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Synthesis and Cytotoxicity of Novel Pyrazole Derivatives Derived from 3-Methyl-1-phenyl-1H-pyrazol-5(4H)-one

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THE 3-Methyl-1-phenyl-1H-pyrazol-5(4H)-one (1) was used to synthesis pyran,and pyridine derivatives through its multi-component reactions with aromatic aldehydes and cyanomethylene reagents. The synthesized products were evaluated for their cytotoxicity against cancer and normal cell lines. 4a, 4c, 6c, 7c, 11b, 11c and 13 showed optimal cytotoxicity among the tested compounds. The toxicity of the most potent cytotoxic compounds was measured using Brine-Shrimp Lethality Assay.

Keywords: Pyrazole, Pyran, Pyridine, Cytotoxicity and Toxicity.

Multicomponent reactions (MCRs) have emerged as a valuable tool in the preparation of structurally diverse chemical libraries of heterocyclic compounds[(1)]. They are inherently atom economical processes in which relatively complex products can be obtained in a one-pot reaction from simple starting materials, and thus they exemplify many of the desired features of an ideal synthesis. MCRs are generally much more environmentally friendly and offer access to large compound libraries with diverse functionalities with the avoidance of protection and deprotection steps for possible combinatorial surveying of structural variations. In view of the increasing interest in the preparation of a large variety of heterocyclic compound libraries, the development of new synthetically valuable MCRs with several diversity points remains a challenge for both academic and industrial institutions[(2)]. Thiophene and its derivatives are an important class of heterocyclic compounds possessing broad biological activities, such as anti-inflammatory[(3)], analgesic[(3)], antioxidant[(4)], antitubercular [(5)], antidepressant [(6)], sedative [(6)], antiamoebic [(7)], oral analgesic[(8)], anti-metabolite[(9)] and antineoplastic properties[(10)]. Many pyrazole derivatives have attracted considerable attention in the recent years for their diverse biological activities[(11-16)]. They are also acknowledged for their anticancer activities [(17-19)]. Celecoxib (1), Sulfaphenazole (2), CDPPB (3), Linazolac (4), Mepiprazole (5), and Rimonabant (6) are some of the pyrazole based drugs available today in the market (Fig. 1) [(20)] .

From the aforementioned reports, it seemed that the development of an efficient, rapid and clean synthetic route towards focused libraries of such

compounds is of great importance to both medicinal and synthetic chemists. Hence in this work, we report a one-pot, three-component reaction for the synthesis of pyran derivatives through the reaction of 3-methyl-phenyl-pyrazol-5(4H)-one (1) with different aromatic aldehydes and cyanomethylene reagents . All the synthesized compounds were characterized using FT-IR, ^{1}H NMR and mass spectrometry and were subjected to screening towards cancer cell lines.

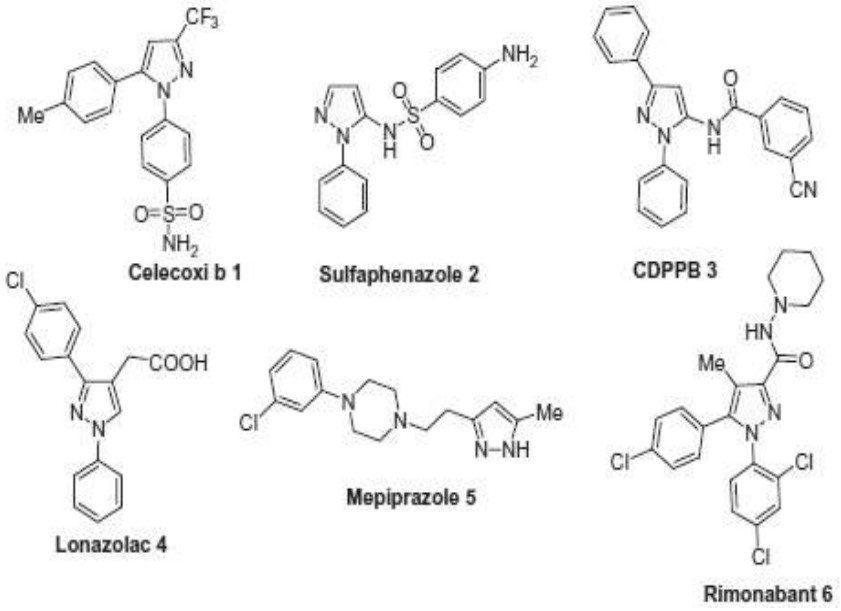


Fig.1. Biologically active pyrazole derivatives.

Results and Discussion

Chemistry

The present investigation emphasized mainly on the synthesis of molecules derived from of 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (1) and evaluation of their cytotoxicity against cancer and normal cell lines. The synthetic strategies adopted for the synthesis of the intermediate and target compounds are depicted in Schemes 1& 2. One pot multi-component reactions (MCR) were utilized to prepare the target compounds. The reaction of the 3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (1) with each of benzaldehyde (2a), 4-methoxybenzaldehyde (2b) or 4-chlorobenzaldehyde (2c) and ethyl cyanoacetate (3) afforded the 6-oxopyranopyrazole derivatives 4a-c. Structures of the latter products were confirmed on the basis of their respective analytical and spectral data. Thus,^{1}H NMR spectrum of compound 4a revealed the presence of a singlet δ at 2.88 ppm for CH_3 and a multiplet δ 7.48-8.09 ppm corresponding to two phenyl protons. Meanwhile, the reaction of compound 1 with either of 2a, 2b or 2c and malononitrile (5) in ethanol containing triethylamine gave the 6-Amino-3-

methyl-4-aryl-1,4-dihydropyrano [2,3-*c*] pyrazole-5- carbonitrile derivatives 6a-c, respectively. The analytical and spectral data of 6a-c were in consistence with their respective structures. Compounds 6a-c reacted with hydrazine hydrate in 1,4-dioxane solution containing sodium acetate to give the 5-amino-3-methyl-4-aryl-4,7-dihydro-1H-pyrano[2,3-c:6,5-c'] dipyrazol--3-amine derivatives 7a-c. The structures of the latter products were based on their respective analytical and spectral data. On the other hand, the reaction of compound 1 with pyridine-3-aldehyde (8) and malononitrile afforded the 6-amino-3-methyl-4-(pyridin-3-yl)-1,4-dihydropyrano [2,3-*c*]pyrazole-5-carbonitrile (9). The structure of the latter product was based on its respective analytical and spectral data. Thus, the ^{1}H NMR spectrum showed the presence of singlet at δ 1.83 ppm corresponding to CH_3 group, a singlet at 4.63 ppm corresponding for pyrane proton and a singlet δ 6.82 ppm for NH_2- and a multiplet δ 7.30-8.39 ppm for phenyl and pyridine protons.

Moreover, the reaction of 1 with the aromatic aldehydes 2a-c and 2-aminoprop-1-ene-1,1,3-tricarbonitrile (10) in ethanol containing a catalytic amount of triethylamine afforded the pyrazolopyrano[2,3-*b*]pyridine-6-carbonitrile derivatives 11a-c. ^{1}H NMR of compound 11a (as an example) showed the presence of a singlet δ 2.78 ppm for CH_3 group, a singlet δ 4.58 ppm for pyrane proton , two singlet δ 4.16-4.88 ppm corresponding to two NH_2 protons and a multiplet δ 7.28-7.73 ppm for phenyl protons .

Moreover, On the other hand, the reaction of compound 6b with phenylisothiocyanate (12) in ethanol afforded the corresponding thiourea derivative 13, the structure of which was based on analytical and spectral data.

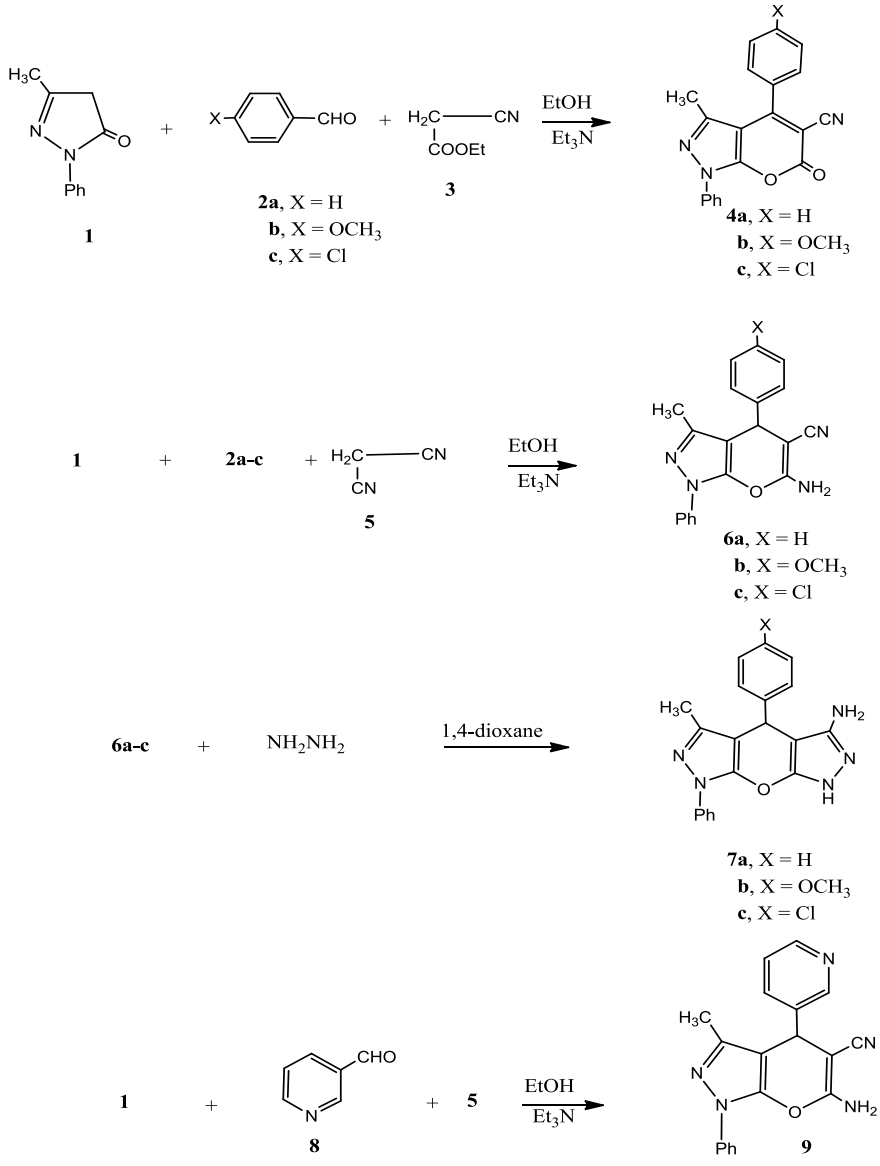
The one-pot reaction of compound 1 with salicylaldehyde and malononitrile gave the annulated 5-Amino-1-methyl-3*H*-chromeno[4',3':4,5]-pyrano[2,3-*c*]pyrazol-6(11*bH*)-one (14). The analytical and spectral data of the latter product was the basis of their structural elucidation. Thus, the ^{1}H NMR spectrum of 14 showed, beside the expected signals , the presence of a singlet at δ 2.76 ppm for CH_3, singlet at δ 4.48 pyran H-4, a singlet at δ 4.26 corresponding to NH_2 group and a multiplet at δ 7.27-7.42 for two phenyl protons .

In vitro cytotoxicity

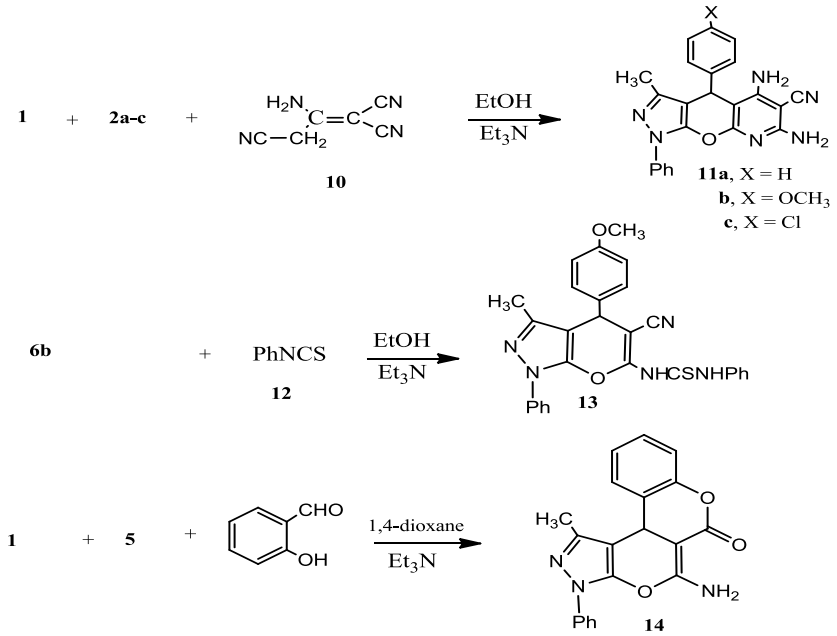
Effect on the growth of human cancer cell lines

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols for their *in vitro* cytotoxicity against six human cancer cell lines including cells derived from human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), nasopharyngeal carcinoma (HONE1), human breast cancer (MCF) and normal fibroblast cells (WI38). (For comparison reasons, CHS 828 was used as standard anticancer drug. All of IC_{50} values in (nM) are listed in Table 1. Many of the synthesized heterocyclic compounds were observed with significant cytotoxicity

against most of the cancer cell lines tested (IC_{50}<1000 nM). Normal fibroblasts cells (WI38) were affected to a much lesser extent (IC_{50}>10,000 nM). Among the tested compounds the 4-(4-Chlorophenyl)-5-methyl -7- phenyl- 4, 7- dihydro-1H-pyrano [2,3-c:6,5-c'] dipyrazol-3-amine (7c) was found to show the highest cytotoxic effect against the six cancer cell lines in the range of IC_{50} 63-1088nM. Broad spectrum antitumor activity was exhibited by compounds 4a,4c, 6c, 7c, 11b , 11c and 13.



Scheme 1



Scheme 2

TABLE 1. Cytotoxicity of the synthesized compounds against a variety of cancer cell lines [a] [IC_{50} [b] (nM)].

Compd	Cytotoxocity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
4a	343	440	120	415	527	231	Na
4b	1280	2237	2337	428	1168	580	Na
4c	60	220	na	227	2354	228	Na
6a	1084	890	3068	399	2280	3365	Na
6b	2420	2445	3017	2320	1820	3444	Na
6c	210	120	283	359	206	2655	Na
7a	2219	2118	1268	2092	1255	1893	2297
7b	1279	230	84	2489	2140	1177	Na
7c	63	28	166	1088	208	38	Na
9	1101	1180	58	2766	180	Na	Na
11a	3124	2670	1165	4321	2166	112	Na
11b	122	90	212	440	1877	436	Na
11c	40	60	152	320	2280	1663	453
13	36	326	122	421	682	1293	1231
14	3255	2674	1374	2693	2227	1438	Na
CHS 828	25	2315	2067	1245	15	18	Na

[a]NUGC, gastric cancer; DLDI, colon cancer; HA22Tand HEPG2, liver cancer; HONEI, nasopharyngeal carcinoma; MCF, breast cancer; WI38, normal fibroblast cells.
[b]The sample concentration that produces a 50% reduction in cell growth.

Structure activity relationship

In the present study, a series of heterocyclic derivatives incorporating a pyrazole moiety were synthesized and evaluated for their cytotoxicty aiming at investigating their SAR. Thus 6-oxopyranopyrazole derivatives 4a-c and their amino analogs 6a-c and 9 were prepared. Referring to the IC_{50} values listed in Table 1, compound 4a bearing a phenyl substituent exhibited significant broad spectrum cytotoxic activity in the range of (IC_{50} 120-527 nM). Meanwhile, 4b bearing a 4-$OCH_3C_6H_4$ group showed selective activity against liver cancer HEPG2 (IC_{50} 428 nM) and breast cancer MCF (IC_{50} 580 nM). The 4-ClC_6H_4 substituted derivative 4c demonstrated better activity compared to 4a and 4b especially against gastric cancer NUGC (IC_{50} 60 nM). Among the 6-amino-4-substituted pyranopyrazole derivatives 6a-c and 9, derivative 6a carrying a 4-C_6H_5 group was found to have selective activity against the human liver cancer cell line HEPG2 (IC_{50} 399 nM) and colon cancer cell line DLDI (IC_{50} 890 nM). However compound 6b bearing 4-$OCH_3C_6H_4$ group was completely devoid of cytotoxic activity. On the other hand, compound 6c bearing the 4-ClC_6H_4 moiety showed high activity against all cancer cell lines except breast cell line MCF in the range of (IC_{50} 120-359 nM). The presence of pyridine ring in compound 9 is most probably responsible for its high potency against human liver cancer cell line HA22T (IC_{50} 58 nM) and nasopharyngeal cancer cell line HONE1 (IC_{50} 180 nM). The previous results suggest that the replacement of the 6-amino group in compounds 6a-c by a 6-oxo group in compounds 4a-c in the latter pyranopyrazole derivatives led to compounds with enhanced cytotoxic effect which might be attributed the presence of the electronegative oxygen moiety. Meanwhile, replacement of the 2-amino group of compound 6b by a phenylthiourea moiety afforded compound 13 which demonstrated a dramatic increase in the cytotoxic activity with the highest activity exhibited against NUGC (IC_{50} 36 nM).

The investigation of the cytotoxicity of the pyrazolo[4',3':5,6]pyrano[2,3-*b*]pyridine derivatives 11a-c revealed that compound 11a bearing a phenyl group exhibited selective activity against MCF (IC_{50} 112 nM). On the other hand, compound 11b bearing the 4-$OCH_3C_6H_4$ group was found to be active against most cancer cell lines with the highest activity against NUGC (IC_{50} 122 nM) and DLDI (IC_{50} 90nM). The 4-ClC_6H_4 substituted derivative 11c showed high cytotoxic activity against four cancer cell lines with potent activity against NUGC (IC_{50} 40 nM) and DLDI (IC_{50} 60 nM).

Experimental

Chemistry

All melting points were determined on a Stuart apparatus and the values given are uncorrected. IR spectra (KBr, cm^{-1}) were determined on a Shimadzu IR 435 spectrophotometer (Faculty of Pharmacy, Cairo University, Egypt). ^1H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 300 MHz (Microanalysisl Center, Cairo University, Egypt) and Bruker Ascend 400 MHz spectrophotometers (Microanalytical Unit, Faculty of Pharmacy, Cairo

University, Egypt) using TMS as internal standard. Chemical shift values are recorded in ppm on δ scale. Mass spectra were recorded on a Hewlett Packard 5988 spectrometer (Microanalysis Center, Cairo University, Egypt). Elemental analyses were carried out at the Microanalysis Center, Cairo University, Egypt; found values were within ±0.35% of the theoretical ones. Progress of the reactions was monitored using thin layer chromatography (TLC) sheets precoated with UV fluorescent silica gel Merck 60F 254 and were visualized using UV lamp.

General procedure for synthesis of compounds 4a-c and 6a-c

To a solution of compound 1 (1.74 g, 0.01 mol) and the appropriate aldehyde (0.01 mol) in ethanol (30 ml) containing triethylamine (1.0 ml) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 1 hr, left to cool and the formed solid product, in each case, was collected by filtration and crystallized from ethanol.

4-Phenyl-3-methyl-6-oxo-1- phenyl-1,6- dihydropyrano [2,3-c]pyrazole-5-carbonitrile (4a): Yield: 80%; m.p.: 220-238 °C; IR (KBr, cm^{-1}) ν: 3036 (CH aromatic), 2980, 2959 (CH aliphatic), 2223 (CN), 1702 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.88 (s, 3H, CH_3), 7.48–8.09 (m, 10H, $2C_6H_5$). MS (*m/z*, %): 328 (M^+, 42). Anal. calculated. for $C_{20}H_{13}N_3O_2$: C, 73.39; H, 4.00; N, 12.84. Found: C, 73.22; H, 3.91; N, 12.66.

4-(4-methoxyphenyl)-3- methyl-6-oxo-1- phenyl-1,6- dihydropyrano-[2,3-c]pyrazole-5- carbonitrile (4b): Yield: 70%; m.p.: 191-193 °C; IR (KBr, cm^{-1}) ν: 3053 (CH aromatic), 2956, 2935 (CH aliphatic), 2220 (CN), 1720 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.83 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 6.88-8.32 (m, 9H, C_6H_5, C_6H_4); MS (*m/z*, %): 358 (M^+, 28). Anal. calculated. for $C_{21}H_{15}N_3O_3$: C, 70.59; H, 4.20; N, 11.76. Found: C, 70.34; H, 4.28; N, 11.93.

4-(4-chlorophenyl)-3- methyl-6-oxo-1- phenyl-1,6- dihydropyrano [2,3-c] pyrazole-5- carbonitrile (4c): Yield: 83% ; m.p.:220-224°C; IR (KBr cm^{-1})ν: 3036 (CH aromatic), 2963 (CH aliphatic), 2220 (CN), 1690 (C=O); ^{1}H NMR (DMSO-d_6) δ: 2.89 (s, 3H, CH_3), 7.25–7.48 (m, 9H, C_6H_5, C_6H_4); Anal. calculated. for $C_{20}H_{12}ClN_3O_2$: C, 66.39; H, 3.32; N, 11.62. Found: C, 66.38; H, 3.49; N, 11.80.

6-Amino-3- methyl-1,4- diphenyl-1,4- dihydropyrano [2,3-c] pyrazole-5-carbonitrile (6a): Yield: 88%; m.p.: 157-159°C; IR (KBr, cm^{-1}) ν: 3480, 3136 (NH_2), 3056 (CH aromatic), 2020 (CN), 1642 (C=C);^{1}H NMR (DMSO-d_6) δ: 3.76 (s, 3H, CH_3), 6.69 (s, 1H, pyran H-4), 5.34 (s, 2H, NH_2, D_2O exchangeable), 7.26–7.36 (m, 10H, $2C_6H_5$) ppm; Anal. calculated. for $C_{20}H_{16}N_4O$: C, 73.17; H, 4.88; N, 17.07. Found: C, 73.22; H, 4.83; N, 16.89.

6-Amino-4- (4-methoxyphenyl)-3- methyl-1-phenyl -1,4-dihydro- pyrano [2,3-c] pyrazole-5-carbonitrile (6b): Yield: 80%; m.p.: 233-235 °C; IR (KBr, cm^{-1}) ν:

3493-3358 (NH_2), 3058 (CH aromatic), 2980 (CH aliphatic), 2220 (CN); ^{1}H NMR (DMSO-d_6) δ: 2.88 (s, 3H, CH_3), 3.16 (s, 3H, OCH_3), 6.73 (s, 1H, pyran H-4), 6.68 (s, 2H, NH_2, D_2O exchangeable), 7.26-7.39 (m, 9H, C_6H_5, C_6H_4) ppm ; Anal. calculated. for $C_{21}H_{18}N_4O_2$: C, 70.39; H, 5.03; N, 15.64. Found: C, 70.42, H, 4.80, N 15.73.

6-Amino-4-(4-chlorophenyl)-3-methyl-1-phenyl-1,4-dihydropyrano-[2,3-c]pyrazole-5-carbonitrile (6c): Yield: 79%; m.p.: 130-136 °C; IR (KBr, cm^{-1}) ν: 3480-3328 (NH_2), 3058 (CH aromatic), 2220 (CN), 1659 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6) δ: 4.74 (s, 3H, CH_3), 6.62 (s, 1H, pyran H-4), 6.85 (s, 2H, NH_2, D_2O exchangeable), 7.26–7.37 (m, 9H, `C_6H_5, C_6H_4) ppm. Anal. calculated. for $C_{20}H_{15}ClN_4O$: C, 66.21; H, 4.17; N, 15.44. Found: C, 66.30; H, 4.08; N, 15.63.

General procedure for the synthesis of the pyrano[2,3-c:6,5-c']dipyrazole derivatives 7a-c

To a solution of either compound 6a (3.28 g, 0.01 mol), 6b (3.58 g, 0.01 mol) or 6c (3.62g, 0.01 mol) in 1,4-dioxane (40 ml) solution containing sodium acetate(4.0g), hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 hr then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product, in each case, was collected by filtration.

5-Methyl-4,7- diphenyl-4,7- dihydro-1H- pyrano [2,3-c:6,5-c'] dipyrazol-3-amine (7a): Yield: 73 %; m.p.: 103-105 °C; IR (KBr, cm^{-1}) ν: 3486- 3350 (NH_2, NH), 3058 (CH aromatic), 1650 (C=N), 1636 (C=C); ^{1}H NMR (DMSO-d_6) δ: 2.83 (s, 3H, CH_3), 6.56 (s, 1H, pyran H-4), 6.73 (s, 2H, NH_2, D_2O exchangeable), 7.25–7.39 (m, 10H, $2C_6H_5$), 10.24 (s, 1H, NH, D_2O exchangeable) ppm. Anal. calculated. for $C_{20}H_{17}N_5O$: C, 69.97; H, 4.96; N, 20.41. Found: C, 69.77; H, 4.73; N, 20.69.

4-(4-Methoxyphenyl)-5- methyl-7- phenyl-4,7- dihydro-1H- pyrano [2,3-c:6,5-c']dipyrazol-3-amine (7b): Yield: 78 %; m.p.: 136-139 °C; IR (KBr, cm^{-1}) ν: 3458-3248 (NH_2,NH), 3053 (CH aromatic), 2987 (CH aliphatic), 1634 (C=C); ^{1}H NMR (DMSO-d_6) δ: 2.68 (s, 3H, CH_3), 3.11 (s, 3H, OCH_3), 6.24 (s, 2H, NH_2, D_2O exchangeable), 6.60 (s, 1H, pyran H-4), 7.27-7.39 (m, 9H, C_6H_5, C_6H_4), 8.27 (s, 1H, NH, D_2O exchangeable) ppm; Anal. calculated. for $C_{21}H_{19}N_5O_2$: C, 67.56; H, 5.09; N, 18.77. Found: C, 67.80, H, 5.29; N, 8.83.

4-(4-Chlorophenyl)-5- methyl-7- phenyl-4,7- dihydro-1H- pyrano [2,3-c:6,5-c'] dipyrazol-3-amine (7c): Yield: 79 %; m.p.: 166-169 °C; IR (KBr, cm^{-1}) ν: 3450-3236 (NH_2, NH), 3058 (CH aromatic), 2988 (CH aliphatic), 1650(C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6) δ: 2.80 (s, 3H, CH_3), 6.64 (s, 1H, pyran H-4), 6.30 (s, 2H, NH_2, D_2O exchangeable), 7.24–7.38 (m, 9H, C_6H_5, C_6H_4), 8.32 (s, 1H, D_2O exchangeable, NH); Anal. calculated. for $C_{20}H_{16}ClN_5O$: C, 63.58; H, 4.24; N, 18.54. Found: C, 63.73; H, 4.35; N, 18.69.

Amino-3-methyl-1- phenyl-4- (pyridin-3-yl)-1,4- dihydropyrano [2,3-c] pyrazole-5- carbonitrile (9)

To a solution of compound 1 (1.74 g, 0.01 mol) in ethanol (40 ml) containing triethylamine (0.50 ml) , pyridine-3-aldehyde (1.7 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 2 hr then left to cool and the formed solid product was collected by filtration and crystallized from ethanol.

Yield: 68%; m.p.: 188-191°C; IR (KBr, cm^{-1}) ν: 3396-3334 (NH_2), 3060 (CH aromatic), 2980, 2926 (CH aliphatic), 2220 (CN); ^{1}H NMR (DMSO-d_6) δ: 1.83 (s, 3H, CH_3), 4.63 (s, 1H, pyran H-4), 6.82 (s, 2H, NH_2, D_2O exchangeable), 7.30–8.39 (m, 9H, C_6H_5, pyridine H) ppm; Anal. calculated. for $C_{19}H_{15}N_5O$: C , 69.30 ; H , 4.56 ; N , 21.28. Found: C, 69.32; H 4.47; N , 21.39.

General procedure for the synthesis of compounds 11a-c

To a solution of compound 1 (1 .74 g, 0.01 mol) in ethanol (30 ml) containing triethylamine (1.0 ml) either benzaldehyde (1.08 g, 0.01 mol), 4-methoxybenzaldehyde (1.36 g, 0.01 mol) or 4-chlorobenzaldehyde (1.42 g, 0.01 mol) and 2-aminoprop-1-ene-1,1,3-tricarbonitrile (1.32 g, 0.01mol) were added. The whole reaction mixture, in each case was heated under reflux for 1 hr then left to cool then poured onto ice/water mixture containing few drops of hydrochloric acid. The formed solid product, in each case, was collected by filtration and crystallized from ethanol.

Diamino-3 -methyl- 1,4- diphenyl- 1,4 - dihydropyrazolo - [4',3':5,6]-pyrano[2,3-b]pyridine-6-carbonitrile (11a): Yield: 83%; m.p.: 166-169 °C; IR (KBr, cm^{-1}) ν: 3393-3239 ($2NH_2$), 3055 (CH aromatic), 2932, 2928 (CH aliphatic), 2220 (CN), 1650 (C=N); ^{1}H NMR (DMSO-d_6) δ: 2.66 (s, 3H, CH_3), 4.58 (s, 1H, pyran H-4), 4.16, 4.44 (2s, 4H, $2NH_2$, D_2O exchangeable), 7.28–7.73 (m, 10H, $2C_6H_5$), Anal.calculated. for $C_{23}H_{18}N_6O$: C, 70.05; H, 4.57; N, 21.32. Found: C, 70.22; H, 4.78; N, 21.29.

5,7-Diamino-4- (4-methoxyphenyl)-3- methyl-1-phenyl-1,4-dihydropyrazolo [4',3':5,6] pyrano[2,3-b]pyridine-6-carbonitrile (11b): Yield: 85%; m.p.: 132-135 °C; IR (KBr, cm^{-1}) ν: 3368-3263 ($2NH_2$), 3056 (CH aromatic), 2953, 2912 (CH aliphatic), 2221 (CN), 1655 (C=N); ^{1}H NMR (DMSO-d_6) δ: 2.83 (s, 3H, CH_3), 3.13 (s, 3H, OCH_3), 4.79 (s, 1H, pyran H-4), 6.77, 6.90 (2s, 4H, $2NH_2$, D_2O exchangeable), 7.26–7.86 (m, 9H, C_6H_5, C_6H_4). Anal. calculated. for $C_{24}H_{20}N_6O_2$: C, 67.92; H, 4.72; N, 19.81 . Found: C, 68.11; H, 4.80; N, 20.03.

5,7-Diamino-4- (4-chlorophenyl)-3- methyl-1- phenyl-1,4- dihydropyrazolo [4',3':5,6] pyrano[2,3-b]pyridine-6-carbonitrile (11c): Yield: 77 %; m.p.: 190-193 °C; IR (KBr, cm^{-1}) ν: 3479-3290 ($2NH_2$), 3053 (CH aromatic), 2990, 2912 (CH aliphatic), 2220 (CN), 1633 (C=C). ^{1}H NMR (DMSO-d_6) δ: 2.78 (s, 3H, CH_3), 4.60 (s, 1H, pyran H-4), 4.88, 7.15 (2s, 4H, $2NH_2$, D_2O exchangeable), 7.27–7.82 (m, 9H, C_6H_5, C_6H_4), Anal. calculated. for $C_{23}H_{17}ClN_6O$: C, 64.41; H, 4.00; N, 19.60. Found: C, 64.66; H, 3.92; N 19.53.

1-(5-Cyano-4-(4-methoxyphenyl)-3-methyl-1- phenyl-1,4- dihydropyrano [2,3-c]pyrazol-6-yl)-3-phenylthiourea (13)

To a solution of compound 6b (3.58 g, 0.01 mol) in ethanol (40 ml) containing triethylamine (1.0 ml), phenylisothiocyanate (1.30 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 hr. The formed solid product was collected by filtration and crystallized from ethanol. Yield: 79 %; m.p.: 166-169 °C; IR (KBr, cm^{-1}) ν: 3374, 3328 (2 NH), 3058 (CH aromatic), 2966, 2929 (CH aliphatic), 2220 (CN), 1180 (C=S); ^{1}H NMR (DMSO-d_6) δ: 2.76 (s, 3H, CH_3), 3.12 (s, 3H, OCH_3), 4.58 (s, 1H, pyran H- 4), 7.28–7.08 (m, 14H, $2C_6H_5$, C_6H_4), 8.28, 8.32 (2s, 2H, 2NH, D_2O exchangeable), Anal. calculated for $C_{28}H_{23}N_5O_2S$: C, 68.15; H, 4.67; N, 14.20. Found: C, 68.22; H, 4.82; N, 14.30.

5-Amino-1-methyl- 3H-chromeno[4',3':4,5]pyrano[2,3-c]pyrazol-6(11 b H)-one (14)

To a solution of compound 1 (0.98 g, 0.01 mol) , salicylaldehyde (1.23 g, 0.01mol) in dioxane (30 ml) containing triethylamine (1.0 ml) and malononitrile (0.66 g, 0.01 mol) was added. The whole reaction mixture, was heated under reflux for 2 hr, left to cool then poured onto ice/water mixture containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from ethanol. Yield: 83%; m.p.: 166-169°C; IR (KBr, cm^{-1}) ν: 3376, 3332 (NH_2), 3054 (CH aromatic), 2989, 2950 (CH aliphatic); ^{1}H NMR (DMSO-d_6) δ: 2.76 (s, 3H, CH_3), 4.48 (s, 1H, pyran H), 4.26 (s, 2H, NH_2, D_2O exchangeable), 7.27–7.42 (m, 9H, C_6H_5, C_6H_4). Anal. calculated. for $C_{20}H_{15}N_3O_3$: C, 69.56; H, 4.35; N, 12.17. Found: C, 69.30; H 4.22; N, 12.38.

Conclusions

The present work showed the synthesis of pyrazole derivatives derived from 3-methyl-1-phenyl-1H-pyrazo l-5(4H)-one. The synthesized compounds were screened against six cancer cell lines and the results showed that compounds 4a, 4c, 6c, 7c, 11b, 11c and 13 are the most potent compounds.

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تشييد وبناء ودراسة السمية لبعض المركبات الجديدة للبيرووزلات ومشتقاتها الناتجة من 3-ميثيل -1-فينيل -1هيدروجين – بيرازول - 5 (4 هيدروجين)- أون

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الهيئة القومية للرقابة والبحوث الدوائية ص ب-29 القاهرة – مصر.

من خلال هذا البحث حاولنا بناء وتشييد بعض المركبات العضوية الحلقية الغير متجانسة الجديدة لمشتقات البيرازول مثل 3-ميثيل-1 -فينيل-بيرازول-5-(4 هيدروجين)-أون .

وقد تم تفاعل تلك المركبات مع الالدهيات الاروماتية وثيانو ميثيلبين معا وايضا مع بيريدو الادهايد ومالونونيتريل لتكوين مركب واحد فقط. وكذلك تفاعل مع مالونونيترايل ,وتفاعل مشتقات مركب 6ج مع الهيدرازين هيدرات وايضا باستخدام استراتجية هى تفاعل متعدد المكونات .وهى أحدى الطرق القيمة الجديدة التى تعتبر من الطرق الاقتصادية وكذلك صديقة للبيئة .وقد تم بناء مركبات معقدة من تفاعل مرة واحدة ومن مركبات بسيطة لعديد من مركبات البيرازول .كل المواد التى تم تشيدها قد اثبتت كيميائيا من خلال استخدام التحليل الاشعة.

وقد خضعت تلك المركبات للتقييم البيولوجى ودراسة مدى السمية لديها ضد الخلايا العادية وكذلك الخلايا الخبيثة. وقد تم قياس مدى السمية باستخدام طريقة براين -شريمب .ولاحظنا ان المركبات 4أ , 4ج ,6ج,7ج,11ب,11ج و13 من ضمن المركبات التى تم تشيدها اظهروا نتائج سمية قوية .