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Potential Inhibitors of Angiogenesis. Part II: 3-(Azolylmethylene)-2,3-dihydrobenzo[*b*]furan-2-ones

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The synthesis and pharmacological evaluation of new 3-(imidazol-4(5)-ylmethylene)-2,3-dihydrobenzo[*b*]-furan-2-ones 8–10 and 3-(3,5-dimethylpyrrol-2-ylmethylene)-2,3-dihydrobenzo[*b*]furan-2-one 11, analogues of SU-5416, as potential inhibitors of angiogenesis, are reported. Compounds 8 and 11 were prepared by a Knoevenagel reaction starting from 2-hydroxyphenylacetic acid 2 and 4-formylimidazole 5 or 2-formyl-3,5-dimethylpyrrole 7, followed by acid-catalysed cyclodehydration. For compounds 9 and 10, an alternative method was used; it consisted in carrying out the Knoevenagel reaction with the 2,3-dihydrobenzo[*b*]-furan-2-ones 3 and 4. The antiangiogenic activity of these compounds was evaluated in the three-dimensional *in vitro* rat aortic rings test at 1 μM. At this concentration, compound 11 induced a decrease of angiogenesis comparable to that observed with SU-5416; the vascular density index at 1 μM of 11 and SU-5416 were 30 ± 10 and 22 ± 4% of control, respectively.

Keywords: Angiogenesis; Vascular density index (VDI); 3-(imidazol-4(5)-ylmethylene)indolin-2-one; 2,3-dihydrobenzo[*b*]furan-2-one; 3-(imidazol-4(5)-ylmethylene)-2,3-dihydrobenzo[*b*]furan-2-one; 3-(3,5-dimethylpyrrol-2-ylmethylene)-2,3-dihydrobenzo[*b*]furan-2-one

INTRODUCTION

Angiogenesis, the formation of new blood vessels from pre-existing vessels, plays a fundamental role in a variety of physiological and pathological processes. In normal physiology, this process occurs during embryonic development, ovulation, endometrial regulation and wound repair. On the other hand, the role of persistent, uncontrolled angiogenesis is observed most dramatically in tumour development where the growth of solid tumours and metastases is dependent on the induction of an ever increasing blood supply. Vascular Endothelial Growth Factor (VEGF), one of the most important activators of angiogenesis, plays an essential role in both physiological and pathological angiogenesis, the VEGF receptor mediating various biological activities of VEGF related to proliferation, differentiation and migration of endothelial cells. Thus, VEGF has become one of the targets in the course of the inhibition of angiogenesis.[1,2] Inhibitors of activators of angiogenesis such as the 4-anilinophthalazine PTK-787[3] (VEGF-receptor tyrosine kinase [TK] inhibitor) as well as the arylidenylindolinones SU-5416[3,4] (VEGF-receptor TK inhibitor) and SU-6668[3] (PDGF-receptor TK inhibitor) elaborated by Sugen, have already been entered into clinical studies (Figure 1).

We have recently reported the synthesis and pharmacological evaluation of 3-(imidazol-4(5)-ylmethylene)indolin-2-ones as potential inhibitors of angiogenesis.[5] Compound **1**, the most active compound on the rat aortic rings test, induced a decrease in angiogenesis comparable to that of SU-5416.

In order to enlarge this study, we are now exploring the effect of the replacement of

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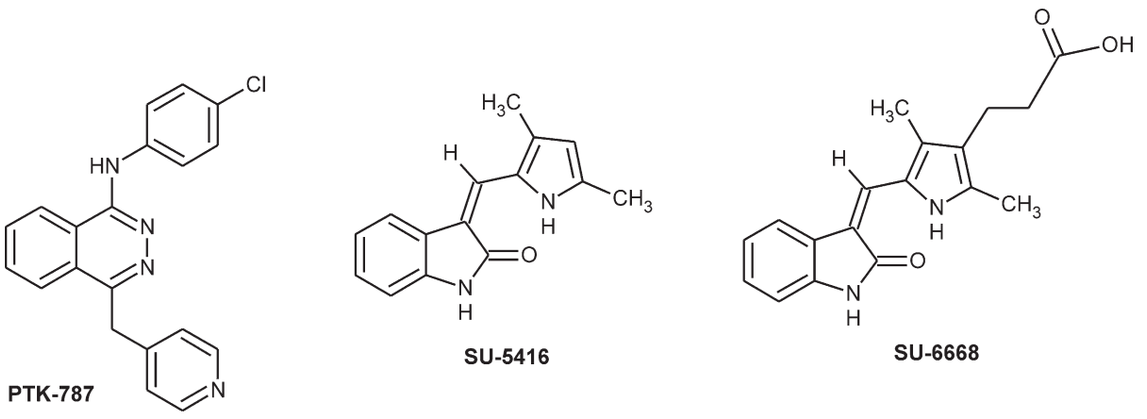

FIGURE 1 Inhibitors of activators of angiogenesis.

the indolin-2-one moiety by a 2,3-dihydrobenzo[*b*]-furan-2-one core (Figure 2).

MATERIALS AND METHODS

Chemistry

Melting points were determined on an Electrothermal IA9000 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker AC 250 spectrometer (250 MHz) (Bruker, Wissembourg, France), using DMSO-d_6 as solvent; chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane as an internal standard. Coupling constants *J* (H–H) are in Hz. IR spectra were recorded on a Perkin-Elmer Paragon PC 1000 spectrometer as KBr pellets or using NaCl pellets for oils (Perkin-Elmer, Courtaboeuf Cedex, France). Chemicals and solvents used were commercially available. 2,3-Dihydrobenzo[*b*]furan-2-one (**3**) was obtained, using the method described by Kadin *et al.* in 89% yield.[6] 4-Formyl-1-methylimidazole (**6**) was prepared by methylation of 4-formylimidazole (**5**), and 2-formyl-3,5-dimethylpyrrole (**7**) was synthesized by a Vilsmeier-Haack reaction,[7] as reported in our previous work.[5]

Method A

To 1.4 ml of acetic anhydride were added 2-hydroxyphenylacetic acid (**2**) (1 eq), 4-formylimidazole (**5**) or 2-formyl-3,5-dimethylpyrrole (**7**) (2 eq) and sodium acetate (0.6 eq). The reaction mixture was heated at 90°C for 19 h. 10 ml of water was then added and the heating maintained for 15 min. The mixture was finally cooled in an ice bath and the precipitate collected by filtration before recrystallization.

(*E*)-3-(Imidazol-4-ylmethylene)-2,3-dihydrobenzo[*b*]furan-2-one (**8**)

Yield: 30%; mp 193°C (methanol); IR (KBr) ν cm^{-1} 1780 (νCO), 1632 (νC=C); ^{1}H NMR (DMSO-d_6), δ ppm, 7.24–7.42 (m, 3H, H_5, H_6 and H_7), 7.73 (s, 1H, $H_{2'}$), 8.07 (s, 1H, Hvinyl), 8.14 (s, 1H, $H_{5'}$), 9.43 (d, $J = 7.6$, 1H, H_4), 12.86 (s, 1H, NH); ^{13}C NMR (DMSO-d_6), δ ppm, 110.10 (CH), 114.98, 118.76 (CH), 123.42, 126.85 (CH), 128.31 (CH), 129.65 (CH), 132.42 (CH), 137.22, 139.16 (CH), 153.07, 170.10 (CO).

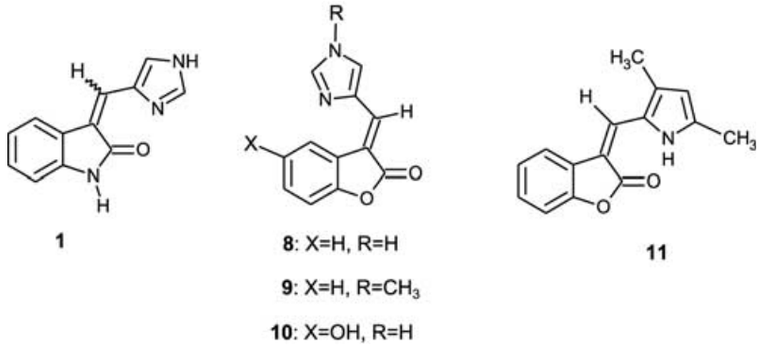

FIGURE 2 Chemical structures of 3-(imidazol-4(5)-ylmethylene)indolin-2-one **1** and the 3-azolylmethylene-2,3-dihydrobenzo[*b*]furan-2-ones **8**–**11**.

(*Z*)-3-(3,5-Dimethylpyrrol-2-ylmethylene)-2,3-dihydrobenzo[*b*]furan-2-one (**11**)

Yield: 17%; mp 204°C (diethyl ether); IR (KBr) $\nu\,cm^{-1}$ 1720 (νCO), 1572 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 2.37 (s, 3H, $CH_3^{3'}$), 2.40 (s, 3H, $CH_3^{5'}$), 6.16 (s, 1H, CHpyrrol), 7.23–7.86 (m, 4H, H_4–H_7), 7.87 (s, 1H, Hvinyl), 12.19 (s, 1H, NH); ^{13}C NMR (DMSO-d_6), δ ppm, 11.81 (CH_3), 13.94 (CH_3), 104.72, 110.16 (CH), 112.66, 118.93 (CH), 121.42 (CH), 123.87 (CH), 126.53 (CH), 126.58 (CH), 127.10, 135.89, 139.83, 150.68, 170.10 (CO).

Method B

To a solution of 2,3-dihydrobenzo[*b*]furan-2-one (**3**) or its 5-hydroxy derivative (**4**) in 10 ml of ethanol were added 4-formylimidazole (**5**) or 4-formyl-1-methylimidazole (**6**) (1 eq) and 0.2 ml of triethylamine. The stirring was maintained for 6 h at room temperature and the final precipitate was collected by filtration before recrystallization from ethanol to afford **9** or **10** as a yellow powder.

(*E*)-3-(1-methylimidazol-4-ylmethylene)-2,3-dihydrobenzo[*b*]furan-2-one (**9**)

Yield: 78%; mp 188–189°C; IR (KBr) $\nu\,cm^{-1}$ 1770 (νCO), 1640 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 3.81 (s, 3H, CH_3), 7.22–7.29 (m, 2H, H_6 and H_7), 7.40 (dd, 1H, $J = 7.6$ and 6.7, 1H, H_5), 7.67 (s, 1H, $H_{2'}$), 8.06 (s, 2H, Hvinyl and $H_{5'}$), 8.35 (d, $J = 7.6$, 1H, H_4); ^{13}C NMR (DMSO-d_6), δ ppm, 33.82 (CH_3), 110.20 (CH), 115.11, 123.30, 123.93 (CH), 127.05 (CH), 129.75 (CH), 131.63 (CH), 132.07 (CH), 137.42, 141.57 (CH), 153.08, 170.03 (CO).

(*E*)-5-hydroxy-3-(imidazol-4-ylmethylene)-2,3-dihydrobenzo[*b*]furan-2-one (**10**)

Yield: 88%; mp >250°C; IR (KBr) $\nu\,cm^{-1}$ 1730 (νCO), 1602 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 6.78 (dd, $J = 8.5$ and 2.15, 1H, H_6), 7.03 (d, $J = 8.5$, 1H, H_7), 7.67 (s, 1H, $H_{2'}$), 8.08 (s, 1H, Hvinyl), 8.11 (s, 1H, $H_{5'}$), 8.88 (d, $J = 2.15$, 1H, H_4), 9.46 (s, 1H, OH), 12.75 (s, 1H, NH); ^{13}C NMR (DMSO-d_6), δ ppm, 106.34, 110.27 (CH), 114.00 (CH), 116.12 (CH), 123.88, 128.04 (CH), 131.97 (CH), 137.26, 138.95 (CH), 146.26, 153.83, 170.70 (CO).

Pharmacology

Three Dimensional In Vitro Rat Aortic Rings Model

Preparation of Three-dimensional Aortic Ring Cultures

Aortic explant cultures were prepared as described by Nicosia *et al.*[8] Briefly, thoracic aortas were rapidly removed from 8- to 12-week-old male Fischer-344 rats sacrificed by CO_2 inhalation and immediately transferred to a culture dish containing cold (4°C) serum-free minimum essential medium (MEM, Life Technologies Ltd, Paisley, Scotland). After gently flushing with 2 × 1 ml medium to remove clotted blood, the periaortic fibroadipose tissue was carefully removed under a dissecting microscope using fine microdissection forceps and scissors. Aortic rings (approximatively 30 per aorta) obtained by sectioning the aorta at 1 mm intervals with a scalpel blade, were extensively rinsed in five washes of MEM. For the preparation of culture wells, 30 ml of sterile 1.5% solution of agarose (type VII, cell culture tested, Sigma, St Quentin Fallavier, France) were poured into 100 mm diameter Petri dishes (cell culture-treated, Costar, Corning Costar Corporation, Cambridge, Massachusetts) and allowed to gel. Agarose rings were obtained by punching two concentric circles in the agarose with punchers of 10 and 17 mm diameter and were transferred to further 100 mm diameter Petri dishes (bacteriological polystyrene, Falcon, Beckton Dickinson, Lincoln Park, New Jersey) with a bent spatula. Four such culture wells were prepared in each dish. Interstitial (type I) collagen gel (1.5 mg/ml) was prepared according to the method of Montesano *et al.*[9] by rapidly mixing, at 4°C, 7.5 volumes of a solution of rat tail collagen (2 mg/ml in acetic acid, Collagen R, Serva, Heidelberg, Germany) with 1 volume of 10 × MEM, 1.5 volumes 15.6 mg/ml $NaHCO_3$ to adjust the pH to 7.4. The bottom of each agarose well was coated with 200 μl of this preparation, which was allowed to gel at 37°C. One aortic ring was then carefully positioned in each well, which was then completely filled with collagen solution.

Media Preparation and Incubation Conditions

All incubations were carried out in MCDB131 (Life technologies Ltd, Paisley, Scotland), a culture medium optimised for the low-serum culture of microvascular endothelial cells[10]: 30 ml per dish for three-dimensional and 7 ml per dish for monolayer culture. To maintain a pH of 7.4 after equilibration at 37°C and 5% CO_2, $NaHCO_3$ must be present in this medium at a concentration of 25 nM. All media were supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin. Cultures were maintained in a humidified incubator at 37°C and 5% CO_2/95% air.

Angiogenesis Quantitation

The vascular density index (VDI) was defined as the number of intersections of the endothelial outgrowth with an imaginery grid placed around the aortic ring with lines at intervals of 100 μm, thus taking into account both the number of vessels and the distance of outgrowth.

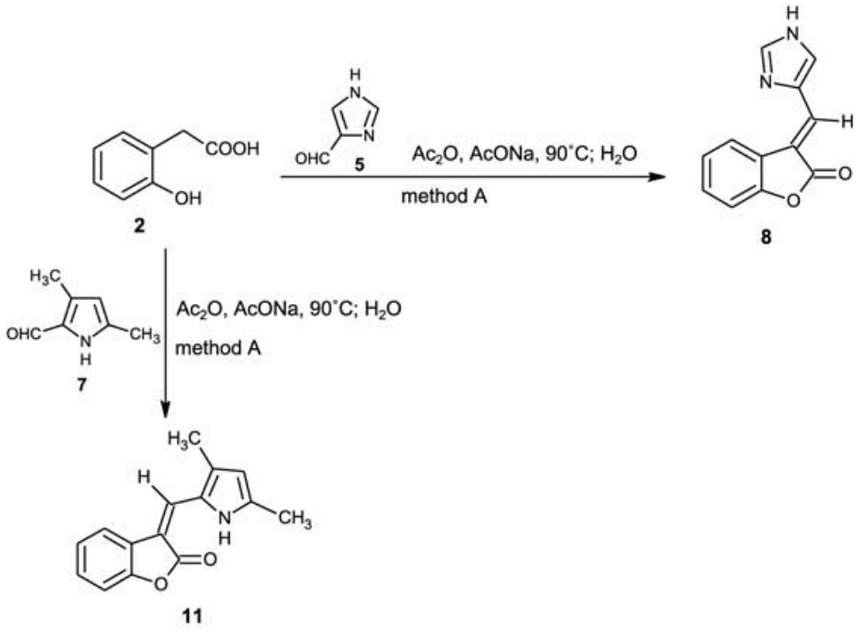

FIGURE 3 Preparation of 3-(imidazol-4(5)-ylmethylene)-2,3-dihydrobenzo[*b*]furan-2-one **8** and 3-[(3,5-dimethylpyrrol-2-yl)methylene]-2,3-dihydrobenzo[*b*]furan-2-one **11** (method A).

RESULTS AND DISCUSSION

Chemistry

3-Arylmethylene-2,3-dihydrobenzo[*b*]furan-2-ones have been previously prepared by the Knoevenagel reaction,[11,12] a Wittig reaction starting from 2,3-dihydrobenzo[*b*]furan-2,3-dione or the corresponding phosphorane,[13–15] or by base-catalysed rearrangement of 3-hydroxyflavanones.[16,17]

In the present study, compounds **8** and **11** were obtained by a Knoevenagel reaction starting from 2-hydroxyphenylacetic acid (**2**) and 4-formylimidazole (**5**), or 2-formyl-3,5-dimethylpyrrole (**7**), followed by cyclodehydration in acidic medium[18,19] (method A) with moderate yields (20–30%) (Figure 3). An alternative method consisted in carrying out an aldolisation-crotonisation reaction starting from 2,3-dihydrobenzo[*b*]furan-2-ones **3** and **4** (method B). By operating under basic catalysis (Et_3N), at room temperature it afforded compounds **9** and **10** in more satisfactory yields (80–90%) (Figure 4).

The stereochemistry of these α,β-unsaturated lactones was determined by vicinal C,H spin coupling constants. The use of ^{13}C n.m.r. ^{13}C-^{1}H coupling constants of the ketonic carbon of the final compounds **8**–**11** afforded the results shown in Figure 5 that allow stereochemistry assignment.

In conclusion, in this series, the pyrrolyl compound **11** presents a *Z*-configuration whereas compounds **8**–**10** have been isolated as the *E*-isomers. These results are in line with the assignment of Kingsbury *et al.* for 4-benzylidene-oxazolin-5-ones or isoxazolin-5-ones and Letcher *et al.* for benzylideneazocinediones.[20,21]

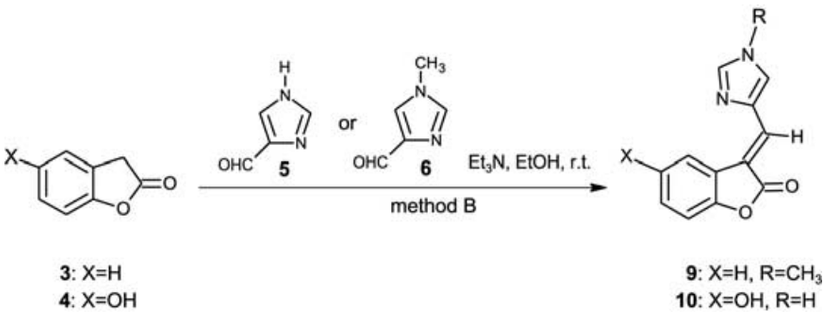

FIGURE 4 Preparation of 3-(imidazol-4(5)-ylmethylene)-2,3-dihydrobenzo[*b*]furan-2-ones **9** and **10** (method B).

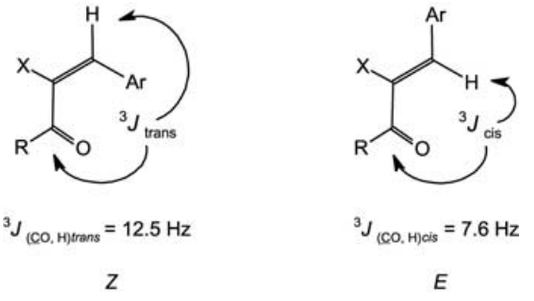

FIGURE 5 Identification of the *Z*- or *E*-configuration by ^3J(C, H) vicinal couplings in the α,β unsaturated lactones **8**–**11**.

TABLE I Evaluation of angiogenesis inhibition by the *in vitro* rat aortic rings test

Compound	VDI (%)* (1 μM)
8	96 ± 6
9	93 ± 49
10	77 ± 19
11	30 ± 10
1	30 ± 18
SU-5416	22 ± 4

* VDI: Vascular Density Index; percentages correspond to the mean of two independent assays.

Pharmacology

Substantial evidence has accumulated over the past 25 years indicating the dependency of solid tumours on angiogenesis. An increase in microvascular density in solid tumours such as breast and prostate carcinoma has been shown to correlate with malignant and metastatic potential. A strategy based on inhibition of VEGF could be successful in interfering, at least in part, with the vascularization and growth of primary tumours as well as with spread and outgrowth of metastases.

To gain a preliminary insight into the inhibitory effect on angiogenesis of the studied α-3-azolylmethylenelactones **8**–**11**, a three dimensional *in vitro* rat aortic rings model was used.[8–10] Angiogenesis quantitation was carried out by determination of the vascular density index (VDI).

Replacement of the indolinone moiety of the β-lactam **1** by a benzofuranone core exerted a detrimental effect, as none of the three studied molecules **8**–**10** was able to significantly inhibit endothelial outgrowth (Table I). Nevertheless, a marked increase of inhibitory activity (VDI: 30 ± 10% of control) was observed with lactone **11** resulting from the replacement of indolin-2-one in SU-5416 by 2,3-dihydrobenzo[*b*]furan-2-one. Although the active indolinone **1** possesses a predominant *E*-configuration (*Z*/*E*: 10/90), preliminary pharmacological results tend to point out the stringent structural requirement for emergence of antiangiogenic activity in this 2,3-dihydrobenzo[*b*]-furan-2-one series.

Our present efforts with the new sub-series of 2,3-dihydrobenzofuranones based on **11** are now mainly concentrated on synthesis of derivatives with various substituents at the homocycle and evaluation of their antiproliferative activity.

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