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ORIGINAL RESEARCH

Uses of dimedone for the synthesis of new heterocyclic derivatives with anti-tumor, c-Met, tyrosine, and Pim-1 kinases inhibitions

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Abstract

The reaction of dimedone (**1**) with any of the diazonium salts **2a–c** to give the arylhydrazone derivatives **3a–c**. The Gewald's reaction of any of compounds **3a–c** using elemental sulfur and either of malononitrile or ethyl cyanoacetate gave the thiophene derivatives **5a–f**, respectively. Compounds **5a**, **5c**, and **5e** underwent a series of heterocclization reactions to give potentially anticancer agents. The newly synthesized compounds were evaluated for their in vitro cytotoxic activity against c-Met kinase, and the six typical cancer cell lines (A549, H460, HT-29, MKN-45, U87MG, and SMMC-7721). All target compounds were initially tested for their anti-proliferative activity against human prostatic cancer PC-3 cell line. The most promising compounds were **5c**, **6c**, **6d**, **8e**, **8f**, **12d**, **14e**, and **14f** were further investigated against tyrosin kinase (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR). Compounds **3c, 5c, 5d, 6c, 6d, 8f, 8g, 8h, 12c, 12d**, **12f**, and **14f** were selected to examine their Pim-1 kinase inhibition activity where compounds **3c**, **6c**, **8g**, **12c**, and **14f** showed high activities.

Keywords Arylhydrazone · Anti-proliferative · Dimedone · Thiophene · Tryrosine kinase

Introduction

As typical reactive 1,3-dicarbonyl compounds, cyclohexane-1,3-dione, and its analogy 5,5-dimethyl cyclohexane-1,3-dione(dimedone) have been widely used in versatile synthetic reactions (Dutra et al. 2014; Yadav et al. 2009). Dimedone is not only a typical reagent for Knoevenagel condensation, but also adds easily to electron-deficient alkenes via Michael addition. On the other hand, its one or two carbonyl groups could take part in substitution and cyclization reactions through the tautomerized enolate form. Thus the cascade reactions of addition, elimination and substitution could be achieved in many reactions involving dimedone. The reactions of cyclohexane-1,3-dione or dimedone with aldehydes have been extensively studied in the past years, from which several types of compounds have been produced according to the reaction conditions (Nadaraj et al. 2009). The normal Knoevenagel condensation of cyclohexane-1,3-done or dimedone with aldehydes have been conducted with numerous methods including promotion via amines (Chassainga et al. 2012), Lewis acids (Amoozadeh et al. 2015), surfactants (Deb Bhuyan 2005), zeolites (Jiao et al. 2011), ionic liquids (Khurana and Magoo 2009). The use of environmentally benign methods like in aqueous medium or in the absence of solvents (Nikoofar and Yielzoleh 2017) and the usage of ultrasound or microwave heating (Neimi and Nazif, 2014) have also been developed in recent years. The reactions usually proceed further through Michael addition reaction of the second molecule of dimedone to yield tetraketones as main products (Silva et al. 2018). On the other hand, tetraketones could be easily converted to 9-substituted 1,8-dioxo-xanthenes by dehydration step (Jiao et al. 2011). 1,8-Dioxo-xanthenes can also be directly prepared from the condensation of two molecules of dimedone with aromatic aldehydes in the presence of different kinds of catalysts (Ilangovan et al. 2011; Jiao et al. 2011) such as Amberlyst (Maripi et al. 2017), triethylbenzylammonium chloride (Darvish et al. 2007), diammonium hydrogen phosphate (Jin et al. 2006), sulfonic acid and ionic liquid under ultrasonic irradiation (Venkatesan et al. 2008; Rostamizadeh et al. 2010). In our continued interest in the design of new multicomponent reactions and the application in the synthesis of heterocyclic compounds (Mohareb et al. 2017, 2018), we found some unprecedented reaction

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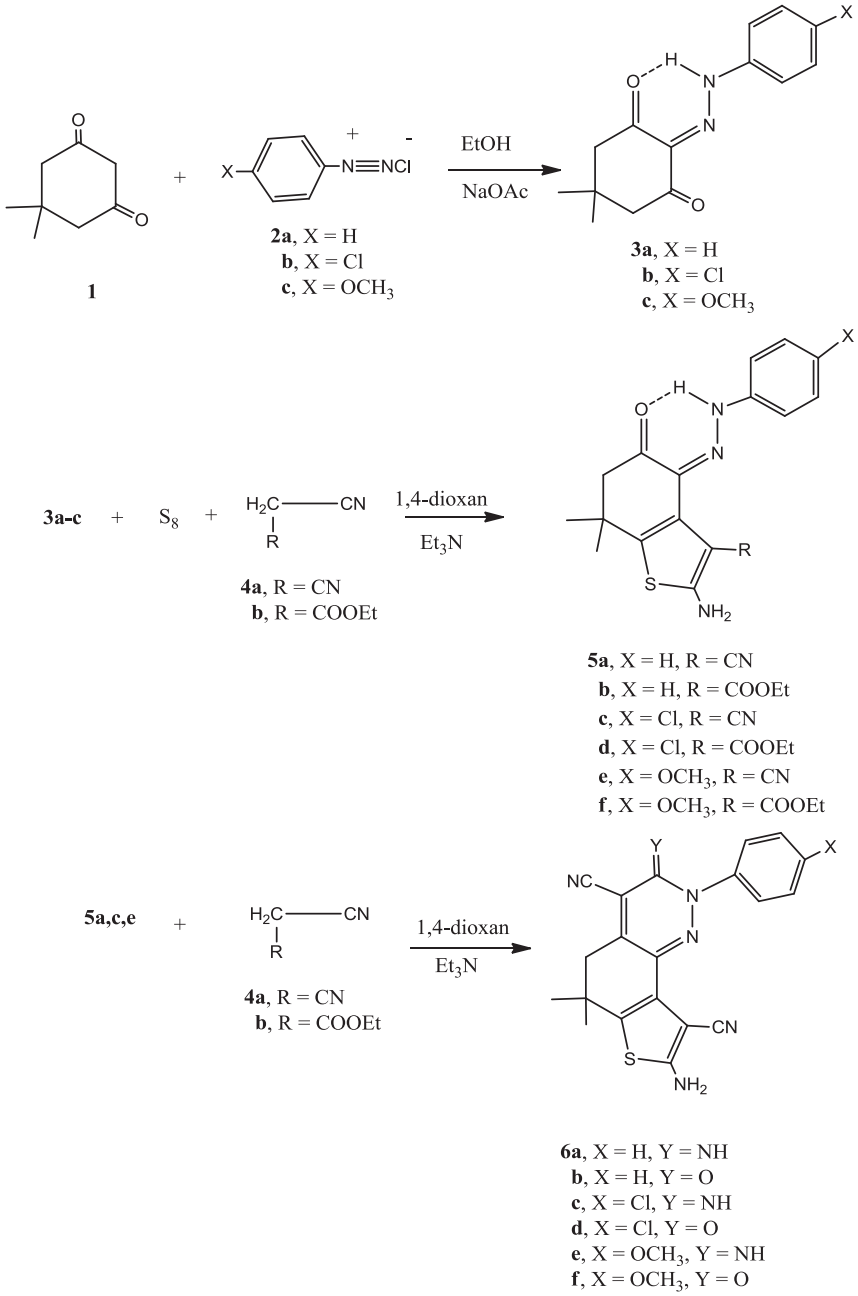
Scheme 1 Synthesis of compounds **3a–c**, **5a–f**, and **6a–f**

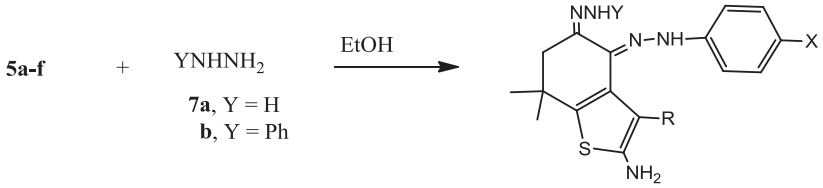


patterns in the reaction of the ary hydrazodimedone derivatives with elemental sulfur and cyanomethylene reagents to produce thiophene derivatives these were capable for further heterocyclization reactions to produce potential antitumor agents.

Results and discussion

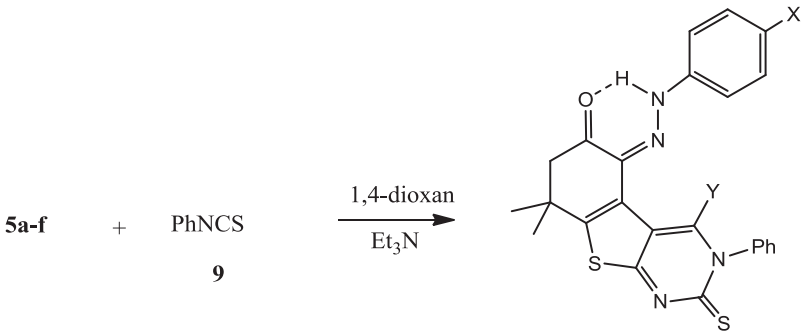
The synthetic route to prepare a new class of biologically active heterocycles compounds linked dimedone moiety starting with the 2-arylhydrazodimedone derivatives. The synthetic pathways employed to prepare the new targeted derivatives are depicted in Schemes 1–3. Thus, the reaction of dimedone (**1**) with the aryldiazonium salts **2a–c** in ethanol containing sodium acetate gave the arylhydrazone derivatives **3a–c**, respectively. Compounds **3a–c** exist in the arylhydrazone form as this structure is stabilized via the intramolecular N-H–O=C- hydrogen bonding (Drew 1982). We speculated that thiophene-fused derivatives can be obtained via the use of the Gewald's thiophene synthesis. Initially, we have investigated the reaction of any of the arylhydrazo compounds **3a–c** with elemental sulfur and either of malonitrile (**4a**) or ethyl cyanoacetate (**4b**) to give the thiophene derivatives **5a–f**, respectively. The structures of compounds **5a–f** were based on their respective analytical

Scheme 2 Synthesis of compounds **8a–m** and **10a–f**



8a-m

8	a	b	c	d	e	f
X	H	H	H	H	Cl	Cl
R	CN	CN	COOEt	COOEt	CN	CN
Y	H	Ph	H	Ph	H	Ph
8	**g**	**h**	**i**	**k**	**l**	**m**
X	Cl	Cl	OCH_3	OCH_3	OCH_3	OCH_3
R	COOEt	COOEt	CN	CN	COOEt	COOEt
Y	H	Ph	H	Ph	H	Ph



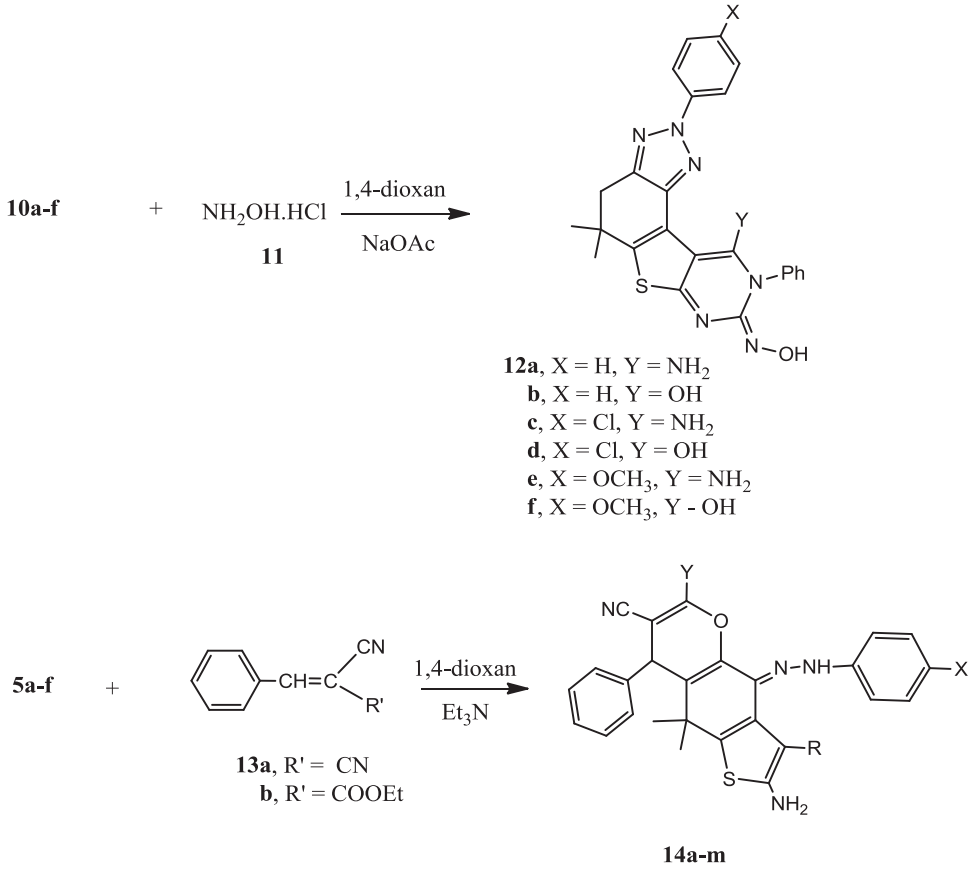
10a, X = H, Y = NH_2
b, X = H, Y = OH
c, X = Cl, Y = NH_2
d, X = Cl, Y = OH
e, X = OCH_3, Y = NH_2
f, X = OCH_3, Y - OH

and spectral data. Thus, the ^{1}H-NMR of **5a** showed, beside the expected signals, the presence of a singlet at δ 4.49 ppm (D_2O exchangeable) indicating the presence of the NH_2 group and a singlet at δ 2.70 ppm equivalent to the CH_2 group. In addition, the ^{13}C-NMR specrum revealed the presence of signals at 142.8, 140.4, 138.7, 136.5, 131.8, 126.4, 124.3, and 120.2 corresponding to the C_6H_5 and tiophene carbons and a signal at 116.7 confirming the presence of the CN group. It is important to note that in such series of dimedone derivatives, although the 5,5-dimethyl groups as in the case of compounds **3a–c** and the 7,7-dimethyl moieties in compounds **5a–f** showed a slight difference through the 1H-NMR like δ 1.08 and 1.09 ppm as in case of **3a**, however, the ^{13}C-NMR both of the two CH_3 groups gave one signal at 24.8. Such findings was in agreement with some reported literature (Drew 1982) although in other reports the ^{1}H-NMR and ^{13}C-NMR showed two signals in each measurement (Gurumurthi et al. 2009).

We then turn our attention to the preparation of cinnoline derivatives through the reaction of any of compounds **5a,c,e** with either of malononitrile (**4a**) or ethyl cyanoacetate (**4b**) to give the 2,3,5,6-tetrahydrothieno[2,3-*h*]cinnoline derivatives **6a–f**, respectively (Scheme 1).

The reaction of any of compounds **6a–f** with either of hydrazine hydrate (**7a**) or phenylhydrazine (**7b**) gave the diarlhydrazone derivatives **8a–m**, respectively. The analytical and spectral data of the latter compounds were consistent with their respective products. Thus, the ^{1}H-NMR of compound **8a** (as an example) revealed the presence the two singlets at δ 4.47 and 5.03 ppm (D_2O exchangeable) corresponding to the two NH_2 groups beside the ^{13}C-NMR spectrum that showed the presence of two signals 179.8 and 176.5 corresponding to the two C=N groups and signals at 179.8, 176.5 (C-4, C-5), 141.6, 140.8, 139.3, 135.9, 134.3, 128.6, 124.1, and 120.1 indicating the presence of C_6H_5 and thiophene carbons.

Scheme 3 Synthesis of compounds **12a–f** and **14a–m**



14	a	b	c	d	e	f
X	H	H	H	H	Cl	Cl
R	CN	CN	COOEt	COOEt	CN	CN
Y	NH_2	OH	NH_2	OH	NH_2	OH
14	**g**	**h**	**i**	**k**	**l**	**m**
X	Cl	Cl	OCH_3	OCH_3	OCH_3	OCH_3
R	COOEt	COOEt	CN	CN	COOEt	COOEt
Y	NH_2	OH	NH_2	OH	NH_2	OH

Encouraged by the initial success, we applied the optimal condition to the synthesis of a series of thienopyrimidino derivatives. Interestingly, the reaction of any of compounds **5a–f** with phenylisothiocyanate (**9**) gave the hexahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-4-yl)yttrium derivatives **10a–f**, respectively (Scheme 2). It is of an interest to mention that compounds **5a–f** and **10a–f** exist in the arylhydrazone form like compounds **3a–c** due to the stability of these tautomeric structures due to the intramolecular H-bonding (Drew 1982).

On the other hand, the reaction of any of compounds **10a–f** with hydroxylamine hydrochloride afforded the triazolo[4″,5″:5′,6′]benzo[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-10-yl)yttrium **12a–f**. Formation of the latter products were explained through the first reaction of the hydroxylamine to give the corresponding oxime derivative followed by dehydration. Finally, the reaction of any of compounds **5a–f** with the cinnamonitrile derivatives **13a** and **13b** gave the thieno[2,3-*g*]chromene derivatives **14a–m**, respectively. Compounds **14a–m** were structurally characterized by various spectroscopic techniques including ^{1}H, ^{13}C-NMR, EI-MS, and IR.

Biology

Materials

Adenosine triphosphate (ATP), DMSO, $MgCl_2$ were obtained from Sigma (St. Louis, MO, USA) kinase buffer

Table 1 c-Met enzymatic activity of the newly synthesized compounds

Compound no	*X*	*Y*	*R*	IC_{50} (nM) c-Met	IC_{50} (nM) PC-3
3a	H	–	–	8.25 ± 2.69	10.52 ± 2.84
3b	Cl	–	–	1.02 ± 0.63	2.48 ± 1.20
3c	OCH_3	–	–	2.14 ± 0.92	3.84 ± 1.27
5a	H	–	CN	12.28 ± 3.91	3.29 ± 1.59
5b	H	–	COOEt	10.62 ± 2.59	4.26 ± 2.83
5c	Cl	–	CN	0.38 ± 0.19	0.22 ± 0.13
5d	Cl	–	COOEt	1.53 ± 0.87	2.62 ± 1.28
5e	OCH_3	–	CN	12.49 ± 3.53	5.73 ± 1.69
5f	OCH_3	–	COOEt	14.02 ± 4.72	1.02 ± 0.82
6a	H	NH	–	18.52 ± 6.93	10.72 ± 2.83
6b	H	O	–	8.16 ± 2.62	10.93 ± 3.29
6c	Cl	NH	–	0.39 ± 0.21	0.63 ± 0.25
6d	Cl	O	–	0.08 ± 0.03	0.19 ± 0.66
6e	OCH_3	NH	–	12.59 ± 2.73	10.91 ± 3.62
6f	OCH_3	O	–	8.28 ± 2.59	7.39 ± 1.42
8a	H	H	CN	12.46 ± 4.63	6.28 ± 2.25
8b	H	Ph	CN	8.28 ± 2.53	9.16 ± 2.72
8c	H	H	COOEt	8.62 ± 2.59	2.03 ± 0.83
8d	H	Ph	COOEt	12.52 ± 4.90	18.39 ± 4.68
8e	Cl	H	CN	0.25 ± 0.06	0.63 ± 0.09
8f	Cl	Ph	CN	0.08 ± 0.006	4.15 ± 1.80
8g	Cl	H	COOEt	20.69 ± 4.80	10.63 ± 2.80
8h	Cl	Ph	COOEt	1.14 ± 0.79	2.38 ± 0.70
8i	OCH_3	H	CN	6.37 ± 1.72	0.74 ± 0.23
8k	OCH_3	Ph	CN	8.39 ± 2.63	1.60 ± 0.69
8l	OCH_3	H	COOEt	14.27 ± 4.72	18.08 ± 5.27
8m	OCH_3	Ph	COOEt	8.06 ± 2.72	6.73 ± 1.52
10a	H	NH_2	–	7.46 ± 2.79	1.81 ± 0.94
10b	H	OH	–	10.28 ± 2.63	8.72 ± 3.92
10c	Cl	NH_2	–	4.73 ± 2.18	8.42 ± 2.93
10d	Cl	OH	–	1.05 ± 0.83	0.62 ± 0.25
10e	OCH_3	NH_2	–	12.52 ± 2.69	5.79 ± 1.80
10f	OCH_3	OH	–	3.72 ± 1.58	1.15 ± 0.82
12a	H	NH_2	–	10.53 ± 2.80	17.19 ± 4.93
12b	H	OH	–	8.69 ± 2.32	9.53 ± 3.86
12c	Cl	NH_2	–	2.39 ± 1.60	3.93 ± 1.72
12d	Cl	OH	–	0.83 ± 0.26	0.87 ± 0.15
12e	OCH_3	NH_2	–	14.73 ± 4.74	8.82 ± 0.62
12f	OCH_3	OH	–	15.26 ± 4.93	12.39 ± 3.49
14a	H	NH_2	CN	6.18 ± 1.59	10.53 ± 2.90
14b	H	OH	CN	8.63 ± 3.53	8.60 ± 3.32
14c	H	NH_2	COOEt	14.93 ± 4.72	18.25 ± 2.39
14d	H	OH	COOEt	10.17 ± 3.80	8.63 ± 2.70
14e	Cl	NH_2	CN	0.06 ± 0.05	0.54 ± 0.32
14f	Cl	OH	CN	0.07 ± 0.006	8.02 ± 2.52
14g	Cl	NH_2	COOEt	2.28 ± 1.63	1.14 ± 0.83
14h	Cl	OH	COOEt	3.79 ± 1.64	0.64 ± 0.04
14i	OCH_3	NH_2	CN	6.73 ± 2.52	2.29 ± 1.59
14k	OCH_3	OH	CN	5.62 ± 2.49	1.07 ± 0.72
14l	OCH_3	NH_2	COOEt	14.63 ± 4.52	16.39 ± 3.63
14m	OCH_3	OH	COOEt	4.82 ± 1.29	6.73 ± 2.80
	–	–		**Foretinib** 1.16 ± 0.17	**SGI-1776** 4.86 ± 0.16

was prepared with 5 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} DTT, or 5 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} $MnCl_2$ and 1 mmol L^{-1} DTT. Receptor tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR were purchased from Carna Biosciences (Kobe, Japan).

HTRF kinase assay

The c-Met kinase activity of all compounds was evaluated (Table 1) using homogeneous time-resolved fluorescence (HTRF) assay as previously reported (Tang et al. 2016; Liu et al. 2016). In addition the most active compounds **5c**, **6c**, **6d**, **8e**, **8f**, **12d**, **14e**, and **14f** were further evaluated against other five tyrosine kinase (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) using the same screening method (Table 3). Briefly, 20 µg/mL poly (Glu, Tyr) 4:1 (Sigma) was pre-coated as a substrate in 384-well plates. Then 50 µL of 10 mM ATP (Invitrogen) solution diluted in kinase reaction buffer (50 µM HEPES, Ph 7.0, 1 M DTT, 1 M $MgCl_2$, 1 M $MnCl_2$, and 0.1% NaN_3) was added to each well. Various concentrations of compounds diluted in 10 µL of 1% DMSO (v/v) were used as the negative control. The kinase reaction was initiated by the addition of purified tyrosine kinase proteins diluted in 39 µL of kinase reaction buffer solution. The incubation time for the reactions was 30 min at 25 °C and stopped by the addition of 5 µL of Streptavidin-XL665 and 5 µL Tk Antibody Cryptate working solution to all of wells. The plates were read using Envision (PerkinElmer) at 320 and 615 nM. The inhibition rate (%) was calculated using the following equation: Percentage inhibition = 100 − [(Activity of enzyme with tested compounds − Min)/(Max − Min)] × 100 (Max: the observed enzyme activity measured in the presence of enzyme, substrates, and cofactors; Min: the observed enzyme activity in the presence of substrates, cofactors and in the absence of enzyme). IC_{50} values were calculated from the inhibition curves.

It has been reported that the c-Met kinase activity correlated to the prostate cancer where the transformation of prostate cancer from the primary androgen-sensitive to the androgen-insensitive status along with the gain of

radioresistance that signaling by the receptor tyrosine kinase (RTK) c-Met played a key role in it. Firstly, an inverse correlation between the expression of androgen receptor (AR) and c-Met has been observed in prostate epithelium and prostate cancer cells (Knudsen et al. 2002; Humphrey et al. 1995). Secondly, AR signaling suppressed c-Met transcription, while the removal of androgen increased c-Met expression (Verras et al. 2007). Thirdly, it is observed that c-Met expression is high in late stage and bone metastatic prostate cancer (Knudsen et al. 2002). Furthermore, a recent study has demonstrated that c-Met expression has a close relationship with the cellular radiosensitivity (Bacco et al. 2011). Based on these reported observations, we studied the activity of the synthesized compounds toward PC-3 prostate cancer cell line, results were demonstrated through Table 1.

In vitro enzymatic assays

All the newly synthesized the aryl hydrazone derivatives were evaluated for their inhibitory activity toward c-Met enzyme (Zhang et al. 2012) using a homogeneous time-resolved fluorescence (HTRF) assay. Taking foretinib as the positive control, the results expressed as IC_{50} were summarized in Table 1. The IC_{50} values are the average of at least three independent experiments.. The anti-proliferative activity of all target compounds against the human prostatic cancer PC-3 cell line were measured using MTT assay (Tiedt et al. 2011; Takayuki et al. 2010) using SGI-1776 as the reference drug. 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 substituents within the thienopyridine moiety together with the hetero cycle ring being attached have a notable influence on the anti-proliferative activity. The most potent compounds were the seven compounds **5c**, **6c**, **8e**, **8f**, **12d**, **14e**, and **14f**.

As illustrated in Table 1, all the tested compounds displayed potent c-Met enzymatic activity with IC_{50} values ranging from 0.08 to 25.28 nM and potent prostate PC-3 cell line with IC_{50} values ranging from 0.06 to 20.69 nM. Compared with foretinib ($IC_{50} = 1.16$ nM), seven compounds (**5c**, **6c**, **8e**, **8f**, **12d**, **14e**, and **14f**) exhibited equivalent or higher potency with IC_{50} values less than 1.00 nM. It is clear from Table 1 that for compounds **3a–c**, compound **3b** ($X = Cl$) showed IC_{50} 1.02 nM against c-Met enzyme while it showed moderate potency toward PC-3 cell line. The reaction of any of compounds **3a–c** with elemental sulfur and either of malononitrile and ethyl cyanoacetate to give the thiophene derivatives **5a–f**, respectively. It is clear that compound **5c** ($X = Cl$, $R = CN$) showed the highes potency among the six compounds against c-Met and PC-3 cell line with IC_{50}'s 0.38 and 0.22 nM, respectively. It was surprised that compound compound **5f** ($X = OCH_3$, $R =$ COOEt) showed low potency against c-Met enzyme but high potency against PC-3 cell line. Compounds **5c** and **5f** showed higher potency than foretinib against the c-Met enzyme. Considering the 2,3,5,6-tetrahydrothieno[2,3-*h*] cinnoline-4-carbonitrile derivatives **6a–f**, it clear tha compounds **6c** and **6d** showed the highest potency against the c-Met enzyme and PC-cell line. Considering the bishydrazone derivatives **8a–m** which were produced from the reaction of any of **5a–f** with either of hydrazine hydrate or phenyhydrazine, it is clear that compounds **8a**, **8b**, **8c**, **8d**, **8g**, **8l**, and **8m** revealed low potencies toward c-Met enzyme and PC-3 cell line. While compounds **8e**, **8f**, and **8h** showed the highest potency anainst c-Met enzyme. Compounds **8e**, **8i**, and **8k** showed high inhibition toward PC-3 cell line. On the other hand for compounds tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidinone derivatives **10a–f**, it is clear that compound **10d** ($X = Cl$, $Y = OH$) showed the highest potency against c-Met enzyme with IC_{50} 1.05 nM while compounds **10a**, **10d**, and **10f** showed the highest potencies against PC-3 cell lines with IC'$_{50}$ 1.81. 0.62 and 1.15 nM, respectively. Considering the nitrogen rich the triazolo[4″,5″:5′,6′]benzo [1′,2′:4,5]thieno[2,3-*d*]pyrimidine derivatives **12a–f**, it is obvious that compounds **12c** and **12d** were the most potent compounds through such series of compounds against the c-Met while compound **12d** was the only compound that showed high cytotoxic compound toward the PC-3 cell line. Finally for the thieno[2,3-*g*]chromene derivatives **14a–m** compounds **14e** and **14f** showed the highest potencies among the new synthesized compounds toward c-Met enzymatic activity. The potencies of compounds **14e** and **14f** were higher than that of the reference drug foretinib with IC_{50}'s 0.06 and 0.07 nM. On the other hand, compounds **4e**, **14g**, **14h**, **14i**, and **14k** were the most potent compounds toward PC-cell line, in addition, thie potency higher than the reference drug SGI-1776 with IC_{50}'s 0.54, 1.14, 0.64, 2.29, and 1.07 nM, respectively.

Inhibition of tyrosine kinases (Enzyme IC50 (nM)

Compounds **5c**, **6c**, **6d**, **8e**, **8f**, **12d**, **14e**, and **14f** 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 **5c** ($X = Cl$, $R = CN$) and **14f** ($X = Cl$, $Y = OH$, $R = CN$) were the most potent compounds among the tested compounds toward the five tyrosin kinases. Compound **8e** showed high potency toward the three kinases c-Kit, VEGFR-2, and PDGFR with IC_{50}'s 0.29, 0.19, and 0.93 nM, respectively. In addition compound **6c** showed activity against VEGFR-2 with IC_{50} 0.96 nM. Compound **8f** showed the lowest potencies among the tested compounds but compounds **6c** and **12d** showed moderate potencies.

Table 2 Inhibition of tyrosine kinases (Enzyme IC_{50} (nM) by compounds **5c**, **6c**, **6d**, **8e**, **8f**, **12d**, **14e**, and **14f**

Compound	c-Kit	Flt-3	VEGFR-2	EGFR	PDGFR
5c	0.08	0.25	0.62	0.27	0.39
6c	1.26	1.22	0.96	4.80	1.31
6d	0.69	0.77	0.38	0.52	0.93
8e	0.29	1.68	0.19	1.83	0.93
8f	6.73	4.82	6.29	8.31	4.90
12d	1.08	0.83	1.52	1.60	2.84
14e	0.49	0.28	0.19	0.83	0.35
14f	0.06	0.51	0.28	0.42	0.80
Foretinib	0.19	0.17	0.20	0.13	0.26

Cell proliferation assay

The anti-proliferative activities of the newly synthesized compounds (Table 3) were evaluated against the five c-Met-dependent cancer cell lines (A549, HT-29, MKN-45, U87MG, and SMMC-7721) and one c-Met-independent cancer cell line (H460) using the standard MTT assay in vitro, with foretinib as the positive control (Li et al. 2013; Fathi et al. 2012). The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximately 4×10^3 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. 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.

In vitro cell assays

All the synthesized compounds were assessed the inhibitory activities against A549 (non-small cell lung cancer), H460 (human lung cancer), HT-29 (human colon cancer), and MKN-45 (human gastric cancer cancer) cancer cell lines together with foretinib as the positive control by a MTT assay. Furthermore, all compounds were further evaluated against U87MG (human glioblastoma) and SMMC-7721 (human liver cancer) cell lines. The results expressed as IC_{50} were summarized in Table 3. The IC_{50} values are the average of at least three independent experiments. The data listed in Table 3 revealed that the compounds possessed moderate to strong cytotoxicity against the five tested cell lines in the single-digit lM range, and high selectivity for inhibition A549, H460, and MKN-45 cells. The promising compounds were **3b**, **5d**, **6c**, **8f**, **8e**, **8f**, **8g**, **8h**, **10c**, **10d**, **12c**, **14f**, and **14g** were more active than foretinib against U87MG cell line with IC_{50} values of 0.59, 0.29, 0.38, 0.79, 0.35, 0.84, 0.80, 0.37, 0.25, 0.78, and 0.78 µM, respectively. The study of structure–activity relationships (SARs) indicated that these analogs showed similar SARs as summarized in the c-Met kinase level. Where compound **3b** showed high potency against the tesed cell line which was attributed to the presence of the 4-chkorophenyl moiety. On the other hand, compound **5d** was the most potent compounds among the series **5a–f** due to the presence of the Cl and COOEt groups. For compounds **6a–f**, it was obvious from Table 3 that compounds **6c** and 6d were the most potent compounds. Considering compounds **10a–m**, it was clear that compounds **8e**, **8f**, **8g**, **8h**, and **8m** were the most cytotoxic compounds. Compounds **10c**, **10f** were the most active compounds among the series of **10a–f**. The same for compounds **12a–f** where comounds **12c** and **12d** were the most active compounds. It is obvious that the presence of the electronegative Cl group and the OH or NH_2 groups were responsible for the high potency of these compounds. Considering the thieno[2,3-*g*]chromene derivatives **14a–m** it is clear from Table 3 that compounds **14e**, **14f**, and **14g** were the most cytotoxic compounds among the twelve compounds. The highcytotoxicity of the three compounds **14e**, **14f** and **14g** was attributed to the presence of the Cl and/or NH_2 or the OH groups.

Inhibition of selected toward Pim-1 kinase

Furthermore, compounds **3c, 5c, 5d, 6c, 6d, 8f, 8g, 8h, 12c, 12d**, **12f**, and **14f** were selected to examine their Pim-1 kinase inhibition activity (Table 4) 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} values were calculated. Compounds **3c**, **6c**, **8g**, **12c**, and **14f** were the most potent to inhibit Pim-1 activity with IC_{50} value of 0.18, 0.16, 0.36. 0.14, 0.17 µM, while **5**, **6d**, **8f**, **8h**, **12d**, **12f**, and **13b** 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, 5c, 5d, 6c, 6d, 8f, 8g, 8h, 12c, 12d**, **12f**, and **14f** were listed in Table 4 which indicated that Pim-1 was a potential target of these compounds.

Experimental

The solvents used through this work were dried prior to their use. All melting points of the synthesized compounds

Table 3 In vitro growth inhibitory effects $IC_{50} \pm SEM$ (μM) of the newly synthesized compounds against cancer cell lines

Compound no	$IC_{50} \pm SEM$ (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
3a	8.38 ± 0.80	9.32 ± 2.08	9.26 ± 2.39	6.16 ± 1.42	8.59 ± 2.59	9.52 ± 3.51
3b	0.26 ± 0.73	0.63 ± 0.25	1.08 ± 0.53	0.42 ± 0.23	0.59 ± 0.25	0.38 ± 0.16
3c	1.47 ± 0.83	0.69 ± 0.32	0.84 ± 0.59	1.65 ± 0.39	0.84 ± 0.42	0.77 ± 0.25
5a	8.55 ± 2.37	9.29 ± 2.63	8.37 ± 3.72	8.69 ± 2.48	9.30 ± 3.27	8.57 ± 2.63
5b	8.32 ± 2.57	8.72 ± 2.68	9.29 ± 2.72	8.83 ± 2.60	9.38 ± 3.80	6.60 ± 1.73
5c	9.28 ± 2.49	8.36 ± 2.59	10.17 ± 3.63	8.39 ± 2.68	9.49 ± 2.29	6.36 ± 2.67
5d	0.26 ± 0.19	0.42 ± 0.22	0.19 ± 0.03	0.82 ± 0.39	0.29 ± 0.17	0.69 ± 0.29
5e	1.20 ± 0.82	1.94 ± 0.82	2.73 ± 0.95	1.62 ± 0.89	1.59 ± 0.93	2.68 ± 0.83
5f	9.53 ± 2.63	8.81 ± 2.92	9.68 ± 2.48	8.63 ± 3.77	8.53 ± 3.56	8.73 ± 2.52
6a	8.57 ± 2.40	8.59 ± 2.41	7.62 ± 1.39	8.72 ± 3.80	9.59 ± 2.53	8.49 ± 2.17
6b	8.59 ± 2.27	7.59 ± 1.63	8.74 ± 2.64	6.52 ± 2.63	8.93 ± 2.73	8.59 ± 1.62
6c	0.34 ± 0.29	0.67 ± 0.53	0.72 ± 0.35	0.39 ± 0.17	0.38 ± 0.24	0.29 ± 0.16
6d	0.42 ± 0.37	0.25 ± 0.16	0.84 ± 0.53	0.42 ± 0.28	1.29 ± 0.39	0.25 ± 0.09
6e	6.26 ± 1.39	8.49 ± 2.23	8.58 ± 2.49	7.39 ± 2.38	8.59 ± 2.49	6.27 ± 1.25
6f	8.42 ± 3.58	7.26 ± 2.59	9.27 ± 2.57	5.16 ± 1.42	6.69 ± 2.54	7.58 ± 2.62
8a	9.53 ± 3.57	9.72 ± 3.89	8.42 ± 2.63	8.38 ± 1.83	8.90 ± 2.85	8.52 ± 3.41
8b	8.59 ± 2.52	8.42 ± 2.49	6.73 ± 1.69	6.73 ± 1.73	6.74 ± 1.52	7.63 ± 2.51
8c	5.42 ± 2.58	5.94 ± 2.39	6.25 ± 2.59	6.39 ± 2.16	5.62 ± 2.37	6.25 ± 1.94
8d	6.42 ± 2.57	2.58 ± 1.82	3.39 ± 1.51	4.27 ± 1.72	6.29 ± 1.85	3.63 ± 1.59
8e	1.27 ± 0.53	0.82 ± 0.57	0.83 ± 0.82	1.72 ± 0.94	0.79 ± 0.26	0.59 ± 0.24
8f	0.26 ± 0.15	0.36 ± 0.14	0.39 ± 0.15	0.15 ± 0.06	0.35 ± 0.15	0.15 ± 0.08
8g	0.67 ± 0.36	0.47 ± 0.25	1.69 ± 0.26	0.92 ± 0.36	0.84 ± 0.29	1.25 ± 0.48
8h	0.25 ± 0.08	0.15 ± 0.07	0.68 ± 0.15	0.49 ± 0.15	0.80 ± 0.32	0.59 ± 0.38
8i	6.42 ± 1.38	7.28 ± 2.59	6.24 ± 2.80	6.25 ± 1.64	7.90 ± 2.35	6.83 ± 2.49
8k	2.27 ± 0.95	2.50 ± 0.81	1.25 ± 0.69	3.62 ± 1.27	2.49 ± 0.57	2.38 ± 0.32
8l	4.61 ± 1.65	3.72 ± 1.42	2.50 ± 0.82	2.63 ± 0.98	3.16 ± 1.80	2.57 ± 1.25
8m	0.93 ± 0.32	1.59 ± 0.95	1.66 ± 0.94	1.37 ± 0.72	2.58 ± 0.96	2.25 ± 1.02
10a	8.42 ± 2.57	9.28 ± 3.52	8.80 ± 3.63	6.70 ± 2.83	7.93 ± 1.27	6.38 ± 1.25
10b	2.34 ± 0.95	3.59 ± 0.93	2.38 ± 0.84	4.53 ± 1.51	3.26 ± 1.42	4.28 ± 2.63
10c	1.72 ± 0.69	1.38 ± 0.28	1.69 ± 0.36	0.69 ± 0.25	0.37 ± 0.15	2.81 ± 0.53
10d	0.63 ± 0.42	0.82 ± 0.49	0.49 ± 0.83	0.08 ± 0.01	0.25 ± 0.03	0.92 ± 0.08
10e	5.25 ± 0.53	7.31 ± 2.69	8.92 ± 2.73	6.27 ± 2.39	7.62 ± 2.49	6.80 ± 2.39
10f	1.06 ± 0.78	1.52 ± 0.73	1.85 ± 0.59	0.93 ± 0.32	0.74 ± 0.29	1.28 ± 0.90
12a	4.27 ± 1.58	3.94 ± 1.80	6.81 ± 2.39	5.62 ± 1.73	6.29 ± 2.32	4.56 ± 1.25
12b	7.29 ± 1.42	8.92 ± 2.69	6.49 ± 2.73	8.28 ± 2.37	6.37 ± 1.62	7.79 ± 1.32
12c	0.98 ± 0.63	1.08 ± 0.76	1.38 ± 0.69	0.89 ± 0.26	0.93 ± 0.69	1.03 ± 0.95
12d	2.16 ± 1.35	1.28 ± 0.63	2.39 ± 0.83	1.92 ± 0.83	3.58 ± 1.62	2.72 ± 0.94
12e	4.52 ± 1.39	3.70 ± 1.53	4.63 ± 2.59	3.22 ± 1.80	4.52 ± 1.63	5.72 ± 1.38
12f	6.48 ± 2.53	6.70 ± 1.93	6.42 ± 1.59	4.84 ± 1.96	4.39 ± 1.73	5.84 ± 2.62
14a	8.36 ± 2.58	6.29 ± 1.47	6.50 ± 1.62	6.49 ± 1.39	7.39 ± 2.92	3.69 ± 0.93
14b	3.22 ± 1.79	7.39 ± 1.25	8.63 ± 2.44	6.39 ± 2.58	4.70 ± 2.09	9.48 ± 3.24
14c	4.58 ± 1.37	3.53 ± 1.52	4.92 ± 1.62	5.63 ± 1.87	6.42 ± 2.74	6.29 ± 2.63
14d	3.69 ± 1.52	4.62 ± 1.39	6.52 ± 2.59	8.32 ± 2.39	4.72 ± 1.49	3.83 ± 1.37
14e	0.38 ± 0.15	0.83 ± 0.52	1.03 ± 0.95	0.54 ± 0.27	1.09 ± 0.82	0.98 ± 0.32
14f	0.32 ± 0.31	0.94 ± 0.25	1.07 ± 0.88	0.63 ± 0.29	0.78 ± 0.36	1.18 ± 0.79
14g	1.80 ± 0.73	0.89 ± 0.30	0.67 ± 0.42	0.93 ± 0.42	0.78 ± 0.39	0.73 ± 0.26

Table 3 (continued)

Compound no	$IC_{50} \pm SEM$ (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
14h	3.38 ± 1.52	2.26 ± 0.53	1.36 ± 0.53	2.63 ± 0.88	1.29 ± 0.25	2.28 ± 0.69
14i	10.17 ± 2.39	9.38 ± 2.42	10.49 ± 3.80	8.52 ± 2.95	8.53 ± 2.27	9.73 ± 2.59
14k	8.25 ± 1.73	10.62 ± 3.60	8.72 ± 2.55	8.20 ± 1.83	6.39 ± 1.72	7.93 ± 1.27
14l	9.87 ± 1.65	6.48 ± 1.33	7.202 ± 2.72	8.73 ± 2.68	6.49 ± 2.42	4.83 ± 1.59
14m	6.39 ± 1.52	1.38 ± 0.69	3.34 ± 1.48	1.89 ± 0.88	2.84 ± 1.52	4.58 ± 1.38
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 4 The inhibitor activity of compounds **3c, 5c, 5d, 6c, 6d, 8f, 8g, 8h, 12c, 12d**, **12f** and **14f** on Pim-1 Kinase

Compound	Inhibition ratio At 10 μM	IC_{50} (μM)
3c	94	0.18
5c	32	>10
5d	96	0.16
6c	92	0.36
6d	21	>10
8f	18	>10
8g	98	0.14
8h	31	>10
12c	97	0.12
12d	28	>10
12f	26	>10
14f	95	0.17
SGI-1776	–	0.048

were recorded on Buchi melting point apparatus D-545; IR spectra (KBr discs) were recorded on Bruker Vector 22 instrument. ^{13}C-NMR and ^{1}H-NMR spectra were recorded on Bruker DPX200 instrument in DMSO-d_6 with TMS as internal standard. Chemical shifts are mentioned in δ (ppm). Mass spectra were measured using EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out using the Microanalytical Data center at Cairo University. The completion of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck).

General procedure for the synthesis of the arylhydrazone derivatives 3a–c

To a solution of dimedone (1.40 g, 0.01 mol) in ethanol (40 mL) containing sodium acetate (4.0 g) any of benzenediazonium chloride (0.01 mol), 4-chlorobenzenediazonium chloride (0.01 mol) or 4-methoxybenzenediazonium chloride (0.01 mol) [prepared through the addition of sodium nitrite (0.70 g, 0.01 mol) to a cold solution 0–5 °C any of aniline (0.92 g, 0.01 mol), 4-chloroaniline (1.27 g, 0.01 mol) or 4-methoxyaniline (1.23 g, 0.01 mol) dissolved in concentrated hydrochloric acid (9.0 mL, 18 mol) was added with continuous stirring] added. The reaction mixture was stirred at room temperature for 2 h and the formed solid product, in each case, was collected by filtration.

5,5-Dimethyl-2-(2-phenylhydrazono)cyclohexane-1,3-dione (3a)

Orange-red crystals (ethanol), yield 70% (1.71 g), m.p. 149–152 °C; (IR (KBr) νmax 3486–3325, 3055, 2984, 1689, 1687, 1635, 1580; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.28 (s, 1H, D_2O exchangeable, NH), 7.26–7.39 (m, 5H, C_6H_5), 2.73, 2.71 (2s, 4H, $2CH_2$), 1.08, 1.09 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 182.3 (C-2, C-4), 133.5, 127.2, 124.8, 120.5 (C_6H_5), 50.3 (C-4, C-6), 24.8 ($2CH_3$); EIMS: *m/z* 244 $[M]^+$ (48%); Analysis Calcd for $C_{14}H_{16}N_2O_2$ (244.29): C, 68.83; H, 6.60; N, 11.47%. Found: C, 68.69; H, 6.49; N, 11.72%.

2-(2-(4-Chlorophenyl)hydrazono)-5,5-dimethylcyclohexane-1,3-dione (3b)

Orange crystals (ethanol), yield 78% (2.16 g), m.p. 168–170 °C; (IR (KBr) νmax 3493–3335, 3050, 2985, 1689, 1688, 1632, and 1583; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.26 (s, 1H, D_2O exchangeable, NH), 7.22–7.49 (m, 4H, C_6H_4), 2.71, 2.70 (2 s, 4H, $2CH_2$), 1.05, 1.07 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 182.3 (C-2, C-4), 135.8, 128.5, 126.7, 121.8 (C_6H_4), 50.1 (C-4, C-6), 24.5 ($2CH_3$); EIMS: *m/z* 278 $[M]^+$ (38%); Analysis Calcd for $C_{14}H_{15}ClN_2O_2$ (278.73): C, 60.33; H, 5.42; N, 10.05%. Found: C, 60.42; H, 5.70; N, 9.85%.

2-(2-(4-Methoxyphenyl)hydrazono)-5,5-dimethylcyclohexane-1,3-dione (3c)

Orange crystals (ethanol), yield 69% (1.89 g), m.p. 144–147 °C; (IR (KBr) νmax 3482–3317, 3050, 2987, 1688, 1686, 1630, 1584; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ =

8.27 (s, 1H, D_2O exchangeable, NH), 7.20–7.46 (m, 4H, C_6H_4), 3.68 (s, 3 H, OCH_3), 2.74, 2.71 (2 s, 4H, $2CH_2$), 1.06, 1.08 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 182.3 (C-2, C-4), 135.5, 127.8, 125.6, 120.3 (C_6H_4), 52.6 (OCH_3), 50.2 (C-4, C-6), 24.6 ($2CH_3$); EIMS: *m/z* 274 $[M]^+$ (28%); Analysis Calcd for $C_{15}H_{18}N_2O_3$ (274.32): C, 65.68; H, 6.61; N, 10.21%. Found: C, 65.73; H, 6.49; N, 10.08%.

General procedure for the synthesis of the 6,7-dihydrobenzo[b]thiophen-5(4*H*)-one derivatives 5a–f

To a solution of any of compound **3a** (2.44 g, 0.01 mol), 3b (2.78 g, 0.01 mol) or **3c** (2.74 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (0.50 g) each of elemental sulfur (0.32 g, 0.01 mol) and either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.3 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 2 h then left to cool and the formed solid product, in each case was collected by filtration.

2-Amino-7,7-dimethyl-5-oxo-4-(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carbonitrile (5a)

Yellow crystals (ethanol), yield 78% (2.52 g), m.p. 213–216 °C; (IR (KBr) νmax 3474–3316, 3056, 2982, 2220, 1688, 1635, 1565; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.26 (s, 1H, D_2O exchangeable, NH), 7.28–7.38 (m, 5H, C_6H_5), 4.49 (s, 2H, D_2O exchangeable, NH_2), 2.70 (s, 2H, CH_2), 1.06, 1.05 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.2 (C-5), 142.8, 140.4, 138.7, 136.5, 131.8, 126.4, 124.3, 120.2 (C_6H_5, C-2, C-3, C-3a, C-7a) 116.7 (CN), 50.3 (C-4, C-6), 24.6 ($2CH_3$), 38.6 (C-7); EIMS: *m/z* 324 $[M]^+$ (32%); Analysis Calcd for $C_{17}H_{16}N_4OS$ (324.40): C, 62.94; H, 4.97; N, 17.27; S, 9.88%. Found: C, 63.15; H, 5.21; N, 17.06; S, 10.02%.

Ethyl 2-amino-7,7-dimethyl-5-oxo-4-(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (5b)

Yellow crystals (ethanol), yield 68% (2.52 g), m.p. 177–179 °C; (IR (KBr) νmax 3481–3324, 3054, 2980, 1687, 1632, 1560; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.28 (s, 1H, D_2O exchangeable, NH), 7.26–7.39 (m, 5H, C_6H_5), 4.46 (s, 2H, D_2O exchangeable, NH_2), 4.22 (q, 2H, J = 7.11 Hz, CH_2), 2.72 (s, 2H, CH_2), 1.13 (t, 3H, J = 7.11 Hz, CH_3), 1.08, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.4 (C-4), 164.6, 168.2 (C-5, ester C=O), 141.8, 138.9, 137..5, 136.8, 132.2, 128.1, 126.7, 120.9 (C_6H_5, C-2, C-3, C-3a, C-7a), 50.3 (C-4, C-6), 38.5 (C-7), 24.6 ($2CH_3$); EIMS: *m/z* 371 $[M]^+$ (28%); Analysis Calcd for $C_{19}H_{21}N_3O_3S$ (371.45): C, 61.44; H, 5.70; N, 11.31; S, 8.63%. Found: C, 61.58; H, 5.39; N, 11.42; S, 8.82%.

2-Amino-4-(2-(4-chlorophenyl)hydrazono)-7,7-dimethyl-5-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (5c)

Yellow crystals (ethanol), yield 80% (2.86 g), m.p. 207–210 °C; (IR (KBr) νmax 3477–3321, 3056, 2980, 2220, 1689, 1630, 1560; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.25 (s, 1H, D_2O exchangeable, NH), 7.47–7.22 (m, 4H, C_6H_4), 4.47 (s, 2H, D_2O exchangeable, NH_2), 2.75 (s, 2H, CH_2), 1.07, 1.02 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.4 (C-4), 164.9 (C-5), 142.6, 141.8, 138.5, 136.9, 130.8, 125.6, 123.8, 120.3 (C_6H_5, C-2, C-3, C-3a, C-7a), 116.8 (CN), 50.2 (C-4, C-6), 38.5 (C-7), 24.6 ($2CH_3$),; EIMS: *m/z* 358 $[M]^+$ (41%); Analysis Calcd for $C_{17}H_{15}ClN_4OS$ (358.85): C, 56.90; H, 4.21; N, 15.61; S, 8.94%. Found: C, 56.76; H, 4.39; N, 15.80; S, 8.76%.

Ethyl 2-amino-4-(2-(4-chlorophenyl)hydrazono)-7,7-dimethyl-5-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (5d)

Yellow crystals (ethanol), yield 73% (2.95 g), m.p. 205–208 °C; (IR (KBr) νmax 3496–3317, 3056, 2983, 1689, 1630, 1552; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.25 (s, 1H, D_2O exchangeable, NH), 7.47–7.23 (m, 4H, C_6H_4), 4.49 (s, 2H, D_2O exchangeable, NH_2), 4.24 (q, 2H, J = 6.83 Hz, CH_2), 2.72 (s, 2H, CH_2), 1.13 (t, 3H, J = 6.83 Hz, CH_3), 1.08, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.7 (C-4), 168.2, 164.7 (C-5, ester CO), 141.8, 140.6, 138.5, 137.3, 131.8, 127.9, 126.4, 120.4 (C_6H_5, C-2, C-3, C-3a, C-7a) 52.6 ($O\underline{CH_2}CH_3$), 50.1 (C-4, C-6), 16.2 ($OCH_2\underline{CH_3}$), 38.7 (C-7), 24.8 ($2CH_3$); EIMS: *m/z* 405 $[M]^+$ (42%); Analysis Calcd for $C_{19}H_{20}ClN_3O_3S$ (405.90): C, 56.22; H, 4.97; N, 10.35; S, 7.90%. Found: C, 56.39; H, 5.16; N, 10.63; S, 8.03%.

2-Amino-4-(2-(4-methoxyphenyl)hydrazono)-7,7-dimethyl-5-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (5e)

Yellow crystals (ethanol), yield 68% (2.40 g), m.p. 258–261 °C; (IR (KBr) νmax 3449–3349, 3054, 2983, 2222, 1687, 1632, 1564; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.26 (s, 1H, D_2O exchangeable, NH), 7.45–7.27 (m, 4H, C_6H_4), 4.49 (s, 2H, D_2O exchangeable, NH_2), 3.69 (s, 3H, OCH_3), 2.79 (s, 2H, CH_2), 1.08, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.6 (C-4), 165.2 (C-5), 142.2, 141.8, 138.2, 134.5, 131.6, 128.3, 125.3, 120.8 (C_6H_4, C-2, C-3, C-3a, C-7a), 116.9 (CN), 52.6 (OCH_3), 50.4 (C-4, C-6), 38.8 (C-7), 24.8 ($2CH_3$),; EIMS: *m/z* 354 $[M]^+$ (36%); Analysis Calcd for $C_{18}H_{18}N_4O_2S$ (354.43): C, 61.00; H, 5.12; N, 15.81; S, 9.05%. Found: C, 60.85; H, 4.94; N, 15.69; S, 8.95%.

Ethyl 2-amino-4-(2-(4-methoxyphenyl)hydrazono)-7,7-dimethyl-5-oxo-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (5f)

Yellow crystals (ethanol), yield 77% (3.08 g), m.p. 156–159 °C; (IR (KBr) νmax 3484–3326, 3056, 2982, 1688, 1630, 1553; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.27 (s, 1H, D_2O exchangeable, NH), 7.39–7.27 (m, 4H, C_6H_4), 4.46 (s, 2H, D_2O exchangeable, NH_2), 4.21 (q, 2H, J = 7.02 Hz, CH_2), 2.76 (s, 2H, CH_2), 1.12 (t, 3H, J = 7.02 Hz, CH_3), 1.05, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.5 (C-4), 165.2, 168.7 (C-5, ester CO),), 142.6, 140.3, 136.8, 134.7, 132.6, 127.5, 125.3, 120.5 (C_6H_4, C-2, C-3, C-3a, C-7a), 52.8 (OCH_3), 52.4 ($O\underline{CH_2}CH_3$), 50.3 (C-4, C-6), 38.9 (C-7), 24.7 ($2CH_3$), 16.8 ($OCH_2\underline{CH_3}$),; EIMS: *m/z* 401 $[M]^+$ (32%); Analysis Calcd for $C_{20}H_{23}N_3O_4S$ (401.48): C, 59.83; H, 5.77; N, 10.47; S, 7.99%. Found: C, 59.73; H, 5.80; N, 10.59; S, 8.15%.

General procedure for the synthesis of the 2,3,5,6-tetrahydrothieno[2,3-*h*]cinnoline derivatives 6a–f

To a solution of any of compounds **5a** (3.24 g, 0.01 mol), **5c** (3.58 g, 0.01 mol), and **5e** (3.54 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (0.50 g, 0.01 mol) either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

8-Amino-3-imino-6,6-dimethyl-2-phenyl-2,3,5,6-tetrahydrothieno[2,3-*h*]cinnoline-4,9-dicarbonitrile (6a)

Yellow crystals (ethanol), yield 78% (2.90 g), m.p. 187–190 °C; (IR (KBr) νmax 3498–3326, 3054, 2983, 2224, 2220, 1632, 1564; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.28 (s, 1H, D_2O exchangeable, NH), 7.38–7.25 (m, 5H, C_6H_5), 4.47 (s, 2H, D_2O exchangeable, NH_2), 2.79 (s, 2H, CH_2), 1.08, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 178.5, 175.4 (C-3, C-9b), 143.8, 142.6, 140.9, 138.3, 137.0, 136.5, 135.3, 127.5, 124.6, 120.5 (C_6H_5, C-2, C-3, C-6a, C-9a, C-4, C-4a), 116.9, 117.3 (2CN), 50.3 (C-4, C-6), 38.4 (C-6), 24.1 ($2CH_3$); EIMS: *m/z* 372 $[M]^+$ (22%); Analysis Calcd for $C_{20}H_{16}N_6S$ (372.45): C, 64.50; H, 4.33; N, 22.56; S, 8.61%. Found: C, 64.68; H, 4.51; N, 22.69; S, 8.79%.

8-Amino-3-oxo-6,6-dimethyl-2-phenyl-2,3,5,6-tetrahydrothieno[2,3-*h*]cinnoline-4,9-dicarbonitrile (6b)

Yellow crystals (ethanol), yield 83% (3.09 g), m.p. 233–236 °C; (IR (KBr) νmax 3474–3352, 3056, 2983, 2221, 2220, 1689, 1636, 1560; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 7.41–7.25 (m, 5H, C_6H_5), 4.49 (s, 2H, D_2O exchangeable, NH_2), 2.73 (s, 2H, CH_2), 1.06, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 178.8, 168.2 (C-3, C-9b), 143.6, 142.2, 140.5, 138.6, 137.2, 136.2, 133.8, 129.9, 126.2, 120.8 (C_6H_5, C-2, C-3, C-6a, C-9a, C-4, C-4a), 116.8, 117.2 (2CN), 50.1 (C-4, C-6), 38.7 (C-6), 24.5 ($2CH_3$); EIMS: *m/z* 373 $[M]^+$ (42%); Analysis Calcd for $C_{20}H_{15}N_5OS$ (373.43): C, 64.33; H, 4.05; N, 18.75; S, 8.59%. Found: C, 64.58; H, 4.19; N, 18.93; S, 8.64%.

8-Amino-2-(4-chlorophenyl)-3-imino-6,6-dimethyl-2,3,5,6-tetrahydrothieno[2,3-*h*]cinnoline-4,9-dicarbonitrile (6c)

Yellow crystals (ethanol), yield 72% (2.92 g), m.p. 177–179 °C; (IR (KBr) νmax 3480–3317, 3054, 2982, 2222, 2220, 1646, 1556; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.41 (s, 1H, D_2O exchangeable, NH), 7.48–7.21 (m, 4H, C_6H_4), 4.43 (s, 2H, D_2O exchangeable, NH_2), 2.77 (s, 2H, CH_2), 1.08, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.6, 175.6 (C-3, C-9b), 143.3, 141.8, 140.2, 139.4, 138.1, 136.8, 134.5, 129.4, 127.8, 122.1 (C_6H_5, C-2, C-3, C-6a, C-9a, C-4, C-4a), 116.7, 117.2 (2CN), 50.3 (C-4, C-6), 38.6 (C-6), 24.3 ($2CH_3$); EIMS: *m/z* 406 $[M]^+$ (38%); Analysis Calcd for $C_{20}H_{15}ClN_6S$ (406.89): C, 59.04; H, 3.72; N, 20.65; S, 7.88%. Found: C, 59.25; H, 3.82; N, 20.73; S, 8.04%.

8-Amino-2-(4-chlorophenyl)-6,6-dimethyl-3-oxo-2,3,5,6-tetrahydrothieno[2,3-*h*]cinnoline-4,9-dicarbonitrile (6d)

Yellow crystals (ethanol), yield 58% (2.36 g), m.p. 177–179 °C; (IR (KBr) νmax 3458–3315, 3055, 2980, 2222, 2220, 1688, 1633, 1562; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 7.46–7.23 (m, 4H, C_6H_4), 4.42 (s, 2H, D_2O exchangeable, NH_2), 2.78 (s, 2H, CH_2), 1.08, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 178.5, 167.9 (C-3, C-9b), 143.3, 142.7, 141.8, 139.2, 137.7, 136.1, 132.3, 127.3, 123.1, 120.5 (C_6H_4, C-2, C-3, C-6a, C-9a, C-4, C-4a), 116.5, 117.1 (2CN), 50.5 (C-4, C-6), 38.3 (C-6), 24.6 ($2CH_3$); EIMS: *m/z* 407 $[M]^+$ (28%); Analysis Calcd for $C_{20}H_{14}ClN_5OS$ (407.88): C, 58.89; H, 3.46; N, 17.17; S, 7.86%. Found: C, 58.66; H, 3.59; N, 16.93; S, 7.69%.

8-Amino-3-imino-2-(4-methoxyphenyl)-6,6-dimethyl-2,3,5,6-tetrahydrothieno[2,3-*h*]cinnoline-4,9-dicarbonitrile (6e)

Yellow crystals (ethanol), yield 78% (3.15 g), m.p. 231–233 °C; (IR (KBr) νmax 3458–3341, 3056, 2982, 2223, 2220, 1642, 1553; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.45 (s, 1H, D_2O exchangeable, NH), 7.43–7.27 (m, 4H, C_6H_4), 4.41 (s, 2H, D_2O exchangeable, NH_2), 3.68 (s, 3H,

OCH_3), 2.79 (s, 2H, CH_2), 1.07, 1.05 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.9, 175.3 (C-3, C-9b), 142.3, 141.5, 140.9, 138.2, 137.8, 135.3, 134.2, 128.1, 127.3, 122.8 (C_6H_5, C-2, C-3, C-6a, C-9a, C-4, C-4a), 116.9, 117.2 (2CN), 52.6 (OCH_3), 50.1 (C-4, C-6), 38.5 (C-6), 24.6 ($2CH_3$); EIMS: *m/z* 402 $[M]^+$ (58%); Analysis Calcd for $C_{21}H_{18}N_6OS$ (402.47): C, 62.67; H, 4.51; N, 20.88; S, 7.97%. Found: C, 62.80; H, 4.92; N, 20.63; S, 8.7%.

8-Amino-2-(4-methoxyphenyl)-6,6-dimethyl-3-oxo-2,3,5,6-tetrahydrothieno[2,3-*h*]cinnoline-4,9-dicarbonitrile (6f)

Yellow crystals (ethanol), yield 88% (3.54 g), m.p. 244–246 °C; (IR (KBr) νmax 3473–3328, 3054, 2983, 2220, 1688, 1642, 1550; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.45 (s, 1H, D_2O exchangeable, NH), 7.46–7.23 (m, 4H, C_6H_4), 4.41 (s, 2H, D_2O exchangeable, NH_2), 3.69 (s, 3H, OCH_3), 2.79 (s, 2H, CH_2), 1.07, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.9, 175.3 (C-3, C-9b), 142.8, 141.6, 140.8, 138.3, 136.4, 135.1, 132.8, 129.6, 126.3, 121.8 (C_6H_5, C-2, C-3, C-6a, C-9a, C-4, C-4a), 116.9, 117.4 (2CN), 50.1 (C-4, C-6), 38.3 (C-6), 24.6 ($2CH_3$); EIMS: *m/z* 403 $[M]^+$ (46%); Analysis Calcd for $C_{21}H_{17}N_5O_2S$ (403.46): C, 62.52; H, 4.25; N, 17.36; S, 7.95%. Found: C, 562.80; H, 4.73; N, 17.46; S, 8.26%.

General procedure for the synthesis of the tetrahydrobenzo [*b*]thiophene derivatives 8a-m

To a solution of any of **5a** (3.24 g, 0.01 mol), **5b** (3.71 g, 0.01 mol), **5c** (3.58 g, 0.01 mol), **5d** (4.05 g, 0.01 mol), **5e** (3.54 g, 0.01 mol) or **5f** (4.01 g, 0.01 mol) in ethanol (40 mL) either of hydrazinehydrate (0.50 mL, 0.01 mol) or phenylhydrazine (1.08 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

2-Amino-5-hydrazono-7,7-dimethyl-4-(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (8a)

Yellow crystals (ethanol), yield 74% (2.50 g), m.p. 196–198 °C; (IR (KBr) νmax 3496–3335, 3054, 2980, 2223, 1655, 1561; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.29, (s, 1H, D_2O exchangeable, NH), 7.39–7.29 (m, 5H, C_6H_5), 4.47, 5.03 (2s, 4H, D_2O exchangeable, $2NH_2$), 2.73 (s, 2H, CH_2), 1.09, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.8, 176.5 (C-4, C-5), 141.6, 140.8, 139.3, 135.9, 134.3, 128.6, 124.1, 120.1 (C_6H_5, C-2, C-3, C-3a, C-7a) 116.9 (CN), 50.3 (C-4), 38.8 (C-7), 24.6 ($2CH_3$); EIMS: *m/z* 338 $[M]^+$ (18%); Analysis Calcd for $C_{17}H_{18}N_6S$ (338.43): C, 60.33; H, 5.36; N, 24.83; S, 9.47%. Found: C, 60.54; H, 5.48; N, 24.65; S, 9.61%.

2-Amino-7,7-dimethyl-4,5-bis(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (8b)

Orange crystals (ethanol), yield 69% (2.85 g), m.p. 155–158 °C; (IR (KBr) νmax 3479–3317, 3055, 2980, 2220, 1653, 1563; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.32, 8.26 (2s, 2H, D_2O exchangeable, 2NH), 7.37–7.24 (m, 10H, $2C_6H_5$), 4.49 (s, 2H, D_2O exchangeable, NH_2), 2.76 (s, 2H, CH_2), 1.07, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.7, 176.3 (C-4, C-5), 142.8, 140.6, 137.8, 135.4, 134.6, 127.3, 126.5, 125.2, 124.8, 123.5, 122.7,120.1 ($2C_6H_5$, C-2, C-3, C-3a, C-7a) 116.5 (CN), 50.2 (C-4), 38.3 (C-7), 24.2 ($2CH_3$); EIMS: *m/z* 414 $[M]^+$ (65%); Analysis Calcd for $C_{23}H_{22}N_6S$ (414.53): C, 66.64; H, 5.35; N, 20.27; S, 7.74%. Found: C, 66.86; H, 5.53; N, 20.38; S, 7.93%.

Ethyl 2-amino-5-hydrazono-7,7-dimethyl-4-(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (8c)

Orange crystals (ethanol), yield 69% (2.85 g), m.p. 155–158 °C; (IR (KBr) νmax 3479–3332, 3053, 2980, 1689, 1653, 1563; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.27 (s, 1H, D_2O exchangeable, NH), 7.39–7.27 (m, 5H, C_6H_5), 4.47, 5.13 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.22 (q, 2H, J = 6.38 Hz, $O\underline{CH_2}CH_3$), 2.73 (s, 2H, CH_2), 1.16 (t, 3H, J = 6.38 Hz, $OCH_2\underline{CH_3}$), 1.08, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.9, 176.5 (C-4, C-5), 164.8 (ester CO), 142.8, 140.6, 137.8, 134.6, 126.5, 124.8, 122.7, 120.1 (C_6H_5, C-2, C-3, C-3a, C-7a), 52.6 ($O\underline{CH_2}CH_3$), 50.5 (C-4), 38.2 (C-7), 24.5 ($2CH_3$), 19.2 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 385 $[M]^+$ (78%); Analysis Calcd for $C_{19}H_{23}N_5O_2S$ (385.48): C, 59.20; H, 6.01; N, 18.17; S, 8.32%. Found: C, 59.30; H, 5.88; N, 18.27; S, 8.60%.

Ethyl 2-amino-7,7-dimethyl-4,5-bis(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (8d)

Reddish brown crystals (ethanol), yield 73% (3.36 g), m.p. 207–210 °C; (IR (KBr) νmax 3459–3341, 3055, 2980, 1688, 1653, 1563; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.27 (s, 1H, D_2O exchangeable, NH), 7.41–7.29 (m, 10H, $2C_6H_5$), 4.43, 5.11 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.22 (q, 2H, J = 7.11 Hz, $O\underline{CH_2}CH_3$), 2.78 (s, 2H, CH_2), 1.13 (t, 3 H, J = 7.11 Hz, $OCH_2\underline{CH_3}$), 1.06, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.5, 176.1 (C-4, C-5), 164.9 (ester CO), 142.9, 141.2, 138.6, 137.4, 134.6, 126.5, 125.8, 124.2, 123.1, 122.3, 121.2, 120.0 ($2C_6H_5$, C-2, C-3, C-3a, C-7a), 52.8 ($O\underline{CH_2}CH_3$), 50.3 (C-4), 38.5 (C-7), 24.2 ($2CH_3$), 19.6 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 461 $[M]^+$ (36%); Analysis Calcd for $C_{25}H_{27}N_5O_2S$ (461.58): C, 65.05; H, 5.90; N, 15.17; S, 6.95%. Found: C, 64.87; H, 5.79; N, 15.35; S, 7.17%.

2-Amino-4-(2-(4-chlorophenyl)hydrazono)-5-hydrazono-7,7-dimethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (8e)

Yellow crystals (ethanol), yield 83% (3.08 g), m.p. 211–214 °C; (IR (KBr) νmax 3477–3321, 3055, 2980, 2220, 1655, 1563; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.3 (s, 1H, D_2O exchangeable, NH), 7.48–7.23 (m, 4H, C_6H_4), 4.45, 5.06 (2s, 4H, D_2O exchangeable, $2NH_2$), 2.76 (s, 2H, CH_2), 1.07, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.6, 176.3 (C-4, C-5), 143.3, 141.6, 137.1, 135.2, 134.0, 127.3, 122.8, 120.6 (C_6H_4, C-2, C-3, C-3a, C-7a) 116.6 (CN), 50.6 (C-4), 38.4 (C-7), 24.9 ($2CH_3$); EIMS: *m/z* 372 $[M]^+$ (42%); Analysis Calcd for $C_{17}H_{17}ClN_6S$ (372.88): C, 54.76; H, 4.60; N, 22.54; S, 8.60%. Found: C, 54.49; H, 4.82; N, 22.80; S, 8.74%.

2-amino-4-(2-(4-chlorophenyl)hydrazono)-7,7-dimethyl-5-(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (8f)

Red crystals (ethanol), yield 72% (3.22 g), m.p. 148–151 °C; (IR (KBr) νmax 3469–3326, 3056, 2982, 2220, 1657, 1561; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.36, 8.29 (2s, 2H, D_2O exchangeable, 2NH), 7.47–7.21 (m, 9H, C_6H_5, C_6H_4), 4.49 (s, 2H, D_2O exchangeable, NH_2), 2.76 (s, 2H, CH_2), 1.07, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.5, 176.1 (C-4, C-5), 142.5, 140.3, 136.9, 135.1, 134.8, 126.7, 126.2, 125.0, 124.9, 123.8, 122.3,120.8 (C_6H_5, C_6H_4, C-2, C-3, C-3a, C-7a) 116.9 (CN), 50.5 (C-4), 38.6 (C-7), 24.3 ($2CH_3$); EIMS: *m/z* 448 $[M]^+$ (26%); Analysis Calcd for $C_{23}H_{21}ClN_6S$ (448.97): C, 61.53; H, 4.71; N, 18.72; S, 7.14%. Found: C, 61.70; H, 4.58; N, 18.90; S, 7.35%.

Ethyl 2-amino-4-(2-(4-chlorophenyl)hydrazono)-5-hydrazono-7,7-dimethyl-4,5,6,7-tetra-hydrobenzo[*b*] thiophene-3-carboxylate (8g)

Orange crystals (ethanol), yield 77% (3.22 g), m.p. 236–239 °C; (IR (KBr) νmax 3458–3341, 3055, 2987, 1650, 1560; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.29 (s, 1H, D_2O exchangeable, NH), 7.48–7.24 (m, 4H, C_6H_4), 4.49, 5.15 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.21 (q, 2H, J = 7.39 Hz, $O\underline{CH_2}CH_3$), 2.70 (s, 2H, CH_2), 1.12 (t, 3H, J = 7.39 Hz, $OCH_2\underline{CH_3}$), 1.07, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.3, 176.1 (C-4, C-5), 165.2 (ester CO), 141.9, 141.2, 136.3, 134.8, 128.9, 125.8, 123.5, 120.3 (C_6H_5, C_6H_4, C-2, C-3, C-3a, C-7a), 52.3 ($O\underline{CH_2}CH_3$), 50.2 (C-4), 38.0 (C-7), 24.3 ($2CH_3$), 19.0 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 419 $[M]^+$ (42%); Analysis Calcd for $C_{19}H_{22}ClN_5O_2S$ (419.93): C, 54.34; H, 5.28; N, 16.68; S, 7.64%. Found: C, 54.62; H, 5.39; N, 16.88; S, 7.80%.

Ethyl 2-amino-4-(2-(4-chlorophenyl)hydrazono)-7,7-dimethyl-5-(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (8h)

Reddish brown crystals (ethanol), yield 65% (3.22 g), m.p. 168–170 °C; (IR (KBr) νmax 3473–3326, 3055, 2980, 1650, 1557; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.29 (s, 1H, D_2O exchangeable, NH), 7.49–7.23 (m, 10H, $2C_6H_5$), 4.48 (s, 2H, D_2O exchangeable, NH_2), 4.23 (q, 2H, J = 5.80 Hz, $O\underline{CH_2}CH_3$), 2.73 (s, 2H, CH_2), 1.12 (t, 3H, J = 5.80 Hz, $OCH_2\underline{CH_3}$), 1.09, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.5, 176.7 (C-4, C-5), 165.3 (ester CO), 143.4, 142.0, 138.8, 137.3, 134.1, 126.8, 124.1, 123.0, 122.9, 122.6, 121.4, 120.3 (C_6H_5, C_6H_4, C-2, C-3, C-3a, C-7a), 52.6 ($O\underline{CH_2}CH_3$), 50.8 (C-4), 38.1 (C-7), 24.0 ($2CH_3$), 19.2 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 496 $[M]^+$ (44%); Analysis Calcd for $C_{25}H_{26}ClN_5O_2S$ (496.02): C, 60.53; H, 5.28; N, 14.12; S, 6.46%. Found: C, 60.74; H, 5.41; N, 14.27; S, 6.80%.

2-Amino-5-hydrazono-4-(2-(4-methoxyphenyl)hydrazono)-7,7-dimethyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (8i)

Yellow crystals (ethanol), yield 70% (2.57 g), m.p. 166–168 °C; (IR (KBr) νmax 3493–3331, 3054, 2982, 2220, 1650, 1553; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.32 (s, 1H, D_2O exchangeable, NH), 7.45–7.25 (m, 4H, C_6H_4), 4.43, 5.02 (2 s, 4H, D_2O exchangeable, $2NH_2$), 3.66 (s, 3H, OCH_3), 2.79 (s, 2H, CH_2), 1.06, 1.01 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.3, 176.1 (C-4, C-5), 143.6, 141.1, 138.5, 136.8, 134.3, 128.8, 123.7, 120.2 (C_6H_4, C-2, C-3, C-3a, C-7a), 117.3 (CN), 50.8 (C-4), 38.2 (C-7), 24.3 ($2CH_3$); EIMS: *m/z* 368 $[M]^+$ (31%); Analysis Calcd for $C_{18}H_{20}N_6OS$ (368.46): C, 58.68; H, 5.47; N, 22.81; S, 8.70%. Found: C, 58.72; H, 5.29; N, 22.69; S, 8.83%.

2-Amino-4-(2-(4-methoxyphenyl)hydrazono)-7,7-dimethyl-5-(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (8k)

Red crystals (ethanol), yield 64% (2.84 g), m.p. 244–247 °C; (IR (KBr) νmax 3482–3340, 3054, 2980, 2220, 1653, 1560; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.39, 8.25 (2s, 2H, D_2O exchangeable, 2NH), 7.49–7.20 (m, 9H, C_6H_5, C_6H_4), 4.44 (s, 2H, D_2O exchangeable, NH_2), 3.72 (s, 3H, OCH_3), 2.73 (s, 2H, CH_2), 1.08, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.8, 176.2 (C-4, C-5), 142.8, 140.6, 136.2, 133.5, 132.6, 128.3, 126.4, 125.2, 124.5, 123.2, 122.6, 120.3 (C_6H_5, C_6H_4, C-2, C-3, C-3a, C-7a) 116.9 (CN), 52.4 (OCH_3), 50.2 (C-4), 38.3 (C-7), 24.5 ($2CH_3$); EIMS: *m/z* 444 $[M]^+$ (28%); Analysis Calcd for

$C_{24}H_{24}N_6OS$ (444.55): C, 64.84; H, 5.44; N, 18.90; S, 7.21%. Found: C, 64.62; H, 5.60; N, 18.83; S, 7.29%.

Ethyl 2-amino-5-hydrazono-4-(2-(4-methoxyphenyl) hydrazono)-7,7-dimethyl-4,5,6,7-tetrahydrobenzo[*b*] thiophene-3-carboxylate (8l)

Orange crystals (ethanol), yield 78% (3.23 g), m.p. 190–194 °C; (IR (KBr) νmax 3492–3348, 3055, 2987, 1688, 1560; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.27 (s, 1H, D_2O exchangeable, NH), 7.46–7.23 (m, 4H, C_6H_4), 4.45, 5.18 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.22 (q, 2H, J = 5.95 Hz, $O\underline{CH_2}CH_3$), 3.76 (s, 3H, OCH_3), 2.68 (s, 2H, CH_2), 1.13 (t, 3H, J = 5.95 Hz, $OCH_2\underline{CH_3}$), 1.09, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.6, 176.3 (C-4, C-5), 164.8 (ester CO), 142.8, 141.8, 136.6, 134.2, 128.7, 125.6, 124.2, 120.6 (C_6H_5, C-2, C-3, C-3a, C-7a), 52.1 ($O\underline{CH_2}CH_3$), 52.6 (OCH_3), 50.0 (C-4), 38.3 (C-7), 24.3 ($2CH_3$), 19.3 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 415 $[M]^+$ (28%); Analysis Calcd for $C_{20}H_{25}N_5O_3S$ (415.51): C, 57.81; H, 6.06; N, 16.85; S, 7.72%. Found: C, 57.68; H, 5.82; N, 16.93; S, 7.83%.

Ethyl 2-amino-4-(2-(4-methoxyphenyl)hydrazono)-7,7-dimethyl-5-(2-phenylhydrazono)-4,5,6,7-tetrahydrobenzo [*b*]thiophene-3-carboxylate (8m)

Orange crystals (ethanol), yield 78% (3.82 g), m.p. 203–206 °C; (IR (KBr) νmax 3483–3342, 3055, 2980, 1689, 1650, 1550; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.32, 8.26 (2s, 2H, D_2O exchangeable, 2NH), 7.49–7.23 (m, 9 H, C_6H_5, C_6H_4), 4.52 (s, 2H, D_2O exchangeable, NH_2), 4.20 (q, 2H, J = 6.22 Hz, $O\underline{CH_2}CH_3$), 3.70 (s, 3H, OCH_3), 2.75 (s, 2H, CH_2), 1.13 (t, 3H, J = 6.22 Hz, $OCH_2\underline{CH_3}$), 1.07, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.3, 176.1 (C-4, C-5), 165.2 (ester CO), 143.2, 141.8, 138.3, 136.1, 134.1, 126.84, 125.8, 123.2, 122.3, 121.9, 121.2, 120.6 (C_6H_5, C_6H_4, C-2, C-3, C-3a, C-7a), 116.9 (CN), 53.6 (OCH_3), 52.3 ($O\underline{CH_2}CH_3$), 50.8 (C-4), 38.3 (C-7), 24.1 ($2CH_3$), 18.5 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 491 $[M]^+$ (24%); Analysis Calcd for $C_{26}H_{29}N_5O_3S$ (491.61): C, 63.52; H, 5.95; N, 14.25; S, 6.52%. Found: C, 63.80; H, 5.72; N, 14.39; S, 6.76%.

General procedure for the synthesis benzo[4,5]thieno[2,3-*d*] pyrimidine derivatives 10a–f

To a solution of any of compounds **5a** (3.24 g, 0.01 mol), **5b** (3.71 g, 0.01 mol), **5c** (3.58 g, 0.01 mol), **5d** (4.05 g, 0.01 mol), **5e** (3.54 g, 0.01 mol), or **5f** (4.01 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (0.50 mL) phenylisothiocyanate was added. The reaction mixture, in each case, was heated under reflux for 6 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

4-Amino-8,8-dimethyl-3-phenyl-5-(2-phenylhydrazono)-2-thioxo-2,3,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-6(5*H*)-one (10a)

Orange crystals (ethanol), yield 83% (3.80 g), m.p. 180–184 °C; (IR (KBr) νmax 3468–3315, 3056, 2980, 1653, 1552, 1205; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.36 (s, 1H, D_2O exchangeable, NH), 7.42–7.29 (m, 10H, $2C_6H_5$), 4.78 (s, 2H, D_2O exchangeable, NH_2), 2.79 (s, 2H, CH_2), 1.08, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 180.1 (C-2), 179.3, 176.1, 165.3 (C-5, C-9a, C-6), 142.8, 141.3, 139.1, 137.1, 134.8, 126.3, 125.2, 124.8, 122.6, 121.8, 121.6, 120.2 ($2C_6H_5$, C-4, C-4a, C-4b, C-8a), 50.6 (C-7), 38.7 (C-8), 24.3 ($2CH_3$); EIMS: *m/z* 459 $[M]^+$ (18%); Analysis Calcd for $C_{24}H_{29}N_5OS_2$ (459.59): C, 62.72; H, 4.61; N, 15.24; S, 13.95%. Found: C, 62.59; H, 4.85; N, 15.41; S, 14.22%.

4-Hydroxy-8,8-dimethyl-3-phenyl-5-(2-phenylhydrazono)-2-thioxo-2,3,7,8-tetrahydro-benzo[4,5]thieno[2,3-*d*]pyrimidin-6(5*H*)-one (10b)

Yellow crystals (acetic acid), yield 79% (3.63 g), m.p. 211–213 °C; (IR (KBr) νmax 3459–3338, 3054, 2980, 1652, 1550, 1207; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.28 (s, 1H, D_2O exchageable, OH), 8.37 (s, 1H, D_2O exchangeable, NH), 7.40–7.26 (m, 10H, $2C_6H_5$), 2.74 (s, 2H, CH_2), 1.06, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 180.4 (C-2), 179.6, 176.5, 165.7 (C-5, C-9a, C-6), 143.2, 142.6, 138.7, 137.5, 134.1, 128.6, 125.1, 124.5, 123.3, 120.8, 119.8, 119.4 ($2C_6H_5$, C-4, C-4a, C-4b, C-8a), 50.8 (C-7), 38.3 (C-8), 24.2 ($2CH_3$); EIMS: *m/z* 460 $[M]^+$ (38%); Analysis Calcd for $C_{24}H_{20}N_4O_2S_2$ (460.57): C, 62.59; H, 4.38; N, 12.16; S, 13.92%. Found: C, 62.42; H, 4.57; N, 12.49; S, 14.16%.

4-Amino-5-(2-(4-chlorophenyl)hydrazono)-8,8-dimethyl-3-phenyl-2-thioxo-2,3,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*] pyrimidin-6(5*H*)-one (10c)

Yellow crystals (acetic acid), yield 68% (3.35 g), m.p. 177–179 °C; (IR (KBr) νmax 3484–3358, 3054, 2980, 1650, 1555, 1205; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.39 (s, 1H, D_2O exchangeable, NH), 7.52–7.23 (m, 9H, C_6H_5, C_6H_4), 4.79 (s, 2H, D_2O exchangeable, NH_2), 2.72 (s, 2H, CH_2), 1.07, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 180.6 (C-2), 179.9, 176.2, 165.4 (C-5, C-9a, C-6), 142.9, 141.4, 139.8, 138.3, 135.6, 130.8, 127.5, 125.2, 123.6, 121.8, 120.5, 120.0 ($2C_6H_5$, C-4, C-4a, C-4b, C-8a), 50.5 (C-7), 38.3 (C-8), 24.6 ($2CH_3$); EIMS: *m/z* 494

$[M]^+$ (42%); Analysis Calcd for $C_{24}H_{20}ClN_5OS_2$ (494.03): C, 58.35; H, 4.08; N, 14.18; S, 12.98%. Found: C, 58.59; H, 4.29; N, 13.87; S, 13.14%.

5-(2-(4-Chlorophenyl)hydrazono)-4-hydroxy-8,8-dimethyl-3-phenyl-2-thioxo-2,3,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-6(5*H*)-one (10d)

Pale yellow crystals (1,4-dioxan), yield 80% (3.95 g), m.p. 215–218 °C; (IR (KBr) νmax 3463–3342, 3056, 2983, 1654, 1550, 1206; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.22 (s, 1H, D_2O exchangeable, OH), 8.35 (s, 1H, D_2O exchangeable, NH), 7.49–7.21 (m, 9H, C_6H_5, C_6H_4), 2.73 (s, 2H, CH_2), 1.08, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 180.3 (C-2), 179.5, 176.8, 165.8 (C-5, C-9a, C-6), 142.7, 142.1, 138.4, 136.8, 135.9, 132.5, 127.8, 126.8, 123.3, 121.5, 120.2, 119.3 ($2C_6H_5$, C-4, C-4a, C-4b, C-8a), 50.8 (C-7), 38.1 (C-8), 24.3 ($2CH_3$); EIMS: *m/z* 495 $[M]^+$ (28%); Analysis Calcd for $C_{24}H_{19}ClN_4O_2S_2$ (495.02): C, 58.23; H, 3.87; N, 11.32; S, 12.96%. Found: C, 58.41; H, 3.69; N, 11.50; S, 13.25%.

4-Amino-5-(2-(4-methoxyphenyl)hydrazono)-8,8-dimethyl-3-phenyl-2-thioxo-2,3,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-6(5*H*)-one (10e)

Yellow crystals (ethanol), yield 80% (3.91 g), m.p. 155–157 °C; (IR (KBr) νmax 3491–3339, 3054, 2985, 1688, 1656, 1551, 1203; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.36 (s, 1H, D_2O exchangeable, NH), 7.43–7.27 (m, 9 H, C_6H_5, C_6H_4), 4.76 (s, 2H, D_2O exchangeable, NH_2), 3.72 (s, 3H, OCH_3), 2.72 (s, 2H, CH_2), 1.09, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 180.3(C-2), 179.5, 176.5, 165.8 (C-5, C-9a, C-6), 142.8, 142.6, 139.2, 137.6, 136.4, 132.3, 127.3, 125.7, 123.2, 121.6, 120.3, 119.6 ($2C_6H_5$, C-4, C-4a, C-4b, C-8a), 52.6 (OCH_3), 50.2 (C-7), 38.1 (C-8), 24.7 ($2CH_3$); EIMS: *m/z* 489 $[M]^+$ (32%); Analysis Calcd for $C_{25}H_{23}N_5O_2S_2$ (489.61): C, 61.33; H, 4.73; N, 14.30; S, 13.10%. Found: C, 61.26; H, 4.53; N, 14.25; S, 13.28%.

4-Hydroxy-5-(2-(4-methoxyphenyl)hydrazono)-8,8-dimethyl-3-phenyl-2-thioxo-2,3,7,8-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyrimidin-6(5*H*)-one (10f)

Pale yellow crystals (1,4-dioxan), yield 69% (3.38 g), m.p. 150–153 °C; (IR (KBr) νmax 3480–3319, 3054, 2981, 1652, 1550; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.29 (s, 1H, D_2O exchangeable, OH), 8.33 (s, 1H, D_2O exchangeable, NH), 7.46–7.28 (m, 9H, C_6H_5, C_6H_4), 3.77 (s, 3H, OCH_3), 2.71 (s, 2H, CH_2), 1.06, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 180.1 (C-2), 179.9, 176.5, 165.4 (C-5, C-9a, C-6), 142.8, 142.3, 138.2, 136.5, 135.3, 132.2, 128.9, 125.6, 124.8, 122.8, 120.9, 120.3 ($2C_6H_5$, C-4, C-4a, C-4b, C-8a), 52.6 (OCH_3), 50.3 (C-7), 38.3 (C-8), 24.1 ($2CH_3$); EIMS: *m/z* 490 $[M]^+$ (18%); Analysis Calcd for $C_{25}H_{22}N_4O_3S_2$ (490.60): C, 61.20; H, 4.52; N, 11.42; S, 13.07%. Found: C, 61.38; H, 4.71; N, 11.62; S, 13.19%.

General procedure for the synthesis of the triazolo [4″,5″:5′,6′]benzo [1′,2′:4,5]thieno[2,3-*d*]pyrimidine derivatives 12a–f

To a solution of any of **10a** (4.59 g, 0.01 mol), **10b** (4.60 g, 0.01 mol), **10c** (4.94 g, 0.01 mol), **10d** (4.54 g, 0.01 mol), **10e** (4.89 g, 0.01 mol), or **10f** (4.90 g, 0.01 mol) in 1,-dioxan (50 mL) containing sodium acetate (2.0 g) hydroxylamine hydrochloride (1.40 g, 0.02 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then poured onto ice/water and the formed solid product was collected by filtration.

10-Amino-5,5-dimethyl-2,9-diphenyl-4,5-dihydro-2*H*-[1,2,3]triazolo[4″,5″:5′,6′]benzo[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-8(9 *H*)-one oxime (12a)

Pale yellow crystals (1,4-dioxan), yield 55% (2.50 g), m.p. 173–176 °C; (IR (KBr) νmax 3570–3329, 3057, 2981, 1656, 1553; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.42 (s, 1H, D_2O exchangeable, OH), 7.46–7.28 (m, 10 H, $2C_6H_5$), 4.57 (s, 2H, D_2O exchageable, NH_2), 2.78 (s, 2H, CH_2), 1.09, 1.02 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 176.5, 175.2, 173.6, 170.2 (C-8, C-6a, C-3a, C-10c), 145.2, 144.6, 141.7, 138.2, 135.1, 130.8, 127.3, 125.2, 124.5, 123.5, 122.6, 120.4 ($2C_6H_5$, C-10, C-10a, C-5a, C-10b), 50.6 (C-4), 38.6 (C-7), 24.3 ($2CH_3$); EIMS: *m/z* 455 $[M]^+$ (20%); Analysis Calcd for $C_{24}H_{21}N_7OS$ (455.53): C, 63.28; H, 4.65; N, 21.52; S, 7.04%. Found: C, 63.52; H, 4.82; N, 21.73; S, 7.27%.

10-Hydroxy-5,5-dimethyl-2,9-diphenyl-4,5-dihydro-2*H*-[1,2,3]triazolo[4″,5″:5′,6′]-benzo[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-8(9 *H*)-one oxime (12b)

Pale yellow crystals (1,4-dioxan), yield 55% (2.50 g), m.p. 203–206 °C; (IR (KBr) νmax 3587–3338, 3054, 2986, 1652, 1553; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.28, 10.47 (2 s, 2H, D_2O exchangeable, 2OH), 7.48–7.25 (m, 10 H, $2C_6H_5$), 2.73 (s, 2H, CH_2), 1.08, 1.04 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 176.8, 175.6, 173.2, 170.8 (C-8, C-6a, C-3a, C-10c), 144.8, 143.3, 140.9, 137.8, 135.1, 132.5, 127.8, 125.5, 123.2, 122.8, 122.3, 120.8 ($2C_6H_5$, C-10, C-10a, C-5a, C-10b), 50.3 (C-4), 38.2 (C-7), 24.1 ($2CH_3$); EIMS: *m/z* 456 $[M]^+$ (28%); Analysis Calcd for $C_{24}H_{20}N_6O_2S$ (456.52): C, 63.14; H, 4.42; N, 18.41; S, 7.02%. Found: C, 63.35; H, 4.36; N, 18.60; S, 7.29%.

10-Amino-2-(4-chlorophenyl)-5,5-dimethyl-9-phenyl-4,5-dihydro-2*H*-[1,2,3]triazolo-[4″,5″:5′,6′]benzo[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-8(9 *H*)-one oxime (12c)

Yellow crystals (1,4-dioxan), yield 82% (4.40 g), m.p. 233–237 °C; (IR (KBr) νmax 3593–3352, 3057, 2986, 1653, 1547; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.45 (s, 1H, D_2O exchangeable, OH), 7.53–7.25 (m, 9 H, C_6H_5, C_6H_4), 4.54 (s, 2H, D_2O exchageable, NH_2), 2.76 (s, 2H, CH_2), 1.07, 1.03 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 176.8, 175.5, 173.2, 170.5 (C-8, C-6a, C-3a, C-10c), 146.3, 143.3, 140.9, 138.6, 133.5, 130.5, 127.0, 125.8, 124.1, 123.7, 123.2, 120.8 (C_6H_5, C_6H_4, C-10, C-10a, C-5a, C-10b), 50.8 (C-4), 38.9 (C-7), 24.2 ($2CH_3$); EIMS: *m/z* 489 $[M]^+$ (35%); Analysis Calcd for $C_{24}H_{20}ClN_7OS$ (489.98): C, 58.83; H, 4.11; N, 20.01; S, 6.54%. Found: C, 58.93; H, 4.29; N, 20.26; S, 6.80%.

2-(4-Chlorophenyl)-10-hydroxy-5,5-dimethyl-9-phenyl-4,5-dihydro-2*H*-[1,2,3]triazolo-[4″,5″:5′,6′]benzo[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-8(9 *H*)-one oxime (12d)

Orange crystals (1,4-dioxan), yield 76% (3.72 g), m.p. 155–158 °C; (IR (KBr) νmax 3565–3314, 3055, 2983, 1650, 1555; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.25, 10.49 (2 s, 2H, D_2O exchangeable, 2OH), 7.53–7.25 (m, 9 H, C_6H_5, C_6H_4), 2.76 (s, 2H, CH_2), 1.07, 1.02 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 176.7, 174.9, 173.6, 170.3 (C-8, C-6a, C-3a, C-10c), 144.5, 142.7, 140.3, 137.5, 134.8, 132.1, 128.2, 125.9, 124.2, 123.3, 121.8, 120.2 ($2C_6H_5$, C-10, C-10a, C-5a, C-10b), 50.6 (C-4), 38.5 (C-7), 24.4 ($2CH_3$); EIMS: *m/z* 490 $[M]^+$ (17%); Analysis Calcd for $C_{24}H_{19}ClN_6O_2S$ (490.96): C, 58.71; H, 3.90; N, 17.12; S, 6.53%. Found: C, 58.48; H, 4.15; N, 17.37; S, 6.72%.

10-Amino-2-(4-methoxyphenyl)-5,5-dimethyl-9-phenyl-4,5-dihydro-2*H*-[1,2,3]triazolo-[4″,5″:5′,6′]benzo[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-8(9 *H*)-one oxime (12e)

Pale brown crystals (1,4-dioxan), yield 80% (3.88 g), m.p. 266–269 °C; (IR (KBr) νmax 3574–3341, 3053, 2982, 1650, 1552; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.47 (s, 1H, D_2O exchangeable, OH), 7.56–7.22 (m, 9 H, C_6H_5, C_6H_4), 4.57 (s, 2H, D_2O exchageable, NH_2), 3.72 (s, 3 H, OCH_3), 2.73 (s, 2H, CH_2), 1.07, 1.05 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 176.3, 173.4, 172.8, 170.6 (C-8, C-6a, C-3a, C-10c), 145.8, 142.7, 141.3, 138.3, 133.2, 130.9, 126.7, 125.2, 124.8, 123.3, 122.6, 121.4 (C_6H_5, C_6H_5, C-10, C-10a, C-5a, C-10b), 52.8 (OCH_3), 50.4 (C-4), 38.3 (C-7), 24.5 ($2CH_3$); EIMS: *m/z* 485 $[M]^+$ (42%); Analysis Calcd for $C_{25}H_{23}N_7O_2S$ (485.56): C, 61.84; H, 4.77; N, 20.19; S, 6.60%. Found: C, 62.08; H, 4.85; N, 20.38; S, 6.84%.

10-Hydroxy-2-(4-methoxyphenyl)-5,5-dimethyl-9-phenyl-4,5-dihydro-2*H*-[1,2,3]triazolo-[4″,5″:5′,6′]benzo[1′,2′:4,5]thieno[2,3-*d*]pyrimidin-8(9 *H*)-one oxime (12f)

Orange crystals (1,4-dioxan), yield 69% (3.35 g), m.p. 211–214 °C; (IR (KBr) νmax 3595–3334, 3055, 2983, 1652, 1550; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.28, 10.43 (2 s, 2H, D_2O exchangeable, 2OH), 7.56–7.22 (m, 9 H, C_6H_5, C_6H_4), 2.73 (s, 2H, CH_2), 1.08, 1.04 (2 s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 176.8, 174.3, 173.4, 171.6 (C-8, C-6a, C-3a, C-10c), 143.9, 141.7, 140.6, 137.3, 134.2, 133.7, 128.0, 125.6, 124.8, 122.2, 121.5, 120.4 (C_6H_5, C_6H_4, C-10, C-10a, C-5a, C-10b), 52.8 (OCH_3), 50.9 (C-4), 38.3 (C-7), 24.2 ($2CH_3$); EIMS: m/z 486 $[M]^+$ (24%); Analysis Calcd for $C_{25}H_{22}N_6O_3S$ (486.55): C, 61.71; H, 4.56; N, 17.27; S, 6.59%. Found: C, 61.93; H, 4.80; N, 17.41; S, 6.83%.

General procedure for the synthesis of the thieno[2,3-*g*]chromene derivatives 14a-m

To a solution of any of compounds **5a** (3.24 g, 0.01 mol), **5b** (3.71 g, 0.01 mol), **5c** (3.58 g, 0.01 mol), **5d** (4.05 g, 0.01 mol), **5e** (3.54 g, 0.01 mol), or **5f** (4.01 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (0.50 mL) either of α-cyanocinnamate (1.54 g, 0.01 mol) or ethyl α-cyanocinnamate (2.01 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 6 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

2,6-Diamino-9,9-dimethyl-8-phenyl-4-(2-phenylhydrazono)-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3,7-dicarbonitrile (14a)

Yellow crystals (1,4-dioxan), yield 67% (3.20 g), m.p. 120–123 °C; IR (KBr) νmax 3468–3339, 3054, 2985, 2223, 2220, 1654, 1553; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.39 (s, 1H, D_2O exchangeable, NH), 6.13 (s, 1H, H-8), 7.41–7.26 (m, 10H, $2C_6H_5$), 4.59, 5.39 (2s, 4H, D_2O exchangeable, $2NH_2$), 1.11, 1.05 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.3 (C-4), 144.2, 143.1, 138.6, 137.8, 134.8, 132.1, 128.6, 125.8, 124.7, 123.2, 122.4, 122.9, 121.6, 120.4, 120.3, 119.6 ($2C_6H_5$, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 38.6 (C-9), 26.9 (C-8), 117.8, 166.7 (2CN), 24.4 ($2CH_3$); EIMS: *m/z* 478 $[M]^+$ (48%); Analysis Calcd for $C_{27}H_{22}N_6OS$ (478.57): C, 67.76; H, 4.63; N, 17.56; S, 6.70%. Found: C, 67.84; H, 4.75; N, 17.80; S, 6.93%.

2-Amino-6-hydroxy-9,9-dimethyl-8-phenyl-4-(2-phenylhydrazono)-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3,7-dicarbonitrile (14b)

Yellow crystals (1,4-dioxan), yield 78% (3.73 g), m.p. 187–190 °C; IR (KBr) νmax 3583–3369, 3056, 2985, 2224, 2220, 1651, 1557; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.29 (s, 1H, D_2O exchangeable, OH), 8.37 (s, 1H, D_2O exchangeable, NH), 6.11 (s, 1H, H-8), 7.44–7.23 (m, 10H, $2C_6H_5$), 4.68 (s, 2H, D_2O exchangeable, NH_2), 1.14, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.6 (C-4), 144.6, 142.9, 138.3, 137.5, 135.6, 132.4, 128.8, 127.6, 124.2, 123.6, 122.8, 122.1, 121.6, 121.3, 120.8, 120.4 ($2C_6H_5$, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 117.5, 116.9 (2CN), 38.9 (C-9), 26.9 (C-8), 24.6 ($2CH_3$); EIMS: *m/z* 479 $[M]^+$ (35%); Analysis Calcd for $C_{27}H_{21}N_5O_2S$ (479.55): C, 67.62; H, 4.41; N, 14.60; S, 6.69%. Found: C, 67.74; H, 4.63; N, 14.79; S, 6.82%.

Ethyl 2,6-diamino-7-cyano-9,9-dimethyl-8-phenyl-4-(2-phenylhydrazono)-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3-carboxylate (14c)

Pale brown crystals (1,4-dioxan), yield 72% (3.78 g), m.p. 233–236 °C; IR (KBr) νmax 3469–3369, 3050, 2982, 2220, 1653, 1550; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.35 (s, 1H, D_2O exchangeable, NH), 6.13 (s, 1H, H-8), 7.41–7.26 (m, 10H, $2C_6H_5$), 4.65, 5.28 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.22 (q, 2H, J = 6.93 Hz, $O\underline{CH_2}CH_3$), 1.15 (t, 3H, J = 6.93 Hz, $OCH_2\underline{CH_3}$), 1.13, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.6 (C-4), 144.6, 142.9, 138.3, 137.5, 135.6, 132.4, 128.8, 126.4, 124.2, 122.8, 122.3, 122.1, 121.6, 121.3, 120.8, 120.4 ($2C_6H_5$, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 117.0 (CN), 52.8 ($O\underline{CH_2}CH_3$), 38.9 (C-9), 26.9 (C-8), 24.6 ($2CH_3$), 19.2 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 525 $[M]^+$ (48%); Analysis Calcd for $C_{29}H_{27}N_5O_3S$ (525.62): C, 66.27; H, 5.18; N, 13.32; S, 6.10%. Found: C, 66.48; H, 5.25; N, 13.57; S, 6.26%.

Ethyl 2-amino-7-cyano-6-hydroxy-9,9-dimethyl-8-phenyl-4-(2-phenylhydrazono)-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3-carboxylate (14d)

Pale brown crystals (1,4-dioxan), yield 78% (4.10 g), m.p. 195–197 °C; IR (KBr) νmax 3573–3324, 3050, 2982, 2221, 1650, 1554; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.42 (s, 1H, D_2O exchangeable, OH), 8.32 (s, 1H, D_2O exchangeable, NH), 6.16 (s, 1H, H-8), 7.44–7.25 (m, 10H, $2C_6H_5$), 4.68 (s, 2H, D_2O exchangeable, NH_2), 4.21 (q, 2H, J = 5.79 Hz, $O\underline{CH_2}CH_3$), 1.15 (t, 3H, J = 5.79 Hz, $OCH_2\underline{CH_3}$), 1.15, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.2 (C-4), 143.8, 142.2, 138.6, 136.3, 135.2, 133.9, 128.3, 126.7, 125.2, 123.3, 122.4, 121.8, 121.3, 121.0, 120.4, 119.6 ($2C_6H_5$, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 116.6 (CN), 52.9 ($O\underline{CH_2}CH_3$), 38.7 (C-9), 26.4 (C-8), 24.5 ($2CH_3$), 19.6 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 526 $[M]^+$ (22%); Analysis Calcd for $C_{29}H_{26}N_4O_4S$ (526.61): C, 66.14; H, 4.98; N, 10.64; S, 6.09%. Found: C, 66.26; H, 5.18; N, 10.19; S, 6.18%.

2,6-Diamino-4-(2-(4-chlorophenyl)hydrazono)-9,9-dimethyl-8-phenyl-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3,7-dicarbonitrile (14e)

Pale orange crystals (1,4-dioxan), yield 65% (3.34 g), m.p. 186–189 °C; (IR (KBr) νmax 3439–3314, 3057, 2982, 2222, 2220, 1654, 1550; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.36 (s, 1H, D_2O exchangeable, NH), 6.12 (s, 1H, H-8), 7.49–7.22 (m, 9H, C_6H_5, C_6H_4), 4.62, 5.35 (2s, 4H, D_2O exchangeable, $2NH_2$), 1.10, 1.04 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.6 (C-4), 143.8, 143.1, 139.2, 135.2, 133.9, 132.0, 128.8, 127.3, 126.8, 125.7, 123.4, 122.1, 122.5, 121.3, 120.1, 119.5 (C_6H_5, C_6H_4, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 117.4, 116.5 (2CN), 38.7 (C-9), 26.3 (C-8), 24.4 ($2CH_3$); EIMS: *m/z* 513 $[M]^+$ (34%); Analysis Calcd for $C_{27}H_{21}ClN_6OS$ (513.01): C, 63.21; H, 4.13; N, 16.38; S, 6.25%. Found: C, 63.47; H, 4.29; N, 16.52; S, 6.41%.

2-Amino-4-(2-(4-chlorophenyl)hydrazono)-6-hydroxy-9,9-dimethyl-8-phenyl-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3,7-dicarbonitrile (14f)

Yellow crystals (1,4-dioxan), yield 80% (4.10 g), m.p. 266–270 °C; (IR (KBr) νmax 3563–3349, 3055, 2983, 2223, 2220, 1654, 1553; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.32 (s, 1H, D_2O exchangeable, OH), 8.39 (s, 1H, D_2O exchangeable, NH), 6.13 (s, 1H, H-8), 7.48–7.21 (m, 9H, C_6H_5, C_6H_4), 4.63 (s, 2H, D_2O exchangeable, NH_2), 1.12, 1.05 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.8 (C-4), 143.3, 142.2, 138.6, 136.9, 133.4, 132.8, 129.0, 127.5, 125.6, 123.1, 122.9, 122.3, 121.4, 121.0, 120.6, 120.1 (C_6H_5, C_6H_4, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 117.1, 116.7 (2CN), 38.9 (C-9), 26.7 (C-8), 24.3 ($2CH_3$); EIMS: *m/z* 514 $[M]^+$ (42%); Analysis Calcd for $C_{27}H_{20}ClN_5O_2S$ (514.00): C, 63.09; H, 3.92; N, 13.63S, 6.24%. Found: C, 62.95; H, 4.19; N, 13.58; S, 6.57%.

Ethyl 2,6-diamino-4-(2-(4-chlorophenyl)hydrazono)-7-cyano-9,9-dimethyl-8-phenyl-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3-carboxylate (14g)

Pale brown crystals (1,4-dioxan), yield 80% (4.48 g), m.p. 189–192 °C; (IR (KBr) νmax 3448–3329, 3050, 2982,

2222, 1650, 1554; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.38 (s, 1H, D_2O exchangeable, NH), 6.15 (s, 1H, H-8), 7.49–7.23 (m, 9H, C_6H_5, C_6H_4), 4.63, 5.25 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.24 (q, 2H, J = 7.19 Hz, $O\underline{CH_2}CH_3$), 1.14 (t, 3H, J = 7.19 Hz, $OCH_2\underline{CH_3}$), 1.13, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.3 (C-4), 143.3, 142.6, 138.8, 136.1, 135.8, 132.2, 128.8, 126.4, 125.3, 124.3, 123.4, 122.8, 121.1, 121.6, 120.3, 119.4 (C_6H_5, C_6H_4, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 117.0 (CN), 52.4 ($O\underline{CH_2}CH_3$), 38.5 (C-9), 26.3 (C-8), 24.3 ($2CH_3$), 19.4 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 560 $[M]^+$ (48%); Analysis Calcd for $C_{29}H_{26}ClN_5O_3S$ (560.07): C, 62.19; H, 4.68; N, 12.50; S, 5.73%. Found: C, 62.30; H, 4.72; N, 12.69; S, 5.82%.

Ethyl 2-amino-4-(2-(4-chlorophenyl)hydrazono)-7-cyano-6-hydroxy-9,9-dimethyl-8-phenyl-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3-carboxylate (14h)

Brown crystals (1,4-dioxan), yield 58% (3.25 g), m.p. 210–213 °C; (IR (KBr) νmax 3559–3346, 3050, 2980, 2220, 1650, 1550; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.44 (s, 1H, D_2O exchangeable, OH), 8.32 (s, 1H, D_2O exchangeable, NH), 6.16 (s, 1H, H-8), 7.44–7.25 (m, 9 H, C_6H_5, C_6H_4), 4.69 (s, 2H, D_2O exchangeable, NH_2), 4.26 (q, 2H, J = 5.79 Hz, $O\underline{CH_2}CH_3$), 1.13 (t, 3H, J = 5.79 Hz, $OCH_2\underline{CH_3}$), 1.17, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 178.8 (C-4), 145.2, 142.7, 138.9, 137.4, 135.8, 133.2, 126.8, 125.4, 124.7, 123.1, 123.0, 122.8, 121.6, 121.2, 120.4, 119.9 (C_6H_5, C_6H_4, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 116.8 (CN), 52.4 ($O\underline{CH_2}CH_3$), 38.9 (C-9), 26.2 (C-8), 24.5 ($2CH_3$), 19.5 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 561 $[M]^+$ (38%); Analysis Calcd for $C_{29}H_{25}ClN_4O_4S$ (561.05): C, 62.08; H, 4.49; N, 9.99; S, 5.72%. Found: C, 61.93; H, 4.73; N, 10.07; S, 5.95%.

2,6-Diamino-4-(2-(4-methoxyphenyl)hydrazono)-9,9-dimethyl-8-phenyl-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3,7-dicarbonitrile (14i)

Pale orange crystals (1,4-dioxan), yield 83% (4.21 g), m.p. 244–248 °C; (IR (KBr) νmax 3459–3316, 3053, 2982, 2225, 2220, 1652, 1550; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.39 (s, 1H, D_2O exchangeable, NH), 6.14 (s, 1H, H-8), 7.46–7.25 (m, 9H, C_6H_5, C_6H_4), 4.64, 5.32 (2s, 4H, D_2O exchangeable, $2NH_2$), 3.71 (s, 3H, OCH_3), 1.08, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.9 (C-4), 145.2, 143.6, 139.6, 135.4, 134.3, 132.6, 127.3, 126.3, 125.9, 124.7, 123.6, 122.9, 122.2, 121.8, 120.7, 120.3 (C_6H_5, C_6H_4, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 117.2, 116.9 (2CN), 52.6 (OCH_3), 38.5 (C-9), 26.5 (C-8), 24.6 ($2CH_3$); EIMS: *m/z* 508 $[M]^+$ (46%); Analysis Calcd for $C_{28}H_{24}N_6O_2S$ (508.59): C, 66.12; H, 4.76; N, 16.52; S, 6.30%. Found: C, 66.03; H, 4.68; N, 16.75; S, 6.52%.

2-Amino-6-hydroxy-4-(2-(4-methoxyphenyl)hydrazono)-9,9-dimethyl-8-phenyl-8,9-dihydro-4*H*-thieno[2,3-*g*]chromene-3,7-dicarbonitrile (14k)

Yellow crystals (1,4-dioxan), yield 77% (3.91 g), m.p. 230–234 °C; (IR (KBr) νmax 3579–3318, 3055, 2982, 2224, 2220, 1653, 1556; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.36 (s, 1H, D_2O exchangeable, OH), 8.33 (s, 1H, D_2O exchangeable, NH), 6.11 (s, 1H, H-8), 7.52–7.24 (m, 9H, C_6H_5, C_6H_4), 4.60 (s, 2H, D_2O exchangeable, NH_2), 3.69 (s, 3H OCH_3), 1.15, 1.03 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.4 (C-4), 145.1, 143.6, 137.9, 134.8, 133.5, 132.1, 127.5, 126.1, 125.9, 123.4, 122.7, 122.1, 121.7, 120.3, 120.1, 119.7 (C_6H_5, C_6H_4, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 117.3, 116.9 (2CN), 52.6 (OCH_3), 38.6 (C-9), 26.3 (C-8), 24.4 ($2CH_3$); EIMS: *m/z* 509 $[M]^+$ (28%); Analysis Calcd for $C_{28}H_{23}N_5O_3S$ (509.58): C, 66.00; H, 4.55; N, 13.74, S, 6.29%. Found: C, 65.80; H, 4.39; N, 13.83; S, 6.41%.

Ethyl 2,6-diamino-7-cyano-4-(2-(4-methoxyphenyl) hydrazono)-9,9-dimethyl-8-phenyl-8,9-dihydro-4*H*-thieno [2,3-*g*]chromene-3-carboxylate (14l)

Pale brown crystals (1,4-dioxan), yield 77% (4.27 g), m.p. 240–243 °C; (IR (KBr) νmax 3443–3329, 3055, 2982, 2222, 1653, 1550; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 8.36 (s, 1H, D_2O exchangeable, NH), 6.12 (s, 1H, H-8), 7.47–7.25 (m, 9H, C_6H_5, C_6H_4), 4.68, 5.28 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.21 (q, 2H, J = 7.36 Hz, $O\underline{CH_2}CH_3$), 3.70 (s, 3H, OCH_3), 1.12 (t, 3H, J = 7.36 Hz, $OCH_2\underline{CH_3}$), 1.09, 1.02 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 179.2 (C-4), 145.6, 143.2, 138.6, 136.6, 135.2, 133.8, 128.5, 127.3, 126.9, 124.6, 123.2, 122.7, 121.4, 121.2, 120.2, 120.0 ($2C_6H_5$, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 116.8 (CN), 52.9 ($O\underline{CH_2}CH_3$), 52.6 (OCH_3), 38.3 (C-9), 26.4 (C-8), 24.5 ($2CH_3$), 19.6 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 555 $[M]^+$ (28%); Analysis Calcd for $C_{30}H_{29}N_5O_4S$ (555.65): C, 64.85; H, 5.26; N, 12.60; S, 5.77%. Found: C, 64.94; H, 5.07; N, 12.48; S, 5.79%.

Ethyl 2-amino-7-cyano-6-hydroxy-4-(2-(4-methoxyphenyl) hydrazono)-9,9-dimethyl-8-phenyl-8,9-dihydro-4*H*-thieno [2,3-*g*]chromene-3-carboxylate (14m)

Brown crystals (1,4-dioxan), yield 78% (4.33 g), m.p. 188–191 °C; (IR (KBr) νmax 3574–3325, 3050, 2980, 2221, 1653, 1551; ^{1}H-NMR (DMSO-d_6, 200 MHz): δ = 10.48 (s, 1H, D_2O exchangeable, OH), 8.31 (s, 1H, D_2O exchangeable, NH), 6.19 (s, 1H, H-8), 7.48–7.25 (m, 9H, C_6H_5,

C_6H_4), 4.71 (s, 2H, D_2O exchangeable, NH_2), 4.23 (q, 2H, J = 6.02 Hz, $O\underline{CH_2}CH_3$), 3.68 (s, 3H, OCH_3), 1.12 (t, 3H, J = 6.02 Hz, $OCH_2\underline{CH_3}$), 1.19, 1.01 (2s, 6H, $2CH_3$); ^{13}C-NMR (DMSO-d_6, 75 MHz): δ = 178.5 (C-4), 144.8, 143.3, 138.6, 136.1, 135.4, 133.8, 127.4, 126.5, 125.2, 123.6, 123.0, 122.5, 121.8, 121.7, 120.4, 119.4 (C_6H_5, C_6H_4, C-2, C-3, C-6, C-7, C-3a, C-4a, C-8a, C-9a), 116.6 (CN), 52.3 ($O\underline{CH_2}CH_3$), 38.6 (C-9), 26.5 (C-8), 24.2 ($2CH_3$), 19.3 ($OCH_2\underline{CH_3}$); EIMS: *m/z* 556 $[M]^+$ (40%); Analysis Calcd for $C_{30}H_{28}N_4O_5S$ (556.63): C, 64.73; H, 5.07; N, 10.07; S, 5.76%. Found: C, 64.69; H, 4.95; N, 10.26; S, 5.83%.

Conclusion

In conclusion, an efficient and practical synthesis of new series of thiophene derivatives were synthesized and characterized. The c-Met kinase activity and the antiproliferative activities of compounds were evaluated against five c-Met-dependent cancer cell lines (A549, HT-29, MKN-45, U87MG, and SMMC-7721) and one c-Met-independent cancer cell line (H460) the results showed that compounds **3c, 5c, 5d, 6c, 6d, 8f, 8g, 8h, 12c, 12d**, **12f**, and **14f** were the most active compounds. The Pim-1 kinase inhibition activity of some selected compounds showed that compounds **3c**, **6c**, **8g**, **12c**, and **14f** were high potencies toward Pim-1 kinases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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