Design, Synthesis, and Structure–Activity Relationships of a Novel Series of 5-Alkylidenepyridazin-3(2*H*)-ones with a Non-cAMP-Based Antiplatelet Activity

Alberto Coelho,[†] Enrique Raviña,*[,‡] Nuria Fraiz,[§] Matilde Yáñez,[§] Reyes Laguna,[§] Ernesto Cano,[§] and Eddy Sotelo*[,†,‡]

Instituto de Farmacia Industrial (IFI), Departamento de Química Orgánica, and Departamento de Farmacología, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela, 15782, Spain

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5-Alkylidenepyridazin-3-ones with four points of diversity (R^2, R_6, X, Y) have been synthesized and evaluated as platelet aggregation inhibitors. Several derivatives eliciting antiplatelet activity in the low micromolar range (e.g., **14e**, **14k**, **14p**, **14v**, $IC_{50} \cong 1\ \mu$ M) were identified. Structure–activity relationships studies on these compounds revealed the key molecular determinants of this new family of antiplatelet agents: (a) two ester groups in the alkoxy moieties; (b) lipophilic substituents at the N2 position of the pyridazin-3-one. The preliminary results of a pharmacological study aimed at determining the mechanism of action of a set of representative compounds revealed that, unlike other pyridazinones, the documented antiplatelet effect is not a consequence of a PDE-III inhibitory activity.

Introduction

Platelets constitute the primary cellular component of hemostasis in mammalian organisms, with their primary physiologic role being to survey the integrity of the circulatory system and respond rapidly at sites of vascular injury.[1] Activation of platelets[2] in vivo is tightly regulated, with circulating platelets remaining quiescent under normal conditions. The interaction of platelets with collagen and/or thrombin initiates a complex biochemical sequence of signal transduction resulting in a functional cell response. Thus, upon vascular damage, platelets are exposed to extracellular matrix proteins such as subendothelial collagen, factors released or generated by activated platelets, and thrombin, which activates platelets. This leads to the formation of a platelet thrombus that prevents further blood loss. Although this mechanism is vital when tissue is damaged, inappropriate activation of platelets can lead to thrombosis; alternatively, failure of platelets to become activated can lead to excessive bleeding. Inhibitors of platelet aggregation currently employed in therapy (Figure 1) are represented by several drug categories[3] such as nonsteroidal anti-inflammatory drugs (NSAIDS),[4] adenosine diphosphate receptor antagonists,[5] and glycoprotein IIb/IIIa (GPIIb/IIIa) antagonists.[6]

A critical analysis of clinical data leads to the conclusion that the therapeutic arsenal currently available to control platelet function is still very limited. Of all the agents in current widespread use (Figure 1), aspirin may be considered to show the most favorable risk/benefit ratio. Despite the documented utility of these drugs, their efficacy and selectivity are not sufficiently high and there are reasons to believe that substantial improvements in antiplatelet therapy can be made. This issue is of primary importance because none of the currently marketed antithrombotic agents, or indeed most of the compounds in advanced clinical trials, are free of significant bleeding risk or meet the basic requirements expected for the ideal antiplatelet agent.[7] Therefore, the search for agents with new mechanisms of action is a goal of great interest not only for their possible use as drugs but also because such compounds could be employed as valuable pharmacological tools to provide comprehensive information regarding platelet function.

Driven by the widely documented PDE III-based antiplatelet activity of a series of 5-arylpyridin-2(1*H*)-ones[8] (Milrinone, Amrinone) and 6-arylpyridazin-3(2*H*)-ones[9] (zardaverine, pimobendan, levosimendan) (Figure 2), our group has been carrying out a project concerning the synthesis and study of the antiplatelet activity of pyridazin-3(2*H*)-ones. Initially, we focused our attention on compounds related to the general structure **I**[10] (incorporating alkoxy or dialkoxy fragments in the phenyl group at position 6 of the heterocycle, Figure 3). Screening of these series highlighted several relatively potent compounds but, most importantly, indicated the significant effect that the substituent at position 5 has on the antiplatelet activity of these compounds. Thus, with the aim of studying in greater detail the effect of the group at position 5 of the heterocyclic core on the antiplatelet activity of this series, the substituents at position 6 of the phenyl group have been removed (compounds **II**, Figure 3).

A wide collection of 5-substituted 6-phenylpyridazin-3(2*H*)-ones (**II**) was obtained and tested as antiplatelet agents. As expected, removal of the substituents in the phenyl ring causes a significant (or complete) decrease in the platelet inhibitory activity, but some compounds in these series retained a modest (150–500 μM) activity.[11] This suggests that other mechanisms could be operating, opening the possibility of identifying novel pyridazinone-based antiplatelet agents with a non-cAMP-based activity. The exhaustive pharmacological studies performed on a collection of around 50 new compounds related to the general structure **II** (including C-, N-, O- and S-based functional groups at position 5) reveal that both the mechanism of action and the antiplatelet activity of compounds in this series are highly dependent on the nature of the substituent present at position 5 of the heterocyclic scaffold.[11]

The systematic studies performed on series **I** and **II** led to the identification of new pyridazin-3(2*H*)-ones that act by at least three different mechanisms of action: PDE III inhibition,[10] modification of the cytosolic calcium levels,[11] and/or changes

* To whom correspondence should be addressed. For E.R.: phone, ++34-981-594628; fax, ++34-981-594912; e-mail, qofara@usc.es. For E.S.: phone, ++34-981-563100; fax, ++34-981-528093; e-mail, qosotelo@usc.es.

[†] Instituto de Farmacia Industrial (IFI).

[‡] Departamento de Química Orgánica.

[§] Departamento de Farmacología.

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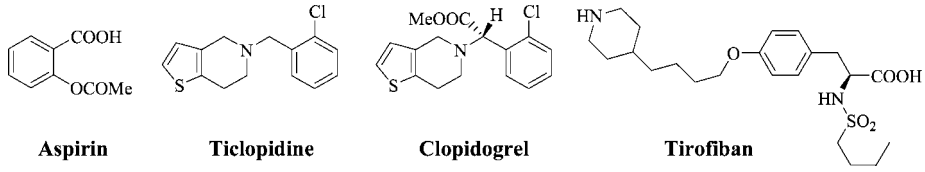


Figure 1. Structures of representative antiplatelet agents in clinical use.

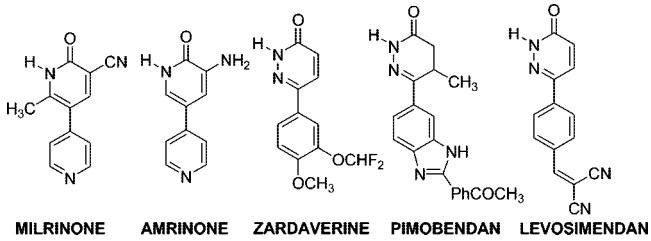


Figure 2. Representative examples of azinone-based inhibitors of PDE III.

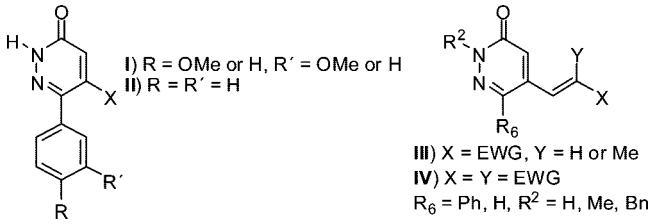


Figure 3. Structures of pyridazin-3-ones **I** and **II** and targeted 5-alkylidenepyridazin-3-ones **III** and **IV**.

in platelet protein phosphorylation.[11,12] Although the different mechanisms of action complicate SAR studies, for the functionally homogeneous series (**II**) it was observed that compounds bearing electron-withdrawing groups at position 5 of the heterocycle generally showed a slightly higher antiplatelet activity.[11] As a consequence, the new series of vinyl analogues of compounds **II** (as represented under the general structures **III** and **IV**, Figure 3) were envisaged. The design of these derivatives was also supported by the presence of similar alkenyl fragments in compounds such as levosimendan (Figure 2) or thyrphostins which, although structurally different, show potent antiplatelet activity. Preliminary results from this work have been published previously.[12,13] We now present a full account of this long-term study, which has enabled the identification of novel and potent antiplatelet agents and the establishment of the most salient features of the SAR in this series.

Results and Discussion

The target 5-alkylidenepyridazin-3-ones **III** and **IV** (Figure 3) bearing different substitution patterns at positions 2 and 6 were accessible from synthons **1** (Figure 4) and **12** (Scheme 7), respectively. These precursors were submitted to the conditions of the Heck alkenylation or Knoevenagel condensation, and experimental details are depicted in the synthetic pathways shown in Schemes 17. 5-Halopyridazin-3-ones **1** constitute the key starting materials employed throughout the project. In order to address the requirements of the preliminary SAR studies, a subset of intermediates **1** (Figure 4) containing different substitution patterns at positions 2 and 6 were obtained by following previously described procedures.[14] Structural elaboration of scaffolds **1** and **12** was achieved by installing or constructing the alkylidene fragments using a subset of olefins (**2**) or malonic acid derivatives (**13**) to provide elements of diversity (Figure 4).

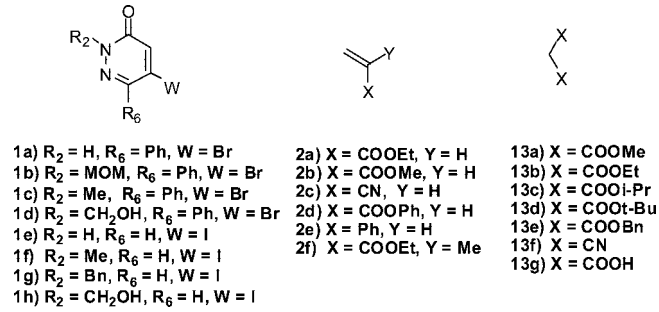
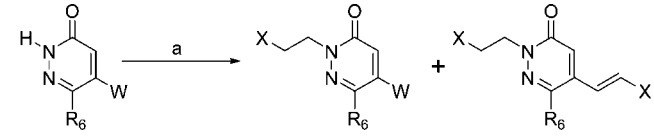


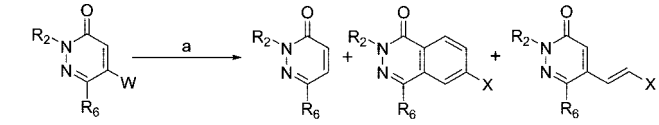
Figure 4. Structures of the scaffolds **1** and the diversity elements **2** and **13**.

Scheme 1[a]



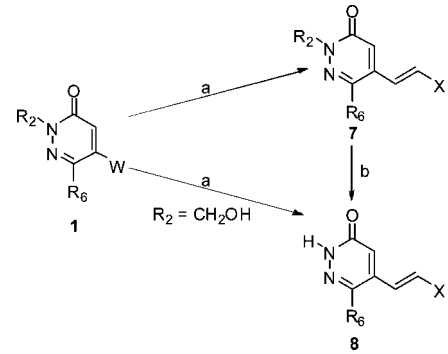
[a] Reagents and conditions: (a) CH_2=CH−X, $Pd(OAc)_2$, PPh_3, TEA, DMF, 110°C, sealed tube.

Scheme 2[a]



[a] Reagents and conditions: (a) CH_2=CH−X, $Pd(OAc)_2$, PPh_3, TEA, DMF, 110°C, sealed tube.

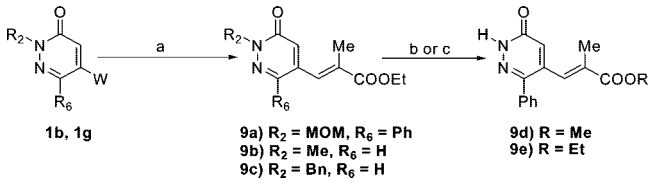
Scheme 3[a]



[a] Reagents and conditions: (a) CH_2=CH−X, $PdCl_2P[(o\text{-tolyl})_3]_2$, TEA, DMF, 110°C, sealed tube; (b) $AlCl_3$, toluene or 6 N HCl, reflux.

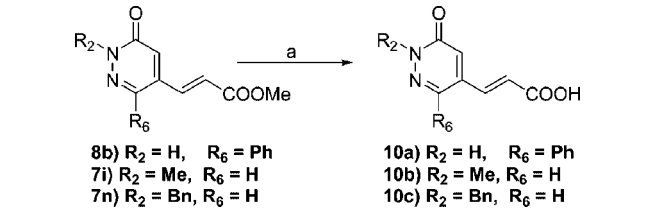
Synthesis of (*E*)-3-(6-Oxopyridazin-4-yl)acrylic Acid Derivatives. Compounds represented under the general structure **III** (Figure 3) were synthesized as reported in Schemes 1−5, with the well-established Heck reaction[15] used as the key transformation. The absence of comprehensive studies on the

Scheme 4[a]



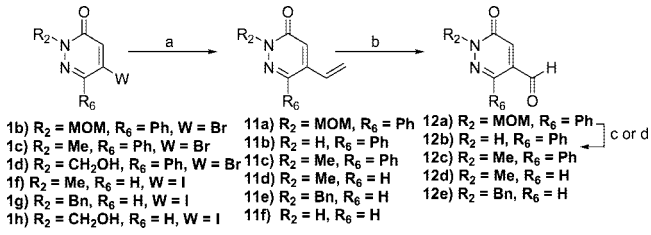
[a] Reagents and conditions: (a) CH_2=C(Me)−COOEt, $PdCl_2P(Ph_3)_2$, TEA, DMF, 110°C, sealed tube; (b) 6 N HCl, reflux; (c) $AlCl_3$, CH_2Cl_2.

Scheme 5[a]



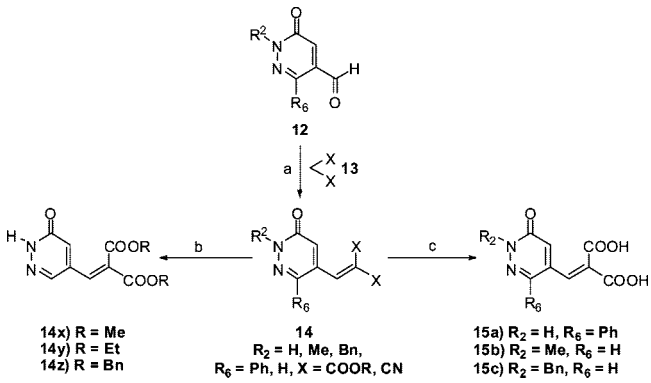
[a] Reagents and conditions: (a) LiOH·H_2O, THF·H_2O.

Scheme 6[a]



[a] Reagents and conditions: (a) $(Bu)_3Sn$−CH=CH_2, $PdCl_2(PPh_3)_2$, toluene (90 °C for W = Br, room temp for W = I); (b) (1) O_3, CH_2Cl_2, −78 °C, (2) $S(Me)_2$; (c) 6 N HCl/MeOH; (d) $AlCl_3/CH_2Cl_2$.

Scheme 7[a]



[a] Reagents and conditions: (a) $AlCl_3$, dioxane, 100°C; (b) 6 N HCl, reflux; or $AlCl_3$, CH_2Cl_2; (c) LiOH·H_2O, THF·H_2O.

Heck olefination on 1,2-diazines, as well as the predicted differences in reactivity within the starting 5-halopyridazin-3(2*H*)-ones **1** (Figure 4), required us to carry out a laborious experimental examination to identify the optimal conditions to perform the Heck alkenylation on this system. Initial experiments were carried out on the simplest members of the starting series **1** [e.g., 5-halopyridazin-3(2*H*)-ones **1a** and **1e**], which were reacted with a slight excess (1.5 equiv.) of the appropriate olefin **2** under classical Heck conditions (Scheme 1).[15] In all cases these experiments failed to give the desired 5-alkenylpyridazin-3(2*H*)-ones **III**, and a variable mixture containing compounds **3** and **4** was always isolated (Scheme 1, Table 1). Modification of the catalytic system [e.g., $Pd(PPh_3)_2Cl_2$, $Pd_2(dba)_3$], base (e.g., Na_2CO_3, DIPEA), or solvent (e.g., acetonitrile, toluene) did not significantly alter the results. Formation of 2-substituted 3-pyridazinones (compounds **3** and **4**, Scheme 1) under these conditions can be rationalized in terms of a conjugate aza-Michael addition between the pyridazin-3(2*H*)-one (**1a**, **1e**) and the highly activated sterically unhindered acrylic acid derivative (**2**). The observed ratio also confirms that the addition of the heterocyclic NH group to the negatively substituted olefin (**2**) is favored over the desired coupling reaction. Isolation of compounds **4** (Scheme 1) can be explained in terms of a consecutive Heck alkenylation on the reactive halogen (Br or I) of the Michael adducts **3** once the tautomeric carbonyl group has been blocked (as a consequence of the previous aza-Michael reaction). It should be pointed that analogous experiments using styrene (a nonactivated olefin) led to recovery of unreacted pyridazinones **1a** and **1e** (Table 1, entries **d**, **g**). The reactivity observed for pyridazin-3(2*H*)-ones **1a** and **1e** toward Heck alkenylation (Scheme 1), although with different behavior, is not dissimilar to that described in the study of other palladium-catalyzed reactions on pyridazin-3(2*H*)-ones.[16]

Table 1. Reaction of Pyridazin-3(2*H*)-ones **1a** and **1e** with **2** under Heck Conditions

entry	X	R_6	W	yield of **3** (%)	yield of **4** (%)
a	COOEt	Ph	Br	70	20
b	COOMe	Ph	Br	63	15
c	CN	Ph	Br	65	18
d	Ph	Ph	Br		
e	COOMe	H	I	73	12
f	COOEt	H	I	58	16
g	Ph	H	I		

Table 2. Heck Alkenylation of Pyridazinones **1**

entry	R_2	R_6	olefin	X	yield of **5** (%)	yield of **6** (%)	yield of **7** (%)
a	MOM	Ph	CH_2=CH−CO_2Et	CO_2Et	50	28	10
b	MOM	Ph	CH_2=CH−CO_2Me	CO_2Me	45	25	8
c	MOM	Ph	CH_2=CH−CN	CN	48	30	10
d	MOM	Ph	CH_2=CH−Ph	Ph			50
e	Bn	H	CH_2=CH−CO_2Et	CO_2Et		7	53

In an effort to circumvent the drawbacks outlined above, position 2 of the heterocyclic core was blocked by the introduction of different groups (e.g., MOM, Me, Bn, CH_2OH). The palladium-catalyzed Heck alkenylation of the heterocycle was then studied employing, as a model system, ethyl acrylate (2 equiv), PPh_3 as a ligand, Et_3N as the base, and $Pd(OAc)_2$ as the palladium source in DMF (Scheme 2). These experiments showed that, as far as the reactive halogen atom involved in the cross-coupling reaction is concerned, the yields of the desired 5-alkenylpyridazinones (**7**) varied from very low (5–14% for W = Br) to satisfactory (50–65% for W = I). These observations are a clear consequence of the reactivity differences between the two different halogen atoms (Br vs I) involved in the palladium-catalyzed cross-coupling reaction, a factor that strongly influences coupling behavior. Thus, for scaffolds **1b–c** (W = Br) the main products isolated from the Heck alkenylation were debrominated pyridazinones **5** (45–50%, Table 2). Surprisingly, in these experiments a small quantity of a compound identified as phthalazinone **6** was also isolated.

An analogous product distribution was obtained when the conditions described above (Scheme 2) were applied to the reaction of **1b–c** with other representative olefins **2** (e.g., methyl acrylate or acrylonitrile). The structures of compounds **5–7** were unambiguously established on the basis of their analytical and spectroscopic data, particularly for the unexpected phthalazi-

nones **6**, whose structural assignment was also supported by additional NMR experiments and single-crystal X-ray analysis of compound **6a**.[13] Preliminary mechanistic proposals to explain the formation of phthalazinones during this reaction have recently been put forward.[13] The low yields generally obtained for compounds **7**, as well as the fact that debrominated pyridazinones **5** were obtained as the main product in the Heck alkenylation for some compounds (e.g., for W = Br), suggests that migratory insertion of the olefin into the σ-heteroarylpalladium(II) complex is not a favored process under the conditions employed. For this reason, different catalysts, phosphines, and solvents from the plethora of protocols and catalytic systems described for the successful Heck alkenylation[15] were tested. The screening of different experimental conditions allowed identification of $PdCl_2P[(o\text{-tolyl})_3]_2$ (5%) as the best and most general catalytic system to perform the Heck coupling on scaffolds **1**, regardless of the halogen atom attached to the heterocycle. The typical procedure also employs DMF or acetonitrile as solvent and 2 equiv of triethylamine. The application of these optimized conditions to a variety of scaffolds **1** and olefins **2** allowed the preparation of a library of 5-alkenyl-6-phenyl-3-pyridazinones **7** in moderate to good yields (Scheme 3, Table 3, entries 1–22).

The preparation of pyridazin-3(2*H*)-ones without alkyl residues on the lactam nitrogen (free NH group, compounds **8**) was easily accomplished by cleavage of the methoxymethyl or benzyl protecting groups in the appropriately blocked 5-alkenylpyridazin-3(2*H*)-ones **7** (Scheme 3). Removal of the ether (MOM) linkage was successfully performed under the simplest cleavage conditions utilizing hydrochloric acid (6 N) or, alternatively, under mild conditions using Lewis acids. For those compounds incorporating a benzyl fragment as the lactam blocker, aluminum chloride proved to be a highly effective and selective reagent to promote cleavage of the protecting group. Alternatively, 5-alkenylpyridazin-3(2*H*)-ones **8** (Scheme 3) were prepared by submitting the starting ene adducts **1d** and **1 h** ($R_2 = CH_2OH$) to the previously optimized conditions for the Heck reaction on scaffolds **1** (Scheme 3). As recently documented,[14a] this one-pot transformation involves the initial Heck olefination of the adduct (**1d** and **1 h**) and subsequent elimination of the thermolabile hydroxymethyl group through a retroene transformation (Scheme 3).

The previously optimized coupling conditions proved to be general in scope not only when applied to different halogens (Br, I) and olefins represented by the general structure **2** but also in allowing the successful introduction of disubstituted alkenes, as exemplified by ethyl methacrylate (Scheme 4). These experiments afforded disubstituted olefins **9**, which were required during the preliminary exploration of the SAR in this series of compounds. Interestingly, acid cleavage (6 N HCl/MeOH) of the methoxymethyl group in compound **9a** gave the methyl ester **9d** because of transesterification of the ethoxy residue in the ester group. Such a transformation, which was not observed in the earlier series (**8**), was circumvented by employing ethanol as the solvent during hydrolytic cleavage or, alternatively, by using aluminum chloride as a milder deblocking agent (Scheme 4).

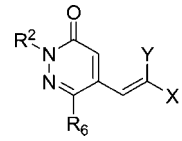
The synthesis of carboxylic acids (**10**, Scheme 5) incorporating representative functional groups at positions 2 and 6 was accomplished, in moderate yields (50–65%), by hydrolysis of the corresponding methyl esters (**8b**, **7i**, or **7n**, Scheme 5) using lithium hydroxide monohydrate in tetrahydrofuran. The exocyclic double bond in all of the previously obtained pyridazinones incorporating alkenyl fragments (including the methacrylates **9**) had an *E* configuration, as evidenced by the NMR spectroscopic data (see Supporting Information).

Synthesis of 2-(6-Oxopyridazin-4-yl)methylenemalonic Acid Derivatives. Heterocyclic carbaldehydes **12** were prepared from 5-halopyridazin-3-ones **1** by a simple and general palladium-assisted pathway (Scheme 6). Functionalization at position 5 of the heterocyclic core by reaction of pyridazinones **1** ($R^2 \neq H$) with tributylvinylstannane under Stille conditions [under reflux or room temperature depending on the reactivity of the halogen (Br or I) at position 5] allowed the preparation of a subset of 5-vinylpyridazin-3(2*H*)-ones **11** Scheme 6). Oxidative cleavage of the vinyl group in compounds **11** by ozonolysis and subsequent treatment with dimethyl sulfide afforded the target 5-formylpyridazin-3(2*H*)-ones **12** in satisfactory yields. Carbaldehyde **12b** (with a free NH group) was obtained by removing the MOM group from **12a** (6 N HCl) or, alternatively, by ozonolysis of the 5-vinylpyridazin-3(2*H*)-one **11b** (Scheme 6). All attempts to develop a similar strategy as described for derivative **12a** in an effort to prepare the 5-formylpyridazin-3(2*H*)-one by cleavage of the benzyl group in compound **12e** were unsuccessful, even when applying Lewis acids ($AlCl_3$ or BBr_3) as cleaving reagents, an approach that worked reasonably well on pyridazinones incorporating sensitive functionalities at position 5. Synthesis of 2,2′-alkylidenepyridazin-3(2*H*)-ones **14** (general structure **IV**, Scheme 7, Table 3, entries 23–53) was achieved in moderate to excellent yields by subjecting aldehydes **12** to Knoevenagel condensations with a subset of malonic acid derivatives (**13**). Classical conditions, using piperidine as the catalyst, were employed for the preparation of the early series (starting from **12b**).[12] However, in an attempt to improve yields and facilitate workup/purification during library production, a high-throughput parallel procedure employing anhydrous aluminum chloride as the catalyst was developed. With the aim of guaranteeing acceptable levels of diversity for the library, different active methylene partners (compounds **13**, Figure 4) were selected and reacted with the heterocyclic carbaldehydes **12**. As observed in Table 3 (entries 23–53), the target compounds **14** were obtained in yields that ranged from acceptable to satisfactory (50–80%).

Taking into account the synthetic difficulties experienced during the preparation of 5-formylpyridazin-3(2*H*)-one, the synthesis of some representative 5-alkylidenepyridazin-3(2*H*)-ones in which the NH group was free (**14x**−**z**) was accomplished by cleavage of the benzyl group from the corresponding esters (Scheme 7). Unfortunately, all attempts to condense carbaldehydes **12** with Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione) afforded complex reaction mixtures in which the main isolated compound was the corresponding acrylic acid (30–43%). Similarly, the use of 1,3-diketones (e.g., pentane-2,4-dione or dimedone) or compounds containing ketonic groups proved to be problematic and afforded the expected Knoevenagel compounds in low yields, probably a consequence of Michael addition to the formed double bond (even when working at 0 °C). Knoevenagel condensation of heterocyclic carbaldehydes **12** with malonic acid under different experimental conditions (e.g., the Doebner modification) was unsuccessful in our hands. As a consequence, dicarboxylic acids **15** (Scheme 7) were obtained by hydrolysis of the corresponding methyl esters under mild conditions ($LiOH \cdot H_2O$).

Structure–Activity Relationships Studies. In order to evaluate the most prominent features of the structure–activity relationship for this series, a focused library of almost 60 new pyridazinones with four points of diversity (R^2, R_6, X, Y) was screened to determine the platelet inhibitory activity

Table 3. Structure and Antiplatelet Activity of Pyridazin-3-ones under Study



entry	compd	R^2	R_6	X	Y	yield (%)	IC_{50} (μM)[c]
1	**7a**	MOM	Ph	COOEt	H	70	*d*
2	**7b**	MOM	Ph	COOMe	H	70	*e*
3	**7c**	MOM	Ph	CN	H	50	*d*
4	**7f**	Me	Ph	COOEt	H	71	*d*
5	**7g**	Me	Ph	COOMe	H	76	*d*
6	**7h**	Me	H	COOEt	H	75	*d*
7	**7i**	Me	H	COOMe	H	60	*d*
8	**7j**	Me	H	CN	H	63	*d*
9	**7k**	Me	H	COOPh	H	60	*d*
10	**7l**	Me	H	Ph	H	50	*d*
11	**7m**	Bn	H	COOEt	H	67	*d*
12	**7n**	Bn	H	COOMe	H	60	*d*
13	**7o**	Bn	H	CN	H	68	221.30 ± 2.20
14	**7p**	Bn	H	COOPh	H	57	*d*
15	**7q**	Bn	H	Ph	H	89	*d*
16	**8a**	H	Ph	COOEt	H	75[a]	*d*
17	**8b**	H	Ph	COOMe	H	78[a]	*d*
18	**8c**	H	Ph	CN	H	77[a]	*d*
19	**8d**	H	Ph	COOPh	H	70[a]	*d*
20	**8e**	H	Ph	Ph	H	70[a]	*d*
21	**8f**	H	H	COOEt	H	60[a]	*d*
22	**8g**	H	H	COOMe	H	62[a]	*d*
23	**9b**	Me	H	COOEt	Me	51	*d*
24	**9c**	Bn	H	COOEt	Me	46	*d*
25	**9d**	H	Ph	COOEt	Me	67[a]	*d*
26	**9e**	H	Ph	COOMe	Me	64[a]	*d*
27	**10a**	H	Ph	COOH	H	57[b]	*d*
28	**10b**	Me	H	COOH	H	50[b]	*d*
29	**10c**	Bn	H	COOH	H	65[b]	*d*
30	**14a**	H	Ph	COOMe	COOMe	73	45.00 ± 4.23
31	**14b**	H	Ph	COOEt	COOEt	65	20.00 ± 1.17
32	**14c**	H	Ph	COO-*i*-Pr	COO-*i*-Pr	80	17.50 ± 1.34
33	**14d**	H	Ph	COO-*t*-Bu	COO-*t*-Bu	76	7.50 ± 0.91
34	**14e**	H	Ph	COOBn	COOBn	78	1.80 ± 0.14
35	**14f**	H	Ph	CN	CN	78	*d*
36	**14g**	Me	Ph	COOMe	COOMe	71	9.20 ± 1.31
37	**14h**	Me	Ph	COOEt	COOEt	70	17.00 ± 3.30
38	**14i**	Me	Ph	COO-*i*-Pr	COO-*i*-Pr	62	4.10 ± 0.28
39	**14j**	Me	Ph	COO-*t*-Bu	COO-*t*-Bu	73	45.00 ± 4.23
40	**14k**	Me	Ph	COOBn	COOBn	69	1.40 ± 0.16
41	**14l**	Me	H	COOMe	COOMe	60	15.60 ± 0.90
42	**14m**	Me	H	COOEt	COOEt	50	12.30 ± 0.90
43	**14n**	Me	H	COO-*i*-Pr	COO-*i*-Pr	72	13.30 ± 1.70
44	**14o**	Me	H	COO-*t*-Bu	COO-*t*-Bu	75	95.00 ± 18.70
45	**14p**	Me	H	COOBn	COOBn	70	1.64 ± 0.160
46	**14q**	Me	H	CN	CN	61	475.25 ± 3.70
47	**14r**	Bn	H	COOMe	COOMe	55	4.86 ± 0.38
48	**14s**	Bn	H	COOEt	COOEt	52	3.62 ± 0.32
49	**14t**	Bn	H	COO-*i*-Pr	COO-*i*-Pr	58	2.90 ± 0.34
50	**14u**	Bn	H	COO-*t*-Bu	COO-*t*-Bu	60	6.25 ± 0.76
51	**14v**	Bn	H	COOBn	COOBn	81	1.36 ± 0.11
52	**14w**	Bn	H	CN	CN	40	125.84 ± 1.10
53	**14x**	H	H	COOMe	COOMe	45[a]	40.25 ± 3.01
54	**14y**	H	H	COOEt	COOEt	60[a]	8.70 ± 1.05
55	**14z**	H	H	COOBn	COOBn	65[a]	3.58 ± 0.37
56	**15a**	H	Ph	COOH	COOH	60[b]	*d*
57	**15b**	Me	H	COOH	COOH	56[b]	*d*
58	**15c**	Bn	H	COOH	COOH	49[b]	*d*
				sulfinpyrazone			509.10 ± 49.00
				milrinone			4.70 ± 0.50

[a] Reported yield corresponds to cleavage of the benzyl group. [b] Reported yield corresponds to hydrolysis of the methyl ester. [c] Mean ± SEM of five separate determinations. IC_{50} value (μ M) interpolated from concentration−inhibition curves. [d] Inactive (significant inhibition of platelet aggregation at concentrations lower than 1000 μM not shown). [e] Promote platelet aggregation.

(Table 3). The pyridazin-3(2*H*)-one template incorporating an exocyclic double bond at position 5 was retained throughout these studies.

The platelet aggregation inhibitory activity was examined on washed human platelets by the turbidimetric method of Born.[17] Since thrombin is the most powerful physiological stimulus for

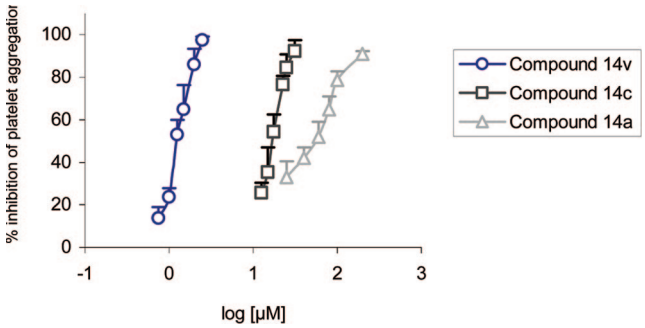


Figure 5. Representative curves for the inhibition of platelet aggregation obtained for compounds **14a**, **14c**, and **14v** using thrombin (0.25 U/mL) as inducer of platelet aggregation.

platelet aggregation (specially in biological buffers) and a commonly employed agonist during the in vitro determination of antiplatelet activity, it was routinely employed during screening. Compounds were tested in DMSO solutions starting at high concentrations (2000 μM). All reported IC_{50} values are the mean of at least five experiments employing human blood from different individuals. Unless otherwise specified, results shown in the text and tables are expressed as the mean ± SEM. Significant differences between two mean values ($p < 0.05$ or $p < 0.01$) were determined by one-way analysis of variance (ANOVA) and/or by Student's t test for nonpaired data. The results of these experiments are summarized in Table 3. Active compounds inhibited platelet aggregation in a dose-dependent manner. Representative curves obtained for compounds **14a**, **14c**, and **14v** are depicted in Figure 5.

Inspection of the biological data (Table 3) shows that structurally novel and highly potent antiplatelet agents were identified during this study. Most of these compounds exhibit IC_{50} values in the low micromolar range (1–20 μM). A general view of Table 3 also reveals the substantial contribution of the two electron-withdrawing groups on the exocyclic ethylenic system to the documented inhibitory activity. Because of the significant number of compounds tested and for the sake of brevity and clarity, the analysis and interpretation of the data will be performed at two levels. First, because of its great importance for the antiplatelet activity in this series, the effect of the substituent in the 5-position will be discussed. Subsequently, the contribution of the substituent on the lactam nitrogen (R^2) and the presence of the phenyl ring at position 6 will be discussed.

The screening of compounds with a single substituent on the exocyclic ethylenic system at position 5 (compounds **7**, **8**, and **10**, Table 3, entries 1–22) revealed that inactive or slightly active derivatives were obtained. These data showed that the antiplatelet activity was effectively unmodified (with most of the compounds remaining inactive) by the attachment of structurally diverse chemical groups to the double bond (X = CN, COOR, COOH, Ph) regardless of the substitution partner at position 2 (H, Me, or Bn) or 6 (H or Ph). The only exception to this observation was the acrylonitrile derivative **7o** (Table 3, entry 13), which showed moderate activity.

Comparison of the antiplatelet data obtained for some representative 5-alkylidenepyridazinones **8** (Figure 6, compounds **8a–c**, Table 3, entries 16–18) with their parent compounds in the previous series[11] (Figure 6, compounds **IIa–c**) revealed that the introduction of a vinyl group as a linker completely removed the weak platelet inhibitory activity (0.5–1 mM) observed in the earlier series (**IIa–c**). Interestingly, the biological evaluation of the collateral phthalazinones **6** revealed

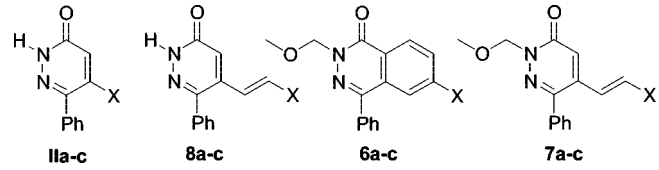


Figure 6. Analysis of structurally related series.

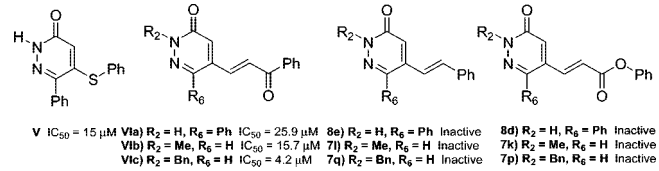


Figure 7. New derivatives designed by starting from the lead compounds **V**[11] and **VI**.[19]

a modest antiplatelet effect [IC_{50} (μ M) **6a** = 142, **6b** = 151, **6c** = 170] that was not completely evident considering the absence of activity observed in the alkenylpryridazin-3(2*H*)-ones **8a–c** (Figure 6, Table 3, entries 16–18) and their closely related analogues 2-methoxymethyl 5-alkylidenepyridazin-3-ones **7a–c** (Figure 6, Table 3, entries 1–3). Thus, the antiplatelet activity described here for compounds **6** could be mechanistically related to the previously documented platelet inhibitory effect in the phthalazinones series.[18]

The pharmacological assay of some 5-alkenylpyridazin-3-ones (compounds **8e**, **8d**, **7l**, **7q**, **7k**, and **7p**, Figure 7), designed using previously identified lead compounds as models (**V** and **VIa–c**, Figure 7) and applying different design criteria, did not give successful results (Figure 7). For example, the styryl derivative **8e**, based on the incorporation of a sulfur/vinyl bioisosteric substitution on the potent derivative **V** (IC_{50} = 15 μ M),[11] was completely inactive; analogous behavior was observed for other 5-styrylpyridazin-3(2*H*)-ones (compounds **7l** and **7q**, Figure 7) regardless of the substitution pattern at 2 or 6. A similar loss of antiplatelet activity was observed on comparing the lead compounds **VIa–c**[19] (Figure 7) with phenyl acrylates **8d**, **7k**, and **7p**. It can be seen that the insertion of an oxygen atom as a linker between the phenyl ring and carbonyl group of the propenone system present in **VI** completely negated the activity. The observed loss of activity in the phenyl acrylate series (**8d**, **7k**, and **7p**) with respect to the 3-phenylpropen-3-ones **VI** (Figure 7) could be interpreted in terms of the lower conjugation between the phenyl and the propenone system, a consequence of the linking oxygen atom. Comparison of the biological data obtained for the styryl derivatives (**8e**, **7l**, and **7q**) and their 3-oxo analogues **VIa–c** enables an evaluation of the importance of the carbonyl group as part of a conjugated system, which should be able to produce a significant interaction with the biological target.

As shown in Table 3, a substantial increase in the potency of the antiplatelet activity is observed for disubstituted 5-alkylidenepyridazin-3-ones represented by the general structure **IV** (compounds **14**), with pyridazin-3-ones that incorporate a benzyloxycarbonyl group on the exocyclic double bond (**14e**, **14k**, **14p**, **14v**, and **14z**) being the most interesting compounds described in this study (exhibiting IC_{50} values in the range 1–2 μM).

Replacement of the hydrogen atom at the end of the exocyclic ethylenic system in compounds **7** and **8** to afford derivatives related to the general structure **IV** (compounds **9**, **14**, and **15**) had a different effect on the antiplatelet activity depending on the nature of the group introduced (Table 3). For example, the

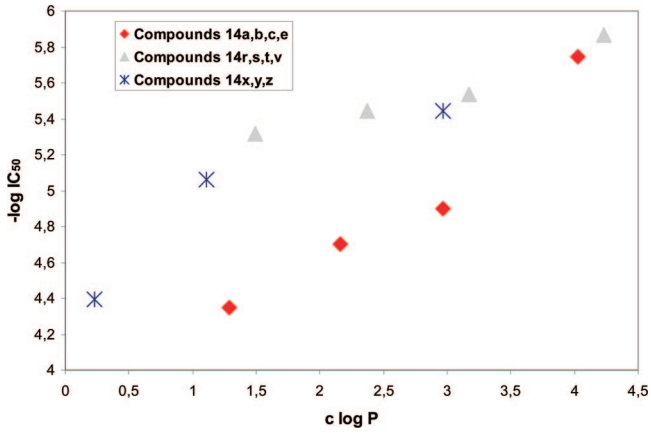


Figure 8. Antiplatelet activity−lipophilicity plot for representative subseries.

attachment of a methyl group gave rise to inactive compounds (**9b–e**), independent of the nature of the alkoxy residue present in the ester moiety (OMe or OEt) or the substitution pattern at position 2 and/or 6 (Table 3, entries 23–26). In contrast, replacement of the proton by electron-withdrawing groups generally led to a significant increase in activity, with IC_{50} values in the low micromolar range (Table 3, entries 30–53). Generally, two oxygenated functional groups are present in the exocyclic system of the most active compounds, the exceptions being dicarboxylic acids **15**, which were all inactive (Table 3, entries 56–58). As shown in Table 3, the presence of cyano groups on the exocyclic olefin afforded inactive (**14f**) or slightly active derivatives (**14q**, **14w**).

In the diester series (Table 3, compounds **14a–e**, **14g–p**, **14r–v**, and **14x–z**) the antiplatelet effect is strongly influenced by the nature of the alkoxy residue in the ester moieties. In general, a weak but progressive increase in potency (2- to 4-fold) is observed as a consequence of the increasing lipophilicity of the alkoxy group on moving through the Me, Et, *i*-Pr, *t*-Bu series (Table 3, entries 30–34, 36–40, 41–45, 47–51, and 53–55). Some reasonable exceptions to this observation were detected for the *t*-Bu and *i*-Pr groups in N-substituted pyridazin-3(2*H*)-ones (Table 3, entries 39, 44, and 50), which had comparatively low activities. Directed by the observed trend (and in order to explore the bulk tolerance of the pharmacophore), the role of a benzyloxy residue in the ester group was investigated (Table 3, entries 34, 40, 45, 51, and 55). In this case, a sharp increase in potency can be observed with respect to previous derivatives (5- to 10-fold) (IC_{50} = 1.36–3.58 μ M) regardless of the substitution pattern at positions 2 (H, Me, or Bn) and 6 (Ph or H).

For a more immediate and efficient demonstration of the previously discussed correlation, the antiplatelet data (IC_{50}) of three representative subseries are presented graphically (Figure 8) as a plot of pIC_{50} ($-\log IC_{50}$) (*Y*-axis) compared to the calculated log *P* [20] (clogP) of the corresponding molecule (*X*-axis).

The respectable activity values of the benzyl esters (**14e**, **14k**, **14p**, **14v**, and **14z**), despite the increased bulk on the exocyclic double bond of the molecule, perfectly correlate with the absence of activity for the carboxylic acids (compounds **15**, Table 3, entries 56–60) and suggest structural elements that would facilitate a better understanding of their action.

In an effort to evaluate the effect of the introduction of alkyl groups at position 2 and in order to determine the importance of the phenyl ring at position 6 of the heterocyclic core, different series of 5-alkenylpyridazin-3(2*H*)ones incorporating these substituents were prepared and tested (Table 3). Alkylation of the heterocyclic lactam in the 5-alkenylpyridazin-3(2*H*)-ones **III** (Table 3, R^2 = H, compounds **8a–g**) had almost no effect on the antiplatelet activity, and the 2-substituted 5-alkenylpyridazin-3(2*H*)-ones (compounds **7a–c** and **7f–q**) were also inactive, regardless of the nature of the X group (COOR, COOH, CN) present in the double bond or the alkyl group introduced at position 2 (MOM, Me, Bn) (Table 3, cf. **8a–g** and the parent derivatives **7**). The only exception to this observation is the (2*E*)-3-(1-benzyl-6-oxo-1,6-dihydropyridazin-4-yl)acrylonitrile **7o**, which showed enhanced activity when compared with **7j** and **8c**. Similarly, the presence/absence of the phenyl ring at position 6 in these compounds does not produce any significant modification in the antiplatelet activity of the series, with most examples remaining inactive (Table 3).

In clear contrast to the result previously discussed for the 5-alkylidenepyridazin-3(2*H*)-ones **III**, the introduction of alkyl groups at position 2 in compounds represented under the structure **IV** (compounds **14** and **15**) generally resulted in an increase in the platelet aggregation inhibitory effect, and this ranged from small to extremely significant. Thus, for those compounds containing a phenyl ring at position 6 (**14a–k**), methylation at position 2 produced a slight (2–5 times) enhancement in the activity (Table 3, compare compounds **14a−e** with **14g−k**), the exception being the *tert*-butoxy ester (**14d** 7.5 μM vs **14j** 45.0 μ M). Similar behavior was observed for those compounds without the phenyl ring at position 6 (**14l–z**); thus, alkylation of N2 resulted in compounds with similar or improved potency. A marked improvement in potency (2- to 8-fold) with respect to the parent compounds was achieved when a benzyl group was placed at position 2 (Table 3, entries 47–51), indicating that lipophilic/hindered groups are also tolerated in this position. The IC_{50} values of these derivatives (**14r–v**) typically range between 6.25 and 1.36 μM, with the 2-(1-benzyl-6-oxo-1,6-dihydropyridazin-4-ylmethylene)malonic acid dibenzyl ester (**14v**) being the most potent (1.35 μM) antiplatelet agent identified during this study.

Analysis of the data presented in Table 3 also leads to the conclusion that, as expected, the phenyl group at position 6 (a remaining structural motif required in the early project to ensure PDE III inhibitory activity) is not a key structural contributor to the observed platelet inhibitory effect in these series. In fact, this group can be removed without significant modification of the activity [Table 3; compare the antiplatelet data obtained for compounds where R_6 = Ph (**14a**, **14b**, **14e**, and **14g−k**) with those obtained for the parent compounds (R_6 = H, **14x−z**, and **14l−p**)]. Moreover, this information suggests that the steric effect produced by the phenyl ring in the active site can be tolerated well. Although the results of this study have demonstrated the noncritical character of the phenyl ring at position 6, the observed steric tolerance of this position could be employed, in further studies, as an additional point of diversity to be properly explored and exploited to improve the pharmacokinetic profile of these series.

The information extracted from the SAR studies allows us to conclude that the antiplatelet activity in these series is a consequence of the presence in the molecule of a highly activated exocyclic double bond. Such activation of the double bond is provided by ester groups that would confer Michael acceptor properties on the molecule. Accordingly, it is expected that the observed antiplatelet activity could be related to the interaction at this level with nucleophilic moieties in the biological target.

Comparison of the antiplatelet activity of the most interesting derivatives discovered during this study (**14e**, **14k**, **14p**, **14v**) with values for reference drugs reveals that the new pyridazin-3(2*H*)-ones documented here are very potent antiplatelet agents. Indeed, their platelet inhibitory effect is markedly superior to the experimental value determined for sulfinpyrazone and slightly higher than the reference compound in the pyridone series (e.g., milrinone) but around 10 times less potent than the currently employed GPIIb/IIIa antagonists (e.g., tirofiban).[7]

The well documented PDE-III inhibitory activity of small organic molecules incorporating a pyridazine-3(2*H*)-one core[9] led us to evaluate a possible cAMP-based activity as the origin of the observed platelet inhibitory effect. With this objective, some representative compounds (**14a**, **14b**, and **14g**), in which structural elements were preserved to allow the interaction of these series with PDE III isoenzyme, were studied to determine a possible PDE III inhibitory activity. These experiments were performed using purified preparations obtained from ventricular tissue of guinea pigs[21] and revealed a very low PDE III inhibitory activity ($IC_{50} = 5–2$ mM), which allows us to exclude this action as the origin of the observed antiplatelet activity. These data are consistent with our own previous findings[10,11] for other pyridazinones and confirm that for these series another pharmacological mechanism could be operating. Further studies are in progress in our laboratories to establish the mechanism of action of these promising new derivatives.

In summary, we have documented the discovery and structural optimization of a new family of potent pyridazinone-based antiplatelet agents. As concluded from the SAR studies, the presence of two lipophilic oxygenated functional groups, particularly benzyloxycarbonyl residues in the exocyclic double bond, is the key structural element for the activity; moreover, the introduction of benzyl groups at position 2 also leads to an improved antiplatelet effect. The preliminary pharmacological characterization of representative derivatives has revealed that, unlike other pyridazinones, its outstanding antiplatelet activity is not sustained by the inhibition of the PDE III. New experiments are in progress to establish a more complete pharmacological profile of its actions.

Experimental Section

Chemistry. Commercially available starting materials, reagents, and solvents were purchased (Sigma-Aldrich) and used without further purification. When necessary, solvents were dried by standard techniques and distilled. After extraction from aqueous phases, the organic solvents were dried over anhydrous sodium sulfate. The reactions were monitored by thin-layer chromatography (TLC) with 2.5 mm Merck silica gel GF 254 strips, and the purified compounds each showed a single spot; unless stated otherwise, UV light and/or iodine vapor was used for detection of compounds. Purification of crude compounds was carried out by flash column chromatography on silica gel 60 (Kieselgel 0.040–0.063 mm, E. Merck) and then recrystallized. The Knoevenagel condensation between 5-formylpyridazin-3(2*H*)-ones (**12**) and methylene active compounds (**13**) was performed on a PLS (6 × 4) organic synthesizer from Advanced Chemtech. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were measured using a Perkin-Elmer 1640 FTIR spectrophotometer with samples as potassium bromide pellets. The NMR spectra were recorded on Bruker AM300 and XM500 spectrometers. Chemical shifts are given as δ values against tetramethylsilane as internal standard, and J values are given in Hz. Mass spectra were obtained on a Varian MAT-711 instrument. High-resolution mass spectra were obtained on an Autospec Micromass spectrometer. Elemental analyses were performed on a Perkin-Elmer 240B apparatus at the Microanalysis Service of the University of Santiago de Compostela. The elemental composition of the new compounds agreed to within ±0.4% of the calculated value. Complete description of synthetic methodologies as well as analytical and spectrocopic data for all described compounds is included in Supporting Information.

Pharmacology. Preparation of Washed Platelets. Human platelet concentrates from blood anticoagulated with citrate−phosphate−dextrose were obtained from the Centro de Transfusión de Galicia (Santiago de Compostela, Spain). Platelets were purified by sedimentation through a discontinuous metrizamide gradient. For this purpose, 8 mL of platelet concentrate was layered onto a 10%/20% (1 mL/1 mL) metrizamide gradient and centrifuged at 1000*g* for 20 min The resulting platelet band was recovered, diluted to 8 mL with washing buffer (NaCl, 140 mM; KCl, 5 mM; trisodium citrate, 12 mM; glucose, 10 mM; sucrose, 12.5 mM; pH 6), and centrifuged again at 1000*g* for 20 min. Finally, the platelet band recovered from this step was resuspended in a modified Tyrode-HEPES buffer (HEPES, 10 mM; NaCl, 140 mM; KCl, 3 mM; MgCl, 0.5 mM; $NaHCO_3$, 5 mM; glucose, 10 mM; pH 7.4), affording a concentration of (2.5–3.5) × 10^8 platelets/mL. The calcium concentration in the extracellular medium was adjusted at 2 mM by the addition of the convenient amount of Ca_2Cl.

Platelet Aggregation Studies. Platelet aggregation was measured in vitro by the turbidimetric method of Born using a dual channel aggregometer (Chrono-log, Havertown, PA). Each test compound was incubated with washed platelets at 37 °C for 5 min. Stimulus was then added to induce platelet aggregation, and the light transmission was monitored over a 5 min period. Platelet aggregation is expressed as the maximum change in light transmission during this period, with a 100% value being obtained when only stimulus, and not compound, was added. Compounds were evaluated in triplicate for each drug concentration, ranging from 200 nM to 2 mM. Concentration–response curves were calculated using the Graphpad Prism software, allowing the estimation of the IC_{50}. Tables 3 reports the IC_{50} values obtained for compounds. Compounds were tested in DMSO solutions starting at high concentrations (2000 μM). All reported IC_{50} values are the mean of at least five experiments employing human blood from different individuals.

Data Presentation and Statistical Analysis. Unless otherwise specified, results shown in the text and tables are expressed as the mean ± SEM. Significant differences between two mean values ($p < 0.05$ or $p < 0.01$) were determined by one-way analysis of variance (ANOVA) and/or by Student's t test for nonpaired data.

Supporting Information Available: Experimental details for the synthesis of compounds described and spectroscopic and elemental analysis data of all compounds prepared. This material is available free of charge via the Internet at http://pubs.acs.org.

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