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Design, Synthesis, and Cytotoxicity of 4-Sulfonamide Substituted Benzamidobenzimidazolones and an Acyl Benzimidazolone

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4-Sulfonamide substituted benzamidobenzimidazolones were designed and docked into the active site model of CDK2, using an oxindole inhibitor as the template. Compounds **6a-6i** were then prepared from the reaction of the sulfonyl chloride **1** with different amines to give the corresponding acids (**2a-2i**), which were converted to their corresponding acyl chlorides (**3a-3i**). Reaction of **3a-3i** with *o*-nitrophenylhydrazine afforded the respective nitro derivatives (**4a-4i**). The nitro groups were then reduced to give the corresponding amines (**5a-5i**), which, upon reaction with ethyl chloroformate, the target compounds (**6a-6i**) were produced. Target benzimidazolone derivatives (**9a-9e**) were also prepared from the reaction of isopropenyl benzimidazolone (**8**) with different sulfonyl or acyl chlorides. The target compounds were then tested by a modified 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay against the cancer cell lines, Hep G2, HT-29, CL1-5 and AGS. Despite similar binding properties of the flexible benzamidobenzimidazolones and rigid cytotoxic oxindole inhibitors at the active site of CDK2, biological screening results indicated that benzamidobenzimidazolones did not exhibit significant cell growth inhibition *in vitro*. Their analogue, 3-acyl benzimidazolone (**12**), however, revealed cytotoxicity similar to that of the reference oxindole inhibitor.

Keywords: Benzimidazolone, Sulfonamide, Cytotoxicity, CDK2

INTRODUCTION

The cell cycle is fundamental to the survival, regulation and proliferation of cells and is highly regulated to ensure that each step progresses in a timely and orderly manner [1]. The progression of cells through the cell cycle arises from the sequential activation and deactivation of several members of the cyclin-dependent kinase (CDK) family [1]. The activation of CDKs is dependent on their interaction with a family of intracellular proteins called cyclins [2]. Cyclins bind CDKs and this interaction is essential for the CDKs (*i.e*. CDK1, CDK2, CDK4 and/or CDK6) activity within the cell. Different cyclins are expressed and degraded at different points in the cell cycle to ensure that activation and inactivation of the CDKs occur in the correct order for progression through the cell cycle. Moreover, CDKs appear to be downstream from a number of oncogene signaling pathways. Deregulation of CDK activity by upregulation of cyclins and/or deletion of endogenous inhibitors appears to be an important axis between

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mitogenic signaling pathways and proliferation of tumor cells. Accordingly, it has been recognized that inhibitors of cell cycle kinases, particularly inhibitors of CDK1, CDK2 and/or CDK4, which operate at the G2/M, G1-S-G2/M and G1-S phase, respectively, should be of value as selective inhibitors of cell proliferation, such as growth of mammalian cancer cells [3,4]. Among natural and synthetic CDK2 inhibitors, oxindole-based CDK2 inhibitors have shown selectivity for CDK2 inhibition [5,6]. Because of the structural resemblance of oxindole to benzimidazol-2-one, we decided to synthesize some 4-sulfonamide substituted benzamidobenzimidazolones and their respective acyl benzimidazolones for cytotoxicity evaluation.

EXPERIMENTAL

General

Chemicals (including **2a**) and solvents were reagent grade and purchased from Aldrich or Fluka Chemical Companies. Melting points were determined using a Mel-temp capillary apparatus, International Devices, USA, and are uncorrected. Infrared spectra were acquired on a Jasco FT/IR-400 spectrophotometer. A Bruker FT-200 DPX instrument was used to acquire ^{1}H NMR spectra using either DMSO-d_6 or $CDCl_3$ as solvents. Mass spectra were determined using a Trio 1000, Fisons mass spectrometer, Manchester, UK. Hydrogenations were performed by use of a Buchiglasuster hydrogenator.

Human hepatocellular carcinoma cells (Hep G2), human colon adenocarcinoma cells (HT 29), human lung carcinoma cells (CL1-5) and human Caucasian gastric adenocarcinoma cells (AGS) were from cultures maintained in the cytotoxicity laboratory of the School of Pharmacy, National Taiwan University. Roswell Park Memorial Institute (RPMI)-1640 culture medium, fetal bovine serum (FBS), L-glutamine, non-essential amino acids (NEAA), penicillin/streptomycin, trypsin-EDTA were purchased from Gibco, USA. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide(MTT) was purchased from Sigma. The absorbance was measured at 540 nm with a microtiter plate reader (Molecular Devices, CA, USA).

4-(Methylaminosulfonyl)benzoic Acid (2b). 4-Carboxy-benzenesulfonyl chloride (**1**, 1.0 g, 4.5 mmol) was added in portions to an ice-cooled methylamine solution (40%, 5.0 ml) while stirring at 5 ºC. After 20 minutes, the solution was acidified using concentrated HCl to give white solid crystals (**2b**, 960 mg, 99% yield) which were filtered and dried, m.p.: 228-230 ºC. IR (KBr): 3284-2982 (NH, CO<u>OH</u>), 1696 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 8.12 (d, 2H, *J* = 8.4 Hz, Ar), 7.88 (2d, 2H, *J* = 8.4 Hz, Ar), 7.00 (q, 1H, *J* = 5 Hz, NH), 2.41 (d, 3H, *J* = 5 Hz, CH_3) ppm.

4-(Dimethylaminosulfonyl)benzoic Acid (2c) [7]**.** 4-Carboxybenzenesulfonyl chloride (**1**, 1.0 g, 4.54 mmol) and dimethylamine were reacted as described for **2b** to give **2c** as white solid crystals (950 mg, 92% yield), m.p.: 218-219 ºC (lit[11]. 99% m.p.: 222-228 ºC). IR (KBr): 3423-2780 (CO<u>OH</u>) 1692 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 8.16 (d, 2H, *J* = 8.2 Hz, Ar), 7.90 (d, 2H, *J* = 8.4 Hz, Ar), 2.62 (s, 6H, $2CH_3$) ppm.

4-(Ethylaminosulfonyl)benzoic Acid (2d). Ethylamine solution (70%, 5 ml) and **1** (1.0 g, 4.5 mmol) were reacted as described for **2b** to give **2d** as snow-white crystals (900 mg, 87% yield), m.p.: 209-210 ºC. IR (KBr): 3285-2944 (NH, CO<u>OH</u>), 1686 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 8.11 (d, 2H, *J* = 8.3 Hz, Ar), 7.89 (d, 2H, *J* = 8.4 Hz, Ar), 7.76 (t, 1H, *J* = 5.6 Hz, NH), 2.79 (q, 2H, *J* = 5.6 Hz, CH_2), 0.95 (t, 3H, *J* = 7.2 Hz, CH_3).

4-(Diethylaminosulfonyl)benzoic Acid [8] **(2e).** 4-Chlorosulfonylbenzoic acid (**1**, 0.5 g, 2.27 mmol) was added in portions to an ice-cooled solution of diethylamine (670 mg, 9.1 mmol) in dry methanol (20 ml) and stirred at room temperature overnight. The solvent was evaporated to give a solid residue which was dissolved in water (10 ml) and acidified using concentrated HCl to give 4-(diethyl aminosulfonyl)benzoic acid (**2e**) as white solid crystals (400 mg, 68.6% yield), m.p.: 195-196 ºC. IR (KBr): 3095-2860 (CO<u>OH</u>), 1700 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 8.11 (d, 2H, *J* = 8.3 Hz, Ar), 7.90 (d, 2H, *J* = 8.3 Hz, Ar), 3.16 (q, 4H, *J* = 7.1 Hz, $2CH_2$), 1.01 (t, 6H, *J* = 7.0 Hz, $2CH_3$) ppm.

4-(Propylaminosulfonyl)benzoic Acid (2f). *n*-Propylamine and **1** (1.0 g, 4.5 mmol) were reacted as described for **2b** to give **2f** as white crystals (940 mg, 86% yield), m.p.: 201-202 ºC. IR (KBr): 3285-2936 (CO<u>OH</u>, NH), 1684 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 13.43 (bs, 1H, COOH), 8.11 (d, 2H, *J* = 8.2 Hz, Ar), 7.88 (d, 2H, *J* = 8.3 Hz, Ar), 7.79 (t, 1H, *J* = 6 Hz, NH), 2.70 (q, 2H, *J* = 6.6 Hz, NH-

CH_2), 1.33 (m, 2H, CH_2), 0.76 (t, 3H, *J* = 7.2 Hz, CH_3) ppm.

4-(Isopropylaminosulfonyl)benzoic Acid (2g). Isopropylamine and **1** (1.0 g, 4.5 mmol) were reacted as described for **2b** to give **2g** as white crystals (920 mg, 84.2% yield), m.p.: 193-194 ºC. IR (KBr): 3442-2938 (NH, COOH), 1698 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 13.41 (s, 1H, COOH), 8.10 (d, 2H, *J* = 8.2 Hz, Ar), 7.90 (d, 2H, *J* = 8.2 Hz, Ar), 7.81 (d, 1H, *J* = 7.4 Hz, NH), 3.27 (m, 1H, CH), 0.92 (d, 6H, *J* = 6.4 Hz, $2CH_3$) ppm.

4-(*n*-Butylaminosulfonyl)benzoic Acid (2h). *n*-Butylamine and **1** (1.0 g, 4.5 mmol) were reacted as described for **2b** to give **2h** as white crystals (900 mg, 77.8% yield), m.p.: 182-183 ºC. IR (KBr): 3291-2873 (NH, COOH), 1685 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 8.08 (d, 2H, *J* = 8.4 Hz, Ar), 7.88 (d, 2H, *J* = 8.4 Hz, Ar), 7.70 (t, 1H, *J* = 5.9 Hz, NH), 2.74 (q, 2H, *J* = 6.2 Hz, NH-CH_2), 1.23 (m, 4H, CH_2-CH_2), 0.76 (t, 3H, *J* = 7.0 Hz, CH_3) ppm.

4-(Piperidylsulfonyl)benzoic Acid [9] **(2i).** Piperidine and **1** (500 mg, 2.27 mmol) were reacted as described for **2b** to give **2i** as white solid crystals (520 mg, 85.2% yield), m.p.: 270-271ºC. IR (KBr): 3096-2848 (COOH) 1603 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 8.15 (d, 2H, *J* = 8.4 Hz, Ar), 7.58 (d, 2H, *J* = 8.3 Hz, Ph-3,5H), 2.87 (m, 4H, Piper-2,6H), 1.43 (m, 6H, Piper) ppm.

4-Sulfamoylbenzoyl-*o*-nitrophenylhydrazine [10,11] **(4a).** A suspension of 4-sulfamoylcarboxylic acid (**2a**, 720 mg, 3.6 mmol) in thionyl chloride (10 ml) was heated under reflux for 3 h to give a clear solution [12]. The solvent was evaporated under reduced pressure to give **3a** as a semisolid residue which was suspended in chloroform (30 ml) and added dropwise to an ice-cooled solution of *o*-nitrophenyl hydrazine (500 mg, 3.26 mmol) and triethylamine (370 mg, 3.6 mmol) in chloroform (10 ml) and stirred 2 h at the same temperature to give a yellow solid. The solid was filtered, washed with water and recrystallized from ethanol to give **4a** as solid yellow crystals (560 mg, 51% yield), m.p.: 238-239 ºC. IR (KBr): 3370, 3336, 3268 (2NH, NH_2) 1640 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 10.98 (s, 1H, NH), 9.49 (s, 1H, NH), 8.11 (m, 3H, arom-3H and arom`-3,5H), 7.96 (2d, 2H, *J* = 8.34 Hz, arom`2,6H), 7.59 (m, 3H, arom-5H and NH_2), 7.20 (d, 1H, *J* = 8.32 Hz, arom-6H), 6.89 (t, 1H, *J* = 7.7 Hz, arom 4H) ppm.

(4-Methylaminosulfonylbenzoyl)-*o*-nitrophenylhydrazine (4b). 4-(Methylaminosulfonyl)benzoic acid (**2b**, 360 mg, 1.67 mmol), thionyl chloride (10 ml), *o*-nitrophenylhydrazine (300 mg, 2 mmol) and triethylamine (200 mg, 2 mmol) were reacted as described for **4a** to afford **4b** as pale grey crystals (330 mg, 57% yield), m.p.: 199-200 ºC. IR (KBr): 3328, 3247 (3NH) 1649 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 11.01 (s, 1H, NH), 9.48 (s, 1H, NH), 8.14 (d, 3H, *J* = 8.4 Hz, arom-3H and arom`-3,5H), 7.92 (d, 2H, *J* = 8.4 Hz, arom`-2,6H), 7.62 (m, 2H, arom-5H and NH), 7.20 (d, 1H, *J* = 8 Hz, arom-6H), 6.89 (t, 1H, *J* = 7.1 Hz, arom-4H, 2.44 (d, 3H, *J* = 4.9 Hz, CH_3) ppm.

(4-Dimethylaminosulfonylbenzoyl)-*o*-nitrophenylhydrazine (4c). 4-(Dimethylaminosulfonyl)benzoic acid (**2c**, 460 mg, 2 mmol), thionyl chloride (10 ml), *o*-nitrophenylhydrazine (306 mg, 2 mmol) and triethylamine (210 mg, 2 mmol) were reacted as described for **4a**. The residue was purified on silica gel column chromatography using hexane-ethyl acetate 1:3 as the eluent to afford **4c** as yellowish-brown solid crystals (150 mg, 21% yield), m.p.: 195-197 ºC. IR (KBr): 3361, 3343 (2 NH) 1685 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 11.04 (s, 1H, NH), 9.49 (s, 1H, NH), 8.15 (m, 3H, arom-3H and arom`-3, 5H), 7.91 (d, 2H, *J* = 8.3 Hz, arom`-2,6H), 7.59 (t, 1H, *J* = 7.6 Hz, arom-5H), 7.20 (d, 1H, arom-6H), 6.89 (t, 1H, *J* = 7.6 Hz, arom-4H), 2.65 (s, 6H, $2CH_3$) ppm.

(4-Ethylaminosulfonylbenzoyl)-*o*-nitrophenylhydrazine (4d). 4-Ethylaminosulfonylbenzoic acid (**2d**, 350 mg, 1.53 mmol), thionyl chloride (10 ml), *o*-nitrophenylhydrazine (215 mg, 1.4 mmol) and triethylamine (142 mg, 1.4 mmol) were reacted as described for **4a** to give **4d** as orange crystals (320 mg, 62.7% yield), m.p.: 170-171 ºC. IR (KBr): 3325, 3263 (3 NH) 1647 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 10.1 (s, 1H, NH), 9.47 (s, 1H, NH), 8.12 (d, 3H, *J* = 8.2 Hz, arom-3H and arom`-3,5H), 7.93 (d, 2H, *J* = 8.2 Hz, arom`-2,6H), 7.76 (t, 1H, *J* = 5.5 Hz, arom-5H), 7.58 (t, 1H, *J* = 7.7 Hz, NH), 7.20 (d, 1H, *J* = 8.5 Hz, arom-6H), 6.89 (t, 1H, *J* = 7.6 Hz, arom-4H), 2.82, (q, 2H, *J* = 6.8 Hz, CH_2), 0.98 (t, 3H, *J* = 7.1 Hz, CH_3) ppm.

(4-Diethylaminosulfonylbenzoyl)-*o*-nitrophenylhydrazine (4e). 4-Diethylaminosulfonylbenzoic acid (**2e**, 300 mg, 1.17 mmol), thionyl chloride (10 ml), *o*-nitrophenylhydrazine (162 mg, 1.06 mmol) and triethylamine (120 mg, 1.1 mmol) were reacted as described for **4a** to give **4e** as orange crystals (290 mg, 70% yield), m.p.: 179-180 ºC. IR (KBr): 3367, 3352 (2NH) 1682 (C=O) cm^{-1}. ^{1}H NMR (DMSO-d_6): δ = 11.01 (s,

1H, NH), 9.47 (s, 1H, NH), 8.12 (d, 3H, *J* = 8.3 Hz, arom 3H and arom`-3,5H), 7.95 (d, 2H, *J* = 8.3 Hz, arom`-2,6H), 7.59 (t, 1H, *J* = 7.1 Hz, arom-5H), 7.19 (d, 1H, *J* = 8.3 Hz, arom-6H), 6.89 (t, 1H, *J* = 7.4 Hz, arom-4H), 3.20 (q, 4H, *J* = 7.1 Hz, $2CH_2$), 1.05 (t, 6H, *J* = 7.1 Hz, $2CH_3$) ppm.

(4-Propylaminosulfonylbenzoyl)-o-nitrophenylhydrazine (4f). 4-Propylaminosulfonylbenzoic acid (**2f**, 450 mg, 1.85 mmol), thionyl chloride (10 ml), *o*-nitrophenylhydrazine (260 mg, 1.7 mmol) and triethylamine (172 mg, 1.7 mmol) were reacted as described for **4a** to give **4f** as orange crystals (340 mg, 53% yield), m.p.: 179-181ºC. IR (KBr): 3365, 3333, 3265 (3NH), 1663 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 10.99 (s, 1H, NH), 9.47 (s, 1H, NH), 8.08 (d, 3H, *J* = 8.4 Hz, arom-3H and arom`-3,5H), 7.93 (d, 2H, *J* = 8.4 Hz, arom`-2, 6H), 7.78 (t, 1H, *J* = 5.7 Hz, arom-5H), 7.59 (t, 1H, *J* = 7.4 Hz, NH), 7.20 (d, 1H, *J* = 8.4 Hz, arom-6H), 6.90 (t, 1H, J = 7.2 Hz, arom-4H), 2.73 (q, 2H, *J* = 6.6 Hz, NH-CH_2), 1.38 (m, 2H, CH_2), 0.79 (t, 3H, *J* = 7.2 Hz, CH_3) ppm.

(4-Isopropylaminosulfonyl-benzoyl)-*o*-nitrophenylhydrazine (4g). 4-Isopropylaminosulfonylbenzoic acid (2.0 g, 450 mg, 1.85 mmol), thionyl chloride (25 ml), *o*-nitrophenylhydrazine (260 mg, 1.7 mmol), and triethylamine (172 mg, 1.7 mmol) were reacted as described for **4a** to give **4g** as orange solid crystals (350 mg, 55% yield), m.p.: 215-216 ºC. IR (KBr): 3357, 3270 (3NH) 1660 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 10.98 (s, 1H, NH), 9.46 (s, 1H, NH), 8.12 (m, 3H, arom-3H and arom`-3,5H), 7.94 (d, 2H, *J* = 8.4 Hz, arom`-2,6H), 7.78 (d, 1H, *J* = 7.2 Hz, NH), 7.58 (t, 1H, *J* = 7.1 Hz, arom-5H), 7.20 (d, 1H, *J* = 7.8 Hz, arom-6H), 6.89 (t, 1H, *J* = 7.1 Hz, arom-4H), 3.33 (m, 1H, CH), 0.96 (d, 6H, *J* = 6.5 Hz, $2CH_3$) ppm.

(4-Butylaminosulfonylbenzoyl)-*o*-nitrophenylhydrazine (4h). 4-Butylaminosulfonylbenzoic acid (**2h**, 400 mg, 1.56 mmol), thionyl chloride (15 ml), *o*-nitrophenylhydrazine (218 mg, 1.42 mmol), and triethylamine (144 mg, 1.42 mmol) were reacted as described for **4a** to give **4h** as pale orange solid crystals (280 mg, 50% yield), m.p.: 174-175 ºC. IR (KBr): 3363, 3267 (3NH) 1683 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 10.98 (s, 1H, NH), 9.48 (s, 1H, NH), 8.12 (d, 3H, *J* = 8.2 Hz, arom-3H and arom`-3,5H), 7.93 (d, 2H, *J* = 8.2 Hz, arom`-2,6H), 7.76 (t, 1H, *J* = 5.6 Hz, NH), 7.58 (t, 1H, *J* = 8.2 Hz, arom-5H), 7.20 (d, 1H, *J* = 8.6 Hz, arom-6H), 6.90 (t, 1H, *J* = 8.7 Hz, arom-4H), 2.76 (q, 2H, *J* = 6.1 Hz, NH-CH_2), 1.25 (m, 4H, CH_2-CH_2), 0.80 (t, 3H, *J* = 7 Hz, CH_3) ppm.

(4-Piperidylsulfonylbenzoyl)-*o*-nitrophenylhydrazine (4i). 4-Piperidylsulfonylbenzoic acid (**2i**, 250 mg, 0.93 mmol), thionyl chloride (10 ml), *o*-nitrophenylhydrazine (130 mg, 0.85 mmol), and triethylamine (86 mg, 0.85 mmol) were reacted as described for **4a** to give **4i** as orange solid crystals (200 mg, 58% yield), m.p.: 224-225 ºC. IR (KBr): 3369, 3340 (2NH), 1680 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.0 (br s, 1H, NH), 9.51 (s, 1H, NH), 8.14 (m, 3H, arom-3H and arom`-3,5H), 7.88 (d, 2H, *J* = 8.2 Hz, arom`-2,6H), 7.58 (t, 1H, *J* = 7.9 Hz, arom-5H), 7.20 (d, 1H, *J* = 8.6 Hz, arom-6H), 6.89 (t, 1H, *J* = 7.7 Hz, arom-4H), 2.93 (m, 4H, Piper-2,6H), 1.48 (m, 6H, Piper) ppm.

(4-Sulfamoylbenzoyl)-*o*-aminophenylhydrazine (5a) and its analogues (5b-5i). All compounds were prepared in the same manner, which is explained for **5a** only. A mixture of 4-sulfamoylbenzoyl-*o*-nitrophenylhydrazine (**4a**, 500 mg, 1.49 mmol) and palladium/charcoal (10%, 200 mg) in methanol (150 ml) was sonicated for 5 minutes and hydrogenated at 4 bars for 2 h. The mixture was filtered through celite and evaporated under reduced pressure to give **5a** as a semisolid which was changed to grey solid crystals (430 mg, 94% yield), m.p.: 110 ºC. IR (KBr): 3258 (br $2NH_2$, 2NH), 1653 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 10.53 (br s, 1H, NH), 7.84 (m, 4H, arom`), 7.52 (s, 2H, SO_2-NH_2), 6.47 (m, 4H, arom), 5.08 (br s, 1H, NH_2) ppm.

1-(4-Aminosulfonylbenzamido)benzimidazolone [13] **(6a).** To a solution of 4-sulfamoylbenzoyl-*o*-aminophenylhydrazine (**5a**, 350 mg, 1.1 mmol) in dry pyridine (20 ml), ethyl chloroformate (136 mg, 1.25 mmol) was added in a drop-wise manner under nitrogen and stirred at room temperature for 4 h, followed by 22 h of reflux. Water (50 ml) was added and the solution was extracted into dichloromethane (3 × 50 ml), dried ($MgSO_4$), filtered, and the solvent evaporated under vacuum to leave a semisolid which was solidified after being azeotroped with toluene. The crude solid was recrystallized from EtOH/EtOAc (1:1) to give **6a** as a solid (200 mg, 55% yield), m.p.: 325-327 ºC. IR (KBr): 3316, 3213 (NH_2, 2NH), 1710, 1686 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.55 (s, 1H, NH), 11.15 (s, 1H, NH), 8.14 (d, 2H, *J* = 8.2 Hz, arom`-3,5H), 8.00 (d, 2H, *J* = 8.16 Hz, arom`2,6H), 7.58 (br s, 2H, NH_2), 7.03 (m, 4H, arom-H) ppm. CI/MS: 333 ($M^+ + 1$).

1-(4-Methylaminosulfonylbenzamido)benzimidazolone (6b). Ethyl chloroformate (110 mg, 1.01 mmol) was reacted with **5b** in dry pyridine (10 ml) under nitrogen, and stirred for 1 h at room temperature. The solution was heated under reflux for 22 h and diluted with water (10 ml), and extracted with dichloromethane (3 × 20 ml). The organic layer was decolorized with charcoal, dried ($MgSO_4$), filtered and evaporated under vacuum and azeotroped with toluene to give a semisolid residue. It was purified on a silica gel column and eluted with $CHCl_3$/MeOH (9:1) to give **6b** as white solid crystals (180 mg, 60% yield), m.p.: 247-248 ºC. IR (KBr): 3265 (br 3NH), 1724, 1682 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.59 (s, 1H, NH), 11.16 (s, 1H, NH), 8.18 (d, 2H, *J* = 8.3 Hz, arom`-3,5H), 7.96 (d, 2H, *J* = 8.4 Hz, arom`-2,6H), 7.68 (q, 1H, *J* = 5 Hz, NH), 7.03 (m, 4H, arom-H), 2.45 (d, 3H, *J* = 5 Hz, CH_3) ppm. CI/MS: 347 ($M^+ + 1$).

1-(4-Dimethylaminosulfonylbenzamido)benzimidazolone (6c). Ethyl chloroformate (50 mg, 0.46 mmol) and **5c** were reacted as described for **6b** to obtain an oily residue, which was purified by column using $CHCl_3$ followed by $CHCl_3$/MeOH (9:1) as the eluent to give **6c** as solid crystals (100 mg, 73% yield), m.p.: 219-220 ºC. IR (KBr): 3263 (br 3NH), 1725, 1692 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.64 (s, 1H, NH), 11.16 (s, 1H, NH), 8.22 (d, 2H, *J* = 8.2 Hz, arom`-3,5H), 7.95 (d, 2H, *J* = 8.2 Hz, arom`-2,6H), 6.96 (m, 4H, arom-H), 2.65 (s, 6H, $2CH_3$) ppm. CI/MS: 361 ($M^+ + 1$).

1-(4-Ethylaminosulfonylbenzamido)benzimidazolone (6d). Ethyl chloroformate (97 mg, 0.89 mmol) and **5d** were reacted as described for **6b** to give an orange oil, which was fractionated on silica gel column using $CHCl_3$/MeOH (15:1) as the eluent to give **6d** as pale cream-colored crystals (100 mg, 37.5% yield), m.p.: 135-136 ºC. IR (KBr): 3259 (br 3NH) 1721, 1678 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.57 (s, 1H, NH), 11.15 (s, 1H, NH), 8.17 (d, 2H, *J* = 8.4 Hz, arom`-3, 5H), 7.97 (d, 2H, *J* = 8.4 Hz, arom`-2,6H), 7.78 (t, 1H, *J* = 5.6 Hz, NH), 7.04 (m, 4H, arom), 2.83, (q, 2H, *J* = 7 Hz, CH_2), 0.98 (t, 3H, *J* = 7.1 Hz, CH_3) ppm. CI/MS: 361 ($M^+ + 1$).

1-(4-Diethylaminosulfonylbenzamido)benzimidazolone (6e). Ethyl chloroformate (71 mg, 0.66 mmol) and **5e** were reacted as described for **6b** to give rise to an orange oil, which was purified by column using $CHCl_3$/MeOH (9:1) as the eluent to give **6e** as solid crystals (160 mg, 73% yield), m.p.: 140-141 ºC. IR (KBr): 3260 (2NH) 1725, 1690 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.6 (s, 1H, NH), 11.16 (s, 1H, NH), 8.17 (d, 2H, *J* = 8.3 Hz, arom`-3,5H), 7.99 (d, 2H, *J* = 8.3 Hz, arom`-2,6H), 7.02 (m, 4H, arom-H), 3.21 (q, 4H, *J* = 7.1 Hz, $2CH_2$), 1.05 (t, 6H, *J* = 7.1 Hz, $2CH_3$) ppm. CI/MS: 389 ($M^+ + 1$).

1-(4-Propylaminosulfonylbenzamido)benzimidazolone (6f). Ethyl chloroformate (102 mg, 0.95 mmol) and **5f** were reacted as described for **6b** to give an orange oil, which was fractionated on silica gel column using $CHCl_3$/MeOH (10:1) as the eluent to give **6f** as white crystals (170 mg, 67% yield), m.p.: 136-137 ºC. IR (KBr): 3263 (br 3NH) 1724, 1682 (2 C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.58 (s, 1H, NH), 11.16 (s, 1H, NH), 8.16 (d, 2H, *J* = 8.0 Hz, arom`-3,5H), 7.98 (d, 2H, *J* = 8.2Hz, arom`-2,6H), 7.80 (t, 1H, *J* = 5.4 Hz, NH), 7.04 (m, 4H, arom), 2.75 (q, 2H, *J* = 6.4Hz, NH-CH_2), 1.38 (q, 2H, *J* = 7.0 Hz, CH_2), 0.79 (t, 3H, *J* = 7.2 Hz, CH_3) ppm. CI/MS: 375 ($M^+ + 1$).

1-(4-Isopropylaminosulfonylbenzamido)benzimidazolone (6g). Ethyl chloroformate (103 mg, 0.95 mmol) and **5g** were reacted as described for **6b** to afford a dark red oil, which was fractionated on silica gel column using $CHCl_3$/MeOH (15:1) as the eluent to give **6g** as white crystals (150 mg, 50% yield), m.p.: 260-261 ºC. IR (KBr): 3269 (br 3NH) 1725, 1685 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 1157 (s, 1H, NH), 11.15 (s, 1H, NH), 8.16 (d, 2H, *J* = 7.2 Hz, arom`-3,5H), 7.99 (d, 2H, *J* = 7.2 Hz, arom`-2,6H), 7.81 (d, 1H, *J* = 5.4 Hz, NH), 7.02 (m, 4H, arom), 3.27 (m, 1H, CH), 0.96 (d, 6H, *J* = 6.4 Hz, $2CH_3$) ppm. CI/MS: 375 ($M^+ + 1$).

1-(4-Butylaminosulfonylbenzamido)benzimidazolone (6h). Ethyl chloroformate (88 mg, 0.82 mmol) and **5h** were reacted as described for **6b** to give an orange oil, which was fractionated on silica gel column using $CHCl_3$/MeOH (15:1) as the eluent to give **6h** as pale red crystals (130 mg, 50% yield), m.p.: 130-132 ºC. IR (KBr): 3263 (br 3NH) 1725, 1683 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.58 (s, 1H, NH), 11.16 (s, 1H, NH), 8.17 (d, 2H, J = 8.4 Hz, arom`-3,5H), 7.97 (d, 2H, *J* = 8.4 Hz, arom`-2,6H), 7.78 (t, 1H, *J* = 5.9 Hz, NH), 7.03 (m, 4H, arom-H), 2.76 (q, 2H, *J* = 6.2 Hz, NH-CH_2), 1.28 (m, 4H, CH_2-CH_2), 0.79 (3H, *J* = 6.8 Hz, CH_3) ppm. CI/MS: 389 ($M^+ + 1$).

1-(4-Piperidylsulfonylbenzamido)benzimidazolone (6i). Ethyl chloroformate (52 mg, 0.48 mmol) and **5i** were reacted as described for **6b** to afford brownish oil, which was

fractionated on a silica gel column using $CHCl_3$/MeOH (15:1) as the eluent to give **6i** as beige crystals (60 mg, 37% yield), m.p.: 209-210 ºC. IR (KBr): 3254 (2NH), 1728, 1696 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 11.63 (s, 1H, NH), 11.17 (s, 1H, NH), 8.21 (d, 2H, *J* = 7.9 Hz, arom`3,5H), 7.92 (d, 2H, *J* = 7.8 Hz, arom`-2,6H), 7.02 (m, 4H, arom-H), 2.95 (m, 4H, Piper-2, 6H), 1.4 (m, 6H, Piper) ppm. CI/MS: 401 ($M^+ + 1$).

2-Methyl-2,3-dehydrobenzimodazole (7). Ethyl acetoacetate (2.62 g, 2 mmol) and *o*-phenylendiamine (2.16 g, 2 mmol) were suspended in xylene (30 ml) containing molecular sieves (2 g), followed by the addition of a catalytic amount of toluene-4-sulfonic acid. The mixture was heated under reflux for 5 h, while the water produced during the reflux was removed using a Dean-Stark apparatus. The hot mixture was filtered. The product, which solidified in the filtrate was kept at room temperature, filtered, washed with xylene (10 ml) and dried to give **7** as pale red crystals (1.4 g, 53% yield), m.p.: 183-184 ºC. IR (KBr): 3262 (NH) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 9.85 (br s, 1H, NH), 7.57 (m, 2H, 5,7-H), 7.22 (m, 2H, 4,6-H), 2.65 (s, 3H, CH_3) ppm.

1-Isopropenyl benzimidazolone (8) [14,15,16]**.** Ethyl acetoacetate (13.8 g, 106 mmol) in xylene (10 ml) was added drop wise within 2.5 h, to a refluxing solution of *o*-phenylendiamine (10.2 g, 94 mmol) in xylene (50 ml) under nitrogen, while the water produced during the reflux was removed using a Dean-Stark trap. It was refluxed for another 3 h and cooled to deposit a solid, which was filtered and dried to give **8** as a white solid (8 g), m.p.: 122-124 ºC. An aqueous solution of sodium hydroxide (16 g, 80 ml) was added to the mother liquor to afford product as its sodium salt, which was then filtered. Acetic acid and water were added to the solid to give a clear solution at pH~6, which was solidified by the addition of more acetic acid. The solid was filtered and washed with acetic acid and dried to give more **8** as pale brown solid crystals (4.1 g, totally 12.1 g, 74% yield). IR (KBr): 3369 (NH) 1694 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 10.83 (br s, 1H, NH), 7.10 (m, 4H, arom-H), 5.41 (s, 1H, *trans*-CH), 5.24 (s, 1H, *cis*-CH), 2.3 (s, 3H, CH_3) ppm.

1-Isopropenyl-3-benzoylbenimidazolone (9a). Sodium hydride (60 mg, from a 80% mixture with paraffin oil, 2 mmol) was washed with hexane and dispersed in DMF (15 ml). 1-Isopropenyl benzimidazolone (**8**, 350 mg, 2 mmol) was added and the mixture was stirred for 15 minutes at room temperature followed by the addition of benzoyl chloride (300 mg, 2.1 mmol) in DMF (5 ml), and stirred overnight. Water (10 ml) was added and the resulting precipitate was filtered and washed with water (2 × 5 ml) and dried to give **9a** as white solid crystals (310 mg, 56% yield), m.p.: 119-120 ºC. IR (KBr): 1735, 1684 (2C=O) cm^{-1}. ^1H NMR ($CDCl_3$): δ = 7.95 (d, 1H, *J* = 8.3 Hz, bz-7H), 7.74 (d, 2H, *J* = 8.1 Hz, Ar), 7.55 (m, 3H, Ar), 7.14 (m, 3H, bz-4,5,6H), 5.40 (s, 1H, *trans*-CH), 5.25 (s, 1H, *cis*-CH), 2.15 (s, 3H, CH_3) ppm. CI/MS: 279 ($M^+ + 1$).

3-(*p*-Toluenesulfonyl)-1-isopropenylbenzimidazol-2-one (9b). A mixture of isopropenylbenzimidazolone (**8**, 350 mg, 2 mmol) and cesium carbonate (650 mg, 2 mmol) in dry DMF (10 ml) was stirred at room temperature for 1 h. A solution of 4-toluensulfonylchloride (380 mg, 2 mmol) in dry DMF (5 ml) was added drop wise and the mixture was stirred at room temperature for 4 h. Water (20 ml) was added to leave a precipitate which was stirred for 0.5 h, filtered and washed with water (20 ml) to give **9b** as beige solid crystals (440 mg, 67% yield) m.p.: 115-116 ºC. IR (KBr): 1733 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 7.94 (d, 2H, *J* = 7.8 Hz, Ph-2,6H), 7.83 (d, 1H, *J* = 6.5 Hz, bz-7H), 7.46 (d, 2H, *J* = 8 Hz, Ar), 7.22 (m, 2H, bz-5,6H), 7.12 (d, 1H, *J* = 8.4 Hz, bz-4H), 5.46 (s, 1H, *trans*-CH), 5.18 (s, 1H, *cis*-CH), 2.38 (s, 3H, Ph-CH_3), 2.01 (s, 3H, isop-CH_3) ppm. CI/MS: 329 ($M^+ + 1$).

3-(*p*-Methoxybenzensulfonyl)-1-isopropenylbenzimidazol-2-one (9c). A mixture of **8** (350 mg, 2 mmol), cesium carbonate (716 mg, 2.2 mmol) and 4-methoxybenzene-sulfonylchloride (496 mg, 2.4 mmol) were reacted as described for **9b** to give **9c** as pale cream-colored solid crystals (350 mg, 50% yield), m.p.: 155-156 ºC. IR (KBr): 1728 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 7.98 (2H, d, *J* = 8.5 Hz, Ph-2,6H), 7.83 (1H, d, *J* = 7.5 Hz, bz-7H), 7.13 (5H, m, Ph-3,5H and bz-4,5,6H), 5.45 (1H, s, *trans*-CH), 5.18 (1H, s, *cis*-CH), 3.82 (3H, s, O-CH3), 2.01 (3H, s, CH_3) ppm. CI/MS: 345 ($M^+ + 1$).

3-(*p*-Fluorobenzensulfonyl)-1-isopropenylbenzimidazol-2-one (9d). A mixture of **8** (350 mg, 2 mmol), cesium carbonate (716 mg, 2.2 mmol) and 4-fluorobenzenesulfonyl chloride (467 mg, 2.4 mmol) were reacted as described for **9b** to give **9d** as solid crystals (260 mg, 39% yield), m.p.: 185-186 ºC. IR (KBr): 1709 (C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 8.12 (m, 2H, Ar), 7.83 (d, 1H, *J* = 8 Hz, bz-7H), 7.51 (m, 2H, Ar),

7.19 (m, 3H, bz-4,5,6H), 5.46 (s, 1H, *trans*-CH), 5.20 (s, 1H, *cis*-CH), 2.07 (s, 3H, CH_3) ppm. CI/MS: 333 ($M^+ + 1$).

1-Isopropenyl-3-(4-propylaminosulfonylbenzoyl) benzimidazolone (9e). A mixture of **8** (300 mg, 1.72 mmol), cesium carbonate (624 mg, 1.9 mmol) and **2f** (500 mg, 2.06 mmol) were reacted, as described for **9b,** to give **9e** as solid crystals (40 mg, 6% yield), m.p.: 144-145 ºC. IR (KBr): 3287 (NH) 1747, 1697 (2C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 7.87 (m, 6H, Ph-2,3,5,6H and bz-5,7H), 7.25 (m, 3H, bz-4,6H and NH), 2.88 (s, 1H, *trans*-CH), 2.72 (m, 3H, *cis*-CH and N-CH_2), 2.06 (s, 3H, isop-CH_3), 1.34 (m, 2H, CH_2-CH_3), 0.84-0.76 (t, 3H, J = 7.4 Hz, CH_3) ppm. CI/MS: 400 ($M^+ + 1$).

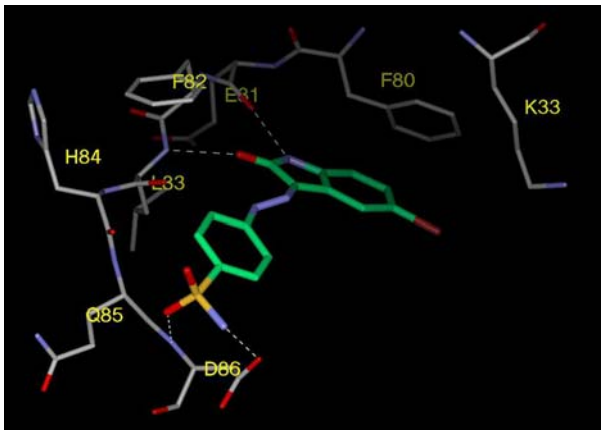
3-(Acetyl)benzimidazolone (11), and 3-(4-propylaminosulfonylbenzoyl)benzimidazolone (12). A solution of **9e** (2.39 g, 6.0 mmol), in MeOH was reacted with O_3 at -75 ºC for 3 h. The O_3 gas was then removed and the solution was purged with N_2 at 25 ºC to remove the extra O_3. Concentrated HCl solution (36%, 5 ml) was added and after 5 h stirring, the solvent was evaporated to afford a residue. Purification by column chromatography using $CHCl_3$/MeOH (8:2) as the eluent gave **11** (m.p.: 165-167, 50% yield), and **12** (m.p.: 219-220, 15% yield). For **12**: IR (KBr): 3285 (NH), 1748, 1712 (2 C=O) cm^{-1}. ^1H NMR (DMSO-d_6): δ = 7.83 (m, 6H, ph-2,3,5,6H and bz-5,7H), 7.25 (m, 4H, bz-4,6H and 2NH), 2.67 (m, 2H, N-CH_2), 1.32 (m, 2H, CH_2CH_3). CI/MS: 360 ($M^+ + 1$).

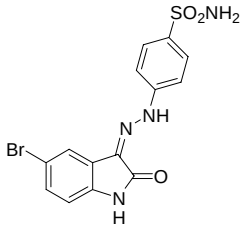
Cell Culture

The cells were grown in RPMI-1640 medium (for CL1-5, and AGS) or MEM (for HepG2) and McCoy's medium (for HT-29), each supplemented with 10% FBS, 0.1 mM NEAA, 100 units/ml penicillin-streptomycin and 2 mM glutamine at 37 °C with 5% CO_2. The cytotoxic effects of **6a-6i**, **9a-9e** and **12** against the tumor cell lines were determined by a rapid colorimetric assay using MTT.

Briefly, after 2-3 subcultures, 80 µl of the cells were seeded in 96 well micro plates and incubated for 24 hours in a humidified atmosphere of 5% CO_2-95% air at 37 °C. Then 20 µl of the stock solutions of each compound (100 µM) were added (final concentration 20 µM) and the micro plates were incubated for another 72 hours at the same conditions. To evaluate cell survival, the contents of each well was removed to another micro plate and incubated with 100 µl of MTT

a





an oxindole CDK2 inhibitor

b

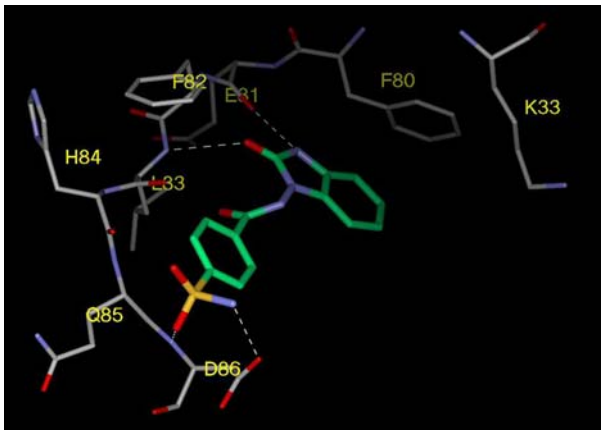


Fig. 1. (a) X-ray crystal structure of CDK2 bound with an oxindole inhibitor in the ATP binding site (PDB code: 1FVT) [18]. The broken lines illustrate hydrogen bonding interactions. (b) The most favorable binding mode of compound **6a** in the ATP binding site of CDK-2 predicted by AutoDock 3.0.

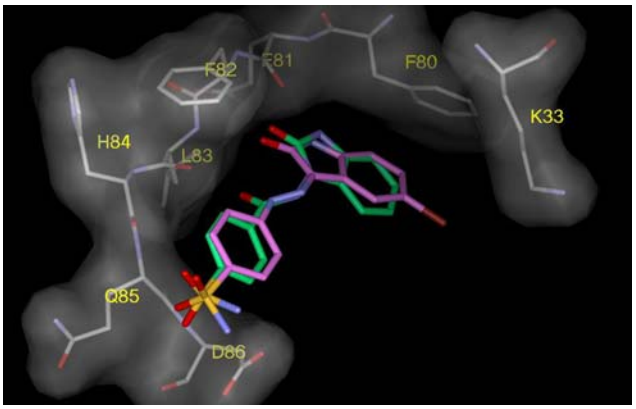


Fig. 2. Superposition of the docking model of compound **6a** (green) and the X-ray crystal structure of CDK2 in complex with the oxindole inhibitor (pink).

solution (5 mg ml^{-1} in PBS solution) for 2 hours. Afterwards, the media in each well was replaced with 100 µl DMSO and pipetted up and down to dissolve the formazan crystals and measured at 540 nm using an ELISA plate reader.

RESULTS AND DISCUSSION

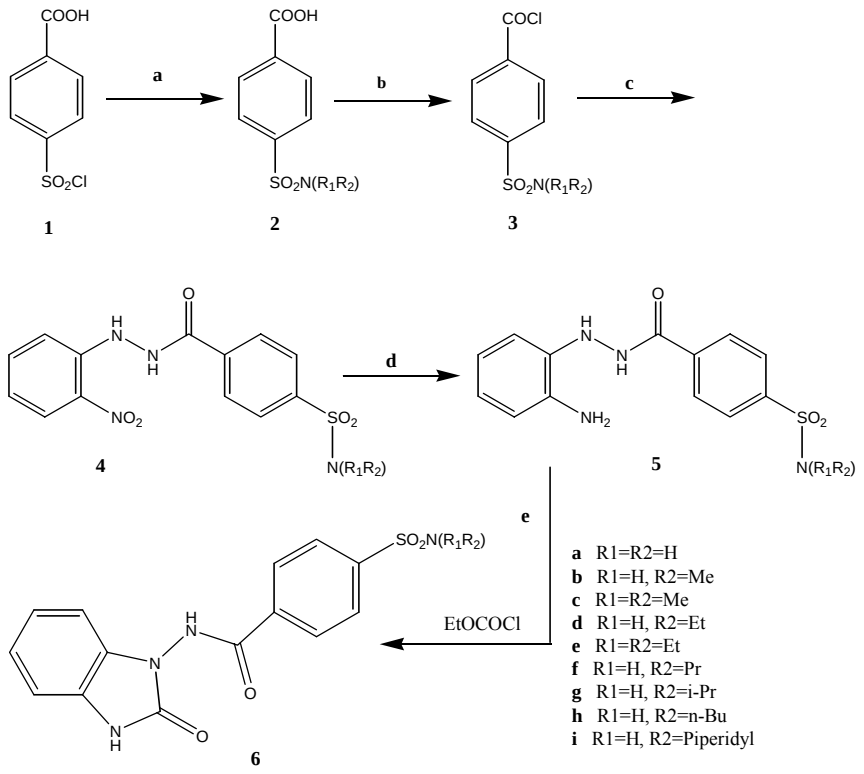
Design

To examine the possible binding interactions of **6a** with CDK2, we carried out a docking study based on the X-ray crystal structure of CDK2 in complex with an oxindole inhibitor (Fig. 1a) [18]. The 3-dimentional structure of **6a** was prepared using the Sybyl program [17] and docked into the ATP binding site of CDK2 using the AutoDock 3.0 program [20]. The most favorable binding mode of **6a** predicted by AutoDock is shown in Fig. 1b. According to the structure superposition (Fig. 2), the bound orientation of the designed molecule **6a** is similar to that of the oxindole derivative at the active site. In particular, the N-H and C=O functionalities in benzimidazolone **6a** have similar orientations to those in oxindole. They are in close proximity to provide the key bounds with Glu-81 and Leu-83, respectively. The sulfonamide group in **6a** has also spatial orientation like the sulfonamide in the oxindole derivative. The lowest energy conformer of **6a** is also similarly accommodated in the active site model of the enzyme. This may fulfill the bounding requirements of the compound with the key amino acids in this region of the active site.

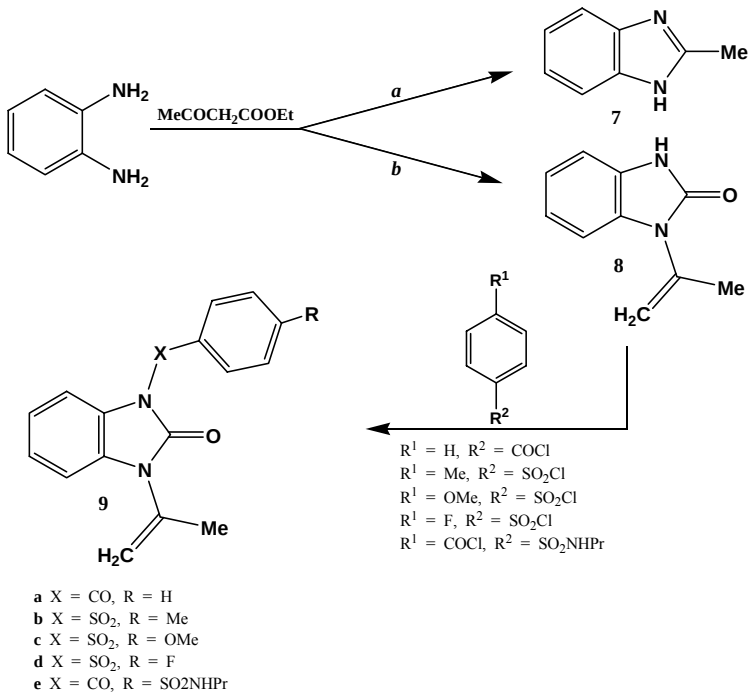
Chemistry

Preparation of 4-sulfonamide substituted benzamido-benzimidazolones is illustrated in Scheme 1. 4-Chlorosulfonylbenzoic acid (**1)** was reacted with different primary or secondary amines to give the corresponding 4-sulfamoylcarboxylic acids **2a-i** in 39-99% yields [7-9]. Acids **2a-i** were refluxed in thionyl chloride to afford the corresponding acyl chlorides **3a-3i**, which upon reaction with a solution of *o*-nitrophenylhydrazine and triethylamine in ether gave the corresponding *N*-(4-sulfamoylbenzoyl)-*o*-nitrophenylhydrazines **4a-4i** in 21-76% yields [10-12]. The nitro derivatives **4a-4i** were hydrogenated in a hydrogenation reactor in the presence of 10% palladium on charcoal to give the corresponding amino derivatives **5a-5i**. Due to their instability, we dissolved **5a-i** in dry pyridine under nitrogen atmosphere and then a solution of ethyl chloroformate was immediately added. After stirring at room temperature for 1-2 h and refluxing for 17-22 h, the corresponding 4-sulfonamide substituted benzamidobenzimidazolones **6a-6i** were obtained with 38-74% yields [13].

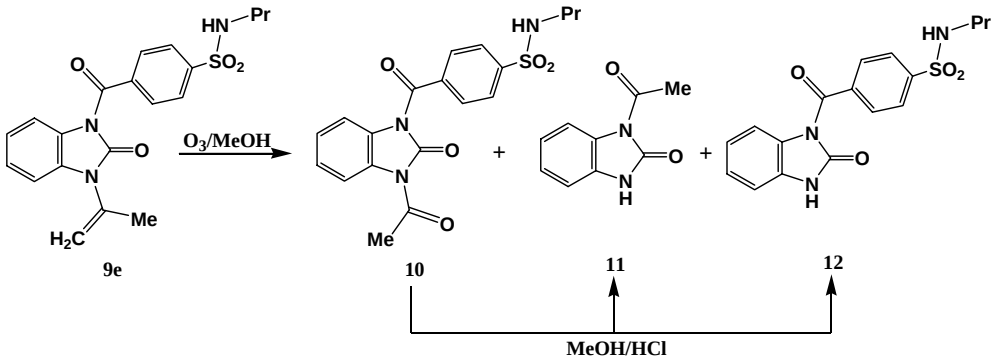
We also decided to synthesize some substituted benzimidazolones possessing a carbonyl or sulfonyl functionality for a structure-activity relationship study. The reaction of acyl or sulfonyl chlorides with benzimidazolone was found to be a tedious task, particularly with regard to selectivity. As shown in Scheme 2, the problems were overcome *via* the preparation of the monosubstituted isopropenylbenzimidazolone **8**. The reaction of *o*-phenylenediamine with ethyl acetoacetate gave benzimidazolone **8** [14-16]. Our initial attempts in preparing **8** resulted in the formation of 2-methylbenzimidazole (**7**). Nonetheless, we were able to obtain 1-isopropenyl-benzimidazolone (**8**) in 74% yield, as described in the experimental section. Then, by reacting of a variety of acyl or sulfonyl chlorides with **8,** we produced the target compounds **9a-9e**. Ozonolysis of **9e** in MeOH at -75 ºC gave a mixture of compounds **10-12**, which upon treatment with methanolic HCl produced acyl benzimidazolones **11** (50% yield) and **12** (15% yield) (Scheme 3).



Scheme 1. General reaction scheme for the preparation of 4-sulfonamide substituted benzamidobenzimidazolones. (*a*) R^1R^2-NH; (*b*) $SOCl_2$, heat; (*c*) *o*-nitrophenylhydrazine; (*d*) H_2, Pd/C (10%); (*e*) pyridine, reflux



Scheme 2. General reaction scheme for the preparation of 2-methylbenzimidazole (**7**), isopropenylbenzimidazolone (**8**), and derivatives **9a-9e**. (*a*) xylene, *p*-TSA, reflux; (*b*) xylene, N_2, $MeCOCH_2COOEt$ (dropwise), reflux; (*c*) RCOCl or RSO_2Cl, dry DMF, Cs_2CO_3 or NaH 80%



Scheme 3. Ozonolysis of **9e** and subsequent methanolysis of the resultant **10** to **11** and **12**

Biology

The cytotoxicity assay is based on the reduction of the soluble yellow MTT tetrazolium salt to a blue insoluble formazan product by mitochondrial succinic dehydrogenase [21,22]. The percentage of cell viability was calculated using the following formula:

%Survival = live cell OD [test]/ live cell OD [control] × 100.

In comparison to an oxindole inhibitor (cell growth inhibition against CL1-5 (63%), Hepa-G2 (65%), HT-29 (70%), and AGS (75%) cell lines), the results of biological screening experiments revealed that compounds **6a-i** were, generally, less active than the reference compound; yet were more cytotoxic (25-30% cell growth inhibition) than compounds **9a-9e** (< 5% cell growth inhibition) at a concentration of 20 μM. The lack of significant cytotoxicity of **6a-i** relative to the rigid oxindole inhibitor may be due to the greater flexibility of the newly synthesized molecules **6a-i**.

On the other hand, the sulfonamide substituted acyl derivative of benzimidazolone (**12)** exhibited cell growth inhibition against CL1-5 (77%), Hepa-G2 (83%), HT-29 (84%) and AGS (89%) cell lines, while the acetyl derivative of benzimidazolone (**11**) as well as the diacetyl derivative of benzimidazolone (**10**) were totally inactive. Due to the favorable molecular dipole, the acyl carbonyl group in **12** is more likely to be oriented in the opposite direction relative to the imidazolone carbonyl group. As such, the acyl benzimidazolone (**12**) is more rigid than the benzamidoimidazolones (*i.e.*, **6a-i**). This rigidity may be essential for a more efficient binding to the active site of the enzyme. Moreover, the lack of activity of the 1-alkenyl-3-acyl substituted **9a-9e**, 1,3-diacyl derivative **10** and 3-acyl derivative **11** means that the presence of a free imidazolone NH group is essential for binding with the enzyme, and the nature of the acyl side chain is important for the exhibition of anticancer activity.

CONCLUSIONS

The newly synthesized flexible benzimidazolone **6a-i**, having a similar orientation to an oxindole inhibitor on the active site of the CDK2 enzyme, did not exhibit significant cell growth inhibition *in vitro*. Nonetheless, the acyl imidazolone **12,** possessing more rigidity and having a free imidazolone NH group as well as an appropriate side chain, exhibited cytotoxicity comparable to that of the reference oxindole inhibitor.

ACKNOWLEDGMENTS

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