Pyridazines. Part 28: 5-Alkylidene-6-phenyl-3(2H)-pyridazinones, a new family of platelet aggregation inhibitors

7 AUTHORS, INCLUDING:

Eddy Sotelo
University of Santiago de Compostela
89 PUBLICATIONS **853** CITATIONS

SEE PROFILE

Matilde Yáñez
University of Santiago de Compostela
81 PUBLICATIONS **1,530** CITATIONS

SEE PROFILE

José Brea
University of Santiago de Compostela
92 PUBLICATIONS **951** CITATIONS

SEE PROFILE

Pyridazines. Part 28: 5-Alkylidene-6-phenyl-3(2H)-pyridazinones, a New Family of Platelet Aggregation Inhibitors☆

Eddy Sotelo,[a] Nuria Fraiz,[b] Matilde Yáñez,[b] Reyes Laguna,[b] Ernesto Cano,[b] José Brea[b] and Enrique Raviña[a],*

[a]*Departamento de Química Orgánica, Laboratorio de Química Farmacéutica, Universidad de Santiago de Compostela, 15782-Santiago de Compostela, Spain*
[b]*Departamento de Farmacología, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782-Santiago de Compostela, Spain*

Abstract—The synthesis and anti-platelet activity of several 5-alkylidene-6-phenyl-3(2H)-pyridazinones are described. The most active compounds are those that contain oxygenated functions (COOR, COMe) on the alkylidene fragment (**6a**, **6b** and **6c**). © 2002 Published by Elsevier Science Ltd.

The activation, adhesion and aggregation of platelets are all important processes in the initiation of thrombus formation at sites showing high-grade stenosis, ruptured atheromatous plaque and endothelial damage within arteries. Platelet-mediated thrombus formation in the coronary artery is a primary factor in the development of thrombotic disorders such as unstable angina, myocardial infarction, stroke and peripheral artery disease.[2,3] The medical need for more efficacious anti-platelet agents and the growing understanding of the role of platelets in vascular injury have catalysed an extensive evaluation of novel approaches to control platelet function.

Current therapeutic-based strategies to inhibit platelet function include the use of Aspirin, Ticlopidine or Sulfinpyrazone (Fig. 1) but some of these drugs are not selective and have a relatively low activity. In the search for new, highly active and selective agents, the field of $GP_{IIb-IIIa}$ has been investigated and several drugs have been identified that have the fibrinogen receptor as a target.[4,5] Nevertheless, several complications involving bleeding have recently been described on using these drugs in clinical trials.[6–8] Thus, the search for new agents with other mechanisms of action is of great interest not only for use as drugs but also because such compounds could be used as pharmacological tools to provide important information regarding platelet function.

Cyclic adenosine monophosphate (c-AMP) phosphodiesterase III (PDE III) has been one of the most studied targets in the search for new anti-platelet agents.[9] Among the large family of PDE III inhibitors, compounds containing the 3(2H)-pyridazinone ring have
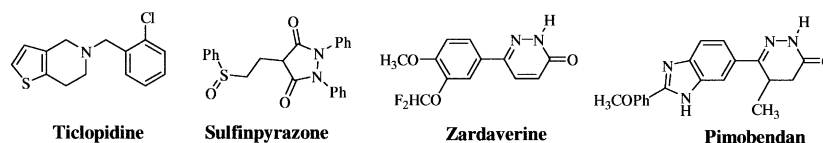
Figure 1.

been extensively studied,[10] as Zardaverine and Pimobendan (Fig. 1).

We recently reported the synthesis and evaluation of the platelet inhibitory activity of a series of 6-aryl-3(2H)-pyridazinones substituted at the 5-position[11–13] (series **I**, Fig. 2). The pharmacological studies on these compounds revealed that both the mechanism of action and the anti-platelet activity of this series are highly dependent on the nature of the chemical group present at the 5-position of the heterocyclic ring.

A diverse range of different mechanisms of action operate for compounds included in series **I** and this situation complicates SAR studies. Despite this complication, we have observed that the most active compounds are those that bear electron-withdrawing groups in the 5-position.[13] As a continuation of our previous work, we wish to report here a convenient methodology for the preparation of new 5-alkylidene-6-phenyl-3(2H)-pyridazinones (series **II**, Fig. 2). The new compounds are vinyl analogues of simple pyridazinones **I**. We also wish to report the results of the anti-platelet activity of the afore-mentioned compounds.

In a recent paper, we reported a procedure for the preparation of aldehyde **5**[14] (Scheme 1). This synthetic route consists of a long multistep process, which involves troublesome procedures that give only a moderate overall yield of compound **5**. For this reason, we now describe a more convenient palladium-assisted approach to this compound, which is the starting material
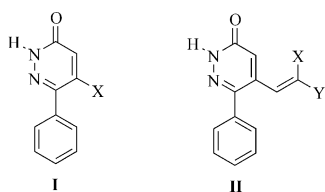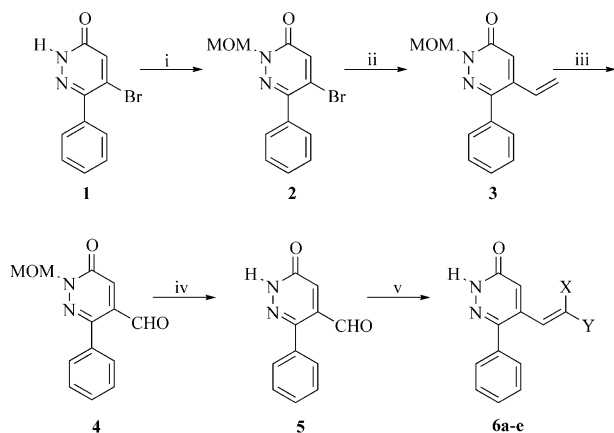
in the preparation of 5-alkylidene derivatives **II** (**6a**–**6e**, Scheme 1).

The reaction sequence is outlined in Scheme 1. Vinylation at position 5 of the pyridazinone ring, through a palladium-catalyzed Stille reaction, requires prior protection of the NH group on the heterocycle as the methoxymethyl (MOM) derivative **2**.[15] Ozonolysis of **3** and subsequent deprotection of the MOM group by treatment with refluxing 6 N hydrochloric acid gave aldehyde **5**. Ozonolysis and deprotection can be achieved in a *one-pot* procedure and so compound **5** has been prepared in 85% yield over three steps. Knoevenagel condensation of aldehyde **5** with several active methylene derivatives in refluxing methanol using catalytic amounts of piperidine leads to the 5-alkylidene-6-phenyl-3(2H)-pyridazinones **6a**–**e**. These compounds (Table 1) were obtained as crystalline solids after purification by column chromatography and recrystallization.[16,17] The stereochemistries of compounds **6a** and **6d** were determined through NMR experiments, with both compounds having an *E*-configuration.[18]

Table 2 shows the results of the experiments to evaluate the anti-platelet activity of compounds **6a–e** by the turbidimetric method of Born[19] employing thrombin as agonist. Three of the five compounds synthesized showed higher antiaggregatory activity—with IC_{50} values in the range 20–45 µM—than the reference drugs (Ticlopidine and Sulfinpyrazone).

The presence of oxygenated functions (COOR, COMe) on the vinyl group gave rise to the highest activity of the series, particularly in esters **6a** and **6c**. The introduction of cyano groups on the alkylidene fragment produces a notable diminution of the anti-platelet effect (**6e**) or

Figure 2.

Table 1. 5-Alkylidene-6-phenyl-3(2H)-pyridazinones **6a**–**e**

Compd	X	Y	Mp (°C solv)	Yield from 5 (%)
6a	COMe	COOMe	193–194 (*i*-PrOH)	70
6b	COOMe	COOMe	178–180 (*i*-PrOH)	75
6c	COOEt	COOEt	201–203 (*i*-PrOH)	70
6d	CN	COOEt	134–135 (*i*-PrOH)	78
6e	CN	CN	186–188 (EtOH)	80

Table 2. Anti-platelet activity (IC_{50}) of compounds **6a–e**[a]

Compd	X	Y	IC_{50} (µM)
6a	COMe	COOMe	25
6b	COOMe	COOMe	45
6c	COOEt	COOEt	20
6d	CN	COOEt	[b]
6e	CN	CN	[c]
Ticlopidine	—	—	1550
Sulfinpyrazone	—	—	696

[a]The anti-platelet activities of the standard and the synthesized compounds were tested by the turbidimetric method in washed platelets using thrombin as agonist. Methods for these assays have been previously published elsewhere.[12] Results shown are means of at least three experiments. The mean standard error of IC_{50} values was 10%.
[b]Promote platelet aggregation.
[c]Promote platelet aggregation or slight inhibition depending on the dose.

Scheme 1. (i) CH_3OCH_2Cl, $DMAP/CH_2Cl_2$; (ii) $PdCl_2(PPh_3)_2$, $Bu_3SnCH=CH_2$/toulene; (iii) O_3/CH_2Cl_2; (iv) 6 N HCl; (v) XCH_2Y, piperidine/methanol.

even inversion of the activity; thus compound **6d** actually stimulates platelet aggregation. Preliminary pharmacological studies were performed in order to evaluate the anti-PDE-III activity of compounds **6a**, **6b**, and **6c**. These values were determined using purified preparations obtained from ventricular tissue of guinea pigs according to a previously reported protocol[20] and reveal that, in contrast to other pyridazinones, the anti-platelet effect observed is not due to the inhibition of the PDE-III (results not shown).

Studies performed on platelet lysates by protein electrophoresis and western blot with an anti-phosphotyrosine antibody show that compounds **6a**, **6b**, and **6c** increase the tyrosine-phosphorylation profile of some platelet proteins (results not shown). This fact could be related to the anti-platelet activity shown by these compounds. The phosphorylation state of any protein is the result of the balance between phosphorylation/dephosphorylation processes. Bearing this point in mind, the increase in the level of tyrosine-phosphorylation shown by these compounds could be explained both by an increase in tyrosine-kinase activity or by an inhibition in tyrosine-phosphatase activity.

In conclusion, we have developed a palladium-assisted synthetic approach to 5-alkylidene-6-phenyl-3(2*H*)-pyridazinones **6a–e**, which show promising anti-platelet activity. Further studies are in progress in our laboratory in order to determine in detail both the mechanism of action and the structural requirements for this series of anti-platelet agents.

Acknowledgements

References and Notes

1. Sotelo, E.; Centeno, B. N.; Rodrigo, J.; Raviña, E. *Tetrahedron* **2002**, *58*, 2389.
2. Davies, M. J.; Thomas, A. C. *Br. Heart J.* **1985**, *53*, 363.
3. Fuster, V.; Badimon, L.; Badimon, J. J.; Chesebro, J. H. *N. Engl. J. Med.* **1992**, *326*, 242.
4. Kitamura, S.; Fukushi, H.; Miyawaki, T.; Kawamura, M.; Konishi, N. *J. Med. Chem.* **2001**, *44*, 2438.
5. Liverton, N. J.; Armstrong, D. J.; Claremon, D. A.; Remy, D. C.; Baldwing, J. J.; Lynch, R. J.; Zhang, G.; Gould, R. J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 483.
6. Aguirre, F. V.; Topol, E. J.; Ferguson, J. J.; Anderson, K.; Blankenship, J. C.; Heuser, R. R.; Sigmon, K.; Tailor, M.; Gottleb, R.; Hannovich, G.; Rosemberg, M.; Donohue, T. J.; Weisman, H. F.; Califf, R. M. *Circulation* **1995**, *91*, 2882.
7. Simpfendorfer, C.; Kottke-Marchant, K.; Lowrie, M.; Andres, R. J.; Burns, D. M.; Miller, D. P.; Cove, C. S.; DeFranco, A. C.; Ellis, S. G.; Moliterno, D. J.; Raymond, R. E.; Sutton, J. M.; Topol, E. J. *Circulation* **1997**, *96*, 76.
8. Chew, D. P.; Bhatt, D. L.; Sapp, S.; Topol, E. J. *Circulation* **2001**, *103*, 201.
9. Moos, W. H.; Humblet, C. C.; Sircar, I.; Rithner, C.; Weishar, R. E.; Bristol, J. A.; McPhail, A. J. *J. Med. Chem.* **1995**, *38*, 1990, and references cited therein.
10. Frank, H.; Heinisch, G. Pharmacologically Active Pyridazines. Part 2. In *Progress in Medicinal Chemistry*; Ellis, G. P., Luscombe, D. K., Eds.; Elsevier: Amsterdam, 1992; Vol. 29, p 141.
11. Laguna, R.; Rodriguez-Liñares, B.; Cano, E.; Estévez, I.; Raviña, E.; Sotelo, E. *Chem. Pharm. Bull.* **1997**, *45*, 1151.
12. Montero-Lastres, A.; Fraiz, N.; Laguna, R.; Cano, E.; Estévez, I.; Raviña, E. *Biol. Pharm. Bull.* **1999**, *22*, 1376.
13. Sotelo, E.; Fraiz, N.; Yañez, M.; Terrades, V.; Laguna, R.; Cano, E.; Raviña, E. *Bioorg. Med. Chem.* In press.
14. Sotelo, E.; Raviña, E.; Estévez, I. *J. Heterocyclic Chem.* **1999**, *36*, 985.
15. Estévez, I.; Coelho, A.; Raviña, E. *Synthesis* **1999**, *9*, 1666.
16. Complete details of the synthesis and spectral data will be published elsewhere in a full paper. All compounds gave satisfactory microanalytical (C, H, N±0.4%) and spectral data (300 MHz ^{1}H NMR and FTIR).
17. **6a**: ^{1}H NMR (DMSO-d_6) δ: 13.20 (bs, 1H), 7.55 (m, 5H+1H), 6.72 (s, 1H), 3.78 (s, 3H), 2.38 (s, 3H). **6b**: ^{1}H NMR (DMSO-d_6) δ: 13.40 (bs, 1H), 7.60–7.45 (m, 5H+1H), 6.68 (s, 1H), 3.73 (s, 3H), 3.67 (s, 3H). **6d**: ^{1}H NMR (DMSO-d_6) δ: 13.56 (bs, 1H), 7.92 (s, 1H), 7.46 (m, 5H), 7.38 (s, 1H), 4.25 (q, $J=7.15$ Hz, 2H), 1.23 (t, $J=7.15$ Hz, 3H). **6e**: ^{1}H NMR (DMSO-d_6) δ: 13.63 (bs, 1H), 8.27 (s, 1H), 7.50 (m, 5H), 7.42 (s, 1H).
18. The *E*-configuration for **6a** and **6d** was assigned unambiguously from the ^{1}H-coupled ^{13}C NMR spectrum, for example for **6a** the vicinal coupling constant of the olefinic proton with the ketone is considerably larger (13.7 Hz) than that with the ester (7.1 Hz).
19. Born, G. V. R. *Nature* **1962**, *194*, 927.
20. Dal Piaz, V.; Giovannoni, M. P.; Castellana, C.; Palacios, J. M.; Beleta, J.; Doménech, T.; Segarra, V. *Eur. J. Med. Chem.* **1998**, *33*, 789.