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New Analogues of the Anticancer E7070: Synthesis and Pharmacology

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Cell cycle control in the G1 phase has attracted considerable attention in recent cancer research, because many of the important proteins involved in G1 progression or G1/S transition have been found to play a crucial role in proliferation, differentiation, transformation, and programmed cell death (apoptosis). E7070 is a novel antitumor sulfonamide, with a unique mode of action that affects G1 progression of the cell cycle. A series of compounds containing an *N*-[1-(3,4,5-trimethoxybenzyl)-1*H*-indol-5-yl]benzene sulfonamide, analogues of E7070, was synthesized and evaluated as potential antitumor agents. Cell cycle analysis with PC3 human prostate cancer cells revealed a cellular accumulation in the G1 phase.

Keywords: E7070; CDKs; Sulfonamide

INTRODUCTION

The term signal transduction is used to describe the molecular processes involved in the communication between the cell and its environment, and in the regulation of cell fate.[1] The rationale for developing signal transduction inhibitors as anticancer agents is clear. New anticancer agents are targeted increasing to the specific abnormalities in the sequence and level of expression of a series of key genes that combine together to drive the progression of human cancer.[2,3] Many of the genetic abnormalities in cancer cells lead to the activation of proliferative signal transduction pathways, deregulation of cell cycle control and the activation of antiapoptotic and cell survival signalling. In addition, process leading to angiogenesis, invasion and metastasis are upregulated.[2]

The new generation of molecular therapeutics targeted specifically to these deregulated pathways should be more effective and less toxic than the broadly antiproliferative cytotoxic drugs which dominate current therapy.[4,5]

Particular attention has been focused on cyclin-dependent kinases. The cyclin-dependent kinases (CDKs) are key regulators of the cell cycle.[6] The cell division cycle is commonly viewed as an orderly progression through four distinct phases: G1 (gap 1), the phase in which the cell prepares for DNA synthesis, S (synthesis), the stage in which DNA is replicated, G2 (gap 2), the phase in which the cell prepares for mitosis, and M (mitosis), the stage that leads to chromosome segregation and daughter cell formation. CDKs provide much of the control that is required for the cell to move through these phases in a coordinated manner. For their activities, CDKs need to form complexes with another set of proteins called cyclins. Thus far, at least ten CDKs and fifteen different cyclins have been reported.[7] In general, cyclin D/CDK4,6 are involved in the G1 phase, cyclin E/CDK2 in the G1 to S transition, cyclin A/CDK1,2 in the S phase as well as in the G2 to M transition. Cyclin-dependent kinases are frequently deregulated in cancer by a variety of means, including overexpression of the cyclin and kinase components, together with mutation or loss of negative regulators such as p16 or p21.[4] Considering these observations, CDKs are attractive targets for the development of antitumor drugs.[8,9]

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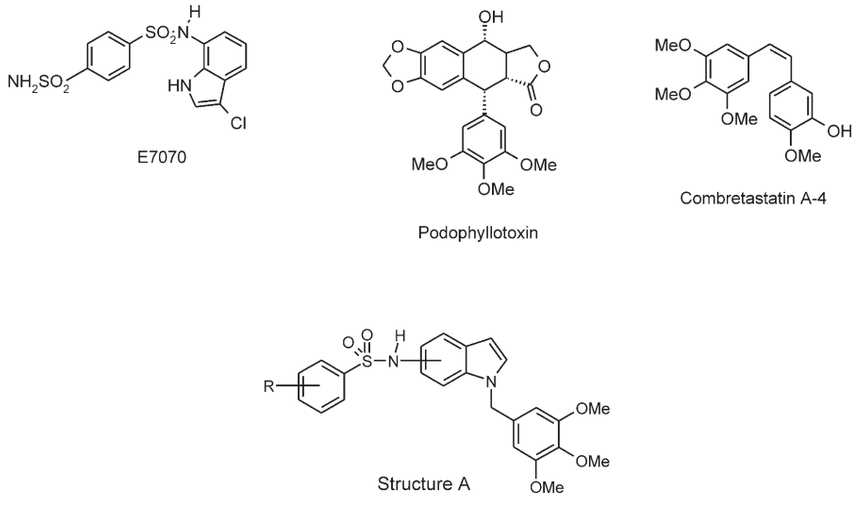


SCHEME 1 Some anticancer agents and the analogues described here.

E7070 is a novel antitumor sulfonamide that affects G1 progression of the cell cycle.[10] This agent was derived from a focused compound library of diarylsulfonamides through intensive chemical synthesis, antitumor screenings and flow cytometric analyses. Preliminary data have indicated that **E7070** may suppress CDK2 activation and cyclin E expression in HCT116 (human colon carcinoma).[11]

Our work has been inspired in part by the discovery of **E7070** as a potential anti-cancer agent acting as an inhibitor at the level of the complex cyclin E/CDK2 and in part by the anti-cancer agents such as the **podophyllotoxin**[12] or **combretastatin**[13] which act as inhibitors of the polymerisation of microtubules.

In order to obtain potential anti-cancer agents acting at the level of CDKs (**structure A**), we envisaged structures which keep an indole ring associated with an aromatic nucleus through a sulfonamide linker (as in **E7070**) in various positions. The indole moiety is *N*-substituted by 3,4,5-trimethoxybenzyl group as in **podophyllotoxin** or **combretastatin**. (Scheme 1)

MATERIALS AND METHODS

General

Melting point were determined on a Büchi SMP-540 apparatus. ^{1}H NMR spectra were recorded on an AC 300 P (300 MHz) spectrometer using d_6-DMSO or $CDCl_3$ as solvents. Chemical shifts are expressed downfield from the internal standard, tetramethylsilane. Coupling constants (J) are expressed in Hz. Key: t = triplet, s = singlet, d = doublet, dd = double doublet, m = multiplet. Mass spectra were recorded on a Funnigan Mat SSQ710 mass spectrometer. Elemental analyses (C, H, N) were determined by the CNRS Center of Analysis, Vernaison, France, and agreed with proposed structures within 0.4% of the theoretical values.

3,4,5-Trimethoxy-1-[(methylsulfonyloxy)methyl]benzene **2**

Methanesulfonyl chloride (80 mmol) was added dropwise to a cold (−5°C) solution of 3,4,5-trimethoxybenzyl alcohol (40 mmol) and triethylamine (90 mmol) in 130 ml of dichloromethane and the mixture stirred for 16 h at room temperature. After dilution with water, the solution was extracted with dichloromethane. The organic layer was washed with water, 10% aqueous solution of hydrochloric acid, dried over magnesium sulfate and evaporated under reduced pressure. The resulting solid was recrystallized from diisopropyl ether to afford 4.97 g (45%) of **2**: As white crystals, m.p. 50–51°C (diisopropyl ether). IR (KBr), cm^{-1}: 1370 and 1180 (SO_2OCH_3); ^{1}H NMR ($CDCl_3$), δ, ppm; 3.00 (s, 3H, Me); 3.86 (s, 3H, *p*-OMe); 3.90 (s, 6H, *m,m′*-OMe); 4.55 (s, 2H, CH_2); 6.61 (s, 2H,H-Trimethoxybenzyl).

*General Procedure for the Preparation of Nitro-1-(3,4,5-trimethoxybenzyl)-1*H*-indole (4a–4c)*

The method adopted for the synthesis of 5-nitro-1-(3,4,5-trimethoxybenzyl)-1*H*-indole **(4a)** is described. To a solution of 5-nitroindole (4 mmol) in 50 ml of acetone, was added potassium carbonate (5 mmol). The mixture was stirred, compound **2** (5 mmol) was added and the mixture refluxed. The reaction medium was cooled, filtered and evaporated under reduced pressure. The residue was taken up in dichloromethane. The organic layer was washed with a 10% aqueous solution of hydrochloric acid

and water. The organic layer, dried over magnesium sulfate, was evaporated under reduced pressure and the residue recrystallized from methanol to afford 0.95 g (70%) of **4a**: As yellow crystals, m.p. 116–117°C (methanol). IR (KBr), cm^{-1}: 1520 and 1370 (NO_2); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.76 (s, 6H, *m*,*m*′-OMe); 3.83 (s, 3H, *p*-OMe); 5.31 (s, 2H, CH_2); 6.31 (s, 2H, H-trimethoxybenzyl); 6.74 (d, 1H, H3-indole, 3.32); 7.27 (d, 1H, H2-indole, 3.32); 7.35 (d, 1H, H7-indole, 9.13); 8.12 (dd, 1H, H6-indole, 9.13 2.08); 8.62 (d, 1H, H4-indole, 2.08).

6-Nitro-1-(3,4,5-trimethoxybenzyl)-1*H*-indole **4b**

As yellow crystals (64%), m.p. 95–96°C (methanol). IR (KBr), cm^{-1}: 1520 and 1350 (NO_2); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.74 (s, 6H, *m*,*m*′-OMe); 3.82 (s, 3H, *p*-OMe); 5.32 (s, 2H, CH_2); 6.33 (s, 2H, H-trimethoxybenzyl); 6.68 (d, 1H, H3-indole, 3.08); 7.42 (d, 1H, H2-indole, 3.08); 7.70 (d, 1H, H4-indole, 8.78); 8.12 (dd, 1H, H5-indole, 8.78 1.76); 8.34 (d, 1H, H4-indole, 1.76).

7-Nitro-1-(3,4,5-trimethoxybenzyl)-1*H*-indole **4c**

As yellow crystals (45%), m.p. 78–79°C (methanol). IR (KBr), cm^{-1}: 1525 and 1350 (NO_2); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.68 (s, 6H, *m*,*m*′-OMe); 3.72 (s, 3H, *p*-OMe); 5.41 (s, 2H, CH_2); 6.20 (s, 2H, H-trimethoxybenzyl); 6.73 (d, 1H, H3-indole, 3.65); 7.15 (t, 1H, H5-indole, 7.63); 7.33 (d, 1H, H2-indole, 3.65); 7.76 (d, 1H, H4-indole, 7.63); 7.90 (d, 1H, H6-indole, 7.63).

General Procedure for the Preparation of Sulfonamides (7a–7j)

The method adopted for the synthesis of *N*-[1-(3,4,5-trimethoxybenzyl)-1*H*-indol-5-yl]benzenesulfonamide **(7a)** is described. To a solution of 5-nitro-1-(3,4,5-trimethoxybenzyl)-1*H*-indole (4 mmol) in 10 ml of propan-2-ol was added Fe powder (0.1 g) and an aqueous solution of NH_4Cl (0.6 N). After stirring at 60°C for 2 h, the insoluble materials were filtered off and washed with ethyl acetate, followed by the immediate addition of benzenesulfonyl chloride (3 mmol), pyridine (4 mmol) and 10 ml of DMF. The reaction mixture was stirred at room temperature for 4 h and evaporated under reduced pressure. The residue was taken up in water, stirred at room temperature for 1h and the precipitate formed was filtered. The product was purified by flash chromatography using dichloromethane as mobile phase and silica gel (60 F_{254}) as solid phase. The resulting solid was recrystallized from ethanol affording 1.26 g (70%) of **7a**: As white crystals, m.p. 135–136°C (ethanol). IR (KBr), cm^{-1}: 3290 (NH), 1350 and 1170 (SO_2N); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.73 (s, 6H, *m*,*m*′-OMe); 3.82 (s, 3H, *p*-OMe); 5.18 (s, 2H, CH_2); 6.25 (s, 2H, H-trimethoxybenzyl); 6.47 (d, 1H, H3-indole, 3.05); 6,54 (s, 1H, SO_2NH); 6.86 (m, 1H, H6-indole); 7.14 (m, 2H, H2/H7-indole); 7.36 (d, 1H, H4-indole, 2.04); 7.40 (m, 2H, H-benzyl); 7.51 (m, 1H, H-benzyl); 7.72 (m, 2H, H-benzyl). EI-MS m/z (%) 452 (M, 85), 181 (100). Anal. ($C_{24}H_{24}N_2O_5S$) C, H, N.

Methyl 2-[(1-(3,4,5-trimethoxybenzyl)-1*H*-indol-5-yl)aminosulfonyl]benzoate **7b**

As white crystals (55%), m.p. 119–120°C (propan-2-ol). IR (KBr), cm^{-1}: 3290 (NH), 1720 (CO), 1350 and 1170 (SO_2N); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.75 (s, 6H, *m*,*m*′-OCH_3); 3.82 (s, 3H, *p*-OCH_3); 4.10 (s, 3H, COOMe); 5.19 (s, 2H, CH_2); 6.30 (s, 2H, H-trimethoxybenzyl); 6.45 (d, 1H, H3-indole, 3.25); 6.97 (m, 1H, H6-indole); 7.10 (d, 1H, H2-indole, 3.25); 7.13 (d, 1H, H7-indole, 8.95); 7.38 (m, 1H, H-benzy); 7.41 (d, 1H, H4-indole, 2.03); 7.55 (m, 1H, H-benzyl); 7.72 (m, 1H, H-benzyl); 7.84 (m, 1H, H-benzyl); 7.96 (s, 1H, SO_2NH). EI-MS m/z (%) 510 (M, 5), 181 (100). Anal. ($C_{26}H_{26}N_2O_7S$) C, H, N.

N-[1-(3,4,5-trimethoxybenzyl)-1*H*-indol-5-yl]benzene-1,4-disulfonamide **7c**

As white crystals (65%), m.p. 156–157°C (ethanol). IR (KBr), cm^{-1}: 3310 (SO_2NH_2), 3290 (NH), 1365 and 1170 (SO_2N); ^1H NMR (d_6-DMSO), δ, ppm; J, Hz: 3.60 (s, 3H, *p*-OCH_3); 3.68 (s, 6H, *m*,*m*′-OCH_3); 5.20 (s, 2H, CH_2); 6.40 (d, 1H, H3-indole, 2.75); 6.57 (s, 2H, H-trimethoxybenzyl); 6.85 (m, 1H, H6-indole); 7.28 (d, 1H, H4-indole, 1.65); 7.44 (d, 1H, H7-indole, 8.79); 7.52 (d, 1H, H2-indole, 2.75); 7.55 (s, 2H, SO_2NH_2); 7.90 (m, 4H, H-benzyl); 10.10 (s, 1H, SO_2NH). EI-MS m/z (%) 531 (M, 55), 181 (100). Anal. ($C_{24}H_{25}N_3O_7S_2$) C, H, N.

4-Methyl-*N*-[1-(3,4,5-trimethoxybenzyl)-1*H*-indol-5-yl]benzenesulfonamide **7d**

As white crystals (69%), m.p. 165–166°C (ethanol). IR (KBr), cm^{-1}: 3295 (NH), 1355 and 1170 (SO_2N); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 2.35 (s, 3H, CH_3); 3.73 (s, 6H, *m*,*m*′-OCH_3); 3.82 (s, 3H, *p*-OCH_3); 5.20 (s, 2H, CH_2); 6.28 (s, 2H, H-trimethoxybenzyl); 6.47 (d, 1H, H3-indole, 3.22); 6.68 (s, 1H, SO_2NH); 6.86 (m, 1H, H6-indole); 7.14 (m, 2H, H2/H7-indole); 7.17 (d, 2H, H-benzyl, 8.26); 7.36 (d, 1H, H4-indole, 1.83); 7.61 (d, 2H, H-benzyl, 8.26). EI-MS m/z (%) 466 (M, 15), 181 (100). Anal. ($C_{25}H_{26}N_2O_5S$) C, H, N.

4-Chloro-*N*-[1-(3,4,5-trimethoxybenzyl)-1*H*-indol-5-yl]benzenesulfonamide **7e**

As white crystals (65%), m.p. 143–144°C (ethanol). IR (KBr), cm^{-1}: 3290 (NH), 1360 and 1170 (SO_2N); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.73 (s, 6H, *m*,*m*′-OCH_3); 3.82 (s, 3H, *p*-OCH_3); 5.21 (s, 2H, CH_2); 6.28 (s, 2H, H-trimethoxybenzyl); 6.47 (d, 1H, H3-indole, 3.10); 6.52 (s, 1H, SO_2NH); 6.85 (m, 1H, H6-indole); 7.14 (d, 1H, H2-indole, 3.10); 7.16 (d, 1H, H7-indole, 8.28); 7.36 (m, 3H, H4-indole/H-benzyl); 7.64 (d, 2H, H-benzyl, 8.10). EI-MS m/z (%) 486 (M, 20), 181 (100). Anal. ($C_{24}H_{23}ClN_2O_5S$) C, H, N.

4-Methoxy-*N*-[1-(3,4,5-trimethoxybenzyl)-1*H*-indol-5-yl]benzenesulfonamide **7f**

As white crystals (70%), m.p. 168–169°C (Ethanol). IR (KBr), cm^{-1}: 3295 (NH), 1355 and 1175 (SO_2N); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.72 (s, 6H, *m*,*m*′-OCH_3); 3.81 (s, 6H, *p*-OCH_3/OCH_3); 5.20 (s, 2H, CH_2); 6.27 (s, 2H, H-trimethoxybenzyl); 6.47 (d, 1H, H3-indole, 3.06); 6.52 (s, 1H, SO_2NH); 6.85 (m, 3H, H6-indole/H-benzyl); 7.14 (m, 2H, H2/H7-indole); 7.36 (d, 1H, H4-indole, 2.04); 7.61 (d, 2H, H-benzyl, 8.06). EI-MS m/z (%) 482 (M, 10), 181 (100). Anal. ($C_{25}H_{26}N_2O_6S$) C, H, N.

4-(Trifluoromethyl)-*N*-[1-(3,4,5-trimethoxybenzyl)-1*H*-indol-5-yl]benzenesulfonamide **7g**

As white crystals (80%), m.p. 162–163°C (ethanol). IR (KBr), cm^{-1}: 3290 (NH), 1355 and 1175 (SO_2N); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.72 (s, 6H, *m*,*m*′-OCH_3); 3.81 (s, 3H, *p*-OCH_3); 5.21 (s, 2H, CH_2); 6.30 (s, 2H, H-trimethoxybenzyl); 6.49 (d, 1H, H3-indole, 3.13); 6.65 (s, 1H, SO_2NH); 6.85 (m, 1H, H6-indole); 7.14 (d, 1H, H2-indole, 3.13); 7.18 (d, 1H, H7-indole, 8.87); 7.38 (d, 1H, H4-indole, 2.09); 7.68 (d, 2H, H-benzyl, 8.34); 7.96 (d, 2H, H-benzyl, 8.34). EI-MS m/z (%) 520 (M, 25), 181 (100). Anal. ($C_{25}H_{23}F_3N_2O_5S$) C, H, N.

2-(Trifluoromethyl)-*N*-[1-(3,4,5-trimethoxybenzyl)-1*H*-indol-5-yl]benzenesulfonamide **7h**

As white crystals (75%), m.p. 157–158°C (ethanol). IR (KBr), cm^{-1}: 3295 (NH), 1355 and 1175 (SO_2N); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.71 (s, 6H, *m*,*m*′-OCH_3); 3.81 (s, 3H, *p*-OCH_3); 5.20 (s, 2H, CH_2); 6.27 (s, 2H, H-trimethoxybenzyl); 6.46 (d, 1H, H3-indole, 2.98); 6.62 (s, 1H, SO_2NH); 6.86 (m, 1H, H6-indole); 7.11 (d, 1H, H2-indole, 2.98); 7.16 (d, 1H, H7-indole, 8.54); 7.36 (d, 1H, H4-indole, 2.03); 7.47 (m, 1H, H-benzyl); 7.62 (m, 1H, H-benzyl); 7.89 (m, 2H, H-benzyl). EI-MS m/z (%) 520 (M, 20), 181 (100). Anal. ($C_{25}H_{23}F_3N_2O_5S$) C, H, N.

Methyl 2-[(1-(3,4,5-trimethoxybenzyl)-1*H*-indol-6-yl)aminosulfonyl]benzoate **7i**

As white crystals (40%), m.p. 110–111°C (propan-2-ol). IR (KBr), cm^{-1}: 3290 (NH), 1720 (CO), 1350 and 1170 (SO_2N); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.74 (s, 6H, *m*,*m*′-OCH_3); 3.83 (s, 3H, *p*-OCH_3); 4.06 (s, 3H, COOMe); 5.18 (s, 2H, CH_2); 6.31 (s, 2H, H-trimethoxybenzyl); 6.44 (d, 1H, H3-indole, 3.27); 6.75 (dd, 1H, H5-indole, 8.72 1.09); 7.13 (d, 1H, H2-indole, 3.27); 7.29 (m, 2H, H7-indole/H-benzyl); 7.40 (d, 1H, H4-indole, 8.72); 7.46 (m, 2H, H-benzyl); 7.72 (d, 1H, H-benzyl, 7.63); 7.99 (s, 1H, SO_2NH). EI-MS m/z (%) 510 (M, 15), 181 (100). Anal. ($C_{26}H_{26}N_2O_7S$) C, H, N.

Methyl 2-[(1-(3,4,5-trimethoxybenzyl)-1*H*-indol-7-yl)aminosulfonyl]benzoate **7j**

As white crystals (45%), m.p. 105–106°C (propan-2-ol). IR (KBr), cm^{-1}: 3290 (NH), 1720 (CO), 1350 and 1170 (SO_2N); ^1H NMR ($CDCl_3$), δ, ppm; J, Hz: 3.78 (s, 6H, *m*,*m*′-OCH_3); 3.83 (s, 3H, *p*-OCH_3); 4.04 (s, 3H, COOMe); 5.18 (s, 2H, CH_2); 6.23 (d, 1H, H6-indole, 7.70); 6.31 (s, 2H,H-trimethoxybenzyl); 6.44 (d, 1H, H3-indole, 3.07); 6.75 (t, 1H, H5-indole, 7.70); 7.19 (d, 1H, H2-indole, 3.07); 7.55 (m, 2H, H4-indole/H-benzyl); 7.69 (m, 1H, H-benzyl); 7.77 (m, 2H, H-benzyl/SO_2NH); 7.94 (dd, 1H, H-benzyl, 7.70 1.03). EI-MS m/z (%) 510 (M, 16), 181 (100). Anal. ($C_{26}H_{26}N_2O_7S$) C, H, N.

Assay for Antiproliferative Activity on Human Cancer PC3 Line

Human prostate cancer PC3 cells were maintained in RPMI 1640 culture medium supplemented with 10% FCS. For growth assays, the cells were seeded onto 96-well plates at a density of approximately 3×10^4 cells/well. After 3 days, the cell medium was changed to serum-free medium and the cells were starved for 24 h for the culture synchronisation. The stimulation of the growth of quiescent cells was then performed by 10 ng/ml EGF plus TSe (50 ng/ml transferrin and 50 pg/ml selenium) and the tested compounds were added to culture medium. After an additional 72 h, the cell growth was estimated by the colorimetric MTT test.[14]

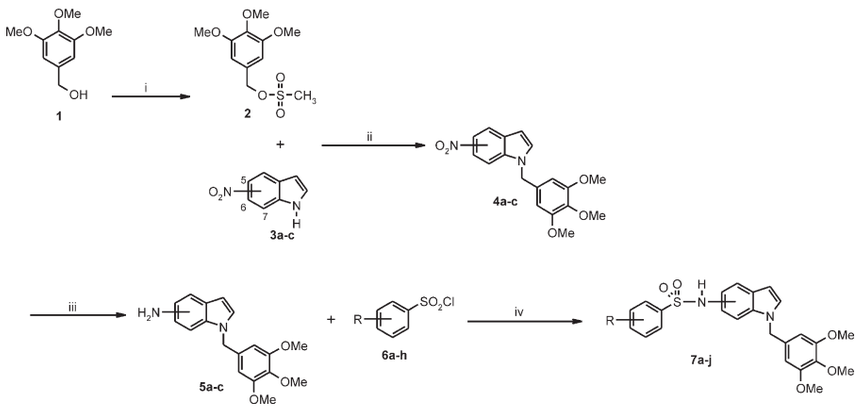
Flow Cytometric Analyses

The PC3 cells were grown at a density of about 5×10^5 cells onto 25 cm^2 dishes, synchronized during 24 h in serum-free medium and stimulated by EGF-TSe in the presence of different tested agents for 3 days. An analysis by FACS was then performed using the method described in the cell cycle test kit from Becton Dickinson. The propidium iodide-stained cell nuclei populations were quantified using the Cellquest logiciel program.

RESULTS AND DISCUSSION

Synthesis

3,4,5-Trimethoxy-1-[(methylsulfonyloxy)methyl]-benzene **2** was synthesized from 3,4,5-trimethoxybenzyl alcohol by reaction with methane sulfonyl chloride in the presence of triethylamine for 16 h at room temperature. Nitro-1-(3,4,5-trimethoxybenzyl)-1*H*-indoles **4a–c** were prepared from the corresponding nitroindoles **3a–c** by *N*-alkylation with 3,4,5-trimethoxy-1-[(methylsulfonyloxy)methyl]benzene **2** in the presence of potassium carbonate for 4 days at reflux. Sulfonyl chlorides were commercially available **6a, 6d, 6e, 6f, 6g, 6h** or previously described such as **6b**[15] or **6c**.[16] The sulfonamides **7a–j** were synthesized in a two step strategy.



Reagents: i. MsCl, Et_3N, CH_2Cl_2; ii. K_2CO_3, Acetone; iii. NH_4Cl, Fe, propan-2-ol; iv. Pyridine, DMF

Compounds	N° of intermediates	Substitution on the indole ring	R	Inhibition (%)*
7a	**3a, 4a, 5a**	5	H (**6a**)	0
7b	**3a, 4a, 5a**	5	2-COOMe (**6b**)	13.64 ± 0.7
7c	**3a, 4a, 5a**	5	4-SO_2NH_2 (**6c**)	0
7d	**3a, 4a, 5a**	5	4-Me (**6d**)	0
7e	**3a, 4a, 5a**	5	4-Cl (**6e**)	17.25 ± 0.8
7f	**3a, 4a, 5a**	5	4-OMe (**6f**)	34.50 ± 1.7
7g	**3a, 4a, 5a**	5	4-CF_3 (**6g**)	0
7h	**3a, 4a, 5a**	5	2-CF_3 (**6h**)	0
7i	**3b, 4b, 5b**	6	2-COOMe (**6b**)	15.90 ± 0.8
7j	**3c, 4c, 5c**	7	2-COOMe (**6b**)	45.50 ± 2.2

*The maximal inhibition of cell growth obtained with 1 μM of tested compounds is shown.

FIGURE 1 Synthesis, structures and inhibition results for the final sulfonamides (**7a–7j**)

Amino-1-(3,4,5-trimethoxybenzyl)-1*H*-indoles **5a–c** were first prepared from nitro-1-(3,4,5-trimethoxybenzyl)-1*H*-indoles **4a–c** by reduction with ammonium chloride solution in the presence of Fe powder. The amino-1-(3,4,5-trimethoxybenzyl)-1*H*-indoles **5a–c** so obtained were not isolated and were directly reacted with sulfonyl chlorides **6a–h** to give the target compounds **7a–j** in good yields (Figure 1).

Pharmacology

In the MTT colorimetric assay, all of the tested products were less active than E7070 (IC_{50} = 0.84 μM). The analogue **7c** of **E7070** had no effect on PC3 cell growth. Suppression or replacement of the sulfonamide group by a methyl group in compounds **7a** and **7d**, did not provide any improvement. Nevertheless in the case of compounds possessing Cl or OMe on the phenyl ring (**7e–7f**), antiproliferative activity was enhanced especially for compound **7f**. To have more information on the importance of the 4 substitution with an electron withdrawing group, the trifluoromethyl derivatives **7g** and **7h** were prepared. Neither of them showed an effect on cellular proliferation.

The recent finding that the polycyclic indolobenzothiazepine dioxide[17] synthesized from the ester **7b** showed good inhibitory properties, prompted us to evaluate **7b**. This methyl ester was tested and was seen that introducing a carboxymethyl moiety, at the 2-position of the phenyl ring, led to an increase in the activity. Effectively, among the tested derivatives, **7b** gave a good result (IC_{50} = 10.70 μM).

In the compounds **7i** and **7j**, the aryl sulfonamide position on the indole was varied with respect to the methyl ester position. These compounds exhibited as for **7b**, an inhibition potency when the tested concentration is close to 10 μM.

At this point, it is important to define the precise target of **7b** and the other compounds. This is in the course of evaluation. It is interesting to note that flow cytometric analyses have already shown a cellular accumulation in the G1 phase and the precise mechanism of this affect is currently under investigation.

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