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Original article

Synthesis and cytotoxicity evaluation of 1-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1*H*-pyrazole-5-carboxylic acid derivatives

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ABSTRACT

Several novel molecules, 1-(3′-(9*H*-carbazol-4-yloxy)-2′-hydroxypropyl)-3-aryl-1*H*-pyrazole-5-carboxylic acid derivatives **3a**–**g** were synthesized and screened to evaluate their cytotoxicity against cancer cells in vitro. The compounds **3a**–**g** has been prepared by the reaction of ethyl 3-aryl-1*H*-pyrazole-5-carboxylate with 4-oxiranylmethoxy-9*H*-carbazole in moderate to excellent yields. The cytotoxicity of synthesized compounds was evaluated by a SRB (sulforhodamine B) assay against cancer cell such as SK–N–SH human neuroblastoma (NB), human A549 lung carcinoma, human breast cancer MCF-7 cell lines. The results showed that seven compounds can suppress SK–N–SH tumor cancer cell growth. Among them, compound **3d** was the most effective small molecule in inhibiting SK–N–SH cell growth.

1. Introduction

Neuroblastoma (NB) is an embryonic malignancy of the sympathetic nervous system arising from neuroblasts and is the most common extracranial solid tumor in child younger than five year of age. Neuroblastoma accounts for 7–10% of all children had cancers, in the majority of patients older than one year of age the disease is fatal [1]. Therefore, new therapeutic targets have been reported that the antitumor efficacy of chemotherapeutic agents correlated with their growth-inhibiting, differentiation-inducing or apoptosis-inducing abilities [2]. The aim of most cancer chemotherapeutic drugs currently in clinical use is to kill malignant tumor cells by inhibiting some of the mechanisms implied in cellular division. Accordingly, the antitumor compounds developed through this approach are cytostatic or cytotoxic. However, the knowledge of tumor biology has exploded during the past decades and this may pave the way for more active, targeted anticancer drugs [3].

More recently a series of phenylcarbazole molecules have been reported as antitumor agent [4], Carbazole sulphonamides are a novel class of *antimitotic* agents against solid tumors [5]. But there were no reports on the synthesis and biological evaluation of 1-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1*H*-pyrazole-5-carboxylic acid.

In our effort to discover and develop tumor growth inhibitors and apoptosis inducers as potential new anticancer agents, we have identified several classes of molecules as novel tumor growth inhibitors of varitriol derivatives [6] and novel antibacterial C-linked imidazole derivatives [7]. In an ongoing study in our laboratory on the design and synthesis of the small molecules, we are interested in extending our small molecule, library to meet the requirement of our research.

We report herein for the first time the synthesis of 1-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1*H*-pyrazole-5-carboxylic acid derivatives in a single step with the aim of evaluating their biological activities in inhibiting SK–N–SH cell growth.

2. Results and discussion

2.1. Chemistry

Synthesis of ethyl 1-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1*H*-pyrazole-5-carboxylic acid (**3a**–**g**) have been achieved in moderate to excellent yields by the reaction between ethyl 3-aryl-1*H*-

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pyrazole-5-carboxylate (**1a**–**g**) and 4-oxiranylmethoxy-9*H*-carbazole (**2**) in the presence of potassium carbonate and tetrabutylammonium iodide at reflux temperature in acetonitrile for 32 h (Scheme 1). The crucial intermediate (**1a**–**g**) were obtained according to the previously reported method [8] (Scheme 2) and 4-oxiranylmethoxy-9*H*-carbazole (**2**) was prepared by the reaction of 4-hydroxy carbazole (**6**) with epichlorohydrine (**7**) in the presence of sodium hydroxide and tetrabutylammonium bromide in water at 40 °C as shown in Scheme 3.

The structures of these compounds were confirmed from their spectral and micro analytical data. Based on [M^++H] 458 its molecular formula was established as $C_{22}H_{19}NO_5$. The IR spectrum of **(3a)** showed absorption due to −OH & −NH stretching at 3400 cm^{-1} and acid carbonyl group at 1713 cm^{-1} indicated that the product formed in the above reaction is a coupled product. Therefore the product was inferred to contain a carbazol aryl pyrazole carboxylic acid structure.

^{1}H NMR spectrum (400 MHz) of **(3a)** recorded in DMSO-d_6 exhibited signals arising due to typical chiral secondary alcohol. The spectrum contained three multiplets, the first one appeared at δ 4.16–4.26 integrating for two protons, the second one appeared at δ 4.49–4.58 integrating for one proton and third one appeared at δ 4.79–4.99 integrating for two protons. The spectrum also revealed the presence of one singlet at δ 3.81 (for 3H) due to $-OCH_3$ group and remaining a multiplet at δ 6.62–8.35 which were assigned to the aromatic 12 protons, respectively.

2.2. Effects of the compounds on the viability of human cancer cells

The newly synthesized 1-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1*H*-pyrazole-5-carboxylic acid derivatives (**3a**–**g**) were evaluated for their cancer activity against the human cancer cell line by sulforhodamine B (SRB) assay method [9,10]. The various human tumor cell growth inhibitors potential was determined as described [11]. The inhibitory activities were presented as micromolar concentrations of the compound that cause 50% inhibition per unit of enzyme (IC_{50}) under the assay conditions. The data obtained from by SRB assay showed the compound **3a**–**g** had inhibitory effects on the growth of SK–N–SH neuroblastoma (NB), human A549 lung carcinoma, human breast cancer MCF-7 cells and standard doxorubicin in dosage and time dependent manner as shown in Fig. 1a, b, c. Compounds **3d** could mild inhibit the cell growth obviously at 0.1 μM after 48h of the treatment. At 0.5 and 5 μM concentration, after 48 h of the treatment compounds **3a**–**g** also suppress the growth of SK–N–SH cell. At 5 μM concentration all the compound showed significantly inhibited the SK–N–SH cell growth (Fig. 1a). Taken altogether compounds **3a** and **3d** are effective compounds in suppressing SK–N–SH cell growth.

3. Conclusion

In conclusion, various substituted 1-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1*H*-pyrazole-5-carboxylic acid derivatives (**3a**–**g**) have been prepared by the reaction of 4-oxiranylmethoxy-9*H*-carbazole with ethyl 3-(4-methoxyphenyl)-1*H*-pyrazole-5-carboxylate and screened in viability assays against SK–N–SH neuroblastoma (**NB**), human A549 lung carcinoma, human breast cancer MCF-7 cells in vitro. In general, most compounds showed significantly cytotoxic effect against human SK–N–SH tumor cell line. Compound **3a** and **3d** were showed significantly inhibit the growth of SK–N–SH cells. The finding suggested that these compounds would be very useful for further investigating the mechanism of cell proliferation, morphology, differennation and apoptosis in our next research project and some of them may be a powerful drug against tumor cancer.

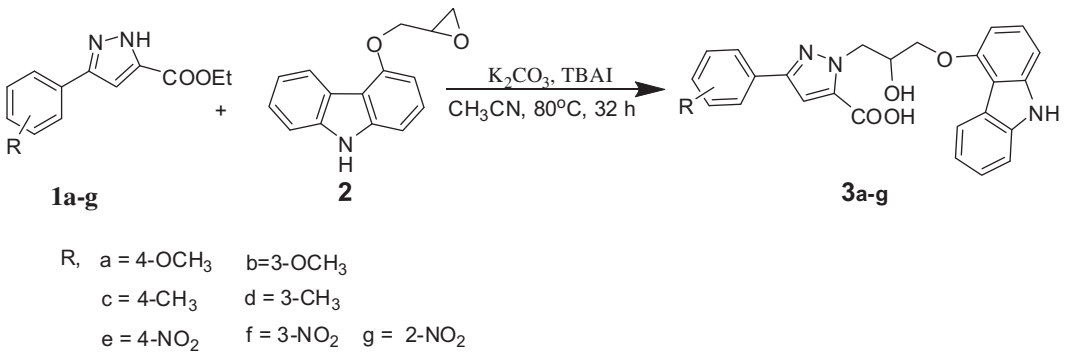
4. Experimental section

4.1. Chemistry

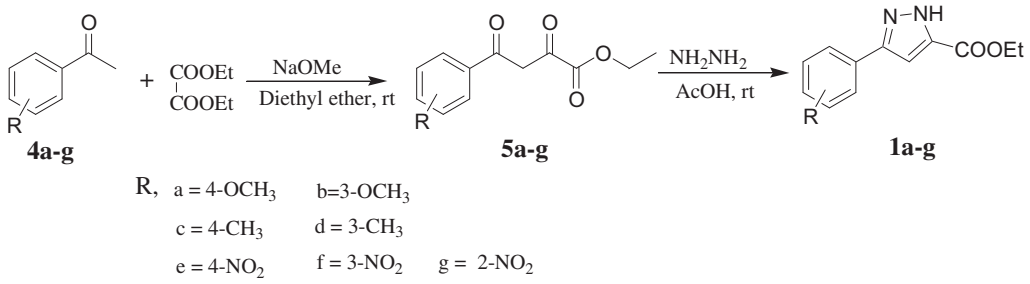
All commercial reagents and solvents were used as received without further purification unless specified. The solvent were used is dried from calcium hydride, calcium chloride for acetonitrile and diethyl ether respectively. The reactions were monitored and Rf value were determined using analytical thin layer chromatography (TLC) with Merck Silica gel 60 and F-254 precoated plates (0.25-mm thickness). Spot on the TLC plates were visualized using ultraviolet light (254 nm). Flash column chromatography was performed with Merck silica gel 60 (100–200 mesh). Melting points were determined in capillaries and are uncorrected. ^{1}H NMR spectra were recorded on Bruker DRX-300, Varian 400 and Varian-500 NMR spectrometers. ^{13}C NMR spectra were recorded on Bruker DRX-300. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS, δ 0.00 or with the solvent reference relative to TMS employed as the internal standard (CDCl3, δ 7.26 ppm, DMSO-$d_6\delta$2.54). Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR 400 spectrometer; data are reported in wave numbers (cm^{-1}). Mass spectra were recorded on Agilent Technologies 1100 Series (Agilent Chemistation Software).

4.2. Preparation of 4-oxiranylmethoxy-9H-carbazole (*2*)

4-Hydroxy carbazole (6.0 g) was added into a round bottom flask containing water (10 mL), epichlorohydrin (6.4 g) and tetrabutylammonium bromide (0.6 g) were then added into the reaction mass under stirring. Sodium hydroxide (50% aqueous) solution (7.8 g) was slowly added in the reaction mixture at 40 °C over



Scheme 1. Synthesis of 1-[3-(9*H*-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1*H*-pyrazole-5-carboxylic acid (**3a**–**g**).



Scheme 2. Synthesis of ethyl 3-aryl-1*H*-pyrazole-5-carboxylate (**1a**–**g**).

a period of 2–3 h. After completion of reaction, ethyl acetate (100 mL) and water (100 mL) were added into the reaction mass. The lower aqueous layer was separated. The ethyl acetate layer was washed with water to obtain neutral pH and dried over anhydrous sodium sulphate. The ethyl acetate is distilled out under vacuum below 50 °C to about 50 mL. The reaction mass was chilled to 0–5 °C and filtered and dried at 50–60 °C to give the compound **2** (5.0 g, 64%), mp 167–169 °C IR (KBr, ν cm^{-1}): 3293 (N–H), 2926 (Ar–H), 1595 (C=C), 1096 (C–O). ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.81–2.88 (dd, J = 2.45, 2.64 Hz, 1H, H_A), 2.90–2.97 (dd, J = 4.34, 4.91 Hz, 1H, H_B), 3.50–3.57 (m, 1H, H_C), 4.04–4.14 (dd, J = 6.23, 6.23 Hz, 1H, H_E), 4.52–4.59 (dd, J = 2.26, 2.26 Hz, 1H, H_D), 6.70 (d, J = 7.93 Hz, 1H, Ar–H), 7.05–7.20 (m, 2H, Ar–H), 7.25–7.39 (m, 2H, Ar–H), 7.46 (d, J = 7.93 Hz, 1H, Ar–H), 8.17(d, J = 7.74 Hz, 1H, Ar–H), 11.27(s, 1H, NH), ESI-MS: m/z = 239 [M$^+$].

4.3. General procedure for the synthesis of ethyl 2,4-dioxo-4-arylbutanoate (**5a**–**g**)

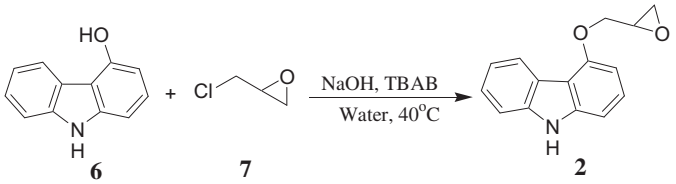
A mixture of a freshly prepared $NaOCH_3$ (0.86 g, 0.016 mol) and diethyl oxalate (1.95 g, 0.013mol) was dissolved in diethyl ether (50 mL) and stirred at room temperature under nitrogen atmosphere. Followed by to this solution was added a 4-methoxyacetophenone **4a** (2.0 g, 0.013mol) in diethyl ether (10 mL). A yellow precipitate immediately formed and the reaction was stirred for 12 h at room temperature. The precipitate was filtered and dissolved in water (100 mL) and the solution was adjusted to pH 3 with acetic acid. The resulting precipitate was filtered and dried to give 3.0 g (92%) of ethyl 2,4-dioxo-4-(methoxyphenyl)butanoate (**5a**).

4.3.1. Ethyl 4-(4-methoxyphenyl)-2,4-dioxobutanoate (**5a**)

Yellow solid in a yield of 92%, mp 56 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (t, J = 7.17 Hz, 3H, CH_3), 3.88 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2), 4.37 (q, J = 7.17 Hz, 2H, OCH_2), 6.94(d, J = 9.06 Hz, 2H, Ar–H), 7.97 (d, J = 9.06 Hz, 2H, Ar–H), ESI-MS: m/z = 273 [M+Na]$^+$.

4.3.2. Ethyl 4-(3-methoxyphenyl)-2,4-dioxobutanoate (**5b**)

Whitish solid in a yield of 89%, mp 72 °C, ^{1}H NMR(300 MHz, $CDCl_3$): δ1.43 (t, J = 7.17 Hz, 3H, CH_3), 3.88 (s, 3H, OCH_3), 3.94 (s, 2H, CH_2), 4.38 (q, J = 7.17 Hz, 2H, OCH_2), 7.10 (d, J = 8.12 Hz, 2H, Ar–H), 7.37 (t, J = 7.93, Hz, 1H, Ar–H), MS: m/z = 251 [M+H]$^+$.



Scheme 3. Synthesis of 4-oxiranylmethoxy-9*H*-carbazole (**2**).

4.3.3. Ethyl 2,4-dioxo-4-p-tolylbutanoate (**5c**)

Whitish solid in a yield of 88%, mp 72–74 °C, ^{1}H NMR(300 MHz, $CDCl_3$):δ 1.40 (t, J = 7.32 Hz, 3H, CH_3), 2.44 (s, 3H, OCH_3), 3.90 (s, 2H, CH_2), 4.34 (q, J = 7.32 Hz, 2H, OCH_2), 7.31(d, J = 7.32 Hz, 2H, Ar–H), 7.90 (d, J = 7.32 Hz, 2H, Ar–H), ESI-MS: m/z = 257 [M+Na]$^+$.

4.3.4. Ethyl 2,4-dioxo-4-m-tolylbutanoate (**5d**)

Reddish solid in a yield of 82%, mp 72 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (t, J = 7.17 Hz, 3H, CH_3), 2.43 (s, 3H, OCH_3), 3.92 (s, 2H, CH_2), 4.37 (q, J = 7.17 Hz, 2H, OCH_2), 7.30–7.40 (m, 2H, Ar–H), 7.72–7.81 (m, 2H, Ar–H), ESI-MS: m/z = 257 [M+Na]$^+$

4.3.5. Ethyl 4-(4-nitrophenyl)-2,4-dioxobutanoate (**5e**)

Yellow solid in a yield of 89%, mp 72–74 °C, ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (t, J = 7.17 Hz, 3H, CH_3), 4.40 (q, J = 7.17 Hz, 2H, OCH_2), 7.06 (s, CH_2), 8.35 (d, J = 8.30 Hz, 2H, Ar–H), 3.90 (s, 2H, CH_2), ESI-MS:m/z = 288 [M+Na]$^+$.

4.3.6. Ethyl 4-(3-nitrophenyl)-2,4-dioxobutanoate (**5f**)

Yellow solid in a yield of 87%, mp 82 °C, ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.45 (t, J = 7.17 Hz, 3H, CH_3), 3.97 (s, 2H, CH_2), 4.42 (q, J = 7.17 Hz, 2H, OCH_2), 7.72 (t, J = 7.93 Hz, 1H, Ar–H), 8.32 (d, J = 8.30 Hz, 1H, Ar–H), 8.45 (d, J = 8.30, Hz, 1H, Ar–H), 8.81(s, 1H, Ar–H); ESI-MS:m/z = 288 [M+Na]$^+$

4.3.7. Ethyl 4-(2-nitrophenyl)-2,4-dioxobutanoate (**5g**)

Yellow oil in a yield of 86%, ^{1}H NMR(300 MHz, $CDCl_3$): δ 1.40 (t, J = 7.17 Hz, 3H, CH_3), 3.92 (s, 2H, CH_2), 4.36 (q, J = 7.17 Hz, 2H, OCH_2), 7.59 (dd, J = 7.17, 5.66 Hz, 1H, Ar–H), 7.67 (dd, J = 7.36, 4.53 Hz, 1H, Ar–H), 7.73 (d, J = 7.36 Hz, 1H, Ar–H), 8.04 (d, J = 7.74 Hz, 1H, Ar–H), ESI-MS: m/z = 266 [M+H]$^+$.

4.4. General procedure for the synthesis of ethyl aryl pyrazole carboxylates (**1a**–**g**)

A mixture **5a** (2.0 g, 0.008 mol) and acetic acid (30 mL) was stirred at room temperature. To this solution was added hydrazine (4.0 mL, 0.08 mol) and the reaction mixture was stirred overnight. The precipitate was filtered, washed with hexane and dried to give following ethyl ester.

4.4.1. Ethyl 3-(4-methoxyphenyl)-1H-pyrazole-5-carboxylate (**1a**)

Yellow solid in a yield of 94%, mp 146–148 °C, IR (KBr, cm^{-1}): 3141 (N–H), 2925 (Ar–H), 1725 (C=O), ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.41 (t, J = 7.17 Hz, 3H, CH_3), 3.83(s, 3H, OCH_3), 4.36 (q, J = 7.16 Hz, 2H, OCH_2CH_3), 6.90 (d, J = 8.05 Hz, 2H, Ar–H), 7.57 (s, 1H, Ar–H), 7.66 (bs, 2H, Ar–H), ESI-MS: m/z = 269 [M+Na]$^+$.

4.4.2. Ethyl 3-(3-methoxyphenyl)-1H-pyrazole-5-carboxylate (**1b**)

Yellow solid in a yield of 83%, mp 154 °C, IR (KBr, cm^{-1}): 3141 (N–H), 2952 (Ar–H), 1729 (C=O), ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.41 (t, J = 7.19 Hz, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.35 (q, J = 7.19 Hz,

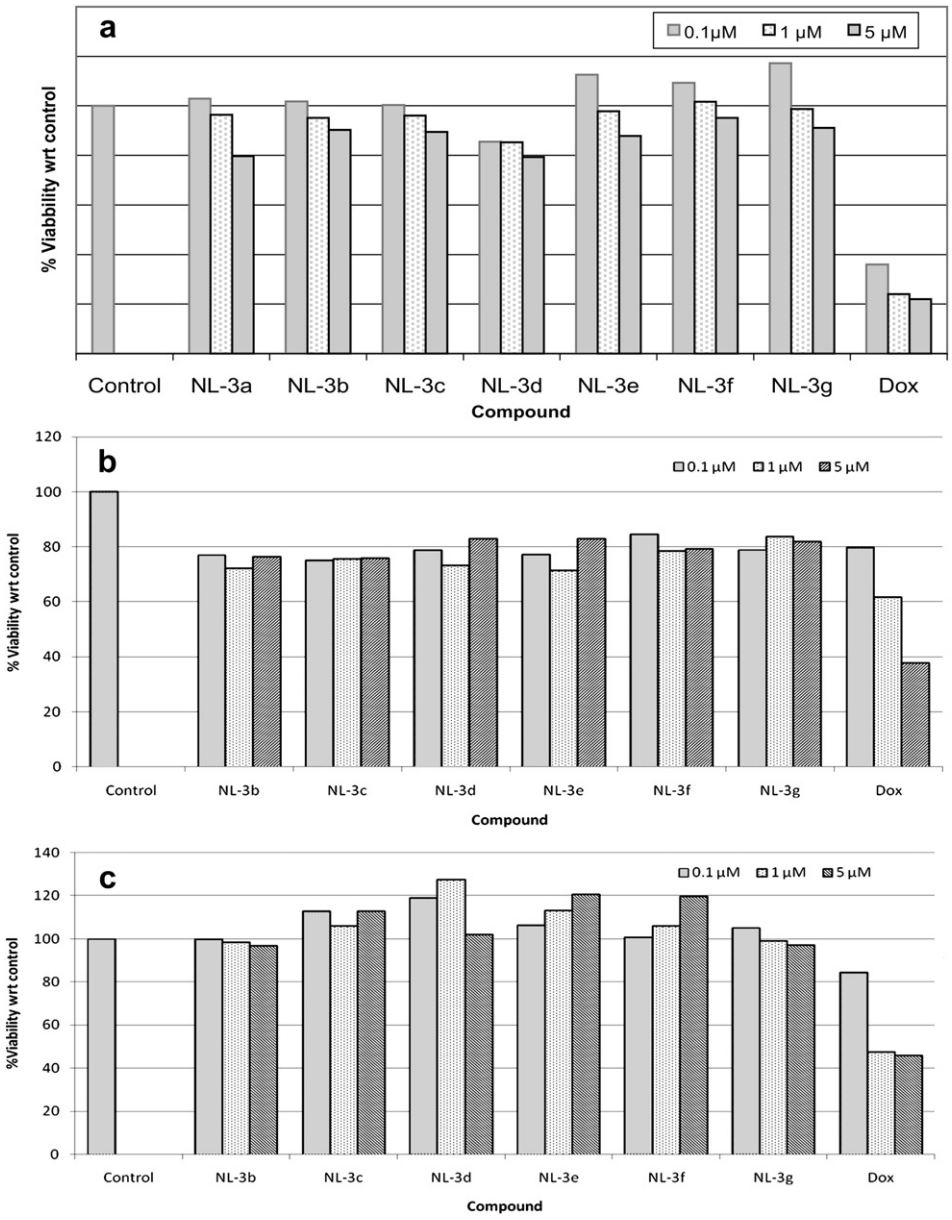


Fig. 1. (a) Analysis of the cytotoxic effect of various derivatives in a human NB cell line (SK–N–SH). SK–N–SH) NB cells were treated with various concentrations (0.1, 1 and 5 μM) of **3a**–**g** for 48 h. A viability assay was carried out. Experiments were performed in triplicate; data are expressed as means of the triplicate determinations of a representative experiment in % cell viability of untreated cells [100%]. (b) Analysis of the cytotoxic effect of various derivatives in a human A549 lung carcinoma cell line. A549 cells were treated with various concentrations (0.1, 1 and 5 μM) of **3a**–**g** for 48 h. A viability assay was carried out. Experiments were performed in triplicate; data are expressed as means of the triplicate determinations of a representative experiment in % cell viability of untreated cells [100%]. (c) Analysis of the cytotoxic effect of various derivatives in a human breast cancer MCF-7 cell line. MCF-7 cells were treated with various concentrations (0.1, 1 and 5 μM) of **3a**–**g** for 48 h. A viability assay was carried out. Experiments were performed in triplicate; data are expressed as means of the triplicate determinations of a representative experiment in % cell viability of untreated cells [100%].

2H, OCH_2CH_3), 6.82 (d, $J = 7.19$ Hz, 1H, Ar–H), 7.00 (d, $J = 5.13$ Hz, 1H, Ar–H), 7.29 (m, 3H, Ar–H), ESI-MS: $m/z = 247$ $[M+H]^+$.

*4.4.3. Ethyl 3-p-tolyl-1H-pyrazole-5-carboxylate (**1c**)*

Whitish solid in a yield of 82%, mp 202 °C, IR (KBr, cm^{-1}): 3220 (N–H), 3005 (Ar–H), 1725 (C=O), ^1H NMR (400 MHz, DMSO-d_6): δ 1.41 (t, $J = 7.16$ Hz, 3H, CH_3), 2.37 (s, 3H, CH_3), 4.35 (q, $J = 7.16$ Hz, 2H, OCH_2CH_3), 7.18 (d, $J = 8.05$ Hz, 2H, Ar–H), 6.95 (s, 1H, Ar–H), 7.62 (b, $J = 8.05$, 2H, Ar–H), ESI-MS: $m/z = 231$ $[M+H]^+$.

*4.4.4. Ethyl 3-m-tolyl-1H-pyrazole-5-carboxylate (**1d**)*

Yellow solid in a yield of 78%, mp 148 °C, IR (KBr, cm^{-1}): 3236 (N–H), 2947 (Ar–H), 1691 (C=O), ^1H NMR (300 MHz, DMSO-d_6): δ 1.41 (t, $J = 7.16$ Hz, 3H, CH_3), 3.37 (s, 3H, CH_3), 4.35 (q, $J = 7.16$ Hz, 2H, OCH_2CH_3), 6.82 (d, $J = 7.16$ Hz, 1H, Ar–H), 7.57 (s, 1H, Ar–H), 7.60 (b, $J = 5.13$ Hz, 1H, Ar–H), 7.80 (m, 3H, Ar–H), ESI-MS: $m/z = 231$ $[M+H]^+$.

*4.4.5. Ethyl 3-(4-nitrophenyl)-1H-pyrazole-5-carboxylate (**1e**)*

Yellow solid in a yield of 98%, mp 210 °C, IR (KBr, cm^{-1}), 3139 (N–H), 2928 (Ar–H), 1733(C=O), ^1H NMR (400 MHz, DMSO-d_6): δ 1.42 (t, $J = 7.17$ Hz, 3H, CH_3); 4.38(q, $J = 7.17$ Hz, 2H, OCH_2CH_3), 7.19 (s, 1H, Ar–H), 8.02 (d, $J = 8.12$ Hz, 2H, Ar–H), 8.25 (d, $J = 8.30$ Hz, 2H, Ar–H), ESI-MS: $m/z = 284$ $[M+Na]^+$.

*4.4.6. Ethyl 3-(3-nitrophenyl)-1H-pyrazole-5-carboxylate (**1f**)*

Yellow solid in a yield of 96%, mp 146 °C, IR (KBr, cm^{-1}) 3304 (N–H), 2984 (Ar–H), 1700 (C=O), ^1H NMR (300 MHz, DMSO-d_6):

δ 1.40 (t, J = 7.08 Hz, 3H, CH_3), 4.36 (q, J = 7.08 Hz, 2H, OCH_2CH_3), 7.32 (s, 1H, Ar–H), 7.69 (t, J = 6.51 Hz, 1H, Ar–H), 8.15 (d, J = 7.36 Hz, 1H, Ar–H), 8.24 (d, J = 7.36 Hz, 1H, Ar–H), 8.66 (s, 1H, Ar–H), ESI-MS: m/z = 262 $[M+H]^+$.

*4.4.7. Ethyl 3-(2-nitrophenyl)-1H-pyrazole-5-carboxylate (**1g**)*

Yellow solid in a yield of 98%, mp 106 °C, IR (KBr, cm^{-1}): 3276 (N–H), 2992 (Ar–H), 1705 (C=O), ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.41 (t, J = 7.17 Hz, 3H, CH_3), 4.36 (q, J = 7.17 Hz, 2H, OCH_2CH_3), 6.88 (s, 1H, Ar–H), 7.50 (t, J = 7.64 Hz, 1H, Ar–H), 7.62 (t, J = 7.45 Hz, 1H, Ar–H), 7.72 (d, J = 7.17 Hz, 2H, Ar–H), ESI-MS: m/z = 262 $[M+H]^+$

*4.5. General procedure for the synthesis 1-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl]-3-aryl-1H-pyrazole-5-carboxylic acid (**3a**–**g**)*

A mixture of 5-(4-methoxyphenyl)-2*H*-pyrazole-3-carboxylic acid ethyl ester **1a** (103 mg, 0.42 mmol), 4-oxiranylmethoxy-9*H*-carbazole (**2,** 100 mg, 0.42 mmol), potassium carbonate (116 mg, 0.82) and tetrabutylammonium iodide (2 mg) were heated in acetonitrile (10 mL) at 80 °C for 32 h under nitrogen atmosphere. The reaction mass were extracted with ethyl acetate. The organic layer dried over sodium sulphate and concentrated crude product obtained was purified by column chromatography over silica gel to give following compounds.

*4.5.1. 1-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl]-3-(4-methoxyphenyl)-1H-pyrazole-5-carboxylic acid (**3a**)*

Brown solid in a yield of 67%, mp 140–142 °C, IR (KBr, ν cm^{-1}): 3400 (O–H, N–H), 2931 (Ar–H), 1713 (C=O), ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.74 s, 1H, OH), 3.81 (s, 3H, OCH_3), 4.16–4.26 (m, 2H, CH_2), 4.49–4.58 (m, 1H, CH), 4.79–4.99 (m, 2H, CH_2), 6.62 (d, J = 8.05 Hz, 1H, Ar–H), 6.93 (d, J = 8.95 Hz, 2H, Ar–H), 7.04–7.15 (m, 3H, Ar–H), 7.21–7.35 (m, 2H, Ar–H), 7.42 (d, J = 7.16 Hz, 1H, Ar–H), 7.73 (d, J = 8.95 Hz, 2H, Ar–H), 8.35 (d, J = 8.05 Hz, 1H, Ar–H), 11.10 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 53.79, 55.03, 68.67, 70.01, 100.28, 103.91, 107.17, 110.19, 111.56, 114.01, 118.57, 121.61, 122.75, 124.47, 125.02, 126.37, 135.33, 138.84, 141.05, 148.80, 154.76, 158.97,160.86, ESI-MS: m/z = 458 $[M+H]^+$.

*4.5.2. 1-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl]-3-(3-methoxyphenyl)-1H-pyrazole-5-carboxylic acid (**3b**)*

Brown solid in a yield of 90%, mp 142 °C, IR (KBr, ν cm^{-1}): 3407 (O–H, N–H), 2932 (Ar–H), 1668 (C=O), ^{1}H NMR (300 MHz, DMSO-d_6): δ2.79 (s, 1H, OH), 3.79 (s, 3H, OCH_3), 4.24 (d, J = 5.09 Hz, 2H, CH_2), 4.56 (m, 1H, CH), 4.99 (m, 2H, CH_2), 6.59 (d, J = 7.93 Hz, 1H, Ar–H), 6.79 (d, J = 7.55 Hz, 1H, Ar–H), 7.07 (m, 3H, Ar–H), 7.24 (m, 2H, Ar–H), 7.42 (d, J = 7.16 Hz, 1H, Ar–H), 7.32 (t, J = 6.98 Hz, 2H, Ar–H), 7.40 (d, J = 7.93 Hz, 1H, Ar–H), 7.82 (s, 1H, Ar–H), 8.36 (d, J = 7.74 Hz, 1H, Ar–H), 10.79 (s, 1H, NH), ESI-MS: m/z = 480$[M+Na]^+$.

*4.5.3. 1-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl]-3-p-tolyl-1H-pyrazole-5-carboxylic acid (**3c**)*

Light brown solid in a yield of 78%, mp 154 °C, IR (KBr, ν cm^{-1}): 3410 (O–H, N–H), 2926 (Ar–H), 1709 (C=O), ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.73 (s, 1H, OH), 2.29 (s, 3H, CH_3), 4.14–4.23 (m, 2H, CH_2), 4.46–4.55 (m, 1H, CH), 4.83–4.99 (m, 2H, CH_2), 6.51 (d, J = 9.25 Hz, 1H, Ar–H), 6.97 (d, J = 8.22 Hz, 1H, Ar–H), 6.99–7.14 (m, 3H, Ar–H), 7.21(t, J = 8.22 Hz, 1H, Ar–H), 7.32 (d, J = 8.22 Hz, 2H, Ar–H), 7.58 (d, J = 8.22 Hz, 2H, Ar–H), 7.62 (s, 1H, Ar–H), 8.29 (d, J = 8 Hz, 1H, Ar–H), 10.55 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 20.82, 53.87, 69.18, 70.07, 100.35, 104.02, 106.67, 110.34, 111.65, 118.66, 121.74, 122.82. 124.56, 125.06, 126.46, 129.25, 130.06, 136.93, 138.47, 138.96, 141.15, 148.65, 154.85, 162.06, ESI-MS: m/z = 464 $[M+Na]^+$.

*4.5.4. 1-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl]-3-m-tolyl-1H-pyrazole-5-carboxylic acid (**3d**)*

Brown solid in a yield of 76%, mp 150 °C, IR (KBr, ν cm^{-1}): 3441 (O–H, N–H), 2925 (Ar–H), 1634 (C=O), ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.78 (s, 1H, OH), 2.39 (s, 3H, CH_3), 4.24 (d, J = 4.91 Hz, 2H, CH_2), 4.53–4.63 (m, 1H, CH), 4.92–4.99 (m, 2H, CH_2), 6.58 (d, J = 7.74 Hz, 1H, Ar–H), 7.01–7.15 (m, 3H, Ar–H), 7.18–7.31 (m, 3H, Ar–H), 7.40 (d, J = 8.12 Hz, 1H, Ar–H), 7.55 (d, J = 7.74 Hz, 1H, Ar–H), 7.60 (s, 1H, Ar–H), 7.82 (s, 1H, Ar–H), 8.35 (d, J = 7.74 Hz, 1H, Ar–H), 10.79 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 21.14, 54.04, 68.94, 70.06,100.48, 104.14, 107.75, 110.43, 111.74, 118.81, 121.83, 122.50, 122.96, 124.71, 125.82, 126.60, 128.67, 132.45, 136.26, 137.98, 139.02, 141.24, 149.08, 154.93, 161.27; ESI-MS: m/z = 464 $[M+Na]^+$

*4.5.5. 1-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl]-3-(4-nitrophenyl)-1H-pyrazole-5-carboxylic acid (**3e**)*

Yellow solid in a yield of 75%, mp 145 °C, IR (KBr, ν cm^{-1}): 3407 (O–H, N–H), 2928(Ar–H), 1707 (C=O), ^{1}H NMR (400 MHz, DMSO-d_6): δ2.79 (s, 1H, OH), 4.16 (dd, 2H, CH_2), 4.45 (m, 1H, CH), 4.93 (dd, 2H, CH_2), 6.56 (d, J = 8.0 Hz, 1H, Ar–H), 7.02 (d, J = 8.05 Hz, 1H, Ar–H), 7.12 (m, 2H, Ar–H), 7.19 (t, J = 8.05, 1H, Ar–H), 7.25 (t, J = 8.05 Hz, 1H, Ar–H), 7.37 (d, J = 8.05 Hz, 1H, Ar–H), 7.92 (d, J = 8.79 Hz, 2H, Ar–H), 8.14 d, J = 8.79 Hz, 2H, Ar–H), 8.31 (d, J = 8.05 Hz, 1H, Ar–H), 10.79 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 54.12, 69.38, 70.04, 100.42, 104.00, 107.53, 110.36, 11.65, 118.69, 121.73, 122.73, 124.07, 124.56, 125.78, 126.46, 138.97, 139.60, 141.15, 142.04, 146.18, 146.32, 154.85, 162.56, ESI-MS: m/z = 495 $[M + +Na]^+$.

*4.5.6. 1-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl]-3-(3-nitrophenyl)-1H-pyrazole-5-carboxylic acid (**3f**)*

Pale yellow solid in a yield of 73%, mp 138 °C, IR (KBr, ν cm^{-1}): 3048 (O–H, N–H), 2928 (Ar–H), 1712 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.81 (s, 1H, OH); 4.28 (d, J = 4.72 Hz, 2H, CH_2), 5.04 (d, J = 5.85 Hz, 2H, CH_2), 4.62 (m, 1H, CH), 6.59 (d, J = 7.93 Hz, 1H, Ar–H), 7.03 (d, J = 7.93 Hz, 1H, Ar–H), 7.12 (t, J = 7.46 Hz, 1H, Ar–H), 7.24 (m, 3H, Ar–H), 7.38 (d, J = 8.12 Hz, 1H, Ar–H), 7.57 (t, J = 7.83 Hz, 2H, Ar–H), 8.12 (d, J = 7.93 Hz, 1H, Ar–H), 8.34 (d, J = 7.74 Hz, 1H, Ar–H), 8.61 (s, 1H, Ar–H), 10.47 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 54.28, 68.77, 70.02, 100.30, 103.97, 108.34, 110.25, 111.58, 118.59, 119.17, 121.65, 122.32, 122.79, 124.50, 126.40, 130.37, 131.50, 134.19, 137.05, 138.88, 141.08, 146.69, 148.26, 154.74, 160.99, ESI-MS: m/z = 495 [M+Na].

*4.5.7. 1-[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl]-3-(3-nitrophenyl)-1H-pyrazole-5-carboxylic acid (**3g**)*

Pale yellow solid in a yield of 76%, mp 139 °C, IR (KBr, ν cm^{-1}): 3416 (O–H, N–H), 2928 (Ar–H), 1704 (C=O), ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.80 (s, 1H, OH), 4.22 (d, J = 5.09 Hz, 2H, CH_2), 4.56 (m, 1H, CH), 4.97 (d, J = 5.66 Hz, 2H, CH_2), 6.60 (d, J = 7.74 Hz, 1H, Ar–H), 6.96 (s, 1H, Ar–H), 7.04 (d, J = 8.12 Hz, 1H, Ar–H), 7.12 (t, J = 7.17 Hz, 1H, Ar–H), 7.19–7.31 (m, 2H, Ar–H), 7.39 (d, J = 7.93 Hz, 1H, Ar–H), 7.49 (t, J = 7.64 Hz, 1H, Ar–H), 7.72(t, J = 7.74 Hz, 2H, Ar–H), 8.32 (d, J = 7.74 Hz, 1H, Ar–H), 10.69 (s, 1H, NH), ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 54.16, 68.83, 69.99, 100.53, 104.14, 109.58, 110.39, 111.71, 118.83, 121.80, 122.94, 123.74, 124.71, 125.38, 126.60, 129.40, 130.38, 132.36, 136.49, 138.99, 141.21, 144.76, 148.62, 154.85, 160.90, ESI-MS: m/z = 495 $[M+Na]^+$.

4.6. In vitro cytotoxic activity evaluation by SRB assay

The newly obtained derivatives were evaluated for cytotoxic activity to cancer cell lines by using the sulforhodamine B (assay) method [8,9]. The cells were grown in minimal essential medium

supplemented with 10% fetal bovine serum (Invitrogen), 100 U/ml Penicillin (Invitrogen) and 100 μg/ml Streptomycin (Invirtogen). The cells were seeded in 24-well microplates and treated with the compounds **3a**–**g** and standard doxorubicin in concentration ranging from 0.1, 1, 5 μM in triplicates, incubated for 48 h at 37 °C under a 5% CO_2 atmosphere. The culture were fixed with cold Trichloroacetic acid (TCA) and stained with 0.4% SRB dissolved in 1% acetic acid. The protein-bound dye is dissolved in 10 ml buffered solution and the optical density was measured by spectrophotmetrically at 565 nm. The % viability were calculated using nonlinear regression analysis. Each value represents the mean of triplicate experiments.

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