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Original article

Synthesis and cytotoxic activity of novel 3-(1*H*-indol-3-yl)-1*H*-pyrazole-5-carbohydrazide derivatives

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ABSTRACT

A series of novel 3-(1*H*-indole-3-yl)-1*H*-pyrazole-5-carbohydrazide derivatives **4Ia–n**, **4IIa–b** and **6** were prepared by hydrazinolysis of ethyl 3-(1*H*-indole-3-yl)-1*H*-pyrazole-5-carboxylate with hydrazine hydrate in excellent yields. These new compounds were fully characterized by spectroscopic methods, and the important intermediates **3Ie**, **3IIc** and **3IId** were further confirmed by X-ray crystallography. All the new compounds were evaluated for their cytotoxic activity against 4 human cancer cell lines by MTT method. Some of them exhibited more potent antiproliferative activity against HepG-2, BGC823 and BT474 cell lines than the positive drug 5-fluorourcail. Flow cytometry analysis showed that **4Ik** and **4Il** arrested the cell cycle at S phase.

1. Introduction

Cancer is one of the most serious clinical problems and among the leading causes of death worldwide [1], and considerable attention has been paid to the discovery of new anticancer agents. Cancer is characterized by uncontrolled cellular growth and proliferation, and therefore finding novel compounds to inhibit the uncontrolled proliferative pathways is believed to be an effective strategy [2–4]. Numerous compounds, both synthetic and naturally derived, have been screened to evaluate their cytotoxic activity. Heterocyclic scaffold is common in many anticancer agents [5–7] and some drugs bear different heterocycles, such as Sunitinib, AG337 and Raltitrexed, etc [8–10] (Fig. 1). Indole derivatives possess interesting biological activities, such as anti-inflammatory [11], antifungal [12], antibacterial [13] and anticancer [14] activities. Meanwhile, pyrazole derivatives are known to be associated with various biological properties, such as anticancer [15], antibacterial [16], antiviral [17], analgesic [18] and anti-inflammatory [19] activities. Recently, some pyrazole carbohydrazide derivatives were reported to have moderate anticancer activity [20]. Therefore, it would be of interest to combine both the indole functionality and pyrazole carbohydrazide into one molecule. We report herein the synthesis and *in vitro* cytotoxic activity of 3-(1*H*-indole-3-yl)-1*H*-pyrazole-5-carbohydrazide derivatives against 4 tumor cell lines, A549, HepG-2, BGC823 and BT474 (Fig. 1).

2. Chemistry

2.1. Synthesis

The synthesis of compounds **4Ia–n** and **4IIa–b** was accomplished as outlined in Scheme 1 starting from compound **1** which can be synthesized according to the procedure described in previous paper [21]. The reaction of the starting material **1** with various halides under the reaction conditions KOH/DMSO or K_2CO_3/CH_3CN gave a mixture of monosubstituted isomers **2Ia–g** and **2IIa–c** (Scheme 1), which were conveniently isolated via column chromatography on silica gel. Alkylation of **2Ia–g** and **2IIa–c** with different halides in the presence of NaH in DMF led to disubstituted intermediates **3Ia–m** and **3IIa–d**, respectively. Treatment of **3Ia–m** and **3IIa–b** with hydrazine hydrate in refluxing methanol afforded the target compounds **4Ia–m** and **4IIa–b**. Hydrazinolysis of the ester intermediates **2Ig** produced monosubstituted compound **4In**. Compound **6** was prepared following the procedure described in Scheme 2.

The structures of novel 3-(1*H*-indol-3-yl) pyrazole-5-carbohydrazide derivatives **4I**, **4II** and **6** were determined by IR,

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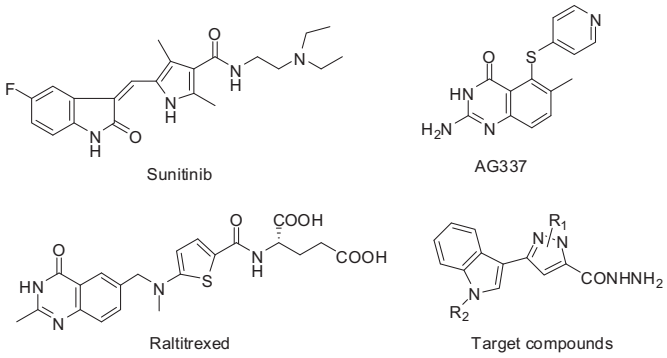
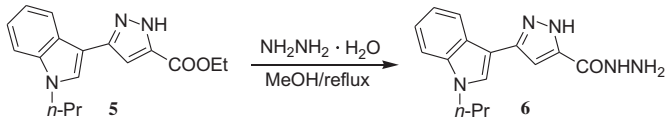


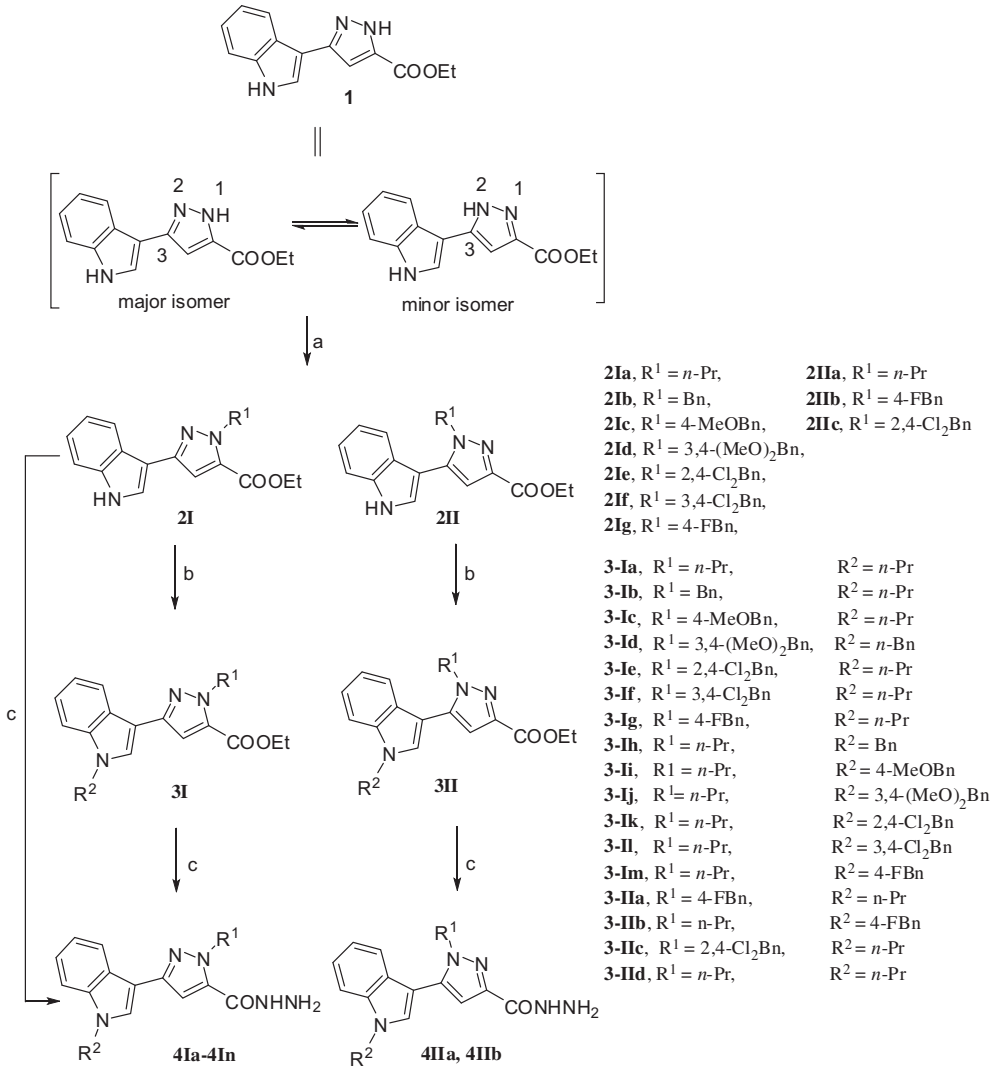
Fig. 1. Structures of some anticancer agents and the target compounds.

^{1}H NMR and HRMS. For example, 1-(2,4-dichlorobenzyl)-3-(1-propyl-1*H*-indol-3-yl)-1*H*-pyrazole-5-carbohydrazide **4Ie** with a quasi-molecular ion peak $[M+H]^+$ at *m*/*z* 442.1189 in the HRMS was in good agreement with the molecular formula $C_{22}H_{22}Cl_2N_5O$. The carbonyl group absorptions in hydrazide moiety appeared at 1653 cm^{-1} in IR spectra and NH and NH_2 bands in $CONHNH_2$ were observed at 3297 cm^{-1} and 3311 cm^{-1}, respectively. The ^{1}H NMR spectra indicated the chemical shift of the NH_2 showed a singlet peak at $\delta = 4.50$ ppm. Three aromatic proton signals in benzyl moiety appeared at $\delta = 7.34$, 6.67 and 7.64 ppm as one double doublet peak ($J = 2.0$, 8.4 Hz) and two doublet peaks ($J = 8.4$ and 2.0 Hz), respectively. The 5- and 6-position aromatic proton signals in indole moiety appeared at the range of $\delta = 7.07$–7.11 and 7.16–7.20 ppm as multiplet peaks. The 4-position and 7-position aromatic proton signals in indole appeared at $\delta = 7.50$ and 8.06 ppm as doublet peaks ($J = 8.0$ Hz). The four singlet signals appearing at $\delta = 5.86$, 7.28, 7.72, and 9.90 ppm were consistent with methylene protons in benzyl group, pyrazole proton, 2-H in indole and NH proton in carbohydrazide moiety, respectively. The *n*-propyl protons had the chemical shifts of 0.85, 1.74–1.84 and 4.17 ppm in the higher field.



Scheme 2. Synthetic route to compound **6**.

Owing to the tautomerism of the pyrazole ring, compound **1** consists of two tautomers in a ratio of 7:3 based on the ^{1}H NMR



Scheme 1. Synthetic route to compounds **4Ia**–**4In**, **4IIa** and **4IIb**. Reagents and conditions: a. R^{1}X, KOH/DMSO or R^{1}X, K_2CO_3/CH_3CN, followed by separation by column chromatography; b. R^{2}X, NaH; c. $NH_2NH_2 \cdot H_2O$, MeOH/reflux.

spectrum, and alkylation at N-H position of pyrazole often results in a mixture of two regioisomers. The regioisomers were assigned according to a known method [21], which is based on the comparison of chemical shifts of CH_2/CH_3 bonded to nitrogen of pyrazole moiety with those reported. For example, in compound **3Ie**, the protons in methylene of benzyl bonded to nitrogen resonated at $\delta = 5.83$ ppm as a singlet, whereas the corresponding protons in **3IIc** appeared at $\delta = 5.54$ ppm (Fig. 2).

2.2. Single-crystal structure

The single crystals of compounds **3Ie**, **3IIc** and **3IId** were obtained by slow evaporation of their solution in petroleum ether and ethyl acetyl at room temperature for a week. The diffraction data were collected with a Bruker SMART CCD diffractometer using graphite-monochromated Mo-$K\alpha$ radiation ($\lambda = 0.71073$ Å) at 298(2) K. The structures were solved by the direct methods with SHELXS-97 program and the refinements on F^2 were performed with SHELXS-97 program [22] by the full-matrix least-squares techniques with anisotropic thermal parameters for non-hydrogen atoms. The crystal structure and crystal packing diagram of compounds **3Ie**, **3IIc** and **3IId** were shown in Figs. 3–5, respectively, while the crystallographic data and structure refinement details were given in Table 1.

In the molecule of **3Ie**, the benzene and pyrrole rings in the indole moiety were coplanar with each other as predicted theoretically, and meanwhile they were coplanar with the pyrazole ring. The benzene ring in the benzyl moiety was essentially orthogonal to the plane formed by the indole and pyrazole rings. However, except for the coplanarity of the benzene and pyrrole rings in the indole moiety, these ring orientations existing in **3Ie** were not observed in its regiomer **3IIc** and **3IId**. There are two intermolecular hydrogen bonds each in the molecules of **3Ie** and **3IIc**, which help to further stabilize the crystals.

The X-ray analysis proved unambiguously the regio-chemistry of the synthesized compounds and was in good agreement with the results by the ^{1}H NMR analysis. As shown in Figs. 3 and 4, compounds **3Ie** and **3IIc** with the same formula were clearly assigned as the N-1 and N-2 isomers, respectively. In conclusion, all the structures of the regioisomers in this series could be definitely assigned by the ^{1}H NMR combined with single-crystal X-ray diffraction.

3. Pharmacology

3.1. Cytotoxic evaluation

The 3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay was employed to evaluate cytotoxic activity of the synthesized compounds against 4 human cancer cell lines including A549, HepG-2, BGC823 and BT474. Cells were incubated with the target compounds at various concentrations for 72 h, and their IC_{50} values (the concentration that causes

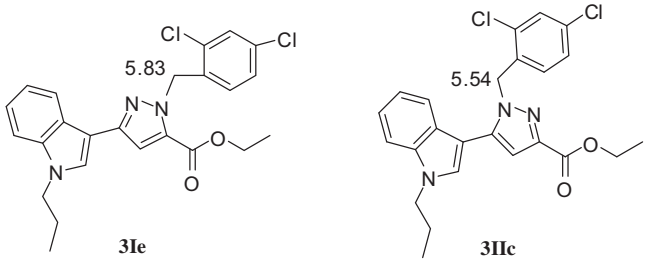


Fig. 2. The chemical shift differences between regioisomers.

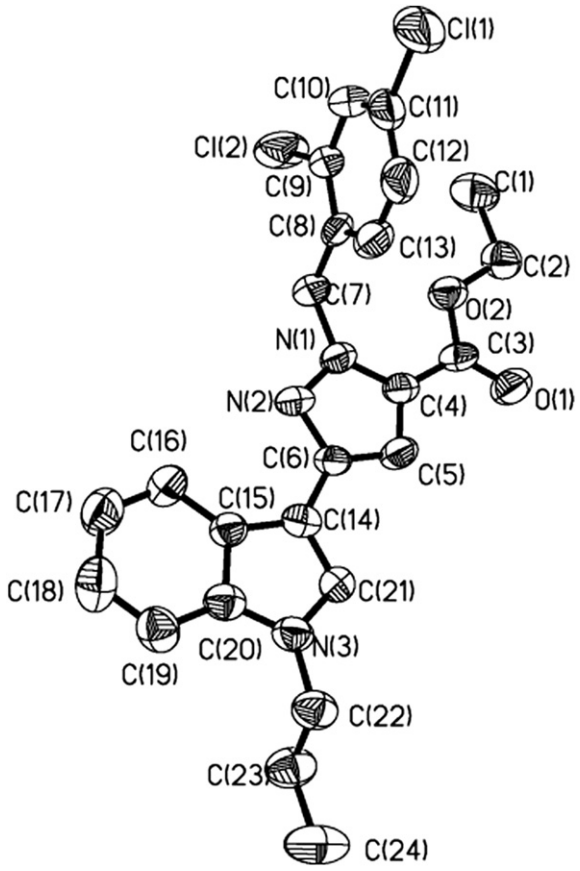


Fig. 3. Molecular structure of compound **3Ie**, with displacement ellipsoids drawn at the 30% probability level and H atoms omitted.

50% of cell growth inhibition) were determined and summaries in Table 2. 5-Fluorourcail was taken as reference drug. The results were expressed as the average of triplicate assays.

3.2. Cell cycle analysis

The results that 3-(1*H*-indol-3-yl)-1*H*-pyrazole-5-carbohydrazide derivatives inhibited the proliferation of 4 human cancer cells led us to further study the effects of them on cell cycle distribution. HepG-2

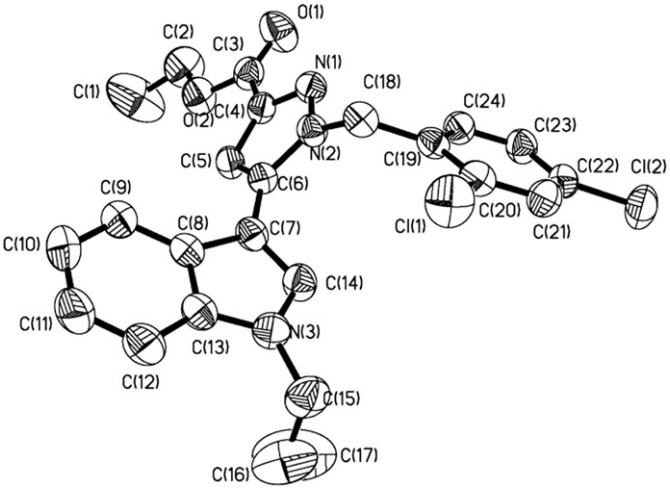


Fig. 4. Molecular structure of compound **3IIc**, with displacement ellipsoids drawn at the 30% probability level and H atoms omitted.

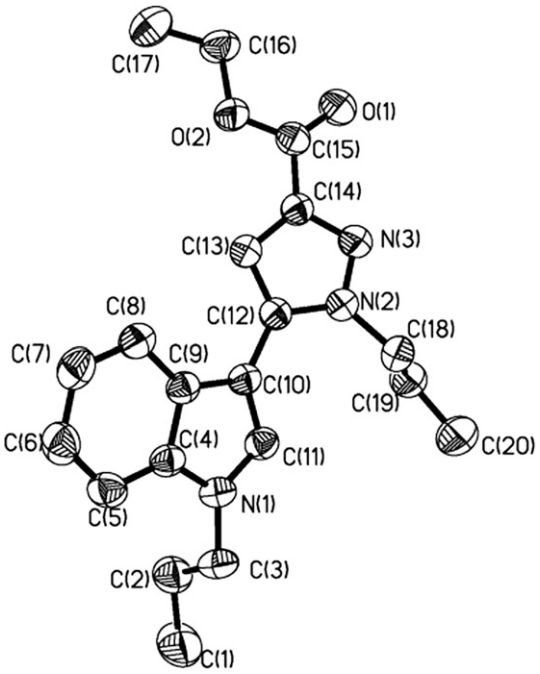


Fig. 5. Molecular structure of compound **3IId**, with displacement ellipsoids drawn at the 30% probability level and H atoms omitted.

cells were treated with different concentrations of compounds **4Ik** and **4Il** for 48 h. 0.1% DMSO was used as vehicle. 5-Fluorouracil, a known inhibitor of thymidylate synthase, was used as the reference drug. Cells were harvested for cell analysis by a flow cytometry.

4. Results and discussion

Seventeen novel 3-(1*H*-indol-3-yl) pyrazole-5-carbohydrazide derivatives were synthesized and identified. In order to investigate the role of the substitution types and patterns on the two N atoms of pyrazole and indole rings for cytotoxic activity, various disubstituted and monosubstituted compounds were prepared.

All the target compounds were evaluated for their cytotoxic activity *in vitro* against four human tumor cell lines, representing different tumor types, namely human alveolar adenocarcinoma cell line (A549), human hepatocellular liver carcinoma cell line (HepG-2), human gastric carcinoma cell line (BGC823) and breast carcinoma cell line (BT474). The results of the cytotoxic studies are shown in Table 2. Most of compounds showed weak cytotoxic activity against A549 cell. When the N atom of indole was occupied by *n*-propyl group, compound **6** with unsubstituted pyrazole ring showed weak activity. The introduction of *n*-propyl, benzyl and monosubstituted benzyl to the pyrazole N atom or the alternation of the substitution position on the pyrazole did not improve the activities as exemplified by analogs **4Ia**–**c** and **4IIa**. However, keeping the *n*-propyl substituent at 1-position of indole but incorporating a disubstituted benzyl group at 1-position of pyrazole produced compounds **4Id**–**f** with significantly improved activities. Compounds **4Ie**, which bore a 2,4-dichlorobenzyl at 1-position of pyrazole, inhibited the growth of A549 with IC_{50} value of 17.64 μM, while compounds **4If** with a 3,4-dichlorobenzyl substituent had IC_{50} value of 14.16 μM against A549. Compounds **4Ih**–**m** and **4IIb** showed lower IC_{50} values than those of their counterparts against A549. Nevertheless, compounds **4Ik** and **4Il**, carrying dichlorobenzyl groups at 1-position of indole, were more potent than other compounds in this series. A comparison between compounds **4Ig** and **4In** showed that elimination of the *n*-propyl substituent at N atom of indole caused little increase in the activity against A549.

It is noticeable that all the disubstituted compounds **4Ia**–**m** had indistinguishable IC_{50} values of 1.3–4.7 μM against HepG-2 cell. In particular, derivatives **4Ik**, **4Il** and **4Im**, with F- or Cl-containing benzyl group on either N atoms of indole and pyrazole, were more potent than the reference drug 5-Fu, which implied that the lipophilic and electron-withdrawing halobenzyl groups were beneficial for the cytotoxic activity against the HepG-2 cell lines. In addition, swapping the position of substituents adjacent to N-1 of pyrazole ring and 1-position of indole ring had minor effects on the

Table 1
The crystallographic data and structure refinement details of **3Ie**, **3IIc** and **3IId**.

Identification code	**3Ie**	**3IIc**	**3IId**
Empirical formula	$C_{24}H_{23}Cl_2N_3O_2$	$C_{24}H_{23}Cl_2N_3O_2$	$C_{20}H_{25}N_3O_2$
Formula weight	456.35	456.35	339.43
Temperature	298(2) K	298(2) K	298(2) K
Wavelength	0.71073 Å	0.71073 Å	0.71073 Å
Crystal system, space group	Triclinic, P-1	Monoclinic, *P*2(1)/n	Monoclinic, *P*2(1)/n
Unit cell dimensions	$a = 8.362(5)$ Å $\alpha = 82.778(10)^\circ$ $b = 8.446(5)$ Å $\beta = 76.757(10)^\circ$ $c = 16.809(10)$ Å $\gamma = 89.921\ (10)^\circ$	$a = 13.895(7)$ Å $\alpha = 90^\circ$ $b = 7.737(4)$ Å $\beta = 91.625(9)^\circ$ $c = 21.551(10)$ Å $\gamma = 90^\circ$	$a = 11.992(3)$ Å $\alpha = 90^\circ$ $b = 8.873(3)$ Å $\beta = 99.934(5)^\circ$ $c = 17.941(5)$ Å $\gamma = 90^\circ$
Volume	1146.0(11) A^3	2316(2) A^3	1880.4(9) A^3
Z, Calculated density	2, 1.323 Mg/m^3	4, 1.309 Mg/m^3	4, 1.199 Mg/m^3
Absorption coefficient	0.309 mm^{-1}	0.306 mm^{-1}	0.079 mm^{-1}
F(000)	476	952	728
Crystal size	0.21 × 0.16 × 0.12 mm	0.22 × 0.14 × 0.08 mm	0.23 × 0.14 × 0.12 mm
Theta range for data collection	2.43–25.05°	1.72–25.05°	1.90–25.05°
Limiting indices	$-9 \le h \le 7$, $-9 \le k \le 0$, $-19 \le l \le 20$	$-16 \le h \le 16$, $-9 \le k \le 8$, $-23 \le l \le 25$	$-14 \le h \le 7$, $-10 \le k \le 10$, $-21 \le l \le 21$
Reflections collected/unique	5844/3910 [*R*(int) = 0.0304]	11,626/4098 [*R*(int) = 0.0430]	9499/3330 [*R*(int) = 0.0261]
Completeness to $\theta = 25.05$	96.6%	99.8%	99.9%
Absorption correction	Semi-empirical from equivalents	Semi-empirical from equivalents	Semi-empirical from equivalents
Max. and min. transmission	0.9639 and 0.9380	0.9760 and 0.9358	0.9906 and 0.9822
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3910/0/281	4098/0/281	3330/0/227
Goodness-of-fit on F^2	1.657	1.015	1.016
Final *R* indices [$I > 2\sigma(I)$]	$R^1 = 0.1516$, $wR^2 = 0.4388$	$R^1 = 0.0456$, $wR^2 = 0.1040$	$R^1 = 0.0364$, $wR^2 = 0.0896$
R indices (all data)	$R^1 = 0.1871$, $wR^2 = 0.4597$	$R^1 = 0.0803$, $wR^2 = 0.1221$	$R^1 = 0.0509$, $wR^2 = 0.0999$
Extinction coefficient	0.025(13)	0.0040(7)	0.0322(19)
Largest diff. peak and hole	1.110 and −0.401 e. $Å^{-3}$	0.245 and −0.203 e. $Å^{-3}$	0.152 and −0.136 e. $Å^{-3}$

Table 2
The characteristics of the target compounds and their IC_{50} values on human cancer lines.

Compd	R^1	R^2	R^1 substitution position	IC_{50} (μM)[a]			
				A549	HepG-2	BGC823	BT474
4Ia	*n*-propyl	*n*-propyl	N-1	169.7 ± 10.34	3.91 ± 0.28	1.94 ± 0.46	3.78 ± 0.76
4Ib	benzyl	*n*-propyl	N-1	–	4.71 ± 0.42	5.15 ± 1.54	3.70 ± 0.05
4Ic	4-MeO-benzyl	*n*-propyl	N-1	69.90 ± 11.86	3.12 ± 0.03	1.89 ± 0.24	5.54 ± 0.55
4Id	3,4-Di-MeO-benzyl	*n*-propyl	N-1	20.37 ± 4.57	2.72 ± 0.27	6.53 ± 0.45	4.57 ± 1.01
4Ie	2,4-Di-Cl-benzyl	*n*-propyl	N-1	17.67 ± 1.42	3.14 ± 0.14	12.80 ± 0.54	5.77 ± 0.72
4If	3,4-Di-Cl-benzyl	*n*-propyl	N-1	14.16 ± 1.52	4.07 ± 0.84	11.06 ± 0.59	6.49 ± 1.13
4Ig	4-F-benzyl	*n*-propyl	N-1	51.80 ± 14.02	1.65 ± 0.10	0.71 ± 0.14	2.33 ± 0.37
4Ih	*n*-propyl	benzyl	N-1	151.0 ± 18.08	3.59 ± 0.80	1.88 ± 0.43	4.68 ± 0.56
4Ii	*n*-propyl	4-MeO-benzyl	N-1	130.9 ± 26.44	3.14 ± 0.75	1.49 ± 0.27	5.44 ± 0.78
4Ij	*n*-propyl	3,4-Di-MeO-benzyl	N-1	52.67 ± 8.36	2.95 ± 0.47	8.78 ± 1.45	6.24 ± 2.19
4Ik	*n*-propyl	2,4-Di-Cl-benzyl	N-1	29.40 ± 2.79	1.32 ± 0.15	4.90 ± 0.67	4.42 ± 1.75
4Il	*n*-propyl	3,4-Di-Cl-benzyl	N-1	24.69 ± 3.68	1.58 ± 0.32	10.22 ± 0.31	3.80 ± 1.07
4Im	*n*-propyl	4-F-benzyl	N-1	54.26 ± 3.78	2.41 ± 0.67	7.23 ± 0.61	1.39 ± 0.18
4In	4-F-benzyl	H	N-1	46.07 ± 2.65	17.23 ± 1.89	8.79 ± 1.27	14.26 ± 3.58
4IIa	4-F-benzyl	*n*-propyl	N-2	63.13 ± 11.12	50.53 ± 3.69	153.0 ± 26.95	83.27 ± 16.92
4IIb	*n*-propyl	4-F-benzyl	N-2	–	106.8 ± 27.70	49.16 ± 5.15	10.48 ± 1.48
6	H	*n*-propyl		51.18 ± 8.65	32.86 ± 4.31	–	17.59 ± 5.67
5-Fu[b]				0.42 ± 0.04	2.70 ± 0.72	6.15 ± 0.59	72.90 ± 13.34

[a] Each experiment was independently performed three times and expressed as means ± SD. "–" means IC_{50} values > 250 μM.
[b] 5-Fu is used as reference drug.

activity. Altering the substitution position on pyrazole ring resulted in a precipitous loss in potency, which could be found in the comparison among **4IIa** and **4IIb** with their counterparts **4Ig** and **4Im**. The poor inhibitory activity of the two monosubstituted derivatives **4In** and **6** indicated that the disubstitution pattern plays an important role for the activity against HepG-2.

Compounds **4Ia**–**4Ic**, **4Ig**–**4Ii** and **4Ik** were found to be more active against BGC823 cell line than the reference drug 5-Fu. When the N atom of indole was attached by *n*-propyl group, compound **4Ig** with 4-F-benzyl group at position-1 of pyrazole displayed the highest antiproliferative activity with IC_{50} value of 0.71 μM which was 8.6-fold more potent than that of 5-Fu. This finding suggested that small and electron-withdrawing group on the benzyl at the N-1 position of pyrazole ring played an important role for the activity. The antiproliferative activity of **4Ic** with a 4-methoxybenzyl group on N-1 of pyrazole was 3-fold higher than that of 5-Fu and better than that of most of the other benzyl-containing derivatives. These results indicated that 4-methoxybenzyl was a more beneficial functional group for the antiproliferative activity against BGC823 cell line. In the series with the *n*-propyl substituent at position-1 of pyrazole and various benzyl groups at position-1 of indole, compound **4Ii** with the electron-donating methoxy group at the 4-position of the benzyl exhibited the best activity against BGC823 with IC_{50} value of 1.49 μM.

As shown in Table 2, all the disubstituted compounds **4Ia**–**4Im** with substituent situated at position-1 of pyrazole were 11- to 50-fold and the monosubstituted compounds were ~5-fold more potent than the reference drug 5-Fu against BT474 cells. Compounds **4Ig** and **4Im** that contain a 4-fluorobenzyl group exhibited high growth inhibitory activity with IC_{50} values of 2.32 and 1.39 μM, respectively. This was to say that a small and electron-withdrawing group at 4-position of benzyl was favorable for the antiproliferative activity. Swapping the position of substituents adjacent to N-1 of pyrazole and N-1 of indole had minor effects on the activity.

To understand the mechanism of the target compounds, cell cycle analysis was performed. The HepG-2 cells were treated with compounds **4Ik** and **4Il** at indicated concentration for 48 h. The eukaryotic cell cycle consists of alternating rounds of DNA replication (S phase) and cell division (M phase) separated by the gap phase G0, G1 and G2. The final results in Fig. 6 suggested that both compounds interfere with the normal cell cycle distribution of this cell line. The result showed that treatment of HepG-2 cells with 1 μM and 2 μM of 5-Fluorourcail arrested the cell cycle at S phase (11.1% and 15.5% increase compared with the blank group, respectively). After the cells were treated with compound **4Ik** at the concentrations of 2 μM and 4 μM for 48 h, there caused 15% and 16.9% increased in the percentage of the cells in S phase in comparison with the blank group, respectively. Upon incubation with compound **4Il** (with 2 and 4 μM) there was an increase of cells in the S phase of about 7.1% and 10.1%, respectively, in comparison with the blank group. The increase in the S phase cell population was accompanied by a decreased in the G1 and G2 phase cell populations. According to these findings, compounds **4Ik** and **4Il** made the HepG-2 cell arrested at S phase.

5. Conclusion

The synthesis and characterization of a new series of 3-(1*H*-indol-3-yl)pyrazole-5-carbohydrazide derivatives were carried out and all the compounds were evaluated for their cytotoxic activity against 4 human cancer cell lines. The majority of the tested compounds possessed antiproliferative activities against all of the tested cancer cell lines, while the HepG-2, BGC823 and BT474 cell lines were more sensitive in response to the growth inhibition than A549 cell line. It is of notice that most of the compounds exhibited more potent cytotoxic activity than the drug 5-Fu against BT474 cell line. Compound **4Im** was the most active and 52-fold more potent than 5-Fu against BT474 with IC_{50} value of 1.39 μM, but was less potent against other three cancer cells. Compound **4Ig** was the most effective against BGC823 cell with IC_{50} value of 0.71 μM, 8.6-fold more potent than 5-Fu against HepG-2 cell, but showed weak activity against A549. Compounds **4Ik** showed the highest potency against HepG-2 with IC_{50} value of 1.32 μM, while it showed modest activity toward A549, BGC823 and BT474 cells. These differences suggested that our compounds displayed selective cytotoxicity in contrast to being a general broad cytotoxic agent. The cell cycle analysis revealed that compounds **4Ik** and **4Il** arrested the HepG-2 cell cycle at S phase in a dose-dependent manner. These preliminary results are beneficial for further lead optimization.

6. Experimental

All reagents were used as purchased from commercial suppliers without further purification unless otherwise noted. Melting points were determined by using a WRS-1B Digital Melting-Point

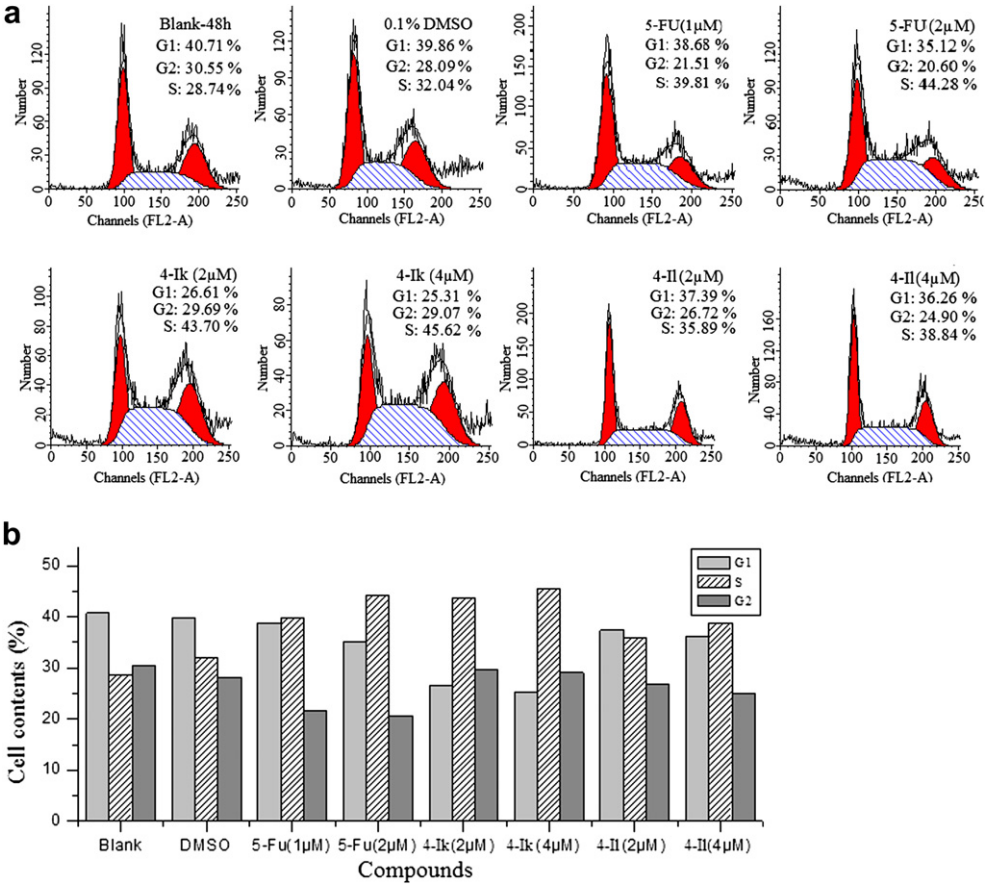


Fig. 6. (a) DNA contents obtained by flow cytometry. (b) FACS of cell cycle distribution of HepG-2 cells after treatment with compounds **4Ik** and **4Il** for 48 h. 5-Fu was used as reference drug and 0.1% DMSO was used as vehicle control.

apparatus and are uncorrected. Thin-layer chromatography (TLC) was performed on Silica Gel F_{254} plates with visualization by UV or iodine vapor. ^{1}H NMR spectra of DMSO-d_6 solutions (TMS as an internal standard) were recorded on Bruker AVANCE II 400 spectrometers. The IR spectra were measured on a Bruker Vector FT-IR spectrophotometer as KBr pellets or film. The high resolution mass spectra were obtained with an Agilent-6510-Q-TOF spectrometer.

Abbreviations: DMF, dimethylformamide; DMSO, dimethylsulfoxide; PE, petroleum ether; EtOAc, ethyl acetate.

6.1. General procedure for the preparation of compounds *2Ia–2If*, *2IIa* and *2IIc*

Compounds **2Ia–d** and **2IIa** were reported in Ref. [21]. Compounds **2Ie–f** and **2IIc** were prepared in a similar way.

As an example, to a stirred mixture of **1** (5.1 g, 20 mmol), KOH (1.23 g, 22 mmol) and KBr (2.6 g, 22 mmol) in dried DMSO (15 ml) under nitrogen was added 2,4-dichlorobenzyl chloride (4.3 g, 22 mmol) at 0 °C and the resulting mixture was stirred at 0 °C for 1.5 h. The mixture was then poured into water, and extracted with CH_2Cl_2 (3 × 50 ml). The combined organic layers were washed with water and brine, dried (Na_2SO_4), and concentrated. The yellow residue was purified by flash column chromatography (silica gel, PE/EtOAc) to give the desired product. For compound **2Ie**, its regioisomer (compound **2IIc**) was also obtained.

6.1.1. Ethyl 1-(2, 4-dichlorobenzyl)-3-(1H-indol-3-yl)-1H-pyrazole-5-carboxylate (*2Ie*)

Yellow solid, yield: 68%; mp: 138.1–139.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.25 (t, J = 7.2 Hz, 3H, $OCH_2\mathbf{CH_3}$), 4.27 (q, J = 7.2 Hz, 2H, $O\mathbf{CH_2}CH_3$), 5.83 (s, 2H, CH_2Ph), 7.02–7.06 (m, 1H, ArH), 7.10–7.14 (m, 1H, ArH), 7.35–7.44 (m, 2H, ArH, 4-H), 7.51–7.54 (m, 1H, ArH), 7.66–7.68 (m, 2H, ArH), 7.92 (d, J = 2.4 Hz, 1H, IndH), 8.08 (d, J = 7.6 Hz, 1H, ArH); IR (KBr, ν/cm^{-1}) 3350, 3011, 2985, 2897, 1645, 1510, 1416, 1341, 1286, 1096, 746.

6.1.2. Ethyl 1-(3, 4-dichlorobenzyl)-3-(1H-indol-3-yl)-1H-pyrazole-5-carboxylate (*2If*)

Yellow solid, yield: 71%; mp: 129.5–130.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, J = 7.2 Hz, 3H, $OCH_2\mathbf{CH_3}$), 4.29 (q, J = 7.2 Hz, 2H, $O\mathbf{CH_2}CH_3$), 5.76 (s, 2H, CH_2Ph), 7.09–7.20 (m, 2H, ArH), 7.27–7.30 (m, 2H, ArH, 4-H), 7.48–7.57 (m, 3H, ArH), 7.90 (d, J = 2.4 Hz, 1H, IndH), 8.14 (d, J = 7.6 Hz, 1H, ArH); IR (KBr, ν/cm^{-1}) 3310, 3080, 2986, 1696, 1580, 1485, 1273, 1085, 760.

6.1.3. Ethyl 1-(2, 4-dichlorobenzyl)-5-(1H-indol-3-yl)-1H-pyrazole-3-carboxylate (*2IIc*)

Light yellow oil, yield: 10%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.29 (t, J = 7.0 Hz, 3H, $OCH_2\mathbf{CH_3}$), 4.30 (q, J = 7.2 Hz, 2H, $O\mathbf{CH_2}CH_3$), 5.54 (s, 2H, CH_2Ph), 6.78 (d, J = 8.0 Hz, 1H, ArH), 6.94 (s, 1H, 4-H), 7.09–7.13 (m, 1H, ArH), 7.19–7.23 (m, 1H, ArH), 7.33–7.36 (m, 1H, ArH), 7.50–7.58 (m, 4-H, ArH, IndH); IR (KBr, ν/cm^{-1}) 3345, 3030, 2980, 2900, 1640, 1515, 1415, 1250, 1011, 785.

6.2. The procedure for compounds 2Ig and 2IIb

Compound **2Ig** was prepared according to the procedure in Ref. [21] and its regioisomer (compound **2IIb**) was obtained as a minor product.

6.2.1. Ethyl 1-(4-fluorobenzyl)-5-(1H-indol-3-yl)-1H-pyrazole-3-carboxylate (2IIb)

Light yellow oil, yield: 10%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.30 (t, J = 7.0 Hz, 3H, OCH$_2$**CH$_3$**), 4.30 (q, J = 7.2 Hz, 2H, O**CH$_2$**CH$_3$), 5.50 (s, 2H, CH$_2$Ph), 6.92 (s, 1H, 4-H), 7.00–7.03 (m, 2H, ArH), 7.08–7.13 (m, 3H, ArH), 7.16–7.20 (m, 1H, ArH), 7.45–7.52 (m, 3H, ArH); IR (KBr, ν/cm^{-1}) 3266, 3068, 2977, 1712, 1578, 1509, 1459, 1263, 1216, 747.

*6.3. General procedure for compounds **3I** and **3II***

Compounds **3Ia**, 3**Ic**, 3**Id**, 3**Ig**, 3**Ii**, 3**Ij** and 3**Im** were reported in Ref. [21]. Compounds 3**Ib**, 3**Ie**, 3**If**, 3**Ih**, 3**Ik**, 3Il and **3IIa**–**d** were prepared in a similar way.

As an example, to a solution of compound **2Ib** (0.345 g, 1 mmol) in dried DMF (1 ml), NaH (60%, 44 mg, 1.1 mmol) was added in portions. After stirring for 10 min below 10 °C, 1-bromoproane (135 mg, 1.1 mmol) was added and stirred for another 1 h at r.t. The mixture was poured into water and extracted with CH_2Cl_2 (3 × 10 ml). The combined organic layers were then washed with water, dried over Na_2SO_4, and the solvent was removed by rotary evaporation. The crude product was purified by silica gel column chromatography to give compound **3Ib**.

*6.3.1. Ethyl 1-benzyl-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carboxylate (**3Ib**)*

White solid, yield: 87%; mp: 104.4–105.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.84 (t, J = 7.2 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.28 (t, J = 7.2 Hz, 3H, OCH$_2$**CH$_3$**), 1.75–1.84 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.14 (t, J = 7.2 Hz, 2H, **CH$_2$**CH$_2$CH$_3$), 4.29 (q, J = 7.2 Hz, 2H, O**CH$_2$**CH$_3$), 5.78 (s, 2H, CH$_2$Ph), 7.08–7.12 (m, 1H, ArH), 7.16–7.27 (m, 5H, ArH, 4-H), 7.30–7.34 (m, 2H, ArH), 7.50 (d, J = 7.6 Hz, 1H, ArH), 7.93 (s, 1H, IndH), 8.14 (d, J = 8.0 Hz, 1H, ArH); IR (KBr, ν/cm^{-1}) 2985, 2937, 1715, 1580, 1480, 1456, 1255, 1097, 740.

*6.3.2. Ethyl 1-(2, 4-dichlorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carboxylate (**3Ie**)*

Light yellow solid, yield: 78%; mp: 126.3–126.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, J = 7.2 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.26 (t, J = 7.2 Hz, 3H, OCH$_2$**CH$_3$**), 1.78–1.83 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.13 (t, J = 7.0 Hz, 2H, **CH$_2$**CH$_2$CH$_3$), 4.27 (q, J = 7.0 Hz, 2H, O**CH$_2$**CH$_3$), 5.83 (s, 2H, **CH$_2$**Ph), 6.71 (d, J = 8.4 Hz, 1H, ArH), 7.05–7.07 (m, 1H, ArH), 7.15–7.18 (m, 1H, ArH), 7.31 (s, 1H, 4-H), 7.36 (dd, J = 1.2, 8.0 Hz, 1H, ArH), 7.50 (d, J = 7.6 Hz, 1H, ArH), 7.66 (d, J = 1.2 Hz, 1H, ArH), 7.96 (s, 1H, IndH), 8.07 (d, J = 7.6 Hz, 1H, ArH); IR (KBr, ν/cm^{-1}) 3045, 2960, 2935, 2872, 1713, 1580, 1460, 1265, 1215, 1132, 740.

*6.3.3. Ethyl 1-(3, 4-dichlorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carboxylate (**3If**)*

Off-white solid, yield: 75%; mp: 118.4–119.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.84 (t, J = 7.2 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.28 (t, J = 7.2 Hz, 3H, OCH$_2$**CH$_3$**), 1.77–1.82 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.14 (t, J = 7.0 Hz, 2H, **CH$_2$**CH$_2$CH$_3$), 4.29 (q, J = 7.0 Hz, 2H, O**CH$_2$**CH$_3$), 5.76 (s, 2H, **CH$_2$**Ph), 7.08–7.12 (m, 2H, ArH), 7.14–7.19 (m, 1H, ArH), 7.26 (s, 1H, 4-H), 7.34 (dd, J = 2.0, 8.4 Hz, 1H, ArH), 7.47–7.50 (m, 1H, ArH), 7.60 (d, J = 8.0 Hz, 1H, ArH), 7.94 (s, 1H, IndH), 8.13 (d, J = 7.6 Hz, 1H, ArH); IR (KBr, ν/cm^{-1}) 3013, 2986, 2922, 1713, 1610, 1580, 1466, 1260, 1217, 741.

*6.3.4. Ethyl 3-(1-benzyl-1H-indol-3-yl)-1-propyl-1H-pyrazole-5-carboxylate (**3Ih**)*

Colorless oil, yield: 93%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.87 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.32 (t, J = 7.0 Hz, 3H, OCH$_2$**CH$_3$**), 1.79–1.87 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.30 (q, J = 7.2 Hz, 2H, O**CH$_2$**CH$_3$), 4.50 (t, J = 7.0 Hz, 2H, **CH$_2$**CH$_2$CH$_3$), 5.42 (s, 2H, **CH$_2$**Ph), 7.09–7.15 (m, 2H, ArH), 7.18 (s, 1H, 4-H), 7.21–7.31 (m, 5H, ArH), 7.50 (d, J = 8.4 Hz, 1H, ArH), 8.03 (s, 1H, IndH), 8.14 (d, J = 7.8 Hz, 1H, ArH); IR (KBr, ν/cm^{-1}) 3103, 2951, 2910, 1700, 1586, 1515, 1480, 1465, 1320, 1260, 745.

*6.3.5. Ethyl 3-[1-(2,4-dichlorobenzyl)-1H-indol-3-y]-1-propyl-1H-pyrazole-5-carboxylate (**3Ik**)*

Light yellow oil, yield: 88%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.87 (t, J = 7.0 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.32 (t, J = 7.0 Hz, 3H, OCH$_2$**CH$_3$**), 1.81–1.85 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.32 (q, J = 7.2 Hz, 2H, O**CH$_2$**CH$_3$), 4.50 (t, J = 7.2 Hz, 2H, **CH$_2$**CH$_2$CH$_3$), 5.51 (s, 2H, **CH$_2$**Ph), 7.06–7.14 (m, 2H, ArH), 7.18 (s, 1H, 4-H), 7.39–7.45 (m, 2H, ArH), 7.49–7.53 (m, 2H, ArH), 7.64 (d, J = 2.0 Hz, 1H, ArH), 7.94 (s, 1H, IndH), 8.14 (d, J = 7.6 Hz, 1H, ArH); IR (KBr, ν/cm^{-1}) 3015, 2980, 2903, 1720, 1595, 1487, 1275, 1213, 1093, 765.

*6.3.6. Ethyl 3-[1-(3,4-dichlorobenzyl)-1H-indol-3-y]-1-propyl-1H-pyrazole-5-carboxylate (**3Il**)*

Light yellow oil, yield: 91%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.88 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.32 (t, J = 7.4 Hz, 3H, OCH$_2$**CH$_3$**), 1.81–1.86 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.32 (q, J = 7.2 Hz, 2H, O**CH$_2$**CH$_3$), 4.50 (t, J = 7.2 Hz, 2H, **CH$_2$**CH$_2$CH$_3$), 5.44 (s, 2H, **CH$_2$**Ph), 7.11–7.20 (m, 4-H, ArH, 4-H), 7.52 (d, J = 8.4 Hz, 1H, ArH), 7.55–7.57 (m, 3H, ArH), 8.05 (s, 1H, IndH), 8.14 (d, J = 7.8 Hz, 1H, ArH); IR (KBr, ν/cm^{-1}) 2967, 2903, 1687, 1610, 1585, 1461, 1371, 1216, 764.

*6.3.7. Ethyl 1-(4-fluorobenzyl)-5-(1-propyl-1H-indol-3-yl)-1H-pyrazole-3-carboxylate (**3IIa**)*

Light yellow oil, yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.78 (t, J = 7.2 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.29, (t, J = 7.2 Hz, 3H, OCH$_2$**CH$_3$**), 1.73–1.78 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.15 (t, J = 7.0 Hz, 2H, **CH$_2$**CH$_2$CH$_3$), 4.29 (q, J = 7.2 Hz, 2H, O**CH$_2$**CH$_3$), 5.50 (s, 2H, CH$_2$Ph), 6.92 (s, 1H, 4-H), 6.99–7.13 (m, 4-H, ArH), 7.22 (t, J = 7.4 Hz, 2H, ArH), 7.50–7.59 (m, 3H, ArH, IndH), 9.47 (brs, 1H, NH); IR (KBr, ν/cm^{-1}) 3015, 2934, 2866, 1720, 1585, 1513, 1456, 1286, 1215, 744.

*6.3.8. Ethyl 5-[1-(4-fluorobenzyl)-1H-indol-3-yl]-1-propyl-1H-pyrazole-3-carboxylate (**3IIb**)*

Light yellow oil, yield: 86%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.72 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.29 (t, J = 7.2 Hz, 3H, OCH$_2$**CH$_3$**), 1.71–1.75 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.18 (t, J = 6.8 Hz, 2H, **CH$_2$**CH$_2$CH$_3$), 4.28 (q, J = 7.2 Hz, 2H, O**CH$_2$**CH$_3$), 5.50 (s, 2H, CH$_2$Ph), 6.84 (s, 1H, ArH), 7.10–7.24 (m, 4-H, ArH), 7.32–7.36 (m, 2H, ArH), 7.50 (d, J = 8.0 Hz, 1H, ArH), 7.57 (d, J = 8.0 Hz, 1H, ArH), 7.91 (s, 1H, IndH); IR (KBr, ν/cm^{-1}) 2989, 2945, 1718, 1585, 1515, 1460, 1275, 1215, 1095, 742.

*6.3.9. Ethyl 1-(2, 4-dichlorobenzyl)-5-(1-propyl-1H-indol-3-yl)-1H-pyrazole-3-carboxylate (**3IIc**)*

Light yellow solid, yield: 85%; mp: 91.0–91.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.76 (t, J = 7.2 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.30 (t, J = 7.0 Hz, 3H, OCH$_2$**CH$_3$**), 1.70–1.78 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.15 (t, J = 6.8 Hz, 2H, **CH$_2$**CH$_2$CH$_3$), 4.29 (q, J = 7.2 Hz, 2H, O**CH$_2$**CH$_3$), 5.54 (s, 2H, CH$_2$Ph), 6.79 (d, J = 8.4 Hz, 1H, ArH), 6.95 (s, 1H, 4-H), 7.09–7.13 (m, 1H, ArH), 7.20–7.24 (m, 1H, ArH), 7.35 (dd, J = 1.6, 8.4 Hz, 1H, ArH), 7.51 (d, J = 8.0 Hz, 1H, ArH), 7.55 (s, 1H, IndH), 7.57–7.58 (m, 1H, ArH); IR (KBr, ν/cm^{-1}) 3035, 2990, 2850, 1650, 1510, 1415, 1215, 1120, 744.

*6.3.10. Ethyl 1-propyl-5-(1-propyl-1H-indol-3-yl)-1H-pyrazole-3-carboxylate (**3IId**)*

Colorless solid, yield: 78%; mp: 104.9–105.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.72 (t, J = 7.4 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 0.84 (t, J = 7.2 Hz, 3H, CH$_2$CH$_2$**CH$_3$**), 1.67–1.74 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 1.78–1.86 (m, 2H, CH$_2$**CH$_2$**CH$_3$), 4.17 (t, J = 7.2 Hz, 2H, **CH$_2$**CH$_2$CH$_3$),

4.22 (t, $J = 6.8$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.29 (q, $J = 7.2$ Hz, 2H, $O\mathbf{CH_2}CH_3$), 6.81 (s, 1H, ArH), 7.10–7.14 (m, 1H, ArH), 7.21–7.25 (m, 1H, ArH), 7.49 (d, $J = 8.0$ Hz, 1H, ArH), 7.59 (d, $J = 8.0$ Hz, 1H, ArH), 7.73 (s, 1H, IndH); IR (KBr, ν/cm^{-1}) 3085, 2950, 2915, 2860, 1700, 1550, 1530, 1440, 1210, 1100, 760.

6.4. General procedure for the synthesis of 3-(1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide derivatives (**4I**, **4II** and **6**)

For example, to a stirred solution of compound **3Ia** (0.339 g, 1 mmol) in methanol (5 ml), 1 ml of 80% hydrazine monohydrate was added. After maintained under reflux until TLC indicated consumption of starting materials, the mixture was quenched with water, and extracted with EtOAc. The combined organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography to give the target compound **4Ia** in high yield.

6.4.1. 1-Propyl-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**4Ia**)

Off-white solid, yield: 95%; mp: 136.5–136.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (m, 6H, $CH_2CH_2\mathbf{CH_3}$), 1.76–1.83 (m, 4-H, $CH_2\mathbf{CH_2}CH_3$), 4.16 (t, $J = 7.0$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.48 (t, $J = 7.0$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.52 (s, 2H, NH_2), 7.08–7.12 (m, 2H, ArH, 4-H), 7.18(dt, $J = 1.2$, 7.2 Hz, 1H ArH), 7.49 (d, $J = 8.4$ Hz, 1H, ArH), 7.69 (s, 1H, IndH), 8.08 (d, $J = 7.6$ Hz, 1H, ArH), 9.78 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3307, 3271, 3207, 2964, 2933, 2873, 1664, 1615, 1583, 1521, 1448, 1315, 1219, 748; HRMS (ESI) calcd for $[M + H]^+$ $C_{18}H_{24}N_5O$: 326.1981, found: 326.1983.

6.4.2. 1-Benzyl-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**4Ib**)

White solid, yield: 94%; mp: 130.0–130.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.83 (t, $J = 7.4$ Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76–1.81 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.16 (t, $J = 6.8$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.53 (s, 2H, NH_2), 5.79 (s, 2H, CH_2Ph), 7.08–7.12 (m, 1H, ArH), 7.15–7.25 (m, 5H, ArH, 4-H), 7.28–7.32 (m, 2H, ArH), 7.50 (d, $J = 8.0$ Hz, 1H, ArH), 7.71 (s, 1H, IndH), 8.07 (d, $J = 7.6$ Hz, 1H, ArH), 9.86 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3305, 3271, 3211, 3142, 3032, 2962, 2931, 2873, 1643, 1620, 1533, 1452, 1323, 1217, 740; HRMS (ESI) calcd for $[M + H]^+$ $C_{22}H_{24}N_5O$: 374.1981, found: 374.1971.

6.4.3. 1-(4-Methoxybenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**4Ic**)

White powder, yield: 96%; mp: 161.0–161.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.84 (t, $J = 7.4$ Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76–1.81 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 3.69 (s, 3H, OCH_3), 4.16 (t, $J = 7.0$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.53 (s, 2H, NH_2), 5.70 (s, 2H, CH_2Ph), 6.86 (d, $J = 8.4$ Hz, 2H, ArH), 7.09–7.23 (m, 5H, ArH, 4-H), 7.49 (d, $J = 8.0$ Hz, 1H, ArH), 7.69 (s, 1H, IndH), 8.08 (d, $J = 7.6$ Hz, 1H, ArH), 9.82 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3311, 3259, 2958, 2931, 2872, 1651, 1616, 1583, 1521, 1448, 1315, 1247, 744; HRMS (ESI) calcd for $[M + H]^+$ $C_{23}H_{26}N_5O_2$: 404.2086, found: 404.2082.

6.4.4. 1-(3,4-Dimethoxybenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**4Id**)

Light yellow solid, yield: 97%; mp: 162.9–163.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.84 (t, $J = 7.4$ Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.74–1.83 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 3.69 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 4.16 (t, $J = 7.0$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.54 (s, 2H, NH_2), 5.68 (s, 2H, CH_2Ph), 6.75 (dd, $J = 2.0$, 8.4 Hz, 1H, ArH), 6.86 (d, $J = 8.4$ Hz, 1H, ArH), 7.00 (d, $J = 2.0$ Hz, 1H, ArH), 7.08–7.13 (m, 3H, ArH, 4-H), 7.49 (d, $J = 8.4$ Hz, 1H, ArH), 7.69 (s, 1H, IndH), 8.10 (d, $J = 7.6$ Hz, 1H, ArH), 9.83 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3278, 3049, 2993, 2958, 2937, 2872, 1645, 1620, 1585, 1519, 1450, 1317, 1263, 732; HRMS (ESI) calcd for $[M + H]^+$ $C_{24}H_{28}N_5O_3$: 434.2192, found: 434.2184.

6.4.5. 1-(2,4-Dichlorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**4Ie**)

Light yellow solid, yield: 93%; mp: 161.3–162.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, $J = 7.4$ Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.74–1.84 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.17 (t, $J = 7.0$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.50 (s, 2H, NH_2), 5.86 (s, 2H, CH_2Ph), 6.67 (d, $J = 8.4$ Hz, 1H, ArH), 7.07–7.11 (m, 1H, ArH), 7.16–7.20 (m, 1H, ArH), 7.28 (s, 1H, 4-H), 7.34 (dd, $J = 2.0$, 8.4 Hz, 1H, ArH), 7.50 (d, $J = 8.0$ Hz, 1H, ArH), 7.64 (d, $J = 2.0$ Hz, 1H, ArH), 7.72 (s, 1H, IndH), 8.06 (d, $J = 8.0$ Hz, 1H, ArH), 9.90 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3311, 3297, 3043, 2964, 2931, 2872, 1653, 1618, 1585, 1535, 1471, 1448, 1313, 1217, 742; HRMS (ESI) calcd for $[M + H]^+$ $C_{22}H_{22}Cl_2N_5O$: 442.1201, found: 442.1189.

6.4.6. 1-(3,4-Dichlorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**4If**)

Light yellow solid, yield: 92%; mp: 173.3–173.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.84 (t, $J = 7.2$ Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.74–1.83 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.17 (t, $J = 6.8$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.55 (s, 2H, NH_2), 5.77 (s, 2H, CH_2Ph), 7.09–7.13 (m, 1H, ArH), 7.16–7.20 (m, 2H, ArH), 7.23 (s, 1H, 4-H), 7.46 (d, $J = 2.0$ Hz, 1H, ArH), 7.51 (d, $J = 8.0$ Hz, 1H, ArH), 7.59 (d, $J = 8.0$ Hz, 1H, ArH), 7.72 (s, 1H, IndH), 8.01 (d, $J = 8.0$ Hz, 1H, ArH), 9.91 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3263, 3045, 2960, 2931, 2872, 1643, 1616, 1581, 1531, 1469, 1446, 1332, 1313, 1213, 1132, 740; HRMS (ESI) calcd for $[M + H]^+$ $C_{22}H_{22}Cl_2N_5O$: 442.1201, found: 442.1190.

6.4.7. 1-(4-Fluorobenzyl)-3-(1-propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**4Ig**)

Light yellow solid, yield: 94%; mp: 122.9–123.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.83 (t, $J = 7.2$ Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.73–1.83 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.16 (t, $J = 7.2$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.55 (s, 2H, NH_2), 5.76 (s, 2H, CH_2Ph), 7.09–7.20 (m, 5H, ArH, 4-H), 7.30 (dd, $J = 1.6$, 5.6 Hz, 2H, ArH), 7.50 (d, $J = 8.0$ Hz, 1H, ArH), 7.71 (s, 1H, IndH), 8.08 (d, J = 7.6 Hz, 1H, ArH), 9.88 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3296, 3211, 3047, 2962, 2926, 2872, 2854, 1662, 1639, 1616, 1583, 1553, 1450, 1327, 1222, 748; HRMS (ESI) calcd for $[M + H]^+$ $C_{22}H_{23}FN_5O$: 392.1886, found: 392.1867.

6.4.8. 3-(1-Benzyl-1H-indol-3-yl)-1-propyl-1H-pyrazole-5-carbohydrazide (**4Ih**)

White solid, yield: 95%; mp: 172.8–173.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, $J = 7.4$ Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76–1.85 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.49 (t, $J = 6.8$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.52 (s, 2H, NH_2), 5.46 (s, 2H, CH_2Ph), 7.09–7.17 (m, 3H, ArH, 4-H), 7.23–7.32 (m, 5H, ArH), 7.49 (d, $J = 7.6$ Hz, 1H, ArH), 7.82 (s, 1H, IndH), 8.10 (d, $J = 7.2$ Hz, 1H, ArH), 9.78 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3313, 3271, 3201, 3115, 3026, 2962, 2924, 2877, 1658, 1616, 1577, 1533, 1469, 1450, 1321, 1211, 1170, 948, 723; HRMS (ESI) calcd for $[M + H]^+$ $C_{22}H_{24}N_5O$: 374.1981, found: 374.1971.

6.4.9. 3-[1-(4-Methoxybenzyl)-1H-indol-3-yl]-1-propyl-1H-pyrazole-5-carbohydrazide (**4Ii**)

Lacteous oil, yield: 91%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, $J = 7.4$ Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.75–1.84 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 3.70 (s, 3H, OCH_3), 4.48 (t, $J = 7.2$ Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.51 (s, 2H, NH_2), 5.36 (s, 2H, CH_2Ph), 6.87 (dd, $J = 2.0$, 6.8 Hz, 2H, ArH), 7.08–7.17 (m, 3H, ArH, 4-H), 7.22 (d, $J = 8.4$ Hz, 1H, ArH), 7.51 (d, $J = 7.6$ Hz, 1H, ArH), 7.78 (s, 1H, IndH), 8.07–8.09 (m, 1H, ArH), 9.78 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3311, 3047, 3007, 2962, 2933, 2873, 2835, 1658, 1614, 1585, 1514, 1465, 1452, 1309, 1249, 1176, 746; HRMS (ESI) calcd for $[M + H]^+$ $C_{23}H_{26}N_5O_2$: 404.2086, found: 404.2076.

*6.4.10. 3-[1-(3,4-Dimethoxybenzyl)-1H-indol-3-yl]-1-propyl-1H-pyrazole-5-carbohydrazide (**4Ij**)*

White solid, yield: 93%; mp: 159.3–161.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, J = 7.6 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.75–1.84 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 3.68 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 4.48 (t, J = 7.2 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.51 (s, 2H, NH_2), 5.34 (s, 2H, CH_2Ph), 6.74 (dd, J = 2.0, 8.4 Hz, 1H, ArH), 6.86 (d, J = 8.4 Hz, 1H, ArH), 7.02 (d, J = 2.0 Hz, 1H, ArH), 7.08–7.18 (m, 3H, ArH, 4-H), 7.53 (d, J = 8.4 Hz, 1H, ArH), 7.78 (s, 1H, IndH), 8.08 (d, J = 7.6 Hz, 1H, ArH), 977 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3317, 3298, 3091, 3047, 2960, 2931, 2872, 2835, 1641, 1616, 1516, 1465, 1450, 1313, 1257, 1234, 1138, 1026, 740; HRMS (ESI) calcd for $[M+H]^+$ $C_{24}H_{28}N_5O_3$: 434.2192, found: 434.2181.

*6.4.11. 3-[1-(2,4-Dichlorobenzyl)-1H-indol-3-yl]-1-propyl-1H-pyrazole-5-carbohydrazide (**4Ik**)*

White solid, yield: 94%; mp: 91.4–91.9 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, J = 7.2 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76–1.85 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.49 (t, J = 7.2 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.52 (s, 2H, NH_2), 5.54 (s, 2H, CH_2Ph), 6.73 (d, J = 8.4 Hz, 1H, ArH), 7.10–7.19 (m, 3H, ArH, 4-H), 7.33 (dd, J = 0.8, 8.4 Hz, 1H, ArH), 7.41 (d, J = 8.0 Hz, 1H, ArH), 7.69 (s, 1H, ArH), 7.75 (s, 1H, IndH), 8.13–8.15 (m, 1H, ArH), 9.79 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3315, 3163, 3088, 3055, 2962, 2931, 2872, 1660, 1627, 1587, 1527, 1469, 1448, 1311, 1174, 740; HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{22}Cl_2N_5O$: 442.1201, found: 442.1186.

*6.4.12. 3-[1-(3,4-Dichlorobenzyl)-1H-indol-3-yl]-1-propyl-1H-pyrazole-5-carbohydrazide (**4Il**)*

White powder, yield: 95%; mp: 86.5–87.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, J = 7.2 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76–1.85 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.49 (t, J = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.52 (s, 2H, NH_2), 5.48 (s, 2H, CH_2Ph), 7.11–7.19 (m, 4-H, ArH, 4-H), 7.50 (d, J = 8.0 Hz, 1H, ArH), 7.55–7.57 (m, 2H, ArH), 7.87 (s, 1H, IndH), 8.11 (d, J = 7.2 Hz, 1H, ArH), 9.80 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3288, 3053, 2960, 2929, 2872, 1651, 1616, 1585, 1527, 1476, 1450, 1334, 1313, 1203, 1176, 744; HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{22}Cl_2N_5O$: 442.1201, found: 442.1196.

*6.4.13. 3-[1-(4-Fluorobenzyl)-1H-indol-3-yl]-1-propyl-1H-pyrazole-5-carbohydrazide (**4Im**)*

Yellow solid, yield: 97%; mp: 113.1–114.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.85 (t, J = 7.0 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.76–1.85 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.49 (t, J = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.52 (s, 2H, NH_2), 5.44 (s, 2H, CH_2Ph), 7.10–7.18 (m, 5H, ArH, 4-H), 7.20–7.31(m, 2H, ArH), 7.50 (d, J = 8.0 Hz, 1H, ArH), 7.83 (s, 1H, IndH), 8.08–8.10 (m, 1H, ArH), 9.79 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3294, 3113, 3049, 2962, 2931, 2872, 1653, 1614, 1585, 1527, 1510, 1465, 1450, 1313, 1222, 1157, 742; HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{23}FN_5O$: 392.1886, found: 392.1885.

*6.4.14. 1-(4-Fluorobenzyl)-3-(1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**4In**)*

Yellow powder, yield: 95%; mp: 198.5–200.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.54 (s, 2H, NH_2), 5.76 (s, 2H, CH_2Ph), 7.05–7.06 (m, 4-H, ArH), 7.20(s, 1H, 4-H), 7.28–7.31 (m, 2H, ArH), 7.41 (d, J = 8.0 Hz, 1H, ArH), 7.66 (d, J = 2.4 Hz, 1H, IndH), 8.7 (d, J = 7.6 Hz, 1H, ArH), 9.79 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3311, 3288, 2956, 2900, 1641, 1604, 1531, 1510, 1450, 1313, 1222, 748; HRMS (ESI) calcd for $[M+H]^+$ $C_{19}H_{17}FN_5O$: 350.1417, found: 350.1413.

*6.4.15. 1-(4-Fluorobenzyl)-5-(1-propyl-1H-indol-3-yl)-1H-pyrazole-3-carbohydrazide (**4IIa**)*

Light yellow oil, yield: 96%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.80 (t, J = 7.8 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.71–1.81 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.15 (t, J = 7.0 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.41 (s, 2H, NH_2), 5.50 (s, 2H, CH_2Ph), 6.89 (s, 1H, 4-H), 7.02–7.13 (m, 5H, ArH), 7.22 (t, J = 7.4 Hz, 1H, ArH), 7.55–7.56 (m, 3H, ArH, IndH), 9.47 (brs, 1H, NH); IR (KBr, ν/cm^{-1}) 3311, 3209, 2966, 2933, 2875, 1660, 1618, 1510, 1442, 1338, 1224, 746; HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{23}FN_5O$: 392.1886, found: 392.1867.

*6.4.16. 5-[1-(4-Fluorobenzyl)-1H-indol-3-yl]-1-propyl-1H-pyrazole-3-carbohydrazide (**4IIb**)*

Light yellow oil, yield: 91%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.73 (t, J = 7.4 Hz, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.69–1.79 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.14 (t, J = 6.8 Hz, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.41 (brs, 2H, NH_2), 5.49 (s, 2H, CH_2Ph), 6.80 (s, 1H, 4-H), 7.10–7.22 (m, 4-H, ArH), 7.32–7.36 (m, 2H, ArH), 7.54–7.58 (m, 2H, ArH), 7.88 (s, 1H, IndH), 9.34 (s, 1H, NH); IR (KBr, ν/cm^{-1}) 3313, 3051, 2964, 2933, 2875, 1662, 1618, 1510, 1463, 1440, 1340, 1222, 1157, 744; HRMS (ESI) calcd for $[M+H]^+$ $C_{22}H_{23}FN_5O$: 392.1886, found: 392.1880.

*6.4.17. 3-(1-Propyl-1H-indol-3-yl)-1H-pyrazole-5-carbohydrazide (**6**) (major/minor = 60:40)*

Compound **5** was prepared according to the procedure in Ref. [21] and subsequent hydrazinolysis produced compound **6**.

White solid, yield: 94%; mp: 194.1–195.5 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.84–0.87 (m, 3H, $CH_2CH_2\mathbf{CH_3}$), 1.77–1.84 (m, 2H, $CH_2\mathbf{CH_2}CH_3$), 4.16–4.19 (m, 2H, $\mathbf{CH_2}CH_2CH_3$), 4.40/4.50 (s/s, 2H, NH_2), 6.94 (s, 1H, ArH, the proton signal of the minor isomer was merged in 7.11–7.24 ppm), 7.11–7.24 (m, 2H, ArH), 7.48–7.51/7.55–7.57 (m/m, 1H, ArH), 7.67–8.08 (m, 2H, ArH), 8.35/8.75 (brs/brs, 1H, NH); IR (KBr, ν/cm^{-1}) 3302, 3278, 3176, 3062, 2960, 2924, 2852, 1660, 1631, 1543, 1469, 1328, 1286, 1193, 734; HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{18}N_5O$: 284.1511, found: 284.1511.

6.5. MTT assay for cell viability

Cell viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. Cancer cells were purchased from American Type Culture Collection (ATCC, USA). The cells were cultured in RPMI-1640 medium, supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 U/ml streptomycin in a humidified environment with 5% CO_2 at 37 °C.

Cells in logarithmic growth were plated in 96-well microtiter plates at following densities of 3500 cells/well for A549, 3500 cells/well for HepG-2, 3500 cells/well for BGC823 and 6000 cells/well for BT474, respectively. After 24 h incubation, cells were treated with vehicle alone (0.4% DMSO) or compounds (drugs were dissolved in DMSO previously) at the concentrations indicated. After three days of incubation (37 °C, 5% CO_2 in a humid atmosphere) 20 μl of MTT (5 mg/ml in PBS) was added to each well and the plate was incubated for a further 3 h (37 °C). To dissolve the resulting MTT-formazan, 150 μl of DMSO was added to each well, followed by thorough mixing with a mechanical plate mixer. Absorbance at 492 nm was measured on a microplate reader, and the data were analyzed by Prism Graphpad software. In all of these experiments, three replicate wells were used to determine each point.

6.6. Flow cytometric analysis of cell cycle distribution

The effects of compounds **4Ik** and **4Il** on cell cycle distribution were studied on HepG-2 cells by flow cytometric analysis after staining with propidium iodide. HepG-2 cells were exposed to 0.1% DMSO, active compounds or 5-Fluorouracil for 48 h. After treatment, cells were harvested by trypsinization, washed twice with ice-cold phosphate buffered saline and fixed in ethanol (75%) at 4 °C for 2 h. Fixed cells were pelleted by centrifugation, washed once with PBS, and treated with 500 μg/ml Rnase in PBS for 30 min at 37 °C. Then the cells were stained with 25 μg/ml propidium

iodide in PBS at 4 °C in the dark for 30 min. The DNA content was analyzed by FACScan flow cytometer (Becton Dickinson, Mountain View, CA) and CELLQUEST software (Becton Dickinson).

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