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MULTI-COMPONENT REACTIONS OF CYCLOHEXAN-1,3-DIONE TO SYNTHESIZE HETEROCYCLIC DERIVATIVES WITH c-MET ENZYMATIC ACTIVITY, ANTI-PROSTATE, ANTI-PROLIFERATIVE AND TYROSINE KINASE ACTIVITIES

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ABSTRACT. We are aiming in this work to synthesize target molecules not only possess anti-tumor activities but also kinase inhibitors. The target molecules were obtained starting from aryl hydrazones of cyclohexan-1,3-dione followed by its heterocyclization reactions to produce anticancer molecules. The multi-component reactions of the arylhydrazocyclohexan-1,3-dione derivatives **3a-c** produced the 1,4,5,6,7,8-hexahydroquinoline derivatives **6a-r** and the 4,5,6,8-tetrahydrochromeno[2,3-*c*]pyrazole derivatives **10a-c**. Other multi-component reactions were demonstrated. The anti-proliferative activity of the synthesized compounds toward the six cancer cell lines namely A549, H460, HT-29, MKN-45, U87MG, and SMMC-7721 was studied. In addition the c-Met enzymatic activities and inhibition toward the prostate cancer cell PC-3 were measured. The results obtained in most cases, indicated that the presence of electronegative Cl group through the molecule favour the inhibitions.

KEY WORDS: Multi-component reactions, Cyclohexan-1,3-dione, Chromene, Chromeno[2,3-*c*]pyrazole, Cytotoxicity

INTRODUCTION

Heterocycles constitute the largest diversity of organic molecules of chemical, biomedical, and industrial significance [1, 2]. They are also among the most frequently encountered scaffolds in numerous drugs and pharmaceutically relevant substances [3-6]. In the past several decades, a significant number of efforts have been made on the discovery and development of more efficient pharmaceuticals, pesticides, insecticides, rodenticides, and weed killers by following well studied natural models and biochemical pathways in living cells [7, 8]. In addition, a series of libraries consisting of heterocycles have been successfully established for the structure activity-relationship studies (SAR) for drug design and synthesis [9]. Meanwhile, the diversity-oriented synthesis (DOS) continues to be an area of importance at the interface of organic synthesis and chemical biology [10-12]. While DOS plays an important role in searching for new bioactive small molecules with functional and stereochemical diversity [13], more efficient multi-component domino reactions (MDRs) for the synthesis of a series of heterocycles, particularly functionalized multi-heterocycles, have been in high demand. In the past several years, the development of new multi-component domino reactions has become an active and challenging topic in modern organic chemistry [14], they can readily provide greater atom-economic access to a diverse spectrum of compounds and their libraries for screening. In addition, hydrazones and their derivatives constitute a versatile class of compounds in organic chemistry. These compounds showed biological properties, such as anti-inflammatory, analgesic, anticonvulsant, anti-tuberculous, antitumor, anti-HIV and antimicrobial activity [15, 16]. Hydrazones are important

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compounds for heterocyclic synthesis due to the presence of C=O, C-N and N-N bonding where the carbon atom of the hydrazone group has both electrophilic and nucleophilic [17]. Due to the large mentioned applications of multi-component reactions together with the chemical reactivity of hydrazones in this context, herein we report on the synthesis and the spectroscopic, structural, and physicochemical characterization of new heterocyclic derivatives incorporating cyclohexanone moiety starting from the arylhydrazono derivatives of cyclohexan-1,3-diones. The anti-proliferative activity of the synthesized compounds toward different cancer cell lines was also explored. This was followed by studying the inhibitions of the most active compounds toward tyrosine kinases and Pim-1 kinase.

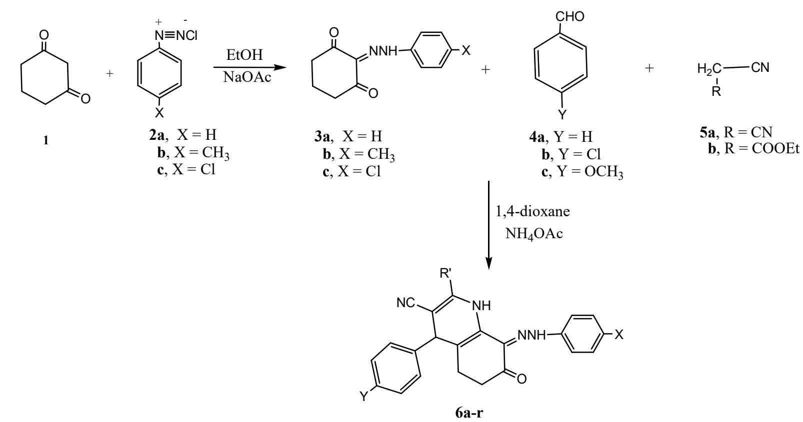
RESULTS AND DISCUSSION

As a continued work through the uses of cyclohexan-1,3-dione to produce heterocyclic compounds characterized by their high anti-proliferative activities. In the present work, we demonstrated the use cyclohexan-1,3-dione to synthesis arylhydrazone derivatives. Thus, the reaction of cyclohexan-1,3-dione (**1**) with either benzene diazoniumchloride (**2a**), 4-methylbenzene diazonium chloride (**2b**) or 4-chlorobenzene diazonium chloride (**2c**) gave the corresponding arylhydrazone derivatives **3a-c** [18]. Initially 2-arylhydrazonocyclohexan-1,3-dione was chosen as the model substrate for the synthesis of fused heterocyclic compounds through studying its multi-component reactions with aromatic aldehydes and cyanomethylene reagents to give biologically active fused pyridine derivatives. The multi-component reactions of either **3a**, **3b** or **3c** with either of benzaldehyde (**4a**), 4-chlorobezaldehyde (**4b**) or 4-methoxybenzaldehyde (**4c**) and either malononitrile (**5a**) or ethyl cyanoacetate (**5b**) in 1,4-dioxane solution containing ammonium acetate gave the 1,4,5,6,7,8-hexahydroquinoline derivatives **6a-r** (Scheme 1). The chemical structures of new compounds were assured by spectral data (IR, ^{1}H, ^{13}C-NMR, MS). Thus, the ^{1}H NMR spectrum of compound **6a** (as an example) showed (beside the expected signals) a singlet at δ 4.58 (D_2O exchangeable) confirming the presence of the NH_2 group and a singlet at δ 5.13 ppm corresponding to the pyridine *H*-4 In addition, the ^{13}C NMR spectrum showed signals at δ 38.3, 41.6 for the two CH_2 groups, a signal at δ 48.8 for the pyridine C-4, a signal at δ 117.0 corresponding to the CN group and two signals at δ 166.3, 167.5 equivalent to the C=N and C=O groups, respectively.

Next, we studied the multi-component reactions of the arylhydrazone derivatives **3a-c** with benzaldehyde (**4a**) and ethyl benzoylacetate (**7**) in ethanol solution containing triethylamine gave the 5,6,7,8-tetrahydro-4*H*-chromene derivatives **8a-c**. Moreover, the multi-component reactions of the arylhydrazone derivatives **3a-c** with benzaldehyde (**4a**) and 3-methyl-1*H*-pyrazol-5(4*H*)-one (**9**) in ethanol solution containing triethylamine gave the 4,5,6,8-tetrahydrochromeno[2,3-*c*]pyrazole derivatives **10a-c** (Scheme 2). The structures of the latter compounds were based on their respective analytical and spectral data. Thus the ^{1}H NMR spectrum of compound **10a** showed (beside the expected signals), a singlet at δ 2.80 ppm for the CH_3 group and a singlet at δ 5.13 ppm indicating the pyran H-4. Moreover, the ^{13}C NMR spectrum revealed the presence of a signal at δ 35.8 corresponding for the CH_3 group, two signals at δ 37.4, 41.5 corresponding to the two CH_2 groups, a signal at δ 50.7 assigning to the pyran C-4, four signals at δ 130.0, 130.6, 131.4, 132.7 for the pyran carbons and three signals at δ 164.5, 165.2, 168.9 for the two C=N and C=O groups.

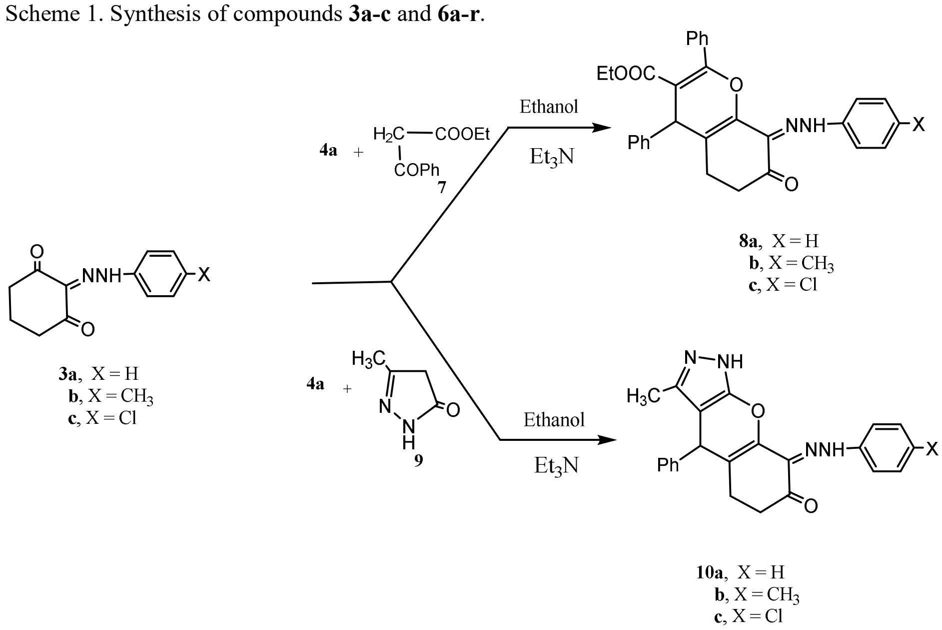
The high yields of such multi-component reaction products encouraged us for further reactions using the arylhydrazone derivatives **3a-c**. Thus, the reaction of either compound **3a**, **3b** or **3c** with benzaldehyde (**4a**) and 3-oxo-*N*,3-diphenylpropanamide (**11**) in 1,4-dioxane containing triethyl amine gave the 5,6,7,8-tetrahydro-4*H*-chromene-3-carboxamide derivatives **12a-c**. The analytical and spectral data of **12a-c** were in agreement with the proposed structures (see experimental section). On the other hand, the multi-component reactions of either **3a**, **3b** or **3c** with benzaldehyde (**4a**) and either 2-cyanoacetamide (**13a**) or 2-cyanoethanethioamide (**13b**) in 1,4-

dioxane solution containing triethylamine gave surprisingly the 1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile derivatives **14a-f** not as the expected chromene derivative (Scheme 3).

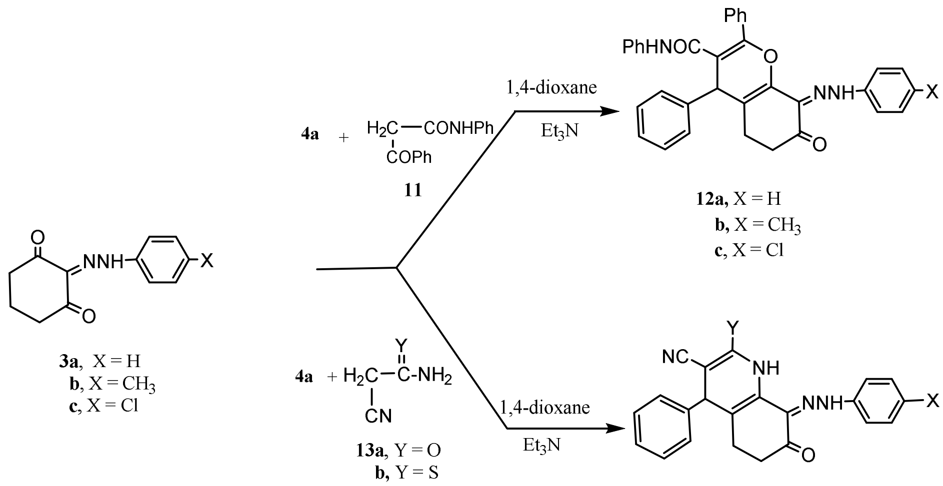


6	a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r
X	H	H	H	H	H	H	CH_3	CH_3	CH_3	CH_3	CH_3	CH_3	Cl	Cl	Cl	Cl	Cl	Cl
Y	H	H	Cl	Cl	OCH_3	OCH_3	H	H	Cl	Cl	OCH_3	OCH_3	H	H	Cl	Cl	OCH_3	OCH_3
R'	NH_2	OH	NH_2	OH	NH_2	OH	NH_2	OH	NH_2	OH	NH_2	OH	NH_2	OH	NH_2	OH	NH_2	OH

Scheme 1. Synthesis of compounds **3a-c** and **6a-r**.



Scheme 2. Synthesis of compounds **8a-c** and **10a-c**.



14	a	b	c	d	e	f
X	H	H	CH	CH_3	Cl	Cl
Y	OH	SH	OH	SH	OH	SH

Scheme 3. Synthesis of compounds **12a-c** and **14a-f**.

Cell proliferation assay

The anti-proliferative activities of the newly synthesized compounds (Table 1) 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 [19-21]. The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximate 4 x 10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 °C for 24 h. The compounds tested at the indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 µg/mL, and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in 100 µL of DMSO each well, and the absorbency at 492 nM (for absorbance of MTT formazan) and 630 nM (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested three times in each cell line and the results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

The mean values of three independent experiments, expressed as IC_{50} values, were presented in Table 1. Most of the synthesized compounds exhibited potent anti-proliferative activity with IC_{50} values less than 30 µM. Generally, the variations of substituent's within the heterocyclic ring being attached have a notable influence on the anti-proliferative activity.

Structure activity relationship

Table 1 showed the cytotoxicity of most of the synthesized compounds toward the six cancer cell lines A549, H460, HT-29, MKN-45, U87MG, and SMMC-7721. The reaction of cyclohexan-1,3-

Table 1. In vitro growth inhibitory effects 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
3a	6.26 ± 2.86	8.36 ± 3.24	5.69 ± 1.39	6.58 ± 1.37	9.62 ± 3.15	6.43 ± 2.25
3b	0.28 ± 0.12	0.33 ± 0.18	0.53 ± 0.13	0.33 ± 0.17	0.61 ± 0.28	0.52 ± 0.16
3c	0.43 ± 0.31	0.51 ± 0.25	0.49 ± 0.28	0.63 ± 0.39	0.82 ± 0.27	0.93 ± 0.39
6b	1.36 ± 0.89	1.61 ± 0.85	0.63 ± 0.25	2.46 ± 0.93	1.53 ± 0.68	1.36 ± 0.27
6c	7.72 ± 2.67	8.25 ± 3.86	6.63 ± 2.34	9.04 ± 1.92	8.62 ± 2.23	9.68 ± 3.25
6d	0.40 ± 0.26	0.36 ± 0.19	0.64 ± 0.28	0.33 ± 0.23	0.23 ± 0.53	0.36 ± 0.13
6f	0.62 ± 0.28	0.83 ± 0.38	0.65 ± 0.26	0.59 ± 0.28	0.62 ± 0.29	0.26 ± 0.28
6g	8.34 ± 2.42	9.56 ± 3.67	7.38 ± 2.42	8.47 ± 2.42	7.28 ± 2.25	8.48 ± 3.82
6h	0.25 ± 0.13	0.30 ± 0.09	0.52 ± 0.17	0.37 ± 0.19	0.34 ± 0.21	0.53 ± 0.17
6i	0.42 ± 0.35	0.60 ± 0.29	0.39 ± 0.28	0.42 ± 0.26	0.64 ± 0.23	0.57 ± 0.23
6l	0.82 ± 0.40	0.77 ± 0.68	0.83 ± 0.26	0.59 ± 0.29	0.53 ± 0.17	0.46 ± 0.18
6m	1.32 ± 0.60	1.15 ± 0.08	2.29 ± 1.02	1.52 ± 0.86	2.28 ± 1.21	1.26 ± 0.84
6n	5.48 ± 1.28	6.79 ± 1.05	5.84 ± 1.69	7.49 ± 2.64	8.09 ± 2.36	6.94 ± 1.68
6o	0.40 ± 0.23	0.32 ± 0.15	0.42 ± 0.20	0.30 ± 0.19	0.73 ± 2.14	0.32 ± 0.19
6p	0.23 ± 0.15	0.33 ± 0.12	0.25 ± 0.19	0.25 ± 0.13	0.42 ± 0.18	0.33 ± 0.21
6q	8.41 ± 2.28	6.35 ± 1.28	9.41 ± 2.16	8.50 ± 2.38	7.42 ± 2.26	8.53 ± 2.29
8a	6.64 ± 1.84	5.26 ± 1.43	6.54 ± 1.71	5.26 ± 1.14	6.28 ± 1.48	6.28 ± 1.32
8b	4.91 ± 1.56	5.41 ± 1.28	3.52 ± 1.15	3.30 ± 1.86	5.02 ± 2.80	4.69 ± 1.38
8c	0.26 ± 0.16	0.32 ± 0.17	0.24 ± 0.19	0.31 ± 0.13	0.36 ± 0.25	0.28 ± 0.19
10a	6.33 ± 1.59	8.40 ± 1.29	6.32 ± 2.17	5.63 ± 0.23	8.53 ± 2.21	5.61 ± 1.26
10b	8.38 ± 3.16	7.26 ± 1.14	9.28 ± 3.19	6.28 ± 1.08	7.89 ± 2.63	9.39 ± 2.37
10c	0.26 ± 0.15	0.49 ± 0.23	0.26 ± 0.09	0.38 ± 0.18	0.27 ± 0.14	0.28 ± 0.08
12a	8.64 ± 2.61	10.3 ± 3.5	7.32 ± 2.83	8.41 ± 2.60	9.38 ± 3.51	8.40 ± 2.62
12b	7.39 ± 2.50	5.48 ± 1.42	5.62 ± 1.63	6.31 ± 1.59	8.13 ± 2.59	5.86 ± 1.52
12c	0.24 ± 0.13	0.28 ± 0.12	0.30 ± 0.16	0.34 ± 0.11	0.25 ± 0.12	0.42 ± 0.15
14a	6.23 ± 1.39	4.23 ± 1.16	4.31 ± 1.18	3.29 ± 1.46	2.61 ± 1.30	3.69 ± 1.13
14b	1.36 ± 0.86	1.06 ± 0.74	2.03 ± 0.88	1.35 ± 0.93	0.79 ± 0.43	1.03 ± 0.39
14c	2.28 ± 0.69	1.32 ± 0.58	1.20 ± 0.68	1.32 ± 0.75	1.24 ± 0.72	0.80 ± 0.42
14d	2.51 ± 1.21	2.16 ± 1.05	1.80 ± 0.79	2.33 ± 1.15	1.60 ± 0.85	0.63 ± 0.27
14e	0.26 ± 0.18	0.36 ± 0.13	0.26 ± 0.15	0.27 ± 0.16	0.39 ± 0.22	0.25 ± 0.13
14f	0.19 ± 0.06	0.20 ± 0.05	0.26 ± 0.13	0.30 ± 0.20	0.35 ± 0.16	0.28 ± 0.15
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

dione (**1**) with the aryldiazonium salts **2a-c** produced the arylhydrazono derivatives **3a-c**, respectively. The two compounds **3b** (X = CH_3) and **3c** (X = Cl) showed the highest cytotoxicity among the three compounds toward the six cancer cell lines. The multi-component reactions of either of **3a-c** with either of the arylaldehydes **4a-c** and either malononitrile or ethyl cyanoacetate to give the 1,4,5,6,7,8-hexahydroquinoline derivatives **6a-r**, respectively. Thirty-one compounds were selected from such series to be tested toward the six cancer cell lines where these showed from moderate to high inhibitions. Compounds **6b** (X = Y = H, R' = OH) , **6d** (X = H, Y = Cl, R' = OH), **6f** (X = H, Y = OCH_3, R' = OH), **6h** (X = CH_3, Y = H, R' = OH) and **6i** (X = CH_3, Y = Cl, R' = NH_2), **6l** (X = CH_3, Y = OCH_3, R' = OH), **6o** (X = Y = Cl, R' = NH_2), and **6p** (X = Y = Cl, R' = OH) were the most cytotoxic compounds among such series of compounds. However, compounds **6m**, **6n** and **8b** showed moderate inhibitions and compounds **6g**, **6q**, **8a**, **10a** and **10b** expressed low inhibitions toward the six cancer cell lines. Considering the 5,6,7,8-tetrahydro-4*H*-chromene derivatives **8a-c** and the 4,5,6,8-tetrahydrochromeno[2,3-*c*]pyrazole derivatives **10a-c** it is clear from table 1 that compounds **8a** (X = H), **8b** (X = CH_3), **10a** (X = H) and **10b** (X = CH_3) decline the inhibitions while compounds **8c** (X = Cl) and **10c** (X = Cl) revealed the highest inhibitions among the three compounds and this is attributed to the presence of the electronegative

Cl group within both compounds. Considering the 5,6,7,8-tetrahydro-4*H*-chromene-3-carboxamide derivatives **12a-c** where compound **12c** exhibited the highest inhibitions. Surprisingly the inhibitions of the 1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile derivatives **14a-f** through such series compounds **14b**, **14c** and **14d** exhibited moderate inhibitions while compounds **14e** (X = Cl, Y' = OH) and **14f** (X = Cl, Y' = SH) exhibited the highest inhibitions. It clear from Table 1 that compounds **3b**, **3c**, **6b**, **6d**, **6f**, **6h**, **6i**, **6l**, **6o**, **6p**, **8c**, **10c**, **12c**, **14e** and **14f** were the most cytotoxic among the tested compounds toward the six cancer cell line.

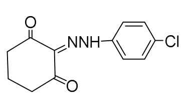
HTRF kinase assay

The c-Met kinase activity of all compounds was evaluated using homogeneous time-resolved fluorescence (HTRF) assay as previously reported [22, 23]. In addition, the most active compounds were further evaluated against other five tyrosine kinase (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) using the same screening method. The experimental procedure applied for the HTRF kinase tests were as reported procedure [24].

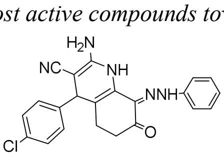
In vitro enzymatic assays

All the newly synthesized quinoline and chromene derivatives were evaluated for their inhibitions toward c-Met enzyme using a homogeneous time-resolved fluorescence (HTRF) assay. Taking foretinib as the positive control, the results expressed as IC_{50} were summarized in Table 2. The IC_{50} values are the average of at least three independent experiments. As illustrated in Table 1, all the tested compounds displayed potent c-Met enzymatic activity with IC_{50} values ranging from 0.03 to 18.29 nM. Compared with foretinib (IC_{50} = 1.16 nM), seventeen of them (**3c**, **6c**, **6e**, **6f**, **6j**, **6n**, **6r**, **8c, 10a**, **10c**, **12c**,**14a, 14b**, **14c**, **14d**, **14e** and **14f**) exhibited equivalent or higher potency with IC_{50} values less than 1.30 nM. On the other hand, compounds **3a**, **3b**, **3c**, **6b**, **6c**, **6d**, **6e**, **6f**, **6h**, **6j**, **6n**, **6o**, **6p**, **6r**, **8c**, **10a**, **10b**, **10c**, **12b, 12c** and **14a-f** showed higher inhibitions toward the PC-3 cell line than the reference SGI-1776 (IC_{50} 4.86 nM). Analyzing the data demonstrated through Table 2 revealed that many compounds displayed potent c-Met enzymatic activity and inhibitions toward the prostate cancer cell line PC-3. Considering the arylhydrazone derivatives **3a-c**, compound **3c** exhibited the highest inhibitions toward c-Met and PC-3 with IC_{50}'s 0.03 and 0.02 nM. For the 1,4,5,6,7,8-hexahydroquinoline derivatives **6a-r**, where compounds **6b**, **6c**, **6d**, **6f**, **6j**, **6n** and **6r** exhibited the highest inhibitions toward c-Met and PC-3. It was found that compounds **6e** and **6h** showed high inhibitions toward c-Met with IC_{50} 1.02, 1.42 nM but low inhibitions toward PC-3 cell line with IC_{50} 3.42 and 3.53 nM, respectively. On the other side, compound **6p** expressed high inhibition toward PC-3 cell line with IC_{50} 0.02 nM but decline inhibition toward c-Met IC_{50} 2.40 nM. For the 5,6,7,8-tetrahydro-4*H*-chromene derivatives **8a-c** and the 4,5,6,8-tetrahydrochromeno[2,3-*c*]pyrazole derivatives **10a-c** where compounds **8c** and **10c** showed the highest inhibitions toward c-Met and PC-3 but compounds **10a** and **10b** exhibited higher inhibitions than that of **8a** and **8b**. On the other hand for compounds **12a-c**, compound **12c** exhibited the highest inhibitions toward c-Met kinase and PC-3 cell line. Surprisingly, the five compounds **14a**, **14c**, **14d**, **14e** and **14f** exhibited high inhibitions toward c-Met kinase and PC-3 cell line.

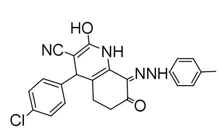
Structures of the most active compounds toward Inhibition against c-Met kinase



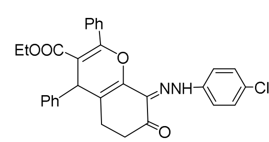
3c IC_{50} 0.03 nM



6c IC_{50} 0.52 nM



6j IC_{50} 0.23 nM



8c IC_{50} 0.39 nM

Table 2. c-Met enzymatic activity of the newly synthesized compounds.

Compound No.	X	Y/Y'	R'	IC_{50} (nM) c-Met	IC_{50} (nM) PC-3
3a	H	-	-	1.89 ± 0.76	2.39 ± 1.06
3b	CH_3	-	-	4.37 ± 1.28	3.52 ±1.32
3c	Cl	-	-	0.03 ± 0.006	0.02 ± 0.01
6a	H	H	NH_2	6.80 ± 2.49	5.72 ± 2.59
6b	H	H	OH	1.32 ± 0.64	1.63 ± 0.89
6c	H	Cl	NH_2	0.52 ± 0.29	0.32 ± 0.22
6d	H	Cl	OH	0.90 ± 0.36	0.69 ± 0.41
6e	H	OCH_3	NH_2	1.02 ± 0.39	3.42 ± 0.69
6f	H	OCH_3	OH	0.92 ± 0.43	0.62 ± 0.15
6g	CH_3	H	NH_2	3.72 ± 1.14	6.42 ± 2.49
6h	CH_3	H	OH	1.42 ± 0.88	3.53 ± 1.29
6i	CH_3	Cl	NH_2	5.27 ± 1.83	7.33 ± 2.82
6j	CH_3	Cl	OH	0.23 ± 0.17	0.36 ± 0.15
6k	CH_3	OCH_3	NH_2	15.31 ± 4.26	12.42 ± 4.28
6l	CH_3	OCH_3	OH	10.31 ± 3.62	4.09 ±1.36
6m	Cl	H	NH_2	4.28 ± 1.53	6.82 ± 2.41
6n	Cl	H	OH	0.29 ± 0.15	0.49 ± 0.26
6o	Cl	Cl	NH_2	1.83 ± 0.67	2.66 ± 1.56
6p	Cl	Cl	OH	2.40 ± 0.53	0.02 ± 0.004
6q	Cl	OCH_3	NH_2	12.56 ± 4.70	8.38 ± 4.72
6r	Cl	OCH_3	OH	1.28 ± 0.98	2.80 ± 1.63
8a	H	-	-	12.32 ± 4.72	8.29 ± 3.52
8b	CH_3	-	-	18.29 ± 6.31	10.17 ± 3.69
8c	Cl	-	-	0.39 ± 0.25	0.21 ± 0.13
10a	H	-	-	1.28 ± 0.52	2.27 ± 0.84
10b	CH_3	-	-	3.61 ± 1.80	2.13 ± 0.83
10c	Cl	-	-	0.13 ± 0.06	0.32 ± 0.16
12a	H	-	-	8.35 ± 2.71	12.52 ± 3.82
12b	CH_3	-	-	10.40 ± 2.64	1.63 ± 0.92
12c	Cl	-	-	0.24 ± 0.15	0.22 ± 0.13
14a	H	OH	-	0.98 ± 0.41	0.84 ± 0.36
14b	H	SH	-	1.23 ± 0.53	3.62 ±1.62
14c	CH_3	OH	-	0.96 ± 0.42	0.70 ± 0.31
14d	CH_3	SH	-	1.16 ± 0.68	1.38 ± 0.92
14e	Cl	OH	-	0.21 ± 0.04	0.13 ± 0.06
14f	Cl	SH	-	0.59 ± 0.23	0.48 ± 0.21
		-	-	**Foretinib** 1.16 ± 0.17	**SGI-1776** 4.86 ± 0.16

Inhibitions of the most active compounds towards tyrosine kinases

The most active compounds **3c**, **6c**, **6d**, **6e**, **6f**, **6j**, **6n**, **6r**, **8c**, **10a**, **10c**, **12c**, **14a**, **14b**, **14c**, **14d**, **14e** and **14f** towards c-Met enzymatic activity were further evaluated against the five tyrosine kinases (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) using the same screening method (Table 3). These receptor tyrosine kinases (RTKs) have been implicated in vascular development by affecting the proliferation and migration of endothelial cells or pericytes. Among them, VEGF is a major regulator of tumor angiogenesis via endothelial cell proliferation and blood vessel permeability [25, 26]. It is clear from Table 3 that compounds **3c**, **6c**, **6e**, **6f**, **6j**, **6n**, **6r**, **8c**, **10c**, **12c**, **14c**, **14d**, **14e** and **14f** were the most potent towards the five tyrosine kinases. Compound **6n** showed high potency towards the four kinases VEGFR-2 with IC_{50} 0.72 nM, while it showed moderate inhibition towards c-Kit, Flt-3 and EGFR kinases with IC_{50}'s 1.07, 1.25 and 1.83 nM. Compound **14b** showed moderate inhibitions toward c-Kit and Flt-3 kinases with IC_{50}'s 1.68 and 1.29 nM, respectively, while it showed high inhibitions toward VEGFR-2, EGFR and PDGFR kinases with IC_{50}'s 0.51, 0.26 and 0.38 nM, respectively. It is clear that compounds **6j** and **14e** exhibited the highest inhibitions among the tested compounds.

Table 3. Inhibitions toward tyrosine kinases [Enzyme IC_{50} (nM)] of selected compounds.

Compound	c-Kit	Flt-3	VEGFR-2	EGFR	PDGFR
3c	0.21	0.34	0.23	0.46	0.29
6c	0.30	0.26	0.31	0.28	0.34
6e	0.30	0.26	0.41	0.53	0.19
6f	0.28	0.23	0.31	0.38	0.41
6j	0.14	0.21	0.54	0.39	0.21
6n	1.07	1.25	0.72	1.83	2.50
6r	0.32	0.29	0.38	0.26	0.33
8c	0.25	0.24	0.19	0.31	0.35
10c	1.26	1.84	2.63	1.52	1.26
12c	0.23	0.38	0.14	0.37	0.51
14b	1.68	1.29	0.51	0.26	0.38
14c	0.42	0.26	0.31	0.24	0.53
14d	0.52	0.21	0.53	0.80	0.46
14e	0.19	0.28	0.27	0.31	0.28
14f	0.33	0.24	0.19	0.32	0.25

Inhibitions of the selected compounds towards Pim-1 kinase

Compounds **3c, 6c**, **6e**, **6f**, **6j**, **6n**, **6r**, **8c**, **10c**, **12c**, **14c**, **14d**, **14e** and **14f** were selected to examine their Pim-1 kinase inhibition activity at a range of 10 concentrations and the IC_{50} values were calculated (Table 4). Compounds **3c, 6c**, **6e**, **6f**, **6j**, **6r**, **8c** and **14d** were the most potent to inhibit Pim-1 activity with IC_{50} values of 0.24, 0.56, 0.23, 0.23, 0.40, 0.48, 0.22 and 0.38 µM, respectively. On the other hand, compounds **6n**, **10c, 12c, 14c, 14e** and **14f** were less effective (IC_{50} > 10 µM). SGI-1776 was used as the positive control with IC_{50} 0.048 µM in the assay. These profiles in combination with cell growth inhibitions data of the selected compounds listed in Table 4 indicated that Pim-1 kinase was a potential target of these compounds.

Table 4. The inhibitor activity of selected compounds towards Pim-1 kinase.

Compound No	Inhibition ratio At 10 µM	IC_{50} (µM)
3c	94	0.24
6c	84	0.56
6e	95	0.23
6f	96	0.23
6j	90	0.40
6n	28	>10
6r	88	0.48
8c	97	0.22
10c	24	>10
12c	16	>10
14c	24	>10
14d	89	0.38
14e	36	>10
14f	28	>10
SGI-1776	-	0.048 ± 0.019

EXPERIMENTAL

Chemistry

Newly synthesized compounds showed melting points that were uncorrected. For all compounds the IR spectra (KBr discs) were measured using a FTIR plus 460 or Pye Unicam SP-1000 spectrophotometer. The spectra ^{1}HNMR were measured using Varian Gemini-300 (300 MHz) and Jeol AS 500 MHz instruments spectra were performed in DMSO-d_6 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. The spectra MS (EI) were measured using Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex Shimadzu instruments. The microanalytical data CHN were obtained from the Micro-analytical Data Unit at Cairo University and were performed on Vario EL III Elemental analyzer. Screening of compounds against the cancer cell lines and tyrosine kinases were performed through The National Cancer Institute at Cairo University. Compounds **3a-c** were prepared according to our reported work [18].

*General procedure for the synthesis of the 1,4,5,6,7,8-hexahydroquinoline derivatives **6a-r**.* Each of either benzaldehyde (1.06 g, 0.01 mol), 4-chlorobenzaldehyde (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde (1.36 g, 0.01 mol) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) were added to a solution of either **3a** (2.16 g, 0.01 mol), **3b** (2.30 g, 0.01 mol) or **3c** (2.50 g, 0.01 mol) in 1,4-dioxane (50 mL) containing ammonium acetate (2.00 g). The whole reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

*2-Amino-7-oxo-4-phenyl-8-(2-phenylhydrazono)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (**6a**).* Pale brown crystals from 1,4-dioxane, yield (2.58 g, 70%), Mp > 300 °C. IR (KBr) υ max cm^{-1}: 3463-3359 (NH_2, NH), 3055 (CH, aromatic), 2221 (CN), 1694 (C=O), 1640 (C=N), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.81-2.99 (2t, 4H, $2CH_2$), 4.58 (s, 2H, D_2O exchangeable NH_2), 5.13 (s, 1H, pyridine H-4), 7.26-7.43 (m, 10H, $2C_6H_5$), 8.29, 8.32 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.3, 41.6 ($2CH_2$), 48.8 (pyridine C-4), 117.0 (CN), 120.1, 120.7, 121.3, 121.8, 123.3, 123.8, 124.2, 125.6 ($2C_6H_5$), 128.1, 129.3, 130.2, 133.8 (pyridine C), 166.3 (C=N), 167.5 (C=O). Anal. calcd. for $C_{22}H_{19}N_5O$: C, 71.53; H, 5.18; N, 18.96%. Found: C, 71.70; H, 5.25; N, 19.14%. MS: *m/z* 369 (M^+, 35%).

*2-Hydroxy-7-oxo-4-phenyl-8-(2-phenylhydrazono)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile (**6b**).* Pale brown from 1,4-dioxane, yield (2.70 g, 73%), Mp 145-147 °C. IR (KBr) υ max cm^{-1}: 3552-3371 (OH, NH), 3055 (CH, aromatic), 2222 (CN), 1697 (C=O), 1643 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.80-3.02 (2t, 4H, $2CH_2$), 5.16 (s, 1H, pyridine H-4), 7.23-7.42 (m, 10H, $2C_6H_5$), 8.29, 8.33 (2s, 2H, D_2O exchangeable, 2NH), 10.40 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.6, 42.5 ($2CH_2$), 48.1 (pyridine C-4), 116.7 (CN), 120.1, 120.4, 121.7, 121.9, 123.4, 124.7, 125.2, 126.9 ($2C_6H_5$), 128.5, 129.6, 130.8, 132.8 (pyridine C), 166.6 (C=N), 168.8 (C=O). Anal. calcd. for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13%. Found: C, 71.60; H, 4.87; N, 15.28%. MS: *m/z* 370 (M^+, 44%).

*2-Amino-4-(4-chlorophenyl)-7-oxo-8-(2-phenylhydrazono)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile (**6c**).* Brown crystals from 1,4-dioxane, yield (2.41 g, 60%), Mp 120-122 °C. IR (KBr) υ max cm^{-1}: 3484-3339 (NH_2, NH), 3055 (CH, aromatic), 2222 (CN), 1693 (C=O), 1642 (C=N), 1633 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.80-2.99 (2t, 4H, $2CH_2$), 4.94 (s, 2H, D_2O exchangeable NH_2), 5.16 (s, 1H, pyridine H-4), 7.23-7.57 (m, 9H, C_6H_5, C_6H_4), 8.36, 8.46 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.2, 41.7 ($2CH_2$), 48.9 (pyridine C-4), 117.8 (CN), 120.3, 120.6, 121.8, 121.9, 122.3, 123.6, 124.7, 126.1 (C_6H_5, C_6H_4), 128.6, 129.2, 130.5, 132.2 (pyridine C), 166.3 (C=N), 167.3 (C=O). Anal. calcd. for $C_{22}H_{18}ClN_5O$: C, 65.43; H, 4.49; N, 17.34%. Found: C, 65.39; H, 4.28; N, 17.57%. MS: *m/z* 403 (M^+, 68%).

4-(4-Chlorophenyl)-2-hydroxy-7-oxo-8-(2-phenylhydrazono)-1,4,5,6,7,8-hexa-hydroquinoline-3-carbonitrile ***(6d)***. Red crystals from 1,4-dioxane, yield (2.68 g, 66%), Mp 148-150 °C. IR (KBr) υ max cm^{-1}: 3561-3373 (OH, NH), 3056 (CH, aromatic), 2222 (CN), 1697 (C=O), 1643 (C=N), 1631 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.80-2.99 (2t, 4H, $2CH_2$), 5.15 (s, 1H, pyridine H-4), 7.25-7.58 (m, 9H, C_6H_5, C_6H_4), 8.28, 8.42 (2s, 2H, D_2O exchangeable, 2NH), 10.49 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.8, 41.5 ($2CH_2$), 48.8 (pyridine C-4), 117.2 (CN), 120.1, 120.8, 121.7, 122.4, 123.6, 124.2, 125.2, 125.8 (C_6H_5, C_6H_4), 128.3, 129.5, 130.5, 132.6 (pyridine C), 166.9 (C=N), 168.4 (C=O). Anal. calcd. for $C_{22}H_{17}ClN_4O_2$: C, 65.27; H, 4.23; N, 13.84%. Found: C, 65.53; H, 4.26; N, 14.02%. MS: *m/z* 404 (M^+, 48%).

2-Amino-4-(4-methoxyphenyl)-7-oxo-8-(2-phenylhydrazono)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile ***(6e)***. Brown crystals from 1,4-dioxane, yield (2.40 g, 60%), Mp > 300 °C. IR (KBr) υ max cm^{-1}: 3459-3324 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1697 (C=O), 1646 (C=N), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.80-2.99 (2t, 4H, $2CH_2$), 3.68 (s, 3H, OCH_3), 4.88 (s, 2H, D_2O exchangeable NH_2), 5.05 (s, 1H, pyridine H-4), 7.25-7.58 (m, 9H, C_6H_5, C_6H_4), 8.30, 8.41 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.6, 40.8 ($2CH_2$), 50.6 (OCH_3), 50.8 (pyridine C-4), 116.8 (CN), 120.1, 121.4, 122.2, 122.6, 123.7, 124.6, 125.5, 126.2 (C_6H_5, C_6H_4), 128.6, 129.08, 130.7, 133.5 (pyridine C), 166.4 (C=N), 167.6 (C=O). Anal. calcd. for $C_{23}H_{21}N_5O_2$: C, 69.16; H, 5.30; N, 17.53%. Found: C, 69.31; H, 5.26; N, 17.42%. MS: *m/z* 399 (M^+, 42%).

2-Hydroxy-4-(4-methoxyphenyl)-7-oxo-8-(2-phenylhydrazono)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile ***(6f)***. Pale brown crystals from 1,4-dioxane, yield (2.80 g, 70%), Mp 143-145 °C. IR (KBr) υ max cm^{-1}: 3568-3341 (OH, NH), 3055 (CH, aromatic), 2222 (CN), 1698 (C=O), 1641 (C=N), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.84-3.03 (2t, 4H, $2CH_2$), 3.72 (s, 3H OCH_3), 5.16 (s, 1H, pyridine H-4), 7.21-7.54 (m, 9H, C_6H_5, C_6H_4), 8.29, 8.43 (2s, 2H, D_2O exchangeable, 2NH), 10.36 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.2, 41.6 ($2CH_2$), 50.6 (OCH_3), 50.8 (pyridine C-4), 116.8 (CN), 120.1, 120.3, 121.5, 123.8, 124.6, 124.8, 125.1, 126.3 (C_6H_5, C_6H_4), 128.6, 129.8, 130.2, 132.3 (pyridine C), 166.5 (C=N), 168.2 (C=O). Anal. calcd. for $C_{23}H_{20}N_4O_3$: C, 68.99; H, 5.03; N, 13.99%. Found: C, 68.63; H, 4.92; N, 14.25%. MS: *m/z* 400 (M^+, 40%).

2-Amino-7-oxo-4-phenyl-8-(2-(p-tolyl)hydrazono)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile ***(6g)***. Brown crystals from 1,4-dioxane, yield (2.29 g, 60%), Mp > 300 °C. IR (KBr) υ max cm^{-1}: 3488-3372 (NH_2, NH), 3055 (CH, aromatic), 2223 (CN), 1697 (C=O), 1643 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.61-3.02 (2t, 4H, $2CH_2$), 2.78 (s, 3H, CH_3), 4.89 (s, 2H, D_2O exchangeable NH_2), 5.16 (s, 1H, pyridine H-4), 7.22-7.49 (m, 9H, C_6H_5, C_6H_4), 8.28, 8.46 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.3, 40.9 ($2CH_2$), 35.6 (CH_3), 50.8 (pyridine C-4), 117.0 (CN), 120.1, 121.3, 122.2, 122.8, 123.1, 124.6, 124.8, 125.9 (C_6H_5, C_6H_4), 129.1, 129.6, 130.5, 131.8 (pyridine C), 166.2 (C=N), 167.4 (C=O). Anal. calcd. for $C_{23}H_{21}N_5O$: C, 72.04; H, 5.52; N, 18.26%. Found: C, 71.92; H, 5.72; N, 18.33%. MS: *m/z* 383 (M^+, 60%).

*2-Hydroxy-7-oxo-4-phenyl-8-(2-(p-tolyl)hydrazono)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile(**6h**)*. Pale brown crystals from 1,4-dioxane, yield (2.64 g, 69%), Mp > 300 °C. IR (KBr) υ max cm^{-1}: 3558-3373 (OH, NH), 3055 (CH, aromatic), 2222 (CN), 1698 (C=O), 1642 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.80-3.21 (2t, 4H, $2CH_2$), 2.76 (s, 3H CH_3), 5.08 (s, 1H, pyridine H-4), 7.24-7.52 (m, 9H, C_6H_5, C_6H_4), 8.32, 8.44 (2s, 2H, D_2O exchangeable, 2NH), 10.41 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.5, 41.6 ($2CH_2$), 36.8 (CH_3), 50.7 (pyridine C-4), 116.2 (CN), 120.3, 120.6, 121.8, 122.2, 123.7, 124.2, 125.8, 126.2 (C_6H_5, C_6H_4), 128.3, 129.1, 132.3, 132.6 (pyridine C), 166.7 (C=N), 168.5 (C=O).

Anal. calcd. for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57%. Found: C, 71.61; H, 5.36; N, 14.70%. MS: *m/z* 384 (M^+, 28%).

*2-Amino-4-(4-chlorophenyl)-7-oxo-8-(2-(p-tolyl)hydrazono)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**6i**).* Pale brown crystals from 1,4-dioxane, yield (3.16 g, 76%), Mp 170-172 °C. IR (KBr) υ max cm^{-1}: 3480-3360 (NH_2, NH), 3055 (CH, aromatic), 2223 (CN), 1694 (C=O), 1643 (C=N), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.68-3.17 (2t, 4H, $2CH_2$), 2.72 (s, 3H, CH_3), 4.68 (s, 2H, D_2O exchangeable NH_2), 5.09 (s, 1H, pyridine H-4), 7.25-7.58 (m, 8H, $2C_6H_4$), 8.31, 8.49 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.2, 41.6 ($2CH_2$), 36.5 (CH_3), 50.9 (pyridine C-4), 116.8 (CN), 120.3, 120.5, 122.5, 122.8, 123.1, 123.6, 125.8, 126.3 ($2C_6H_4$), 127.8, 128.8, 130.2, 131.6 (pyridine C), 166.3 (C=N), 168.6 (C=O). Anal. calcd. for $C_{23}H_{20}ClN_5O$: C, 66.10; H, 4.82; N, 16.76%. Found: C, 65.91; H, 4.63; N, 16.82%. MS: *m/z* 417 (M^+, 55%).

*4-(4-Chlorophenyl)-2-hydroxy-7-oxo-8-(2-(p-tolyl)hydrazono)-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**6j**).* Yellow crystals from 1,4-dioxane, yield (2.31 g, 55%), Mp 131-133 °C. IR (KBr) υ max cm^{-1}: 3539-3342 (OH, NH), 3055 (CH, aromatic), 2221 (CN), 1692 (C=O), 1645 (C=N), 1631 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.83-2.96 (2t, 4H, $2CH_2$), 2.72 (s, 3H CH_3), 5.11 (s, 1H, pyridine H-4), 7.21-7.47 (m, 8H, 2 C_6H_4), 8.26, 8.42 (2s, 2H, D_2O exchangeable, 2NH), 10.31 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.4, 42.8 ($2CH_2$), 36.2 (CH_3), 51.2 (pyridine C-4), 117.6 (CN), 120.0, 120.6, 122.8, 123.2, 125.0, 125.2, 126.0, 126.5 (2 C_6H_4), 130.2, 132.8, 134.8, 136.5 (pyridine C), 166.8 (C=N), 168.5 (C=O). Anal. calcd. for $C_{23}H_{19}ClN_4O_2$: C, 65.81; H, 4.29; N, 9.82%. Found: C, 65.54; H, 4.51; N, 9.68%. MS: *m/z* 418 (M^+, 50%).

*2-Amino-4-(4-methoxyphenyl)-7-oxo-8-(2-(p-tolyl)hydrazono)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile (**6k**).* Brown crystals from 1,4-dioxane, yield (3.22 g, 78%), Mp > 300 °C. IR (KBr) υ max cm^{-1}: 3482-3348 (NH_2, NH), 3055 (CH, aromatic), 2222 (CN), 1696 (C=O), 1643 (C=N), 1631 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.62-3.19 (2t, 4H, $2CH_2$), 2.78 (s, 3H, CH_3), 3.69 (s, 3H, OCH_3), 4.52 (s, 2H, D_2O exchangeable NH_2), 5.06 (s, 1H, pyridine H-4), 7.21-7.50 (m, 8H, $2C_6H_4$), 8.34, 8.41 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.3, 40.7 ($2CH_2$), 36.8 (CH_3), 50.2 (OCH_3),51.3 (pyridine C-4), 116.9 (CN), 119.6, 120.2, 122.6, 123.2, 124.7, 125.0, 125.2, 126.3(2 C_6H_4),128.7, 130.1, 130.2, 132.5 (pyridine C), 166.4 (C=N), 168.6 (C=O). Anal. calcd. for $C_{24}H_{23}N_5O_2$: C, 69.72; H, 5.61; N, 16.94%. Found: C, 69.58; H, 5.76; N, 17.17%. MS: *m/z* 413 (M^+, 66%).

*2-Hydroxy-4-(4-methoxyphenyl)-7-oxo-8-(2-(p-tolyl)hydrazono)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile (**6l**).* Orange crystals from 1,4-dioxane, yield (2.98 g, 72%), Mp 175-177 °C. IR (KBr) υ max cm^{-1}: 3573-3347 (OH, NH), 3054 (CH, aromatic), 2222 (CN), 1696 (C=O), 1640 (C=N), 1634 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.79-3.13 (2t, 4H, $2CH_2$), 2.69 (s, 3H CH_3), 3.70 (s, 3H, OCH_3), 5.05 (s, 1H, pyridine H-4), 7.24-7.49 (m, 8H, 2 C_6H_4), 8.30, 8.45 (2s, 2H, D_2O exchangeable, 2NH), 10.34 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.5, 40.9 ($2CH_2$), 36.7 (CH_3), 50.3 (OCH_3), 50.5 (pyridine C-4),116.5 (CN), 120.1, 120.4, 121.8, 122.6, 123.5, 124.8, 125.6, 125.9 (2 C_6H_4), 128.3, 129.6, 130.7, 131.3 (pyridine C), 166.9 (C=N), 168.3 (C=O). Anal. calcd. for $C_{24}H_{22}N_4O_3$: C, 69.55; H, 5.35; N, 13.52%. Found: C, 69.68; H, 5.47; N, 13.80%. MS: *m/z* 414 (M^+, 32%).

*2-Amino-8-(2-(4-chlorophenyl)hydrazono)-7-oxo-4-phenyl-1,4,5,6,7,8-hexa-hydroquinoline-3-carbonitrile (**6m**).* Orange crystals from 1,4-dioxane, yield (2.21 g, 55%), Mp > 300 °C. IR (KBr) υ max cm^{-1}: 3479-3341 (NH_2, NH), 3055 (CH, aromatic), 2222 (CN), 1694 (C=O), 1646 (C=N), 1635 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.64-3.02 (2t, 4H, $2CH_2$), 4.87 (s, 2H, D_2O

exchangeable NH_2), 5.08 (s, 1H, pyridine H-4), 7.22-7.53 (m, 9H, C_6H_5, C_6H_4), 8.33, 8.42 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.3, 41.8 (2CH_2), 50.8 (pyridine C-4), 117.0 (CN), 120.1, 120.4, 121.3, 122.7, 122.8, 123.9, 124.6, 125.7 (C_6H_5, C_6H_4), 129.3, 129.7, 130.3, 131.8 (pyridine C), 167.6 (C=N), 168.2 (C=O). Anal. calcd. for $C_{22}H_{18}ClN_5O$: C, 65.43; H, 4.49; N, 17.34%. Found: C, 65.72; H, 4.39; N, 17.52%. MS: *m/z* 403 (M^+, 48%).

*8-(2-(4-Chlorophenyl)hydrazono)-2-hydroxy-7-oxo-4-phenyl-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**6n**).* Yellow crystals from 1,4-dioxane, yield (2.42 g, 60%), Mp 162-164 °C. IR (KBr) υ max cm^{-1}: 3528-3358 (OH, NH), 3054 (CH, aromatic), 2222 (CN), 1697 (C=O), 1640 (C=N), 1633 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.83-3.32 (2t, 4H, 2CH_2), 5.08 (s, 1H, pyridine H-4), 7.25-7.49 (m, 9H, C_6H_5, C_6H_4), 8.30, 8.42 (2s, 2H, D_2O exchangeable, 2NH), 10.41 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.2, 41.3 (2CH_2), 50.7 (pyridine C-4), 117.3 (CN), 120.2, 121.2, 121.8, 122.9, 123.4, 124.4, 125.1, 125.8 (C_6H_5, C_6H_4), 128.7, 128.8, 130.8, 131.9 (pyridine C), 167.0 (C=N), 168.9 (C=O). Anal. calcd. for $C_{22}H_{17}ClN_4O_2$: C, 65.27; H, 4.23; N, 13.84%. Found: C, 65.14; H, 4.40; N, 14.21%. MS: *m/z* 404 (M^+, 35%).

*2-Amino-4-(4-chlorophenyl)-8-(2-(4-chlorophenyl)hydrazono)-7-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile (**6o**).* Orange crystals from 1,4-dioxane, yield (3.32 g, 76%), Mp 185-187 °C. IR (KBr) υ max cm^{-1}: 3473-3352 (NH_2, NH), 3053 (CH, aromatic), 2220 (CN), 1696 (C=O), 1641 (C=N), 1635 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.63-3.13 (2t, 4H, 2CH_2), 4.89 (s, 2H, D_2O exchangeable NH_2), 5.08 (s, 1H, pyridine H-4), 7.24-7.53 (m, 8H, 2C_6H_4), 8.33, 8.42 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 36.7, 41.2 (2CH_2), 50.8 (pyridine C-4), 117.1 (CN), 120.2, 120.7, 122.4, 122.6, 123.8, 124.3, 125.3, 126.4 (2C_6H_4), 128.8, 129.5, 130.4, 131.6 (pyridine C), 167.3 (C=N), 168.8 (C=O). Anal. calcd. for $C_{22}H_{17}Cl_2N_5O$: C, 60.29; H, 3.91; N, 15.98%. Found: C, 60.37; H, 3.78; N, 16.25%. MS: *m/z* 438 (M^+, 48%).

*4-(4-Chlorophenyl)-8-(2-(4-chlorophenyl)hydrazono)-2-hydroxy-7-oxo-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile (**6p**).* Pale yellow crystals from 1,4-dioxane, yield (2.97 g, 68%), Mp 171-173 °C. IR (KBr) υ max cm^{-1}: 3538-3349 (OH, NH), 3055 (CH, aromatic), 2220 (CN), 1701 (C=O), 1646 (C=N), 1632 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.83-3.21 (2t, 4H, 2CH_2), 5.05 (s, 1H, pyridine H-4), 7.23-7.59 (m, 8H, 2 C_6H_4), 8.26, 8.43 (2s, 2H, D_2O exchangeable, 2NH), 10.35 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.8, 41.5 (2CH_2), 51.4 (pyridine C-4), 117.2 (CN), 120.3, 120.8, 121.5, 121.9, 123.6, 124.8, 125.1, 126.3 (2 C_6H_4), 129.2, 129.8, 130.3, 132.4 (pyridine C), 166.6 (C=N), 168.9 (C=O). Anal. calcd. for $C_{22}H_{16}Cl_2N_4O_2$: C, 60.15; H, 3.67; N, 12.75%. Found: C, 60.26; H, 3.59; N, 12.92%. MS: m/z 439 (M^+, 48%).

*2-Amino-8-(2-(4-chlorophenyl)hydrazono)-4-(4-methoxyphenyl)-7-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (**6q**).* Orange crystals from 1,4-dioxane, yield (3.03 g, 70%), Mp > 300 °C. IR (KBr) υ max cm^{-1}: 3483-3329 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1687 (C=O), 1641 (C=N), 1633 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.58-3.14 (2t, 4H, 2CH_2), 3.64 (s, 3H, OCH_3), 4.86 (s, 2H, D_2O exchangeable NH_2), 5.13 (s, 1H, pyran H-4), 7.26-7.55 (m, 8H, 2C_6H_4), 8.32. 8.42 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.3, 41.8 (2CH_2), 50.1 (OCH_3), 51.6 (pyran C-4), 117.0 (CN), 120.3, 120.5, 121.8, 122.9, 124.3, 124.6, 125.1, 126.0 (2C_6H_4), 128.1, 129.6, 130.2, 131.3 (pyran C), 165.4 (C=N), 168.9 (C=O). Anal. calcd. for $C_{23}H_{20}ClN_5O_2$: C, 63.67; H, 4.65; N, 16.14%. Found: C, 63.92; H, 4.59; N, 16.26%. MS: *m/z* 433 (M^+, 66%).

*8-(2-(4-Chlorophenyl)hydrazono)-2-hydroxy-4-(4-methoxyphenyl)-7-oxo-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile (**6r**).* Pale yellow crystals from 1,4-dioxane, yield (3.38 g, 78%), Mp 171-

173 °C. IR (KBr) υ max cm^{-1}: 3573-3352 (OH, NH), 3055 (CH, aromatic), 2220 (CN), 1688 (C=O), 1641 (C=N), 1633 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.78-3.12 (2t, 4H, $2CH_2$), 3.76 (s, 3H, OCH_3), 5.07 (s, 1H, pyridine H-4), 7.21-7.53 (m, 8H, 2 C_6H_4), 8.26, 8.42 (2s, 2H, D_2O exchangeable, 2NH), 10.36 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 38.2, 41.5 ($2CH_2$), 50.4 (OCH_3), 50.8 (pyridine C-4), 116.7 (CN), 120.3, 120.8, 121.2, 122.9, 123.0, 123.7, 125.2, 126.5 (2 C_6H_4), 128.4, 129.4, 130.2, 131.6 (pyridine C), 164.3 (C=N), 168.8 (C=O). Anal. calcd. for $C_{23}H_{19}ClN_4O_3$: C, 63.52; H, 4.40; N, 12.88%. Found: C, 63.63; H, 4.52; N, 13.05%. MS: *m/z* 434 (M^+, 86%).

*General procedure for the synthesis of the 5,6,7,8-tetrahydro-4H-chromene derivatives **8a-c.*** Each of benzaldehyde (1.06 g, 0.01 mol) and ethyl benzoylacetate (1.92 g, 0.01 mol) were added to a solution of either **3a** (2.16 g, 0.01 mol), **3b** (2.30 g, 0.01 mol) or **3c** (2.50 g, 0.01 mol) in absolute ethanol (50 mL) containing triethylamine (2.00 mL). The whole reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

*Ethyl 7-oxo-2,4-diphenyl-8-(2-phenylhydrazono)-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (**8a**).* Orange crystals from ethanol, yield (3.15 g, 66%), Mp 142-144 °C. IR (KBr) υ max cm^{-1}: 3439-3353 (NH), 3055 (CH, aromatic), 1699, 1688 (2C=O), 1641 (C=N), 1632 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.13 (t, 3H, J = 7.22 Hz, $OCH_2\underline{CH_3}$), 2.63-3.20 (2t, 4H, $2CH_2$), 4.21 (q, 2H, J = 7.22 Hz, $O\underline{CH_2}CH_3$), 5.11 (s, 1H, pyran H-4), 7.24-7.53 (m, 15H, $3C_6H_5$), 8.31 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.8 ($OCH_2\underline{CH_3}$), 37.2, 41.8 ($2CH_2$), 50.1 (pyran C-4), 50.8 ($\underline{OCH_2}CH_3$), 120.3, 120.6, 121.0, 121.7, 122.5, 122.8, 123.1, 123.4, 124.3, 125.2, 125.6, 126.7 ($3C_6H_5$), 130.3, 130.5, 131.6, 133.8 (pyran C), 165.8 (C=N), 166.3, 168.7 (2C=O). Anal. calcd. for $C_{30}H_{26}N_2O_4$: C, 75.30; H, 5.48; N, 5.85%. Found: C, 75.51; H, 5.62; N, 5.73%. MS: *m/z* 478 (M^+, 56%).

*Ethyl 7-oxo-2,4-diphenyl-8-(2-(p-tolyl)hydrazono)-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (**8b**).* Orange crystals from ethanol, yield (3.64 g, 74%), Mp 186-188°C. IR (KBr) υ max cm^{-1}: 3469-3336 (NH), 3055 (CH, aromatic), 1694, 1689 (2C=O), 1643 (C=N), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.12 (t, 3H, J = 7.59 Hz, $OCH_2\underline{CH_3}$), 2.72 (s, 3H, CH_3), 2.63-3.20 (2t, 4H, $2CH_2$), 4.21 (q, 2H, J = 7.59 Hz, $O\underline{CH_2}CH_3$), 5.13 (s, 1H, pyran H-4), 7.21-7.58 (m, 14H, $2C_6H_5$, C_6H_4), 8.32 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.8 ($OCH_2\underline{CH_3}$), 34.2 (CH_3), 36.5, 40.6 ($2CH_2$), 50.3 (pyran C-4), 50.8 ($\underline{OCH_2}CH_3$), 120.1, 120.5, 122.3, 122.5, 123.8, 123.9, 124.0, 124.2, 124.8, 125.1, 125.3, 126.9 ($2C_6H_5$, C_6H_4), 130.6, 131.7, 132.5, 133.9 (pyran C), 165.2 (C=N), 166.6, 168.9 (2C=O). Anal. calcd. for $C_{31}H_{28}N_2O_4$: C, 75.59; H, 5.73; N, 5.69%. Found: C, 75.31; H, 5.49; N, 5.82%. MS: *m/z* 492 (M^+, 48%).

*Ethyl 8-(2-(4-chlorophenyl)hydrazono)-7-oxo-2,4-diphenyl-5,6,7,8-tetrahydro-4H-chromene-3-carboxylate (**8c**).* Orange crystals from ethanol, yield (3.68 g, 72%), Mp 173-175 °C. IR (KBr) υ max cm^{-1}: 3469-3336 (NH), 3055 (CH, aromatic), 1694, 1689 (C=O), 1643 (C=N), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.12 (t, 3H, J = 6.80 Hz, $OCH_2\underline{CH_3}$), 2.61-3.23 (2t, 4H, $2CH_2$), 4.23 (q, 2H, J = 6.80 Hz, $O\underline{CH_2}CH_3$), 5.12 (s, 1H, pyran H-4), 7.24-7.62 (m, 14H, $2C_6H_5$, C_6H_4), 8.32 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 16.8 ($OCH_2\underline{CH_3}$), 36.2, 39.1 ($2CH_2$), 50.4 (pyran C-4), 51.0 ($\underline{OCH_2}CH_3$), 120.4, 120.8, 122.2, 123.0, 123.6, 123.8, 124.2, 124.6, 125.0, 125.7, 126.2, 126.7 ($2C_6H_5$, C_6H_4), 130.4, 131.6, 132.8, 133.3 (pyran C), 165.7 (C=N), 166.2, 168.5 (2C=O). Anal. calcd. for $C_{30}H_{25}ClN_2O_4$: C, 70.24; H, 4.91; N, 5.46%. Found: C, 70.37; H, 5.16; N, 5.72%. MS: *m/z* 512 (M^+, 32%).

*General procedure for the synthesis of the 4,5,6,8-tetrahydrochromeno[2,3-c]pyrazole derivatives **10a-c.*** Each of benzaldehyde (1.06 g, 0.01 mol) and 3-methyl-1*H*-pyrazol-5(4*H*)-one

(0.98 g, 0.01 mol) were added to a solution of either **3a** (2.16 g, 0.01 mol), **3b** (2.30 g, 0.01 mol) or **3c** (2.50 g, 0.01 mol) in absolute ethanol (50 mL) containing triethylamine (2.00 mL). The whole reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

*3-Methyl-4-phenyl-8-(2-phenylhydrazono)-4,5,6,8-tetrahydrochromeno[2,3-c]pyrazol-7(1H)-one (**10a**).* Orange crystalsfrom ethanol, yield (2.30 g, 60%), Mp 135-137 °C. IR (KBr) υ max cm^{-1}: 3453-3329 (NH), 3055 (CH, aromatic), 1699 (C=O), 1641 (C=N), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.80 (s, 3H, CH_3), 2.60-3.29 (2t, 4H, $2CH_2$), 5.13 (s, 1H, pyran H-4), 7.26-7.45 (m, 10H, $2C_6H_5$), 8.27, 8.40 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 35.8 (CH_3), 37.4, 41.5 ($2CH_2$), 50.7 (pyran C-4), 120.1, 120.3, 121.9, 122.6, 123.8, 124.1,125.3, 126.1 ($2C_6H_5$), 130.0, 130.6, 131.4, 132.7 (pyran C), 164.5, 165.2 (2C=N), 168.9 (C=O). Anal. calcd. for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57%. Found: C, 71.75; H, 5.39; N, 14.80%. MS: *m/z* 384 (M^+, 38%).

*3-Methyl-4-phenyl-8-(2-(p-tolyl)hydrazono)-4,5,6,8-tetrahydrochromeno[2,3-c]pyrazol-7(1H)-one (**10b**).* Orange crystals from ethanol, yield (2.78 g, 70%), Mp 180-182 °C. IR (KBr) υ max cm^{-1}: 3481-3325 (NH), 3055 (CH, aromatic), 1697 (C=O), 1644 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.68, 2.73 (2s, 6H, $2CH_3$), 2.62-3.30 (2t, 4H, $2CH_2$), 5.11 (s, 1H, pyran H-4), 7.24-7.42 (m, 9H, C_6H_5, C_6H_4), 8.24, 8.43 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 32.3, 35.6 ($2CH_3$), 37.1, 41.2 ($2CH_2$), 50.9 (pyran C-4), 119.6, 120.8, 121.3, 122.9, 123.5, 124.7,125.5, 126.0 (C_6H_5, C_6H_4), 130.3, 131.1, 131.6, 132.9 (pyran C), 164.2, 165.8 (2C=N), 168.5 (C=O). Anal. calcd. for $C_{24}H_{22}N_4O_2$: C, 72.34; H, 5.57; N, 14.06%. Found: C, 72.53; H, 5.44; N, 14.25%. MS: *m/z* 398 (M^+, 40%).

*8-(2-(4-Chlorophenyl)hydrazono)-3-methyl-4-phenyl-4,5,6,8-tetrahydrochromeno-[2,3-c]pyrazol-7(1H)-one (**10c**).* Pale brown crystals from ethanol, yield (2.29 g, 55%), Mp 159-162 °C. IR (KBr) υ max cm^{-1}: 3471-3332 (NH), 3055 (CH, aromatic), 1701 (C=O), 1644 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.83 (s, 3H, CH_3), 2.62-3.15 (2t, 4H, $2CH_2$), 5.12 (s, 1H, pyran H-4), 7.22-7.54 (m, 9H, C_6H_5, C_6H_4), 8.31, 8.42 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 34.3 (CH_3), 37.1, 41.9 ($2CH_2$), 50.5 (pyran C-4), 120.3, 120.8, 121.2, 121.8, 122.5, 124.6,125.0, 125.7 (C_6H_5, C_6H_4), 130.2, 130.3, 131.1, 132.2 (pyran C), 164.8, 165.7 (2C=N), 168.6 (C=O). Anal. calcd. for $C_{23}H_{19}ClN_4O_2$: C, 65.95; H, 4.57; N, 13.38%. Found: C, 66.15; H, 4.70; N, 13.52%. MS: *m/z* 418 (M^+, 21%).

*General procedure for the synthesis of the 5,6,7,8-tetrahydro-4H-chromene derivatives **12a-c.*** Each of benzaldehyde (1.06 g, 0.01 mol) and 3-oxo-*N*,3-diphenylpropanamide (2.39 g, 0.01 mol) were added to a solution of either **3a** (2.16 g, 0.01 mol), **3b** (2.30 g, 0.01 mol) or **3c** (2.50 g, 0.01 mol) in 1,4-dioxane (50 mL) containing triethylamine (2.00 mL). The whole reaction mixture was heated under reflux for 5 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

*7-Oxo-N,2,4-triphenyl-8-(2-phenylhydrazineylidene)-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide (**12a**).* Orange crystals from 1,4-dioxane, yield (3.67 g, 70%), Mp 180-182 °C. IR (KBr) υ max cm^{-1}: 3478-3339 (NH), 3055 (CH, aromatic), 1689 (C=O), 1641 (C=N), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 2.61-3.23 (2t, 4H, $2CH_2$), 5.15 (s, 1H, pyran H-4), 7.21-7.56 (m, 20H, $4C_6H_5$), 8.23, 8.33 (s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 37.4, 41.6 ($2CH_2$), 50.1 (pyran C-4), 119.8, 120.1, 120.8, 121.2, 121.5, 122.3, 122.5, 123.0, 123.6, 124.2, 124.6, 124.8, 125.2, 125.8, 126.2, 126.9 ($4C_6H_5$), 130.3, 130.5, 131.6, 133.8 (pyran C), 165.9 (C=N), 167.3, 168.6 (2C=O). Anal. calcd. for $C_{34}H_{27}N_3O_3$: C, 77.70; H, 5.18; N, 7.99%. Found: C, 77.89; H, 5.42; N, 8.03%. MS: *m/z* 525 (M^+, 42%).

*7-Oxo-N,2,4-triphenyl-8-(2-(p-tolyl)hydrazineylidene)-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide **(12b)**.* Yellow crystals from 1,4-dioxane, yield (3.66 g, 68%), Mp 135-137 °C. IR (KBr) υ max cm^{-1}: 3495-3346 (NH), 3054 (CH, aromatic), 1698, 1689 (2C=O), 1641 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 2.78 (s, 3H, CH_3), 2.61-3.24 (2t, 4H, $2CH_2$), 5.16 (s, 1H, pyran H-4), 7.25-7.56 (m, 19H, $3C_6H_5$, C_6H_4), 8.25, 8.32 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d$_6$, 75 MHz): δ 34.2 (CH_3), 36.5, 40.6 ($2CH_2$), 50.3 (pyran C-4),120.2, 120.4, 121.3, 121.8, 122.3, 122.4, 123.2, 123.5, 123.8, 124.0, 124.2, 124.8, 125.1, 125.3, 126.0, 126.5 ($3C_6H_5$, C_6H_4), 130.3, 131.2, 132.3, 133.6 (pyran C), 165.5 (C=N), 164.6, 168.7 (2C=O). Anal. calcd. for $C_{35}H_{29}N_3O_3$: C, 77.90; H, 5.42; N, 7.79%. Found: C, 78.21; H, 5.36; N, 7.80%. MS: *m/z* 539 (M^+, 34%).

*8-(2-(4-Chlorophenyl)hydrazineylidene)-7-oxo-N,2,4-triphenyl-5,6,7,8-tetrahydro-4H-chromene -3-carboxamide **(12c)**.* Orange crystals from 1,4-dioxane, yield (3.80 g, 68%), Mp 155-157 °C. IR (KBr) υ max cm^{-1}: 3469-3339 (NH), 3055 (CH, aromatic), 1695, 1688 (2C=O), 1640 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 2.63-3.25 (2t, 4H, $2CH_2$), 5.09 (s, 1H, pyran H-4), 7.21-7.58 (m, 19H, $3C_6H_5$, C_6H_4), 8.25, 8.31 (2s, 2H, D_2O exchangeable, 2NH); ^{13}C NMR (DMSO-d$_6$, 75 MHz): δ 36.2, 39.1 ($2CH_2$), 50.7 (pyran C-4), 119.3, 120.1, 120.5, 121.3, 121.6, 121.9, 122.2, 123.0, 123.8, 123.8, 124.2, 124.6, 125.2, 125.5, 126.0, 126.3 ($3C_6H_5$, C_6H_4), 130.1, 131.2, 132.4, 133.1 (pyran C), 165.4 (C=N), 166.2, 168.7 (2C=O). Anal. calcd. for $C_{34}H_{26}ClN_3O_3$: C, 72.92; H, 4.68; N, 7.50%. Found: C, 73.26; H, 4.72; N, 7.74%. MS: *m/z* 560 (M^+, 36%).

*General procedure for the synthesis of the 1,4,5,6,7,8-hexahydroquinoline derivatives **14a-f**.* Each of benzaldehyde (1.06 g, 0.01 mol) and either 2-cyanoacetamide (0.84 g, 0.01 mol) or 2-cyanoethanethioamide (1.00 g, 0.01 mol) were added to a solution of either **3a** (2.16 g, 0.01 mol), **3b** (2.30 g, 0.01 mol) or **3c** (2.50 g, 0.01 mol) in 1,4-dioxane (50 mL) containing triethylamine (2.00 mL). The whole reaction mixture was heated under reflux for 2 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

*2-Hydroxy-7-oxo-4-phenyl-8-(2-phenylhydrazineylidene)-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile **(14a)**.* Yellow crystals from 1,4-dioxane, yield (2.77 g, 75%), Mp 170-172 °C. IR (KBr) υ max cm^{-1}: 3564-3372 (OH, NH),3055 (CH, aromatic), 2220 (CN), 1689 (C=O), 1640 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 2.60-3.28 (2t, 4H, $2CH_2$), 5.13 (s, 1H, pyridine H-4), 7.25-7.48 (m, 10H, $2C_6H_5$), 8.28, 8.34 (2s, 2H, D_2O exchangeable, 2NH), 10.23 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d$_6$, 75 MHz): δ 36.2, 39.5 ($2CH_2$), 50.4 (pyridine C-4), 116.7 (CN), 119.6, 120.1, 122.2, 123.0, 123.8, 123.6, 124.2, 124.6 ($2C_6H_5$), 130.3, 131.6, 132.1, 133.8 (pyridine C), 165.6 (C=N) 168.9 (C=O). Anal. calcd. for $C_{22}H_{18}N_4O_2$: C, 71.34; H, 4.90; N, 15.13%. Found: C, 71.59; H, 4.78; N, 15.08%. MS: *m/z* 370 (M^+, 48%).

*2-Mercapto-7-oxo-4-phenyl-8-(2-phenylhydrazineylidene)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile **(14b)**.* Yellow crystals from 1,4-dioxane, yield (2.54 g, 66%), Mp 180-184 °C. IR (KBr) υ max cm^{-1}: 3487-3339 (NH), 3055 (CH, aromatic), 2222 (CN), 1688 (C=O), 1640 (C=N), 1630 (C=C), 1205 (SH); ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 2.62-3.27 (2t, 4H, $2CH_2$), 5.15 (s, 1H, pyridine H-4), 7.22-7.52 (m, 10H, $2C_6H_5$), 8.26, 8.38 (2s, 2H, D_2O exchangeable, 2NH), 10.41 (s, 1H, D_2O exchangeable, SH); ^{13}C NMR (DMSO-d$_6$, 75 MHz): δ 36.2, 39.8 ($2CH_2$), 50.6 (pyridine C-4), 116.9 (CN), 119.4, 120.5, 121.8, 122.7, 123.6, 124.0, 124.6, 124.8 ($2C_6H_5$), 130.0, 131.7, 132.5, 133.3 (pyridine C), 165.4 (C=N) 168.5 (C=O). Anal. calcd. for $C_{22}H_{18}N_4OS$: C, 68.37; H, 4.69; N, 14.50; S, 8.30%. Found: C, 68.44; H, 4.82; N, 14.36; S, 8.49%. MS: *m/z* 386 (M^+, 36%).

*2-Hydroxy-7-oxo-4-phenyl-8-(2-(p-tolyl)hydrazineylidene)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile (**14c**).* Yellow crystals from 1,4-dioxane, yield (2.30 g, 60%), Mp 205-207 °C. IR (KBr) υ max cm^{-1}: 3587-3352 (OH, NH), 3055 (CH, aromatic), 2220 (CN), 1689 (C=O), 1642 (C=N), 1632 (C=C); ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 2.64-3.28 (2t, 4H, 2CH$_2$), 2.87 (s, 3H, CH$_3$), 5.18 (s, 1H, pyridine H-4), 7.24-7.56 (m, 9H, C$_6$H$_5$, C$_6$H$_4$), 8.23, 8.35 (2s, 2H, D$_2$O exchangeable, 2NH), 10.32 (s, 1H, D$_2$O exchangeable, OH); ^{13}C NMR (DMSO-d$_6$, 75 MHz): δ 36.0, 39.6 (2CH$_2$), 36.9 (CH$_3$), 50.8 (pyridine C-4), 116.8 (CN), 119.1, 119.6, 121.6, 122.3, 123.2, 124.2, 124.8, 125.3 (C$_6$H$_5$,C$_6$H$_4$), 130.2, 131.5, 132.2, 135.6 (pyridine C), 165.2 (C=N) 168.6 (C=O). Anal. calcd. for $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.34; N, 14.57 %. Found: C, 71.68; H, 5.24; N, 14.30%. MS: *m/z* 384 (M^+, 56%).

*2-Mercapto-7-oxo-4-phenyl-8-(2-(p-tolyl)hydrazineylidene)-1,4,5,6,7,8-hexahydro-quinoline-3-carbonitrile (**14d**).* Yellow crystals from 1,4-dioxane, yield (2.54 g, 64%), Mp 180-184 °C. IR (KBr) υ max cm^{-1}: 3487-3339 (NH), 3055 (CH, aromatic), 2222 (CN), 1688 (C=O), 1640 (C=N), 1630 (C=C), 1205 (SH); ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 2.62-3.27 (2t, 4H, 2CH$_2$), 2.88 (s, 3H, CH$_3$), 5.15 (s, 1H, pyridine H-4), 7.22-7.52 (m, 9H, C$_6$H$_5$,C$_6$H$_4$), 8.26, 8.38 (2s, 2H, D$_2$O exchangeable, 2NH), 10.41 (s, 1H, D$_2$O exchangeable, SH); ^{13}C NMR (DMSO-d$_6$, 75 MHz): δ 36.2, 39.8 (2CH$_2$), 36.8 (CH$_3$), 50.6 (pyridine C-4), 116.7 (CN), 119.4, 120.5, 121.8, 122.7, 123.6, 124.0, 124.6, 124.8 (C$_6$H$_5$,C$_6$H$_4$), 130.0, 131.7, 132.5, 133.3 (pyridine C), 165.4 (C=N) 168.5 (C=O). Anal. calcd. for $C_{23}H_{20}N_4OS$: C, 68.98; H, 5.03; N, 13.99; S, 8.00%. Found: C, 68.73; H, 4.92; N, 14.18; S, 8.21%. MS: *m/z* 400 (M^+, 28%)

*8-(2-(4-Chlorophenyl)hydrazineylidene)-2-hydroxy-7-oxo-4-phenyl-1,4,5,6,7,8-hexa-hydroquinoline-3-carbonitrile (**14e**).* Yellow crystals from 1,4-dioxane, yield (2.22 g, 55%), Mp 235-237 °C. IR (KBr) υ max cm^{-1}: 3551-3348 (OH, NH), 3055 (CH, aromatic), 2220 (CN), 1688 (C=O), 1642 (C=N), 1632 (C=C); ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 2.62-3.20 (2t, 4H, 2CH$_2$), 5.18 (s, 1H, pyridine H-4), 7.21-7.58 (m, 9H, C$_6$H$_5$, C$_6$H$_4$), 8.22, 8.38 (2s, 2H, D$_2$O exchangeable, 2NH), 10.34 (s, 1H, D$_2$O exchangeable, OH); ^{13}C NMR (DMSO-d$_6$, 75 MHz): δ 36.2, 39.5 (2CH$_2$), 50.4 (pyridine C-4), 116.8 (CN), 119.2, 119.8, 120.2, 121.8, 122.2, 124.6, 124.9, 125.6 (C$_6$H$_5$,C$_6$H$_4$), 130.2, 131.8, 132.6, 135.1 (pyridine C), 165.6 (C=N) 168.8 (C=O). Anal. calcd. for $C_{22}H_{17}ClN_4O_2$: C, 65.27; H, 4.23; N, 13.84%. Found: C, 65.41; H, 4.56; N, 13.96%. MS: *m/z* 404 (M^+, 48%).

*8-(2-(4-Chlorophenyl)hydrazineylidene)-2-mercapto-7-oxo-4-phenyl-1,4,5,6,7,8-hexa-hydroquinoline-3-carbonitrile (**14f**).* Yellow crystals from 1,4-dioxane, yield (2.94 g, 70%), Mp 222-225 °C. IR (KBr) υ max cm^{-1}: 3489-3348 (NH), 3055 (CH, aromatic), 2220 (CN), 1688 (C=O), 1640 (C=N), 1630 (C=C), 1205 (SH); ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ = 2.64-3.32 (2t, 4H, 2CH$_2$), 5.15 (s, 1H, pyridine H-4), 7.22-7.52 (m, 9H, C$_6$H$_5$, C$_6$H$_4$), 8.26, 8.38 (2s, 2H, D$_2$O exchangeable, 2NH), 10.41 (s, 1H, D$_2$O exchangeable, SH); ^{13}C NMR (DMSO-d$_6$, 75 MHz): δ 36.2, 39.8 (2CH$_2$), 50.6 (pyridine C-4), 116.7 (CN), 119.2, 120.3, 120.7, 122.8, 123.4, 124.2, 124.3, 124.9 (C$_6$H$_5$, C$_6$H$_4$), 130.4, 131.2, 132.4, 133.7 (pyridine C), 165.2 (C=N) 168.7 (C=O). Anal. calcd. for $C_{22}H_{17}ClN_4OS$: C, 62.78; H, 4.07; N, 13.31; S, 7.62%. Found: C, 62.90; H, 4.25; N, 13.26; S, 7.80%. MS: *m/z* 420 (M^+, 68%).

CONCLUSION

In this work the adopted the synthesis of heterocyclic compounds starting from arylhydrazone derivatives of cyclohexan-1,3-dione through different multi-component reactions. Different measurements were carried out to evaluate the target molecules as anticancer agents. Seven compounds were the most common potent compounds toward the cancer cell lines, c-Met kinase, PC-3 cell line, tyrosine kinases. Whereas, eight compounds were the most common potent compounds toward Pim-1 kinase. The results obtained in this work encourage further work in the future since many compounds were considered as promising anticancer agents.

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