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Original article

Pyrazole derivatives as inhibitors of arachidonic acid-induced platelet aggregation

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ABSTRACT

Antiplatelet drugs are promising therapeutics to intervene with platelet aggregation in arterial thrombosis, most prominently in myocardial infarction and ischemic stroke. Here, we describe the synthesis and structure–activity relationships of potent inhibitors of platelet aggregation based on the 1,5-diarylpyrazol-3-carboxamide scaffold. Analogs from this series demonstrated potent anti-aggregatory activities against arachidonic acid-induced platelet aggregation, as measured by turbidimetric method of Born. 1,5-Diarylpyrazole-3-carboxamides obtained with small-basic amines (**7**, **8**, **50**, **51**, **61**, **62**) displayed the strongest activity with IC_{50} values in low nanomolar range (5.7–83 nM). On the basis of their high potency in cellular environment, these straightforward pyrazole derivatives may possess potential in the design of more potent compounds for intervention with cardiovascular diseases.

1. Introduction

Platelets are key mediators of haemostasis at sites of vascular injury, however they also mediate pathologic thrombosis. Thrombus formation through activation of platelets in response to rupture of an atherosclerotic plaque or endothelial cell erosion is the major cause of atherothrombotic disease [1–4]. Therefore, treating patients under thrombotic risk with rapidly acting antiplatelet drugs is of clinical importance for prevention of morbidity and mortality [2,5]. However, most of the currently available antiplatelet drugs, i.e. aspirin and clopidogrel, have limited efficacy and deleterious side effects [6,7]. Almost half of the world population is resistant to these drugs correlating to an increased risk of cardiovascular events such as recurrent myocardial infarction and stroke [8,9]. Although the use of dual antiplatelet therapy with aspirin and clopidogrel has demonstrated significant clinical benefit to reduce cardiac syndromes after coronary procedures and interventions, atherothrombotic disease still remains the leading cause of deaths in Western populations [10]. Therefore, current limitations of antiplatelet therapy continuously trigger the search for novel and potent antiplatelet agents to decrease the residual risk for ischemic events [3,5].

The pyridazine and pyrazole cores were often used as versatile scaffolds to develop new compounds with wide range of biological activities and shown that certain compounds having these scaffolds endowed with inhibitory activity on platelet aggregation [11–16]. In addition, synthesis of diverse pools of small drug-like molecules possessing vicinal diaryl template was the focus of many medicinal chemists and our research on vicinal diaryl systems produced a large number of compounds endowed with interesting pharmacological properties against cyclooxygenase and lipoxygenase pathways [17–21]. Especially, 1-(pyridazin-3-yl)pyrazole-3-carboxylic acid derivatives having a vicinal diaryl motif endowed with good inhibitory activity against cyclooxygenase enzymes and also for the inhibition of 5-lipoxygenase-mediated leukotriene formation [17,20]. Encouraged with the well-documented antiplatelet properties associated with these heterocyclic cores, we screened our own chemical library for their ability to inhibit arachidonic acid (AA)-induced platelet aggregation leading to the identification of ethyl 5-(4-chlorophenyl)-1-(6-chloropyridazin-3-yl)-1*H*-pyrazole-3-carboxylate (**I**, Fig. 1) as a developable scaffold (67% inhibition at 100 μM). In this communication, we present the synthesis and preliminary results of the structure–activity relationships (SAR) on newly synthesized pyrazole-3-carboxamide derivatives having the general structure of **I**.

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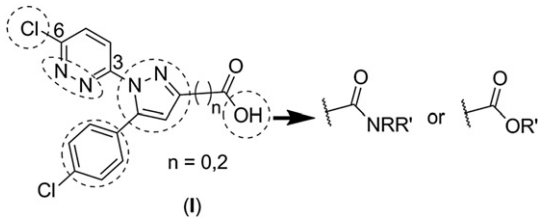


Fig. 1. Structure of the starting compound and features modified during SAR.

2. Results and discussion

2.1. Chemistry

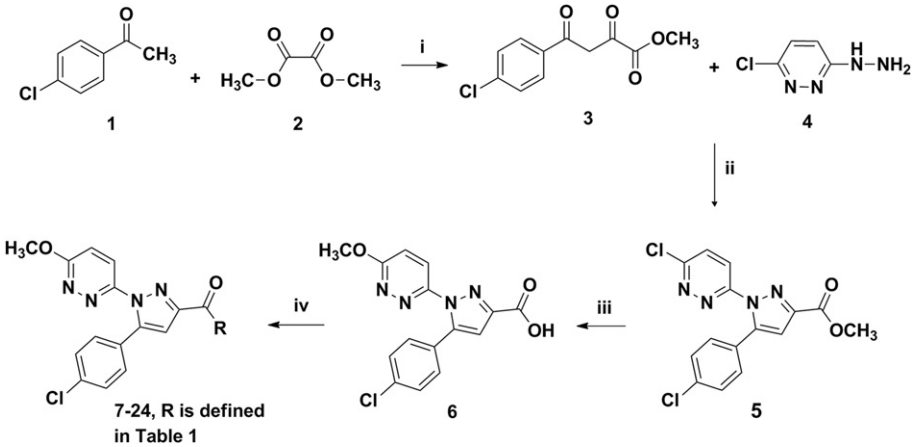
The pyrazole derivatives studied in this communication were synthesized as outlined in Schemes 1–6 and the isoxazole derivatives were prepared as shown in Scheme 7. All compounds were purified by automated flash chromatography and checked for purity with UPLC before being tested in biological assays (purity was >97%). The structures of these compounds were confirmed by high resolution mass spectrometry (HRMS), elemental analysis, IR, ^{13}C NMR and ^{1}H NMR spectral data.

As shown in Scheme 1, the synthesis of methyl 4-(4-chlorophenyl)-2,4-dioxobutanoate (**3**) was carried out by Claisen condensation of the commercially available acetophenone with dimethyl oxalate in the presence of a strong base [22]. The 1,5-diarylpyrazole derivative (**5**) was then conveniently synthesized by condensation of diketone (**3**) with 3-chloro-6-hydrazinylpyridazine (**4**) in methanol in the presence of 0.5 eq HCl. Nucleophilic substitution of 6-chloropyridazine (**5**) with sodium methoxide followed by successive ester hydrolysis yielded 6-methoxypyridazine analog in acid form (**6**). The structure of 1,5-diarylpyrazole scaffold of **6** was confirmed using X-ray diffraction as shown in Fig. 2.

The first series of amide derivatives (**7**–**15**) were obtained by coupling of **6** with various amines using ethyl chloroformate as the carboxyl group activator in the presence of triethylamine. The same acid derivative was also reacted with various phenols and *i*-pentanol using *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) as the carboxyl group activator to obtain the corresponding ester derivatives (**16**–**24**). Preparation of 6-methylsulfonylpyridazine derivative (**25**) was achieved by reaction of **5** with sodium methane thiolate to obtain the methylthio intermediate, which was subsequently used to produce methylsulfonyl (**25**) under controlled oxidation with *m*-chloroperbenzoic acid [20]. Compound **5** was also converted to the corresponding pyridazinone (**28**) (a predominant form of hydroxypyridazines) derivative, by refluxing in glacial acetic acid in the presence of sodium acetate [20] (Scheme 2).

Carboxamide derivatives lacking the pyridazine ring at 1-position of pyrazole were prepared as outlined in Scheme 3. Synthesis of 3-phenylpyrazole-5-carboxylate (**31**) was accomplished by reaction of **3** with hydrazine hydrate in acetic acid [23]. Compound **31** was then methylated by methyl iodide to obtain *N*-methylpyrazole derivative (**32**) in analogy to the published procedures [24–26]. Since the reaction of unsymmetrical 1,3-diketones with hydrazine may lead to a tautomeric pyrazole (1*H* or 2*H*) derivative, the methylation of **31** may produce 1- or 2-methylpyrazole regioisomers. However, the structural verification of 1-methylpyrazole regioisomer was fully elucidated using HMBC spectra of **32** in analogy to procedures described before. The HMBC spectra of **32** indicated a remarkable $^{3}J_{CH}$ correlation of three protons at δ 3.8 ppm (N1-CH_3) and δ 7.78 ppm (*o*-ph-H) with quaternary sp^2 carbon signals at δ 138.3 ppm (C5) and 148.2 ppm (C3), respectively (Figure S1, Supplementary data). Both compounds (**31** and **32**) were further hydrolyzed in THF–water mixture in the presence of LiOH to carboxylic acid derivatives, which were reacted with selected amines to obtain target carboxamides (**33**–**36**). The synthesis of analogs which lack the 5-phenyl ring of pyrazole was outlined in Scheme 4. As shown, methyl 2,4-dioxopentanoate (**38**), which was prepared from acetone and dimethyl oxalate, was reacted with 6-chloro-3-hydrazinopyridazine (**4**) in the presence of catalytic amount of HCl to obtain the carboxylate **39**. As one may expect, the reaction of unsymmetrical 1,3-diketones with *N*-aryl-substituted hydrazines can give two regioisomeric products which cannot tautomerize. In the case of the reaction of **38** with 6-chloro-3-hydrazinopyridazine (**4**), the structure of the resulting derivative (**39**) was verified on the basis of observed $^{1}H-^{13}C$ correlations in HSQS and HMBC spectra (Figure S2, Supplementary data). As shown, methyl protons at δ 2.8 ppm demonstrated $^{2}J_{CH}$ correlation with the sp^2 carbon (C5) at δ 143.9 ppm indicating that the ring closure occurred to put the methyl at 5-position of pyrazole. Subsequent nucleophilic substitution of **39** with sodium methoxide and ester hydrolysis produced the intermediate carboxylic acid derivative **40**, which was used to prepare the target amide derivatives (**41**–**42**).

Chain elongated amide derivatives (**45**–**46**) were synthesized as demonstrated in Scheme 5. The starting intermediate 1,5-diarylpyrazole-3-propanoic acid (**44**) was prepared by condensation of 4,6-dioxo-6-phenylhexanoic acid (**43**) with HCl salt of



Scheme 1. Synthesis of amide and ester derivatives of 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1*H*-pyrazole-3-carboxylic acid. Reagents and conditions: *i*. $NaOCH_3$, MeOH, rt; *ii*. MeOH, HCl, rt; *iii*. a) $NaOCH_3$, MeOH, Δ, b) H_2O, Δ; *iv*. Amine derivatives, ethyl chloroformate, Et_3N, CH_2Cl_2, rt, or Phenol derivatives, EDC, DMAP, CH_2Cl_2, rt.

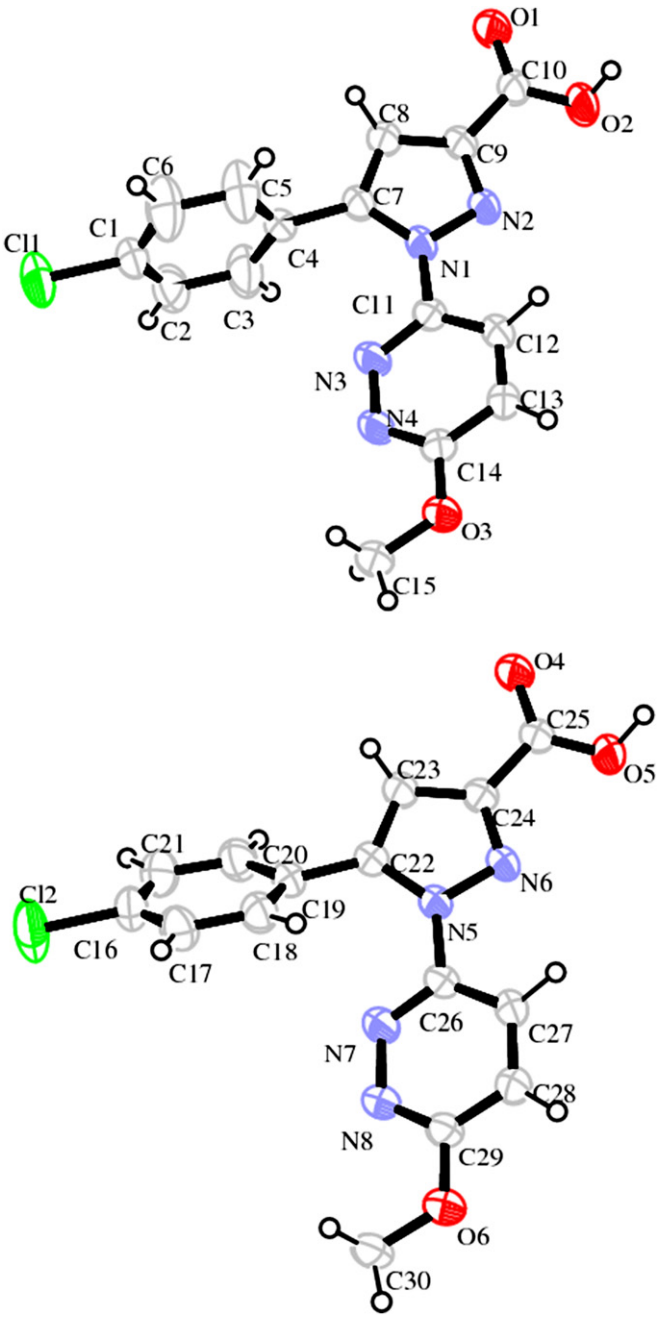
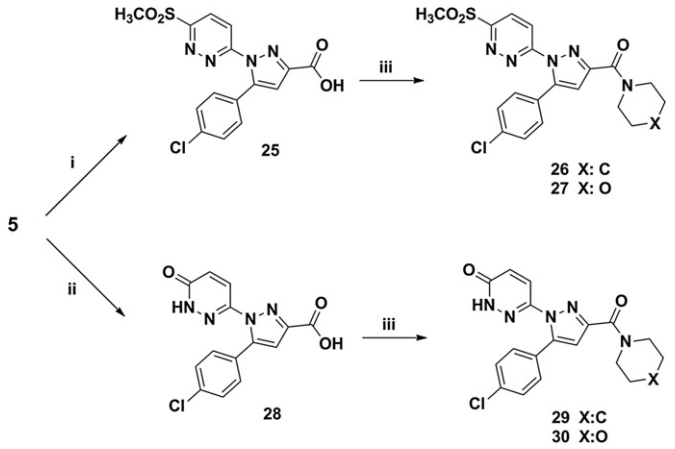


Fig. 2. X-ray crystal structures of **6**. Displacement ellipsoids are drawn at 50% probability level.



Scheme 2. Synthesis of methylsulfonylpyridazine and pyridazinone derivatives. Reagents and conditions: *i*. a) $NaSCH_3$, THF, Δ, b) NaOH, H_2O, Δ, c) MCPA; CH_2Cl_2, MeOH, 0 °C → rt, *ii*. a) CH_3COOH, CH_3COONa, Δ; b) LiOH, THF, H_2O, 70 °C; *iii*. Piperidine or morpholine, EDC, DMAP, CH_2Cl_2, rt.

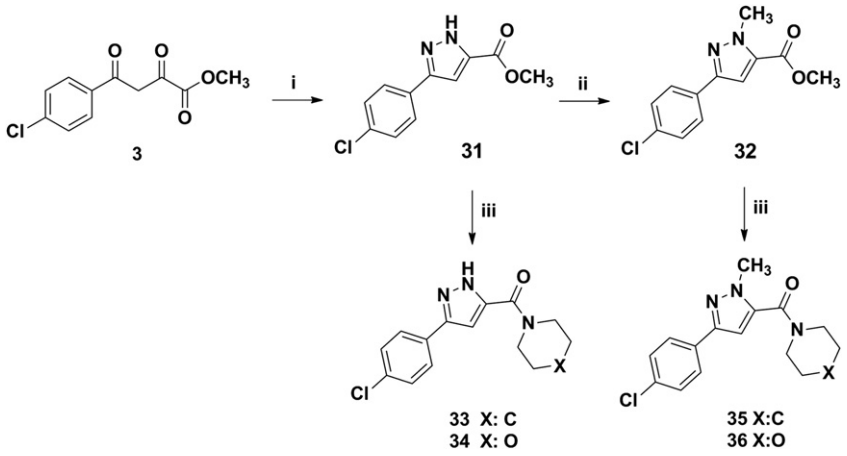
3-chloro-6-hydrazinopyridazine (**4**) in alcohol/triethylamine as previously reported by our group [17,20]. Amidation of compound **44** with selected amines generated the corresponding amide derivatives (**45**–**46**). Isosteric replacement of the pyridazine ring with phenyl ring was realized by condensation of the HCl salt of 4-methoxyphenylhydrazine with **3**, ester hydrolysis and subsequent amidation with selected amine derivatives (**50**–**51**, Scheme 6).

Isoxazole derivatives were prepared as shown in Scheme 7. The keto-enol ester **54**, which was obtained by reaction of 4-methoxyacetophenone with diethyloxalate, was reacted with hydroxylamine hydrochloride to produce the isoxazole **55**. Bromination at the 4-position of the isoxazole (**56**) was achieved with *N*-bromosuccinimide with the addition of a catalytic amount of ceric ammonium nitrate. Compound **56** underwent a palladium-catalyzed Suzuki cross-coupling reaction with 4-chlorophenylboronic acid to generate **57**, which was hydrolyzed (**58**) and reacted with selected amines to furnish the desired amide derivatives **59** and **60**. Lastly, the pyrrolidine amides (**61**–**62**) of methoxypyridazine (**6**) and methoxyphenyl (**49**) carboxylic acid intermediates were also prepared. All starting carboxylic acid intermediates **25**, **28**, **31**, **32**, **40**, **44**, **49** and **58** were reacted with piperidine, morpholine and pyrrolidine to produce the corresponding amides (**26**–**27**, **29**–**30**, **33**–**36**, **41**–**42**, **45**–**46**, **50**–**51**, **59**–**60**, **61**–**62** were prepared from pyrrolidine) under conditions using EDC as the carboxylate activator.

2.2. Structure–activity relationships

In order to determine the most eminent features of the SAR in this series, we initially modified the carboxyl side chain of the starting compound (**6**) by amidation/esterification with various amines/phenols of different size, basicity and hydrophobicity with the aim to improve potency and to trace a tentative SAR profile for potential anti-platelet agents. Next, we introduced polar substituents at 6-position of pyridazine, replaced pyridazine with phenyl and extended the carbon chain in the carboxyl arm of central pyrazole. Furthermore, the pyrazole derivatives lacking either the 1-pyridazine or 5-phenyl groups were synthesized. The final area of our interest in SAR studies was the pyrazole to isoxazole scaffold change. By these derivatives, our aim was to explore how antiplatelet activity would be affected by the presence of a side chain of varying magnitude and basicity at 3-position of pyrazole and to understand the potential influence of vicinal diaryl pattern about the central heterocycle as well as the necessity of central pyrazole on their biological profiles.

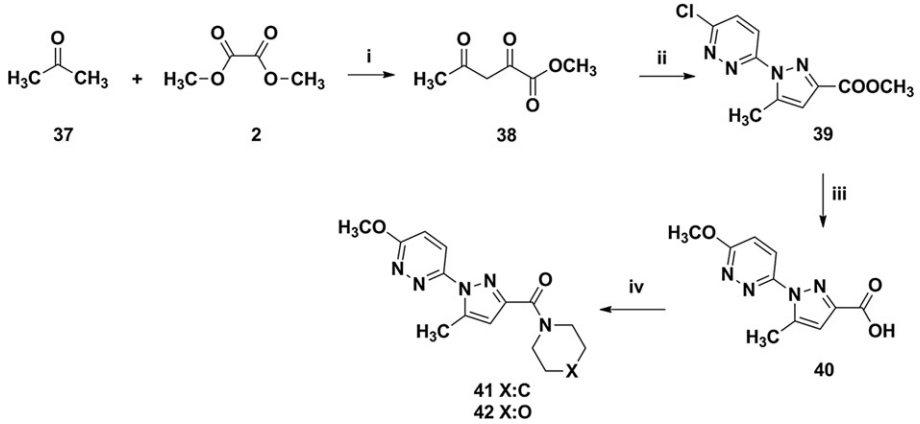
In an initial screening round, compounds **7**–**24** were tested at 1 and 10 μM for their ability to inhibit platelet aggregation by the turbidimetric method of Born [27] employing AA, ADP and collagen as platelet aggregation inducers (Table 1). Since we intended to identify potent platelet aggregation inhibitors with improved efficiency versus the starting compound (**I**), higher concentrations than 10 μM were not screened as they appeared not pharmacologically relevant and encouraging. Although limited number of compounds precludes a detailed SAR, a few general features can be deduced to be taken into account for further work. Analysis of the aggregation data obtained indicated that amide derivatives as



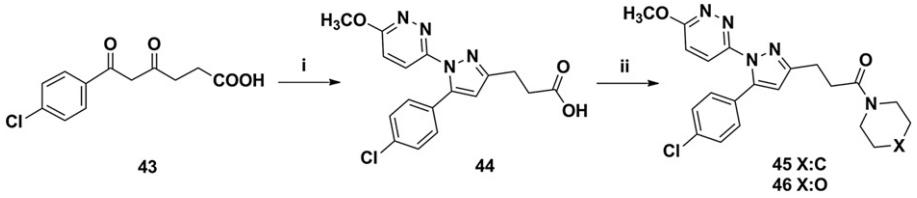
Scheme 3. Synthesis of derivatives lacking pyridazine nucleus. Reagents and conditions: *i.* $NH_2NH_2{\cdot}H_2O$, CH_3COOH, Δ; *ii.* CH_3I, K_2CO_3, DMF, 50 °C; *iii.* a) LiOH, H_2O, THF, 70 °C, b) $SOCl_2$, CH_2Cl_2, Δ, c) Piperidine or morpholine, DIEA, CH_2Cl_2, rt.

compared to ester derivatives caused greater inhibition of AA-induced platelet aggregation at 1 μM (Table 1). Furthermore, in this series, introduction of smaller carboxamide side chains led to an increased inhibitory activity against AA-induced aggregation of platelets. Especially, a substantial increase in potency was associated with amidation with small basic amines, i.e. piperidine and morpholine (compounds **7** and **8**). In addition, secondary amide derivatives produced by pyridin-4-amine (**9**) and anilines (**10**–**11**) were also potent inhibitors of AA-induced platelet aggregation. However, larger phenylpiperazine amides (**12**–**14**) led to a diminished activity at 1 μM while still maintaining the antiplatelet properties at 10 μM. Surprisingly, in the case of 2-pyrimidinylpiperazine derivative (**15**), a loss of potency was observed at both concentrations. Lipophilic ester derivatives (**16**–**24**) were much less active at 1 μM as compared to amide derivatives. When the antiplatelet activity of compounds was tested against collagen- and ADP-induced platelet aggregation, none of the compounds showed inhibitory activity against ADP-induced aggregation, while some (**7**–**11**, **16**, **24**) were able to maintain their antiplatelet properties against collagen-induced aggregation at 10 μM. The most active derivatives showing more than 50% inhibition at 10 μM against collagen-induced aggregation (**7**–**11**, **16** and **24**) were rescreened at 1 μM to conclude that compounds **7**–**9** with smaller basic amide side chains were also strong inhibitors of collagen-induced platelet aggregation although with a reduced potency as compared to the inhibitory activities of same compounds against AA-induced platelet aggregation (Table 1).

To determine the exact potency of those compounds that showed significant inhibition of AA- and collagen-induced platelet aggregation at 1 μM, selected (potent) compounds (**7**–**11**, **13**–**14**, **24** for AA-induced aggregation; **9**–**11** for collagen-induced aggregation) were further investigated in concentration-response studies. As shown in Table 2, the IC_{50} values of selected compounds against AA-induced platelet aggregation were in the range of 0.041–5.49 μM. Compounds **7**–**9** also significantly inhibited the collagen-induced platelet aggregation with IC_{50} values of 1.98, 2.5 and 2.8 μM, respectively. In agreement with previous reports [28,29], the IC_{50} values for the reference drugs aspirin and indomethacine were 7.76 and 1.62 μM for AA-induced platelet aggregation, respectively, under our assay conditions. Among all compounds studied for inhibition of AA-induced platelet aggregation, piperidine (**7**), morpholine (**8**), *N*-pyridin-4-yl (**9**), *N*-(4-methoxyphenyl)- (**10**), and *N*-(4-chlorophenyl)- (**11**) carboxamides were identified as potent antiplatelet agents, with **7** and **8** being the most potent ones with an inhibitory activity about forty and twenty times than that of indomethacin, respectively (Table 2). The above results indicated that amide bond formation with small-sized and more basic amines played a key role in enhancing antiplatelet activity (comparing **7**–**9** with **10**–**11**). However, amidation with less basic and larger phenylpiperazine derivatives (**13**–**14**) greatly reduced the potency. It was interesting to



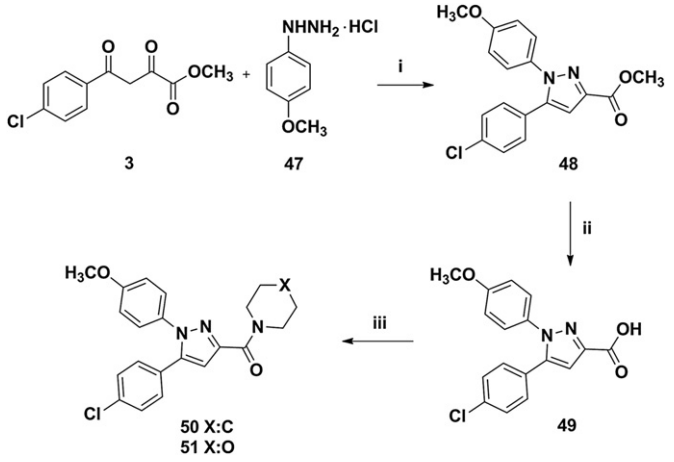
Scheme 4. Synthesis of derivatives lacking phenyl nucleus. Reagents and conditions: *i.* $NaOCH_3$, MeOH, rt; *ii.* compound **4**, HCl, MeOH, rt; *iii.* a) $NaOCH_3$, MeOH, Δ, b) H_2O, Δ; *iv.* Piperidine or morpholine, EDC, DMAP, CH_2Cl_2, rt.



Scheme 5. Synthesis of carboxyl side-chain elongated derivatives. Reagents and conditions: *i.* a) HCl salt of compound **4**, Et_3N, MeOH, Δ, b) CH_3ONa, MeOH, Δ; *ii.* Piperidine or morpholine, EDC, DMAP, CH_2Cl_2, rt.

see that esterification with *i*-pentanol (**24**) also resulted in potent antiplatelet activity as compared to positive control.

On the basis of the results from the initial screening, these first series of compounds were determined to be more potent inhibitors of AA-induced platelet aggregation, and the carboxamide formation with smaller basic amines remarkably influenced the activity and selectivity of the pyrazole molecules toward this pathway. It is well known that the carbonyl oxygen of carboxamides is the center of basicity in the processes of protonation and H-bond formation [30]. Therefore, it might be speculated that formation of carboxamides with basic amines such as piperidine and morpholine results in a substantial increase in the basicity of this center, most probably correlating with the potency increase in this type of pyrazole compounds. For this reason, these carboxamide moieties in molecules were not altered for further SAR approaches (Schemes 2–7) while concentrating on modifications of the 1,5-diarylpyrazole skeleton. Our first attempt was to use the most active derivatives (**7** and **8**) to pursue SAR on the modification of 6-methoxy of pyridazine ring (Scheme 2). On this basis, moving from methoxy to methylsulfonyl (**26**–**27**) or carbonyl functionality (**29**–**30**) led to a considerable loss of activity indicating that polar substituents at this position are not well tolerated (Table 2). We next examined the necessity of the vicinal diaryl pattern of the most active derivatives (**7**–**8**) by successive removal of either aryl groups. Removal of the pyridazine nucleus strongly disfavored the antiplatelet activity of the resulting carboxamide derivatives (**33**–**36**; $IC_{50} > 20$ μM). Likewise, replacement of 5-phenyl with methyl (**41**–**42**) also caused a dramatic loss of antiplatelet activity ($IC_{50} > 20$ μM). We also investigated the effects of introducing a longer amide chain at 3-position of pyrazole. When the direct connection of amide bond to the central pyrazole was separated by introduction of a carbon spacer (**45**–**46**), compounds were still active, albeit the potency was decreased ($IC_{50} = 0.92$ and 1.08 μM). Taken together, vicinally diaryl substituted pyrazole skeleton with a carboxamide connected directly to the pyrazole clearly govern the potent inhibition of AA-induced platelet aggregation. Since amidation of the carboxylic acid side chain with piperidine (**7**) and morpholine (**8**) in methoxypyridazine series contributed greatly to the efficiency, we next evaluated the methoxyphenyl analogs (**50** and **51**) of respective amide derivatives, as more lipophilic counterparts. Apparently, introducing more lipophilicity to molecules even increased the potency leading to **50** ($IC_{50} = 0.0079$ μM) and **51** ($IC_{50} = 0.0073$ μM) with respect to corresponding methoxypyridazine analogs (**7**, $IC_{50} = 0.041$ μM and **8**, $IC_{50} = 0.083$ μM) (Table 2). In this regard, we also prepared additional pyrrolidine carboxamides (**61** and **62**) of methoxypyridazine (**6**) and methoxyphenyl (**49**) intermediates, obtaining a great contribution to antiplatelet activity with IC_{50} values of 0.015 μM and 0.0057 μM, respectively (Fig. 3, Table 2). Meantime, we also investigated the SAR for the five-membered heteroaryl ring segment in the current methoxyphenyl derivatives. As a result, the pyrazole to isoxazole switch caused about 600 (**59**, $IC_{50} = 5.03$ μM) and 2500 (**60**, $IC_{50} = 18.4$ μM) fold loss of potency irrespective of the carboxamide side chain (Table 2), indicating that pyrazole core was the structural basis for peak activities in low nanomolar ranges against AA-induced platelet aggregation.



Scheme 6. Replacement of pyridazine residue by a phenyl group. Reagents and conditions: *i.* a) Et_3N, MeOH, Δ; *ii.* a) LiOH, THF, H_2O, Δ; *iii.* Piperidine or morpholine, EDC, DMAP, CH_2Cl_2, rt.

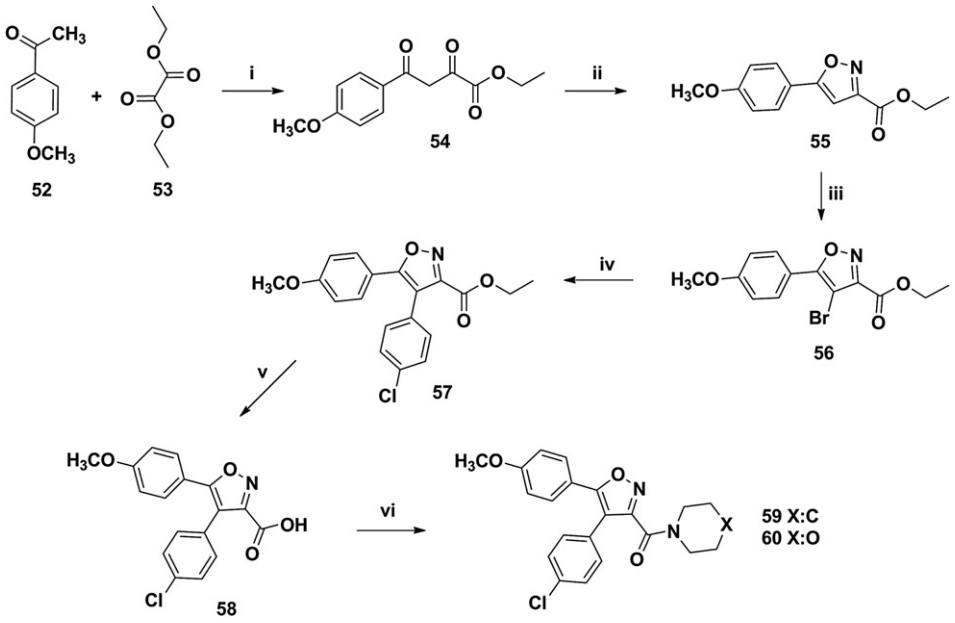
3. Conclusions

We have designed, synthesized and performed biological evaluation of a small set of potential antiplatelet pyrazole derivatives by modifying the scaffold of starting compound (**I**) from our own chemical library. Our efforts for increasing the inhibitory efficiency of (**I**) against platelet aggregation helped for comprehension of the key features of these pyrazole-based compounds to direct the synthesis toward the most potent inhibitors against AA-induced platelet aggregation. In particular, the different carboxamide side-chains at 3-position of the pyrazole ring caused a great disparity in activity among this class of compounds, where only **7**–**9** with small-sized carboxamide side arm exhibited significant inhibitory activity on platelet aggregation induced by both AA and collagen, specifically favoring the potent inhibition of AA-induced platelet aggregation. By means of our SAR efforts, a satisfactory explanation of the necessary scaffold for these pyrazole-based antiplatelet compounds was provided. As a final outcome, we present compounds **61** and **62** (Fig. 3) as novel potent anti-platelet agents with IC_{50} values in the range of two or one-digit nanomolar range (IC_{50} values of 15 and 5.7 nM, respectively). Further studies will be dedicated to evaluate the effect of pyridazine and phenyl substitutions and replacement of pyridazine nucleus with different heterocycles with a detailed analysis of their molecular pharmacology and selectivity toward other platelet-aggregation pathways.

4. Experimental section

4.1. Chemistry

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Varian Mercury 400 MHz High Performance Digital FT-NMR



Scheme 7. Synthesis of isoxazole derivatives. Reagents and conditions: *i.* $NaOC_2H_5$ EtOH, rt; *ii.* $NH_2OH{\cdot}HCl$, EtOH, Δ; *iii.* NBS, CAN, AcCN, Δ, *iv.* $Pd(PPh)_3Cl_2$, *p*-chlorophenyboronic acid, 80 °C; *v.* LiOH, H_2O, THF, Δ; *vi.* EDC, DMAP, CH_2Cl_2, rt.

spectrometer using tetramethylsilane as the internal standard at the NMR facility of Faculty of Pharmacy, Ankara University. All chemical shifts were recorded as δ (ppm). Because of the tautomeric effect of the pyrazole ring in compounds **33**–**34**, before obtaining the ^{13}C NMR spectra, this compounds were dissolved in DMSO-d_6, followed by a tiny amount of dry NaH, and 2–3 drops of D_2O were added to the NMR tube and stirred well in order to prevent the tautomeric effects to obtain a clear ^{13}C NMR spectra. The structure verification of the compounds **35** and **39** was done by means of HSQC and HMBC spectra (Varian Mercury 400 FT-NMR). 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). X-ray diffraction data were recorded on an STOE IPDS II single crystal diffractometer (STOE & Cie GmbH, Darmstadt, Germany). Melting points were determined with an SMP-II Digital Melting Point Apparatus and are uncorrected (Schorpp Geaetetechnik, Germany). IR spectra were obtained using a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer equipped with a Universal ATR Sampling Accessory and only carbonyl stretching frequencies were given. Flash chromatography was performed with a Combiflash®Rf automated flash chromatography system with RediSep columns (Teledyne-Isco, Lincoln, NE, USA) using hexane–ethyl acetate or dichloromethane-methanol solvent gradients. The purity of the final compounds was determined to be >97% by UPLC with UV detector. Compounds **3**, **4**, **31**, **32**, **38**, **43**, **44**, **54**, **55** and **56** were previously reported and synthesized in analogy to the described procedures by using conventional methods [17,20,22–26,31]. All biochemicals used in this study were purchased from Sigma–Aldrich Chemical Company (St Louis, MO, USA).

Table 1
Structure and activity of compounds **7**–**24**: Percent reduction of platelet aggregation induced by agonists AA (700 μM), collagen (2 μg/ml) and ADP (10 μM).

Cmpd no	R	AA		Collagen	ADP
		1 μM	10 μM	10 μM	10 μM
7	Piperidine	92.89	94.77	71.13 (76.78)[a]	NI[b]
8	Morpholine	92.74	94.81	70.87 (74.21)[a]	11.34
9	4-Aminopiperidine	84.87	80.53	73.56 (77.59)[a]	8.14
10	4-Methoxyaniline	94.23	79.93	72.14 (43.56)[a]	3.92
11	4-Chloroaniline	80.93	74.31	70.48 (25.71)[a]	7.93
12	4-F-phenylpiperazine	NI	79.74	32.25	NI
13	4-CH_3-phenylpiperazine	3.96	97.59	20.12	NI
14	4-CF_3-piridin-2-ylpiperazine	10.86	65.46	16.02	6.8
15	2-Pyrimidinylpiperazine	NI	NI	22.86	2.7
16	4-Heptyloxyphenol	3.87	93.03	61.89 (32.24)[a]	NI
17	4-Methoxyphenol	6.05	84.25	24.16	NI
18	4-Methylphenol	23.55	95.00	48.51	NI
19	4-Cyclopentylphenol	NI	86.70	19.87	NI
20	4-(Imidazol-1-yl)phenol	5.25	90.05	2.11	NI
21	3-*i*-Propylphenol	8.97	84.00	42.27	NI
22	2-Cl-3-pyridinol	NI	86.52	8.49	NI
23	2-Naphthol	2.36	86.31	22.44	3.34
24	*i*-Pentanol	19.00	91.16	66.7 (34.16)[a]	6.77

[a] Percent inhibition at 1 μM.
[b] No detectable inhibition.

4.1.1. *Methyl 5-(4-chlorophenyl)-1-(6-chloropyridazine-3-yl)-1H-pyrazole-3-carboxylate (**5**)*

To a mixture of methyl 4-(4-chlorophenyl)-2,4-dioxobutanoate (**3**) (0.005 mol) and 6-chloro-3-hydrazino-pyridazine (**4**) (0.005 mol) in 25 ml methanol, conc. HCl (0.0025 mol) was added. After

Table 2
IC_{50} values for antiplatelet activity of selected compounds against AA-induced platelet aggregation.

Cmpd no	IC_{50} (μM)	Cmpd no	IC_{50} (μM)
7	0.041	**35**	>10
8	0.083	**36**	>10
9	0.11	**41**	>10
10	0.40	**42**	>10
11	0.61	**45**	0.92
13	4.67	**46**	1.08
14	5.49	**50**	0.0079
24	1.33	**51**	0.0073
26	19.6	**59**	5.03
27	>20	**60**	18.4
29	2.09	**61**	0.015
30	12.6	**62**	0.0057
33	>10	**Aspirin**	7.76
34	>10	**Indomethacin**	1.62

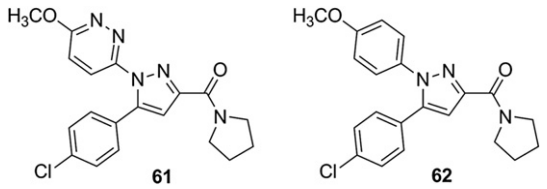


Fig. 3. Structures of the most active compounds **61** and **62** against AA-induced platelet aggregation.

stirring at rt for 5 h, the obtained precipitate was filtrated, washed with methanol, and dried. Yield 75%; Mp 162–163 °C; IR (ATR): 3129, 3084, 2977, 1726, 1094 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.87 (3H, s), 7.25 (1H, s), 7.37 (2H, d, $J = 8.4$ Hz), 7.43 (2H, d, $J = 8.4$), 8.18 (1H, d, $J = 8.8$ Hz), 8.23 (1H, d, $J = 9.6$ Hz); ^{13}C NMR (DMSO-d_6): δ 52.86, 112.11, 127.68, 128.67, 129.22, 131.19, 132.58, 134.50, 145.21, 145.88, 155.72, 156.56, 162.12; HRMS (m/z): $[M + H]^+$ calcd for $C_{15}H_{11}Cl_2N_4O_2$: 349.0259; found 349.0257.

*4.1.2. 5-(4-Chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylic acid (**6**)*

The solution of compound **5** (0.015 mol) and sodium methoxide (0.045 mol) in 20 ml methanol was heated at reflux for 2.5 h. After addition of 40 ml water, the reaction mixture was heated at reflux for another hour, diluted with water, and acidified. The precipitate formed was washed with water and recrystallized from ethanol–water mixture. Yield 82%; Mp 225–226 °C; IR (ATR): 3381, 2976, 2600, 1694, 1089 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.04 (3H, s), 7.16 (1H, s), 7.34 (2H, d, $J = 8.8$ Hz), 7.45 (2H, d, $J = 8.8$), 7.52 (1H, d, $J = 9.2$ Hz), 8.35 (1H, d, $J = 9.6$ Hz), 13.23 (1H, br s); ^{13}C NMR (DMSO-d_6): δ 55.74, 111.31, 120.92, 128.44, 128.94, 129.25, 131.03, 134.19, 144.65, 146.30, 152.79, 163.33, 165.36; HRMS (m/z): $[M + H]^+$ calcd for $C_{15}H_{12}ClN_4O_3$: 331.0598; found 331.0602; Anal. Calcd for $C_{15}H_{11}ClN_4O_3$: C, 54.47; H; 3.35; N, 16.94; found C, 54.04; H, 3.31; N, 16.85.

*4.1.3. Synthesis of amide derivatives **7**–**15** (procedure 1)*

Compound **2** (1.0 mmol) in 10 ml dichloromethane at 0 °C (ice-bath) was treated with triethylamine (2.2 mmol) and ethyl chloroformate (1.1 mmol). After stirring the reaction mixture at 0 °C under an argon atmosphere for 30 min, the appropriate amine derivative (1.1 mmol) was added, and the final mixture was stirred at room temperature for overnight. After diluting with 15 ml dichloromethane, the mixture was extracted with 1 N HCl (3 × 30 ml), 5% $NaHCO_3$ (3 × 30 ml) and water (2 × 30 ml). The organic phase was dried over sodium sulfate, and evaporated to dryness. The crude product was purified by flash chromatography or recrystallized from the appropriate solvent.

*4.1.4. Synthesis of ester derivatives **16**–**24** (procedure 2)*

To a mixture of compound **2** (0.001 mol), a phenol/alcohol derivative (0.0011 mol) and DMAP (0.0002 mol) in 30 ml dichloromethane, *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (1.1 mmol) was added, and stirred at room temperature overnight (dicyclohexylcarbodiimide (DCC) was used instead of EDC for compounds **13** and **15**). The reaction mixture was diluted with dichloromethane and washed with 0.5 N HCl (2 × 30 ml), 1% NaOH solution (2 × 30 ml) and water (2 × 30 ml). After drying over sodium sulfate, solvent was evaporated off under reduced pressure and the crude product was purified by flash chromatography.

*4.1.5. 1-{[1-(6-Methoxypyridazine-3-yl)-5-(4-chlorophenyl)-1H-pyrazole-3-yl]carbonyl}piperidine (**7**)*

Prepared from **2** and piperidine using the procedure 1. Flash chromatography hexane:EtOAc; yield 77%; Mp 154–155 °C. IR (ATR): 2976, 1614, 1092 cm^{-1}. ^1H NMR (DMSO-d_6): δ 1.51–1.61 (6H, m), 3.59 (2H, s), 3.75 (2H, s), 3.99 (3H, s), 6.95 (1H, s), 7.31 (2H, m), 7.42 (2H, m), 7.47 (1H, d, $J = 8.8$ Hz), 7.98 (1H, d, $J = 9.6$ Hz). ^{13}C NMR (DMSO-d_6): δ 24.75, 26.09, 27.05, 43.37, 48.17, 55.69, 110.80, 120.91, 128.27, 129.12, 129.21, 131.00, 134.08, 143.76, 149.21, 152.77, 161.87, 165.19; HRMS (m/z): $[M + H]^+$ calcd for $C_{20}H_{21}ClN_5O_2$: 398.1384; found 398.1371. Anal. Calcd for $C_{20}H_{20}ClN_5O_2$: C, 60.38; H, 5.07; N, 17.60; found C, 59.51; H, 4.86; N, 17.11.

*4.1.6. 4-{[1-(6-Methoxypyridazine-3-yl)-5-(4-chlorophenyl)-1H-pyrazole-3-yl]carbonyl}morpholin (**8**)*

Prepared from **2** and morpholin using the procedure 1. Flash chromatography hexane:EtOAc; yield 67%; Mp 208–209 °C; IR (ATR): 2976, 1622, 1088 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.60–3.64 (6H, m), 3.91 (2H, m), 3.99 (3H, s), 7.01 (1H, s), 7.31 (2H, d, $J = 8.4$ Hz), 7.42 (2H, d, $J = 8.4$), 7.48 (1H, d, $J = 9.2$ Hz), 8.00 (1H, d, $J = 9.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 43.01, 47.86, 55.71, 66.80, 67.16, 111.30, 120.93, 128.36, 129.01, 129.25, 131.01, 134.14, 143.88, 148.62, 152.72, 161.86, 165.25. HRMS (m/z): $[M + H]^+$ calcd for $C_{19}H_{19}ClN_5O_3$: 400.1176; found 400.1129. Anal. Calcd for $C_{19}H_{18}ClN_5O_3$: C, 57.07; H, 4.54; N, 17.52; found C, 56.67; H, 4.37; N, 17.33.

*4.1.7. 5-(4-Chlorophenyl)-1-(6-methoxypyridazin-3-yl)-N-(pyridin-4-yl)-1H-pyrazole-3-carboxamide (**9**)*

Prepared from **2** and pyridine-4-amine using the procedure 1. Flash chromatography CH_2Cl_2:MeOH; yield 37%; Mp 247–248 °C. IR (ATR): 3382, 3130, 2976, 1694, 1091 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.02 (3H, s), 7.27 (1H, s), 7.35 (2H, d, $J = 8.4$ Hz), 7.45 (2H, d, $J = 8.8$ Hz), 7.55 (1H, d, $J = 9.2$ Hz), 7.84 (2H, d, $J = 6.4$ Hz), 8.12 (1H, d, $J = 9.2$ Hz), 8.46 (2H, d), 10.68 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ 55.79, 110.08, 114.83, 120.85, 128.75, 129.31, 131.10, 134.37, 145.18, 146.04, 148.23, 151.01, 152.72, 160.91, 165.45. HRMS (m/z): $[M + H]^+$ calcd for $C_{20}H_{16}ClN_6O_2$: 407.1023; found: 407.1035. Anal. Calcd for $C_{20}H_{15}ClN_6O_2$: C, 59.05; H, 3.72; N, 20.66; found C, 58.89; H, 3.66; N, 20.57.

*4.1.8. 5-(4-Chlorophenyl)-N-(4-methoxyphenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxamide (**10**)*

Prepared from **2** and *p*-anisidine using the procedure 1. Recrystallized from methanol; yield 42%; Mp 209–210 °C. IR (ATR): 3360, 3136, 3061, 2975, 1675, 1091 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.82 (3H, s), 4.16 (3H, s), 6.91 (2H, d, $J = 9.2$ Hz), 7.13 (1H, s), 7.14 (1H, d, $J = 9.2$ Hz), 7.25 (2H, d, $J = 8.8$ Hz), 7.34 (2H, d, $J = 8.8$ Hz), 7.61 (2H, d, $J = 8.8$ Hz), 7.68 (1H, d, $J = 9.2$ Hz), 8.65 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ 54.96, 55.09, 109.04, 113.69, 120.03, 121.92, 127.78, 128.29, 128.48, 130.30, 131.48, 133.45, 144.07, 148.27, 152.04, 155.57, 158.88, 164.56. HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{19}ClN_5O_3$: 436.1176; found 436.1183; Anal. Calcd for $C_{22}H_{18}ClN_5O_3$: C, 60.62; H, 4.16; N, 16.07; found: C, 60.36; H, 4.17; N, 15.99.

*4.1.9. N,5-Bis(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxamide (**11**)*

Prepared from **2** and 4-chloroaniline using the procedure 1. Recrystallized with ether-petroleum ether; yield 61%; Mp 254–255 °C. IR (ATR): 3258, 3120, 3070, 2977, 1671, 1090 cm^{-1}. ^1H NMR (DMSO-d_6): δ 4.02 (3H, s), 7.23 (1H, s), 7.35 (2H, d, $J = 8.8$ Hz), 7.39 (2H, d, $J = 8.8$ Hz), 7.44 (2H, d, $J = 8.8$ Hz), 7.54 (1H, d, $J = 9.6$ Hz), 7.85 (2H, d, $J = 8.8$ Hz), 8.12 (1H, d, $J = 9.2$ Hz), 10.45 (1H, s, NH). ^{13}C NMR (DMSO-d_6): δ 55.77, 109.94, 120.84, 122.65, 128.16, 128.66, 128.92, 129.25, 129.28, 131.08, 134.29, 138.19, 144.99, 148.66, 152.77, 160.14, 165.39. HRMS (m/z): $[M + H]^+$ calcd for $C_{21}H_{16}Cl_2N_5O_2$: 440.0681; found 440.0663; Anal. Calcd for $C_{21}H_{15}Cl_2N_5O_2$: C, 57.29; H, 3.43; N, 15.91; found C, 56.77; H, 3.48; N, 15.78.

*4.1.10. 1-{[1-(6-Methoxypyridazine-3-yl)-5-(4-chlorophenyl)-1H-pyrazole-3-yl]carbonyl}-4-(4-fluorophenyl)piperazine (**12**)*

Prepared from **2** and 1-(4-fluorophenyl)piperazine using the procedure 1. Flash chromatography hexane:EtOAc; yield 47%; Mp 193–194 °C; IR (ATR): 2977, 1618, 1090 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.15 (2H, t), 3.20 (2H, t), 3.99 (2H, t), 4.13 (3H, s), 4.22 (2H, t), 6.89–7.01 (5H, m), 7.12 (1H, d, $J = 9.6$ Hz), 7.26 (2H, m), 7.33 (2H, m) 7.70 (1H, d, $J = 9.6$ Hz). ^{13}C NMR (DMSO-d_6): δ 42.50, 47.05, 49.95, 50.51, 55.71, 111.29, 115.94, 116.15, 118.42, 118.50, 120.91, 128.33, 129.03, 129.24, 131.03, 134.16, 143.92, 148.37, 148.79, 152.76, 155.84, 158.18, 161.79, 165.27. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{25}H_{23}ClFN_6O_2$: 493.1555; found 493.1560; Anal. Calcd for $C_{25}H_{22}ClFN_6O_2$: C, 60.91; H, 4.50; N, 17.05; found: C, 60.63; H, 4.62; N, 16.65.

*4.1.11. 1-{[1-(6-Methoxypyridazine-3-yl)-5-(4-chlorophenyl)-1H-pyrazole-3-yl]carbonyl}-4-(4-methylphenyl)piperazine (**13**)*

Prepared from **2** and 1-(4-methylphenyl)piperazine using the procedure 1. Flash chromatography hexane:EtOAc; yield 59%; Mp: 193–194 °C. IR (ATR): 3069, 2977, 1639, 1090 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.28 (3H, s), 3.17 (2H, t), 3.23 (2H, t), 3.98 (2H, t), 4.13 (3H, s), 4.21 (2H, t), 6.87 (2H, d, $J = 8.0$ Hz), 6.92 (1H, s), 7.09–7.13 (3H, m), 7.27 (2H, d, $J = 8.8$ Hz), 7.33 (2H, d, $J = 8.8$ Hz), 7.72 (1H, d, $J = 9.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 20.73, 42.52, 47.08, 49.68, 50.27, 55.70, 111.28, 116.91, 120.90, 128.32, 128.98, 129.05, 129.23, 130.12, 131.02, 134.16, 143.91, 148.83, 149.39, 152.76, 161.79, 165.26. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{26}H_{26}ClN_6O_2$: 489.1806; found 489.1788; Anal. Calcd for $C_{26}H_{25}ClN_6O_2$: C, 63.86; H, 5.15; N, 17.19; found C, 63.61; H, 5.17; N, 17.02.

*4.1.12. 1-{[1-(6-Methoxypyridazine-3-yl)-5-(4-chlorophenyl)-1H-pyrazole-3-yl]carbonyl} 4-(5-trifluoromethylpyridine-2-yl) piperazine (**14**)*

Prepared from **2** and 1-(5-trifluoromethylpyridine-2-yl)piperazine using the procedure 1. Flash chromatography hexane:EtOAc; yield 71%; Mp 198–199 °C; IR (ATR): 3129, 2978, 1612, 1079 cm^{-1}. ^1H NMR (DMSO-d_6): δ 3.71–3.76 (6H, m), 4.00 (3H, s), 4.05 (2H, m), 6.95 (1H, d, $J = 8.8$ Hz), 7.04 (1H, s), 7.33 (2H, d, $J = 8.4$ Hz), 7.44 (2H, d, $J = 8.4$ Hz), 7.49 (1H, d, $J = 9.6$ Hz), 7.82 (1H, dd, $J_1 = 8.8$ Hz, $J_2 = 2.4$ Hz), 8.04 (1H, d, $J = 8.8$ Hz), 8.42 (1H, s). ^{13}C NMR (DMSO-d_6): δ 42.33, 44.45, 45.31, 46.64, 55.73, 67.16, 107.09, 111.38, 120.95, 128.34, 129.02, 129.25, 131.03, 134.16, 135.33, 143.91, 145.91, 148.74, 152.75, 160.68, 161.97, 165.27. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{25}H_{22}ClF_3N_7O_2$: 544.1476; found 544.1464; Anal. Calcd for $C_{25}H_{21}ClF_3N_7O_2$: C, 55.20; H, 3.89; N, 18.03; found C, 55.04; H, 3.97; N, 17.76.

*4.1.13. 1-{[1-(6-Methoxypyridazine-3-yl)-5-(4-chlorophenyl)-1H-pyrazole-3-yl]carbonyl}-4-(pyrimidine-2-yl)piperazine (**15**)*

Prepared from **2** and 1-(2-pyrimidyl)piperazine using the procedure 1. Flash chromatography CH_2Cl_2:MeOH; Recrystallized with methanol; yield 63%; Mp 241–242 °C; IR (ATR): 3056, 2976, 1628, 1092 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.91–3.97 (8H, m), 4.14 (3H, s), 6.55 (1H, t, $J = 4.8$ Hz), 6.94 (1H, s), 7.13 (1H, d, $J = 9.6$ Hz), 7.27 (2H, m), 7.34 (2H, m), 7.75 (1H, d, $J = 9.6$ Hz), 8.34 (2H, d, $J = 4.8$ Hz). ^{13}C NMR (DMSO-d_6): δ 42.50, 43.76, 44.56, 46.99, 55.72, 111.18, 111.35, 120.95, 128.33, 129.11, 129.24, 131.06, 134.16, 143.91, 148.80, 152.78, 158.69, 161.81, 162.07, 165.27. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{23}H_{22}ClN_8O_2$: 477.1554; found 477.1534; Anal. Calcd for $C_{23}H_{21}ClN_8O_2$: C, 57.92; H, 4.44; N, 23.50; found C, 58.16; H, 4.57; N, 22.96.

*4.1.14. 4-Propoxyphenyl 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylate (**16**)*

Prepared from **2** and 4-propoxyphenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH; Recrystallized with methanol; yield 26%; Mp 197–198 °C. IR (ATR): 3133, 3072, 2974, 1727, 1091 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.04 (3H, t, $J = 7.4$ Hz), 1.82 (2H, m), 3.93 (2H, t, $J = 6.6$ Hz), 4.13 (3H, s), 6.93 (2H, d), 7.15 (4H, m), 7.28 (2H, d, $J = 8.4$ Hz), 7.35 (2H, d, $J = 8.4$ Hz), 7.93 (1H, d, $J = 9.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 11.09, 22.73, 55.81, 70.03, 111.88, 115.79, 120.99, 123.31, 128.57, 128.62, 129.34, 131.11, 134.45, 144.06, 144.53, 145.14, 152.73, 157.28, 160.82, 165.55. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{24}H_{22}ClN_4O_4$: 465.1330; found 465.1318. Anal. Calcd for $C_{24}H_{21}ClN_4O_4$: C, 62.00; H, 4.55; N, 12.05; found C, 61.58; H, 4.58; N, 11.99.

*4.1.15. 4-Methoxyphenyl 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylate (**17**)*

Prepared from **2** and 4-methoxyphenol using the procedure 2. Recrystallized with methanol; yield 82%; Mp 221–222 °C; IR (ATR): 2976, 1730, 1092 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.83 (3H, s), 4.13 (3H, s), 6.94 (2H, d, $J = 8.8$ Hz), 7.15–7.19 (4H, m), 7.28 (2H, d, $J = 8.4$ Hz), 7.35 (2H, d, $J = 8.4$ Hz), 7.93 (1H, d, $J = 9.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 55.79, 56.17, 111.83, 115.31, 120.93, 123.27, 128.53, 128.61, 129.31, 131.11, 134.45, 144.22, 144.53, 145.16, 152.73, 157.85, 160.78, 165.57. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{22}H_{18}ClN_4O_4$: 437.1017; found 437.0997; Anal. Calcd for $C_{22}H_{17}ClN_4O_4$: C, 60.49; H, 3.92; N, 12.83; found C, 60.16; H, 4.03; N, 12.67.

*4.1.16. 4-Methylphenyl 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylate (**18**)*

Prepared from **2** and 4-methylphenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 38%; Mp 245–246 °C; IR (ATR): 3141, 3054, 2977, 1731, 1091 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.37 (3H, s), 4.13 (3H, s), 7.12–7.17 (4H, m), 7.23 (2H, d, $J = 8.4$ Hz), 7.28 (2H, d, $J = 8.4$ Hz), 7.35 (2H, d, $J = 8.4$ Hz), 7.93 (1H, d, $J = 9.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 21.11, 55.81, 111.85, 120.94, 122.15, 128.57, 128.62, 129.33, 130.70, 131.14, 134.48, 136.10, 144.51, 145.20, 148.67, 152.75, 160.61, 165.60. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{22}H_{18}ClN_4O_3$: 421.1067; found 421.1074; Anal. Calcd for $C_{22}H_{17}ClN_4O_3$: C, 62.79; H, 4.07; N, 13.31; found C, 62.65; H, 4.17; N, 13.26.

*4.1.17. 4-Cyclopentylphenyl 5-(4-chlorophenyl)-1-(6-methoxy-pyridazin-3-yl)-1H-pyrazole-3-carboxylate (**19**)*

Prepared from **2** and 4-cyclopentylphenyl using the procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 81%; Mp 212–213 °C; IR (ATR): 3141, 3063, 2974, 1733, 1090 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.55–1.85 (6H, m), 2.09 (2H, m), 3.01 (1H, m), 4.13 (3H, s), 7.15–7.19 (4H, m), 7.26–7.30 (4H, m), 7.35 (2H, d, $J = 8.4$ Hz), 7.93 (1H, d, $J = 9.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 25.70, 34.96, 45.45, 55.82, 110.91, 111.87, 120.97, 122.14, 128.60, 128.73, 129.34, 131.15, 134.48, 144.50, 144.57, 145.20, 148.80, 152.75, 160.65, 165.60. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{26}H_{24}ClN_4O_3$: 475.1537; found 475.1520; Anal. Calcd for $C_{26}H_{24}ClN_4O_3$: C, 65.75; H, 4.88; N, 11.80; found C, 65.60; H, 4.84; N, 11.64.

*4.1.18. 4-(1H-Imidazol-1-yl)phenyl 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylate (**20**)*

Prepared from **2** and 4-(1*H*-imidazol-1-yl)phenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH; recrystallized with ethanol; yield 31%; Mp 207–208 °C. IR (ATR): 2976, 1728, 1093 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.15 (3H, s), 7.17 (1H, s, $J = 9.6$ Hz), 7.20 (1H, s), 7.23 (1H, m), 7.28–7.30 (3H, m), 7.36 (2H, d, $J = 8.0$ Hz), 7.40 (2H, d, $J = 8.4$ Hz), 7.47 (2H, d, $J = 8.4$ Hz), 7.86 (1H, s), 7.91 (1H, d, $J = 9.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 55.04, 111.23, 118.14, 120.24, 121.59, 123.19, 127.78, 127.80, 128.58, 129.87, 130.34, 133.70, 134.82, 135.62, 143.47, 144.45, 148.43, 151.93, 159.68, 164.79. HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{24}H_{18}ClN_6O_3$: 473.1129; found 473.1107; Anal. Calcd for $C_{24}H_{17}ClN_6O_3$: C, 60.96; H, 3.62; N, 17.77; found C, 60.42; H, 3.73; N, 17.50.

*4.1.19. 3-Isopropylphenyl 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylate (**21**)*

Prepared from **2** and 3-isopropylphenol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 24%; Mp 167–168 °C; IR (ATR): 3146, 3076, 2974, 1732, 1092 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.27 (6H, d, $J = 6.4$ Hz), 2.95 (1H, m), 4.13 (3H, s), 7.07–7.37 (10H, m), 7.94 (1H, d, $J = 8.8$ Hz). ^{13}C NMR (DMSO-d_6): δ 24.39, 33.91, 55.80, 111.88, 119.86, 120.20, 120.99, 124.91, 128.57, 128.59, 129.34, 130.14, 131.11, 134.44, 144.49, 145.16, 150.90, 151.18, 152.72, 160.52, 165.55. HRMS (m/z): $[M + H]^+$ calcd for $C_{24}H_{22}ClN_4O_3$: 449.1380; found 449.1384; Anal. Calcd for $C_{24}H_{21}ClN_4O_3$: C, 64.21; H, 4.72; N, 12.48; found C, 63.94; H, 4.78; N, 12.38.

*4.1.20. 2-Chloropyridin-3-yl 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylate (**22**)*

Prepared from **2** and 2-chloropyridin-3-ol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH; recrystallized with ethanol; yield 16%; Mp 187–188 °C. IR (ATR): 1746, 1091 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.14 (3H, s), 7.17, (1H, d, $J = 9.6$ Hz), 7.22 (1H, s), 7.28 (2H, d, $J = 8.8$ Hz), 7.36 (3H, m), 7.70 (1H, m), 7.93 (1H, d, $J = 9.2$ Hz), 8.37 (1H, m). ^{13}C NMR (DMSO-d_6): δ 55.84, 112.19, 121.01, 125.42, 128.40, 128.67, 129.36, 131.18, 134.17, 134.57, 143.19, 143.73, 144.06, 145.49, 148.20, 152.66, 159.28, 165.63. HRMS (m/z): $[M + H]^+$ calcd for $C_{20}H_{14}Cl_2N_5O_3$: 442.0474; found 442.0463; Anal. Calcd for $C_{20}H_{13}Cl_2N_5O_3$: C, 54.32; H, 2.96; N, 15.84; found C, 54.07; H, 2.92; N, 15.67.

*4.1.21. Naphthalen-2-yl 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylate (**23**)*

Prepared from **2** and naphthalen-2-ol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 44%; Mp 216–217 °C. IR (ATR): 3152, 3058, 2977, 1729, 1091 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.14 (3H, s), 7.17 (1H, d, $J = 9.6$ Hz), 7.22 (1H, s), 7.34 (2H, m), 7.37 (2H, m), 7.40 (1H, dd, $J_1 = 8.8$ Hz, $J_2 = 2.4$ Hz), 7.47–7.54 (2H, m), 7.74 (1H, d, $J = 2.4$ Hz), 7.84–7.93 (3H, m), 7.95 (1H, d, $J = 9.6$ Hz). ^{13}C NMR (DMSO-d_6): δ 55.81, 111.99, 119.37, 121.00, 122.13, 126.71, 127.50, 128.26, 128.44, 128.59, 129.35, 130.25, 131.13, 131.86, 134.04, 134.47, 144.39, 145.24, 148.49, 152.73, 160.70, 165.57. HRMS (m/z): $[M + H]^+$ calcd for $C_{25}H_{18}ClN_4O_3$: 457.1067; found 457.1060; Anal. Calcd for $C_{25}H_{17}ClN_4O_3$: C, 65.72; H, 3.75; N, 12.26; found C, 65.22; H, 3.73; N, 12.15.

*4.1.22. 3-Methylbutyl 5-(4-chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazole-3-carboxylate (**24**)*

Prepared from **2** and isoamylalcohol using the procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 79%; Mp 149–150 °C. IR (ATR): 3131, 3072, 2971, 1706, 1090 cm^{-1}. ^1H NMR (DMSO-d_6): δ 0.97 (6H, d, $J = 6.4$ Hz), 1.51–1.91 (3H, m), 4.12 (3H, s), 4.43 (2H, t, $J = 6.8$ Hz), 7.01 (1H, s), 7.14 (1H, d, $J = 9.6$ Hz), 7.25 (2H, d, $J = 8.4$ Hz), 7.33 (2H, d, $J = 8.4$ Hz), 7.91 (1H, d, $J = 9.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 22.99, 25.26, 37.57, 55.77, 63.88, 111.20, 120.94, 128.53, 128.73, 129.27, 131.07, 134.33, 144.85, 145.27, 152.75, 161.90, 165.47. HRMS (m/z): $[M + H]^+$ calcd for $C_{20}H_{22}ClN_4O_3$: 401.1380; found 401.1380; Anal. Calcd for $C_{20}H_{21}ClN_4O_3$: C, 59.92; H, 5.28; N, 13.98; found C, 59.25; H, 5.46; N, 13.70.

*4.1.23. Synthesis of 5-(4-chlorophenyl)-1-(6-(methylsulfonyl) pyridazin-3-yl)-1H-pyrazole-3-carboxylic acid (**25**)*

Compound **5** (0.003 mol) was mixed with sodium thiomethoxide (0.009 mol) in THF (35 ml) under argon atmosphere and refluxed for 3 h. After that, sodium hydroxide (0.0033 mol) and 25 ml water were added and refluxed for another 2 h. The solution was poured into iced-water and acidified with HCl, the precipitate was filtered to furnish the crude intermediate 5-(4-chlorophenyl)-1-(6-(methylthio)pyridazin-3-yl)-1*H*-pyrazole-3-carboxylic acid, which was submitted to next step without further purification to obtain methylsulfonyl derivative (**25**) as follows: To the solution of methylthio intermediate in dichloromethane–methanol mixture (5:1), *m*-chloroperbenzoic acid (70%, 0.0016 mol) was added portion wise at 0 °C. The reaction mixture was stirred for 20 min at 0 °C and kept stirred overnight at room temperature. After evaporation of reaction media, the residue was suspended in ether and the precipitate was filtered and recrystallized from ethanol. Yield 74%; Mp 232–233 °C. ^1H NMR (DMSO-d_6): δ 3.46 (3H, s), 7.20 (1H, s), 7.46 (4H, m), 8.51 (1H, d, $J = 8.8$ Hz), 8.53 (1H, d, $J = 8.8$ Hz). HRMS (m/z): $[M + H]^+$ calcd for $C_{15}H_{12}ClN_4O_4S$: 379.0268; found 379.0257.

*4.1.24. (5-(4-Chlorophenyl)-1-(6-(methylsulfonyl)pyridazin-3-yl)-1H-pyrazol-3-yl)(piperidin-1-yl)methanone (**26**)*

Prepared from **25** and piperidine using procedure 2. Flash chromatography hexane:EtOAc; yield 65%; Mp 216–217 °C. ^1H NMR (DMSO-d_6): δ 1.57–1.73 (6H, m), 3.38 (3H, s), 3.77 (4H, dt), 6.84 (1H, s), 7.29 (2H, d, $J = 8.4$ Hz), 7.38 (2H, d, $J = 8.8$ Hz), 8.27 (1H, d, $J = 9.2$ Hz), 8.32 (1H, d, $J = 9.2$ Hz). ^{13}C NMR (DMSO-d_6): δ 23.92, 25.29, 26.26, 40.59, 42.59, 47.42, 111.81, 124.81, 127.60, 128.32, 128.67, 130.52, 133.58, 143.87, 149.91, 156.76, 160.77, 160.91. HRMS (m/z): $[M + H]^+$ calcd for $C_{20}H_{21}ClN_5O_3S$: 446.1054; found 446.1033.

*4.1.25. (5-(4-Chlorophenyl)-1-(6-(methylsulfonyl)pyridazin-3-yl)-1H-pyrazol-3-yl)(morpholino)methanone (**27**)*

Prepared from **25** and morpholine using procedure 2. Flash chromatography hexane:EtOAc; yield 86%; Mp 248–249 °C. ^1H NMR (DMSO-d_6): δ 3.39 (3H, s), 3.74 (2H, t), 3.76–3.84 (4H, m), 4.04 (2H, t), 6.93 (1H, s), 7.28 (2H, d, $J = 8.4$ Hz), 7.38 (2H, d, $J = 8.4$ Hz), 8.26 (1H, d, $J = 8.8$ Hz), 8.29 (1H, d, $J = 8.8$ Hz). ^{13}C NMR (DMSO-d_6): δ 41.37, 43.02, 47.87, 66.78, 67.16, 113.02, 125.79, 128.41, 129.13, 129.32, 131.30, 134.42, 144.72, 150.06, 157.52, 161.57, 161.78. HRMS (m/z): $[M + H]^+$ calcd for $C_{19}H_{19}ClN_5O_4S$: 448.0846; found 448.0846.

*4.1.26. 5-(4-Chlorophenyl)-1-(6-oxo-1,6-dihydropyridazin-3-yl)-1H-pyrazole-3-carboxylic acid (**28**)*

A solution of compound **5** (0.003 mol) and sodium acetate (0.006 mol) in 50 ml glacial acetic acid was refluxed for 10 h. After cooling, it was poured into ice-water (100 ml) and the precipitate formed was filtered off, washed with water, dried and recrystallized from ethanol to obtain methyl 5-(4-chlorophenyl)-1-(6-oxo-1,6-dihydropyridazin-3-yl)-1*H*-pyrazole-3-carboxylate (yield 91%), which was subsequently saponified (0.003 mol) with lithium hydroxide (0.008 mol) in THF/water (1:1) mixture at 70 °C for 3 h. After acidification, the precipitate was filtered and recrystallized from ethanol to furnish **28**. Yield 85%; Mp 280–281 °C. ^1H NMR (DMSO-d_6): δ 7.12–7.15 (2H, d, $J = 10.0$ Hz), 7.44 (2H, d, $J = 8.4$ Hz), 7.51 (2H, d, $J = 8.4$ Hz), 7.82 (1H, d, $J = 10.0$ Hz), 13.4 (1H, s); HRMS (m/z): $[M + H]^+$ calcd for $C_{14}H_{10}ClN_4O_3$: 317.0441; found 317.0446.

*4.1.27. 6-(5-(4-Chlorophenyl)-3-(piperidine-1-carbonyl)-1H-pyrazol-1-yl)pyridazin-3(2H)-one (**29**)*

Prepared from **28** and piperidine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 55%; Mp 228–229 °C. IR (ATR): 3155, 3032, 2952, 1680, 1600 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.70 (6H, m), 3.80 (4H, m), 6.80 (1H, s), 7.06 (1H, d, $J = 10.0$ Hz), 7.26 (2H, d, $J = 8.8$ Hz), 7.37 (2H, d, $J = 8.4$ Hz), 7.72 (1H, d, $J = 10.0$ Hz), 10.54 (1H, s). ^{13}C NMR (DMSO-d_6): δ 24.85, 25.89, 26.98, 43.88, 48.47, 111.40, 128.19, 129.13, 130.23, 131.01, 132.66, 135.49, 141.94, 143.85, 149.49, 159.94, 162.06. HRMS (m/z): $[M + H]^+$ calcd for $C_{19}H_{19}ClN_5O_2$: 384.1227; found 384.1222.

*4.1.28. 6-(5-(4-Chlorophenyl)-3-(morpholine-4-carbonyl)-1H-pyrazol-1-yl)pyridazin-3(2H)-one (**30**)*

Prepared from **28** and morpholine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 61%; Mp 249–250 °C. IR (ATR): 3185, 3112, 2966, 1678, 1617 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.73–4.08 (8H, m), 6.90 (1H, s), 7.07 (1H, d, J = 10.0 Hz), 7.25 (2H, d, J = 8.8 Hz), 7.38 (2H, d, J = 8.8 Hz), 7.65 (1H, d, J = 10.0 Hz), 10.70 (1H, s). ^{13}C NMR (DMSO-d_6): δ 43.01, 47.85, 66.80, 67.16, 110.99, 128.57, 129.41, 130.93, 131.86, 132.93, 134.34, 141.46, 143.60, 148.46, 160.54, 161.77. HRMS (m/z): $[M + H]^+$ calcd for $C_{18}H_{17}ClN_5O_3$: 386.1026; found 386.1020.

*4.1.29. Synthesis of (3-(4-chlorophenyl)-1H-pyrazol-5-yl)(piperidin-1-yl)methanone (**33**)*

After hydrolysis of the ester, thionyl chloride (0.025 mol) and catalytic amount of DMF were added to the solution of acid derivative (0.01 mol) in CH_2Cl_2 under argon atmosphere and refluxed for 3 h. Then, to the reaction mixture, diisopropylethylamine (0.015 mol) and piperidine (0.01 mol) were added and stirred overnight at room temperature. After the reaction was complete, the solvent was evaporated and the residue was purified by flash chromatography using CH_2Cl_2:MeOH solvent gradient. Yield 59%; Mp 223–224 °C. IR (ATR): 3098, 2938, 1578 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.69 (6H, m), 3.76 (4H, m), 6.73 (1H, s), 7.37 (2H, d, J = 8.8 Hz), 7.70 (2H, d, J = 8.8 Hz). ^{13}C NMR (DMSO-d_6 + NaH + 3 drops D_2O): δ 25.10, 25.90, 26.64, 43.10, 48.53, 104.28, 126.57, 128.76, 129.48, 136.18, 147.70, 148.40, 165.86. HRMS (m/z): $[M + H]^+$ calcd for $C_{15}H_{17}ClN_3O$: 290.1060; found 290.1053.

*4.1.30. (3-(4-Chlorophenyl)-1H-pyrazol-5-yl)(morpholino) methanone (**34**)*

Prepared from **31** acid and morpholine as described for **33**. Flash chromatography CH_2Cl_2:MeOH; yield 59%; Mp 248–248.5 °C. IR (ATR): 3132, 2976, 1591 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.76 (4H, m), 3.88 (4H, m), 6.80 (1H, s), 7.40 (2H, d, J = 8.0 Hz), 7.64 (2H, d, J = 8.0 Hz). ^{13}C NMR (DMSO-d_6 + NaH + 3 drops D_2O): δ 43.39, 45.72, 66.61, 67.33, 105.02, 126.48, 128.69, 129.31, 136.41, 147.80, 148.47, 165.35. HRMS (m/z): $[M + H]^+$ calcd for $C_{14}H_{15}ClN_3O_2$: 292.0853; found 292.0843.

*4.1.31. (3-(4-Chlorophenyl)-1-methyl-1H-pyrazol-5-yl)(piperidin-1-yl)methanone (**35**)*

Prepared from **32** acid and piperidine as described for **33**. Flash chromatography CH_2Cl_2:MeOH; yield 47%; Mp 119.5–121 °C. ^1H NMR ($CDCl_3$): δ 1.65 (6H, m), 3.60 (4H, m), 3.98 (3H, s), 6.53 (1H, s), 7.35 (2H, d, J = 8.4 Hz), 7.70 (2H, d, J = 8.8 Hz). ^{13}C NMR (DMSO-d_6): δ 24.61, 25.94, 26.77, 38.45, 43.03, 48.48, 103.81, 127.51, 129.39, 132.23, 132.87, 138.30, 148.19, 160.41. HRMS (m/z): $[M + H]^+$ calcd for $C_{16}H_{19}ClN_3O$: 304.1217; found 304.1220.

*4.1.32. (3-(4-Chlorophenyl)-1-methyl-1H-pyrazol-5-yl)(morpholino) methanone (**36**)*

Prepared from **32** acid and morpholine as described for **33**. Flash chromatography CH_2Cl_2:MeOH; yield 81%; Mp 158–159 °C. ^1H NMR ($CDCl_3$): δ 3.70 (8H, m), 4.10 (3H, s), 6.55 (1H, s), 7.36 (2H, d, J = 8.0 Hz), 7.70 (2H, d, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ 38.66, 48.06, 55.44, 66.83, 72.30, 104.64, 127.51, 129.40, 132.15, 132.90, 137.41, 148.13, 160.66. HRMS (m/z): $[M + H]^+$ calcd for $C_{15}H_{17}ClN_3O_2$: 306.1009; found 306.1010.

*4.1.33. 1-(6-Methoxypyridazin-3-yl)-5-methyl-1H-pyrazole-3-carboxylic acid (**40**)*

Compound **38** (0.003 mol) and **4** (0.0032 mol) were dissolved in methanol and HCl (0.003 mol) was added. The reaction mixture was stirred at rt for 6 h. The precipitate formed was filtered to give **39**, which was used in the synthesis of **40**. Yield 63%; ^1H NMR ($CDCl_3$): δ 2.80 (3H, s), 3.98 (3H, s), 6.77 (1H, s), 7.68 (1H, d, J = 8.0 Hz), 8.27 (1H, d, J = 8.0 Hz). ^{13}C NMR ($CDCl_3$): δ 15.16, 52.54, 111.93, 123.73, 130.72, 143.97, 145.69, 155.55, 156.04, 162.45. HRMS (m/z): $[M + H]^+$ calcd for $C_{10}H_{10}ClN_4O_2$: 253.0492; found 253.0496). **39** (0.006 mol) and sodium methoxide (0.018 mol) in methanol were refluxed for 2 h. After evaporating the solvent to one-third, the water was added and refluxed for another hour and acidified with HCl. The yellow precipitate was recrystallized from acetone–water mixture to furnish **40** as a white solid. Yield 89%; Mp 211–211.5 °C. ^1H NMR ($CDCl_3$): δ 4.10 (3H, s), 6.78 (1H, s), 7.50 (1H, d, J = 9.6 Hz), 8.07 (1H, d, J = 9.2 Hz); HRMS (m/z): $[M + H]^+$ calcd for $C_{10}H_{11}N_4O_3$: 235.0831; found 235.0832.

*4.1.34. (1-(6-Methoxypyridazin-3-yl)-5-methyl-1H-pyrazol-3-yl)(piperidin-1-yl)methanone (**41**)*

Prepared from **40** and piperidine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 53%; Mp 98.4–99.1 °C. ^1H NMR ($CDCl_3$): δ 1.65 (6H, m), 2.73 (3H, s), 3.74 (4H, m), 4.17 (3H, s), 6.50 (1H, s), 7.12 (1H, d, J = 9.6 Hz), 8.03 (1H, d, J = 9.6 Hz). ^{13}C NMR (DMSO-d_6): δ 14.19, 24,77, 26.09, 27.02, 43.31, 48.10, 55.57, 110.60, 121.14, 126.14, 141.82, 148.67, 153.47, 162.21, 164.66. HRMS (m/z): $[M + H]^+$ calcd for $C_{15}H_{20}N_5O_2$: 302.1617; found 302.1611.

*4.1.35. (1-(6-Methoxypyridazin-3-yl)-5-methyl-1H-pyrazol-3-yl)(morpholino) methanone (**42**)*

Prepared from **40** and morpholine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 46%; Mp 104.5–105.5 °C. ^1H NMR ($CDCl_3$): δ 2.60 (3H, s), 3.60–3.64 (6H, m), 3.91 (2H, t), 4.08 (3H, s), 6.66 (1H, s), 7.48 (1H, d, J = 9.2 Hz), 8.06 (1H, d, J = 9.6 Hz). ^{13}C NMR (DMSO-d_6): δ 14.14, 42.98, 47.82, 55.60, 66.83, 67.19, 111.18, 121.14, 126.30, 141.98, 148.12, 153.43, 162.14, 164.75. HRMS (m/z): $[M + H]^+$ calcd for $C_{14}H_{18}N_5O_3$: 304.1410; found 304.1412.

*4.1.36. 3-(5-(4-Chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazol-3-yl)-1-(piperidin-1-yl)propan-1-one (**45**)*

Prepared from **44** and piperidine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 89%; Mp 130–131 °C. ^1H NMR ($CDCl_3$): δ 1.53–1.63 (6H, m), 2.77 (2H, t, J = 7.8 Hz), 3.08 (2H, t, J = 7.8 Hz), 3.43 (2H, t, J = 5.4 Hz), 3.58 (2H, t, J = 5.4 Hz), 4.09 (3H, s), 6.39 (1H, s), 7.07 (1H, d, J = 9.2 Hz), 7.22 (2H, d, J = 8.4 Hz), 7.29 (2H, d, J = 8.8 Hz), 7.75 (1H, d, J = 9.6 Hz). ^{13}C NMR (DMSO-d_6): δ 24.20, 24.77, 26.02, 26.72, 32.11, 42.70, 46.51, 55.52, 109.50, 120.69, 127.70, 129.09, 130.19, 130.70, 133.55, 143.65, 153.01, 154.77, 164.69, 169.98. HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{25}ClN_5O_2$: 426.1697; found 426.1679.

*4.1.37. 3-(5-(4-Chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazol-3-yl)-1-morpholinopropan-1-one (**46**)*

Prepared from **44** and morpholine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 68%; Mp 127–128 °C. ^1H NMR ($CDCl_3$): δ 2.78 (2H, t, J = 7.2 Hz), 3.09 (2H, t, J = 6.4 Hz), 3.50 (2H, m), 3.66 (6H, m), 4.10 (3H, s), 6.39 (1H, s), 7.07 (1H, d, J = 9.2 Hz), 7.22 (2H, d, J = 8.8 Hz), 7.30 (2H, d, J = 8.4 Hz), 7.72 (1H, d, J = 9.2 Hz). ^{13}C NMR (DMSO-d_6): δ 23.98, 31.87, 42.22, 46.02, 55.50, 66.79, 109.47, 120.69, 127.68, 129.09, 130.15, 130.69, 133.56, 143.66, 152.98, 155.62, 164.69, 170.66. HRMS (m/z): $[M + H]^+$ calcd for $C_{21}H_{23}ClN_5O_3$: 428.1489; found 428.1496.

*4.1.38. 5-(4-Chlorophenyl)-1-(4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid (**49**)*

Compound **48** [32] (0.003 mol) and lithium hydroxide (0.008 mol) in THF/water mixture (1:1) were refluxed for 4 h. After cooling, the reaction mixture was diluted with water, acidified and the precipitate was filtered and recrystallized from ethanol. Yield

95%; Mp 92–93 °C. ^{1}H NMR (DMSO-d_6): δ 3.77 (3H, s), 6.99 (2H, d, J = 8.4), 7.07 (1H, s), 7.23–7.26 (4H, m), 7.42 (2H, d, J = 8.4 Hz). HRMS (m/z): $[M + H]^+$ calcd for $C_{17}H_{14}ClN_2O_3$: 329.0693; found 329.0702.

*4.1.39. (5-(4-Chlorophenyl)-1-(4-methoxyphenyl)-1H-pyrazol-3-yl)(piperidin-1-yl)methanone (**50**)*

Prepared from **49** and piperidine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 77%; Mp 118–119 °C. ^{1}H NMR ($CDCl_3$): δ 1.60–1.68 (6H, m), 3.73 (2H, m), 3.82 (3H, s), 3.93 (2H, m), 6.83 (1H, s), 6.86 (2H, d, J = 9.2 Hz), 7.15 (2H, d, J = 8.4 Hz), 7.18 (2H, d, J = 8.8 Hz), 7.27 (2H, d, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6): δ 24.83, 26.14, 27.10, 43.44, 48.10, 56.13, 109.71, 115.06, 127.69, 128.93, 129.37, 130.97, 132.86, 134.05, 142.63, 147.87, 159.64, 162.20. HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{23}ClN_3O_2$: 396.1479; found 396.1462.

*4.1.40. (5-(4-Chlorophenyl)-1-(4-methoxyphenyl)-1H-pyrazol-3-yl)(morpholino) methanone (**51**)*

Prepared from **49** and morpholine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 56%; Mp 142–143 °C. ^{1}H NMR ($CDCl_3$): δ 3.73–3.80 (6H, m), 3.83 (3H, s), 4.18–4.20 (2H, m), 6.88 (2H, d, J = 9.2 Hz), 6.93 (1H, s), 7.15 (2H, d, J = 8.4 Hz), 7.18 (2H, d, J = 8.4 Hz), 7.28 (2H, d, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6): δ 43.06, 47.87, 56.15, 66.88, 67.21, 110.23, 115.08, 127.76, 128.82, 129.39, 130.99, 132.79, 134.12, 142.79, 147.32, 159.73, 162.16. HRMS (m/z): $[M + H]^+$ calcd for $C_{21}H_{21}ClN_3O_3$: 398.1271; found 398.1278.

*4.1.41. Ethyl 4-(4-chlorophenyl)-5-(4-methoxyphenyl)isoxazole-3-carboxylate (**57**)*

4-Chlorophenylboronic acid (2 mmol) was added to a mixture of **56** (1.7 mmol) and sodium bicarbonate (5.1 mol) in DMF (15 ml). After addition of water (5 ml), the resulting suspension was degassed by evacuation-nitrogen purge (three cycles) and then bubbled with nitrogen gas for 10 min. Dichlorobis(triphenylphosphine) palladium(II) (0.085 mmol) was added, and the reaction mixture was heated under nitrogen atmosphere at 80 °C for 3 h. The mixture was allowed to cool, water (100 ml) was added, and extracted with EtOAc (3 × 50 ml), and filtered through a Celite pad. The organic phase was washed with water (2 × 100 ml) and with aqueous sodium chloride solution (100 ml) and then dried over anhydrous Na_2SO_4 and filtered. The filtrate was evaporated in vacuo, and the crude product was purified by automated flash chromatography (hexane–EtOAc). Yield 65%. Mp 125–126 °C. ^{1}H NMR (DMSO-d_6): δ 1.15 (3H, t, J = 7.2 Hz), 3.77 (3H, s), 4.23 (2H, q, J = 7.2 Hz), 7.02 (2H, d, J = 8.4 Hz), 7.41 (4H, d, J = 8.4 Hz), 7.52 (2H, d, J = 8.0 Hz). HRMS (m/z): $[M + H]^+$ calcd for $C_{19}H_{17}ClNO_4$: 358.0846; found 358.0840.

*4.1.42. 4-(4-Chlorophenyl)-5-(4-methoxyphenyl)isoxazole-3-carboxylic acid (**58**)*

Prepared from **57** as described for **49**. Yield 80%. Mp 157–159 °C. ^{1}H NMR (DMSO-d_6): δ 3.77 (3H, s), 7.01 (2H, d, J = 8.4 Hz), 7.39 (4H, d, J = 8.0 Hz), 7.51 (2H, d, J = 8.0 Hz). HRMS (m/z): $[M + H]^+$ calcd for $C_{17}H_{13}ClNO_4$: 330.0533; found 330.0526.

*4.1.43. (4-(4-Chlorophenyl)-5-(4-methoxyphenyl)isoxazol-3-yl)(piperidin-1-yl) methanone (**59**)*

Prepared from **58** and piperidine using procedure 2. Flash chromatography hexane:EtOAc; yield 41%; Mp 132–133 °C. ^{1}H NMR ($CDCl_3$): δ 1.30–1.33 (2H, m), 1.53–1.59 (4H, m), 3.29 (2H, t, J = 5.4 Hz), 3.63 (2H, t, J = 5.4 Hz), 3.83 (3H, s), 6.89 (2H, d, J = 9.2 Hz), 7.31–7.37 (4H, m), 7.50 (2H, d, J = 9.2 Hz). ^{13}C NMR (DMSO-d_6): δ 24.30, 25.80, 26.60, 42.64, 47.88, 56.06, 112.64, 115.37, 119.29, 128.28, 129.38, 129.85, 131.71, 133.93, 159.35, 161.71, 165.78. HRMS (m/z): $[M + H]^+$ calcd for $C_{22}H_{22}ClN_2O_3$: 397.1319; found 397.1318.

*4.1.44. (4-(4-Chlorophenyl)-5-(4-methoxyphenyl)isoxazol-3-yl)(morpholino)methanone (**60**)*

Prepared from 58 and morpholine using procedure 2. Flash chromatography hexane:EtOAc; yield 63%; Mp 169–170 °C. ^{1}H NMR ($CDCl_3$): δ 3.41–3.46 (4H, m), 3.64–3.66 (2H, m), 3.70–3.73 (2H, m), 3.83 (3H, s), 6.89 (2H, d, J = 9.2 Hz), 7.32 (2H, d, J = 8.4 Hz), 7.38 (2H, d, J = 8.8 Hz), 7.49 (2H, d, J = 9.2 Hz). ^{13}C NMR (DMSO-d_6): δ 42.46, 47.41, 56.07, 66.50, 66.88, 112.95, 115.40, 119.24, 128.18, 129.35, 129.89, 131.82, 134.01, 158.73, 159.63, 161.75, 165.99. HRMS (m/z): $[M + H]^+$ calcd for $C_{21}H_{20}ClN_2O_4$: 399.1112; found 399.1126.

*4.1.45. (5-(4-Chlorophenyl)-1-(6-methoxypyridazin-3-yl)-1H-pyrazol-3-yl)(pyrrolidin-1-yl)methanone (**61**)*

Prepared from **6** and pyrrolidine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 75%; Mp 185–186 °C. ^{1}H NMR ($CDCl_3$): δ 1.95 (4H, m), 3.70 (2H, t, J = 6.8 Hz), 3.98 (2H, t, J = 6.4 Hz), 4.12 (3H, s), 7.01 (1H, s), 7.10 (1H, d, J = 9.2 Hz), 7.25 (2H, d, J = 8.8 Hz), 7.32 (2H, d, J = 8.8 Hz), 7.75 (1H, d, J = 9.6 Hz). ^{13}C NMR (DMSO-d_6): δ 24.11, 26.74, 47.26, 49.01, 55.71, 111.27, 120.97, 128.20, 129.20, 131.04, 134.08, 143.72, 149.99, 152.86, 160.74, 165.21. HRMS (m/z): $[M + H]^+$ calcd for $C_{19}H_{19}ClN_5O_2$: 384.1227; found 384.1212.

*4.1.46. (5-(4-Chlorophenyl)-1-(4-methoxyphenyl)-1H-pyrazol-3-yl)(pyrrolidin-1-yl) methanone (**62**)*

Prepared from **49** and pyrrolidine using procedure 2. Flash chromatography CH_2Cl_2:MeOH; yield 88%; Mp 154.5–155 °C (lit. Mp 152 °C) [33]. ^{1}H NMR ($CDCl_3$): δ 1.94 (4H, m), 3.69 (2H, t, J = 6.8 Hz), 3.83 (3H, s), 4.01 (2H, t, J = 6.4 Hz), 6.87 (2H, d, J = 8.8 Hz), 7.00 (1H, s), 7.16 (2H, d, J = 8.4 Hz), 7.20 (2H, d, J = 8.8 Hz), 7.28 (2H, d, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6): δ 23.30, 25.97, 46.40, 48.22, 55.35, 109.19, 114.29, 126.82, 128.17, 128.60, 130.19, 132.19, 133.27, 141.82, 147.88, 158.87, 160.38. HRMS (m/z): $[M + H]^+$ calcd for $C_{21}H_{21}ClN_3O_2$: 382.1322; found 382.1321.

4.2. Antiplatelet activity

Test compounds were investigated by the turbidimetric Born method for their antiplatelet activities [27]. Platelet-reach plasma (PRP) and platelet-poor plasma (PPP) were obtained at the Blood Center of the Gazi University Hospital Ankara (Turkey) or prepared in house from the venous blood of healthy volunteers. In brief, freshly drawn venous human citrated (3.2% sodium citrate, 9:1) blood from healthy subjects, who had not taken any nonsteroidal anti-inflammatory or other drugs with antiplatelet activity for fifteen days, was centrifuged at 800 rpm or at 3500 rpm (Digicen 21R, Orto Alresa, Madrid, Spain) to obtain platelet-rich plasma (PRP) or platelet-poor plasma (PPP), respectively. Platelet numbers were determined with a cell counter (Swelab Alfa, Boule Medical AB, Stockholm, Sweden) and adjusted to 3.8×10^8 platelets/ml with PPP. Platelet aggregation was measured turbidimetrically with the APACT aggregometer (Automated Platelet Aggregation and Coagulation Tracer, Biochemica GmbH, Flacht, Germany) with software APACT professional version 1.1.

The test compound (or the standard inhibitor) is dissolved in dimethylsulfoxide (DMSO). To eliminate solvent effects, the final concentration of DMSO was fixed at <1%. Then 0.5 μL of the test solution is given to 199 μL PRP in the test cuvette and incubated 3 min at 37.4 °C. Then, 10 μL of the inducer (final concentrations of the inducers: arachidonic acid, 700 μM; ADP, 10 μM; collagen, 2 μg/ml) is added and the change in light transmission was recorded. The percentage of aggregation is determined as the ratio of heights of the aggregation curves with and without the test compound. Each curve is corrected automatically for the light absorption of platelet-poor plasma (PPP) of the same donor. Percent platelet aggregation

values of PRP were determined at a series of concentrations of selected active compounds and from the determined values, the IC_{50} values with respect to each tested compound were calculated by means of the software Sigma Plot (version 9). All experiments were performed in triplicate.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.03.048.

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