European Journal of Medicinal Chemistry 64 (2013) 42–53

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Pyrazole derivatives as inhibitors of arachidonic acid-induced platelet aggregation

Serkan Levent [a], Burcu Çalışkan [a], Murat Çiftçi [a], Yeşim Özkan [b], İdil Yenicesu [c], Hüseyin Ünver [d], Erden Banoglu [a,*]

[a] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Gazi University, Taç Sok. No:3, Etiler, Yenimahalle, Ankara 06330, Turkey
[b] Department of Biochemistry, Faculty of Pharmacy, Gazi University, Yenimahalle, Ankara 06330, Turkey
[c] Pediatric Hematology Unit and Blood Bank, Faculty of Medicine, Gazi University, Ankara, Turkey
[d] Department of Physics, Faculty of Science, Ankara University, Tandoğan, Ankara 06100, Turkey

ARTICLE INFO

Article history:
Received 25 December 2012
Received in revised form
20 March 2013
Accepted 24 March 2013
Available online 6 April 2013

Keywords:
Pyrazole
Carboxamide
Antiplatelet
Arachidonic acid

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**.

* Corresponding author. Tel.: +90 3122023236; fax: +90 3122235018.
E-mail addresses: banoglu@gazi.edu.tr, ebanoglu@gmail.com (E. Banoglu).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.03.048

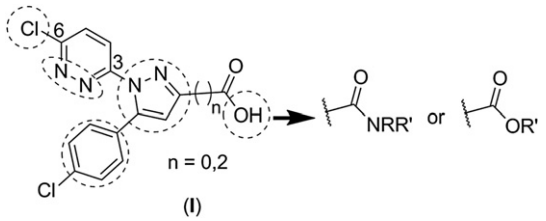


Fig. 1. Structure of the starting compound and features modified during SAR.

2. Results and discussion

2.1. Chemistry

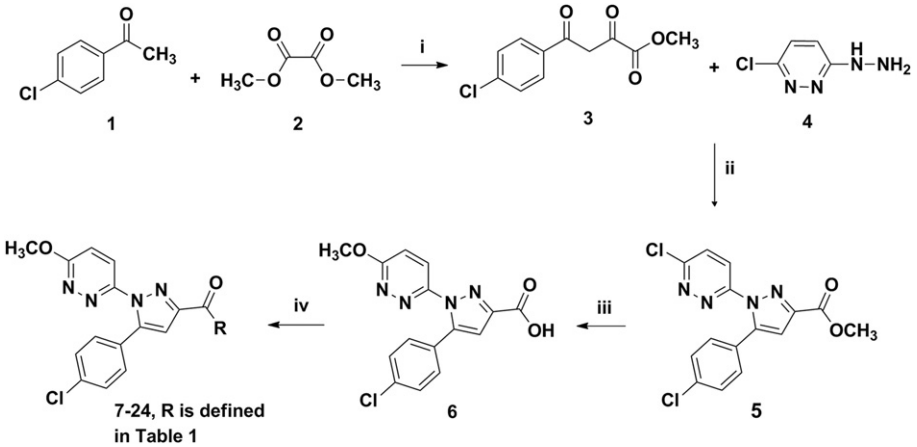
The pyrazole derivatives studied in this communication were synthesized as outlined in Schemes 1–6 and the isoxazole derivatives were prepared as shown in Scheme 7. All compounds were purified by automated flash chromatography and checked for purity with UPLC before being tested in biological assays (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.