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Synthesis and biological evaluation of novel Psoralen amide derivatives as potent cytotoxic agents

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ABSTRACT
A new series of novel psoralen (**1)** derivatives of different amides were synthesized. All the derivatives were subjected to 3-(4,5-Dimethylthiazol-yl)-diphenyl tetrazoliumbromide (MTT) cytotoxicity screening against a panel of four different human cancer cell lines viz. Pancreatic (MIAPaCa-2), Leukemia (HL-60 and MOLT-4) and Colon (Colo-205) to check their cytotoxic potential. Interestingly, among the tested molecules, most of the analogs displayed better cytotoxic activity than the parent psoralen (**1)**. Among all the tested amide analogs, compound **10** showed the best activity with IC_{50} of 1.4, and 1.8 µM against Leukemia (HL-60 and MOLT-4)cancer cell lines respectively. Among all the tested cancer cell lines, (HL-60 and MOLT-4) cancer cell line was sensitive towards most of these derivatives, while as, Pancreatic (MIAPaCa-2) cancer cell line was least affected cell line by these derivatives. The present study resulted in identification of broad-spectrum cytotoxic activity of analogs bearing electron withdrawing substituents. Most of the synthesized psoralen derivatives possess acceptable physicochemical properties and are suitable for being further developed as a novel anti-cancer agent in the future.

Keywords: Naturalproducts,Psoralen,Amide

I. INTRODUCTION

Natural products (NPs) have been used to treat human disease for thousands of years and play an increasingly important role in drug discovery and development [1,2].NPs with a coumarin moiety have been reported to display numerous biological and pharmacological activities including anticancer, [3,4] antioxidant, [5,6] anti-inflammatory, [7] antimicrobial, [8] antiviral [9] and enzymatic inhibitory activities [10]. Natural coumarins and their synthetic derivatives are of great interest due to their widespread pharmacological properties. Therefore, to achieve better therapeutic impact, i.e., lower sensitivity and higher efficacy, chemical modification of NPs apparently looks an interesting proposition. Some new intersecting derivatives bearing coumarin ring including the furanocoumarins (imperatorin), pyranocoumarins (seselin) and coumarin sulfamates (coumates) have been found to be useful in photochemotherapy, antitumor and anti-HIV therapy [11,12].

The furanocoumarins are a therapeutically important subtype as they have various clinical applications. Psoralens are naturally occurring plant biosynthetic metabolites that have been used since ancient times in photochemotherapy to treat several skin disorders including mycosis fungoides, psoriasis and vitiligo [13]. Recently psoralens have been used in the regulation of human cervical carcinoma cell proliferation in conjunction with anti-sense technology [14].Psoralens has been used as most effective ones having antifungal and anti-TB activity [15]. Methoxsalen (8-methoxypsoralen) is found in the seeds of the ammimajus (Umbelliferae) and exhibited potent mechanism-based microsomal P 450 inhibitor in vitro [16] and single-dose methoxsalen effects on human cytochrome P 450 2A6 activity [17].Breast cancer is currently a major global health concern with high incidence and mortality rate in women, as there were 2.26 million cases reported in 2020, with the ASR (Age Standardized Incidence Rate per 100,000 people) as high as 47.8, according to the World Health Organization (WHO) [18]. Both

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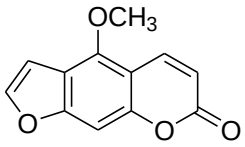
chemotherapy and phototherapy have been regularly used for the treatment of breast cancer, especially in the breast-conserving therapy (BCT), and in combination with surgery [19]. However, the effectiveness drops dramatically if the tumors are detected in the later stages. Moreover, these methods also suffer from a certain degree of limitations such as toxic side effects stemming from the limited selectivity of the currently available anti-cancer drugs[20,21], as well as the lack of efficacy due to the development of resistance in some breast cancerstrains[22]. Consequently, the development of novel anti-breast cancer agents with high efficacy, selectivity, and biocompatibility is highly in demand.

Apart from photoactivation, psoralen derivatives have been demonstrated to possess moderate activity against breast cancer cell lines even in the absence of radiation [23]. Previous studies on the synthesis of novel psoralen derivatives focused on the structural modification at the C-5 position with substituents ranging from immine (II)[24], thiazolidine (III)[24], thiourea (IV)[24], amine (V)[25], and amide (VI)[25].The amide derivative VIa bearing adamantoyl group exhibited promising activity towards both estrogenic (MCF-7) and non-estrogenic (MDA-MB-231) breast cancer cell lines, while little to no activity was found in the naphthoyl derivative VIb. The stark contrast in the activity, together with the lack of the scope of substituents encouraged us to further explore this amide series.

The potential of psoralens has not been fully exploited despite its biological importance; therefore, more efforts are invested towards the building of diverse libraries around its chemical structure and their biological profiles are in demand.



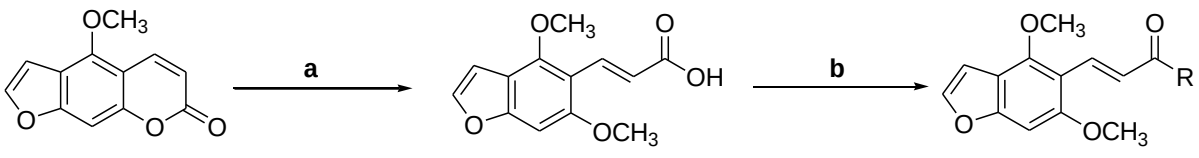
1

Figure 1

Based on the above cited findings and inspiration from the potential biological activities of psoralens, it makes it a very promising molecule for its development as lead compound in the field of drug discovery. The biological studies of psoralens carried out in last few years have provided an additional dimension to the bioactivity profile of the title compound. Therefore, it was thought worthwhile to carry out chemical modifications of this compound with focus to obtain more potent and less toxicanalogs which may qualify as a potential lead compounds as anti-cancer agents and provide information about the SAR of the compound. We directed this work towards the synthesis of a diverse series of novel derivatives having anti-cancer effects using psoralen**1** as a key starting material. The targeted compounds **3-10** were synthesized as depicted in (Scheme 1). This work provides the initial report on structure activity relationship of lactone ring opened coumarins in general and 5-methoxypsoralen (**1**) in particular.

Chemistry

Initially, 5-methoxy psoralen**1** as starting material was subjected to lactone ring opening in DMSO using NaOH as base yielding a trans (E) product which simultaneously undergoes alkylation at OH group in presence of methyl iodide to form trans (E)-3-(4,6-dimethoxybenzofuran-5-yl) acrylic acid **2**.The proposed structure (**2**) was confirmed by spectral data analysis. The trans (E) behaviour of the protons of α, β-unsaturated system was depicted by the presence of two doublets at δ 6.29 and 7.81with the coupling constant (J) of 16.04 Hz each. Compound**2** under reflux conditions was allowed to react with thionyl chloride in DCM (i.e. conversion of acid to acid chloride) and the contents concentrated on rotavapor and in situ appropriate addition of various amines in DCM under dry conditions resulting in the formation of different amides (**3-10**) in good to excellent yields (Scheme 1). The structures of all the synthesised amide derivatives were characterised by analytical and spectral data analysis (^{1}H NMR, ^{13}C NMR, IR, and MS).



1 **2** **3-10**

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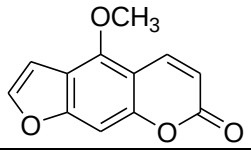
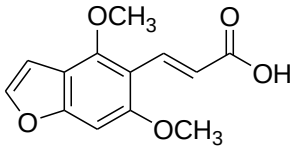
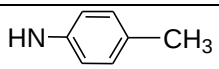
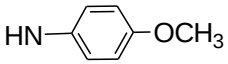
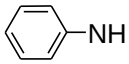
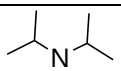
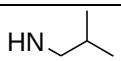
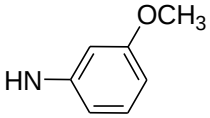
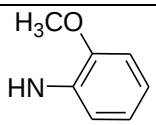
Scheme 1. Reagents and conditions:(**a**) aq. NaOH/DMSO, CH_3I, rt (**b**) $SOCl_2$/DCM, reflux, Amines.

Table-1: Novel amide derivatives of psoralen

Entry	SM/R	M. P. ° C
1		187
2		181
3		152
4		161
5		167
6		155
7		145
8		160
9		171
10		168

Biological

Anti-proliferative activity

There are immeasurable numbers of synthetic drugs to treat the diseased condition, but the treatment is not satisfactory, often due to their severe adverse effects, making provision for the synthesis of new and safer ones. Presently, scientists are keen to evaluate drugs from plant origin, due to their specific curative properties, healthy action, and safe and non-toxic effects. The biological studies of coumarins and related molecules containing coumarin ring; carried out in last few years have provided an additional dimension to the bioactivity. The potential of coumarins has not been fully exploited despite their biological importance; therefore, more efforts toward the building of diverse libraries around its chemical structure and their biological profile are in demand. Thus, based on the reported anti-cancer activity of coumarins, psoralen (**1**), its amide derivatives(**3-10**) were tested for cytotoxic activity against a panel of four different human cancer cell lines viz. Pancreatic (MIAPaCa-2), Leukemia (HL-60 and MOLT-4) and Colon (Colo-205)usingMTT[3-(4,5-Dimethylthiazol-yl)-diphenyl tetrazolium bromide]cytotoxicity screening assay. Preliminary cytotoxicity screening of the analogs was carried out at 60 µM concentration and cell death was determined. BEZ-235 (0.01µM) and Paclitaxel (1µM) were used as positive controls in this assay. The analogs which exhibited significant cytotoxic effect, greater than

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50% growth inhibition at the preliminary screening concentration were further assayed at different concentrations (0.06-60 µM) to generate the IC_{50} values (Table 2). The values are the average of triplicate analysis.

Among all the tested amide analogs, compound **10** exhibited the best results against leukemia (HL-60 and MOLT-4) cancer cell cancer cell lines with IC_{50} of 1.4, and 1.8 µM respectively. Compound **10**having methoxy group at orthoposition displayed better results in comparison to corresponding meta andpara-analogs. Compound**10**bearing a methoxy group at ortho position of R moiety showed potent cytotoxic effect against Leukemia (HL-60 and MOLT-4) cancer cell lines withIC_{50} of 1.4, and 1.8 µM, respectively. The other synthesized derivatives were moderately active against all the tested cancer cell lines. These results demonstrate the broad-spectrum cytotoxic efficiency of compounds bearing electron withdrawing groups, besides the selective activity of analogs with electron donating moieties. Among all the tested cancer cell lines, (HL-60 and MOLT-4) cancer cell line was sensitive towards most of the derivatives, while as, Pancreatic (MIA PaCa-2) cancer cell line was least affected cell line by these derivatives. From these results, we come to the conclusion that it is not only the effect of a particular group but also its position plays a significant role on the bioactivity profile. However, in vivo studies are warranted to investigate the mechanisms of action responsible for the cytotoxic activity of the most active derivatives.

Table-2: IC_{50} values of analogs against colon (colo-205), colon (HCT-116), breast (MCF-7), lung (NCI-322), lung (A549), prostate (PC-3) and Skin (A-431) cancer lines using MTT assay.

Entry	Pancreatic (MIA PaCa-2)	Leukemia (HL-60)	Leukemia (MOLT-4)	Colon (Colo-205)
	IC_{50}			
1	>60	27.4	45.1	>60
2	>60	>60	>60	>60
3	>60	>60	>60	>60
4	>60	>60	20.8	>60
5	>60	>60	>60	>60
6	>60	>60	>60	>60
7	>60	45.1	22.3	46.1
8	>60	12.5	21.2	>60
9	>60	50.1	47.9	>60
10	>60	1.4	1.8	>60

IC_{50} values are expressed in µM concentration.
BEZ-235 (0.01µM) was used as positive control.

II. CONCLUSION

In conclusion, the current study seems to be the first report involving synthesis of ring opening amides derivatives of psoralen**1**and their cytotoxic activity studies using MTT assay. We have demonstrated the cytotoxic activity of a novel library of amides derivatives of psoralen**1**and characterized by spectral data analysis. All the derivatives displayed significant broad-spectrum cytotoxic activity against the tested cancer cell lines. The most active derivative **10** exhibited potent anticancer activity with IC_{50} of 1.4, and 1.8 µM against Leukemia (HL-60 and MOLT-4) cancer cell lines respectively. However further studies especially the in vivo studies need to be carried out for revealing the exact mechanism of action.

Experimental

All reagents used for chemical synthesis were purchased from Sigma-Aldrich. Solvents were distilled before use. All the reactions were monitored by TLC on silica gel F254 plates (E. Merck) using 2% ceric ammonium sulfate solution for detection of the spots. Column chromatography was carried out for purification of the products. All NMR spectra were recorded on Bruker DPX 200, DPX 400 and DPX 500 instruments using $CDCl_3$ as the solvent with TMS as internal standard. The chemical shifts are expressed in delta whereas coupling constants in Hertz. Mass spectra were recorded on ESI-esquire 3000 BrukerDaltonics instrument. IR recorded on FT Bruker (270-30) spectrophotometer. The purity of all the

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compounds was determined by RP-HPLC.Melting points of compounds were recorded on Buchi melting point apparatus B-542.

Synthesis of (E)-3-(4,6-dimethoxybenzofuran-5-yl) acrylic acid 2 (Scheme 1)

To a solution of 5-methoxypsoralen (**1**) (1 g, 1eq.) in DMSO (10 ml), crushed NaOH pellets (1.5 g, 1.5eq.) were added and stirred for 30-40mins at 25 º C. Solution of propargyl bromide (1.1 ml, 1eq.) in DMSO was then added slowly to the mixture and the suspension was stirred for 2-3 h. Progress of reaction was monitored using TLC at regular intervals. After the completion of reaction, the reaction mixture was extracted with ethyl acetate(3 ×30 ml) and the combined organic layer was dried over sodium sulphate and purified through column chromatography to give pure product **2** in 95% yield.^{1}H NMR (400 MHz, $CDCl_3$): δ 3.81 (3H, s), 3.89 (3H, s), 6.29 (1H, d, J=16Hz), 6.86 (1H, d, J=2.28 Hz), 7.09 (1H, s), 7.44 (1H, d, J=2.65 Hz), 6.29 (1H, d, J=15.55 Hz);^{13}C NMR (100 MHz, $CDCl_3$): δ 56.33, 60.65, 93.82, 105.66, 112.53, 116.54, 123.36, 135.23, 146.77, 152.57, 156.57, 157.35, 169.01;IR (KBr) max^{cm-1}: 2941, 1682, 1608, 1593, 1486, 1384, 1366, 1314, 1266, 1162, ESI-MS (m/z): 249.07 (M+ H) $^{+}$.

General procedure for synthesis amide derivatives (3-10)

Thionyl chloride (1.2 eq.) freshly prepared was added to compound **2** dissolved in DCM, and the contents were refluxed for 1h under nitrogen conditions. Then the contents concentrated on rotavapor and re-constituted in DCM (10 ml). To this, appropriate amines (1eq.) were added using dry DCM as solvent, and the contents were stirred for 1h. Progress of reaction was monitored using TLC at regular intervals. After the completion of reaction, the reaction mixture was extracted with DCM (3 ×30 ml) and the combined organic layer was dried over sodium sulphate and purified through column chromatography to give pureamides (**3-10**) in excellent yields of 90-95%.

Spectral data of compounds

4-methoxy-7H-furo[3,2-g] chromen-7-one: 1

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.91 (3H, s), 6.26 (1H, d, J=9.45 Hz), 6.93 (1H, d, J=2.21 Hz), 7.09 (1H, s), 7.61 (1H, d, J=2.67 Hz), 7.99 (1H, d, J=9.50 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 59.6, 105.6, 114.7, 118.5, 121.2, 125.6, 130.3, 142.1, 145.2, 146.4, 147.3, 160.0. IR (KBr) max^{cm-1}: 1717, 1607, 1500, 1495, 1385, 1365; ESI-MS (m/z): 217.06 (M+ H) $^{+}$.

(E)-3-(4,6-dimethoxybenzofuran-5-yl) acrylic acid: 2

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.81 (3H, s), 3.89 (3H, s), 6.29 (1H, d, J=16Hz), 6.86 (1H, d, J=2.28 Hz), 7.09 (1H, s), 7.44 (1H, d, J=2.65 Hz), 6.29 (1H, d, J=15.55 Hz);^{13}C NMR (100 MHz, $CDCl_3$): δ 56.33, 60.65, 93.82, 105.66, 112.53, 116.54, 123.36, 135.23, 146.77, 152.57, 156.57, 157.35, 169.01;IR (KBr) max^{cm-1}: 2941, 1682, 1608, 1593, 1486, 1384, 1366, 1314, 1266, 1162, ESI-MS (m/z): 249.07 (M+ H) $^{+}$.

(E)-3-(4,6-dimethoxybenzofuran-5-yl)-1-(piperidin-1-yl) prop-2-en-1-one: 3

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.65 - 1.78 (m, 6H), 3.36 – 3.40 (m, 4H),3.79 (3H, s), 3.87 (3H, s), 6.69 (1H, d, J=16.04Hz), 7.02 (1H, d, J=2.28 Hz), 7.09 (1H, s), 7.49(1H, d, J=2.30 Hz), 7.68 (1H, d, J=15.75 Hz);^{13}C NMR (100 MHz, $CDCl_3$): δ 23.42, 24.78, 24.99, 45.20, 45.59, 56.79, 60.45, 92.82, 105.66, 112.53, 116.54, 123.44, 140.43, 146.77, 153.43, 155.57, 157.75, 168.89;IR (KBr) max^{cm-1}: 2915, 2851, 1731, 1637, 1595, 1485, 1443, 1381, 1362, 1091, 1022; ESI-MS (m/z): 316.17 (M+ H) $^{+}$.

(E)-3-(4,6-dimethoxybenzofuran-5-yl)-N-p-tolylacrylamide: 4

^{1}H NMR (400 MHz, $CDCl_3$): δ 2.33 (s, 3H), 3.80 (3H, s), 3.84 (3H, s), 6.31 (1H, d, J=16.03Hz), 6.67 (1H, d, J=2.34 Hz), 6.98 (1H, s), 7.20 (2H, d, J=8.06 Hz), 7.38 (2H, d, J=7.79 Hz), 7.59 (1H, d, J=2.64 Hz), 7.74 (1H, d, J=15.95 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.13, 57.79, 60.65, 88.82, 104.43, 110.47, 116.54, 119.70, 120.08, 129.74, 134.10, 136.37, 138.86, 146.77, 152.57, 156.57, 157.65, 167.89; IR (KBr) max^{cm-1}:2925, 1660, 1605, 1562, 1490, 1442, 1386, 1278, 1248, 1160, 1088, 1026, 830; ESI-MS (m/z): 338.15 (M+ H) $^{+}$.

(E)-3-(4,6-dimethoxybenzofuran-5-yl)-N-(4-methoxyphenyl) acrylamide: 5

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.79 (s, 3H), 3.83 (3H, s), 3.88 (3H, s), 6.65 (1H, d, J=16.68Hz), 6.66 (1H, d, J=2.59 Hz), 6.87 (1H, s), 6.95 (2H, d, J=8.24 Hz), 7.40 (2H, d, J=8.81 Hz), 7.47 (1H, d, J=2.95Hz), 8.12 (1H, d, J=16.75 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.04, 56.79, 60.65, 93.82, 106.66, 112.57, 114.59, 116.54, 120.08, 122.37, 131.19, 138.86, 146.77, 152.57, 156.57, 156.78, 157.35, 166.56;IR (KBr) max^{cm-1}: 2935, 2837, 1681, 1620, 1593, 1485, 1462, 1365, 1273, 1247, 1170, 1090;ESI-MS (m/z): 354.12 (M+ H) $^{+}$.

(E)-3-(4,6-dimethoxybenzofuran-5-yl)-N-phenyl acrylamide: 6

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^{1}H NMR (400 MHz, $CDCl_3$): δ 3.84 (s, 3H), 3.86 (3H, s), 6.66 (1H, d, J=2.65 Hz), 6.78 (1H, d, J=15.92 Hz), 6.86 (1H, s), 7.01 (1H, m), 7.33 (2H, m), 7.43 (2H, m),7.45 (1H, d, J=2.78 Hz), 7.97 (1H, d, J=16.05 Hz);^{13}C NMR (100 MHz, $CDCl_3$): δ 56.79, 59.65, 93.82, 106.66, 112.53, 116.54, 120.08, 121.97, 124.36, 129.14, 137.27, 138.86, 146.77, 152.57, 156.35, 157.95, 166.66;IR (KBr) max^{cm-1}:2998, 2793, 1741, 1617, 1555, 1542, 1497, 1403, 1384, 1366;ESI-MS (m/z): 324.13 (M+ H)$^{+}$.

(E)-N,N-diisopropyl-3-(4,6-dimethoxybenzofuran-5-yl)acrylamide: 7

^{1}H NMR (400 MHz, $CDCl_3$): δ1.34 (12H, bs), 3.81 (s, 3H), 3.84 (3H, s), 4.25 (2H, m), 6.70 (1H, d, J=16.12 Hz), 7.01 (1H, s), 7.04 (1H, d, J=2.83 Hz), 7.55 (1H, d, J=16.05 Hz), 7.66 (1H, d, J=2.78 Hz);^{13}C NMR (100 MHz, $CDCl_3$): δ21.07, 47.86, 56.10, 56.37, 93.82, 106.42, 112.25,117.28, 125.34, 142.63, 146.77, 152.57, 156.25, 157.40, 168.36;IR (KBr) max^{cm-1}:2966, 2928, 1732, 1641, 1607, 1484, 1440, 1386, 1373, 1335, 1300, 1270, 1251, 1211, 1161, 1117, 1045, 989;ESI-MS (m/z): 332.15 (M+ H)$^{+}$.

(E)-N-isobutyl-3-(4,6-dimethoxybenzofuran-5-yl) acrylamide: 8

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (6H, d, J= 6.65 Hz), 2.71(m,1H), 3.21 (2H, d, J= 6.35 Hz), 3.79 (s, 3H), 3.82 (3H, s), 6.36 (1H, d, J=16.81Hz), 6.66 (1H, d, J=2.23 Hz), 6.97 (1H, s), 7.27 (1H, d, J=16.65 Hz), 7.59 (1H, d, J=2.38Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.10, 28.18, 48.54, 56.79, 59.65, 94.02, 107.02, 112.53, 116.54, 120.94, 144.30, 147.17, 153.17, 157.15, 158.03, 168.88; IR (KBr) max^{cm-1}:2959, 2871, 1650, 1614, 1594, 1556, 1485, 1386, 1364, 1337, 1272, 1255, 1180, 1170, 1020, 990; ESI-MS (m/z): 304.15 (M+ H)$^{+}$.

(E)-3-(4,6-dimethoxybenzofuran-5-yl)-N-(3-methoxyphenyl) acrylamide: 9

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.73 (s, 3H), 3.81 (3H, s), 3.84 (3H, s), 6.35 (1H, d, J=16.13Hz), 6.66 (1H, d, J=2.59 Hz), 6.69 (1H, m), 6.93 (1H, m), 6.97 (1H, s), 7.23-7.31 (2H, m), 7.60 (1H, d, J=2.95Hz), 7.65 (1H, d, J=16.75 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 56.14, 57.32, 59.34, 93.82, 105.16, 109.99, 112.53, 114.84, 116.54, 120.08, 129.47, 138.86, 139.25, 146.57, 153.57, 156.17, 157.35, 160.43, 166.56;IR (KBr) max^{cm-1}: 2964, 2867, 1651, 1620, 1593, 1462, 1365, 1247, 1170, 1090;ESI-MS (m/z): 354.12 (M+ H)$^{+}$.

(E)-3-(4,6-dimethoxybenzofuran-5-yl)-N-(2-methoxyphenyl) acrylamide: 10

^{1}H NMR (400 MHz, $CDCl_3$): δ 3.79 (s, 3H), 3.84 (3H, s), 3.86 (3H, s), 6.65 (1H, d, J=2.35 Hz), 6.76 (1H, d, J=15.93Hz), 6.86 (1H, s), 6.95-7.06 (3H, m), 7.45 (1H, d, J=2.36Hz), 7.55 (1H, m), 7.96 (1H, d, J=16.05 Hz);^{13}C NMR (100 MHz, $CDCl_3$): δ 56.79, 60.64, 93.82, 105.66, 112.50, 116.54, 120.08, 121.37, 122.52, 125.59, 129.08, 138.16, 146.77, 148.95, 152.57, 156.57, 157.35, 167.09;IR (KBr) max^{cm-1}: 2977, 2887, 1681, 1620, 1593, 1462, 1365, 1273, 1247, 1170, 1090;ESI-MS (m/z): 354.12 (M+ H)$^{+}$.

Biology

RPMI-1640 medium, Penicillin, streptomycin, fetal calf serum, sodium bicarbonate, phosphate buffer saline, trypsin, gentamycin sulphate, trypan blue, ethanol, DMSO, paraformaldehydewere purchased from Sigma Chemicals Co. Glacial acetic acid from Fischer scientific, PBS and trichloroacetic acid (TCA) from Merck specialties private limited. All the human cancer cell lines Pancreatic (MIA PaCa-2), Leukemia (HL-60 and MOLT-4) and Colon (Colo-205) were obtained from National Center for Cell Science, Ganeshkhind, Pune-4111007 (India) and National Cancer Institute, Biological Testing Branch DTP/ DCTD/ NCI, Frederick Cancer Research and Development Center, Fairview Center, Suite 205, 1003 West 7th Street, Frederick, MD 21701-8527 (USA).

MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] assay

To determine IC_{50} value, all the derivatives were evaluated against Pancreatic (MIAPaCa-2), Leukemia (HL-60 and MOLT-4) and Colon (Colo-205) at $3x10^3$ cells per mL per 100 µL per well. Priorly cells were plated into a 96 well tissue culture plate and incubated in Dulbecco's modified eagle's media containing 10% fetal calf serum, supplemented with 100 units/mL penicillin,100 mg/L streptomycin in a humidified atmosphere in 5.0 % CO_2 at 37 °C. Fetal calf serum was reduced to 3% for the experiments with the test material (0.6-60 µM) for 48 h and 4 h before completion of 48 h MTT (50µg/well). Inhibition of formation of colored MTT formazan was taken as an index of cytotoxicity activity. The amount of colored formazan derivative was determined by measuring optical density (OD) using TECAN microplate reader (Infinite M200 PRO) at 570 nm. Further the IC_{50} values on the cancer cells of different tissue origin used for screening were determined by non-linear regression analysis using graph pad prism software.

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