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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, biological evaluation, and molecular modeling studies of novel heterocyclic compounds as anti-proliferative agents

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Abstract Two novel series of heterocyclic compounds have been synthesized. In first series, isatin was allowed to react with substituted aromatic/cyclic carbonyl compounds to get desired mannich bases (**2a**–**e**). In second series, 4,5-disubstituted oxazoles (**6a**–**p**) were synthesized. Eight compounds (**2c**, **6a**, **6e**, **6f**, **6i**, **6j**, **6m**, and **6n**) were screened for anticancer activity in 60 cell lines. Compound **2c**, 1-[(4,7,7-trimethyl-3-oxobicyclo[2.2.1]heptan-2-yl)methyl]indoline-2, 3-dione, showed maximum activity and thus, selected for further evaluation at five dose level screening. Furthermore, molecular docking studies of compounds **2c** into the colchicine-binding site of tubulin, revealed possible mode of inhibition by the compound.

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Introduction

Malignant tumor is one of the most serious threats against human health in the world, and the clinical prognosis remains relatively poor. Therefore, it needs to develop the new therapeutic strategies for the improvement of drugs that are currently in use. There has been more and more interest in the search for antitumor compounds with high efficacy and low toxicity in recent years. There are three major approaches in the drug discovery: empiric screening, rational design of new compounds, and chemical modification of known compounds with established pharmacological actions. During the course of identifying compounds active for the prevention and treatment of cancer, stilbene derivatives, such as plant micro-components resveratrol and combretastatin A-4 (CA-4, Fig. 1), have emerged to hold promise for their potent in vitro and in vivo anticancer bioactivities (Murias *et al*., 2005; Pettit *et al*., 2005; Latruffe *et al*., 2002; Bhat and Pezzuto 2001; Ciolino and Yeh 2001; Pettit *et al*., 1998; Jang *et al*., 1997; Pettit *et al*., 1989; Lin *et al*., 1988). The anticancer potential of CA-4 is attributed to the finding that, this compound binds to the colchicine-binding site of tubulin, which in turn inhibits the microtubule assembly (Lin *et al*., 1989). CA-4 is active in *cis* form. The reason for this is that in *cis* conformation the two phenyl rings are noncoplanar and within an appropriate distance (Gaukroger *et al*., 2001; Pettit *et al*., 1995; Lin *et al*., 1988). In fact, the anticancer potential of resveratrol, CA-4 and their derivatives has been widely studied, but it is still important to contribute for an ample knowledge on the biological effects of related compounds (Kang *et al*., 2003).

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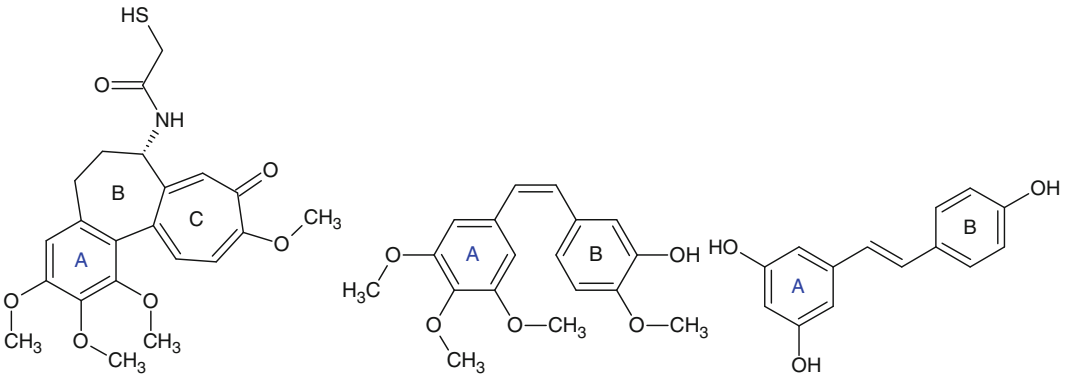


DAMA-Colchicine (COL) **Combretastatin A-4 (CA-4)** **Resveratrol**

Fig. 1 Chemical structures of antitubulin agent **COL** and cytotoxic stilbenes (**CA-4** and resveratrol)

Different authors worldwide have reported anticancer activity of N-substituted isatin derivatives (Vine *et al*., 2009; Solomon *et al*., 2009). Many CA-4 related heterocyclic compounds, containing fused ring system, have shown potent anticancer activity (Dupeyre *et al*., 2006; Liou *et al*., 2004; Bailly *et al*., 2003; Tahir *et al*., 2003; Flynn *et al*., 2002; Xia *et al*., 1998). It has been revealed that potent activity of some of these heterocyclic compounds is based on the fact that two phenyl rings, present in their structure, are noncoplanar and at an appropriate distance, as in CA-4 (Bailly *et al*., 2003). Therefore, we can presume that mannich bases of isatin, formed by using cyclic ketones, have some structure resemblance with CA-4 and hence, their mechanism of action in terms of cytotoxic potential may also be similar. Thus, this rational gave us immense confidence to synthesize some novel mannich bases of isatin by using cyclic ketones, which might be having potential anticancer activity.

CA-4 does not show in vivo efficacy due to isomerization of *cis*-double bond to the more thermally stable *trans*-isomer, which is inactive (Pettit *et al*., 1998). Isomerization of *cis* to *trans* form can be prevented by forming locked *cis*-type bridge between the two phenyl rings. Various researchers have reported structurally rigid analogs having locked *cis*-type bridge, and many of them have shown potent activity (Peifer *et al*., 2006; Kaffy *et al*., 2006; Simoni *et al*., 2005; Wang *et al*., 2002; Ohsumi *et al*., 1998). Therefore, to further explore in this area, we planned to synthesize some novel analogs, in which *cis*-type bridge is locked by forming an oxazole ring, to find novel anticancer agents.

The data, of these synthesized compounds, was submitted to National Cancer Institute (NCI) for anti-proliferative activity. Selection of structures, for anticancer screening at NCI, is based on their ability to add diversity to the NCI small molecule compound collection. Novel heterocyclic ring systems, compounds with drug-like properties utilizing the concept of privileged scaffolds or structures based on computer-aided design are particularly encouraged for submission. Highly flexible acyclic analogs with accompanying entropic liabilities or without prior activity data are generally not accepted. Structures containing problematic linkages or functional groups for successful drug development (e.g., nitro, nitroso, –N–N–, –N=N–, imine, semicarbazone) are also not accepted. From an analog series, representative compounds which will most efficiently provide the greatest information are selected. As per the protocol of NCI, only eight representative compounds, of the series, were selected and granted NSC codes viz; **NSC 742530**, **NSC 753088**, **NSC 753089**, **NSC 753090**, **NSC 753091**, **NSC 753092**, **NSC 753093**, **NSC 753094** and screened at NCI, USA for anti-proliferative activity at a single high dose (10^{-5} M) in full NCI cell panel. Compound **2c** (**NSC 742530**) exhibited the best result at single dose and was selected for further evaluation at five dose level screening. This article presents the in vitro anticancer profile of all the selected compounds including the most potent compound of the series (**2c**), 1-[(4,7,7-trimethyl-3-oxobicyclo[2.2.1]heptan-2-yl)methyl] indoline-2,3-dione (**NSC 742530**), against NCI's human cancer cell lines. In addition, molecular docking study was also taken into account to explore the possible binding mode of these compounds.

Experimental

Chemistry

All the chemicals used were laboratory grade and procured from Fisher Scientific, S.D. Fine Chemicals and CDH. Melting points were determined by open tube capillary method and are uncorrected. Thin layer chromatography

(TLC) plates (silica gel G) were used to confirm the purity of commercial reagents used, compounds synthesized and to monitor the reactions as well. Two different solvent systems; petroleum ether:toluene:acetic acid in ratio 5:4:1 (for compounds of Scheme 1) and *n*-hexane:ethyl acetate:methanol in ratio 10:10:1 (for compounds of Scheme 2) were used to run the TLC. The spots were located under iodine vapors/UV light. IR spectra were obtained on a Shimadzu 8400S FT-IR spectrometer (KBr Pellets). ^{1}H NMR spectra were recorded on a 300 MHz dpx 300, spectrometer-using TMS as internal standard in dimethyl sulfoxide (DMSO). ^{13}C NMR spectra were recorded on an AC 300 BRUKER spectrometer. Mass spectra's were recorded on an API 3000 LC/MS/MS Q3 (SHIMADZU) spectrometer.

General procedure for synthesis of N-substituted isatin derivatives (**2a**–**e**)

A mixture of isatin (0.326 M), paraformaldehyde (0.33 mol), and ketones (**1a**–**e**, 0.25 M) was taken in 250 ml round bottom flask attached to a reflux condenser. To it 40 ml of 95 % ethanol, containing 0.5 ml of concentrated hydrochloric acid, was added, and mixture was refluxed on a water bath for 2 h. Resulting hot solution was filtered through a hot water funnel, and to the filtrate 200 ml of acetone was added. Solution was cooled to room temperature and left overnight in refrigerator. Crystals of the crude product were filtered by using vacuum pump and washed with 10 ml of acetone, and dried. Product was purified by re-crystallization from ethanol–acetone (1:5).

*1-((1-Methyl-4-oxopiperidin-3-yl)methyl)indoline-2,3-dione (**2a**)*

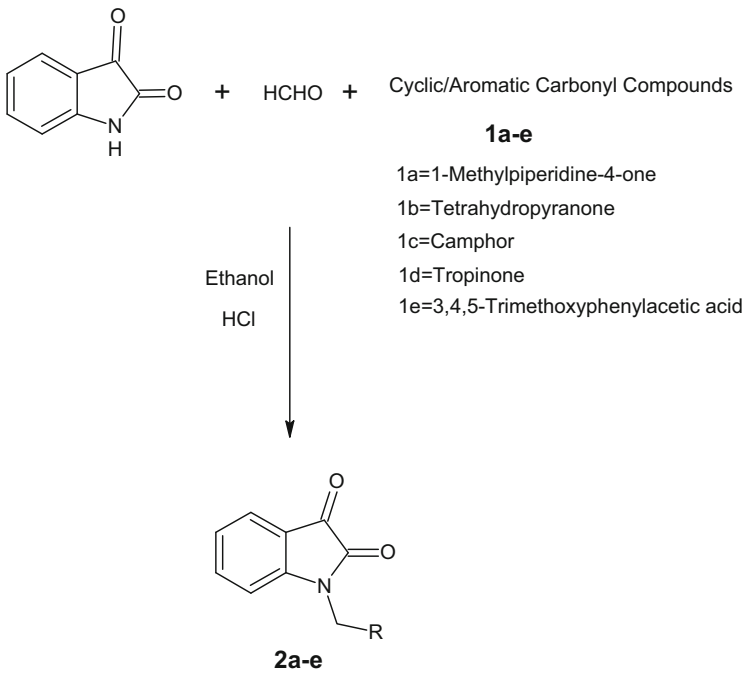
FTIR (KBr) cm^{-1} 3034 and 753 (Ar–H), 2996, 2947, 2859, 1454, 1422 and 1326 (C–H), 1732, 1722, 1681 (C=O), 1602 (C=C of Ar), 1175 (NR_3). ^{1}H NMR (DMSO) δ 7.73–7.41 (3H, m), 7.19 (1H, d), 4.15 (1H, dd), 3.90 (1H, dd), 3.29 (1H, quintet), 2.69–2.43 (6H, m), 2.21 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 38.8, 45.3, 48.7, 49.8, 55.6, 59.4, 117.5, 121.9, 124.6, 130.0, 134.7, 148.3, 160.4, 184.6, 211.7. MS (TISI) 272.3 (M^+). Anal. Calcd: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.18; H, 5.84; N, 10.33.

*1-((2-Oxotetrahydro-2H-pyran-3-yl)methyl)indoline-2,3-dione (**2b**)*

FTIR (KBr) cm^{-1} 3033 and 751 (Ar–H), 2948, 2849, 1467 and 1327 (C–H), 1726, 1719, 1685 (C=O), 1604 (C=C of Ar), 1108 (C–O). ^{1}H NMR (DMSO) δ 7.71–7.38 (3H, m), 7.21 (1H, d), 4.35–4.23 (3H, m), 4.01 (1H, dd), 3.08 (1H, quintet), 2.05–1.84 (4H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 19.7, 28.1, 43.3, 49.6, 71.9, 117.7, 122.0, 124.4, 130.2, 134.3, 148.6, 160.7, 172.5, 184.5. MS (TISI) 259.2 (M^+). Anal. Calcd: C, 64.86; H, 5.05; N, 5.40. Found: C, 66.83; H, 5.04; N, 5.43.

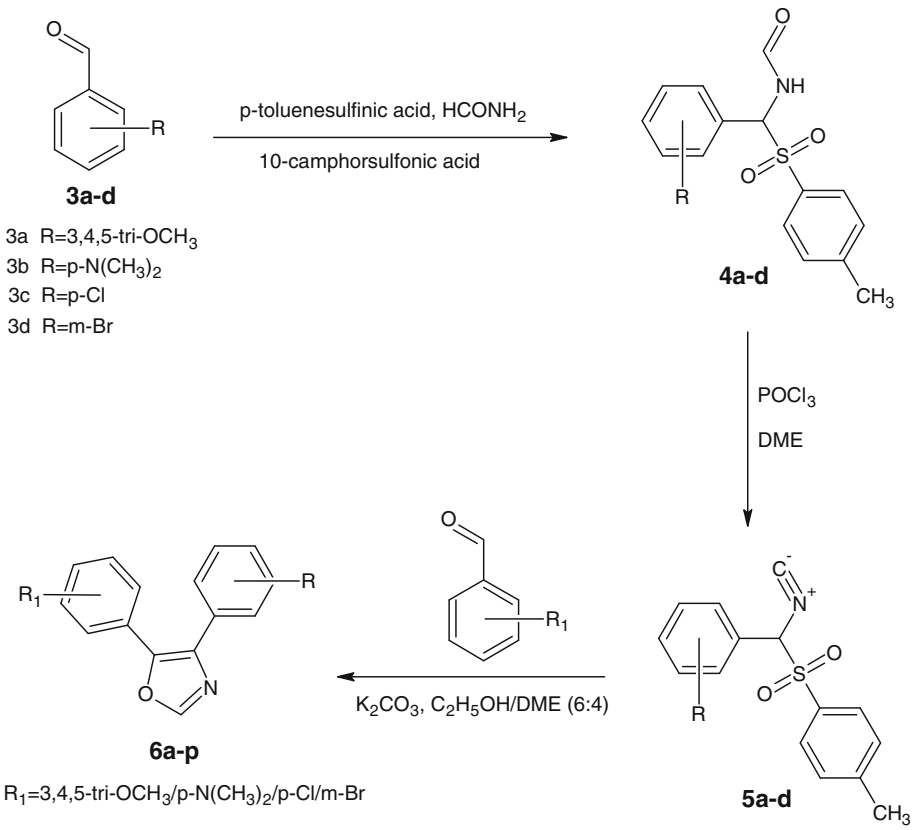
*1-((4,7,7-Trimethyl-3-oxobicyclo[2.2.1]heptan-2-yl)methyl)indoline-2,3-dione (**2c**).*

FTIR (KBr) cm^{-1} 3039 and 759 (Ar–H), 2957, 2926, 2869, 1457, 1423, 1343 and 1323 (C–H), 1742, 1727, 1689



Scheme 1 Synthesis route of N-substituted isatin derivatives

Scheme 2 Synthesis route of 4,5-disubstituted oxazole derivatives



(C=O), 1599 (C=C of Ar). ^{1}H NMR (DMSO) δ 7.75–7.39 (3H, m), 7.19 (1H, d), 4.11 (1H, dd), 3.87 (1H, dd), 2.76 (1H, q), 2.04–1.39 (5H, m), 1.27 (3H, s), 0.97 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 15.5, 22.0, 31.3, 36.5, 37.3, 46.5, 51.6, 59.8, 117.4, 122.2, 124.7, 130.3, 134.3, 148.4, 160.6, 184.9, 222.9, 258.1. MS (TISI) 311.3 (M^+). Anal. Calcd: C, 66.16; H, 5.92; N, 10.29. Found: C, 66.18; H, 5.84; N, 10.33.

*1-((8-Methyl-3-oxo-8-azabicyclo[3.2.1]octan-2-yl)methyl)indoline-2,3-dione (**2d**)*

FTIR (KBr) cm^{-1} 3043 and 755 (Ar–H), 2999, 2936, 2857, 1459, 1420 and 1318 (C–H), 1739, 1725, 1683 (C=O), 1595 (C=C of Ar), 1184 (NR_3). ^{1}H NMR (DMSO) δ 7.79–7.45 (3H, m), 7.23 (1H, d), 4.03 (1H, dd), 3.81 (1H, dd), 3.19 (1H, q), 2.69–2.40 (4H, m), 2.23 (3H, s), 1.71 (2H, m), 1.41 (2H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.6, 31.2, 38.4, 43.5, 44.3, 52.1, 58.7, 64.7, 117.5, 122.1, 124.4, 130.2, 134.6, 148.4, 160.5, 185.1, 211.5. MS (TISI) 298.3 (M^+). Anal. Calcd: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.48; H, 6.09; N, 9.33.

*3-(2,3-Dioxoindolin-1-yl)-2-(3,4,5-trimethoxyphenyl)propanoic acid (**2e**)*

FTIR (KBr) cm^{-1} 3037, 827 and 749 (Ar–H), 2941, 2843, 1459 and 1321 (C–H), 1725, 1707, 1679 (C=O), 1601 (C=C of Ar), 1578 and 1418 (COO^-), 1241 and 1003 (C–O). ^{1}H NMR (DMSO) δ 12.04 (1H, s), 7.68–7.39 (3H, m), 7.18 (1H, d), 6.54 (2H, s), 4.19 (1H, t), 3.93 (1H, dd), 3.81 (3H, s), 3.78 (6H, s), 3.63 (1H, dd). ^{13}C NMR (75 MHz, DMSO-d_6) δ 43.6, 51.3, 56.3, 56.9, 106.8, 107.4, 117.6, 122.2, 124.7, 129.1, 130.4, 134.8, 138.3, 148.6, 151.2, 160.5, 177.1, 184.3. MS (TISI) 385.3 (M^+). Anal. Calcd: C, 62.33; H, 4.97; N, 3.63. Found: C, 62.38; H, 4.99; N, 3.61.

General procedure for synthesis of *N*-{[(4-methylphenyl)sulfonyl](phenyl)methyl}formamide derivatives (**4a–d**)

A solution of toluenesulfinic acid (22.3 g, 0.15 mol), **3a** or **3b** or **3c** or **3d** (0.18 mol), and camphorsulfonic acid (3.48 g, 1.5 mmol) in formamide (40 ml) was stirred vigorously at 65 °C for 16 h, cooled, and filtered. The resulting solid was washed with methanol and dried to provide the desired compound.

*N-{[(4-methylphenyl)sulfonyl](3,4,5-trimethoxyphenyl)methyl}formamide (**4a**)*

FTIR (KBr) cm^{-1} 3436 and 1515 (N–H), 3027, 840 and 748 (Ar–H), 2935, 1460 and 1371 (CH_3), 2893 and 1336 (C–H), 1672 (C=O), 1598 (C=C of Ar), 1326 and 1144

(S=O), 1233 and 999 (C–O). ^{1}H NMR (DMSO) δ 8.17 (1H, s), 7.85 (2H, d), 7.30 (2H, d), 6.41 (1H, s), 6.07 (2H, s), 3.89 (6H, s), 3.78 (3H, s), 2.29 (3H, s), 1.97 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.2, 56.4, 56.8, 74.7, 104.0, 128.5, 129.8, 135.8, 136.9, 137.2, 143.5, 150.8, 163.2. Anal. Calcd: C, 56.98; H, 5.58; N, 3.69. Found: C, 57.04; H, 5.54; N, 3.66.

*N-{[4-(dimethylamino)phenyl] [(4-methylphenyl)sulfonyl]methyl}formamide (**4b**)*

FTIR (KBr) cm^{-1} 3434 and 1515 (N–H), 3028, 844 and 749 (Ar–H), 2937, 1456 and 1375 (CH_3), 2897 and 1336 (C–H), 1673 (C=O), 1600 (C=C of Ar), 1327 and 1144 (S=O), 1173 (C-NR_2). ^{1}H NMR (DMSO) δ 8.20 (1H, s), 7.84 (2H, d), 7.28 (2H, d), 6.84 (2H, d), 6.49 (2H, d), 6.38 (1H, s), 2.93 (6H, s), 2.26 (3H, s), 2.01 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.7, 40.6, 74.5, 114.0, 127.6, 128.2, 128.6, 130.4, 131.5, 135.7, 143.5, 147.3, 163.3. Anal. Calcd: C, 61.42; H, 6.06; N, 8.43. Found: C, 61.39; H, 6.10; N, 8.41.

*N-{(4-chlorophenyl) [(4-methylphenyl)sulfonyl]methyl}formamide (**4c**)*

FTIR (KBr) cm^{-1} 3431 and 1517 (N–H), 3033, 847 and 750 (Ar–H), 2938, 1454 and 1373 (CH_3), 2895 and 1334 (C–H), 1675 (C=O), 1602 (C=C of Ar), 1326 and 1143 (S=O), 719 (C–Cl). ^{1}H NMR (DMSO) δ 8.14 (1H, s), 7.80 (2H, d), 7.32 (2H, d), 7.17 (2H, d), 6.97 (2H, d), 6.45 (1H, s), 2.37 (3H, s), 1.94 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.3, 74.3, 128.1, 128.5, 128.9, 130.5, 132.4, 135.6, 140.7, 143.5, 163.1. Anal. Calcd: C, 55.64; H, 4.36; N, 4.33. Found: C, 55.59; H, 4.33; N, 4.31.

*N-{(3-bromophenyl) [(4-methylphenyl)sulfonyl]methyl}formamide (**4d**)*

FTIR (KBr) cm^{-1} 3433 and 1514 (N–H), 3034, 846 and 748 (Ar–H), 2936, 1458 and 1370 (CH_3), 2897 and 1333 (C–H), 1673 (C=O), 1601 (C=C of Ar), 1327 and 1143 (S=O), 595 (C–Br). ^{1}H NMR (DMSO) δ 8.19 (1H, s), 7.86 (2H, d), 7.30–7.24 (4H, m), 7.05–6.98 (2H, m), 6.40 (1H, s), 2.34 (3H, s), 2.03 (1H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.4, 74.5, 122.6, 126.0, 128.7, 129.4, 130.4, 130.6, 131.6, 135.3, 143.8, 144.9, 163.4. Anal. Calcd: C, 48.92; H, 3.83; N, 3.80. Found: C, 48.95; H, 3.83; N, 3.78.

General procedure for synthesis of 1-{[isocyano(phenyl)methyl]sulfonyl}-4-methylbenzene derivatives (**5a**–**d**)

A solution of **4a** or **4b** or **4c** or **4d** (36.3 mmol) in 200 ml of 1,2-dimethoxyethane (DME) was cooled to −10 °C. To this solution was added phosphorus oxychloride (10.3 ml, 110 mmol) followed by dropwise addition of triethylamine (25.3 ml, 181.5 mmol) in DME (20 ml). The reaction mixture was stirred at −5 °C for 3 h, then poured into ice cold water (500 ml), and extracted with ethylacetate (3 × 120 ml). The combined extracts were washed with 10 % sodium bicarbonate and brine, dried with magnesium sulfate, concentrated in vacuo to 20 % of the original volume, and filtered to afford the desired compound.

*5-{Isocyano[(4-methylphenyl)sulfonyl]methyl}-1,2,3-trimethoxybenzene (**5a**)*

FTIR (KBr) cm^{-1} 3024, 837 and 747 (Ar–H), 2930, 1456 and 1372 (CH_3), 2898 and 1332 (C–H), 2171 (NC), 1596 (C=C of Ar), 1329 and 1145 (S=O), 1236 and 1002 (C–O). ^{1}H NMR (DMSO) δ 7.87 (2H, d), 7.33 (2H, d), 6.23 (1H, s), 6.09 (2H, s), 3.84 (6H, s), 3.73 (3H, s), 2.28 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.3, 56.3, 56.7, 80.6, 106.4, 128.0, 128.5, 130.4, 132.2, 135.7, 136.3, 143.6, 150.5, 157.2. Anal. Calcd: C, 59.82; H, 5.30; N, 3.88. Found: C, 59.88; H, 5.28; N, 3.84.

*4-{Isocyano[(4-methylphenyl)sulfonyl]methyl}-N,N-dimethylaniline (**5b**)*

FTIR (KBr) cm^{-1} 3027, 836 and 749 (Ar–H), 2933, 1457 and 1372 (CH_3), 2896 and 1331 (C–H), 2173 (NC), 1597 (C=C of Ar), 1330 and 1146 (S=O), 1168 (C-NR_2). ^{1}H NMR (DMSO) δ 7.89 (2H, d), 7.36 (2H, d), 6.83 (2H, d), 6.49 (2H, d), 6.24 (1H, s), 2.97 (6H, s), 2.22 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.4, 40.4, 79.6, 114.4, 127.5, 128.5, 130.3, 130.6, 135.6, 143.8, 146.7, 157.1. Anal. Calcd: C, 64.94; H, 5.77; N, 8.91. Found: C, 64.91; H, 5.76; N, 8.94.

*(4-Chlorophenyl)(isocyano)methyl 4-methylphenyl sulfone (**5c**)*

FTIR (KBr) cm^{-1} 3031, 839 and 750 (Ar–H), 2933, 1456 and 1376 (CH_3), 2899 and 1335 (C–H), 2177 (NC), 1600 (C=C of Ar), 1327 and 1146 (S=O), 723 (C–Cl). ^{1}H NMR (DMSO) δ 7.86 (2H, d), 7.30 (2H, d), 7.13 (2H, d), 6.96 (2H, d), 6.27 (1H, s), 2.28 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.6, 79.7, 128.4, 128.5, 130.1, 130.3, 131.1, 135.6, 135.7, 143.7, 157.0. Anal. Calcd: C, 58.92; H, 3.96; N, 4.58. Found: C, 58.97; H, 3.93; N, 4.57.

*(3-Bromophenyl)(isocyano)methyl 4-methylphenyl sulfone (**5d**)*

FTIR (KBr) cm^{-1} 3034, 840 and 751 (Ar–H), 2936, 1459 and 1376 (CH_3), 2896 and 1332 (C–H), 2175 (NC), 1603

(C=C of Ar), 1326 and 1144 (S=O), 589 (C–Br). ^{1}H NMR (DMSO) δ 7.83 (2H, d), 7.32–7.23 (4H, m), 7.07–7.01 (2H, m), 6.26 (1H, s), 2.29 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 24.5, 79.4, 123.4, 128.1, 128.3, 128.5, 130.4, 130.7, 133.7, 135.8, 140.0, 144.1, 157.2. Anal. Calcd: C, 51.44; H, 3.45; N, 4.00. Found: C, 51.42; H, 3.47; N, 4.02.

General procedure for synthesis of 4,5-disubstituted oxazoles (**6a**–**p**)

A mixture of **5a** or **5b** or **5c** or **5d** (1.2 mmol), substituted benzaldehyde (1 mmol), and potassium carbonate (2.4 mmol) in 10 ml of methanol and 3 ml of DME was refluxed for 2 h. After it was cooled to room temperature, the solution was concentrated in vacuo and diluted with ethyl acetate. The solution was washed with water and brine, dried with magnesium sulfate, filtered and concentrated in vacuo. The residue was purified with column chromatography by eluting with methanol to give the product.

*4,5-Bis(3,4,5-trimethoxyphenyl)oxazole (**6a**)*

FTIR (KBr) cm^{-1} 3033, 837 and 751 (Ar–H), 2936 and 1456 (CH_3), 1658 (C=N–C), 1593 (C=C of Ar), 1238 and 1002 (C–O). ^{1}H NMR (DMSO) δ 7.98 (1H, s), 6.85 (4H, s), 3.86 (12H, s), 3.76 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.4, 56.7, 104.7, 124.2, 127.6, 136.5, 139.6, 145.1, 151.0, 151.8. MS (TISI) 401.1 (M^+). Anal. Calcd: C, 62.83; H, 5.78; N, 3.49. Found: C, 62.81; H, 5.79; N, 3.45.

*N,N-dimethyl-4-[4-(3,4,5-trimethoxyphenyl)oxazol-5-yl]aniline (**6b**)*

FTIR (KBr) cm^{-1} 3025, 835 and 752 (Ar–H), 2938, 2903, 1454 and 1378 (CH_3), 1673 (C=N–C), 1602 (C=C of Ar), 1240 and 995 (C–O), 1169 (C-NR_2). ^{1}H NMR (DMSO) δ 7.98 (1H, s), 7.63 (2H, d), 6.84–6.71 (4H, m), 3.83 (6H, s), 3.72 (3H, s), 3.09 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.9, 56.3, 56.6, 104.5, 115.1, 119.7, 127.6, 128.1, 136.8, 139.1, 145.2, 149.8, 151.0, 151.7. MS (TISI) 354.1 (M^+). Anal. Calcd: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.79; H, 6.27; N, 7.91.

*5-(4-Chlorophenyl)-4-(3,4,5-trimethoxyphenyl)-1,3-oxazole (**6c**)*

FTIR (KBr) cm^{-1} 2943, 1458 and 1392 (CH_3), 1687 (C=N–C), 1589 (C=C of Ar), 1233 and 992 (C–O), 845 and 757 (Ar–H), 729 (C–Cl). ^{1}H NMR (DMSO) δ 7.95 (1H, s), 7.77 (2H, d), 7.51 (2H, d), 6.83 (2H, s), 3.85 (6H, s), 3.75 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.4, 56.9, 104.9, 124.5, 128.7, 129.3, 129.9, 134.6, 136.2, 139.1, 145.6, 151.2, 151.9. MS (TISI) 345.0 (M^+). Anal. Calcd: C, 62.52; H, 4.66; N, 4.05. Found: C, 62.54; H, 4.67; N, 4.07.

*5-(3-Bromophenyl)-4-(3,4,5-trimethoxyphenyl)-1,3-oxazole (**6d**)*

FTIR (KBr) cm^{-1} 3026, 833 and 750 (Ar–H), 2941 and 1455 (CH_3), 1675 (C=N–C), 1601 (C=C of Ar), 1235 and 998 (C–O), 593 (C–Br). ^{1}H NMR (DMSO) δ 8.12 (1H, d), 7.97 (1H, s), 7.54–7.40 (3H, m), 6.85 (2H, s), 3.82 (6H, s), 3.72 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.6, 56.7, 104.4, 123.8, 124.1, 126.6, 131.5, 131.9, 132.5, 133.4, 136.5, 139.3, 145.4, 150.7, 151.5. MS (TISI) 389.0 (M^+). Anal. Calcd: C, 55.40; H, 4.13; N, 3.59. Found: C, 55.41; H, 4.14; N, 3.56.

*N,N-dimethyl-4-[5-(3,4,5-trimethoxyphenyl)-1,3-oxazol-4-yl]aniline (**6e**)*

FTIR (KBr) cm^{-1} 3030, 835 and 750 (Ar–H), 2939, 2901, 1454 and 1375 (CH_3), 1676 (C=N–C), 1601 (C=C of Ar), 1240 and 997 (C–O), 1171 (C-NR_2). ^{1}H NMR (DMSO) δ 7.95 (1H, s), 7.64 (2H, d), 6.85–6.69 (4H, m), 3.85 (6H, s), 3.75 (3H, s), 3.15 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.5, 56.3, 56.6, 104.3, 114.1, 122.6, 124.4, 128.7, 136.9, 145.6, 149.8, 151.0, 151.5. MS (TISI) 354.1 (M^+). Anal. Calcd: C, 67.78; H, 6.26; N, 7.90. Found: C, 67.79; H, 6.23; N, 7.91.

*4,4′-(1,3-Oxazole-4,5-diyl)bis(N,N-dimethylaniline) (**6f**)*

FTIR (KBr) cm^{-1} 3027, 812 and 727 (Ar–H), 2901 and 1371 (CH_3), 1661 (C=N–C), 1598 (C=C of Ar), 1231 (C–O), 1165 (C-NR_2). ^{1}H NMR (DMSO) δ 7.96 (1H, s), 7.63 (4H, d), 6.84 (4H, d), 3.13 (12H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.6, 114.4, 119.7, 122.0, 128.3, 136.5, 145.7, 149.2, 149.4, 151.8. MS (TISI) 307.1 (M^+). Anal. Calcd: C, 74.24; H, 6.89; N, 13.67. Found: C, 74.25; H, 6.90; N, 13.68.

*4-[5-(4-Chlorophenyl)-1,3-oxazol-4-yl]-N,N-dimethylaniline (**6g**)*

FTIR (KBr) cm^{-1} 3034, 819 and 749 (Ar–H), 2903 and 1377 (CH_3), 1661 (C=N–C), 1605 (C=C of Ar), 1234 (C–O), 1173 (C-NR_2), 717 (C–Cl). ^{1}H NMR (DMSO) δ 7.95 (1H, s), 7.82 (2H, d), 7.63 (2H, d), 7.51 (2H, d), 6.83 (2H, d), 3.15 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.7, 114.6, 122.8, 128.0, 128.1, 128.9, 129.9, 134.4, 136.2, 145.5, 149.3, 151.6. MS (TISI) 298.1 (M^+). Anal. Calcd: C, 68.34; H, 5.06; N, 9.38. Found: C, 68.35; H, 5.07; N, 9.36.

*4-[5-(3-Bromophenyl)-1,3-oxazol-4-yl]-N,N-dimethylaniline (**6h**)*

FTIR (KBr) cm^{-1} 3029, 822 and 747 (Ar–H), 2907 and 1371 (CH_3), 1655 (C=N–C), 1598 (C=C of Ar), 1231 (C–O), 1165 (C-NR_2), 595 (C–Br). ^{1}H NMR (DMSO) δ 8.11 (1H, d), 7.96 (1H, s), 7.76–7.32 (5H, m), 6.83 (2H, d), 3.15 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.6, 114.3, 122.8, 123.7, 126.2, 128.7, 131.3, 131.5, 132.2, 133.4, 136.4, 145.0, 149.9, 151.5. MS (TISI) 342.0 (M^+). Anal. Calcd: C, 59.49; H, 4.41; N, 8.16. Found: C, 59.47; H, 4.39; N, 8.15.

*4-(4-Chlorophenyl)-5-(3,4,5-trimethoxyphenyl)-1,3-oxazole (**6i**)*

FTIR (KBr) cm^{-1} 3032, 841 and 754 (Ar–H), 2947 and 1453 (CH_3), 1684 (C=N–C), 1589 (C=C of Ar), 1236 and 993 (C–O), 721 (C–Cl). ^{1}H NMR (DMSO) δ 8.08 (2H, d), 7.97 (1H, s), 7.53 (2H, d), 6.82 (2H, s), 3.84 (6H, s), 3.73 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.3, 56.6, 104.8, 124.4, 128.2, 129.1, 131.5, 134.6, 136.5, 139.8, 145.7, 151.1, 151.3. MS (TISI) 345.0 (M^+). Anal. Calcd: C, 62.52; H, 4.66; N, 4.05. Found: C, 62.54; H, 4.67; N, 4.087.

*4-[4-(4-Chlorophenyl)-1,3-oxazol-5-yl]-N,N-dimethylaniline (**6j**)*

FTIR (KBr) cm^{-1} 3026, 821 and 749 (Ar–H), 2905 and 1375 (CH_3), 1664 (C=N–C), 1601 (C=C of Ar), 1236 (C–O), 1173 (C-NR_2), 719 (C–Cl). ^{1}H NMR (DMSO) δ 8.10 (2H, d), 7.95 (1H, s), 7.64 (2H, d), 7.51 (2H, d), 6.83 (2H, d), 3.13 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.5, 115.1, 119.7, 128.2, 128.9, 129.2, 131.3, 134.4, 136.9, 145.0, 149.4, 151.6. MS (TISI) 298.1 (M^+). Anal. Calcd: C, 68.34; H, 5.06; N, 9.38. Found: C, 68.35; H, 5.07; N, 9.39.

*4,5-Bis(4-chlorophenyl)-1,3-oxazole (**6k**)*

FTIR (KBr) cm^{-1} 3030, 823 and 750 (Ar–H), 1668 (C=N–C), 1599 (C=C of Ar), 1240 (C–O), 721 (C–Cl). ^{1}H NMR (DMSO) δ 8.08 (2H, d), 7.96 (1H, s), 7.79 (2H, d), 7.62 (2H, d), 7.52 (2H, d). ^{13}C NMR (75 MHz, DMSO-d_6) δ 128.3, 128.7, 129.8, 131.6, 134.1, 136.4, 145.5, 152.2. MS (TISI) 289.0 (M^+). Anal. Calcd: C, 62.09; H, 3.13; N, 4.83. Found: C, 62.07; H, 3.14; N, 4.85.

*5-(3-Bromophenyl)-4-(4-chlorophenyl)-1,3-oxazole (**6l**)*

FTIR (KBr) cm^{-1} 3027, 818 and 753 (Ar–H), 1673 (C=N–C), 1603 (C=C of Ar), 1241 (C–O), 717 (C–Cl), 596 (C–Br). ^{1}H NMR (DMSO) δ 8.15 (1H, d), 8.08 (2H, d), 7.95 (1H, s), 7.72–7.30 (5H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 123.8, 126.8, 128.4, 128.9, 129.6, 131.2, 131.4, 132.4, 133.5, 134.2, 136.7, 145.2, 152.5. MS (TISI) 334.9 (M^+). Anal. Calcd: C, 53.84; H, 2.71; N, 4.19. Found: C, 53.85; H, 2.72; N, 4.16.

*4-(3-Bromophenyl)-5-(3,4,5-trimethoxyphenyl)-1,3-oxazole (**6m**)*

FTIR (KBr) cm^{-1} 3033, 834 and 750 (Ar–H), 2945 and 1456 (CH_3), 1677 (C=N–C), 1598 (C=C of Ar), 1235 and 1007 (C–O), 595 (C–Br). ^{1}H NMR (DMSO) δ 7.96 (1H, s), 7.79 (1H, d), 7.59–7.35 (3H, m), 6.83 (2H, s), 3.83 (6H, s). 3.72 (3H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 56.0, 56.3, 104.5, 123.4, 124.8, 126.8, 131.3, 131.5, 133.3, 135.6, 136.6, 139.0, 145.1, 151.7, 151.9. MS (TISI) 389.0 (M^+). Anal. Calcd: C, 55.40; H, 4.13; N, 3.59. Found: C, 55.41; H, 4.14; N, 3.60.

*4-[4-(3-Bromophenyl)-1,3-oxazol-5-yl]-N,N-dimethylaniline (**6n**)*

FTIR (KBr) cm^{-1} 3024, 823 and 749 (Ar–H), 2907 and 1373 (CH_3), 1657 (C=N–C), 1593 (C=C of Ar), 1231 (C–O), 1167 (C-NR_2), 595 (C–Br). ^{1}H NMR (DMSO) δ 7.95 (1H, s), 7.78 (1H, d), 7.69–7.33 (5H, m), 6.84 (2H, d), 3.14 (6H, s). ^{13}C NMR (75 MHz, DMSO-d_6) δ 40.7, 114.7, 120.2, 123.1, 126.2, 128.9, 131.4, 131.6, 133.4, 135.7, 136.4, 145.0, 149.3, 151.1. MS (TISI) 342.0 (M^+). Anal. Calcd: C, 59.49; H, 4.41; N, 8.16. Found: C, 59.49; H, 4.42; N, 8.14.

*4-(3-Bromophenyl)-5-(4-chlorophenyl)-1,3-oxazole (**6o**)*

FTIR (KBr) cm^{-1} 3025, 823 and 751 (Ar–H), 1670 (C=N–C), 1603 (C=C of Ar), 1239 (C–O), 719 (C–Cl), 598 (C–Br). ^{1}H NMR (DMSO) δ 7.98 (1H, s), 7.85 (1H, d), 7.77 (2H, d), 7.68–7.31 (5H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 123.8, 126.7, 128.7, 129.1, 131.3, 131.5, 132.9, 134.6, 135.9, 136.6, 145.5, 151.7. MS (TISI) 334.9 (M^+). Anal. Calcd: C, 53.84; H, 2.71; N, 4.19. Found: C, 53.86; H, 2.72; N, 4.17.

*4,5-Bis(3-bromophenyl)-1,3-oxazole (**6p**)*

FTIR (KBr) cm^{-1} 3029, 827 and 752 (Ar–H), 1665 (C=N–C), 1605 (C=C of Ar), 1239 (C–O), 596 (C–Br). ^{1}H NMR (DMSO) δ 8.09 (1H, d), 7.97 (1H, s), 7.78 (1H, d), 7.69–7.31 (6H, m). ^{13}C NMR (75 MHz, DMSO-d_6) δ 123.5, 126.3, 131.2, 131.4, 132.1, 133.3, 135.5, 136.9, 145.8, 151.6. MS (TISI) 378.9 (M^+). Anal. Calcd: C, 47.53; H, 2.39; N, 3.70. Found: C, 47.54; H, 2.40; N, 3.71.

Pharmacological studies

In vitro cancer screen

The human tumor cell lines of the cancer screening panel were grown in RPMI-1640 medium containing 5 % fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96-well microtiter plates in 100 µl at plating densities ranging from 5,000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line were fixed in situ with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 µg/ml gentamicin. Additional four, tenfold or ½ log serial dilutions were made to provide a total of five drug concentrations plus control. Aliquots of 100 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 100 µl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 min at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each well, and plates were incubated for 10 min at room temperature. After staining, unbound dye was removed by washing five times with 1 % acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology was the same except that the assay was terminated by fixing settled cells at the bottom of the wells by gently adding 50 µl of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (*C*), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as:

$$[(\mathrm{Ti} - \mathrm{Tz})/(C - \mathrm{Tz})] \times 100 (\text{concentrations for which Ti} \geq \mathrm{Tz})$$
$$[(\mathrm{Ti} - \mathrm{Tz})/\mathrm{Tz}] \times 100 (\text{concentrations for which Ti} < \mathrm{Tz})$$

Three dose–response parameters were calculated for each experimental agent. GI_{50} was calculated from $[(\mathrm{Ti} - \mathrm{Tz})/(C - \mathrm{Tz})] \times 100 = 50$, which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from $\mathrm{Ti} = \mathrm{Tz}$. The concentration of drug resulting in a 50 % reduction in the measured protein at the end of the drug treatment as compared to that at the beginning (LC_{50}) indicating a net loss of cells following treatment was calculated from $[(\mathrm{Ti} - \mathrm{Tz})/\mathrm{Tz}] \times 100 = -50$. Values were calculated for each of these three parameters if the level of activity was reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as greater or less than the maximum or minimum concentration tested (Boyd and Paull 1995; DeVita *et al*., 1989; Alley *et al*., 1988).

Molecular modeling

The 3D structure of the most active compound of the series (**2c**) as well as combretastatin (**CA-4**), were built using SYBYL 7.1 molecular modeling package (SYBYL 7.1 2005) installed on a silicon graphics fuel work station running IRIX 6.5. The full optimizations of these built structures were carried out by AM1 method implemented in MOPAC interface of SYBYL. The following parameters were set while performing the minimization. Spin state of wave function was set to singlet and normal SCF convergence was used for the optimization, rest all other parameters were set as default.

For the purpose of molecular docking, crystal structure of tubulin (PDB ID: 3SA0) (Ravelli *et al*., 2004) solved at 3.58 Å resolution was utilized. Ligand docking was accomplished by the Grid-based Ligand Docking with Energetics (GLIDE) (GLIDE 2009) program incorporated in Schrödinger package. GLIDE algorithm is based on a systematic search of positions, orientations, and conformations of the ligand in the receptor binding site using funnel type approach.

Protein and ligand preparation

The X-ray crystallographic structures of α,β-tubulin (PDB ID: 1SA0; 3.58 Å resolution) in complex with DAMA-colchicine and with the stathmin-like domain (SLD) of RB3 was downloaded from Protein Data Bank (PDB). Tubulin dimer 1 (chains A and B) was extracted along with the associated GTP, GDP, DAMA-colchicine (**COL**), and Mg. While other two chains (C and D) and SLD of RB3 was removed.

Further, the tubulin dimer structure was prepared for the docking study using the protein preparation wizard tool implemented in Maestro. The right bond orders were assigned and the hydrogen atoms were added to the protein. The orientation of hydroxyl groups, amide groups of Asn and Gln, and charge state of His residues were optimized using the utility protassign. The final step was a restrained minimization by the impref utility until the average root-mean-square-deviation (RMSD) of the hydrogen atoms reached 0.3 Å, leaving heavy atoms in place. Ligands were prepared using LigPrep by generating all possible low energy ionization, tautomeric, and stereoisomeric states within the range of pH 7.0 ± 2.0 using OPLS_2005 force field.

Grid generation and protocol setting

An interaction grid was generated for protein structure by using the receptor grid generation wizard of GLIDE 5.5, by taking bound inhibitor DAMA-colchicine as the reference structure for defining active site. The grid box was centered on the bound ligand in the protein structure by selecting the ligand from the workspace, and used its centroid (XYZ coordinates: 117.1827, 89.9909, 6.2823) for grid generation. The grid box was extended 10 Å from the center, with the outer box extending an additional 10 Å, completely covering the active site cavity.

Docking and scoring of compounds using GLIDE

To set a docking protocol, the bound ligand (DAMA-colchicine) was extracted from the prepared protein structure. The atom and bond type of the extracted ligand were corrected, and was subjected to re-docking by implementing abovementioned grid parameters, and finally RMSD was determined.

After the validation of docking protocol, the set of prepared ligands were docked into the colchicine-binding site of α,β-tubulin by using GLIDE 5.5 at SP (Standard Precision) mode. The docking and scoring algorithms of GLIDE have been fully described elsewhere (Friesner *et al*., 2004; Halgren *et al*., 2004). All docking calculations were performed using the OPLS-2005, an implementation of the optimized potential for liquid simulation − all atom force field (Schrödinger, Portland, OR). All the other parameters were kept at default for the docking.

Results and discussion

Chemistry

The synthesis of N-substituted isatin derivatives and 4,5-disubstituted oxazole derivatives, (**2a**–**e**) and (**6a**–**p**), respectively, described in this study are outlined in Schemes 1 and 2. The chemical structure and physical data of these compounds is presented in Table 1. N-substituted isatin derivatives (**2a**–**e**) were synthesized by mannich reaction of isatin with aromatic/cyclic carbonyl compounds in the presence of HCl and ethanol. 4,5-Disubstituted oxazole derivatives (**6a**–**p**) were synthesized by reaction of substituted benzaldehydes with *p*-toluenesulfinic acid and formamide in the presence of 10-camphorsulfonic acid, followed by dehydration and subsequent reaction with substituted benzaldehydes. N-substituted isatin derivatives were recrystallized with ethanol, while 4,5-disubstituted oxazole derivatives were recrystallized with methanol.

The purity of the compounds was checked by TLC. Spectral data ^{1}H and ^{13}C NMR, and IR of all the synthesized compounds, and mass of final compounds, were recorded and found in full agreement with the proposed structures. Structural formulae of the compounds were further confirmed by elemental analysis.

Pharmacological evaluation

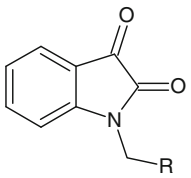
Primary single high dose (10^{-5} M) full NCI 60 cell panel in vitro assay

All the eight selected compounds, submitted to NCI for in vitro anticancer assay, were evaluated for their antiproliferative activity. Primary in vitro one dose anticancer assay was performed in full NCI 60 cell panel representing leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate in accordance with the protocol of the NCI, USA. The compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, SRB. Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. There after obtaining the results for one dose assay, analysis of historical Developmental Therapeutics Program (DTP) was performed and compound **2c** (**NSC 742530**), which satisfied pre-determined threshold inhibition criteria was selected for NCI full panel 5 dose assay. Data of 5 dose assay for compound **2c** is shown in Table 2.

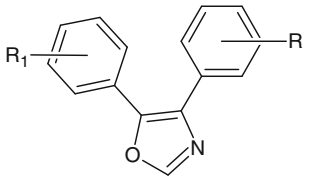
In vitro 5 dose full NCI 60 cell panel assay

All the cell lines (about 60), representing nine tumor subpanels, were incubated at five different concentrations (0.01, 0.1, 1, 10, and 100 mM). The outcomes were used to create log concentration vs. % growth inhibition curves, and three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the

Table 1 Physical properties of the synthesized compounds



(**2a-e**)



(**6a-p**)

Compound code	NSC number	R	R_1	Molecular formula	Molecular weight	Melting point
2a	–	1-Methyl-4-oxopiperidin-3-yl	–	$C_{15}H_{16}N_2O_3$	272.30	>250
2b	–	3-Oxotetrahydro-2H-pyran-2-yl	–	$C_{14}H_{13}NO_4$	259.26	>250
2c	742530	4,7,7-Trimethyl-3-oxobicyclo[2.2.1]heptan-1-yl	–	$C_{19}H_{21}NO_3$	311.37	>250
2d	–	8-Methyl-3-oxo-8-azabicyclo[3.2.1]octan-2-yl	–	$C_{17}H_{18}N_2O_3$	298.34	>250
2e	–	(3,4,5-Trimethoxyphenyl) carboxymethyl	–	$C_{20}H_{19}NO_7$	385.37	>250
6a	753088	3,4,5-Trimethoxy	3,4,5-Trimethoxy	$C_{21}H_{23}NO_7$	401.40	>250
6b	–	3,4,5-Trimethoxy	*p*-Dimethylamino	$C_{20}H_{22}N_2O_4$	354.39	108–110
6c	–	3,4,5-Trimethoxy	*p*-Chloro	$C_{18}H_{16}ClNO_4$	345.77	163–165
6d	–	3,4,5-Trimethoxy	*m*-Bromo	$C_{18}H_{16}BrNO_4$	390.22	155–157
6e	753089	*p*-Dimethylamino	3,4,5-Trimethoxy	$C_{20}H_{22}N_2O_4$	354.39	149–151
6f	753092	*p*-Dimethylamino	*p*-Dimethylamino	$C_{19}H_{21}N_3O$	307.38	145–147
6g	–	*p*-Dimethylamino	*p*-Chloro	$C_{17}H_{15}ClN_2O$	298.76	152–154
6h	–	*p*-Dimethylamino	*m*-Bromo	$C_{17}H_{15}BrN_2O$	343.21	167–169
6i	753090	*p*-Chloro	3,4,5-Trimethoxy	$C_{18}H_{16}ClNO_4$	345.77	179–181
6j	753093	*p*-Chloro	*p*-Dimethylamino	$C_{17}H_{15}ClN_2O$	298.76	190–192
6k	–	*p*-Chloro	*p*-Chloro	$C_{15}H_9Cl_2NO$	290.14	140–142
6l	–	*p*-Chloro	*m*-Bromo	$C_{15}H_9BrClNO$	334.59	182–184
6m	753091	*m*-Bromo	3,4,5-Trimethoxy	$C_{18}H_{16}BrNO_4$	390.22	171–173
6n	753094	*m*-Bromo	*p*-Dimethylamino	$C_{17}H_{15}BrN_2O$	343.21	138–140
6o	–	*m*-Bromo	*p*-Chloro	$C_{15}H_9BrClNO$	334.59	131–133
6p	–	*m*-Bromo	*m*-Bromo	$C_{15}H_9Br_2NO$	379.04	202–204

compound causing 50 % decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in TGI and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50 % loss of initial cells at the end of the incubation period of 48 h. Compound **2c** (**NSC 742530**) under investigation exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} values between "1.53 and 26.9 μM" (Table 2). With regard to the sensitivity against individual cell line (Table 2) the compound showed highest activity against leukemia SR cell line with GI_{50} 1.53 μM and least for melanoma MALME-3M cell line with GI_{50} 26.9 μM. Obtained data revealed that compound sensitivity is more in leukemic (GI_{50} 1.53–7.46 μM) and colon cancer (GI_{50} 1.95–13.5 μM except HCT-15) cell lines, and least in non-small cell lung cancer (GI_{50} 13.5–23.4 μM) and melanoma (GI_{50} 10.4–26.9 μM) cell lines. The criterion for selectivity of a compound depends upon the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines

Table 2 NCI developmental therapeutics program, in vitro testing results of compound **NSC 742530** (compound **2c**) in μM

Panel	Cell line	GI_{50}^{c}		TGI^{d}	LC_{50}^{e}
		Concentration per cell line	Subpanel MID[b]		
Leukemia	CCRF-CEM	7.46	3.51	28.6	>100
	HL-60 (TB)	4.40		15.9	59.8
	K-562	2.46		8.20	96.9
	MOLT-4	3.51		10.6	91.1
	RPMI-8226	3.22		17.2	>100
	SR	1.53		4.02	13.4
Non-small cell	A549/ATCC	16.9	17.78	32.9	64.1
Lung cancer	EKVX	19.6		37.9	73.3
	HOP-62	15.6		30.1	58.1
	HOP-92	17.9		38.3	81.9
	NCI-H226	21.8		61.0	>100
	NCI-H23	14.5		29.6	60.4
	NCI-H322M	23.4		38.3	62.7
	NCI-H460	16.9		36.5	78.4
	NCI-H522	13.5		29.4	64.1
Colon cancer	COLO 205	12.6	8.27	29.2	67.4
	HCC-2998	13.5		27.5	56.2
	HCT-116	1.95		3.76	7.25
	HCT-15	17.1		34.7	70.4
	HT29	5.45		20.4	65.5
	KM12	4.12		12.5	45.8
	SW-620	3.21		9.25	35.5
CNS cancer	SF-268	15.8	14.33	36.0	81.9
	SF-295	11.13		24.3	52.5
	SF-539	12.8		25.8	51.8
	SNB-19	13.8		26.6	51.6
	SNB-75	17.1		33.1	64.2
	U251	15.4		29.3	56.0
Melanoma	LOX IMVI	10.4	17.21	24.5	58.0
	M-14	15.9		33.5	70.9
	MALME-3M	26.9		44.8	74.5
	MDA-MB-435	15.6		32.9	69.2
	SK-MEL-2	19.0		41.7	91.3
	SK-MEL-28	19.7		39.5	78.9
	SK-MEL-5	17.3		33.0	63.0
	UACC-257	13.9		31.1	69.3
	UACC-62	16.2		30.6	57.7
Ovarian cancer	OVCAR-3	15.6	13.26	33.5	72.2
	OVCAR-4	18.8		44.1	>100
	OVCAR-5	14.8		28.4	54.6
	OVCAR-8	2.86		6.97	38.7
	NCI/ADR-RES	12.7		35.3	98.0
	SK-OV-3	14.8		28.5	54.8

Table 2 continued

Panel	Cell line	GI_{50}^{c}		TGI^{d}	LC_{50}^{e}
		Concentration per cell line	Subpanel MID[b]		
Renal cancer	786-0	16.8	13.67	30.8	56.4
	ACHN	16.2		29.7	54.5
	CAKI-1	16.3		31.3	60.1
	RXF 393	5.46		16.9	44.0
	SN12C	15.3		31.6	65.0
	TK-10	14.8		28.5	54.8
	UO-31	13.2		26.2	51.8
Prostate cancer	PC-3	11.9	13.4	25.9	56.4
	DU-145	14.9		28.1	53.0
Breast cancer	MCF7	5.80	13.38	24.6	90.0
	MDA-MB-231/ATCC	15.1		30.0	59.8
	HS 578T	13.8		32.6	81.2
	BT-549	12.3		24.8	49.8
	T-47D	20.6		46.7	>100
MID[a]		13.21			

[a] Average sensitivity of all cell lines in μM

[b] Average sensitivity of all cell lines of a particular sub panel in μM

[c] Concentration of the compound causing 50 % decrease in net cell growth

[d] Concentration of the compound resulting in total growth inhibition

[e] Concentration of the compound causing net 50 % loss of initial cells at the end of the incubation period of 48 h

toward the test agent) by their individual subpanel MID (the average sensitivity of all cell lines of a particular subpanel toward the test agent). Ratio between 3 and 6 refer to moderate selectivity; ratios >6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria were rated non-selective (Rostom 2006). As per this criterion, compound in the study was found to be mild selective toward leukemia subpanel only. Whereas, on overall basis it was found to be non-selective.

Molecular modeling studies

The colchicine-binding site

The X-ray crystal structure of tubulin in complex with *N*-deacetyl-*N*-(2-mercaptoacetyl)-colchicine (DAMA-colchicine, Fig. 1) and with the SLD of RB3 (PDB ID: 1SA0), at 3.5 Å resolution was reported by Ravelli *et al*., 2004. The same group also determined the 4.2 Å structure of a ternary tubulin-podophyllotoxin: RB3-SLD complex (PDB ID: 1SA1), and found that podophyllotoxin also binds to tubulin at the same site as colchicine. Thus, it can compete with the colchicine to occupy the binding pocket in tubulin (Wilson *et al*., 1974). The X-ray structure 1SA0 shows the interaction of RB3-SLD with two tubulin heterodimers (α,β-subunits), adopting a hook-like shape and extending from head to tail of a complex in a curved manner. This type of arrangement suggested that the stathmin plays an important role in the regulation of microtubule assembly, by not allowing the tubulin heterodimers to be incorporated in the microtubule.

Molecular modeling studies were performed to investigate the binding ability of the N-substituted isatin derivative (**2c**) to the colchicine-binding site of α,β-tubulin. Before performing any molecular docking study of ligands of interest, the first most imperative step is to set up a docking protocol and its validation.

Thus, in order to check whether the selected docking process was adequate enough for finding the correct binding mode of ligands binding to the colchicine-binding site, DAMA-colchicine (**COL**) was extracted from the original X-ray crystallographic tubulin structure (PDB ID: 1SA0), and re-docked using GLIDE 5.5 (GLIDE 2009). The set docking protocol was successfully able to dock the extracted bound ligand inside the protein with an almost identical binding mode (GLIDE score −8.39), when compared with the co-crystallized X-ray structure. The superimposition of the best scored docked conformer and X-ray structure bound DAMA-colchicine showed the

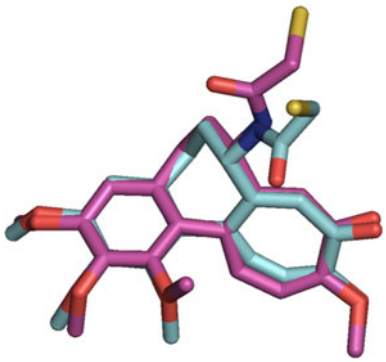


Fig. 2 Superimposition of co-crystallized X-ray bound (PDB ID: 1SA0; *cyan*) and docked (*magenta*) **COL** (RMSD 1.33 Å)

RMSD of 1.33 Å and is depicted in Fig. 2. It indicates that the docking protocol is acceptable and can be further extended to dock other ligands.

The detailed analyses of the docked **COL** revealed that it binds at the α/β interface, but mostly buried in the intermediate domain of β-subunit, which is surrounded by the strands S8 and S9, loop T7 and helices H7 and H8. However, in addition to the interactions of **COL** with β-subunit, it was also found to interact with loop T5 (Ser178, Thr179, Ala180, Val181) of the neighboring α-subunit; consistent with the fact that colchicine stabilizes the tubulin α,β-heterodimer (Shearwin and Timasheff 1994). Thus, binding model of **COL** correlates well with biochemical data showing that mutants of tubulin formed by substitution at Valβ318 have a reduced sensitivity to colchicine (Burns 1992), and that the oxygen atom of methoxy groups in ring A can form strong hydrogen bonding (cross-linked) with the side chain of Cysβ241 (Bai *et al*., 2000).

Docking of CA-4

CA-4, a natural *cis*-stilbene product is the most promising compound for the treatment of solid tumors (Pettit *et al*., 1989). Its water-soluble phosphate prodrug (**CA4P**) is currently in phase II and III clinical trials (Patterson and Rustin 2007; Lippert 2007; Hinnen and Eskens 2007; Cai 2007; Horsman and Siemann 2006; West and Price 2004). The enormous amount of literature for the **CA-4** (Fig. 1) and its analogs suggested that, these compounds also inhibit tubulin polymerization by occupying the same binding pocket similar to that of **COL** (Simoni *et al*., 2006; Tron *et al*., 2006; Woods *et al*., 1995).

In order to compare the docking score and binding mode of **CA-4** with the **COL** and synthesized potent anticancer compound **2c**, **CA-4** was docked into the colchicine-binding site using our adopted docking methodology.

The combination of GLIDE scores and visual inspection of the docked conformation was taken into account to select the most plausible binding mode. The best docked

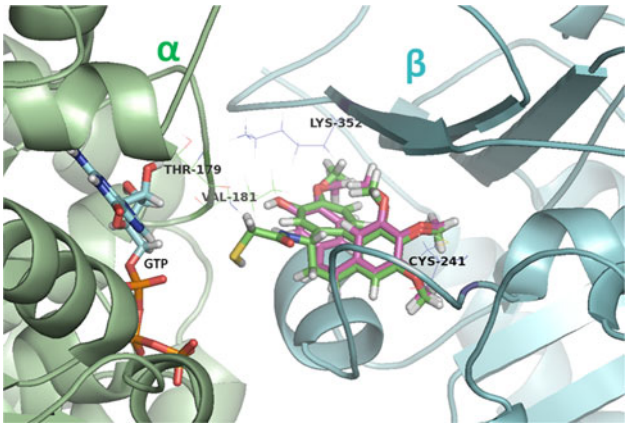


Fig. 3 Comparison of **CA-4** (*magenta*) and **COL** (*green*) binding models inside the α,β-tubulin binding pocket (PDB ID: 1SA0)

conformer of **CA-4** showed GLIDE score of −8.30 which is almost comparable to that of **COL** (−8.39). The comparison of the binding mode of **CA-4** and **COL** is presented in Fig. 3. This figure clearly indicates that **CA-4** binds to the colchicine-binding site in a similar fashion and orientation like **COL** (rings A and B of **CA-4** and rings A and C of **COL** almost superimposes).

Moreover, we also observed that the interaction pattern of docked **CA-4** is parallel to the **COL**. The methoxy groups at ring A of **CA-4** forms strong hydrogen bonding with the side chain of Cysβ241. The distance of these methoxy groups from –SH of Cysβ241 are 5.43 Å (3-O····S), 2.95 Å (4-O····S), and 3.10 Å (5-O·····S), respectively, while the three methoxy groups at ring A of **COL** are 3.19 Å (2-O·····S), 2.93 Å (3-O·····S), and 5.49 Å (4-O·····S), respectively, away from the same residue. The binding pose of docked **CA-4** showed that the 3-hydroxyl group of the ring B of **CA-4** forms hydrogen bonding (3.09 Å) with the main chain carbonyl group of Thr179 of α-tubulin subunit. The same hydroxyl moiety may also be involved in a hydrogen bonding (3.55 Å) with the side chain of Lys352 of β-tubulin. In addition, the 4-methoxy group of the ring B of **CA-4** might also form long range hydrogen bond (4.07 Å) with the main chain amide nitrogen of Lysβ352. Thus, our proposed binding model is consistent with the fact that **CA-4** induces GTPase activity (Pandit *et al*., 2006). Beside this, some weaker interactions were also observed. The Ala180 of α-tubulin subunit interacts by the weak CH·····O hydrogen bonds, Leuβ255 by CH·····π interaction with ring A. The side chains of the residues Alaβ316 and Valα181 may be involved in hydrophobic, van der Waals interactions.

Docking of synthesized, active compound ***2c***

Molecular docking studies showed that the synthesized, bioactive isatin derivative (**2c**) occupied the

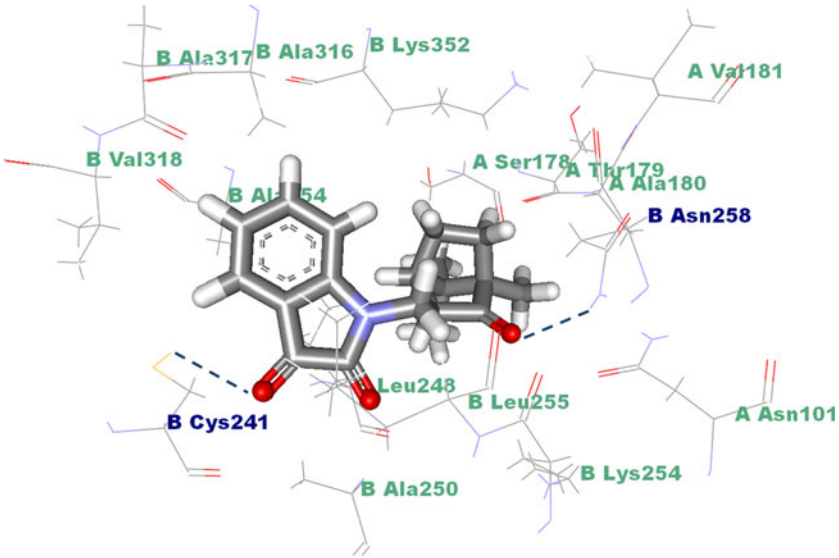


Fig. 4 Binding model of compound **2c** and its hydrogen bonding interactions (*blue dashed lines*) with tubulin (PDB ID: 1SA0) binding pocket (*blue color residues*). *A* and *B* in the amino acid residues label signifies α- and β-tubulin, respectively

colchicine-binding site of α,β-tubulin (mostly buried in the β-subunit). The binding model of compounds **2c** and its interactions with the tubulin heterodimer are presented in Fig. 4. The best docked conformer of compound **2c** showed GLIDE score of 6.51. In consistent with the most prominent interaction of tubulin inhibitors (**COL**, **CA-4** and its analogs), the O_3 atom of indoline ring in compound **2c** also formed hydrogen bonding interaction (2.72 Å) with the side chain of Cys241 of β-tubulin subunit. Weaker C–H·····π interactions were observed between the benzene of indoline ring and Leuβ248 and Leuβ255 hydrophobic residues. The same benzene ring also involved in hydrophobic interaction with Valβ318. The oxobicycloheptane ring in compound **2c** showed multiple interactions with the residues of α,β-tubulin subunit: (1) the carbonyl group present in the ring showed two hydrogen bonding interactions (2.54 and 3.10 Å) with the side chain of Asnβ258; (2) hydrophobic interaction with the Leuβ248 and; (3) weak C–H·····O type of interaction with side and main chain of Serα178 and Thrα179, respectively (distance $\leq$ 3.0 Å) (Fig. 4).

Overall, the docking simulation of compound **2c** shows similar interactions with the tubulin heterodimers as revealed by previous molecular modeling studies of tubulin inhibitors (La Regina *et al*., 2007; Do Yoon *et al*., 2006; De Martino *et al*., 2006; De Martino *et al*., 2004). Thus, this compound is showing anticancer activity most plausibly via blocking the colchicine-binding site of α,β-tubulin subunit and making interactions with its key residues.

Conclusion

To summarize, we have reported the synthesis of novel isatin derivatives (**2a**–**e**) based on mannich reaction, and 4,5-disubstituted oxazole derivatives (**6a**–**p**) synthesized by reaction of substituted benzaldehydes with *p*-toluenesulfinic acid and formamide in the presence of 10-camphorsulfonic acid, followed by dehydration and subsequent reaction with substituted benzaldehydes. Data of the synthesized compounds was submitted to NCI, and only eight compounds (**2c**, **6a**, **6e**, **6f**, **6i**, **6j, 6m**, and **6n**) were subsequently selected for their in vitro anticancer activity. On the basis of results obtained, it was found that compound **2c**, 1-[(4,7,7-trimethyl-3-oxobicyclo[2.2.1]heptan-2-yl)methyl]indoline-2,3-dione (**NSC 742530**) was most active compound of the series and also satisfied pre-determined threshold inhibition criteria. Hence, it was subjected to NCI full panel 5 dose assays for further evaluation. The result of these assays indicated that compound **2c** exhibited significant anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} values between "1.53 and 26.9 μM." Molecular docking studies, of compound **2c**, revealed possible mode of action, plausibly by blocking the colchicine-binding site of α,β-tubulin heterodimer. The binding model of the compound **2c**, suggest that, this compounds form key interactions with the tubulin α,β-subunits in the similar fashion to that of DAMA-colchicine (**COL**) and CA-4. Finally, it is conceivable that further derivatization of such compounds will be of interest with the hope to get more selective anticancer agents.

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