Synthesis, crystal structure and evaluation of cancer inhibitory activity of 4-[indol-3-yl-methylene]-1*H*-pyrazol-5(4*H*)-one derivatives

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A series of 4-(1*H*-indol-3-yl-methylene)-1*H*-pyrazol-5(4*H*)-one derivatives have been synthesised. The *Z* structure of 4-[(1-methyl-1*H*-indol-3-yl)methylene]-3-phenyl-1-*p*-tolyl-1*H*-pyrazol-5-one was determined by X-ray crystallography. The antitumour activity was evaluated against five cancer cells by MTT assay. [(1*H*-Indol-3-yl)methylene]-1-(2,4-dinitrophenyl)-3-methyl-1*H*-pyrazole-5-one and 4-{4-[(1-benzyl-1*H*-indol-3-yl)methylene]-3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-1-yl}-benzoic acid have similar anticancer activity with 5-UF on the test cancer cells (exception of A375). Almost all the target compounds displayed antitumour activity against A549 and PC-9, and those with benzyl at 1-position of indole had higher activity against PC-9 (IC50 value lower than 30 μM). Those with benzyl at the indole and carboxyl at the phenyl part of of pyrazole were more active against PC-9 and A549 cells, providing a good indication for subsequent optimisation as lung cancer inhibitory agents.

Keywords: 4-(indol-3-yl-methylene)-1*H*-pyrazol-5(4*H*)-one, 1-aryl-1*H*-pyrazole-5(4*H*)-one, indole derivative, cancer inhibitory activity

The indole unit is the key building block for a variety of compounds which have crucial roles in the functions of biologically important molecules. Indole and its derivatives have a wide range of biological activities,[1] and there has been a growing interest in their synthesis and biological studies. Introduction of different groups to the modified indole structure can produce a series of compounds with multiple activitives,[2] such as antihypertensive drugs (for example, indapamide,[3] indorenate),[4] neuropsychiatric disorders drugs (tryptophan and 5-hydroxytryptophan),[5] plant growth regulator (tryptamine),[6] anticancer drugs (Sunitinib). SU5416, SU668, SU11248 (Sunitinib, Fig. 1) have been developed as anticancer agents by Sugen,[7] and SU11248 is mainly used for treatment of gastrointestinal stromal tumours and metastatic renal cell carcinoma.[8,9] Other drugs such as indomethacin (immunosuppressive activity inhibitor),[10,11] indirubin (topo II inhibitor)[12] had also been used.

Pyrazole derivatives have been widely used in drugs, such as COX-2 inhibitor (celecoxib),[13,14] free radical scavenger (3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one), and acaricides (MK-239). Diana *et al*.[15] synthesised several *Nortopsentins*' analogues (3,5-bis(3′-indolyl)pyrazole), and one compound had low GI_{50} towards nine cancer cell lines, having GI_{50} in the range 1.63–9.64 μM towards all the sub-panel cell lines.

Reddy *et al*.[16] synthesised substituted (*Z*)-5-(*N*-benzylindol-3-ylmethlene)imidazolidine-2,4-diones. Among these compounds, two had IC_{50} values of 4.4 and 5.2 μM against MCF7 cell, respectively, compared to 5-fluorouracil (IC_{50}=15.2 μM). Singh *et al*.[17] reported a series of indole–pyrazole moieties compounds having tumour growth inhibitory activities. Penthala *et al*.[18] synthesised a series of structurally related *N*-heterocyclic indol-3-yl-methylene derivatives, and found that such compounds exhibited substantial activity *in vitro* against human tumour cell lines. Cummings *et al*.[19] reported a novel small molecule natural product analogues capable of discriminating between serotonin 5-HT1A, 5-HT2A, 5-HT2C receptor subtypes.

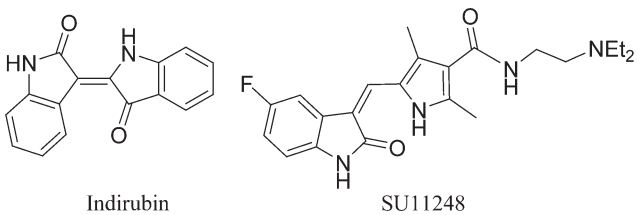

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Fig. 1 Some indole derivatives as anticancer agents.

Based on the structural analysis of the above compounds, and to study their SARS, *N*-substituted indole-3-carbaldehyde and 1-aryl-3-methylpyrazol-5-one and 1,3-diarylpyrazol-5-one were combined with the methylene bond. A series of compounds with indole and pyrazole moieties were designed and synthesised, and their tumour inhibitory activity was evaluated against A549, HCT116, HepG2, PC-9, A375 by the MTT assay.

Results and discussion

All intermediates and the target compounds were prepared with good yield using general procedures (Scheme 1). During the synthesis of **4d**, ethyl bromoacetate was used as the starting material, but there was some hydrolysed product under the reaction conditions. It was therefore replaced by *tert*-butyl bromoacetate for this synthesis, and *tert*-butyl 2-(3-formyl-1H-indole-1-yl)acetate (**4d**) was prepared with high yield. The target compounds **5–33** were obtained by Aldol condensation from intermediate **3** or **4a–d** with **2a–g** catalysed by drops of piperidine, and purified by recrystallisation from ethanol.

The geometry of the double bond in the representative compound **10** was determined by X-ray crystallographic analysis. The geometry of the other target compounds can be speculated from the crystal structure of **10** and analogues reported by Reddy and Singh,[15,16] when hydrogen at the double bond (C=CH) in ^{1}H NMR displayed singlet (δ ranges from 8.08 to 8.18), the geometry was *Z*-form, if it displayed as a doublet or a multiplet (because of the surrounding aryl hydrogens), the geometry of the double bond is ambiguous.

The X-ray crystallographic analysis of compound **10** ($C_{26}H_{21}N_3O$) showed the *Z*-structure (Fig. 2), the bond length between C(9)–C(17) is 1.375 Å, which indicated a double bond, and the bond angle of C(9)–C(17)–C(18) is 133.2°, this *Z*-structure is consistent with that reported by Singh.[17] The other parameters of crystal of compound **10** related to its structure are listed in Table 1. The crystal of compound **10** is monoclinic, $P2_1$ space group, whose structural parameters are a = 13.8705(6) Å, b = 14.5185(7) Å, c = 15.2310(8) Å, α = 90.00 °, β = 93.711(4) °, γ = 90.00°, U = 3060.8(3) Å^3, Z=6.

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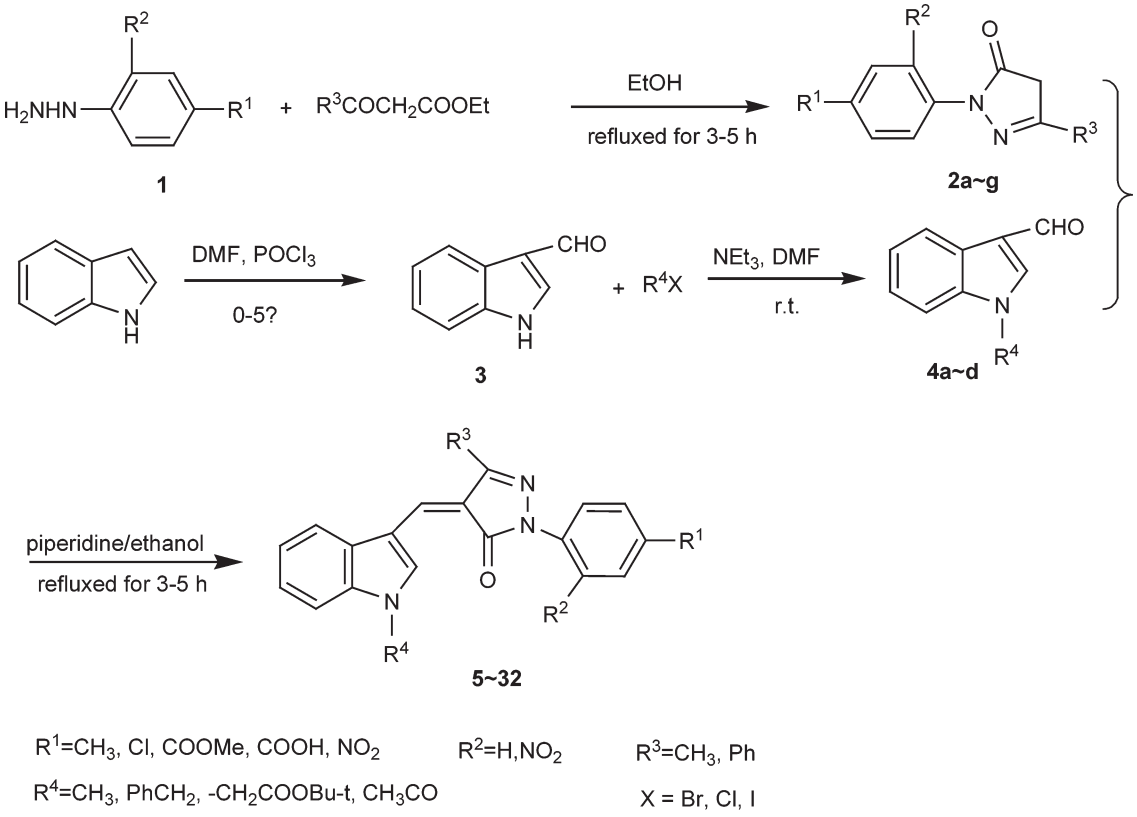


Scheme 1 The general synthetic route for compounds **5–32**.

As shown in Table 2, all the synthesised compounds are inactive against A375 and HepG2 cancer cells (IC_{50} > 70 μM, with the exception of **28** with IC_{50} values of 41.15 μM and 46.53 μM against these two cancer cells, and **14** with IC_{50} value of 28.03 μM against HepG2, respectively). The compounds **7**, **14** and **28**, which contain 2,4-dinitrophenyl at 1-position of pyrazole moiety, displayed moderate activity against HCT116 (IC_{50} = 36.07 μM for **7**, 29.87 μM for **14** and 40.43 μM for **28**, respectively). Almost all the target compounds displayed activity against A549 and PC-9 cancer lines, and the PC-9 cell is more sensitive to them. Compounds with benzyl at 1-position of indole have a higher activity against PC-9 (IC_{50} value lower than 30 μM for **5**, **17**, **26** and **28**), and those containing 4-carboxylphenyl and 2,4-dinitrophenyl at 1-position of pyrazole showed potential inhibitory activity over A549 and PC-9.

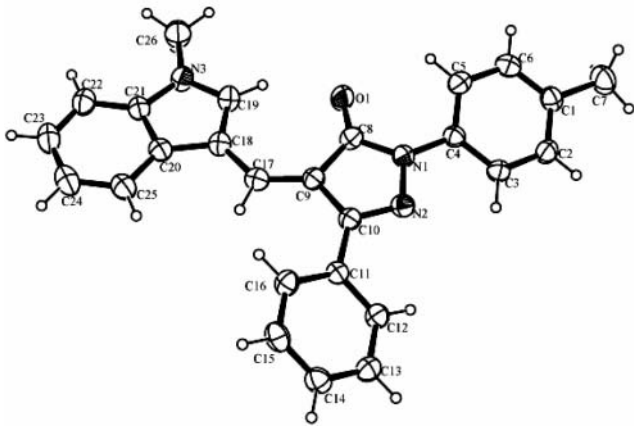


Fig. 2 Partially labeled ORTEP plot of $C_{26}H_{21}N_3O$ (**10**).

Experimental

All starting materials and solvents were used as-received. The melting points of products were determined on a XRC-1 micro melting point apparatus and are uncorrected. TLC was performed on 0.20 mm silica gel GF_{254} plates (Qingdao Ocean Chemical Factory, Shandong, China). The purities of all synthetic molecules in the present work were confirmed as ≥97% by HPLC with a photodiode array detector (Waters, Milford, MA) and the chromatographic column was an Atlantis C18 (150 mm×4.6 mm, i.d. 5 μm, Waters, Milford, Ireland). NMR were recorded on 400 MHz Varian spectrometer (Varian, Palo Alto, CA) model Gemini 400 and reported in parts per million with TMS as internal standard. The IR spectra were recorded on VECTOR22 (Brucker, German). High resolution electron impact (EI) ionisation mass spectra were recorded at 25 eV on a Waters Q-TOF Premier station (magnetic sector instrument) at a resolution of greater than 10000. Crystal structure was determined on Xcalibur E X-ray single crystal diffractometer (oxford, British).

According to the literature procedures,[20] the target compounds **5–32** were obtained in 72–82% yield by the synthetic route shown in Scheme 1, *i.e.* by Aldol condensation from **3** or **4** with **2** catalysed by drops of piperidine.

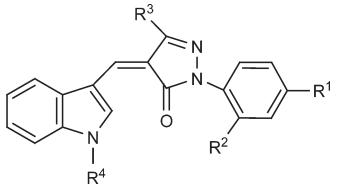
Synthesis of compounds **2a–g***; general procedure*

A mixture of ethyl acetoacetate or ethyl benzoylacetate (0.1 mol) and substituted phenylhydrazine (0.1 mol) in ethanol was refluxed at 90 °C for 3–5 h. The mixture was then cooled to room temperature with stirring to afford a solid which was filtered and washed with

Table 1 Selected bond lengths (Å) and bond angles (°) for compound **10**

Atom–atom(–atom)	Bond lengths (Å) or bond angles (°)
C(8)–C(9)	1.466(4)
C(9)–C(17)	1.375(4)
C(17)–C(18)	1.409(4)
C(9)–C(17)–C(18)	133.2(3)
C(17)–C(9)–C(8)	126.7(3)
C(17)–C(9)–C(10)	128.8(3)

Table 2 IC_{50} value of compounds **5–32** against tumour cells *in vitro*



Compound	IC_{50}[a,b](μM)				
	A549	HCT116	HepG2	PC-9	A375
5 (R^1=Cl, R^2=H, R^3= R^4=Me)	51.55	66.09	>80	45.1	>80
6 (R^1= R^2=NO_2, R^3= Me, R^4=Bn)	53.63	>80	>80	22.42	>80
7 (R^1= R^2=NO_2, R^3=R^4=Me)	>80	>80	>80	42.78	>80
8 (R^1=R^3=Me, R^2=R^4=H)	43.55	>80	>80	36.85	>80
9 (R^1=R^3=R^4=Me, R^2=H,)	62.26	>80	>80	39.33	>80
10 (R^1=R^4=Me, R^2=H, R^3=Ph)	47.78	>80	>80	40.06	>80
11 (R^1=Me, R^2=H, R^3=Ph, R^4=Bn)	61.9	>80	>80	26.61	>80
12 (R^1=Me, R^4=R^2=H, R^3=Ph)	27.25	>80	>80	26.41	>80
13 (R^1=Cl, R^2=H, R^3=Ph, R^4=Bn)	74.51	>80	>80	31.51	>80
14 (R^1=R^2=NO_2, R^3= Me, R^4=H,)	34.7	29.87	28.03	42.47	>80
15 (R^1=CO_2CH_3, R^2=H, R^3=R^4=Me)	48.66	59.52	>80	51.06	>80
16 (R^1=CO_2CH_3, R^2=H, R^3=Me, R^4=CH_2CO_2Bu-t)	35.1	52.8	>80	34.63	>80
17 (R^1= CO_2CH_3, R^2=H, R^3= Me, R^4=Bn,)	65.75	55.96	>80	24.01	>80
18 (R^1=CO_2H, R^3= Me, R^2=R^4=H)	44.12	56.08	>80	44.22	>80
19 (R^1=CO_2H, R^2=H, R^3= R^4=Me)	37.21	50.1	>80	34.95	>80
20 (R^1=CO_2H, R^2=H, R^3=Me, R^4=Ac)	NT[c]	NT	NT	NT	NT
21 (R^1=Cl, R^2=H, R^3= Ph, R^4=Ac)	55.67	>80	>80	37.72	>80
22 (R^1=Cl, R^2=H, R^3=Ph, R^4=CH_2CO_2Bu-t,)	>80	>80	>80	60.37	>80
23 (R^1=Cl, R^2=H, R^3=Me, R^4=Ac)	67.72	>80	>80	57.2	>80
24 (R^1=Cl, R^2=H, R^3=Ph, R^4=Me)	61.77	>80	>80	52.02	>80
25 (R^1=CO_2CH_3, R^2=R^4=H, R^3=Me)	>80	55.56	>80	43.03	>80
26 (R^1=Cl, R^2=H, R^3=Me, R^4=Bn)	70.82	78.63	>80	30.03	>80
27 (R^1=Cl, R^2=R^4=H, R^3=Me)	36.81	63.59	75.18	38.26	>80
28 (R^1=CO_2H, R^2=H, R^3=CH_3, R^4=Bn)	37.24	40.43	46.53	21.62	>80
29 (R^1=CO_2CH_3, R^2=H, R^3=Me, R^4=Ac)	56.82	54.89	>80	41.24	>80
30 (R^1=CO_2H, R^2=H, R^3= Me, R^4=CH_2CO_2Bu-t)	44.88	56.54	>80	39.39	73.69
31 (R^1= Me, R^2=H, R^3=Ph, R^4=Ac)	66.23	>80	>80	49.32	>80
32 (R^1=Me, R^2=H, R^3=Ph, R^4=CH_2CO_2Bu-t)	69.48	78.46	>80	59.2	>80
5-FU	35.05	31.72	46.31	23.47	46.55
SU11248	12.35	18.35	13.24	10.97	11.58

[a] The IC_{50} values represent the concentration that causes 50% growth inhibition.
[b] The IC_{50} values were the mean values of three repeated experiments.
[c] Not tested.

cooled ethanol. The crude product was recrystallised from ethanol to afford **2** as a white solid (characteristic data are shown in Table 3).

1-(2,4-Dinitrophenyl)-3-methyl-1H-pyrazol-5(4H)-one (**2g**): ^{1}H NMR (DMSO-d_6): δ 2.12 (s, 3H, CH_3), 3.54 (s, 2H, CH_2), 7.84 (d, 1H, *J* = 8.0 Hz, ArH), 8.41 (dd, 1H, *J* = 2.0 Hz, *J* = 8.0 Hz, ArH), 8.88 (d, 1H, *J* = 2.4 Hz, ArH), 10.82 (s, 1H, OH).

Synthesis of indole-3-carboxaldehyde (**3**)
Compound **3** was synthesised by Vilsmeir reaction,[24] and recrystallisation from ethanol afforded a pale yellow solid with 85% yield, m.p. 194–195 °C (lit.[25] 193–195 °C).

Synthesis of compound **4a–d***; general procedure*
A solution of 1.2 equimolar amount of the appropriate R^4X in DMF was slowly added to a stirred mixture of compound **3** (5 g, 34 mmol) and K_2CO_3 (5 g) in dry DMF (20 mL). The reaction mixture was maintained at room temperature until TLC indicated the end of reaction. Then water (80 mL) was added and the mixture was extracted with ethyl acetate (3×10 mL). The organic phase was combined, washed twice with brine (30 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to afford the target compound **4** as a white solid.

N-Methyl-1H-indole-3-carbaldehyde (**4a**): Yield: 91%; m.p. 66–67 °C (lit.[26] 68–70 °C); ^{1}H NMR (DMSO-d_6): δ 4.15 (s, 3H, CH_3), 7.20–7.30 (m, 2H, ArH), 7.46 (d, *J* = 7.6 Hz, ArH), 7.98 (d, 1H, *J* = 8.0 Hz, ArH), 8.19 (s, 1H,indole-2-H), 9.94 (s, 1H, CHO).

N-Acetyl-1H-indole-3-carbaldehyde (**4b**): Yield: 82%; m.p. 158–161 °C (lit.[27] 159–162 °C). ^{1}H NMR (DMSO-d_6): δ 2.56 (s, 3H, CH_3), 7.24–7.35 (m, 2H, ArH), 7.49 (d, 1H, *J* = 8.0 Hz, ArH), 8.02 (d, 1H, *J* = 8.0 Hz, ArH), 8.27 (s, 1H, indole-2-H), 9.91 (s, 1H, CHO).

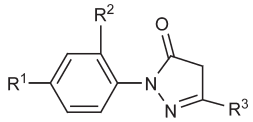
N-Benzyl-1H-indole-3-carbaldehyde (**4c**): Yield: 87%; m.p. 105–107 °C (lit.[28]: m.p. 111 °C, or 102–104 °C). ^{1}H NMR (DMSO-d_6): δ 5.50 (s, 2H, CH_2), 7.22–7.38 (m, 7H, ArH), 7.49 (d, 1H, *J* = 8.0 Hz, ArH), 8.09 (d, 1H, *J* = 8.0 Hz, ArH), 8.24 (s, 1H, indole-2-H), 9.92 (s, 1H, CHO).

tert-Butyl 2-(3-formyl-1H-indol-1-yl)acetate (**4d**): Yield: 92%; m.p. 120–122 °C. IR (KBr, cm^{-1}): 1695, 1742; ^{1}H NMR (DMSO-d_6): δ 1.43 (s, 9H, $3CH_3$), 5.18 (s, 2H, CH_2), 7.24–7.33 (m, 2H, ArH), 7.51 (d, 1H, *J* = 8.0 Hz, ArH), 8.10 (d, 1H, *J* = 7.6 Hz, ArH), 8.27 (s, 1H, indole-2-H), 9.94 (s, 1H, CHO). ESI-HRMS: Calcd for $C_{15}H_{17}NO_3$ (MH^+): 260.3078, found: 260.3072.

Synthesis of compound **5–32***; general procedure*
Piperidine (2–3 drops) was added to a mixture of compound **2** or **3** and **4** (1:1 equiv.) in ethanol. The mixture was refluxed for 3–5 h. The precipitate thus obtained was collected by filtration after cooled, washed with cold water, finally washed with methanol and dried to afford the crude product. Crystallisation from methanol or methanol and DMF (3:1) afforded the target compound **5–32** as an orange crystalline product.

1-(4-Chlorophenyl)-3-methyl-4[(1-methy-1H-indol-3-yl)methylene]-1H-pyrazol-5(4H)-one (**5**): Yield: 78%; m.p. 196–198 °C; ν_{max} (KBr, cm^{-1}): 1674 (CO); ^{1}H NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 4.02 (s, 3H, CH_3), 7.38–7.40 (m, 2H, ArH), 7.48 (d, 2H, *J* = 8.4 Hz, ArH), 7.67 (d, 1H, *J* = 8.4 Hz, ArH), 8.06 (d, 2H, *J* = 8.4 Hz, ArH), 8.11 (s, 1H, =CH), 8.20 (d, 1H, *J* = 7.2 Hz, ArH), 9.78 (s, 1H, ArH). ESI-HRMS: Calcd for $C_{20}H_{16}ClN_3O$ (MH^+): 350.1060; found: 350.1056 (100%), 352.1036 (38%).

Table 3 Some characteristic data of the compounds **2a–g**



2a-g

Product		M.p./°C	Yield/%
2a	R^1=CH_3, R^2=H, R^3=CH_3	134–135 (lit.[21]134–134.5)	65
2b	R^1= Cl, R^2=H, R^3= CH_3	172–174 (lit.[21] 172–173)	60
2c	R^1=COOH, R^2=H, R^3=CH_3	283–285 (lit.[21] 285)	67
2d	R^1=$COOCH_3$, R^2=H, R^3=CH_3	127–128 (lit.[21]127–127.5)	66
2e	R^1=CH_3, R^2=H, R^3=Ph	141–142 (lit.[21]142–144)	75
2f	R^1=Cl, R^2=H, R^3=Ph	161–163 (lit.[21]162.5–164)	70
2g	R^1=R^2=NO_2, R^3=CH_3	180–181(lit.[22] 144–145, lit.[23] 201–202)	60

4-[(1-Benzyl-1H-indol-3-yl)methylene]-1-(2,4-dinitrophenyl)-3-methyl-1H-pyrazol-5(4H)-one (**6**): Yield: 80%; m.p. 206–208 °C; ν_{max} (KBr, cm^{-1}): 1691 (CO), 1523 (NO_2), 1333 (NO_2); ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 5.71 (s, 2H, CH_2), 7.28–7.40 (m, 7H, ArH), 7.69–7.71 (m, 1H, ArH), 8.11 (s, 1H, =CH), 8.25 (m, 2H, ArH), 8.60 (dd, 1H, *J* = 2.4 Hz, *J* = 8.4 Hz, ArH), 8.76 (d, 1H, *J* = 2.4 Hz, ArH), 9.72 (s, 1H, indole-CH). ESI-HRMS: Calcd for $C_{26}H_{19}N_5O_5$ (MH^+): 482.1464; found: 482.1467.

1-(2,4-Dinitrophenly)-3-methyl-4-[(1-methyl-1H-indol-3-yl)methylene]-1H-pyrazol-5(4H)-one (**7**): Yield: 82%; m.p. 258–260 °C; ν_{max} (KBr, cm^{-1}): 1688 (CO), 1534 (NO_2), 1329 (NO_2); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 4.02 (s, 3H, CH_3), 7.41 (m, 2H, ArH), 7.70 (m, 1H, ArH), 8.11 (s, 1H, =CH), 8.23 (brs, 2H, ArH), 8.60 (dd, 1H, *J* = 2.4 Hz, *J* = 8.4 Hz, ArH), 8.75 (d, 1H, *J* = 2.4 Hz, ArH), 9.57 (s, 1H, =CH). ESI-HRM: Calcd for $C_{20}H_{15}N_5O_5$ (MH^+): 406.1151; found: 406.1147.

4-[(1H-Indol-3-yl)methylene]-3-methy-1-p-tolyl-1H-pyrazol-5-one (**8**): Yield: 78%; m.p. 204–206 °C; ν_{max} (KBr, cm^{-1}): 3267 (NH), 1651 (CO); ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 7.23 (d, 2H, *J* = 8.4 Hz, ArH), 7.31–7.33 (m, 2H, ArH), 7.59 (m, 1H, ArH), 7.72 (d, 1H, *J* = 7.2 Hz, ArH), 7.88 (d, 2H, *J* = 8.4 Hz, ArH), 8.11 (s, 1H, =CH), 8.16–8.19 (m, 1H, ArH), 9.82 (s, 1H, indolyl-2-CH), 12.71 (s, 1H, NH). ESI-HRMS: Calcd for $C_{20}H_{17}N_3O$ ($M+Na^+$): 338.1269; found: 338.1261.

3-Methyl-4-[(1-methyl-1H-indol-3-yl)methylene]-1-p-tolyl-1H-pyrazol-5-one (**9**): Yield: 77%; m.p. 176–178 °C; ν_{max} (KBr, cm^{-1}): 1668 (CO); ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 4.02 (s, 3H, CH_3), 7.23 (d, 2H, *J* = 8.0 Hz, ArH), 7.37–7.40 (m, 2H, ArH), 7.67 (d, 1H, *J* = 7.2 Hz, ArH), 7.89 (d, 2H, *J* = 8.4 Hz, ArH), 8.08 (s, 1H, =CH), 8.20 (d, 1H, *J* = 8.4 Hz, ArH), 9.80 (s, 1H, indolyl=CH). ESI-HRMS: Calcd for $C_{21}H_{19}N_3O$ (MH^+): 330.1606; found: 330.1610.

(4Z)-4-[(1-Methyl-1H-indol-3-yl)methylene]-3-phenyl-1-p-tolyl-1H-pyrazol-5-one (**10**): Yield: 78%; m.p. 206–208 °C; ν_{max} (KBr, cm^{-1}): 1674 (CO); ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 4.06 (s, 3H, CH_3), 7.28 (d, 2H, *J* = 8.4 Hz, ArH), 7.33–7.42 (m, 2H, ArH), 7.59–7.65 (m, 3H, ArH), 7.69 (d, 1H, *J*= 7.6 Hz, ArH), 7.79 (m, 3H, ArH), 7.97 (d, 2H, *J* = 8.4 Hz, ArH), 8.10 (s, 1H, =CH), 9.88 (s, 1H, indolyl=CH). ESI-HRMS: Calcd for $C_{26}H_{21}N_3O$ (MH^+): 392.1763; found: 392.1776.

4-[(1-Benzyl-1H-indol-3-yl)methylene]-3-phenyl-1-p-tolyl-1H-pyrazol-5-one (**11**): Yield: 80%; m.p. 210–212 °C; ν_{max} (KBr, cm^{-1}): 1673 (CO); ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 5.74 (s, 2H, CH_2), 7.28–7.39 (m, 9H, ArH), 7.59–7.65 (m, 3H, ArH), 7.69–7.71 (m, 1H, ArH), 7.78–7.82 (m, 3H, ArH), 7.95 (d, 2H, *J* = 8.4 Hz, ArH), 8.12 (s, 1H, =CH), 10.03 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{32}H_{25}N_3O$ (MH^+): 468.2076; found: 468.2076.

4-[(1H-Indol-3-yl)methylene]-3-phenyl-1-p-tolyl-1H-pyrazol-5-one (**12**): Yield: 80%; m.p. 174–176 °C; ν_{max} (KBr, cm^{-1}): 3257 (NH), 1658 (CO); ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, CH_3), 7.28–7.34 (m, 4H, ArH), 7.57–7.65 (m, 4H, ArH), 7.74–7.81 (m, 3H, ArH), 7.96 (d, 2H, *J* =8.4 Hz, ArH), 8.13 (s, 1H, =CH), 9.90 (s, 1H, indolyl-2-CH), 12.78 (s, 1H, NH). ESI-HRMS: Calcd for $C_{25}H_{19}N_3O$ (MH^+): 378.1606; found: 378.1603.

4-[(1-Benzyl-1H-indol-3-yl)methylene]-1-(4-chlorophenyl)-3-phenyl-1H-pyrazole-5-one (**13**): Yield: 78%; m.p. 248–250 °C; ν_{max} (KBr, cm^{-1}): 1675 (CO); ^{1}H NMR (DMSO-d_6): δ 5.75 (s, 2H, CH_2), 7.30–7.39 (m, 9H, ArH), 7.54 (m, 1H, ArH), 7.6–7.66 (m, 3H, ArH), 7.71 (m, 1H, ArH), 7.80 (m, 3H, ArH), 8.12 (s, 1H, =CH), 8.14 (d, 2H, *J* = 8.4 Hz, ArH), 10.02 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{31}H_{22}ClN_3O$ (MH^+): 488.1530; found: 488.1518 (100%), 490.1498 (35%).

4-[(1H-Indol-3-yl)methylene]-1-(2,4-dinitrophenyl)-3-methyl-1H-pyrazole-5-one (**14**): Yield: 86%; m.p. 158–162 °C; ν_{max} (KBr, cm^{-1}): 3357 (NH), 1694 (CO), 1527 (NO_2), 1330 (NO_2); ^{1}H NMR (DMSO-d_6): δ 2.47 (s, 3H, CH_3), 7.35 (m, 2H, ArH), 7.62 (m, 1H, ArH), 8.09 (d, 1H, *J* = 8.4 Hz, =CH), 8.21 (m, 1H, ArH), 8.27 (s, 1H, ArH), 8.61 (d, 1H, *J* = 8.4 Hz, ArH), 8.76 (s, 1H, ArH), 9.63 (s, 1H, indolyl-2-CH), 12.80 (s, 1H, NH). ESI-HRMS: Calcd for $C_{19}H_{13}N_5O_5$ (MH^+): 392.0995; found: 392.0998.

Methyl 4-{3-methyl-4-[(1-methyl-1H-indol-3-yl)methylene]-5-oxo-4,5-dihydro-1H-pyrazole-1-yl} benzoate (**15**): Yield: 80%; m.p. 190–192 °C; ν_{max} (KBr, cm^{-1}): 1712 (ester CO), 1679 (CO); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 4.05 (s, 3H, CH_3), 7.38–7.44 (m, 2H, ArH), 7.69 (d, 1H, *J* = 7.6 Hz, ArH), 8.04 (d, 2H, *J* = 8.4 Hz, ArH), 8.15 (s, 1H, =CH), 8.22 (d, 3H, *J* = 8.4 Hz, ArH), 9.79 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{22}H_{19}N_3O_3$ (MH^+): 374.1505; found: 374.1511.

Methyl 4-{4-[[1-(2-tert-butoxy-2-oxoethyl)-1H-indol-3-yl]methylene]-3-methyl-5-oxo-4,5-dihydro-1H- pyrazol-1-yl}benzoate (**16**): Yield: 76%; m.p. 220–222 °C; ν_{max} (KBr, cm^{-1}): 1741 (ester CO), 1708 (ester CO), 1682 (CO); ^{1}H NMR (DMSO-d_6): δ 1.45 (s, 9H, $3CH_3$), 2.46 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 5.39 (s, 2H, CH_2), 7.37–7.40 (m, 2H, ArH), 7.60 (m, 1H, ArH), 8.05 (d, 2H, *J* = 8.4 Hz, ArH), 8.17 (s, 1H, =CH), 8.24 (m, 3H, ArH), 9.78 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{27}H_{27}N_3O_5$ (MH^+): 474.2029; found: 474.2036.

Methyl 4-{4-[(1-benzl-1H-indol-3-yl)methylene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}benzoate (**17**): Yield: 79%; m.p. 236–238 °C; ν_{max} (KBr, cm^{-1}): 1708 (ester CO), 1678 (CO); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 5.73 (s, 2H, CH_2), 7.30–7.38 (m, 7H, ArH), 7.69 (m, 1H, ArH), 8.04 (d, 2H, *J* = 8.4 Hz, ArH), 8.17 (s, 1H, =CH), 8.25 (m, 3H, ArH), 9.93 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{28}H_{23}N_3O_3$ (MH^+): 450.1818; found: 450.1808.

4-{4-[(1H-Indol-3-yl)methylene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}benzoic acid (**18**): Yield: 80%; m.p. >300 °C; ν_{max} (KBr, cm^{-1}): 3195 (NH, COOH), 1691 (CO),; ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 7.32–7.35 (m, 2H, ArH), 7.60–7.62 (m, 1H, ArH), 8.01 (d, 2H, *J* = 8.4 Hz, ArH), 8.18 (m, 4H, ArH, =CH), 9.81 (s, 1H, indolyl-2-CH), 12.75 (s, 1H, NH), 12.81 (s, 1H, COOH). ESI-HRMS: Calcd for $C_{20}H_{15}N_3O_3$ (MH^+): 346.1192; found: 346.1194.

4-{3-Methyl-4-[(1-methyl-1H-indol-3-yl)methylene]-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}benzoic acid (**19**): Yield: 82%; m.p. >300 °C; ν_{max} (KBr, cm^{-1}): 3409 (COOH), 1685 (CO); ^{1}H NMR (DMSO-d_6): δ 2.44 (s, 3H, CH_3), 4.04 (s, 3H, CH_3), 7.37–3.43 (m, 2H, ArH), 7.68 (m, 1H, ArH), 8.01–8.05 (m, 3H, ArH), 8.13 (s, 1H, =CH), 8.22 (m, 2H, ArH), 9.78 (s, 1H, indolyl-2-CH), 12.88 (s, 1H, COOH). ESI-HRMS: Calcd for $C_{21}H_{17}N_3O_3$ (MH^+): 360.1348; found: 360.1339.

4-{4-[(1-Acetyl-1H-indol-3-yl)methylene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazolyl}benzoic acid (**20**): Yield: 79%; m.p. >300 °C; ^{1}H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 3.86 (s, 3H, CH_3), 7.34 (dd, 2H, *J* = 2.4 Hz, *J* = 7.2 Hz, ArH), 7.60 (m, 1H, CH), 7.93 (d, 2H,

J = 8.4 Hz, ArH), 7.99 (s, 1H, CH), 8.02 (m, 2H, CH), 8.09 (s, 1H, =CH), 9.81 (s, 1H, indolyl-2-CH), 12.80 (s, 1H, COOH). ESI-HRMS: Calcd for $C_{22}H_{17}N_3O_4$ (MH^+–CH_2CO): 346.1192; found: 346.1190, 384.0748 (M+K^+-CH_2CO).

4-[(1-Acetyl-1H-indol-3-yl)methylene]-1-(4-chlorophenyl)-3-phenyl-1H-pyrazol-5-one (**21**): Yield: 76%; m.p. 266–268 °C; ν_{max} (KBr, cm^{-1}): 1719 (acetyl CO), 1679 (CO); ^{1}H NMR (DMSO-d_6): δ 2.86 (s, 3H, CH_3), 7.43–7.51 (m, 2H, ArH), 7.58 (d, 2H, *J* = 8.4 Hz, ArH), 7.65 (m, 3H, ArH), 7.85 (m, 3H, ArH), 8.10 (s, 1H, =CH), 8.13 (d, 2H, *J* = 7.2 Hz, ArH), 8.41 (d, 1H, *J* = 7.2 Hz, ArH), 10.16 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{26}H_{18}ClN_3O_2$ (MH^+): 440.1166; found: 440.1161 (100%), 442.1147 (38%).

tert-Butyl 2-{3-[(1-(4-chlorophenyl)-5-oxo-3-phenyl-1H-pyrazol-4(5H)-ylidene)methyl]-1H-indol-1- yl}acetate (**22**): Yield: 78%; m.p. 274–276 °C; ν_{max} (KBr, cm^{-1}): 1747 (ester CO), 1673 (CO); ^{1}H NMR (DMSO-d_6): δ 1.45 (s, 9H, $3CH_3$), 5.40 (s, 2H, CH_2), 7.32–7.41 (m, 2H, ArH), 7.53–7.56 (m, 2H, ArH), 7.60–7.67 (m, 4H, ArH), 7.78– 7.84 (m, 3H, ArH), 8.14 (m, 3H, ArH, =CH), 9.86 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{30}H_{26}ClN_3O_3$ (MH^+): 512.1741; found: 512.1730 (100%), 514.1765 (38%).

4-[(1-Acetyl-1H-indol-3-yl)methylene]-1-(4-chlorophenyl)-3-methyl-1H-pyrazol-5(4H)-one (**23**): Yield: 82%; m.p. 236–240 °C; ν_{max} (KBr, cm^{-1}): 1718 (acetyl CO), 1675 (CO); ^{1}H NMR (DMSO-d_6): δ 2.46 (s, 3H, CH_3), 2.82 (s, 3H, $COCH_3$), 7.49 (m, 2H, ArH), 7.52 (d, 2H, *J* = 8.4 Hz, ArH), 8.03 (d, 2H, *J* = 8.4 Hz, ArH), 8.15 (s, 1H, =CH), 8.28 (m, 1H, ArH), 8.40 (m, 1H, ArH), 10.11 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{21}H_{16}ClN_3O_2$ (MH^+): 378.1009; found: 378.1007 (100%), 380.1011 (38%).

1-(4-Chlorophenyl)-4-[(1-methyl-1H-indol-3-yl)methylene]-3-phenyl-1H-pyrazol-5(4H)-one (**24**): Yield: 79%; m.p. 216–218 °C; ν_{max} (KBr, cm^{-1}): 1674 (CO); ^{1}H NMR (DMSO-d_6): δ 4.05 (s, 3H, CH_3), 7.35 (d, 1H, *J* = 7.2 Hz, CH), 7.40 (d, 1H, *J* = 7.2 Hz, CH), 7.54 (d, 2H, *J* = 9.2 Hz, CH), 7.60–7.70 (m, 3H, ArH), 7.69 (d, 1H, *J* = 7.6 Hz, ArH), 7.78 (m, 3H, ArH), 8.10 (s, 1H, =CH), 8.15 (d, 2H, *J* = 8.4 Hz, ArH), 9.86 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{25}H_{18}ClN_3O$ (MH^+): 412.1217; found: 412.1208 (100%), 414.1221 (38%).

Methyl 4-{4-[(1H-indol-3-yl)methylene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}benzoate (**25**): Yield: 77%; m.p. 280–282 °C; ν_{max} (KBr): 3257 (NH), 1713 (ester CO), 1663 (CO); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 7.35 (dd, 2H, *J* = 2.4 Hz, *J* = 7.2 Hz, CH), 7.612 (dd, 1H, *J* = 2.4 Hz, *J* = 7.2 Hz, CH), 8.04 (d, 2H, *J* = 8.4 Hz, CH), 8.18–8.23 (m, 4H, ArH, =CH), 9.81 (s, 1H, indolyl-2-CH), 12.75 (s, 1H, NH). ESI-HRMS: Calcd for $C_{21}H_{17}N_3O_3$ (MH^+): 360.1348; found: 360.1353.

4-[(1-Benzyl-1H-indol-3-yl)methylene]-1-(4-chlorophenyl)-3-methy-1H-pyrazol-5-one (**26**): Yield: 76%; m.p. 240–242 °C; ν_{max} (KBr, cm^{-1}): 1670 (CO); ^{1}H NMR (DMSO-d_6): δ 2.43 (s, 3H, CH_3), 5.71 (s, 2H, CH_2), 7.28–7.38 (m, 7H, ArH), 7.49 (d, 2H, *J* = 8.4 Hz, ArH), 7.68 (m, 1H, ArH), 8.05 (d, 2H, *J* = 8.4 Hz, ArH), 8.14 (s, 1H, =CH), 8.23 (m, 1H, ArH), 9.93 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{26}H_{20}ClN_3O$ (MH^+): 426.1373; found: 426.1381 (100%), 428.1335 (38%).

4-[(1H-Indol-3-yl)methylene]-1-(4-chlorophenyl)-3-methyl-1H-pyrazol-5-one (**27**): Yield: 80%; m.p. 280–282 °C; ν_{max} (KBr, cm^{-1}): 3242 (NH), 1658 (CO); ^{1}H NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 7.33 (dd, 2H, *J* = 2.4 Hz, *J* = 7.2 Hz, Ph), 7.49 (d, 2H, *J* = 7.6 Hz, ArH), 7.60 (m, 1H, ArH), 8.06 (d, 2H, *J* = 8.4 Hz, ArH), 8.15 (s, 1H, =CH), 8.18 (m, 1H, ArH), 9.81 (s, 1H, indolyl-2-CH), 12.70 (s, 1H, NH). ESI-HRMS: Calcd for $C_{19}H_{14}ClN_3O$ (MH^+): 336.0904; found: 336.0908 (100%), 338.0898 (38%).

4-{4-[(1-Benzyl-1H-indol-3-yl)methylene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}benzoic acid (**28**): Yield: 82%; m.p. 298–302 °C; ν_{max} (KBr, cm^{-1}): 3434 (COOH), 1681 (CO); ^{1}H NMR (DMSO-d_6): δ 2.45 (s, 3H, CH_3), 5.73 (s, 2H, CH_2), 7.3–7.38 (m, 7H, ArH), 7.69 (d, 1H, *J* = 7.6 Hz, ArH), 8.01 (d, 2H, *J* = 8.4 Hz, ArH), 8.16 (s, 1H, =CH), 8.19 (s, 2H, ArH), 8.24 (d, 1H, *J* = 7.2 Hz, ArH), 9.94 (s, 1H, indolyl-2CH), 12.79 (s, 1H, COOH). ESI-HRMS: Calcd for $C_{27}H_{21}N_3O_3$ (MH^+): 436.1661; found: 436.1668.

Methy 4-{4-[(1-acetyl-1H-indol-3-yl)methylene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}benzoate (**29**): Yield: 81%; m.p. 238–240 °C; ν_{max} (KBr, cm^{-1}): 1718 (acetyl CO), 1676 (CO); ^{1}H NMR (DMSO-d_6): δ 2.50 (s, 3H, CH_3), 2.84 (s, 3H, $COCH_3$), 3.86 (s, 3H, CH_3), 7.49 (brs, 2H, ArCH), 8.07 (m, 2H, CH), 8.18 (2s, 3H, =CH, ArH), 8.28 (m, 1H, CH), 8.39 (m, 1H, CH), 10.09 (s, 1H, indolyl-2CH). ESI-HRMS: Calcd for $C_{23}H_{19}N_3O_4$ (MH^+): 402.1454; found, 402.1456.

4-{4-[[1-(2-tert-Butoxy-2-oxoethyl)-1H-indol-3-yl]methylene]-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl}benzoic acid (**30**): Yield: 79%; m.p. >300 °C; ν_{max} (KBr, cm^{-1}): 3458 (COOH), 1738 (COOH), 1679 (CO); ^{1}H NMR (DMSO-d_6): δ 1.45 (s, 9H, CH_3), 2.46 (s, 3H, CH_3), 5.37 (s, 2H, CH_2), 7.38 (m, 2H, ArH), 7.58 (m, 1H, ArH), 8.02 (d, 2H, *J* = 8.4 Hz, ArH), 8.01–8.27 (m, 4H, ArH, =CH), 9.78 (s, 1H, indolyl-2-CH), 12.80 (s, 1H, COOH). ESI-HRMS: Calcd for $C_{26}H_{25}N_3O_5$ (MH^+): 460.1872; found: 460.1863.

4-[(1-Acetyl-1H-indol-3-yl)methylene]-3-phenyl-1-p-tolyl-1H-pyrazol-5-one (**31**): Yield: 79%; m.p. 244–246 °C; ν_{max} (KBr, cm^{-1}): 1721 (acetyl CO), 1675 (CO); ^{1}H NMR (DMSO-d_6): δ 2.36 (s, 3H, CH_3), 2.85 (s, 3H, CH_3), 7.32 (d, 2H, *J* = 8.0 Hz, ArH), 7.42–7.51 (m, 2H, ArH), 7.64 (m, 3H, ArH), 7.84 (m, 3H, ArH), 7.92 (d, 2H, *J* = 8.0 Hz, ArH), 8.11 (s, 1H, =CH), 8.41 (d, 1H, *J* = 8.0 Hz, ArH), 10.18 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{27}H_{21}N_3O_2$ (MH^+): 420.1712; found: 420.1717.

tert-Butyl 2-{3-[(5-oxo-3-phenyl-1-p-tolyl-1H-pyrazol-4(5H)-ylidene)methyl]-1H-indol-1-yl}-acetate (**32**): Yield: 76%; m.p. 264–266 °C; ν_{max} (KBr, cm^{-1}): 1721 (ester CO), 1675 (CO); ^{1}H NMR (DMSO-d_6): δ 1.45 (s, 9H, CH_3), 2.34 (s, 3H, CH_3), 5.39 (s, 2H, CH_2), 7.28–7.39 (m, 4H, ArH), 7.60 (m, 4H, ArH), 7.80 (m, 3H, ArH), 7.96 (d, 2H, *J* = 8.0 Hz, ArH), 8.12 (s, 1H, =CH), 9.87 (s, 1H, indolyl-2-CH). ESI-HRMS: Calcd for $C_{31}H_{29}N_3O_3$ (MH^+): 492.2287; found: 492.2276, 530.1829 (M+K^+).

Crystal structure of ***9***

A saturated solution of compound **9** in CH_2Cl_2-DMF (1:2) at room temperature enabled slow solvent evaporation to improve crystal growth. A suitable crystal was selected and placed on Xcalibur Eos diffractometer. The crystal was kept at 293.15 K during data collection. Using Olex2,[29] the structure was resolved with the ShelXS structure solution program using direct methods and refined with the ShelXL refinement package using least squares minimisation (crystal information is shown in Table 4).[30]

Bioactivity assay

All the target compounds were evaluated for their antitumour activity against A549, HCT116, HepG2, PC-9 and A375 cancer cells by the MTT assay with 5-FU and sunitinib as positive controls. Cells (4–5×10^3/well) were seeded in 96-well plates and cultured for 24 h, followed by various concentrations of compounds treatment for 48 h. MTT (10 μL of 10 mg mL^{-1}) was added per well and incubated for

Table 4 Crystal structure determination information of **10**

Identification code	*(4Z)*-4-((1-Methyl-1*H*-indol-3-yl)methylene)-3-phenyl-1-*p*-tolyl-1*H*-pyrazol-5-one
Empirical formula	$C_{26}H_{21}N_3O$
Mr	391.46
Crystal system	Monoclinic
Space group	$P2_1$
Volume/Å^3	3060.8(3)
Z	6
μ(Mo Kα)	0.079
ρ_{calcd}/mg mm^{-3}	1.274
m mm^{-1}	0.079
F(000)	1236
Crystal size/mm^3	0.38 × 0.30 × 0.25
2Θ range for data collection	5.94 to 50°
Index ranges	$-14 \le h \le 16$, $-15 \le k \le 17$, $-17 \le l \le 18$
Reflections collected	12662
Independent reflections	8028[R(int) = 0.0205]
Data/restraints/parameters	8028/1/817
Goodness-of-fit on F^2	1.031
Final R indexes [I>=2σ (I)]	R^1 = 0.0428, wR^2 = 0.0845
Final R indexes [all data]	R^1 = 0.0630, wR^2 = 0.0955
Largest diff. peak/hole/e Å^{-3}	0.120/–0.158
Flack parameter	0.7(12)
Program	Olex2, ShelXS, ShelXL
Crystal chromatography data deposited accession number	CCDC873677

another 2.5 h at 37 °C, then the supernatant fluid was removed and DMSO was added 150 μL/well for 15–20 min. The light absorptions (OD) were measured at 570 nm with SpectraMAX M5 microplate spectrophotometer (Molecular Devices). The effect of compounds on tumour cells viability was expressed by the IC_{50} of each cell lines, the IC_{50} values are shown in Table 2.

Conclusion

A series of 4-(indol-3-yl-methylene)-1*H*-pyrazol-5(4*H*)-one compounds have been synthesised and evaluated for their anti-proliferative activity against five cancer cells in culture. All compounds exhibited anti-proliferative activity against the PC-9 cell line, and most compounds also were active against the A549 cell line. We consider analogues containing carboxyl or 2,4-dinitro at 1-phenyl of pyrazole moieties and benzyl at 1-position of indole worthy of consideration for further structural optimisation and development as potential anticancer agents for the treatment of lung cancer.

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