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Rational design and synthesis of 1-(arylideneamino)-4-aryl-1*H*-imidazole-2-amine derivatives as antiplatelet agents

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Abstract: Based on the previous studies indicating the pharmacophoric role of hydrazone group and azole rings for antiplatelet aggregation activity, a few series of compounds with both hydrazone and azole (imidazole) ring in their structures were synthesized and their platelet aggregation inhibitory effect was evaluated. Two compounds among the 1-(arylideneamino)-4-aryl-1*H*-imidazole-2-amine derivatives compound **4a** and **4p** showed IC_{50} values comparable to that of aspirin against collagen as platelet aggregation inducer. Structural comparison of the synthesized compounds revealed that those compounds with para-substituted phenyl ring on imidazole ring are among the most active compounds against the platelet aggregation induced by arachidonic acid (AA) and the presence of thiophene ring in these compounds (**6b**, **6c** and **6d**) will maximize their antiplatelet activity.

Introduction

Cardiovascular diseases (CVDs) such as coronary heart disease (heart attack), cerebrovascular disease (stroke) and deep vein thrombosis are one of the main causes of death in the world. It is estimated that CVDs are responsible for 17.5 million deaths (31%) every year. Over 75% of CVD deaths occur in low- and middle-income countries.[1]

Platelet activation and aggregation play a pivotal role in CVDs and their complications. Antiplatelet drugs are widely used in the treatment of CVDs and prevention of heart attack and stroke.

Platelet activation can be initiated upon exposure to various endogenous agonists such as thrombin, thromboxane A2 (TXA_2), collagen and/or adenosine 5'-diphosphate (ADP).[2]

Many antiplatelet drugs have been developed based on different mechanisms of action such as: aspirin (TXA_2 pathway blocker), clopidogrel and ticlopidine (ADP receptor antagonist)[3], tirofiban (GPIIa/IIIb antagonist), cilostazol (phosphodiesterase inhibitor)[3], dipyridamole,[4] ozagrel and dazoxiben (TXA_2 synthase inhibitors).[5,6]

Alongside the advantages of antiplatelet drugs, they have some limitations such as gastric erosion, bleeding, variable response in patients, drug resistance and interaction.[3,7] Therefore, attempts to develop new efficient antiplatelet drugs is ongoing.[8]

Recently several imidazole derivatives were also synthesized as antiplatelet agents. Rehse and steege have reported imidazole-4-carboxylic acid derivatives with antiplatelet activity in a low micromolar range.[9] A series of fluorinated imidazole-2-thiones were prepared with concentration dependent antiplatelet aggregation activity on the thrombin and ADP-induced platelet aggregation by Momin et al.[10]

A review among the compounds with antiplatelet aggregation activity reveals that hydrazone moiety plays a pharmacophoric role in many of these compounds. Chelucci et al reported drugs that hybrid with N-acyl hydrazone subunit show antiplatelet and antithrombotic activities.[11] Todeschini et al. reported a series of 2-pyridylhydrazones in 1998 with moderate inhibitory activity against arachidonic acid (AA).[12] Todeschini et al. disclosed the synthesis and pharmacological profile of N-phenylpyrazole arylhydrazones derivatives and described the pharmacophoric contribution of both the functionalized arylhydrazone units and the N-heterocyclic moiety in antiplatelet aggregation activities.[12] Jordao et al have also introduced a group of N-acylheteroarylhydrazones efficient inhibitors of AA, ADP and/or adrenaline pathways.[13] It was reported by Watala et al. that resorcylidene aminoguanidine showed unique antithrombotic activity in vivo.[14]

In our previous works we synthesized a group of indole hydrazone derivatives **1** (Figure 1) with efficient platelet aggregation inhibitory effect.[15-17] Classic bioisosteric replacement of phenyl ring with heteroaromatic rings gave us compound **2** (Figure 1) with IC_{50} = 2.2 µM against the platelet aggregation induced by AA.[18]

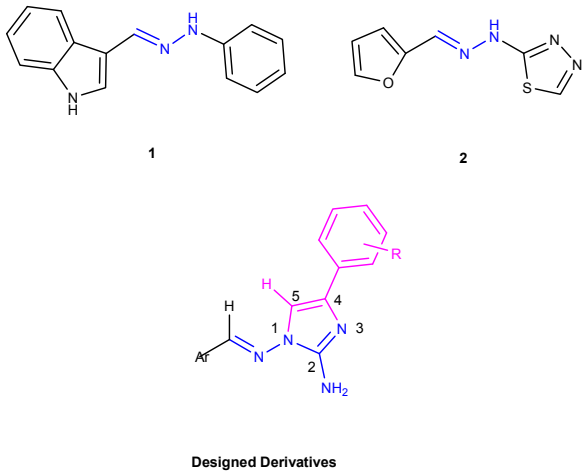


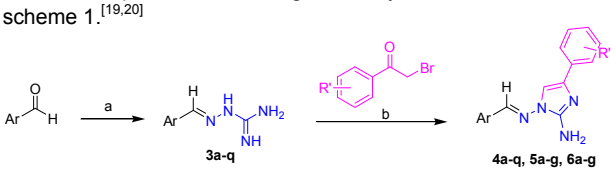
Figure 1. Structure of **1**, **2** and designed derivatives.

In continuation of our quest to find new structures with potent antiplatelet aggregation activity we decided to take advantage of the possible pharmacophoric contribution of both hydrazone unit and N-heterocyclic rings by molecular hybridization of the two moieties. Therefore we designed and synthesized a new series of hydrazinic group of 1-(arylideneamino)-4-aryl-1*H*-imidazole-2-amine.

Results and Discussion

Chemistry

The guanylhydrazones **3a-q** were prepared by the reaction of aminoguanidine with appropriate aromatic aldehydes. The obtained hydrazone derivatives **3a-q** were converted to compounds **4a-q** in the presence of phenacyl bromide in ethanol at room temperature according to the synthetic route shown in scheme 1.[19,20]



Scheme 1. *Reagents and conditions:* a) Aminoguanidine bicarbonate, HCl, 1 h; b) NaOH, EtOH, 40-45 °C, 24 h.

The double bond geometry of **4a** was characterized by nuclear overhauser effect (NOE) experiments. The stereochemistry determined to be *E* according to the strong NOE effect between the HC=N and proton of imidazole ring.(Figure 2) This finding was also consistent with previous studies.[20,21]

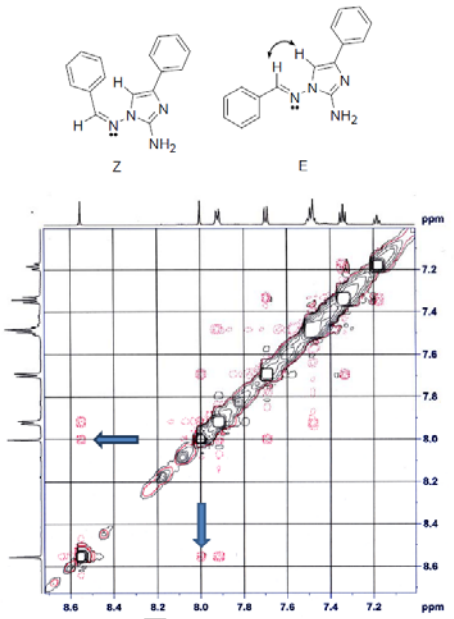


Figure 2. NOE spectrum of compound **4a**. The off-diagonal signal which correlate peaks at 8.0 and 8.57 ppm indicates the spacial correlation of HC=N and H-5 of imidazole ring.

The compounds **5a-g** and **6a-g** with substituent on phenyl ring at position 4 of imidazole ring were prepared by reaction of **3a** and **3p** with different phenacyl bromide derivatives.

Pharmacology: Antiplatelet activity

The antiplatelet activity of synthesized compounds were evaluated according to Born method[22] by using ADP, collagen and AA as platelet aggregation inducers. The obtained IC_{50} values are reported in Table 1 and 2 as mean ± SE (n=3).

Structure activity relationships

As shown in Table 1 all guanylhydrazone compounds displayed poor antiplatelet activity against ADP with the exception of **3f**, **3i** and **3j** which showed a moderate activity (IC_{50} = 230-240 µM). In order to investigate the pharmacophoric contribution of azole ring in exerting antiplatelet activity, guanylhydrazone derivatives were converted to imidazole derivatives via a ring closure reaction. Thus compound **4a-q** were synthesized and their antiplatelet activity was evaluated (Table 1). The obtained data demonstrated that antiplatelet activity against the aggregation induced by ADP did not improve after ring closure.

As presented in Table 1, among the guanylhydrazone compounds when collagen was used as platelet aggregation inducer, only 2-fluorophenyl derivative **3b** exhibited good antiplatelet activity (IC_{50} = 23.98 µM). Compounds **3f**, **3g**, **3i**, **3j**, **3m** and **3p** with IC_{50} values range between 50 to 90 µM, show moderate activities. Ring closure and converting the guanylhydrazones to imidazole derivatives resulted in improvement in activity of compounds **4a** (IC_{50} = 11.9 µM) and **4p** (IC_{50} = 10.65 µM) against the platelet aggregation induced by collagen. These IC_{50} values are comparable to that of aspirin (9.7 µM). This result showed that replacement of phenyl ring with the bioisostere ring, thienyl, did not affect the activity against collagen. Compounds **4f**, **4g**, **4i** and **4l** exhibited modest activity against the platelet aggregation induced by collagen (IC_{50} = 33-74 µM). Most of the imidazole derivatives exhibited an antiplatelet activity very similar to their corresponding guanylhydrazone compounds.

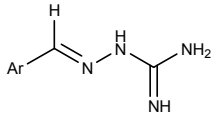
Guanylhydrazone compounds **3g** (IC_{50} = 153.7 µM) and **3m** (IC_{50} = 155.4 µM) showed the highest antiplatelet activity against AA among other guanylhydrazone derivatives. Interestingly, the presence of imidazole ring improves the antiplatelet activity against AA induced platelet aggregation. The antiplatelet activity induced by AA of all imidazole derivatives with halogen substituent (**4b-j**) at ortho, meta and para position of the phenyl ring decreases as the size of the halogen increases from fluorine to bromine. Moreover, addition of methoxy and methyl group at para position of phenyl ring in **4l** and **4k** improve the antiplatelet activity against the AA pathway, whereas introduction of hydroxyl groups at the C-4 position of phenyl group decreased the antiplatelet activity. The antiplatelet activity of imidazole derivatives with a thienyl group **4p** improved when compared to imidazole derivatives with furanyl **4o** and 2-pyridyl **4q** groups. Compound **4e** (IC_{50} = 27.00 µM) and **4p** (IC_{50} = 26.61 µM) with phenyl and thienyl ring were the most active compounds against the platelet aggregation induced by AA.

As Compound **4p** was the most active compound against the platelet aggregation induced by both collagen and AA and compound **4a** showed activity against collagen comparable to that of **4p**, we decided to investigate the importance of substitution at the phenyl ring attached to imidazole ring for

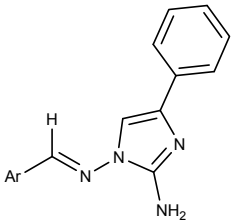
these series of compounds. Compounds **5a-g** with various substituted phenyl on the imidazole ring were synthesized and their antiplatelet activity was evaluated. As indicated in Table 2, the antiplatelet activity of phenyl imidazole derivatives **5a-g** didn't improve against ADP but the activity decreased significantly against collagen (**4a** against **5a-g**, $P < 0.001$). Introduction of methyl **5c** and methoxy **5d** group onto the para position of phenyl ring of imidazole led to a significant increase in antiplatelet activity against AA (**4a** against **5c-d**; $P < 0.005$). However the compounds containing fluoro **5a** at para position of phenyl ring show moderate activity (IC_{50} = 41.9 µM). The compounds with a chlorine group at the ortho and meta position **5e-g** show no improvement in activity.

Thienylimidazole derivatives **6a-g** were synthesized with different substituents on phenyl ring at position 4 of imidazole as shown in Table 2. All the synthesized compounds show no activity against ADP and the activity decreased significantly against collagen (**4p** against **6a-g**; $P < 0.01$). Compounds **6b**, **6c** and **6d** with chlorine, methyl and methoxy at the para position of phenyl ring showed increased activity against AA. The IC_{50} of **6b**, **6c** and **6d** were between 2.68 to 3.36 µM and comparable to that of indomethacin with IC_{50} value of 1.67 µM (Figure 3).

Table 1: In vitro antiplatelet activity using arachidonic acid (AA), adenosine diphosphate (ADP) and Collagen as platelet aggregation inducers. The obtained IC_{50} values are reported as mean (n=3).



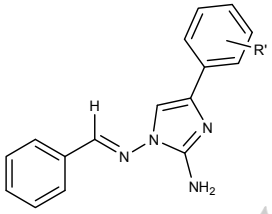
3a-q



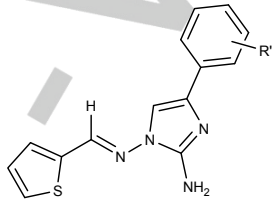
4a-q

Compd	Ar	IC_{50} (µM) ADP (5 µM)	Collagen (2.5 µg/ml)	AA (1.35 mM)	Compd	IC_{50} (µM) ADP (5 µM)	Collagen (2.5 µg/ml)	AA (1.35 mM)
3a	phenyl	>500	>100	>500	**4a**	>500	**11.90±2.18**	177.9±10.2
3b	2-fluorophenyl	>500	**23.98±6.42**	>500	**4b**	>500	>100	112.1±4.1
3c	2-chlorophenyl	473.8±7.7	>100	>500	**4c**	>500	76.7±7.8	145.0±4.3
3d	2-bromophenyl	>500	>100	>500	**4d**	>500	>100	375.3±7.0
3e	3-fluorophenyl	>500	>100	>500	**4e**	458.0±8.8	>100	**27.00±4.0**
3f	3-chlorophenyl	**231.9±5.5**	89.1±4.4	241.0±8.7	**4f**	>500	51.3±5.7	>500
3g	3-bromophenyl	>500	81.3±1.5	**153.70±5.9**	**4g**	>500	65.3±7.5	>500
3h	4-fluorophenyl	>500	>100	>500	**4h**	>500	>100	40.0±2.5
3i	4-chlorophenyl	**241.7±8.3**	57.5±3.9	383.0±10.0	**4i**	>500	74.2±9.4	109.8±7.8
3j	4-bromophenyl	**230.4±8.3**	66.1±4.8	219.0±10.6	**4j**	>500	>100	>500
3k	4-methylphenyl	>500	>100	>500	**4k**	>500	>100	254.8±7.1
3l	4-metoxyphenyl	>500	>100	474.0±11.6	**4l**	>500	33.1±4.0	91.4±1.6
3m	4-hydroxyphenyl	>500	56.2±2.8	**155.40±3.7**	**4m**	>500	>100	>500
3n	3,4,5-trimethoxyphenyl	>500	>100	>500	**4n**	>500	>100	>500
3o	2-furanyl	>500	>100	>500	**4o**	>500	>100	210.0±7.6
3p	2-thienyl	>500	70.0±4.3	>500	**4p**	>500	**10.65±0.63**	**26.61±3.26**
3q	2-pyridyl	>500	>100	>500	**4q**	>500	>100	260.8±2.7
Indomethacin		>500	1.2±0.3	1.67±0.67				
Aspirin		>500	9.7±0.7	0.24±0.05				

Table 2: In vitro antiplatelet activity using arachidonic acid (AA), adenosine diphosphate (ADP) and Collagen as platelet aggregation inducers. The obtained IC_{50} values are reported as mean (n=3).



5a-g



6a-g

		IC_{50} (µM)		
Compd	R′	ADP (5 µM)	Collagen (2.5 µg/ml)	AA (1.35 mM)
5a	4 -F	>1000	>500	41.9±1.9
5b	4- Cl	>1000	>500	310.0±11.2
5c	4- CH_3	226.0±7.4	73.3±3.3	**10.76±1.02**
5d	4- OCH_3	496.0±7.7	60.7±3.8	**15.78±2.69**
5e	2- Cl	>1000	396.0±8.5	152.8±5.2
5f	2- Br	>1000	>500	>500
5g	3- Cl	>1000	>500	295.4±7.8
6a	4 -F	>1000	96.6±8.1	311.0±8.4
6b	4- Cl	>1000	264.0±15.4	**2.78±0.46**
6c	4- CH_3	>1000	125.2±4.9	**2.68±0.22**
6d	4- OCH_3	402.0±11.8	166.5±7.1	**3.36±0.40**
6e	2- Br	>1000	103.0±6.1	42.6±4.2
6f	3- Cl	>1000	445.0±12.8	316.0±7.9
6g	3- Br	>1000	262.0±5.8	94.1±3.9
Indomethacin		>500	1.2±0.3	1.67±0.67
Aspirin		>500	9.7±0.7	0.24±0.05

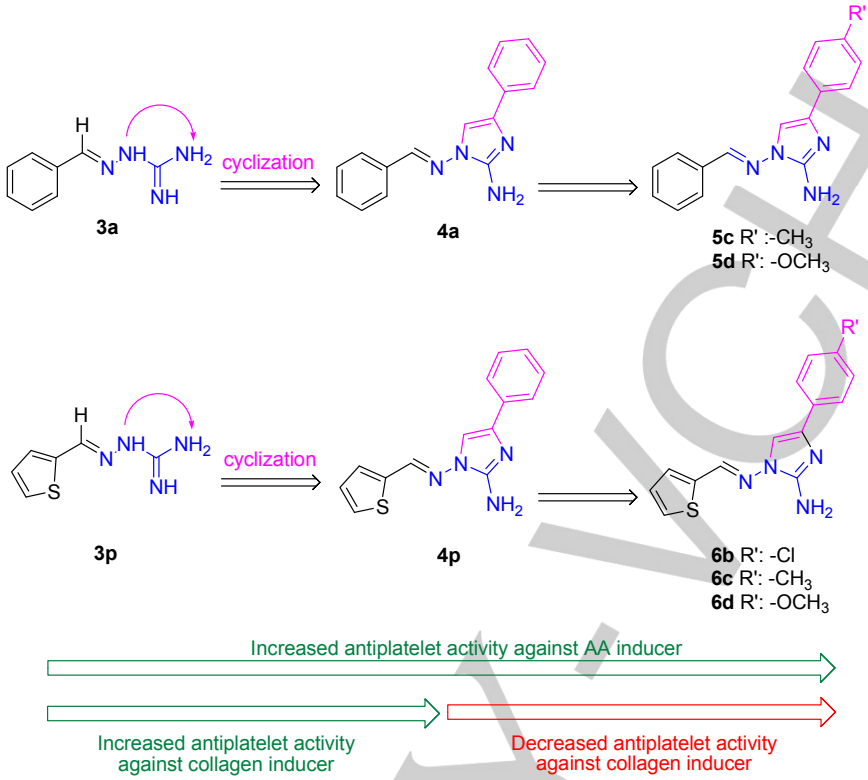


Figure 3. Structure activity relationship (SAR) of synthesized compounds.

Lipinski rule of five

To predict if a chemical compound could be used orally, the most active derivatives **5c**, **5d** and **6b–d** against AA were analyzed according to the Lipinski "rule of five"[23] using OSIRIS Datawarrior (version 4.2.2)[24] (Table 3). In lipinski rule of five the clogP is an indicator for the lipophilicity of compounds. Based on the calculated clogP values of compounds **5c**, **5d** and **6b–d** (clog P between 2.85-3.53), they might be able to pass through the biological membranes. The results of calculating Lipinski's criteria of drug likeness for the most active derivatives (Table 3) indicate that all of these compounds fall within the rule of five and thus have properties which would make them kinetically proper drug candidates.

Table 3: The calculated parameters of Lipinski rule of five for the most active derivatives against arachidonic acid (AA).

		Lipinski rule of five[a]			
Compound	**IC_{50} (μM)**	**HBA**	**HBD**	**Mw**	**clog P**
5c	10.76	4	1	276	3.40
5d	15.78	5	1	292	2.99
6b	2.78	4	1	302	3.53
6c	2.68	4	1	282	3.27
6d	3.36	5	1	298	2.85
Indomethacin	1.67	5	1	357	4.00

[a] number of hydrogen bond acceptors (HBA) ≤ 10, number of hydrogen bond donors (HBD) ≤ 5, clog P ≤ 5 and Molecular weight (Mw) ≤ 500

Docking studies

On the basis of the antiplatelet aggregation test, the most active derivatives **6b, 6c** and **6d** against AA were selected for molecular docking studies on their possible potential target i.e. cyclooxygenase 1 (COX-1). The best docked pose with the lowest energy calculated by AutoDock version 4.2 was selected and analyzed with PyMol and Ligplot software.

The position of compounds **6b**, **6c** and **6d** in the binding site was shown in Figure 4. It was observed that all of the **6b**, **6c** and **6d** displayed strong hydrogen bound between the NH_2 group of

imidazole ring and hydroxyl oxygen of Ser530. In compounds **6b** and **6c** another hydrogen bond was established between the imidazole nitrogen atom and the hydroxyl group of Ser530.
In the compounds **6b** and **6c** the thiophene rings were in the same position and oriented toward Tyr355, Leu359 and Val116. On the contrast, in **6d** with methoxy substituent the thiophene ring is directed to the opposite site and display hydrophobic contacts with Trp387, Leu384 and Met522.

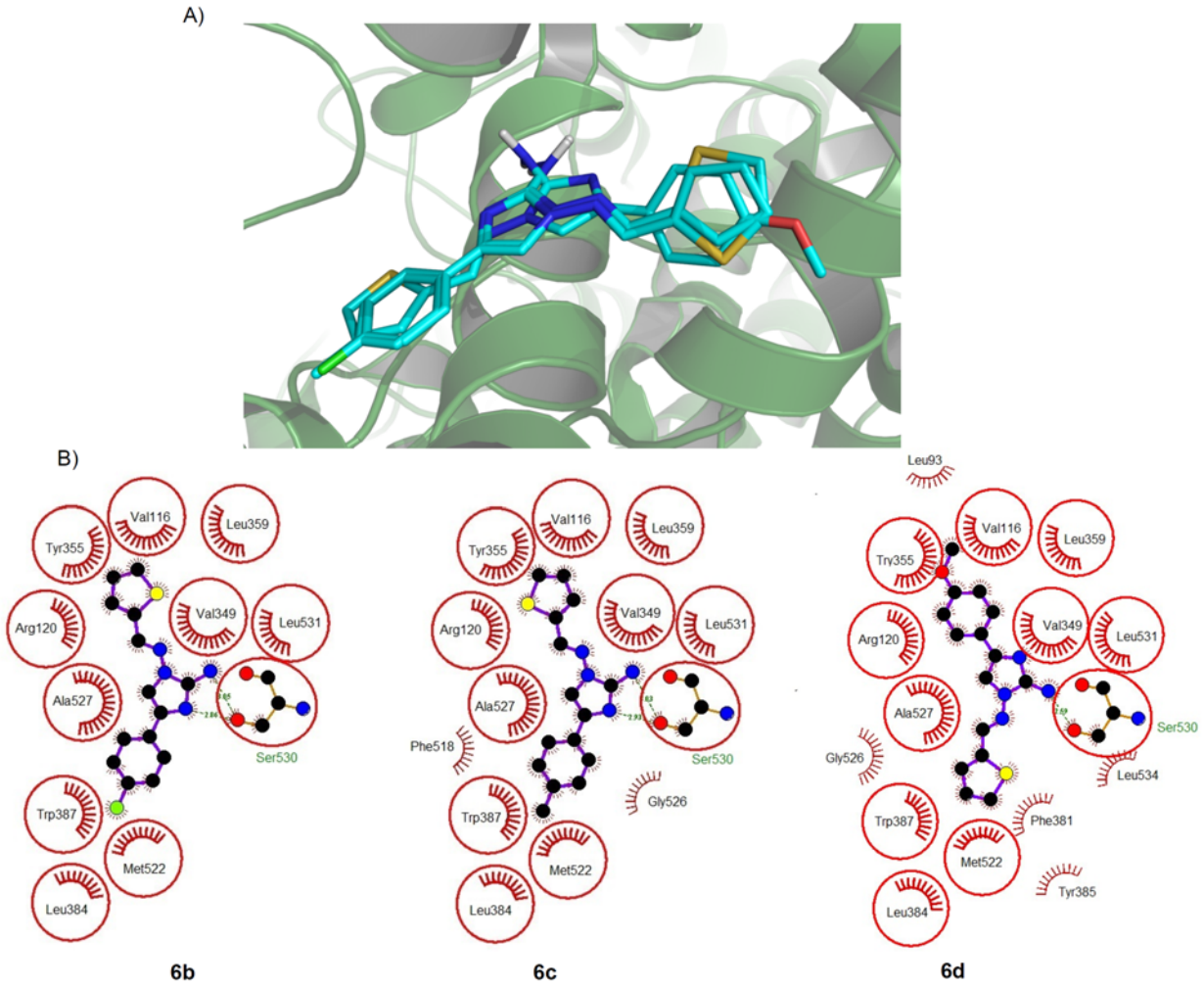


Figure 4. Interaction of Compounds **6b**, **6c** and **6d** with COX-1 active site. The residues are showed in green lines. Picture was generated with PyMOL (A). Schematic diagram of interactions of **6b**, **6c** and **6d** with COX-1 active site generated by Ligplot. Hydrogen bonds are showed with green dashed lines. Residues involved in hydrophobic interaction with the ligands are shown with red rays. The red cycles showed the same residues which interact with ligands (B).

Conclusions

A series of guanylhydrazone and 1-(arylideneamino)-4-aryl-1*H*-imidazole-2-amine derivatives were synthesized and their antiplatelet activity on platelet rich plasma (PRP) was evaluated by using ADP, collagen and AA as platelet aggregation inducers**.** The obtained data demonstrated that synthesized derivatives did not show significant activity against ADP. The structure activity relationship studies showed that compounds **4a** and **4p** with phenyl ring at position 4 of imidazole were active against collagen as platelet aggregation inducer while, substitutions at

para position of phenyl at position 4 of imidazole ring decreased the antiplatelet activity against collagen. As for the activity against collagen, substitution on the phenyl ring attached to imidazole ring could not be tolerated. Whereas derivatives with substituents such as chlorine, methyl and methoxy at para position of phenyl at position 4 of imidazole ring were the most active compounds against AA. The presence of thiophene ring in these compounds will maximize their anitplatelet activity. The compound **6c** has an IC_{50} comparable to indomethacin. The results of this study are helpful for our ongoing studies to design and obtain promising compounds with antiplatelet effects.

Experimental Section

General methods

All the chemicals and solvents were purchased from Merck (Darmstadt, Germany). The ^{1}H-NMR spectra were recorded on a 500 MHz Bruker spectrometer. NOE spectrum was recorded on a 600 MHz Bruker spectrometer. The positive ESI-MS mass spectra were recorded on an Agilent 6410 triple quadrupole mass spectrometer. IR spectra were recorded on Perkin Elmer IR spectrophotometer as potassium bromide discs. The melting points of the compounds were obtained on 9100 Electrothermal melting point apparatus. Elemental analyses were performed by a Costech ECS 4010 CHNS analyzer. For all the compounds the calculated values agreed with the measured values to within 0.4%. All the compounds used in platelet aggregation assay were > 98% pure as determined by ^{1}H NMR spectroscopy.

General procedure for the synthesis of guanylhydrazones

The guanylhydrazones **3a-q** were prepared by the procedure described in the literature.[19,20,25] In Brief, a mixture of aminoguanidine bicarbonate (1 mmol) and appropriate aromatic aldehydes in the presence of HCl (37%) was stirred for 30 min at room temperature. Then a solution of 5% aqueous NaOH (7 mL) was added. The obtained precipitate was collected by filtration.

(*E*)-2-benzylidenehydrazine-1-carboximidamide (3a)

White solid. Yield: (137.7 mg, 85%); mp: 180-182 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=6.16 (bs, 4H, NH_2), 7.28 (t, *J*=7.2 Hz, 1H, H-4); 7.35 (t, *J*=7.6 Hz, 2H, H-3,H-5), 7.71 (d, *J*=7.2 Hz, 2H, H-2, H-6), 8.02 ppm (s, 1H, HC=N); IR (KBr): ü=3465, 3423, 3312, 1648, 1580, 1480, 1437, 1144, 1016, 936, 758, 686 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 163.1; Anal. Calcd for $C_8H_{10}N_4$: C 59.24, H 6.21, N 34.54, found: C 59.31, H 6.21, N 34.48.

(*E*)-2-(2-fluorobenzylidene)hydrazine-1-carboximidamide (3b)

White solid. Yield: (158.4 mg, 88%); mp: 183-185 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=5.59 (s, 2H, NH_2), 5.98 (s, 2H, NH_2), 7.18 (m, 2H, H-4, H-5); 7.29 (dd, *J*=14.1 Hz, *J*=8.5 Hz,1H, H-3), 8.06 (dt, *J*=8.0 Hz, *J*=1.5 Hz, 1H, H-6), 8.12 ppm (s,1H, HC=N); IR (KBr): ü=3488, 3447, 3373, 1648, 1600, 1522, 1239, 1156, 767 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 181; Anal. Calcd for $C_8H_9FN_4$: C 53.33, H 5.03, N 31.09, found C 53.35, H 5.02, N 31.10.

(*E*)-2-(2-chlorobenzylidene)hydrazine-1-carboximidamide (3c)

Yellow solid. Yield: (172.5 mg, 88%); mp: 145-146 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=5.58 (s, 2H, NH_2), 5.97 (s, 2H, NH_2), 7.23 (m, 2H, H-4, H-5), 7.34 (dd, *J*=7.5 Hz, *J*=1.7 Hz, 1H, H-3), 8.08 (dd, *J*=7.5 Hz, *J*=1.9 Hz, 1H, H-6), 8.23 ppm (s, 1H, HC=N); IR (KBr): ü=3455, 3327, 3284, 1628, 1600, 1544, 1162, 748 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 197.1; Anal. Calcd for $C_8H_9ClN_4$: C 48.87, H, 4.61, N 28.49, found C 48.86, H, 4.62, N 28.47.

(*E*)-2-(2-bromobenzylidene)hydrazine-1-carboximidamide (3d)

Cream solid. Yield: (216.0 mg, 90%); mp: 161-168 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=5.57 (s, 2H, NH_2), 5.97 (s, 2H, NH_2), 7.13 (t, *J*=7.5 Hz, 1H, H-5), 7.27 (t, *J*=7.6 Hz, 1H, H-4), 7.52 (d, *J*=7.6 Hz, 1H, H-3), 8.067 (dd, *J*=8.0 Hz, *J*=1.5 Hz, 1H, H-6), 8.18 ppm (s, 1H, N=CH); IR (KBr): ü=3472, 3394, 3317, 1638, 1608, 1573, 1439, 1169, 760 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 241, 243; Anal. Calcd for $C_8H_9BrN_4$: C 39.86, H 3.76, N 23.24, found C 39.85, H 3.75, N 23.22.

(*E*)-2-(3-fluorobenzylidene)hydrazine-1-carboximidamide (3e)

White solid. Yield: (162.0 mg, 90%); mp: 153-156 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=5.48 (s, 2H, NH_2), 5.94 (s, 2H, NH_2), 6.99 (t, *J*=7.7 Hz;1H, H-5), 7.30 (dd, *J*=14.0 Hz, *J*=7.7 Hz, 1H, H-4), 7.37 (d, *J*=7.3 Hz; 1H, H-6), 7.54 (d, *J*=10.0 Hz, 1H, H-2), 7.92 ppm (s, 1H, N=CH); IR (KBr): ü=3377, 3209, 3163, 1688, 1636, 1599, 1274, 1137, 942, 799, 686 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 181; Anal. Calcd for $C_8H_9FN_4$: C 53.33, H 5.03, N 31.09, found C 53.30, H 5.02, N 31.11.

(*E*)-2-(3-chlorobenzylidene)hydrazine-1-carboximidamide (3f)

White solid. Yield: (172.5 mg, 88%); mp: 164-166 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=6.2 (bs, 4H, NH_2), 7.29 (d, *J*=7.9 Hz, 1H, H-4), 7.35 (t, *J*=7.7 Hz, 1H, H-5), 7.58 (d, *J*=7.6 Hz, 1H, H-6); 7.83 (s, 1H, H-2), 7.97 ppm (s, 1H, N=CH); IR (KBr): ü=3475, 3443, 1643, 1595, 1349, 1148, 937, 789, 682 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 197.1; Anal. Calcd for $C_8H_9ClN_4$: C 48.87, H 4.61, N 28.49, found C 48.89, H 4.60, N 28.50.

(*E*)-2-(3-bromobenzylidene)hydrazine-1-carboximidamide (3g)

White solid. Yield: (220.8 mg, 92%); mp: 178-180 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=5.54 (s, 2H, NH_2), 7.27 (t, *J*=7.8 Hz, 1H, H-5), 7.40 (d, *J*=7.7 Hz, 1H, H-4), 7.60 (d, *J*=7.6 Hz, 1H, H-6), 7.94 ppm (s, 2H, H-2, N=CH); IR (KBr): ü=3452, 3295, 3187, 1652, 1531, 1338, 1150, 933, 801, 682 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 241.3, 243; Anal. Calcd for $C_8H_9BrN_4$: C 39.86, H 3.76, N 23.24, found C 39.84, H 3.76, N 23.25.

(*E*)-2-(4-fluorobenzylidene)hydrazine-1-carboximidamide (3h)

White solid. Yield: (162.0 mg, 90%); mp: 182-186 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=5.41 (s, 2H, NH_2), 5.86 (s, 2H, NH_2), 7.11 (t, *J*=8.9 Hz, 2H, H-3, H-5), 7.67 (dd, *J*=8.8 Hz, *J*=5.8 Hz, 2H, H-2, H-6), 7.93 ppm (s, 1H, N=CH); IR (KBr): ü=3495, 3449, 1650, 1595, 1245, 1163, 847, 682 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 181; Anal. Calcd for $C_8H_9FN_4$: C 53.33, H 5.03, N 31.09, found C 53.36, H 5.03, N 31.08.

(*E*)-2-(4-chlorobenzylidene)hydrazine-1-carboximidamide (3i)

White solid. Yield: (184.2 mg, 94%); mp: 206-208 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=5.52 (s,2H, NH_2), 5.94 (s, 2H, NH_2), 7.37 (d, *J*=8.5 Hz, 2H, H-3, H-5), 7.69 (d, *J*=8.5 Hz, 2H, H-2 ,H-6), 7.96 ppm (s, 1H, N=CH); IR (KBr): ü=3471, 3435, 3357, 1643, 1595, 1151, 1090, 824 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 197.1; Anal. Calcd for $C_8H_9ClN_4$: C 48.87, H 4.61, N 28.49, found C 48.90, H 4.61, N 28.48.

(*E*)-2-(4-bromobenzylidene)hydrazine-1-carboximidamide (3j)

White solid. Yield: (220.8 mg, 90%); mp: 207-209 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=5.50 (s, 2H, NH_2), 5.91 (s, 2H, NH_2), 7.45 (d, *J*=8.4 Hz, 2H, H-3, H-5), 7.58 (d, *J*=8.4 Hz, 2H, H-2, H-6), 7.90 ppm (s, 1H, N=CH); IR (KBr): ü=3466, 3321, 1639, 1627, 1590, 1390, 1164, 932, 819 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 241.3, 243; Anal. Calcd for $C_8H_9BrN_4$: C 39.86, H 3.76, N 23.24, found C 39.85, H 3.75, N 23.24.

(*E*)-2-(4-methylbenzylidene)hydrazine-1-carboximidamide (3k)

Yellow solid. Yield: (153.12 mg, 87%); mp: 202-206 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ=2.30 (s, 3H, CH_3), 5.42 (s, 2H, NH_2), 5.83 (s, 2H, NH_2), 7.14 (d, *J*=8.0 Hz, 2H, H-3, H-5), 7.56 (d, *J*=8.0 Hz, 2H, H-2, H-6), 7.95 ppm (s, 1H, N=CH); IR (KBr): ü=3473, 3432, 3357, 3092, 1646, 1584, 1349, 1147, 930, 817 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 177; Anal. Calcd for $C_9H_{12}N_4$: C 61.34, H 6.86, N 31.79, found C 61.38, H 6.85, N 31.77.

(*E*)-2-(4-methoxybenzylidene)hydrazine-1-carboximidamide (3l)

White solid. Yield: (161.3 mg, 84%); mp: 187-191 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 3.72 (s, 3H, OCH_3), 5.32 (s, 2H, NH_2), 5.77 (s, 2H, NH_2), 6.85 (d, *J*=8.7 Hz, 2H, H-3, H-5), 7.55 (d, *J*=8.7 Hz, 2H, H-2, H-6), 7.90 ppm (s, 1H, N=CH); IR (KBr): ü=3475, 3350, 1662, 1597, 1248, 1176, 1026, 829 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 193; Anal. Calcd for $C_9H_{12}N_4O$: C 56.24, H 6.29, N 29.15, found C 56.25, H 6.30, N 29.14.

(*E*)-2-(4-hydroxybenzylidene)hydrazine-1-carboximidamide (3m)

Yellow solid. Yield: (154.9 mg, 87%); mp (as HCl): 245-250 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.79 (d, *J*=9.7 Hz, 2H, H-3, H-5), 7.5 (bs, 2H, NH_2), 7.62 (d, *J*=9.1 Hz, 2H, H-2, H-6), 7.8 (bs, 2H, NH_2), 8.01 (s, 1H, N=CH), 11.80 ppm (s, 1H, OH); IR (KBr): ü=3424, 3280, 1680, 1636, 1509, 1425, 1264, 1094, 833 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 179; Anal. Calcd for $C_8H_{10}N_4O$: C 53.92, H 5.66, N31.44, found C 53.92, H 5.67, N 31.42.

(*E*)-2-(3,4,5-trimethoxybenzylidene)hydrazine-1-carboximidamide (3n)

Pale yellow solid. Yield: (219.2 mg, 87%); mp: 175-178 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 3.61 (s, 3H, OCH_3), 3.75 (s, 6H, OCH_3), 5.35 (s, 2H, NH_2), 5.87 (s, 2H, NH_2), 6.93 (s, 2H, H-2, H-6), 7.86 ppm (s, 1H, N=CH); IR (KBr): ü=3496, 3412, 3381, 3152, 1653, 1577, 1230, 1126, 936, 824, 792 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 253; Anal. Calcd for $C_{11}H_{16}N_4O_3$: C 52.37, H 6.39, N 22.21, found C 52.42, H 6.37, N 22.20.

(*E*)-2-(furan-2-ylmethylene)hydrazine-1-carboximidamide (3o)

Orange solid. Yield: (118.6 mg, 78%); mp: 175-178 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 5.50 (s, 2H, NH_2), 5.76 (s, 2H, NH_2), 6.52 (dd, *J*=3.1 Hz, *J*=1.6 Hz, 1H, H-4), 6.61 (d, *J*=3.2 Hz, 1H, H-5), 7.64 (s, 1H, H-3), 7.85 ppm (s, 1H, N=CH); IR (KBr): ü=3448, 3301, 3119, 1611, 1547, 1486, 1336, 1162, 944, 729 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 153; Anal. Calcd for $C_6H_8N_4O$: C 47.36, H 5.30, N 36.82, found C 47.36, H 5.29, N 36.83.

(*E*)-2-(thiophen-2-ylmethylene)hydrazine-1-carboximidamide (3p)

Yellow solid. Yield: (146.2 mg, 87%); mp: 107-109 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 5.51 (s, 2H, NH_2), 5.70 (s, 2H, NH_2), 7.03 (dd, *J*=4.3 Hz, *J*=3.5 Hz, 1H, H-4), 7.14 (d, *J*=5.0 Hz, 1H, H-5), 7.37 (d, *J*=5.0 Hz, 1H, H-3), 8.14 ppm (s, 1H, N=CH); IR (KBr): ü=3488, 3449, 3353, 1648, 1597, 1159, 932, 773, cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 169; Anal. Calcd for $C_6H_8N_4S$: C 42.84, H 4.79, N 33.31, S 19.06, found C 42.81, H 4.80, N 33.32, S 19.07.

(*E*)-2-(pyridin-2-ylmethylene)hydrazine-1-carboximidamide (3q)

Yellow solid. Yield: (156.5 mg, 96%); mp: 219-221 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 5.68 (s, 2H, NH_2), 6.06 (s, 2H, NH_2), 7.20 (dd, *J*=6.7 Hz, *J*=5.1 Hz,1H, H-5), 7.69 (t, *J*=7.8 Hz, 1H, H-4), 7.96 (s, 1H, N=CH), 8.06 (d, *J*=7.9 Hz, 1H, H-3), 8.45 ppm (d, *J*=5.1 Hz, 1H, H-6); IR (KBr): ü=3439, 3335, 1646, 1562, 1143, 962, 822 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 164; Anal. Calcd for $C_7H_9N_5$: C 51.52, H 5.56, N 42.92, found C 51.50, H 5.57, N 42.93.

General procedure for the synthesis of 2-amino-4-aryl-1-arylideneaminoimidazoles

A solution of NaOH (1 mmol) in ethanol was added dropwise to a solution of phenacylbromide or phenacylbromide derivatives (1 mmoL) and benzilydene aminoguanidine (1 mmoL) in ethanol. The mixture was warmed to 40-45°C and stirred at room temperature overnight.[25] The obtained precipitate **4-6** was filtered off and purified by plate chromatography or recrystallization in ethanol.

(*E*)-1-(benzylideneamino)-4-phenyl-1*H*-imidazol-2-amine (4a)

Yellow solid. Yield: (11.3 mg, 4.3%); mp: 197 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.17 (s, 2H, NH_2); 7.19 (t, *J*=7.3 Hz, 1H, H-4'), 7.35 (t, *J*=7.7 Hz, 2H, H-3, H-5), 7.5 (m, 3H, H-4, H-3', H5'), 7.71 (d, *J*=7.4 Hz, 2H, H-2', H-6'), 7.93 (d, 2H, H-2, H-6), 8.00 (s, 1H, H-imidazole), 8.56 ppm (s, 1H, N=CH); IR (KBr): ü=3433, 3295, 3135, 3066, 1644, 1469, 764, 689 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 263; Anal. Calcd for $C_{16}H_{14}N_4$: C 73.26, H 5.38, N 21.36, found C 73.24, H 5.39, N 21.37.

(*E*)-1-((2-fluorobenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4b)

Yellow solid. Yield: (7.6 mg, 2.7%); mp: 190 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.23 (s, 2H, NH_2), 7.15 (t, *J*=7.3 Hz, 1H, H-4'), 7.3 (m, 4H,

H-3', H-5', H-4, H-5), 7.49 (dd, *J*=13.2, *J*=6.5 Hz, 1H, H-3), 7.69 (d, *J*=7.4 Hz, 2H, H-2', H-6'), 8.13 (s, 1H, H-imidazole), 8.25 (t, *J*=7.3 Hz, 1H, H-6), 8.57 ppm (s, 1H, N=CH); IR (KBr): ü=3403, 3064, 1632, 1469, 1237, 768 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 281; Anal. Calcd for $C_{16}H_{13}FN_4$: C 68.56, H 4.67, N 19.99, found C 68.57, H 4.68, N 19.98.

(*E*)-1-((2-chlorobenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4c)

Yellow solid. Yield: (22.2 mg, 7.5%); mp: 207 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.28 (s, 2H, NH_2); 7.19 (t, *J*=7.3 Hz, 1H, H-4'), 7.34 (t, *J*=7.8 Hz, 2H, H-3', H- 5'), 7.45 (t, *J*=7.4 Hz, 1H, H-5), 7.49 (t, *J*=7.8 Hz, 1H, H-4), 7.57 (dd, *J*=5.7 Hz, *J*=1.2 Hz, 1H, H-3), 7.72 (d, *J*=7.6 Hz, 2H, H-2', H-6'), 8.15 (s, 1H, H-imidazole), 8.35 (dd, *J*=7.7 Hz, *J*=1.7 Hz, 1H, H-6), 8.65 ppm (s, 1H, N=CH); IR (KBr): ü=: 3412, 933, 1635, 1470, 1127, 764 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 297; Anal. Calcd for $C_{16}H_{13}ClN_4$: C 64.76, H 4.42, N 18.88, found C 64.78, H 4.41, N 18.87.

(*E*)-1-((2-bromobenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4d)

Yellow solid. Yield: (23.8 mg, 7.5%); mp: 192 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.28 (s, 2H, NH_2), 7.20 (t, *J*=7.3 Hz, 1H, H-4'), 7.34 (t, *J*=7.7 Hz, 2H, H-3', H-5'), 7.41 (t, *J*=7.4 Hz, 1H, H-5), 7.49 (t, *J*=7.6 Hz, 1H, H-4), 7.74 (d, *J*=7.9 Hz, 1H, H-3), 7.77 (d, *J*=8.2 Hz, 2H, H-2', H-6'), 8.10 (s, 1H, H-imidazole), 8.31 (dd, *J*=7.8 Hz, *J*=1.6 Hz, 1H, H-6), 8.57 ppm (s, 1H, N=CH); IR (KBr): ü=3400, 3268, 3140, 2931, 1630, 1454, 763 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 341, 343; Anal. Calcd for $C_{16}H_{13}BrN_4$: C 56.32, H 3.84, N 16.42, found C 56.33, H 3.83, N 16.43.

(*E*)-1-((3-fluorobenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4e)

Yellow solid. Yield: (9.2 mg, 3.3%); mp: 193 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.25 (s, 2H, NH_2), 7.14 (t, *J*=7.3 Hz, 1H, H-4'), 7.3 (m, 3H, H-3', H-5', H-5), 7.50 (dd, *J*=14.0 Hz, *J*=7.6 Hz, 1H, H-4), 7.62 (d, *J*=7.6 Hz, 1H, H-6), 7.66 (d, *J*=7.3 Hz, 2H, H-2', H-6'), 7.84 (d, *J*=9.9 Hz, 1H, H-2), 7.92 (s, 1H, H-imidazole), 8.51 ppm (s, 1H, N=CH); IR (KBr): ü=3422, 3083, 1655, 1592, 1486, 812, 716, 692 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 281; Anal. Calcd for $C_{16}H_{13}FN_4$: C 68.56, H 4.67, N 19.99, found C 68.61, H 4.66, N 19.97.

(*E*)-1-((3-chlorobenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4f)

Yellow solid. Yield: (29.6 mg, 10%); mp: 199 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.31 (s, 2H, NH_2), 7.20 (t, *J*=7.3 Hz, 1H, H-4'), 7.35 (t, *J*=7.7, 2H, H-3', H-5'), 7.52 (d, *J*=5.1 Hz, 2H, H-4, H-6), 7.7 (d, *J*=7.3 Hz, 2H, H-2', H-6'),7.79 (t, *J*=4.8 Hz, 1H, H-5), 7.94 (s, 1H, H-imidazole), 8.10 (s, 1H, H-2) 8.54 ppm (s, 1H, N=CH); IR (KBr): ü=3426, 3069, 1639, 1466, 936, 796, 683 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 297; Anal. Calcd for $C_{16}H_{13}ClN_4$: C 64.76, H 4.42, N 18.88, found C 64.74, H 4.41, N 18.89.

(*E*)-1-((3-bromobenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4g)

Yellow solid. Yield: (207.0 mg, 61%); mp: 203 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.61 (s, 2H, NH_2), 7.23 (t, *J*=7.2 Hz, 1H, H-4'), 7.37 (t, *J*=7.3 Hz, 2H, H-3', H-5'), 7.46 (t, *J*=7.7 Hz, 1H, H-5), 7.67 (d, *J*=8.1 Hz, 1H, H-4), 7.70 (d, *J*=7.7 Hz, 2H, H-2', H-6'), 7.84 (d, *J*=7.5 Hz, 1H, H-6), 8.01 (s, 1H, H-imidazole), 8.25 (s, 1H, H-2), 8.57 ppm (s, 1H, N=CH); IR (KBr): ü=3425, 3295, 3051, 1648, 1482, 1074, 799, 705 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 341, 343; Anal. Calcd for $C_{16}H_{13}BrN_4$: C 56.32, H 3.84, N 16.42, found C 56.34, H 3.85, N 16.41.

(*E*)-1-((4-fluorobenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4h)

Yellow solid. Yield: (16.8 mg, 6%); mp: 210 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.16 (s, 2H, NH_2), 7.15 (t, *J*=7.3 Hz, 1H, H-4'), 7.3 (m, 4H, H-2', H-6', H-3', H-5'), 7.66 (d, *J*=8.0 Hz, 2H, H-2 H-6), 7.92 (s, 1H, H-imidazole), 7.95 (t, *J*=7.5 Hz, 2H, H-3, H-5), 8.52 ppm (s, 1H, N=CH); IR (KBr): ü=3425, 3291, 3221, 3133, 3079, 1648, 1468, 835, 698 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 281; Anal. Calcd for $C_{16}H_{13}FN_4$: C 68.56, H 4.67, N 19.99, found C 68.55, H 4.67, N 20.00.

(*E*)-1-((4-chlorobenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4i)

Yellow solid. Yield: (35.5 mg, 12%); mp: 211 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.23 (s, 2H, NH_2), 7.19 (t, *J*=7.8 Hz, 1H, H-4'), 7.35 (t, *J*=9.5, 2H, H-3', H-5'), 7.56 (d, *J*=8.4 Hz, 2H, H-3, H-5), 7.70 (d, *J*=8.1 Hz, 2H, H-2', H-6'), 7.94 (d, *J*=8.4 Hz, 2H, H-2, H-6), 7.96 (s, 1H, H-imidazole), 8.55 ppm (s, 1H, N=CH); IR (KBr): ü=3419, 3286, 3214, 3128, 1644, 1461, 1085, 824, 693 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 297; Anal. Calcd for $C_{16}H_{13}ClN_4$: C 64.76, H 4.42, N 18.88, found C 64.62, H 4.43, N 19.00.

(*E*)-1-((4-bromobenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4j)

Yellow solid. Yield: (33.2 mg, 9.8%); mp: 214 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.19 (s, 2H, NH_2), 7.15 (t, *J*=7.3 Hz, 1H, H-4'), 7.31 (t, *J*=7.7, 2H, H-3', H-5'), 7.66 (m, 4H, H-3, H-5, H-2', H-6'), 7.83 (d, *J*=8.4 Hz, 2H, H-2, H-6), 7.92 (s, 1H, H-imidazole), 8.50 ppm (s, 1H, N=CH); IR (KBr): ü=3436, 3313, 3140, 3085, 1654, 1474, 1075, 830, 703 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 341, 343; Anal. Calcd for $C_{16}H_{13}BrN_4$: C 56.32, H 3.84, N 16.42, found C 56.32, H 3.82, N 16.44.

(*E*)-1-((4-methylbenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4k)

Yellow solid. Yield: (8.0 mg, 3%); mp: 218 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 2.37 (s, 3H, CH_3), 6.12 (s, 2H, NH_2), 7.15 (t, *J*=7.3 Hz, 1H, H-4'), 7.31 (d, *J*=8.0 Hz, 2H, H-3, H-5), 7.34 (t, *J*=7.5 Hz, 2H, H-3', H-5'), 7.70 (d, *J*=7.7 Hz, 2H, H-2', H-6'), 7.81 (d, *J*=8.0, 2H, H-2, H-6), 7.98 (s, 1H, H-imidazole), 8.52 ppm (s, 1H, N=CH); IR (KBr): ü=3414, 3154, 3071, 1648, 1483, 1332, 827, 710 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 276.9; Anal. Calcd for $C_{17}H_{16}N_4$: C 73.89; H, 5.84, N, 20.27, found C 73.87; H, 5.85, N, 20.28.

(*E*)-1-((4-methoxybenzylidene)amino)-4-phenyl-1*H*-imidazol-2-amine (4l)

Brown solid. Yield: (5.3 mg, 2%); mp: 198 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 3.84 (s, 3H, OCH_3), 6.91 (bs, 2H, NH_2), 7.08 (d, *J*=8.7 Hz, 2H, H-3, H-5), 7.27 (t, *J*=7.3, 1H, H-4'), 7.41 (t, *J*=7.7 Hz, 2H, H-3', H-5'), 7.70 (d, *J*=7.4 Hz, 2H, H-2', H-6'), 7.91 (d, *J*=8.7 Hz, 2H, H-2, H-6), 8.16 (s, 1H, H-imidazole), 8.62 ppm (s, 1H, N=CH); IR (KBr): ü=3399, 3352, 3198, 3070, 1664, 1601, 1478, 1256, 1171, 837, 703 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 293; Anal. Calcd for $C_{17}H_{16}N_4O$: C 69.85, H 5.52, N 19.17, found C 69.81, H 5.53, N 19.18.

(*E*)-4-(((2-amino-4-phenyl-1*H*-imidazol-1-yl)imino)methyl)phenol (4m)

Yellow solid. Yield: (4.7 mg, 1.7%); mp: 197 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 5.97 (bs, 2H, NH_2), 6.83 (d, *J*=8.5 Hz, 2H, H-3, H-5), 7.13 (t, *J*=7.3 Hz, 1H, H-4'), 7.29 (t, *J*=7.7 Hz, 2H, H-3', H-5'), 7.65 (d, *J*=7.4 Hz, 2H, H-2', H-6'), 7.71 (d, *J*=8.6 Hz, 2H, H-2, H-6), 7.91 (s, 1H, H-imidazole), 8.40 ppm (s, 2H, N=CH, OH); IR (KBr): ü=3352, 3247, 2968, 1642, 1599, 1235, 1107, 841 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 279; Anal. Calcd for $C_{16}H_{14}N_4O$: C 69.05, H 5.07, N 20.13, found C 69.06, H 5.08, N 20.12.

(*E*)-4-phenyl-1-((3,4,5-trimethoxybenzylidene)amino)-1*H*-imidazol-2-amine (4n)

Brown solid. Yield: (9.9 mg, 2.8%); mp: 145 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 3.6 (s, 3H, OCH_3), 3.8 (s, 6H, OCH3), 6.22 (s, 2H, NH_2), 7.14 (t, *J*=7.1 Hz, 1H, H-4'), 7.19 (s, 2H, H-2, H-6), 7.32 (t, *J*=7.5 Hz, 2H, H-3', H-5'), 7.65 (d, *J*=7.6 Hz, 2H, H-2', H-6'), 7.90 (s, 1H, H-imidazole), 8.44 ppm (s, 1H, N=CH); IR (KBr): ü=3352, 2939, 1686, 1465, 1128, 783, 702 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 353; Anal. Calcd for $C_{19}H_{20}N_4O_3$: C 64.76, H 5.72, N 15.90, found C 64.72, H 5.73, N 15.91.

(*E*)-1-((furan-2-ylmethylene)amino)-4-phenyl-1*H*-imidazol-2-amine (4o)

Brown solid. Yield: (10.1 mg, 4%); mp: 134 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 5.92 (s, 2H, NH_2), 6.67 (moderately broad singlet, 1H, furan H-4), 7.0 (d, *J*=3.3 Hz, 1H, furan H-5), 7.16 (t, *J*=8.7 Hz, 1H, H-4'), 7.30 (t, *J*=7.5 Hz, 2H, H-3', H-5'), 7.64 (d, *J*=7.8 Hz, 2H, H-2', H-6'), 7.88 (s, 1H, furan H-3), 7.93 (s, 1H, H-imidazole), 8.41 ppm (s, 1H, N=CH); IR (KBr): ü=3453, 3308, 2945, 1636, 1482, 1158, 699 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 253; Anal. Calcd for $C_{14}H_{12}N_4O$: C 66.65, H 4.79, N 22.21, found C 66.68, H 4.77, N 22.20.

(*E*)-4-phenyl-1-((thiophen-2-ylmethylene)amino)-1*H*-imidazol-2-amine (4p)

Yellow solid. Yield: (10.7 mg, 4%); mp: 188 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 5.95 (s, 2H, NH_2), 7.2 (m, 2H, H-4', thiophene H-4), 7.34 (t, *J*=7.6 Hz, 2H, H-3', H-5'), 7.56 (d, *J*=3.4 Hz, 1H, thiophene H-5), 7.68 (d, *J*=7.5 Hz, 2H, H-2', H-6'), 7.77 (d, *J*=4.9 Hz, 1H, thiophene H-3), 7.95 (s, 1H, H-imidazole), 8.78 ppm (s, 1H, N=CH); IR (KBr): ü=3457, 3311, 3153, 3095, 1660, 1483, 1332, 788, 708 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 269; Anal. Calcd for $C_{14}H_{12}N_4S$: C 62.66, H 4.51, N 20.88, S 11.95, found C 62.09, H 4.50, N 20.87, S 11.94.

(*E*)-4-phenyl-1-((pyridin-2-ylmethylene)amino)-1*H*-imidazol-2-amine (4q)

Brown solid. Yield: (10.0 mg, 3.8%); mp: 197 °C dec; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 7.23 (t, *J*=6.9 Hz, 1H, H-4'), 7.51 (t, *J*=7.3 Hz, 2H, H-3', H-5'), 7.56 (t, *J*=5.8 Hz, 1H, pyridine H-5), 7.73 (d, *J*=7.5 Hz, 2H, H-2', H-6'), 8.01 (t, *J*=7.5 Hz, 1H, pyridine H-4), 8.35 (bs, 2H, NH_2), 8.47 (d, *J*=7.8 Hz, 1H, pyridine H-3), 8.63 (s, 1H, H-imidazole), 8.74 (s, 1H, pyridine H-6), 8.78 ppm (s, 1H, N=CH); IR (KBr): ü=3364, 3261, 3209, 3082, 2880, 1662, 1522, 1220, 1182, 765 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 264; Anal. Calcd for $C_{15}H_{13}N_5$: C 68.42, H, 4.98, N, 26.60, found C 68.31, H, 4.99, N, 26.70.

(*E*)-1-(benzylideneamino)-4-(4-fluorophenyl)-1*H*-imidazol-2-amine (5a)

Yellow solid. Yield: (14.0 mg, 5%); mp: 183-186 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.14 (s, 2H, NH_2), 7.14 (t, *J*=6.7 Hz, 2H, H-2', H-6'), 7.46 (m, 3H, H-3, H-4, H-5), 7.68 (dd, *J*=8.5 Hz, *J*=5.6 Hz, 2H, H-3', H-5'), 7.88 (d, *J*=6.7 Hz, 2H, H-2 ,H-6), 7.94 (s, 1H, H-imidazole), 8.50 ppm (s, 1H, N=CH); IR (KBr): ü=3400, 3278, 3078, 1638, 1563, 1493, 1218, 839 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 281 Anal. Calcd for $C_{16}H_{13}FN_4$: C 68.56, H 4.67, N 19.99, found C 68.55, H 4.68, N 19.98.

(*E*)-1-(benzylideneamino)-4-(4-chlorophenyl)-1*H*-imidazol-2-amine (5b)

Brown solid. Yield: (8.9 mg, 3%); mp: 195 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 6.17 (s, 2H, NH_2), 7.35 (d, *J*=8.5 Hz, 2H, H-3', H-5'), 7.47 (m, 3H, H-3, H-4, H-5), 7.66 (d, *J*=8.5 Hz, 2H, H-2', H-6') 7.9 (d, *J*=7.8 Hz, 2H, H-2, H-6), 8.02 (s, 1H, H-imidazole), 8.59 ppm (s, 1H, N=CH); IR (KBr): ü=3399, 3276, 3125, 3080, 1638, 1469, 1090, 833 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 297; Anal. Calcd for $C_{16}H_{13}ClN_4$: C 64.76, H 4.42, N 18.88, found C 64.72, H 4.43, N 18.89.

(*E*)-1-(benzylideneamino)-4-(*p*-tolyl)-1*H*-imidazol-2-amine (5c)

Yellow solid. Yield: (5.5 mg, 2%); mp: 210-212 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 2.25 (s, 3H, CH_3), 6.10 (s, 2H, NH_2), 7.12 (d, *J*=8.0 Hz, 2H, H-3', H-5'), 7.46 (m, 3H, H-3, H-4, H-5), 7.56 (d, *J*=8.0 Hz, 2H, H-2', H-4'), 7.88 (dd, *J*=7.7 Hz, *J*= 1.9 Hz, 2H, H-2, H-6), 7.9 (s, 1H, H-imidazole), 8.5 ppm (s, 1H, N=CH); IR (KBr): ü=3410, 3293, 3082, 1645, 1472, 1381, 832 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 277; Anal. Calcd for $C_{17}H_{16}N_4$: C 73.89, H 5.84, N 20.27, found C 73.97, H 5.83, N 20.20.

(*E*)-1-(benzylideneamino)-4-(4-methoxyphenyl)-1*H*-imidazol-2-amine (5d)

Yellow solid. Yield: (5.8 mg, 2%); mp: 198-200 °C; ^{1}H NMR (500 MHz, [D_6]DMSO): δ= 3.72 (s, 3H, OCH_3), 6.14 (s, 2H, NH_2), 6.9 (d, *J*=6.9 Hz, 2H, H-3', H-5'), 7.45 (m, 3H, H-3, H-4, H-5), 7.59 (d, 2H, *J*=6.8 Hz, H-2', H-6'), 7.83 (s, 1H, H-imidazole), 7.71 (dd, *J*=7.7 Hz, *J*=1.9 Hz; 2H, H-2, H-6), 8.48 ppm (s, 1H, N=CH); IR (KBr): ü=3439, 3298, 3093, 1643, 1510, 1483, 1254, 1185, 1042, 955, 850 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 293; Anal. Calcd for $C_{17}H_{16}N_4O$: C 69.85, H 5.52, N 19.17, found C 69.89, H 5.53, N 19.10.

(*E*)-1-(benzylideneamino)-4-(2-chlorophenyl)-1*H*-imidazol-2-amine (5e)

Yellow solid. Yield: (8.9 mg, 3%); mp: 190-191 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 6.18 (s, 2H, NH$_2$), 7.18 (t, *J*=7.6 Hz, 1H, H-5'), 7.32 (t, *J*=7.7 Hz, 1H, H-4'), 7.42 (d, *J*=7.9 Hz, 1H, H-6'), 7.46 (m, 3H, H-3, H-4, H-5), 7.94 (dd, *J*=7.6 Hz, *J*=1.6 Hz, 2H, H-2, H-6), 8.06 (dd, *J*=7.9 Hz, *J*=1.4 Hz, 1H, H-3'), 8.08 (s, 1H, H-imidazole), 8.67 ppm (s, 1H, N=CH); IR (KBr): ü=3455, 3307, 3104, 1657, 1476, 1327, 781, 693 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 297; Anal. Calcd for $C_{16}H_{13}ClN_4$: C 64.76, H 4.42, N 18.88, found C 64.72, H 4.41, N 18.90.

(*E*)-1-(benzylideneamino)-4-(2-bromophenyl)-1*H*-imidazol-2-amine (5f)

Yellow solid. Yield: (6.8 mg, 2%); mp: 180 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 6.19 (bs, 2H, NH$_2$), 7.12 (t, *J*=6.6 Hz, 1H, H-5'), 7.36 (t, *J*=7.9 Hz, 1H, H-4'), 7.46 (m, 3H, H-3, H-4, H-5), 7.62 (d, *J*=7.6 Hz, 1H, H-6'), 7.95 (m, 3H, H-2, H-6, H-3'), 8.11 (s, 1H, H-imidazole), 8.64 ppm (s, 1H, N=CH); IR (KBr): ü=3441, 3307, 1650, 1478, 1218, 743, 699 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 341, 343; Anal. Calcd for $C_{16}H_{13}BrN_4$: C 56.32, H 3.84, N 16.42, found C 56.30, H 3.85, N 16.41.

(*E*)-1-(benzylideneamino)-4-(3-chlorophenyl)-1*H*-imidazol-2-amine (5g)

Brown solid. Yield: (6.5 mg, 2.2%); mp: 197-200 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 6.19 (s, 2H, NH$_2$), 7.2 (dd, *J*=7.8 Hz, *J*=1.3 Hz, 1H, H-6'), 7.34 (t, *J*=7.8 Hz, 1H, H-5'), 7.47 (m, 3H, H-3, H-4, H-5), 7.60 (d, *J*=7.8 Hz, 1H, H-4'), 7.68 (s, 1H, H-2'), 7.88 (dd, *J*=7.3 Hz, *J*=1.5 Hz, 2H, H-2, H-6), 8.1 (s, 1H, H-imidazole), 8.52 ppm (s, 1H, N=CH); IR (KBr): ü=3398, 3057, 1636, 1471, 1208, 1068, 760, 684 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 297; Anal. Calcd for $C_{16}H_{13}ClN_4$: C 64.76, H 4.42, N 18.88, found C 64.84, H 4.42, N 18.81.

(*E*)-4-(4-fluorophenyl)-1-((thiophen-2-ylmethylene)amino)-*1H*-imidazol-2-amine (6a)

Yellow solid. Yield: (5.7 mg, 2%); mp: 148-150 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 5.93 (s, 2H, NH$_2$), 7.15 (m, 3H, thiophene H-4, H-2', H-6'), 7.51 (d, *J*=3.4 Hz, 1H, thiophene H-5), 7.66 (dd, *J*=7.6 Hz, *J*=4.7 Hz, 2H, H-3', H-5'), 7.72 (d, *J*=5.0 Hz, 1H, thiophene H-3), 7.90 (s, 1H, H-imidazole), 8.72 ppm (s, 1H, N=CH); IR (KBr): ü=3391, 3125, 3062, 1636, 1562, 1470, 1210 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 287; Anal. Calcd for $C_{14}H_{11}FN_4S$: C 58.73, H 3.87, N 19.57, S 11.20, found C 58.72, H 3.88, N 19.58, S 11.19.

(*E*)-4-(4-chlorophenyl)-1-((thiophen-2-ylmethylene)amino)-1*H*-imidazol-2-amine (6b)

Yellow solid. Yield: (6.6 mg, 2.2%); mp: 208-210 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 5.97 (s, 2H, NH$_2$), 7.16 (dd, *J*=4.9 Hz, *J*=1.3 Hz, 1H, thiophene H-4), 7.37 (d, *J*=8.5 Hz, 2H, H-2', H-6'); 7.52 (d, *J*=3.1 Hz, 1H, thiophene H-5), 7.64 (d, *J*=8.5 Hz, 2H, H-3', H-5'), 7.73 (d, *J*=5.0 Hz, 1H, thiophene H-3), 7.97 (s, 1H, H-imidazole), 8.73 ppm (s, 1H, N=CH); IR (KBr): ü=3395, 3120, 1634, 1470, 1085, 829, 694 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 303; Anal. Calcd for $C_{14}H_{11}ClN_4S$: C 55.54, H 3.66, N 18.50, S 10.59, found C 55.54, H 3.67, N 18.49, S 10.60.

(*E*)-1-((thiophen-2-ylmethylene)amino)-4-(p-tolyl)-1*H*-imidazol-2-amine (6c)

Yellow solid. Yield: (8.5 mg, 3%); mp: 202-203 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 2.25 (s, 3H, CH$_3$), 5.89 (s, 2H, NH$_2$), 7.11 (d, *J*=7.9 Hz, 2H, H-3', H-5'), 7.16 (dd, *J*=4.9 Hz, *J*=1.3 Hz, 1H, thiophene H-4), 7.51 (d, *J*=3.1 Hz, 1H, thiophene H-5), 7.53 (d, *J*=8.0 Hz, 2H, H-2', H-6'), 7.72 (d, *J*=5.0 Hz, 1H, thiophene H-3), 7.85 (s, 1H, H-imidazole), 8.71 ppm (s, 1H, N=CH); IR (KBr): ü=3409, 3032, 1644, 1479, 827 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 283; Anal. Calcd for $C_{15}H_{14}N_4S$: C 63.81, H 5.00, N 19.84, S 11.35, found C 63.80, H 5.01, N 19.83, S 11.36.

(*E*)-4-(4-methoxyphenyl)-1-((thiophen-2-ylmethylene)amino)-1*H*-imidazol-2-amine (6d)

Yellow solid. Yield: (9.5 mg, 3.2%); mp: 192 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 3.72 (s, 3H, OCH$_3$), 5.89 (s, 2H, NH$_2$), 6.89 (d, *J*=8.8 Hz, 2H, H-3', H-5'), 7.15 (dd, *J*=4.9 Hz, *J*=1.3 Hz, 1H, thiophene H-4), 7.50 (d, *J*=3.5 Hz, 1H, thiophene H-5), 7.57 (d, *J*=8.7 Hz, 2H, H-2', H-6'), 7.71 (d, *J*=5.0 Hz, 1H, thiophene H-3), 7.77 (s, 1H, H-imidazole), 8.69 ppm (s, 1H, N=CH); IR (KBr): ü=3424, 3104, 1653, 1488, 1260 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 299; Anal. Calcd for $C_{15}H_{14}N_4OS$: C 60.38, H 4.73, N 18.78, S 10.75, found C 60.40, H 4.72, N 18.79, S 10.74.

(*E*)-4-(2-bromophenyl)-1-((thiophen-2-ylmethylene)amino)-1*H*-imidazol-2-amine (6e)

Yellow solid. Yield: (5.2 mg, 1.5%); mp: 189-190 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 5.94 (s, 2H, NH$_2$), 7.10 (t, *J*=7.6 Hz, 1H, H-5'), 7.16 (t, *J*=4.9 Hz, 1H, thiophene H-4), 7.35 (t, *J*=7.1 Hz, 1H, H-4'), 7.57 (d, *J*=3.4 Hz, 1H, thiophene H-5), 7.60 (d, *J*=7.5 Hz, 1H, H-6'), 7.74 (d, *J*=5.0 Hz, 1H, thiophene H-3), 7.97 (dd, *J*=7.9 Hz, *J*=1.7 Hz, 1H, H-3'), 8.06 (s, 1H, H-imidazole), 8.86 ppm (s, 1H, N=CH); IR (KBr): ü=3444, 1654, 1484, 1225, 746 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 347, 349; Anal. Calcd for $C_{14}H_{11}BrN_4S$: C 48.43, H 3.19, N 16.14, S 9.23, found C 48.42, H 3.19, N 16.15, S 9.24.

(*E*)-4-(3-chlorophenyl)-1-((thiophen-2-ylmethylene)amino)-1*H*-imidazol-2-amine (6f)

Yellow solid. Yield: (5.4 mg, 2%); mp: > 300 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 5.97 (s, 2H, NH$_2$), 7.18 (m, 2H, thiophene H-4, H-4'), 7.33 (t, *J*=7.8 Hz, 1H, H-5'), 7.52 (d, *J*=3.1 Hz, 1H, thiophene H-5), 7.58 (d, *J*=8.0 Hz, 1H, H-6'), 7.65 (s, 1H, H-2'), 7.75 (d, *J*=5.0 Hz, 1H, thiophene H-3), 8.07 (s, 1H, H-imidazole), 8.75 ppm (s, 1H, N=CH); IR (KBr): ü=3409, 3032, 1644, 1479, 1471, 1445, 827 cm^{-1}; ESI-MS *m/z* [*M*+H$^+$] = 303; Anal. Calcd for $C_{14}H_{11}ClN_4S$: C 55.54, H 3.66, N 18.50, S 10.59, found C 55.54, H 3.65, N 18.51, S 10.58.

(*E*)-4-(3-bromophenyl)-1-((thiophen-2-ylmethylene)amino)-1*H*-imidazol-2-amine (6g)

Yellow solid. Yield: (5.2 mg, 2%); mp: 189-193 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ= 5.97 (s, 2H, NH$_2$), 7.17 (t, *J*=3.7 Hz, 1H, thiophene H-4), 7.27 (t, *J*=7.9 Hz, 1H, H-5'), 7.32 (d, *J*=7.9 Hz, 1H, H-6'), 7.52 (d, *J*=3.6 Hz, 1H, thiophene H-5), 7.62 (d, *J*=7.1 Hz, 1H, H-4'), 7.74 (d, *J*= 4.8 Hz,

1H, thiophene H-3), 7.81 (s, 1H, H-2'), 8.04 (s, 1H, H-imidazole), 8.73 ppm (s, 1H, N=CH); IR (KBr): ü=3401, 3100, 2933, 1634, 1471, 1206 cm^{-1}; ESI-MS *m/z* [*M*+H^+] = 347, 349; Anal. Calcd for $C_{14}H_{11}BrN_4S$: C 48.43, H 3.19, N 16.14, S 9.23, found C 48.44, H 3.19, N 16.13, S 9.22.

Platelet aggregation studies

Platelet aggregation studies were performed on APACT-4004 aggregometer (LABiTec, Ahrensburg, Germany) according to Born method as described before.[16,22,26]

Briefly, platelet rich plasma (PRP) was obtained by centrifugation of human citrated blood at 100 g for 10 min. To obtain platelet poor plasma (PPP) the residual blood was centrifuged at 1500 g for 15 min. PRP (200 µL) and synthesized compounds were incubated at 37°C for 5 min before activation by the addition of ADP, collagen and AA.

The final concentrations of ADP, collagen and AA were 5 µM, 2.5 µg/mL and 1.35 mM, respectively. DMSO used as solvent of synthesized compounds and blank sample (0.5% v/v) in aggregation studies. The stock solutions of synthesized compounds (5 mM) were prepared. These compounds were screened at 1 mM concentration and the active ones (> 50% inhibition) were diluted to obtain IC_{50}. The IC_{50} values were calculated by Graphpad Prism Version 3.02. [16,22,26] Student's t-test ($P < 0.05$) was used for statistical analysis.

Docking studies

The crystal structure of COX-1 complex (PDB code: 3N8Y[27]) was obtained from the RCSB Protein Data Bank. The docking studies were performed with AutoDock version 4.2[28] (autodock.scripps.edu). Protein was prepared by removing water molecules and ligands, and adding the polar hydrogens and Gasteiger partial charges. The structure of compounds was generated and minimized using molecular mechanic AMBER method with the algorithm Polak-Ribiere through Hyperchem 8.0 software (Gainesville, FL, USA).

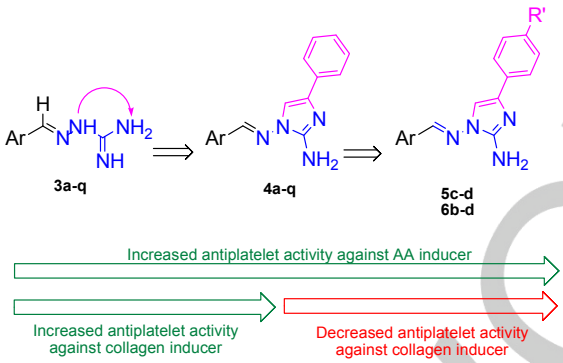
The grid box of 40 × 40 × 40 Å with a spacing of 0.375 Å was chosen. Center of the native ligand in active site was selected as center of grid box. The population size and the maximum number of evaluations were 150 and 2500000, respectively. The results were analyzed using PyMol[29] and Ligplot[30] software.

Keywords: 2-Aminoimidazole, Antiplatelet, Cyclization, Hydrazone

References:

[1] WHO, "WHO | New initiative launched to tackle cardiovascular disease, the world's number one killer," can be found under http://www.who.int/cardiovascular_diseases/global-hearts/Global_hearts_initiative/en/, **2016**.
[2] S. Chackalamannil, *J. Med. Chem.* **2006**, *49*, 5389–5403.
[3] R. Hall, C. D. Mazer, *Anesth. Anal.* **2011**, *112*, 292–318.
[4] Y. Ren, K. Patel, T. Crane, *J. Extra Corpor. Technol.* **2010**, *42*, 103–113.
[5] I. A. F. B. Silveira, L. G. Paulo, A. L. P. Miranda, S. O. Rocha, A. C. C. Freitas, E. J. Barreiro, *J. Pharm. Pharmacol.* **1993**, *45*, 646–649.
[6] P. C. Sathler, M. Santana, A. L. Lourenço, C. R. Rodrigues, P. Abreu, L. M. Cabral, H. C. Castro, *J. Enzyme Inhib. Med. Chem.* **2013**, *6366*, 1–5.
[7] R. Guthrie, *Adv. Ther.* **2011**, *28*, 473–482.
[8] L. Zhao, H. Jin, L. Sun, H. Piao, Z. Quan, *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5027–5029.
[9] K. Rehse, J. Steege, *Arch. Pharm.* **2005**, *338*, 539–547.
[10] M. I. K. Momin, D. Ramjugernath, R. A. Mosa, A. R. Opoku, N. A. Koorbanally, *Med. Chem. Res.* **2015**, *24*, 2075–2084.
[11] R. Chelucci, L. Dutra, M. Lopes Pires, T. de Melo, P. Bosquesi, M. Chung, J. dos Santos, *Molecules.* **2014**, *19*, 2089–2099.
[12] A. R. Todeschini, A. L. P. De Miranda, K. C. M. Da Silva, S. C. Parrini, E. J. Barreiro, *Eur. J. Med. Chem.* **1998**, *33*, 189–199.
[13] A. K. Jordão, V. F. Ferreira, E. S. Lima, M. C. B. V de Souza, E. C. L. Carlos, H. C. Castro, R. B. Geraldo, C. R. Rodrigues, M. C. B. Almeida, A. C. Cunha, *Bioorg. Med. Chem.* **2009**, *17*, 3713–3719.
[14] C. Watala, M. Dobaczewski, P. Kazmierczak, J. Gebicki, M. Nocun, I. Zitnanova, O. Ulicna, Z. Durackova, I. Waczulíková, J. Carsky, et al., *Vascul. Pharmacol.* **2009**, *51*, 275–283.
[15] N. Kalhor, M. Mardani, S. Abdollahzadeh, M. Vakof, M. E. Zadeh, K. H. M. E. Tehrani, F. Kobarfard, S. Mohebbi, *Bull. Korean. Chem. Soc.* **2015**, *36*, 2632–2639.
[16] V. Mashayekhi, K. Haj Mohammad Ebrahim Tehrani, S. Amidi, F. Kobarfard, *Chem. Pharm. Bull.* **2013**, *61*, 144–150.
[17] S. S. Mirfazli, M. Khoshneviszadeh, M. Jeiroudi, A. Foroumadi, F. Kobarfard, A. Shafiee, *Med. Chem. Res.* **2016**, *25*, 1–18.
[18] K. Haj Mohammad Ebrahim Tehrani, S. Sardari, V. Mashayekhi, M. Esfahani Zadeh, P. Azerang, F. Kobarfard, *Chem. Pharm. Bull.* **2013**, *61*, 160–166.
[19] W. Holzer, Z. Györgydeák, *Monatsh. Chem.* **1992**, *123*, 1163–1173.
[20] W.-T. Li, D.-R. Hwang, J.-S. Song, C.-P. Chen, J.-J. Chuu, C.-B. Hu, H.-L. Lin, C.-L. Huang, C.-Y. Huang, H.-Y. Tseng, et al., *J. Med. Chem.* **2010**, *53*, 2409–2417.
[21] Z. Györgydeák, G. Szabó, W. Holzer, *Monatsh. Chem.* **2004**, *135*, 173–184.
[22] G. V. R. Born, *Nature.* **1962**, *194*, 927–929.
[23] C. A. Lipinski, *Drug. Discov. Today. Technol.* **2004**, *1*, 337–341.
[24] T. Sander, J. Freyss, M. von Korff, C. Rufener, *J. Chem. Inf. Model.* **2015**, *55*, 460–473.
[25] M. Z. Krimer, F. Z. Makaev, E. P. Styngach, A. G. Koretskii, S. I. Pogrebnoi, A. I. Kochug, *Chem. Heterocycl. Compd.* **1996**, *32*, 1035–1039.
[26] S. Amidi, F. Kobarfard, A. B. Moghaddam, K. Tabib, Z. Soleymani, *Iran J. Pharm. Res.* **2013**, *12*, 89–101.
[27] R. S. Sidhu, J. Y. Lee, C. Yuan, W. L. Smith, *Biochem.* **2010**, *49*, 7069–7079.
[28] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, *J. Comput. Chem.* **2009**, *30*, 2785–2791.
[29] Schrodinger, LLC, The PyMol Molecular Graphics System, **2008**, *Version 1.1eval.*
[30] R. A. Laskowski, M. B. Swindells, *J. Chem. Inf. Model.* **2011**, *51*, 2778–2786.

Entry for the Table of Contents



Cyclization of aromatic guanylhydrazones with phenacyl bromide increased antiplatelet activity against arachidonic acid (AA) and collagen as platelet aggregation inducers. The substituents phenyl ring at position 4 of imidazole decreased the activity against collagen while the phenyl ring with para substituent increased the activity against AA. These changes did not affect the antiplatelet activity against adenosine diphosphate (ADP) as platelet aggregation inducers.