3-chloropropyl)piperazine (0.005 mol of each) was added. The reaction mixture was refluxed for 18 h, cooled and filtered. The filtrate was evaporated under vacuum till near dryness, ice cold water and brine were added where a white solid was precipitated. The precipitate was filtered, washed with water and 10% NaOH solution, dried over anhydrous calcium chloride and crystallized from acetone.

3.2.1. Compound **IIIa**

It was separated as yellow crystals (74% yield), m.p. 190 °C. MS (EI): *m/z* 344 (M^+, 61.2%), IR (FT): 3500 (NH), 1656 (CO). ^{1}H NMR; 1.84 (p, ***J*** = 7 Hz, 2H, -S-CH_2-CH_2-CH_2-), 2.15 (s, 3H, CH_3), 2.42 (t, ***J*** = 7 Hz, 2H, -S-CH_2-CH_2-CH_2-), 3.10–3.16 (m, 10H, 4 CH_2 piperazine,-S-CH_2-CH_2-CH_2-), 5.92 (s, 1H, pyrimidyl-H), 6.76–7.20 (m, ***J*** = 7 Hz, 5H, Ar-H).
$C_{18}H_{24}N_4OS$

3.2.2. Compound **IIIb**

It was separated as white crystals (85% yield), m.p. 180 °C. MS (EI): *m/z* 379 (M^+, 1.11%), IR (FT): 3470 (NH), 1681 (CO). ^{1}H NMR; 1.85 (p, ***J*** = 7 Hz, 2H, -S-CH_2-CH_2-CH_2-), 2.15 (s, 3H, CH_3), 2.40 (t, ***J*** = 7 Hz, 2H, -S-CH_2-CH_2-CH_2-), 3.09–3.17 (m, 10H, 4 CH_2 piperazine, -S-CH_2-CH_2-CH_2-), 5.92 (s, 1H, pyrimidyl-H), 6.77–7.19 (m, 4H, Ar-H).
$C_{18}H_{23}ClN_4OS$

3.2.3. Compound **IIIc**

It was separated as white crystals (92% yield), m.p. 132 °C. MS (EI): *m/z* 407 (M^+, 2.3%), IR (FT): 3358 (NH), 1710 (CO). ^{1}H NMR; 0.88 (t, ***J*** = 7 Hz, 3H, CH_3-CH_2-CH_2-), 1.62 (sextet, ***J*** = 7 Hz, 2H, CH_3-CH_2-CH_2-), 1.83 (p, ***J*** = 7 Hz, 2H, -S-CH_2-CH_2-CH_2-), 2.35 (t, ***J*** = 7 Hz, 2H, -S-CH_2-CH_2-CH_2-), 2.40-2.48 (m, 2H, CH_3-CH_2-CH_2-), 3.10–3.15 (m, 10H, 4 CH_2 piperazine, -S-CH_2-CH_2-CH_2-), 5.90 (s, 1H, pyrimidyl-H), 6.74–7.18 (m, 4H, Ar-H).
$C_{20}H_{27}ClN_4OS$

3.3. Preparation of 7-methyl-5[2′(4″-aryl-piperazine-1″-yl)-2′-oxoalkylthio] [1,2,4]triazolo[4,3-c]pyrimidine (VII)

To a mixture of the triazolopyrimidine derivative **VI** (0.005 mol of each) and K_2CO_3 (1.38 g, 0.01 mol) in DMF (5 mL), the appropriate 4-aryl-1-chloroacyl-piperazine (0.005 mol of each) was added. The reaction mixture was refluxed for 24 h, cooled and filtered. Water (10 mL) was added to the filtrate, and the resulting white suspension was extracted with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with brine (10 mL X 2), dried over anhydrous magnesium sulphate, and concentrated *in vaccuo*. The resulting solid was recrystallized from ether.

3.3.1. Compound **VIIa**

It was separated as yellowish white crystals (57% yield), m.p. 60 °C. MS (EI): *m/z* 368 (M^+, 7%), IR (FT): 1710 (CO). ^{1}H NMR; 2.50 (s, 3H, CH_3), 3.14–3.24 (m, 4H, $2CH_2$ piperazine), 3.65–3.78 (m, 4H, $2CH_2$ piperazine), 4.46 (s, 2H, -CO-CH_2-S-), 6.82–7.24 (m, 5H, Ar-H), 7.46 (s, 1H, pyrimidyl-H), 8.58 (s, 1H, triazolyl-H).
$C_{18}H_{20}N_6OS$

3.3.2. Compound **VIIb**

It was separated as yellowish white crystals (63% yield), m.p. 194 °C. MS (EI): *m/z* 368 (M^+, 7%), IR (FT): 1710 (CO). ^{1}H NMR; 1.61 (d, ***J*** = 10 Hz, 3H, S-CH-CH_3), 2.51 (s, 3H, CH_3), 3.17–3.27 (m, 4H, $2CH_2$ piperazine), 3.68–3.84 (m, 4H, $2CH_2$ piperazine), 5.26 (q, ***J*** = 10 Hz, 1H,, -CO-CH-S-), 6.83–7.25 (m, 5H, Ar-H), 7.51 (s, 1H, pyrimidyl-H), 8.60 (s, 1H, triazolyl-H).
$C_{19}H_{22}N_6OS$

3.4. Preparation of 4-(3,5-dimethylpyrazol-1-yl)-6-methyl-2-mercaptopyrimidine (VIII)

The hydrazino pyrimidine derivative **V** (10 g, 0.06 mol) was refluxed for 12 h with acetylacetone (60 mL, 0.3 mol). The precipitate formed was filtered, washed and crystallized from dry acetone to give 4-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6-methylpyrimidine-2-thiol (**VIII**) as yellow crystals (7.9 g, 64%), m.p. 251 °C. MS (EI): *m/z* 220 (M^+, 50.6%), IR (FT): 1466 (SH). ^{1}H NMR; 2.24 (s, 3H, CH_3), 2.32 (s, 3H, CH_3), 2.62 (s, 3H, CH_3), 6.21 (s, 1H, pyrazolyl-H), 7.13 (s, 1H, pyrimidyl-H).
$C_{10}H_{12}N_4S$

3.5. Preparation of 4-(3,5-dimethylpyrazol-1-yl)-6-methyl-2[2′(4″-phenyl-piperazin-1″-yl)-2′-oxoethylthio]pyrimidine (IX)

The title compound was prepared from 4-(3,5-dimethyl-1*H*-pyrazol-1-yl)-6-methylpyrimidine-2-thiol (**VIII**) (1 g, 5 mmol) and 4-phenyl-1-chloroacetylpiperazine (0.88 g, 5 mmol) using the same general procedure described for the preparation of **IIa–f** to give the crude product as yellow solid which is further recrystallized from acetone/ether to provide (1.6 g, 83%) of the desired compound **IX**, m.p.:162 °C. MS (EI): *m/z* 422 (M^+, 3.2%), IR (FT): 1651 (CO). ^{1}H NMR; 2.21 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 3.13–3.23 (d, 4H, $2CH_2$ piperazine), 3.63–3.70 (d, 4H, $2CH_2$ piperazine), 4.29 (s, 2H, -S-CH2-C=O), 6.21 (s, 1H, pyrazolyl-H), 6.83 (t, 1H, Ar-H), 7.00 (d, 2H, Ar-H), 7.25 (t, 2H, Ar-H), 7.46 (s, 1H, pyrimidyl-H).
$C_{22}H_{26}N_6OS$

3.6. Preparation of 4-hydroxy-6-alkyl-2-[(2″-cyanobiphenyl-4′-yl) methylthio]-pyrimidines X

To a mixture of the corresponding pyrimidine derivatives **Ia**, **b** (0.002 mol of each) and K_2CO_3 (0.55 g, 0.004 mol) in DMF (5 mL), 4′- (bromomethyl)-2-cyano-biphenyl (0.544 g, 0.002 mol) was added. The reaction mixture was refluxed for 24 h, cooled and filtered. The filtrate was evaporated under vacuum almost to dryness, ice cold water and brine were added where a white solid was precipitated. The precipitate was filtered, washed with water and 10% NaOH solution and dried over anhydrous magnesium sulphate.

3.6.1. Compound **Xa**

It was separated as yellow crystals (27% yield), m.p. 260 °C. MS (EI): *m/z* 333 (M^+, 100%). IR(FT): 3524(NH), 2222(CN), 1655(CO). ^{1}H NMR; 2.26 (s, 3H, CH_3), 4.45 (s, 2H, S-CH_2-), 5.98 (s, 1H, pyrimidyl-H), 7.35–7.94 (m, 8H, Ar-H).
$C_{19}H_{15}N_3OS$

3.6.2. Compound **Xb**

It was separated as yellow crystals (23% yield), m.p. 172 °C. MS (EI): *m/z* 361 (M^+, 100%). IR(FT): 3550(NH), 2258(CN), 1652(CO). ^{1}H NMR; 0.863 (t, J = 7 Hz, 3H, CH_3-CH_2-CH_2), 1.640 (sextet, J = 7 Hz, 2H, CH_3-CH_2-CH_2), 2.386 (t, J = 7 Hz, 2H, CH_3-CH_2-CH_2), 4.44 (s, 2H, S-CH_2) 5.91(s, 1H, pyrimidyl-H), 7.51–7.95 (m, 8H, Ar-H).
$C_{21}H_{19}N_3OS$

3.7. Preparation of 4-hydroxy-6-alkyl-2-[(2″-carboethoxybiphenyl-4′-yl)methyl-thio]pyrimidines (XIa,b)

The title compounds were prepared from 4′-bromomethyl-biphenyl-2-carboxylic acid ethyl ester (2 g, 19.3 mmol) and (I) (1.2 g, 17.5 mmol) using the same procedure described in the preparation of **X** to give the crude product as brown oil which was purified by flash column chromatography using *n*-hexane/chloroform (3:1) giving the ester **XI** as a pale yellow oil.

3.7.1. Compound **XIa**

It was separated as pale yellow oil (25% yield). MS (EI): *m/z* 380 (M^+, 0.61%). IR(FT): 3560(OH), 1705(CO). ^{1}H NMR; 1.26 (t, 3H, $\underline{CH_3}$-CH_2-C=O), 2.40 (s, 3H, CH_3), 2.9 (q, 2H, CH_3-$\underline{CH_2}$-C=O), 3.45 (s, 2H, S-CH_2-), 5.67 (s, 1H, pyrimidyl-H), 7.23–7.67(m, 8H, Ar-H).

3.7.2. Compound **XIb**

It was separated as pale yellow oil (28% yield). MS (EI): *m/z* 408 (M^+, 1.02%). IR(FT): 3620(OH), 1710(CO). ^{1}H NMR; 0.97 (two overlapped triplets, 6H, CH_3-CH_2-CH_2, CH_3-CH_2-C=O), 1.19 (m, 2H, CH_3-CH_2-CH_2), 2.34 (t, 2H, CH_3-CH_2-CH_2), 2.88 (q, 2H, CH_3-CH_2-C=O), 3.36 (s, 2H, S-CH_2-), 5.56 (s, 1H, pyrimidyl-H), 7.16–7.60 (m, 8H, Ar-H).

3.8. Pharmacological tests

In vivo biological evaluation of the effect of the tested compounds on the arterial blood pressure of normotensive adult rats were carried out according to Ismail et al. (2002).

3.8.1. Materials and method

Experiments were carried out in unanaesthetized normotensive rats according to a reported method (Banappa and Basangouda 2009). Pre-trained, seventy-two normal rats were used in this set of experiment, animals were divided to 12 equal groups and intraperitoneally (i.p.) injected as follows: group 1, kept as a normal control (0.1 ml DMSO, i.p.); while groups 2-12 were given (1 mg/kg in 0.1 ml DMSO, i.p.) the tested compounds, and the standard drugs; prazosin and losartan. The systolic blood pressure (SBP) was measured in the animals by the tail-cuff method (Beunag 1973) after 1 hour of treatment. SBP was measured using the tail-cuff method in conscious rats. Each rat was put in a thermostatted box (UGO BASILE Heating Box 8450) at 39 °C for 10 min, and SBP was then measured in unanaesthetized rats (Riva Rocci Sphygmomanometer, 58500 UGO BASILE, ITALY). All results are expressed throughout as means ± S.E.M. For comparison of responses with control group, means were analyzed by one-way analysis of variance (ANOVA) and LSD. Statistical significance was determined at $P < 0.05$.

3.8.2. Molecular modeling experiments

All molecular modeling work was performed on Silicon Graphic (SGI), Fuel workstation (500 MHz, R 14000 ATM processor, 512 MB memory) using the Catalyst package of Molecular Simulation (version 4.8), under an IRIX 6.8 operating system, at Faculty of Pharmacy, Ain Shams University. A generalized visualizer, confirm, info, HipHop, Compare/fit, force field was used throughout. Molecules were built within the catalyst and conformational models for each compound were generated automatically using the poling algorithm. This emphasizes representative coverage over a 20 kcal mol^{-1} energy range above the estimated global energy minimum and the best searching procedure was chosen.

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