Bull. Chem. Soc. Ethiop. **2023**, 37(2), 405-425.
DOI: https://dx.doi.org/10.4314/bcse.v37i2.12

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

ANTI-PROLIFERATIVE ACTIVITIES OF 4*H*-PYRAN DERIVATIVES SYNTHESIZED FROM BENZOYLACETONE

Rafat M. Mohareb[1*], Bishoy A. Ibrahim[1] and Fatma Omar Al Farouk[2]

[1]Department of Chemistry, Faculty of Science, Cairo University, Egypt
[2]Faculty of Pharmacy, University of Hertfordshire, New Cairo Capital, Egypt

(Received September 13, 2022; Revised November 10, 2022; Accepted November 11, 2022)

ABSTRACT. Benzoylacetone (**1**) underwent a series of multi-component reactions with aromatic aldehydes and malononitrile or ethyl cyanoacetate to produce the pyran derivatives **4a-f**. The latter compounds reacted with malononitrile or ethyl cyanoacetate to yield the condensation products **5a-m**. On the other hand, the reaction of **4a-f** with either the diazonium salts **6a-c** yielded the arylhydrazone derivatives **7a-i**. The multi-component reaction of (**1**) with aromatic adehydes and cyclohexan-1,3-dione produced the pyran derivatives **9a-c**. Compound **1** underwent the Gewald's reactions with elemental sulfur and malononitrile or ethyl cyanacetate yielding the thiophene derivatives **10a,b**. Evaluations of the synthesized products were carried out against some selected cancer cell lines and the most active compounds were further evaluated against the seventeen cancer cell lines classified according to the disease. Morphological changes of A549 cell line by the effect of compound **7k** was studied using microenvironment of the lung tissue where an excellent results was obtained.

KEY WORDS: Benzoylacetone, Multi-component reactions, Pyran, Thiophene, Arylhydrazone, Cytotoxicity

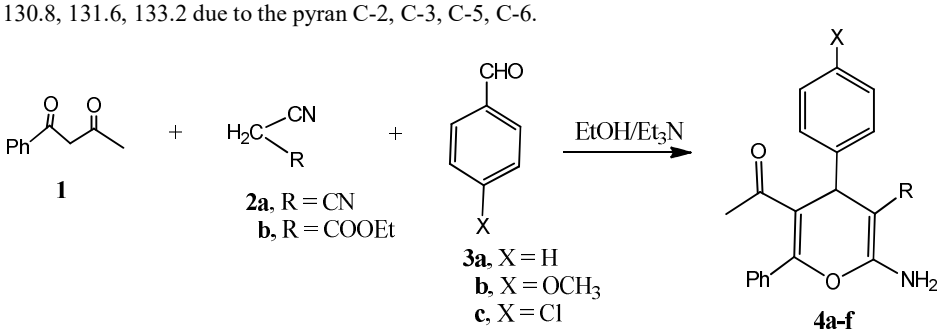
INTRODUCTION

Within the field of pharmaceutical and medicinal chemistry [1] the pyran derivatives were of the most important bioactive compounds. They attract the attention in recent years as a result of their wide range of biological activities among which their antitumor [2], COX-2 and HDAC inhibitory [3], anti-microbial [4], anti-mycobacterial [5], anti-inflammatory [6], antiviral [7], antifungal [8], anticonvulsant [9], antioxidant [10], analgesic [11, 12] and antinociceptive [13]. According to WHO cancer can be defined as a generic term for a large group of diseases that can impair any part of the body beside it was known as malignant tumours and neoplasms. Metastasis is another term for defining cancer which involve the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. As the result of the large spread of cancer and the anticancer activities of 4*H*-pyran derivatives, many reports concerned with the synthesis and anticancer activities of such group of compounds [14, 15]. Recently, we were focused through the production of a large number of 4*H*-pyran compounds via the multicomponent reactions of aromatic aldehyde, 1,3-dicarbonyl compounds and active methylene reagents followed by anti-proliferative evaluations [16, 17]. The best way to get poly-substituted 4*H*-pyran derivatives is the use of multi-component reactions (MCRs) [18, 19]. There are also many reports showed that compounds containing pyran nucleus could be a worthy choice for cyctotoxic activity against various human tumor cell lines together with their pharmacological activities [20-25]. In the light of the above findings, we demonstrated herein the synthesis of some new 4*H*-pyran and arylhydrazone derivatives that cannot be obtained via another way. In addition, the evaluation of the newly synthesized products towards human cancer cell lines and cancer cell lines classified according to the disease was done. The originality of this work appeared through the investigating of new heterocyclic compounds that can be used as anti-cancer agents.

*Corresponding author. E-mail: raafat_mohareb@cu.edu.eg

RESULTS AND DISCUSSION

This work demonstrated the uses of benzoylacetone as starting compound for the synthesis of many heterocyclic derivatives and the progress of reactions were outlined through Schemes 1-4. The multi-component reaction of benzoylacetone (**1**) with each of malononitrile (**2a**) or ethyl cyanoacetate (**2b**) and benzaldehyde (**3a**), 4-methoxybenzaldehyde (**3b**) or 4-chlorobenzaldehyde (**3c**) using absolute ethanol together with a catalytic amount of triethylamine yielded the 1-(6-amino-2-phenyl-4-aryl-4*H*-pyran-3-yl)ethanone derivatives **4a-f** (Scheme 1). The analytical and spectral data were the tools that were used to confirm the structures of compounds **4a-f** together with the studying of their reactions with some chemical reagents. Thus, the reaction of compounds **4a-f** with malononitrile (**2a**) or ethyl cyanoacetate (**2b**) in an oil bath at 120 ºC produced the Knoevenagel condensation products **5a-m** (Scheme 2). The structures of compounds **5a-m** were based on analytical and spectral data. Thus, the ^{1}H NMR spectrum of **5a** (as an example) revealed the presence of a singlet at δ 2.92 for the CH_3 group, a singlet at δ 4.87 ppm (D_2O exchangeable) confirming the presence of the NH_2 group, a singlet at δ 6.50 ppm due to the presence of the pyran *H*-4 and a multiplet at δ 7.24-7.56 ppm which was attributed to the two C_6H_5 groups. Beside, the ^{13}C NMR spectrum showed the presence of a signal at δ 38.7 for the CH_3 group, two signals at δ 86.3, 89.4 confirming the C=C moiety, a signal at δ 90.2 for the pyran C-4, three signals at δ 116.8, 117.0, 117.2 confirming the presence of the three CN groups, signals at δ 120.3, 120.5, 121.4, 122.6, 123.2, 123.8, 124.3, 125.9 for the two C_6H_5 groups and four signals at δ 128.2, 130.8, 131.6, 133.2 due to the pyran C-2, C-3, C-5, C-6.

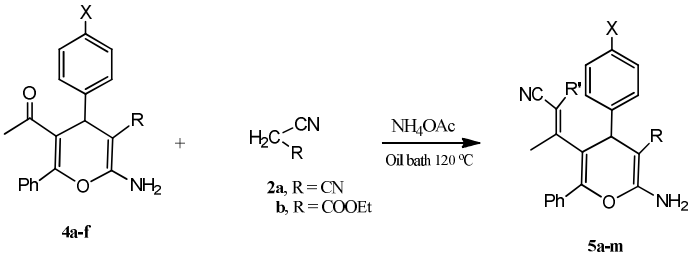


4	a	b	c	d	e	f
X	H	H	OCH_3	OCH_3	Cl	Cl
R	CN	COOEt	CN	COOEt	CN	COOEt

Scheme 1. Synthesis of compounds **4a-f**.

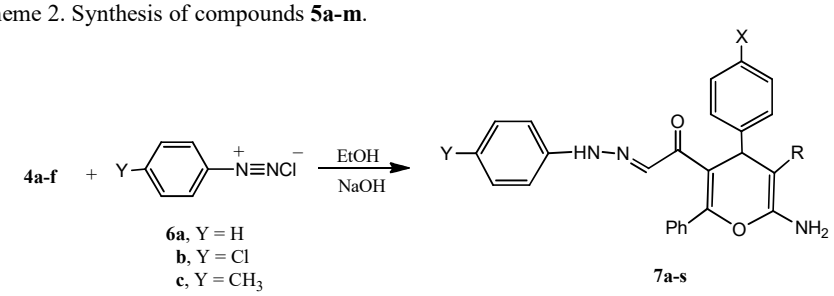
The azomethine -NHN=CH group, hydrazones and their derivatives represents an important class of compounds with many applications as anticancer activities against different cancer cell lines [26-29] for that reason many hydrazone derivatives were synthesized. To our knowledge azomethine were previously prepared from the reaction of carbaldehyde with hydrazine [30, 31], however, in this work we succeeded to get such compounds through the reaction of diazonium salts with the acetyl group of compounds **4a-f**. Moreover, we obtained a variety of arylhydrazone

derivatives characterized with different substituents not only at the heterocyclic ring but also at the aryl moiety where this enabled study of structure activity relationship when tested against



5	a	b	c	d	e	f	g	h	i	k	l	m
X	H	H	H	H	Cl	Cl	Cl	Cl	OCH_3	OCH_3	OCH_3	OCH_3
R	CN	COOEt	CN	COOEt	CN	COOEt	CN	COOEt	CN	COOEt	CN	COOEt
R'	CN	CN	COOEt	COOEt	CN	CN	COOEt	COOEt	CN	CN	COOEt	COOEt

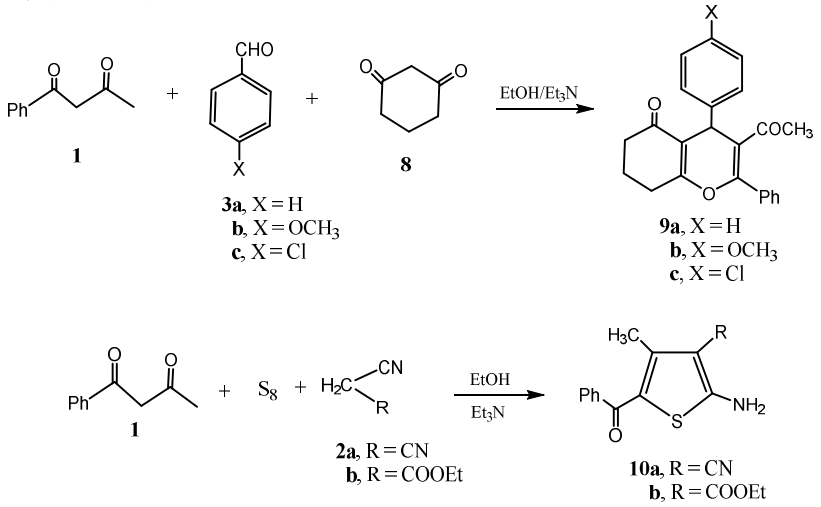
Scheme 2. Synthesis of compounds **5a-m**.



7	a	b	c	d	e	f	g	h	i
X	H	H	H	H	H	H	Cl	Cl	Cl
R	CN	COOEt	CN	COOEt	CN	COOEt	CN	COOEt	CN
Y	H	H	Cl	Cl	CH_3	CH_3	H	H	Cl
7	**k**	**l**	**m**	n	o	p	q	r	s
X	Cl	Cl	Cl	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
R	COOEt	CN	COOEt	CN	COOEt	CN	COOEt	CN	COOEt
Y	Cl	CH_3	CH_3	H	H	Cl	Cl	CH_3	CH_3

Scheme 3. Synthesis of compounds **7a-s**.

cancer cell lines. In these terms the reaction of compounds **4a-f** with benzenediazonium chloride (**6a**), 4-chlorobenzenediazonium chloride (**6b**) or 4-methylbenzenediazonium chloride (**6c**) in the presence of ethanolic sodium acetate solution produced the arylhydrazone derivatives **7a-s** (Scheme 3). Their respective structures were based on their agreement with the analytical and spectral data (see experimental section). Similarly, the multi-component reactions of benzoylacetone with each of benzaldehyde (**3a**), 4-methoxybenzaldehyde (**3b**) or 4-chlorobenzaldehyde and cyclohexan-1,3-dione (**8**) yielded the 3-acetyl-2-phenyl-7,8-dihydro-4*H*-chromen-5(6*H*)-one derivatives **9a-c**. The reaction of compound **1** with elemental sulfur and malononitrile (**2a**) or ethyl cyanoacetate (**2b**) afforded the thiophene derivatives **10a** and **10b**, respectively (Scheme 4).



Scheme 4. Synthesis of compounds **9a-c** and **10a,b**.

Biology

Anticancer evaluations of the newly synthesized compounds

Using Foretinib as the positive control in such measurements, it was noticed that many of the synthesized compounds exhibited high potent anti-proliferative activity. Some of the synthesized compounds exhibited higher activity than the positive control Foretinib. The variations of substituents within the ring system beside the nature of the heterocyclic compound had a notable impact on the anti-proliferative activity. Through such measurements the IC_{50}'s of Foretinib demonstrated in Table 1 against the cancer cell lines were considered identical to the previously reported work [32-34].

SAR's (structure activity relationship) of the synthesized compounds

Table 1 demonstrated that many of the synthesized compounds revealed high inhibitions toward the used cancer cell lines. The most cytotoxic compounds were the twenty five compounds **4a**, **4b**, **4e**, **5a**, **5d**, **5e**, **5f**, **5g**, **5h**, **5i**, **7c**, **7e**, **7f**, **7g**, **7h**, **7i**, **7k**, **7l**, **7m**, **7o**, **7p**, **7q**, **9a**, **9c** and **10a**. Considering the pyran derivatives **4a-f**, where compounds **4a**, **4b**, **4e** and **4f** were the most cytotoxic compounds. The high inhibitions of such compounds were attributed to the presence of the electron withdrawn moieties like the CN, COOEt and the Cl groups. On the other hand, the low inhibitions of compounds **4c** and **4d** were attributed to the presence of the electron donating

OCH_3 group. Considering the pyran derivatives **5a-m**, where the data in Table 1 demonstrated that compounds **5a**, **5c**, **5d**, **5e**, **5f**, **5g**, **5h** and **5i** were the most cytotoxic compounds among these compounds. Table 1 showed that compounds **5e** (X = Cl, R = R'= CN), **5f** (X = Cl, R = COOEt, R' = CN) and **5g** (X = Cl, R = CN, R' = COOEt) exhibited higher inhibitions than the reference

Table 1. *In vitro* cytotoxic inhibitions IC_{50} ± SEM (μM) of the newly synthesized compounds against cancer cell lines.

Compound No	IC_{50} ± SEM (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
4a	1.06± 0.78	1.26 ± 0.87	0.96 ± 0.44	0.55±0.32	0.78± 0.31	0.65 ± 0.26
4b	2.18±1.25	1.29±0.84	2.63±0.78	2.36±1.01	4.56±1.80	3.72±1.53
4c	7.52 ± 2.39	6.43 ± 2.30	8.52 ± 3.52	7.58 ± 3.73	9.83 ± 3.62	5.81±1.32
4d	7.42 ± 2.32	8.15 ± 2.43	5.59 ± 2.52	6.37 ± 1.67	5.56 ± 1.76	8.53 ± 1.43
4e	0.24 ±0.18	0.33±0.14	0.37 ±0.25	0.36 ±0.18	0.48 ±0.29	0.32 ±0.15
4f	3.67± 1.56	2.38 ±0.38	1.48 ± 0.88	2.71 ± 1.15	1.80 ±0.79	1.58 ±0.78
5a	1.23± 0.68	1.26 ± 0.63	0.93 ± 0.42	0.45 ± 0.23	1.68 ± 0.62	0.65± 0.36
5b	4.70±1.26	2.12±0.92	3.56±1.32	2.27±0.89	3.27±1.62	1.43±0.96
5c	3.45±1.32	2.41± 0.82	3.53± 1.09	4.28 ± 1.38	3.28±1.43	2.23± 1.25
5d	2.36 ±1.14	2.31 ±0.86	3.41±1.32	3.27 ± 1.43	3.38±1.26	2.41 ±1.06
5e	0.24 ± 0.11	0.35 ± 0.20	0.43 ± 0.21	0.29 ± 1.60	0.39 ± 0.13	0.43 ± 0.52
5f	0.83± 0.53	0.28± 0.14	0.22± 0.11	0.46 ± 0.20	0.23± 0.19	0.62± 0.18
5g	0.42 ±0.18	0.18±0.03	0.51±0.15	0.32±0.14	0.53±0.25	0.29±0.15
5h	0.94 ± 0.35	1.27 ± 0.69	0.58±0.31	0.64± 0.28	0.63 ± 0.27	1.24 ± 0.42
5i	2.26 ± 1.12	1.36 ±0.85	2.46 ± 1.02	0.89±0.34	1.23 ±0.75	2.13±0.79
5k	5.83 ±1.42	7.40 ± 2.35	8.22± 2.53	5.41 ±1.36	6.03 ±2.50	8.43 ± 2.34
5l	4.82± 1.18	6.58 ± 1.23	7.42± 2.19	6.31± 1.28	4.35 ± 1.16	4.58± 1.15
5m	8.42±2.37	6.53±2.63	5.80±2.47	7.41±3.52	8.92±2.59	4.36±1.62
7a	3.41±1.25	4.61±1.28	4.82±1.52	2.16±0.73	2.37±1.29	3.42±1.53
7b	6.41±2.36	5.22±1.68	8.91±2.04	7.62±1.59	8.04±2.16	9.15±2.38
7c	0.26±0.12	0.26±0.15	0.33±0.14	0.25±0.08	0.38±0.21	0.42±0.21
7d	3.80±1.42	4.07±1.26	3.59±1.32	5.73±1.68	3.85±1.73	4.73±1.37
7e	1.58 ± 0.73	1.65 ± 0.73	0.79± 0.36	1.64 ± 0.98	1.16 ± 0.59	2.36 ± 1.15
7f	0.57±0.26	0.62±0.30	0.73±0.29	0.83±0.31	0.58±0.42	0.64±0.28
7g	1.08±0.69	0.82±0.26	0.63±0.37	0.38±0.26	1.82±0.79	0.63±0.31
7h	0.33±0.12	0.28±0.15	0.28±3.19	6.28±1.08	7.89±2.63	9.39±2.37
7i	1.21±0.59	2.36 ±0.87	3.45 ± 1.34	2.36 ±0.82	1.54 ±0.63	2.26 ± 1.07
7k	0.19 ± 0.02	0.24 ±0.08	0.34 ±0.12	0.13±0.02	0.16±0.09	0.23±0.15
7l	1.12±0.58	1.02 ±0.61	0.34±0.32	0.21±0.14	0.32±0.26	0.39±0.13
7m	0.28± 0.12	0.29± 0.14	0.34± 0.12	0.53 ± 0.26	0.41 ± 0.24	0.36 ± 0.14
7n	7.41 ± 2.43	6.37 ± 2.51	8.28 ± 2.51	5.28 ± 1.43	4.36 ± 2.16	5.37 ± 1.70
7o	0.43±0.26	0.23±0.13	0.35 ± 0.17	0.25 ± 0.19	0.36 ± 0.16	0.29±0.12
7p	1.35±0.72	1.09±0.18	2.63± 1.28	1.38 ±0.53	2.31 ±0.79	1.42 ±0.74
7q	2.28 ±1.14	2.82± 0.78	1.75 ± 0.83	0.90± 0.32	1.74± 0.69	1.04±0.84
7r	6.41 ± 2.53	8.49 ±2.64	7.26 ± 1.69	8.39 ± 2.26	8.73 ± 2.18	5.76 ± 2.19
7s	6.29±2.14	5.37±1.42	4.69±1.84	3.59±1.73	8.40 ± 2.69	5.73 ± 2.63
9a	1.49±0.65	2.69 ± 1.05	1.69 ±0.80	2.85 ± 1.81	1.53 ±0.79	0.82 ±0.29
9b	8.76± 2.34	9.62 ± 3.27	8.43± 2.26	7.51± 1.23	6.60± 2.29	8.39±3.24
9c	0.12±0.05	0.12±0.03	0.15±0.09	0.28±0.13	0.34±0.16	0.25±0.04
10a	0.26±0.13	0.34±0.15	0.37±0.16	0.22±0.08	0.48±0.16	0.37±0.27
10b	6.42± 2.80	5.63± 2.03	8.81± 3.68	6.68±1.23	5.62±1.66	8.46± 3.52
Foretinib[a]	0.08 ± 0.01	0.18 ± 0.03	0.15 ± 0.023	0.03±0.0055	0.90 ± 0.13	0.44 ± 0.062

[a]IC_{50}'s of Foretinib against cancer cell lines was taken identical to the previously reported work [32-34].

Foretinib against U87MG and SMMC-7721 cell lines. On the other hand, compound **5h** (X = Cl, R = R' = COOEt) showed higher inhibition than the reference only against U87MG cell line. It was surprisingly; within such series of compounds that compound **5i** with the electron donating OCH_3 group was the high cytotoxic derivative. It seemed that the presence of the two CN group had a great effect for its high inhibitions beside the conjugate effect of the OCH_3 group with aryl moiety. Preferably among the arylhydrazone derivatives **7a-s**, compounds **7c**, **7e**, **7f**, **7g**, **7h**, **7i**, **7k**, **7l**, **7m**, **7o** and **7p** were the most cytotoxic compounds. Compounds **7c** (X = H, R = CN, Y = Cl), **7g** (X = Cl, R = CN, Y = H), **7h** (X = Cl, R = COOEt, Y = H), **7i** (X = Cl, R = CN, Y = Cl), **7k** (X = Cl, R = COOEt, Y = Cl), **7l** (X = Cl, R = CN, Y = CH_3), **7m** (X = Cl, R = COOEt, Y = CH_3) and **7p** (X = OCH_3, R = CN, Y = Cl) exhibited good inhibitions against the six cancer cell lines. It is of interest to mention that compounds **7c**, **7o** and **7m** exhibited higher inhibitions than the reference Foretinib against the three cancer cell lines MKN-45, U84MG and SMMC-7721. On the other hand, the presence of an electron donating methyl group (Y = CH_3) in compounds **7e**, **7f** and **7i**, and methoxy group (X = OCH_3) in derivatives **7o** and **7p** resulted in high inhibitions for these compounds against A549, H460 and SMMC-7721 cell lines. Tabel 1 showed that compounds with the CH_3 moiety like**7e**, **7f** and **7i** relatively exhibited lower inhibitions than compounds **7o** and **7p** with the OCH_3 group. Our explanation for such finding was based on the conjugation effect of the OCH_3 group with the double bonds of the phenyl group which might enhance the inhibitions. For the 4*H*-chromen-5(6*H*)-one derivatives **9a-c**, compounds **9a** (X = H) and **9c** (X = Cl) were of high inhibitions while compound **9b** (X = OCH_3) exhibited low inhibitions this can be attributed to the steric effect of the OCH_3 group which is larger H or Cl atom. Considering the thiophene derivatives **10a,b** where compound **10a** substituted with a relatively smaller CN exhibited higher inhibition than **10b** substituted with a larger COOEt moiety.

Throughout these evaluations Foretinib was used as the reference drug, some of the tested compound revealed higher inhibitions than the reference. Thus, against H460 cell line compound **5g** showed IC_{50} = 0.18 µM exactly like Foretinib and compound **9c** exhibited IC_{50} = 0.12 µM which was higher than the reference. Moreover, against HT29 cell line compound **9c** expressed IC_{50} = 0.15 µM like Foretinib (IC_{50} = 0.15 µM). Interestingly, compounds **4a**, **4e**, **5e**, **5f**, **5g**, **5h**, **7c**, **7f**, **7k**, **7l**, **7m**, **7o**, **9c** and **10a** revealed higher inhibitions than Foretinib. Moreover, against U87MG cell line compounds **4a**, **4e**, **5e**, **5f**, **5g**, **7c**, **7k**, **7l**, **7m**, **7o**, **9c** and **10a** were of high inhibitions greater than Foretinib. In addition, against SMMC-7721 cell line, twelve compounds exhibited higher inhibitions than the reference drug like compounds **4e**, **5e**, **5g**, **7c**, **7h**, **7k**, **7l**, **7m**, **7o**, **9a**, **9c** and **10a**.

In vitro anticancer activities of the synthesized compounds

Against a panel of approximately seventeen tumor cell lines at 10-fold dilutions of five concentrations (100, 10, 1.0, 0.1 and 0.01 µM) [35], the arylhydrazone derivatives **7c**, **7k**, **7l, 7m** and **7o** were selected for this assay. The tested compounds exhibited inhibitions (GI_{50} < 5 µM) against the cancer cell lines that are classified into groups according to the type of disease, the data were shown through Table 2. There are two factors in this work affecting inhibitions of compounds **7c**. **7k**, **7l**, **7m** and **7o**, the substituent at C-3 of the pyran ring and the substituents at the 4-position of the two aryl group. Table 2 explored that all tested compounds exhibited notable inhibitions toward the cell lines categorized according to the type of the disease. Compound **7c** (R = CN, X = H, Y = Cl) exhibited high inhibitions toward SR, HI-60, HOP-62, UACC-62 and UACC-62 cell lines with IG_{50}'s 0.52, 0.48, 0.40 and 0.57 µM. Moreover, compound **7k** (R = COOEt, X = Y = Cl) showed high inhibitions toward HI-60, K-526, HOP-62, NCI-H460, HCT-116, HCT-15, SF-295, U251, UACC-62, cell lines with IG_{50}'s 0.25, 0.29, 0.36, 0.48, 0.58, 0.31, 0.36 and 0.42 µM, respectively. Compound **7l** (R = CN, X = Cl, Y = CH_3) showed high inhibitions toward RPMI-8262, HI-60, HI-60, HOP-62, HCT-116, SF-295, MDA-MB-435, UACC-62, OVCAR-3 and 786-0 cell lines with IC_{50}'s 0.59, 0.29, 0.34, 0.31, 0.52, 0.31, 0.38, 0.24 and 0.48

μM. Compound **7m** (R = COOEt, X = Cl, Y = CH_3) showed high inhibitions toward K-526, HCT-116, HCT-15, MDA-MB-435 and 786-0 cell lines with IC_{50}'s 0.26, 0.37, 0.42 and 0.50 μM, respectively. Finally, compound **7o** (R = COOEt, X = OCH_3, Y = H) exhibited high inhibitions toward CCRF-CEM, K-526, NCI-H460 and U251 cell lines with IC_{50}'s 0.47, 0.33, 0.51 and 0.52 μM, respectively.

Table 2. Evaluations of compounds **7c**, **7k**, **7l, 7m** and **7o** toward the seventeen cancer cell lines (GI_{50} < 5 μM).

Type of disease	Cell line	**7c**	**7k**	**7l**	**7m**	**7o**
Leukemia	CCRF-CEM	1.08	0.95	1.52	3.61	0.47
Leukemia	RPMI-8262	0.86	0.72	0.59	1.47	1.29
Leukemia	SR	0.52	1.03	1.72	2.62	0.74
Leukemia	HI-60 (TB)	0.48	0.25	0.29	2.31	3.62
Leukemia	K-526	0.93	0.29	2.42	0.26	0.33
NCS-Lung cancer	HOP-62	0.40	0.36	0.34	1.28	2.40
NCS-Lung cancer	NCI-H460	0.70	0.48	1.36	1.92	0.51
Colon cancer	HCT-116	0.63	0.58	0.31	0.62	1.50
Colon cancer	HCT-15	0.85	0.31	3.48	0.37	0.61
Colon cancer	KM12	1.06	1.16	2.85	2.36	2.71
CNS cancer	SF-295	0.73	0.36	0.52	2.70	1.28
CNS cancer	U251	0.62	0.48	2.37	1.93	0.52
Melanoma	LOX IMVI	0.85	1.55	1.31	3.26	3.31
Melanoma	MDA-MB-435	0.68	0.72	0.31	0.42	0.71
Melanoma	UACC-62	0.57	0.42	0.38	0.50	1.26
Ovarian cancer	OVCAR-3	0.62	1.35	0.24	0.94	0.39
Renal cancer	786-0	0.69	0.73	0.48	0.26	1.17

Determination of morphological changes of A549 cell line

The lung is a unique organ that should be protected against inhaled pathogens and toxins, without unbalance immune responses or compromise its vital function. For that matter microenvironment of the lung tissue is regulated through complex and refined cell interactions [36, 37]. For that reason we studied the effect of compound **7k** toward A549 cell line which was selected for studying the morphological changes. There are many reports concerned with morphological changes of other cell lines [38, 39]. To understand the ability of compound **7k** in apoptosis induction, various qualitative (morphological) and quantitative assays were performed on the A549 lung cancer cell line. The changes in the morphological features of A549 cells were observed after the treatment with compound **7k** at different concentrations along with the untreated control cells. Further, images reported in Figure 1A were captured using phase-contrast microscopy after 72 h; reveal the characteristic apoptotic features like changes in morphology (shape, shrinkage) of the cell, reduction in the number of live cells. In the present study, A549 cells after treatment with compound **7k** for 72 h exhibited the formation of apoptotic features such as the appearance of membrane blebs and inverse proportion in the number of cells with a concentration of compound tested as indicated in Figure 1B. Compound **7k** was treated A549 cells after 72 h, on staining with DAPI visualized the chromatin condensation, pyknotic (inset of 1.25 μM), and condensed (bright colored: inset of 2.5 μM) nuclei formation as depicted in Figure 1C. Through figure 1the effect of compound **7k** on A549 cells marked by the red cells indicating reduction of the size of the cancer cell line .

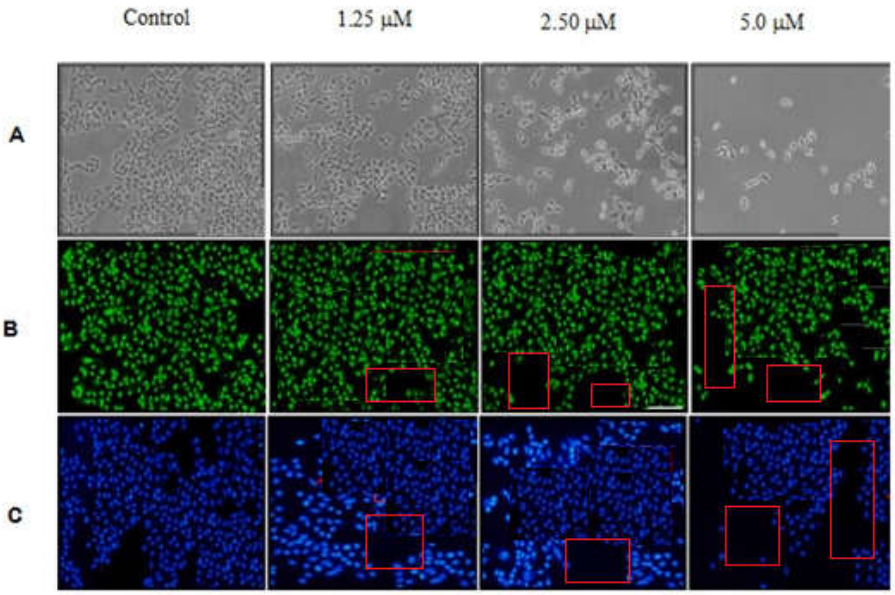


Figure 1. Microscopic observation of the effect of different concentrations (1.25, 2.5, and 5 µM) of compound **7k** in comparison to control (untreated) on A549 cells after 72 h of treatment. The red scale bar denotes 50 µM in all the images. (A) Morphological changes were observed through phase-contrast microscopy. (B) Morphological changes such as membrane blebs were visualized through a fluorescent microscope by performing acridine orange staining. (C) Nuclear changes were observed by DAPI staining using a fluorescent microscope. Insets at different concentrations of treatment indicate the changes induced by treatment and red-colored arrows specify the area of effect.

EXPERIMENTAL

Chemistry

Melting points were recorded using an electrothermal digital melting point apparatus. Using a FTIR plus 460 or PyeUnicam SP-1000 spectrophotometer the IR spectra were recorded. The ^{1}H NMR spectra were measured using Varian Gemini-300 (300 MHz) instrument. Moreover, the mass spectra were measured using Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex Shimadzu instruments. The CHNS microanalyses were using Vario EL III Elemental analyzer. The anti-proliferative evaluations were measured by the aid of the National Cancer Research Centre in Cairo.

*The synthesis of the pyran derivatives **4a-f***

Malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) and benzaldehyde (1.06 g, 0.01 mol), 4-methoxybenzaldehyde (1.37 g, 0.01 mol) or 4-chlorobenzaldehyde (1.40, 0.01 mol) were added to a solution of benzoylacetone (1.62 g, 0.01 mol) in absolute ethanol (50 mL, 0.01 mol) containing triethylamine (1.0 mL). The reaction mixture was heated under reflux for 3 h then left to cool and the formed solid product was collected by filtration.

*5-Acetyl-2-amino-4,6-diphenyl-4H-pyran-3-carbonitrile (**4a**)*. Dark orange crystals, yield (2.21 g, 70%), mp 100-102 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3472-3328 (NH_2), 3055 (CH, aromatic), 2220 (CN), 1705 (C=O), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.67 (s, 3H, CH_3), 4.80 (s, 2H, D_2O exchangeable, NH_2), 6.52 (s, 1H, pyran H-4), 7.26-7.59 (m, 10H, $2C_6H_5$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.8 (CH_3), 90.5 (pyran C-4), 117.3 (CN), 120.2, 121.3, 121.8, 122.4, 123.0, 123.6, 125.8, 127.6 ($2C_6H_5$), 129.3, 130.5, 131.5, 132.8 (pyran C-2, C-3, C-5, C-6), 166.2 (C=O). Anal. calcd. for $C_{20}H_{16}N_2O_2$: C, 75.93; H, 5.10; N, 8.86%. Found: C, 75.82; H, 5.23; N, 8.71%. *m/z* 316 (M^+, 46%).

*Ethyl 5-acetyl-2-amino-4,6-diphenyl-4H-pyran-3-carboxylate (**4b**)*. Pale yellow, yield (2.46 g, 68%), mp 56-58 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3395-3341 (NH_2), 3055 (CH, aromatic), 1705, 1685 (2C=O), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 6.90 Hz, $OCH_2\underline{CH_3}$), 2.66 (s, 3H, CH_3), 4.25 (q, 2H, *J* = 6.90 Hz, $O\underline{CH_2}CH_3$), (4.86 (s, 2H, D_2O exchangeable, NH_2), 6.47 (s, 1H, pyran H-4), 7.23-7.49 (m, 10H, $2C_6H_5$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.2 ($OCH_2\underline{CH_3}$), 36.7 (CH_3), 50.2 ($O\underline{CH_2}CH_3$), 90.3 (pyran C-4), 120.6, 121.1, 121.6, 122.6, 123.2, 123.8, 124.5, 126.3 ($2C_6H_5$), 129.6, 130.8, 131.2, 132.7 (pyran C-2, C-3, C-5, C-6), 165.8, 166.4 (2C=O). Anal. calcd. for $C_{22}H_{21}NO_4$: C, 72.71; H, 5.82; N, 3.85%. Found: C, 72.69; H, 5.93; N, 4.03%. m/z 363 (M^+, 58%).

*5-Acetyl-2-amino-4-(4-methoxyphenyl)-6-phenyl-4H-pyran-3-carbonitrile (**4c**)*. Pale yellow crystals, yield (2.67 g, 60%), mp 97-100 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3378-3329 (NH_2), 3053 (CH, aromatic), 2220 (CN), 1701 (C=O), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.69 (s, 3H, CH_3), 3.63 (s, 3H, OCH_3), 4.84 (s, 2H, D_2O exchangeable, NH_2), 6.45 (s, 1H, pyran H-4), 7.23-7.49 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.7 (CH_3), 50.8 (OCH_3), 90.1 (pyran C-4), 116.8 (CN), 120.3, 121.4, 120.0, 122.6, 123.5, 124.1, 124.5, 125.2 (C_6H_5, C_6H_4), 129.9, 131.2, 132.2, 133.1 (pyran C-2, C-3, C-5, C-6), 166.2 (C=O). Anal. calcd. for $C_{21}H_{18}N_2O_3$: C, 72.82; H, 5.24; N, 8.09%. Found: C, 72.66; H, 5.18; N, 8.21%. *m/z* 346 (M^+, 46%).

*Ethyl 5-acetyl-2-amino-4-(4-methoxyphenyl)-6-phenyl-4H-pyran-3-carboxylate (**4d**)*. Orange crystals, yield (2.55 g, 65%), mp 75-78 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3380-3336 (NH_2), 3053 (CH, aromatic), 1700, 1688 (2C=O), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.15 (t, 3H, *J* = 6.52 Hz, $OCH_2\underline{CH_3}$), 2.68 (s, 3H, CH_3), 3.70 (s, 3H, OCH_3), 4.23 (q, 2H, *J* = 6.52 Hz, $O\underline{CH_2}CH_3$), (4.84 (s, 2H, D_2O exchangeable, NH_2), 6.45 (s, 1H, pyran H-4), 7.21-7.55 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5 ($OCH_2\underline{CH_3}$), 36.3 (CH_3), 50.1 ($O\underline{CH_2}CH_3$), 52.3 (OCH_3), 90.4 (pyran C-4), 120.3, 120.8, 121.2, 122.8, 123.5, 124.1, 124.3, 126.1 (C_6H_5, C_6H_4), 129.8, 130.6, 131.1, 132.4 (pyran C-2, C-3, C-5, C-6), 166.3, 166.8 (2C=O). Anal. calcd. for $C_{23}H_{23}NO_5$: C, 70.21; H, 5.89; N, 3.56%. Found: C, 70.39; H, 5.67; N, 3.80%. *m/z* 393 (M^+, 70%).

*5-Acetyl-2-amino-4-(4-chlorophenyl)-6-phenyl-4H-pyran-3-carbonitrile (**4e**)*. Orange crystals, yield (2.55 g, 73%), mp 86-88 °C (ethanol). IR (KBr) ν_{max} cm^{-1}: 3468-3336 (NH_2), 3055 (CH, aromatic), 2220 (CN), 1702 (C=O), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.68 (s, 3H, CH_3), 4.83 (s, 2H, D_2O exchangeable, NH_2), 6.50 (s, 1H, pyran H-4), 7.22-7.56 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.6 (CH_3), 90.3 (pyran C-4), 117.1 (CN), 120.1, 120.6, 121.3, 122.6, 123.5, 124.3, 125.8, 126.3 (C_6H_5, C_6H_4), 129.6, 130.6, 131.8, 132.3 (pyran C-2, C-3, C-5, C-6), 166.8 (C=O). Anal. calcd. for $C_{20}H_{15}ClN_2O_2$: C, 68.48; H, 4.31; N, 7.99%. Found: C, 68.59; H, 4.60; N, 7.70%. m/z 350 (M^+, 26%).

*Ethyl 5-acetyl-2-amino-4-(4-chlorophenyl)-6-phenyl-4H-pyran-3-carboxylate (**4f**)*. White crystals, yield (3.31 g, 79%), mp 72-74 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3379-3352 (NH_2), 3055 (CH, aromatic), 1702, 1685 (2C=O), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.15 (t, 3H, *J* = 5.83 Hz, $OCH_2\underline{CH_3}$), 2.68 (s, 3H, CH_3), 4.23 (q, 2H, *J* = 5.83 Hz, $O\underline{CH_2}CH_3$), 4.85 (s, 2H,

D_2O exchangeable, NH_2), 6.49 (s, 1H, pyran H-4), 7.21-7.54 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5 ($OCH_2\underline{CH_3}$), 36.6 (CH_3), 50.1 ($O\underline{CH_2}CH_3$), 90.6 (pyran C-4), 120.4, 121.3, 121.8, 122.2, 122.5, 123.8, 124.8, 127.1 ($2C_6H_5$), 129.5, 130.4, 131.8, 132.8 (pyran C-2, C-3, C-5, C-6), 165.8, 166.6 (2C=O). Anal. calcd. for $C_{22}H_{20}ClNO_4$: C, 66.42; H, 5.07; N, 3.52%. Found: C, 66.59; H, 5.13; N, 3.74%. *m/z* 397 (M^+, 70%).

The synthesis of compounds ***5a-m***

Ammonium acetate (1.45 g, 0.02 mol) was added to either **4a** (3.16 g, 0.01 mol), **4b** (3.26 g, 0.01 mol), **4c** (3.46 g, 0.01 mol), **4d** (3.93 g, 0.01 mol), **4e** (3.50 g, 0.01 mol) or 4f (3.97 g, 0.01 mol) followed by addition of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) to the respective mixture. The whole reaction mixture was heated in an oil bath for 20 min then was left to cool producing a solid product that was collected by filtration after treatment with diethyl ether.

*2-(1-(6-Amino-5-cyano-2,4-diphenyl-4H-pyran-3-yl)ethylidene)malono-nitrile (**5a**)*. Orange crystals, yield (2.40 g, 66%), mp 225-228 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3493-3358 (NH_2), 3055 (CH, aromatic), 2222-2220 (3CN), 1632 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.92 (s, 3H, CH_3), 4.87 (s, 2H, D_2O exchangeable, NH_2), 6.50 (s, 1H, pyran H-4), 7.24-7.56 (m, 10H, $2C_6H_5$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.7 (CH_3), 86.3, 89.4 (C=C), 90.2 (pyran C-4), 116.8, 117.0, 117.2 (3CN), 120.3, 120.5, 121.4, 122.6, 123.2, 123.8, 124.3, 125.9 ($2C_6H_5$), 128.2, 130.8, 131.6, 133.2 (pyran C-2, C-3, C-5, C-6). Anal. calcd. for $C_{23}H_{16}N_4O$: C, 75.81; H, 4.43; N, 15.38%. Found: C, 75.62; H, 4.60; N, 15.43%. *m/z* 364 (M^+, 70%).

*Ethyl 2-amino-5-(1,1-dicyanoprop-1-en-2-yl)-4,6-diphenyl-4H-pyran-3-carboxylate (**5b**)*. Pale yellow crystals, yield (2.83 g, 69%), mp 120-122 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3387-3339 (NH_2), 3055 (CH, aromatic), 2223, 2221 (2CN), 1684 (CO), 1632 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.15 (t, 3H, *J* = 7.21 Hz, $OCH_2\underline{CH_3}$), 2.66 (s, 3H, CH_3), 4.23 (q, 2H, *J* = 7.21 Hz, $O\underline{CH_2}CH_3$), 4.88 (s, 2H, D_2O exchangeable, NH_2), 6.50 (s, 1H, pyran H-4), 7.24-7.45 (m, 10H, $2C_6H_5$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5 ($OCH_2\underline{CH_3}$), 38.9 (CH_3), 50.1 ($O\underline{CH_2}CH_3$), 86.7, 88.3 (C=C), 90.2 (pyran C-4), 116.8, 117.1 (2CN), 120.4, 121.5, 121.9, 122.3, 123.7, 123.3, 124.8, 126.1 ($2C_6H_5$), 129.3, 130.5, 131.5, 132.8 (pyran C-2, C-3, C-5, C-6), 165.4 (CO). Anal. calcd. for $C_{25}H_{21}N_3O_3$: C, 72.98; H, 5.14; N, 10.21%. Found: C, 72.65; H, 5.25; N, 10.08%. *m/z* 411 (M^+, 70%).

*Ethyl 3-(6-amino-5-cyano-2,4-diphenyl-4H-pyran-3-yl)-2-cyanobut-2-enoate (**5c**)*. Pale brown crystals, yield (2.58 g, 63%), mp 222-225 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3377-3342 (NH_2), 3055 (CH, aromatic), 2222, 2220 (2CN), 1688 (CO), 1632 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 7.01 Hz, $OCH_2\underline{CH_3}$), 2.68 (s, 3H, CH_3), 4.24 (q, 2H, *J* = 7.01 Hz, $O\underline{CH_2}CH_3$), 4.87 (s, 2H, D_2O exchangeable, NH_2), 6.56 (s, 1H, pyran H-4), 7.25-7.48 (m, 10H, $2C_6H_5$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.8 ($OCH_2\underline{CH_3}$), 38.6 (CH_3), 50.2 ($O\underline{CH_2}CH_3$), 86.4, 88.6 (C=C), 90.8 (pyran C-4), 120.2, 121.8, 121.7, 122.3, 123.9, 124.1, 124.7, 126.6 ($2C_6H_5$), 129.4, 130.2, 131.8, 133.4 (pyran C-2, C-3, C-5, C-6). Anal. calcd. for $C_{25}H_{21}N_3O_3$: C, 72.98; H, 5.14; N, 10.21%. Found: C, 72.75; H, 5. 32; N, 10.41%. *m/z* 411 (M^+, 58%).

*Ethyl 2-amino-5-(3-cyano-4-ethoxy-4-oxobut-2-en-2-yl)-4,6-diphenyl-4H-pyran-3-carboxylate (**5d**)*. Yellow crystals, yield (3.25 g, 71%), mp 133-137 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3359-3322 (NH_2), 3056 (CH, aromatic), 2220 (CN), 1689, 1687 (2CO), 1636 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.14, 1.1.6 (2t, 6H, *J* = 6.11, 6.70 Hz, two $OCH_2\underline{CH_3}$), 2.66 (s, 3H, CH_3), 4.23, 4.26 (2q, 4H, *J* = 6.11, 6.70 Hz, two $O\underline{CH_2}CH_3$), 4.83 (s, 2H, D_2O exchangeable, NH_2), 6.57 (s, 1H, pyran H-4), 7.25-7.49 (m, 10H, $2C_6H_5$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16,3, 16.6 (two $OCH_2\underline{CH_3}$), 38.9 (CH_3), 50.8, 50.1 (two $O\underline{CH_2}CH_3$), 90.6 (pyran C-4), 116.7 (CN),

120.3, 121.4, 121.8, 122.2, 122.9, 123.1, 124.8, 125.9 ($2C_6H_5$), 129.7, 130.2, 131.8, 133.2 (pyran C-2, C-3, C-5, C-6), 165.8, 166.1 (2CO). Anal. calcd. for $C_{27}H_{26}N_2O_5$: C, 70.73; H, 5.72; N, 6.11%. Found: C, 70.69; H, 5.53; N, 6.04%. *m/z* 458 (M^+, 60%).

2-(1-(6-Amino-4-(4-chlorophenyl)-5-cyano-2-phenyl-4H-pyran-3-yl)ethylidene)-malononitrile ***(5e)***.Yellowish brown crystals, yield (2.62 g, 66%), mp 215-218 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3481-3335 (NH_2), 3055 (CH, aromatic), 2223-2220 (3CN), 1635 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.96 (s, 3H, CH_3), 4.85 (s, 2H, D_2O exchangeable, NH_2), 6.53 (s, 1H, pyran H-4), 7.23-7.59 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.9 (CH_3), 86.5, 87.2 (C=C), 90.3 (pyran C-4), 116.5, 116.8, 117.2 (3CN), 120.1, 120.3, 121.6, 122.8, 123.8, 123.8, 124.4, 126.3 (C_6H_5, C_6H_4), 128.6, 130.4, 131.3, 133.5 (pyran C-2, C-3, C-5, C-6). Anal. calcd. for $C_{23}H_{15}ClN_4O$: C, 69.26; H, 3.79; N, 14.05%. Found: C, 69.53; H, 3.83; N, 14.29%. *m/z* 398 (M^+, 55%).

Ethyl 2-amino-4-(4-chlorophenyl)-5-(1,1-dicyanoprop-1-en-2-yl)-6-phenyl-4H-pyran-3-carboxylate ***(5f)***. Pale brown crystals, yield (2.93 g, 66 %), mp 120-122 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3386-3329 (NH_2), 3054 (CH, aromatic), 2223, 2220 (2CN), 1690 (CO), 1637 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 7.27 Hz, $OCH_2\underline{CH_3}$), 2.65 (s, 3H, CH_3), 4.23 (q, 2H, *J* = 7.27 Hz, $O\underline{CH_2}CH_3$), 4.84 (s, 2H, D_2O exchangeable, NH_2), 6.55 (s, 1H, pyran H-4), 7.24-7.57 (m, 9H, C_6H_5, C_6H_4), 165.8 (CO); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.6 ($OCH_2\underline{CH_3}$), 38.5 (CH_3), 50.4 ($O\underline{CH_2}CH_3$), 86.4, 87.5 (C=C), 90.7 (pyran C-4), 116.9, 117.3 (2CN), 120.1, 121.4, 121.8, 123.5, 123.9, 124.5, 125.1, 125.4 ($2C_6H_5$), 129.9, 130.2, 131.8, 132.7 (pyran C-2, C-3, C-5, C-6), 166.4 (CO). Anal. calcd. for $C_{25}H_{20}ClN_3O_3$: C, 67.34; H, 4.52; N, 9.42%. Found: C, 67.16; H, 4.80; N, 9.60%. *m/z* 445 (M^+, 49%).

Ethyl 3-(6-amino-4-(4-chlorophenyl)-5-cyano-2-phenyl-4H-pyran-3-yl)-2-cyanobut-2-enoate ***(5g)***. Brown crystals, yield (3.29 g, 74%), mp 198-200 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3391-3342 (NH_2), 3056 (CH, aromatic), 2222, 2220 (2CN), 1688 (CO), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.14 (t, 3H, *J* = 6.72 Hz, $OCH_2\underline{CH_3}$), 2.68 (s, 3H, CH_3), 4.25 (q, 2H, *J* = 5.80 Hz, $O\underline{CH_2}CH_3$), 4.85 (s, 2H, D_2O exchangeable, NH_2), 6.59 (s, 1H, pyran H-4), 7.21-7.58 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.8 ($OCH_2\underline{CH_3}$), 38.7 (CH_3), 50.2 ($O\underline{CH_2}CH_3$), 86.4, 87.5 (C=C), 90.8 (pyran C-4), 116.3, 117.1 (2CN), 120.3, 120.8, 121.6, 122.3, 123.2, 123.5, 124.8, 125.6 ($2C_6H_5$), 129.7, 130.4, 131.8, 132.5 (pyran C-2, C-3, C-5, C-6), 166.1 (CO). Anal. calcd. for $C_{25}H_{20}ClN_3O_3$: C, 67.34; H, 4.52; N, 9.42%. Found: C, 67.52; H, 4.70; N, 9.57%. *m/z* 445 (M^+, 60%).

Ethyl 2-amino-4-(4-chlorophenyl)-5-(3-cyano-4-ethoxy-4-oxobut-2-en-2-yl)-6-phenyl-4H-pyran-3-carboxylate ***(5h)***. Yellow crystals, yield (2.83 g, 62%), mp 83-85 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3379-3341 (NH_2), 3055 (CH, aromatic), 1688, 1685 (2CO), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.14, 1.1.6 (2t, 6H, *J* = 6.11, 6.70 Hz, two $OCH_2\underline{CH_3}$), 2.66 (s, 3H, CH_3), 4.23, 4.26 (2q, 4H, *J* = 6.11, 6.70 Hz, two $O\underline{CH_2}CH_3$), 4.83 (s, 2H, D_2O exchangeable, NH_2), 6.57 (s, 1H, pyran H-4), 7.25-7.49 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16,3, 16.6 (two $OCH_2\underline{CH_3}$), 38.9 (CH_3), 50.8, 50.1 (two $O\underline{CH_2}CH_3$), 86.5, 87.8 (C=C), 90.6 (pyran C-4), 116.9 (CN), 120.3, 121.4, 121.8, 122.2, 122.9, 123.1, 124.8, 125.9 (C_6H_5, C_6H_4), 129.7, 130.2, 131.8, 133.2 (pyran C-2, C-3, C-5, C-6), 165.8, 166.2 (2CO). Anal. calcd. for $C_{27}H_{25}ClN_2O_5$: C, 65.79; H, 5.11; N, 5.68%. Found: C, 65.82; H, 5.31; N, 5.80%. *m/z* 492 (M^+, 60%).

2-(1-(6-Amino-5-cyano-4-(4-methoxyphenyl)-2-phenyl-4H-pyran-3-yl)ethylidene)-malononitrile ***(5i)***. Brown crystals,yield (2.95 g, 75%), mp 94-96 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3458-3320 (NH_2), 3055 (CH, aromatic), 2222-2220 (3CN), 1635 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.92 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 4.87 (s, 2H, D_2O exchangeable, NH_2), 6.58 (s,

1H, pyran H-4), 7.21-7.57 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 39.3 (CH_3), 50.6 (OCH_3), 86.5, 87.8 (C=C), 90.4 (pyran C-4), 116.7, 116.9, 117.1 (3CN), 120.3, 120.6, 121.8, 122.3, 122.7, 123.9, 124.2, 125.7 (C_6H_5, C_6H_4), 128.4, 130.2, 131.6, 133.7 (pyran C-2, C-3, C-5, C-6). Anal. calcd. for $C_{24}H_{18}N_4O_2$: C, 73.08; H, 4.60; N, 14.20%. Found: C, 72.83; H, 4.49; N, 14.38%. *m/z* 394 (M^+, 49%).

*Ethyl 2-amino-5-(1,1-dicyanoprop-1-en-2-yl)-4-(4-methoxyphenyl)-6-phenyl-4H-pyran-3-carboxylate (**5k**)*. Orange crystals, yield (2.99 g, 68%), mp 210-212 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3394-3342 (NH_2), 3056 (CH, aromatic), 2221 (CN), 1688 (CO), 1635 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 6.80 Hz, $OCH_2\underline{CH_3}$), 2.67 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 4.24 (q, 2H, *J* = 6.80 Hz, $O\underline{CH_2}CH_3$), 4.89 (s, 2H, D_2O exchangeable, NH_2), 6.52 (s, 1H, pyran H-4), 7.26-7.54 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5 ($OCH_2\underline{CH_3}$), 38.7 (CH_3), 50.2 ($O\underline{CH_2}CH_3$), 50.8 (OCH_3), 86.4, 87.7 (C=C), 90.5 (pyran C-4), 116.7, 117.1 (2CN), 120.3, 121.6, 121.9, 123.9, 123.7, 124.3, 125.2, 125.6 (C_6H_5, C_6H_4), 130.4, 130.8, 131.5, 132.9 (pyran C-2, C-3, C-5, C-6), 166.2 (CO). Anal. calcd. for $C_{26}H_{23}N_3O_4$: C, 70.73; H, 5.25; N, 9.52%. Found: C, 70.92; H, 4.98; N, 9.67%. *m/z* 441 (M^+, 62%).

*Ethyl 3-(6-amino-5-cyano-4-(4-methoxyphenyl)-2-phenyl-4H-pyran-3-yl)-2-cyanobut-2-enoate (**5l**)*. Pale brown crystals, yield (3.71 g, 72%), mp 80-82 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3358-3322 (NH_2), 3055 (CH, aromatic), 2223, 2220 (2CN), 1686 (CO), 1635 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 6.22 Hz, $OCH_2\underline{CH_3}$), 2.69 (s, 3H, CH_3), 3.71 (s, 3H, OCH_3), 4.24 (q, 2H, *J* = 6.22 Hz, $O\underline{CH_2}CH_3$), 4.87 (s, 2H, D_2O exchangeable, NH_2), 6.54 (s, 1H, pyran H-4), 7.23-7.59 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.7 ($OCH_2\underline{CH_3}$), 38.6 (CH_3), 50.2 ($O\underline{CH_2}CH_3$), 50.5 (OCH_3), 86.4, 87.9 (C=C), 90.5 (pyran C-4), 116.9, 117.3 (2CN), 120.2, 120.8, 121.5, 123.8, 123.3, 124.6, 125.4, 125.8 (C_6H_5, C_6H_4), 130.1, 130.6, 131.8, 132.7 (pyran C-2, C-3, C-5, C-6), 166.2 (CO). Anal. calcd. for $C_{26}H_{23}N_3O_4$: C, 70.73; H, 5.25; N, 9.52%. Found: C, 70.92; H, 4.98; N, 9.67%. *m/z* 441 (M^+, 82%).

*Ethyl 2-amino-5-(3-cyano-4-ethoxy-4-oxobut-2-en-2-yl)-4-(4-methoxy-phenyl)-6-phenyl-4H-pyran-3-carboxylate (**5m**)*. Brown crystals, yield (3.41 g, 70%), mp 68-71°C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3384-3352 (NH_2), 3055 (CH, aromatic), 1702, 1688 (2CO), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.14, 1.1.7 (2t, 6H, *J* = 6.40, 7.10 Hz, two $OCH_2\underline{CH_3}$), 2.68 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 4.23, 4.25 (2q, 4H, *J* = 6.40, 7.10 Hz, two $O\underline{CH_2}CH_3$), 4.85 (s, 2H, D_2O exchangeable, NH_2), 6.54 (s, 1H, pyran H-4), 7.23-7.54 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.4, 16.6 (two $OCH_2\underline{CH_3}$), 38.7 (CH_3), 50.8, 50.1 (two $O\underline{CH_2}CH_3$), 50.8 (OCH_3), 86.4, 87.9 (C=C), 90.8 (pyran C-4), 117.0 (CN), 120.2, 121.6, 121.9, 122.4, 122.5, 123.1, 124.7, 125.8 ($2C_6H_5$), 129.8, 130.4, 132.6, 133.6 (pyran C-2, C-3, C-5, C-6), 165.4, 166.3 (2CO). Anal. calcd. for $C_{28}H_{28}N_2O_6$: C, 68.84; H, 5.78; N, 5.73%. Found: C, 68.93; H, 5. 63; N, 5.82%. *m/z* 488 (M^+, 68%).

*The synthesis of the arylhydrazone derivatives **7a-l**.*

The diazonium salts (0.01 mol) [prepared by the addition of a solution of sodium nitrite (0.70 g, 0.01 mol) in water (10 mL) to a cold solution (0-5 ºC) of aniline (0.93 g, 0.01 mol), 4-chloroaniline (1.27 g, 0.01 mol) or 4-methylaniline (1.07 g, 0.01 mol) dissolved in hydrochloric acid (10 mL, 18 mol) with continuous stirring] was added to a solution of compounds **4a** (3.16 g, 0.01 mol), **4b** (3.63, 0.01 mol), **4c** (3.46 g, 0.01 mol), **4d** (3.50 g, 0.01 mol), **4e** (3.50 g, 0.01 mol) or **4f** (3.97 g, 0.01 mol) in ethanol (50 mL)through which sodium acetate (3.0 g) was added with stirring. The reaction mixture was left at room temperature for additional 2 h and the formed solid product was collected by filtration.

*2-Amino-4,6-diphenyl-5-(2-(2-phenylhydrazono)acetyl)-4H-pyran-3-carbonitrile (**7a**)*. Red crystals, yield (3.23 g, 77%), mp 87-89 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3492-3337 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1680 (C=O), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 4.84 (s, 2H, D_2O exchangeable, NH_2), 6.56 (s, 1H, pyran H-4), 6.80 (s, 1H, CH=N), 7.26-7.48 (m, 15H, $3C_6H_5$), 8.25 (s, 1H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 90.5 (pyran C-4), 117.1 (CN), 120.2, 120.8, 121.1, 121.8, 122.2, 122.6, 123.3, 123.4, 124.6, 125.8, 126.2, 127.3 ($3C_6H_5$), 129.6, 130.6, 131.8, 133.2 (pyran C-2, C-3, C-5, C-6), 165.8 (C=O), 174.8 (C=N). Anal. calcd. for $C_{26}H_{20}N_4O_2$: C, 74.27; H, 4.79; N, 13.33%. Found: C, 74.38; H, 4.83; N, 13.50%. *m/z* 420 (M^+, 72%).

*Ethyl 2-amino-4,6-diphenyl-5-(2-(2-phenylhydrazono)acetyl)-4H-pyran-3-carboxylate (**7b**)*. Pale yellow crystals, yield (2.80 g, 60%), mp 135-135 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3483-3368 (NH, NH_2), 3055 (CH, aromatic), 1750, 1688 (2CO), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 6.88 Hz, $OCH_2\underline{CH_3}$), 4.23 (q, 2H, *J* = 6.88 Hz, $O\underline{CH_2}CH_3$), 4.89 (s, 2H, D_2O exchangeable, NH_2), 6.56 (s, 1H, pyran H-4), 6.83 (s, 1H, N=CH), 7.22-7.48 (m, 15H, $3C_6H_5$), 8.36 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.6 ($OCH_2\underline{CH_3}$), 50.1 ($O\underline{CH_2}CH_3$), 90.6 (pyran C-4), 120.2, 120.4, 121.8, 121.7, 122.3, 122.6, 123.9, 124.1, 124.7, 125.2, 125.4, 126.6 ($3C_6H_5$), 129.1, 130.6, 131.2, 133.6 (pyran C-2, C-3, C-5, C-6), 165.8, 166.3 (2CO), 170.2 (C=N). Anal. calcd. for $C_{28}H_{25}N_3O_4$: C, 71.93; H, 5.39; N, 8.99%. Found: C, 72.22; H, 5. 47; N, 9.14%. *m/z* 467 (M^+, 42%).

*2-Amino-5-(2-(2-(4-chlorophenyl)hydrazono)acetyl)-4,6-diphenyl-4H-pyran-3-carbonitrile (**7c**)*. Red crystals from, yield (3.04 g, 67%), mp 88-90 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3469-3348 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1683 (C=O), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 4.86 (s, 2H, D_2O exchangeable, NH_2), 6.54 (s, 1H, pyran H-4), 6.83 (s, 1H, CH=N), 7.22-7.54 (m, 14H, $2C_6H_5$, C_6H_4), 8.23 (s, 1H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 90.6 (pyran C-4), 117.3 (CN), 120.3, 120.5, 121.4, 122.2, 122.5, 123.6, 123.8, 124.2, 124.7, 125.4, 126.5, 126.9 ($2C_6H_5$, C_6H_4), 129.3, 130.3, 131.7, 133.8 (pyran C-2, C-3, C-5, C-6), 165.5 (C=O), 174.4 (C=N). Anal. calcd. for $C_{26}H_{19}ClN_4O_2$: C, 68.65; H, 4.21; N, 12.32%. Found: C, 68.42; H, 4.48; N, 12.51%. *m/z* 454 (M^+, 31%).

*Ethyl 2-amino-5-(2-(2-(4-chlorophenyl)hydrazono)acetyl)-4,6-diphenyl-4H-pyran-3-carboxylate (**7d**)*. Pale yellow crystals, yield (3.00 g, 60%), mp 145-147 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3446-3385 (NH, NH_2), 3055 (CH, aromatic), 1680 (CO), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 5.92 Hz, $OCH_2\underline{CH_3}$), 4.22 (q, 2H, *J* = 5.92 Hz, $O\underline{CH_2}CH_3$), 4.86 (s, 2H, D_2O exchangeable, NH_2), 6.54 (s, 1H, pyran H-4), 6.83 (s, 1H, N=CH), 7.23-7.56 (m, 14H, $2C_6H_5$, C_6H_4), 8.34 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5 ($OCH_2\underline{CH_3}$), 50.3 ($O\underline{CH_2}CH_3$), 90.4 (pyran C-4), 120.1, 120.6, 121.5, 121.8, 122.1, 122.7, 123.6, 124.3, 124.8, 125.4, 125.2, 126.3 ($3C_6H_5$), 129.5, 130.4, 132.7, 133.2 (pyran C-2, C-3, C-5, C-6), 166.0, 166.5 (2CO), 170.8 (C=N). Anal. calcd. for $C_{28}H_{24}ClN_3O_4$: C, 67.00; H, 4.82; N, 8.37%. Found: C, 67.23; H, 5.02; N, 8.58%. *m/z* 501 (M^+, 38%).

*2-Amino-4,6-diphenyl-5-(2-(2-(p-tolyl)hydrazono)acetyl)-4H-pyran-3-carbonitrile (**7e**)*. Brown crystals, yield (3.12 g, 72%), mp 100-103 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3492-3329 (NH, NH_2), 3054 (CH, aromatic), 2220 (CN), 1687 (C=O), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.64 (s, 3H, CH_3), 4.89 (s, 2H, D_2O exchangeable, NH_2), 6.56 (s, 1H, pyran H-4), 6.85 (s, 1H, CH=N), 7.24-7.49 (m, 14H, $2C_6H_5$, C_6H_4), 8.25 (s, 1H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.8 (CH_3), 90.3 (pyran C-4), 117.1 (CN), 120.4, 120.8, 121.1, 122.5, 123.2, 123.2, 123.7, 124.1, 124.6, 125.2, 126.3, 126.5 ($2C_6H_5$, C_6H_4), 129.4, 130.6, 131.4, 133.7 (pyran C-2, C-3, C-5, C-6), 165.8 (C=O), 174.6 (C=N). Anal. calcd. for $C_{27}H_{22}N_4O_2$: C, 74.64; H, 5.10; N, 12.89%. Found: C, 74.80; H, 4.90; N, 12.71%. *m/z* 434 (M^+, 60%).

*Ethyl 2-amino-5-(2-(2-(4-chlorophenyl)hydrazono)acetyl)-4,6-diphenyl-4H-pyran-3-carboxylate (**7f**)*. Orange crystals, yield (3.00 g, 60%), mp 166-168 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3446-3385 (NH, NH_2), 3055 (CH, aromatic), 1680 (CO), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 5.92 Hz, $OCH_2\underline{CH_3}$), 2.80 (s, 3H, CH_3), 4.22 (q, 2H, *J* = 5.92 Hz, $O\underline{CH_2}CH_3$), 4.86 (s, 2H, D_2O exchangeable, NH_2), 6.54 (s, 1H, pyran H-4), 6.83 (s, 1H, N=CH), 7.23-7.56 (m, 14H, $2C_6H_5$, C_6H_4), 8.34 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5 ($OCH_2\underline{CH_3}$), 36.2 (CH_3), 50.3 ($O\underline{CH_2}CH_3$), 90.4 (pyran C-4), 120.1, 120.6, 121.5, 121.8, 122.1, 122.7, 123.6, 124.3, 124.8, 125.4, 125.2, 126.3 ($3C_6H_5$), 129.5, 130.4, 132.7, 133.2 (pyran C-2, C-3, C-5, C-6), 166.2, 166.8 (2CO), 170.6 (C=N). Anal. calcd. for $C_{29}H_{27}N_3O_4$: C, 72.33; H, 5.65; N, 8.73%. Found: C, 72.26; H, 5.42; N, 8.80%. *m/z* 481 (M^+, 58%).

*2-Amino-4-(4-chlorophenyl)-6-phenyl-5-(2-(2-phenylhydrazono)acetyl)-4H-pyran-3-carbonitrile (**7g**)*. Red crystals, yield (2.86 g, 63%), mp 116-118 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3488-3347 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1685 (C=O), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 4.82 (s, 2H, D_2O exchangeable, NH_2), 6.53 (s, 1H, pyran H-4), 6.85 (s, 1H, CH=N), 7.22-7.58 (m, 14H, $2C_6H_5$, C_6H_4), 8.27 (s, 1H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 90.6 (pyran C-4), 117.4 (CN), 120.0, 120.6, 121.3, 121.8, 122.5, 122.9, 123.1, 123.4, 124.4, 125.7, 126.7, 127.1 ($3C_6H_5$), 129.8, 130.9, 131.2, 133.4 (pyran C-2, C-3, C-5, C-6), 165.6 (C=O), 174.4 (C=N). Anal. calcd. for $C_{26}H_{19}ClN_4O_2$: C, 68.65; H, 4.21; N, 12.32%. Found: C, 68.70; H, 4.41; N, 12.60%. *m/z* 454 (M^+, 60%).

*Ethyl 2-amino-4-(4-chlorophenyl)-6-phenyl-5-(2-(2-phenylhydrazono)-acetyl)-4H-pyran-3-carboxylate (**7h**)*. Brown crystals, yield (3.40 g, 68%), mp 102-104 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3469-3348 (NH, NH_2), 3055 (CH, aromatic), 1687 (CO), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.15 (t, 3H, *J* = 5.98 Hz, $OCH_2\underline{CH_3}$), 4.22 (q, 2H, *J* = 5.98Hz, $O\underline{CH_2}CH_3$), 4.87 (s, 2H, D_2O exchangeable, NH_2), 6.54 (s, 1H, pyran H-4), 6.85 (s, 1H, N=CH), 7.24-7.53 (m, 14H, $2C_6H_5$, C_6H_4), 8.34 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.7 ($OCH_2\underline{CH_3}$), 50.3 ($O\underline{CH_2}CH_3$), 90.8 (pyran C-4), 120.1, 120.6, 121.5, 121.9, 122.5, 122.7, 123.4, 124.6, 124.7, 125.7, 125.9, 126.2 ($2C_6H_5$, C_6H_4), 129.4, 130.8, 131.5, 133.8 (pyran C-2, C-3, C-5, C-6), 166.2, 1665 (2CO), 170.5 (C=N). Anal. calcd. for $C_{28}H_{24}ClN_3O_4$: C, 67.00; H, 4.82; N, 8.37%. Found: C, 67.28; H, 4.93; N, 8.49%. *m/z* 501 (M^+, 58%).

*2-Amino-4-(4-chlorophenyl)-5-(2-(2-(4-chlorophenyl)hydrazono)acetyl)-6-phenyl-4H-pyran-3-carbonitrile (**7i**)*. Red crystals, yield (3.52 g, 72%), mp 95-97 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3468-3353 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1687 (C=O), 1633 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 4.83 (s, 2H, D_2O exchangeable, NH_2), 6.51 (s, 1H, pyran H-4), 6.84 (s, 1H, CH=N), 7.24-7.56 (m, 13H, C_6H_5, $2C_6H_4$), 8.27 (s, 1H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 90.6 (pyran C-4), 116.8 (CN), 120.2, 121.1, 121.3, 122.2, 122.5, 123.0, 123.5, 124.2, 124.6, 125.7, 126.4, 127.0 (C_6H_5, $2C_6H_4$), 129.6, 130.7, 131.6, 133.1 (pyran C-2, C-3, C-5, C-6), 165.8 (C=O), 174.2 (C=N). Anal. calcd. for $C_{26}H_{18}Cl_2N_4O_2$: C, 63.81; H, 3.71; N, 11.45%. Found: C, 64.21; H, 3.90; N, 11.53%. *m/z* 489 (M^+, 55%).

*Ethyl 2-amino-4-(4-chlorophenyl)-5-(2-(2-(4-chlorophenyl)hydrazono)-acetyl)-6-phenyl-4H-pyran-3-carboxylate (**7k**)*. Orange crystals, yield (2.94 g, 55%), mp 85-87 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3483-3336 (NH, NH_2), 3055 (CH, aromatic), 1688, 1685 (CO), 1633 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 5.98 Hz, $OCH_2\underline{CH_3}$), 4.26 (q, 2H, *J* = 5.98 Hz, $O\underline{CH_2}CH_3$), 4.85 (s, 2H, D_2O exchangeable, NH_2), 6.56 (s, 1H, pyran H-4), 6.82 (s, 1H, N=CH), 7.21-7.58 (m, 13H, C_6H_5, $2C_6H_4$), 8.36 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5 ($OCH_2\underline{CH_3}$), 50.1 ($O\underline{CH_2}CH_3$), 90.6 (pyran C-4), 120.3, 120.8, 121.2, 121.7, 122.9, 123.1, 123.4, 124.5, 124.9, 125.2, 125.8, 126.0 (C_6H_5, $2C_6H_4$), 129.2, 130.5, 131.3, 133.5 (pyran

C-2, C-3, C-5, C-6), 165.8, 166.8 (2CO, 170.3 (C=N). Anal. calcd. for $C_{28}H_{23}Cl_2N_3O_4$: C, 62.69; H, 4.32; N, 7.83%. Found: C, 62.73; H, 4.46; N, 8.02%. *m/z* 536 (M^+, 49%).

*2-Amino-4-(4-chlorophenyl)-6-phenyl-5-(2-(2-(p-tolyl)hydrazono)acetyl)-4H-pyran-3-carbonitrile (**7l**)*. Red crystals, yield (3.08 g, 66%), mp 110-112 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3478-3341 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1686 (C=O), 1633 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.69 (s, 3H, CH_3), 4.79 (s, 2H, D_2O exchangeable, NH_2), 6.53 (s, 1H, pyran H-4), 6.82 (s, 1H, CH=N), 7.22-7.58 (m, 13H, C_6H_5, $2C_6H_4$), 8.26 (s, 1H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.2 (CH_3), 90.4 (pyran C-4), 117.2 (CN), 120.0, 121.4, 121.8, 122.4, 122.7, 123.0, 123.8, 124.3, 124.6, 125.9, 126.1, 126.8 (C_6H_5, $2C_6H_4$), 129.4, 130.8, 131.3, 133.6 (pyran C-2, C-3, C-5, C-6), 165.5 (C=O), 174.1 (C=N). Anal. calcd. for $C_{27}H_{21}ClN_4O_2$: C, 69.15; H, 4.51; N, 11.95%. Found: C, 68.93; H, 4.63; N, 11.72 %. *m/z* 468 (M^+, 78%).

*Ethyl 2-amino-4-(4-chlorophenyl)-5-(2-(2-(4-chlorophenyl)hydrazono)-acetyl)-6-phenyl-4H-pyran-3-carboxylate (**7m**)*. Orange crystals, yield (2.88 g, 56%), mp 70-72 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3496-3327 (NH, NH_2), 3055 (CH, aromatic), 1688 (CO), 1631 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 6.80Hz, $OCH_2\underline{CH_3}$), 2.80 (s, 3H, CH_3), 4.23 (q, 2H, *J* = 6.80 Hz, $O\underline{CH_2}CH_3$), 4.87 (s, 2H, D_2O exchangeable, NH_2), 6.58 (s, 1H, pyran H-4), 6.80 (s, 1H, N=CH), 7.23-7.54 (m, 13H, C_6H_5, $2C_6H_4$), 8.36 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.7 ($OCH_2\underline{CH_3}$), 36.7 (CH_3), 50.3 ($O\underline{CH_2}CH_3$), 90.8 (pyran C-4), 120.5, 120.9, 121.1, 121.3, 122.2, 122.6, 123.2, 124.6, 125.0, 125.6, 126.0, 126.3 (C_6H_5, $2C_6H_4$), 129.6, 130.8, 131.3, 133.2 (pyran C-2, C-3, C-5, C-6), 166.1, 166.6 (2CO), 170.2 (C=N).. Anal. calcd. for $C_{29}H_{26}ClN_3O_4$: C, 67.50; H, 5.08; N, 8.14%. Found: C, 67.41; H, 4.84; N, 8.30%. *m/z* 515 (M^+, 68%).

*2-Amino-4-(4-methoxyphenyl)-6-phenyl-5-(2-(2-phenylhydrazono)acetyl)-4H-pyran-3-carbonitrile (**7n**)*. Red crystals, yield (2.70 g, 60%), mp 75-77 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3492-3357 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (C=O), 1633 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 3.70 (s, 3H, OCH_3), 4.82 (s, 2H, D_2O exchangeable, NH_2), 6.56 (s, 1H, pyran H-4), 6.81 (s, 1H, CH=N), 7.22-7.58 (m, 14H, $2C_6H_5$, C_6H_4), 8.26 (s, 1H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 50.8 (OCH_3), 90.4 (pyran C-4), 117.2 (CN), 120.0, 121.4, 121.8, 122.4, 122.7, 123.0, 123.8, 124.3, 124.6, 125.9, 126.1, 126.8 ($2C_6H_5$, C_6H_4), 129.4, 130.8, 131.3, 133.6 (pyran C-2, C-3, C-5, C-6), 165.8 (C=O), 174.4 (C=N). Anal. calcd. for $C_{27}H_{22}N_4O_3$: C, 71.99; H, 4.92; N, 12.44%. Found: C, 72.25; H, 4.68; N, 12.59%. *m/z* 450 (M^+, 42%).

*Ethyl 2-amino-4-(4-methoxyphenyl)-6-phenyl-5-(2-(2-phenylhydrazono)-tyl)-4H-pyran-3-carboxylate (**7o**)*. Orange crystals , yield (3.47 g, 70%), mp 120-122 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3480-3331 (NH, NH_2), 3055 (CH, aromatic), 1688 (CO), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.15 (t, 3H, *J* = 6.59 Hz, $OCH_2\underline{CH_3}$), 3.72 (s, 3H, OCH_3), 4.22 (q, 2H, *J* = 6.59 Hz, $O\underline{CH_2}CH_3$), 4.85 (s, 2H, D_2O exchangeable, NH_2), 6.59 (s, 1H, pyran H-4), 6.82 (s, 1H, N=CH), 7.21-7.49 (m, 14H, $2C_6H_5$, C_6H_4), 8.38 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5 ($OCH_2\underline{CH_3}$), 50.1 ($O\underline{CH_2}CH_3$), 50.6 (OCH_3), 90.5 (pyran C-4), 120.2, 120.7, 121.6, 121.9, 122.0, 122.8, 123.5, 124.2, 124.5, 125.3, 126.2, 126.4 ($2C_6H_5$, C_6H_4), 129.4, 130.6, 131.8, 133.6 (pyran C-2, C-3, C-5, C-6), 165.5, 1667 (2C=O), 174.3 (C=N).. Anal. calcd. for $C_{29}H_{27}N_3O_5$: C, 70.01; H, 5.47; N, 8.45%. Found: C, 70.24; H, 5.26; N, 8.60%. *m/z* 497 (M^+, 57%).

*2-Amino-5-(2-(2-(4-chlorophenyl)hydrazono)acetyl)-4-(4-methoxyphenyl)-6-phenyl-4H-pyran-3-carbonitrile (**7p**)*. Red crystals, yield (2.83 g, 55%), mp130-134 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3492-3357 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (C=O), 1633 (C=C); ^{1}H

NMR (DMSO-d_6, 300 MHz): δ = 3.68 (s, 3H, OCH_3), 4.83 (s, 2H, D_2O exchangeable, NH_2), 6.56 (s, 1H, pyran H-4), 6.81 (s, 1H, CH=N), 7.25-7.56 (m, 13H, C_6H_5, $2C_6H_4$), 8.30 (s, 1H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 50.5 (OCH_3), 90.2 (pyran C-4), 117.0 (CN), 120.2, 120.6, 121.6, 122.7, 122.9, 123.0, 123.6, 124.6, 124.8, 125.3, 126.0, 126.5 (C_6H_5, $2C_6H_4$), 129.8, 130.6, 131.7, 133.4 (pyran C-2, C-3, C-5, C-6), 165.6 (C=O), 174.0 (C=N). Anal. calcd. for $C_{27}H_{21}ClN_4O_3$: C, 66.87; H, 4.36; N, 11.55%. Found: C, 66.94; H, 4.52; N, 11.30%. *m/z* 484 (M^+, 70%).

*Ethyl 2-amino-5-(2-(2-(4-chlorophenyl)hydrazono)acetyl)-4-(4-methoxy-phenyl)-6-phenyl-4H-pyran-3-carboxylate (**7q**)*. Brown crystals, yield (3.18 g, 60%), mp 142-144 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3496-3353 (NH, NH_2,), 3055 (CH, aromatic), 1688 (CO), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 6.59 Hz, $OCH_2\underline{CH_3}$), 3.69 (s, 3H, OCH_3), 4.22 (q, 2H, *J* = 6.59 Hz, $O\underline{CH_2}CH_3$), 4.87 (s, 2H, D_2O exchangeable, NH_2), 6.55 (s, 1H, pyran H-4), 6.81 (s, 1H, N=CH), 7.23-7.56 (m, 13H, C_6H_5, $2C_6H_4$), 8.38 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.7 ($OCH_2\underline{CH_3}$), 50.1 ($O\underline{CH_2}CH_3$), 50.4 (OCH_3), 90.3 (pyran C-4), 120.4, 120.8, 121.3, 121.6, 122.4, 122.6, 123.2, 124.4, 124.1, 125.8, 126.3, 126.5 (C_6H_5, $2C_6H_4$), 129.6, 130.4, 131.2, 133.8 (pyran C-2, C-3, C-5, C-6), 165.6 (C=O), 174.3 (C=N).. Anal. calcd. for $C_{29}H_{26}ClN_3O_5$: C, 65.47; H, 4.93; N, 7.90%. Found: C, 65.58; H, 5.07; N, 8.18%. *m/z* 531 (M^+, 68%).

*2-Amino-4-(4-methoxyphenyl)-6-phenyl-5-(2-(2-(p-tolyl)hydrazono)-acetyl)-4H-pyran-3-carbo-nitrile (**7r**)*. Break red crystals, yield (2.83 g, 55%), mp 94-96 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3492-3357 (NH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (C=O), 1633 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.83 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 4.83 (s, 2H, D_2O exchangeable, NH_2), 6.56 (s, 1H, pyran H-4), 6.81 (s, 1H, CH=N), 7.25-7.56 (m, 13H, C_6H_5, $2C_6H_4$), 8.30 (s, 1H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.7 (CH_3), 50.5 (OCH_3), 90.2 (pyran C-4), 117.0 (CN), 120.2, 120.6, 121.6, 122.7, 122.9, 123.0, 123.6, 124.6, 124.8, 125.3, 126.0, 126.5 (C_6H_5, $2C_6H_4$), 129.8, 130.6, 131.7, 133.4 (pyran C-2, C-3, C-5, C-6), 165.6 (C=O), 174.0 (C=N). Anal. calcd. for $C_{28}H_{24}N_4O_3$: C, 72.40; H, 5.21; N, 12.06%. Found: C, 72.58; H, 5.37; N, 11.93%. *m/z* 464 (M^+, 66%).

*Ethyl 2-amino-4-(4-methoxyphenyl)-6-phenyl-5-(2-(2-(p-tolyl)hydrazono)-acetyl)-4H-pyran-3-carboxylate (**7s**)*. Orange crystals, yield (3.21 g, 63%), mp 150-152 °C (1,4-dioxane), IR (KBr) ν_{max} cm^{-1}: 3471-3348 (NH, NH_2,), 3055 (CH, aromatic), 1701, 1687 (2CO), 1633 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.16 (t, 3H, *J* = 7.04 Hz, $OCH_2\underline{CH_3}$), 2.66 (s, 3H, CH_3), 3.68 (s, 3H, OCH_3), 4.25 (q, 2H, *J* = 7.04 Hz, $O\underline{CH_2}CH_3$), 4.89 (s, 2H, D_2O exchangeable, NH_2), 6.53 (s, 1H, pyran H-4), 6.87 (s, 1H, N=CH), 7.21-7.59 (m, 13H, C_6H_5, $2C_6H_4$), 8.38 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.7 ($OCH_2\underline{CH_3}$), 36.8 (CH_3), 50.1 ($O\underline{CH_2}CH_3$), 50.4 (OCH_3), 90.6 (pyran C-4), 120.1, 120.5, 120.8, 121.2, 122.7, 123.1, 123.6, 124.8, 124.4, 125.7, 126.3, 126.2 (C_6H_5, $2C_6H_4$), 129.3, 130.1, 131.7, 133.3 (pyran C-2, C-3, C-5, C-6), 165.8, 166.2 (2C=O), 174.3 (C=N).. Anal. calcd. for $C_{30}H_{29}N_3O_5$: C, 70.43; H, 5.71; N, 8.21%. Found: C, 70.57; H, 5.63; N, 8.36%. *m/z* 511 (M^+, 78%).

*Synthesis of compounds **9a-c***

To a solution of benzoylacetone (1.62 g, 0.01 mol) in absolute ethanol (50 mL, 0.01 mol) containing triethylamine (1.0 mL) cyclohexan-1,3-dione (0.98 g, 0.01 mol) and benzaldehyde (1.06 g, 0.01 mol), 4-methoxybenzaldehyde (1.37 g, 0.01 mol) or 4-chlorobenzaldehyde (1.40, 0.01 mol) were added. The reaction mixture was heated under the reflux conditions for 3 h then was left to cool and the formed solid product was collected by filtration.

*3-Acetyl-2,4-diphenyl-7,8-dihydro-4H-chromen-5(6H)-one (**9a**)*. Pale yellow, yield (2.06 g, 60%), mp 190-192 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3055 (CH, aromatic), 1702, 1690 (2C=O), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.78-1.93 (m, 6H, 3CH_2), 2.69 (s, 3H, CH_3), 6.56 (s, 1H, pyran H-4), 7.28-7.42 (m, 10H, 2C_6H_5); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.9 (CH_3), 36.2, 38.0, 52.6 (3CH_2), 90.6 (pyran C-4), 120.6, 120.8, 121.5, 122.3, 123.2, 123.8, 125.5, 127.3 (2C_6H_5), 129.6, 130.2, 131.8, 133.3 (pyran C-2, C-3, C-5, C-6), 165.8, 166.4 (2C=O). Anal. calcd. for $C_{23}H_{20}O_3$: C, 80.21; H, 5.85%. Found: C, 80.29; H, 5.68 %. *m/z* 344 (M^+, 86%).

*3-Acetyl-4-(4-methoxyphenyl)-2-phenyl-7,8-dihydro-4H-chromen-5(6H)-one (**9b**)*. White crystals, yield (2.43 g, 65%), mp 141-143 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3055 (CH, aromatic), 1703, 1689 (2C=O), 1633 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.75-1.97 (m, 6H, 3CH_2), 2.65 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 6.54 (s, 1H, pyran H-4), 7.25-7.46 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.7 (CH_3), 36.1, 38.3, 52.5 (3CH_2), 50.8 (OCH_3), 90.4 (pyran C-4), 120.4, 120.9, 121.2, 122.6, 123.4, 123.9, 125.3, 126.1 (C_6H_5, C_6H_4), 129.4, 130.5, 131.4, 133.1 (pyran C-2, C-3, C-5, C-6), 165.7, 166.6 (2C=O). Anal. calcd. for $C_{24}H_{22}O_4$: C, 76.99; H, 5.92%. Found: C, 77.25; H, 5.80%. *m/z* 374 (M^+, 75%).

*3-Acetyl-4-(4-chlorophenyl)-2-phenyl-7,8-dihydro-4H-chromen-5(6H)-one (**9c**)*. Pale yellow, yield (2.64 g, 70%), mp 158-160 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3055 (CH, aromatic), 1703, 1690 (2C=O), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.75-1.96 (m, 6H, 3CH_2), 3.68 (s, 3H, CH_3), 6.54 (s, 1H, pyran H-4), 7.24-7.46 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.7 (CH_3), 36.1, 38.0, 52.9 (3CH_2), 90.3 (pyran C-4), 120.3, 120.5, 121.2, 122.5, 123.6, 124.9, 125.1, 126.4 (C_6H_5, C_6H_4), 129.8, 130.6, 132.4, 133.6 (pyran C-2, C-3, C-5, C-6), 165.4, 166.5 (2C=O). Anal. calcd. for $C_{23}H_{19}ClO_3$: C, 72.92; H, 5.06%. Found: C, 73.17; H, 5.15%. *m/z* 378 (M^+, 47%).

*Synthesis of the thiophene derivatives **10a,b***

Elemental sulfur (0.32 g, 0.01 mol) together with malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) were added to a solution of compound **1** (1.62 g, 0.01 mol) in ethanol (50 mL) for which triethylamine (1.0 mL) was added. The reaction mixture was heated under the reflux condition for 1 h then left to cool. The solid product produced after pouring onto ice/water containing a few drops of hydrochloric acid was collected by filtration and dried.

*2-Amino-5-benzoyl-4-methylthiophene-3-carbonitrile (**10a**)*. Pale brown, yield (3.40 g, 77%), mp 120-122 °C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3372-3336 (NH_2), 3055 (CH, aromatic), 2220 (CN), 1680 (C=O), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.68 (s, 3H, CH_3), 4.89 (s, 2H, D_2O exchangeable, NH_2), 7.28-7.39 (m, 5H, C_6H_5); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.7 (CH_3), 117.1 (CN), 121.1, 122.6, 123.2, 124.5 (C_6H_5), 166.4 (C=O). Anal. calcd. for $C_{13}H_{10}N_2OS$: C, 64.44; H, 4.16; N, 11.56; S, 13.23%. Found: C, 64.58; H, 4.08; N, 11.70, S 13.17%. *m/z* 242 (M^+, 30%).

*Ethyl 2-amino-5-benzoyl-4-methylthiophene-3-carboxylate (**10b**)*. Pale brown, yield (2.13 g, 73%), mp 68-70°C (ethanol), IR (KBr) ν_{max} cm^{-1}: 3380-3341 (NH_2), 3055 (CH, aromatic), 1686, 1680 (2C=O), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 1.13 (t, 3H, *J* = 5.72 Hz, $OCH_2\underline{CH_3}$), 2.67 (s, 3H, CH_3), 4.22 (q, 2H, *J* = 5.72 Hz, $O\underline{CH_2}CH_3$), 4.89 (s, 2H, D_2O exchangeable, NH_2), 7.28-7.39 (m, 5H, C_6H_5); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.5 ($OCH_2\underline{CH_3}$), 36.8 (CH_3), 50.2 ($O\underline{CH_2}CH_3$), 120.8, 122.3, 123.6, 124.8 (C_6H_5), 164.8, 166.4 (2C=O). Anal. calcd. for $C_{15}H_{15}NO_3S$: C, 62.26; H, 5.23; N, 4.84; S, 11.08%. Found: C, 62.51; H, 5.01; N, 11.26; S, 11.13%. *m/z* 289 (M^+, 22%).

Procedure of cell proliferation

Using the standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [40, 41] the tested compounds were evaluated *in vitro* as anti-proliferative agents. The human lung carcinoma (A549), lung cancer (H460), human colorectal (HT29), gastric cancer cell (MKN-45), glioma cell line (U87MG) and cellosaurus cell line (SMMC-7721) were used in such evaluations (Table 1). The procedure used for such measurements were carried out according to our previously reported work [42, 43]. The mean values of three independent experiments, expressed as IC_{50} values, were presented in Table 1. The IC_{50}'s presented in this were considered as the mean values of three independent experiments.

CONCLUSION

In conclusion, a series of new pyran, chromen-5-one and thiophene derivatives were synthesized and identified. The products were evaluated as novel anti-cancer agents toward the six cancer cell lines namely the human lung carcinoma (A549), lung cancer (H460), human colorectal (HT29), gastric cancer cell (MKN-45), glioma cell line (U87MG) and cellosaurus cell line (SMMC-7721). The preliminary investigation showed that the pyran derivatives **4a**, **4b**, **4e**, **5a**, **5d**, **5e**, **5f**, **5g**, **5h**, **5i**; the arylhydrazone derivatives **7c**, **7e**, **7f**, **7g**, **7h**, **7i**, **7k**, **7l**, **7m**, **7o**, **7p**, **7q**; the chromen-5-one derivatives **9a**, **9c** and the thiophene derivative **10a** exhibited from good to excellent potency against the six used cell lines. In most cases, the analysis of SARs indicated that compounds with the 4-chloro on the phenyl ring and the CN or COOEt on the pyran ring were more active than those with other substituents. Screening of compounds **7c**, **7k**, **7l, 7m** and **7o** against cancer cell lines classified according to the disease revealed that all of them expressed high inhibitions. Morphological changes of A549 cell line by the effect of compound **7k** was studied using microenvironment of the lung tissue where an excellent result was obtained. In our laboratory, further studies on the structural optimization about these derivatives are still underway for future work.

ACKNOWLEDGEMENTS

R.M. Mohareb would like to express high great thanks to the Alexander von Humboldt Foundation in Bon, Germany for affording regular fellowships to him that help for completing this work.

REFERENCES

1. Usachev, B.I. 1-6-(Trifluoromethyl)-2H-pyran-2-ones: Promising CF3-bearing conjugated cyclic diene and electrophilic building-blocks. *J. Fluorine Chem.* **2015**, 175, 36-46.
2. Dong, Y.; Goto, K.N.; Lai, C.Y.; Natschke, S.L.; Bastow, K.F.; Lee, K.F. Antitumor agents 287. Substituted 4-amino-2H-pyran-2-one (APO) analogs reveal a new scaffold from neo-tanshinlactone with in vitro anticancer activity. *Bioorg. Med. Chem. Lett.* **2011**, 21, 2341-2344.
3. Wu, L.Q.; Ma, X.; Zhang, C.; Liu Z.P. Design, synthesis, and biological evaluation of 4-substituted-3,4-dihydrobenzo[h]quinoline-2,5,6(1H)-triones as NQO1-directed antitumor agents. *Eur. J. Med. Chem.* **2020**, 198, 112396.
4. Saundane, A.R.; Vijaykumar, K.; Vaijinath, A.V. 4-Synthesis of novel 2-amino-4-(5′-substituted 2′-phenyl-1H-indol-3′-yl)-6-aryl-4H-pyran-3-carbonitrile derivatives as antimicrobial and antioxidant agents. *Bioorg. Med. Chem. Lett.* **2013**, 23, 1978-1984.
5. Almansour, A.I.; Kumar, R.S.; Arumugam, N.; Sriram, D. A solvent free, four-component synthesis and 1,3-dipolar cycloaddition of 4(H)-pyrans with nitrile oxides: Synthesis and

discovery of antimycobacterial activity of enantiomerically pure 1,2,4-oxadiazoles. *Eur J. Med. Chem.* **2012**, 53, 416-423.
6. El-Sayed, N.N.E.; Abdelaziz, M.A.; Wardakhan, W.W.; Mohareb, R.M. The Knoevenagel reaction of cyanoacetylhydrazine with pregnenolone: Synthesis of thiophene, thieno[2,3-d]pyrimidine, 1,2,4-triazole, pyran and pyridine derivatives with anti-inflammatory and anti-ulcer activities. *Steroids* **2016**, 107, 98-111.
7. Fan, X.; Feng, D.; Qu, Y.; Zhang, X.; Wang, J.; Loiseau, P.M.; Andrei, G.; Snoeck, R. Clercq, E.D. Practical and efficient synthesis of pyrano[3,2-c]pyridone, pyrano[4,3-b]pyran and their hybrids with nucleoside as potential antiviral and antileishmanial agents. *Bioorg. Med. Chem. Lett.* **2010**, 20, 809-813.
8. Ram, V.J.; Goel, A.; Shukla, P.K.; Kapil, A. Synthesis of thiophenes and thieno[3,2-c]pyran-4-ones as antileishmanial and antifungal agents. *Bioorg. Med. Chem. Lett.* **1997**, 7, 3101-3106.
9. Zhao, Z.; Bai, Y.; Xie, J.; Chen, X.; He, X.; Sun, Y.; Bai, Y.; Zhang, Y.; Wu, S.; Zheng, X. Excavating precursors from the traditional Chinese herb *Polygala tenuifolia* and *Gastrodia elata*: Synthesis, anticonvulsant activity evaluation of 3,4,5-trimethoxycinnamic acid (TMCA) ester derivatives. *Bioorg. Chem.* **2019**, 88, 102832.
10. Saundane, A.R.; Vijaykumar, K.; Vaijinath, A.V. Synthesis of novel 2-amino-4-(5′-substituted 2′-phenyl-1H-indol-3′-yl)-6-aryl-4H-pyran-3-carbonitrile derivatives as anti-microbial and antioxidant agents. *Bioorg. Med. Chem. Lett.* **2013**, 23, 1978-1984.
11. Capim, S.L.; Gonçalves, G.M.; Santos, C.M.; Marinho, B.G.; Vasconcellos, M.L.A. High analgesic and anti-inflammatory in vivo activities of six new hybrids NSAIAs tetrahydropyran derivatives. *Bioorg. Med. Chem.* **2013**, 21, 6003-6010.
12. Zhao, W.; Wang, B.; Liu, Y.; Fu, L.; Sheng, L.; Zhao, H.; Lu, Y.; Zhang, D. Design, synthesis, and biological evaluation of novel 4H-chromen-4-one derivatives as antituberculosis agents against multidrug-resistant tuberculosis. *Eur. J. Med. Chem.* **2020**, 189, 112075.
13. Capim, S.L.; Carneiro, P.H.P.; Castro, P.C.; Barros, M.R.M.; Marinho, B.G.; Vasconcellos, M.L.A. Design, Prins-cyclization reaction promoting diastereoselective synthesis of 10 new tetrahydropyran derivatives and in vivo antinociceptive evaluations. *Eur. J. Med. Chem.* **2012**, 58, 1-11.
14. Cottu, P.; Tredan, O.; Livartowski, A.; Pero, D.; Gilberg, M.; Ghorbal, R.; Dupin, J.; Maillard, C. PCN190 Kador: A French retrospective study describing the therapeutic management of patients WHO received trastuzumab based neoadjuvant treatment for HER2-positive early breast cancer (EBC). *Value in Health* **2020**, 23, S456.
15. Burgera, M.; Van der Aa, M.N.M.; Van Oers, J.M.M.; Brinkmann, A.; Kwast, T.H.; Steyerberg, E.C.; Stoehr, R.; Kirkels, W.J.; Denzinger, S.; Wild, P.J.; Wieland, W.F.; Hofstaedter, F.; Hartmann, A.; Zwarthoff, E.C. *Eur. Urology* **2008**, 54, 835-844.
16. El-Shwiniy, W.H. ; Shehab, W.S. Zordok, W.A. Synthesis, structural elucidation, molecular modeling and antimicrobial studies of 6-(2-hydroxyphenyl-imine)-2-thioxotetra-hydropyrimidin-4(1*H*)-one (L) Schiff base metal complexes. *J. Mol. Struct.* **2020**, 1199, 126993.
17. Abdo, N.Y.M.; Samir, E.M.; Mohareb, R.M. Synthesis and evaluation of novel 4H-pyrazole and thiophene derivatives derived from chalcone as potential anti-proliferative agents, Pim-1 kinase inhibitors, and PAINS. *J. Heterocycl. Chem.* **2020**, 57, 1993-2009.
18. Mohareb, R.M.; Megally, N.A.; Al-drazkazali, W.N. Uses of cyclohexan-1,3-dione for the synthesis of thiazole, pyrazole, thiophene, isoxazole and pyran derivatives with antitumor activities. *Lett. Drug Des. Disc.* **2020**, 17, 595-607.
19. Honarmand, M.; Tzani, A.; Detsi, A. A choline chloride-based deep eutectic solvent promoted three-component synthesis of tetrahydrobenzo[b]pyran and pyrano[2,3-d] pyrimidinone (thione) derivatives. *J. Mol. Liq.* **2019**, 290, 111358.

20. Yu, B.; Yu, D.Q.; Liu, H.M. Spirooxindoles: Promising scaffolds for anticancer agents. *Eur. J. Med. Chem.* **2015**, 97, 673-698.
21. Shi, X.J.; Yu, B.; Wang, J.W.; Qi, P.P.; Tang, K.; Huang, X.; Liu, H.M. Structurally novel steroidal spirooxindole by 241 potently inhibits tumor growth mainly through ROS-mediated mechanisms. *Sci. Rep.* **2016**, 6, 31607.
22. Mohammadi, Z.G.; Hosseini, M.N.; Lashgari, N.; Badiei, A.; Amanlou, M.; Bazl, R. Convenient one-pot synthesis of spirooxindole-4H-pyrans in the presence of SBA-Pr-NH2 and evaluation of their urease inhibitory activities. *J. of Nanostructures* **2012**, 2, 489-500.
23. Ahmed, E.M.; Khalil, N.A.; Zaher, A.F.; Alhamaky, S.M.; El-Zoghbib, M.S. Synthesis, molecular modeling and biological evaluation of new benzo[4,5]thieno[3,2-*b*]pyran derivatives as topoisomerase I-DNA binary complex poisons. *Bioorg. Chem.* **2021**, 112, 104915.
24. Tan, B.; Zhang, X.; Quan, X.; Zheng, G.; Li, X.; Zhao, L.; Li, W.L. Design, synthesis and biological activity evaluation of novel 4-((1-cyclopropyl-3-(tetrahydro-2H-pyran-4-yl)-1H-pyrazol-4-yl) oxy) pyridine-2-yl) amino derivatives as potent transforming growth factor-β (TGF-β) type I receptor inhibitors. *Bioorg. Med.Chem. Lett.* **2020**, 30, 127339.
25. Biglari, M.; Shirini, F.; Mahmoodi, N.O.; Zabihzadeh, M.; Mashhadinezh, M. A choline chloride-based deep eutectic solvent promoted three-component synthesis of tetrahydrobenzo[*b*]pyran and pyrano[2,3-*d*] pyrimidinone (thione) derivatives. *J. Mol. Struc*. **2020**, 1205, 127652.
26. Abou-Seri, S.M.; Eissa, A.A.M.; Behery, M.G.M.; Omar, F.A. Synthesis, in vitro anticancer activity and in silico studies of certain isoxazole-based carboxamides, ureates, and hydrazones as potential inhibitors of VEGFR2. *Bioorg. Chem.* **2021**, 116, 105334.
27. Şenkardeş, S.; Türe, A.; Ekrek, S.; Durak, A.T.; Abbak, M.; Çevik, Ö.; Kaşkatepe, B.; Küçükgüzel, I.; Küçükgüzel, Ş.G. Novel 2,6-disubstituted pyridine hydrazones: Synthesis, anticancer activity, docking studies and effects on caspase-3-mediated apoptosis. *J. Mol. Struct.* **2021**, 1223, 128962.
28. Labib, M.B.; Philoppes, J.N.; Lamie, P.F.; Ahmed, E.R. Azole-hydrazone derivatives: Design, synthesis, in vitro biological evaluation, dual EGFR/HER2 inhibitory activity, cell cycle analysis and molecular docking study as anticancer agents. *Bioorg. Chem.,* **2018**, 76, 67-80.
29. Nasr, T.; Bondock, S.; Youns, M. Anticancer activity of new coumarin substituted hydrazide-hydrazone derivatives. *Eur. J. Med. Chem.* **2014**, 76, 539-548.
30. Afkhami, A.; Zarei, A.R. Simultaneous spectrophotometric determination of hydrazine and phenylhydrazine based on their condensation reactions with different aromatic aldehydes in micellar media using H-point standard addition method. *Talanta* **2004**, 62, 559-565.
31. Timchenko, Y.V.; Stavrianidi, A.N.; Smolenkov, A.D.; Pirogov, A.V.; Shpigun, O.A. A novel simple and sensitive approach for determination of 1,1-dimethylhydrazine in aqueous samples by high performance liquid chromatography with ultraviolet and tandem mass spectrometric detection after derivatization with unsubstituted aromatic aldehydes. *Chemosphere* **2021**, 280, 130747.
32. Li, S.; Zhao, Y.; Wang, K.; Gao, Y.; Han, J.; Cui, B.; Gong, P. Discovery of novel 4-(2-fluorophenoxy)quinoline derivatives bearing 4-oxo-1,4-dihydrocinnoline-3-carboxamide moiety as c-Met kinase inhibitors. *Bioorg. Med. Chem*. **2013**, 21, 2843-2855.
33. Mohareb, R.M.; Ibrahim, B.A. Synthesis of heterocyclic derivatives as potential cytotoxic compounds evaluated hepatocellular and cervical carcinoma cell lines. *Bull Chem. Soc. Ethiop.* **2023**, 37, 141-158.
34. Alahmdi, M.I.; Mohareb, R.M.; Mahmoud, M.A.; Alkhamis, K.; Abo-Dya, N.E.; Zidan, N.S.; Khasim, S.; Alsharif, M.A. Anti-proliferative activities of thiophenes, pyrans and pyridines derived from 1,3-dicarbonyl compounds. *Chemistry Select* **2021**, 6, 12094-12100.

35. Mamizadeh, Z.; Kalani, M.R.; Parsania, M.; Dallal, M.M.S.; Moradi, A. NEBL and AKT1 maybe new targets to eliminate the colorectal cancer cells resistance to oncolytic effect of vesicular stomatitis virus M-protein. *Molec. Therapy Oncolytics* **2021**, 23, 593-601.
36. Gokce, M.; Utku, S.; Kupeli, E. Synthesis and analgesic and antiinflammatory activities 6-substituted-3(2H)-pyridazinone-2-acetyl-2-(psubstituted/nonsubstituted benzal)hydrazone derivatives. *Eur. J. Med. Chem.* **2009**, 44, 3760-3764.
37. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Wolff, A.V.; Goodrich, M.G.; Cambell, H.; Mayo, J.; Boyd, M. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Nat. Cancer Inst.* **1991**, 83, 757-766.
38. Mohareb, R.M.; Ibrahim, R.A.; Alwan, E.S. Multi-compnent reactions of cyclohexan-1,3-dione to synthesis heterocyclic derivatives with c-Met enzymatic activity and anti-prostate anti-proliferative and tyrosine kinase activities. *Bull. Chem. Soc. Ethiop.* **2022**, 36, 119-136.
39. Mohareb, R.M.; Mohamed, A.A.; Ibrahim, R.A. Uses of Chalcone acetophenone to synthesis heterocyclic compounds with cytotoxic and c-Met kinase acativities. *Bull. Chem. Soc. Ethiop.* **2022**, 36, 149-172.
40. X. Han, A. Alu, H. Liu, Y. S. X. Wei, L. Cai, Y. Wei, Biomaterial-assisted biotherapy: A brief review of biomaterials used in drug delivery, vaccine development, gene therapy, and stem cell therapy. *Bioact. Mater.* **2022**, 17, 29-48.
41. Mendieta, I.; Nieto, M.R.; Anita, R.E.; Luis, J.; Arredondo, M.; Alcocer, G.G.; Laura Berumen, C. Ultrastructural changes associated to the neuroendocrine transdifferentiation of the lung adenocarcinoma cell line A549. *Acta Histochem.* **2021**, 123, 151797.
42. Gao, J.; Zhao, Y.; Wang, C.; Ji, H.; Yu, J.; Liu, C.; Liu, A. A novel synthetic chitosan selenate (CS) induces apoptosis in A549 lung cancer cells via the Fas/FasL pathway. *Int. J. Biol. Macromol*. **2020**, 158, 689-697.
43. Nunhart, P.; Konkoľová, E.; Janovec, L.; Jendželovský, R.; Vargová, J.; Ševc, J.; Matejová, M.; Miltáková, B.; Fedoročko, P.; Kozurkova, M. Fluorinated 3,6,9-trisubstituted acridine derivatives as DNA interacting agents and topoisomerase inhibitors with A549 antiproliferative activity. *Bioorg. Chem.* **2020**, 94, 103393.