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Original article

Synthesis and evaluation of indole, pyrazole, chromone and pyrimidine based conjugates for tumor growth inhibitory activities – Development of highly efficacious cytotoxic agents

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ABSTRACT

Based upon the lead compounds **10** and **11**, a number of conjugates were synthesized by the combination of chromone-pyrimidine, chromone-indolinone, chromone-pyrazole, indole-pyrimidine, indole-indolinone and indole-pyrazole moieties. Evaluation of these compounds for tumor growth inhibitory activities over 60 human tumor cell lines provided highly efficacious compounds **15**, **41**, **43**, **66**, **69**, and **72** with an average GI_{50} over all the 60 human tumor cell lines as 3.2 μM, 3.1 μM, 1.7 μM, 2.6 μM, 50.1 μM and 2.0 μM, respectively.

1. Introduction

As the number of deaths due to cancer alone is more than those caused by AIDS, malaria, and tuberculosis combined, it is turning into one of the most overwhelming health problems worldwide. In the last 30 years [1,2] the number of patients with tumors get doubled, by 2020 it will double once more, and by 2030 it will be triple and if new treatments are not found, by 2030, there will be 27 million people with cancer and 17 million deaths annually. The practice of chemotherapy of cancer suffers from various drawbacks *viz.* the participation of a number of enzymes like ribonucleotide reductase (RNR), thymidylate synthase (TS), thymidylate phosphorylase (TP), topoisomerase II (topoII) etc. at different stages of development of cancer [3,4], survival of cancer cells even under anaerobic conditions [5–7], and ultimately the problem of multidrug resistance [8–14] developed in the cancerous cells towards chemotherapeutic agents. The understanding about the participation of the enzymes at various stages of cancer led to the development of a number of anticancer agents, some of which are currently in clinical use. The drugs like 5-fluorouracil (**1**, GI_{50} = 17.1 μM, TS inhibitor) [15,16], indomethacin (**2**, GI_{50} = 64.3 μM, COX-2 inhibitor) [17–20], taxol (**3**, GI_{50} = 0.01 μM, topoII inhibitor) [21–23], doxorubicin (**4**, GI_{50} = 0.096 μM, topoII inhibitor) [24], mitoxantrone (**5**, GI_{50} = 0.059 μM, topoII inhibitor) [25–27], indirubin (**6** IC_{50} = 10 μM for CDK1) [28,29], celecoxib (**7**, GI_{50} = 15.9 μM, COX-2 inhibitor) [30–33], and hydrazinecarbothioamide (**8**, GI_{50} = 9.17 μM, DNA antimetabolite) [34] (Chart 1) have created some hope for the life of cancer patients. Flavonoids, [35] the most common group of polyphenolic compounds in the human diet and found ubiquitously in plants, are also known for significant anticancer activity [36]. Therefore, the available anticancer drugs have distinct mechanism of action which may vary in their effects on different types of normal and cancer cells. However, the chemotherapeutic agents under present use suffer from various drawbacks, such as their toxicity to normal cells, bone marrow depression, the adverse effect to the gastrointestinal tract [37] and above all the cost factor. This undoubtedly underscores the need of developing new chemotherapeutic agents for more effective and economical treatment of cancer.

Compounds **10** and **11** [38] (Chart 2) were identified as highly promising candidates for tumor growth inhibitory activities out of which compound **11** showed significantly high maximum tolerable dose (MTD) and presently under investigation for *in-vivo* anticancer activities. Owing to the importance of hybrid molecules [39–41] and to improve the anticancer activities of compounds **10** and **11**, new compounds have been developed (Chart 2) by combining the appropriate fragments of some of the anticancer drugs shown in Chart 1. Some pyrazoles and indolinones-carrying pyridine, naphthyl and styryl moieties were also included in the present investigations.

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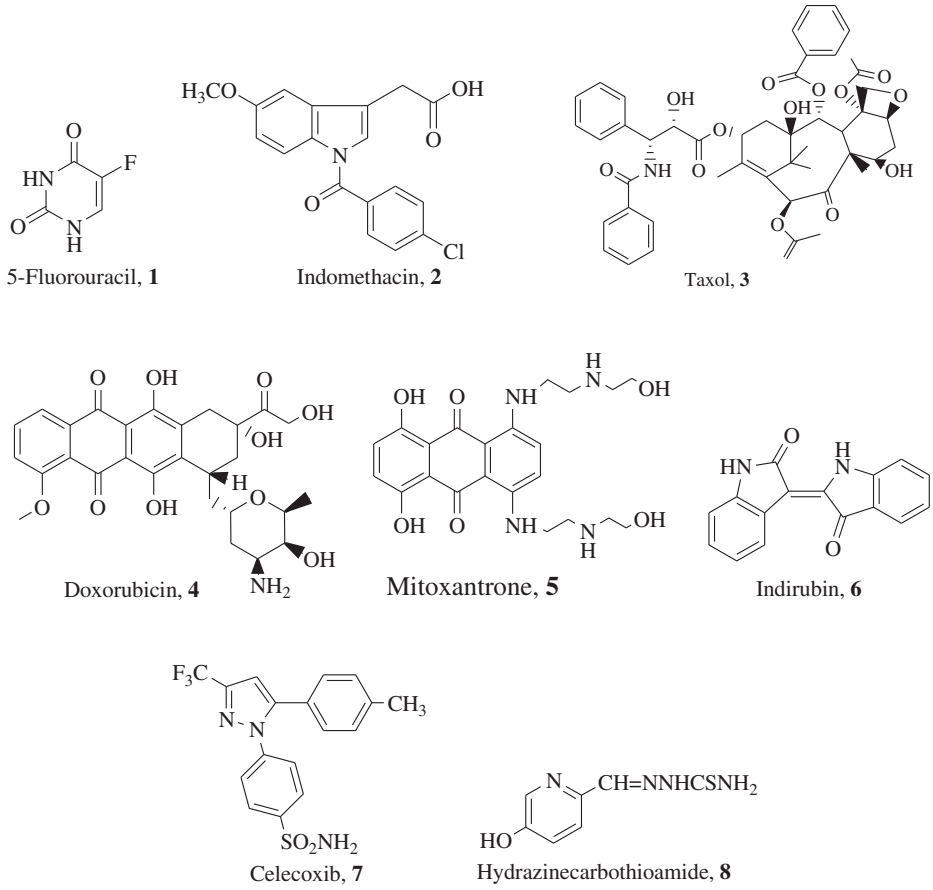


Chart 1. Some clinically used anticancer drugs.

Tumor growth inhibitory activities of these compounds were tested at National Cancer Institute (NCI), Bethesda, USA and led to the identification of new molecules which showed better *in-vitro* activities than 5-fluorouracil, indomethacin and celecoxib.

2. Results and discussion

2.1. MTD test on compounds *10* and *11*

The MTD of compounds **10** and **11** (Chart 2) was determined by Nontumored Animal Toxicity Assay. Intraperitoneous (IP) injections of 400 mg/kg, 200 mg/kg and 100 mg/kg were given to the female athymic nude mice and monitored their weight loss. It was observed that after 19 days there was no survival where compound **10** was injected indicating that all the three doses of compound **10** were toxic while in case of compound **11**, there was no loss and therefore its MTD is 400 mg/kg. The experiment was repeated with compound **10**, this time two doses of 50 mg/kg and 25 mg/kg were given through IP injection to two mice. Now there was no loss after 17 days indicating 50 mg/kg as the MTD of compound **10** (Table S1).

2.2. Hollow fiber assay

The preliminary *in-vivo* activity of compound **10** was demonstrated by hollow fiber assay, which provided quantitative indices of drug efficacy of this compound. A panel of 12 tumor cell lines *viz.* NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX, UACC-62, OVCAR-3, OVCAR-5, U251 and SF-295 was used (supplementary information, Table S2). Based on the MTD, each mice was administered by IP and subcutaneous (SC) injection at 2 dose levels. The fibers were collected from the mice on the day following the fourth compound treatment and subjected to the stable endpoint MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay. The optical density of each sample was determined spectrophotometrically at 540 nm and the mean of each treatment group was calculated. Out of the maximum possible score of 96 for an agent (12 cell lines × 2 sites × 2 dose levels × 2 [score]), compound **10** showed IP score 2 (out of 48), SC score 6 (out of 48) and it did not cause cell killing. *In-vivo* investigation on compound **11** is underway.

2.3. Synthesis and tumor growth inhibitory activities of new conjugates

Encouraged by the tumor growth inhibitory activities and toxicity data of compounds **10** and **11**, a library of compounds was synthesized by the combination of two biologically active heterocycles. Since microwave heating [42,43] is continuing to be an attractive application and has become widely accepted non-conventional energy source for performing organic reactions due to the dramatically short reaction time, improved yields and environmentally benign reactions; here also, the C–C bond formation between the two selected moieties were carried under microwaves. In the synthesis of indole-based compounds, Knoevenegal condensation under microwave irradiation was preceded by the *N*-substitution of indole.

For the first set of compounds, the pyrimidine/indolinone/pyrazole moiety was combined with chromone. A finely ground mixture of 3-formyl chromone (**12**, 1 mmol) and barbituric acid (1.2 mmol) on irradiating in microwave oven for 1 min gave product

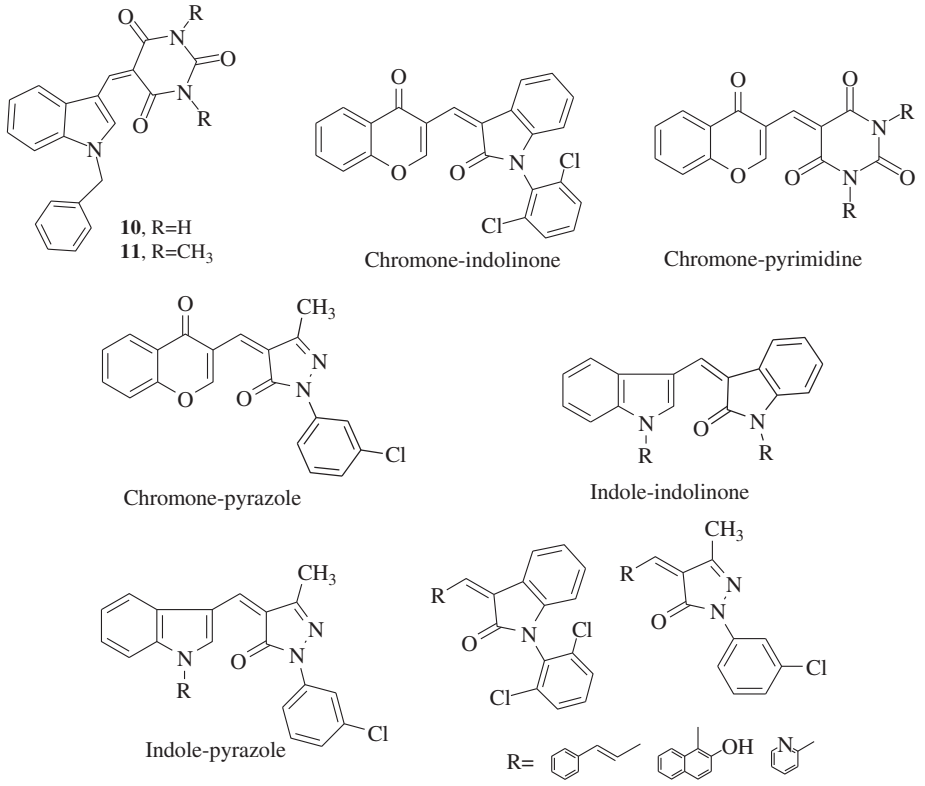
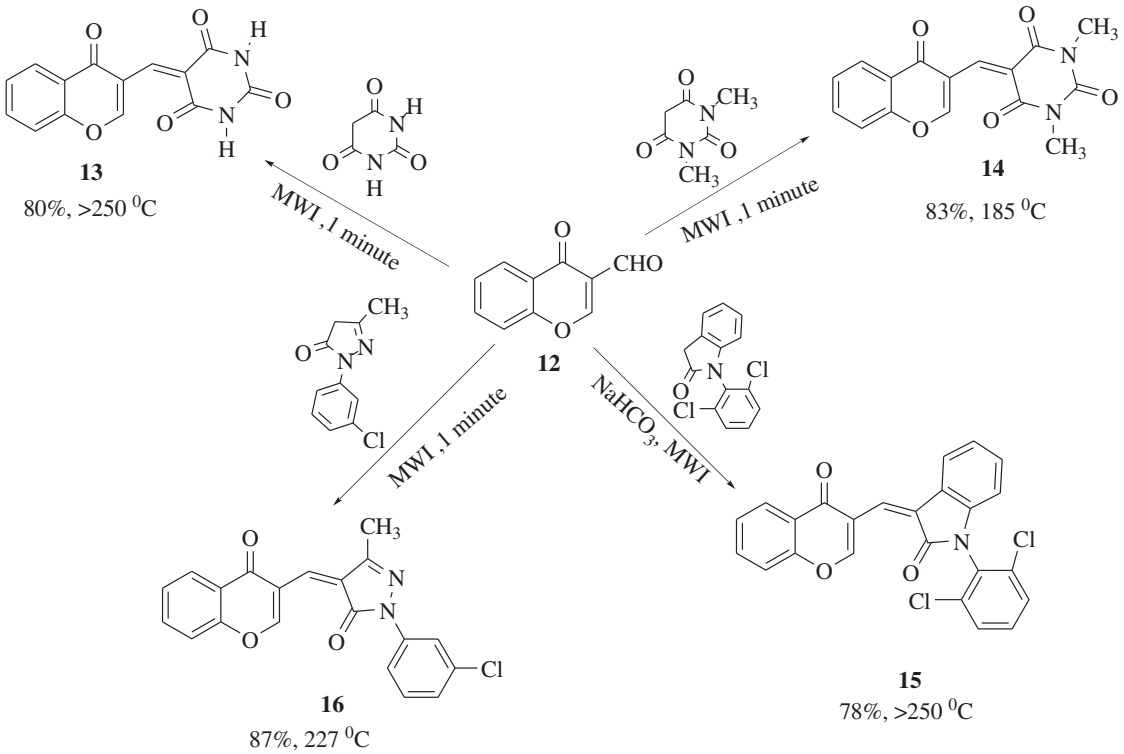


Chart 2. New designed Hybrid molecules.

13 in 80% yield as creamish white solid, mp >250 °C. Similar reactions of **12** with 1,3-dimethylbarbituric acid, indolinone and pyrazole provided the required products **14**, **15** and **16** respectively (Scheme 1).

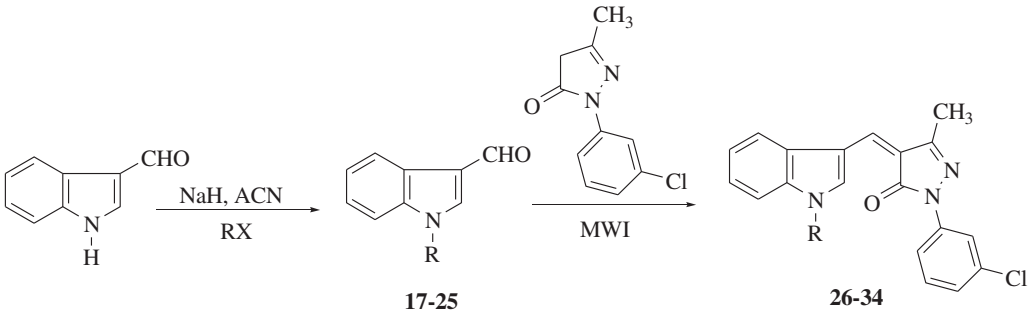
Compounds **14**, **15** and **16** were investigated for their tumor growth inhibitory activities at 10^{-5} M concentration on all the 60 human tumor cell lines. Compounds **14** and **16** showed 30–40% inhibition only at RPMI-8226, SR, M14 and UO-31 cell lines of



Scheme 1. Synthesis of chromone-based compounds.

Table 1
GI_{50} (μM) and LC_{50} (μM) values for compounds **15**, **41**, **43**, **66**, **69** and **72**.

Panel/cell line	**15**		**41**		**43**		**66**		**69**		**72**	
	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}	GI_{50}	LC_{50}
Leukemia												
CCRF-CEM	2.0	>100	0.34	>100	2.3	>100	3.1	>100	97.7	>100	3.2	>100
HL-60 (TB)	2.0	>100	2.4	>100	1.5	9.3	3.1	>100	>100	>100	3.8	>100
K-562	2.0	>100	3.1	>100	2.2	>100	2.8	>100	44.6	>100	3.9	>100
MOLT-4	2.9	>100	2.8	>100	1.5	>100	3.1	>100	>100	>100	4.4	>100
RPMI-8226	1.2	69.1	3.4	>100	1.9	>100	2.8	>100	>100	>100	2.2	>100
SR	1.4	>100	2.2	>100	2.3	>100	2.3	>100	–	–	–	–
Non-small cell lung cancer												
A549/ATCC	4.2	>100	3.2	>100	2.6	>100	16.2	>100	>100	>100	1.9	43.6
EKVX	2.8	>100	2.8	>100	1.6	>100	3.0	>100	>100	>100	2.0	>100
HOP-62	4.7	>100	12.8	>100	1.3	5.4	3.1	57.5	31.6	>100	2.3	89.1
HOP-92	–	–	1.5	>100	1.9	6.3	4.6	>100	–	–	0.01	9.1
NCI-H226	20.8	>100	7.4	83.1	1.6	7.7	2.6	64.5	47.8	>100	–	–
NCI-H23	4.4	>100	4.1	50.1	1.6	6.9	2.3	>100	>100	>100	3.5	>100
NCI-H322M	5.8	>100	2.9	>100	1.9	54.9	28.1	>100	>100	>100	2.8	28.8
NCI-H460	2.0	>100	1.9	7.9	1.6	6.9	1.6	6.4	>100	>100	1.8	7.0
NCI-H522	–	–	4.2	54.9	1.8	70.7	2.6	>100	–	–	1.6	7.5
Colon cancer												
COLO 205	3.8	>100	7.6	54.9	1.8	5.8	2.3	>100	>100	>100	2.3	>100
HCC-2998	4.2	>100	6.0	52.4	1.6	5.7	1.9	6.3	–	–	–	–
HCT-116	1.9	>100	1.6	6.9	1.2	5.0	1.5	6.1	>100	>100	1.7	6.9
HCT-15	0.6	>100	3.4	>100	1.1	7.4	2.2	>100	>100	>100	2.0	28.8
HT29	3.0	>100	3.8	54.9	1.8	6.3	2.9	>100	>100	>100	2.1	9.1
KM12	3.0	>100	1.8	8.9	1.8	7.4	1.6	5.8	>100	>100	1.8	6.3
SW-620	5.2	>100	2.9	52.4	1.9	6.6	1.7	6.7	>100	>100	1.9	6.7
CNS cancer												
SF-268	3.3	>100	2.4	57.5	1.5	8.1	2.3	>100	97.7	>100	3.6	40.7
SF-295	4.2	>100	2.2	66.1	1.7	7.0	3.1	>100	22.9	>100	1.7	13.8
SF-539	3.0	>100	5.0	66.1	1.5	5.7	2.5	>100	0.01	>100	2.4	>100
SNB-19	–	–	7.2	>100	2.7	63.1	5.6	>100	45.70	>100	2.2	32.3
SNB-75	1.9	>100	–	–	1.6	36.3	7.0	>100	0.9	>100	1.4	32.3
U251	1.4	>100	3.4	54.9	1.5	8.5	1.7	23.9	40.7	>100	1.8	7.4
Melanoma												
LOX IMVI	1.5	57.5	1.6	6.6	1.5	6.0	1.4	6.7	>100	>100	1.7	6.4
MALME-3M	2.4	>100	2.6	58.8	1.0	7.2	2.7	>100	29.5	>100	1.9	16.9
M14	5.2	>100	2.3	33.8	–	–	1.6	7.5	>100	>100	1.7	7.7
MDA-MB-435	2.6	>100	2.2	51.2	1.7	6.9	2.5	>100	>100	>100	1.8	61.6
SK-MEL-2	2.7	61.6	–	–	1.9	6.7	3.8	>100	21.8	>100	2.3	24.5
SK-MEL-28	3.8	>100	–	–	2.1	5.4	2.0	8.9	–	–	–	–
SK-MEL-5	2.4	>100	7.6	45.7	1.5	5.4	2.2	20.8	>100	>100	1.6	5.7
UACC-257	4.5	>100	5.0	95.4	2.3	9.1	2.1	>100	>100	>100	1.7	18.2
UACC-62	2.1	>100	4.9	61.6	1.3	5.2	1.7	8.5	39.8	>100	1.6	6.9
Ovarian cancer												
IGROV1	1.6	97.9	–	–	1.2	8.5	1.6	16.5	26.9	>100	1.7	–
OVCAR-3	2.6	>100	1.6	8.1	1.8	7.7	2.1	8.9	58.8	>100	1.7	6.9
OVCAR-4	3.6	>100	–	–	2.3	38.9	3.3	>100	>100	>100	3.7	>100
OVCAR-5	20.8	>100	8.9	>100	–	–	2.8	>100	>100	>100	2.4	>100
OVCAR-8	5.4	>100	4.1	>100	3.2	70.7	2.6	>100	>100	>100	2.1	>100
NCI/ADR-RES	3.3	>100	3.4	>100	–	–	3.3	>100	>100	>100	3.0	>100
SK-OV-3	35.4	>100	8.3	>100	2.1	>100	3.2	>100	26.3	>100	3.4	74.1
Renal cancer												
786–0	3.0	>100	3.3	91.2	1.7	5.8	2.1	10.2	21.3	>100	1.9	6.6
A498	4.3	>100	1.2	21.8	3.1	7.5	17.7	>100	>100	>100	2.9	72.4
ACHN	4.1	>100	3.1	>100	2.3	39.8	1.7	6.1	16.9	>100	1.8	7.2
CAKI-1	2.4	>100	1.9	>100	1.4	>100	1.9	>100	>100	>100	1.3	7.4
RXF 393	1.8	69.1	4.1	43.6	2.0	7.5	2.1	7.2	–	–	–	–
SN12C	3.3	>100	3.9	70.7	1.5	5.4	2.4	>100	>100	>100	2.2	58.8
TK-10	3.3	>100	10.4	>100	2.1	27.5	3.1	>100	34.6	>100	3.0	50.1
UO-31	1.8	>100	1.1	48.9	1.3	7.4	1.6	7.5	>100	>100	1.4	6.0
Prostate cancer												
PC-3	1.3	>100	2.4	>100	1.3	6.4	2.9	>100	>100	>100	2.2	93.3
DU-145	4.6	>100	2.7	46.7	1.7	6.1	2.8	53.7	70.7	>100	2.1	9.1
Breast cancer												
MCF7	3.2	>100	–	–	1.7	8.1	1.1	>100	–	–	–	–
MDA-MB- 31/ATCC	3.8	>100	3.1	>100	1.5	6.4	2.2	>100	36.3	>100	2.0	29.5
HS 578T	9.1	>100	2.2	>100	–	–	1.6	>100	32.3	>100	5.6	>100
BT-549	2.8	>100	2.4	37.1	1.6	11.2	1.6	6.1	27.5	>100	2.4	23.4
T-47D	3.0	>100	4.1	>100	1.9	28.8	2.2	>100	>100	>100	2.3	>100
MDA-MB-468	2.4	>100	4.4	69.1	–	–	1.6	39.8	17.7	>100	0.9	7.4
Mean	3.2	97.7	3.1	58.8	1.7	14.1	2.6	45.7	50.1	>100	2.0	27.5



Scheme 2. Synthesis of indole-pyrazole conjugates.

Leukemia, melanoma and renal cancer (Table S3). Compound **15** showed 50–90% tumor growth inhibition in most of the cell lines with the mean of percent growth over all the cell lines as 40.79 (Table S3). It was further tested at 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} M concentrations and found to exhibit an average GI_{50} (over all the 60 cell lines) 3.2 μM. Compound **15** also showed specificity for certain cell lines like RPMI-8226, SR, HCT-15, U251, LOX IMVI and PC-3 where it exhibited GI_{50} 1.2 μM, 1.4 μM, 0.6 μM, 1.4 μM, 1.5 μM, and 1.3 μM, respectively (Table 1). LC_{50} of compound **15** was 97.7 μM indicating very low toxicity of this compound. Therefore, on the basis of appreciable tumor growth inhibitory activity of compound **15**, it seems that the presence of indole moiety as one of the two heterocycles of these hybrid molecules is essential. In the next sets of molecules, following compounds **10**, **11** and **15**, the indole moiety was retained and combined with pyrazole/indolinone heterocycles.

Treatment of indole-3-carboxaldehyde with NaH in CH_3CN followed by the addition of alkyl/acyl/tosyl halide provided *N*-substituted indole-3-caboxaldehydes **18**–**25** (Scheme 2). Further reaction of compounds **17**–**25** (1 mmol) with pyrazole (1.2 mmol) in microwave oven gave solid compounds **26**–**34** in 1–5 min (Scheme 2, Table 2).

Compounds **26**, **27**, **29**, **30**, **32** and **33** were investigated at 10^{-5} M concentration for tumor growth inhibitory activities over 60 human tumor cell lines. Compounds **26**, **27**, **29**, **32** and **33** did not exhibit anticancer activities as the mean of percent growth of tumor cells over all the 60 cell lines is almost 100 in presence of these compounds (Table S3). Though the mean of percent growth of tumor cells in presence of compound **30** is 89.25, it showed 83% inhibition of tumor growth at NCI-H226 cell line of Non-small cell lung cancer (Table S3). Therefore, the results of the six compounds investigated in this set indicated that the presence of benzyl group at $N-1$ of indole (compound **30**) is leading to some activity in the compound. To see if the presence of more benzyl groups improves the activity of the compound, **10** was modified to compound **35** by introducing benzyl groups at $N-1$ and $N-3$ of pyrimidine moiety (Scheme 3). Compound **35** showed 43–75% tumor growth inhibition at NCI-H460, COLO 205, SF-295, SNB-75, UACC-257, UACC-62, OVCAR-4, SK-OV-3, RXF 393, TK-10, T-47D cell lines of Non-small cell lung cancer, colon, central nervous system (CNS), melanoma, ovarian, renal, and breast cancer with a mean of growth percent of 74.71 (Table S3). Less tumor growth inhibitory activity of compound **35** than that of compounds **10** and **11** indicates that the presence of many hydrophobic substituents in the compound may not be desirable for anticancer activities.

The third category of compounds was built by the combination of two indole moieties (indirubin and sunitinib analogues [44–47]). Reactions of *N*-substituted indole-3-carboxaldehydes and oxindole gave compounds **36**–**42** (Scheme 4, Table 3), *N*-substituted indole-3-carboxaldehyde and indolinone provided compounds **43**–**51** (Scheme 5, Table 4) and the combination of indole-3-carboxaldehye with oxindole [48,49] followed by the introduction of various substituents at nitrogen of two heterocycles resulted in the formation of compounds **52**–**60** (Scheme 6, Table 5).

Compounds **38**, **40**, **41**, **43**, **44**, **46**, **49**, **54**, **55**, **56**, **58** and **59** were investigated by NCI for their tumor growth inhibitory activities. Compounds **38** and **40** showed 30–60% inhibition with their mean of growth percent as 88.85 and 97.46 respectively (Table S4). Compound **41** with 2,6-dichlorobenzoyl group showed notable results with 33–82% tumor growth inhibition and specificity for certain cell lines like CCRF-CEM, HOP-92, HCT-116, KM12, LOX IMVI, OVCAR-3, A489, CAKI-1 and UO-31 where it exhibited GI_{50} 0.34 μM, 1.5 μM, 1.6 μM, 1.8 μM, 1.6 μM, 1.6 μM, 1.2 μM, 1.9 μM, and 1.1 μM, respectively (Table 1). Compound **43** showed 75–99% tumor growth inhibition in most of the cell lines with mean of growth percent inhibition as −18.77% and average GI_{50} (over all the 60 cell lines) 1.7 μM. Compound **43** also showed specificity for HL-60 (TB), MOLT-4, HOP-62, HCT-116, SF-268, SF-539, U251, LOX IMVI, MALME-3M, SK-MEL-3M, UACC-62, IGROV1, CAKI-1, UO-31, and PC-3 cell lines with GI_{50} in the range 1.2 μM–1.5 μM (Table 1). The tumor growth inhibition by compounds **44**, **46** and **49** range from 20 to 66% at HOP-92, HCT-15, OVCAR-4, A498, UO-31, PC-3 and T-47D cell lines of Non-small cell lung cancer, colon, ovarian, renal, prostate and breast cancer with their mean of growth percent inhibitions as 84.76, 88.62, and 102.10, respectively (Table S4). Compounds **54**, **55**, **56**, **58** and **59** exhibited 33–76% tumor growth inhibition at certain cell lines with a mean of growth percent as 99.11, 88.44, 88.46, 75.24, and 94.54, respectively. Therefore, after the combination of two indole moieties, it is the nature of the substituent which also contributes to the activity of the compounds. Here, the presence of 2,6-dichlorobenzoyl and 2,6-dichlorophenyl substituent at one of the two nitrogens led to better tumor growth inhibitory activities of compounds **41** and **43** in comparison to their analogues.

Two more categories of compounds were prepared by introducing appropriate substituents on pyrazole and indolinone moieties. A mixture of pyrazole and an aldehyde after irradiation under microwaves provided the desired products (**66**–**70**) in quantitative yields (Scheme 7, Table 6). The mixture of indolinone and aldehydes (as used in Scheme 7) on irradiating under microwaves in presence of $NaHCO_3$ provided compounds **71**–**74** (Scheme 8, Table 7). It is noteworthy that the Knoevenegal condensation of indolinone (formation of **15**, Schemes 5 and 8) requires $NaHCO_3$.

Compounds **66**, **67**, **69** and **72** were investigated for their tumor growth inhibitory activities. Compound **67** did not produce desirable results, the mean of percent growth of tumor cells over all the 60 cell lines was 108.97 (Table S5). Compounds **66**, **69** and **72** showed 74–99%, 50–99% and 87–99% inhibition in most of the cell lines with the mean of percent growth over all the cell lines as 33.13, 25.40, and 5.01, respectively (Table S5). These were passed to

Table 2
Percentage yields and melting points for indole–pyrazole conjugates **26**–**34**.

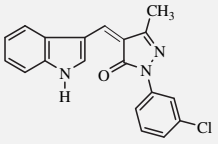
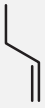
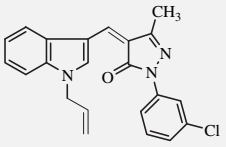
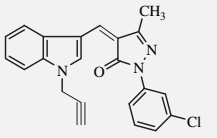
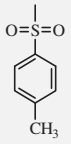
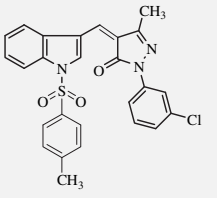
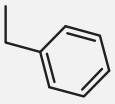
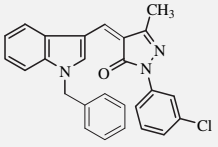
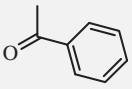
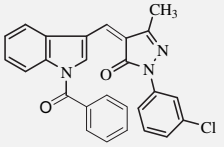
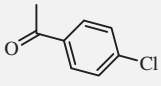
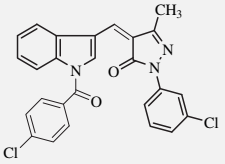
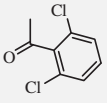
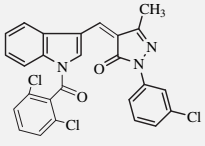
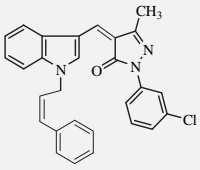
Reactant	R	Product	% yield	Mp (°C)
17	H	 **26**	70	>240
18		 **27**	89	145
19		 **28**	86	193
20		 **29**	86	215 –218
21		 **30**	95	182
22		 **31**	87	>240
23		 **32**	80	>240
24		 **33**	94	>240

Table 2 (*continued*)

Reactant	R	Product	% yield	Mp (°C)
25		 **34**	88	105

further testing at 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} M concentrations and exhibited an average GI_{50} (over all the 60 cell lines) 2.6 μM, 50.1 μM, and 2.0 μM, respectively. Compound **66** also showed specificity for certain cell lines like SR, NCI-H23, NCI-H460, COLO 205, HCC-2998, HCT-116, HCT-15, KM12, SW-620, SF-268, U251, LOX IMVI, M14, SK-MEL-28, SK-MEL-5, UACC-257, UACC-62, IGROV1, OVCAR-3, 786-0, ACHN, CAKI-1, RXF 393, UO-31, MCF7, MDA-MB-231/ATCC, HS 578T, BT-549, T-47D, and MDA-MB-468 with GI_{50} in the range 1.1 μM–2.3 μM (Table 1). Compound **69** showed GI_{50} 0.01 μM at SF-539 cell line of CNS cancer and 0.9 μM at SNB-75 cell line of CNS cancer. Compound **72** also showed specificity for RPMI-8226, A549/ATCC, EKVX, HOP-92, NCI-H460, NCI-H522, COLO 205, HCT-116, HCT-15, HT29, KM12, SW-620, SF-295, SNB-19, SNB-75, U251, LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-5, UACC-257, UACC-62, IGROV1, OVCAR-3, OVCAR-8, 786-0, ACHN, CAKI-1, SN12C, UO-31, PC-3, DU-145, MDA-MB-231/ATCC, and MDA-MB-468 cell lines with GI_{50} ranging from 0.01 μM to 2.3 μM (Table 1). A comparison of the anticancer activities of compounds **66**, **67**, **69**, and **72** showed that the combination of cinnamyl-pyrazole and pyridinyl-indolinone, in the form of compounds **66** and **72**, result in better anticancer activities.

2.4. Docking studies

In order to support the experimental results, the docking [50] of the most active compounds (**15**, **41**, **43**, **66** and **72**) in the active sites of RNR, TS, TP and COX-2 were performed (supporting information, Figs. S1–S20). It was found that each compound shows interactions with more than one enzyme out of RNR, TS, TP and COX-2.

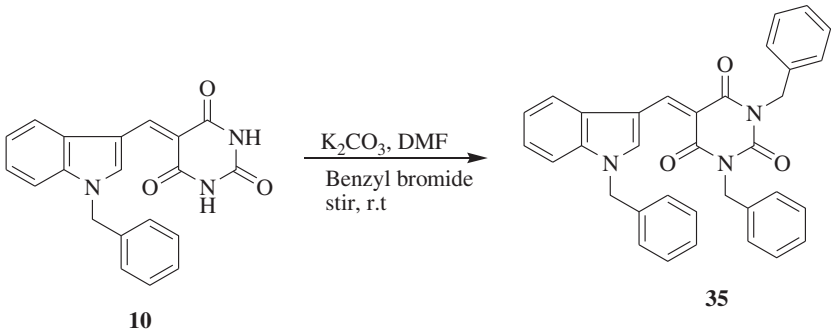
3. Conclusions

Investigations of a series of indole, pyrimidine, pyrazole and chromone based conjugates for tumor growth inhibitory activities have identified compounds **15**, **41**, **43**, **66**, and **72** with GI_{50} (over 60 human tumor cell lines) in the range 1.7 μM–3.2 μM and *in-vitro* therapeutic indices (LC_{50}/GI_{50}) 30.5, 18.9, 10.0, 17.5, and 13.7, respectively. It seems as indole/indolinone along with the presence of benzyl/2,6-dichlorophenyl/2,6-dichlorobenzoyl substituent is essential as one of the two heterocyclic moieties of these hybrid molecules (because all of them except compounds **66** and **69** carry indole/indolinone as one of the two fragments) for their appreciable tumor growth inhibitory activities.

4. Experimental

4.1. Chemistry

Melting points were determined in capillaries and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on JEOL 300 MHz and 75 MHz NMR spectrometer, respectively using $CDCl_3$ and/or DMSO-d_6 as solvent. Chemical shifts are given in ppm with TMS as



Scheme 3. Synthesis of compound **35.**

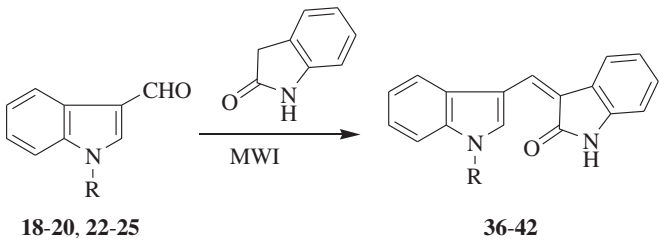
an internal reference. *J*-values are given in Hertz. Signals are abbreviated as singlet, s; doublet, d; double-doublet, dd; triplet, t; multiplet, m. In ^{13}C NMR spectral data, +ve, −ve terms correspond to CH_3, CH, CH_2 signals in DEPT-135 NMR spectra. Chromatography was performed with silica 100–200 mesh and reactions were monitored by thin layer chromatography (TLC) with silica plates coated with silica gel HF-254. The mass spectra were recorded using JEOL SX102/Da-600 instrument. Elemental analysis was performed on Thermoelectron FLASH EA1112 CHN analyzer. Reactions under microwaves were performed using domestic microwave oven (INALSA model 1MW17EG) with microwave power 700 W and operating frequency 2450 MHz.

*Preparation of N-substituted indole-3-carboxaldehyde (***18**–**25***) (Procedure A).* *N*-substituted indole-3-carboxaldehyde was prepared by the treatment of indole-3-carboxaldehyde (1 mmol) with NaH (1.5 mmol) in CH_3CN followed by the addition of alkyl/acyl/tosyl halides (1.5 mmol). After the completion of the reaction as indicated *via* TLC, the reaction mixture was filtered and the filtrate was evaporated to give solid compounds in quantitative yields.

Preparation of conjugate molecules **26**–**42**, **66**–**70** *(Procedure B).* A finely ground mixture of indole-3-carboxaldehyde (**17**–**25**) (1 mmol) and 1-(3-chlorophenyl)-3-methyl-2-pyrazolin-5-one/oxindole (1.2 mmol) on irradiating in microwave oven gave solid products which were washed with diethyl ether to get pure products **26**–**42**. Similar reactions of 1-(3-chlorophenyl)-3-methyl-2-pyrazolin-5-one with cinnamaldehyde/pyridine-2 carboxaldehyde/p-nitrobenzaldehyde/naphthaldehyde/anthranaldehyde (1 mmol) gave products **66**–**70**.

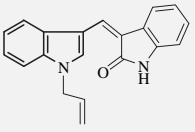
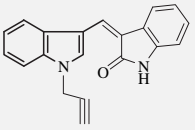
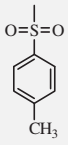
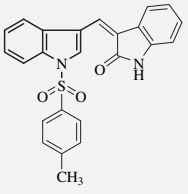
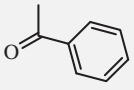
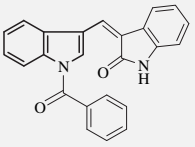
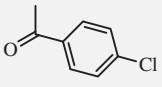
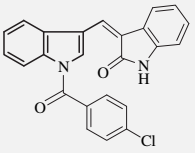
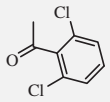
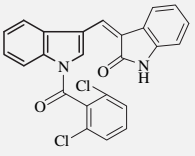
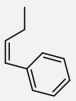
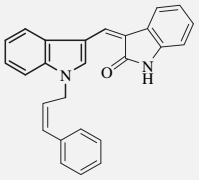
Preparation of conjugates **43**–**51** *and* **71**–**74** *(Procedure C).* Indole-3-carboxaldehyde (**17**–**25**) (1 mmol) and indolinone (1.2 mmol) were irradiated under microwaves in the presence of catalytic amount of $NaHCO_3$. The reaction mixture was extracted with ethyl acetate/chloroform ($CHCl_3$). The combined organic layers were dried over sodium sulphate and concentrated by evaporating the solvent to give the desired products **43**–**51**. Similar reactions of indolinone with cinnamaldehyde/pyridine-2 carboxaldehyde/p-nitrobenzaldehyde/anthranaldehyde (1 mmol) gave products **71**–**74**.

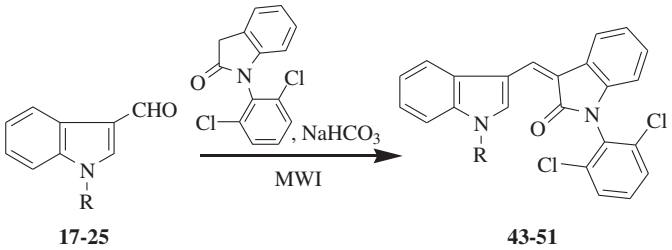
Preparation of indole-oxindole conjugates **52**–**60** *(Procedure D).* A finely ground mixture of indole-3-carboxaldehyde (**17**–**25**)



Scheme 4. Synthesis of indole-oxindole conjugates.

Table 3
Percentage yields and melting points for indole–oxindole conjugates **36**–**42**.

Reactant	R	Product	% yield	Mp (°C)
18		**36** 	90	212
19		**37** 	83	180
20		**38** 	76	232
22		**39** 	85	>240
23		**40** 	83	>240
24		**41** 	80	196
25		**42** 	89	230



17-25 43-51

Scheme 5. Synthesis of indole-indolinone conjugates.

(1 mmol) and oxindole (1.2 mmol) on irradiating in microwave oven gave solid product which was washed with diethyl ether to get pure product **52**. Further the *N*-substitutions of compound **52** were performed following the procedure A to get solid compounds **53**–**60**.

*4.1.1. 5-((4-Oxo-4H-chromen-3-yl)methylene)pyrimidine-2,4,6 (1H,3H,5H)-trione (**13**)*

Creamish white solid, 80% yield, mp >250 °C; ν_{max} (KBr): 1713 (C═O), 3380 (NH); FAB mass *m/z* 285 (M + 1); (Found: C 58.97, H 2.78, N 9.60; $C_{14}H_8N_2O_5$ requires C 59.16, H 2.84, N 9.86).

*4.1.2. 1,3-Dimethyl-5-((4-oxo-4H-chromen-3-yl)methylene) pyrimidine-2,4,6(1H,3H,5H)-trione (**14**)*

Creamish solid, 83% yield, mp 185 °C; ν_{max} (KBr): 1650 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.45 (s, 6H, 2×CH_3), 7.46–7.55 (m, 2H, ArH), 7.73 (dd, $J^3 = 7.2$ Hz, $J^4 = 1.5$ Hz, 1H, ArH), 8.28 (dd, $J^3 = 7.8$ Hz, $J^4 = 1.5$ Hz, 1H, ArH), 8.88 (s, 1H), 9.79 (s, 1H, 2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 28.4 (+ve, CH_3), 29.0 (+ve, CH_3), 117.9 (+ve, CH), 118.34 (+ve, CH), 118.36 (+ve, CH), 123.5 (+ve, CH), 126.3 (+ve, CH), 126.5 (+ve, CH), 134.5 (C), 148.7 (C), 151.0 (C), 155.6 (C), 161.2 (C), 161.7 (C), 163.50 (C), 175.2 (C); FAB mass *m/z* 312 (M + 1); (Found: C 60.86, H 4.13, N 9.27; $C_{16}H_{12}N_2O_5$ requires C 61.54, H 3.87, N 8.97).

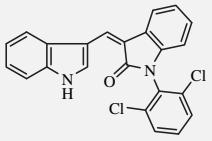
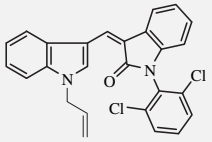
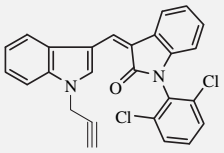
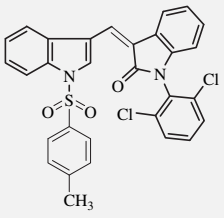
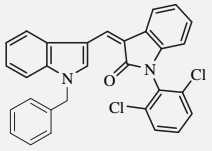
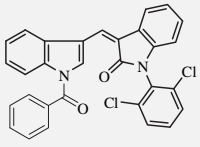
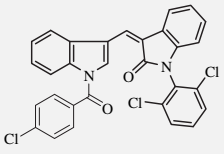
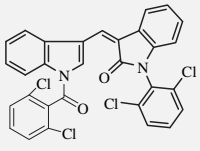
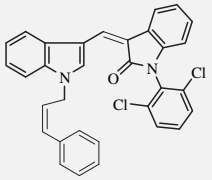
*4.1.3. (3Z)-1-(2,6-Dichlorophenyl)-3-((4-oxo-4H-chromen-3-yl) methylene)indolin-2-one (**15**)*

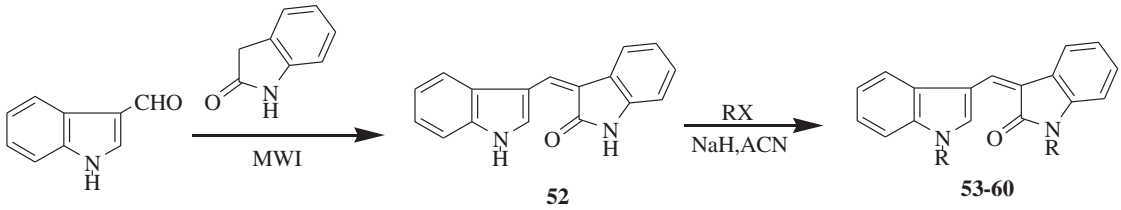
Orange solid, 78% yield, mp >250 °C; ν_{max} (KBr): 1720, 1680 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.44 (d, $J = 7.8$ Hz, 1H, ArH), 7.16 (t, $J = 7.6$ Hz, 1H, ArH), 7.24 (t, $J = 7.7$ Hz, 1H, ArH), 7.41 (t, $J = 8.2$ Hz, 1H, ArH), 7.46 (t, $J = 7.6$ Hz, 1H, ArH), 7.51 (d, $J = 7.8$ Hz, 1H, ArH), 7.54 (d, $J = 8.2$ Hz, 2H, ArH), 7.71 (t, $J = 7.6$ Hz, 1H, ArH), 7.77 (d, $J = 7.6$ Hz, 1H, ArH), 8.18 (s, 1H), 8.32 (d, $J = 8.1$ Hz1H, ArH), 10.29 (s, 1H, ArH); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 109.0 (+ve, CH), 109.1 (C), 118.3 (+ve, CH), 118.5 (C), 120.1 (C), 122.9 (+ve, CH), 123.0 (+ve, CH), 123.7 (+ve, CH), 124.2 (+ve, CH), 124.7 (C), 125.4 (+ve, CH), 125.7 (+ve, CH), 126.4 (+ve, CH), 127.3 (+ve, CH), 127.9 (+ve, CH), 128.9 (+ve, CH), 129.0 (C), 129.1 (C), 130.4 (+ve, CH), 130.8 (+ve, CH), 134.1 (+ve, CH), 135.7 (C), 140.2 (C), 156.0 (C), 161.3 (C), 165.6 (C), 176.0 (C). FAB mass *m/z* 434 (M + 1), 434:436:438 (9:6:1); (Found: C 63.73, H 3.24, N 3.75; $C_{24}H_{13}Cl_2NO_3$ requires C 66.38, H 3.02, N 3.23).

*4.1.4. (4Z)-1-(3-Chlorophenyl)-3-methyl-4-((4-oxo-4H-chromen-3-yl)methylene)-1H-pyrazol-5(4H)-one (**16**)*

Orange solid, 87% yield, mp 227 °C; ν_{max} (KBr): 1705 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.39 (s, 3H, CH_3), 7.17–7.18 (m, 1H, ArH), 7.33 (t, $J = 8.1$ Hz, 1H, ArH), 7.50–7.58 (m, 2H, ArH), 7.74–7.79 (m, 1H, ArH), 7.88–7.91 (m, 1H, ArH), 8.01–8.04 (m, 2H, ArH), 8.29 (dd, $J^3 = 8.1$ Hz, $J^4 = 1.5$ Hz, 1H, ArH), 10.75 (s, 1H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 22.5 (+ve, CH_3), 117.93 (+ve, CH), 118.33 (+ve, CH), 122.3 (+ve, CH), 123.59 (+ve, CH), 126.5 (+ve, CH), 130.3 (+ve, CH), 130.6 (+ve, CH), 135.2 (+ve, CH), 150.5 (+ve, CH), 155.6

Table 4
Percentage yields and melting points for indole–indolinone conjugates **43**–**51**.

Product	% yield	Mp (°C)
 **43**	95	242
 **44**	83	178
 **45**	85	168
 **46**	78	>240
 **47**	81	232
 **48**	90	170
 **49**	75	>260
 **50**	82	250
 **51**	91	190



Scheme 6. Synthesis of indole-oxindole conjugates.

(+ve, CH), 118.38 (C), 126.3 (C), 134.53 (C), 138.7 (C), 148.5 (C), 161.2 (C), 165.7 (C), 175.2 (C); FAB mass *m/z* 365 (M + 1), 365:367 (3:1); (Found: C 66.13, H 3.73, N 7.70; $C_{20}H_{13}ClN_2O_3$ requires C 65.85, H 3.59, N 7.68).

*4.1.5. (4Z)-4-((1H-Indol-3-yl)methylene)-1-(3-chlorophenyl)-3-methyl-1H-pyrazol-5(4H)-one (**26**)*

Reddish orange solid, 70% yield, mp >240 °C; ν_{max} (KBr): 1640 (C═O), 3200 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.38 (s, 3H, CH_3), 7.16–7.35 (m, 6H, ArH), 7.37 (t, J = 8.7 Hz, 2H, ArH), 7.85–8.00 (m, 1H, ArH), 8.00 (t, J = 2.1 Hz, 1H, indole2-H); FAB mass *m/z* 335 (M^+), 335:337 (3:1); (Found: C 67.77, H 4.24, N 12.45; $C_{19}H_{14}ClN_3O$ requires C 67.96, H 4.20, N 12.51).

*4.1.6. (4Z)-4-((1-Allyl-1H-indol-3-yl)methylene)-1-(3-chlorophenyl)-3-methyl-1H-pyrazol-5(4H)-one (**27**)*

Shiny orange solid, 89% yield, mp 145 °C; ν_{max} (KBr): 1680 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$):δ 2.41 (s, 3H, CH_3), 4.8 (dd, J^2 = 5.4 Hz, J^3 = 1.8 Hz, 2H, CH_2), 5.3 (ddd, J^2 = 16.8 Hz, J^3 = 10.2 Hz, J^4 = 0.3 Hz, 2H, CH_2), 6.05 (multiplet of 12 lines, 1H, CH), 7.11 (dd, J^3 = 6.9 Hz, J^4 = 0.9 Hz, 1H, ArH), 7.14–7.45 (m, 4H, ArH), 7.82 (s, 1H, =H), 7.88–7.90 (m, 1H, ArH), 7.96 (dd, J^3 = 6.6 Hz, J^4 = 0.9 Hz, 1H, ArH), 8.11 (t, J = 2.1 Hz, 1H, ArH), 9.85 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 13.1 (+ve, CH_3), 50.1 (−ve, CH_2), 111.2 (C), 112.0 (+ve, CH), 116.7 (−ve, CH_2), 118.0 (+ve, CH), 118.8 (+ve, CH), 118.9 (+ve, CH), 119.3 (+ve, CH), 122.7 (+ve, CH), 123.7 (+ve, CH), 124.1 (+ve, CH), 129.2 (C), 129.7 (C), 131.4 (C), 134.4 (+ve, CH), 135.5 (C), 136.6 (C), 140.0 (+ve, CH), 140.7 (C), 150.9 (C), 163.4 (C); FAB mass *m/z* 375 (M^+), 375:377 (3:1); (Found: C 69.97, H 4.74, N 10.95; $C_{22}H_{18}ClN_3O$ requires C 70.30, H 4.83, N 11.18).

*4.1.7. (4Z)-1-(3-Chlorophenyl)-3-methyl-4-((1-(prop-2-ynyl)-1H-indol-3-yl)methylene)-1H-pyrazol-5(4H)-one (**28**)*

Reddish solid, 86% yield, mp 193 °C; ν_{max} (KBr): 1650 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41 (s, 3H, CH_3), 2.52 (t, J = 2.7 Hz, 1H, ≡H), 5.03 (d, J = 2.7 Hz, 2H, CH_2), 7.14 (dd, J^3 = 6.9, J^4 = 0.9 Hz, 1H, ArH), 7.25–7.43 (m, 4H, ArH), 7.55 (dd, J^3 = 5.1 Hz, J^4 = 1.5 Hz, 1H, ArH), 7.78 (s, 1H, =H), 7.88 (dd, J^3 = 4.2 Hz, J^4 = 2.7 Hz, 1H, ArH), 7.96 (dd, J^3 = 6.6 Hz, J^4 = 0.9 Hz, 1H, ArH), 9.91 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 13.1 (+ve, CH_3), 37.2 (−ve, CH_2), 75.2 (+ve, CH), 75.8 (C), 110.9 (C), 112.2 (+ve, CH), 116.6 (+ve, CH), 118.1 (+ve, CH), 118.7 (+ve, CH), 120.0 (+ve, CH), 122.9 (+ve, CH), 124.0 (+ve, CH), 124.2 (C), 129.2 (+ve, CH), 129.7 (+ve, CH), 134.4 (C), 135.3 (+ve, CH), 136.1 (C), 139.9 (C), 150.8 (C), 163.3 (C); FAB mass *m/z* 373 (M^+), 373:375 (3:1); (Found: C 70.49, H, 4.53, N 11.02; $C_{22}H_{16}ClN_3O$ requires C 70.68, H 4.31, N, 11.24).

*4.1.8. (4Z)-1-(3-Chlorophenyl)-3-methyl-4-((1-tosyl-1H-indol-3-yl)methylene)-1H-pyrazol-5(4H)-one (**29**)*

Orange solid, 86% yield, mp 215–18 °C; ν_{max} (KBr): 1660 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.16 (dd, J^3 = 7.8 Hz, J^4 = 0.9 Hz, 1H, ArH), 7.27 (d, J = 8.4 Hz, 2H, ArH), 7.34–7.44 (m, 3H, ArH), 7.61 (s, 1H, =H), 7.75 (dd, J^3 = 6.45 Hz, J^4 = 2.7 Hz, 1H, ArH), 7.94 (d, J = 8.4 Hz, 2H, ArH), 8.01 (dd, J^3 = 7.8 Hz, J^4 = 2.1 Hz, 1H, ArH), 8.05 (dd, J^3 = 6.6 Hz, J^4 = 2.4 Hz, 1H, ArH), 8.10 (t, J = 2.1 Hz, 1H, ArH), 9.90 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 13.1 (+ve, CH_3), 21.6 (+ve, CH_3), 114.0 (C), 115.2 (+ve, CH), 116.6 (+ve, CH), 118.2 (+ve, CH), 118.7 (+ve, CH), 124.3 (+ve, CH), 124.5 (+ve, CH), 125.2 (+ve, CH), 125.6 (C), 127.3 (+ve, CH), 129.81 (+ve, CH), 129.85 (+ve, CH), 130.2 (+ve, CH), 133.1 (+ve, CH), 134.4 (C), 134.5 (C), 134.6 (C), 136.0 (C), 139.5 (C), 145.9 (+ve, CH), 150.3 (C), 162.5 (C); FAB mass *m/z* 490 (M + 1), 490:492 (3:1); (Found: C 63.59, H 4.00, N 8.40, S 6.47; $C_{26}H_{20}ClN_3O_3S$ requires C 63.73, H 4.11, N 8.58, S 6.54).

*4.1.9. (4Z)-4-((1-benzyl-1H-indol-3-yl)methylene)-1-(3-chlorophenyl)-3-methyl-1H-pyrazol-5(4H)-one (**30**)*

Reddish orange solid, 95% yield, mp 182 °C; ν_{max} (KBr): 1660 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.42 (s, 3H, CH_3), 5.49 (s, 2H, CH_2), 7.13–7.35 (m, 9H, ArH), 7.84 (s, 1H, =H), 7.88–8.11 (m, 4H ArH), 9.98 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 13.1 (+ve, CH_3), 51.5 (−ve, CH_2), 109.4 (C), 111.4 (+ve, CH), 112.1 (C), 116.6 (+ve, CH), 118.0 (+ve, CH), 118.7 (+ve, CH), 119.5 (C), 120.7 (C), 122.7 (+ve, CH), 123.8 (+ve, CH), 124.1 (+ve, CH), 126.3 (C), 126.7 (+ve, CH), 128.1 (+ve, CH), 128.9 (+ve, CH), 129.3 (C), 129.7 (+ve, CH), 129.8 (+ve, CH), 134.4 (C), 134.5 (C), 135.2 (C), 135.4 (+ve, CH), 136.6 (C), 139.9 (C), 141.0 (+ve, CH), 150.8 (C), 161.3 (C), 163.3 (C); FAB mass *m/z* 426 (M + 1), 426:428 (3:1); (Found: C 72.94, H 4.88, N 9.58; $C_{26}H_{20}ClN_3O$ requires C 73.32, H 4.73, N 9.87).

*4.1.10. Compound **31***

Reddish orange solid, 87% yield, mp >240 °C; ν_{max} (KBr): 1670 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, CH_3), 7.11–7.14 (m, 1H, ArH), 7.22–7.52 (m, 4H, ArH), 7.61–7.74 (m, 5H, ArH), 7.86–8.01 (m, 3H, ArH), 8.46–8.49 (m, 1H, =H), 9.96 (s, 1H, indole2-H); FAB mass *m/z* 441 (M + 2), 441:443 (3:1); (Found: C 70.74, H 4.32, N 9.80; $C_{26}H_{18}ClN_3O_2$ requires C 70.99, H 4.12, N 9.55).

*4.1.11. Compound **32***

Reddish orange solid, 80% yield, mp >240 °C; ν_{max} (KBr): 1620 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.43 (s, 3H, CH_3), 7.13–7.16 (m, 1H, ArH), 7.25–7.52 (m, 4H, ArH), 7.60–8.01 (m, 7H, ArH), 8.43–8.47 (m, 1H, =H), 9.92 (s, 1H, indole2-H); FAB mass *m/z* 473 (M^+), 473:475:477 (9:6:1); (Found: C 65.48, H 3.54, N 8.53; $C_{26}H_{17}Cl_2N_3O_2$ requires C 65.83, H 3.61, N 8.86).

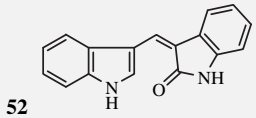
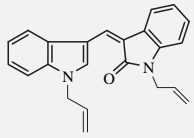
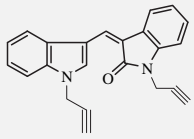
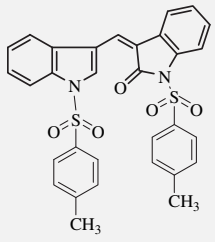
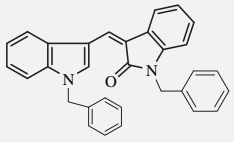
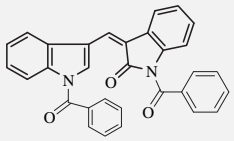
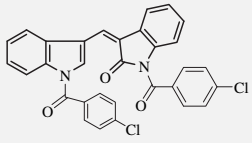
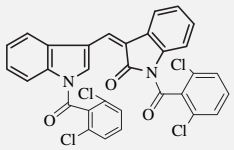
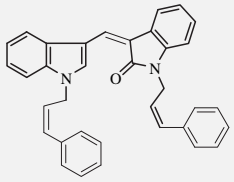
*4.1.12. Compound **33***

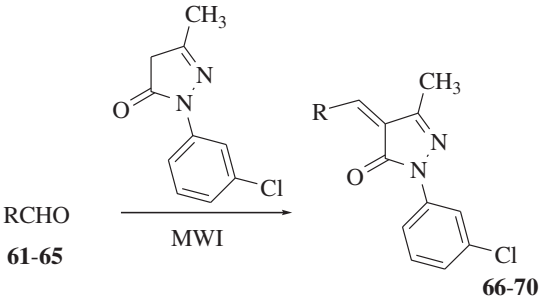
Reddish orange solid, 94% yield, mp >240 °C; ν_{max} (KBr): 1680 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 7.06–7.50 (m, 8H, ArH), 7.70–7.78 (m, 3H, ArH), 8.57 (s, 1H, =H), 9.44 (s, 1H, indole2-H); FAB mass *m/z* 508 (M^+), 508:510:512:514 (27:27:9:1); (Found: C 61.58, H 3.14, N 8.33; $C_{26}H_{16}Cl_3N_3O_2$ requires C 61.38, H 3.17, N 8.26).

*4.1.13. (4Z)-1-(3-Chlorophenyl)-4-((1-cinnamyl-1H-indol-3-yl) methylene)-3-methyl-1H-pyrazol-5(4H)-one (**34**)*

Reddish orange solid, 88% yield, mp 105 °C; ν_{max} (KBr): 1620 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41 (s, 3H, CH_3), 5.05 (dd,

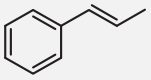
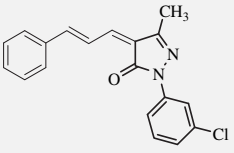
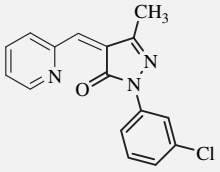
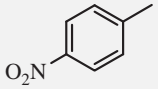
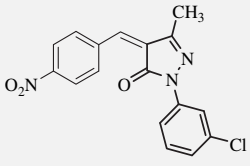
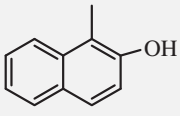
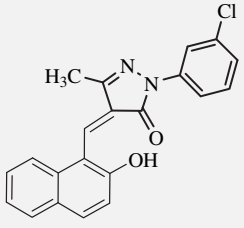
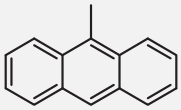
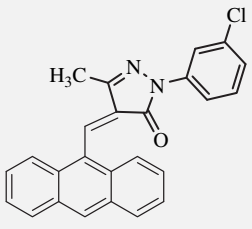
Table 5
Percentage yields and melting points for indole–oxindole conjugates **52**–**60**.

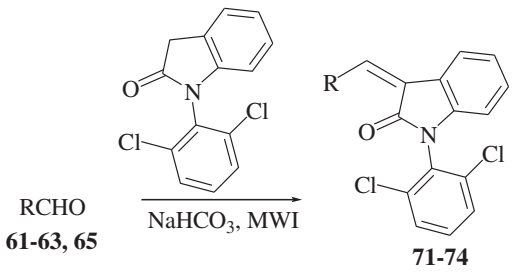
Product	% yield	Mp (°C)
 **52**	95	220
 **53**	82	210
 **54**	81	>240
 **55**	73	>250
 **56**	85	148
 **57**	87	238
 **58**	81	>250
 **59**	87	185
 **60**	92	160



Scheme 7. Synthesis of aromatic aldehyde-pyrazole conjugates.

Table 6
Percentage yields and melting points for aromatic aldehyde–pyrazole conjugates **66**–**70**.

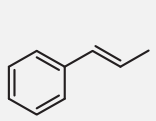
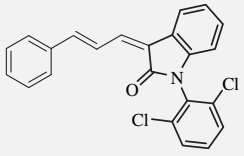
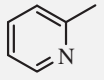
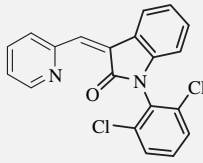
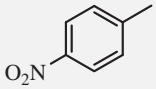
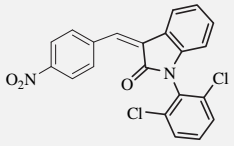
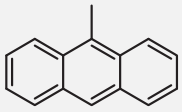
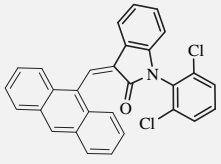
Reactant	R	Product	% yield	Mp (°C)
61		 **66**	95	135
62		 **67**	94	192
63		 **68**	96	158
64		 **69**	90	200
65		 **70**	94	145



Scheme 8. Synthesis of aromatic aldehyde-indolinone conjugates.

J^2 = 6 Hz, J^3 = 1.2 Hz, 2H, CH_2), 6.31–6.56 (m, 1H, =H), 6.62 (s, 1H, =H), 7.09–7.20 (m, 1H, ArH), 7.21–7.38 (m, 4H, ArH), 7.47–7.49 (m, 3H, ArH), 7.81–7.90 (m, 3H, ArH), 7.95–7.98 (m, 1H, ArH), 7.98 (s, 1H, =H), 8.10 (t, J = 1.8 Hz, 1H, ArH), 9.90 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 12.8 (+ve, CH_3), 49.9 (−ve, CH_2), 109.5 (C), 111.3 (+ve, CH), 118.1 (+ve, CH), 118.7 (+ve, CH), 120.8 (+ve, CH), 122.7 (+ve, CH), 122.8 (+ve, CH), 123.8 (+ve, CH), 124.1 (+ve, CH), 126.4 (+ve, CH), 126.6 (+ve, CH), 128.2 (+ve, CH), 128.6 (+ve, CH), 129.7 (+ve, CH), 129.9 (+ve, CH), 133.9 (+ve, CH), 135.5 (+ve, CH), 138.4 (+ve, CH), 140.7 (+ve, CH), 118.8 (C), 119.4 (C), 129.3 (C), 134.4 (C), 134.6 (C), 135.7 (C), 136.7 (C), 138.6 (C), 140.0 (C), 153.0 (C), 161.5 (C), 163.4 (C); FAB mass *m/z* 451 (M^+), 451:453 (3:1); (Found: C 74.62, H 4.91, N 9.68; $C_{28}H_{22}ClN_3O$ requires C 74.41, H 4.91, N 9.30).

Table 7
Percentage yields and melting points for aromatic aldehyde–indolinone conjugates **71**–**74**.

Reactant	R	Product	% yield	Mp (°C)
61		 **71**	95	203
62		 **72**	86	188
63		 **73**	83	190
65		 **74**	89	177

*4.1.14. 1,3-Dibenzyl-5-((1-benzyl-1H-indol-3-yl)methylene) pyrimidine-2,4,6(1H,3H,5H)-trione (**35**)*

Yellow solid, 80% yield, mp 192 °C; ν_{max} (KBr): 1630 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.20 (s, 4H, 2×CH_2), 5.48 (s, 2H, CH_2), 7.18 (dd, J^3 = 7.2 Hz, J^4 = 1.8 Hz, 2H, ArH), 7.25–7.50 (m, 16H, ArH), 7.99 (d, J = 7.8 Hz, 1H, ArH), 9.06 (s, 1H, =H), 9.69 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 44.5 (−ve, CH_2), 45.3 (−ve, CH_2), 51.8 (−ve, CH_2), 108.4 (C), 111.3 (+ve, CH), 112.4 (C), 118.8 (+ve, CH), 123.3 (+ve, CH), 124.2 (+ve, CH), 126.8 (+ve, CH), 127.4 (+ve, CH), 127.5 (+ve, CH), 128.3 (+ve, CH), 128.4 (+ve, CH), 128.6 (+ve, CH), 129.0 (+ve, CH), 130.7 (C), 134.8 (C), 136.7 (C), 137.1 (C), 142.9 (+ve, CH), 146.8 (+ve, CH), 151.5 (C), 161.7 (C), 163.5 (C); FAB mass *m/z* 527 (M + 2); (Found: C 77.85, H 4.94, N 8.25; $C_{34}H_{27}N_3O_3$ requires C 77.70, H 5.18, N 7.99).

*4.1.15. (Z)-3-((1-Allyl-1H-indol-3-yl)methylene)indolin-2-one (**36**)*

Yellow solid, 90% yield, mp 212 °C; ν_{max} (KBr): 1670 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.8 (d, J = 3 Hz, 2H, CH_2), 5.2 (dd, J^2 = 9 Hz, J^3 = 3 Hz, 2H, CH_2), 6.02–6.09 (m, 1H, CH), 6.8 (d, J = 8.4 Hz, 1H, ArH), 7.0 (t, J = 4.5 Hz, 1H, ArH), 7.1 (t, J = 4.5 Hz, 1H, ArH), 7.2–7.4 (m, 2H, ArH), 7.6 (d, J = 4.5 Hz, 1H, ArH), 7.7–7.9 (m, 2H, ArH), 7.9 (s, 1H, =H), 9.4 (s, 1H, indole2-H); FAB mass *m/z* 301 (M + 1); (Found: C 79.78, H 5.38, N 9.17; $C_{20}H_{16}N_2O$ requires C 79.98, H 5.37, N 9.33).

*4.1.16. (Z)-3-((1-(prop-2-ynyl)-1H-indol-3-yl)methylene)indolin-2-one (**37**)*

Dark red solid, 83% yield, mp 180 °C; ν_{max} (KBr): 1680 (C=O), 3450 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.4 (s, 1H, ≡H), 4.9 (d, J = 2.4 Hz, 2H, CH_2), 6.8–8.2 (m, 9H, ArH+ =H), 10.0 (s, 1H, indole2-H); FAB mass *m/z* 298 (M^+); (Found: C 80.58, H 4.68, N 9.37; $C_{20}H_{14}N_2O$ requires C 80.52, H 4.73, N 9.39).

*4.1.17. (Z)-3-((1-Tosyl-1H-indol-3-yl)methylene)indolin-2-one (**38**)*

Dark red solid, 76% yield, mp 232 °C; ν_{max} (KBr): 1668 (C=O), 3430 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 6.88–7.43 (m, 2H, ArH), 7.59 (t, J = 8.4 Hz, 1H, ArH), 7.71–8.11 (m, 9H, ArH), 8.35 (s, 1H, =H), 9.74 (s, 1H, indole2-H); FAB mass *m/z* 414 (M^+); (Found: C 69.58, H 4.68, N 6.37, S 7.80; $C_{24}H_{18}N_2O_3S$ requires C 69.55, H 4.38, N 6.76, S 7.74).

*4.1.18. Compound **39***

Yellow solid, 85% yield, mp >240 °C; ν_{max} (KBr): 1660 (C=O), 3432 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.8 (d, 1H, J = 7.5 Hz, ArH), 7.04–7.24 (m, 4H, ArH), 7.45–7.48 (m, 2H, ArH), 7.59–7.66 (m, 3H, ArH), 7.87 (t, J = 6.6 Hz, 3H, ArH), 8.50–8.53 (m, 1H, ArH), 9.54 (s, 1H, indole2-H); FAB mass *m/z* 364 (M^+); (Found: C 79.20, H 4.52, N 7.76; $C_{24}H_{16}N_2O_2$ requires C 79.11, H 4.43, N 7.69).

*4.1.19. Compound **40***

Yellow solid, 83% yield, mp > 240 °C; ν_{max} (KBr): 1680 (C=O), 3452 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.8 (d, J = 7.5 Hz, 1H, ArH), 7.19–7.21 (m, 4H, ArH), 7.37–7.60 (m, 4H, ArH), 7.67–7.86 (m, 3H, ArH), 8.04 (d, J = 8.4 Hz, 1H, ArH), 9.54 (s, 1H, indole2-H); FAB mass *m/z* 398 (M^+), 398:400 (3:1); (Found: C 72.29, H 4.02, N 7.16; $C_{24}H_{15}ClN_2O_2$ requires C 72.27, H 3.79, N 7.02).

*4.1.20. Compound **41***

Yellow solid, 80% yield, mp 196 °C; ν_{max} (KBr): 1704 (C=O), 3460 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.75–6.84 (m, 2H, ArH), 7.07–7.71 (m, 9H, ArH), 7.78 (s, 1H, =H), 8.68 (d, J = 7.5 Hz, 1H, indole2-H); FAB mass *m/z* 433 (M^+), 433:435:437 (9:6:1); (Found: C 66.57, H 3.32, N 6.56; $C_{24}H_{14}Cl_2N_2O_2$ requires C 66.53, H 3.26, N 6.47).

*4.1.21. (3Z)-3-((1-Cinnamyl-1H-indol-3-yl)methylene)indolin-2-one (**42**)*

Dark yellow solid, 89% yield, mp 230 °C; ν_{max} (KBr): 1690 (C=O), 3430 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.01–5.04 (m, 2H, CH_2), 6.36–6.40 (m, 1H, =H), 6.61 (d, J = 6 Hz, 1H, =H), 6.88 (d, J = 6.0 Hz, 1H, ArH), 7.06–7.64 (m, 10H, ArH), 7.79–7.81 (m, 2H, ArH), 7.96 (s, 1H, =H), 9.58 (s, 1H, indole2-H); FAB mass *m/z* 440 (M^+); (Found: C 82.98, H 5.36, N 7.40; $C_{26}H_{20}N_2O$ requires C 82.95, H 5.35, N 7.44).

*4.1.22. (Z)-3-((1H-Indol-3-yl)methylene)-1-(2,6-dichlorophenyl) indolin-2-one (**43**)*

Dark yellow solid, 95% yield, mp 242 °C; ν_{max} (KBr): 1670 (C=O), 3450 (NH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.48 (d, J = 8.1 Hz, 1H, ArH), 7.15–7.57 (m, 8H, ArH), 7.74 (d, J = 8.7 Hz, 1H, ArH), 7.95 (d, J = 7.5 Hz, 1H, ArH), 8.14 (s, 1H, =H), 9.28 (bs, 1H, NH), 9.56 (d, J = 3 Hz, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 110.6 (C), 111.1 (+ve, CH), 118.2 (+ve, CH), 119.0 (+ve, CH), 119.0 (+ve, CH), 120.1 (+ve, CH), 120.2 (C), 121.1 (+ve, CH), 122.2 (+ve, CH), 126.1 (C), 127.3 (+ve, CH), 127.9 (+ve, CH), 127.9 (+ve, CH), 128.0 (C), 128.9 (+ve, CH), 129.4 (+ve, CH), 129.4 (+ve, CH), 130.8 (+ve, CH), 135.5 (C), 139.7 (+ve, CH), 140.0 (C), 144.9 (C), 159.3 (C); FAB mass *m/z* 405 (M + 1), 405:407:409 (9:6:1); (Found: C 67.95, H 3.30, N 7.12; $C_{23}H_{14}Cl_2N_2O$ requires C 68.16, H 3.48, N 6.91).

*4.1.23. (Z)-3-((1-Allyl-1H-indol-3-yl)methylene)-1-(2,6-dichlorophenyl)indolin-2-one (**44**)*

Dark yellow solid, 83% yield, mp 178 °C; ν_{max} (KBr): 1680 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.3 (ddd, J^2 = 16.8 Hz, J^3 = 10.2 Hz, J^4 = 0.3 Hz, 2H, CH_2), 6.03–6.12 (multiplet of 12 lines, 1H, CH), 6.45 (d, J = 6.9 Hz, 2H, CH_2), 7.00–7.55 (m, 9H, ArH), 7.82 (d, J = 7.2 Hz, 1H, ArH), 7.91 (d, J = 7.2 Hz, 1H, ArH), 7.95 (s, 1H, =H), 8.24 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 49.3 (−ve, CH_2), 108.9 (+ve, CH), 110.2 (+ve, CH), 111.5 (C), 118.0 (C), 118.4 (+ve, CH), 120.3 (+ve, CH), 120.8 (C), 121.4 (+ve, CH), 122.1 (−ve, CH_2), 122.5 (C), 123.2 (+ve, CH), 126.7 (C), 128.1 (+ve, CH), 128.3 (C), 128.9 (+ve, CH), 130.2 (+ve, CH), 130.4 (+ve, CH), 130.9 (C), 131.1 (+ve, CH), 132.3 (+ve, CH), 135.8 (C), 136.5 (C), 141.1 (C), 167.9 (C); FAB mass *m/z* 445 (M^+), 445:447:449 (9:6:1); (Found: C 69.88, H 3.54, N 6.49; $C_{26}H_{18}Cl_2N_2O$ requires C 70.12, H 4.07, N 6.29).

*4.1.24. (Z)-1-(2,6-Dichlorophenyl)-3-((1-(prop-2-ynyl)-1H-indol-3-yl)methylene)indolin-2-one (**45**)*

Dark red solid, 85% yield, mp 168 °C; ν_{max} (KBr): 1678 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.56 (t, J = 2.7 Hz, 1H, ≡H), 5.01 (d, J = 2.4 Hz, 2H, CH_2), 6.45 (d, J = 7.5 Hz, 1H, ArH), 7.01–7.56 (m, 8H, ArH), 7.82 (d, J = 6.3 Hz, 1H, ArH), 7.94 (d, J = 6.9 Hz, 1H, ArH), 8.11 (s, 1H, =H), 8.22 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 36.5 (−ve, CH_2), 75.0 (+ve, CH), 77.1 (C), 108.7 (+ve, CH), 109.0 (C), 109.9 (+ve, CH), 110.5 (C), 111.9 (C), 118.2 (C), 120.5 (+ve, CH), 121.5 (C), 121.7 (+ve, CH), 122.2 (+ve, CH), 122.4 (+ve, CH), 123.5 (+ve, CH), 128.3 (+ve, CH), 128.4 (C), 128.9 (+ve, CH), 129.8 (+ve, CH), 130.2 (+ve, CH), 130.5 (+ve, CH), 135.9 (C), 136.1 (C), 141.3 (C), 167.8 (C); FAB mass *m/z* 443 (M^+), 443:445:447 (9:6:1); (Found:. C 70.30, H 3.32, N 6.28; $C_{26}H_{16}Cl_2N_2O$ requires C 70.44, H 3.64, N 6.32).

*4.1.25. (Z)-1-(2,6-Dichlorophenyl)-3-((1-tosyl-1H-indol-3-yl) methylene)indolin-2-one (**46**)*

Yellow solid, 78% yield, mp >240 °C; ν_{max} (KBr): 1690 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 6.29 (d, J = 7.5 Hz, 1H, ArH), 6.96–7.86 (m, 14H, ArH), 7.88 (s, 1H, =H), 9.66 (s, 1H, indole2-H); FAB mass *m/z* 558 (M + 1), 558:560:562 (9:6:1); (Found: C 64.55, H 3.88, N 5.12, S 6.06; $C_{30}H_{20}Cl_2N_2O_3S$ requires C 64.40, H 3.60, N 5.01, S 5.73).

*4.1.26. (Z)-3-((1-Benzyl-1H-indol-3-yl)methylene)-1-(2,6-dichlorophenyl)indolin-2-one (**47**)*

Dark yellow solid, 81% yield, mp 232 °C; ν_{max} (KBr): 1690 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.37 (s, 2H, CH_2), 6.48 (d, J = 7.8 Hz, 1H, ArH), 7.16–7.55 (m, 13H, ArH), 7.76 (d, J = 6.0 Hz, 1H, ArH), 7.95 (d, J = 7.8 Hz, 1H, ArH), 8.13 (s, 1H, =H), 9.61 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 51.2 (−ve, CH_2), 96.1 (C), 108.6 (+ve, CH), 110.9 (+ve, CH), 111.4 (C), 117.7 (C), 118.1 (+ve, CH), 121.1 (+ve, CH), 122.2 (+ve, CH), 123.0 (C), 125.3 (C), 126.6 (+ve, CH), 126.8 (+ve, CH), 127.8 (+ve, CH), 127.9 (+ve, CH), 128.8 (+ve, CH), 128.9 (+ve, CH), 129.5 (C), 130.4 (+ve, CH), 135.9 (C), 136.1 (C), 136.2 (C), 137.8 (+ve, CH), 138.9 (C), 157.9 (C), 166.0 (C); FAB mass *m/z* 495 (M^+), 495:497:499 (9:6:1); (Found: C 72.43, H 3.78, N 6.02; $C_{30}H_{20}Cl_2N_2O$ requires C 72.73, H 4.07, N 5.65).

*4.1.27. Compound **48***

Yellow solid, 90% yield, mp 170 °C; ν_{max} (KBr): 1690 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.38 (m, 1H, ArH), 7.13–7.92 (m, 13H, ArH), 8.01 (s, 1H, =H), 8.38–8.41 (m, 2H, ArH), 9.47 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 108.9 (+ve, CH), 109.2 (+ve, CH), 116.4 (+ve, CH), 117.8 (+ve, CH), 118.9 (+ve, CH), 120.1 (+ve, CH), 122.4 (+ve, CH), 123.1 (+ve, CH), 124.3 (+ve, CH), 124.6 (+ve, CH), 125.1 (+ve, CH), 125.4 (+ve, CH), 126.0 (+ve, CH), 127.6 (+ve, CH), 128.4 (+ve, CH), 128.71 (+ve, CH), 128.77 (+ve, CH), 128.8 (+ve, CH), 128.9 (C), 129.1 (C), 129.9 (C), 130.4 (C), 130.6 (C), 132.4 (C), 132.7 (C), 133.9 (C), 135.7 (C), 136.0 (C), 165.0 (C), 168.8 (C); FAB mass *m/z* 508 (M^+), 508:510:512 (9:6:1); (Found: C 70.71, H 3.43, N 5.63; $C_{30}H_{18}Cl_2N_2O_2$ requires C 70.74, H 3.56, N 5.50).

*4.1.28. Compound **49***

Yellow solid, 75% yield, mp >260 °C; ν_{max} (KBr): 1685 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.42 (d, J = 7.5 Hz, 1H, ArH), 6.43–7.99 (m, 14H, ArH), 8.35 (d, J = 8.7 Hz, 1H, =H), 9.47 (s, 1H, indole2-H); FAB mass *m/z* 543 (M^+), 543:545:547:549 (27:27:9:1); (Found: C 66.00, H 2.84, N 5.24; $C_{30}H_{17}Cl_3N_2O_2$ requires C 66.26, H 3.15, N 5.15).

*4.1.29. Compound **50***

Yellow solid, 82% yield, mp 250 °C; ν_{max} (KBr): 1690 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.38 (d, J = 7.8 Hz, 1H, ArH), 7.08–7.70 (m, 11H, ArH), 7.82 (s, 1H, =H), 7.86–7.89 (m, 1H, ArH), 8.68–8.71 (m, 1H, ArH), 8.96 (s, 1H, indole2-H); FAB mass *m/z* 578 (M^+) 576:578:580:582:584 (81:108:54:12:1); (Found: C 62.15, H 2.82, N 4.85; $C_{30}H_{16}Cl_4N_2O_2$ requires C 62.31, H 2.79, N 4.84).

*4.1.30. (3Z)-1-(2,6-Dichlorophenyl)-3-((1-cinnamyl-1H-indol-3-yl) methylene)indolin-2-one (**51**)*

Dark yellow solid, 91% yield, mp 190 °C; ν_{max} (KBr): 1700 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.97 (multiplet of 8 lines, 2H, CH_2), 6.28–6.44 (m, 1H, =H), 6.45–6.56 (m, 2H, =H + ArH), 7.13–7.53 (m, 13H, ArH), 7.72–7.98 (m, 2H, ArH), 8.11 (s, 1H, =H), 9.53 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 49.6 (−ve, CH_2), 108.6 (+ve, CH), 110.8 (+ve, CH), 111.4 (C), 117.5 (C), 118.1 (+ve, CH), 121.6 (+ve, CH), 122.2 (+ve, CH), 122.9 (+ve, CH), 123.6 (+ve, CH), 125.4 (C), 126.5 (+ve, CH), 126.8 (+ve, CH), 127.9 (+ve, CH), 128.5 (+ve, CH), 128.6 (+ve, CH), 128.9 (+ve, CH), 129.5 (C), 130.4 (+ve, CH), 133.1 (+ve, CH), 131.2 (C), 135.9 (C), 136.0 (C), 136.2 (C), 137.3 (+ve, CH), 138.9 (C), 166.0 (C); FAB mass *m/z* 521 (M^+) 521:523:525 (9:6:1); (Found: C 73.50, H 4.52, N 5.56; $C_{32}H_{22}Cl_2N_2O$ requires C 73.71, H 4.25, N 5.37).

*4.1.31. (Z)-3-((1H-indol-3-yl)methylene)indolin-2-one (**52**) [48,49]*

Yellow solid, 95% yield, mp 220 °C; ν_{max} (KBr):1685 (C=O), 3470 (NH). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.82–6.92 (m, 1H, ArH), 6.97–7.32 (m, 3H, ArH), 7.41–7.47 (m, 1H, ArH), 7.58–7.93(m, 3H, ArH), 7.98 (s, 1H, =H), 9.51 (s, 1H, indole2-H), 10.05 (s, 1H, NH);

(Found: C 78.46, H 4.62, N 10.79; $C_{32}H_{22}Cl_2N_2O$ requires C 78.44, H 4.65, N 10.76).

4.1.32. (Z)-1-Allyl-3-((1-allyl-1H-indol-3-yl)methylene)indolin-2-one (**53**)

Yellow solid, 82% yield, mp 210 °C; ν_{max} (KBr): 1680 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.48–4.51 (m, 2H, CH_2), 4.83–4.85 (m, 2H, CH_2), 5.14–5.27 (m, 4H, 2×CH_2), 5.87–6.03 (m, 2H, 2×CH), 6.84 (d, J = 7.5 Hz, 1H, ArH), 7.05–7.40 (m, 5H, ArH), 7.64 (d, J = 7.2 Hz, 1H, ArH), 7.91–7.94 (m, 1H, ArH), 7.99 (s, 1H, =H), 9.50 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 42.0 (−ve, CH_2), 49.7 (−ve, CH_2), 77.2 (+ve, CH), 108.3 (+ve, CH), 110.6 (+ve, CH), 111.2 (C), 116.9 (+ve, CH), 117.7 (+ve, CH), 118.0 (+ve, CH), 118.2 (+ve, CH), 118.3 (C), 121.4 (−ve, CH_2), 122.7 (−ve, CH_2), 125.1 (C), 126.7 (+ve, CH), 126.8 (+ve, CH), 129.3 (C), 132.0 (+ve, CH), 132.2 (+ve, CH), 136.1 (C), 136.8 (+ve, CH), 139.9 (C), 166.9 (C); FAB mass m/z 341 (M + 1); (Found: C 81.11, H 5.74, N 8.19; $C_{23}H_{20}N_2O$ requires C 81.15, H 5.92, N 8.23).

4.1.33. (Z)-1-(Prop-2-ynyl)-3-((1-(prop-2-ynyl)-1H-indol-3-yl) methylene)indolin-2-one (**54**)

Dark red solid, 81% yield, mp >240 °C; ν_{max} (KBr): 1680 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.57 (s, 1H, ≡H), 2.21 (s, 1H, ≡H), 4.66 (d, J = 2.7 Hz, 2H, CH_2), 5.74 (d, J = 6.6 Hz, 2H, CH_2), 7.04–7.35 (m, 5H, ArH), 7.63–7.70 (m, 2H, ArH), 7.88–7.91 (m, 1H, ArH), 7.93 (s, 1H, =H), 9.49 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 28.9 (−ve, CH_2), 39.1 (−ve, CH_2), 71.7 (C), 77.4 (+ve, CH), 88.4 (+ve, CH), 97.2 (+ve, CH), 108.5 (+ve, CH), 111.1 (+ve, CH), 118.1 (+ve, CH), 119.4 (C), 121.9 (+ve, CH), 122.1 (+ve, CH), 123.3 (+ve, CH), 126.2 (+ve, CH), 127.2 (+ve, CH), 133.9 (+ve, CH), 139.1 (C), 203.7 (C); FAB mass m/z 337(M + 1); (Found: C 81.94, H 4.59, N 8.25; $C_{23}H_{16}N_2O$ requires C 82.12, H 4.79, N 8.33).

4.1.34. (Z)-1-Tosyl-3-((1-tosyl-1H-indol-3-yl)methylene)indolin-2-one (**55**)

Yellow solid, 73% yield, mp >250 °C; ν_{max} (KBr): 1663 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H, CH_3), 2.43 (s, 3H, CH_3), 7.21 (t, J = 6 Hz, 1H, ArH), 7.28–7.40 (m, 8H, ArH), 7.60 (d, J = 7.4 Hz, 1H, ArH), 7.71 (s, 1H, =H), 7.74 (d, J = 7.6 Hz, 2H, ArH), 7.92 (d, J = 6 Hz, 2H, ArH), 8.04 (d, J = 8.2 Hz, 1H, ArH), 8.08 (d, J = 8.3 Hz, 1H, ArH), 9.52 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 21.6 (+ve, CH_3), 21.7 (+ve, CH_3), 113.7 (+ve, CH), 113.8 (+ve, CH), 115.1 (C), 118.2 (+ve, CH), 118.7 (+ve, CH), 121.9 (C), 123.9 (+ve, CH), 124.2 (+ve, CH), 124.5 (C), 125.3 (+ve, CH), 125.7 (+ve, CH), 127.2 (+ve, CH), 127.9 (+ve, CH), 129.2 (+ve, CH), 129.7 (+ve, CH), 130.1 (+ve, CH), 130.2 (C), 132.9 (C), 134.3 (C), 134.7 (C), 135.6 (C), 137.3 (C), 145.4 (C), 145.5 (C); FAB mass m/z 521 (M^+); (Found: C 65.43, H 3.94, N 4.49, S 10.93; $C_{31}H_{24}N_2O_5S_2$ requires C 65.47, H 4.25, N 4.93, S 11.28).

4.1.35. (Z)-1-Benzyl-3-((1-benzyl-1H-indol-3-yl)methylene) indolin-2-one (**56**)

Yellow solid, 85% yield, mp 148 °C; ν_{max} (KBr): 1680 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.09 (s, 2H, CH_2), 5.47 (s, 2H, CH_2), 6.73–6.87 (m, 3H, ArH), 7.07–7.40 (m, 11H, ArH), 7.66–7.86 (m, 3H, ArH), 7.97 (d, J = 6 Hz, 1H, ArH), 8.06 (s, 1H, =H), 9.68 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 43.6 (−ve, CH_2), 51.1 (−ve, CH_2), 108.6 (+ve, CH), 110.9 (+ve, CH), 111.4 (C), 117.8 (+ve, CH), 118.6 (C), 118.1 (+ve, CH), 121.51 (+ve, CH), 121.55 (+ve, CH), 123.0 (+ve, CH), 125.2 (C), 126.71 (+ve, CH), 126.73 (+ve, CH), 126.8 (+ve, CH), 126.9 (+ve, CH), 127.1 (+ve, CH), 127.2 (+ve, CH), 127.3 (+ve, CH), 127.9 (+ve, CH), 128.71 (+ve, CH), 128.75 (+ve, CH), 128.92 (+ve, CH), 128.94 (+ve, CH), 129.5 (C), 136.31 (C), 136.36 (C), 136.68 (C), 137.3 (+ve, CH), 140.0 (C), 167.3 (C); FAB mass m/z 440 (M^+); (Found: C 84.42, H 5.49, N 6.25; $C_{31}H_{24}N_2O$ requires C 84.52, H 5.49, N 6.36).

4.1.36. Compound **57**

Yellow solid, 87% yield, mp 238 °C; ν_{max} (KBr): 1699 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.26–7.40 (m, 5H, ArH), 7.45–7.54 (m, 6H, ArH), 7.60–7.82 (m, 6H, ArH), 7.88 (s, 1H, =H), 7.92 (d, J = 6 Hz, 1H, ArH), 9.22 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 114.8 (C), 115.1 (+ve, CH), 116.7 (+ve, CH), 117.8 (+ve, CH), 118.6 (+ve, CH), 122.9 (C), 124.5 (+ve, CH), 124.6 (+ve, CH), 125.0 (C), 125.7 (+ve, CH), 126.2 (+ve, CH), 127.9 (+ve, CH), 128.1 (+ve, CH), 128.4 (+ve, CH), 128.8 (+ve, CH), 128.9 (+ve, CH), 129.2 (+ve, CH), 129.3 (+ve, CH), 129.4 (+ve, CH), 129.8 (+ve, CH), 130.2 (C), 133.1 (C), 132.0 (+ve, CH), 132.4 (+ve, CH), 134.7 (+ve, CH), 134.9 (C), 135.7 (C), 138.2 (C); FAB mass m/z 468 (M^+); (Found: C 79.19, H 4.31, N 5.69; $C_{31}H_{20}N_2O_3$ requires C 79.47, H 4.30, N 5.98).

4.1.37. Compound **58**

Yellow solid, 81% yield, mp >250 °C; ν_{max} (KBr): 1680 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.82–7.09 (m, 4H, ArH), 7.14–7.92 (m, 11H, ArH), 8.08 (s, 1H, =H), 9.50 (d, J = 3.6 Hz, 1H, ArH), 10.04 (s, 1H, indole2-H); FAB mass m/z 536 (M^+), 536:538:540 (9:6:1); (Found: C 69.15, H 3.64, N 5.51; $C_{31}H_{18}Cl_2N_2O_3$ requires C 69.28, H 3.38, N 5.21).

4.1.38. Compound **59**

Yellow solid, 87% yield, mp 185 °C; ν_{max} (KBr): 1690 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.20–7.58 (m, 4H, ArH), 7.69–7.84 (m, 6H, ArH), 8.39 (d, J = 8.1 Hz, 2H, ArH), 8.62–8.65 (m, 4H, ArH); FAB mass m/z 607 (M + 1), 605:607:609:611:613 (81:108:54:12:1); (Found: C 61.46, H 2.34, N, 4.26; $C_{31}H_{16}Cl_4N_2O_3$ requires C 61.41, H 2.66, N 4.62).

4.1.39. (3Z)-1-Cinnamyl-3-((1-cinnamyl-1H-indol-3-yl)methylene) indolin-2-one (**60**)

Yellow solid, 92% yield, mp 160 °C; ν_{max} (KBr): 1690 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.66 (d, J = 4.8 Hz, 2H, CH_2), 5.03 (d, J = 5.1 Hz, 2H, CH_2), 6.22–6.39 (m, 2H, 2×CH), 6.53–6.62 (m, 2H, 2×CH), 6.92–7.47 (m, 16H, ArH), 7.66 (d, J = 6.9 Hz, 1H, ArH), 7.93–7.96 (m, 1H, ArH), 8.02 (s, 1H, =H), 9.58 (s, 1H, indole2-H); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 41.7 (−ve, CH_2), 49.5 (−ve, CH_2), 108.4 (+ve, CH), 110.7 (C), 111.3 (+ve, CH), 117.8 (+ve, CH), 118.1 (+ve, CH), 118.5 (+ve, CH), 121.4 (C), 122.9 (C), 123.6 (C), 125.2 (C), 126.4 (+ve, CH), 126.8 (+ve, CH), 127.6 (+ve, CH), 128.0 (+ve, CH), 128.4 (+ve, CH), 128.5 (+ve, CH), 128.6 (+ve, CH), 132.2 (+ve, CH), 133.1 (+ve, CH), 136.2 (C), 136.4 (C), 136.8 (C); FAB mass m/z 492 (M^+); (Found: C 85.27, H 5.85, N 5.59; $C_{35}H_{28}N_2O$ requires C 85.34, H 5.73, N 5.69).

4.1.40. (4Z)-1-(3-Chlorophenyl)-3-methyl-4-((E)-3-phenylallylidene)-1H-pyrazol-5(4H)-one (**66**)

Reddish orange solid, 95% yield, mp 135 °C; ν_{max} (KBr): 1753 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.35 (s, 3H, CH_3), 7.11–7.14 (m, 1H, ArH), 7.15–7.44 (m, 6H, ArH), 7.64–7.67 (m, 2H, ArH), 7.89–7.93 (m, 1H, ArH), 8.04 (t, J = 2.1 Hz, 1H, ArH), 8.54 (dd, J = 15.4 Hz, J = 11.4 Hz, 1H, CH); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 12.7 (+ve, CH_3), 116.2 (+ve, CH), 118.3 (+ve, CH), 123.1 (+ve, CH), 124.4 (+ve, CH), 125.3 (C), 128.3 (+ve, CH), 128.6 (+ve, CH), 129.0 (+ve, CH), 129.7 (+ve, CH), 130.9 (+ve, CH), 134.5 (C), 135.4 (C), 139.4 (C), 144.9 (+ve, CH), 149.8 (+ve, CH), 149.9 (C), 163.1 (C); FAB mass m/z 323 (M + 1) 323:325 (3:1); (Found: C 70.71, H 4.70, N 8.39; $C_{19}H_{15}ClN_2O$ requires C 70.70, H 4.68, N 8.68).

4.1.41. (4Z)-1-(3-Chlorophenyl)-3-methyl-4-((pyridin-2-yl) methylene)-1H-pyrazol-5(4H)-one (**67**)

Reddish orange solid, 94% yield, mp 192 °C; ν_{max} (KBr): 1650 (C=O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.71 (s, 3H, CH_3), 7.11–7.19 (m, 2H, ArH), 7.24–7.35 (m, 3H, ArH), 7.58–7.64 (m, 1H, ArH), 7.72–7.76 (m, 1H, ArH), 7.90 (t, J = 2.1 Hz, 1H, ArH), 8.50–8.52 (m, 1H, ArH);

^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 11.8 (+ve, CH_3), 48.5 (+ve, CH), 98.3 (C), 118.5 (+ve, CH), 120.7 (+ve, CH), 122.9 (+ve, CH), 124.7 (+ve, CH), 125.1 (+ve, CH), 129.7 (+ve, CH), 134.4 (C), 138.6 (+ve, CH), 140.1 (C), 146.5 (+ve, CH), 147.5 (C), 152.5 (C), 162.3 (C); FAB mass *m/z* 297 (M + 1) 297:299 (3:1); (Found: C 64.21, H 4.06, N 13.84; $C_{16}H_{12}ClN_3O$ requires C 64.54, H 4.06, N 14.11).

*4.1.42. (4Z)-4-(4-Nitrobenzylidene)-1-(3-chlorophenyl)-3-methyl-1H-pyrazol-5(4H)-one (**68**)*

Reddish orange solid, 96% yield, mp 158 °C; ν_{max} (KBr): 1650 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.81 (s, 3H, CH_3), 6.69–6.72 (m, 1H, ArH), 7.26–7.34 (m, 3H, ArH), 7.51–7.73 (m, 2H, ArH), 8.08–8.15 (m, 2H, ArH), 8.48 (d, *J* = 8.7 Hz, 1H, ArH); FAB mass *m/z* 341 (M^+) 341:343 (3:1); (Found: C 59.63, H 3.31, N 12.16; $C_{17}H_{12}ClN_3O_3$ requires C 59.75, H 3.54, N 12.30).

*4.1.43. (4Z)-1-(3-Chlorophenyl)-4-((2-hydroxynaphthalen-3-yl) methylene)-3-methyl-1H-pyrazol-5(4H)-one (**69**)*

Orange solid, 90% yield, mp 200 °C; ν_{max} (KBr): 1685 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.38 (s, 3H, CH_3), 7.23–7.46 (m, 6H, ArH), 7.62–7.79 (m, 2H, ArH), 7.82–7.88 (m, 2H, ArH), 7.97–8.00 (m, 1H, ArH); FAB mass *m/z* 362 (M^+) 362:364 (3:1); (Found: C 69.43, H 4.11, N 7.66; $C_{21}H_{15}ClN_2O_2$ requires C 69.52, H 4.17, N 7.72).

*4.1.44. (4Z)-4-((Anthracen-10-yl)methylene)-1-(3-chlorophenyl)-3-methyl-1H-pyrazol-5(4H)-one (**70**)*

Reddish orange solid, 94% yield, mp 145 °C; ν_{max} (KBr): 1690 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.59 (s, 3H, CH_3), 7.04–7.07 (m, 1H, ArH), 7.17–7.7.40 (m, 1H, ArH), 7.50–7.58 (m, 5H, ArH), 7.76–7.79 (m, 1H, ArH), 7.92–8.09 (m, 4H, ArH), 8.58 (d, *J* = 3.6 Hz, 1H, ArH), 8.78 (s, 1H, CH); FAB mass *m/z* 396 (M^+) 396:398 (3:1); (Found: C 75.33, H 4.31, N 7.06; $C_{25}H_{17}ClN_2O$ requires C 75.66, H 4.32, N 7.06).

*4.1.45. (3Z)-1-(2,6-Dichlorophenyl)-3-((E)-3-phenylallylidene) indolin-2-one (**71**)*

Shiny yellow solid, 95% yield, mp 203 °C; ν_{max} (KBr):1700 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.42–6.45 (m, 1H, =H), 7.12–7.25 (m, 4H, ArH), 7.34–7.46 (m, 5H, 4× ArH+, =H), 7.49–7.84 (m, 5H, ArH); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 109.2 (+ve, CH), 122.4 (+ve, CH), 123.2 (+ve, CH), 123.7 (+ve, CH), 124.4 (C), 127.7 (+ve, CH), 128.8 (+ve, CH), 128.91(+ve, CH), 128.94 (+ve, CH), 129.7 (+ve, CH), 130.6 (+ve, CH), 135.71 (C), 135.73 (C), 135.9 (C), 136.9 (+ve, CH), 141.5 (C), 144.8 (+ve, CH), 167.1(C); FAB mass *m/z* 392 (M^+) 392:394:396 (9:6:1); (Found: C 70.30, H 3.56, N 3.63; $C_{23}H_{15}Cl_2NO$ requires C 70.42, H 3.85, N 3.37).

*4.1.46. (Z)-1-(2,6-Dichlorophenyl)-3-((pyridin-2-yl)methylene) indolin-2-one (**72**)*

Shiny yellow solid, 86%, mp 188 °C; ν_{max} (KBr): 1668 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.41 (d, *J* = 7.6 Hz, 1H, ArH), 7.13 (t, *J* = 7.6 Hz, 1H, ArH), 7.15–7.41 (m, 3H, ArH), 7.53 (d, *J* = 8.1 Hz, 2H, ArH), 7.66 (d, *J* = 7.7 Hz, 1H, ArH), 7.82–7.85 (m, 2H, ArH), 8.91 (d, *J* = 4.5 Hz, 1H, ArH), 9.12 (d, *J* = 7.6 Hz, 1H, ArH); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 109.2 (+ve, CH), 122.4 (+ve, CH), 123.2 (+ve, CH), 123.7 (+ve, CH), 124.4 (C), 127.7 (+ve, CH), 128.8 (+ve, CH), 128.91 (+ve, CH), 128.94 (+ve, CH), 129.7 (+ve, CH), 130.6 (+ve, CH), 135.71 (C), 135.73 (C), 135.9 (C), 136.9 (+ve, CH), 141.5 (C), 144.8 (+ve, CH), 167.1(C); FAB mass *m/z* 366 (M^+) 366:368:370 (9:6:1); (Found: C 65.15, H 3.28, N 7.38; $C_{20}H_{12}Cl_2N_2O$ requires C 65.41, H 3.29, N 7.63).

*4.1.47. (Z)-3-(4-Nitrobenzylidene)-1-(2,6-dichlorophenyl)indolin-2-one (**73**)*

Yellow solid, 83% yield, mp 190 °C; ν_{max} (KBr): 1706 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.41 (d, *J* = 7.8 Hz, 1H, ArH), 6.86–6.97 (m, 1H, ArH), 7.13–7.54 (m, 5H, ArH), 7.84–7.93 (m, 3H, ArH), 8.35 (d, *J* = 8.7 Hz, 2H, ArH); FAB mass *m/z* 411 (M^+) 411:413:415 (9:6:1); (Found: C 61.36, H 2.83, N 6.68; $C_{21}H_{12}Cl_2N_2O_3$ requires C 61.33, H 2.94, N 6.81).

*4.1.48. (Z)-3-((Anthracen-10-yl)methylene)-1-(2,6-dichlorophenyl) indolin-2-one (**74**)*

Shiny yellow solid, 89% yield, mp 177 °C; ν_{max} (KBr): 1680 (C═O); ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.04 (d, *J* = 7.5 Hz, 1H, ArH), 6.37 (d, *J* = 7.5 Hz, 1H, ArH), 6.47–6.53 (m, 1H, ArH), 7.01–7.24 (m, 1H, ArH), 7.37 (s, 1H, =H), 7.39–7.57 (m, 6H, ArH), 8.05–8.13 (m, 4H, ArH), 8.57 (s, 1H, ArH), 8.71 (s, 1H, ArH); ^{13}C NMR (normal/DEPT-135) ($CDCl_3$): δ 108.9 (+ve, CH), 121.1 (C), 122.6 (+ve, CH), 124.4 (+ve, CH), 125.6 (+ve, CH), 125.7 (+ve, CH), 126.6 (+ve, CH), 128.3 (C), 128.5 (+ve, CH), 128.91 (+ve, CH), 128.95 (C), 129.0 (C), 129.6 (C), 130.5 (C), 130.6 (C), 130.7 (+ve, CH), 131.2 (C), 134.5 (+ve, CH), 135.8 (C), 142.3 (C), 166.2 (C); FAB mass *m/z* 466 (M^+) 466:468:470 (9:6:1); (Found: C 74.87, H 3.53, N 3.09; $C_{29}H_{17}Cl_2NO$ requires C 74.69, H 3.67, N 3.00).

4.2. In-vitro tumor growth inhibitory activities

In-vitro tumor growth inhibitory activities of these compounds were investigated at NCI, Bethesda on 60 cell line panel of human cancer cells using standard procedure. Compounds were first tested at 10^{-5} M for the growth inhibitory activities at entire 60 cell line panel. Compounds with considerable activities at 10^{-5} M concentration (as per the standard of NCI) were subjected to detailed tumor growth inhibitory studies at five concentrations viz. 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} M.

4.3. Maximum tolerable dose test (acute toxicity description)

A single mouse was given a single injection [Intravenous injection (IV)] of 400 mg/kg; a second mouse received a dose of 200 mg/kg and a third mouse received a single dose of 100 mg/kg. Dose volumes were generally 0.1 ml/10 gm body weight. The mice were observed for a period of 2 weeks. They were sacrificed if they lost more than 20% of their body weight or if there were other signs of significant toxicity. If all 3 mice were sacrificed, then the next 3 dose levels (50, 25, 12.5 mg/kg) were tested in a similar way. The process was repeated until a tolerated dose was found. The maximum tolerated dose was used to calculate the amount of material given to experimental mice during antitumor testing.

4.4. In-vivo anticancer activities

A panel of 12 tumor cell lines *viz*. NCI-H23, NCI-H522, MDA-MB-231, MDA-MB-435, SW-620, COLO 205, LOX, UACC-62, OVCAR-3, OVCAR-5, U251 and SF-295, cultivated in RPMI-1640 containing 10% FBS and 2 mM glutamine was used. The cell suspension ($2–10 \times 10^6$ cells/ml) was flushed into 1 mm (internal diameter) polyvinylidene fluoride hollow fibers with molecular weight exclusion of 500,000 Da. The hollow fibers were heat-sealed at 2 cm intervals and the samples generated from these seals were placed into tissue culture medium and incubated at 37 °C in 5% CO_2 for 24–48 h prior to implantation. Samples of each tumor cell line preparation were quantitated for viable cell mass by a stable endpoint MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay before and after the administration of test agent. The optical density of each sample was determined spectrophotometrically at 540 nm and the mean of each treatment group was calculated. A 50% or greater reduction in percent net growth in the treated samples compared to the vehicle control samples was considered a positive result.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2010.08.004.

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