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NOVEL SYNTHESIS OF PYRAN-3-HYDRAZIDE DERIVATIVES AND THEIR USES TO THE SYNTHESIS HYDRAZIDE-HYDRAZONE, PYRAZOLE AND THIAZOLE DERIVATIVES WITH ANTICANCER ACTIVITIES

Eman M. Samir[1] and Rafat M. Mohareb[2*]

[1]Egyptian Drug Authority (EDA), National Organization for Drug Control and Research (NODCAR), P.O. 29, Cairo, Egypt
[2]Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt

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ABSTRACT. The multi-component reaction of ethyl acetoacetate with each of malononitrile (**3**) benzaldehyde (**1**) in ethanol containing triethylamine gave the ethyl 6-amino-5-cyano-2-methyl-4-phenyl-4*H*-pyran-3-carboxylate (**4**). The latter compound reacted with hydrazine hydrate to give the hydrazide derivative **6**. Compound **6** underwent a series of hetero-cyclization reactions to give pyrzole, hydraide-hydrazone, thiazole derivatives. The produced compounds tested against cancer cell lines six cancer cell lines and showed that compounds **8b**, **10b**, **11a**, **17a**, **21** and **24a** were the most cytotoxic compounds. Further tests of the latter compounds toward the five tyrosine kinases and Pim-1 kinase showed that compounds **10b**, **21** and **24a** were the most potent of the tested compounds and compounds **10a**, **11a** and **17a** were of the highest inhibitions toward Pim-1 kinase. The high inhibitions of most of the tested compounds toward the selected cancer cell lines and the tyrosine kinases encourage for future work to be done.

KEY WORDS: Hydrazide, Thiophene, Pyrazole, Pyran, Cytotoxicity, Tyrosine kinases

INTRODUCTION

It was well known that multi-component reactions (MCR) were easy way to mix three or more components together in a one pot reaction to produce high yield products in a very short time. Many compounds were available in literature were found to be easily obtained within a very short reaction time [1-5]. One of the most popular class of compounds obtained the multi-component reaction was the pyran derivatives. Such compounds exhibited wide range of biological activities like antitumor, antibacterial, antibiotic, hypolipidemic, antiallergic, and immune-modulating activities [6]. Among the 6-membered oxygen-comprising heterocycles are the 2-Amino-4*H*-pyrans as they show high potencies as antimicrobial and anticancer agents [7, 8]. We were concerned within the recent years through heterocyclic transformations using cyclohexan-1,3-dione as starting material involved through many multi-component reactions [9-13]. In addition, a number of hydrazide–hydrazone derivatives have been claimed to possess interesting bioactivity such as antibacterial–antifungal, anticonvulsant, antiinflammatory, antimalarial, analgesic, antiplatelets, antituberculosis and anticancer activities [14-21].

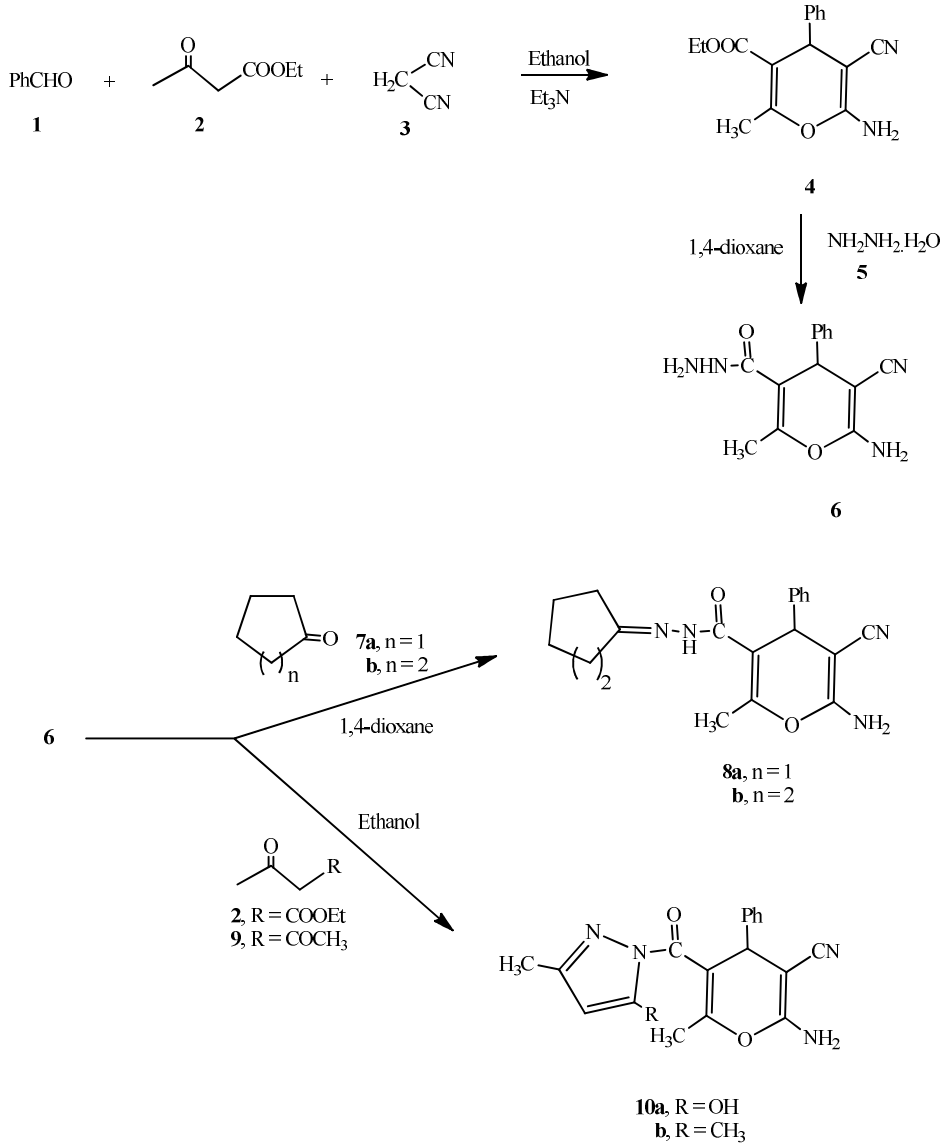
In the aim of producing anti-cancer agents, in the present work we were demonstrating the multi-component reactions of ethyl acetoacetate to produce a polysubstituted pyran derivative followed by its conversion into pyran-3-hydrazide followed by its transformation into the corresponding hydrazide-hydrazone derivatives. Based on the different active centers of the latter product, it was involved in many heterocyclization reaction to produce biologically active compounds that can be used as anticancer agents in the future.

RESULTS AND DISCUSSION

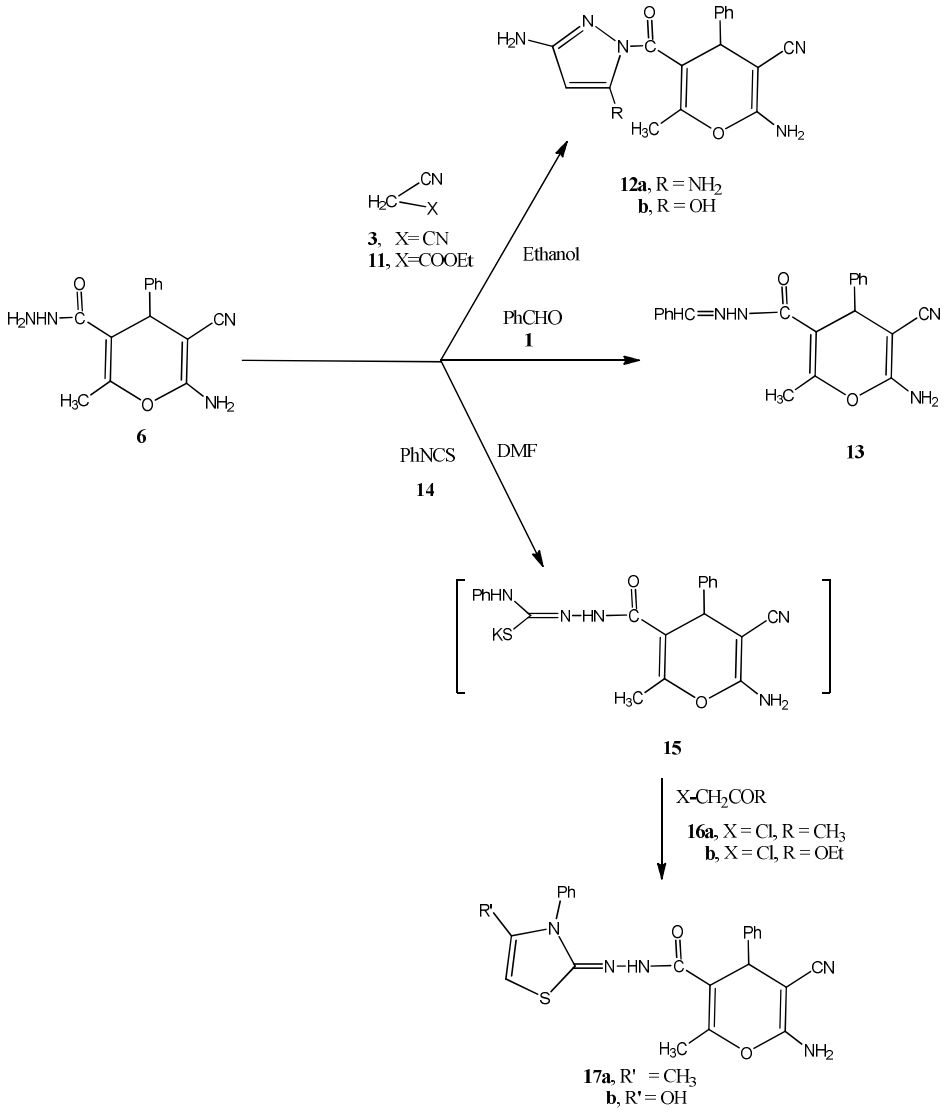
In the present work benzaldehyde (**1**) underwent a multi-component reaction with ethyl acetoacetate (**2**) and malononitrile (**3**) in ethanol containing triethylamine gave the ethyl 6-amino-

*Corresponding author. E-mail: raafat_mohareb@yahoo.com

5-cyano-2-methyl-4-phenyl-4*H*-pyran-3-carboxylate (**4**). The structure of the latter product was confirmed on the basis of its analytical and spectral data (see experimental section). Compound **4** reacted with hydrazine hydrate to give the pyran-3-hydrazide derivative **6**. The reactivity of compound **6** toward different chemical reagents was studied in order to produce biologically active compounds. It was reported that hydrazide-hydrazone derivatives are good candidate as anti-cancer agents [22-24]. This encouraged us to synthesis hydrazide-hydrazone derivatives from compound **6**. Thus, the reaction of compound **6** with either cyclopentanone (**7a**) or cyclohexanone (**7b**) gave the hydrazide-hydrazone derivatives **8a** and **8b**, respectively [25]. The structures of the



Scheme 1. Synthesis of compounds **4**, **6**; **8a**,**b** and **10a**,**b**.

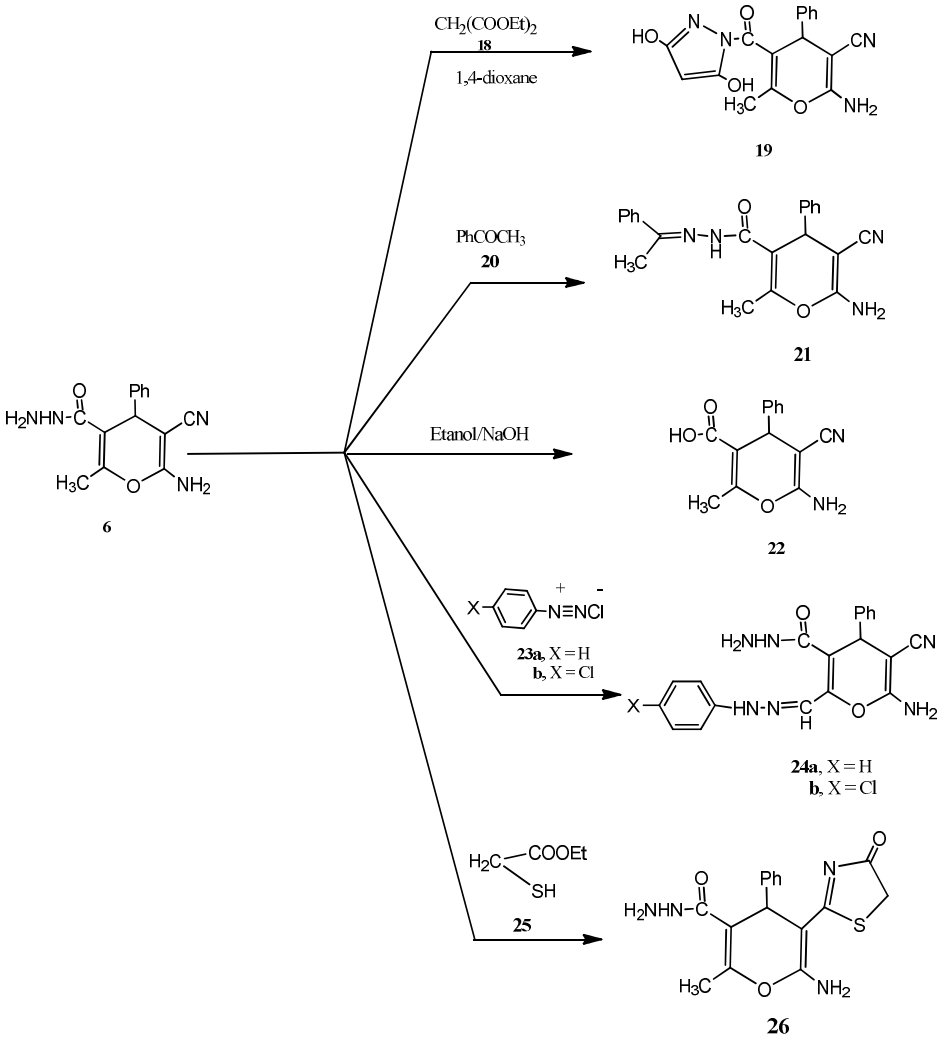


Scheme 2. Synthesis of compounds **12a,b**, 15; and **17a**,**b**.

latter compounds were confirmed on the basis of their respective analytical and spectral data. Thus, the ^{1}H NMR of compound **8a** showed the presence of two multiplets at δ 1.69-1.85 and 2.49-2.63 ppm equivalent to the four CH_2 groups, a singlet at δ 4.93 ppm corresponding to the NH_2 group, a singlet at δ 5.09 ppm indicating the pyran H-4, a multiplet at δ 7.26-7.45 ppm for the C_6H_5 group and a singlet (D_2O exchangeable for the NH group. In addition, the ^{13}C NMR spectrum showed the presence of four signals at δ 23.2, 28.2, 30.5, 31.6 according to the $4CH_2$ groups, a signal at δ 36.6 equivalent to the CH_3 group, a signal at δ 51.1 proving the pyran C-4, a signal at δ 116.7 for the CN group, four signals at δ 120.5, 121.8, 122.5, 124.6 equivalent to the

C_6H_5 group, four signals at δ 132.1, 133.2, 134.9, 138.6 indicating the pyran C and two signals at δ 164.6, 166.4 corresponding to the C=N and C=O groups, respectively.

Compound **6** reacted with either ethyl acetoacetate (**2**) or acetylacetone **(9**) to give the pyrazole derivatives **10a** and **10b**, respectively (Scheme 1).



Scheme 3. Synthesis of compounds **19**, **21**, **22**, **24a**,**b** and **26**.

Moreover, the reaction of compound **6** with either malononitrile (**3**) or ethyl cyanoacetate (**11**) afforded the pyrazole derivatives **12a** and **12b**, respectively. The reaction of compound (**6**) with benzaldehyde (**1**) gave pyrazole derivatives **13** [26].

Our research group was involved through a comprehensive program involving the reaction of hydrazides and active methylene reagents with phenylisothiocyanate in dimethylformamide solution containing potassium hydroxide to give the intermediate potassium sulphide salt. The

latter underwent a series of heterocyclization reaction upon reaction with α-halocarbonyl compounds to produce thiophene or thiazole derivatives [27-29]. In continuation of this program the reaction of compound **6** with phenylisothiocyanate (**14**) in dimethylformamide containing potassium hydroxide gave the intermediate potassium sulphide salt **15**. The latter reacted with either chloroacetone or ethyl chloroacetate to give the thiazole derivatives **17a** and **17b**, respectively (Scheme 2).

In addition, the reaction of compound **6** with diethylmalonate gave the pyrazole derivative **19**. The reaction of compound **6** with acetophenone (**20**) gave the hydrazide-hydrazone derivative **21**. Compound **6** underwent hydrolysis when reacted with ethanolic sodium hydroxide to give the pyran-3-carboxylic acid **22**. The methyl group present in compound **6** showed interesting activity toward hydrazone formation, this is attributed to the presence of the CH_3 group in ortho position to electronegative COOEt group. Thus, compound **6** reacted with either benzenediazonium chloride **23a,b** or 4-chlorobenzenediazonium chloride to give the corresponding arylhydrazone derivatives **24a** and **24b**, respectively. Finally, the reaction of compound **6** with thioglycollic acid (**25**) in ethanol containing triethylamine gave the thiazole derivative **26** (Scheme 3).

Cell proliferation assay

The anti-proliferative activities of the newly synthesized compounds (Table 3) were evaluated against the six cancer cell lines A549, HT-29, MKN-45, U87MG, and SMMC-7721 and H460 using the standard MTT assay in vitro, with foretinib as the positive control [30-32].

The mean values of three independent experiments, expressed as IC_{50} values, were presented in Table III. Most of the synthesized compounds exhibited potent anti-proliferative activity with IC_{50} values less than 30 μM. Generally, the variations of substituents' within the heterocyclic moiety together with the nature of the heterocyclic ring being attached have a notable influence on the anti-proliferative activity.

Table 1. In vitro growth inhibitory effects IC_{50} (μM) of the selected compounds against cancer cell lines ± SEM.

Compound No	IC_{50} ± SEM (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
8a	2.37± 0.39	4.58 ± 1.25	2.31 ± 1.49	5.62 ± 1.48	6.39 ± 1.42	2.87 ±1.22
8b	0.23± 0.15	0.89 ± 0.41	0.38 ±0.17	0.26± 0.12	1.22 ± 0.65	0.73± 0.22
10a	5.29 ±1.48	5.36 ±2.32	3.41 ±1.23	1.53 ±0.92	3.26 ±1.06	4.53 ±1.33
10b	1.25 ± 0.63	0.72 ± 0.28	0.35 ± 0.28	0.53 ± 0.26	0.65 ± 0.42	0.18 ± 0.05
11a	1.29 ± 0.74	1.42 ± 0.47	1.28 ± 0.63	2.28 ± 1.15	1.63 ± 0.46	2.98 ± 1.63
12b	6.82 ±1.53	4.53 ± 2.15	5.24± 2.31	4.22 ± 2.08	2.35 ± 2.31	4.28 ± 1.29
13	6.45 ± 2.58	8.25 ± 2.61	6.53 ± 2.49	6.77 ± 1.27	4.19± 2.08	6.40 ± 3.30
16	4.63 ± 1.82	2.93± 1.64	5.28 ± 1.63	6.48 ± 1.73	5.27± 1.43	2.29 ± 0.59
17a	0.61 ± 0.28	0.58± 0.29	0.48±0.26	0.62 ± 0.35	0.52 ± 0.26	1.59±0.26
17b	4.53 ±1.28	6.70 ± 1.83	6.27 ± 2.31	5.83±1.43	5.28 ± 2.34	2.34 ± 2.26
19	6.08 ± 1.28	5.61± 1.72	8.08 ± 2.27	1.68± 0.49	4.22± 1.53	6.45± 2.14
21	0.28 ± 0.18	0.64 ± 0.25	0.53 ± 0.29	0.31 ± 0.18	0.62 ± 0.26	0.58 ± 0.26
22	4.26± 1.43	4.42 ± 2.39	3.42 ± 1.57	4.52 ± 1.59	2.42 ± 0.93	3.38 ± 0.46
24a	0.34 ±0.18	0.37±0.25	0.52±0.14	0.33 ±0.18	0.92 ± 0.32	0.83± 0.26
24b	4.24 ± 3.18	4.25 ± 2.43	6.52 ± 2.32	7.48 ± 2.62	8.58 ± 2.73	5.82 ± 2.23
26	6.27± 2.12	5.42 ± 2.38	4.36 ± 2.59	5.75 ± 1.48	6.20 ± 2.38	3.68 ± 1.42
Foretinib	0.08 ± 0.01	0.18 ± 0.03	0.15 ± 0.023	0.03±0.0055	0.90 ± 0.13	0.44 ± 0.062

Structure activity relationship

It is clear from Table 1 that compounds **8b**, **10b**, **17a**, **21** and **24a** were the most active compounds among the tested compounds. Considering the hydrazide-hydrazone derivatives **8a** and **8b**, it is

clear that compound **8b** with the cyclohexylidene moiety showed more inhibitions than compound **8a** with the cyclopentylidene moiety. Considering the pyrazole derivatives **10a** and **10b**, surprisingly compound **10b** (R = CH_3) exhibited more inhibitions than **10a** (R = OH) although the latter contains the electronegative OH group. Similarly, compound **12b** exhibited low inhibitions against the six cancer cell lines although it has OH group within its structure. On the other hand, the pyran derivatives **13** and **15** showed low inhibitions against the six cancer cell lines. On the other hand, for the pyran derivatives **17a** and **17b**, it is obvious that compound **17a** (R = CH_3) exhibited higher inhibitions than **17b** (R = OH). It is clear from Table 1 that the pyran derivative **21** has higher inhibitions than the pyran derivatives **19** and **22**. The high inhibitions of **21**was attributed to the presence of the hydrazide-hydrazone moiety within its structure. For the pyran derivative **22**, it was obvious that it had moderate inhibitions toward the cancer cell lines, in addition its highest inhibition was toward the U87MG cell line with IC_{50} 2.42 µM. Considering the arylhydrazone derivatives **24a** and **24b**, it was clear that compound **24a** (X = H) exhibited higher inhibitions that **24b** (X = Cl). Finally, the thiazole derivative **26** exhibited from low to moderate inhibitions against the six cancer cell lines. From the demonstration of such activities of the compounds, it is clear that in most cases the presences of certain heterocyclic and/or certain moiety was the most controlling factor through the high inhibition of the compounds. For example, for compounds **8a,b** and **21** the high inhibition was attributed to the presence of the hydrazide-hydrazone moiety. On the other hand, for compounds **17a** and **17b** the presence of thiazole ring in their structure was responsible for their activities. For compounds **24a** and **24b**, the presence of the hydrazide group at C-5 and the hydrazone group at C-6 of the pyran ring increase the inhibitions of such compounds.

Inhibition of selected compounds against tyrosine kinases

The most potent compounds toward the cancer cell lines compounds **8b**, **10b**, **11a**, **17a**, **21** and **24a** were further investigated towards the five tyrosine kinases c-kit, FIT-3, VEGFR-2, EGFR and PDGFR and the data were expressed through Table 2. It is obvious from the Table that compound **8b** showed high inhibitions toward EGFR and PDGFR kinases with IC_{50}'s 0.78 and 0.64 nM, respectively while it showed moderate inhibitions toward c-Kit and Flt-3 kinases with IC_{50}'s 1.27 and 1.46 nM, respectively. On the other hand, compounds **10b**, **21** and **24a** expressed high inhibitions toward VEGFR-2, EGFR and PDGFR kinases and moderate inhibitions toward c-Kit and Flt-3 kinases. Interestingly, compounds **11a**, **17a** showed high inhibitions toward the five tyrosine kinases.

Table 2. Inhibition of tyrosine kinases (Enzyme IC_{50} (nM) for compounds **8b**, **10b**, **11a**, **17a**, **21**and **24a**.

Compound	c-Kit	Flt-3	VEGFR-2	EGFR	PDGFR
8b	1.27	1.46	1.38	0.78	0.64
10b	1.28	1.21	0.93	0.28	0.70
11a	0.27	0.28	0.31	0.58	0.44
17a	0.23	1.06	0.68	0.48	0.49
21	2.52	2.47	1.79	1.32	0.93
24a	1.24	1.28	0.87	0.72	0.49
Foretinib	0.19	0.17	0.20	0.13	0.26

Inhibition of Pim-1 kinase for compounds 10b, 13b, 18b, 18a and 20

Compounds **8b**, **10a**, **11a and 17a** were selected to examine their Pim-1 kinase inhibition activity (Table 3) as these compounds showed high inhibition towards the tested cancer cell lines at a range of 10 concentrations and the IC_{50} values were calculated. Compounds **10a, 11a and 17a** were the most potent to inhibit Pim-1 kinase with IC_{50} value of 0.32, 0.49 and 0.28 and µM,

respectively. On the other hand, compounds **8b** was less effective (IC_{50} > 10 μM). These profiles in combination with cell growth inhibition data of compounds **8b**, **10a**, **11a and 17a** were listed in Table 3 and indicated that Pim-1 was a potential target of these compounds where SGI-1776 was used as positive control with IC_{50} 0.048 μM in the assay.

Table 3. Inhibition of Pim-1 kinase for compounds **8b**, **10a**, **11a and 17a**

Compound	Inhibition ratio At 10 μM	IC_{50} (μM)
8b	38	>100
10a	94	0.32
11a	90	0.49
17a	96	0.28
SGI-1776	-	0.048

It is clear from Table 3 that compounds **10a**, **11a** and **17a** are of the highest inhibitions toward Pim-1 kinase with IC_{50}'s 0.32, 0.49 and 0.28 μM, respectively.

EXPERIMENTAL

Chemistry

Through this work the melting points of the synthesized compounds were recorded on Buchi melting point apparatus D-545. The IR spectra (KBr discs) were measured on Bruker Vector 22 instrument. ^{13}C NMR and ^{1}H NMR spectra were measured on Bruker DPX300 instrument in DMSO-d_6 with TMS as internal standard. Mass spectra were measured using EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were measured using the Micro-analytical Data center at Cairo University. All reactions was monitored by TLC on 2 x 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck) for getting complete reactions.

*Ethyl 6-amino-5-cyano-2-methyl-4-phenyl-4H-pyran-3-carboxylate (**4**).* A mixture of benzaldehyde (1.06 g, 0.01 mol), ethyl acetoacetate (1.30 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) in absolute ethanol (60 mL) containing triethylamine (1.0 mL) was heated under reflux for 3 h then was left to cool. The solid product produced upon cooling was collected by filtration. Yellow crystals from ethanol, yield (1.99 g, 70%), mp 190-192 °C. IR (KBr) υ max cm^{-1}: 3469, 3342 (NH_2), 3055 (CH, aromatic), 2920, 2867 (CH_2, CH_3), 2220 (CN), 1703 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.13 (t, 3H, *J* = 7.26 Hz, $OCH_2\underline{CH_3}$), 2.70 (s, 3H, CH_3), 4.22 (q, 2H, *J* = 7.26 Hz, $O\underline{CH_2}CH_3$), 4.92 (s, 2H, D_2O exchangeable, NH_2), 5.08 (s, 1H, pyran H-4), 7.23-7.55 (m, 5H, C_6H_5); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.8 ($OCH_2\underline{CH_3}$), 36.8 (CH_3), 50.2 (OCH_2CH_3), 51.0 (pyran C-4), 116.8 (CN), 120.4, 121.8, 124.6, 126.4 (C_6H_5), 132.6, 134.2, 136.0, 138.4 (pyran C), 164.8 (C=O). Anal. calcd. for $C_{16}H_{16}N_2O_3$ (284.31): C, 67.59; H, 5.67; N, 9.85 %. Found: C, 67.36; H, 5.73; N, 10.02%. MS: m/z 284 [M^+, 65%].

*6-Amino-5-cyano-2-methyl-4-phenyl-4H-pyran-3-carbohydrazide (**6**).* To a solution of compound **4** (2.84 g, 0.01 mol) in 1,4-dioxane (40 mL) hydrazine hydrate was added. The reaction mixture was heated under reflux for 4 h then was poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration. Yellow crystals from ethanol, yield (2.02 g, 75%), Mp 143-145 °C. IR (KBr) υ max cm^{-1}: 3478, 3351(NH_2), 3055 (CH, aromatic), 2920, 2867 (CH_2, CH_3), 2220 (CN), 1689 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.73 (s, 3H, CH_3), 4.96, 5.21 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.10 (s, 1H, pyran H-4), 7.24-7.48 (m, 5H, C_6H_5), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-

d$_6$, 75 MHz): δ 36.4 (CH_3), 51.3 (pyran C-4), 116.8 (CN), 120.2, 122.4, 123.9, 125.8 (C_6H_5), 132.3, 133.8, 135.6, 138.2 (pyran C), 163.8 (C=O). Anal. calcd. for $C_{14}H_{14}N_4O_2$ (270.29): C, 62.21; H, 5.22; N, 20.73 %. Found: C, 62.08; H, 5.41; N, 20.59%. MS: m/z 270 [M^+, 80%].

General procedure for the synthesis of the hydrazide-hydrazone derivatives ***8a,b****.* To a solution of compound **6** (2.70 g, 0.01 mol) in 1,4-dioxane (40 mL) either cyclopentanone (0.84 g, 0.01 mol) or cyclohexanone (0.98 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then was left to cool and the formed solid product was collected by filtration.

6-Amino-5-cyano-N'-cyclopentylidene-2-methyl-4-phenyl-4H-pyran-3-carbohydrazide ***(8a)****.* Brown crystals from ethanol, yield (2.28 g, 68%), mp 70-75 °C. IR (KBr) υ max cm^{-1}: 3461-3339 (NH, NH_2), 3055 (CH, aromatic), 2928, 2869 (CH_2, CH_3), 2220 (CN), 1688 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ = 1.69-1.85 (m, 4H, $2CH_2$), 2.49-2.63 (m, 4H, $2CH_2$), 2.76 (s, 3H, CH_3), 4.93 (s, 2H, D_2O exchangeable, NH_2), 5.09 (s, 1H, pyran H-4), 7.26-7.45 (m, 5H, C_6H_5), 8.32 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-*d*$_6$, 75 MHz): δ 23.2, 28.2, 30.5, 31.6 ($4CH_2$), 36.6 (CH_3), 51.1 (pyran C-4), 116.7 (CN), 120.5, 121.8, 122.5, 124.6 (C_6H_5), 132.1, 133.2, 134.9, 138.6 (pyran C), 164.6 (C=N), 166.4 (CO). Anal. calcd. for $C_{19}H_{20}N_4O_2$ (336.39): C, 67.84; H, 5.99; N, 16.66 %. Found: C, 68.02; H, 5.83; N, 16.80%. MS: m/z 336 [M^+, 68%].

6-Amino-5-cyano-N'-cyclohexylidene-2-methyl-4-phenyl-4H-pyran-3-carbohydrazide ***(8b)****.* Browm crystals from ethanol, yield (2.59 g, 74%), mp 100 °C. IR (KBr) υ max cm^{-1}: 3473-3362 (NH, NH_2), 3055 (CH, aromatic), 2952, 2841 (CH_2, CH_3), 2220 (CN), 1702 (C=O), 1632 (C=C); ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ = 1.49-1.87 (m, 6H, $3CH_2$), 2.32-2.72 (m, 4H, $2CH_2$), 2.78 (s, 3H, CH_3), 4.96 (s, 2H, D_2O exchangeable, NH_2), 5.12 (s, 1H, pyran H-4), 7.25-7.49 (m, 5H, C_6H_5), 8.30 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-*d*$_6$, 75 MHz): δ 24.6, 26.8, 28.5, 30.8, 32.4 ($5CH_2$), 36.4 (CH_3), 51.3 (pyran C-4), 116.9 (CN), 120.2, 122.6, 123.8, 125.0 (C_6H_5), 132.3, 133.5, 134.6, 138.2 (pyran C), 164.9 (C=N), 168.3 (CO). Anal. calcd. for $C_{20}H_{22}N_4O_2$ (350.41): C, 68.55; H, 6.33; N, 15.99%. Found: C, 68.43; H, 6.25; N, 16.25%. MS: m/z 350 [M^+, 45%].

General procedure for the synthesis of the 1H-pyrazole-1-carbonyl)-6-methyl-4-phenyl-4H-pyran derivatives ***10a,b****.* Either of ethyl acetoacetate (1.30 mL, 0.01 mol) or acetylacetone (1.00 mL, 0.01 mol) in absolute ethanol (50 mL) was added to a solution of compound **6** (2.70 g, 0.01 mol) in absolute ethanol (40 mL). The reaction mixture, in each case, was heated under reflux for 2 h then poured onto ice/water mixture and the formed solid product was collected by filtration.

2-Amino-5-(5-hydroxy-3-methyl-1H-pyrazole-1-carbonyl)-6-methyl-4-phenyl-4H-pyran-3-carbonitrile ***(10a)****.* Brown crystals from ethanol, yield (2.21g, 66%), mp 80 °C. IR (KBr) υ max cm^{-1}: 3529, 3328 (OH, NH_2), 3055 (CH, aromatic), 2928, 2863 (CH_2, CH_3), 2220 (CN), 1688 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ = 2.78, 2.84 (2s, 6H, $2CH_3$), 4.94 (s, 2H, D_2O exchangeable, NH_2), 5.08, 6.11 (2s, 2H, pyran H-4), 7.26-7.46 (m, 5H, C_6H_5), 10.26 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-*d*$_6$, 75 MHz): δ 36.2, 37.8 ($2CH_3$), 51.4 (pyran C-4), 116.6 (CN), 120.4, 121.8, 124.6, 125.2 (C_6H_5), 132.0, 133.2, 134.8, 135.4, 137.6, 138.1 (pyran, pyrazole C), 163.9 (C=N), 166.2 (C=O). Anal. calcd. for $C_{18}H_{16}N_4O_3$ (336.34): C, 64.28; H, 4.79; N, 16.66%. Found: C, 64.35; H, 4.93; N, 16.80%. MS: m/z 336 [M^+, 54%].

2-Amino-5-(3,5-dimethyl-1H-pyrazole-1-carbonyl)-6-methyl-4-phenyl-4H-pyran-3-carbonitrile ***(10b)****.* Deep yellow crystals from ethanol, yield (2.33 g, 70%), mp 90-92 °C. IR (KBr) υ max cm^{-1}: 3479, 3342 (NH_2), 3055 (CH, aromatic), 2960, 2883 (CH_2, CH_3), 2222 (CN), 1689 (C=O), 1632 (C=C); ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ = 2.74, 2,80, 2.82 (3s, 9H, $3CH_3$), 4.93 (s, 2H, D_2O exchangeable, NH_2), 5.11, 6.16 (2s, 2H, pyran H-4 pyrazole H-4), 7.23-7.48 (m, 5H, C_6H_5); ^{13}C NMR (DMSO-*d*$_6$, 75 MHz): δ 36.1, 37.3, 38.2 ($3CH_3$), 51.2 (pyran C-4), 116.9 (CN), 120.1,

121.3, 123.9, 125.6 (C_6H_5), 132.1, 132.9, 133.4, 134.5, 137.2, 138.4 (pyran, pyrazole C), 164.0 (C=N), 167.8 (C=O). Anal. calcd. for $C_{19}H_{18}N_4O_2$ (334.37): C, 68.25; H, 5.43; N, 16.76%. Found: C, 68.44; H, 5.72; N, 16.58%. MS: m/z 334 [M^+, 68%].

*General procedure for the synthesis of the pyrazole-1-carbonyl)-6-methyl-4-phenyl-4H-pyran derivatives **12a,b**.* To a solution of compound **6** (2.70 g, 0.01 mol) in absolute ethanol (50 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 2 h then poured onto ice/water mixture containing a few drops of hydrochloric acid. The produced solid product, in each case, was collected by filtration.

*2-Amino-5-(3,5-diamino-1H-pyrazole-1-carbonyl)-6-methyl-4-phenyl-4H-pyran-3-carbonitrile (**12a**)* . Brown crystals from ethanol, yield (2.55 g, 76%), mp 100 °C. IR (KBr) υ max cm^{-1}: 3468, 3362 (OH, NH_2), 3057 (CH, aromatic), 2980, 2869 (CH_2, CH_3), 2220 (CN), 1689 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.76 (s, 3H, CH_3), 4.90, 4.96, 5.25 (3s, 6H, D_2O exchangeable, $3NH_2$), 5.09, 6.14 (2s, 2H, pyran H-4 pyrazole H-4), 7.24-7.43 (m, 5H, C_6H_5); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.8 (CH_3), 51.3 (pyran C-4), 116.6 (CN), 120.4, 121.8, 124.6, 125.2 (C_6H_5), 132.0, 133.2, 133.2, 134.8, 137.6, 138.0 (pyran, pyrazole C), 164.2 (C=N), 166.2 (C=O). Anal. calcd. for $C_{17}H_{16}N_6O_2$ (336.35): C, 60.71; H, 4.79; N, 24.99%. Found: C, 60.93; H, 4.46; N, 25.27%. MS: m/z 336 [M+, 75%].

*2-Amino-5-(3-amino-5-hydroxy-1H-pyrazole-1-carbonyl)-6-methyl-4-phenyl-4H-pyran-3-carbonitrile (**12b**).* Deep yelow crystals from ethanol, yield (2.42 g, 72%), mp 97-100 °C. IR (KBr) υ max cm^{-1}: 3574, 3368(OH, NH_2), 3055 (CH, aromatic), 2982 (CH_3), 2220 (CN), 1689 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.78 (s, 3H, CH_3), 4.94, 5.23 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.06, 6.12 (2s, 2H, pyran H-4 pyrazole H-4), 7.22-7.40 (m, 5H, C_6H_5), 10.22 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.4 (CH_3), 51.4 (pyran C-4), 116.9 (CN), 120.7, 122.5, 124.1, 125.8 (C_6H_5), 132.1, 133.6, 133.8, 134.4, 137.2, 138.3 (pyran, pyrazole C), 164.5 (C=N), 166.8 (C=O). Anal. calcd. for $C_{17}H_{15}N_5O_3$ (337.33): C, 60.53; H, 4.48; N, 20.76%. Found: C, 60.72; H, 4.30; N, 20.51%. MS: m/z 337 [M^+, 50%].

*6-Amino-N'-benzylidene-5-cyano-2-methyl-4-phenyl-4H-pyran-3-carbohydrazide (**13**).* To a solution of compound **6** (2.70 g, 0.01 mol) in 1,4-dioxane (40 mL) benzaldehyde (1.08 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then was poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration. Brown crystals from ethanol, yield (2.43 g, 68%), mp 90-93 °C. IR (KBr) υ max cm^{-1}: 3488, 3360 (NH_2), 3055 (CH, aromatic), 2920 (CH_3), 2220 (CN), 1688 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.71 (s, 3H, CH_3), 4.96 (s, 2H, D_2O exchangeable, NH_2), 5.12 (s, 1H, pyran H-4), 6.80 (s, 1H, CH=N), 7.25-7.49 (m, 10H, $2C_6H_5$), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.46 (CH_3), 51.2 (pyran C-4), 116.8 (CN), 120.2, 121.4, 121.8, 122.4, 123.9, 124.0, 1246, 125.9 (C_6H_5), 132.2, 133.4, 136.8, 138.9 (pyran C), 164.4 (C=O). Anal. calcd. for $C_{21}H_{18}N_4O_2$ (358.39): C, 70.38; H, 5.06; N, 15.63%. Found: C, 70.15; H, 5.26; N, 15.42%. MS: m/z 358 [M^+, 65%].

*General procedure for the synthesis of the phenylthiazol-2(3H)-ylidene)-4-phenyl-4H-pyran derivatives **17a,b**.* To a solution of compound **6** (2.70 g, 0.01 mol) in dimethylformamide (30 mL) containing potassium hydroxide (0.80 g, 0.02 mol) phenylisothiocyanate (1.30 g, 0.01 mol) was added. The reaction mixture was stirred at room temperature for 24 h then either chloroacetone (0.92 g, 0.01 mol) or α-chloroethyl acetate (1.67 g, 0.01 mol) was added, The whole reaction mixture was stirred at room temperature for another 24 h then was poured onto ice/water mixture

containing drops of hydrochloric acid (till pH 6) and the formed solid product, in each case, was collected by filtration.

*6-Amino-5-cyano-2-methyl-N'-(4-methyl-3-phenylthiazol-2(3H)-ylidene)-4-phenyl-4H-pyran-3-carbohydrazide (**17a**).* Brown crystals from acetic acid, yield (2.65 g, 60%), mp 160-162 °C. IR (KBr) υ max cm^{-1}: 3474, 3382 (NH, NH_2), 3054 (CH, aromatic), 2984 (CH_3), 2222 (CN), 1688 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.74, 2.86 (2s, 6H, $2CH_3$), 4.96 (s, 2H, D_2O exchangeable, NH_2), 5.09, 6.53 (2s, 2H, pyran H-4, thiazole H-5), 7.22-7.46 (m, 10H, $2C_6H_5$), 8.26 (s, 1H, D_2O exchaneable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.2, 38.5 ($2CH_3$), 51.1 (pyran C-4), 116.8 (CN), 120.1, 121.3, 121.9, 123.8 124.2, 125.6,125.8, 126.1 ($2C_6H_5$), 132.2, 133.5, 133.3, 134.2, 135.0, 137.3 (pyran, thiazole C), 166.3 (C=N), 166.8 (C=O). Anal. calcd. for $C_{24}H_{21}N_5O_2S$ (443.52): C, 64.99; H, 4.77; N, 15.79; S, 7.23%. Found: C, 65.22; H, 4.80; N, 15.62; S, 7.08%. MS: m/z 443 [M^+, 46%].

*6-Amino-5-cyano-N'-(4-hydroxy-3-phenylthiazol-2(3H)-ylidene)-2-methyl-4-phenyl-4H-pyran-3-carbohydrazide (**17b**).* Brown crystals from acetic acid, yield (3.11 g, 70%), mp 165 °C. IR (KBr) υ max cm^{-1}: 3582, 3359(OH, NH, NH_2), 3055 (CH, aromatic), 2986 (CH_3), 2220 (CN), 1688 (C=O), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.78 (s, 3H, CH_3), 4.98 (s, 2H, D_2O exchangeable, NH_2), 5.12, 6.46 (2s, 2H, pyran H-4, thiazole H-5), 7.24-7.52 (m, 10H, $2C_6H_5$), 8.29 (s, 1H, D_2O exchaneable, NH), 10.22 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.8 (CH_3), 51.6 (pyran C-4), 116.9 (CN), 120.5, 121.8, 122.6, 123.9 124.5, 125.1,125.3, 126.6 ($2C_6H_5$), 131.8, 132.6, 133.0, 134.7, 135.2, 142.9 (pyran, thiazole C), 166.6 (C=N), 166.5 (C=O). Anal. calcd. for $C_{23}H_{19}N_5O_3S$ (445.49): C, 62.01; H, 4.30; N, 15.72; S, 7.20%. Found: C, 61.80; H, 4.41; N, 15.92; S, 7.35%. MS: m/z 445 [M^+, 28%].

*2-Amino-5-(3,5-dihydroxy-1H-pyrazole-1-carbonyl)-6-methyl-4-phenyl-4H-pyran-3-carbonitrile (**19**).* To a solution of compound **6** (2.70 g, 0.01 mol) in 1,4-dioxane (40 mL) diethylmalonate (1.60 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then was left to cool. The reaction mixture was evaporated under vacuum and the remaining product was triturated with ethanol and the formed solid product was collected by filtration. Brown crystals from 1,4-dioxane, yield (2.44 g, 72%), mp 43-45 °C. IR (KBr) υ max cm^{-1}: 3571, 3342 (OH, NH_2), 3057 (CH, aromatic), 2986 (CH_3), 2220 (CN), 1689 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.72 (s, 3H, CH_3), 4.81 (s, 2H, D_2O exchangeable, NH_2), 5.12, 6.58 (2s, 2H, pyran H-4 pyrazole H-4), 7.23-7.48 (m, 5H, C_6H_5), 10.30, 10.36 (2s, 2H, 2OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.3 (CH_3), 51.2 (pyran C-4), 116.9 (CN), 120.1, 121.4, 123.9, 124.8 (C_6H_5), 131.2, 132.6, 133.7, 134.2, 137.3, 141.5 (pyran, pyrazole C), 164.6 (C=N), 167.8(C=O). Anal. calcd. for $C_{17}H_{14}N_4O_4$ (338.32): C, 60.35; H, 4.17; N, 16.56%. Found: C, 60.49; H, 4.36; N, 16.72%. MS: m/z 338 [M^+, 60%].

*6-Amino-5-cyano-2-methyl-4-phenyl-N'-(1-phenylethylidene)-4H-pyran-3-carbohydrazide (**21**).* To a solution of compound **6** (2.70 g, 0.01 mol) in 1,4-dioxane (40 mL) acetophenone (1.20 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1 h then was left to cool. The reaction mixture was evaporated under vacuum and the remaining product was triturated with ethanol and the formed solid product was collected by filtration. Brown crystals from ethanol, yield (2.45 g, 66%), mp 65-68 °C. IR (KBr) υ max cm^{-1}: 3462-3358 (NH, NH_2), 3055 (CH, aromatic), 2972 (CH_3), 2222 (CN), 1689 (C=O), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.71, 2.86 (2s, 6H, $2CH_3$), 4.93 (s, 2H, D_2O exchangeable, NH_2), 5.10 (s, 1H, pyran H-4),7.21-7.49 (m, 10H, $2C_6H_5$), 8.40 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.4, 38.8 ($2CH_3$), 51.2 (pyran C-4), 116.9 (CN), 120.4, 122.7, 124.3, 125.9 (C_6H_5), 132.3, 132.6, 133.8, 134.9, (pyran), 164.6 (C=N), 168.1(C=O). Anal. calcd. for $C_{22}H_{20}N_4O_2$ (372.42): C, 70.95; H, 5.41; N, 15.04%. Found: C, 71.26; H, 5.55; N, 14.82%. MS: m/z 372 [M^+, 80%].

*6-Amino-5-cyano-2-methyl-4-phenyl-4H-pyran-3-carboxylic acid (**22**).* To a solution of compound **6** (2.70 g, 0.01 mol) in ethanol (40 mL) sodium hydroxide (5 mL, 10%) solution was added. The reaction mixture was heated under reflux for 4 h then was poured onto ice/water containing hydrochloric acid (till pH 6) and the formed solid product was collected by filtration. Pale brown crystals from ethanol, yield (1.92 g, 75%), mp 180-182 °C. IR (KBr) υ max cm^{-1}: 3580-3328 (OH, NH_2), 3055 (CH aromatic), 2965 (CH_3), 2220 (CN), 1693 (C=O), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.59 (s, 3H, CH_3), 4.72 (s, 2H, D_2O exchangeable, NH_2), 5.13 (s, 1H, pyran H-4), 7.25-7.41 (m, 5H, C_6H_5), 10.22 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.5 (CH_3), 51.3 (pyran C-4), 116.8 (CN), 120.2, 121.6, 123.9, 126.2 (C_6H_5), 132.2, 134.3, 136.1, 138.6 (pyran C), 164.5 (C=O). Anal. calcd. for $C_{14}H_{12}N_2O_3$ (256.26): C, 65.62; H, 4.72; N, 10.93%. Found: C, 65.72; H, 4.90; N, 10.75%. MS: m/z 256 [M^+, 42%].

*General procedure for the synthesis of the arylhydrazone derivatives **24a,b**.* To a cold solution of compound **6** (2.70 g, 0.01 mol) in ethanol (60 mL) containing sodium hydroxide (10 mL, 10%) either of benzenediazonium chloride (0.01 mol) or 4-chlorobenzenediazonium chloride (0.01 mol) [prepared by the addition of sodium nitrite solution (1.70 g, 0.02 mol dissolved in water, 10 mL) to a cold solution of either aniline (0.92 g, 0.01 mol) or 4-chloroaniline (1.26 g, 0.01 mol) dissolved in concentrated hydrochloric acid (10 mL, 12 M) with continuous stirring] was added with continuous stirring. The reaction mixture, in each case, was stirred at room temperature for an additional two hours and the formed solid product, was collected by filtration.

*6-Amino-5-cyano-4-phenyl-2-((2-phenylhydrazineylidene)-methyl)-4H-pyran-3-carbohydrazide (**24a**).* Deep red crystals from ethanol, yield (2.81 g, 75%), mp 80-82 °C. IR (KBr) υ max cm^{-1}: 3495-3362 (NH, NH_2), 3055 (CH aromatic), 2965 (CH_3), 2222 (CN), 1688 (C=O), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 4.69, 5.26 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.23 (s, 1H, CH), 5.11 (s, 1H, pyran H-4), 7.23-7.52 (m, 10H, $2C_6H_5$), 8.22, 8.35 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 51.2 (pyran C-4), 116.6 (CN), 120.5, 121.2, 121.4, 122.0, 122.7, 123.9, 124.1, 126.8 ($2C_6H_5$), 132.6, 133.7, 136.5, 138.2 (pyran C), 164.3 (C=O), 168.3 (C=N). Anal. calcd. for $C_{20}H_{18}N_6O_2$ (374.40): C, 64.16; H, 4.85; N, 22.45%. Found: C, 64.30; H, 4.62; N, 22.09%. MS: m/z 374 [M^+, 56%].

*6-Amino-2-((2-(4-chlorophenyl)hydrazineylidene)methyl)-5-cyano-4-phenyl-4H-pyran-3-carbohydrazide (**24b**).* Deep brown crystals from ethanol, yield (2.85 g, 70%), mp 90-93 °C. IR (KBr) υ max cm^{-1}: 3479-3341 (NH, NH_2), 3055 (CH aromatic), 2969 (CH_3), 2220 (CN), 1689 (C=O), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 4.72, 5.31 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.26 (s, 1H, CH), 5.13 (s, 1H, pyran H-4), 7.22-7.50 (m, 9H, C_6H_5, C_6H_4), 8.24, 8.38 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 51.6 (pyran C-4), 117.1 (CN), 120.3, 121.6, 122.9, 123.3, 123.7, 124.2, 124.6, 126.1 (C_6H_5, C_6H_4), 132.2, 133.4, 136.1, 138.6 (pyran C), 164.6 (C=O), 168.7 (C=N). Anal. calcd. for $C_{20}H_{17}ClN_6O_2$ (408.84): C, 58.75; H, 4.19; N, 20.56%. Found: C, 58.49; H, 4.31; N, 20.80%. MS: m/z 408 [M^+, 60%].

*6-Amino-2-methyl-5-(4-oxo-4,5-dihydrothiazol-2-yl)-4-phenyl-4H-pyran-3-carbohydrazide (**26**).* To a solution of compound **6** (2.70 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (2.0 mL) ethyl glycolate (1.20 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then was poured onto ice/water and the formed solid product was collected by filtration. Brown crystals from ethanol, yield (2.06 g, 60%), mp 170-172 °C. IR (KBr) υ max cm^{-1}: 3480-3339 (NH, NH_2), 3055 (CH, aromatic), 2986 (CH_3), 1702, 1689 (2C=O), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.71 (s, 3H, CH_3), 3.93 (s, 2H thiazole CH_2), 4.93, 5.11 (2s, 4H, D_2O exchangeable, $2NH_2$), 5.10 (s, 1H, pyran H-4), 7.24-7.45 (m, 5H, C_6H_5), 8.32 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.6 (CH_3), 51.1 (pyran C-4), 80.3 (s, 2H,

thiazole CH_2), 120.4, 121.3, 124.6, 125.2 (C_6H_5), 132.1, 132.4, 133.7, 134.2 (pyran), 166.8 (C=N), 167.2, 168.8 (2C=O). Anal. calcd. for $C_{16}H_{16}N_4O_3S$ (344.39): C, 55.80; H, 4.68; N, 16.27; S, 9.31%. Found: C, 55.59; H (4.71; N, 16.39; S, 9.25%. MS: m/z 344 [M^+, 72%].

CONCLUSION

Throughout this work we aimed to study the effect of electronegative substituents to either aryl group and/or heterocyclic ring for inhibitions toward cancer cell lines. The target molecules were synthesised using ethyl acetoacetate. Their structures were confirmed by multiple techniques and the synthesized compounds were screened for cytotoxic activity against a panel of six human cancer cell lines using MTT assay. The produced compounds tested against cancer six cancer cell lines and showed that compounds **8b**, **10b**, **11a**, **17a**, **21** and **24a** were the most cytotoxic compounds. Further tests of the latter compounds toward the five tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR and Pim-1 kinase showed that compounds **10b**, **21** and **24a** were the most potent of the tested compounds and compounds **10a**, **11a** and **17a** were of the highest inhibitions toward Pim-1 kinase

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