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SYNTHESIS OF NOVEL PYRAZOLE DERIVATIVES BEARING 1,2,4-TRIAZOLE MOIETY AS POTENTIAL ANTICANCER AGENTS

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*A series of novel 3-(1H-pyrazol-3-yl)-4H-1,2,4-triazole derivatives were synthesized and the structure of the prepared compounds was fully characterized by ^{1}H NMR, ^{13}C NMR, and HRESI-MS. Six of the prepared compounds were screened for in-vitro cytotoxicity against different cancer cell lines at National Cancer Institute (NCI), USA. Compound **7e** exhibited a broad-spectrum of anticancer activity against different cancer cell lines without pronounced selectivity. Moreover, the anticancer activity of the prepared compounds was also evaluated against different human cancer cell lines including breast MCF-7, lung A549 as well as the human normal melanocyte (HFB4) using doxorubicin as a reference drug. Compounds **7l** and **7o** exhibited remarkable anticancer activity similar to or more potent than doxorubicin against breast MCF-7 and Lung A549 cell lines.*

INTRODUCTION

Cancer is one of the leading causes of morbidity and mortality worldwide[1]. Treatment of cancer is associated with various side effects which include bone marrow, depression, alopecia, and hepatotoxicity. In addition, the development of resistance against the existing anticancer drugs and cytotoxicity of anticancer drugs to the normal cells are other major problems in cancer therapy so the development of new anticancer therapeutic agents with improved efficacy and minimal side effects is one of the fundamental goals in medicinal chemistry.

Several pyrazole derivatives have been reported to possess diverse pharmacological activities such as anti-inflammatory[2-4], analgesic[5], anticancer[6-12], antiviral[13], antimicrobial[14&15], antitubercular[16&17], antihyperglycemic[18], antidepressant[19], anticonvulsant[20&21], and antihepatotoxic[22]. Additionally, the pyrazole ring is a prominent structural motif found in numerous pharmaceutically active compounds such as selective COX-2 inhibitor (Celecoxib)[23], non-steroidal anti-inflammatory drug (Lonazolac)[24], phosphodiesterase inhibitor (Sildenafil)[25], clinically approved anticancer agents (Ruxolitinib)[26] and antiobesity cannabinoid drug (Rimonabant)[27] (Fig. 1). A cannabinoid based medicine is already licensed for treatment of the nausea and vomiting associated with chemotherapy in cancer patients[28]. Cannabinoids possess antineoplastic effect against types of growth, such as thyroid epithelioma, skin carcinomas, uterine carcinoma, breast cancer, prostate carcinoma, and neuroblastoma[29]. Cannabinoids might offer a relatively comprehensive medical treatment for cancer patients as they could inhibit tumour cell proliferation, induce appetite, and reduce inflammation and pain.

Meanwhile, 1,2,4-triazoles are an important class of heterocyclic compounds with a wide range of biological activities such as antimicrobial[30&31], antitubercular[32], anticancer[33-38], antiviral[39], anticonvulsant[40],

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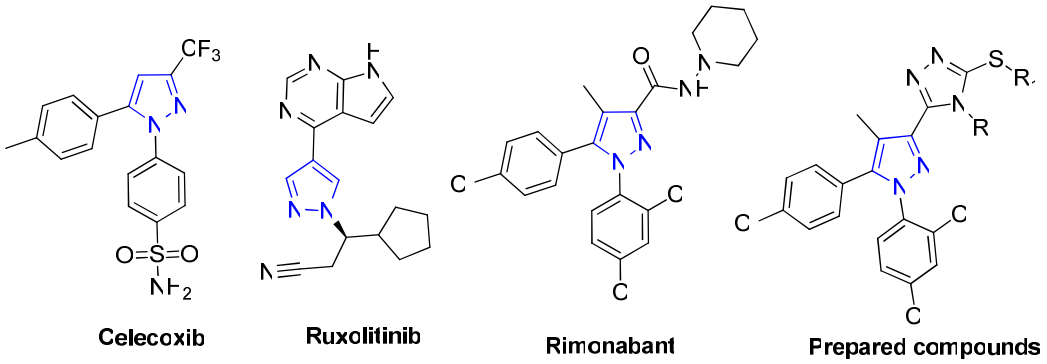


Fig. 1: Structures of some pharmaceutically active compounds containing pyrazole moiety and general structure of the prepared 3-(1*H*-pyrazol-3-yl)-4*H*-1,2,4-triazoles.

antiinflammatory[41], analgesic[42], antihypertensive[43], and antidepressant[44]. Furthermore, the 1,2,4-triazole nucleus has been incorporated into a variety of therapeutically interesting drug candidates including antifungal (Fluconazole), antiviral (Ribavarin), antimigraine (Rizatriptan), and antianxiety compounds (Alprazolam)[36&45].

Based on the above mentioned studies, the present work gathers the two bioactive entities, 1,5-diarylpyrazole functionality which the main nucleus in Rimonabant and the 1,2,4-triazole moiety in one compact structure for the purpose of synergistic effect. The synthesis and *in-vitro* anticancer activities of the prepared 3-(1*H*-pyrazol-3-yl)-4*H*-1,2,4-triazole derivatives were reported here.

MATERIALS AND METHODS

All the chemicals used were of analytical grade and purified by standard methods prior to use. Silica gel column chromatography was carried out using kieselgel 60 (merck). TLC analysis was performed on aluminium-backed plates coated with silica gel 60 F_{254} (Merck). Melting points were determined using a Gallen Kamp melting point apparatus and are uncorrected. Components were visualized using potassium permanganate solution and UV light. NMR Spectra were taken using a Varian Unity INOVA 400 MHz spectrometer for proton and 101 MHz for carbon at university of Aberdeen. All numbers referring to NMR data obtained are in parts per million (ppm). High resolution mass spectrometric data were obtained using Thermo Instruments MS system (LTQ XL/LTQ Orbitrap Discovery) coupled to a Thermo Instruments HPLC system (Accela PDA detector, Accela PDA autosampler and Pump) at university of Aberdeen.

Chemistry

Synthesis of lithium Salt of 4-(4-chlorophenyl)-3-methyl-2,4-dioxobutyric acid ethyl ester (2)

To a magnetically stirred solution of lithium bis(trimethylsilyl)amide (LHMDS) (32 mL, 1.0 M in THF, 32 mmol) in diethyl ether (80 mL) at -78 °C a solution of 4-chloropropiophenone **1** (4 g, 24 mmol) in diethyl ether (20 mL) was added in a drop wise manner under nitrogen atmosphere. After the mixture was stirred at the ambient temperature for period of 1 h, diethyl oxalate (4 g, 27.78 mmol) was added in a drop wise manner. The reaction mixture was allowed to warm to room temperature and stirred for another 16 h. The formed precipitate was collected by filtration, washed with diethyl ether, and dried under vacuum to afford the crude lithium salt **2** as a yellowish solid.

Synthesis of ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylate (3)

To a solution of the lithium salt **2** (5 g, 18.18 mmol) in ethanol (50 mL) 2,4-dichlorophenylhydrazine hydrochloride (4.67 g, 21.82 mmol) was added at room temperature. The resulting mixture was stirred for additionally 20 h. The formed precipitate

was collected by filtration, washed with ethanol and Et_2O, and dried under vacuum to give a light-yellow solid of the hydrazone. The solid was dissolved in acetic acid (30 mL) and heated to reflux for 24 h. The reaction mixture was poured into ice water, and extracted with ethyl acetate (3 x 40 mL). The EtOAc extract was washed successively with water, saturated aqueous sodium bicarbonate, brine, dried over $MgSO_4$, and evaporated under vacuum to provide a crude product which was purified by flash chromatography on silica gel with ethyl acetate/ n-hexane (1:6) to give the pyrazole-3-carboxylic acid ethyl ester **3** as a white solid. Yield, m.p., elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data are listed in tables I and II.

Synthesis of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carbohydrazide (4)

A mixture of pyrazole-3-carboxylic acid ethyl ester **3** (5 g, 12.25 mmol) and 80% hydrazine hydrate (4.7 mL, 122.5 mmol) in ethanol (50 mL) was heated to reflux for 5 hours. The solvent was concentrated in vacuum and the resulting residue was taken up in water, filtered, washed with water, and dried in vacuum to provide the title compound **4** as white solid. Yield, m.p., elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data are listed in tables I and II.

General procedure A for synthesis of 2-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carbonyl)-*N*-subsituted hydrazine carbothioamide (5a-c)

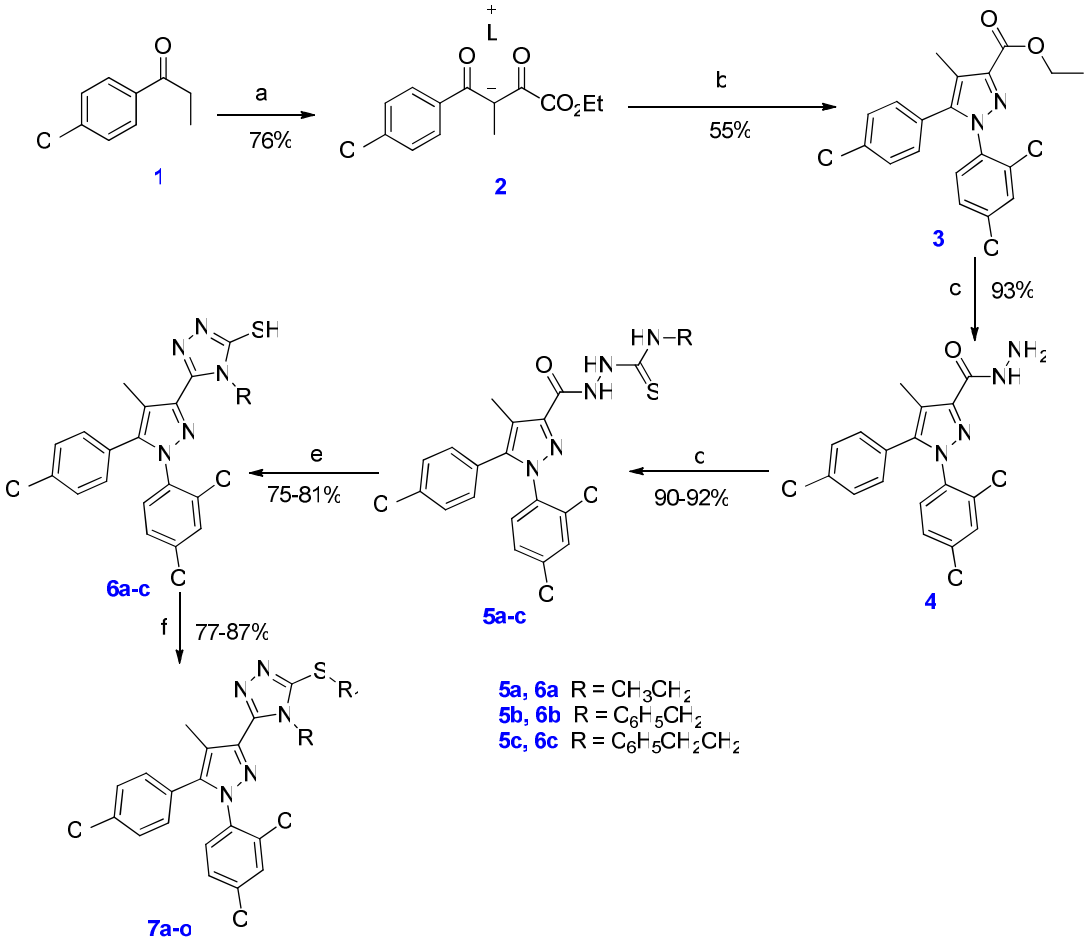
A mixture of **4** (1.5 g, 3.81 mmol) and an appropriate isothiocyanate (3.81 mmol) in EtOH (30 mL) was heated at reflux for 2 h. The solvent was concentrated in vacuum and the resulting residue was taken up in water, filtered off, washed with water, and dried to give the title compounds which were used for next step without further purification. Yields, m.p., elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data are listed in tables I and II.

General procedure B for synthesis of 5-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazol-3-yl)-4-subsituted-4*H*-1,2,4-triazole-3-thiol (6a-c)

A mixture of the appropriate thiosemicarbazides **5a-c** (4 mmol) and 2N NaOH (30 mL) was refluxed for 3h. After cooling, the reaction mixture was acidified to pH 6 with 2M HCl and the resulting precipitate was filtered off and washed with water to afford a crude product which was purified by suspended in few mL of Et_2O then the separated precipitate was filtered and washed with Et_2O to give pure desired compound. Yields, m.p., elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data are listed in tables I and II.

General procedure C for synthesis of the final target compounds (7a-o)

A mixture of the appropriate 1,2,4-triazole-3-thiol derivatives **6a-c** (0.5 mmol), an appropriate haloalkane (0.6 mmol) and K_2CO_3 (0.06 g, 0.6 mmol) in acetone (20 mL) was heated under reflux for 1 h. After removal of the organic solvent in vacuo and addition of water, the resulting residue was extracted by EtOAc. The organic layer was washed with brine, dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to afford the crude product which was purified by column chromatography on silica using EtOAc/hexane (1:2) to give the desired compound. Yields, m.p., elemental analyses, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data are listed in tables I and II.



Reagents and conditions: (a) diethyl oxalate, LHMDS, -78°C to room temp, 16 h; (b) 2,4-dichlorophenylhydrazine hydrochloride, EtOH, room temp, 20 h, then acetic acid, reflux, 24 h; (c) NH_2NH_2. H_2O (85%), ethanol, reflux, 5 h; (d) an appropriate isothiocyanate, ethanol, reflux, 2 h; (e) 2N NaOH, reflux, 3 h; (f) an appropriate haloalkane, K_2CO_3, acetone, reflux, 1 h.

Compd.	R	R_1	Compd.	R	R_1
7a	CH_3CH_2	$C_6H_5CH_2$	**7i**	$C_6H_5CH_2$	*p*-$OCH_3C_6H_4CH_2$
7b	CH_3CH_2	$C_6H_5CH_2CH_2$	**7j**	$C_6H_5CH_2$	*p*-$CH_3C_6H_4CH_2$
7c	CH_3CH_2	$C_6H_5CH_2CH_2CH_2$	**7k**	$C_6H_5CH_2CH_2$	$C_6H_5CH_2$
7d	CH_3CH_2	*p*-$OCH_3C_6H_4CH_2$	**7l**	$C_6H_5CH_2CH_2$	$C_6H_5CH_2CH_2$
7e	CH_3CH_2	*p*-$CH_3C_6H_4CH_2$	**7m**	$C_6H_5CH_2CH_2$	$C_6H_5CH_2CH_2CH_2$
7f	$C_6H_5CH_2$	$C_6H_5CH_2$	**7n**	$C_6H_5CH_2CH_2$	*p*-$OCH_3C_6H_4CH_2$
7g	$C_6H_5CH_2$	$C_6H_5CH_2CH_2$	**7o**	$C_6H_5CH_2CH_2$	*p*-$CH_3C_6H_4CH_2$
7h	$C_6H_5CH_2$	$C_6H_5CH_2CH_2CH_2$	-	-	-

Scheme 1: Synthetic route of target compounds 3-(1*H*-pyrazol-3-yl)-4*H*-1,2,4-triazoles **7a-o**.

Table I: Physicochemical properties of compounds **3, 4, 5a-c, 6a-c** and **7a-o.**

Compd.	R	R^1	Yield (%)	m.p. (°C)	M.F. (M.Wt.)
3	--	--	55	120-121	$C_{19}H_{15}C_{l3}N_2O_2$ (409.69)
4	--	--	93	102-104	$C_{17}H_{13}Cl_3N_4O$ (395.67)
5a	CH_3CH_2	--	92	143-145	$C_{20}H_{18}Cl_3N_5OS$ (482.81)
5b	$C_6H_5CH_2$	--	90	138-140	$C_{25}H_{20}Cl_3N_5OS$ (544.88)
5c	$C_6H_5CH_2CH_2$	--	91	125-127	$C_{26}H_{22}Cl_3N_5OS$ (558.91)
6a	CH_3CH_2	--	81	234-236	$C_{20}H_{16}Cl_3N_5S$ (464.80)
6b	$C_6H_5CH_2$	--	80	242-244	$C_{25}H_{18}Cl_3N_5S$ (526.87)
6c	$C_6H_5CH_2CH_2$	--	75	224-226	$C_{26}H_{20}Cl_3N_5S$ (540.89)
7a	CH_3CH_2	$C_6H_5CH_2$	87	72-74	$C_{27}H_{22}Cl_3N_5S$ (554.92)
7b	CH_3CH_2	$C_6H_5CH_2CH_2$	86	130-132	$C_{28}H_{24}Cl_3N_5S$ (568.95)
7c	CH_3CH_2	$C_6H_5CH_2CH_2CH_2$	77	oil	$C_{29}H_{26}Cl_3N_5S$ (582.97)
7d	CH_3CH_2	*p*-$OCH_3C_6H_4CH_2$	86	oil	$C_{28}H_{24}Cl_3N_5OS$ (584.95)
7e	CH_3CH_2	*p*-$CH_3C_6H_4CH_2$	88	90-92	$C_{28}H_{24}Cl_3N_5S$ (568.95)
7f	$C_6H_5CH_2$	$C_6H_5CH_2$	87	65-67	$C_{32}H_{24}Cl_3N_5S$ (616.99)
7g	$C_6H_5CH_2$	$C_6H_5CH_2CH_2$	82	61-62	$C_{33}H_{26}Cl_3N_5S$ (631.02)
7h	$C_6H_5CH_2$	$C_6H_5CH_2CH_2CH_2$	78	oil	$C_{34}H_{28}Cl_3N_5S$ (645.04)
7i	$C_6H_5CH_2$	*p*-$OCH_3C_6H_4CH_2$	87	132-134	$C_{33}H_{26}Cl_3N_5OS$ (647.02)
7j	$C_6H_5CH_2$	*p*-$CH_3C_6H_4CH_2$	85	72 -74	$C_{33}H_{26}C_{l3}N_5S$ (631.02)
7k	$C_6H_5CH_2CH_2$	$C_6H_5CH_2$	86	59-60	$C_{33}H_{26}Cl_3N_5S$ (631.02)
7l	$C_6H_5CH_2CH_2$	$C_6H_5CH_2CH_2$	78	90-92	$C_{34}H_{28}Cl_3N_5S$ (645.04)
7m	$C_6H_5CH_2CH_2$	$C_6H_5CH_2CH_2CH_2$	78	118-120	$C_{35}H_{30}Cl_3N_5S$ (659.07)
7n	$C_6H_5CH_2CH_2$	*p*-$OCH_3C_6H_4CH_2$	86	163-165	$C_{34}H_{28}Cl_3N_5OS$ (661.04)
7o	$C_6H_5CH_2CH_2$	*p*-$CH_3C_6H_4CH_2$	83	58-60	$C_{34}H_{28}Cl_3N_5S$ (645.04)

Table II: Spectral characterization of compounds **3, 4, 5a-c, 6a-c** and **7a-o.**

Compd.	^{1}H NMR (δ ppm)	^{13}C NMR (δ ppm)	HRMES (calculated for $[M+H]^+$ /found)
3	(400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.33 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.29–7.24 (m, 3H, Ar-H), 7.05 (d, *J* = 8.8 Hz, 2H, Ar-H), 4.42 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 2.31 (s,	(101 MHz, $CDCl_3$) δ 162.71, 142.94, 142.85, 136.01, 135.88, 134.97, 133.01, 130.86, 130.72, 130.06, 128.86, 127.75, 127.02, 119.11, 60.95, 14.42, 9.66.	409.0272 409.0277

Table II: Continued.

Compd.	^{1}H NMR (δ ppm)	^{13}C NMR (δ ppm)	HRMES (calculated for $[M+H]^+$ /found)
	3H, $C\underline{H_3}$), 1.39 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H_3}$).		
4	(400 MHz, DMSO-d_6) δ 9.46 (s, 1H, NH), 7.77–7.67 (m, 2H, Ar-H), 7.56 (dd, *J* = 8.7, 2.3 Hz, 1H, Ar-H), 7.44 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.23 (d, *J* = 8.1 Hz, 2H, Ar-H), 4.15 (s, 2H, NH_2), 2.24 (s, 3H, CH_3).	(101 MHz, DMSO-d_6) δ 161.51, 144.27, 142.20, 135.78, 135.00, 133.72, 132.09, 131.84, 131.21, 129.62, 128.74, 128.32, 127.23, 116.19, 9.00.	395.0228 395.0216
5a	(400 MHz, DMSO-d_6) δ 10.07 (s, 1H, N$\underline{H}$NHCSNH), 9.22 (s, 1H, NHN$\underline{H}$CSNH), 7.96 (t, *J* = 5.7 Hz, 1H, NHNHCSN$\underline{H}$), 7.77 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.70 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.60 (dd, *J* = 8.5, 2.3 Hz, 1H, Ar-H), 7.46 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.23 (d, *J* = 8.3 Hz, 2H, Ar-H), 3.47 (p, *J* = 7.2 Hz, 2H, $C\underline{H_2}CH_3$), 2.25 (s, 3H, CH_3), 1.06 (t, *J* = 7.2 Hz, 3H, $CH_2C\underline{H_3}$).	(101 MHz, DMSO-d_6) δ 189.08, 161.62, 143.56, 142.41, 135.64, 135.13, 133.84, 131.89, 131.83, 131.19, 129.68, 128.81, 128.42, 127.06, 117.27, 38.56, 14.49, 9.13.	482.0370 482.0358
5b	(400 MHz, DMSO-d_6) δ 10.26 (s, 1H, N$\underline{H}$NHCSNH), 9.47 (s, 1H, NHN$\underline{H}$CSNH), 8.54 (t, *J* =7.9 Hz, 1H, NHNHCSN$\underline{H}$), 7.77–7.67 (m, 2H, Ar-H), 7.59 (dd, *J* = 8.5, 2.3 Hz, 1H, Ar-H), 7.45 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.38–7.16 (m, 7H, Ar-H), 4.77 (d, *J* = 5.4 Hz, 2H, $NHC\underline{H_2}$), 2.28 (s, 3H, CH_3).	(101 MHz, DMSO-d_6) δ 190.30, 143.64, 142.45, 139.44, 138.12, 135.65, 135.19, 133.91, 131.92, 131.21, 129.69, 128.84, 128.27, 127.98, 127.37, 127.12, 127.00, 126.54, 117.36, 47.76, 9.13.	544.0527 544.0511
5c	(400 MHz, DMSO-d_6) δ 10.13 (s, 1H, N$\underline{H}$NHCSNH), 9.34 (s, 1H, NHN$\underline{H}$CSNH), 8.03 (t, *J* = 5.8 Hz, 1H, NHNHCSN$\underline{H}$), 7.78 (d, *J* = 2.1 Hz, 1H, Ar-H), 7.71 (dd, *J* = 8.6, 1.6 Hz, 1H, Ar-H), 7.61 (dd, *J* = 8.5, 2.0 Hz, 1H, Ar-H), 7.47 (dd, *J* = 8.5, 1.8 Hz, 2H, Ar-H), 7.31–7.16 (m, 7H, Ar-H), 3.64 (q, *J* = 7.5 Hz, 2H, $NHC\underline{H_2}CH_2$), 2.82 (t, *J* = 7.5 Hz, 2H, $NHCH_2C\underline{H_2}$), 2.27 (s, 3H, CH_3).	(101 MHz, DMSO-d_6) δ 143.50, 142.42, 139.37, 135.62, 135.14, 133.84, 131.89, 131.82, 131.20, 129.69, 128.82, 128.62, 128.43, 128.39, 128.35, 127.04, 126.05, 117.28, 109.53, 45.34, 34.91, 9.14.	558.0683 558.0667
6a	(400 MHz, DMSO-d_6) δ 7.81 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.66 (d, *J* = 8.6 Hz, 1H, Ar-H), 7.55 (dd, *J* = 8.5, 2.3 Hz, 1H, Ar-H), 7.47 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.26 (d, *J* = 8.2 Hz, 2H, Ar-H), 4.36 (q, *J* = 7.0 Hz, 2H, $C\underline{H_2}CH_3$), 2.23 (s, 3H, CH_3), 1.21 (t, *J* = 7.0 Hz, 3H, $CH_2C\underline{H_3}$).	(101 MHz, DMSO-d_6) δ 166.90, 144.57, 141.93, 139.74, 135.75, 134.94, 133.84, 132.04, 131.54, 131.27, 129.88, 128.82, 128.42, 127.07, 115.72, 39.68, 13.80, 9.65.	464.0265 464.0251
6b	(400 MHz, DMSO-d_6) δ 7.78 (s, 1H, Ar-H), 7.58 (s, 2H, Ar-H), 7.45 (d, *J* = 8.2 Hz, 2H, Ar-H), 7.26–7.17 (m, 7H, Ar-H), 5.63 (s, 2H, NCH_2), 2.14 (s, 3H, CH_3).	(101 MHz, DMSO-d_6) δ 167.82, 144.91, 142.11, 138.78, 136.21, 135.48, 135.13, 133.96, 131.86, 131.53, 131.23, 129.80, 128.82, 128.44, 128.16, 127.41, 127.31, 126.75, 115.99, 47.20, 9.45.	526.0421 526.0405
6c	(400 MHz, DMSO-d_6) δ 7.84 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.73 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.62 (dd, *J* = 8.5, 2.3 Hz, 1H, Ar-H), 7.48 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.26 (d, *J* = 8.1 Hz, 2H, Ar-H), 7.16–7.12 (m, 3H, Ar-H), 7.06–6.99 (m, 2H, Ar-H), 4.57 (t, *J* = 7.5 Hz, 2H, $NC\underline{H_2}CH_2$), 3.01 (t, *J* = 7.5 Hz, 2H, $NCH_2C\underline{H_2}$), 2.09 (s, 3H, CH_3).	(101 MHz, DMSO-d_6) δ 166.83, 144.86, 142.11, 138.83, 137.67, 135.64, 135.21, 133.98, 132.03, 131.67, 131.21, 129.84, 128.89, 128.63, 128.50, 128.24, 126.86, 126.43, 115.93, 45.82, 33.45, 9.44.	540.0578 540.0560
7a	(400 MHz, $CDCl_3$) δ 7.33 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.29 (d, *J* = 7.0 Hz, 2H, Ar-H), 7.21–	(101 MHz, $CDCl_3$) δ 150.74, 149.11, 141.94, 140.82, 136.76, 136.08, 135.51,	554.0734 554.0721

Table II: Continued.

Compd.	^{1}H NMR (δ ppm)	^{13}C NMR (δ ppm)	HRMES (calculated for $[M+H]^+$ /found)
	7.12 (m, 6H, Ar-H), 7.07 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.00 (d, *J* = 8.3 Hz, 2H, Ar-H), 4.43 (s, 2H, SCH_2), 4.17 (q, *J* = 7.1 Hz, 2H, $C\underline{H_2}CH_3$), 2.33 (s, 3H, CH_3), 1.12 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H_3}$).	134.79, 132.87, 130.79, 130.38, 130.32, 129.06, 128.88, 128.61, 127.78, 127.67, 127.34, 117.03, 40.49, 37.77, 15.09, 10.12.	
7b	(400 MHz, $CDCl_3$) δ 7.35 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.24–7.05 (m, 9H, Ar-H), 7.05–6.98 (m, 2H, Ar-H), 4.27 (q, *J* = 7.1 Hz, 2H, $C\underline{H_2}CH_3$), 3.47 (t, *J* = 7.7 Hz, 2H, $SC\underline{H_2}CH_2$), 3.05 (t, *J* = 7.7 Hz, 2H, $SCH_2C\underline{H_2}$), 2.35 (s, 3H, CH_3), 1.23 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H_3}$).	(101 MHz, $CDCl_3$) δ 151.10, 149.13, 141.98, 140.90, 139.67, 136.15, 135.55, 134.83, 132.94, 130.84, 130.42, 130.38, 128.92, 128.76, 128.47, 127.82, 127.39, 126.54, 117.05, 40.56, 35.93, 34.08, 15.16, 10.19.	568.0891 568.0876
7c	(400 MHz, $CDCl_3$) δ 7.34 (d, *J* = 2.7 Hz, 1H, Ar-H), 7.23–7.03 (m, 9H, Ar-H), 7.00 (d, *J* = 8.3 Hz, 2H, Ar-H), 4.30 (q, *J* = 7.1 Hz, 2H, $C\underline{H_2}CH_3$), 3.23 (t, *J* = 7.6 Hz, 2H, $SC\underline{H_2}CH_2CH_2$), 2.69 (t, *J* = 7.6 Hz, 2H, $SCH_2CH_2C\underline{H_2}$), 2.33 (s, 3H, CH_3), 2.06 (p, *J* = 7.6 Hz, 2H, $SCH_2C\underline{H_2}CH_2$), 1.24 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H_3}$).	(101 MHz, $CDCl_3$) δ 151.15, 149.08, 141.95, 140.99, 140.89, 136.12, 135.51, 134.79, 132.91, 130.81, 130.40, 130.34, 128.88, 128.47, 128.39, 127.79, 127.37, 125.98, 117.03, 40.54, 34.64, 32.29, 31.13, 15.16, 10.14.	582.1047 582.1036
7d	(400 MHz, $CDCl_3$) δ 7.36 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.24–7.19 (m, 4H, Ar-H), 7.15 (dd, *J* = 8.4, 2.2 Hz, 1H, Ar-H), 7.08 (d, *J* = 8.4 Hz, 1H, Ar-H), 7.02 (d, *J* = 8.1 Hz, 2H, Ar-H), 6.76–6.69 (m, 2H, Ar-H), 4.40 (s, 2H, SCH_2), 4.19 (q, *J* = 7.1 Hz, 2H, $C\underline{H_2}CH_3$), 3.66 (s, 3H, OCH_3), 2.33 (s, 3H, CH_3), 1.15 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H_3}$).	(101 MHz, $CDCl_3$) δ 159.15, 150.94, 149.08, 141.99, 140.86, 136.13, 135.57, 134.85, 132.93, 130.83, 130.41, 130.38, 130.34, 128.93, 128.71, 127.82, 127.38, 117.08, 114.06, 55.26, 40.53, 37.42, 15.15, 10.14.	584.0840 584.0828
7e	(400 MHz, $CDCl_3$) δ 7.41 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.30–7.19 (m, 5H, Ar-H), 7.15 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.10–7.05 (m, 4H, Ar-H), 4.47 (s, 2H, SCH_2), 4.26 (q, *J* = 7.1 Hz, 2H, $C\underline{H_2}CH_3$), 2.41 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 1.21 (t, *J* = 7.1 Hz, 3H, $CH_2C\underline{H_3}$).	(101 MHz, $CDCl_3$) δ 150.89, 149.08, 141.91, 140.91, 137.36, 136.10, 135.49, 134.78, 133.64, 132.87, 130.79, 130.39, 130.31, 129.29, 128.98, 128.88, 127.78, 127.37, 116.99, 40.44, 37.46, 21.10, 15.10, 10.12.	568.0891 568.0878
7f	(400 MHz, $CDCl_3$) δ 7.32–7.23 (m, 3H, Ar-H), 7.23–7.06 (m, 9H, Ar-H), 7.02 (dd, *J* = 8.5, 1.2 Hz, 1H, Ar-H), 6.99–6.96 (m, 4H, Ar-H), 5.45 (s, 2H, NCH_2), 4.38 (s, 2H, SCH_2), 2.30 (s, 3H, CH_3).	(101 MHz, $CDCl_3$) δ 151.82, 149.45, 142.11, 140.85, 136.59, 136.06, 135.71, 135.67, 134.91, 132.89, 130.83, 130.48, 130.34, 129.21, 128.94, 128.69, 128.52, 127.83, 127.78, 127.45, 127.33, 117.30, 48.29, 38.05, 10.14.	616.0891 616.0876
7g	(400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.21–7.00 (m, 14H, Ar-H), 7.00–6.94 (m, 2H, Ar-H), 5.54 (s, 2H, NCH_2), 3.38 (t, *J* = 7.6 Hz, 2H, $SC\underline{H_2}CH_2$), 2.98 (t, *J* = 7.6 Hz, 2H, $SCH_2C\underline{H_2}$), 2.32 (s, 3H, CH_3).	(101 MHz, $CDCl_3$) δ 152.04, 149.41, 142.08, 140.89, 139.63, 136.04, 135.76, 135.63, 134.87, 132.87, 130.82, 130.46, 130.30, 128.91, 128.75, 128.50, 128.46, 127.80, 127.77, 127.44, 127.31, 126.53, 117.23, 48.30, 35.85, 34.42, 10.16.	630.1047 630.1034
7h	(400 MHz, $CDCl_3$) δ 7.29 (d, *J* = 2.4 Hz, 1H, Ar-H), 7.23–7.00 (m, 14H, Ar-H), 7.00–6.94 (m, 2H, Ar-H), 5.57 (s, 2H, NCH_2), 3.14 (t, *J* = 7.6 Hz, 2H, $SC\underline{H_2}CH_2CH_2$), 2.63 (t, *J* = 7.6 Hz, 2H, $SCH_2CH_2C\underline{H_2}$), 2.31 (s, 3H, CH_3), 2.00 (p, *J* = 7.6 Hz, 2H, $SCH_2C\underline{H_2}CH_2$).	(101 MHz, $CDCl_3$) δ 152.16, 149.39, 142.08, 141.00, 140.90, 136.05, 135.79, 135.63, 134.87, 132.88, 130.82, 130.46, 130.31, 128.91, 128.51, 128.41, 127.80, 127.78, 127.42, 127.32, 126.01, 117.25, 48.32, 34.61, 32.65, 31.01, 10.13.	644.1204 644.1190

Table II: Continued.

Compd.	^{1}H NMR (δ ppm)	^{13}C NMR (δ ppm)	HRMES (calculated for $[M+H]^+$ /found)
7i	(400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.23–7.15 (m, 5H, Ar-H), 7.14–7.10 (m, 3H, Ar-H), 7.03 (d, *J* = 8.5 Hz, 1H, Ar-H), 6.70–6.95 (m, 3H, Ar-H), 6.79 (dd, *J* = 8.6, 2.0 Hz, 1H, Ar-H), 6.72 (dd, *J* = 8.3, 1.8 Hz, 2H, Ar-H), 5.46 (s, 2H, NCH_2), 4.34 (s, 2H, SCH_2), 3.67 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3).	(101 MHz, $CDCl_3$) δ 159.16, 151.90, 149.30, 142.08, 140.75, 136.00, 135.65, 134.88, 132.84, 130.79, 130.42, 130.39, 130.29, 128.90, 128.59, 128.47, 127.78, 127.74, 127.41, 127.27, 117.27, 114.04, 113.87, 55.26, 48.26, 37.72, 10.05.	646.0996 646.0982
7j	(400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.22–7.08 (m, 8H, Ar-H), 7.06–6.94 (m, 7H, Ar-H), 5.46 (s, 2H, NCH_2), 4.36 (s, 2H, SCH_2), 2.30 (s, 3H, CH_3), 2.21 (s, 3H, CH_3).	(101 MHz, $CDCl_3$) δ 151.97, 149.37, 142.12, 140.84, 137.53, 136.06, 135.70, 135.69, 134.92, 133.44, 132.89, 130.84, 130.48, 130.34, 129.38, 129.14, 128.95, 128.51, 127.83, 127.79, 127.48, 127.33, 117.32, 48.32, 37.87, 21.21, 10.13.	630.1047 630.1050
7k	(400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.31 (dd, *J* = 8.0, 1.5 Hz, 2H, Ar-H), 7.27–7.10 (m, 7H, Ar-H), 7.09–6.98 (m, 5H, Ar-H), 6.92–6.84 (m, 2H, Ar-H), 4.43 (s, 2H, SCH_2), 4.34 (t, *J* = 7.6 Hz, 2H, $NC\underline{H_2}CH_2$), 2.81 (t, *J* = 7.6 Hz, 2H, $NCH_2C\underline{H_2}$), 2.29 (s, 3H, CH_3).	(101 MHz, $CDCl_3$) δ 151.01, 149.27, 142.16, 140.86, 137.42, 136.82, 136.22, 135.79, 134.93, 133.03, 130.81, 130.58, 130.36, 129.19, 128.99, 128.91, 128.72, 128.43, 127.90, 127.81, 127.37, 126.70, 117.17, 46.63, 38.00, 35.96, 10.15.	630.1047 630.1033
7l	(400 MHz, $CDCl_3$) δ 7.39 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.28–7.09 (m, 9H, Ar-H), 7.09–6.98 (m, 5H, Ar-H), 6.95–6.92 (m, 2H, Ar-H), 4.43 (t, *J* = 7.6 Hz, 2H, $NC\underline{H_2}CH_2$), 3.45 (t, *J* = 7.5 Hz, 2H, $SC\underline{H_2}CH_2$), 3.05 (t, *J* = 7.6 Hz, 2H, $NCH_2C\underline{H_2}$), 2.93 (t, *J* = 7.5 Hz, 2H, $SCH_2C\underline{H_2}$), 2.30 (s, 3H, CH_3).	(101 MHz, $CDCl_3$) δ 151.35, 149.25, 142.15, 140.93, 139.68, 137.46, 136.25, 135.78, 134.92, 133.05, 130.82, 130.59, 130.36, 128.98, 128.95, 128.81, 128.53, 128.46, 127.90, 127.39, 126.72, 126.59, 117.12, 46.68, 36.01, 35.99, 34.29, 10.17.	644.1204 644.1194
7m	(400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 2.2 Hz, 1H, Ar-H), 7.26–6.99 (m, 14H, Ar-H), 6.96–6.93 (m, 2H, Ar-H), 4.45 (t, *J* = 7.5 Hz, 2H, $NC\underline{H_2}CH_2$), 3.20 (t, *J* = 7.6 Hz, 2H, $SC\underline{H_2}CH_2CH_2$), 2.95 (t, *J* = 7.5 Hz, 2H, $NCH_2C\underline{H_2}$), 2.69 (t, *J* = 7.6 Hz, 2H, $SCH_2CH_2C\underline{H_2}$), 2.28 (s, 3H, CH_3), 2.05 (p, *J* = 7.6 Hz, 2H, $SCH_2C\underline{H_2}CH_2$).	(101 MHz, $CDCl_3$) δ 151.41, 149.20, 142.12, 141.02, 140.93, 137.45, 136.23, 135.73, 134.88, 133.02, 130.79, 130.57, 130.33, 128.95, 128.93, 128.52, 128.45, 127.87, 127.37, 126.71, 126.03, 117.10, 46.66, 36.01, 34.68, 32.48, 31.17, 10.13.	658.1360 658.1348
7n	(400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 1.8 Hz, 1H, Ar-H), 7.29–7.18 (m, 5H, Ar-H), 7.14 (d, *J* = 8.5 Hz, 1H, Ar-H), 7.10–6.99 (m, 5H, Ar-H), 6.91–6.88 (m, 2H, Ar-H), 6.74 (dd, *J* = 8.5, 1.5 Hz, 2H, Ar-H), 4.41 (s, 2H, SCH_2), 4.36 (t, *J* = 7.6 Hz, 2H, $NC\underline{H_2}CH_2$), 3.67 (s, 3H, OCH_3), 2.84 (t, *J* = 7.6 Hz, 2H, $NCH_2C\underline{H_2}$), 2.29 (s, 3H, CH_3).	(101 MHz, $CDCl_3$) δ 159.29, 151.22, 149.21, 142.23, 140.83, 137.44, 136.26, 135.86, 135.00, 133.08, 130.85, 130.62, 130.48, 130.42, 129.05, 128.96, 128.71, 128.49, 127.94, 127.40, 126.76, 117.25, 114.17, 55.36, 46.70, 37.67, 36.01, 10.17.	660.1153 660.1139
7o	(400 MHz, $CDCl_3$) δ 7.40 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.29–7.18 (m, 5H, Ar-H), 7.14 (dd, *J* = 8.5, 1.5 Hz, 1H, Ar-H), 7.11–6.98 (m, 7H, Ar-H), 6.91–6.88 (m, 2H, Ar-H), 4.43 (s, 2H, SCH_2), 4.37 (t, *J* = 7.6 Hz, 2H, $NC\underline{H_2}CH_2$), 2.84 (t, *J* = 7.6 Hz, 2H, $NCH_2C\underline{H_2}$), 2.29 (s, 3H, CH_3), 2.22 (s, 3H, CH_3).	(101 MHz, $CDCl_3$) δ 151.23, 149.22, 142.21, 140.86, 137.64, 137.45, 136.26, 135.84, 134.99, 133.64, 133.08, 130.85, 130.61, 130.41, 129.46, 129.17, 129.04, 128.96, 128.48, 127.94, 127.40, 126.75, 117.24, 46.71, 37.78, 35.99, 21.24, 10.17.	644.1204 644.1193

Anticancer screening in National Cancer Institute (NCI)

The methodology of the NCI anticancer screening has been described in detail elsewhere (http://www.dtp.nci.nih.gov)[46-48]. Briefly, the primary anticancer assay was performed at approximately 60 human tumor cell lines panel derived from nine neoplastic diseases in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, reported elsewhere. Tested compounds were added to the culture at a single concentration (10^{-5} M) and the cultures were incubated for 48 h. End point determinations were made with a protein binding dye, SRB. Results for each tested compound were reported as the percent of growth of the treated cells when compared to the untreated control cells. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. The cytotoxic and/or growth inhibitory effects of the most active selected compound were tested *in-vitro* against the full panel of about 60 human tumor cell lines at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. A 48-h continuous drug exposure protocol was followed and an SRB protein assay was used to estimate cell viability or growth. Using the seven absorbance measurements [time zero (Tz), control growth in the absence of drug (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentrations levels. Percentage growth inhibition was calculated as: [(Ti − Tz)/(C − Tz)] x 100 for concentrations for which Ti ≥ Tz, and [(Ti − Tz)/Tz] x 100 for concentrations for which Ti < Tz. Three-dose response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each compound. Growth inhibition of 50% (GI_{50}) was calculated from [(Ti − Tz)/(C−Tz)] x 100 = 50, which is the drug concentration resulting in a 50% lower net protein increase in the treated cells (measured by SRB staining) as compared to the net protein increase seen in the control cells. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti = Tz. The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment was calculated from [(Ti −Tz)/Tz] x 100 = −50. Values were calculated for each of these three parameters if the level of activity is reached; however, if the effect was not reached or was exceeded, the value for that parameter was expressed as more or less than the maximum or minimum concentration tested. The logGI_{50}, logTGI, and logLC_{50} were then determined. LogGI_{50}, log TGI, and log LC_{50} are the logarithm molar concentrations producing 50% growth inhibition (GI_{50}), a total growth inhibition (TGI), and a 50% cellular death (LC_{50}), respectively. The lowest values are obtained with the most sensitive cell lines.

Anticancer screening in National Research Centre, Egypt

Chemicals: Fetal bovine serum (FBS) and L-glutamine, were obtained from Gibco Invitrogen Company (Scotland, UK). Dulbecco's modified Eagle's (DMEM) medium was provided from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and Sulfo-Rhodamine-B stain (SRB) (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) were obtained from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used in this study were of analytical grade and purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Cell lines and culturing: Anticancer activity screening for the tested compounds breast MCF-7, lung A549 cancer cell lines as well as the normal cell line (human normal melanocyte, HFB4) were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal calf serum (GIBCO), penicillin (100 U/mL) and streptomycin (100 µg/mL) at 37 °C in humidified atmosphere containing 5% CO_2. Cells at a concentration of 0.50 x 10^6 were grown in a 25 cm^2 flask in 5 mL of complete culture medium.

***In-vitro* cytotoxicity assay:** The cytotoxicity activity was measured *in-vitro* using the Sulforhodamine-B stain (SRB) assay according to the previous reported standard procedure[49].

Cells were inoculated in 96-well microtiter plate (10^4 cells/ well) for 24 h before treatment with the tested compounds to allow attachment of cell to the wall of the plate. The tested compounds were dissolved in DMSO at 1 mg/mL immediately before use and diluted to the appropriate volume just before addition to the cell culture. Different concentration of tested compounds and doxorubicin were added to the cells. Triplicate wells were prepared for each individual dose. Cells were incubated with the compounds for 48 h at 37°C and in atmosphere of 5% CO_2. After 48 h cells were fixed, washed, and stained for 30 min with 0.4% (w/v) SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris-EDTA buffer. Colour intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in table III. The results were compared to the antiproliferative effects of the reference control doxorubicin.

RESULTS AND DISCUSSION

1- Chemistry

The synthetic route of the designated compounds was shown in scheme 1. 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylate **3** was synthesised through treatment of 1-(4-chlorophenyl)propanone **1** with diethyl oxalate in the presence of LHMDS as a base to afford lithium salt **2** in 80% yield, which in turn was coupled with 2,4-dichlorophenylhydrazine hydrochloride in ethanol followed by intramolecular cyclization in acetic acid under refluxing conditions to provide the pyrazole-3-carboxylic acid ethyl ester **3** in 55% yield over two steps[50]. The ^{1}H-NMR spectrum of **3** showed a singlet equivalent to three protons at δ 2.31 ppm which assigned to methyl groupand a quartet at 4.42 ppm and a triplet at 1.39 ppm related to ethoxy moiety as well as aromatic protons appeared at expected chemical shift. The key hydrazide intermediate **4** was prepared in a high yield by reflux pyrazole-3-carboxylic acid ester **3** with hydrazine in ethanol. The structure of compound **4** was confirmed by NMR and ESI-HRMS. Heating at reflux a mixture of the hydrazide **4** and an appropriate isothiocyanate in ethanol afforded substituted thiosemicarbazides **5a-c** which was used for next step without further purification. A solution of **5a-c** in 2 N NaOH was stirred under reflux for 3 h to yield 1,2,4-triazole-3-thiol derivatives **6a-c**. All of the synthetic compounds of this series gave satisfactory spectroscopic and HRESI-MS data, which were in full accordance with their depicted structures. The ^{1}H NMR spectrum of **6a** as a representative example of this series revealed in addition to aromatic proton the appearance of ethyl group signals at δ 4.36, 1.21ppm. The structure of **6a** has been also confirmed by HRESI-MS data. The synthesis of the final compounds **7a-o** was accomplished by refluxing 1,2,4-triazole-3-thiols **6a-c** with benzyl bromide, phenethyl bromide, 3-phenylpropyl bromide, 4-methoxybenzyl chloride, or 4-methylbenzyl bromide in the presence of K_2CO_3 in acetone. All the final structures **7a-o** were verified using ^{1}H NMR, ^{13}C NMR and HRESI-MS. Analysis of the ^{1}H NMR spectrum of **7a** as an example of this series showed the appearance of a signal at 4.43 ppm assigned to SCH_2 group, ethyl protons at 4.17 (q) and 1.12 (t) ppm and a singlet signal integrating for three protons at 2.33 ppm which was attributed to methyl group as well as aromatic protons at the expected chemical shift. Additional confirmation of the structure of **7a** was provided by HRESI-MS data, which showed a peak at m/z 554.0721 for $[M+H]^+$, which consistent with the molecular formula $C_{27}H_{23}Cl_3N_5S$.

2- Screening of anticancer activity by NCI

***In-vitro* one-dose full NCI 60 cell panel assay**

Compounds **7a**, **7b**, **7d-g** were selected by the National Cancer Institute (NCI) according to the protocol of the Drug Evaluation Branch of the National Cancer Institute, Bethesda, USA for *in-vitro* anticancer screening[46]. Primary *in-vitro* one-dose anticancer assay was performed in full NCI 60 cell lines derived from nine tumor subpanels, including leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate, and breast cancer cell lines. The selected compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48 h. End point determination was

made with a protein binding dye sulforhodamine B (SRB). Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells.

Compound **7e** achieved remarkable cell growth inhibition activity against most of the tested cell lines (Table III). A complete cell death was recorded for leukemia HL-60(TB), RPMI-8226, non-small cell lung cancer HOP-92, NCI-H522, colon cancer COLO 205, melanoma, SK-MEL-5, UACC-257, ovarian cancer SK-OV-3, breast cancer BT-549, T-47D cell lines. Compound **7e** indicated a remarkable cell growth inhibition activity against most of the tested cell lines including leukemia K-562, MOLT-4, SR, non-small cell lung cancer A549/ATCC, EKVX, HOP-62, NCI-H226, NCI-H23, NCI-H460, colon cancer HCC-2998, HCT-116, HCT-15, HT29, KM12, SW-620, CNS cancer SF-268, SF-295, SF-539, SNB-19, SNB-75, U251, melanoma LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, UACC-62, ovarian cancer OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, renal cancer 786-0, A498, ACHN, RXF 393, SN12C, TK-10, UO-31, prostate cancer PC-3, DU-145, breast cancer MCF7, MDA-MB-231/ATCC, MDA-MB-468 cell lines. Compound **7e** revealed moderate cell growth inhibition against melanoma SK-MEL-28, ovarian cancer IGROV1, renal cancer CAKI-1, breast cancer HS 578T cell lines. The results also indicated that **7a** revealed a remarkable cell growth inhibition activity only against prostate cancer PC-3 cell line and exhibited moderate cell growth inhibition against non-small cell lung cancer EKVX, colon cancer HCT-116, HCT-15, CNS cancer SF-295, renal cancer ACHN, UO-31, breast cancer MCF7, T-47D, MDA-MB-468 cell lines. The obtained results indicate that compound **7e** exhibited the highest ability to inhibit the proliferation of different cancer cell lines (Table III) compared to compounds **7a**, **7b**, **7d, 7f-g**.

From the obtained results; several conclusions could be deduced, a 1,5-diarylpyrazole functionality attached to the 1,2,4-triazole moiety might contribute to the anticancer activity of the synthesized compounds. It could be noted that compound **7e** with weak electron donating groups (CH_3) in 4-position of benzyl moiety at 5-position of 1,2,4-triazole is more active than compound **7d** with strong electron donating groups (OCH_3) Moreover, the presence of ethyl at 4-position of 1,2,4-triazole is preferable over the presence of benzyl group (compound **7e** has superior anticancer activity against different cancer cell lines over all tested compounds by NCI).

Table III: One-dose growth (%) of nine different cancer cell types for compounds **7a, 7b**, **7d-g**.

Panel/cell line	Growth (%) in one-dose assay					
	7a	**7b**	**7d**	**7e**	**7f**	**7g**
Leukemia						
CCRF-CEM	81.95	NT	85.38	NT	NT	NT
HL-60(TB)	61.45	67.83	76.73	-13.10	62.12	82.38
K-562	53.97	48.11	77.68	8.88	49.93	59.13
MOLT-4	51.41	41.59	79.22	2.46	39.89	64.63
RPMI-8226	56.85	54.07	60.90	-3.78	51.43	74.78
SR	57.72	37.63	77.13	6.14	39.72	46.40
Non-Small Cell Lung Cancer						
A549/ATCC	56.12	65.06	87.08	9.87	56.19	69.23
EKVX	42.68	52.56	58.03	10.41	52.60	69.65
HOP-62	85.07	101.43	91.93	9.33	80.67	87.53
HOP-92	57.34	84.96	85.40	-17.19	67.18	96.46
NCI-H226	59.60	57.99	68.27	11.63	64.49	62.80
NCI-H23	51.90	62.25	64.87	3.53	59.70	72.66
NCI-H322M	76.44	NT	94.02	NT	NT	NT
NCI-H460	64.44	73.73	75.16	8.69	68.08	78.60
NCI-H522	62.21	61.12	64.07	-17.45	53.74	61.33
Colon Cancer						
COLO 205	77.57	77.72	84.48	-34.35	77.74	83.34
HCC-2998	86.09	110.06	94.82	23.36	79.38	97.90
HCT-116	40.34	39.08	47.27	1.74	47.55	37.04

Table III: Continued.

Panel/cell line	Growth (%) in one-dose assay					
	7a	**7b**	**7d**	**7e**	**7f**	**7g**
HCT-15	45.07	45.62	74.04	11.58	59.79	56.57
HT29	73.56	67.15	67.37	2.65	49.53	75.18
KM12	56.67	69.54	66.39	15.84	63.02	77.81
SW-620	71.46	83.34	77.33	20.37	72.94	83.80
CNS Cancer						
SF-268	59.11	77.35	83.61	22.84	75.89	76.91
SF-295	45.48	65.75	83.04	9.65	61.23	82.90
SF-539	90.97	85.86	98.20	19.78	82.34	83.76
SNB-19	75.41	76.75	87.25	22.07	78.83	67.98
SNB-75	71.33	90.68	85.07	23.30	67.79	89.17
U251	65.72	54.74	87.72	14.44	66.42	52.20
Melanoma						
LOX IMVI	56.79	63.77	75.35	7.19	60.05	60.72
MALME-3M	76.86	95.99	84.80	7.80	87.94	91.77
M14	62.22	72.78	80.62	12.27	71.58	82.08
MDA-MB-435	71.41	83.18	91.13	19.69	80.64	90.01
SK-MEL-2	66.11	53.80	76.22	4.93	65.79	68.05
SK-MEL-28	85.86	79.20	96.85	30.32	86.82	87.58
SK-MEL-5	57.73	74.26	66.51	-92.32	73.08	72.65
UACC-257	85.19	77.84	98.23	-1.54	72.19	75.98
UACC-62	60.77	68.63	78.80	21.19	57.52	68.37
Ovarian Cancer						
IGROV1	84.42	94.08	97.46	30.85	92.03	87.56
OVCAR-3	56.55	69.06	65.91	15.82	68.30	70.94
OVCAR-4	53.20	75.61	66.43	16.89	56.54	68.70
OVCAR-5	81.13	81.72	96.02	26.76	88.64	87.41
OVCAR-8	76.89	84.55	95.86	12.85	83.56	91.25
NCI/ADR-RES	64.57	72.64	87.65	**52.12**	77.40	91.21
SK-OV-3	86.05	79.07	86.39	-7.13	75.46	NT
Renal Cancer						
786-0	73.38	72.89	84.09	12.17	74.07	79.51
A498	85.98	96.56	83.13	18.85	95.57	95.24
ACHN	46.12	60.98	80.48	2.32	65.48	76.66
CAKI-1	57.13	64.39	69.94	40.52	56.85	74.01
RXF 393	80.08	66.04	90.81	8.71	84.66	83.26
SN12C	50.22	70.33	90.92	1.66	67.90	86.09
TK-10	84.75	75.30	81.96	18.10	75.58	74.53
UO-31	43.91	53.53	79.11	9.59	60.23	72.72
Prostate Cancer						
PC-3	25.66	39.80	48.82	5.63	40.69	49.39
DU-145	79.10	84.67	90.63	23.38	85.00	92.86
Breast Cancer						
MCF7	43.89	58.70	62.67	4.46	57.33	71.96
MDA-MB-231/ATCC	71.62	81.06	73.01	5.05	71.21	78.19
HS 578T	76.73	97.95	91.56	38.41	91.19	92.62
BT-549	83.19	93.35	91.65	-7.34	81.77	100.14
T-47D	35.76	42.20	61.89	-8.15	41.25	51.29
MDA-MB-468	45.14	45.88	62.48	6.59	58.89	68.15
Mean	64.77	70.37	79.50	8.80	67.82	75.98
Range	65.31	72.43	50.96	144.44	55.85	63.10

NT: not tested.

***In-vitro* five-dose full NCI 60 cell panel assay**

Compound **7e** (NSC: D-785448/1) was satisfied the threshold inhibition criteria and selected for advanced five-dose testing against the full panel of 60 human tumor cell lines. All the 60 cell lines representing nine tumor subpanels were incubated at five different concentrations (0.01, 0.1, 1, 10 and 100 μM). The outcomes were used to create log concentration versus % growth inhibition

curves and three response parameters (GI_{50}, TGI, and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50% decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition (TGI) and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50% loss of initial cells at the end of the incubation period of 48 h. The results in table IV indicated that compound **7e** exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} ranging from 0.43 to 3.55 µM.

The criterion for selectivity of a compound depends upon the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines toward the test agent) (µM) by their individual subpanel MID (µM). Ratios between 3 and 6 refer to moderate selectivity; ratios > 6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria rated non selective. In this context, compound **7e** was found to have broad spectrum antitumor activity against the nine tumour subpanels tested with no selectivity toward the tested cell lines (selectivity ratios ranging between 0.80 and 1.39 at the GI_{50} level).

Table IV: NCI *in-vitro* testing results of compound **7e** at five-dose level in mM.

Panel/cell line	GI_{50}			TGI	LC_{50}
	Conc. per cell line	Subpanel MID[b]	Selectivity ratio		
Leukemia					
CCRF-CEM	2.51	1.51	1.38	13.5	> 100
HL-60(TB)	1.60			5.47	35.60
K-562	0.84			10.40	44.20
MOLT-4	2.15			11.00	46.70
RPMI-8226	0.47			3.05	90.20
Non-Small Cell Lung Cancer					
A549/ATCC	2.82	2.11	0.99	11.30	34.70
EKVX	2.88			13.00	38.40
HOP-62	2.73			11.90	38.10
HOP-92	0.53			4.22	30.90
NCI-H226	2.26			13.90	51.30
NCI-H23	2.24			12.5	42.80
NCI-H322M	2.71			13.00	36.90
NCI-H460	1.46			10.50	38.10
NCI-H522	1.35			5.20	23.60
Colon Cancer					
COLO 205	1.51	1.69	1.23	3.39	7.61
HCC-2998	2.35			13.20	39.00
HCT-116	0.57			10.60	36.30
HCT-15	2.70			11.40	35.70
HT29	1.11			10.60	34.70
KM12	1.04			6.31	31.90
SW-620	2.56			12.60	39.30
CNS Cancer					
SF-268	3.06	2.32	0.90	14.90	46.40
SF-295	2.40			11.70	35.80
SF-539	3.02			11.80	35.50
SNB-19	2.30			13.30	38.70
SNB-75	2.21			11.70	35.60
U251	0.93			11.80	35.40
Melanoma					
LOX IMVI	2.72			10.70	34.30
MALME-3M	2.15			8.88	32.20
M14	1.99			10.80	34.80

Table IV: Continued.

Panel/cell line	GI_{50}			TGI	LC_{50}
	Conc. per cell line	Subpanel MID[b]	Selectivity ratio		
MDA-MB-435	2.85	2.34	0.89	11.50	34.80
SK-MEL-2	3.01			12.50	37.90
SK-MEL-28	3.11			15.30	41.30
SK-MEL-5	1.40			2.74	5.38
UACC-257	1.86			4.73	15.40
UACC-62	2.01			10.60	34.90
Ovarian Cancer					
IGROV1	2.62	2.60	0.80	15.40	42.80
OVCAR-3	1.98			11.60	35.70
OVCAR-4	1.79			12.40	38.80
OVCAR-5	3.55			15.20	40.80
OVCAR-8	2.12			10.70	36.60
NCI/ADR-RES	3.54			14.60	44.30
SK-OV-3	2.58			8.18	28.80
Renal Cancer					
786-0	2.69	2.49	0.84	12.20	36.40
A498	1.37			7.88	30.60
ACHN	2.74			10.40	35.50
CAKI-1	2.83			12.60	36.50
RXF 393	2.69			10.90	36.10
SN12C	2.31			10.80	37.20
TK-10	2.61			10.90	35.30
UO-31	2.66			10.80	34.00
Prostate Cancer					
PC-3	0.49	1.90	1.09	10.40	38.50
DU-145	3.30			13.80	40.40
Breast Cancer					
MCF7	2.26	1.79	1.16	11.40	47.10
MDA-MB-231/ATCC	2.35			8.54	32,10
HS 578T	3.30			18.40	92.10
BT-549	1.79			5.75	23.60
T-47D	0.43			10.10	42.10
MDA-MB-468	0.62			10.60	39.20
MID[a]		2.08			

3- Screening of anticancer activity by National Research Centre, Egypt

The cytotoxicity of the synthetic final compounds except **7e** was tested using SRB assay as described by Skehan *et al*[49] against breast MCF-7, lung A549 cell lines as well as human normal melanocyte (HFB4) cells using doxorubicin as a reference drug and DMSO as a control (Table V). Moreover, the tumor cells showed normal growth in culture system and DMSO did not seem to have any noticeable effect on cellular growth. The results revealed that compounds **7l** and **7o** exhibited higher potency against MCF-7 and A549 cells with IC_{50}: 2.85±0.35 and 2.84±0.37 µg/mL against MCF-7 and IC_{50}: 4.11±0.50 and 3.90±0.48 µg/mL against A549 cells, respectively, which is lower than of doxorubicin as shown in table III. Moreover, the results showed that compounds **7m** and **7n** were found to be equipotent to doxorubicin against MCF-7 cells with IC_{50}: 3.70±4.00 and 3.30±0.36 µg/ml and against A549 cells with IC_{50}: 8.86±0.90 and 6.86±0.87 µg/mL, respectively. The rest of compounds revealed moderate to low anticancer effect as shown in table V. Additionally, while compounds **7c-d, 7l-o** exhibited no activity against the growth of normal HFB4 cells, the rest compounds revealed high toxicity on the normal cells.

From the above mentioned results, it is clear that in general, biological activity against cancer cells depends on the presence of 1,5-diarylpyrazole nucleus and the chemical nature of the substituents R, R1 at triazole moiety. The compounds **7l-o** with phenethyl group at 4-position of 1,2,4-triazole were highly active against two cancer cell lines (MCF-7 and

A549). These results also confirmed that the presence of either benzyl group with electron donating groups (CH_3 and OCH_3) at its *p*-position or phenethyl group is preferable over unsubstituted benzyl group for anticancer activity.

Table V: *In-vitro* cytotoxicity activity of the tested compounds **7a-d**, **7f-o** on different cell lines.

Compd.	IC_{50} (µg/mL)		
	MCF-7	**A549**	**HFB4**
7a	N.A.	N.A.	8.03±0.80
7b	N.A.	N.A.	9.76±0.74
7c	N.A.	N.A.	N.A.
7d	N.A.	N.A.	N.A.
7f	23.90±3.31	62.30±7.11	4.63±0.50
7g	13.80±2.50	21.31±2.60	40.73±5.20
7h	26.20±3.40	41.11±4.26	6.71±0.75
7i	18.56±2.20	16.96±2.46	8.11±0.73
7j	36.80±3.96	23.29±2.94	7.62±0.85
7k	19.27±2.11	19.80±2.48	27.53±2.92
7l	2.84±0.37	3.90±0.48	80.13±9.00
7m	3.70±4.00	8.86±0.90	66.89±7.00
7n	3.30±0.36	6.86±0.87	65.94±8.77
7o	2.85±0.35	4.11±0.50	76.10±8.66
Doxorubicin	2.86±0.31	4.16±0.40	88.70±9.11

Data were expressed as Mean ± Standard error (S.E.) of three independent experiments.
N.A. is no activity.

Conclusions

The present study reports the design and synthesis of novel series of 3-(1*H*-pyrazol-3-yl)-4*H*-1,2,4-triazole derivatives **7a-o** as potential anticancer agents. The prepared compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and HRESI-MS. The target compounds were evaluated for their anticancer activity. One-dose *in-vitro* anticancer test results indicated that compounds **7e** exhibited the highest ability to inhibit the proliferation of different cancer cell lines. *In-vitro* five-dose full NCI 60 cell panel assay revealed that **7e** exhibited a broad-spectrum antitumor activity against the nine tumour subpanels without pronounced selectivity. The studies confirmed that compound **7e** is a potent lead compound for drug discovery and requires further optimization. In addition, compounds **7l** and 7**o** were found to be highly potent and similar to doxorubicin against MCF-7 cells and A549 cells.

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نشرة العلوم الصيدلية
جامعة أسيوط

تشييد مشتقات جديدة من البيرازول حاملات ١،٢،٤-تريازول كمضادات محتملة للسرطان

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تم في هذا البحث تشييد مشتقات جديدة من ٣-(١يد-بيرازول-٣-ويل)-٤يد-١،٢،٤-تريازول وقد تم التأكد من الصيغة البنائية للمركبات المشيدة بواسطة الرنين النووي المغناطيسي لعنصري الهيدروجين والكربون بالإضافة لمطياف الكتلة عالي الدقة وتم اختبار الفاعلية البيولوجية لست من المركبات المشيدة كمضادات للسرطان ضد العديد من الخلايا السرطانية في المعهد الوطني للسرطان (NCI)، الولايات المتحدة الأمريكية. وقد أثبت المركب **7e** فاعلية بيولوجية عالية ضد العديد من الخلايا السرطانية المختبرة بدون انتقائية واضحة ضد الخلايا السرطانية المختبرة. وقد تم أيضاً اختبار العديد من المركبات المشيدة ضد الخلايا السرطانية للإنسان وتشمل خلايا الثدي السرطانية MCF-7 وخلايا الرئة السرطانية A549 وكذا خلايا الجلد البشرية العادية HFB4 وتم مقارنة النتائج بعقار الدوكسوروبيسين وقد أظهرت النتائج أن المركبات **7l** & **7o** لها فاعلية بيولوجية عالية كمضادات للسرطان تضاهي أو تزيد عن عقار الدوكسوروبيسين ضد خلايا الثدي السرطانية MCF-7 وخلايا الرئة السرطانية A549.