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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and in vitro cytotoxicity study of 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones

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Abstract A series of 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones **3a–l** were synthesized in good to excellent yield by Michael addition of indole **1** with α,β-unsaturated ketones **2a–l** in presence of indium(III) sulphate (20 mol%). The structure of the title compounds were established by ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. All the synthesized compounds were evaluated for in vitro cytotoxicity against five different cancer cell lines such as ACHN (human kidney adenocarcinoma), Panc1 (pancreatic), Calu1 (lung), H460 (non small cell lung), HCT116 (human colon cancer cell) and MCF10A (normal breast epithelium) using propidium iodide staining assay protocol. The result showed that the compounds **3e** and **3l** have excellent cytotoxic activity with the IC_{50} value ranging from 1.4–2.7 to 2.4–3.4 μM, respectively, in comparison with the other compounds, Flavopiridol and Gemcitabine were employed as a positive control. The findings conferred 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones seem to be promising candidates for the development of new anticancer drugs.

Keywords Michael addition · Indium(III) sulphate · Indole · Friedel–Crafts alkylation · Cytotoxic activity · SAR analysis

Introduction

Indole nucleus has been a structural subunit of many natural and pharmaceutical agents (Kameshwara *et al*., 2011; Abdel-Rahman, 2010). In addition, several indole derivatives have been found to exhibit anticancer (Ekhlass, 2010; Vishal *et al*., 2012; Chen *et al*., 1996), antioxidant (Suzen and Buyukbingol, 2000), antirhemuatoidal and anti-HIV (Buyukbingol *et al*., 1994; Suzen and Buyukbingol, 1998) activities. Natural product (+)-(S)-kurasoin B (**1**), analogues of 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones isolated from soil fungus *Paecilomyces* sp. (FO-3684) has been reported as selective inhibitors of farnesyltransferase (Rodney and Fernandes, 2008). Magdy and Atef (2009) and Magdy *et al*. (2010) studied that indolylchalcones were found to be promising candidates for in vitro antitumor activity, as they were previously reported to posses anticancer, immunosuppressant and therapeutic activities for autoimmune diseases (Shum-ichi *et al*., 1999). Various indolylchalcones containing methoxy group exhibits potent and selective anticancer activity (Dalip *et al*., 2010). More recently 3-(5-methoxy,2-methyl-1*H*-indol-3-yl)-1-(4-pyridinyl)-2-propen-1-one (**2**) served as a novel molecule for methuosis (a type of nonapoptotic cell death) (Michael *et al*., 2012).

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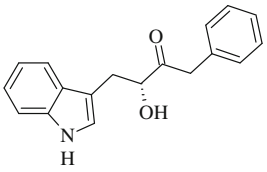
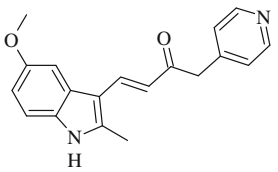


(+)-(S)-kurasoin B (1)



MOMIPP (2)

The structure of compounds **1** and **2** resembles our synthesized compounds, which prompted us to carry out their in vitro cytotoxicity studies. This study was supported by our previous study on investigation of new anticancer molecules (Bindu *et al*., 2012a, b). Hence, herein we report the synthesis and cytotoxic studies of 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones **3a–l**. The cytotoxic study was carried out in vitro against five human cancer cell lines such as ACHN, Panc1, Calu1, H460 and HCT116. The normal breast epithelium cells (MCF10A) were used to identify the selectivity of the compounds towards highly proliferating cancer cells. Although many methods have been reported to prepare various 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones (Brindaban *et al*., 2005; Bimal *et al*., 2005; Bei-Yao *et al*., 2010; Arrigo *et al*., 2009; Jianwei *et al*., 2010; Xiang *et al*., 2011; Zhi-Liang *et al*., 2008), these methods suffer due to some draw backs such as low yield, long reaction time, solubility of the catalyst in solvents make difficulty in isolation of the products, their reusability and chance of polymerization or dimerization of the reactants. In this context, it is worthy to note that the indium(III) sulphate is an effective milder Lewis acid catalyst which offers the conjugate addition of indole to α,β-unsaturated ketones smoothly to give good yield and also the catalyst could be recycled and reused two to three times without the loss of its catalytic activity.

Results and discussion

Chemistry

Initially, the conjugate addition of indole to α,β-unsaturated ketones was carried out by varying percentage of indium(III) sulphate (10, 20, 30 and 50 %) with different solvent under reflux temperature. In our observations, 20 mol% of the indium(III) sulphate in ethanol was sufficient for the synthesis of 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones with high yield (Table 1). The variation in the percentage of indium(III) sulphate had only marginal difference in the yields and reactions time. Hence 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones **3a–l**, (Scheme 1, Table 2) were synthesized via two component one pot reaction of Indole **1** with different α,β-unsaturated ketones **2a–l** in presence of 20 mol% Indium(III) sulphate in ethanol.

The relevance in using indium(III) sulphate for conjugate addition is of its insolubility in ethanol as compared with indium(III) chloride and indium(III) bromide as they are soluble and cannot be reusable (Brindaban *et al*., 2005; Bimal *et al*., 2005; Bei-Yao *et al*., 2010). Thus indium(III) sulphate catalysed method found to be economical and efficient. Easy isolation of the products and the recovery of the catalyst make it environmental friendly procedure. Hence, we optimized the amount of indium(III) sulphate i.e. 20 mol% of the catalyst was only used in entire reaction and evaluated the reusability of catalyst in the model reaction **3a**. Accordingly, we tested the reusability in which the 20 mol% catalyst was taken for the first reaction was filtered, washed with EtOH, oven dried and reused for the reaction to get satisfactory yield of 92 %. In the third run of catalyst, yield was 89 %.

The catalytic activity of $In_2(SO_4)_3$ in the conjugated addition of indole to α,β-unsaturated ketones **2a–l** is as

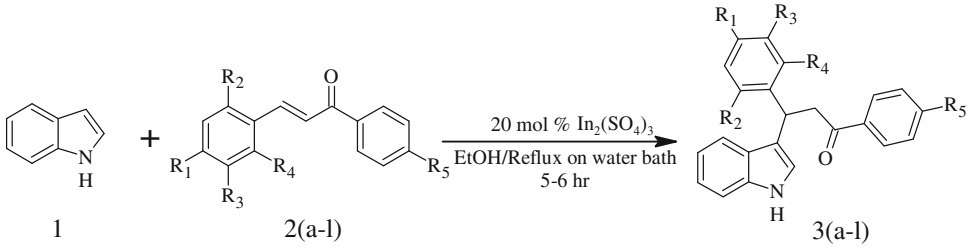
Table 1 Effect of solvent and catalyst on reaction time and yield

Entry	Solvent	$In_2(SO_4)_3$ (mol%)	Time (h)	Yield (%)[a]
1	Ethanol	10	6	85
2	Ethanol	20	6	94
3	Ethanol	30	6	90
4	Ethanol	50	6	91
5	Methanol	10	6	82
6	Acetonitrile[b]	10	6–20	30

[a] Yield after column chromatography

[b] Not a good solvent since many undesired side products were observed by TLC

Scheme 1 Synthesis of 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones **3a–l**



1 2(a-l) 3(a-l)

R_1= H, F, Cl, OMe R_3= OMe
R_2= F, Cl R_4= F, OMe R_5= H, OMe, F, Cl, Br

Table 2 $In_2(SO_4)_3$ mediated conjugate addition of indole with α,β-unsaturated ketones

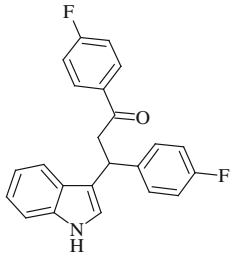
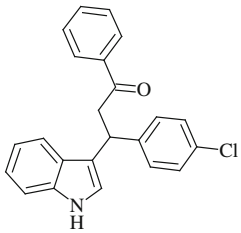
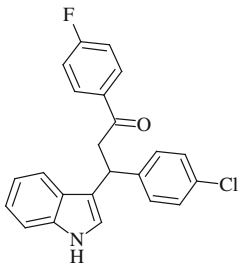
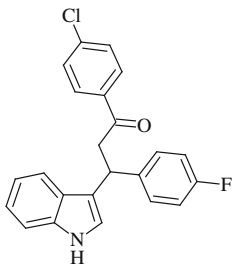
Entry	Products[a]	Time (h)	Yield (%)[b]	M.P/L (°C)
3a		6	94	118–120
3b		6	89	115–117/117–119
3c		6	90	220–221
3d		6	87	70–73

Table 2 continued

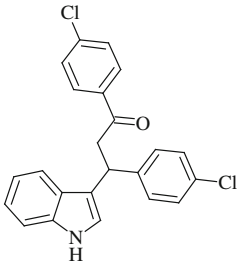
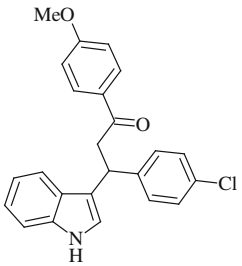
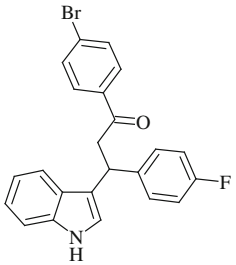
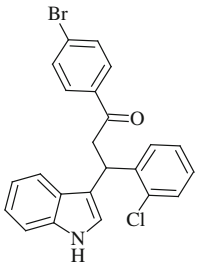
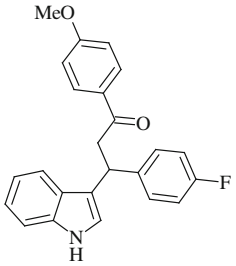
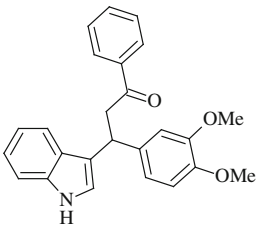
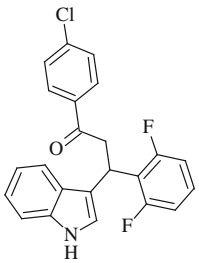
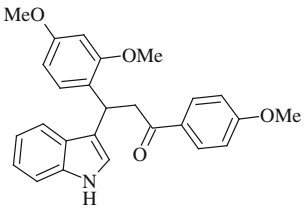
Entry	Products[a]	Time (h)	Yield (%)[b]	M.P/L (°C)
3e		6	90	221–225
3f		5	91	198–200/204–205
3g		6	85	160–162
3h		6	91	183–185

Table 2 continued

Entry	Products[a]	Time (h)	Yield (%)[b]	M.P/L (°C)
3i		6	90	177–179/172–175
3j		5	88	200–202
3k		6	87	156–159
3l		6	89	198–201

All reactions were carried out at reflux temperature using 20 mol% $In_2(SO_4)_3$ in ethanol

[a] All products were characterized by ^1H and ^{13}C NMR and mass spectroscopy

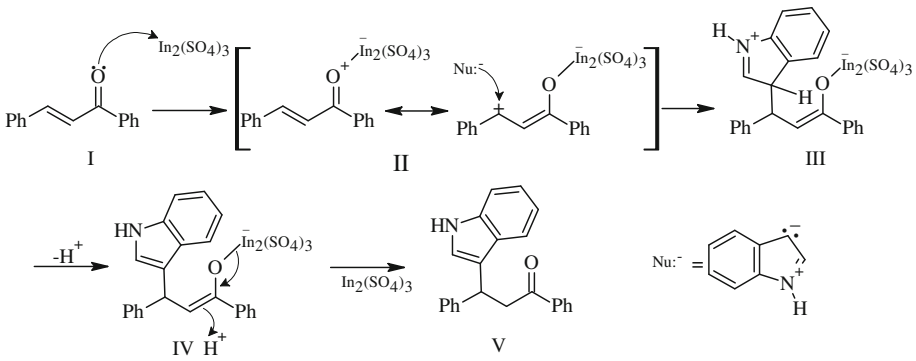
[b] Isolated yields

shown in Scheme 2. Initially the molecule of $In_2(SO_4)_3$ attack the carbonyl oxygen to produce intermediate **II.** Thus the 1,4-conjugate addition of indole at 3rd position was facilitated which led to the formation of **III**. Subsequently, the intermediate **III** underwent cleavage to furnish the final product **V** (Marco *et al*., 2002).

Biological activity

The synthesized 3-(1*H*-indol-3-yl)-1,3-diphenylpropan-1-ones **3a–l** were tested for their in vitro cytotoxic effect against five cancer cell lines such as ACHN, Panc1, Calu1, H460 HCT116 and MCF10A. Gemcitabine and Flavopyridol were

Scheme 2 The plausible reaction mechanism for the Indium(III) sulphate catalysed Michael addition reaction



employed as positive control. According to the PI staining assay, the IC_{50} (the concentration of compound required to inhibit 50 % of cell growth) was determined. The results are summarized in Table 3.

As shown in Table 3 most of the tested compounds showed moderate to high cytotoxic activity against ACHN, Panc1, Calu1, H460 and HCT116 cell lines. It was note worthy that the cytotoxic effect was more pronounced against H460 cell line compared with other cell line, wherein IC_{50} values at 1.7–4.8 μM in comparison with positive control Gemcitabine at 0.27 μM and Flavopyridol at 0.58 μM, respectively. Among the tested compounds **3a–l**, the compound **3e** having chloro groups and compound **3l** with methoxy groups are found to be the most promising compounds in this series when compared to positive control. Furthermore, the compound **3e** showed high potent activity against Panc1, Calu1, H460 cell line with IC_{50} values at 1.4, 1.8 and 1.7 μM which was comparable with positive control Gemcitabine at 0.34, 0.45, 0.27 μM and Flavopyridol at 0.56, 0.64, 0.58 μM, respectively.

Also found that the compounds **3a–d** and positive control exhibited less cytotoxicity against the normal MCF10A cell line at $IC_{50} > 10$ μM. whereas the compounds **3e–l** showed moderate cytotoxic effect with IC_{50} values at 2.1–8.7 μM on normal MCF10A cell line.

The structural activity relationship (SAR) analysis of compounds **3a–l** containing chloro, fluoro, bromo and methoxy functional groups in phenyl rings at C2, C3, C4 and C6 positions shows cytotoxic effect on cancer and normal cell lines are listed in Table 3. The resultant values of each compound depend on their functional groups which show moderate to high inhibitory activity in all cancer cell lines with reference to Gemcitabine and Flavopiridol with % SAR activation of 0.434 and 0.52 respectively. Since the synthesized compounds **3a–d** and **3f–k** show high to moderate % SAR activation compare to standard drugs no significant activity found on tested cancer cell lines (Fig. 1). The % SAR activation value of compounds **3e** having chloro substitution and compound **3l** with methoxy substitution lies in the range of standard drug i.e. 0.460 and 0.407 respectively shows significant anti cancer effect on tested cell lines. Finally it has been concluded that the target compounds having chloro substituent in 1,3-bis(4-chlorophenyl)-3-(1*H*-indol-3-yl)propan-1-one **3e** and methoxy substituent in 3-(2,4-dimethoxyphenyl)-3-(1*H*-indol-3-yl)-1-(4-methoxyphenyl)propan-1-one **3l** are the probable lead molecules which significantly act against cancer cell lines.

Experimental

Chemistry

The chemicals and reagents obtained from HiMedia, Sigma-Aldrich Chemical Company were used as received. Melting points were uncorrected, determined in open capillary. Purity of the compounds was checked by TLC on silica gel, and compounds were purified by using column chromatography. FTIR spectra were recorded in KBr pellet using Shimadzu-8400S spectrometer in the range 400–4,000 cm^{-1}, ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker supercon FT NMR (400 MHz) spectrometer in $CDCl_3$ or DMSO-d_6 and TMS as an internal standard. The chemical shifts are expressed in δ units. Mass spectra were recorded on a JEOL SX 102/DA-6000 (10 kV) FAB mass spectrometer. The purity of the compound is between 96 and 100 %.

*Typical procedure for the synthesis of 1,3-bis(4-fluorophenyl)-3-(1H-indol-3-yl)propan-1-one (**3a**)*

To the stirred solution of chalcone (0.25 g, 1 mmol), indole (0.12 g, 1 mmol) in ethanol (5 mL) was added indium(III) sulphate (0.10 g, 20 mol%) and the reaction mixture was refluxed on water bath until completion of the reaction as indicated by TLC (pet ether/ethyl acetate 8:2 v/v). The catalyst was recovered by filtration and washed with ethanol (3 × 3 mL), dried in oven. Then the combined filtrate was quenched with water and extracted with ethyl acetate (10 × 3 mL). The organic phase was combined, dried over anhyd Na_2SO_4. Evaporation of organic phase offered crude product which was then purified by column chromatography over silica gel (pet ether/ethyl acetate 8:2 v/v) and recrystallization from ethanol to get pure product **3a** (0.349 g, 94 %), as crystalline solid, mp 118–120 °C. Similarly remaining compounds (**3b–l**) were also prepared.

Table 3 In vitro cytotoxic activities of compounds **3a–f**, against five cancer and normal MCF10A cell lines

Compound	Cell line (IC_{50}, μM ± SD)						SAR analysis
	ACHN	Panc1	Calu1	H460	HCT116	MCF10A	(% of activation)
3a	6.3 ± 2.82	4.3 ± 1.08	4.5 ± 1.16	2.4 ± 0.62	5.2 ± 1.53	>10 ± 3.2	0.970
3b	3.3 ± 0.18	4.4 ± 1.18	5.4 ± 2.06	4.0 ± 1.57	4.7 ± 1.03	>10 ± 3.2	0.831
3c	5.9 ± 2.42	3.8 ± 0.58	3.6 ± 0.26	3.6 ± 0.57	4.3 ± 0.63	>10 ± 3.2	0.933
3d	3.7 ± 0.22	3.2 ± 0.01	3.3 ± 0.03	3.6 ± 0.57	4.2 ± 0.53	>10 ± 3.2	0.807
3e	2.3 ± 1.18	1.4 ± 1.81	1.8 ± 1.53	1.7 ± 1.32	2.7 ± 0.97	6.8 ± 0	0.460
3f	3.1 ± 0.38	3.2 ± 0.01	3.4 ± 0.06	3.3 ± 0.27	3.5 ± 0.17	8.7 ± 1.9	0.756
3g	3.8 ± 0.32	4.1 ± 0.88	3.4 ± 0.06	2.9 ± 0.12	4.5 ± 0.83	6.5 ± 0.3	0.768
3h	2.9 ± 0.58	4.2 ± 0.98	3.7 ± 0.36	4.8 ± 1.77	4.8 ± 1.13	4.6 ± 2.2	0.886
3i	4.3 ± 0.82	5.1 ± 1.88	4.6 ± 1.26	2.6 ± 0.42	3.1 ± 0.57	3.9 ± 2.9	0.816
3j	3.2 ± 0.28	2.7 ± 0.51	3.1 ± 0.23	3.5 ± 0.47	3.8 ± 0.13	2.7 ± 4.1	0.806
3k	3.4 ± 0.08	2.4 ± 0.81	3.2 ± 0.13	2.7 ± 0.32	2.8 ± 0.87	3.1 ± 3.7	0.223
3l	2.6 ± 0.88	2.4 ± 0.81	2.7 ± 0.63	3.1 ± 0.07	3.4 ± 0.27	2.1 ± 4.7	0.407
Positive control 1 Gemcitabine	0.17 ± 3.29	0.34 ± 2.85	0.45 ± 2.87	0.27 ± 2.73	0.24 ± 3.39	>10 ± 3.2	0.434
Positive control 2 Flavopiridol	0.45 ± 3.03	0.56 ± 2.65	0.64 ± 2.69	0.58 ± 2.44	0.72 ± 2.95	>10 ± 3.2	0.52

Spectral data

*1,3-Bis(4-fluorophenyl)-3-(1H-indol-3-yl)propan-1-one (**3a**)* IR (KBr): *v* 3402, 2923, 1666, 1010 cm^{-1}, ^1H NMR (400 MHz, DMSO-d_6) δ: 10.86 (s, 1H), 8.12–8.08 (m, 2H), 7.45–7.41 (m, 3H), 7.36–7.30 (m, 4H), 7.06–7.01 (m, 3H), 6.90 (t, $J = 1.8$ Hz, 1H), 4.87 (t, $J = 1.8$ Hz, 1H), 3.91 (dd, $J = 7.2$, 7.2 Hz, 1H), 3.81 (dd, $J = 8.0$, 8.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 197.4 (CO–C_1), 168.6 (C_4–Ar_1), 168.0 (C_4–Ar_3), 136.8 (C_1–Ar_1), 134.8 (C_1–Ar_3), 131.5 (C_9–Ind), 131.5 ($C_{3,5}$–Ar_1), 131.4, 130.0 ($C_{2,6}$–Ar_3, $C_{2,6}$–Ar_1), 126.7, 122.3, 121.9, 119.0, 118.7, 114.9, 111.8 ($C_{8,2,5,6,4,3,7}$–Ind), 45.3 (CH–C_3), 37.7 (CH_2–C_2). MS: *m/z* = 360.1 (M+), 361.1 (M+1). Elemental analysis calcd for $C_{23}H_{17}F_2NO$ = C, 76.44, H, 4.74, N, 3.88 % Found C, 76.14, H, 4.79, N, 3.81 %.

*3-(4-Chlorophenyl)-3-(1H-indol-3-yl)-1-phenylpropan-1-one (**3b**) (Chauan-Ji and Chen-Jiang, 2010)* ^1H NMR (300 MHz, $CDCl_3$) δ: 8.00 (s, 1H), 7.78 (d, $J = 8.55$ Hz, 2H), 7.58 (d, $J = 8.58$ Hz, 2H), 7.26–7.41 (m, 3H), 7.17 (t, $J = 8.07$ Hz, 1H), 7.04 (t, $J = 7.92$ Hz, 1H), 6.92–6.95 (m, 4H), 5.03 (t, $J = 7.14$ Hz, 1H), 3.77 (dd, $J = 6.45$, 16.62 Hz, 1H), 3.65 (dd, $J = 8.01$, 16.59 Hz, 1H). ^{13}C NMR (300 MHz, $CDCl_3$) δ: 197.3 (CO–C_1), 135.6 (C_1–Ar_1), 131.8 (C_9–Ind), 129.5, 129.2 ($C_{3,5}$–Ar_3, $C_{2,6}$–Ar_1), 129.0, 128.2 ($C_{2,6}$–Ar_3, $C_{3,5}$–Ar_1), 126.3, 122.2, 121.1, 119.5, 119.3, 115.0, 111.1 ($C_{8,2,5,6,4,3,7}$–Ind), 45.0 (CH–C_3), 37.4 (CH_2–C_2). MS: *m/z* = 361.1 (M+1). Elemental analysis calcd for $C_{23}H_{18}ClNO$ = C, 76.77, H, 5.04, N, 3.89 % Found C, 76.69, H, 5.01, N, 3.81 %.

*3-(4-Chlorophenyl)-1-(4-fluorophenyl)-3-(1H-indol-3-yl) propan-1-one (**3c**)* IR (KBr): *v* 3386, 2923, 1658, 1087 cm^{-1},^1H NMR (300 MHz, DMSO-d_6) δ: 10.90 (s, 1H), 8.06–8.12 (m, 2H), 7.25–7.43(m, 9H), 7.00–7.05 (m, 1H), 6.87–6.92 (m, 1H), 4.86 (t, $J = 7.20$ Hz, 1H), 3.92 (dd, $J = 6.9$, 17.4 Hz, 1H), 3.82 (dd, $J = 7.8$, 17.4 Hz, 1H). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 197.2 (CO–C_1), 167.1 (C_4–Ar_1), 163.8 (C_4–Ar_3), 144.7 (C_1–Ar_3), 136.8 (C_9–Ind), 134.0 ($C_{2,6}$–Ar_1), 131.5 (C_1–Ar_1), 131.4 ($C_{2,6}$–Ar_3), 130.1, 130.7 ($C_{3,5}$–Ar_1, $C_{3,5}$–Ar_3), 128.4, 122.4, 121.5, 119.0, 118.0, 115.9, 111.8 ($C_{8,2,5,6,4,3,7}$–Ind), 44.4 (CH–C_3), 37.4 (CH_2–C_2). MS: *m/z* = 376.2 (M−1). Elemental analysis calcd for $C_{23}H_{17}ClFNO$ = C, 73.11, H, 4.54, N, 3.71 % Found C, 73.09, H, 4.59, N, 3.68 %.

*1-(4-Chlorophenyl)-3-(4-fluorophenyl)-3-(1H-indol-3-yl) propan-1-one (**3d**)* IR (KBr): *v* 3409, 2922, 1666, 1234 cm^{-1}, ^1H NMR (300 MHz, DMSO-d_6) δ: 10.88 (d, $J = 12.90$ Hz, 1H), 8.03 (d, $J = 8.40$ Hz, 2H), 7.57 (d, $J = 8.40$ Hz, 2H), 7.27–7.45 (m, 5H), 7.04 (t, $J = 8.40$ Hz, 3H), 6.88 (q, $J = 12.90$ Hz, 1H), 4.88 (t, $J = 7.20$ Hz, 1H), 3.94 (dd, $J = 6.9$, 17.1 Hz, 1H), 3.81 (dd, $J = 7.8$, 17.4 Hz, 1H). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 197.8 (CO–C_1), 162.5 (C_4–Ar_3), 159.3 (C_4–Ar_1), 141.7 ($C_{2,6}$–Ar_1), 138.5 ($C_{3,5}$–Ar_1), 136.8 (C_9–Ind), 135.9 (C_1–Ar_3), 130.4 (C_1–Ar_1), 130.0 ($C_{3,5}$–Ar_3), 129.9 ($C_{2,6}$–Ar_3), 126.7, 122.4, 121.5, 119.1, 118.7, 115.0, 111.8 ($C_{8,2,5,6,4,3,7}$–Ind), 44.7 (CH–C_3), 37.3 (CH_2–C_2). MS: *m/z* = 376.8 (M−1). Elemental analysis calcd for $C_{23}H_{17}ClFNO$ = C,73.11, H,4.54, N,3.71 % Found C, 73.15, H, 4.58, N, 3.68 %.

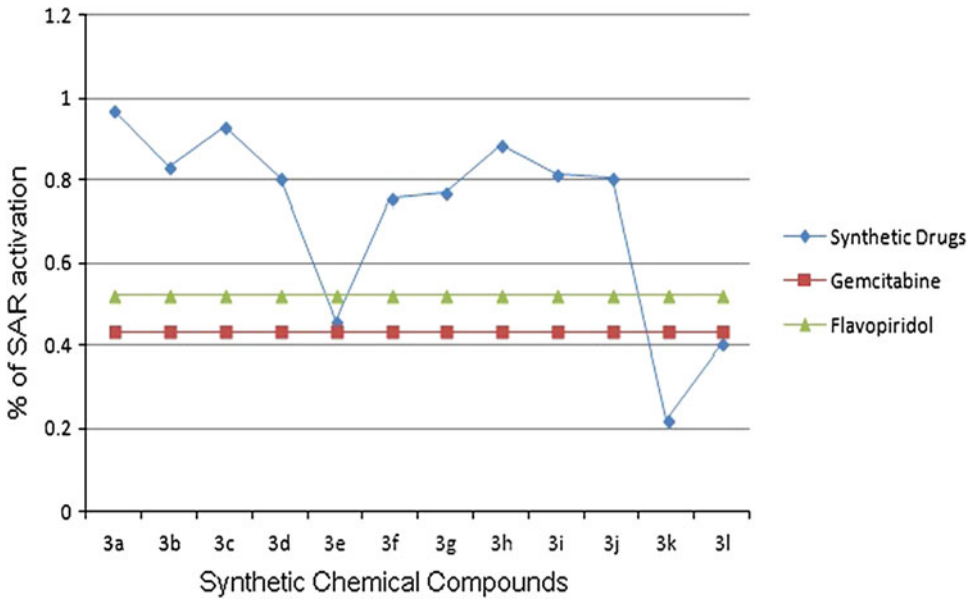


Fig. 1 % SAR activation of synthesized compounds **3a–f** in comparison with positive control Gemcitabine and Flavopiridol

*1,3-Bis(4-chlorophenyl)-3-(1H-indol-3-yl)propan-1-one (**3e**)* IR (KBr): *v* 3448, 2930, 1681, 1095 cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$) δ: 8.03 (s, 1H), 7.86 (d, $J = 8.52$ Hz, 2H), 7.41 (q, $J = 2.55$ Hz, 3H), 7.37 (q, $J = 8.55$ Hz, 2H), 7.11–7.15 (m, 4H), 5.03 (t, $J = 7.02$ Hz, 1H), 3.78 (dd, $J = 6.39$, 16.77 Hz, 1H), 3.66 (dd, $J = 8.04$, 16.8 Hz, 1H). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 197.3 (CO–C_1), 161.5 (C_4–Ar_1), 158.9 (C_4–Ar_3), 141.8 ($C_{3,5}$–Ar_1), 141.6 ($C_{3,5}$–Ar_3), 139.4 (C_1–Ar_3), 134.9 (C_1–Ar_1), 136.3 (C_9–Ind), 130.9 ($C_{2,6}$–Ar_3), 131.0 ($C_{2,6}$–Ar_1), 129.9, 121.9, 117.5, 117.4, 116.8, 115.5, 110.3 ($C_{8,2,5,6,4,3,7}$–Ind), 44.6 (CH–C_3), 37.8 (CH_2–C_2). MS: *m/z* = 396.0 (M+2). Elemental analysis calcd for $C_{23}H_{17}Cl_2NO$ = C, 70.06, H, 4.35, N, 3.55 % Found C, 70.11, H, 4.40, N, 3.47 %.

*3-(4-Chlorophenyl)-3-(1H-indol-3-yl)-1-(4-methoxyphenyl) propan-1-one (**3f**) (Chauan-Ji and Chen-Jiang, 2010)* ^1H NMR (400 MHz, $CDCl_3$) δ: 7.99 (s, 1H), 7.91–7.94 (d, $J =$ 12 Hz, 2H), 7.00–7.41 (m, 9H), 6.90–6.93 (d, $J = 12$ Hz, 2H), 5.04 (t, $J = 7.16$ Hz, 1H), 3.00 (dd, $J = 6.4$, 6.36 Hz, 1H), 3.65 (dd, $J = 8.08$, 8.08 Hz, 1H), 3.87 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 197.8 (CO–C_1), 161.5 (C_4–Ar_1), 158.9 (C_4–Ar_3), 141.8 ($C_{3,5}$–Ar_3), 141.6 ($C_{3,5}$–Ar_1), 139.4 (C_1–Ar_3), 134.9 (C_1–Ar_1), 136.3 (C_9–Ind), 130.9 ($C_{2,6}$–Ar_1), 131.0 ($C_{2,6}$–Ar_3), 129.9, 127.0, 117.5, 117.4, 116.8, 115.5, 110.3 ($C_{8,2,5,6,4,3,7}$–Ind), 55.8 (OCH_3), 44.6 (CH–C_3), 37.8 (CH_2–C_2). MS: *m/z* = 412.3 (M+23). Elemental analysis calcd for $C_{24}H_{20}ClNO_2$ = C, 73.94, H, 5.17, N, 3.59 % Found C, 73.89, H, 5.21, N, 3.63 %.

*1-(4-Bromophenyl)-3-(4-fluorophenyl)-3-(1H-indol-3-yl) propan-1-one (**3g**)* IR (KBr): *v* 3409, 2923, 1681, 1218, 1072 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ: 7.98 (s, 1H), 7. 77 (d, $J = 8.8$ Hz, 2H), 7.57 (d, $J = 8.8$ Hz, 2H), 7.39–7.26 (m, 4H), 7.18–7.14 (m, 1H), 7.05–6.91 (m, 4H), 5.02 (t, $J = 7.2$ Hz, 1H), 3.77 (dd, $J = 6.4$ 16.8 Hz, 1H), 3.65 (dd, $J = 7.6$, 16.8 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 197.3 (CO–C_1), 139.6 ($C_{3,5}$–Ar_1), 136.6 (C_9–Ind), 135.8 (C_1–Ar_1), 131.9 ($C_{2,6}$–Ar_1), 129.5 ($C_{2,6}$–Ar_3), 128.2 122.3, 121.2, 119.5, 119.1, 115.1, 111.2 ($C_{8,2,5,6,4,3,7}$–Ind), 45.1 (CH–C_3), 37.6 (CH_2–C_2). MS: *m/z* = 422.2 (M+), 423.3 (M+1). Elemental analysis calcd for $C_{23}H_{17}BrFNO$ = C, 65.42, H, 4.06, N, 3.32 % Found C, 65.36, H, 4.11, N, 3.36.

*1-(4-Bromophenyl)-3-(2-chlorophenyl)-3-(1H-indol-3-yl) propan-1-one (**3h**)* IR (KBr): *v* 3409, 2923, 1681, 1095 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ: 8.02 (s, 1H), 7. 89 (d, $J = 8.4$ Hz, 2H), 7.78 (d, $J = 8.8$ Hz, 2H), 7.72 (s, 1H), 7.67–7.56 (m, 5H), 7.49–7.45 (m, 3H), 5.02 (t, $J =$ 7.2 Hz, 1H), 3.76 (dd, $J = 6.4$, 16.8 Hz, 1H), 3.64 (dd, $J = 8$, 16.4 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 197.8 (CO–C_1), 139.8 (C_1–Ar_3), 136.6 (C_9–Ind), 136.3 (C_1–Ar_1), 135.7 ($C_{3,5}$–Ar_1), 131.6 ($C_{2,6}$–Ar_1), 129.7 (C_4–Ar_3), 128.7 ($C_{3,5}$–Ar_3), 128.4 (C_2–Ar_3), 127.6, 123.2, 121.8, 119.3, 118.9, 115.4, 111.0 ($C_{8,2,5,6,4,3,7}$–Ind), 45.4 (CH–C_3), 37.9 (CH_2–C_2). MS: *m/z* = 439.8 (M+1). Elemental analysis calcd for $C_{23}H_{17}BrClNO$ = C, 62.96, H, 3.91, N, 3.19 % Found C, 62.99, H, 3.88, N, 3.23 %.

*3-(4-Fluorophenyl)-3-(1H-indol-3-yl)-1-(4-methoxyphenyl) propan-1-one (**3i**) (Chauan-Ji and Chen-Jiang, 2010)* ^1H NMR (400 MHz, $CDCl_3$) δ: 7.97 (s, 1H), 7. 91 (d, $J =$ 8.4 Hz, 2H), 7.39 (d, $J = 8$ Hz, 2H), 7.34–7.27 (m, 3H), 7.15(t, $J = 7.2$ Hz, 1H), 7.03–6.90 (m, 6H), 5.04 (t, $J =$ 7.6 Hz, 1H), 3.85 (s, 3H), 3.74 (dd, $J = 6.4$, 16.8 Hz, 1H), 3.63 (dd, $J = 8$, 16.4 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 197.1 (CO–C_1), 164.2 (C_4–Ar_1), 149.5 (C_4–Ar_3),

139.0 ($C_{3,5}$–Ar_1), 138.4 (C_1–Ar_1), 137.3 (C_9–Ind), 135.7 (C_1–Ar_3), 130.1 ($C_{3,5}$–Ar_3) 128.1 ($C_{2,6}$–Ar_3), 127.2 ($C_{2,6}$–Ar_1), 122.5, 122.3, 119.4, 119.3, 118.9, 110.4 ($C_{8,2,5,6,4,3,7}$–Ind), 56.3 (OCH_3), 45.7 (CH–C_3), 37.8 (CH_2–C_2). MS: $m/z = 374.0$ (M+ 1). Elemental analysis calcd for $C_{24}H_{20}FNO_2$ = C, 77.19, H, 5.40, N, 3.75 % Found C, 77.23, H, 5.36, N, 3.70 %.

3-(3,4-Dimethoxyphenyl)-3-(1H-indol-3-yl)-1-phenylpropan-1-one ***(3j)*** IR (KBr): ν 3379, 2932, 1681, 1257 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ: 7.98 (s, 1H), 7. 85 (d, $J = 6.4$ Hz, 2H), 7.44 (d, $J = 8$ Hz, 1H), 7.40–7.38 (m, 2H), 7.33 (d, $J = 8.4$ Hz, 1H), 7.18–7.14 (m, 1H), 7.05–7.01 (m, 1H), 6.9 (s, 1H), 6.86–6.84 (m, 2H), 6.75 (d, $J = 8.8$ Hz, 1H), 4.98 (t, $J = 7.2$ Hz, 1H), 3.81 (s, 3H), 3.78 (s, 3H), 3.72 (dd, $J = 6.4$, 16.4 Hz, 1H), 3.65 (dd, $J = 8.4$, 17.6 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 198.0 (CO–C_1), 149.2 (C_4–Ar_3), 147.9 (C_3–Ar_3), 139.9 (C_1–Ar_1), 136.9 (C_4–Ar_1), 135.9 (C_1–Ar_3), 129.9 ($C_{3,5}$–Ar_1), 126.9 (C_5–Ar_3), 122.6 (C_2–Ind), 121.7 (C_2–Ar_3), 119.9, 119.9, 119.8, 111.4 ($C_{6,4,3,7}$–Ind), 56.2 ($2OCH_3$), 45.6 (CH–C_3), 38.4 (CH_2–C_2). MS: $m/z = 384.0$ (M−1). Elemental analysis calcd for $C_{25}H_{23}NO_3$ = C, 77.90, H, 6.01, N, 3.63 % Found C, 77.93, H, 6.09, N, 3.59 %.

1-(4-Chlorophenyl)-3-(2,6-difluorophenyl)-3-(1H-indol-3-yl) propan-1-one ***(3k)*** IR (KBr): ν 3417, 2962, 1681, 1265, 1087 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$) δ: 8.03 (s, 1H), 7. 91–7. 89 (m, 2H), 7.68 (d, $J = 8$ Hz, 2H), 7.42–7.39 (m, 2H), 7.33 (d, 8 Hz, 1H), 7.19–7.15 (m, 2H), 7.12–7.07 (m, 2H), 6.82 (t, $J = 8.4$ Hz, 1H), 5.46 (t, $J = 7.6$ Hz, 1H), 4.06 (dd, $J = 8.8$, 17.2 Hz, 1H), 3.85 (dd, $J = 6$, 17.6 Hz, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 197.4 (CO–C_1), 163.0 (C_2–Ar_3), 160.6 (C_6–Ar_3), 160.5 (C_4–Ar_1), 139.9 (C_1–Ar_3), 136.4 (C_9–Ind), 135.5 (C_1–Ar_1), 129.9 ($C_{3,5}$–Ar_1), 128.3 ($C_{3,5}$–Ar_3), 128.3 (C_4–Ar_3), 122.6, 122.0, 120.1, 119.2, 117.3, 111.5 ($C_{5,2,6,4,3,7}$–Ind), 42.7 (CH–C_3), 37.5 (CH_2–C_2). MS: $m/z = 418.8$ (M+23). Elemental analysis calcd for $C_{23}H_{16}ClF_2NO$ = C, 69.79, H, 4.07, N, 3.54 % Found C, 69.81, H, 4.11, N, 3.50 %.

3-(2,4-Dimethoxyphenyl)-3-(1H-indol-3-yl)-1-(4-methoxyphenyl)propan-1-one ***(3l)*** IR (KBr): ν 3332, 2931, 1674, 1242 cm^{-1}, ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.91$ (s, 1H), 7.92 (d, $J = 8.8$ Hz, 2H), 7.45 (d, $J = 8.0$ Hz, 1H), 7.32 (d, $J = 8.4$ Hz, 1H), 7.15 (t, $J = 7.2$ Hz, 1H), 7.02 (t, $J = 7.2$ Hz, 1H), 6.9 (s, 1H), 6.91–6.85 (m, 4H), 6.74 (d, $J = 8.8$ Hz, 1H), 5.00 (t, $J = 7.6$ Hz, 1H), 3.85 (s, 3H), 3.81 (s, 3H), 3.76 (s, 3H), 3.71–3.66 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) $\delta = 197.5$ (CO–C_1), 146.6 ($C_{2,4}$–Ar_3), 137.1 (C_1–Ar_3), 136.0 (C_9–Ind), 134.7 ($C_{3,5}$–Ar_3), 132.3 (C_6–Ar_3), 130.1 (C_1–Ar_1), 130.0 ($C_{2,6}$–Ar_1), 129.9 ($C_{3,5}$–Ar_1), 127.3, 122.8, 121.8, 120.1, 119.8, 118.8, 111.5 ($C_{5,2,6,4,3,7}$–Ind), 45.2 ($2OCH_3$), 40.3 (CH–C_3), 38.2 (OCH_3, CH_2–C_2). MS: $m/z = 438.0$ (M+23). Elemental analysis calcd for $C_{26}H_{25}NO_4$ = C, 75.16, H, 6.06, N, 3.37 % Found C, 75.21, H, 6.16, N, 3.30 %.

Cytotoxic study

Propidium iodide (PI) staining assay (Zakiah et al., 2012)

PI was used to stain the nuclear changes of living and apoptotic cells. Briefly, ACHN, Panc1, Calu1, H460 and HCT116 cancer cell lines along with normal MCF10A cells (2×10^6 cells/well) were incubated for 24 h in 5 % CO_2 at 37 °C with different concentrations of the synthesized compounds **3a**–**l**. Gemcitabine and Flavopyridol were used as positive controls. The cells were further incubated for another 48 h, harvested, homogenised in 200 µL of 1 % formaldehyde and again incubated for 15 min. The cells were washed twice with cold PBS (Phosphate Buffered Saline), and then 1 mL of 10 µg/mL PI was added into each well and incubated at 37 °C for 5 min in dark to allow nuclear penetration. After being washed with cold PBS, the cells were detected by blue filter (515 nm) fluorescent microscope (Olympus Corp., Shibuya-ku, Tokyo, Japan) at 400× magnification. The activity of derived compounds of inhibitory constants was predicted based on standard deviation.

Conclusion

In conclusion, we demonstrated an efficient and simple procedure for the Michael addition of indole to α,β-unsaturated ketones catalysed by indium(III) sulphate. The resultant compounds were tested to identify the cytotoxic effect on cancer cell lines. The bioactivity of cell lines compared with standard shows **3e** and **3l** have significant effect with all tested cancer cell lines with IC50 values ranging from 1.4 to 2.7 µM % SAR activation value of 0.46 and 2.4–3.4 µM % SAR activation of 0.407, respectively. The standard compounds Gemcitabine and Flavopiridol show % SAR activation of 0.434 and 0.53.

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