Synthesis and Cytotoxic Activity of Novel Pyrazoline Derivatives against Human Lung Tumor Cell Line (A549)

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In the present investigation, a series of 5-(-4-(substituted)phenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-toluidino methane thione and 5-(substituted)phenyl-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-methoxy anilino methane thiones were synthesized and were examined against human lung tumor cell line (A549) *in vitro* using the MTT assay system. Among those tested, 5-(4-flurophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-toluidino methane thione & 5-(4-chlorophenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-methoxy anilino methane thione showed more potent cytotoxicity against human lung tumor cell line (A549) than the other synthesized compounds.

Keywords: Cytotoxicity; Pyrazoline; Anti-proliferation.

INTRODUCTION

Effective treatment of cancer results from destruction of the cancer cells, which is a result of the cytotoxicity of a drug against proliferating cells. Most of the clinically available anticancer drugs interfere with DNA function to exert their cytotoxicity. DNA intercalators represent an important class of anticancer drugs.[1] Lung cancer is the most frequent cause of cancer-related death and accounts for more than a million deaths yearly worldwide with non-small cell lung cancer (NSCLC) accounting for 75-85% of lung cancer.[2,3] The pathogenesis of lung cancer involves the accumulation of multiple molecular abnormalities, particularly with the exposure to smoking. These alterations include the genetic mutations, chromosomal changes with consequent inactivation of tumor suppressor genes and over activity of signal transduction cascades. Apoptosis (programmed cell death), not only occurs naturally during development and differentation, but also acts to eliminate damaged cells after injury.[4] Molecular studies of lung cancer have provided new avenues for early diagnosis and therapeutic strategies; however, certain patients are still plagued by rapid disease recurrence and progression, and there has been no significant improvement in their overall survival. Therefore, it remains a disease with poor prognosis and the primary cause of cancer-related death in both men and women despite recent advances made in drug development. The development or presence of resistance to chemotherapeutic agents is a major obstacle to the effective treatment of lung cancer. Identifying the molecular determinants of sensitivity and resistance to chemotherapy is expected to improve the therapeutic efficacy. A literature survey reveals pyrazoline derivatives are active against many tumor cell lines.[5,6] The current work describes the synthesis of novel pyrazoline moiety with encouraging cytotoxic activity against human lung tumor cell lines (A 549).

RESULTS AND DISCUSSION

Chemistry

5-(4-(Substituted)phenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-toluidinomethanethione and 5-(substituted)phenyl-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-methoxyanilinomethanethione (**5a-k**) and (**6a-k**) described in this study are shown in Tables 1 and 2, and a reaction sequence for the preparation is outlined in Scheme I. The chalcones were

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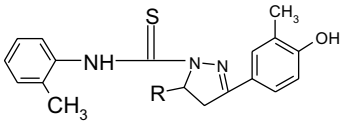
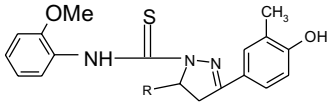
Table 1. Physical constants of the synthesized compounds



5a-k



6a-k

Compound	R	Yield (%)	M.P (°C)	Mol. Formula	Mol. Wt
5a	4-Methoxy phenyl-	74	144	$C_{25}H_{25}N_3O_2S$	431.55
5b	4-Chloro phenyl-	70	131	$C_{24}H_{22}N_3OSCl$	435.97
5c	4-Dimethylamino phenyl-	72	104	$C_{26}H_{28}N_4OS$	444.59
5d	Phenyl-	80	121	$C_{24}H_{23}N_3OS$	401.52
5e	3,4-Dimethoxy phenyl-	82	102	$C_{26}H_{27}N_3O_3S$	461.57
5f	3,4,5-Trimethoxy phenyl-	85	103	$C_{27}H_{29}N_3O_4S$	491.60
5g	4-Fluoro phenyl-	92	196	$C_{24}H_{22}N_3OSF$	420.52
5h	2-Chloro phenyl-	85	115	$C_{24}H_{22}N_3OSCl$	435.97
5i	2,6-Dichloro phenyl-	77	164	$C_{24}H_{21}N_3OSCl_2$	470.41
5j	3-Nitro phenyl-	82	104	$C_{24}H_{22}N_4O_3S$	446.52
5k	Furfuryl-	90	205	$C_{22}H_{21}N_3O_2S$	392.48
6a	4-Methoxy phenyl-	82	124	$C_{25}H_{25}N_3O_3S$	447.55
6b	4-Chloro phenyl-	80	153	$C_{24}H_{22}N_3O_2SCl$	451.96
6c	4-Dimethylamino phenyl-	75	115	$C_{26}H_{28}N_4O_2S$	460.59
6d	Phenyl-	80	234	$C_{24}H_{23}N_3O_2S$	417.52
6e	3,4-Dimethoxy phenyl-	77	197	$C_{26}H_{27}N_3O_4S$	477.57
6f	3,4,5-Trimethoxy phenyl-	72	106	$C_{27}H_{29}N_3O_5S$	507.60
6g	4-Fluoro phenyl-	82	142	$C_{24}H_{22}N_3O_2SF$	436.52
6h	2-Chloro phenyl-	72	156	$C_{24}H_{22}N_3O_2SCl$	451.96
6i	2,6-Dichloro phenyl-	70	172	$C_{24}H_{21}N_3O_2SCl_2$	486.41
6j	3-Nitro Phenyl-	44	194	$C_{24}H_{22}N_4O_4S$	462.52
6k	Furfuryl-	80	169	$C_{22}H_{21}N_3O_3S$	407.48

prepared by reacting 3-methyl-4-hydroxy acetophenone with an appropriate aldehyde in the presence of base by conventional Claisen-Schmidt condensation. Reaction between newly synthesized chalcones with hydrazine hydrate in ethanol led to synthesis of novel pyrazolines (**4a-k**), which on treatment with various aryl isothiocyanates afforded respective 3,5-disubstituted pyrazolines (**5a-k**) & (**6a-k**) in 65-85% yield. The purity of the compounds was checked by TLC and elemental analyses. Spectral data (^{1}H-NMR & IR) of all the synthesized compounds were in full agreement with the proposed structures.

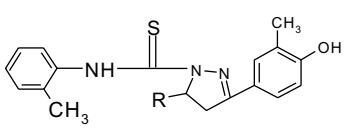
Antiproliferative Activity

The ring substituted pyrazoline derivatives (**5a-k**) and (**6a-k**) were tested for their anti-proliferative activity against *in-vitro* human lung tumor cell lines (A 549) using the MTT assay system. The resulting $_pIC_{50}$ values of cancer cell growth inhibition by the novel pyrazoline analogues are summarized in Table 2. Among the newly synthesized compounds, electron rich group substituted analogue **7** (p-fluro) showed potent inhibitory activities with a $_pIC_{50}$ value of 1.073. Next in the order **2** (p-chloro) showed good inhibitory activity against human lung tumor cell lines (A 549), with a $_pIC_{50}$ value of 1.011. However, furfuryl, 3-NO_2, 2-Cl and 2,6-dichlorosubstituted pyrazolines resulted decreases in activity. On the other hand, the methoxy group mono substituted at the 4-position, disubstituted at the 3,4-position and tri-substituted 3,4,5-position resulted in decreases of the inhibitory activity. These reports clearly showed that the presence of fluro groups at the pyrazoline nucleus causes remarkable improvement in antiproliferative activity.

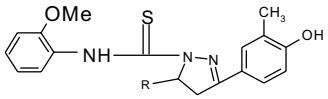
CONCLUSION

Among the newer derivatives, compounds **5g** and **5b**

Table 2. Anti-proliferative activity of the synthesized compounds against human lung tumor cell lines (A 549)



5a-k



6a-k

Compound	R	IC_{50}	μM/mL	$_{P}IC_{50}$ Experimental
5a	4-Methoxy phenyl-	90.5	0.2097	0.6783
5b	4-Chloro phenyl-	42.5	0.0974	1.0110
5c	4-Dimethylamino phenyl-	72.5	0.1630	0.7876
5d	Phenyl-	114	0.2839	0.5468
5e	3,4-Dimethoxy phenyl-	146	0.3163	0.4998
5f	3,4,5-Trimethoxy phenyl-	164	0.3336	0.4767
5g	4-Fluoro phenyl-	35.5	0.0844	1.0730
5h	2-Chloro phenyl-	72.5	0.1662	0.7791
5i	2,6-Dichloro phenyl-	84.25	0.1790	0.7469
5j	3-Nitro phenyl-	98.75	0.2211	0.6553
5k	Furfuryl-	72.5	0.1847	0.7334
6a	4-Methoxy phenyl-	94	0.2100	0.6777
6b	4-Chloro phenyl-	86	0.1902	0.7206
6c	4-Dimethylamino phenyl-	116	0.2518	0.5988
6d	Phenyl-	122	0.2922	0.5343
6e	3,4-Dimethoxy phenyl-	85	0.1779	0.7496
6f	3,4,5-Trimethoxy phenyl-	94	0.18577	0.7323
6g	4-Fluoro phenyl-	60	0.1374	0.8618
6h	2-Chloro phenyl-	82	0.1814	0.7412
6i	2,6-Dichloro phenyl-	74	0.1521	0.8177
6j	3-Nitro phenyl-	102	0.2205	0.6565
6k	Furfuryl-	72	0.1766	0.7527

showed promising activity and the rest showed moderate to good activity against human lung tumor cell lines (A 549). It is conceivable that derivatives showing anti-proliferative activity can be further modified to exhibit better anticancer chemotherapeutic agents. Further studies to acquire more information about quantitative structure-activity relationships (QSAR) are in progress in our laboratory.

EXPERIMENTAL

All chemicals were supplied by E. Merck (GERMANY) and S.D fine chemicals (India). Melting points were determined by open tube capillary method and are uncorrected. Purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in the solvent system toluene-ethyl formate-formic acid (5:4:1) and benzene-methanol (8:2); the spots were located under iodine vapors or UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets). ^{1}H-NMR spectra were recorded on a Bruker AC 300 MHz spectrometer using TMS as internal standard in DMSO/$CDCl_3$.

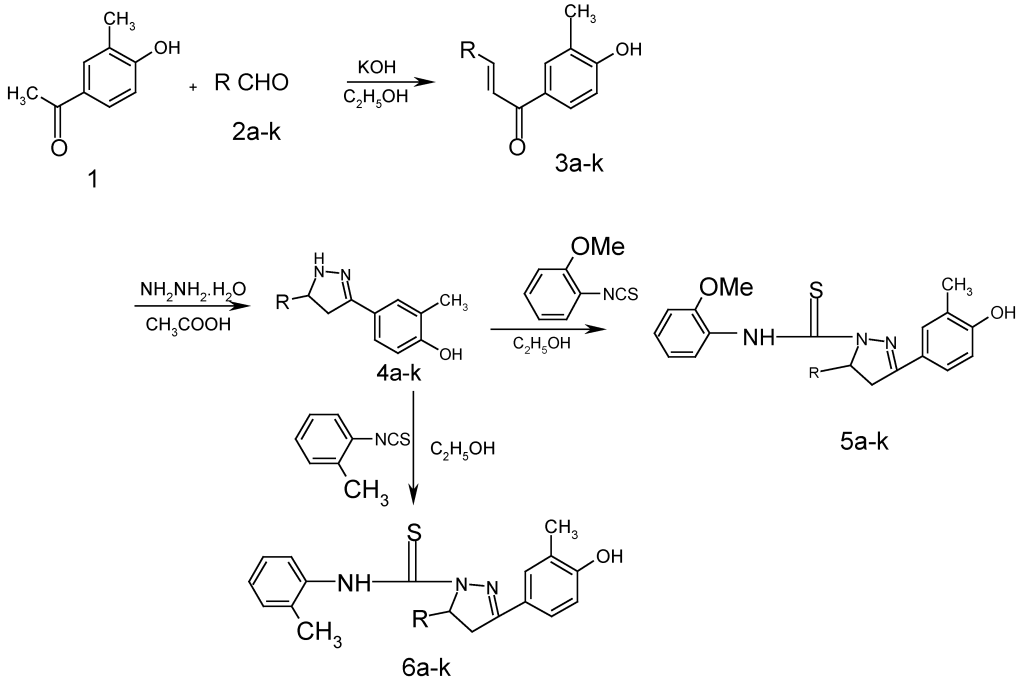
General Procedure

4-Hydroxy-3-methyl acetophenone was dissolved in ethanol. Then, a solution of sodium hydroxide (30%, 5 mL) and suitable substituted aldehydes in 10 mL of Pet. ether was added to the resulting solution with continuous stirring. The resulting solution was allowed to stand overnight. After 4 h stirring, it was then poured into ice-cold water then neutralize with Hcl. The solid separate was filtered off, dried and purified from ethanol (**3a-k**).

General procedure for 4-[5-(substituted)phenyl-4,5-dihydro-1*H*-3-pyrazolyl]-2-methylphenol (4a-k)

To a solution of chalcone (**3a-k**) in ethanol, hydrazine hydrate (99%) was added dropwise. The reaction mixture

Scheme I Protocol for the synthesis



was heated under reflux for 7 hr and then cooled and poured onto crushed ice. The solid pyrazoline product was filtered and recrystalized from ethanol (**4a-k**).

General procedure for 5-(4-(substituted)phenyl)-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-toluidinomethane thione

To a solution of chalcone (**4a-k**) in ethanol (20 mL) was added 2-methyl aryl isothiocyanate (0.01 mol), and the reaction mixture was refluxed for 4 hr. The reaction mixture was cooled and then poured onto crushed ice. Then solid mass separate out was filtered, washed with water and purified from ethanol (**5a-k**).

(**5a**) IR: (KBr) cm^{-1} 3307 (OH), 1596 (C=N), 1320 (C-N), 1130 (C=S), 3224 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (IH, s, NH), 9.7 (1H, s, OH), 7.2-7.4 (11H, m, aromatic), 3.9 (3H, s, OCH_3), 2.8 (6H, s, CH_3), 2.2 (2H, s, CH_2), Cal/Anal [C (69.58) 69.56, H (5.84) 5.86, N (9.74) 9.72].

(**5b**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 770 (C-Cl), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (IH, s, NH), 9.5 (1H, s, OH), 7.2-7.4 (11H, m, aromatic), 2.8 (6H, s, CH_3), 2.2 (2H, s, CH_2). Cal/Anal [C (66.12) 66.10, H (5.09) 5.07, N (9.64) 9.60].

(**5c**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 8.7 (1H, s, OH), 2.8 (6H, s, CH_3), 7.2-7.4 (11H, m, aromatic), 5.2 (1H, s, NH), 3.9 (6H, s, $N(CH_3)_2$), 2.2 (2H, s, CH_2). Cal/Anal [C (70.24) 70.24, H (6.35) 6.34, N (12.60) 12.56].

(**5d**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (IH, s, NH), 9.7 (1H, s, OH), 7.2-7.8 (12H, m, aromatic), 2.8 (6H, s, CH_3), 2.2 (2H, s, CH_2). Cal/Anal [C (71.79) 71.76, H (5.77) 5.74, N (10.46) 10.42].

(**5e**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.2 (1H, s, NH), 8.7 (1H, s, OH), 7.2-7.4 (10H, m, aromatic), 3.9 (6H, s, $OCH_3 \times 2$), 2.8 (6H, s, CH_3), 2.2 (2H, s, CH_2). Cal/Anal [C (67.66) 67.63, H (5.90) 5.85, N (9.10) 9.08].

(**5f**) IR: (KBr) cm^{-1} 3317 (OH), 1596 (C=N), 1320 (C-N), 1132 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.4 (1H, s, NH), 8.7 (1H, s, OH), 7.2-7.4 (9H, m, aromatic), 3.9 (9H, s, $OCH_3 \times 3$), 5.2 (1H, s, NH) 2.8 (6H, s, CH_3), 2.2 (2H, s, CH_2).Cal/Anal [C (65.97) 65.94, H (5.95) 5.93, N (8.55) 8.53].

(**5g**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 824 (C-F), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (1H, s, NH), 8.7 (1H, s, OH), 7.2-7.9

(11H, m, aromatic), 2.8 (6H, s, CH_3), 2.2 (2H, s, CH_2), Cal/Anal [C (68.71) 68.72, H (5.29) 5.26, N (10.02) 10.00].

(**5h**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 770 (C-Cl), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (1H, s, NH), 9.4 (1H, s, OH), 7.2-7.4 (11H, m, aromatic), 2.8 (6H, s, CH_3), 2.2 (2H, s, CH_2). Cal/Anal [C (66.12) 66.10, H (5.09) 510, N (9.64) 9.62].

(**5i**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 770 (C-Cl), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (1H, s, NH), 9.7 (1H, s, OH), 7.2-7.4 (10H, m, aromatic), 2.8 (6H, s, CH_3), 2.2 (2H, s, CH_2), Cal/Anal [C (61.28) 61.26, H (4.50) 4.52, N (8.93) 8.90].

(**5j**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 13.5 (1H, s, NH), 8.6 (1H, s, OH), 2.4 (6H, s, CH_3), 7.2-7.4 (11H, m, aromatic), 2.1 (2H, s, CH_2). Cal/Anal [C (64.56) 64.53, H (4.97) 4.94, N (12.55) 12.53].

(**5k**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (1H, s, NH), 9.2 (1H, s, OH), 2.8 (6H, s, CH_3), 7.8-8.0 (Furan), 7.2-7.4 (7H, m, aromatic), 2.2 (2H, s, CH_2). Cal/Anal [C (67.50) 67.46, H (5.41) 5.40, N (10.73) 10.72].

General procedure for 5-(substituted)phenyl-3-(4-hydroxy-3-methylphenyl)-4,5-dihydro-1*H*-1-pyrazolyl-2-methoxyanilino methane thione

To a solution of chalcone (**4a-k**) in ethanol (20 mL) was added 2-methoxy-aryl isothiocyanate (0.01 mol), and the reaction mixture was refluxed for 4 hr. The reaction mixture was cooled and then poured onto crushed ice. Then solid mass separate out was filtered, washed with water and purified from ethanol (**6a-k**).

(**6a**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (1H, s, NH), 9.7 (1H, s, OH), 6.5-8.4 (11H, m, aromatic), 5.3 (1H, s, CH), 2.5 (3H, s, CH_3), 3.3 (6H, s, OCH_3 × 2), 1.6 (2H, s, CH_2). Cal/Anal [C (67.09) 67.04, H (5.63) 5.62, N (9.39) 9.36].

(**6b**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 770 (C-Cl), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (1H, s, NH), 9.9 (1H, s, OH), 6.5-8.4 (11H, m, aromatic), 5.3 (1H, s, CH), 2.5 (3H, s, CH_3), 3.8 (3H, s, OCH_3), 2.5 (2H, s, CH_2). Cal/Anal [C (63.78) 63.75, H (4.91) 4.90, N (9.30) 9.28].

(**6c**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.0 (1H, s, NH), 9.7 (1H, s, OH), 2.8 (3H, s, CH_3), 7.2-7.4 (11H, m, aromatic), 3.9 (3H, s, OCH_3), 3.9 (6H, s, N(CH_3 × 2), 4.4 (1H, s, CH), 2.2 (2H, s, CH_2). Cal/Anal [C (63.40) 63.38, H (5.73) 5.70, N (11.37) 11.35].

(**6d**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.10 (1H, s, NH), 9.7 (1H, s, OH), 7.2-7.4 (12H, m, aromatic), 4.24 (1H, s, CH), 3.9 (3H, s, OCH_3), 2.8 (3H, s, CH_3), 2.2 (2H, s, CH_2). Cal/Anal [C (69.04) 69.00, H (5.55) 5.53, N (10.06)10.02].

(**6e**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.10 (1H, s, NH), 8.7 (1H, s, OH), 2.8 (3H, s, CH_3), 7.2-7.4 (11H, m, aromatic), 3.9 (9H, s, OCH_3 × 3), 4.24 (1H, s, CH), 2.2 (2H, s, CH_2). Cal/Anal [C (65.39) 65.39, H (5.70) 5.64, N (8.80) 8.76].

(**6f**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.10 (1H, s, NH), 9.7 (1H, s, OH), 2.8 (3H, s, CH_3), 7.2-7.4 (11H, m, aromatic), 3.9 (9H, s, OCH_3 × 4), 4.24 (1H, s, CH), 2.2 (2H, s, CH_2). Cal/Anal [C (63.89) 63.86, H (5.76) 5.72, N (8.28) 8.24].

(**6g**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 820 (C-F), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.10 (1H, s, NH), 9.4 (1H, s, OH), 2.8 (6H, s, CH_3), 3.9 (9H, s, OCH_3), 7.2-7.4 (11H, m, aromatic), 2.2 (2H, s, CH_2), 5.2 (1H, s, NH). Cal/Anal [C (66.19) 66.16, H (5.09) 5.06, N (9.65) 9.64].

(**6h**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 13.0 (1H, s, NH), 9.2 (1H, s, OH), 7.8-8.0 (Furan), 7.2-7.4 (7H, m, aromatic), 3.8 (9H, s, OCH_3), 2.5 (6H, s, CH_3), 2.2 (2H, s, CH_2). Cal/Anal [C (64.85) 64.82, H (5.19) 5.16, N (10.31)10.29].

(**6i**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 770 (C-Cl), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 12.10 (1H, s, NH), 12.0 (1H, s, OH), 2.8 (6H, s, CH_3), 3.9 (9H, s, OCH_3), 7.2-7.4 (11H, m, aromatic), 2.2 (2H, s, CH_2), 5.2 (1H, s, NH). Cal/Anal [C (63.78) 63.75, H (4.91) 4.88, N (9.30) 9.28].

(**6j**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 770 (C-Cl), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm: 10.10 (1H, s, NH), 9.7 (1H, s, OH), 2.8 (6H, s, CH_3), 3.9 (9H, s, OCH_3), 7.2-7.4 (10H, m, aromatic), 2.2 (2H, s, CH_2), 5.2 (IH, s, NH). Cal/Anal [C (59.26) 59.23, H (4.35) 4.36,N (8.64) 8.60].

(**6k**) IR: (KBr) cm^{-1} 3307 (OH), 1590 (C=N), 1320 (C-N), 1130 (C=S), 3220 (NH); ^{1}H-NMR (DMSO-d_6) ppm:

10.10 (1H, s, NH), 9.7 (1H, s, OH), 2.8 (6H, s, CH_3), 3.9 (9H, s, OCH_3), 7.2-7.4 (11H, m, aromatic), 2.2 (2H, s, CH_2). Cal/Anal [C (62.32) 62.30, H (4.79) 4.76, N (12.11) 12.10].

Biological Assay

Cytotoxic Assay

The cytotoxicity assay was carried out using 0.1-mL of cell suspension containing 10,000 cells seeded in each well of a 96 well microtiter plate (Nunc and Tarsons). Fresh medium containing different concentrations of the extracts was added 24 h after seeding. Control cells were incubated without the test extracts and with DMSO (solvent). The very little percentage of DMSO present in the wells (maximal 0.2%) did not affect the experimental results. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for a period of 3 days. Twelve wells were used for each concentration of the extracts. Morphological changes were examined using an inverted microscope. The cells were observed at different time intervals during the incubation period in the presence or absence of the compound. Cellular viability was determined by using the standard MTT (3-(4,5-dimethylthiozole-2-yl)-2,5-diphenyl tetrazolium bromide) assay.[7]

MTT Assay

MTT assay is based on the reduction of the soluble MTT into a blue purple formazan product mainly by mitochondrial reductase activity inside living cells. The number of viable cells were found to be proportional to the extent of formazan production for the cell lines used in this study. After incubation, the solutions in four wells of each concentration were discarded and 50 µl of a solution of 2 mg/mL of MTT (Sigma Chemicals Co., St. Louis, MO, USA) in DMEM (without phenol red) was added and the cultures were incubated for an additional 3 h at 37 °C. The supernatant was removed and the cells were treated with propanol (100 µl/well) and kept aside for 10 min at room temperature. The absorbance was read on a microtiter plate reader (Bio-Rad, Model 550) at a wavelength of 540 nm, and the mean absorbance from four wells was recorded. Mean absorbance taken from cells grown in the absence of the extracts was taken as 100% cell survival (control). The percentage inhibition was calculated using the following formula.

$$\text{Growth Inhibition \%} = 100 \left[\frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\right]$$

The percentage inhibition was plotted against concentration, and the CTC50 (concentration required to reduce viability by 50%) value for each cell line was calculated.

Data Set

These cytotoxic values (IC_{50}) were transformed to pIC_{50} (-log IC_{50}, concentration in molarity) for statistical analysis. The cytotoxic data of these compounds are listed.

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