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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel 1,3-diarylpyrazole acrylamides: synthesis, antiplatelet activity screening, and in silico evaluation studies

Sultan Nacak Baytas · Nazan Inceler · Yesim Ozkan · Serdar Unlu · M. Fethi Sahin

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Abstract (*E*)-3-[3-(pyridin-4-yl)-1-phenyl-1*H*-pyrazole-4-yl]acryl amides were evaluated for their antiplatelet activities. Compounds **4o** and **4r** were found as active derivatives showing a potent inhibitory activity on the arachidonic acid-induced aggregation, with IC_{50} of 20.2 and 30.3 μM, respectively. Compounds **4j**, **4k**, and **4u** presented significant inhibitor effect on collagen-induced platelet aggregation (73.1, 82.5, and 86.7 %, respectively). All synthesized compounds demonstrated good drug-likeness and drug-score values in silico evaluations.

Keywords Antiplatelet activity · 1,3-Diarylpyrazoles · Acryl amides · Arachidonic acid · Collagen

Introduction

Cardiovascular diseases are responsible for the largest number of natural deaths worldwide. Cardiac conditions involving thrombotic disorders include acute myocardial infarction, valvular heart disease, unstable angina, and atrial fibrillation (Yusuf *et al.,* 2001). Platelet adhesion and aggregation are key events in homeostasis and thrombosis (clot formation). Hyperactivity of platelets increases the risk of various vascular diseases, such as unstable angina, acute myocardial infarction, transient ischemic attacks, stroke, and complications following percutaneous coronary intervention (Fuster *et al.,* 1992; Davis and Thomas, 1985; Trip *et al.,* 1990; Jackson and Schoenwaelder, 2003). Thus, antiplatelet therapy is a basic tool in the prevention and treatment of atherothrombotic diseases. In injured vessels, blood aggregation is generated as a physiological defense reaction by releasing biologically active compounds such as adenosine diphosphate (ADP), thrombin, and prostaglandin endoperoxide. Agonists such as thrombin, arachidonic acid (AA), thromboxane A_2 (TxA_2), platelet-activating factor (PAF), and collagen are able to induce platelet aggregation. Among these agonists, AA is one of the most powerful agonists for platelet activation (Fuster *et al.,* 1992; Arita and Hansaki, 1989; Needleman *et al.,* 1986). Currently, different antiplatelet agents such as ticlopidine, clopidogrel, glycoprotein IIb/IIIa inhibitors, and acetylsalicylic acid (ASA) are available for clinical use. However, they have certain disadvantages such as notable side effects (i.e., gastric erosion, agranulocytosis) and inefficient therapy (Storey, 2001; Schros, 1995; Bennett, 2001; Berger, 1999; McKee *et al.,* 2002). Therefore, studies for developing safer antiplatelet agents with fewer side effects have recently been the goal of many researchers.

Essential structural features of TxA_2 synthetase inhibitors are a basic nitrogen atom of a substituted pyridine or imidazole ring, and carboxylic acid group separated by an unsaturated trans-alkyl chain (De Leval *et al.,* 2004). It is found that there should be appropriate length between nitrogen atom of pyridine residue, which is known to selectively inhibit TxA_2 synthetase via making chelate with iron, and carboxylic acid moiety for selective TxA_2

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synthetase inhibitor (Lizuka *et al.,* 1981; Tanouchi *et al.,* 1981). Ozagrel is a cinnamic acid derivative having imidazol-1-yl substituent (Fig. 1) that acts as a selective inhibitor of TxA_2 synthetase with an IC_{50} of 11 nM. (Silveira *et al.,* 1993). In particular, an *N*-Phenylpyrazole arylhydrazone derivative compound (Fig. 1) possesses analgesic, anti-inflammatory, and antiplatelet activities. These types of compounds were found to be very effective in antiplatelet activity on arachidonic acid-induced platelet aggregation in rabbit citrated platelet rich plasma possibly acting at the arachidonic acid cascade level (Loo *et al.,* 1987).

Microwave irradiation dominates over usual conventional methods due to its non-hazardous and eco-friendly nature. The microwave induced organic reactions have received considerable attention due to their simplicity and operational convenience. The use of microwave irradiation as a source of heat in synthetic chemistry offers a promising alternative (Jyothi *et al.,* 2007; Kalluraya *et al.,* 2008). Microwave-assisted heating under controlled conditions is a priceless technology because it often dramatically reduces reaction times, with increased yield (Escalante and Diaz-Coutino, 2009). Nowadays, microwave-assisted organic synthesis has become a new era in the field of synthetic chemistry (Algul *et al.,* 2009).

In our previous study (Baytas *et al.,* 2012), a series of (*E*)-3-[3-(2,3-dihydro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1-phenyl-1*H*-pyrazole-4-yl]acrylamides (**A** in Fig. 1) have been synthesized using both conventional and microwave-assisted methods and evaluated for their in vitro inhibitory activities on COX-1 and COX-2 isoforms using human whole blood assay as well as their antiplatelet profile against human platelet aggregation using arachidonic acid, as agonists. Especially, a 4-pyridylpiperazin amide derivative exhibited the best inhibitory activity on platelet aggregation. Based on these results, we aimed to introduce pyridine moiety to central pyrazole ring instead of benzoxazole ring, and searched its effect on platelet aggregation. In the search for new antiplatelet agents, we describe herein the design, synthesis, and antiplatelet activities of new (*E*)-3-[3-(pyridin-4-yl)-1-phenyl-1*H*-pyrazole-4-yl]acryl amides, bearing a central pyrazole ring substituted with phenyl, pyridine moieties, and 3-oxoprop-1-en-1-yl fragment at 1,3, and 4 positions, respectively (Fig. 1). Finally, these derivatives were submitted to an in silico oral biodisponibility screening to analyze their overall potential to qualify for a drug.

Results

Chemistry

The synthesis of (*E*)-3-[1-phenyl-3-(pyridin-4yl)-1*H*-pyrazole-4-yl]acrylamides, starting from 3-[1-phenyl-3-(pyridin-4-yl)-1*H*-pyrazol-4-yl]acrylic acid **3** and amine derivatives, has been accomplished as outlined in Scheme 1. At first, the hydrazone derivative **1** was generated by carrying out a condensation reaction in the presence of 4-acetylpyridine, phenylhydrazine, and acetic acid in refluxing ethanol. This hydrazone derivative was then

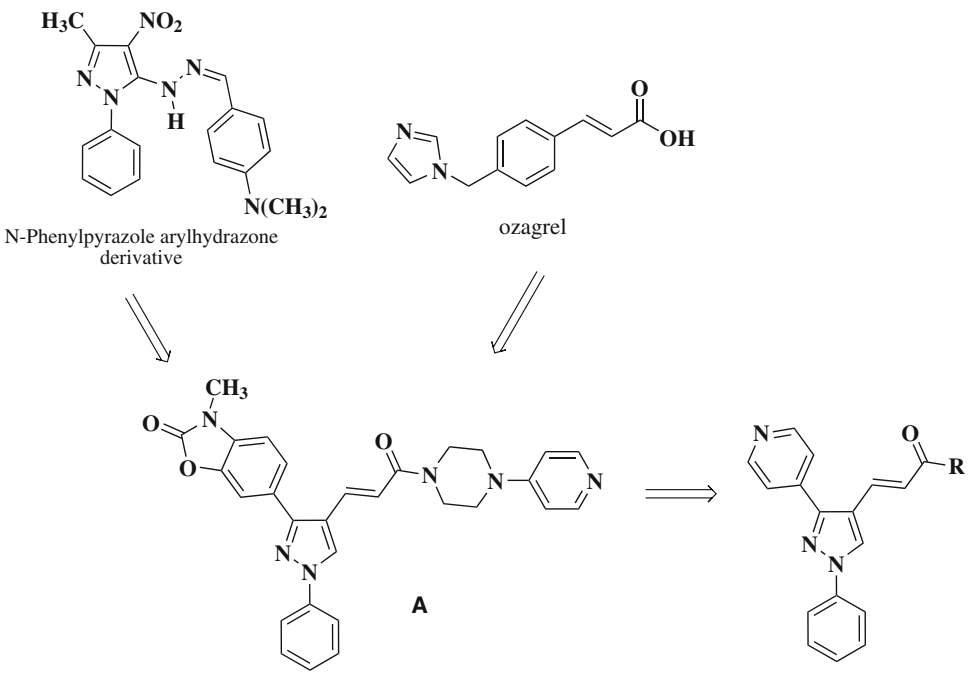
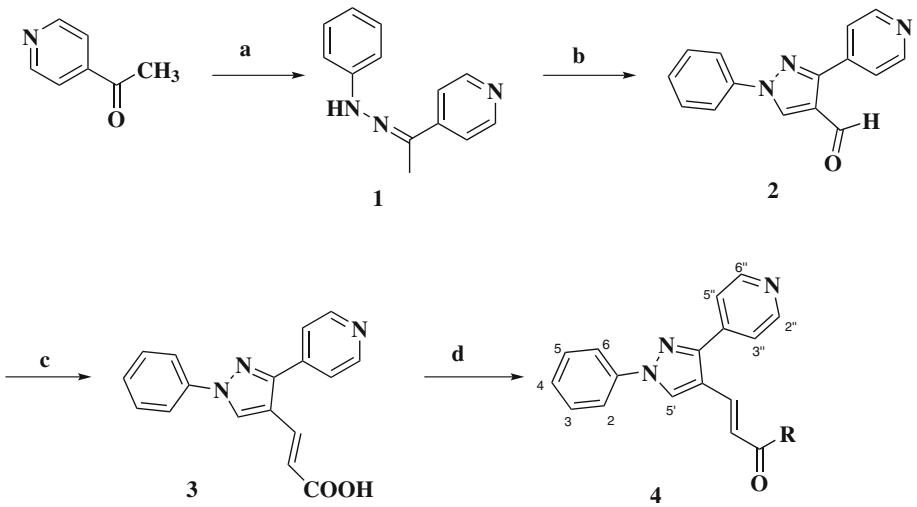


Fig. 1 Structure of Ozagrel, the lead compound (*A*) and general structure of the synthesized compounds



Scheme 1 *a* Phenyl hydrazine, acetic acid, ethanol, 2 h reflux; *b Method A*; dimethyl formamide, $POCl_3$, 50 °C, 4 h; *Method B*; dimethyl formamide, $POCl_3$, MW irradiation, 210 W, 50 °C, 20 min; *c Method A*; malonic acid, pyridine, piperidine, 115 °C, 5 h; *Method B*; malonic acid, pyridine, piperidine, MW irradiation, 600 W, 115 °C, 15 min; *d* appropriate amine derivative, ethyl chloroformate, triethylamine, dichloromethane, rt, overnight

reacted with $POCl_3$ and DMF using two different methods (i.e., conventional synthesis and reaction under microwave conditions) resulting in 1,3-diaryl pyrazole **2** with aldehyde group at the 4 position. 1-Phenyl-3-(pyridin-4-yl)-1*H*-pyrazole-4-carbaldehyde **2** was then treated with malonic acid in pyridine to prepare the corresponding α,β-unsaturated carboxylic acid via Knoevenagel condensation reaction. We focused our attention on the microwave-assisted synthesis technique after obtaining compounds **2** and **3** by classical heating method. Thus, synthesis of compounds **2** and **3** is performed in a microwave oven due to its low cost and ready availability. In order to synthesize compound **2**, phosphoroxy chloride ($POCl_3$) was added dropwise to an ice-cold stirred solution of 4-(1-(2-phenylhydrazono)ethyl)pyridine **1** in DMF. The reaction mixture was allowed to attain room temperature, and then heated at 50 °C using conventional method or in microwave oven. Following the work-up, the desired compounds were obtained. Comparing the two methods utilized, the microwave-assisted synthesis technique dramatically cut down reaction time (i.e., for compound 2; conventional method 5 h, microwave-assisted synthesis 20 min), and increased the product yields as shown in experimental part (conventional method yield 80 %, microwave-assisted synthesis yield 92 %). By treatment of **3** with appropriate amines in the presence of triethylamine and ethyl chloroformate which was used as the carboxylate activator, the resulting amide derivatives **4** were prepared in good yield (40–88 %). Compounds were purified by automated flash chromatography and checked for purity with UPLC before being tested in biological assays (purity was >97 %). The structures of these compounds were confirmed by high-resolution mass spectrometry (HRMS), IR, ^{1}H-, and ^{13}C-NMR spectral data (Table 1).

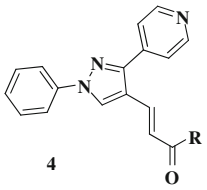
The IR spectra of hydrazone **1** showed disappearance of the carbonyl peak of acetyl group of 4-acetylpyridine; secondary N–H stretching band was observed at 3274 cm^{-1} as singlet. The IR spectra of compound **2** exhibited a characteristic strong absorption for aldehyde carbonyl group at 1669 cm^{-1}. In the IR spectra of compound **3,** strong absorption band at 1681 cm^{-1} and intense O–H stretching absorption in the region of 3300–2500 cm^{-1} for α,β-unsaturated carboxylic acid were observed. Final amide derivatives exhibited a characteristic strong absorption in the area of 1664–1641 cm^{-1} attributable to the C=O of amide group.

The ^{1}H-NMR spectrum of the compound **2** showed the disappearance of the methyl proton signal and N–N–H signal. In the ^{1}H-NMR spectra of compound **2**, two singlets were displayed due to aldehyde group at 10.04 ppm and pyrazole-H_5 at 9.43 ppm, each showing the integration for one proton. In the ^{1}H-NMR spectra of compound **3**, signal of the carboxylic acid was not observed due to proton exchange. One of the olefinic protons of alkyl chain was observed as doublet at 6.51 ppm with coupling constant 16 Hz indicating the (*E*) isomer. In the ^{1}H-NMR spectra of final amide derivatives **4a–v,** that olefinic proton was observed at about 7.26–7.17 ppm as doublet with coupling constant 15.2 Hz. Pyrazole-H_5 gave a singlet at about 9.23–8.99 ppm.

Biological evaluation

The antiplatelet effects obtained in the Born test (Born and Cross, 1963), with compounds **3, 4a–v** are summarized in Fig. 2 and Table 2. We used arachidonic acid or collagen as inducer of the platelet aggregation. The antiplatelet activity was measured in vitro on the responses induced by AA (700 μM) or collagen (5 μg/ml) on citrated platelet-rich human plasma. The initial screening at 100 μM concentrations revealed the compound **4o** and **4r** as the most important derivatives in antiplatelet activity

Table 1 Synthesized 1, 3-pyrazole acryl amide derivatives **4a–v**



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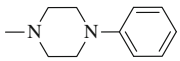
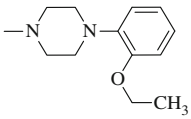
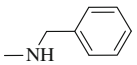
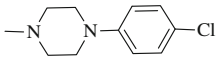
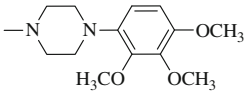
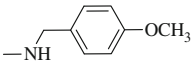
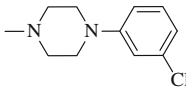
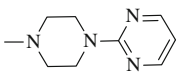
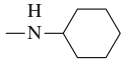
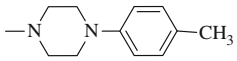
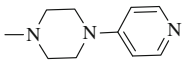
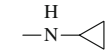
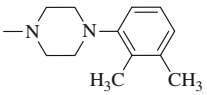
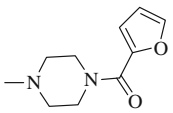
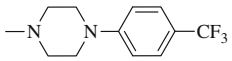
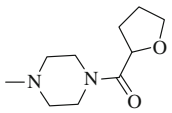
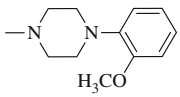
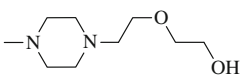
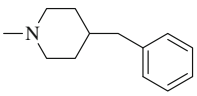
Comp.	R	Comp.	R	Comp.	R
4a		**4h**		**4o**	
4b		**4i**		**4p**	
4c		**4j**		**4r**	
4d		**4k**		**4s**	
4e		**4l**		**4t**	
4f		**4m**		**4u**	
4g		**4n**		**4v**	

screening studies. Compound **4o,** bearing benzylamine moiety at amide portion, was the most active derivative showing a potent inhibitory activity on the AA-induced aggregation, with an IC_{50} of 20.2 μM (Table 2). The inhibitor effect of this compound on the collagen-induced aggregation was less pronounced (inhibition 54.9 %) (Fig. 2). Compound **4r,** a non aromatic amide having cyclohexyl substitution at the amide portion, also displayed potency on AA-induced aggregation test and its IC_{50} value was 30.3 μM. Compound **4r** presented a high activity in the collagen-induced assay (71.8 %). As concerns substituted piperazine amides **4a**–**n**, six compounds (**4f**, **4h**, **4j**, **4k**, **4l**, and **4m**) out of 14 prove to be effective as inhibitor on the AA-induced aggregation, although with lower potency than the **4o** and **4r** (inhibition 5–11 %). Interestingly, these piperazine amides showed moderate to high inhibitor activity on the collagen-induced aggregation (25.0, 42.0, 73.1, 82.5, 50.0, and 68.9 %, respectively). Compounds **4t** and **4u** were weakly active at 100 μM concentration on AA-induced aggregation. Moreover, **4t** and **4u** were very active at the screening concentration in the collagen-induced test (56.5 and 86.7 %, respectively). The rest of the compounds were quite inactive at test conditions.

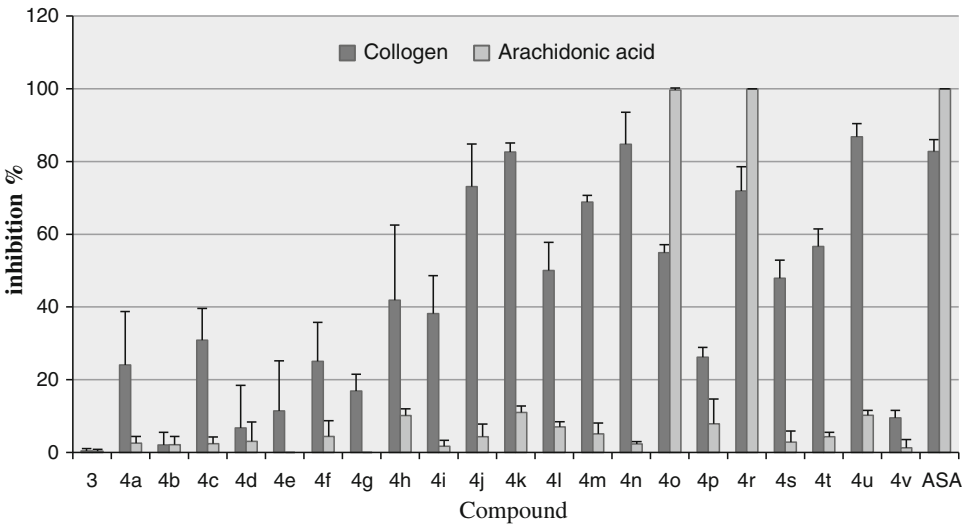


Fig. 2 Effect of (*E*)-3-[1-Phenyl-3-(pyridin-4-yl)-1*H*-pyrazol-4-yl]acryl amide derivatives **3**, **4a–v** (100 μM) and aspirin on in vitro platelet aggregation of human citrated platelet-rich plasma induced by arachidonic acid and collagen

Table 2 IC_{50} (μM) of compound **4r** and **4o** on AA-induced platelet aggregation

Compound	**4o**	**4r**	ASA
IC_{50} (μM)	20.2	30.3	7.7

Lipinski's Rule of five and drug-likeness profile

The synthesized compounds **(4a–v)** were submitted for an in silico evaluation. To predict drug-like properties of synthesized compounds, we analyzed these derivatives according to the rule-of-five developed by Lipinski *et al.,* (2001). Predictions of ADME properties for the compounds are given in Table 3. Calculated physicochemical properties (http://www.molinspiration.com) showed that most of the compounds fulfilled the Lipinski 'rule-of-five'. The compounds **4f** and **4i** violated only the criteria for molecular weight. Theoretically, these compounds should present good passive oral absorption, and differences in their bioactivities cannot be attributed to this property. All compounds exhibited favorable *c*log *P* values. The polar surface areas of synthesized compounds were relatively small in comparison with the average value for acceptable drug molecules (<90Å^2).

Currently, there are many approaches to assess a compound drug-likeness based on topological descriptors, fingerprints of molecular drug-likeness structure keys (Tetko, 2005). In this study, we used the Osiris program (http://www.organic-chemistry.org/prog/peo) for calculating the fragment-based drug-likeness of the compounds (Table 3). Interestingly, all compounds demonstrated good drug-likeness values (from 6.92 to 0.15) except compounds **4f** (having trifluoromethylphenylpiperazine ring) and **4r** (bearing cyclohexyl moiety) (−3.44 and −4.17, respectively).

In this study, we also examined the drug-score. The drug score combines drug-likeness, *c*log *P*, log S, molecular weight, and toxicity risks in one handy value, and may be used to judge the compound's overall potential to qualify for a drug. The results showed that the all synthesized compounds including the two potent ones (i.e., **4r** and **4o**) demonstrated good drug-score values (Table 3). Moreover, we used the Osiris program for prediction of the overall toxicity of the derivatives as it may point to the presence of some fragments generally responsible for the mutagenic, tumorigenic, irritant, or reproductive effects in these molecules. All compounds presented a low in silico toxicity risk profile.

Discussion

Antiplatelet drugs are intended to prevent and/or reverse platelet aggregation in arterial thrombosis, most prominently in myocardial infarction and ischemic stroke. Aspirin is widely used to reduce the risk of myocardial infarction and thromboembolic disease. The mechanism of action of ASA is related to its capacity to permanently inactivate the platelet PGHS by acetylation of the serine amino acid residue (Mason *et al.,* 2005; Patrignani, 2003). Different antiplatelet agents such as ticlopidine, clopidogrel, glycoprotein IIb/IIIa inhibitors are also available for clinical use. But, they have certain disadvantages such as notable side effects (i.e., gastric erosion, agranulocytosis) and inefficient therapy.

In our previous study, we verified the antiplatelet activity of (*E*)-3-[3-(2,3-dihydro-3-methyl-2-oxo-3*H*-benzoxazole-6-yl)-1-phenyl-1*H*-pyrazole-4-yl]acrylamides (Baytas *et al.,* 2012) and determined the 6-(4-((*E*)-3-(4-(Pyridin-4-yl)

Table 3 Calculated ADME properties of synthesized compounds

Compd.	Predicted oral bioavailability						Drug-likeness	Drug-score
	HBA[a]	HBD[b]	M.W[c]	clog *P*[d]	LogS[e]	TPSA[f]		
4a	6	0	435.5	3.45	−3.76	54.26	6.92	0.71
4b	6	0	469.9	4.13	−4.50	54.26	5.8	0.58
4c	6	0	469.9	4.11	−4.50	54.26	6.11	0.58
4d	6	0	449.5	3.90	−4.11	54.26	3.95	0.65
4e	6	0	463.5	4.36	−4.45	54.26	5.32	0.59
4f	6	0	503.5	4.35	−4.54	54.26	−3.44	0.28
4g	7	0	465.5	3.46	−3.78	63.49	6.46	0.67
4h	7	0	479.5	3.84	−4.08	63.49	4.69	0.62
4i	9	0	525.6	3.28	−3.82	81.96	5.65	0.60
4j	8	0	437.4	2.17	−3.03	80.04	6.52	0.77
4k	7	0	436.5	2.17	−2.97	67.15	5.23	0.77
4l	8	0	453.5	2.05	−3.25	84.47	5.23	0.74
4m	8	0	457.5	1.45	−2.58	80.57	3.99	0.76
4n	8	1	447.5	1.02	−1.63	83.72	2.75	0.78
4o	5	1	380.4	3.02	−4.03	59.81	0.15	0.57
4p	6	1	410.4	3.08	−4.05	69.05	1.33	0.64
4r	5	1	372.4	3.53	−4.28	59.81	−4.17	0.36
4s	5	1	330.3	1.99	−3.47	59.81	0.81	0.71
4t	6	0	360.4	1.71	−2.41	60.26	1.48	0.79
4u	5	0	358.4	2.77	−3.30	51.02	0.24	0.63
4v	5	0	448.5	4.44	−4.60	51.02	3.39	0.55

[a] Number of hydrogen bond acceptor

[b] Number of hydrogen bond donor

[c] Molecular weight

[d] Calculated lipophilicity

[e] Solubility parameter

[f] Topological polar surface area ($\mathring{A}^2$)

piperazin-1-yl)-3-oxoprop-1-enyl)-1-phenyl-1*H*-pyrazole-3-yl)-3-methyl-2-oxo-3*H*-benzoxazolone as lead compound (**A** in Fig. 1). With respect to the knowledge that the TxA_2 synthetase inhibitors have substituted pyridine or imidazole ring, we aimed at modifying structural features of the lead compound. Our strategy to optimize the activity was the replacement of the benzoxazolone ring of the central pyrazole with the pyridine ring. In particular, the structural changes in the 1,3-diaryl part of compounds did yield substantial improvement of inhibition of platelet aggregation comparison with the previously prepared derivative **A** (Fig. 1).

It has been also determined the suitability antiplatelet purposes where a good absorption after oral administration is obligatory. Physicochemical properties of synthesized compounds were evaluated in silico, and found that all compounds should present good passive oral absorption. All synthesized compounds demonstrated good drug-likeness and drug-score values. The drug-score combines drug likeness, *c*logP, logS, molecular weight, and toxicity risks in one handy value that may be used to judge the drug potential of a compound. Although the Osiris risk alerts are not a fully reliable toxicity prediction, the theoretical low-toxicity profile of these compounds reinforces further synthetic and biological exploration for the development of new antiplatelet drugs.

Conclusion

A series of new 1,3-diarylpyrazole-4-acrylic acid derivatives as inhibitors of AA-induced and collagen-induced platelet aggregation were investigated with a focus on the role of the pyridine replacement of one of the aryl groups in the diarylpyrazole template. Furthermore, the microwave-assisted technique performed, presented the minimum use of the hazardous phosphorus oxychloride, short reaction time and increased yield compared to conventional method. Based on our biological and theoretical results, we identified some of the synthesized 1,3-diarylpyrazole

derivatives as potential lead compounds and significant inhibitors (**4o,** IC_{50} of 20.2 μM; **4r,** IC_{50} of 30.3 μM on AA-induced platelet aggregation; **4j** 73.1 %, **4k** 82.5 %, and **4u** 86.7 % inhibition on collagen-induced platelet aggregation), to be further in vitro and in vivo investigated.

Experimental

Chemistry

The chemicals were purchased from the commercial vendors and were used without purification. Thin-layer chromatography (TLC) was performed on Merck 60F254 plates. Reactions were monitored by TLC on silica gel, with detection by UV light (254 nm) or charring Dragendorff reagent (Stahl, 1969). Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected (Schorpp Geaetetechnik, Germany). Elementary analyses were performed on a Leco CHNS 932 analyzer and satisfactory results ± 0.4 % of calculated values (C, H, N) were obtained. IR spectra were obtained in-house using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory. ^{1}H-NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 400 MHz FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of Faculty of Pharmacy, Ankara University; values are given in δ (ppm) and J values are in Hz. ^{13}C-NMR spectra were recorded in $CDCl_3$ on a Varian Mercury 300 MHz FT-NMR spectrometer using tetramethylsilane as the internal standard at the NMR facility of FARGEM (Pharmaceutical Research and Development Center) Inc. High-resolution mass spectra data (HRMS) were collected in-house using a Waters LCT Premier XE Mass Spectrometer (high sensitivity orthogonal acceleration time-of-flight instrument) operating in ESI (+) method, also coupled to an AQUITY ultra performance liquid chromatography system (Waters Corporation, Milford, MA, USA). All compounds were of >97 % purity. Flash chromatography was performed with a Combiflash®Rf automated flash chromatography system with RediSep columns (Teledyne-Isco, Lincoln, NE, USA) using dichloromethane-methanol solvent gradients. Microwave-assisted reactions were carried out with a Milestone MicroSYNTH microwave synthesis system. Calculation of important physicochemical properties (log$P$, number of hydrogen bond donors and acceptors and total polar surface area) was performed using mol inspiration cheminformatics software at URL http://www.molinspiration.com. Determination of drug-likeness and drug score was done using Osiris program at URL http://www.organic-chemistry.org/prog/peo.

4-(1-(2-Phenylhydrazono)ethyl)pyridine 1

A solution of 4-acetylpyridine (0.052 mol), phenyl hydrazine (6.27 g, 0.058 mol), and acetic acid (2 ml, 0.035 mol) in ethanol was stirred for 2 h at reflux, and then evaporated. The precipitated was filtered off and dried. Yield 85 %, m.p. 147 °C [Lit. m.p. (Chu and Teague, 1958): 148–149 °C]; IR (FTIR/FTNIR-ATR): 3227 cm^{-1} (N–H), 1941 cm^{-1} (C=N); ^{1}H-NMR ($CDCl_3$) δ: 8.59 (2H, d, $J = 6.4$ Hz), 7.65 (2H, d, $J = 6$ Hz), 7.59 (1H, s), 7.31 (2H, t, $J = 7.6$ Hz), 7.21 (2H, d, $J = 7.6$ Hz), 6.93 (1H, dd, $J = 6.8$ Hz, $J = 7.6$ Hz), 2.22 (3H, s); ^{13}C-NMR ($CDCl_3$) δ: 170.2, 150.6, 142.3, 138.7, 130.4, 125.7, 125.2, 120.0, 114.3, 114.1, 17.1; HRMS $C_{13}H_{14}N_3$ $[M+H]^+$ *Calc*. 212.1188, Found *m/z* 212.1180; Anal. *Calc*.(%) for $C_{13}H_{13}N_3$. C: 73.91 H: 6.20 N: 19.89, Found C: 74.21 H: 6.22 N: 19.38.

1-Phenyl-3-(pyridin-4-yl)-1H-pyrazole-4-carbaldehyde ***2***

Method A In a dry flask, phosphoroxy chloride ($POCl_3$) (16.77 ml, 0.18 mol) was added dropwise to an ice-cold stirred solution of 4-(1-(2-phenylhydrazono)ethyl)pyridine (12.71 g, 0.06 mol) in 100 ml DMF. The reaction mixture was allowed to attain room temperature, and then heated at 50 °C for about 5 h. The resulting mixture was poured onto crushed ice, neutralized with dilute NaOH and left overnight. The yellow precipitate obtained was purified by crystallization in acetone–water mixture. Yield 12 g (80 %).

Method B In a dry flask, $POCl_3$ (16.77 ml, 0.18 mol) was added dropwise to an ice-cold stirred solution of 4-(1-(2-phenylhydrazono)ethyl)pyridine (12.71 g, 0.06 mol) in 100 ml DMF. The reaction mixture was allowed to attain room temperature, and then flask was placed in MicroSYNTH microwave synthesis system and irradiated at 210 W for 20 min while the temperature was set to 50 °C. The resulting mixture was poured onto crushed ice, neutralized with dilute sodium hydroxide and left standing overnight. The yellow precipitate obtained was prufied by crystallization in acetone–water mixture. Yield 13.8 (92 %), m.p. 147–149 °C [Lit. mp (Badadhe *et al.,* 2011): 148 °C]; IR (FTIR/FTNIR-ATR): 1669 cm^{-1} (C=O); ^{1}H-NMR (DMSO- d_6) δ: 10.04 (1H, s), 9.43 (1H, s), 8.73 (2H, d, $J = 1.6$ Hz), 8.02-7.97 (4H, m), 7.60 (2H, dd, $J = 7.6$ Hz, $J = 8.4$ Hz), 7.46 (1H, dd, $J = 7.2$ Hz, $J = 7.6$ Hz); ^{13}C-NMR (DMSO- d_6) δ: 185.0, 150.7, 150.3, 139.2, 139.1, 136.9, 130.4, 128.7, 123.4, 123.3, 120.0; HRMS $C_{15}H_{12}N_3O$ $[M+H]^+$ *Calc*. 250.0980, Found *m/z* 250.0980. Anal. *Calc*. (%) for $C_{15}H_{11}N_3O$ C: 72.28 H: 4.45 N: 16.86; Found C: 72.20 H: 4.42 N: 17.07.

(E)-3-[1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]acrylic acid ***3***

Method A To a solution of 1-phenyl-3-(pyridin-4-yl)-1*H*-pyrazole-4-carbaldehyde **2** (2.49 g, 0.01 mol) in pyridine (30 ml), malonic acid (4.16 g, 0.04 mol) and piperidine (1.5 ml, 0.015 mol) were added, and the reaction mixture was refluxed for 5 h. On cooling, the reaction mixture was poured onto a solution (100 ml) of crushed ice and concentrated HCl (50 % by volume) mixture, then, pH was adjusted to 7. The resulting precipitate was filtered off, washed with acidified water, and dried. Yield 2.3 g (63.7 %).

Method B The dry flask charged with 1-phenyl-3-(pyridin-4-yl)-1*H*-pyrazole-4-carbaldehyde **2** (2.49 g, 0.01 mol), malonic acid (4.16 g, 0.04 mol), piperidine (1.5 ml, 0.015 mol), and 30 ml pyridine was placed in MicroSYNTH microwave synthesis system and irradiated at 600 W for 15 min while the temperature was set to 115 °C. The resulting mixture was poured onto a solution (100 ml) of crushed ice and concentrated HCl (50 % by volume) mixture, then, pH was adjusted to 7. The resulting precipitate was filtered off, washed with acidified water, and dried. Yield 78 %, m.p. 287–288 °C; IR (FTIR/FTNIR-ATR): 1681 cm^{-1} (C=O), 2440 cm^{-1} (C=C); ^{1}H-NMR (DMSO- d_6) δ: 9.27 (1H, s), 8.73 (2H, d, $J = 6$ Hz), 7.95 (2H, d, $J = 8.4$ Hz), 7.66 (2H, d, $J = 6.4$ Hz), 7.60–7.53 (3H, m), 7.41 (1H, t, $J = 7.6$ Hz), 6.49 (1H, d, $J = 16$ Hz); ^{13}C-NMR (DMSO- d_6) δ: 168.2, 150.9, 149.9, 140.1, 139.4, 133.9, 130.3, 129.6, 128.0, 123.3, 120.3, 119.5, 118.3; HRMS $C_{17}H_{14}N_3O$ $[M+H]^+$ *Calc*. 292.1086, Found *m/z* 292.1089; Anal *Calc*. (%) for $C_{17}H_{13}N_3O$ C: 70.09 H: 4.50 N: 14.42; Found C: 69.92 H: 4.65 N: 14.34.

General procedure for the preparation of (E)-3-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]acrylamides ***4***

To the solution of acid derivatives (1 mmol), triethylamine (2 mmol) and ethyl chloroformate (1 mmol), were added, followed by stirring at 0 °C for 30 min. After addition of the appropriate amine derivative (1.2 mmol), the mixture was stirred for an additional 1 h at 0 °C. Then, the reaction mixture was warmed to room temperature, and kept stirring overnight. After the solvent was evaporated under reduced pressure, acetone was added, filtered, and evaporated. Residue was dissolved in DCM and the organic phase was washed with a 1 % $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated under vacuum. The residue was purified by flash column chromatography (Combiflash®Rf) using DCM-MeOH as eluents.

1-Phenyl-4-[(2E)-3-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]prop-2-enoyl] piperazine ***4a*** Elution with DCM-MeOH (0–5 %) yielded **4a** as a white solid. (Yield 48 %, m.p. 208–209 °C); IR (FTIR/FTNIR-ATR): 1645 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.71 (2H, d, $J = 6.4$ Hz), 8.25 (1H, s), 7.80 (1H, d, $J = 15.2$ Hz), 7.79 (2H, d, $J = 7.6$ Hz), 7.67 (2H, d, $J = 6.0$ Hz), 7.56–7.53 (2H, m), 7.39 (1H, t, $J = 7.6$ Hz), 7.32 (2H, t, $J = 7.2$ Hz), 6.96–6.90 (3H, m), 6.79 (1H, d, $J = 15.2$ Hz), 3.90 (2H, s), 3.75 (2H, s), 3.24–3.21 (4H, m); ^{13}C-NMR ($CDCl_3$) δ: 165.1, 160.5, 151.0, 149.7, 146.1, 141.1, 139.9, 139.4, 139.3, 132.7, 129.9, 129.5, 127.8, 126.9, 123.1, 120.9, 119.6, 118.0, 116.9, 50.7, 49.5, 45.9, 42.3; HRMS $C_{27}H_{26}N_5O$ $[M+H]^+$ *Calc.* 436.2137, Found *m/z* 436.2122.

1-(4-Chlorophenyl)-4-[(2E)-3-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]prop-2-enoyl]piperazine ***4b*** Elution with DCM-MeOH (0–2 %) gave **4b** as a white solid (Yield 76 %, m.p. 219–221 °C); IR (FTIR/FTNIR-ATR): 1642 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8,72 (2H, d, $J = 5.6$ Hz), 8.25 (1H, s), 7.82 (1H, d, $J = 15.2$ Hz), 7.78–7.76 (2H, m), 7.66 (2H, d, $J = 6$ Hz), 7.53–7.50 (2H, m), 7.39–7.36 (1H, m), 7.24 (2H, d, $J = 8.4$ Hz), 6.86 (2H, d, $J = 8.4$ Hz), 6.74 (1H, d, $J = 15.2$ Hz), 3.88 (2H, s), 3.74 (2H, s), 3.18 (4H, t, $J = 5.2$ Hz); ^{13}C-NMR ($CDCl_3$) δ: 165.1, 160.5, 149.7, 149.6, 141.1, 139.9, 132.8, 129.8, 129.3, 127.8, 126.9, 125.8, 123.1, 119.6, 119.1, 118.0, 50.7, 49.5, 45.9, 42.3; HRMS $C_{27}H_{25}ClN_5O$ $[M+H]^+$ *Calc.* 470.1748, Found *m/z* 470.1730.

1-(3-Chlorophenyl)-4-[(2E)-3-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]prop-2-enoyl]piperazine ***4c*** Elution with DCM-MeOH (0–2 %) yielded **4c** as a white solid (Yield 60 %, m.p. 231–232 °C); IR (FTIR/FTNIR-ATR): 1644 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.72 (2H, d, $J = 5.6$ Hz), 8.25 (1H, s), 7.82 (1H, d, $J = 15.2$ Hz), 7.79–7.77 (2H, m), 7.66 (2H, d, $J = 6$ Hz), 7.51 (2H, m), 7.38 (1H, m), 7.2 (1H, t, $J = 8$ Hz), 6.89–6.78 (3H, m), 6.74 (1H, d, $J = 15.2$ Hz), 3.88 (2H, s), 3.74 (2H, s), 3.23 (4H, m); ^{13}C-NMR ($CDCl_3$) δ: 165.1, 152.0, 149.6, 149.5, 141.2, 139.4, 135.2, 132.8, 130.4, 129.9, 127.9, 127.0, 123.2, 120.4, 119.6, 119.1, 117.9, 116.6, 116.5, 114.6, 49.5, 49.0, 45.6, 42.3; HRMS $C_{27}H_{25}N_5OCl$ $[M+H]^+$ *Calc.* 470.1748, Found *m/z* 470.1736.

1-(4-Methylphenyl)-4-[(2E)-3-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]prop-2-enoyl]piperazine ***4d*** Elution with DCM-MeOH (0–2 %) gave **4d** as a white solid (Yield 60 %, m.p. 241–242 °C); IR (FTIR/FTNIR-ATR): 1643 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d_6) δ: 9.28 (1H, s), 8.73 (2H, d, $J = 6.4$ Hz), 7.94 (2H, d, $J = 9.6$ Hz), 7.65 (2H, d, $J = 6.4$ Hz), 7.59 (2H, m), 7.55 (1H, d, $J = 15.2$ Hz), 7.42 (1H, m), 7.28 (1H, d, $J = 15.2$ Hz),

7.05 (2H, d, $J = 8.4$ Hz), 6.90 (2H, d, $J = 8.4$ Hz), 3.83 (2H, s), 3.72 (2H, s), 3.11 (4H, m), 2.21 (3H, s); ^{13}C-NMR ($CDCl_3$) δ: 165.2, 149.6, 149.5, 139.4, 135.2, 132.8, 130.4, 129.9, 127.9, 127.0, 125.5, 123.2, 119.6, 118.1, 117.3, 50.7, 49.5, 45.9, 42.3, 20.9; HRMS $C_{28}H_{28}N_5O$ $[M+H]^+$ *Calc.* 450.2294, Found *m/z* 450.2272.

1-(2,3-Dimethylphenyl)-4-[(2E)-3-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]prop-2-enoyl]piperazine ***4e*** Elution with DCM-MeOH (0–5 %) gave **4e** as a white solid (Yield 41 %, m.p. 207–209 °C); IR (FTIR/FTNIR-ATR): 1645 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.71 (2H, d, $J = 6.0$ Hz), 8.24 (1H, s), 7.82 (1H, d, $J = 15.2$ Hz), 7.79 (2H, d, $J = 7.6$ Hz), 7.68 (2H, d, $J = 6.4$ Hz), 7.52 (2H, m), 7.39 (1H, t, $J = 7.6$ Hz), 7.11 (1H, m), 6.95 (1H, d, $J = 7.2$ Hz), 6.89 (1H, d, $J = 8.4$ Hz), 6.81 (1H, d, $J = 15.2$ Hz), 3.74 (4H, s), 2.91 (4H, s), 2.29 (3H, s), 2.26 (3H, s); ^{13}C-NMR ($CDCl_3$) δ: 165.2, 150.9, 149.6, 141.1, 139.9, 138.4, 132.4, 127.8, 127.2, 126.1, 125.8, 123.2, 119.6, 119.5, 118.3, 117.0, 52.6, 52.2, 46.3, 42.5, 20.4, 14.1; HRMS $C_{29}H_{30}N_5O$ $[M+H]^+$ *Calc.* 464.2450, Found *m/z* 464.2443.

1-[(2E)-3-[1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]prop-2-enoyl]-4-[3-(trifluoromethyl)phenyl] piperazine ***4f*** Elution with DCM-MeOH (0–5 %) yielded **4f** as a white solid (Yield 40 %, m.p. 182–185 °C); IR (FTIR/FTNIR-ATR): 1647 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.71 (2H, d, $J = 6.0$ Hz), 8.25 (1H, s), 7.83 (1H, d, $J = 15.2$ Hz), 7.78 (2H, d, $J = 7.2$ Hz), 7.66 (2H, d, $J = 6.0$ Hz), 7.52 (2H, m), 7.40–7.36 (2H, m), 7.15 (2H, d, $J = 7.6$ Hz), 7.09 (1H, d, $J = 8.4$ Hz), 6.78 (1H, d, $J = 15.2$ Hz), 3.91 (2H, s), 3.76 (2H, s), 3.29–3.26 (4H, t, $J = 5.2$ Hz); ^{13}C-NMR ($CDCl_3$) δ: 165.1, 151.1, 149.2, 144.6, 141.5, 139.3, 132.9, 131.6, 131.5, 130.5, 129.9, 127.9, 127.0, 123.2, 119.6, 119.5, 119.2, 117.9, 117.0, 112.9, 49.9, 49.5, 45.9, 42.3; HRMS $C_{28}H_{25}F_3N_5O$ $[M+H]^+$ *Calc.* 504.1986, Found *m/z* 504.1971.

1-(2-Methoxyphenyl)-4-[(2E)-3-[1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl]prop-2-enoyl]piperazine ***4g*** Elution with DCM-MeOH (0–5 %) gave **4g** as a white solid (Yield 72 %, m.p. 152–153 °C); IR (FTIR/FTNIR-ATR): 1645 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.71 (2H, d, $J = 6.4$ Hz), 8.24 (1H, s), 7.79 (1H, d, $J = 15.2$ Hz), 7.78 (2H, d, $J = 7.6$ Hz), 7.66 (2H, d, $J = 6.0$ Hz), 7.52 (2H, m), 7.38 (1H, t, $J = 7.6$ Hz), 7.05 (1H, m), 6.94–6.89 (3H, m), 6.78 (1H, d, $J = 15.2$ Hz), 3.93 (2H, s), 3.90 (3H, s), 3.77 (2H, s), 3.10 (4H, m); ^{13}C-NMR ($CDCl_3$) δ: 165.1, 152.4, 149.6, 145.6, 141.2, 140.5, 139.4, 132.5, 129.9, 127.8, 126.9, 123.9, 123.1, 121.2, 119.6, 119.3, 118.7, 118.2, 111.4, 55.6, 52.2, 51.8, 46.2, 42.6; HRMS $C_{28}H_{28}N_5O_2$ $[M+H]^+$ *Calc.* 466.2243, Found *m/z* 466.2241.

1-(2-Ethoxyphenyl)-4-[(2E)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-enoyl]piperazine ***4h*** Elution with DCM-MeOH (0–5 %) yielded **4h** as a white solid (Yield 62 %, m.p. 133–135 °C); IR (FTIR/FTNIR-ATR): 1643 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.72 (2H, d, $J = 6.0$ Hz), 8.24 (1H, s), 7.81 (1H, d, $J = 15.2$ Hz), 7.77 (2H, d, $J = 7.2$ Hz), 7.66 (2H, d, $J = 6.0$ Hz), 7.53 (2H, m), 7.38 (1H, t, $J = 7.6$ Hz), 7.01–6.87 (4H, m), 6.82 (1H, d, $J = 15.2$ Hz), 4.12–4.07 (2H, m), 3.92 (2H, s), 3.78 (2H, s), 3.12 (4H, t, $J = 4.8$ Hz), 1.48 (3H, m); ^{13}C-NMR ($CDCl_3$) δ: 165.1, 150.2, 149.5, 149.1, 141.6, 140.4, 139.3, 132.3, 129.9, 129.0, 127.8, 126.9, 126.2, 123.8, 123.2, 121.1, 119.6, 119.5, 118.6, 118.4, 112.5, 63.8, 51.3, 50.5, 46.2, 42.6, 15.1; HRMS $C_{29}H_{30}N_5O_2$ $[M+H]^+$ *Calc.* 480.2400, Found *m/z* 480.2381.

1-[(2E)-3-(1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-enoyl]-4-(2,3,4-trimethoxybenzyl)piperazine ***4i*** Elution with DCM-MeOH (0–5 %) gave **4i** as a white solid (Yield 60 %, m.p. 148–150 °C); IR (FTIR/FTNIR-ATR): 1649 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.71 (2H, d, $J = 6.0$ Hz), 8.21 (1H, s), 7.78 (2H, d, $J = 7.6$ Hz), 7.76 (1H, d, $J = 15.2$ Hz), 7.66 (2H, d, $J = 6.0$ Hz), 7.52 (2H, m), 7.38 (1H, m), 6.99 (1H, d, $J = 8$ Hz), 6.74 (1H, d, $J = 15.2$ Hz), 6.66 (1H, d, $J = 8.4$ Hz), 3.89 (3H, s), 3.88 (3H, s), 3.86 (3H, s), 3.73 (2H, s), 3.57 (2H, s), 3.51 (2H, s), 2.50 (4H, m); ^{13}C-NMR ($CDCl_3$) δ: 165.1, 152.8, 150.5, 142.4, 140.3, 139.9, 139.4, 133.8, 129.9, 127.7, 126.8, 123.0, 119.6, 118.8, 117.9, 107.1, 61.4, 61.0, 56.6, 56.2, 53.1, 52.5, 45.6; HRMS $C_{31}H_{34}N_5O_4$ $[M+H]^+$ *Calc.* 540.2611, Found *m/z* 540.2608.

2-{4-[(2E)-3-(1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-enoyl]piperazine-1-yl}pyrimidine ***4j*** Elution with DCM-MeOH (0–2 %) yielded **4j** as a white solid (Yield 78 %, m.p. 246–248 °C); IR (FTIR/FTNIR-ATR): 1648 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d_6) δ: 9.25 (1H, s), 8.71 (2H, d, $J = 6.0$ Hz), 8.37 (2H, d, $J = 4.4$ Hz), 7.89 (2H, d, $J = 8.0$ Hz), 7.63 (2H, d, $J = 6.0$ Hz), 7.56 (2H, t, $J = 8.0$ Hz), 7.51 (1H, d, $J = 15.2$ Hz), 7.39 (1H, m), 7.27 (1H, d, $J = 15.2$ Hz), 6.65 (1H, m), 3.78–3.64 (8H, m); ^{13}C-NMR ($CDCl_3$) δ: 165.3, 161.8, 158.0, 149.9, 140.9, 139.4, 132.8, 129.9, 129.8, 127.9, 126.9, 123.1, 119.6, 117.9, 110.8, 45.8, 44.0, 43.8, 42.6; HRMS $C_{25}H_{24}N_7O$ $[M+H]^+$ *Calc.* 438.2029, Found *m/z* 438.2019.

1-[(2E)-3-(1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-enoyl]-4-pyridin-4-ylpiperazine ***4k*** Elution with DCM-MeOH (0–5 %) gave **4k** as a white solid (Yield 88 %, m.p. 228–230 °C); IR (FTIR/FTNIR-ATR): 1645 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d_6) δ: 9.28 (1H, s), 8.74 (2H, d, $J = 6.0$ Hz), 8.20 (2H, d, $J = 6.4$ Hz), 7.94 (2H, d, $J = 9.6$ Hz), 7.66 (2H, d, $J = 6.4$ Hz), 7.60 (2H, m), 7.55

(1H, d, $J = 15.2$ Hz), 7.43 (1H, t, $J = 6.8$ Hz), 7.27 (1H, d, $J = 15.2$ Hz), 6.88 (2H, d, $J = 6.8$ Hz), 3.83–3.71 (4H, m), 3.43 (4H, m); ^{13}C-NMR ($CDCl_3$) δ: 165.3, 154.8, 150.2, 149.7, 140.2, 139.9, 133.4, 129.9, 127.8, 127.0, 123.0, 119.6, 118.8, 117.1, 108.5, 46.0, 45.4, 42.1, 41.8; HRMS $C_{26}H_{25}N_6O$ $[M+H]^+$ *Calc.* 437.2090, Found *m/z* 437.2082.

1-(2-Furoyl)-4-[(2E)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-enoyl]piperazine ***4l*** Elution with DCM-MeOH (0–5 %) yielded **4 l** as a white solid (Yield 86 %, m.p. 183–185 °C); IR (FTIR/FTNIR-ATR): 1647 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d_6) δ: 9.26 (1H, s), 8.74 (2H, d, $J = 6.4$ Hz), 7.92 (2H, d, $J = 7.6$ Hz), 7.87 (1H, d, $J = 2.4$ Hz), 7.65 (2H, d, $J = 6.4$ Hz), 7.59 (2H, m), 7.54 (1H, d, $J = 15.2$ Hz), 7.42 (1H, t, $J = 7.2$ Hz), 7.25 (1H, d, $J = 15.2$ Hz), 7.06 (1H, d, $J = 3.6$ Hz), 6.66 (1H, t, $J = 5.2$ Hz), 3.78–3.67 (8H, m); ^{13}C-NMR ($CDCl_3$) δ: 177.2, 165.1, 159.8, 149.9, 147.6, 144.3, 140.7, 139.3, 133.3, 129.9, 127.9, 127.0, 123.1, 119.1, 119.0, 117.4, 111.8, 45.8, 44.0, 43.8, 42.6; HRMS $C_{26}H_{24}N_5O_3$ $[M+H]^+$ *Calc.* 454.1879, Found *m/z* 454.1871.

1-[(2E)-3-(1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-enoyl]-4-(tetrahydrofuran-2-yl-carbonyl) piperazine ***4m*** Elution with DCM-MeOH (0–5 %) gave **4 m** as a white solid (Yield 43 %, m.p. 177–178 °C); IR (FTIR/FTNIR-ATR): 1646 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.71 (2H, d, $J = 6.0$ Hz), 8.25 (1H, s), 7.81 (1H, d, $J = 15.2$ Hz), 7.78 (2H, d, $J = 7.6$ Hz), 7.65 (2H, d, $J = 5.6$ Hz), 7.52 (2H, m), 7.39 (1H, m), 6.75 (1H, d, $J = 15.2$ Hz), 4.61 (1H, s), 3.92–3.53 (10H, m), 2.34–1.90 (4H, m); ^{13}C-NMR ($CDCl_3$) δ: 177.2, 165.1, 149.9, 147.6, 140.7, 139.3, 133.3, 129.9, 127.9, 123.6, 119.6, 118.7, 69.8, 55.6, 45.8, 44.0, 43.8, 42.6, 26.0; HRMS $C_{26}H_{28}N_5O_3$ $[M+H]^+$ *Calc.* 458.2192, Found *m/z* 458.2177.

2-(2-{4-[(2E)-3-(1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)prop-2-enoyl]piperazine-1-yl}ethoxy)ethanol ***4n*** Elution with DCM-MeOH (0–5 %) yielded **4n** as a white solid (Yield 41 %, m.p. 113–115 °C); IR (FTIR/FTNIR-ATR): 1641 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.70 (2H, d, $J = 6.4$ Hz), 8.23 (1H, s), 7.78 (2H, d, $J = 7.2$ Hz), 7.77 (1H, d, $J = 15.6$ Hz), 7.66 (2H, d, $J = 5.6$ Hz), 7.52 (2H, m), 7.39 (1H, t, $J = 7.6$ Hz), 6.73 (1H, d, $J = 15.6$ Hz), 3.78 (1H, s), 3.73 (2H, m), 3.69 (4H, m), 3.64 (4H, m), 2.64 (2H, m), 2.58 (4H, t, $J = 5.2$ Hz); ^{13}C-NMR ($CDCl_3$) δ: 165.1, 151.2, 150.3, 149.9, 141.1, 139.4, 132.7, 129.9, 129.8, 126.9, 123.1, 123.0, 119.6, 118.9, 117.5, 72.69, 64.5, 61.9, 57.8, 56.8, 49.9; HRMS $C_{25}H_{30}N_5O_3$ $[M+H]^+$ *Calc.* 448.2349, Found *m/z* 448.2332.

(2E)-N-benzyl-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)acrylamide ***4o*** Elution with DCM-MeOH (0–5 %) gave **4o** as a white solid (Yield 48 %, m.p. 205–207 °C); IR (FTIR/FTNIR-ATR): 1651 cm^{-1} (C=O), 3247 cm^{-1} (N–H); ^{1}H-NMR (DMSO-d_6) δ: 9.06 (1H, s), 8.73 (2H, d, $J = 5.6$ Hz), 8.68 (1H, m), 7.97 (2H, d, $J = 10.0$ Hz), 7.67 (2H, d, $J = 6.0$ Hz), 7.57 (2H, m), 7.51 (1H, d, $J = 15.6$ Hz), 7.42 (1H, m), 7.35–7.24 (5H, m), 6.58 (1H, d, $J = 15.6$ Hz), 4.39 (2H, d, $J = 6.4$ Hz); ^{13}C-NMR ($CDCl_3$) δ: 165.7, 149.9, 139.3, 139.0, 137.9, 130.7, 129.8, 129.2, 128.9, 128.1, 127.8, 126.7, 123.1, 121.7, 119.5, 118.2, 114.1, 44.0; HRMS $C_{24}H_{21}N_4O$ $[M+H]^+$ *Calc.* 381.1715, Found *m/z* 381.1705.

(2E)-N-(4-Methoxybenzyl)-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)acrylamide ***4p*** Elution with DCM-MeOH (0–5 %) yielded **4p** as a white solid (Yield 45 %, m.p. 221–223 °C); IR (FTIR/FTNIR-ATR): 1650 cm^{-1} (C=O), 3282 cm^{-1} (N–H); ^{1}H-NMR (DMSO-d_6) δ: 9.04 (1H, s), 8.73 (2H, d, $J = 6.0$ Hz), 8.60 (1H, m), 7.95 (2H, d, $J = 8.8$ Hz), 7.66 (2H, d, $J = 6.0$ Hz), 7.56 (2H, m), 7.49 (1H, d, $J = 15.6$ Hz), 7.40 (1H, t, $J = 7.6$ Hz), 7.22 (2H, d, $J = 9.2$ Hz), 6.90 (2H, d, $J = 8.4$ Hz), 6.55 (1H, d, $J = 15.6$ Hz), 4.32 (2H, d, $J = 6.0$ Hz), 3.73 (3H, s); ^{13}C-NMR ($CDCl_3$) δ: 165.4, 159.2, 149.9, 149.6, 141.8, 139.3, 130.6, 130.3, 129.9, 129.6, 127.9, 127.1, 123.2, 122.0, 119.6, 118.6, 114.3, 55.6, 43.7; HRMS $C_{25}H_{23}N_4O_2$ $[M+H]^+$ *Calc.* 411.1821, Found *m/z* 411.1806.

(2E)-N-Cyclohexyl-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)acrylamide ***4r*** Elution with DCM-MeOH (0–5 %) gave **4r** as a white solid (Yield 60 %, m.p. 234–235 °C); IR (FTIR/FTNIR-ATR): 1649 cm^{-1} 3277 cm^{-1} (N–H); ^{1}H-NMR ($CDCl_3$) δ: 8.70 (2H, d, $J = 6.0$ Hz), 8.17 (1H, s), 7.75 (2H, d, $J = 8.0$ Hz), 7.70 (1H, d, $J = 15.6$ Hz), 7.65 (2H, d, $J = 6.0$ Hz), 7.52 (2H, m), 7.38 (1H, m), 6.23 (1H, d, $J = 15.2$ Hz), 4.45 (1H, d, $J = 8.0$ Hz), 3.92 (1H, m), 2.00–1.62 (6H, m), 1.45–1.12 (4H, m); ^{13}C-NMR ($CDCl_3$) δ: 166.5, 149.9, 143.9, 140.4, 139.9, 133.4, 129.9, 129.3,127.9, 126.3, 121.1, 52.1, 32.3, 26.2, 25.0; HRMS $C_{23}H_{25}N_4O$ $[M+H]^+$ *Calc.* 373.2028, Found *m/z* 373.2010.

(2E)-N-Cyclopropyl-3-(1-phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl)acrylamide ***4s*** Elution with DCM-MeOH (0–5 %) yielded **4s** as a white solid (Yield 61 %, m.p. 224–225 °C); IR (FTIR/FTNIR-ATR): 1664 cm^{-1} (C=O), 3238 cm^{-1} (N–H); ^{1}H-NMR ($CDCl_3$) δ: 8.69 (2H, d, $J = 6.4$ Hz), 8.16 (1H, s), 7.75 (2H, m), 7.68 (1H, d, $J = 13.6$ Hz), 7.63 (2H, d, $J = 6.0$ Hz), 7.52 (2H, t, $J = 8.0$ Hz), 7.38 (1H, m), 6.19 (1H, d, $J = 16.0$ Hz), 5.77 (1H, s), 2.87 (1H, m), 0.86–0.54 (4H, m); ^{13}C-NMR

($CDCl_3$) δ: 167.2, 149.9, 149.3, 139.3, 130.1, 129.9, 127.8, 127.1, 123.1, 121.2, 119.5, 118.6, 114.0, 23.1, 6.9; HRMS $C_{20}H_{19}N_4O$ $[M+H]^+$ *Calc* 331.1559, Found *m/z* 331.1545.

4-[(2E)-3-(1-Phenyl-3-(pyridin-4-yl)-1H-pyrazol-4-yl) prop-2-enoyl]morpholine ***4t*** Elution with DCM-MeOH (0–5 %) gave **4t** as a white solid (Yield 40 %, m.p. 236–238 °C); IR (FTIR/FTNIR-ATR): 1647 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.72 (2H, d, J = 6.0 Hz), 8.23 (1H, s), 7.81 (1H, d, J = 15.2 Hz), 7.78 (2H, d, J = 7.6 Hz), 7.66 (2H, d, J = 6.0 Hz), 7.52 (2H, m), 7.39 (1H, m), 6.72 (1H, d, J = 15.2 Hz), 3.73 (6H, s), 3.59 (2H, s); ^{13}C-NMR ($CDCl_3$) δ: 162.8, 149.9, 141.8, 139.9, 132.7, 129.9, 128.9, 127.9, 126.7, 123.2, 119.6, 118.9, 117.2, 114.0, 66.6, 46.2; HRMS $C_{21}H_{21}N_4O_2$ $[M+H]^+$ *Calc.* 361.1655, Found *m/z* 361.1644.

4-{4-[(1E)-3-Oxo-3-(piperidin-1-yl)prop-1-en-1-yl]-1-phenyl-1H-pyrazol-3-yl}pyridine ***4u*** Elution with DCM-MeOH (0–5 %) yielded **4u** as a white solid (Yield 44 %, m.p. 192–194 °C); IR (FTIR/FTNIR-ATR):1646 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.71 (2H, d, J = 6.4 Hz), 8.22 (1H, s), 7.78 (2H, d, J = 7.2 Hz), 7.66 (1H, d, J = 16.0 Hz), 7.65 (2H, d, J = 6.4 Hz), 7.52 (2H, m), 7.38–7.34 (1H, m), 6.78 (1H, d, J = 15.2 Hz), 3.66 (2H, s), 3.52 (2H, s), 1.69–1.61 (6H, m); ^{13}C-NMR ($CDCl_3$) δ: 165.0, 149.9, 149.7, 140.8, 139.4, 131.7, 129.8, 127.6, 126.8, 123.0, 119.5, 119.3, 118.8, 114.1, 47.2, 43.6, 27.0, 25.8, 24.8; HRMS $C_{22}H_{23}N_4O$ $[M+H]^+$ *Calc.* 359.1872, Found *m/z* 359.1871.

4-{4-[(1E)-3-(4-Benzylpiperidin-1-yl)-3-oxoprop-1-en-1-yl]-1-phenyl-1H-pyrazol-3-yl}pyridine ***4v*** Elution with DCM-MeOH (0–5 %) gave **4v** as a white solid (Yield 56 %, m.p. 162–163 °C); IR (FTIR/FTNIR-ATR): 1645 cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$) δ: 8.71 (2H, d, J = 6.4 Hz), 8.21 (1H, s), 7.84 (2H, d, J = 7.2 Hz), 7.75 (1H, d, J = 15.2 Hz), 7.66 (2H, d, J = 6.4 Hz), 7.52 (2H, t, J = 8.0 Hz), 7.38 (1H, m), 7.31 (1H, m), 7.21 (2H, t, J = 7.6 Hz), 7.15 (2H, d, J = 8.0 Hz), 6.76 (1H, d, J = 15.2 Hz), 4.72–3.96 (2H, d, J = 12.8 Hz), 3.06–2.99 (2H, m), 2.62–1.73 (6H, m), 1.26-1.22 (2H, m); ^{13}C-NMR ($CDCl_3$) δ: 165.0, 149.8, 141.0, 139.7, 137.8, 131.3, 129.8, 129.3, 128.5, 126.8, 126.3, 123.1, 119.6, 119.3, 118.8, 114.1, 46.3, 43.1, 42.9, 38.5; HRMS $C_{29}H_{29}N_4O$ $[M+H]^+$ *Calc.* 449.2341, Found *m/z* 449.2329.

Biological assays

Antiplatelet activity

Preparation of platelet rich plasma (PRP) and platelet poor plasma (PPP) Freshly drawn venous human citrated blood (sodium citrate 3.2 %, 1:9 v/v) from healthy subjects, who had not taken drugs with antiplatelet activity for 10 days, was centrifuged at 800 rpm for 10 min to obtain PRP. After separation, samples were centrifuged at 3,500 rpm for 10 min to obtain PPP. Platelet count in PRP was adjusted to 3.8×10^8 platelets/ml using PPP. Throughout the experiments, the volunteers were treated under the audit of Gazi University's Commission of Ethics according to the suggested international ethical guidelines (Permission No: 303).

Platelet aggregation studies Platelet aggregation was measured by using an aggregometer (APACT 4004, LABi-Tec, Ahrensburg, Germany) according to the turbidimetric method described by Born *et al.,* (1963). The test compound (or the standard inhibitor, aspirin) was dissolved in DMSO. PRP was incubated at 37 °C with constant stirring at 1,100 rpm and stimulated with arachidonic acid (AA, final concentration 700 µM) or collagen (final concentration 5 µM). 199 µl sample of PRP was placed in the cuvette of aggregometer and incubated for 5 min with 0.5 µl of test compound (or standard inhibitor, or DMSO) before addition of 10 µl inducer. The changes in optical density were monitored for 3 min. All experiments were performed in triplicate. Inhibition of platelet aggregation was expressed as percentage of inhibition using the following equation:

$$\%\text{Inhibition} = \left(1 - \frac{\text{Maximum aggregation of compound treated PRP}}{\text{Maximum aggregation of DMSO treated PRP}}\right) \times 100$$

Lipinski's rule of five and drug-likeness profile In order to explore the bioavailability of synthesized derivatives, theoretical calculations were carried out to predict some physicochemical properties of synthesized compounds. The bioavailability of the compounds was assessed using ADME (absorption, distribution, metabolism, and elimination) prediction methods. In particular, we calculated the compliance of compounds to the Lipinski's rule of five (Lipinski *et al.,* 2001). Lipinski's 'rule-of-five' and the later addition of other parameters such as polar surface area (PSA) (Ertl *et al.,* 2000) describes molecular properties important for drug pharmacokinetics in the human body. This approach has been widely used as a filter for substances that would likely be further developed in drug design programs. Poor absorption and permeation are more likely to occur when there are more than five hydrogen-bond donors (HBD), more than ten hydrogen-bond acceptors (HDA), the molecular mass (MM) is greater than 500, or the logP value (*c*log*P*) is greater than five. The topological polar surface area (TPSA) should be smaller than

90 Å (Brueggemeier *et al.,* 2005). Molecules violating more than one of these rules may have problems with bioavailability.

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