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2-Substituted 4-, 5-, and 6-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]pyridazin-3(2*H*)-ones and 2-substituted 4,5-bis[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]pyridazin-3(2*H*)-ones as potent platelet aggregation inhibitors: Design, synthesis, and SAR studies

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Abstract—A set of regioisomeric 2-substituted pyridazin-3(2*H*)-ones containing a 3-oxo-3-phenylprop-1-en-1-yl fragment at either position 4, 5 or 6 and 2-substituted pyridazin-3(2*H*)-ones containing the same fragment both at positions 4 and 5 have been synthesized and evaluated as antiplatelet agents. The study allows the identification of a new highly potent platelet aggregation inhibitor (**4c**).

Platelets, or thrombocytes, are anucleated cells that circulate in blood as sentinels of vascular integrity.[1] They show no interaction with the inner surface of normal vessels but adhere promptly where endothelial cells are altered or extracellular matrix substrates are exposed. Since under pathological conditions platelets are a major contributor to thrombosis and heart disease the inhibition of platelet function represents a well-established approach to prevent these diseases.[2] The therapeutic family of antiplatelet agents (Fig. 1) is represented by drugs as ticlopidine, clopidogrel, aspirin, dipiridamole, and tirofiban, being the combination of clopidogrel and aspirin the current gold standard of antiplatelet therapy.[3] However, the number of agents available is limited and most importantly the drugs in use today show deleterious side effects (e.g., gastrointestinal bleeding, neutropenia, thrombocytopenia, hematuria or cholestatic jaundice). Substantial improvements in antiplatelet therapy are therefore much-needed.[3]

The discovery of the first naturally occurring pyridazine derivative (Pyridazomycin)[4] meant a milestone in the recognition of the potential of the 1,2-diazine core as a valuable unit in medicinal chemistry. In particular, 3-oxo derivatives [pyridazin-3(2*H*)-one scaffold] have shown a wide range of biological actions[5] (e.g., antinociceptive, antiviral, antibacterial, cytotoxic, fungicidal, bronchospasmolytic, analgesic, hypolipidemic), especially on targets that play a key role in cardiovascular diseases [e.g., phosphodiesterase III (PDE III) or α-adrenergic receptors].[6] In this context, the versatility of this heterocyclic skeleton in the search for new antiplatelet agents is well documented.[7]

In the course of our studies in this field we have recently identified[8] a novel family of pyridazinone-based anti-

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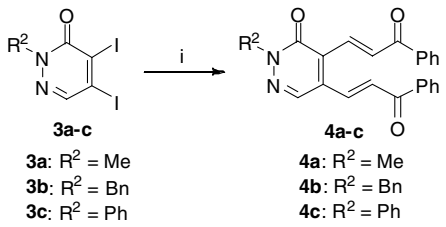
platelet agents that can be formally considered as hybrid structures since they incorporate at the 5 position of the heterocyclic nuclei an α,β unsaturated moiety present in chalcones, another chemotype eliciting antiplatelet activity[9].

In the present study, we describe the design, the synthesis, and the biological evaluation of a series of regioisomeric pyridazin-3(2*H*)-ones including the preliminary interpretation of their structure–activity relationships. The set consists of pyridazin-3(2*H*)-ones containing a 3-oxo-3-phenylprop-1-en-1-yl fragment at either 4, 5 or 6 position, as well as pyridazin-3(2*H*)-ones that contain this pharmacophoric unit at positions 4 and 5 (Schemes 1 and 2.

Pyridazin-3(2*H*)-ones (**1** and **3**), containing different substituents at the 2 position (Me, Bn or Ph) and a halogen atom at the 4, 5 or 6 position or both at positions 4 and 5, were synthesized using previously described procedures.[10,11a] 6-Chloro-2-phenylpyridazin-3(2*H*)-one (**1i**) was synthesized from 6-chloropyridazin-3(2*H*)-one via a chemoselective Cu-catalyzed arylation reaction with iodobenzene.[12] The active Cu-complex was formed in situ from Cu(I)Cl and 8-hydroxychinoline. The target compounds (**2** and **4)** were readily accessible through Sonogashira reaction of the proper precursor (**1a**–**i** or **3a**–**c**) with 1-phenylprop-2-yn-1-ol as alkyne (Schemes 1 and 2).[8,11] Such a transformation involves the initial alkynylation of the heterocycle followed by a base-promoted isomerization of the cross-coupling product [2-substituted 4-, 5-, or 6-(3-hydroxy-3-phenylprop-1-yn-1-yl)pyridazin-3(2*H*)-ones and 2-substituted 4,5-bis(3-hydroxy-3-phenylprop-1-yn-1-yl)pyridazin-3(2*H*)-ones] affording the *E*-enone as the main product.[11]



Scheme 2. Synthesis of 2-substituted 4,5-bis[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]pyridazin-3(2*H*)-ones **4a**–**c**. Reagents and conditions: (i) CH ≡ CCH(OH)Ph, $PdCl_2$ $(PPh_3)_2$, Et_3N, CuI, DMF, 60 °C.

When the Sonogashira reaction yielded an *E*/*Z* mixture, the *E* compound was isolated by recrystallization from isopropanol or preparative chromatography. The spectroscopic data of representative compounds (R^2 = Ph) are described in the reference section of his article.[13]

The obtained compounds (**2a**–**i** and **4a**–**c**) as well as three reference derivatives [milrinone, sulfinpyrazone, and (2*E*)-1,3-diphenylprop-2-en-1-one (the simplest member of the chalcone series)] were evaluated in vitro by the turbidimetric method of Born,[14] using thrombin as platelet aggregation inductor. The compounds were tested in DMSO solutions starting at high concentrations (2000 μM). All reported IC_{50} values (Table 1) are means of at least five experiments employing human blood from different individuals. Unless indicated otherwise, results shown in the text and in Table 1 are expressed as means ± SEM. Significant differences between two means ($p < 0.05$ or $p < 0.01$) were determined by one-way analysis of variance (ANOVA) and/or by Student's *t*-test for non-paired data.

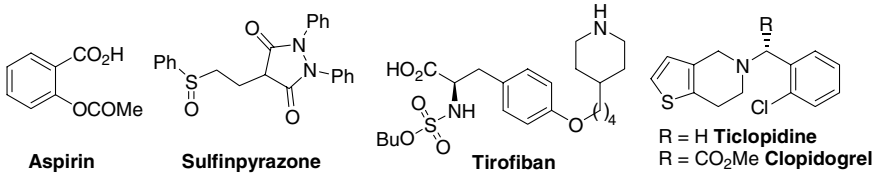
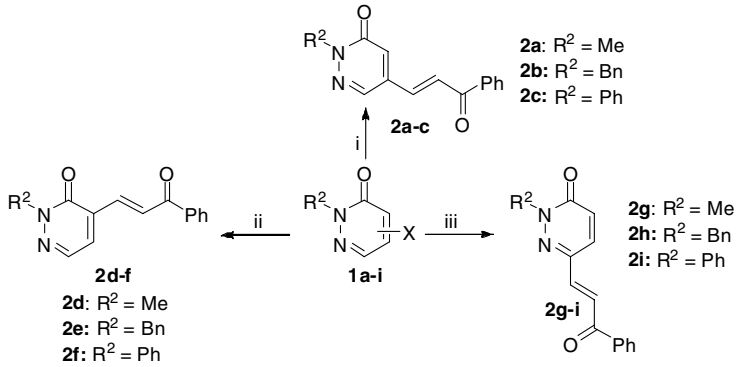
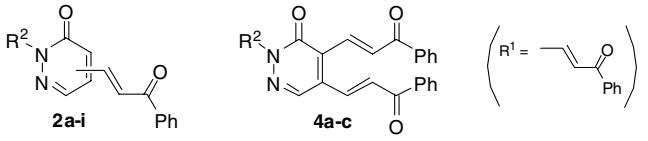


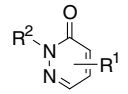
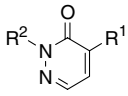
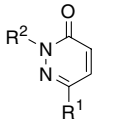
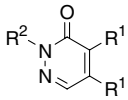
Figure 1. Structures of the most prominent antiplatelet agents currently employed.



Scheme 1. Synthesis of 2-substituted 4-, 5-, and 6-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]pyridazine-3(2*H*)-ones **2a**–**i**. Reagents and conditions: (i) X = I: CH ≡ CCH(OH)Ph, $PdCl_2$ $(PPh_3)_2$, Et_3N, CuI, DMF, 60 °C; (ii) X = I: CH ≡ CCH(OH)Ph, $PdCl_2(PPh_3)_2$, Et_3N, CuI, THF, 55 °C; (iii) X = Cl: CH ≡ CCH(OH)Ph, $PdCl_2$ $(PPh_3)_2$, Et_3N, CuI, DMF, 100 °C, pressure tube.

Table 1. Antiplatelet activity of pyridazin-3(2*H*)-ones **2**, **4** and some reference compounds



Compound		R^2	mp (°C)	Yield (%) and [*E*/*Z*] ratio	IC_{50} (μM) or inhibition (%) at 100 μM
2a		Me[11a]	192–194	89 [1/0]	15.70 ± 0.70
2b		Bn[11a]	174–175	70 [1/0]	4.20 ± 0.30
2c		Ph[11a]	156–157	77 [1/0]	3.30 ± 0.40
2d		Me	142–143	67 [1/0]	37.11 ± 2.61
2e		Bn	161–163	78 [1/0]	40%[a]
2f		Ph	175–177	44 [3/2]	17.56 ± 2.01
2g		Me	204–205	47 [4/1]	13.44 ± 1.62
2h		Bn	198–200	82 [3/2]	60 ± 11.27
2i		Ph	119–120	58 [1/0]	11.96 ± 0.68
4a		Me	176–178	62 [1/0]	6.15 ± 1.60
4b		Bn	181–183	43 [1/0]	[b]
4c		Ph	192–194	74 [1/0]	1.98 ± 0.66
		Milrinone			4.7 ± 0.50
		Sulfinpyrazone			509.1 ± 49.00
		(2*E*)-1,3-diphenylprop-2-en-1-one			162.56 ± 12.56

[a] Precipitate at lower concentrations.
[b] Precipitate under test conditions.

Analysis of the biological data (Table 1) reveals that the new chemotypes under study elicit a potent antiplatelet effect as most of them exhibit IC_{50} values in the low micromolar range (1–20 μM). Although the present limited number of compounds precludes detailed SAR studies, a few general features emerging from this preliminary exploration can be withdrawn and taken into account for designing future series.

Comparison of the obtained platelet inhibitory activity (Table 1) suggests that the optimum position for the 3-oxo-3-phenylprop-1-en-1-yl fragment is the 5 position of the pyridazinone core. The shift of such a pharmacophoric unit to position 4 or 6 of the heterocyclic nucleus (compounds **2d**–**f** and **2g**–**i**) results in a still interesting but slightly attenuated (2- to 4-fold) antiplatelet activity when compared to the 2-substituted 5-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]pyridazin-3(2*H*)-ones (**2a**–**c**). This can be due to a different interaction with the biological target.

Table 1 shows that the activity of the compounds is not only dependent on the position of the 3-oxo-3-phenylprop-1-en-1-yl fragment on the heterocyclic ring since a significant modulation of the platelet inhibitory effect is observed when the substituent at the 2 position is altered. A phenyl ring in the structure gives the most potent derivatives of each subseries (compounds **2c**, **2f**, and **2i**). The biological evaluation of the 4,5-bis[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]pyridazin-3(2*H*)-ones (**4**) confirmed the hypothesis that duplication of the 3-oxo-3-phenylprop-1-en-1-yl residue on the heterocycle generates compounds with improved activity (2- to 6-fold when compared with their parent compounds **2a**–**c** or **2d**–**f**). Within these series the outstanding platelet inhibitory effect (1.98 μM) of the 4,5-bis[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-2-phenylpyridazin-3(2*H*)-one (**4c**) should be highlighted.

Comparison of the antiplatelet activity of the most interesting derivatives described in this paper with values determined for some reference drugs (milrinone or sulfinpyrazone) reveals that the new structures are potent antiplatelet agents with an activity highly or slightly superior to, respectively, sulfinpyrazone and milrinone (Table 1). Moreover, the significantly superior platelet inhibitory effect determined for compounds **2a**–**i** with respect to (2*E*)-1,3-diphenylprop-2-en-1-one, the simplest representative of the chalcone family, unequivocally confirms the relevance of the pyridazinone core as a key pharmacophoric unit in these series.

In summary, the design, the synthesis, and the biological evaluation of several 2-substituted pyridazinones incorporating a 3-oxo-3-phenylprop-1-en-1-yl fragment at either position 4, 5 or 6 (compounds **2**) or at both positions 4 and 5 (compounds **4**) have been described. This study allowed the identification of several new highly potent antiplatelet agents and also the establishment of the preliminary relevant features of the SAR in these series. Further studies are in progress in our laboratories to exploit these results for the synthesis of a larger library of hybrid structures.

Acknowledgments

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13. All described compounds gave satisfactory microanalytical (C, H, N ± 0.4%) and spectral data (300 or 400 MHz ^{1}H NMR and FTIR). Spectral data for representative compounds (R^2 = Ph): 5-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-2-phenylpyridazin-3(2*H*)-one (**2c**): ^{1}H NMR: δ_H (300 MHz, DMSO-d_6): 8.15 (d, J = 2.1 Hz, 1H), 8.01 (d, J = 8.9 Hz, 2H), 7.83–7.64 (m, 3H), 7.52 (d, J = 14.9 Hz, 1H), 7.48–7.37 (m, 6H), 7.16 (d, J = 2.1 Hz, 1H). 4-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-2-phenylpyridazin-3(2*H*)-one (**2f**): ^{1}H NMR: δ_H (400 MHz, DMSO-d_6): 8.75 (d, J = 15.2 Hz, 1H), 8.10 (m, 2H), 8.02 (d, J = 4.2 Hz, 1H), 7.55 (m, 10H). 6-[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl]-2-phenylpyridazin-3(2*H*)-one (**2i**):

^{1}H NMR: δ_H (400 MHz, DMSO-d_6): 8.33 (d, J = 9.8 Hz, 1H), 8.17 (dd, J = 8.3, 1.4 Hz, 2H), 8.06 (d, J = 15.9 Hz, 1H), 7.71 (tt, J = 8.0, 1.4 Hz, 1H), 7.61 (m, 4H), 7.54 (m, 2H), 7.47 (d, J = 15.9 Hz, 1H), 7.46 (m, 1H), 7.22 (dd, J = 9.8, 0.5 Hz, 1H). 4,5-bis[(1*E*)-3-oxo-3-phenylprop-1-en-1-yl)-2-phenylpyridazin-3(2*H*)-one (**4c**): ^{1}H NMR: δ_H (300 MHz, $CDCl_3$): 8.80 (d, J = 15.3 Hz, 1H), 8.18 (s, 1H), 8.11–8.04 (m, 5H), 7.96 (d, J = 15.3 Hz, 1H), 7.67–7.41 (m, 12H).

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