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# New pyridazinone derivatives with vasorelaxant and platelet antiaggregatory activities ☆

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ABSTRACT

New 6-substituted and 2,6-disubstituted pyridazinone derivatives were obtained starting from easily accessible alkyl furans by using oxidation with singlet oxygen to give 4-methoxy or 4-hydroxybutenolides, key intermediates of this synthetic strategy. The new pyridazinone derivatives have been studied as vasorelaxant and antiplatelet agents. Analysis of biological data revealed the silyl ethers (**4a–i**) and *N*,*O*-dibenzyl derivatives (**6g–i**) as the most active compounds.



The pyridazine nucleus represents a versatile scaffold to develop new pharmacologically active compounds. This nitrogen heterocycle is included in chemicals with a wide range of biological activities and can also be used to link other pharmacophoric groups.[1] For instance, the pyridazinone derivatives are known as drugs with interesting effects in cardiovascular system due to their platelet aggregation inhibition as well as their antihypertensive activity and cardiotonic properties (Fig. 1).[1e,2–7]

In general, the 6-arylpyridazinone structure was considered essential for the activity on cardiovascular system, especially to ensure inhibition of some key targets as phosphodiesterase III (PDE III),[8,1e,2] and this can be the reason why most of pyridazinone derivatives known include aryl residues at position 6. However, in the last few years new pyridazinone derivatives with activity on cardiovascular system were described in which the phenyl group at C6 was removed or replaced, such as $\alpha_1$-adrenoreceptor antagonists or platelet aggregation inhibitors.[9,1e,2] Thus, replacement of aryl by alkenyl fragments or their inclusion in other positions of pyridazine ring gave rise to potent antiplatelet agents whose activity is not related with the inhibition of the PDE III.[1e,2]

Bearing in mind the good properties described by pyridazinone based compounds for the control of cardiovascular diseases, in particular as vasodilators and antiplatelet agents and looking for new modifications on the pyridazinone nucleus that could give rise

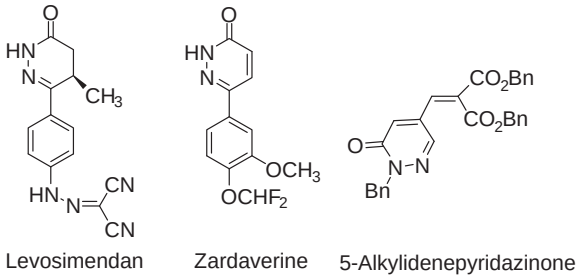


**Figure 1.** Representative pyridazinone derivatives with activity on cardiovascular system.

to derivatives with novel mechanisms of action we have designed and synthesized a preliminary series of derivatives of structure **I**. These compounds show two positions for structural diversity, C6 and N2 (Fig. 2), since both positions seem very important to modulate the effect of pyridazinone derivatives on cardiovascular system.[10,1e]

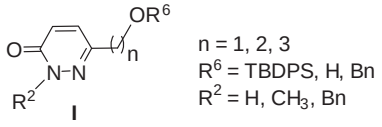


**Figure 2.** General structure of pyridazinone analogues synthesized.

☆ In memory of Professor Francisco Orallo.

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The new pyridazinone analogues proposed will allow us to explore how vasorelaxant and antiplatelet activity is affected by the presence at C6 of an alkyl chain of varying magnitude (1, 2 or 3 carbon atoms), functionalized with alcohol or ether groups, as well as by inclusion of a methyl or a benzyl group at N2. The selection of substituents at N2 was based on the activity data previously known (Fig. 2).[1e,2]

The pyridazinone derivatives studied in this work were synthesized as outlined in Schemes 1 and 2.

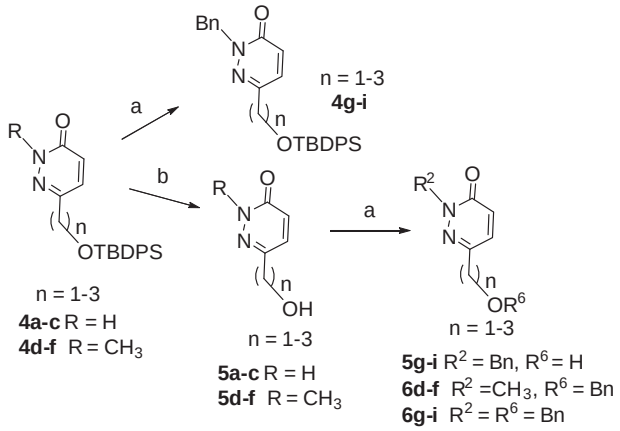
According to Scheme 1, the pyridazinone core was obtained in moderate yield in two steps starting from the adequate 2-alkylfuran. Thus, oxidation of alkylfurans **1** with singlet oxygen[11] gave an appropriate butenolide suitable to react with hydrazine or methyl hydrazine.[12] The 2-alkylfuran **1a**, precursor of 6-methyl derivatives, was easily prepared by reaction of furfuryl alcohol with *tert*-butyldiphenylsilyl chloride (TBDPSCl) and imidazol in DMF[13] while its homologues, the alkylfurans **1b** and **1c**, were obtained following previously described synthetic approaches.[11,14]

Once obtained the 2-alkylfurans **1a–c**, two strategies are followed to build the heterocyclic ring. The first one involves oxidation of compounds **1** with singlet oxygen and subsequent treatment with acetic anhydride, to give the 4-methoxybutenolide intermediates **2** in moderate to good yields (56–89%).[15,11,14] The second one, involves oxidation of **1** with oxygen singlet in the presence of the Hünig's base to afford the 4-hydroxybutenolides **3**,[16] which can be used in the following step without purification.[17] However, to our surprise, the latter methodology has not been adequate to provide the 4-hydroxybutenolide **3a**, which has been obtained in a yield significantly lower than the corresponding 4-methoxybutenolide **2a**. These poor results are in contrast with previous observations and could be related to the possible instability of compound **3a**.[16]

Both intermediates, compounds **2** and **3**, by reaction with hydrazine or methyl hydrazine in ethanol afforded the 6- and 2,6-substituted pyridazinones respectively (**4a–f**, 11–45% yields).[18] The low yield obtained in some cases could be explained taking into account the high reactivity of the α,β-unsaturated system.[11,14]

It is noteworthy that both types of butenolides are suitable to provide the desired compounds, although the hydroxy derivatives, probably due to their equilibrium with the acyclic form (γ-keto acid), are more reactive, allowing milder reaction conditions and better yields, in general.

Treatment of 6-silyloxy derivatives **4a–c** with benzyl bromide at room temperature in the presence of NaH and $Bu_4NI$ catalytic gave the expected *N*-benzyl derivatives **4g–i**.[19] In addition, compounds **4a–f** by treatment with TBAF provided the hydroxy derivatives **5a–f** in very good yields (86–99%),[20] which have been satisfactorily transformed into the *N*-benzyl-6-hydroxyalkyl derivatives **5g–i**, into the *O*-benzyl derivatives **6d–f**, as well as into the *N*,*O*-dibenzyl derivatives **6g–i** using the standard procedure




**Scheme 2.** Reagents and conditions: (a) NaH, BnBr, $Bu_4NI$, THF, rt; 68% (**4g**), 70% (**4h**), 61% (**4i**), 45% (**5g**), 23% (**5h**), 49% (**5i**), 57% (**6d**), 99% (**6e**), 87% (**6f**), 40% (**6g**), 61% (**6h**), 30% (**6i**); (b) TBAF, THF, rt, 86% (**5a**), 94% (**5b**), 99% (**5c**), 99% (**5d**), 99% (**5e**), 98% (**5f**).

above indicated (Scheme 2).[21] The acid character of the pyridazinone hydrazidic hydrogen makes possible a selective benzylation at N2 for compounds **5a–c** using one equivalent of benzyl bromide. However, when excess benzyl halide was used, although the *N*-benzyl derivative was initially obtained, increased reaction times led to mixtures of *N*-benzyl and *N*,*O*-dibenzyl derivatives, in varying proportions, whose separation was possible by column chromatography.

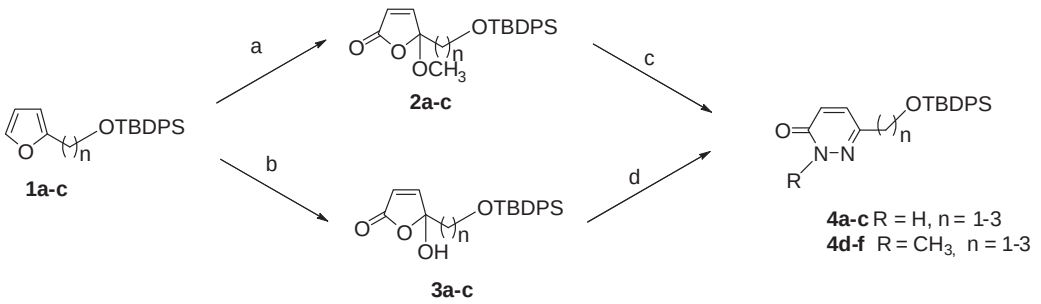
The new pyridazinone derivatives synthesized, compounds **4**–**6** as well as the reference drug (milrinone) were screened for vasorelaxant activity as reported previously,[22] using intact rat aortic rings pre-contracted with noradrenaline (NA, 1 μM).

The cumulative addition of milrinone and the compounds **4–6** (1–100 μM) caused a concentration-dependent relaxation of the contractions induced by NA. The corresponding $IC_{50}$ values are shown in Table 1.

Many of synthesized pyridazinones produced vasorelaxation in micromolar range (32.5–78.25 μM), being less efficient than milrinone. The *N*,*O*-dibenzyl derivatives, compounds **6g–i**, were the most active compounds. The following effective compounds are the silyl ethers **4**, in particular the *N*-benzyl derivative **4i**. On the other hand, all hydroxyl derivatives (**5a–i**) were inactive, with $IC_{50}$ values greater than 100 μM.

Platelet aggregation studies of compounds **4–6** were performed in washed human platelets following the Born's turbidimetric method,[23] and using thrombine (0.25 U/mL) and collagen (2.5 μg/mL) as platelet aggregation inductors.[22b]

Most of new pyridazinones selectively inhibited aggregation induced by collagen in the low μM range (1.80–69.6 μM), since none




**Scheme 1.** Reagents and conditions: (a) (1) $O_2$, *hv*, rose bengal, MeOH, −78 °C; (2) $Ac_2O$, Py, DMAP, rt, 56% (**2a**); (b) $O_2$, *hv*, rose bengal, DIPEA, MeOH, −78 °C; (c) $NH_2NH_2{\cdot}H_2O$ or $CH_3NHNH_2$, EtOH, reflux, 14% (**4a**), 41% (**4d**); (d) $NH_2NH_2{\cdot}H_2O$ or $CH_3NHNH_2$, EtOH, 0 °C to rt, 34% (**4b**), 45% (**4c**), 11% (**4e**), 31% (**4f**), yields for two steps.

**Table 1**
Vasorelaxant activity ($IC_{50}$ in μM)[a] of tested compounds

| Compound | Noradrenaline (NA, 1 μM) |
|---|---|
| **4a** | 62.61 ± (5.12) |
| **4b** | 56.2 ± (5.6) |
| **4c** | 78.25 ± (6.89) |
| **4d** | 58.4 ± (4.4) |
| **4e** | 47.0 ± (3.1) |
| **4f** | >100 |
| **4g** | >100 |
| **4h** | >100 |
| **4i** | 39.9 ± (8.6) |
| **5a** | >100 |
| **5b** | >100 |
| **5c** | >100 |
| **5d** | >100 |
| **5e** | >100 |
| **5f** | >100 |
| **5g** | >100 |
| **5h** | >100 |
| **5i** | >100 |
| **6d** | >100 |
| **6e** | >100 |
| **6f** | 76.4 ± (7.1) |
| **6g** | 35.3 ± (5.7) |
| **6h** | 32.3 ± (2.5) |
| **6i** | 32.5 ± (4.3) |
| Milrinone | 0.122 ± (0.008) |

[a] Values are means of five experiments, standard deviation is given in parentheses.

of them inhibited thrombin-induced aggregation at concentrations below 100 μM. This selectivity was also developed by several chalcones,[24] some zoanthamine-type alkaloids,[25] and also by aspirin,[26] used as standard drug for this study. Table 2 shows the antiplatelet activity data against collagen ($IC_{50}$ values) of the target compounds and the reference drug, obtained from experiments conducted at concentration intervals of 1.25–10 μM and of 25–100 μM, depending on the compound.

Analysis of these last biological data also revealed the silyl ethers (**4a–i**) and *N*,*O*-dibenzyl derivatives (**6g–i**) as the most active compounds of these series, being more potent than aspirin.

**Table 2**
Antiplatelet activity ($IC_{50}$ in μM)[a] of tested compounds

| Compound | Collagen (2.5 μg/mL) |
|---|---|
| **4a** | 1.80 ± (0.11) |
| **4b** | 3.87 ± (0.38) |
| **4c** | 2.13 ± (0.16) |
| **4d** | 4.55 ± (0.30) |
| **4e** | 3.17 ± (0.16) |
| **4f** | 2.34 ± (0.6) |
| **4g** | 4.6 ± (0.4) |
| **4h** | 4.8 ± (0.2) |
| **4i** | 3.9 ± (0.2) |
| **5a** | >100 |
| **5b** | 64.3 ± (5.2) |
| **5c** | >100 |
| **5d** | 69.6 ± (5.0) |
| **5e** | 60.4 ± (3.1) |
| **5f** | >100 |
| **5g** | >100 |
| **5h** | >100 |
| **5i** | 4.7 ± (0.3)[b] |
| **6d** | >100 |
| **6e** | >100 |
| **6f** | 50.7 ± (2.3) |
| **6g** | 8.0 ± (0.4) |
| **6h** | 4.11 ± (0.38) |
| **6i** | 5.9 ± (0.5) |
| Aspirin | 38.5 ± (2.9) |

[a] Values are means of five experiments, standard deviation is given in parentheses.
[b] Active in 50% of the studied plasmas.

Since all tested compounds showed a selective effect towards the aggregation induced by collagen, exploratory studies were performed in order to evaluate the $TXA_2$ pathway. In addition, biological tests were carried out using purified enzymes according to a previously reported protocol.[27] Thus, preliminary studies performed with compound **4e** revealed moderate COX-1 inhibition (results not shown) that can explain, at least in part, the antiplatelet effect observed.

In conclusion, 24 new pyridazinone derivatives were obtained starting from easily accessible alkyl furans by using oxidation with singlet oxygen to give 4-methoxy or 4-hydroxybutenolides, key intermediates of this synthetic strategy. The new molecules have been characterised as vasorelaxant and platelet antiaggregatory agents. The target compounds could show a pharmacological profile as antiplatelet drugs similar to that of aspirin. Further studies are in progress to clarify the mechanisms by which the new pyridazinone analogues produce their vasorelaxant and antiplatelet effects.

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## References and notes

1. (a) Rival, Y.; Hoffmann, R.; Didier, B.; Rybaltchenko, V.; Bourguignon, J. J.; Wermuth, C. G. *J. Med. Chem.* **1998**, *41*, 311; (b) Contreras, J. M.; Parrot, I.; Sippl, W.; Rival, Y.; Wermuth, C. G. *J. Med. Chem.* **2001**, *44*, 2707; (c) Montesano, F.; Barlocco, D.; Dal Piaz, V.; Leonardi, A.; Poggesi, E.; Fanelli, F.; De Benedetti, P. G. *Bioorg. Med. Chem.* **1998**, *6*, 925; (d) Ramalho, T. C.; Rocha, M.; da Cunha, E.; Freitas, M. P. *Expert Opin. Ther. Patent* **2009**, *19*, 1; (e) Coelho, A.; Raviña, E.; Fraiz, N.; Yañez, M.; Laguna, R.; Cano, E.; Sotelo, E. *J. Med. Chem.* **2007**, *50*, 6476; (f) Biancanali, C.; Giovannoni, M. P.; Pieretti, S.; Cesari, N.; Graciano, A.; Vergelli, C.; Cilibrizzi, A.; di Gianuario, A.; Colucci, M.; Mangano, G.; Garrone, B.; Polenzani, L.; dal Piaz, V. *J. Med. Chem.* **2009**, *52*, 7397.
2. Meyers, C.; Yañez, M.; Elmaatougi, A.; Verhelst, T.; Coelho, A.; Fraiz, N.; Lemière, G. L. F.; García-Mera, X.; Laguna, R.; Cano, E.; Maes, B. U. W.; Sotelo, E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 793.
3. Siddiqui, A.; Mishra, R.; Shaharyar, M. *Eur. J. Med. Chem.* **2010**, *45*, 2283.
4. Schudt, C.; Winder, S.; Mueller, B.; Ukena, D. *Biochem. Pharmacol.* **1991**, *42*, 153.
5. von der Saal, W.; Holck, J. P.; Kampe, W.; Alfred Mertens, A.; Muller-Beckmannf, B. *J. Med. Chem.* **1989**, *32*, 1481.
6. Pollesello, P.; Ovaska, M.; Kaivola, J.; Tilgmann, C.; Lundström, K.; Kalkkinen, N.; Ulmanen, I.; Nissinen, E.; Taskinen, J. *J. Biol. Chem.* **1994**, *269*, 28584.
7. Amin, E. N.; Abdel-Alim, A.-A. M.; Abdel-Moty, S. G.; El-Shorbagi, A.-N. A.; Abdel-Rahman, M. Sh. *Arch. Pharmacol. Res.* **2010**, *33*, 25.
8. (a) Bristol, J. A.; Sircar, I.; Moos, W. H.; Evans, D. B.; Weishaan, R. E. *J. Med. Chem.* **1984**, *27*, 1099; (b) Moos, W. H.; Humblet, C. C.; Sircar, I.; Rithner, C.; Weishaar, R. E.; Bristol, J. A.; McPhail, A. T. *J. Med. Chem.* **1987**, *30*, 1963.
9. (a) Betti, L.; Corelli, F.; Floridi, M.; Giannaccini, G.; Maccari, L.; Manetti, F.; Strappaghetti, G.; Botta, M. *J. Med. Chem.* **2003**, *46*, 3555; (b) Betti, L.; Floridi, M.; Giannaccini, G.; Manetti, F.; Strappaghetti, G.; Tafi, A.; Botta, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 171.
10. Manetti, F.; Corelli, F.; Strappaghetti, G.; Botta, M. *Curr. Med. Chem.* **2002**, *9*, 1303.
11. Pérez, M.; Canoa, P.; Gómez, G.; Terán, C.; Fall, Y. *Tetrahedron Lett.* **2004**, *45*, 5207.
12. (a) Escobar, C.; Fariña, F.; Sañudo, J. M. *An. Quim.* **1971**, *67*, 43; (b) Bourgignon, J. J.; Wermuth, C. G. *J. Org. Chem.* **1981**, *46*, 4889; (c) Mylari, B. L.; Zembrowski, W. J.; Beyer, T. A.; Aldinger, C. E.; Siegel, T. W. *J. Med. Chem.* **1992**, *35*, 2155.
13. Nicolau, K. C.; Aversa, R. J.; Jin, J.; Rivas, F. *J. Am. Chem. Soc.* **2010**, *132*, 6855.
14. Estévez, L.; Besada, P.; Fall, Y.; Teijeira, M.; Terán, C. *Synthesis* **2010**, 425.
15. *5-(tert-Butyldiphenylsilyloxymethyl)-5-methoxy-5H-furan-2-one* (**2a**): $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 7.62 (m, 4H), 7.40 (m, 6H), 7.09 (d, $J$ = 7.5 Hz, 1H), 6.29 (d, $J$ = 5.7 Hz, 1H), 3.88 (s, 2H), 3.24 (s, 3H), 1.01 (s, 9H). $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 169.8, 152.1, 135.6, 132.4, 129.9, 127.8, 126.1, 110.3, 65.2, 51.4, 26.7, 19.2.
16. Teijeira, M.; Suárez, P. L.; Gómez, G.; Terán, C.; Fall, Y. *Tetrahedron Lett.* **2005**, *46*, 5889.
17. *All hydroxybutenolides* **3** *exhibited satisfactory* $^1$*H NMR data. Selected spectroscopic data for compound* **3b**: $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 7.73 (m, 4H), 7.46 (m, 6H), 7.29 (d, $J$ = 5.6 Hz, 1H), 6.09 (d, $J$ = 5.6 Hz, 1H), 4.31 (m, 1H), 3.89 (m, 1H), 2.34 (m, 1H), 1.90 (m, 1H), 1.11 (s, 9H).
18. *Spectroscopic data for a representative compound* **4b**: $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 7.58 (m, 4H), 7.40 (m, 6H), 7.18 (d, $J$ = 9.7 Hz, 1H), 6.84 (d, $J$ = 9.7 Hz, 1H), 3.92

(t, $J$ = 6.1 Hz, 2H), 2.79 (t, $J$ = 6.1 Hz, 2H), 1.00 (s, 9H). $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 160.9, 147.0, 135.5, 134.9, 133.2, 129.8, 129.6, 127.7, 62.6, 37.5, 26.8, 19.1.

19. *Spectroscopic data for a representative compound* **4i**: $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 7.63 (m, 4H), 7.38 (m, 8H), 7.26 (m, 3H), 6.99 (d, $J$ = 9.5 Hz, 1H), 6.82 (d, $J$ = 9.5 Hz, 1H), 5.24 (s, 2H), 3.70 (t, $J$ = 6.0 Hz, 2H), 2.68 (t, $J$ = 7.6 Hz, 2H), 1.87 (m, 2H), 1.05 (s, 9H). $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 159.7, 147.6, 136.5, 135.5, 133.7, 132.6, 130.1, 129.6, 128.6, 128.5, 127.7, 127.6, 62.6, 55.1, 30.9, 30.8, 26.9, 19.2.
20. *Spectroscopic data for a representative compound* **5d**: $^1$H NMR ($CD_3OD$) $\delta$ (ppm): 7.55 (d, $J$ = 9.5 Hz, 1H), 6.97 (d, $J$ = 9.5 Hz, 1H), 4.47 (s, 2H), 3.73 (s, 3H). $^{13}$C NMR ($CD_3OD$) $\delta$ (ppm): 162.6, 149.8, 133.5, 130.3, 63.5, 40.6.
21. *Spectroscopic data for representative compound* **5h**: $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 7.42 (m, 2H), 7.34 (m, 3H), 7.11 (d, $J$ = 9.6 Hz, 1H), 6.92 (d, $J$ = 9.6 Hz, 1H), 5.31 (s, 2H), 3.96 (t, $J$ = 5.8 Hz, 2H), 2.84 (t, $J$ = 5.8 Hz, 2H). $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 159.6, 146.1, 136.3, 133.0, 130.5, 128.7, 128.8, 60.4, 54.9, 36.8. *Compound* **6h**: $^1$H NMR ($CDCl_3$) $\delta$ (ppm): 7.42 (m, 2H), 7.31 (m, 8H), 7.21 (d, $J$ = 9.5 Hz, 1H), 6.89 (d, $J$ = 9.5 Hz, 1H), 5.31 (s, 2H), 4.52 (s, 2H), 3.77 (t, $J$ = 6.2 Hz, 2H), 2.89 (t, $J$ = 6.2 Hz, 1H). $^{13}$C NMR ($CDCl_3$) $\delta$ (ppm): 159.8, 145.9, 138.0, 136.5, 133.2, 129.8, 128.6, 128.5, 128.4, 127.7, 127.6, 73.1, 68.4, 55.1, 35.1.
22. (a) Orallo, F.; Álvarez, E.; Camiña, M.; Leiro, J. M.; Gómez, E.; Fernández, P. *Mol. Pharmacol.* **2002**, *61*, 294; (b) Vilar, S.; Quezada, E.; Santana, L.; Uriarte, E.; Yañez, M.; Fraiz, N.; Alcaide, C.; Cano, E.; Orallo, F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 257.
23. Born, G. V. R. *Nature* **1962**, *194*, 927.
24. Lin, C. N.; Hsieh, H. K.; Ko, H. H.; Hsu, M. F.; Lin, H. C.; Chang, Y. L.; Chung, M. I.; Kang, J. J.; Wang, J. P.; Teng, C. M. *Drug Dev. Res.* **2001**, *53*, 9.
25. Villar, R. M.; Gil-Longo, J.; Daranas, A. H.; Souto, M. L.; Fernández, J. J.; Peixinho, S.; Barral, M. A.; Santafé, G.; Rodríguez, J.; Jiménez, C. *Bioorg. Med. Chem.* **2003**, *11*, 2301.
26. De Meyers, S. F.; Vanhoorelbeke, K.; Bross, K.; Salles, I. I.; Deckmyn, H. *Br. J. Haematol.* **2008**, *142*, 515.
27. Alcaro, S.; Artese, A.; Botta, M.; Zizzari, A. T.; Orallo, F.; Ortuso, F.; Schenone, S.; Brullo, C.; Yáñez, M. *ChemMedChem* **2010**, *5*, 1242.