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Potential Inhibitors of Angiogenesis. Part I: 3-(Imidazol-4(5)-ylmethylene)indolin-2-ones

EMMANUELLE BRAUD[a], MURIEL DUFLOS[a], MARIE-RENEE NOURRISSON[a], ALAIN TONNERRE[a], CARINE PICOT[a], GUILLAUME LE BAUT[a,*], PIERRE RENARD[b], BRUNO PFEIFFER[b] and GORDON TUCKER[c]

[a]*Laboratoire de Chimie Organique et de Chimie Thérapeutique, UPRES EA 1155, Faculté de Pharmacie, 1 rue Gaston Veil, F-44035 Nantes, France;* [b]*Laboratoire SERVIER, 1 rue Carle Hébert, F-92415 Courbevoie, France;* [c]*Institut de Recherches SERVIER, Division Recherche Cancérologie, 125 Chemin de Ronde, 78290 Croissy sur Seine, France*

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The synthesis and pharmacological evaluation of new 3-(imidazol-4(5)-ylmethylene)indolin-2-ones, analogues of SU-5416, are reported. The final compounds 20–51 were obtained by Knoevenagel coupling between the substituted indolin-2-ones 1–15 and either the formylimidazole derivatives 16–18 or 2-formyl-3,5-dimethylpyrrole 19. Methylation at the nitrogen atom of the indolin-2-one and/or imidazole moities was carried out in the presence of the couple NaH/DMF. A Mannich reaction afforded the 1-dimethylaminomethyl derivatives 43 and 48. The antiangiogenic activity of these compounds was evaluated in a three dimensional *in vitro* rat aortic ring assay. In this test, compound 20 induced a decrease of angiogenesis comparable to that observed with SU-5416; the vascular density indexes at 1 μM were 30 ± 18 and 22 ± 4 % of control, respectively. The compounds were also evaluated, in an independent manner, as inhibitors of the human EGF-receptor tyrosine kinase activity. As expected, only minor activities were observed with four compounds, out of thirty-one, exerting inhibitory effects in the range of 40–55 % at 10 μM concentration.

Keywords: Cancer; Angiogenesis; Epidermal growth factor (EGF); Vascular density index (VDI); Indolin-2-one; 3-(Imidazol-4(5)-ylmethylene)indolin-2-one

INTRODUCTION

Angiogenesis is fundamental to healing, reproduction and embryonic development. In 1971, Folkman demonstrated that tumor growth depends on angiogenesis.[1–3] Indeed, the formation of new blood vessels through the vascularisation process provides nutrients to proliferating cancer cells thus favouring tumour growth. Since then, the angiogenesis process has been widely studied.[4–7]

Various inhibitors of angiogenesis are at the moment under investigation in patients with advanced cancer. They are classified through their mechanism of action.[8] Thus, the main steps of the angiogenic process can be interrupted using natural substances that directly inhibit endothelial cells such as angiostatin and endostatin,[8] squalamin and TNP-470, a synthetic analog of fumagillin. A second strategy consists in elaborating synthetic drugs that block the matrix breakdown through the inhibition of metalloproteinases (collagenases and gelatinases) such as BMS-275291.[8] Molecules that block the action of integrins present on the endothelial cell surface, such as the cyclopentapeptide EMD-121974 or drugs that inhibit the calcium influx such as CAI are also promising new antiangiogenic agents. Finally, inhibitors of activators of angiogenesis such as the arylidenylindolinones SU-5416[9,10] (VEGF-receptor tyrosine kinase [TK] inhibitor) and SU-6668[9] (PDGF-receptor TK inhibitor) elaborated by Sugen, as well as the 4-anilinophthalazine PTK-787[9] (VEGF-receptor TK inhibitor) have entered in clinical studies (Figure 1).

Among activators of angiogenesis, the epidermal growth factor (EGF) and the vascular endothelial growth factor (VEGF) are two regulators of the angiogenesis process in pathologic conditions. The signal transduction of vascular processes occurs when the (V)EGF binds on its receptor. Tyrosine kinase inhibitors of the (V)EGF-receptor prevent the signalling of (V)EGF on its receptor by binding, in a competitive manner, on the ATP site of the kinase domain of the receptor.[11–17]

*Corresponding author. Fax: +33-240412876. E-mail: guillaume.lebaut@sante.univ-nantes.fr

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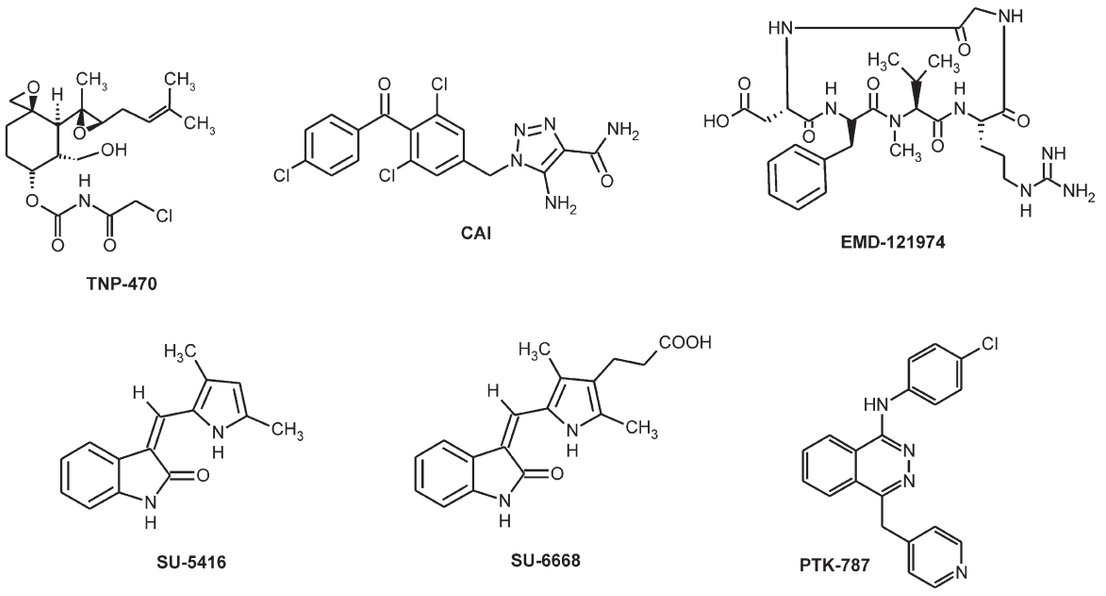


FIGURE 1 Inhibitors of activators of angiogenesis.

Thus, referring to the structure of SU-5416 and SU-6668 and to our previous work,[18] we investigated the access to new 3-(imidazol-4(5)-ylmethylene)indolin-2-ones as potential inhibitors of activators of angiogenesis (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 (Perkin-Elmer, Courtaboeuf Cedex, France). Chemicals and solvents used were commercially available. The indolin-2-ones **2**–**15** were prepared according to previously described methods (Figure 5). The 4- and 6-halogenated indolin-2-ones **2**–**4** were obtained following the Sandmeyer reaction starting from the corresponding 3-halogenoanilines.[19] 4-Trifluoromethylindolin-2-one **5** was synthetized using the same strategy. N-methoxyindolin-2-ones **13**–**15** were obtained via the N-methoxyphenylacetamide derivatives.[20,21] The 5-chloro and 5-methoxyindolin-2-ones, **6** and **7**, were synthesized by a nucleophilic rearrangement of *N*-methoxyindolin-2-one **13**.[22] The 6-phenyl-and 6-trifluoromethylindolin-2-ones, **8** and **9**, were prepared from the corresponding 2-nitrophenylacetic acids.[23,24] 4-Aminoindolin-2-one **10** was obtained by hydrogenolysis of the 3-methylthioindolin-2-one.[25] Acetamide **11** was prepared by direct acetylation of **10**. 5-Bromoindolin-2-one **12** was obtained according to Sumpter *et al.*[26] 2-Formyl-3,5-dimethylpyrrole **19** was prepared by a Vilsmeier–Haack reaction.[27]

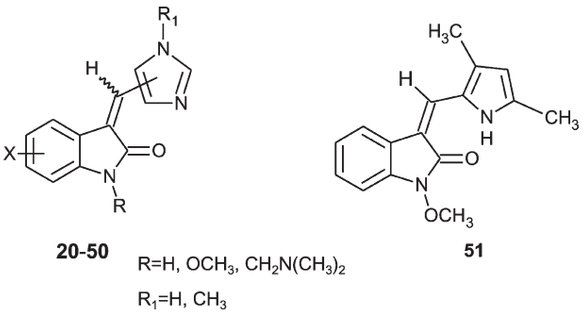


FIGURE 2 3-(Imidazol-4(5)-ylmethylene)indolin-2-ones **20**–**50** and 3-(3,5-dimethylpyrrol-2-ylmethylene)indolin-2-one **51**.

4-Formyl-1-methylimidazole (17) and 5-formyl-1-methylimidazole (18)

To a solution of 1 g of **16** (10.41 mmol) in 20 ml of DMF was added 0.46 g of NaH (11.5 mmol). The reaction mixture was stirred at room temperature for 45 min and 0.65 ml of methyl iodide (10.41 mmol) was added. The stirring was maintained for 2 h and 20 ml of water were added to the solution. The solvent was finally evaporated under reduced pressure and the residue was chromatographed on silica gel (CH_2Cl_2/EtOH, 95:5) to afford pure **17** and **18** as white powders.

4-Formyl-1-methylimidazole (**17**)

Yield: 46%; mp 59–61°C (from hexane) (lit.[28] 65–66°C). IR (KBr) 1678 (νCO) cm^{-1}; ^1H NMR (DMSO-d_6), δ ppm, 3.76 (s, 3H, CH_3), 7.85 (s, 1H, H_5), 8.05 (s, 1H, H_2), 9.72 (s, 1H, CHO).

5-Formyl-1-methylimidazole (**18**)

Yield: 25%; mp 49–51°C (from hexane) (lit.[29] 53–54°C). IR (KBr) 1678 (νCO) cm^{-1}; ^1H NMR (DMSO-d_6), δ ppm, 3.89 (s, 3H, CH_3), 7.90 (s, 1H, H_4), 8.03 (s, 1H, H_2), 9.77 (s, 1H, CHO).

Method A

The imidazoles **16** or **17** or **18** or **19** (1.16 eq) and 0.6 ml of piperidine were added to a solution of indolin-2-one **1**–**15** (1 eq) in 30 ml of ethanol. The reaction mixture was heated under reflux for 2 h. After cooling, the yellow or orange precipitate was filtered and recrystallized from ethanol.

(*Z*,*E*)-3-(Imidazol-4-ylmethylene)indolin-2-one (**20**)

Yield: 80%; mp 228–230°C; IR (KBr) ν cm^{-1} 1690 (νCO), 1610 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 6.93 (d, $J = 6.9$, 1H, H_7), 7.05 (dd, $J = 7.1$, 1H, H_6), 7.23 (d, $J = 6.7$, 1H, H_5), 7.68–7.71 (m, 2H, $H_{2'}$ and H_4), 7.88 (s, 1H, H vinyl), 8.06 (s, 1H, $H_{5'}$), 11.05 (s, 1H, NH ind), 13.76 (s, 1H, NH imid). *Z*: 6.94–7.23 (m, 2H, H_5 and H_7), 7.03–7.08 (m, 1H, H_6), 7.68–8.06 (m, 3H, H vinyl, H_4 and $H_{2'}$), 8.95 (s, 1H, $H_{5'}$), 10.60 (s, 1H, NH ind), 12.59 (s, 1H, NH imid).

(*Z*)-4-Bromo-3-(imidazol-4-ylmethylene)indolin-2-one (**21**)

Yield: 68%; mp >300°C; IR (KBr) ν cm^{-1} 1685 (νCO), 1617 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 6.95 (d, $J = 6.7$, 1H, H_7), 7.15 (dd, $J = 6.7$ and 7.6, 1H, H_6), 7.25 (d, $J = 7.6$, 1H, H_5), 8.05 (s, 1H, $H_{2'}$), 8.15 (s, 1H, H vinyl), 8.62 (s, 1H, $H_{5'}$ or $H_{4'}$), 11.21 (s, 1H, NH ind), 13.54 (s, 1H, NH imid).

(*Z*,*E*)-4-Amino-3-(imidazol-4-ylmethylene)indolin-2-one (**22**)

Yield: 82%; mp 238–241°C; IR (KBr) ν cm^{-1} 1676 (νCO), 1592 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 5.72 (s, 2H, NH_2), 6.22–7.34 (m, 3H, H_5, H_6 and H_7), 7.55 (s, 1H, $H_{2'}$), 7.95 (s, 1H, H vinyl), 8.03 (s, 1H, $H_{5'}$), 11.13 (s, 1H, NH ind), 13.82 (s, 1H, NH imid). *Z*: 5.72 (s, 2H, NH_2), 6.22–7.34 (m, 3H, H_5, H_6 and H_7), 7.68 (s, 1H, $H_{2'}$), 8.55 (s, 2H, H vinyl and $H_{5'}$), 10.90 (s, 1H, NH ind), 13.82 (s, 1H, NH imid).

(*E*)-4-Acetylamino-3-(imidazol-4-ylmethylene)indolin-2-one (**23**)

Yield: 78%; mp >300°C; IR (KBr) ν cm^{-1} 1686 (νCO), 1606 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 2.19 (s, 3H, CH_3), 6.92 (m, 1H, H_6), 7.19 (m, 1H, H_5), 7.65 (s, 1H, $H_{2'}$), 7.76 (s, 1H, H vinyl), 8.0 (m, 1H, H_7), 8.04 (s, 1H, $H_{5'}$), 9.94 (s, 1H, NH), 11.14 (s, 1H, NH ind), 13.79 (s, 1H, NH imid).

(*Z*)-5-Bromo-3-(imidazol-4-ylmethylene)indolin-2-one (**24**)

Yield: 73%; mp >300°C; IR (KBr) ν cm^{-1} 1693 (νCO), 1615 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 6.89 (d, $J = 8.3$, 1H, H_7), 7.38 (d, $J = 8.3$, 1H, H_6), 7.67 (s, 1H, H_4), 7.96 (s, 1H, $H_{2'}$), 8.04 (s, 1H, H vinyl), 8.08 (s, 1H, $H_{5'}$), 11.16 (1s, 1H, NH ind), 13.66 (s, 1H, NH imid).

(*E*)-5-Chloro-3-(imidazol-4-ylmethylene)indolin-2-one (**25**)

Yield: 77%; mp >250°C; IR (KBr) ν cm^{-1} 1696 (νCO), 1627 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 6.88 (d, $J = 7.3$, 1H, H_7), 7.23 (d, $J = 7.3$, 1H, H_6), 7.84 (s, 1H, $H_{2'}$), 8.01 (s, 1H, H vinyl), 8.14 (s, 1H, $H_{5'}$), 9.51 (s, 1H, H_4), 10.56 (1s, 1H, NH ind), 13.65 (s, 1H, NH imid); ^{13}C NMR (DMSO-d_6), δ ppm, 110.54 (C_7), 120.96 (C_3), 124.59 (C_{3a}), 126.44 (C_6), 127.17 (C_4), 127.67 (C_5), 128.12 ($C_{5'}$), 128.96 (CH vinyl), 137.29 ($C_{4'}$), 138.70 ($C_{2'}$), 140.70 (C_{7a}), 170.03 (CO).

(*Z*,*E*)-6-Chloro-3-(imidazol-4-ylmethylene)indolin-2-one (**26**)

Yield: 41%; mp >250°C; IR (KBr) ν cm^{-1} 1685 (νCO), 1602 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 6.90 (m, 2H, H_5 and H_7), 7.70–8.05 (m, 3H, H vinyl, $H_{2'}$ and $H_{5'}$), 9.10 (d, $J = 8.5$, 1H, H_4), 11.20 (s, 1H, NH ind), 13.62 (s, 1H, NH imid). *Z*: 6.94 (s, 1H, H_7), 7.09 (d, $J = 7.9$, 1H, H_5), 7.70–7.74 (m, 2H, $H_{2'}$ and H_4), 7.93 (s, 1H, H vinyl), 8.05 (s, 1H, $H_{5'}$), 11.20 (s, 1H, NH ind), 13.62 (s, 1H, NH imid); ^{13}C NMR (DMSO-d_6), δ ppm *E*: 107.58 (C_7), 119.22 (C_3), 119.45 (C_{3a}), 123.48 (C_5), 126.79 (C_6), 128.83 (C_4), 129.52 ($C_{5'}$), 134.56 (CH vinyl), 136.44 ($C_{4'}$), 139.77 ($C_{2'}$), 143.26 (C_{7a}), 165.52 (CO).

(*Z*,*E*)-6-Bromo-3-(imidazol-4-ylmethylene)indolin-2-one (**27**)

Yield: 37%; mp >300°C; IR (KBr) ν cm^{-1} 1678 (νCO), 1602 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 7.02 (s, 1H, H_7), 7.20–7.26 (m, 1H, H_5), 7.83 (s, 1H, $H_{2'}$), 7.90 (s, 1H, H vinyl), 8.08 (s, 1H, $H_{5'}$ or $H_{4'}$), 10.73 (s, 1H, NH ind), 12.65 (s, 1H, NH imid). *Z*: 7.07 (s, 1H, H_7), 7.24 (d, $J = 7.3$, 1H, H_5), 7.68 (d, $J = 7.3$, 1H, H_4), 7.70 (s, 1H, $H_{2'}$), 7.95 (s, 1H, H vinyl), 8.95 (s, 1H, $H_{5'}$ or $H_{4'}$), 11.17 (s, 1H, NH ind), 13.62 (s, 1H, NH imid).

(*Z*)-3-(Imidazol-4-ylmethylene)-6-phenylindolin-2-one (**28**)

Yield: 73%; mp >300°C; IR (KBr) ν cm^{-1} 1684 (νCO), 1607 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 7.15 (s, 1H, H_7), 7.35–7.43 (m, 2H, H_5 and H_4 phenyl), 7.5 (dd, $J = 7$ and 7.6, 2H, H_3 phenyl and H_5 phenyl), 7.68 (d, $J = 7.6$, 2H, H_2 phenyl and H_6 phenyl), 7.79 (d, $J = 7.9$, 1H, H_4), 7.91 (s, 1H, $H_{2'}$),

8.09 (s, 1H, $H_{5'}$), 11.13 (s, 1H, NH ind), 13.74 (s, 1H, NH imid).

(*Z*)-3-(1-Methylimidazol-4-ylmethylene)indolin-2-one (**29**)

Yield: 58%; mp 261–263°C; IR (KBr) ν cm^{-1} 1685 (νCO), 1622 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 3.80 (s, 3H, CH_3), 6.86 (d, $J = 7.6$, 1H, H_7), 6.96 (dd, $J = 7.4$ and 7.6, 1H, H_6), 7.20 (dd, $J = 7.4$ and 7.35, 1H, H_5), 7.67 (s, 1H, $H_{2'}$), 7.82 (s, 1H, H vinyl), 8.91 (s, 1H, $H_{5'}$), 10.62 (s, 1H, NH).

(*Z*)-3-(1-Methylimidazol-4-ylmethylene)-4-trifluoromethylindolin-2-one (**30**)

Yield: 58%; mp >300°C; IR (KBr) ν cm^{-1} 1700 (νCO), 1608 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 3.82 (s, 3H, CH_3), 7.19 (d, $J = 6.4$, 1H, H_7), 7.38 (dd, $J = 6.4$ and 7.6, 1H, H_6), 7.41–7.44 (m, 1H, H_5), 7.87 (s, 1H, $H_{2'}$), 7.92 (s, 1H, H vinyl), 9.01 (s, 1H, $H_{5'}$), 11.06 (s, 1H, NH).

(*Z*)-4-Bromo-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**31**)

Yield: 88%; mp >300°C; IR (KBr) ν cm^{-1} 1692 (νCO), 1585 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 3.81 (s, 3H, CH_3), 6.91 (d, $J = 7.3$, 1H, H_7), 7.13 (dd, $J = 7.3$ and 8.5, 1H, H_6), 7.23 (d, $J = 8.5$, 1H, H_5), 7.85 (s, 1H, $H_{2'}$), 8.63 (s, 1H, H vinyl), 8.97 (s, 1H, $H_{5'}$), 10.91 (s, 1H, NH).

(*Z*,*E*)-6-Chloro-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**32**)

Yield: 42%; mp 285–287°C; IR (KBr) ν cm^{-1} 1692 (νCO), 1614 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 3.80 (s, 3H, CH_3), 7.07 (s, 1H, H_7), 7.47 (s, 1H, $H_{2'}$), 7.96 (s, 1H, H vinyl), 7.97 (d, $J = 8.2$, 1H, H_5), 7.99 (s, 1H, $H_{5'}$), 9.32 (d, $J = 8.2$, 1H, H_4), 11.60 (s, 1H, NH). *Z*: 3.80 (s, 3H, CH_3), 6.87 (s, 1H, H_7), 7.02 (d, $J = 7.9$, 1H, H_5), 7.73 (s, 1H, $H_{2'}$), 7.75 (d, $J = 7.9$, 1H, H_4), 7.83 (s, 1H, H vinyl), 8.90 (s, 1H, $H_{5'}$), 10.77 (s, 1H, NH).

(*Z*,*E*)-3-(1-Methylimidazol-4-ylmethylene)-6-trifluoromethylindolin-2-one (**33**)

Yield: 75%; mp >250°C; IR (KBr) ν cm^{-1} 1697 (νCO), 1625 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 3.79 (s, 3H, CH_3), 7.08 (s, 1H, H_7), 7.31–7.39 (m, 1H, H_5), 7.61 (s, 1H, $H_{2'}$), 8.04 (s, 2H, $H_{5'}$ and H vinyl), 9.50 (d, $J = 8.2$, 1H, H_4), 10.73 (s, 1H, NH). *Z*: 3.82 (s, 3H, CH_3), 7.08 (s, 1H, H_7), 7.33 (d, $J = 7.6$, 1H, H_5), 7.87 (s, 1H, $H_{2'}$), 7.88 (d, $J = 7.6$, 1H, H_4), 7.90 (s, 1H, H vinyl), 7.98 (s, 1H, $H_{5'}$), 10.91 (s, 1H, NH).

(*Z*,*E*)-6-Bromo-3-(1-methylimidazol-5-ylmethylene)indolin-2-one (**34**)

Yield: 37%; mp 275–278°C; IR (KBr) ν cm^{-1} 1707 (νCO), 1607 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 4.01 (s, 3H, CH_3), 7.06 (s, 1H, H_7), 7.07 (d, $J = 7.9$, 1H, H_5), 7.25 (d, $J = 7.9$, 1H, H_4), 7.41 (s, 1H, $H_{2'}$), 8.96 (s, 1H, $H_{4'}$), 10.93 (s, 1H, NH). *Z*: 3.86 (s, 3H, CH_3), 7.06–7.27 (m, 1H, H_7), 7.18 (d, $J = 7.5$, 1H, H_5), 7.87 (d, $J = 7.5$, 1H, H_4), 8.06 (s, 1H, $H_{2'}$), 8.48 (s, 1H, H vinyl), 8.80 (s, 1H, $H_{4'}$), 10.84 (s, 1H, NH).

(*Z*,*E*)-1-Methoxy-3-(imidazol-4-ylmethylene)indolin-2-one (**35**)

Yield: 83%; mp 169–170°C; IR (KBr) cm^{-1} 1686 (νCO), 1602 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 4.05 (s, 3H, CH_3), 7.11–7.40 (m, 3H, H_5, H_6 and H_7), 7.80–7.83 (m, 1H, H_4), 7.80–8.08 (m, 3H, H vinyl, $H_{2'}$ and $H_{5'}$), 13.34 (s, 1H, NH). *Z*: 4.05 (s, 3H, CH_3), 7.11–7.40 (m, 4H, H_4, H_5, H_6 and H_7), 7.80–8.08 (m, 2H, H vinyl and $H_{2'}$), 8.94 (s, 1H, $H_{5'}$), 12.73 (s, 1H, NH).

(*Z*)-4-Bromo-3-(imidazol-4-ylmethylene)-1-methoxyindolin-2-one (**36**)

Yield: 76%; mp 230–231; IR (KBr) ν cm^{-1} 1702 (νCO), 1616 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 4.04 (s, 3H, OCH_3), 7.16 (d, $J = 7.9$, 1H, H_7), 7.28 (dd, $J = 7.3$ and 7.9, 1H, H_6), 7.36 (d, $J = 7.3$, 1H, H_5), 8.06 (s, 2H, H vinyl and $H_{2'}$), 8.74 (s, 1H, $H_{5'}$), 13.34 (s, 1H, NH); ^{13}C NMR (DMSO-d_6), δ ppm, 64.31 (OCH_3), 106.96 (C_7), 115.34 (C_4), 120.22 (C_3), 121.42 (C_{3a}), 126.49 (C_5), 127.92 (C_6), 129.48 ($C_{5'}$), 137.45 ($C_{4'}$), 138.90 (CH vinyl), 141.47 ($C_{2'}$), 142.03 (C_{7a}), 167.26 (CO).

(*Z*,*E*)-6-Bromo-1-methoxy-3-(imidazol-4-ylmethylene)indolin-2-one (**37**)

Yield: 85%; mp 190–192°C; IR (KBr) ν cm^{-1} 1686 (νCO), 1606 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 4.00 (s, 3H, OCH_3), 7.26 (s, 1H, H_7), 7.34 (d, $J = 7.9$, 1H, H_5), 7.68 (s, 1H, $H_{2'}$), 8.09 (s, 1H, H vinyl), 8.11 (s, 1H, $H_{5'}$), 9.38 (d, $J = 7.9$, 1H, H_4). *Z*: 4.04 (s, 3H, OCH_3), 7.26–7.35 (m, 2H, H_4 and H_7), 7.68–7.78 (m, 2H, H_5 and $H_{2'}$), 8.01 (s, 1H, H vinyl), 8.04 (s, 1H, $H_{5'}$).

(*E*)-1-Methoxy-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**38**)

Yield: 59%; mp 205–207°C; IR (KBr) ν cm^{-1} 1706 (νCO), 1610 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 3.80 (s, 3H, CH_3), 4.00 (s, 3H, OCH_3), 7.06 (d, $J = 7.6$, 1H, H_7), 7.15 (dd, $J = 6.9$ and 7.6, 1H, H_6), 7.36 (dd, $J = 7.6$ and 6.9, 1H, H_5), 7.58 (s, 1H, $H_{2'}$), 8.01 (s, 1H, H vinyl), 8.02 (s, 1H, $H_{5'}$), 9.37 (d, $J = 7.6$, 1H, H_4).

(*Z*)-4-Bromo-1-methoxy-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**39**)

Yield: 52%; mp 224–226°C; IR (KBr) ν cm^{-1} 1698 (νCO), 1617 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 3.83 (s, 3H, CH_3), 4.03 (s, 3H, OCH_3), 7.13 (d, $J = 7.6$, 1H, H_7), 7.24 (d, $J = 7.9$, 1H, H_5), 7.33 (dd, $J = 7.6$ and 7.9, 1H, H_6), 7.89 (s, 1H, $H_{2'}$), 8.74 (s, 1H, H vinyl), 8.97 (s, 1H, $H_{5'}$).

(*Z*,*E*)-6-Bromo-1-methoxy-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**40**)

Yield: 57%; mp 220–223°C; IR (KBr) ν cm^{-1} 1706 (νCO), 1626 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, *E*: 3.79 (s, 3H, CH_3), 4.00 (s, 3H, OCH_3), 7.26

(s, 1H, H_7), 7.33 (d, $J = 8$, 1H, H_5), 7.61 (s, 1H, $H_{2'}$), 8.04 (s, 2H, H vinyl and $H_{5'}$), 9.30 (d, $J = 8$, 1H, H_4). *Z*: 3.79 (s, 3H, CH_3), 4.00 (s, 3H, OCH_3), 7.44 (s, 1H, H_7), 7.63 (s, 1H, $H_{2'}$), 7.78–7.85 (m, 1H, H_5), 7.85–8.10 (m, 3H, H_4, H vinyl and $H_{5'}$).

(*Z*)-1-Methoxy-3-(3,5-dimethylpyrrol-2-ylmethylene)indolin-2-one (**51**)

Yield: 54%; mp 135–136°C; IR (KBr) ν cm^{-1} 1672 (νCO), 1567 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 2.36 (s, 3H, $CH_3^{3'}$), 2.39 (s, 3H, $CH_3^{5'}$), 4.04 (s, 3H, OCH_3), 6.1 (s, 1H, CH), 7.09–7.15 (m, 2H, H_6 and H_7), 7.26 (dd, $J = 7$ and 7.9, 1H, H_5), 7.69 (s, 1H, H vinyl), 7.88 (d, $J = 7$, 1H, H_4), 13.05 (s, 1H, NH).

Method B

To a solution of 3-(imidazol-4(5)-ylmethylene)indolin-2-one (1 eq) dissolved in DMF was added 1.1 or 2.2 eq of NaH. After 30 min at room temperature, methyl iodide (1 or 2 eq) was added and the stirring maintained for 2 h. The reaction mixture was then diluted into water and the precipitate filtered before being recrystallized from ethanol.

(*Z*)-1-Methyl-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**41**)

Yield: 11%; mp 190–193°C; IR (KBr) ν cm^{-1} 1696 (νCO), 1605 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 3.28 (s, 3H, CH_3 ind), 3.81 (s, 3H, CH_3 imid), 7.02–7.09 (m, 2H, H_6 and H_7), 7.3 (dd, $J = 7.9$ and 7.3, 1H, H_5), 7.73 (s, 1H, $H_{2'}$), 7.76 (d, $J = 7.9$, 1H, H_4), 7.83 (s, 1H, H vinyl), 8.97 (s, 1H, $H_{5'}$).

(*Z*)-1-Methyl-3-(1-methylimidazol-4-ylmethylene)-4-trifluoromethylindolin-2-one (**42**)

Yield: 71%; mp 196–200°C; IR (KBr) ν cm^{-1} 1704 (νCO), 1612 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 3.34 (s, 3H, CH_3 ind), 3.83 (s, 3H, CH_3 imid), 7.39–7.52 (m, 3H, H_5, H_6 and H_7), 7.88 (s, 1H, $H_{2'}$), 7.97 (s, 1H, H vinyl), 9.07 (s, 1H, $H_{5'}$).

(*Z*)-5-Bromo-1-methyl-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**44**)

Yield: 82%; mp >300°C; IR (KBr) ν cm^{-1} 1699 (νCO), 1624 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 3.23 (s, 3H, CH_3 ind), 3.79 (s, 3H, CH_3 imid), 6.87 (d, $J = 7.6$, 1H, H_7), 7.37 (d, $J = 7.6$, 1H, H_6), 7.96 (s, 2H, H_4 and $H_{2'}$), 8.01 (s, 1H, H vinyl), 8.05 (s, 1H, $H_{5'}$).

(*Z*)-5-Chloro-1-methyl-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**45**)

Yield: 37%; mp 198–201°C; IR (KBr) ν cm^{-1} 1680 (νCO), 1617 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 3.31 (s, 3H, CH_3 ind), 3.81 (s, 3H, CH_3 imid), 7.04 (d, $J = 8.2$, 1H, H_7), 7.31 (d, $J = 8.2$, 1H, H_6), 7.86 (s, 2H, H_4 and $H_{2'}$), 7.91 (s, 1H, H vinyl), 8.99 (s, 1H, $H_{5'}$).

(*Z*,*E*)-5-Methoxy-1-methyl-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**46**)

Yield: 50%; mp 180–183°C; IR (KBr) ν cm^{-1} 1675 (νCO), 1623 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, *E*: 3.20 (s, 3H, CH_3 ind), 3.80 (s, 6H, OCH_3 and CH_3 imid), 6.84–6.94 (m, 2H, H_6 and H_7), 7.51 (s, 1H, H_4), 7.94 (s, 1H, $H_{2'}$), 8.00 (s, 2H, H vinyl and $H_{5'}$). *Z*: 3.23 (s, 3H, CH_3 ind), 3.80 (s, 6H, OCH_3 and CH_3 imid), 6.84–6.94 (m, 2H, H_6 and H_7), 7.47 (d, $J = 1.8$, 1H, H_4), 7.77 (s, 1H, $H_{2'}$), 7.84 (s, 1H, H vinyl), 9.16 (s, 1H, $H_{5'}$).

(*E*)-6-Bromo-1-methyl-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**47**)

Yield: 61%; mp 223–226°C; IR (KBr) ν cm^{-1} 1701 (νCO), 1601 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 3.24 (s, 3H, CH_3 ind), 3.79 (s, 3H, CH_3 imid), 7.27 (m, 2H, H_5 and H_7), 7.58 (s, 1H, $H_{2'}$), 8.00 (s, 2H, H vinyl and $H_{5'}$), 9.3 (d, $J = 8.5$, 1H, H_4).

(*Z*,*E*)-1-Methyl-3-(1-methylimidazol-4-ylmethylene)-6-phenylindolin-2-one (**49**)

Yield: 50%; mp 243–246°C; IR (KBr) ν cm^{-1} 1687 (νCO), 1602 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, *E*: 3.33 (s, 3H, CH_3 ind), 3.8 (s, 3H, CH_3 imid), 7.32–7.55 (m, 7H, 5 H phenyl, H_5 and H_7), 7.81 (s, 1H, $H_{2'}$), 7.97 (s, 1H, H vinyl), 8.02 (s, 1H, $H_{5'}$), 9.40 (d, $J = 7$, 1H, H_4). *Z*: 3.33 (s, 3H, CH_3 ind), 3.8 (s, 3H, CH_3 imid), 7.32–7.80 (m, 10H, 5 H phenyl, H_4, H_5, H_7, H vinyl and $H_{2'}$), 8.02 (s, 1H, $H_{5'}$).

(*Z*,*E*)-1-Methyl-3-(1-methylimidazol-4-ylmethylene)-6-trifluoromethylindolin-2-one (**50**)

Yield: 52%; mp 190–192°C; IR (KBr) ν cm^{-1} 1701 (νCO), 1616 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, *E*: 3.31 (s, 3H, CH_3 ind), 3.81 (s, 3H, CH_3 imid), 7.35–7.49 (m, 2H, H_5 and H_7), 7.89 (s, 1H, $H_{2'}$), 7.95 (s, 1H, H vinyl), 8.07 (s, 1H, $H_{5'}$), 9.55 (d, $J = 8.1$, 1H, H_4). *Z*: 3.35 (s, 3H, CH_3 ind), 3.83 (s, 3H, CH_3 imid), 7.35–7.49 (m, 3H, H_4, H_5 and H_7), 7.69 (s, 1H, $H_{2'}$), 8.07 (s, 1H, H vinyl), 9.05 (s, 1H, $H_{5'}$).

Method C

To a solution of 3-(imidazol-4(5)-ylmethylene)indolin-2-one 31 (or 34) (1 eq) and 0.1 ml of formaldehyde in THF was added 1 eq of a solution of dimethylamine (40% in water) in THF. The reaction mixture was then stirred at room temperature for 2 h and the solvent finally evaporated in vacuo. The final compound was recrystallized from ethanol.

(*Z*)-4-Bromo-1-dimethylaminomethyl-3-(1-methylimidazol-4-ylmethylene)indolin-2-one (**43**)

Yield: 77%; mp 168–171°C; IR (KBr) ν cm^{-1} 1692 (νCO), 1610 (νC═C); ^{1}H NMR (DMSO-d_6), δ ppm, 2.26 (s, 6H, 2 CH_3), 3.82 (s, 3H, CH_3), 4.49 (s, 2H, CH_2), 7.19–7.32 (m, 3H, H_5, H_6 and H_7), 7.87 (s, 1H, $H_{2'}$), 8.74 (s, 1H, H vinyl), 8.99 (s, 1H, $H_{5'}$); ^{13}C NMR

(DMSO-d_6) δ ppm, 33.79 (NCH_3), 42.98 (2 NCH_3), 62.31 (CH_2), 109.60 (C_7), 115.15 (C_4), 119.67 (C_3), 120.51 (C_{3a}), 127.00 (C_5), 128.87 (C_6), 129.84 ($C_{5'}$), 134.82 ($C_{4'}$), 136.15 (CH vinyl), 139.82 ($C_{2'}$), 143.39 (C_{7a}), 166.27 (CO).

(*E*)-6-Bromo-1-dimethylaminomethyl-3-(1-methyl-imidazol-4-ylmethylene)indolin-2-one (**48**)

Yield: 46%; mp 189–191°C; IR (KBr) ν cm^{-1} 1698 (νCO), 1598 (νC=C); ^1H NMR (DMSO-d_6), δ ppm, 2.5 (s, 6H, 2 CH_3), 3.79 (s, 3H, CH_3), 4.42 (s, 2H, CH_2), 7.28 (dd, $J = 8.2$ and 1.5, 1H, H_5), 7.32 (d, $J = 1.5$, 1H, H_7), 7.59 (s, 1H, $H_{2'}$), 8.01 (s, 2H, H vinyl and $H_{5'}$), 9.34 (d, $J = 8.2$, 1H, H_4).

Pharmacology

Inhibition of Cellular EGF Tyrosine Kinase Activity

Purpose

The evaluation of the effects of compounds on the activity of the human EGF-tyrosine kinase was quantified by measuring the phosphorylation of poly GAT (Glu, Ala, Tyr) using a purified A-431 cell membrane preparation as the enzyme source.

Growth of A-431 Cells and Preparation of Membrane Fraction

Briefly, the cells were grown following the method described by Carpenter *et al.*[30] to high density in 100 mm Falcon dishes containing Dulbecco's modified Eagle's medium supplemented with 10% calf serum and gentamycin. A membrane fraction was prepared by the procedure of Thom *et al.*[31] In brief, the cells were scraped from dishes, concentrated into a small volume by centrifugation and lysed by dilution into a hypotonic borate/ EDTA buffer, pH 10.2. The lysed cells were filtered through a nylon screen and a crude membrane fraction was obtained by centrifugation at $25,000 \times g$ for 30 min. The pelleted material was resuspended, layered over 35% (w/w) sucrose and centrifuged ($40,000 \times g$ for 45 min) in a swinging bucket rotor. The material at the buffer sucrose interface was collected with a Pasteur pipette, suspended in 10 nM Hepes, pH 7.4, and recentrifuged ($75,000 \times g$ for 30 min). The final membrane preparation was resuspended in the Hepes buffer (approximately 10 mg of protein/ml), divided into small aliquots, frozen on dry ice, and stored at −70°C.

Human EGF-tyrosine Kinase Assay

The experiments were carried out in 96-well MultiScreen filter plates presoaked with 50 mM Hepes/Tris (pH 7.4) for 30 min. The test compound, reference compound or water (control) were mixed with the membrane preparation (5–10 μg) in a buffer containing 50 mM Hepes/Tris (pH 7.4), 0.2% Triton X-100, 6% glycerol, 90 μM Na_3VO_4, 1 mM DTT and 48 μg poly GAT in the absence (basal control) or presence (stimulated control) of 0.4 μM EGF. Phosphorylation was initiated by addition of 0.25 μCi [$\gamma^{33}P$] ATP, (Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, England), 10 μM ATP and 15 mM $MgCl_2$. The mixture was incubated for 60 min at 30°C after which time the reaction was stopped by addition of 500 mM H_3PO_4. After 20 min at room temperature, the samples were filtered and rinsed several times with 125 mM H_3PO_4 using a vacuum manifold system (Millipore). The plates were dried, then the radioactivity associated with poly GAT retained on the filters was measured in a scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 20, Packard). The results were expressed as a percent inhibition of the control enzyme activity. The standard inhibitory reference compound was PD 153035, which was tested in each experiment at several concentrations to obtain an inhibition curve which allowed calculation of its IC_{50} value: 5 nM.

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.*[32] 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) was 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.*[33] 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

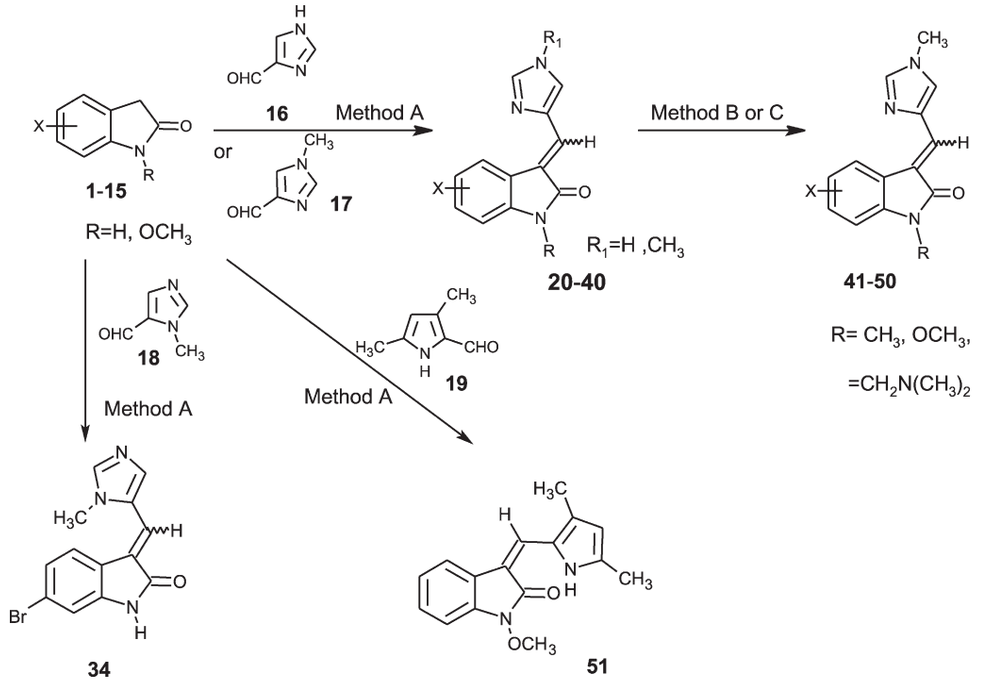


FIGURE 3 Preparation of 3-(imidazol-4(5)-ylmethylene)indolin-2-ones **20**–**50** and 3-(3,5-dimethylpyrrol-2-ylmethylene)indolin-2-one **51**. Method A: piperidine, ethanol, reflux, 2 h, 37–85%; Method B: (i) NaH, DMF, rt, 30 min, (ii) CH_3I, rt, 2 h, 11–82%; Method C: HCHO (37%), $NH(CH_3)_2$ (40% in water), THF, rt, 2 h, 46–77%.

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:[34] 30 ml per dish for three-dimensional 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. Results are expressed as percent of control cultures.

RESULTS AND DISCUSSION

Chemistry

3-(Imidazol-4(5)-ylmethylene)indolin-2-ones **20**–**51** were prepared by the Knoevenagel reaction, (see Figure 3), by condensation of the formyl derivatives **16**–**19** with indolin-2-ones **1**–**15** in the presence of piperidine and in refluxing ethanol following a method previously reported by Andreani *et al.*[35,36] Indolin-2-ones **2**–**15** were obtained by reduction of isatines or 3-methylthioindolinone from the corresponding anilines[19,25] or by reduction of 2-nitrophenylacetic acids[23,24] or N-methoxyphenylacetamides.[20–22]

Compounds **20**–**40**, monomethylated at the N^1-imidazole ring, were obtained starting from **17** or **18**. Simultaneous N-methylation at indolinone and imidazole rings was carried out using the couple NaH/DMF in the presence of 2.2 equivalents of CH_3I (method B). N^1-Aminomethyl derivatives **43** and **48** were obtained by the Mannich reaction in THF at room temperature (method C).

The Knoevenagel reaction leads either to a mixture of both *Z* and *E* isomers or to a single (*Z* or *E*) isomer. Although, it could be established by ^{1}H n.m.r. data study, that δ of the imidazole $H_{5'}$ was, generally speaking, located near 9 ppm in the *Z* isomers and 8.1 ppm in the *E* forms, (as previously described in 3-arylidenylindolinones),[37]

TABLE I Inhibition of the EGF-receptor tyrosine kinase

No.	R	R_1	X	Molecular formula MW	Yield (%) method	Mp (°C)*	% of *Z*/*E* isomer	% of inhibition at 10 μM
20	H	H	H	$C_{12}H_9N_3O$ 211.22	80 A	228–230	10/90	54
21	H	H	4-Br	$C_{12}H_8BrN_3O$ 290.11	68 A	>300	100/0	36
22	H	H	4-NH_2	$C_{12}H_{10}N_4O$ 226.23	82 A	238–241	45/55	12
23	H	H	4-NHAc	$C_{14}H_{12}N_4O_2$ 268.27	78 A	>300	0/100	0
24	H	H	5-Br	$C_{12}H_8BrN_3O$ 290.11	73 A	>300	100/0	28
25	H	H	5-Cl	$C_{12}H_8ClN_3O$ 245.66	77 A	>250	0/100	34
26	H	H	6-Cl	$C_{12}H_8ClN_3O$ 245.66	41 A	>250	5/95	41
27	H	H	6-Br	$C_{12}H_8BrN_3O$ 290.11	37 A	>300	20/80	37
28	H	H	6-C_6H_5	$C_{18}H_{13}N_3O$ 287.32	73 A	>300	100/0	37
29	H	CH_3	H	$C_{13}H_{11}N_3O$ 225.24	58 A	261–263	100/0	53
30	H	CH_3	4-CF_3	$C_{14}H_{10}F_3N_3O$ 293.24	58 A	>300	100/0	46
31	H	CH_3	4-Br	$C_{13}H_{10}BrN_3O$ 304.14	88 A	>300	100/0	10
32	H	CH_3	6-Cl	$C_{13}H_{10}ClN_3O$ 259.69	42 A	285–287	15/85	0
33	H	CH_3	6-CF_3	$C_{14}H_{10}F_3N_3O$ 293.24	75 A	>250	75/25	34
34	H	CH_3	6-Br	$C_{13}H_{10}BrN_3O$ 304.14	37 A	275–278	40/60	13
35	OCH_3	H	H	$C_{13}H_{11}N_3O_2$ 241.24	83 A	169–170	25/75	0
36	OCH_3	H	4-Br	$C_{13}H_{10}BrN_3O_2$ 320.14	76 A	230–231	100/0	34
37	OCH_3	H	6-Br	$C_{13}H_{10}BrN_3O_2$ 320.14	85 A	190–192	85/15	24
38	OCH_3	CH_3	H	$C_{14}H_{13}N_3O_2$ 255.27	59 A	205–207	0/100	16
39	OCH_3	CH_3	4-Br	$C_{14}H_{12}BrN_3O_2$ 334.17	52 A	224–226	100/0	0
40	OCH_3	CH_3	6-Br	$C_{14}H_{12}BrN_3O_2$ 334.17	57 A	220–223	95/5	0
41	CH_3	CH_3	H	$C_{14}H_{13}N_3O$ 239.27	11 B	190–193	100/0	0
42	CH_3	CH_3	4-CF_3	$C_{15}H_{12}F_3N_3O$ 307.27	71 B	196–200	100/0	8
43	$CH_2N(CH_3)_2$	CH_3	4-Br	$C_{16}H_{17}BrN_4O$ 412.26	77 C	168–171	100/0	0
44	CH_3	CH_3	5-Br	$C_{14}H_{12}BrN_3O$ 318.17	82 B	>300	100/0	26
45	CH_3	CH_3	5-Cl	$C_{14}H_{12}ClN_3O$ 273.72	37 B	198–201	100/0	0
46	CH_3	CH_3	5-OCH_3	$C_{15}H_{15}N_3O_2$ 269.3	50 B	180–183	85/15	0
47	CH_3	CH_3	6-Br	$C_{14}H_{12}BrN_3O$ 318.17	61 B	223–226	0/100	0
48	$CH_2N(CH_3)_2$	CH_3	6-Br	$C_{16}H_{17}BrN_4O$ 361.24	46 C	189–191	0/100	14
49	CH_3	CH_3	6-C_6H_5	$C_{20}H_{17}N_3O$ 315.37	50 B	243–246	80/20	0
50	CH_3	CH_3	6-CF_3	$C_{15}H_{12}F_3N_3O$ 307.27	52 B	190–192	55/45	18
SU5416				$C_{15}H_{14}N_2O$ 238.28	66 A	225–226	100/0	30

* All compounds were recrystallized from ethanol.

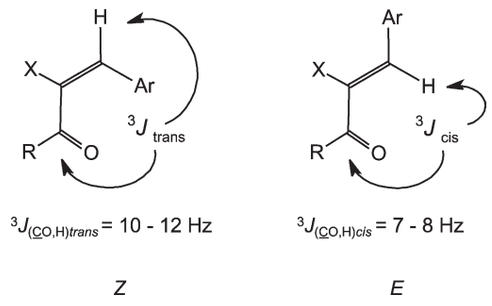


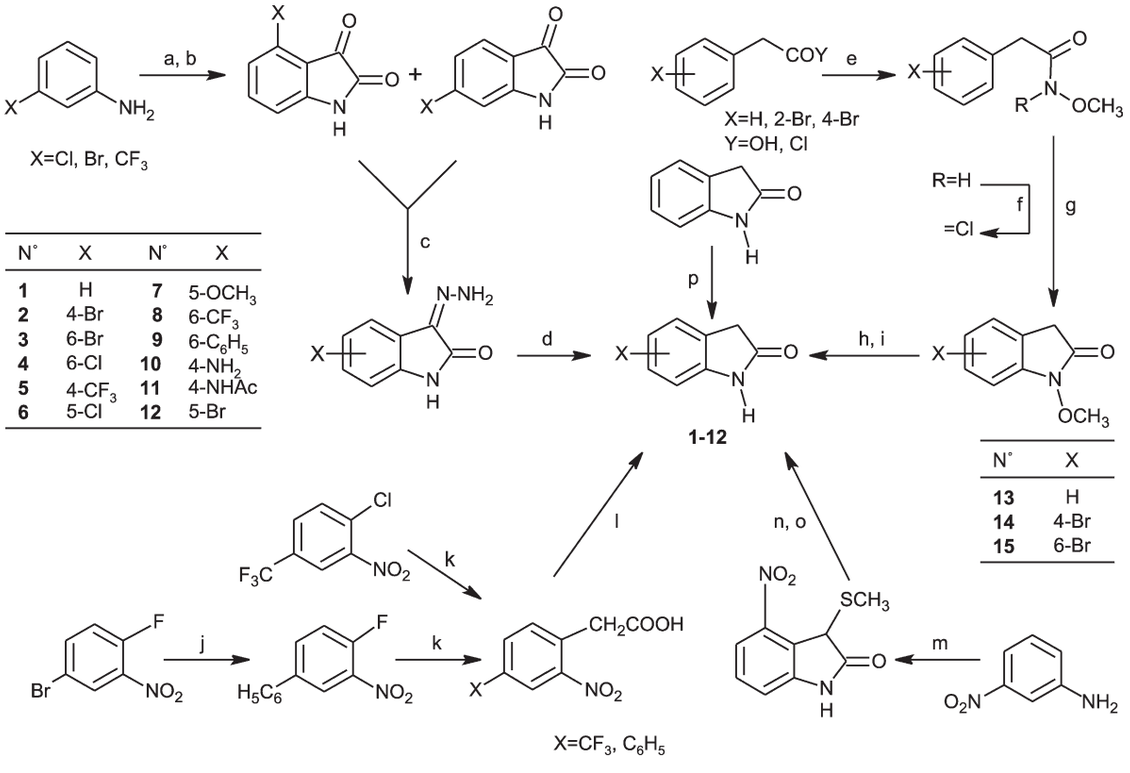
FIGURE 4 Identification of the *Z*- or *E*-configuration by ^3J(C, H) vicinal couplings.

no clear-cut difference was observed in some compounds. The stereochemistry of these α,β-unsaturated lactams was ascertained by vicinal C,H spin coupling constants. The use of ^{13}C n.m.r. ^{13}C-^1H coupling constants of the ketonic carbon of the studied 3-(imidazol-4(5)-ylmethylene)indolin-2ones afforded results (Table I) that can be summarized in the following diagnostic correlation for determining their stereochemistry: Thus, the coupling constant of the *E* derivatives **25** and **26** was 7.6 Hz and that of the *Z* derivatives **36** and **43** was 10.0 Hz and 10.5 Hz, respectively (Figure 4).

These results are in line with the assignment of Kingsbury *et al.* for α-phenylchalcones and Letcher *et al.* for 5-methyl-5,6,11,12-tetrahydro-6,12-dioxo-11(4-methylbenzylidene)-dibenz[*b*,*f*]azocines.[38,39] (Figure 5)

Pharmacology

Inhibitory properties against a cell membrane-based EGF receptor TK assay were determined for the indolin-2-ones **20**–**50** and the results are gathered in Table I. Determination of inhibition percentages was carried out at 10 μM, in duplicate. **SU-5416**, which is a specific inhibitor of the VEGF receptor TK activity, has only moderate inhibitory effect against the EGF receptor TK (30% inhibition at 10 μM). As expected we also observed modest activities with our compounds since only four compounds, **20**, **26**, **29** and **30**, exhibit inhibition in the range of 40–55% at 10μM. Interestingly, these compounds showed comparable potency against the EGF receptor TK, regardless of the predominant *Z*-*E* configuration. Indeed, it was previously hypothetized that



N°	X	N°	X
1	H	**7**	5-OCH_3
2	4-Br	**8**	6-CF_3
3	6-Br	**9**	6-C_6H_5
4	6-Cl	**10**	4-NH_2
5	4-CF_3	**11**	4-NHAc
6	5-Cl	**12**	5-Br

N°	X
13	H
14	4-Br
15	6-Br

FIGURE 5 Preparation of substituted indolin-2-ones **2**–**15**. Reagents and conditions: (a) $CCl_3CH(OH)_2$, Na_2SO_4, NH_2OH,HCl, H_2O, 65°C, 6 h, 80–98%; (b) conc. H_2SO_4, 75°C, 45 min, 25–44%; (c) NH_2–NH_2,H_2O, EtOH, reflux, 2 h, 64–95%; (d) Na/EtOH, reflux, 15 min, 64–97%; (e) NH_2OCH_3,HCl, Na_2CO_3, toluene, 0–5°C, 5 h, or NH_2OCH_3,HCl, Et_3N, DCC, CH_2Cl_2, r.t., 16 h, 70–90%; (f) ^{t}BuOCl, $CHCl_3$, 0°C, 30 min, 96–100%; (g) Ag_2CO_3, TFA, 0°C, 30 min, 25–64%; (h) conc. HCl, 70°C, 1.5 h, 60%; (i) conc. H_2SO_4, MeOH, reflux, 1 h, 49%; (j) phenylboronic acid, $NaHCO_3$, toluene/ethanol, reflux, 2 h, 72%; (k) NaH, DMSO, $CH_2(CO_2Et)_2$, 100°C, 2 h, then KOH, EtOH/H_2O, reflux, 1.5 h, HCl 6M 35–79%; (l) Fe, CH_3CO_2H, reflux, 2 h, 64–77%; (m) ^{t}BuOCl, $CH_3SCH_2CO_2Et$, Et_3N, CH_2Cl_2, −65°C, 1 h, 60%; (n) H_2, Ni Raney, EtOH, 60°C, 2.5 h, 90%; (o) $(CH_3CO)_2O$, r.t., 15 min, 71%; (p) NBS, CH_3CN, −10°C, 1 h, then 0°C, 2 h, 52%.

isomerization to the *Z* form could intervene during interaction with the VEGF receptor TK.[40] Compound **20** analogues containing an halogen substituent on the homocycle were less potent. Attachment of a methyl group at the N-1 imidazole of **20**, leading to **29**, did not modify the level of activity: 54 and 53%, respectively; on the contrary, a detrimental effect was observed in the sub-series of the corresponding halogenated derivatives: **21** → **31**, **26** → **32**, **27** → **34**. Only the 4-CF_3 congener, **30**, remained efficient (46%). Generally speaking N-1 and N-1′ disubstitution by CH_3, OCH_3 or CH_2–$N(CH_3)_2$ and CH_3, respectively, induced a dramatic decrease or even a suppression of the inhibitory activity.

Four of our compounds were evaluated in a three dimensional *in vitro* rat aortic ring model in order to investigate their effects on microvascular growth. Angiogenesis quantitation was carried out by determination of the vascular density index (VDI). Compound **20** exhibits a significant angiogenesis inhibitory activity, at 1 μM, with a VDI of 30 ± 18% of control, comparable to that of **SU-5416** (22 ± 4%). In contrast, the indolin-2-ones **35** and **38**, which possess a methoxy group at the indolinone nitrogen, were totally inefficient and introduction of the same group at N-1 of **SU-5416** (leading to **51**) induced a clear-cut decrease in antiangiogenic activity (VDI: 64 ± 5% of control). This last result is in line with previous work and tends to confirm that the proton at the N-1 position of **SU-5416** could be involved in a critical hydrogen-bonding interaction with a carbonyl oxygen within the catalytic domain.[27]

In conclusion, these results bring to the fore that replacement of the pyrrole ring of **SU-5416** by an imidazole moiety allows emergence of EGF RTK inhibitory activity. Investigations aimed at replacing the indolinone core by a dihydrobenzofuranone one are now in progress and the corresponding pharmacomodulation results will be published in due course.

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