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ARTICLE

Synthesis and evaluation of novel 4*H*-pyrazole and thiophene derivatives derived from chalcone as potential anti-proliferative agents, Pim-1 kinase inhibitors, and PAINS

Nadia Y. Megally Abdo[1] | **Eman M. Samir**[2] | **Rafat M. Mohareb**[3]

[1]Chemistry Department, Faculty of Education, Alexandria University, Alexandria, A. R. Egypt

[2]Department of Organic Chemistry, National Organization for Drug Control & Research (NODCAR), P.O. 29, Cairo, A. R. Egypt

[3]Department of Chemistry, Faculty of Science, Cairo University, Cairo, A. R. Egypt

Correspondence
Nadia Y. Megally Abdo, Chemistry Department, Faculty of Education, Alexandria University, Alexandria 21526, Egypt.
Email: nadiamegally@yahoo.com; nadiamegally@gmail.com

Abstract

The chalcone derivatives **3a-d** reacted with either malononitrile or ethyl cyanoacetate in ethanol in the presence of catalytic amount of ammonium acetate in an oil bath at 120°C to give the Knowevenagel condensation products **5a-h**. The latter compounds reacted with hydrazine hydrate and afforded the 4*H*-pyrazole derivatives **7a-h**, respectively. The reaction of compounds **7a-h** with ethyl cyanoacetate in dimethylformamide under refluxing condition afforded the cyanoacetamido derivatives **8a-h**, respectively. When compounds **8a-h** reacted with elemental sulfur and either of malononitrile or ethyl cyanoacetate in ethanol containing triethylamine, the thiophene derivatives **9a-h** and **10a-h**, respectively, were obtained. The structure of the newly synthesized compounds was established by the analytical and spectral data. All the newly synthesized compounds were evaluated against the six cancer cell lines: A549, HT-29, MKN-45, U87MG, and SMMC-7721 and H460. Compounds **3c**, **5h**, **7g**, **7h**, **8f**, **9e**, **9g**, and **10g** were selected to examine their Pim-1 kinase inhibition activity as these compounds showed high inhibition toward the c-Met kinase and the tested cancer cell lines. Furthermore, compounds **3b**, **3c**, **5g**, **5h**, **7f**, **7g**, **7h**, **8e**, **8f**, **8g**, **8h**, **9e**, **9f**, **9g**, **9h**, **10g** and **10h** were selected to be tested for pan-assay interference compounds analysis (PAINS). Almost all the tested compounds showed zero PAINS alert and can be used as drug compounds in the future.

1 | INTRODUCTION

Because of the impressive anti-infective activities[1] of chalcones, they are considered as important classes of compounds. Benzylidene acetophenone scaffold is the 1,3-diphenyl-2*E*-propene-1-one (chalcones), which is considered as one of the most important classes of natural products across the plant kingdom. It contains two aromatic nuclei that are joined by a three-carbon α,β-unsaturated carbonyl bridge. Generally, chalcones can be obtained synthetically by condensing aryl ketones with aromatic aldehydes in the presence of a catalyst or condensing agents.[2] Chalcone compounds were used in many heterocyclic reactions to produce a variety of biological active target molecules.[3–5] The produced molecules showed analgesic,[6] arthritis,[7] anti-inflammatory,[8] antimalarial,[9,10] antipyretic,[11] antibacterial,[12] antiviral,[13,14] and anticancer[15–17] effects. Many reported works also showed that chalcones have anti-proliferative and cytotoxic effects.[18–20] There are many studies through

which chalcones are characterized by high cytotoxicities and inhibitions toward different cancer cell lines.[21–23] Originally, chalcones were first isolated from flavonoid biosynthesis in plants[24]; afterwards, they were structurally modified through the synthesis of different polyfunctionally substituted compounds.[25] Basically, it has been shown that the removal of α,β-unsaturated carbonyl system could decrease their biological activities.[26] A number of synthetic modifications, such as fused heterocyclic compounds,[27] biphenyl-based[28] coumarin-based chalcones,[29] or other substitutions,[30–34] have also been reported to affect the biological activities including anticancer activities of chalcones.[35–37]

In the present work, because of the high pharmaceutical activities of chalcones[38] as described before, we tried to make different heterocyclization reactions of some chalcones, which leads to the preparation of pyrazole and thiophene rings together with their antitumor evaluations.

2 | RESULTS AND DISCUSSION

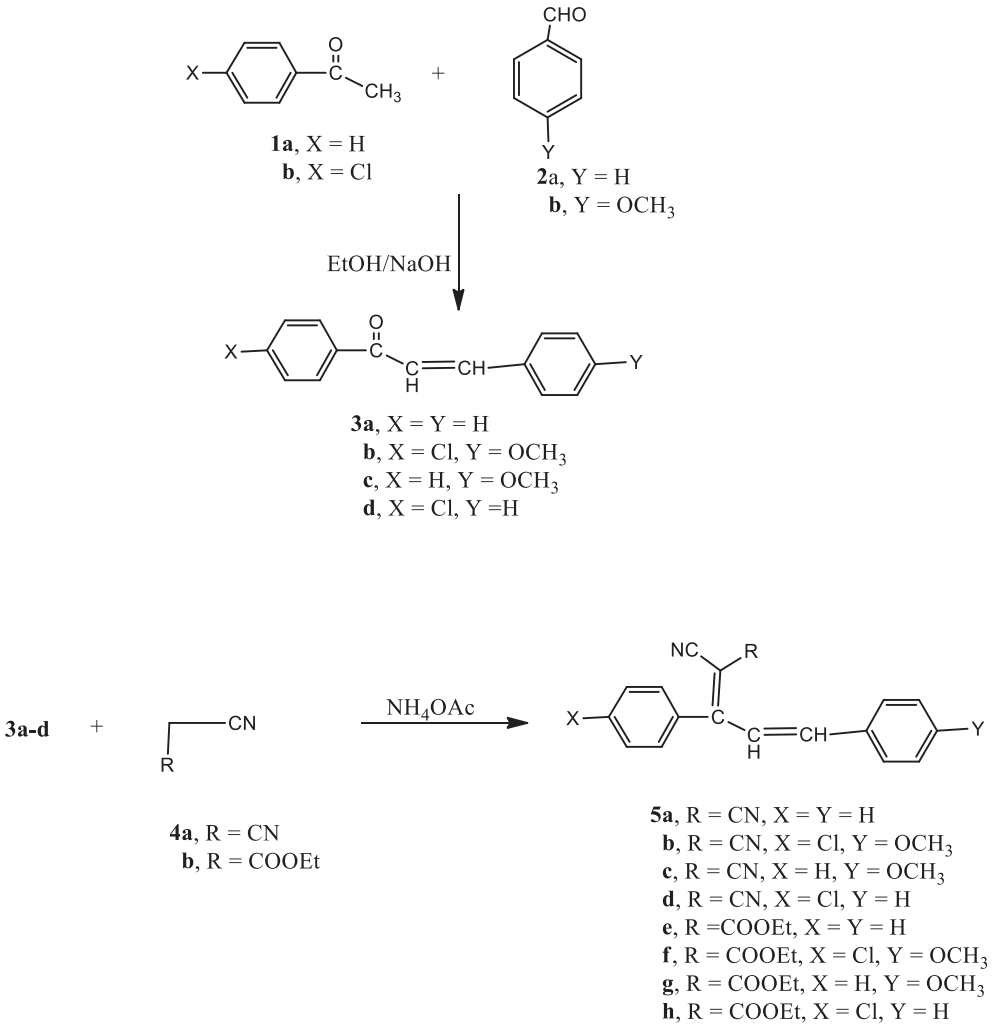
2.1 | Chemistry

In this work, we demonstrated a series of reactions starting from different charlcones that underwent a series of heterocyclization reaction to produce biologically active target molecules. Thus, the reaction of either of acetophenone (**1a**) or 4-chloroacetophenone (**1b**) with either of benzaldehyde (**2a**) or 4-methoxybenzaldehyde (**2b**) in ethanol containing sodium hydroxide gave the chalcone derivatives **3a-d**.[39–41] It has been reported the reaction of chalcone derivatives with either malononitrile or ethyl cyanoacetate in varieties of reaction conditions to produce each time specific product. Thus, carrying the reaction in toluene containing $Al(O^iPr)_3$, the product mainly according to Michael addition[42] followed by cyclization to pyridine in ethanolic sodium ethoxide solution. In other report, carrying the reaction in MW and in the presence of morphiline the product is the dicyanobenzene derivative.[43] Moreover, carrying the reaction in ethanol containing piperidine gave pyran derivative.[44] Our result for this reaction is clearly in conflict with previous results as the result of using different reaction conditions. These significant discrepancies and our extensive work on the synthesis of the Knoevenagel condensation products prompted us to carefully reinvestigate the product of this reaction using ammonium acetate. Thus, the reaction of any of the chalcone derivatives **3a**, **3b**, **3c**, or **3d** with either of malononitrile (**4a**) or ethyl cyanoacetate (**4b**) in the presence of ammonium acetate in an oil bath at 120°C gave the Knowevenagel condensation products **5a-h** (Scheme 1). The structures of compounds **5a-h** were confirmed on the basis of their respective analyses. Thus, the ^{1}H NMR spectrum of compound **5a** showed the presence of two doublets at δ = 6.38, 6.41 ppm corresponding to the two vinyl protons beside multiplet at δ = 7.26 to 7.39 ppm for the two C_6H_5 protons. Moreover, the ^{13}C NMR spectrum revealed the presence of signals at 89.3, 90.5 (CH═CH), 116.5, 116.9 (2CN), and 131.2, 134.2 (C═C) beside the signals corresponding to the two phenyl carbons. Further confirmation for these structures were obtained through the studying of their reactivities toward some chemical reagents.

Thus, the reaction of any of compounds **5a-h** with hydrazine hydrate gave the pyrazole derivatives **7a-h**, respectively. The analytical and spectral data of the latter products were consistent with their respective structures (see Section 4). The 2-amino group of the pyrazole derivatives **7a-h** showed interesting reactivity toward amide formation. Thus, the reaction of any of compounds **7a-h** with one fold of ethyl cyanoacetate in dimethylformamide under refluxing condition gave the monocyanoacetamido derivatives **8a-h**, respectively (Scheme 2). However, the use of two folds of ethyl cyanoacetate in case of **7a**, **7b**, **7c**, and **7d** gave unidentified mixture of products, and our trials to separate the bis product failed. Our explanation for such findings is that for compounds **7a**, **7b**, **7c**, and **7d**, it seemed that there is a tautomeric structure through which the second NH_2 group exist in the imino form instead of pure NH_2 group. The ^{1}H NMR spectrum of compound **8a** (as an example) revealed the presence of two singlets at δ = 3.80 and 8.26 ppm corresponding to the CH_2 and NH (D_2O exchangeable) groups of the cyanoacetamido moiety. In addition to the presence of a singlet at δ = 4.56 ppm for the two protons of the NH_2 (D_2O exchangeable), two doublets at δ = 6.34, 6.43 ppm indicating the presence of the CH═CH group and a multiplet at δ = 7.25 to 7.39 ppm for the two C_6H_5 protons. Moreover, the ^{13}C NMR spectrum showed the presence of signals at 27.2 (CH_2), 89.3, 90.5 (CH═CH), 117.1 (CN), 131.5, 134.4 (C═C), 168.1 (CO), and 172.4, 176.2 (2C═N) beside signals corresponding to the two phenyl carbons.

The cyanomethyl moiety present in compounds **8a-h** showed interesting reactivity toward the Gewald's thiophene synthesis.[45–47] Thus, the reaction of any of compounds **8a-h** with elemental sulfur and malononitrile (**4a**) in ethanol containing triethylamine gave the thiophene derivatives **9a-h**. The analytical and spectral data of **9a-h** were in agreement with their respective structures. It is important to note that in these reactions, we used the

SCHEME 1 Synthesis of compounds **3a-d** and **5a-h**



1a, X = H
b, X = Cl

2a, Y = H
b, Y = OCH_3

3a, X = Y = H
b, X = Cl, Y = OCH_3
c, X = H, Y = OCH_3
d, X = Cl, Y =H

4a, R = CN
b, R = COOEt

5a, R = CN, X = Y = H
b, R = CN, X = Cl, Y = OCH_3
c, R = CN, X = H, Y = OCH_3
d, R = CN, X = Cl, Y = H
e, R =COOEt, X = Y = H
f, R = COOEt, X = Cl, Y = OCH_3
g, R = COOEt, X = H, Y = OCH_3
h, R = COOEt, X = Cl, Y = H

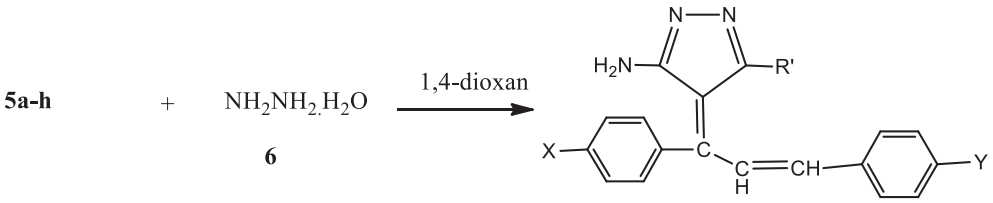
ideal conditions of Gewald's thiophene reaction because of reacting two molecules of different cyanomethylene compounds with elemental sulfur in the presence of triethylamine as a catalyst and the possibility of Micheal addition reaction occurred through intermediate steps. Thus, the ^{1}H NMR spectrum of **9a** (as an example) showed the presence of three singlets at δ = 4.46, 4.56, 5.21 ppm corresponding to the presence of the three NH_2 groups beside another singlet at δ = 8.29 ppm (D_2O exchangeable) for the NH proton. In addition to that the presence of two doublets at δ = 6.32, 6.48 ppm corresponding to the two vinyl protons and multiplet at δ = 7.22 to 7.36 ppm for the two C_6H_5 protons. Moreover, the ^{13}C NMR spectrum revealed the presence of signals at 89.1, 90.8 (CH═CH), 117.0 (CN), 131.1, 134.7 (C═C), 167.4 (CO), 172.1, 176.6 (2C═N) beside twelve signals corresponding to the two phenyl and thiophene carbons. Similarly, the reaction of any of compounds **8a-h** with elemental sulfur and ethyl cyanoacetate (**4b**) in ethanol containing triethylamine gave the thiophene derivatives **10a-h** (Scheme 3).

2.2 | Biology

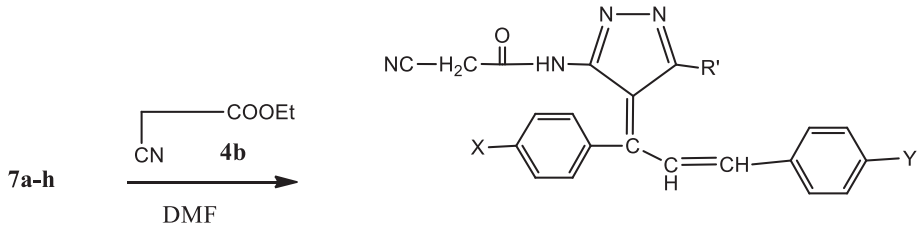
2.2.1 | Cell proliferation assay

The newly synthesized compounds were screened for anti-proliferative activities (Table 1) against the six cancer cell lines A549, HT-29, MKN-45, U87MG, and SMMC-7721 and H460 by applying the standard MTT assay in vitro, using foretinib as the positive control.[48–50]

The cancer cell lines were cultivated in a minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). In the MEM medium, 4×10^3 cells were plated onto each well of a 96-well plate and kept in 5% CO_2 at 37°C for 24 hours. At the specified final concentrations, the compounds tested were added to the culture medium, and the cell cultures were sustained for 72 hours. To each well fresh, MTT was added at a terminal concentration of 5 μg/mL and kept in dark with the cells at 37°C for 4 hours. An enzyme-linked immunosorbent assay (ELISA) reader was used to measure the absorbance (the absorbance of MTT formazan at 492 nM



7a, R' = NH_2, X = Y = H
b, R' = NH_2, X = Cl, Y = OCH_3
c, R' = NH_2, X = H, Y = OCH_3
d, R' = NH_2, X = Cl, Y = H
e, R' = OH, X = Y = H
f, R' = OH, X = Cl, Y = OCH_3
g, R' = OH, X = H, Y = OCH_3
h, R' = OH, X = Cl, Y = H



8a, R' = NH_2, X = Y = H
b, R' = NH_2, X = Cl, Y = OCH_3
c, R' = NH_2, X = H, Y = OCH_3
d, R' = NH_2, X = Cl, Y = H
e, R' = OH, X = Y = H
f, R' = OH, X = Cl, Y = OCH_3
g, R' = OH, X = H, Y = OCH_3
h, R' = OH, X = Cl, Y = H

SCHEME 2 Synthesis of compounds **7a-h** and **8a-h**

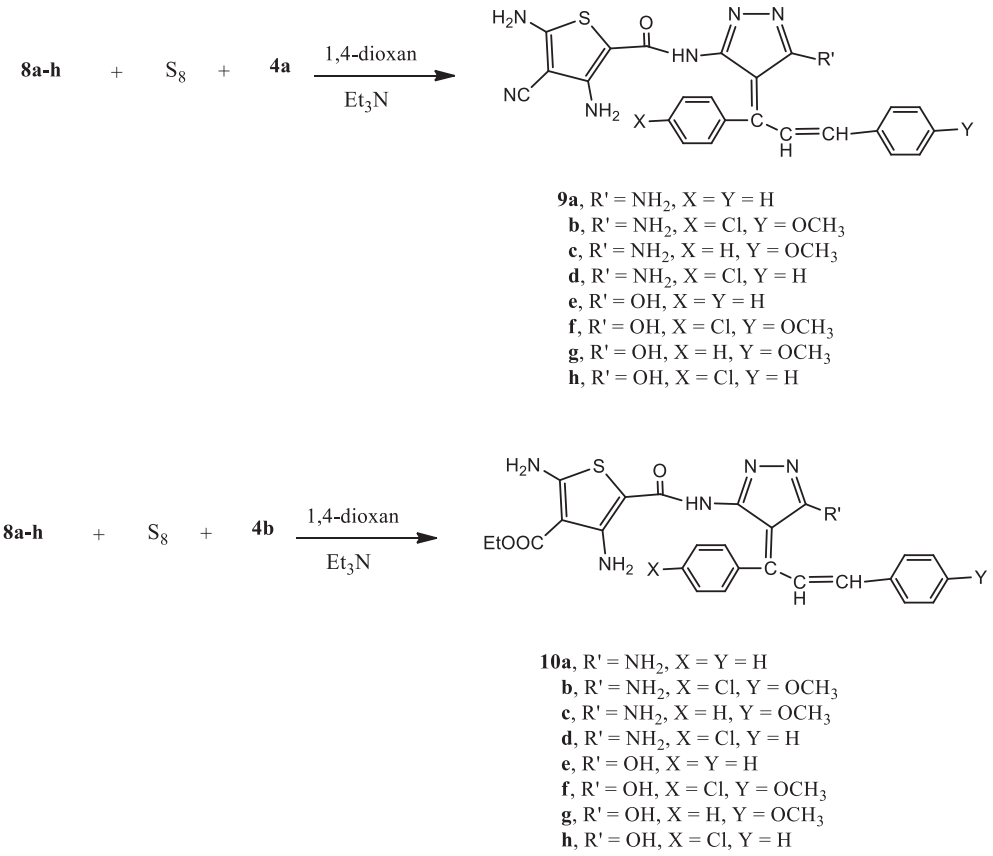
and for the reference wavelength at 630 nM) after dissolving the formazan crystals in 100 μL of DMSO each well.

The mean values of three independent experiments, expressed as IC_{50} values (inhibitory concentration 50%), were calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software and presented in Table 1. Most of the synthesized compounds exhibited potent anti-proliferative activity with IC_{50} values less than 12.00 μM. Generally, the variations of substituents within the pyrazole and thiophene moieties have a notable influence on the anti-proliferative activity.

2.2.2 | Structure activity relationship

It is clear from Table 1 that compounds **3b**, **3c**, **3d**, **5f**, **5g**, **5h**, **7f**, **7g**, **7h**, **8e**, **8f**, **8g**, **8h**, **9d-9h**, **10e**, **10g**, and **10h** were the most cytotoxic compounds against the six cancer cell lines. Considering the chalcones **3a-d**, it is clear that compounds **3b**, **3c**, and **3d** were the most active compounds through this series of compounds, in addition compound **3c** (X═H, Y═OCH_3) was of the highest cytotoxicity. For the reaction of compounds **3a-d** with either malononitrile or ethyl cyanoacetate to give compounds **5a-h**, it is obvious that compounds **5f** (R═COOEt, X═Cl, Y═OCH_3), **5g** (R═COOEt, X═H, Y═OCH_3), and **5h** (R═COOEt, X═Cl, Y═H) were the most inhibit compounds. Similarly, for the pyrazole derivatives **7a-h**, it is clear that compounds **7f** (R′═OH, X═Cl, Y═OCH_3), **7g** (R′═OH, X═H, Y═OCH_3), and **7h** (R′═OH, X═Cl, Y═H) were the most cytotoxic compounds. On the other hand, the *N*-cyanoacetamido derivatives **8a-h**, it is of great value to note that compounds **8e** (R′═OH, X═Y═H), **8f** (R′═OH, X═Cl, Y═OCH_3), **8g** (R′═OH, X═H, Y═OCH_3), and **8h** (R′═OH, X═Cl, Y═H) were of the highest inhibitions among the eight compounds. Moreover, for the thiophene derivatives **9a-h**, compounds **9d-h** were the most active compounds. The cytotoxicity of such compounds was attributed to the presence of the pyrazole ring together with the thiophene moieties. However, the thiophene derivatives **10a-h**, compounds **10e**, **10g**, and **10h** were of the highest cytotoxicity. It is obvious that the presence of the OH group through the three compounds

SCHEME 3 Synthesis of compounds **9a-h** and **10a-h**



9a, R' = NH_2, X = Y = H
b, R' = NH_2, X = Cl, Y = OCH_3
c, R' = NH_2, X = H, Y = OCH_3
d, R' = NH_2, X = Cl, Y = H
e, R' = OH, X = Y = H
f, R' = OH, X = Cl, Y = OCH_3
g, R' = OH, X = H, Y = OCH_3
h, R' = OH, X = Cl, Y = H

10a, R' = NH_2, X = Y = H
b, R' = NH_2, X = Cl, Y = OCH_3
c, R' = NH_2, X = H, Y = OCH_3
d, R' = NH_2, X = Cl, Y = H
e, R' = OH, X = Y = H
f, R' = OH, X = Cl, Y = OCH_3
g, R' = OH, X = H, Y = OCH_3
h, R' = OH, X = Cl, Y = H

together with the thiophene and pyrazole moieties was the reason for such activities.

In summary, compounds **3b**, **3c**, **3d**, **5g**, **5h**, **7f**, **7g**, **7h**, **8e**, **8f**, **8g**, **8h**, **9e**, **9f**, **9g**, **9h**, **10g**, and **10h** were of the higher cytotoxicity than the reference foretinib against U87MG cell line. On the other hand, compounds **3c**, **8g**, and **9h** with IC_{50}'s 0.23 μM, 0.26 μM, and 0.32 μM were higher cytotoxic than the reference foretinib against SMMC-7721 cell line.

2.2.3 | Inhibition of the most active compounds against tyrosine kinases (Enzyme IC_{50} [nM])

The most active compounds **3b**, **3c**, **5g**, **5h**, **7f**, **7g**, **7h**, **8e**, **8f**, **8g**, **8h**, **9e**, **9f**, **9g**, **9h**, **10g**, and **10h** were further evaluated against the five tyrosine kinase (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) using the screening method (Table 2). In summary, 20 μg/mL poly(Glu, Tyr) 4:1 (Sigma) was precoated as a substrate in 384-well plates. To each well, 50 μL of 10 M ATP (Invitrogen) solution diluted in kinase reaction buffer (50μM HEPES, Ph 7.0, 1M DTT, 1M $MgCl_2$, 1M $MnCl_2$, and 0.1% NaN_3) was added. The concentrations of compounds diluted in 10 μL of 1% DMSO (v/v) were used. Purified tyrosine kinase proteins diluted in 39 μL was added to the kinase reaction, which was initiated by the kinase buffer solution.

Compounds **3b, 3c, 5g, 5h, 7f, 7g, 7h, 8e, 8f, 8g, 9e, 9f, 9g, 9h, 10g**, and **10h** were selected for inhibition of the five tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR. It clear from Table 2 that compounds **3c, 5h, 7g, 7h, 8f, 9e, 9g**, and **10g** were the most potent of the tested compounds toward the five tyrosine kinases. Compound **5g** showed high potency toward the two kinases Flt-3 and PDGFR with IC_{50}'s 0.62 nM and 0.73 nM, respectively. In addition, compound **7f** showed activity toward the three kinases VEGFR-2, EGFR, and PDGFR with IC_{50}'s 0.72 nM, 0.59 nM, and 0.31 nM, respectively. Compounds **3b** and **10h** showed the lowest potency among the tested compounds.

2.2.4 | Inhibition of selected compounds toward Pim-1 kinase

Furthermore, compounds **3c, 5h, 7g, 7h, 8f, 9e, 9g**, and **10g** were selected to examine their Pim-1 kinase inhibition activity (Table 3) as these compounds showed high inhibition toward the c-Met kinase and the tested cancer cell lines at a range of 10 concentrations, and the IC_{50}

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

	IC_{50} ± SEM, μM					
Compound No	**A549**	**H460**	**HT29**	**MKN-45**	**U87MG**	**SMMC-7721**
3a	2.61 ± 1.23	4.26 ± 0.09	2.43 ± 1.28	3.65 ± 1.26	2.26 ± 1.05	3.57 ± 1.96
3b	1.65 ± 0.81	1.29 ± 0.78	1.29 ± 0.81	0.68 ± 0.29	0.72 ± 0.38	1.36 ± 0.86
3c	0.22 ± 0.13	0.39 ± 0.21	0.43 ± 0.18	0.63 ± 0.34	0.28 ± 0.09	0.23 ± 0.08
3d	0.75 ± 0.34	0.53 ± 0.19	0.28 ± 0.09	0.26 ± 0.07	0.35 ± 0.21	1.24 ± 0.89
5a	8.42 ± 2.43	8.52 ± 2.79	7.56 ± 2.69	8.37 ± 2.49	6.46 ± 2.31	8.27 ± 3.17
5b	6.16 ± 2.32	6.27 ± 2.31	6.46 ± 2.29	4.63 ± 1.28	6.53 ± 2.41	5.41 ± 1.28
5c	1.64 ± 0.84	2.87 ± 1.26	2.39 ± 0.84	1.93 ± 1.08	2.62 ± 1.12	2.86 ± 1.06
5d	3.21 ± 1.48	5.73 ± 1.69	6.42 ± 2.42	5.68 ± 1.32	4.28 ± 1.28	5.43 ± 2.42
5e	6.24 ± 3.21	5.39 ± 2.28	6.39 ± 2.24	8.18 ± 2.90	8.27 ± 2.73	5.42 ± 2.27
5f	0.79 ± 0.24	0.53 ± 0.22	0.83 ± 0.39	1.08 ± 0.55	1.22 ± 0.86	0.48 ± 0.21
5g	0.24 ± 0.18	0.56 ± 0.31	0.83 ± 0.53	0.58 ± 0.22	0.28 ± 0.16	0.58 ± 0.21
5h	0.37 ± 0.19	0.53 ± 0.26	0.31 ± 0.19	0.68 ± 0.28	0.35 ± 0.28	0.86 ± 0.29
7a	9.29 ± 2.39	8.28 ± 2.68	6.33 ± 1.28	4.32 ± 2.83	6.74 ± 1.38	6.80 ± 2.49
7b	6.28 ± 2.79	5.78 ± 1.28	6.35 ± 2.58	5.29 ± 2.80	6.84 ± 1.82	6.14 ± 2.35
7c	2.68 ± 1.34	2.73 ± 1.94	5.28 ± 1.84	6.26 ± 1.79	6.23 ± 1.46	5.49 ± 2.24
7d	4.21 ± 1.83	3.62 ± 1.61	3.68 ± 1.32	4.58 ± 1.39	2.92 ± 0.73	3.18 ± 0.49
7e	1.28 ± 0.82	2.61 ± 1.39	1.59 ± 0.39	2.53 ± 1.06	3.63 ± 1.23	2.76 ± 0.92
7f	0.53 ± 0.25	0.38 ± 0.19	0.63 ± 0.35	0.72 ± 0.31	0.58 ± 0.24	0.69 ± 0.13
7g	0.52 ± 0.31	0.59 ± 0.23	0.69 ± 0.31	1.03 ± 0.68	0.53 ± 0.23	0.69 ± 0.28
7h	0.62 ± 0.39	0.72 ± 0.31	0.53 ± 0.36	0.64 ± 0.29	0.58 ± 0.31	0.64 ± 0.23
8a	7.32 ± 2.18	8.62 ± 3.62	6.23 ± 1.82	5.32 ± 1.62	4.28 ± 1.62	6.59 ± 1.28
8b	6.82 ± 1.48	6.74 ± 2.84	8.23 ± 2.63	7.68 ± 1.84	8.39 ± 2.59	5.74 ± 1.93
8c	4.56 ± 1.24	3.36 ± 1.63	4.63 ± 1.36	5.53 ± 1.35	4.96 ± 1.48	5.24 ± 2.69
8d	3.46 ± 1.42	3.24 ± 1.80	2.29 ± 1.02	3.60 ± 1.86	4.59 ± 1.34	3.25 ± 1.68
8e	1.21 ± 0.86	0.93 ± 0.46	0.62 ± 0.31	1.46 ± 0.87	0.79 ± 0.35	0.69 ± 0.23
8f	1.09 ± 0.53	1.21 ± 0.69	1.32 ± 0.68	0.92 ± 0.44	0.79 ± 0.38	1.20 ± 0.53
8g	0.82 ± 0.44	0.39 ± 0.21	0.64 ± 0.41	0.27 ± 0.08	0.49 ± 0.28	0.26 ± 0.19
8h	0.71 ± 0.32	0.83 ± 0.46	0.71 ± 0.32	0.33 ± 0.17	0.28 ± 0.07	0.57 ± 0.29
9a	10.58 ± 4.25	12.49 ± 4.28	10.57 ± 2.73	9.52 ± 2.84	11.62 ± 2.93	8.39 ± 2.52
9b	5.69 ± 1.04	7.40 ± 2.16	6.39 ± 2.42	8.29 ± 2.63	8.26 ± 2.92	6.80 ± 2.71
9c	12.28 ± 4.06	8.48 ± 2.53	8.62 ± 2.70	8.31 ± 3.70	9.66 ± 2.17	7.63 ± 2.17
9d	1.06 ± 0.59	1.15 ± 0.68	2.79 ± 1.15	0.89 ± 0.27	0.92 ± 0.37	1.05 ± 0.36
9e	0.88 ± 0.25	0.69 ± 0.17	0.46 ± 0.23	0.92 ± 0.26	0.56 ± 0.29	0.44 ± 0.16
9f	0.68 ± 0.21	0.73 ± 0.26	1.05 ± 0.42	0.87 ± 0.30	0.61 ± 0.24	0.49 ± 0.26
9g	0.37 ± 0.09	0.26 ± 0.18	0.57 ± 0.31	0.48 ± 0.14	0.28 ± 0.16	0.46 ± 0.20
9h	0.28 ± 0.08	0.29 ± 0.05	0.80 ± 0.24	0.42 ± 0.28	0.38 ± 0.18	0.32 ± 0.17
10a	8.42 ± 2.65	8.69 ± 2.43	7.80 ± 2.54	9.17 ± 2.74	8.36 ± 2.48	9.32 ± 2.57
10b	5.85 ± 1.69	8.59 ± 2.69	8.73 ± 2.80	8.59 ± 2.49	9.36 ± 3.41	7.09 ± 2.42
10c	9.42 ± 2.64	8.69 ± 2.43	6.84 ± 2.48	9.42 ± 2.56	8.52 ± 2.39	7.93 ± 2.31
10d	6.20 ± 2.53	7.08 ± 2.35	8.04 ± 3.54	9.22 ± 3.69	8.95 ± 2.70	7.49 ± 2.53
10e	1.08 ± 0.64	0.85 ± 0.28	0.94 ± 0.42	0.85 ± 0.32	0.92 ± 0.37	0.49 ± 0.16

(Continues)

TABLE 1 (Continued)

Compound No	$IC_{50} \pm SEM$, μM					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
10f	2.57 ± 1.04	2.75 ± 0.92	1.69 ± 0.73	2.68 ± 1.49	1.69 ± 0.77	1.48 ± 0.83
10g	1.02 ± 0.28	1.27 ± 0.82	0.58 ± 0.30	0.87 ± 0.16	0.55 ± 0.21	0.74 ± 0.25
10h	0.36 ± 0.12	0.48 ± 0.16	0.69 ± 0.25	0.56 ± 0.23	0.49 ± 0.21	0.62 ± 0.36
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

TABLE 2 Inhibition of tyrosine kinases enzyme IC_{50} (nM) of compounds **3b, 3c, 5g, 5h, 7f, 7g, 7h, 8e, 8f, 8g, 9e, 9f, 9g, 9h, 10g**, and **10h**

Compound	c-Kit	Flt-3	VEGFR-2	EGFR	PDGFR
3b	2.58	1.69	2.42	2.80	1.63
3c	0.68	0.37	0.92	0.47	0.58
5g	1.05	0.62	1.15	1.62	0.73
5h	0.35	0.62	0.49	0.81	0.27
7f	1.16	1.05	0.72	0.59	0.31
7g	0.26	0.35	0.72	0.29	0.83
7h	0.33	0.51	1.08	0.28	0.52
8e	1.15	1.27	1.08	0.59	1.42
8f	0.26	0.19	0.42	0.38	0.61
8g	2.65	1.37	1.22	1.59	1.26
9e	0.26	0.72	0.31	0.47	0.33
9f	1.36	1.27	1.47	1.09	0.96
9g	0.40	0.37	0.29	0.18	0.42
9h	1.36	1.80	1.27	1.00	0.66
10g	0.27	0.17	1.97	0.81	1.05
10h	4.68	5.77	6.80	4.29	5.39

TABLE 3 The inhibition activity of compounds **3c, 5h, 7g, 7h, 8f, 9e, 9g**, and **10g** toward Pim-1 Kinase

Compound	Inhibition Ratio At 10μM	IC_{50}, μM
3c	21	>10
5h	96	0.24
7g	86	0.30
7h	18	>10
8f	16	>10
9e	92	0.23
9g	89	0.32
10g	24	>10

values were calculated. Compounds **5h, 7g, 9e**, and **9g** were the most potent to inhibit Pim-1 activity with IC_{50} value of 0.24 μM, 0.30 μM, 0.23 μM, and 0.32 μM, respectively. On the other hand, compounds **3c, 7h, 8f**, and **10g** were less effective (IC_{50} > 10 μM). SGI-1776 was used as positive control with IC_{50} 0.048 μM in the assay. These profiles in combination with cell growth inhibition data of compounds **3c, 5h, 7g, 7h, 8f, 9e, 9g**, and **10g** were listed in Table 3 indicated that Pim-1 was a potential target of these compounds.

2.2.5 | PAINS analysis

Compounds **3b, 3c, 5g, 5h, 7f, 7g, 7h, 8e, 8f, 8g, 8h, 9e, 9f, 9g, 9h, 10g**, and **10h** were selected to be tested for pan-assay interference compounds analysis (PAINS). Successful drug discovery process involves high-throughput screening (HTS). During HTS, it is predicted that successes from HTS operations include primarily false positives between the actual hits if it is found.[51,52] Compounds can be regarded as false positives because of

TABLE 4 Drug like the character of different compounds and standard drugs foretinib and SGI-1776

Compound	Druglikeness Rule	Medicinal Chemistry Rules			
	Lvio.a/No. of vio.[a]	Vvio.b/No. of vio.[b]	Gvio.c/No. of vio.[c]	Lead likeliness /No.	PAINS alertd of vio.
3b	None	None	None	None	0
3c	None	None	None	None	0
5g	None	None	None	1	1
5h	None	None	None	2	0
7f	None	None	None	None	0
7g	None	None	None	None	0
7h	None	None	None	None	0
8e	None	None	None	1	0
8f	None	None	None	3	1
8g	None	None	None	1	0
8h	None	None	None	None	0
9e	None	None	None	None	0
9f	None	None	None	None	0
9g	None	None	None	2	1
9h	None	None	None	1	
10g	None	None	None	None	0
10h	None	None	None	1	0
Foretinib	None	None	None	1	0
SGI-1776	None	None	None	2	0

Abbreviations: Gvio., Ghose filter; Lvio., Lipinski's rule; Vvio., Veber rules; PAINS, pan-assay interference compounds analysis.

a number of reasons like binding interactions by forming aggregates,[53–55] by being protein-reactive entities[56–58] or by directly interfering with assay signaling. PAINS are chemical entities that are frequently false positives in HTS. PAINS have a tendency to nonspecifically react with several biological targets moderately, then specifically disturbing one preferred.[59] A number of disorderly functional groups are collected by numerous PAINS.[60] Unwanted compounds may negatively influence not only enzyme assays but also phenotypic screens and show biological activity for the wrong reason.[61] The PAINS violations of proposed compounds and reference drugs are given in Table 4. Almost all the compounds showed zero PAINS alert and can be used as lead compounds.

3 | CONCLUSION

The well-known chalcones **3a-d** were considered as the key starting material through their reactions with different reagents to synthesize the 4*H*-pyrazole derivatives **7a-h** and the thiophene derivatives **9a-h** and **10a-h**, respectively. The structure of the newly synthesized compounds was established by the analytical and spectral data. All the newly synthesized compounds were evaluated against the six cancer cell lines A549, HT-29, MKN-45, U87MG, and SMMC-7721 and H460. Compounds **3c, 5h, 7g, 7h, 8f, 9e, 9g**, and **10g** were selected to examine their Pim-1 kinase inhibition activity as these compounds showed high inhibition toward the c-Met kinase and the tested cancer cell lines. Furthermore, compounds **3b, 3c, 5g, 5h, 7f, 7g, 7h, 8e, 8f, 8g, 8h, 9e, 9f, 9g, 9h, 10g**, and **10h** were selected to be tested for PAINS. Almost all the tested compounds showed zero PAINS alert and can be used as lead compounds.

4 | EXPERIMENTAL

4.1 | Chemistry

The obtained compounds showed their melting points using electrothermal digital melting point apparatus and are uncorrected. IR spectra (KBr discs) were measured on a Fourier transform infrared (FTIR) plus 460 or Pye Unicam SP-1000 spectrophotometer (Pye Unicam, UK, Cambridge). ^{1}H NMR spectra were obtained using Varian Gemini-300 (300 MHz, Varian UK) using DMSO-d_6 as a

solvent and tetramethylsilane @@(TMS) as internal standard chemical shifts are expressed as δ = ppm. The mass spectra were measured with Hewlett Packard 5988 A GC/MS system (Hewlett Packard, Agilent, USA) instrument. Analytical data were obtained from on Vario EL III Elemental CHNS analyzer. The antitumor evaluation has been carried out through the National Cancer Research Center in Cairo, Egypt, where the IC_{50} values were calculated. Compounds **3a-d** were synthesized according to the reported procedures.[39–41]

4.1.1 | General procedure for the synthesis of the penta-2,4-dienenitrile derivatives 5a-h

Either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) and ammonium acetate (1.00 g) were added to any of compounds **3a** (2.08, 0.01 mol), **3b** (2.72 g, 0.01 mol), **3c** (2.38 g, 0.01 mol), or **3d** (2.42 g, 0.01 mol). The reaction mixture in each case was heated in an oil bath at 120°C for 1 hour then was left to cool. The solid product, formed in each case, upon trituration with diethyl ether was collected by filtration.

2-(1,3-Diphenylallylidene)malononitrile (5a)

Pale yellow crystals from ethanol, yield (1.81 g, 71%), mp: 180°C to 183°C. IR (KBr) υ_{max} cm^{-1}: 3055 (CH, aromatic), 2223, 2220 (2CN), 1632 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 6.38, 6.41 (2d, 2H, J = 9.89 Hz, CH═CH), 7.26-7.39 (m, 10H, $2C_6H_5$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.3, 90.5 (CH═CH), 116.5, 116.9 (2CN), 120.6, 122.4, 123.5, 124.1, 124.8, 125.4, 127.3, 128.3 ($2C_6H_5$), 131.2, 134.2 (C═C). Anal. calculated for $C_{18}H_{12}N_2$: C, 84.35; H, 4.72; N, 10.93. Found: C, 84.66; H, 5.01; N, 11.23. MS: *m/e* 256 (M^+, 28%).

2-(1-(4-Chlorophenyl)-3-(4-methoxyphenyl)allylidene) malononitrile (5b)

Pale yellow crystals from ethanol, yield (2.11 g, 66%), mp: 166°C to 169°C. IR (KBr) υ_{max} cm^{-1}: 3056 (CH, aromatic), 2221, 2220 (2CN), 1634 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.69 (s, 3H, OCH_3), 6.32, 6.40 (2d, 2H, J = 9.91 Hz, CH═CH), 7.23-7.42 (m, 8H, $2C_6H_4$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.8 (OCH_3), 89.3, 90.5 (CH═CH), 116.5, 116.9 (2CN), 120.4, 121.8, 122.9, 123.6, 126.2, 127.8, 128.1, 129.7 ($2C_6H_4$), 131.1, 134.6 (C═C). Anal. calculated for $C_{19}H_{13}ClN_2O$: C, 71.14; H, 4.08; N, 8.73. Found: C, 71.29; H, 3.82; N, 8.90. MS: *m/e* 320 (M^+, 38%).

2-(3-(4-Methoxyphenyl)-1-phenylallylidene) malononitrile (5c)

Yellow crystals from ethanol, yield (1.97 g, 77%), mp: 203°C to 206°C. IR (KBr) υ_{max} cm^{-1}: 3053 (CH, aromatic), 2222, 2220 (2CN), 1631 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.66 (s, 3H, OCH_3), 6.30, 6.43 (2d, 2H, J = 10.17 Hz, CH═CH), 7.24-7.46 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.4 (OCH_3), 89.6, 90.7 (CH═CH), 116.6, 116.8 (2CN), 120.3, 122.6, 122.8, 123.5, 125.9, 126.3, 127.4, 128.2 (C_6H_5, C_6H_4), 131.1, 134.6 (C═C). Anal. calculated for $C_{19}H_{14}N_2O$: C, 79.70; H, 4.93; N, 9.78. Found: C, 79.59; H, 5.22; N, 10.02. MS: *m/e* 286 (M^+, 42%).

2-(1-[4-Chlorophenyl]-3-phenylallylidene) malononitrile (5d)

Pale yellow crystals from ethanol, yield (1.97 g, 68%), mp: 155°C to 157°C. IR (KBr) υ_{max} cm^{-1}:3053 (CH, aromatic), 2225, 2220 (2CN), 1631 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 6.34, 6.45 (2d, 2H, J = 10 Hz, CH═CH), 7.22-7.48 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.1, 90.7 (CH═CH), 116.6, 116.8 (2CN), 120.3, 121.9, 122.8, 123.6, 124.1, 126.2, 127.1, 127.8 (C_6H_5, C_6H_4), 131.2, 134.3 (C═C). Anal. calculated for $C_{18}H_{11}ClN_2$: C, 74.36; H, 3.81; N, 9.63. Found: C, 74.52; H, 4.04; N, 9.82. MS: *m/e* 290 (M^+, 28%).

Ethyl 2-cyano-3,5-diphenylpenta-2,4-dienoate (5e)

Pale yellow crystals from ethanol, yield (2.30 g, 76%), mp: 196°C to 198°C. IR (KBr) υ_{max} cm^{-1}: 3056 (CH, aromatic), 2221 (CN), 1710 (CO), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.13 (t, 3H, J = 7.18 Hz, CH_3), 4.22 (q, 2H, J = 7.18 Hz, CH_2), 6.32, 6.41 (2d, 2H, J = 10 Hz, CH═CH), 7.29-7.38 (m, 10H, $2C_6H_5$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.3 ($OCH_2\underline{CH_3}$), 50.4 ($O\underline{CH_2}CH_3$), 89.3, 90.5 (CH═CH), 116.8 (CN), 120.1, 121.6, 123.0, 123.8, 125.6, 126.8, 127.4, 128.2 ($2C_6H_5$), 131.1, 134.5 (C═C), 168.9 (CO). Anal. calculated for $C_{20}H_{17}NO_2$: C, 79.19; H, 5.65; N, 4.62. Found: C, 79.08; H, 5.48; N, 4.80. MS: *m/e* 303 (M^+, 42%).

Ethyl 3-(4-chlorophenyl)-2-cyano-5-(4-methoxyphenyl) penta-2,4-dienoate (5f)

Pale yellow crystals from ethanol, yield (2.93 g, 80%), mp: 233°C to 235°C. IR (KBr) υ_{max} cm^{-1}: 3055 (CH, aromatic), 2220 (CN),1714 (CO), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.14 (t, 3H, J = 6.72 Hz, CH_3), 3.70 (s, 3H, OCH_3), 4.23 (q, 2H, J = 6.72 Hz, CH_2), 6.31, 6.44 (2d, 2H, J = 9.95 Hz, CH═CH), 7.25-7.49 (m, 8H, $2C_6H_4$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.6 ($OCH_2\underline{CH_3}$), 50.5 (OCH_3), 52.8 ($O\underline{CH_2}CH_3$), 89.1, 90.6 (CH═CH), 116.9 (CN), 119.8, 120.2, 121.8, 122.7, 124.9,

126.5, 128.3, 128.8 ($2C_6H_4$), 131.4, 134.2 (C═C), 167.1 (CO). Anal. calculated for $C_{21}H_{18}ClNO_3$: C, 68.57; H, 4.93; N, 3.81. Found: C, 68.32; H, 5.08; N, 4.02. MS: *m/e* 367 (M^+, 38%).

Ethyl 2-cyano-5-(4-methoxyphenyl)-3-phenylpenta-2,4-dienoate (5g)

Pale yellow crystals from ethanol, yield (2.09 g, 63%), mp: 205°C to 208°C. IR (KBr) υ_{max} cm^{-1}: 3055 (CH, aromatic), 2220 (CN), 1709 (CO), 1632 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.12 (t, 3H, *J* = 7.02 Hz, CH_3), 3.68 (s, 3H, OCH_3), 4.22 (q, 2H, *J* = 7.02 Hz, CH_2), 6.30, 6.45 (2d, 2H, *J* = 10.1 Hz, CH═CH), 7.23-7.44 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.7 ($OCH_2\underline{CH_3}$), 50.1 (OCH_3), 52.4 ($O\underline{CH_2}CH_3$), 89.2, 90.4 (CH═CH), 116.5 (CN), 119.9, 120.8, 121.3, 123.5, 124.4, 125.8, 128.1, 128.6 (C_6H_5, C_6H_4), 131.3, 134.5 (C═C), 168.9 (CO). Anal. calculated for $C_{21}H_{19}NO_3$: C, 75.66.; H, 5.74; N, 4.20. Found: C, 75.42; H, 5.63; N, 4.31. MS: *m/e* 333 (M^+, 41%).

Ethyl 3-(4-chlorophenyl)-2-cyano-5-phenylpenta-2,4-dienoate (5h)

Dark yellow crystals from ethanol, yield (2.46 g, 73%), mp: 148°C to 151°C. IR (KBr) υ_{max} cm^{-1}: 3055 (CH, aromatic), 2220 (CN), 1710 (CO), 1633 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.12 (t, 3H, *J* = 7.38 Hz, CH_3), 4.22 (q, 2H, *J* = 7.38 Hz, CH_2), 6.32, 6.43 (2d, 2H, *J* = 10.21 Hz, CH═CH), 7.21-7.49 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.7 ($OCH_2\underline{CH_3}$), 50.3 ($O\underline{CH_2}CH_3$), 89.3, 90.2 (CH═CH), 116.4 (CN), 120.6, 121.93, 122.5, 123.8, 124.4, 126.8, 127.1, 127.9 (C_6H_5, C_6H_4), 131.6, 134.2 (C═C), 169.5 (CO). Anal. calculated for $C_{20}H_{16}ClNO_2$: C, 71.11; H, 4.77; N, 4.15%. Found: C, 70.92; H, 4.80; N, 4.31%. MS: *m/e* 337 (M^+, 24%).

4.1.2 | General procedure for the synthesis of the pyrazole derivatives 7a-h

Hydrazine hydrate (0.50 mL, 0.01 mol) was added to a solution of any of compounds **5a** (2.56 g, 0.01 mol), **5b** (3.20 g, 0.01 mol), **5c** (2.86 g, 0.01 mol), **5d** (2.90 g, 0.01 mol), **5e** (3.03 g, 0.01 mol), **5f** (3.67 g, 0.01 mol), **5g** (3.33 g, 0.01 mol) or **5h** (3.37 g, 0.01 mol) in 1,4-dioxan (40 mL). The reaction mixture in each case was heated under reflux for 3 hours then poured onto a solution of ice/water mixture containing a few drops of hydrochloric acid and the formed precipitate was collected by filtration.

4-(1,3-Diphenylallylidene)-4H-pyrazole-3,5-diamine (7a)

White crystals from 1,4-dioxan, yield (2.01 g, 70%), mp: 266°C to 268°C. IR (KBr) υ_{max} cm^{-1}: 3487, 3368 ($2NH_2$), 3054 (CH, aromatic), 1653 (C═N), 1632 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 4.59, 5.03 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.35, 6.43 (2d, 2H, *J* = 9.88 Hz, CH═CH), 7.28-7.36 (m, 10H, $2C_6H_5$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.1, 90.7 (CH═CH), 120.8, 121.5, 123.8, 124.7, 125.3, 126.8, 127.8, 128.1 ($2C_6H_5$), 131.8, 134.6 (C═C), 172.8, 176.2 (2C═N). Anal. calculated for $C_{18}H_{16}N_4$: C, 74.98; H, 5.59; N, 19.43. Found: C, 75.03; H, 5.38; N, 19.39. MS: *m/e* 288 (M^+,36%).

4-(1-(4-Chlorophenyl)-3-(4-methoxyphenyl)allylidene)-4H-pyrazole-3,5-diamine (7b)

Yellow crystals from 1,4-dioxan, yield (2.28 g, 65%), mp: 196°C to 198°C. IR (KBr) υ_{max} cm^{-1}: 3498, 3346 ($2NH_2$), 3055 (CH, aromatic), 1650 (C═N), 1631 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.69 (s, 3H, OCH_3), 4.73, 5.08 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.38, 6.41 (2d, 2H, *J* = 9.97 Hz, CH═CH), 7.25-7.48 (m, 8H, $2C_6H_4$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.6 (OCH_3), 89.4, 90.9 (CH═CH), 120.3, 122.8, 122.9, 123.1, 124.6, 126.5, 127.3, 129.4 ($2C_6H_4$), 131.3, 134.2 (C═C), 172.5, 176.2 (2C═N). Anal. calculated for $C_{19}H_{17}ClN_4O$: C, 64.68; H, 4.86; N, 15.88. Found: C, 64.70; H, 4.57; N, 16.16. MS: *m/e* 352 (M^+, 24%).

4-(3-(4-Methoxyphenyl)-1-phenylallylidene)-4H-pyrazole-3,5-diamine (7c)

Yellow crystals from 1,4-dioxan, yield (2.01 g, 70%), mp: 222°C to 225°C. IR (KBr) υ_{max} cm^{-1}: 3439, 3372 ($2NH_2$), 3055 (CH, aromatic), 1650 (C═N), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.67 (s, 3H, OCH_3), 4.71, 5.12 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.36, 6.43 (2d, 2H, *J* = 9.93 Hz, CH═CH), 7.23-7.46 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.4 (OCH_3), 89.2, 90.5 (CH═CH), 120.1, 121.6, 122.7, 123.5, 123.9, 126.2, 128.1, 128.6 (C_6H_5, C_6H_4), 131.5, 134.7 (C═C),172.8, 176.2 (2C═N). Anal. calculated for $C_{19}H_{18}N_4O$: C, 71.68; H, 5.70; N, 17.60. Found: C, 71.83; H, 5.92; N, 17.41. MS: *m/e* 318 (M^+, 65%).

4-(1-(4-Chlorophenyl)-3-phenylallylidene)-4H-pyrazole-3,5-diamine (7d)

White crystals from 1,4-dioxan, yield (2.54 g, 79%), mp: 177°C to 179°C. IR (KBr) υ_{max} cm^{-1}: 3457, 3338 ($2NH_2$), 3055 (CH, aromatic), 1650 (C═N), 1632 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 4.56, 5.02 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.36, 6.45 (2d, 2H, *J* = 9.87 Hz, CH═CH), 7.24-7.48 (m, 9H, C_6H_5, C_6H_4); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.3, 90.2 (CH═CH), 120.5, 121.6, 123.2, 124.3, 126.2, 126.9, 127.5, 129.9 (C_6H_5, C_6H_4), 131.2, 134.3 (C═C), 172.8, 176.0 (2C═N). Anal. calculated for $C_{18}H_{15}ClN_4$: C, 66.98; H, 4.68; N, 17.36. Found: C, 67.25; H, 4.36; N, 17.48. MS: *m/e* 322 (M^+, 42%).

5-Amino-4-(1,3-diphenylallylidene)-4H-pyrazol-3-ol (7e)
Pale yellow crystals from ethanol, yield (1.93 g, 67%), mp: 210°C to 212°C. IR (KBr) υ_{max} cm^{-1}: 3527-3368 (OH, NH_2), 3055 (CH, aromatic), 1650 (C═N), 1632 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 4.58 (s, 2H, D_2O exchangeable, NH_2), 6.38, 6.42 (2d, 2H, J = 9.87 Hz, CH═CH), 7.29-7.38 (m, 10H, $2C_6H_5$), 10.31 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.6, 90.6 (CH═CH), 119.3, 120.8, 122.6, 125.9, 127.2, 127.8, 128.1, 129.3 ($2C_6H_5$), 131.4, 134.6 (C═C), 172.6, 176.2 (2C═N). Anal. calculated for $C_{18}H_{15}N_3O$: C, 74.72; H, 5.23; N, 14.52. Found: C, 74.39; H, 5.41; N, 14.73. MS: *m/e* 289 (M^+, 27%).

5-Amino-4-(1-(4-chlorophenyl)-3-(4-methoxyphenyl) allylidene)-4H-pyrazol-3-ol (7f)
Yellow crystals from ethanol, yield (2.64 g, 75%), mp: 177°C to 179°C. IR (KBr) υ_{max} cm^{-1}: 3548-3373 (OH, NH_2), 3055 (CH, aromatic), 1651 (C═N), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.68 (s, 3H, OCH_3), 4.56 (s, 2H, D_2O exchangeable, NH_2), 6.36, 6.45 (2d, 2H, J = 10.13 Hz, CH═CH), 7.22-7.48 (m, 8H, $2C_6H_4$), 10.33 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.6 (OCH_3), 89.4, 90.4 (CH═CH), 120.8, 120.9, 121.2, 123.6, 125.8, 126.3, 129.6, 130.1 ($2C_6H_4$), 131.2, 134.8 (C═C), 172.8, 176.2 (2C═N). Anal. calculated for $C_{19}H_{16}ClN_3O_2$: C, 64.50; H, 4.56; N, 11.88. Found: C, 64.81; H, 4.69; N, 11.64. MS: *m/e* 353 (M^+, 32%).

5-Amino-4-(3-(4-methoxyphenyl)-1-phenylallylidene)-4H-pyrazol-3-ol (7g)
Pale yellow crystals from ethanol, yield (2.23 g, 70%), mp: 231°C to 234°C. IR (KBr) υ_{max} cm^{-1}: 3561-3358 (OH, NH_2), 3056 (CH, aromatic), 1648 (C═N), 1632 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.69 (s, 3H, OCH_3), 4.59 (s, 2H, D_2O exchangeable, NH_2), 6.34, 6.42 (2d, 2H, J = 9.84 Hz, CH═CH), 7.24-7.49 (m, 9H, C_6H_5, C_6H_4), 10.31 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.4 (OCH_3), 89.2, 90.7 (CH═CH), 120.6, 121.3, 122.8, 123.2, 124.6, 127.1, 127.8, 129.5 (C_6H_5, C_6H_4), 131.4, 134.5 (C═C), 172.9, 175.9 (2C═N). Anal. calculated for $C_{19}H_{17}N_3O_2$: C, 71.46; H, 5.37; N, 13.16. Found: C, 71.38; H, 5.49; N, 12.92. MS: *m/e* 319 (M^+, 58%).

5-Amino-4-(1-(4-chlorophenyl)-3-phenylallylidene)-4H-pyrazol-3-ol (7h)
Yellowish brown crystals from 1,4-dioxan, yield (2.51 g, 79%), mp: 188°C to 191°C. IR (KBr) υ_{max} cm^{-1}: 3536-3331 (OH, NH_2), 3054 (CH, aromatic), 1642 (C═N), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 4.53 (s, 2H, D_2O exchangeable, NH_2), 6.36, 6.41 (2d, 2H, J = 10 Hz, CH═CH), 7.22-7.48 (m, 9H, C_6H_5, C_6H_4), 10.33 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.5, 90.3 (CH═CH), 120.2, 120.8, 121.5, 122.8, 123.9, 125.8, 126.2, 128.9 (C_6H_5, C_6H_4), 131.1, 134.6 (C═C) 172.5, 176.3 (2C═N). Anal. calculated for $C_{18}H_{14}ClN_3O$: C, 66.77; H, 4.36; N, 12.98. Found: C, 66.92; H, 4.60; N, 12.72. MS: *m/e* 323 (M^+, 38%).

4.1.3 | General procedure for the synthesis of the 2-cyano-*N*-(4*H*-pyrazol-3-yl) acetamide derivatives 8a-h

Ethyl cyanoacetate (1.07 g, 0.01 mol) was added to a solution of any of compounds **7a** (2.88, 0.01 mol), **7b** (3.52 g, 0.01 mol), **7c** (3.18 g, 0.01 mol), **7d** (3.22 g, 0.01 mol), **7e** (2.89 g, 0.01 mol), **7f** (3.53 g, 0.01 mol), **7g** (3.19 g, 0.01 mol) or **7h** (3.23 g, 0.01 mol) in dimethylformamide (40 mL). The reaction mixture, in each case, was heated under reflux for 3 hours then was left to cool, and the solid product produced upon pouring onto ice/water was collected by filtration.

N-*(5-Amino-4-(1,3-diphenylallylidene)-4H-pyrazol-3-yl)-2-cyanoacetamide (8a)*
Yellow crystals from 1,4-dioxan, yield (2.55 g, 72%), mp: 201°C to 204°C. IR (KBr) υ_{max} cm^{-1}: 3469-3331 (NH_2, NH), 3055 (CH, aromatic), 2258 (CN), 1681 (CO), 1651 (C═N), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.80 (s, 2H, CH_2), 4.56 (s, 2H, D_2O exchangeable, NH_2), 6.34, 6.43 (2d, 2H, J = 10.07 Hz, CH═CH), 7.25-7.39 (m, 10H, $2C_6H_5$), 8.26 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 27.2 (CH_2), 89.3, 90.5 (CH═CH), 117.1 (CN), 120.4, 121.7, 122.2, 123.4, 125.9, 126.1, 127.3, 128.5 ($2C_6H_5$), 131.5, 134.4 (C═C),168.1 (CO), 172.4, 176.2 (2C═N). Anal. calculated for $C_{21}H_{17}N_5O$: C, 70.97; H, 4.82; N, 19.71. Found: C, 71.08; H, 4.69; N, 19.53. MS: *m/e* 355 (M^+,36%).

N-*(5-Amino-4-(1-(4-chlorophenyl)-3-(4-methoxyphenyl) allylidene)-4H-pyrazol-3-yl)-2-cyanoacetamide (8b)*
Yellow crystals from 1,4-dioxan, yield (2.84 g, 68%), mp: 180°C to 183°C. IR (KBr) υ_{max} cm^{-1}: 3485-3359 (NH_2, NH), 3055 (CH, aromatic), 2250 (CN), 1685 (CO),1650 (C═N), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.68 (s, 3H, OCH_3), 3.83 (s, 2H, CH_2), 4.71 (s, 2H, D_2O exchangeable, NH_2), 6.35, 6.39 (2d, 2H, J = 10 Hz, CH═CH), 7.21-7.49 (m, 8H, $2C_6H_4$), 8.26 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 27.6 (CH_2), 52.3 (OCH_3), 89.2, 90.7 (CH═CH), 117.0 (CN), 120.1, 121.5, 122.6, 123.8, 124.4, 125.6, 126.8, 127.3 ($2C_6H_4$), 131.6, 134.5 (C═C),167.9 (CO), 172.5, 176.6 (2C═N). Anal. calculated for $C_{22}H_{18}ClN_5O_2$: C, 62.93; H,

4.32; N, 16.68. Found: C, 63.28; H, 4.49; N, 16.53. MS: *m/e* 419 (M^+, 30%).

N-*((5-Amino-4-(3-(4-methoxyphenyl)-1-phenylallylidene)-4H-pyrazol-3-yl)-2-cyanoacetamide (8c)*

Yellow crystals from 1,4-dioxan, yield (3.08 g, 80%), mp: 166°C to 168°C. IR (KBr) υ_{max} cm^{-1}: 3461-3349 (NH_2, NH), 3055 (CH, aromatic), 2253 (CN), 1681 (CO), 1650 (C═N), 1632 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.69 (s, 3H, OCH_3), 3.84 (s, 2H, CH_2), 4.74, (s, 2H, D_2O exchangeable, NH_2), 6.36, 6.46 (2d, 2H, *J* = 9.94 Hz, CH═CH), 7.21-7.49 (m, 9H, C_6H_5, C_6H_4), 8.29 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 27.6 (CH_2), 52.4 (OCH_3), 89.1, 90.8 (CH═CH), 116.9 (CN), 120.4, 121.3, 122.9, 124.1, 125.3, 126.7, 127.8, 128.9 (C_6H_5, C_6H_4), 131.3, 134.8 (C═C), 168.4 (CO), 172.5, 176.6 (2C═N). Anal. calculated for $C_{22}H_{19}N_5O_2$: C, 68.56; H, 4.97; N, 18.17. Found: C, 68.73; H, 5.21; N, 18.26. MS: *m/e* 385 (M^+, 42%).

N-*(5-Amino-4-(1-(4-chlorophenyl)-3-phenylallylidene)-4H-pyrazol-3-yl)-2-cyanoacetamide (8d)*

Yellowish brown crystals from 1,4-dioxan, yield (2.76 g, 71%), mp: 201°C to 204°C. IR (KBr) υ_{max} cm^{-1}: 3472-3358 (NH_2, NH), 3055 (CH, aromatic), 2248 (CN), 1680 (CO), 1650 (C═N), 1631 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.72 (s, 2H, CH_2), 4.58 (s, 2H, D_2O exchangeable, NH_2), 6.33, 6.48 (2d, 2H, *J* = 10.1 Hz, CH═CH), 7.21-7.47 (m, 9H, C_6H_5, C_6H_4), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 27.8 (CH_2), 89.5, 90.6 (CH═CH), 116.9 (CN), 120.8, 122.9, 123.1, 125.8, 126.5, 127.3, 127.8, 128.2 (C_6H_5, C_6H_4), 131.6, 134.5 (C═C), 168.5 (CO), 172.8, 176.4 (2C═N). Anal. calculated for $C_{21}H_{16}ClN_5O$: C, 64.70; H, 4.14; N, 17.96. Found: C, 64.52; H, 4.38; N, 17.58. MS: *m/e* 389 (M^+, 36%).

*2-Cyano-*N*-(4-(1,3-diphenylallylidene)-5-hydroxy-4H-pyrazol-3-yl)acetamide (8e)*

Yellow crystals from 1,4-dioxan, yield (2.77 g, 78%), mp: 189°C to 191°C. IR (KBr) υ_{max} cm^{-1}: 3558-3342 (OH, NH), 3056 (CH, aromatic), 2254 (CN), 1679 (CO), 1650 (C═N), 1632 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.75 (s, 2H, CH_2), 6.34, 6.46 (2d, 2H, *J* = 10.09 Hz, CH═CH), 7.26-7.42 (m, 10H, $2C_6H_5$), 8.19 (s, 1H, D_2O exchangeable, NH), 10.28 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 27.9 (CH_2), 89.4, 90.9 (CH═CH), 116.8 (CN), 120.9, 121.8, 122.3, 123.7, 125.8, 126.5, 128.5, 128.8 ($2C_6H_5$), 131.3, 134.4 (C═C), 167.7 (CO), 172.6, 176.1 (2C═N). Anal. calculated for $C_{21}H_{16}N_4O_2$: C, 70.77; H, 4.53; N, 15.72. Found: C, 70.42; H, 4.68; N, 15.80. MS: *m/e* 356 (M^+, 42%).

N-*(4-(1-(4-Chlorophenyl)-3-(4-methoxyphenyl) allylidene)-5-hydroxy-4H-pyrazol-3-yl)-2-cyanoacetamide (8f)*

Yellow crystals from 1,4-dioxan, yield (2.89 g, 69%), mp: 113°C to 115°C. IR (KBr) υ_{max} cm^{-1}: 3568-3360 (OH, NH), 3055 (CH, aromatic), 2255 (CN), 1684 (CO),1651 (C═N), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.68 (s, 3H, OCH_3), 3.76 (s, 2H, CH_2), 6.36, 6.45 (2d, 2H, *J* = 9.96 Hz, CH═CH), 7.22-7.48 (m, 8H, $2C_6H_4$), 8.33 (s, 1H, D_2O exchangeable, NH), 10.33 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 27.6 (CH_2), 52.1 (OCH_3) 89.2, 90.4 (CH═CH), 117.2 (CN), 120.8, 120.9, 121.2, 123.6, 125.8, 126.3, 129.6, 130.1 ($2C_6H_4$), 131.4, 134.5 (C═C), 168.3 (CO), 172.8, 176.2 (2C═N). Anal. calculated for $C_{22}H_{17}ClN_4O_3$: C, 62.79; H, 4.07; N, 13.31. Found: C, 62.59; H, 4.28; N, 13.52. MS: *m/e* 420 (M^+, 28%).

*2-Cyano-*N*-(5-hydroxy-4-(3-(4-methoxyphenyl)-1-phenylallylidene)-4H-pyrazol-3-yl)acetamide (8g)*

Pale yellow crystals from ethanol, yield (2.54 g, 66%), mp: 177°C to 179°C. IR (KBr) υ_{max} cm^{-1}: 3583-3339 (OH, NH), 3054 (CH, aromatic), 2255 (CN), 1687 (CO), 1643 (C═N), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.67 (s, 3H, OCH_3), 3.80 (s, 2H, CH_2), 6.32, 6.45 (2d, 2H, *J* = 10.11 Hz, CH═CH), 7.21-7.47 (m, 9H, C_6H_5, C_6H_4), 8.25 (s, 1H, D_2O exchangeable, NH), 10.33 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 27.4 (CH_2), 52.3 (OCH_3), 89.5, 90.9 (CH═CH), 116.6 (CN), 119.8, 121.6, 122.3, 123.9, 125.3, 126.8, 127.3, 128.2 (C_6H_5, C_6H_4), 131.4, 134.7 (C═C), 167.8 (CO),172.3, 176.6 (2C═N). Anal. calculated for $C_{22}H_{18}N_4O_3$: C, 68.38; H, 4.70; N, 14.50. Found: C, 68.52; H, 4.93; N, 14.68. MS: *m/e* 386 (M^+, 38%).

N-*(4-(1-(4-Chlorophenyl)-3-phenylallylidene)-5-hydroxy-4H-pyrazol-3-yl)-2-cyanoacetamide (8h)*

Pale brown crystals from 1,4-dioxan, yield (2.61 g, 67%), mp: 232°C to 235°C. IR (KBr) υ_{max} cm^{-1}: 3547-3361 (OH, NH), 3055 (CH, aromatic), 2248 (CN), 1684 (CO), 1642 (C═N), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 3.80 (s, 2H, CH_2), 6.34, 6.45 (2d, 2H, *J* = 9.87 Hz, CH═CH), 7.20-7.42 (m, 9H, C_6H_5, C_6H_4), 8.28 (s, 1H, D_2O exchangeable, NH), 10.33 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 27.8 (CH_2), 89.5, 90.6 (CH═CH), 116.9 (CN), 120.4, 120.3, 121.8, 122.6, 123.7, 125.3, 125.8, 128.3 (C_6H_5, C_6H_4), 131.3, 134.7 (C═C), 168.2 (CO),172.3, 176.8 (2C═N). Anal. calculated for $C_{21}H_{15}ClN_4O_2$: C, 64.54; H, 3.87; N, 14.34. Found: C, 64.73; H, 4.13; N, 14.28. MS: *m/e* 390 (M^+, 26%).

4.1.4 | General procedure for the synthesis of the thiophene derivatives 9a-h

Elemental sulfur (0.32 g, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) were added to any of **8a** (4.55 g, 0.01 mol), **8b** (4.19 g, 0.01 mol), **8c** (3.85 g, 0.01 mol), **8d** (3.89 g, 0.01 mol), 8e (3.56 g, 0.01 mol), **8f** (4.20 g, 0.01 mol), **8g** (3.86 g, 0.01 mol) or **8h** (3.90 g, 0.01 mol) in 1,4-dioxan (50 mL) containing triethylamine (1.0 mL). The reaction mixture, in each case, was heated under reflux for 2 hours then poured onto ice/water containing a few drops of hydrochloric acid, and the formed solid product was collected by filtration.

*3,5-Diamino-*N*-(5-amino-4-(1,3-diphenylallylidene)-4*H*-pyrazol-3-yl)-4- cyano-thiophene-2-carboxamide (9a)*

Pale brown crystals from ethanol, yield (2.71 g, 60%), mp: 158°C to 160°C. IR (KBr) υ_{max} cm^{-1}: 3483-3329 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1690 (CO), 1648 (C═N), 1630 (C═C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 4.46, 4.56, 5.21 (3s, 6H, D_2O exchangeable, $3NH_2$), 6.32, 6.48 (2d, 2H, J = 10.07 Hz, CH═CH), 7.22-7.36 (m, 10H, $2C_6H_5$), 8.29 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.1, 90.8 (CH═CH), 117.0 (CN), 120.8, 121.0, 121.6, 122.8, 124.3, 126.8, 127.5, 128.2, 130.5, 132.4, 135.9, 137.0 ($2C_6H_5$, thiophene C), 131.1, 134.7 (C═C),167.4 (CO),172.1, 176.6 (2C═N). Anal. calculated for $C_{24}H_{19}N_7OS$: C, 63.56; H, 4.22; N, 21.62; S, 7.07. Found: C, 63.70; H, 4.39; N, 21.80; S.7.28. MS: *m/e* 453 (M^+, 26%).

*3,5-Diamino-*N*-(5-amino-4-(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-allylidene)-4*H*-pyrazol-3-yl)-4-cyanothiophene-2-carboxamide (9b)*

Yellow crystals from 1,4-dioxan, yield (3.87 g, 75%), mp: 155°C to 158°C. IR (KBr) υ_{max} cm^{-1}: 3449-3326 (NH_2, NH), 3055 (CH, aromatic), 2220 (CN), 1683 (CO), 1650 (C═N), 1630 (C═C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 3.68 (s, 3H, OCH_3), 4.58, 4.71, 5.22 (3s, 6H, D_2O exchangeable, $3NH_2$), 6.37, 6.38 (2d, 2H, J = 10.07 Hz, CH═CH), 7.21-7.52 (m, 8H, $2C_6H_4$), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.1 (OCH_3), 89.2, 90.7 (CH═CH), 116.8 (CN), 120.4, 122.3, 122.6, 123.8, 124.4, 125.9, 126.3, 127.3, 130.3, 132.6, 135.9, 137.4 ($2C_6H_4$, thiophene C), 131.6, 134.5 (C═C), 167.7 (CO), 172.2, 176.8 (2C═N). Anal. calculated for $C_{25}H_{20}ClN_7O_2S$: C, 57.97; H, 3.89; N, 18.93; S, 6.19. Found: C, 58.27; H, 4.02; N, 19.18; S, 6.27. MS: *m/e* 517 (M^+, 26%).

*3,5-Diamino-*N*-(5-amino-4-(3-(4-methoxyphenyl)-1-phenylallylidene)-4*H*-pyrazol-3-yl)-4-cyanothiophene-2-carboxamide (9c)*

Yellow crystals from 1,4-dioxan, yield (3.38 g, 70%), mp: 185°C to 188°C, IR (KBr) υ_{max} cm^{-1}: 3478-3353 (NH_2, NH), 3055 (CH, aromatic), 2223 (CN), 1687 (CO), 1648 (C═N), 1630 (C═C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 3.67 (s, 3H, OCH_3), 4.48, 4.74, 5.28 (3s, 6H, D_2O exchangeable, $3NH_2$), 6.35, 6.48 (2d, 2H, J = 9.89 Hz, CH═CH), 7.23-7.48 (m, 9H, C_6H_5, C_6H_4), 8.26 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.2 (OCH_3), 89.3, 90.6 (CH═CH), 116.6 (CN), 120.2, 122.6, 122.5, 123.6, 125.1, 126.5, 127.3, 128.4, 130.2, 132.6, 134.9, 136.1 (C_6H_5, C_6H_4, thiophene C), 131.6, 134.9 (C═C), 167.1 (CO), 172.2, 176.8 (2C═N). Anal. calculated for $C_{25}H_{21}N_7O_2S$: C, 62.10; H, 4.38; N, 20.28; S, 6.63. Found: C, 61.89; H, 4.66; N, 20.41; S, 7.01. MS: *m/e* 483 (M^+, 68%).

*3,5-Diamino-*N*-(5-amino-4-(1-(4-chlorophenyl)-3-phenylallylidene)-4*H*-pyrazol-3-yl)-4-cyanothiophene-2-carboxamide (9d)*

Brown crystals from 1,4-dioxan, yield (3.01 g, 62%), mp: 166°C to 169°C. IR (KBr) υ_{max} cm^{-1}: 3482-3337 (NH_2, NH), 3055 (CH, aromatic), 2222 (CN), 1688 (CO), 1650 (C═N), 1631 (C═C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 4.39, 4.58, 5.28 (3s, 6H, D_2O exchangeable, $3NH_2$), 6.30, 6.48 (2d, 2H, J = 10.13 Hz, CH═CH), 7.22-7.53 (m, 9H, C_6H_5, C_6H_4), 8.26 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.5, 90.4 (CH═CH), 116.7 (CN), 120.3, 121.8, 122.6, 125.5, 126.3, 127.1, 128.4, 129.0, 130.6, 132.7, 134.8, 138.2 (C_6H_5, C_6H_4, thiophene C), 131.3, 134.7 (C═C), 166.9 (CO), 172.3, 176.6 (2C═N). Anal. calculated for $C_{24}H_{18}ClN_7OS$: C, 59.07; H, 3.72; N, 20.09; S, 6.57%. Found: C, 58.84; H, 3.69; N, 19.82; S, 6.39. MS: *m/e* 487 (M^+, 26%).

*3,5-Diamino-4-cyano-*N*-(4-(1,3-diphenylallylidene)-5-hydroxy-4*H*-pyrazol-3-yl)thiophene-2-carboxamide (9e)*

Yellow crystals from 1,4-dioxan, yield (3.49 g, 77%), mp: 133°C to 136°C. IR (KBr) υ_{max} cm^{-1}: 3548-3329 (OH, NH_2, NH), 3053 (CH, aromatic), 2220 (CN), 1685 (CO), 1650 (C═N), 1630 (C═C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 4.38, 4.58 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.31, 6.48 (2d, 2H, J = 10.12 Hz, CH═CH), 7.29-7.39 (m, 10H, $2C_6H_5$), 8.42 (s, 1H, D_2O exchangeable, NH), 10.26 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.1, 90.9 (CH═CH), 116.7 (CN), 120.5, 121.6, 122.6, 123.9, 124.5, 127.3, 128.1, 128.6, 130.4, 132.7, 134.5, 138.0 ($2C_6H_5$, thiophene C), 131.6, 134.8 (C═C),167.5 (CO), 172.3, 176.4 (2C═N). Anal. calculated for $C_{24}H_{18}N_6O_2S$: C, 63.42; H, 3.99; N, 18.49; S, 7.05. Found: C, 63.58; H, 4.05; N, 18.39; S, 6.88. MS: *m/e* 454 (M^+, 30%).

*3,5-Diamino-*N*-(4-(1-(4-chlorophenyl)-3-(4-methoxyphenyl)allylidene)-5-hydroxy-4*H*-pyrazol-3-yl)-4-cyanothiophene-2-carboxamide (9f)*

Yellow crystals from 1,4-dioxan, yield (3.57 g, 69%), mp: 148°C to 150°C. IR (KBr) υ_{max} cm^{-1}: 3563-3352 (OH,

NH_2, NH), 3055 (CH, aromatic), 2223 (CN), 1686 (CO), 1650 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 3.68 (s, 3H, OCH_3), 4.58, 4.79 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.34, 6.47 (2d, 2H, J = 10.05 Hz, CH=CH), 7.25-7.46 (m, 8H, $2C_6H_4$), 8.31 (s, 1H, D_2O exchangeable, NH), 10.34 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.5 (OCH_3) 89.2, 90.6 (CH=CH), 117.0 (CN), 120.6, 120.3, 121.2, 124.2, 125.8, 126.7, 128.6, 128.7, 130.2, 132.6, 133.9, 135.1 ($2C_6H_4$, thiophene C), 131.2, 134.7 (C=C), 167.8 (CO), 172.8, 176.6 (2C=N). Anal. calculated for $C_{25}H_{19}ClN_6O_3S$: C, 57.86; H, 3.69; N, 16.19; S, 6.18. Found: C, 57.62; H, 3.49; N, 16.05; S, 6.27. MS: *m/e* 518 (M^+, 55%).

*3,5-Diamino-4-cyano-*N*-(5-hydroxy-4-(3-(4-methoxyphenyl)-1-phenylallylidene)-4*H*-pyrazol-3-yl)thiophene-2-carboxamide (9g)*

Yellow crystals from ethanol, yield (2.90 g, 60%), mp: 199°C to 202°C. IR (KBr) υ_{max} cm^{-1}: 3569-3335 (OH, NH_2, NH), 3054 (CH, aromatic), 2223 (CN), 1686 (CO), 1640 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 3.69 (s, 3H, OCH_3), 4.58, 4.68 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.31, 6.46 (2d, 2H, J = 9.89 Hz, CH=CH), 7.23-7.49 (m, 9H, C_6H_5, C_6H_4), 8.23 (s, 1H, D_2O exchangeable, NH), 10.35 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 52.3 (OCH_3), 89.5, 90.9 (CH=CH), 116.6 (CN), 119.2, 121.4, 122.8, 123.9, 125.3, 126.8, 127.3, 128.5, 131.8, 132.4, 133.8, 135.1 (C_6H_5, C_6H_4, thiophene C), 131.6, 134.8 (C=C), 166.8 (CO), 172.4, 176.2 (2C=N). Anal. calculated for $C_{25}H_{20}N_6O_3S$: C, 61.97; H, 4.16; N, 17.34; S, 6.62. Found: C, 62.08; H, 4.26; N, 17.59; S, 6.81. MS: *m/e* 484 (M^+, 38%).

*3,5-Diamino-*N*-(4-(1-(4-chlorophenyl)-3-phenylallylidene)-5-hydroxy-4*H*-pyrazol-3-yl)-4-cyanothiophene-2-carboxamide (9h)*

Orange crystals from 1,4-dioxan, yield (2.83 g, 58%), mp: 177°C to 180°C. IR (KBr) υ_{max} cm^{-1}: 3537-3379 (OH, NH_2, NH), 3055 (CH, aromatic), 2222 (CN), 1681 (CO), 1641 (C=N), 1628 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 4.55, 4.80 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.34, 6.48 (2d, 2H, J = 10.09 Hz, CH=CH), 7.22-7.47 (m, 9H, C_6H_5, C_6H_4), 8.26 (s, 1H, D_2O exchangeable, NH), 10.32 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 89.5, 90.6 (CH=CH), 117.0 (CN), 120.8, 122.6, 124.2, 124.6, 126.1, 125.3, 125.8, 128.7, 129.0, 130.8, 132.5, 134.2 (C_6H_5, C_6H_4, thiophene C), 131.1, 134.5 (C=C), 167.3 (CO), 172.4, 176.8 (2C=N). Anal. calculated for $C_{24}H_{17}ClN_6O_2S$: C, 58.95; H, 3.50; N, 17.19; S, 6.56. Found: C, 58.72; H, 3.78; N, 17.05; S, 6.73. MS: *m/e* 488 (M^+, 18%).

4.1.5 | General procedure for the synthesis of the thiophene derivatives 10a-h

Elemental sulfur (0.32 g, 0.01 mol) and ethyl cyanoacetate (1.07 g, 0.01 mol) were added to any of **8a** (4.55 g, 0.01 mol), **8b** (4.19 g, 0.01 mol), **8c** (3.85 g, 0.01 mol), **8d** (3.89 g, 0.01 mol), **8e** (3.56 g, 0.01 mol), **8f** (4.20 g, 0.01 mol), **8g** (3.86 g, 0.01 mol) or **8h** (3.90 g, 0.01 mol) in 1,4-dioxan (50 mL) containing triethylamine (1.0 mL). The reaction mixture, in each case, was heated under reflux for 2 hours then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

*Ethyl 2,4-diamino-5-(5-amino-4-(1,3-diphenylallylidene)-4*H*-pyrazol-3-yl)carbamoyl) thiophene-3-carboxylate (10a)*

Pale brown crystals from ethanol, yield (3.60 g, 72%), mp: 223°C to 226°C. IR (KBr) υ_{max} cm^{-1}: 3469-3342 (NH_2, NH), 3055 (CH, aromatic), 1718, 1681 (2CO),1643 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 1.13 (t, 3H, J = 7.22 Hz, CH_3), 4.23 (q, 2H, J = 7.22 Hz, CH_2), 4.45, 4.51, 5.28 (3s, 6H, D_2O exchangeable, $3NH_2$), 6.30, 6.46 (2d, 2H, J = 10.13 Hz, CH=CH), 7.26-7.39 (m, 10H, $2C_6H_5$), 8.27 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.8 ($OCH_2\underline{CH_3}$), 58.6 ($O\underline{CH_2}CH_3$), 89.3, 90.6 (CH=CH), 120.6, 120.8, 121.3, 122.5, 124.8, 126.8, 127.2, 128.3, 130.8, 132.6, 133.9, 135.7 ($2C_6H_5$, thiophene C), 131.6, 134.8 (C=C), 167.9, 169.1 (2CO),172.9, 177.1 (2C=N). *Anal.* Calculated for $C_{26}H_{24}N_6O_3S$: C, 62.38; H, 4.83; N, 16.79; S, 6.41. Found: C, 62.48; H, 4.65; N, 16.83; S, 6.29. MS: *m/e* 500 (M^+, 42%).

*Ethyl 2,4-diamino-5-(5-amino-4-(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-allylidene)-4*H*-pyrazol-3-yl) carbamoyl)thiophene-3-carboxylate (10b)*

Yellow crystals from 1,4-dioxan, yield (4.62 g, 82%), mp: 208°C to 210°C. IR (KBr) υ_{max} cm^{-1}: 3468-3351 (NH_2, NH), 3054 (CH, aromatic), 1720, 1684 (2CO), 1650 (C=N), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 1.12 (t, 3H, J = 7.09 Hz, CH_3), 3.69 (s, 3H, OCH_3), 4.22 (q, 2H, J = 7.09 Hz, CH_2), 4.54, 4.68, 5.20 (3s, 6H, D_2O exchangeable, $3NH_2$), 6.37, 6.38 (2d, 2H, J = 9.87 Hz, CH=CH), 7.21-7.52 (m, 8H, $2C_6H_4$), 8.29 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.8 ($OCH_2\underline{CH_3}$), 52.3 (OCH_3), 58.4 ($O\underline{CH_2}CH_3$), 89.0, 90.5 (CH=CH), 119.6, 122.8, 123.4, 124.2, 124.8, 126.3, 126.6, 127.7, 130.5, 134.2, 135.7, 137.8 ($2C_6H_4$, thiophene C), 131.4, 134.1 (C=C),167.4, 168.9 (2CO), 172.8, 176.4 (2C=N). Anal. calculated for $C_{27}H_{25}ClN_6O_4S$: C, 57.39; H, 4.46; N, 14.87; S, 5.67. Found: C, 57.41; H, 4.65; N, 14.68; S, 5.83. MS: *m/e* 565 (M^+, 42%).

Ethyl 2,4-diamino-5-(5-amino-4-(3-(4-methoxyphenyl)-1-phenylallylidene)-4H-pyrazol-3-yl)carbamoyl) thiophene-3-carboxylate (10c)

Yellow crystals from 1,4-dioxan, yield (3.49 g, 66%), mp: 233°C to 235°C, IR (KBr) υ_{max} cm^{-1}: 3459-3342 (NH_2, NH), 3054 (CH, aromatic), 1722, 1690 (2CO), 1644 (C═N), 1630 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.13 (t, 3H, J = 6.77 Hz, CH_3), 3.69 (s, 3H, OCH_3), 4.22 (q, 2H, J = 6.77 Hz, CH_2), 4.43, 4.78, 5.27 (3s, 6H, D_2O exchangeable, $3NH_2$), 6.31, 6.48 (2d, 2H, J = 9.96 Hz, CH═CH), 7.25-7.50 (m, 9H, C_6H_5, C_6H_4), 8.28 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.6 ($OCH_2\underline{CH_3}$), 52.4 (OCH_3), 58.1 ($O\underline{CH_2}CH_3$), 89.3, 90.8 (CH═CH), 120.5, 122.9, 123.5, 124.9, 125.7, 126.8, 127.3, 128.4, 130.2, 132.3, 134.5, 135.8 (C_6H_5, C_6H_4, thiophene C), 131.8, 135.3 (C═C), 167.7, 168.7 (2CO), 172.5, 176.6 (2C═N). Anal. calculated for $C_{27}H_{26}N_6O_4S$: C, 61.12; H, 4.94; N, 15.84; S, 6.04. Found: C, 61.36; H, 4.79; N, 15.69; S, 6.29. MS: *m/e* 530 (M^+, 48%).

Ethyl 2,4-diamino-5-(5-amino-4-(1-(4-chlorophenyl)-3-phenylallylidene)-4H-pyrazol-3-yl)carbamoyl) thiophene-3-carboxylate (10d)

Brown crystals from 1,4-dioxan, yield (3.21 g, 60%), mp: 244°C to 247°C. IR (KBr) υ_{max} cm^{-1}: 3479-3341 (NH_2, NH), 3056 (CH, aromatic), 1723, 1687 (2CO), 1650 (C═N), 1631 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.12 (t, 3H, J = 6.52 Hz, CH_3), 4.22 (q, 2H, J = 6.52 Hz, CH_2), 4.34, 4.58, 5.25 (3s, 6H, D_2O exchangeable, $3NH_2$), 6.32, 6.46 (2d, 2H, J = 9.91 Hz, CH═CH), 7.22-7.48 (m, 9H, C_6H_5, C_6H_4), 8.24 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.8 ($OCH_2\underline{CH_3}$), 58.1 ($O\underline{CH_2}CH_3$), 89.2, 90.6 (CH═CH), 120.6, 122.0, 122.9, 124.1, 125.9, 126.8, 127.1, 128.7, 130.9, 132.4, 134.2, 138.9 (C_6H_5, C_6H_4, thiophene C), 131.6, 134.5 (C═C), 167.5, 169.2 (2CO), 172.1, 176.8 (2C═N). Anal. calculated for $C_{26}H_{23}ClN_6O_3S$: C, 58.37; H, 4.33; N, 15.71; S, 5.99%. Found: C, 58.51; H, 4.63; N, 15.80; S, 6.24. MS: *m/e* 535 (M^+, 34%).

Ethyl 2,4-diamino-5-(4-(1,3-diphenylallylidene)-5-hydroxy-4H-pyrazol-3-yl)carbamoyl)thiophene-3-carboxylate (10e)

Yellow crystals from 1,4-dioxan, yield (3.40 g, 68%), mp: 158°C to 161°C. IR (KBr) υ_{max} cm^{-1}: 3559-3372 (OH, NH_2, NH), 3058 (CH, aromatic), 1718, 1691 (2CO),1650 (C═N), 1629 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.12 (t, 3H, J = 7.11 Hz, CH_3), 4.22 (q, 2H, J = 7.11 Hz, CH_2), 4.38, 4.58 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.35, 6.48 (2d, 2H, J = 9.96 Hz, CH═CH), 7.27-7.42 (m, 10H, $2C_6H_5$), 8.46 (s, 1H, D_2O exchangeable, NH), 10.28 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.8 ($OCH_2\underline{CH_3}$), 58.3 ($O\underline{CH_2}CH_3$), 89.3, 90.9 (CH═CH), 120.1, 121.9, 122.6, 123.9, 125.9, 127.6, 127.8, 128.1, 130.6, 133.9, 134.1, 138.8 ($2C_6H_5$, thiophene C), 131.3, 134.1 (C═C), 168.1, 169.3 (2CO), 172.5, 176.2 (2C═N). Anal. calculated for $C_{26}H_{23}N_5O_4S$: C, 62.26; H, 4.62; N, 13.96; S, 6.39. Found: C, 62.48; H, 4.80; N, 14.27; S, 6.47. MS: *m/e* 501 (M^+, 28%).

Ethyl 2,4-diamino-5-(4-(1-(4-chlorophenyl)-3-(4-methoxyphenyl)-allylidene)-5-hydroxy-4H-pyrazol-3-yl)carbamoyl)thiophene-3-carboxylate (10f)

Yellow crystals from 1,4-dioxan, yield (4.13 g, 73%), mp: 240°C to 244°C. IR (KBr) υ_{max} cm^{-1}:3570-3362 (OH, NH_2, NH), 3055 (CH, aromatic), 1724, 1685 (2CO), 1649 (C═N), 1626 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.12 (t, 3H, J = 7.69 Hz, CH_3), 3.66 (s, 3H, OCH_3), 4.24 (q, 2H, J = 7.69 Hz, CH_2), 4.56, 4.73 (s, 4H, D_2O exchangeable, $2NH_2$), 6.34, 6.49 (2d, 2H, J = 9.96 Hz, CH═CH), 7.25-7.54 (m, 8H, $2C_6H_4$), 8.29 (s, 1H, D_2O exchangeable, NH), 10.33 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.6 ($OCH_2\underline{CH_3}$), 52.8 (OCH_3), 58.5 ($O\underline{CH_2}CH_3$), 89.2, 90.9 (CH═CH), 120.8, 121.0, 121.2, 123.6, 125.2, 126.9, 128.3, 128.4, 130.8, 132.3, 133.6, 135.5 ($2C_6H_4$, thiophene C), 131.5, 134.6 (C═C), 168.1, 169.3 (2CO), 172.3, 176.2 (2C═N). Anal. calculated for $C_{27}H_{24}ClN_5O_5S$: C, 57.29; H, 4.27; N, 12.37; S, 5.66. Found: C, 57.51; H, 4.08; N, 12.45; S, 5.80. MS: *m/e* 566 (M^+, 36%).

Ethyl 2,4-diamino-5-(5-hydroxy-4-(3-(4-methoxyphenyl)-1-phenylallylidene)-4H-pyrazol-3-yl)carbamoyl) thiophene-3-carboxylate (10g)

Yellow crystals from ethanol, yield (3.71 g, 70%), mp: 155°C to 158°C. IR (KBr) υ_{max} cm^{-1}: 3541-3357 (OH, NH_2, NH), 3054 (CH, aromatic), 1718, 1692 (2CO), 1640 (C═N), 1632 (C═C); ^1H NMR (DMSO-d_6, 200 MHz): δ = 1.14 (t, 3H, J = 6.80 Hz, CH_3), 3.68 (s, 3H, OCH_3), 4.22 (q, 2H, J = 6.80 Hz, CH_2), 4.54, 4.66 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.35, 6.46 (2d, 2H, J = 10.06 Hz, CH═CH), 7.21-7.55 (m, 9H, C_6H_5, C_6H_4), 8.26 (s, 1H, D_2O exchangeable, NH), 10.33 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.6 ($OCH_2\underline{CH_3}$), 52.5 (OCH_3), 58.7 ($O\underline{CH_2}CH_3$), 89.2, 90.7 (CH═CH), 120.8, 121.6, 122.3, 123.9, 125.8, 126.5, 127.3, 128.7, 131.9, 132.7, 133.2, 135.9 (C_6H_5, C_6H_4, thiophene C), 131.3, 134.6 (C═C), 167.9, 168.7 (2CO), 172.1, 176.5 (2C═N). Anal. calculated for $C_{27}H_{25}N_5O_5S$: C, 61.00; H, 4.74; N, 13.17; S, 6.03. Found: C, 60.84; H, 4.86; N, 13.49; S, 6.29. MS: *m/e* 531 (M^+, 42%).

Ethyl 2,4-diamino-5-(4-(1-(4-chlorophenyl)-3-phenylallylidene)-5-hydroxy-4H-pyrazol-3-yl) carbamoyl)thiophene-3-carboxylate (10h)

Yellow crystals from 1,4-dioxan, yield (4.07 g, 76%), mp: 177°C to 180°C. IR (KBr) υ_{max} cm^{-1}: 3562-3359 (OH,

NH_2, NH), 3055 (CH, aromatic), 1720, 1689 (2CO), 1644 (C=N), 1624 (C=C); ^{1}H NMR (DMSO-d_6, 200 MHz): δ = 1.12 (t, 3H, J = 6.38 Hz, CH_3), 4.21 (q, 2H, J = 6.38 Hz, CH_2), 4.55, 4.83 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.32, 6.45 (2d, 2H, J = 10.09 Hz, CH=CH), 7.24-7.49 (m, 9H, C_6H_5, C_6H_4), 8.24 (s, 1H, D_2O exchangeable, NH), 10.30 (s, 1H, D_2O exchangeable, OH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ = 16.8 ($OCH_2\underline{CH_3}$), 58.5 ($O\underline{CH_2}CH_3$), 89.1, 90.4 (CH=CH), 120.4, 121.3, 124.7, 126.9, 127.3, 125.0, 125.2, 128.1, 129.4, 131.6, 133.3, 134.8 (C_6H_5, C_6H_4, thiophene C), 131.4, 134.7 (C=C), 167.5, 168.6 (2CO), 172.2, 176.5 (2C=N). Anal. calculated for $C_{26}H_{22}ClN_5O_4S$: C, 58.26; H, 4.14; N, 13.07; S, 5.98. Found: C, 58.47; H, 4.26; N, 12.98; S, 6.15. MS: *m/e* 536 (M^+, 26%).

ORCID

Nadia Y. Megally Abdo https://orcid.org/0000-0003-0130-5060
Rafat M. Mohareb https://orcid.org/0000-0003-3922-803X

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