Abstract

Several cycline dependent kinase 2 (CDK2) inhibitors with different chemical structures have been introduced. The hinge region of CDK2 (residues 81–84) contains a set of hydrogen bond donor and acceptor sites some of which must be satisfied for potent inhibitor binding. The benzimidazolone skeleton may provide such interactions. Accordingly, 3-sulfonamide substituted benzamido-benzimidazolones 24-31 were prepared starting from benzoic acid to give the acyl chloride 1 which was reacted with different amines to afford the acids 2-9. The acids were changed to their corresponding acyl chlorides 10-17. Reaction of 10-17 with o-nitropheyl hydrazine gave the nitro derivatives 18-25 followed by reduction of the nitro groups to give 26-33 which were then reacted with ethyl chloroformate to give the target compounds 34-41. The 3-pyridyl derivative 47 was prepared starting with chlorosulfonyl benzoyl chloride to give the acid 43 which was changed to the corresponding acyl, nitro and amino derivatives 44, 45 and 46, respectively, followed by the final ring closure reaction to give 47. The dibenzimidazolinoe derivative 49 was also obtained from the reaction of isopropenyl-benzimidazolone 48 and 3-chloro sulfonyl benzoyl chloride. The target compounds were then tested against the cancer cell lines, Hepa G2, HT-29, CL1-5 and AGS. Results indicated that the target compounds did not show reasonable cell growth inhibition comparing to the positive and negative controls.

Keywords: Benzimidazolone; Sulfonamide; Cytotoxicity; CDK2; Design.

Introduction

Cyclin-dependent kinases (CDKs) are a prominent family of protein kinases, playing important role in the growth, development, proliferation, and death of eukaryotic cells. Along with their regulatory subunit cyclins, CDKs serve the function of orderly coordinating events which move cells through the cell cycle and insure the genetic integrity of daughter cells. The importance of CDK2 for cell cycle progression has led to an active pursuit of small molecule inhibitors of this enzyme as a possible treatment against cancer and other hyperproliferative disorders (1-4).

To date, several natural and synthetic CDK2 inhibitors have been introduced 1-6. Oxindole-based CDK2 inhibitors have shown selectivity for CDK2 inhibition (6-8).

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According to the structural resemblance of oxindole and benzimidazol-2-one, some 3-sulfonamide substituted benzamido-benzimidazolones were prepared and tested against different cancer cell lines.

**Experimental**

All chemicals and solvents were reagent grade and purchased from Aldrich, Acros or Fluka companies. Melting points were determined using a Mel-temp capillary apparatus, international devices, USA and are uncorrected. Infrared spectra acquired on a Jasco FT/IR-400 spectrometer. A Bruker FT-200 DPX instrument was used to acquire 1H NMR spectra using either DMSO or CDCl₃ as solvents. Hydrogenations were performed in 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) medium, Eagle’s minimal essential medium (MEM), McCoy’s culture medium, foetal bovin serum (FBS), L-glutamine, non-essential amino acids (NEAA), penicillin/streptomycin and trypsin-EDTA were purchased from Gibco-USA. MTT was from Sigma. The absorbance was measured at 540 nm with a Microtiter Plate Reader (Molecular Devices, CA, USA).

**Synthetic methods**

3-Chlorosulfonyl benzoic acid: [1] (9).

Benzoic acid (5 g, 40.9 mmole) and chlorosulfonic acid (20 mL) was reacted as described in the literature to give [1] as white solid 5.1 g, 56.5 % mp: 129-131 ºC (lit mp: 133-134 ºC).

IR (KBr): 3096, 2840, 2670, 2558, 1705, 1598, 1416, 1372, 1293, 1270, 1183, 1080, 930, 851, 821, 754, 671, 653, 588, 550, 472 cm⁻¹.

3-Sulfamoyl benzoic acid: [2] (10, 11).

Ammonia solution (25%, 9 mL) was added to 3-chlorosulfonyl benzoic acid [1] (3.3 g, 15 mmole) and the mixture was stirred at room temperature for 20 minutes followed by acidification of the resulting solution with 12 N hydrochloric acid to give a white solid (mixture of the acid and its corresponding amide). The solid was dissolved in sodium hydroxide (10%, 15 ml) and heated under reflux for 1 h. The solution was then cooled and acidified using concentrated hydrochloric acid to give [2] a white solid 1.74 g, 58 % mp: 251-252 C.

IR (KBr): 3365, 3252, 3098, 2976, 2886, 2670, 2565, 1685, 1600, 1561, 1444, 1420, 1321, 1164, 1134, 1090, 919, 753, 662, 589, 565, 469 cm⁻¹.


Methyl amine (40%, 6 mL) was added drop wise to 3-chlorosulfonyl benzoic acid [1] (1g, 4.5 mmole) and stirred at room temperature for 20 minutes. Water (5 mL) was then added and the resulting solution was acidified using concentrated HCl to leave a white precipitate which was filtered and dried (3) 700 mg, 73% mp: 217-218 ºC.

IR (KBr): 3281, 3076, 3006, 2884, 1685, 1600, 1419, 1327, 1297, 1172, 1131, 937, 864, 678, 589, 565 cm⁻¹.

3-(Dimethylaminosulfonyl)benzoic acid: [4] (9).

3-Chlorosulfonyl benzoic acid [1] (1 g, 4.54 mmole) was added in small portions to a solution of dimethyl amine (40%, 6 mL) and stirred at room temperature for 5 hours while TLC showed that the starting material finished. It was then diluted with water (6 mL) and acidified using concentrated HCl to leave a solid which
was filtered and washed with water (5 mL) and dried to give [4] a white solid 1 g, 96% mp: 142-143 °C.

IR (KBr): 3423, 2968, 2845, 2781, 2609, 2561, 1681, 1601, 1577, 1457, 1419, 1341, 1317, 1172, 1132, 1086, 952, 857, 745, 583, 512, 421 cm⁻¹.

1HNMR (DMSO-d6): 8.26 (1H, s, ph-H2), 8.20 (1H, d, J=8 Hz, ph-H6), 7.99 (1H, d, J=8 Hz, ph-H4), 7.61 (1H, t, J=8.0 Hz, ph-H5), 2.62 (6H, s, 2 CH₃) ppm.

3-(Ethylaminosulfonyl)benzoic acid: [5]
A Mixture of 3-Chlorosulfonyl benzoic acid [1] (1 g, 4.5 mmole) and ethylamine (70%, 6 mL) was reacted as explained for [4] to give [5] as snow-white crystals 830 mg, 80.5% mp: 198-199 °C.

IR (KBr): 3428, 3268, 3074, 2986, 2900, 2663, 2550, 1685, 1598, 1426, 1327, 1295, 1170, 1134, 1077, 952, 828, 754, 679, 569, 467 cm⁻¹.

1HNMR (DMSO-d6): 13.49 (1H, br s, COOH), 8.31 (1H, s, ph-H2), 8.16(1H, d, J=7.9 Hz, ph-H6), 8.01 (1H, d, J=8.0 Hz, ph-H4), 7.75 (2H, m, ph-H5 and NH), 2.76 (2H, q, J=7.1 Hz, CH2), 0.95 (3H, t, J=7.2 Hz, CH3) ppm.

3-(N-Diethylaminosulfonyl) benzoic acid: (6)
A solution of diethyl amine (5 mL) and 3-chlorosulfonyl benzoic acid [1] (1 g, 4.5 mmole) was reacted as explained for (4) to give (6) as a pale cream solid 800 mg, 69.2% mp: 153-154 °C.

IR (KBr): 3072, 2976, 2938, 2874, 2821, 2679, 2561, 1684, 1601, 1446, 1339, 1299, 1202, 1170, 1017, 934, 799, 736, 691, 655, 579, 470 cm⁻¹.

1HNMR (DMSO-d6): 8.33 (1H, s, ph-H2), 8.15 (1H, d, J=7.9 Hz, ph-H6), 8.02 (1H, d, J=8.0 Hz, ph-H4), 7.74 (2H, m, ph-H5 and NH), 3.22 (1H m, CH), 0.92 (6H, d, J=6.5 Hz, 2 CH3) ppm.

3-(Propylaminosulfonyl)benzoic acid: (7)
3-Chlorosulfonyl benzoic acid [1] (1 g, 4.5 mmole) and n-propylamine (6 mL) was reacted as described for (4) to give (7) as white crystals 950 mg, 87% mp: 186-187 °C.

IR (KBr): 3269, 3076, 2965, 2877, 1658, 1600, 1432, 1329, 1297, 1170, 1133, 1089, 754, 678, 567, 447 cm⁻¹.

1HNMR (DMSO-d6): 8.31 (1H, s, COOH), 8.31 (1H, s, ph-H2), 8.16 (1H, d, J=7.1Hz, ph-H4), 8.01 (1H, d, J=7.1 Hz, ph-H6), 7.72 (2H, m, ph-H5 and NH), 2.70 (2H, q, J=6.2 Hz, NH-CH2), 1.35 (2H, q, J=7 Hz, CH2-CH3), 0.77 (3H, t, J=7.2 Hz, CH3) ppm.

3-(Isopropylaminosulfonyl)benzoic acid: (8)
3-Chlorosulfonyl benzoic acid (1) (1 g, 4.5 mmole) and isopropylamine (6 mL) was reacted as explained for (4) to give (8) as white crystals 900 mg, 82.3% mp: 251-252 °C.

IR (KBr): 3270, 3076, 2966, 2877, 2665, 2552, 1685, 1600, 1437, 1407, 1330, 1298, 1170, 1134, 1076, 928, 755, 678, 567, 475, 436 cm⁻¹.

1HNMR (DMSO-d6): 8.33 (1H, s, ph-H2), 8.15 (1H, d, J=7.9 Hz, ph-H6), 8.02 (1H, d, J=8.0 Hz, ph-H4), 7.74 (2H, m, ph-H5 and NH), 3.22 (1H m, CH), 0.92 (6H, d, J=6.5 Hz, 2 CH3) ppm.

3-(n-Butylaminosulfonyl)benzoic acid: (9)
A solution of 3-chlorosulfonyl benzoic acid (1) (1 g, 4.5 mmole) and n-butylamine (6 mL) was reacted as described for (4) to give (9) as white crystals 1 g, 86.5% mp: 193-194 °C.

IR (KBr): 3270, 3076, 2961, 2934, 2873, 2664, 2549, 1685, 1599, 1433, 1407, 1329, 1298, 1170, 1134, 1077, 933, 754, 678, 568, 475, 421 cm⁻¹.

1HNMR (DMSO-d6): 13.50 (1H, br s, COOH), 8.30 (1H, s, ph-H2), 8.15 (1H, d, J=7.9 Hz, ph-H6), 8.00 (1H, d, J=8Hz, ph-H4), 7.70 (2H, m, ph-H5 and NH), 2.72 (2H, q, J=6.2 Hz, NH-CH2), 1.22 (4H, m, CH2-CH2), 0.75 (3H, t, J= 6 Hz, CH3) ppm.

3-Sulfamoyl benzoyl-o-nitrophenylhydrazine: (18)
To an ice cooled suspension of 3-sulfamoyl benzoic acid [2] (0.5 g, 2.49 mmole) in dry THF (10 mL) was added triethyl amine (251 mg, 2.49 mmole) followed by the drop wise addition of pivaloyl chloride (300 mg, 2.49 mmole) in dry THF (5mL) to give a suspension which was kept at room temperature for 2 hours. A solution of
o-nitro phenyl hydrazine (320 mg, 2.1 mmole) in dry THF (5mL) was then added drop wise to the previous suspension and stirred overnight at room temperature. The solvent was evaporated under reduced pressure to leave an oily residue which was fractionated on a column of silica gel using hexane: ethyl acetate 1/1 (to elute two first spots), 1/2 (to remove the third impurity) and finally with ethyl acetate to give (18) as a yellowish-brown solid 220 mg, 31.2% mp: 203-204 °C.

IR (KBr): 3365, 3319, 3258, 3187, 2962, 2934, 1686, 1600, 1573, 1496, 1417, 1324, 1274, 1160, 1134, 901, 742, 675, 601, 490 cm⁻¹.

1HNMR (DMSO-d₆): 11.03 (1H, s, NH), 9.49 (1H, s, NH), 8.38 (1H, s, arom`-H2), 8.18 (3H, m, arom-H3 and arom`-H3,6), 7.65 (4H, m, arom-H5, arom`-H5 and NH₂), 7.17 (1H, d, J=8 Hz, arom-H6), 6.89 (1H, t, J=7.2 Hz, arom-H4) ppm.

N-(3-Methyl amino sulfonylbenzoyl) o-nitrophenyl hydrazine:

14. Thionyl chloride (3 mL) was added to a suspension of 3-(N-methyl sulfamoyl) benzoic acid (3) (600 mg, 2.8 mmole) in chloroform (10 mL) and the mixture was refluxed for 1h. The solvent was then evaporated under vacuum to leave (11) as pale yellow semisolid which was dissolved in chloroform (10 mL) and added immediately to a solution of o-nitrophenylhydrazine (360 mg, 2.34 mmole) and triethyl amine (288 mg, 2.8 mmole) in chloroform (10 mL) at room temperature to leave a suspension. The solvent was evaporated to leave sticky dark red oil which was added to a gradient column of silica gel and eluted with hexane: ethyl acetate 1/1 until all yellow and orange bonds eluted. It was then eluted using hexane: ethyl acetate 1/2 and ethyl acetate to give (19) as red brown oil which was solidified (brown solid) 290 mg, 29.6% mp: 80-81 °C.

IR (KBr): 3297, 3035, 2923, 1671, 1614, 1575, 1519, 1416, 1325, 1271, 1231, 1159, 918, 840, 780, 741, 683, 583 cm⁻¹.

1HNMR (DMSO-d₆): 11.06 (1H, s, NH), 9.49 (1H, s, NH), 8.32 (1H, s, arom`-H2), 8.22 (1H, d, J=7.8 Hz, arom`-H4), 8.13 (1H, d, J=8.4 Hz, arom`-H6), 7.78 (1H, t, J=7.6 Hz, arom-H5), 7.62 (2H, m, arom`-H5 and NH), 7.19 (1H, d, J=8.4 Hz, arom-H6), 6.89 (1H, t, J=7.6 Hz, arom-H4), 2.44 (3H, d, J=5 Hz, CH₃) ppm.

3-(Dimethyl amino sulfonylbenzoyl) o-nitrophenyl hydrazine:

A suspension of 3-(dimethylaminosulfonyl) benzoic acid (4) (460 mg, 2 mmole) in thionyl chloride (6 mL) was heated under reflux for 45 minutes and the solvent evaporated under vacuum to leave (12) as white residue solid. The residue was dispersed in ether (10 mL) and added drop wise to an ice cooled solution of o-nitrophenylhydrazine (306 mg, 2 mmole) and triethyl amine (210 mg, 2 mmole) in ether (30 mL). It was then stirred at room temperature overnight. The solvent was evaporated under reduced pressure and the residue was purified on gradient silica gel column using, hexane: ethyl acetate 1/1 until all yellow bond reached to bottom) and 1/1 as eluents. The solvent was evaporated to give (20) as sticky pale-yellow solid 330 mg, 49.4 % mp: 96-97 °C.

IR (KBr): 3352, 3175, 2970, 1673, 1614, 1576, 1519, 1343, 1274, 1233, 1159, 955, 862, 782, 743, 708, 582, 417 cm⁻¹.

1HNMR (DMSO-d₆): 11.10 (1H, s, NH), 9.49 (1H, s, NH), 8.27 (2H, m, arom`-H2 and arom-H3), 8.12 (1H, d, J=8.1 Hz, arom`-H2), 7.98 (1H, t, J=7.9 Hz, arom-H5), 7.58 (1H, t, J=7.2 Hz, arom`-H5), 7.20 (1H, d, J=8.4 Hz, arom-H6), 6.89 (1H, t, J=7.4 Hz, arom-H4), 2.65 (6H, s, 2 CH₃) ppm.

N-(3-Ethyl amino sulfonyl-benzoyl)-o-nitrophenyl hydrazine:

3-Ethylaminosulfonylbenzoic acid (5) (350 mg, 1.53 mmole), thionyl chloride, o-nitrophenylhydrazine (215 mg, 1.4 mmole) and triethyl amine (142 mg, 1.4 mmole) were reacted as described for 20 and the residue was fractionated on an air pressured gradient silica gel column using hexane: ethyl acetate 1/1 (to remove all yellow bonds) and 1/1 to separate the product (21) as an orange solid 340 mg, 66.7 % mp: 155-156 °C.

IR (KBr): 3329, 3087, 2980, 2938, 2875, 1673, 1614, 1576, 1519, 1416, 1326, 1274,
1233, 1158, 950, 863, 742, 684, 585, 461 cm\(^{-1}\).

\(^4\)HNMR (DMSO-d\(_6\)):\ 11.05 (1H, s, NH), 9.48 (1H, s, NH), 8.32 (1H, s, arom-H2), 8.21 (1H, d, \(J=7.7\) Hz, arom-H4), 8.12 (1H, d, \(J=8.4\) Hz, arom-H6), 8.02 (1H, d, \(J=8.8\) Hz, arom-H4), 7.78 (1H, t, \(J=7.8\) Hz, arom-H5), 7.59 (1H, t, \(J=7.9\) Hz, arom-H5), 7.18 (1H, d, \(J=8.4\) Hz, arom-H6), 6.89 (1H, t, \(J=7.9\) Hz, arom-H5), 3.20 (4H, q, \(J=7.1\) Hz, 2 CH2), 1.06 (6H, t, \(J=7.1\) Hz, 2 CH3) ppm.

**N-(3-Diethyl amino sulfonyl-benzoyl)-o-nitro phenyl hydrazine:** (22)

3-Diethylyaminosulfonylbenzoic acid (6) (300 mg, 1.17 mmole) thionyl chloride, o-nitrophenylhydrazine (162 mg, 1.06 mmole) and triethylamine (120 mg, 1.1 mmole) were reacted as described for 20 and the residue was fractionated on an air pressured gradient silica gel column using hexane: ethyl acetate 3/1 (to remove all yellow bonds), 2/1 and 3/2 to separate the product (22) as orange sticky oil which was gradually solidified 200 mg, 48.2%, mp: 84-85 °C.

IR (KBr): 3352, 3079, 2976, 2935, 2874, 1671, 1614, 1519, 1491, 1328, 1274, 1201, 1152, 1016, 937, 742, 695, 579, 460, 431 cm\(^{-1}\).

\(^4\)HNMR (DMSO-d\(_6\)):\ 11.08 (1H, s, NH), 9.47 (1H, s, NH), 8.31 (1H, s, arom-H2), 8.18 (1H, d, \(J=8.1\) Hz, arom-H3), 8.13 (1H, d, \(J=8.4\) Hz, arom-H4), 8.00 (1H, d, \(J=8.4\) Hz, arom-H6), 7.77 (2H, m, arom-H5 and arom-H5), 7.59 (1H, t, \(J=7.8\) Hz, NH), 7.17 (1H, d, \(J=8.5\) Hz, arom-H6), 6.90 (1H, t, \(J=7.6\) Hz, arom-H4), 2.73 (2H, q, \(J=6.6\) Hz, CH2-CH3), 1.37 (2H, q, \(J=7\) Hz, CH2-CH3), 0.78 (3H, t, \(J=7.2\) Hz, CH3) ppm.

**N-(3-Propyl amino sulfonyl-benzoyl)-o-nitro phenyl hydrazine:** (23)

3-Propylaminosulfonylbenzoic acid (7) (450 mg, 1.85 mmole), thionyl chloride, o-nitrophenylhydrazine (260 mg, 1.7 mmole) and triethyl amine (172 mg, 1.7 mmole) were reacted as described for 20 and the residue was fractionated on a gradient silica gel column (under air pressure) using hexane: ethyl acetate 3/1 (until almost two first yellow bonds eluted), 2/1 and 1/1 as eluents to leave an orange oil which was dissolved in chloroform and evaporated again to give (23) as orange foam-solid 420 mg, 65.4% mp: 155-156 °C.

IR (KBr): 3371, 3290, 3081, 2976, 2934, 2873, 1661, 1614, 1496, 1419, 1325, 1273, 1235, 1158, 1013, 739, 684, 475 cm\(^{-1}\).

\(^4\)HNMR (DMSO-d\(_6\)):\ 11.04 (1H, s, NH), 9.08 (1H, s, NH), 8.34 (1H, s, arom-H2), 8.18 (2H, m, arom-H3 and arom-H5), 8.02 (1H, d, \(J=8.4\) Hz, arom-H6), 7.50 (1H, t, \(J=7.8\) Hz, arom-H5), 7.58 (1H, t, \(J=7.6\) Hz, arom-H5), 7.18 (1H, d, \(J=8.4\) Hz, arom-H6), 6.88 (1H, t, \(J=7.9\) Hz, arom-H5), 3.30 (1H, m, CH), 0.94 (6H, d, \(J=6.5\) Hz, 2 CH3) ppm.

**N-(3-Isopropyl amino sulfonyl-benzoyl)-o-nitro phenyl hydrazine:** (24)

3-Isopropylamino sulfonylbenzoic acid (8) (450 mg, 1.85 mmole), thionyl chloride, o-nitrophenylhydrazine (260 mg, 1.7 mmole) and triethyl amine (172 mg, 1.7 mmole) were reacted as described for 20 and the residue was fractionated on a gradient silica gel column (under air pressure) using hexane: ethyl acetate 3/1 (until almost two first yellow bonds eluted), 2/1 and 1/1 as eluents to leave an orange oil which was dissolved in chloroform and evaporated again to give (24) as orange foam-solid 420 mg, 65.4% mp: 155-156 °C.

IR (KBr): 3371, 3290, 3081, 2976, 2934, 2873, 1661, 1614, 1496, 1419, 1325, 1273, 1235, 1158, 1013, 739, 684, 475 cm\(^{-1}\).

\(^4\)HNMR (DMSO-d\(_6\)):\ 11.04 (1H, s, NH), 9.48 (1H, s, NH), 8.34 (1H, s, arom-H2), 8.18 (2H, m, arom-H3 and arom-H5), 8.02 (1H, d, \(J=8.4\) Hz, arom-H6), 7.50 (2H, m, arom-H5 and NH), 7.58 (1H, t, \(J=7.2\) Hz, arom-H5), 7.18 (1H, d, \(J=7.7\) Hz, arom-H6), 6.88 (1H, t, \(J=7.9\) Hz, arom-H4), 3.30 (1H, m, CH), 0.94 (6H, d, \(J=6.5\) Hz, 2 CH3) ppm.

**N-(3-Butyl amino sulfonyl-benzoyl)-o-nitro phenyl hydrazine:** (25)

3-Butylaminosulfonylbenzoic acid (9) (400 mg, 1.56 mmole), thionyl chloride, o-nitrophenyl hydrazine (218 mg, 1.42 mmole) and triethyl amine (144 mg, 1.42 mmole) were reacted as described for 20 and the residue was fractionated on a gradient silica gel column (under air pressure) using hexane: ethyl acetate 3/1 (until almost two first yellow bonds eluted), 2/1 and 1/1 as eluents to leave an orange oil which was
dissolve in chloroform and evaporated again to give (25) as orange sticky solid 420 mg, 75.5% mp: 78-79 °C.

IR (KBr): 3284, 2959, 2871, 1672, 1614, 1576, 1519, 1490, 1415, 1326, 1233, 1158, 1083, 920, 863, 740, 682, 593, 560, 481 cm⁻¹.

1HNMR (DMSO-d6): 11.04 (1H, s, NH), 9.47 (1H, s, NH), 8.32 (1H, s, arom`-H2), 8.19 (1H, d, J=8 Hz, arom-H3), 8.12 (1H, d, J=8.0 Hz, arom`-H4), 8.00 (1H, d, J=7.8 Hz, arom`-H6), 7.58 (1H, t, J=7.4 Hz, NH), 7.17 (1H, d, J=8.4 Hz, arom-H6), 6.89 (1H, t, J=7.8 Hz, arom-H4), 2.75 (2H, q, J=6.3 Hz, NH-CH2), 1.26 (4H, m, CH2CH2), 0.78 (3H, t, J=7.0 Hz, CH3) ppm.

3-Sulfamoyl benzoyl o-aminophenyl hydrazine: (26) and other amino derivatives 27-33

A mixture of 3-sulfamoylbenzoyl-o-nitrophenylhydrazine (18) (200 mg, 0.6 mmole) and palladium/charcoal 10% (50 mg) in methanol (120 mL) was sonicated for 10 min and hydrogenated at 4 bars for 2.5 hours. The mixture was then filtered through celite and evaporated under reduced pressure to give (26) as semisolid which was directly used for the next step without further purification.

Compounds 27-33 were prepared from the hydrogenation of 0.66, 0.25, 0.82, 0.48, 1.1, 0.93 and 0.97 mmoles of 19-25, respectively, as described above for 26 and used immediately for the next steps without further purification. In case of the 3-ethyl derivative (29) 6 bars pressure for 10 hours was used to complete the reduction.

1-(3-Aminosulfonyl-benzamido)benzimidazolone: (34)

Ethyl chloroformate (86 mg, 0.79 mmole) and crude (27) were reacted as described for (34) to give a semisolid residue. It was then purified on a silica gel column eluted with chloroform: methanol (9/1) as eluent to give (35) as white solid 90 mg, 39.4% (from 19) mp: 203-204 °C.

IR (KBr): 3268, 3072, 3033, 2819, 1726, 1682, 1600, 1576, 1509, 1485, 1308, 1272, 1161, 1095, 921, 814, 746, 685, 585, 486 cm⁻¹.

1HNMR (DMSO-d6): 11.66 (1H, s, NH), 11.16 (1H, s, NH), 8.36 (1H, s, arom`-H2), 8.25 (1H, d, J=7.5 Hz, arom`-H6), 8.04 (1H, d, J=7.8 Hz, arom`-H6), 7.84 (1H, t, J=7.8 Hz, arom`-H5), 7.67 (1H, q, J=5Hz, NH), 7.05 (4H, m, arom-H), 2.45 (3H, d, J=5Hz, CH3) ppm.

1-(3-Dimethylaminosulfonyl-benzamido) benzimidazolone: (36)

Ethyl chloroformate (32 mg, 0.3 mmole) and crude (28) were reacted as described for (34) to leave a brownish oil which was fractionated on a gradient silica gel column using chloroform: methanol 15/1 and 9/1 as eluents to give (36) as white crystals 70 mg, 77.8 % (from 20) mp: 159-160 °C.

IR (KBr): 3254, 3072, 2965, 2884, 1726, 1682, 1602, 1538, 1519, 1479, 1340, 1269, 1164, 955, 817, 750, 705, 686, 582, 414 cm⁻¹.

1HNMR (DMSO-d6): 11.70 (1H, s, NH), 11.17 (1H, s, NH), 8.31 (2H, m, arom`-H2,4), 8.04 (1H, d, J=7.7 Hz, arom`-H6), 7.87 (1H, t, J=7.6 Hz, arom`-H5), 7.04 (4H, m, arom-H), 2.67 (6H, s, 2 CH3) ppm.
1-(3-Ethylaminosulfonyl-benzamido)benzimidazolone: (37)

Ethyl chloroformate (106 mg, 0.99 mmole) and crude (29) were reacted as described for (34) to leave dark orange oil which was fractionated on a gradient silica gel column using chloroform: methanol 15/1 (to elute two first yellow bonds) and 9/1 as eluents to give (37) as white solid 170 mg, 57.6% (from 21) mp: 145-146 °C.

IR (KBr): 3263, 2981, 2879, 1723, 1681, 1599, 1524, 1485, 1427, 1307, 1272, 1160, 1098, 746, 685, 586, 510, 458, 428 cm⁻¹.

¹HNMR (DMSO-d6): 11.65 (1H, s, NH), 11.16 (1H, s, NH), 8.39 (1H, s, arom’-H2), 8.24 (1H, d, J=7.9 Hz, arom’-H4), 8.06 (1H, d, J=8 Hz, arom’-H6), 7.79 (2H, m, arom’-H5 and NH), 7.03 (2H, m, arom-H), 2.83 (2H, q, J=7.1 Hz, CH₂), 0.97 (3H, t, J=7.2 Hz, CH₃) ppm.

1-(3-Diethylaminosulfonyl-benzamido) benzimidazolone: (38)

Ethylchloroformate (63 mg, 0.58 mmole) and crude (30) were reacted as described for (34) to leave an orange oil which was purified on a gradient column using hexane: ethyl acetate 1/2, 1/3 and 1/4 as eluents to give (38) as white solid 140 mg, 75.2% (from 22) mp: 247-248 °C.

IR (KBr): 3263, 3157, 3067, 3028, 2977, 1723, 1681, 1599, 1524, 1481, 1425, 1307, 1277, 1270, 1161, 1115, 1012, 748, 685, 590, 512, 431 cm⁻¹.

¹HNMR (DMSO-d6): 11.69 (1H, s, NH), 11.17 (1H, s, NH), 8.38 (1H, s, arom’-H2), 8.23 (1H, d, J=7 Hz, arom’-H4), 8.07 (1H, d, J=7 Hz, arom’-H6), 7.80 (2H, m, arom’-H5 and NH), 7.15-6.95 (4H, m, arom-H), 3.38 (1H, m, CH), 0.97-0.94 (6 H, d, J=6 Hz, 2 CH₃) ppm.

1-(3-Propylaminosulfonyl-benzamido) benzimidazolone: (39)

Ethylchloroformate (144 mg, 1.33 mmole) and crude (31) were reacted as described for (34) to leave orange oil which was fractionated on silica gel column using chloroform: methanol 15/1 and 10/1 as eluents to give (39) as white crystals 250 mg, 60.8% (from 23) mp: 127-129 °C.

IR (KBr): 3264, 2967, 1877, 1723, 1684, 1600, 1485, 1307, 1160, 922, 745, 685, 587, 467 cm⁻¹.

¹HNMR (DMSO-d6): 11.63 (1H, s, NH), 11.13 (1H, s, NH), 8.36 (1H, s, arom’-H2), 8.21 (1H, d, J=7.6Hz, arom’-H4), 8.03 (1H, d, J=7.8 Hz, arom’-H6), 7.79 (2H, m, arom’-H5 and NH), 7.03 (4H, m, arom-H), 2.72 (2H, q, J=6.4 Hz, NH-CH₂), 1.36 (2H, q, J=7 Hz, CH₂CH₃), 0.77 (3H, t, J=6.8 Hz, CH₃) ppm.

1-(3-Butylaminosulfonyl-benzamido) benzimidazolone: (41)

Ethyl chloroformate (126 mg, 1.17 mmole) and crude (33) were reacted as described for (34) to leave an orange oil which was fractionated on a silica gel column using chloroform: methanol 15/1 as eluent to give (41) as pale-ream crystals 220 mg, 58.4% (from 25) mp: 127-129 °C.

IR (KBr): 3271, 2959, 2872, 1724, 1683, 1600, 1523, 1486, 1427, 1304, 1271, 1159, 1085, 920, 816, 744, 685, 587, 511, 449, 420 cm⁻¹.

¹HNMR (DMSO-d6): 11.65 (1H, s, NH), 11.16 (1H, s, NH), 8.40 (1H, s, arom’-H2), 8.23 (1H, d, J=7 Hz, arom’-H4), 8.07 (1H, d, J=7 Hz, arom’-H6), 7.80 (2H, m, arom’-H5 and NH), 7.15-6.95 (4H, m, arom-H), 3.38 (1H, m, CH), 0.97-0.94 (6 H, d, J=6 Hz, 2 CH₃) ppm.

3-(2-Pyridylaminosulfonyl)benzoic acid: (43)

Chlorosulfonylbenzoyl chloride (1 g,
4.2 mmole) was added to a solution of 2-aminopyridine (790 mg, 8.4 mmole) in dry pyridine (8 mL) and stirred at room temperature for 0.5 h. The solvent was evaporated to small volume, water added (15 mL) and the mixture stirred for 10 minutes to leave a solid which was filtered (may have some corresponding pyridyl amide 42). The solid was dispersed in sodium hydroxide solution (10%, 10 mL) and heated under reflux for 1 h. The resulting solution was then cooled and acidified using concentrated HCl to give (43) as white solid which was filtered and dried 710 mg, 60.8% mp: 232-233 °C.

IR (KBr): 3123, 3054, 2889, 2820, 2757, 2561, 1696, 1600, 1393, 1359, 1311, 1268, 1168, 999, 945, 780, 761, 681, 594, 513, 421 cm⁻¹.

1HNMR (DMSO-d₆): 8.37 (1H, s, ph-H₂), 8.08 (2H, m, ph-H₄,₆), 7.96 (1H, d, J=5 Hz, pyr-H₃), 7.72 (3H, m, ph-H₅, pyr-H₅ and NH), 7.18 (1H, d, J=8.6 Hz, pyr-H₆), 6.84 (1H, t, J=6.4 Hz, pyr-H₄) ppm.

N-[3-(2-Pyridylaminosulfonyl)-benzoyl]-o-nitrophenylhydrazine: (45)

A suspension of 3-(2-pyridylaminosulfonyl)benzoic acid (43) (320 mg, 1.15 mmole) in thionyl chloride (6 mL) was heated under reflux for 3.5 hours. The solvent was evaporated to give (44) as a solid residue which was dispersed in ether/dichloromethane (1/1, 40 mL) and added drop wise to a solution of o-nitrophenylhydrazine (176 mg, 1.15mmole) and triethyl amine (116 mg, 1.15 mmole) in ether (20 mL) and stirred at room temperature overnight to give a suspension. The solvent was evaporated under reduced pressure and the residue was fractionated on silica gel column using chloroform (until elution of the first orange bond) and chloroform: methanol 15/1 as eluents to give (45) as orange crystal 260 mg, 54.7% mp: 220-221 °C.

IR (KBr): 3338, 3126, 3056, 2933, 1683, 1613, 1519, 1388, 1356, 1271, 1232, 1135, 1005, 961, 777, 741, 684, 592, 435 cm⁻¹.

1HNMR (DMSO-d₆): 11.03 (1H, br s, NH), 9.46 (1H, s, NH), 8.39 (1H, s, arom' -H₂), 8.09 (3H, m, arom-H₃ and arom’-H₄,₆), 7.96 (1H, d, J=5.2 Hz, pyr-H₃), 7.72 (2H, m, arom-H₅ and arom’-H₅), 7.58 (1H, t, J=7.7 Hz, pyr-H₅), 7.19- (2H, m, pyr-H₆ and arom-H₆), 6.89 (2H, m, pyr-H₄ and arom-H₄) ppm.

N-[3-(2-Piridylaminosulfonyl)-benzoyl]-o-aminophenylhydrazine: (46)

A mixture of N-[3-(2-piridylaminosulfonyl)-benzoyl]-o-nitro phenyl hydrazine (45) (200 mg, 0.48 m mole) and palladium/charcoal (10%, 80 mg) in methanol (130 mL) was sonicated for 5 minutes and hydrogenated at 5 bars for 1:15 h. The mixture was then filtered through celite and the solvent evaporated under reduced pressure to leave (46) as brown oil which was used immediately for the next step without further purification.

1-[3-(2-piridylaminosulfonyl)-benzamido] benzimidazolone: (47)

Ethyl chloroformate (63 mg, 0.58 mmole) and crude (46) were reacted as described for (34) to leave a brownish oil which was fractionated on silica gel column using chloroform: methanol 15/1 and 9/1 as eluents to give (47) as white crystals 100 mg, 51 % (from 45) mp: 318-320 °C.

IR (KBr): 3262, 3057, 2937, 2830, 1729, 1698, 1631, 1557, 1388, 1267, 1139, 1080, 960, 775, 745, 682, 591, 459, 437 cm⁻¹.

1HNMR (DMSO-d₆): 11.62 (1H, s, NH), 11.14 (1H, s, NH), 8.15 (2H, m, arom-H₄,₆), 7.95 (1H, d, J=5.1 Hz, pyr-H₃), 7.75 (2H, m, pyr-H₅ and arom’-H₅), 7.23 (1H, d, J=8.7 Hz, pyr-H₆), 7.03 (4H, m, arom-H), 6.85 (1H, t, J=6.3, pyr-H₄) ppm.

1-Isopropenyl benzimidazolone: (48)

Ethyl acetooacetate (13.8 g, 106 mmole) in xylene (10 mL) was added drop wise in a course of 2.5 h, to a refluxing solution of o-phenylenediamine (10.2 g, 94 mmole) in xylene (50 mL) under nitrogen, while the water which was produced was removed using a Dean-Stark water separator. It was refluxed for further 3h and cooled to deposit a solid which was filtered and dried to give (45) as white solid 8g, mp:122-124 °C. An aqueous solution of sodium hydroxide (16g/ 80 mL) was then added to the mother liquor to precipitate the product as its sodium salt which was filtered. Acetic acid and water were then added to the solid to give a clear
solution at pH~6 which was solidified again by the addition of more acetic acid. The solid was filtered and washed with acetic acid and dried to give the second crop of (48) as pale brown solid 4.1 g (totally 12.1 g, 74%).

IR (KBr): 3369, 3140, 3067, 2981, 2843, 2760, 1694, 1610, 1479, 1425, 1386, 1255, 1189, 1119, 728, 678, 574, 492, 422 cm⁻¹.

¹H NMR (CDCl₃): 10.83 (1H, br s, NH), 7.11 (4H, m, arom-H), 5.41 (1H, s, trans-CH), 5.24 (1H, s, cis-CH), 2.3 (3H, s, CH₃) ppm.

1-[(1-Isopropenyl)benzimidazolin-2-one-3-carboxy]-3-[(1-isopropenyl)benzimidazolin-2-one-3-sulfonyl]benzene: (49)

To a hexane washed suspension of sodium hydride (60 mg, from 80%, 2 mmole) in dry DMF (10 mL) was added isopropenyl benzimidazolone (48) (350 mg, 2 mmole) and stirred for 30 minutes followed by the drop wise addition of 3-chlorosulfonylbenzoyl chloride (240 mg, 1 mmole) in DMF (5 mL). A catalytic amount of sodium iodide was then added and the mixture was stirred at room temperature for 20 hours. The solid precipitate which was produced by the addition of water (10 mL) was separated and dried to give (49) as white solid 340 mg, 33.1% mp: 192-193 ºC.

IR (KBr): 3079, 1737, 1690, 1660, 1585, 1526, 1482, 1386, 1352, 1264, 1174, 1077, 902, 756, 670, 601, 550, 420 cm⁻¹.

¹H NMR (CDCl₃): 8.47 (1H, s, ph-H2), 8.38 (1H, d, J=8.0 Hz, ph-H4), 7.98 (3H, m, ph-H6 and 2 bzmizd-H4), 7.64 (1H, t, J=7.8 Hz, ph-H5), 7.15 (6H, m, bzmizd-H), 5.37 and 5.35 (2H, 2 s, 2 trans-CH), 5.20 and 5.17 (2H, 2 s, 2 cis-CH), 2.10 and 2.07 (6H, 2 s, 2 CH₃) ppm.

In-vitro cytotoxicity assay

The cells were grown in RPMI-1640 (for CL1-S, and AGS), MEM (for HepG2) and McCoy’s mediums (for HT-29) supplemented with 10% heat-inactivated foetal bovine serum (50 mL), 0.1 mM non-essential amino acids (5 mL), 100 units/mL penicillin-streptomycin (5 mL) and 2 mM glutamine (5 mL) to provide a total volume of 500 mL. The completed mediums (pH=7.4) were sterilized by filtering through 0.22 μ Sterioprop microbiological filters and kept at 4°C before use. The cytotoxic effects of 34-41, 47 and 49 against the tumor cell lines were determined by a rapid colorimetric cell lines using MTT. The results were compared with the negative control (containing no drug). Colchicin was used as a positive control. In this assay, mitochondrial enzyme activity of viable cells would metabolically reduce the yellow color soluble MTT into an insoluble blue colored formazan product which in turn can be dissolved in DMSO and measured spectrophotometrically using an ELISA plate reader at 540 nm.

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% CO2 - 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 further incubated for 72 hours at the same conditions. To evaluate cell survival, the content of each well was sucked out and incubated with 100 µL of MTT solution (5 mg/ml 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 formazan crystals and measured at 540 nm using an ELISA plate reader. All experiments were performed in triplicate and in three different days. The percentage of cell viability was calculated using the following formula and shown in scheme 1:

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

Results and discussion

Design

The 3D structures of the oxindole and the benzimidazolone 34 was modeled using the Nemesis program. The compounds were then subjected to energy minimization and the lowest energy conformers of each compound were used for superimposition as shown in figure 1. The benzimidazolone system is superimposed almost completely on the oxindole ring system while the other parts of the molecules extended along each other. In another attempt the benzimidazolone 34 was docked into the crystal structure of the CDK2 enzyme using AUTODOCK program. The lowest energy and the sixth-ranked energy
orientations of 34 are shown in figure 2. The results indicated that although the lowest energy orientation of this compound is accommodated in different way comparing to the oxindole derivative the sixth-ranked energy orientation of 34 may extended in the active site more similar to the oxindole derivative 8.

**Chemistry**

The general reaction scheme for the preparation of 3-sulfonamide substituted benzamido-benzimidazolones is shown in figure 3.

Benzoic acid was heated in chlorosulfonic acid to give 3-chlorosulfonyl benzoic acid (1) in 57% yield. The sulfonyl chloride (1) was then reacted with different primary and secondary amines followed by dilution with water and acidification with concentrated hydrochloric acid to give the corresponding 3-sulfamoylbenzoic acids 2-9 in 58-96% yields. The acids 2-9 were then refluxed in thionyl chloride for 15-60 minutes to provide the acyl chlorides 10-17 which were reacted immediately with a solution of o-nitro phenylhydrazine in the presence of triethylamine in ether to give the corresponding N-(3-sulfamoylbenzoyl)-o-nitrophenyl hydrazines 18-25 in 30-76% yields. These nitro derivatives were then hydrogenated in a hydrogenation reactor using hydrogen gas at 4-5 bars pressure and palladium charcoal 10% as catalyst to give the corresponding amino derivatives 26-33 which were immediately dissolved in pyridine followed by the addition of ethyl chloroformate solution under nitrogen atmosphere. After being kept at room temperature for 1-2 h, the solutions were heated under reflux for 15-24 hours to give the corresponding 3-sulfonamide substituted benzamido-benzimidazolones 34-41 in 15-77% yields (from their corresponding nitro derivatives).

Preparation of 3-(2-pyridine)substituted
Figure 2. Lowest and sixth-ranked docking energy orientation of 34 in the crystal structure of CDK2 using AUTODOCK program.

Figure 3. General reaction scheme for the preparation of 3-sulfonamide substituted benzamido-benzimidazolones.
(a) Chlorosulfonic acid, reflux (b) 1-R1R2-NH, 2-HCl (c) SOCl2, reflux (d) o-nitrophenyl hydrazine, TEA, ether (e) H2, Pd/C, methanol
Sulfamoyl benzoic acid was not successful using the corresponding chlorosulfonylbenzoic acid, as 2-amino pyridine is a weaker base or nucleophile comparing to other aliphatic amines to attack these weak sulphonyl chlorides. However, 1-[3-(2-pyridylaminosulfonyl)-benzamido]benzimidazolone 47 was prepared according to the general reaction scheme depicted in figure 4.

The commercially available 3-chlorosulfonylbenzoyl chloride was reacted with 2-aminopyridine in pyridine to give the corresponding pyridyl amide 42. The product was then hydrolysed in refluxing sodium hydroxide solution (10%) to give 3-(2-pyridylaminosulfonyl)benzoic acid 43 in 61% yield. The acid 43 was then changed to its corresponding acyl chloride derivative 44 in the usual manner described before which in turn was reacted with o-nitrophenyl hydrazine and TEA to provide N-[3-(2-pyridylamino sulfonyl)-benzoyl]-o-nitro phenyl hydrazine 45 in 55% yield. The nitro derivative 45 was then subjected to hydrogenation in the usual manner to give the amine 46 which was reacted immediately, under nitrogen, with ethyl chloroformate in the usual way to give 1-[3-(2-pyridylamino sulfonyl)

Figure 4. General reaction scheme for the preparation of 3-(2-pyridyl) sulfonamide substituted benzamido-benzimidazolone (47).
(a) Pyridine, (b) NaOH 10%, reflux, (c) SOCl₂, reflux (d) Ether, TEA, RT, (e) H₂ Pd/C 10%, methanol, (f) Ethyl chloroformate, pyridine, reflux

Sulfamoyl benzoic acid was not successful using the corresponding chlorosulfonylbenzoic acid, as 2-amino pyridine is a weaker base or nucleophile comparing to other aliphatic amines to attack these weak sulphonyl chlorides. However, 1-[3-(2-pyridylaminosulfonyl)-benzamido]benzimidazolone 47 was prepared according to the general reaction scheme depicted in figure 4.

The commercially available 3-chlorosulfonylbenzoyl chloride was reacted with 2-aminopyridine in pyridine to give the corresponding pyridyl amide 42. The product was then hydrolysed in refluxing sodium hydroxide solution (10%) to give 3-(2-pyridylamino sulfonyl)benzoic acid 43 in 61% yield. The acid 43 was then changed to its corresponding acyl chloride derivative 44 in the usual manner described before which in turn was reacted with o-nitrophenyl hydrazine and TEA to provide N-[3-(2-pyridylamino sulfonyl)-benzoyl]-o-nitro phenyl hydrazine 45 in 55% yield. The nitro derivative 45 was then subjected to hydrogenation in the usual manner to give the amine 46 which was reacted immediately, under nitrogen, with ethyl chloroformate in the usual way to give 1-[3-(2-pyridylamino sulfonyl)-benzamido]benzimidazolone 47.

Figure 5. General reaction scheme for the preparation of di-isopropenyl benzimidazolone 49.
(a) NaH 80%,Dry DMF, RT.
benzamido)] benzimidazolone 47 in 51% (from 45) yield.

1-[[(1-Isopropenyl)benzimidazolin-2-one-3-carboxy]-3-[(1-isopropenyl)benzimidazolin-2-one-3-sulfonyl]benzene 49 was obtained from the reaction of 3-chlorosulfonylbenzoyl chloride and 2-equimolar of 1-isopropenylbenzimidazolone 48 and sodium hydride in 33% yield as depicted in figure 5.

Cell culture and MTT assay

The cells were grown in appropriate mediums supplemented with 10% foetal bovine serum, 0.1 mM non-essential amino acids (NEM), 100 units/ml penicillin-streptomycin and 2 mM glutamine at 37°C with 5% CO2.

Cytotoxicity was then determined by dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. This assay is based on the reduction of the soluble yellow MTT tetrazolium salt to a blue insoluble formazan product by mitochondrial succinic dehydrogenase. In brief, the cells were cultured in 96-well microtiter plates at 37°C in a 5% CO2/95% air humidified atmosphere. After overnight culture, cells were treated with the compounds (20 µM) or 0.4% DMSO (control) for 72 h. Plates were then incubated for 2 h with MTT solution (final concentration 0.5 mg/ml). Culture medium in each well was discarded and replaced with 100 µl DMSO. The absorbance of each well was measured at 540 nm with a microtiter plate reader. The results are shown in scheme 1 for CL1-5, Hepa-G2, HT-29 and AGS cell lines, respectively.

The results indicated that although these compounds could accommodate in the active site of the CDK-2 enzyme, however the preliminary screening on 4 different cell lines indicated no significant cell growth inhibition. There are several possibilities as the compounds could not be able to fulfill the binding requirement for potent inhibitor binding or may not be able to penetrate through plasma membrane. It may also proposed that these benzimidazolones can bind to the target, but not to the active site, the compound-target affinity was too low, the compounds degraded after penetration because of some enzymatic reactions or they are not very stable when added to the culture medium. The lack of significant cytotoxicity of the benzimidazolones relative to the rigid oxindole inhibitor may also be due to the more flexibility of the newly synthesized molecules.

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