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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Uses of dimedone to synthesis pyrazole, isoxazole and thiophene derivatives with antiproliferative, tyrosine kinase and Pim-1 kinase inhibitions

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Abstract

We are aiming in this work to synthesize target molecules not only possess antitumor activities but also kinase inhibitors. The target molecules were obtained from dimedone, which reacted with triethoxymethane to produce a product that is capable for many heterocyclization reactions to give fused pyrazole, thiophene and isoxazole derivatives. Compounds **7b**, **7c**, **7d**, **9b**, **11**, **12c**, **12d**, **14b**, **16b**, **17c**, **17d**, **18c**, **18d**, and **18e** were the most cytotoxic compounds, their further tests toward the five tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR and Pim-1 kinase showed that compounds **7b**, **7d**, **11**, **12c**, **14b**, **16b**, **17d**, **18d**, and **18e** were the most potent of the tested compounds toward the five tyrosine kinases and compounds **7b**, **7d**, **14b**, **16b**, and **18e** were of the highest inhibitions toward Pim-1 kinase. PAINS the most cytotoxic compounds showed zero PAINS alert, therefore, these compounds can be used as useful drugs in the future.

Keywords Pyrazole · Isoxazole · Thiophene · Cytotoxicity · Tyrosine kinase

Introduction

Cyclohexane-1,3-dione, commonly known as dimedone, belongs to the family of cyclic 1,3-diketones class of organic compounds. A wide range of applications of cyclohexan-1,3-dione includes its use as reagent for various analytical techniques (Bodini et al. 1990; Benitez and Allison 1974). In addition, they are used as a versatile synthon for of several spiro and heterocyclic compounds (Ashry et al. 2009), such as xanthene derivatives, which have emerged as an important class of compound because of their industrial importance (Saitoh et al. 2002) and other synthetic applications (Cremlyn and Saunders 1993). The versatile chemistry (Greenhill et al. 1977; Guan-Wu and Chun-Bao 2006; James et al. 2006; Majumdar and Samanta 2001, 2002) and ready availability of cyclohexane-1,3-diones (Ilkhanizadeh et al. 2019; Bonsignore et al. 1993; Deng et al. 1999) and its derivatives make them suitable precursors for the preparation of divergent organic compounds, for example chromene derivatives, which possess anticancer, antioxidant, spasmolytic, antianaphylactic, anti-HIV and antibacterial activities (Ilkhanizadeh et al. 2019; Sonderegger et al. 2003), oxozolidinones with antibacterial activity (Cui et al. 2005; Jae-Min et al. 1997; Jo et al. 2004; Pae et al. 1999) substituted xanthene derivatives with several uses in dyes (Banerjee and Mukherjee 1981; Menchen et al. 2003), laser technology (Ahmad et al. 2002), fluorescent compounds (Knight and Stephens 1989), and more importantly, have been reported to show a variety of biological activity (Hideo and Teruomi 1981; Poupelin et al. 1978; Lambert et al. 1997). In addition, acridine and its derivatives display antimalarial, anticancer, antibacterial, and mutagenic properties, while phenylbutazones exhibit unique pharmacological uses for pain treatment associated with Tietze's syndrome and rheumatoid arthritis (Dewse and Potter 1975). On the other hand, with the increasing environmental concerns, green chemistry has attracted major scientific and commercial interest in recent years. Multicomponent one-pot reactions are an efficient and

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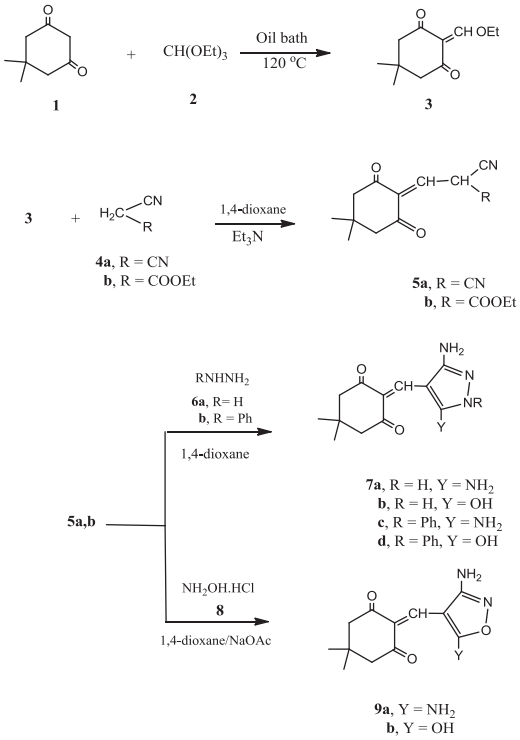
economical procedure, with wide uses in the preparation of heterocycles molecules (Ramazani and Rezaei 2010; Ramazani et al. 2011, 2012). On the other hand, organic transformation takes place in aqueous media gives a clean, environmentally safe, and cost-effective approach. Recently, Barakat et al. reported examples for MCR (one-pot fission) for example reaction of substituted cyclohexanedione with alkanal mediated by diethylamine in water (Al-Majid et al. 2013, 2017; Barakat et al. 2013, 2014a, 2014b; Al-Najjar et al. 2014).

Recently our research group reported several reactions of dimedone to produce thaizoles and hydrazone derivatives. The produced compounds showed high antiproliferative activities against cancer cell lines together with high inhibitions toward tyrosine kinases (Mohareb et al. 2019a, 2019b, 2018). This encouraged us to continue this goal through the reaction of dimedone with triethoxymethane and using the produced molecule as a suitable starting material for subsequent heterocyclization to produce a variety of fused derivatives. The antiproliferative activities of the synthesized compounds and their inhibitiory activity toward tyrosine kinases were demonstrated.



Scheme 1 Synthesis of compounds **5a**, **b**; **7a–d,** and **9a**, **b**

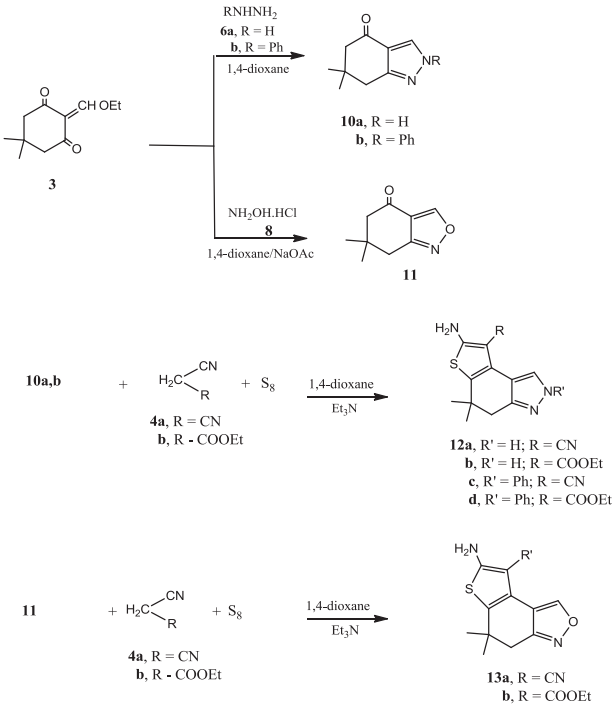
Results and discussion

The synthetic route to prepare a new class of biologically active heterocycles compounds corporated dimetone moiety starting from 2-ethoxymythyleno was discussed. The synthetic pathways employed to prepare the new target derivatives are depicted in Schemes 1–4. Thus, the reaction of dimedone (**1**) with triethoxymethane in an oil bath at 120 °C gave the 2-(ethoxymethylene)-5,5-dimethylcyclohexane-1,3-dione (**3**). Compound **3** reacted with either malononitrile (**4a**) or ethyl cyanoacetate (**4b**) to afford the alkylated products **5a** and **5b**, respectively. The structures of compounds **5a**, **b** were based on their respective analytical and spectral data (see "Experimental" section). Compounds **5a**, **b** were used to synthesize pyrazole derivatives through the reaction of either **5a** or **5b** with either hydrazine hydrate (**6a**) or phenylhydrazine (**6b**) to give the pyrazole derivatives **7a–d**, respectively. On the other hand, the reaction of either compound **5a** or **5b** with hydroxylamine hydrochloride (**8**) in 1,4-dioxane containing sodium acetate gave the isoxazole derivatives **9a** and **9b**, respectively (Scheme 1).

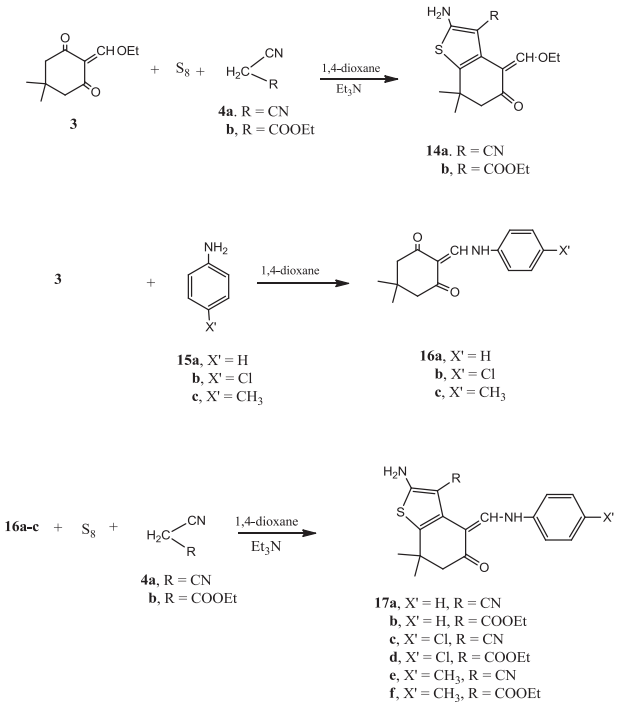
The high yields of compounds **7a–d** and **9a**, **b** encouraged us to continue such series of reactions but using 2-(ethoxymethylene)-5,5-dimethylcyclohexane-1,3-dione (**3**). Thus, the reaction of compound **3** with either hydrazine hydrate (**6a**) or phenylhydrazine (**6b**) gave the 1*H*-indazole derivatives **10a** and **10b**, respectively. On the other hand, the reaction of compound **3** with hydroxylamine hydrochloride (**8**) in 1,4-dioxane containing sodium acetate gave the isoxazole derivative **11**.

The reaction of either **10a** or **10b** with elemental sulfur and either malononitrile (**4a**) or ethyl cyanoacetate (**4b**) in 1,4-dioxane solution containing triethylamine gave the thieno[3,2-*e*]indazole derivatives **12a–d**, respectively. The chemical structures of the latter products were assured by spectral data (IR, ^{1}H, ^{13}C NMR, MS). Thus, the ^{1}H NMR spectrum of compound **12a** (as an example) showed the presence of one NH_2 group at δ 4.83 ppm (D_2O exchangeable), presence of one NH group at δ 8.29 (D_2O exchangeable) and a singlet at δ 2.36 ppm indicating one CH_2 group. In addition, the ^{13}C NMR spectrum revealed, beside the expected signals, a signal at δ 118.2 due to presence CN group, two signals at δ 136.9, 132.6 for pyrazole C-4, C-5 and four signals at δ 138.4, 135.2, 132.8, 130.2 for thiophene carbons. Similarly, the reaction of the isoxazle derivative **11** with elemental sulfur and either malononitrile (**4a**) or ethyl cyanoacetate (**4b**) in 1,4-dioxane solution containing triethylamine gave the dihydrothieno [3′,2′:3,4]benzo[1,2-*c*]isoxazole derivatives **13a** and **13b**, respectively (Scheme 2).

The reaction of compound **3** with elemental sulfur and either malononitrile (**4a**) or ethyl cyanoacetate (**4b**) in 1,4-dioxane solution containing triethylamine gave the 6,7-dihydrobenzo[*b*]thiophene derivatives **14a** and **14b**, respectively. On the other hand, compound **3** reacted with either aniline (**15a**), 4-chloroaniline (**15b**), or



Scheme 2 Synthesis of compounds **10a**, **b**; **11**; **12a–d**, and **13a**, **b**



Scheme 3 Synthesis of compounds **14a**, **b**; **16a–c**, and **17a–f**

4-methylaniline (**15c**) to produce the arylaminomethylene cyclohexane derivatives **16a–c**, respectively. With the optimized reaction conditions for thiophene formation in hand the scope of this reaction was examined. This protocol was successful for one pot formation of benzo [*b*] thiophene derivatives. Therefore, the reaction of either **16a**, **16b**, or **16c** with elemental sulfur and either malononitrile or ethyl cyanoacetate in 1,4-dioxane solution containing triethylamine gave the arylaminomethylene-6,7-dihydrobenzo[*b*] thiophene derivatives **17a–f**, respectively (Scheme 3). The analytical and spectral data of the latter product were in agreement with their respective structures. Thus, the ^{1}H NMR spectrum of compound **17a** (as an example) showed, beside the expected signals, the presence of one NH_2 group at δ 4.88 ppm (D_2O exchangeable), the presence of one NH group at δ 8.40 (D_2O exchangeable), a singlet at δ 2.35 ppm indicating one CH_2 group In addition, ^{13}C NMR showed signals at δ 138.1, 136.7, 132.4, 130.8 due to the thiophene carbons and a signal at δ 116.5 indicating the presence of CN group.

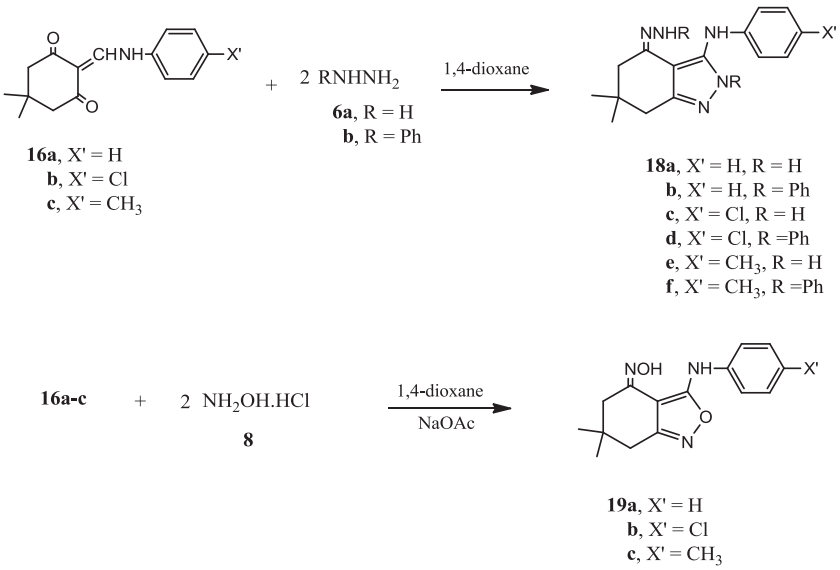
Continuing heterocyclizations of compounds were adopted through the reaction of either **16a**, **16b**, or **16c** with either hydrazine hydrate or phenylhydrazine in 1,4-dioxane, which produced the indazole **18a–f**, respectively. On the other hand, the reaction of either **16a**, **16b**, or **16c** with hydroxylamine hydrochloride (**8**) in 1,4-dioxane containing sodium acetate gave the corresponding benzo[*c*]isoxazole derivatives **19a–c**, respectively (Scheme 4). Basically, in this work we synthesized a series of fused heterocyclic compounds derived from dimedone and their evaluation through cancer cell lines gave results that can encourage continuing work in the future as these compounds can be used as synthons for the production of anticancer agents.

Biology section

Cell proliferation assay

The antiproliferative activities of the newly synthesized compounds were evaluated against the five c-Met-dependent cancer cell lines namely A549 (nonsmall cell lung cancer), HT29 (human colon cancer), MKN-45 (human gastric cancer), U87MG (human glioblastoma), SMMC-7721 (human liver cancer), and one c-Met-independent cancer cell line H460 (human lung cancer) using the standard MTT assay in vitro with foretinib as the positive control. The results expressed as IC_{50} were summarized in Table 1 and the values are the average of at least three independent experiments. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The number of assay steps has been minimized as much as possible to expedite sample processing. The MTT reagent yields low background absorbance values in the absence of cells. For each cell type the linear relationship between cell number and signal

Scheme 4 Synthesis of compounds **18a–f** and **19a–c**



produced is established, thus allowing an accurate quantification of changes in the rate of cell proliferation. The MTT reagent is ready to use and stable at 4 °C in the dark for up to 18 months, provided there is no contamination. Care should be taken not to contaminate the MTT Reagent with cell culture medium during pipetting. We recommend that the appropriate volume required for each experiment be removed and aseptically placed into a separate clean tube and the stock bottle returned to 4 °C in the dark. Plate cells at 1000–100,000 per well, incubate for 6–24 h to which add 10 µL MTT reagent incubate for 2–4 h until purple precipitate is visible then add 100 µL detergent reagent. Finally leave at room temperature in the dark for 2 h and record absorbance at 570 nm. The mean values of three independent experiments, expressed as IC_{50} values, were presented in Table 1. HTRF assay utilizes the signal generated by the fluorescence resonance energy transfer between donor and acceptor molecules in close proximity. Dual-wavelength detection helps to eliminate media interference, and the final signal is proportional to the extent of product formation. Thus far, the reported applications of this technology for in vitro kinase assays have mainly focused on high-throughput screening (HTS). The MTT assay is a colorimetric assay for assessing cell metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate. With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport. However, this traditionally assumed explanation is currently contended as proof has also been found of MTT reduction to formazan in lipidic cellular structures without apparent involvement of oxidoreductases. Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are done in the dark since the MTT reagent is sensitive to light. Within this protocol, replace serum-containing media with serum-free media and MTT reagent in cell cultures incubate for 3 h at 37 °C add MTT solvent and incubate for 15 min analyze with microplate reader.

All the synthesized compounds were tested to know their inhibitory activities against the six cancer cell lines. From the data listed in Table 1 it was noticed that about 30% of the synthesized compounds showed high cytotoxicities against the six cancer cell lines. Compounds were **7b**, **7d**, **9b**, **11**, **12c**, **12d**, **14b**, **16b**, **17c**, **17d**, **18c**, **18d**, and **18e** were the most cytotoxic compounds among the tested compounds. On the other hand, compounds **5b**, **7c**, **9a**, **12a**, **13b**, **14a**, **16c**, and **17e** showed moderate cytotoxicity against the six cancer cell lines.

Structure activity relationship

From Table 1 it is clear that many compounds of the newly synthesized products showed high cytotoxicity against the six cancer cell lines. The ethoxymethylene derivative **3** showed low inhibitions toward the six cancer cell lines. On the other hand, the alkylated products **5a** (R=CN) and **5b** (R=COOEt) revealed that compound **5b** was of moderate cytotoxicities against the six cancer cell lines but it is higher than that of compound **5a**. Considering the pyrazole derivatives **7a–d**, it is clear that **7a** (Y=NH_2 and R=H) which is of low inhibitions and compounds **7b** (Y=OH, R=H) and

Table 1 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-77217721
3	6.89 ± 1.70	8.63 ± 2.42	8.38 ± 2.08	6.49 ± 1.29	5.41 ± 1.38	6.80 ± 1.59
5a	4.23 ± 1.25	5.64 ± 1.83	6.31 ± 2.51	6.52 ± 2.46	6.19 ± 2.38	5.19 ± 1.29
5b	2.19 ± 0.86	2.82 ± 1.06	3.14 ± 1.07	2.83 ± 1.02	3.63 ± 1.27	1.59 ± 0.83
7a	6.35 ± 2.40	5.69 ± 1.39	7.28 ± 2.61	6.93 ± 2.72	6.35 ± 1.80	5.39 ± 1.68
7b	0.38 ± 0.21	0.29 ± 0.08	0.36 ± 0.20	0.38 ± 0.16	0.42 ± 0.18	0.36 ± 0.231
7c	1.62 ± 0.93	1.47 ± 0.83	0.96 ± 0.29	0.93 ± 0.41	1.83 ± 0.79	0.74 ± 0.36
7d	0.25 ± 0.16	0.25 ± 0.08	0.42 ± 0.19	0.47 ± 0.28	0.39 ± 0.26	0.73 ± 0.29
9a	1.14 ± 0.32	1.27 ± 0.68	1.38 ± 0.83	0.96 ± 0.36	1.42 ± 0.83	1.61 ± 0.73
9b	0.56 ± 0.28	0.61 ± 0.28	0.72 ± 0.25	0.36 ± 0.13	0.53 ± 0.17	0.49 ± 0.25
10a	3.42 ± 1.39	2.80 ± 1.04	3.39 ± 1.82	2.31 ± 1.26	2.94 ± 1.25	2.77 ± 1.26
10b	5.28 ± 2.31	6.36 ± 2.34	5.48 ± 1.39	6.27 ± 2.42	5.29 ± 1.63	4.09 ± 2.17
11	0.25 ± 0.06	0.29 ± 0.13	0.43 ± 0.15	0.37 ± 0.15	0.26 ± 0.12	0.52 ± 0.12
12a	1.43 ± 0.69	1.82 ± 0.96	1.95 ± 0.43	2.49 ± 1.84	1.24 ± 0.96	1.73 ± 0.49
12b	4.43 ± 1.31	4.71 ± 1.25	5.69 ± 2.38	4.93 ± 1.39	4.80 ± 2.27	2.93 ± 1.39
12c	0.43 ± 0.18	0.36 ± 0.18	0.35 ± 0.29	0.29 ± 0.18	0.52 ± 0.17	0.43 ± 0.29
12d	0.63 ± 0.31	0.72 ± 0.35	0.43 ± 0.25	0.62 ± 0.19	0.41 ± 0.26	0.83 ± 0.15
13a	4.80 ± 1.46	5.86 ± 1.39	4.69 ± 1.08	5.36 ± 1.93	6.29 ± 2.73	8.06 ± 2.53
13b	1.28 ± 0.72	1.63 ± 0.96	0.79 ± 0.19	0.83 ± 0.41	1.63 ± 0.69	0.92 ± 0.63
14a	1.62 ± 0.84	0.83 ± 0.39	1.64 ± 0.81	1.69 ± 0.80	0.83 ± 0.42	1.80 ± 0.93
14b	0.91 ± 0.52	0.41 ± 0.28	0.52 ± 0.13	1.30 ± 0.86	1.02 ± 0.85	0.63 ± 0.36
16a	4.51 ± 1.62	5.29 ± 1.28	6.48 ± 2.25	8.43 ± 2.25	6.38 ± 2.42	6.59 ± 1.73
16b	0.34 ± 0.19	0.63 ± 0.35	0.64 ± 0.65	0.41 ± 0.63	0.53 ± 0.26	0.39 ± 0.24
16c	1.40 ± 0.28	2.26 ± 1.06	2.28 ± 1.01	1.46 ± 0.85	1.25 ± 0.68	0.92 ± 0.53
17a	8.52 ± 2.68	7.39 ± 2.48	8.30 ± 3.62	9.30 ± 2.69	8.53 ± 2.80	7.36 ± 2.98
17b	6.29 ± 2.59	5.49 ± 1.73	6.92 ± 1.68	8.71 ± 2.59	6.83 ± 2.15	8.76 ± 2.41
17c	0.42 ± 0.20	0.37 ± 0.18	0.43 ± 0.16	0.29 ± 0.14	0.58 ± 0.19	0.36 ± 0.12
17d	0.32 ± 0.26	0.63 ± 0.18	0.35 ± 0.16	0.49 ± 0.25	0.46 ± 0.22	0.29 ± 0.15
17e	3.28 ± 1.19	2.73 ± 1.09	4.61 ± 1.06	1.36 ± 0.84	2.45 ± 0.69	3.26 ± 1.30
17f	6.38 ± 2.52	6.59 ± 1.36	8.64 ± 2.60	8.36 ± 1.28	7.83 ± 1.69	8.42 ± 2.65
18a	4.62 ± 1.54	3.26 ± 1.03	4.52 ± 1.41	5.36 ± 1.64	4.26 ± 1.21	5.26 ± 1.51
18b	5.34 ± 1.38	2.56 ± 1.27	4.38 ± 1.42	3.46 ± 1.26	5.28 ± 1.26	4.48 ± 1.62
18c	1.03 ± 0.84	0.36 ± 0.24	0.64 ± 0.39	0.56 ± 0.37	0.64 ± 0.15	0.48 ± 0.25
18d	0.28 ± 0.13	0.36 ± 0.13	0.29 ± 0.11	0.36 ± 0.15	0.31 ± 0.29	0.24 ± 0.13
18e	0.32 ± 0.18	0.35 ± 0.08	0.42 ± 0.24	0.43 ± 0.24	0.28 ± 0.08	0.36 ± 0.28
18f	4.29 ± 2.59	3.39 ± 1.39	5.42 ± 2.38	7.32 ± 2.42	4.74 ± 2.28	1.70 ± 0.73
19a	6.26 ± 2.68	4.53 ± 2.15	5.24 ± 2.31	4.22 ± 2.08	2.35 ± 2.31	4.28 ± 1.29
19b	9.52 ± 2.51	6.32 ± 2.82	5.18 ± 1.49	7.64 ± 2.46	5.83 ± 1.58	8.72 ± 2.60
19c	4.28 ± 1.23	5.68 ± 1.72	6.69 ± 2.17	1.38 ± 0.49	4.22 ± 1.53	6.42 ± 2.18
Foretinib	0.08 ± 0.01	0.18 ± 0.03	0.15 ± 0.023	0.03 ± 0.005	0.90 ± 0.13	0.44 ± 0.062

7d (Y=OH, R=Ph) are of high cytotoxicities while compound **7c** (Y=NH_2, R=Ph) showed moderate inhibitions.

Considering the isoxazole derivatives **9a**, **b** where compound **9b** (Y=OH) was of higher cytotoxicities than compound **9a** (Y=NH_2). On the other side, compound **9a** gave high inhibitions toward MKN-45 cell line with IC_{50} 0.92 μM. Considering the fused pyrazole derivatives **10a** and **10b**, it was obvious from Table 1 that both of them showed low inhibitions. On the other hand, the fused isoxazole derivative **11** showed high inhibitions toward the six cancer cell lines. On the other side, investigations of the thieno[3,2-*e*]indazole derivatives **12a–d**, surprisingly, compound **12a** (R=CN, R′=H) showed slight decline in the activity observed and compound **12b** (R=COOEt, R′=H)

exhibited low inhibitions. Interestingly, compounds **12c** (R=CN, R′=Ph) and **12d** (R=COOEt, R′=Ph) was found to be the most potent analog of this series toward the six cancer cell lines. Considering the dihydrothieno[3′,2′:3,4] benzo[1,2-*c*]isoxazole derivatives **13a** and **13b**, it is clear from Table 1 that compound **13a** (R=CN) showed lesser activity than compound **13b** (R=COOEt). Surprisingly, the 6,7-dihydrobenzo[*b*]thiophene derivatives **14a**, **b** showed from moderate to high inhibitions. On the other hand, for the arylaminomethylene cyclohexane derivatives **16a–c** compound **16b** (X=Cl) exhibited the highest cytotoxicities among the three compounds. The reaction of compounds **16a–c** with elemental sulfur and either malononitrile or ethyl cyanoacetate to form the 4-(arylaminomethylene)-benzo[*b*]thiophene derivatives **17a–f** where compounds **17c** (R=CN, X=Cl) and **17d** (R=COOEt, X=Cl) exhibited the highest cytotoxicities among the six compounds. For the N-aryl-tetrahydro-indazol-3-amine derivatives **18a–f**, it is clear from Table 1 that compounds **18c** (R=H, X=Cl), **18d** (R=Ph, X=Cl), and **18e** (R=H, X=Me) are of the highest cytotoxicities among the six compounds. Surprisingly, all compounds of the 6,7-dihydrobenzo[*c*]isoxazole derivatives **19a–c** exhibited noticeable decline of inhibitions toward the six cancer cell lines. It is of great value to mention that some compounds showed inhibitions higher than that of foretinib (IC_{50} 0.90 μM) against the U87MG cell line compounds **7b**, **9b**, **11**, **12c**, **12d**, **14a**, **17c**, **17d**, **18c**, **18d**, and **18e** showed higher inhibition than foretinib with IC_{50}'s 0.42, 0.39, 0.53, 0.26, 0.52, 0.53, 0.58, 0.46, 0.64, 0.31, and 0.28 μM. On the other hand, compounds **7b**, **12c**, **16b**, **17d**, **18d**, and **18e** with IC_{50}'s 0.36, 0.43, 0.39, 0.36, 0.29, and 0.24 μM, respectively, showed higher inhibitions than foretinib againat (IC_{50} 0.44 μM) the SMMC-7721 cell line.

The above activity pattern showed that that chloro group was playing a crucial role in the activity. In summary, compounds **7b**, **7c**, **7d**, **9b**, **11**, **12c**, **12d**, **14b**, **16b**, **17c**, **17d**, **18c**, **18d**, and **18e** were the most cytotoxic compounds.

Inhibition of tyrosine kinases (Enzyme IC_{50} (nM)) and normal cell line WI38

Compounds **7b**, **7c**, **7d**, **9b**, **11**, **12c**, **12d**, **14b**, **16b**, **17c**, **17d**, **18c**, **18d**, and **18e** were selected for inhibition of the five tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR and evaluation toward WI38, the normal fibroblast cells due to their high inhibitions toward the six cancer cell lines. These receptor tyrosine kinases have been implicated in vascular development by affecting the proliferation and migration of endothelial cells or pericytes. Among them, VEGF is a major regulator of tumor angiogenesis via endothelial cell proliferation and blood vessel permeability (DiSalvo et al. 1995; Senger et al. 1983). VEGF is expressed in most human cancers such as breast, kidney, and colon where patients with tumors showing elevated VEGF expression have a poor prognosis (Ferrara 2002). It clear from Table 2 that compounds **7b**, **7d**, **11**, **12c**, **14b**, **16b**, **17d**, **18d**, and **18e** were the most potent of the tested compounds toward the five tyrosin kinases. Compound **9b** showed high potency toward PDGFR kinase with IC_{50}'s 0.93 μM. In addition compound **18e** showed high inhibitions toward Flt-3, VEGFR-2, and PDGFR tyrosine kinases with IC_{50}'s 0.39, 0.59, and 0.52 μM. It is clear from Table 2 that all the tested compounds have low toxicity against the WI38, normal fibroblast cells except compound **17d** has moderate cytoxicity.

Table 2 Inhibition of tyrosine kinases (Enzyme IC_{50} (μM)) and WI38 by compounds **7b**, **7c**, **7d**, **9b**, **11**, **12c**, **12d**, **14b**, **16b**, **17c**, **17d**, **18c**, **18d**, and **18e**

Compound	c-Kit	Flt-3	VEGFR-2	EGFR	PDGFR	WI38
7b	0.28	0.32	0.28	0.61	0.43	28.21
7c	1.30	1.24	0.53	0.62	0.71	36.27
7d	0.26	0.42	0.30	0.62	0.40	44.02
9b	1.61	1.25	2.25	1.08	0.93	62.49
11	0.62	0.42	0.24	0.38	0.29	28.17
12c	0.82	0.43	0.63	0.29	0.43	34.58
12d	1.08	1.37	2.73	2.61	1.59	42.63
14b	0.36	0.52	0.41	0.68	0.39	58.70
16b	0.46	0.26	0.29	0.37	0.32	39.05
17c	1.03	0.24	0.53	1.28	1.22	23.41
17d	0.16	0.28	0.17	0.29	0.34	2.80
18c	1.09	2.85	1.41	1.38	2.77	33.72
18d	0.26	0.45	0.19	0.35	0.28	42.83
18e	1.42	0.39	0.59	1.60	0.52	38.52

Inhibition of selected compounds toward Pim-1 kinase

Furthermore, compounds **7b**, **7d**, **11**, **12c**, **14b**, **16b**, **17d**, **18d**, and **18e** were selected to examine their Pim-1 kinase inhibition activity (Table 3) as these compounds showed high inhibition toward the tested cancer cell lines at a range of ten concentrations and the IC_{50} values were calculated. Compounds **7b**, **7d**, **14b**, **16b**, and **18e** were the most potent to inhibit Pim-1 kinase with IC_{50} value of 0.26, 0.23, 0.21, 0.28, and 0.28 μM, respectively. On the other hand, compounds **11**, **12c**, **17d**, and **18d** were of decline potencies ($IC_{50} > 10$ μM). Through this assay, SGI-1776 was used as positive control with IC_{50} 0.048 μM. These profiles in combination with cell growth inhibition data of compounds **7b**, **7d**, **11**, **12c**, **14b**, **16b**, **17d**, **18d**, and **18e** were listed in Table 3 indicated that Pim-1 was a potential target of these compounds.

Table 3 The inhibitor activity of compounds **7b**, **7d**, **11**, **12c**, **14b**, **16b**, **17d**, **18d**, and **18e** toward Pim-1 kinase

Compound	Inhibition ratio At 10 μM	IC_{50} (μM)
7b	90	0.26
7d	92	0.23
11	24	>10
12c	20	>10
14b	94	0.21
16b	88	0.28
17d	26	>10
18d	19	>10
18e	88	0.28
SGI-1776	–	0.048

Pan Assay Interference Compounds (PAINS)

Successful drug discovery process involves HTS. During HTS it is predicted that successes from HTS operations include primarily false positives between the actual hits if it is found (Jonathan and Georgina 2010; McGovern 2002). Compounds can be regarded as false positives due to a number of reasons like binding interactions by forming aggregates (McGovern and Shoichet 2003; Feng and Shoichet 2006; Feng et al. 2005) by being protein-reactive entities (Metz et al. 2007; Huth et al. 2007; Jonathan and Michael 2014) or by directly interfering with assay signaling. PAINS are chemical entities that are frequently false positive in HTS. PAINS have a tendency to nonspecifically react with several biological targets moderately, then specifically disturbing one preferred target (Jayme and Michael 2014). A number of disorderly functional groups are collected by numerous PAINS (Martin and Stephane 2016). Unwanted compounds may negatively influence not only enzyme assays but also phenotypic screens and show biological activity for the wrong reason (Cheng and Li 2012). PAINS violations of proposed compounds and reference drugs are given in Table 4. Almost all the compounds showed zero PAINS alert and thus can be used in the future as good antitumor agents.

Experimental

Dry solvents were used through this work and all melting points of the synthesized compounds were recorded on Buchi melting point apparatus D-545. The IR spectra (KBr disks) were recorded on Bruker Vector 22 instrument. ^{13}C NMR and ^{1}H NMR spectra were measured on Bruker DPX200 instrument in DMSO-d_6 with TMS as internal standard. Mass spectra were measured using EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were measured using the Micro-Analytical Data center at Cairo University. All reactions were monitored by TLC on 2 × 5 cm precoated silica gel 60 F254 plates of thickness of 0.25 mm (Merck) for getting complete reactions

Table 4 PAINS evaluation of selected compounds using standard drugs foretinib and SGI-1776

Compound	Drug likeness 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. of vio	PAINS alert[d]
7b	None	None	None	None	0
7c	None	None	None	None	0
7d	None	None	None	None	1
9b	None	None	None	1	0
11	None	None	None	3	1
12c	None	None	None	None	0
12d	None	None	None	3	0
14b	None	None	None	2	1
16b	None	None	None	1	0
17c	None	None	None	3	0
17d	None	None	None	None	0
18c	None	None	None	None	1
18d	None	None	None	1	0
18e	None	None	None	2	1
Foretinib	None	None	None	None	0
SGI-1776	None	None	None	None	0

[a]*Lvio.* Lipinski's rule

[b]*Vvio.* Veber rules

[c]*Gvio.* Ghose filter

[d]*PAINS* Pan Assay Interference Compounds Analysis

General procedure for the synthesis of 2-(ethoxymethylene)-5,5-dimethylcyclohexane-1, 3-dione (3)

To a dry solid of dimedone (1.40 g, 0.01 mol) triethoxymethane (1.48 g, 0.01 mol) was added. The reaction mixture was heated in an oil bath at 120 °C for 1 h then left to cool. The solidified product was triturated with diethyl ether and the participated crystals were collected by filtration.

White crystals from ethanol; m.p. 215–217 °C; yield 72%. IR (KBr) cm^{-1}: 3055, 2986, 1689, 1688, 1632. ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.63 (s, 1H, CH), 3.46 (q, 2H, $J = 6.35$ Hz, CH_2), 2.31, 2.28 (2s, 4H, $2CH_2$), 1.12 (t, 3H, $J = 6.35$ Hz, CH_2), 1.09, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 166.6$, 164.4 (C-1, C-3), 108.8, 103.5 (CH=C), 58.4 ($O\underline{CH_2}CH_3$), 50.6 (C-4), 36.2 (C-5), 28.6 (C-4), 24.4 ($2CH_3$), 16.2 ($OCH_2\underline{CH_3}$); EIMS:

m/z 196 $[M]^+$ (32%); analysis Calcd for $C_{11}H_{16}O_3$ (196.24): C, 67.32; H, 8.22%. Found: C, 67.27; H, 8.36%.

General procedure for the synthesis of the 2,6-dioxocyclohexylidene derivatives 5a, b

To a solution of compound **3** (1.96 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1 h then left to cool and the formed solid product was collected by filtration, dried and crystallized from ethanol to give **5a**, **b**, respectively.

2-((4,4-Dimethyl-2,6-dioxocyclohexylidene)methyl) malononitrile (5a)

Yellow crystals from ethanol; m.p. 233–236 °C; yield 62%. IR (KBr) cm^{-1}: 3055, 2985, 2223, 2220, 1690, 1688, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 4.92, 6.61 (2d, 2H, CH=CH), 2.36, 2.23 (2s, 4H, $2CH_2$), 1.08, 1.04 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 166.6$ (C-1), 116.8, 117.0 (2CN), 109.7, 60.8 (CH=CH), 56.6 (C-2), 36.5 (C-5), 28.8 (C-4), 24.8 ($2CH_3$); EIMS: m/z 216 $[M]^+$ (40%); analysis Calcd for $C_{12}H_{12}N_2O_2$ (216.24): C, 66.65; H, 5.59; N, 12.96%. Found: C, 66.72; H, 5.45; N, 13.16%.

Ethyl 2-cyano-3-(4,4-dimethyl-2, 6-dioxocyclohexylidene)propanoate (5b)

Yellow crystals from ethanol; m.p. 247–250 °C; yield 73%. IR (KBr) cm^{-1}: 3054, 2983, 2220, 1705–1687, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 4.90, 6.63 (2d, 2H, CH=CH), 4.22 (q, 2H, $J = 7.25$ Hz, $O\underline{CH_2}CH_3$), 2.38, 2.21 (2s, 4H, $2CH_2$), 1.13 (t, 3H, $J = 7.23$ Hz, $OCH_2\underline{CH_3}$), 1.09, 1.03 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta =$ 166.8 (C-1), 164.6, 163.8 (C-3, ester CO), 116.8 (CN), 109.6, 60.5 (CH=CH), 56.8 (C-2), 52.3 ($O\underline{CH_2}CH_3$), 36.3 (C-5), 28.6 (C-4), 24.7 ($2CH_3$), 16.5 ($OCH_2\underline{CH_3}$); EIMS: m/z 263$[M]^+$ (32%); analysis Calcd for $C_{14}H_{17}NO_4$ (263.29): C, 63.87; H, 6.51; N, 5.32%. Found: C, 64.02; H, 6.39; N, 5.52%.

General procedure for the synthesis of the pyrazole derivatives 7a–d

To a solution of either **5a** (2.16 g, 0.01 mol), **5b** (2.63 g, 0.01 mol) in ethanol (40 mL) either of hydrazine hydrate (0.50 mL, 0.01 mol) or phenylhydrazine (1.80 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then was left to cool and the formed solid product was collected by filtration upon pouring onto ice/water mixture containing a few drops of hydrochloric acid.

2-((3,5-Diamino-1*H*-pyrazol-4-yl)methylene)-5, 5-dimethylcyclohexane-1,3-dione (7a)

Orange crystals from 1,4-dioxane; m.p. 270–273 °C; yield 68%. IR (KBr) cm^{-1}: 3489–3341, 3055, 2985, 1690, 1687, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 8.26 (s, 1H, D_2O exchangeable, NH), 6.68 (s, 1H, CH), 4.87, 5.21 (2s, 4H, D_2O exchangeable, $2NH_2$), 2.36, 2.24 (2s, 4H, $2CH_2$), 1.07, 1.03 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta =$ 171.8 (C=N), 166.8 (C-1), 164.3 (C-3), 136.0, 132.4 (pyrazole C-4, C-5), 108.2 ($\underline{CH}$=C), 56.8 (C-2), 36.3 (C-5), 28.5 (C-4), 24.8 ($2CH_3$); EIMS: m/z 248 $[M]^+$ (32%); analysis Calcd for $C_{12}H_{16}N_4O_2$ (248.28): C, 58.05; H, 6.50; N, 22.57%. Found: C, 57.86; H, 6.37; N, 22.38%.

2-((5-Amino-3-hydroxy-1*H*-pyrazol-4-yl)methylene)-5,5-dimethylcyclohexane-1,3-dione (7b)

Reddish brown crystals from 1,4-dioxane; m.p. 237–240 °C; yield 70%. IR (KBr) cm^{-1}: 3548–3327, 3055, 2984, 1693, 1687, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 10.31 (s, 1H, D_2O exchangeable, OH), 8.28 (s, 1H, D_2O exchangeable, NH), 6.67 (s, 1H, CH), 4.73 (s, 2H, D_2O exchangeable, NH_2), 2.36, 2.26 (2s, 4H, $2CH_2$), 1.08, 1.03 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 171.6$ (C=N), 166.5 (C-1), 164.1 (C-3), 136.0, 132.6 (pyrazole C-4, C-5), 108.4 ($\underline{CH}$=C), 56.6 (C-2), 36.3 (C-5), 28.4 (C-4), 24.8 ($2CH_3$); EIMS: m/z 249 $[M]^+$ (36%); analysis Calcd for $C_{12}H_{15}N_3O_3$ (249.27): C, 57.82; H, 6.07; N, 16.86%. Found: C, 57.62; H, 6.27; N, 16.73%.

2-((3,5-Diamino-1-phenyl-1*H*-pyrazol-4-yl) methylene)-5,5-dimethylcyclo-hexane-1,3-dione (7c)

Red crystals from 1,4-dioxane; m.p. 215–217 °C; yield 72%. IR (KBr) cm^{-1}: 3474–3362, 3055, 2985, 1689, 1687, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 7.28–7.41 (m, 5H, C_6H_5), 6.64 (s, 1H, CH), 4.89, 5.24 (2s, 4H, D_2O exchangeable, $2NH_2$), 2.38, 2.23 (2s, 4H, $2CH_2$), 1.08, 1.02 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 171.6$ (C=N), 166.7 (C-1), 164.6 (C-3), 136.3, 132.6 (pyrazole C-4, C-5), 127.8, 125.4, 122.8, 120.4 (C_6H_5), 108.8 ($\underline{CH}$=C), 56.8 (C-2), 36.2 (C-5), 28.8 (C-4), 24.6 ($2CH_3$); EIMS: m/z 324 $[M]^+$ (38%); analysis Calcd for $C_{18}H_{20}N_4O_2$ (324.38): C, 66.65; H, 6.21; N, 17.27%. Found: C, 66.80; H, 6.36; N, 17.42%.

2-((5-Amino-3-hydroxy-1-phenyl-1H-pyrazol-4-yl) methylene)-5,5-dimethylcyclo-hexane-1,3-dione (7d)

Orange crystals from ethanol; m.p. 257–259 °C; yield 78%. IR (KBr) cm^{-1}: 3483-3352, 3055, 2983, 1688, 1687, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 10.24 (s, 1H, D_2O exchangeable, OH), 7.25–7.43 (m, 5H, C_6H_5), 6.66 (s, 1H,

CH), 4.86 (s, 2H, D_2O exchangeable, NH_2), 2.39, 2.25 (2s, 4H, $2CH_2$), 1.08, 1.02 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 171.8$ (C=N), 166.4 (C-1), 164.8 (C-3), 136.7, 132.2 (pyrazole C-4, C-5), 126.9, 125.1, 123.5, 120.2 (C_6H_5), 108.6 (CH=C), 56.6 (C-2), 36.4 (C-5), 28.6 (C-4), 24.8 ($2CH_3$); EIMS: m/z 325 $[M]^+$ (26%); analysis Calcd for $C_{18}H_{19}N_3O_3$ (325.36): C, 66.45; H, 5.89; N, 12.91%. Found: C, 66.63; H, 6.15; N, 12.68%.

General procedure for the synthesis of the isoxazole derivatives 9a, b

To a solution of either compound **5a** (2.16 g, 0.01 mol), **5b** (2.63 g, 0.01 mol) in ethanol (40 mL) containing sodium acetate (1.00 g) hydroxylamine hydrochloride (0.78 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water and the formed solid product was collected by filtration.

2-((3,5-Diaminoisoxazol-4-yl)methylene)-5, 5-dimethylcyclohexane-1,3-dione (9a)

White crystals from 1,4-dioxane; m.p. 270–273 °C; yield 75%. IR (KBr) cm^{-1}: 3487–3328, 3055, 2985, 1690, 1688, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 6.66 (s, 1H, CH), 4.89, 5.23 (2s, 4H, D_2O exchangeable, $2NH_2$), 2.37, 2.23 (2s, 4H, $2CH_2$), 1.08, 1.05 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 171.4$ (C=N), 166.5 (C-1), 164.5 (C-3), 132.6, 136.3 (isoxazole C-4, C-5), 108.6 (CH=C), 56.5 (C-2), 28.9 (C-4), 36.5 (C-5), 24.7 ($2CH_3$); EIMS: m/z 249 $[M]^+$ (42%); analysis Calcd for $C_{12}H_{15}N_3O_3$ (249.27): C, 57.82; H, 6.07; N, 16.86%. Found: C, 57.63; H, 6.15; N, 16.69%.

2-((5-Amino-3-hydroxyisoxazol-4-yl)methylene)-5, 5-dimethylcyclohexane-1,3-dione (9b)

White crystals from 1,4-dioxane; m.p. 240–243 °C; yield 78%. IR (KBr) cm^{-1}: 3567–3342, 3052, 2987, 1692, 1688, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 10.30 (s, 1H, D_2O exchangeable, OH), 6.66 (s, 1H, CH), 4.86 (s, 2H, D_2O exchangeable, NH_2), 2.37, 2.21 (2s, 4H, $2CH_2$), 1.08, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 171.8$ (C=N), 166.3 (C-1), 164.2 (C-3), 132.8, 136.1 (isoxazole C-4, C-5), 108.3 (CH=C), 56.7 (C-2), 36.3 (C-5), 28.6 (C-4), 24.8 ($2CH_3$); EIMS: m/z 250 $[M]^+$ (36%); analysis Calcd for $C_{12}H_{14}N_2O_4$ (250.25): C, 57.59; H, 5.64; N, 11.19%. Found: C, 57.73; H, 5.72; N, 11.30%.

General procedure for the synthesis of the indazole derivatives 10a, b

Either of hydrazine hydrate (0.1 mL, 0.02 mol) or phenylhydrazine (3.16 g, 0.02 mol) was added to a solution of compound **3** (2.63 g, 0.01 mol) in ethanol (40 mL). The reaction mixture, in each case, was heated under reflux for 3 h then left to cool and the formed solid product was collected by filtration upon pouring onto ice/water mixture containing a few drops of hydrochloric acid.

6,6-Dimethyl-6,7-dihydro-2H-indazol-4(5H)-one (10a)

Brown crystals from 1,4-dioxane; m.p. 160–163 °C; yield 60%. IR (KBr) cm^{-1}: 3462–3359, 3052, 2985, 1688, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 8.26 (s, 1H, D_2O exchangeable, NH), 6.73 (s, 1H, pyrazole H-3), 2.39, 2.27 (2s, 4H, $2CH_2$), 1.09, 1.03 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.6$, 172.5 (2C=N), 165.8 (C-4), 136.6, 132.2 (pyrazole C-4, C-5), 56.8 (C-2), 36.3 (C-6), 28.6 (C-7), 24.8 ($2CH_3$); EIMS: m/z 164 $[M]^+$ (36%); analysis Calcd for $C_9H_{12}N_2O$ (164.20): C, 65.83; H, 7.37; N, 17.06%. Found: C, 65.69; H, 715; N, 17.28%.

6,6-Dimethyl-2-phenyl-6,7-dihydro-2H-indazol-4 (5H)-one (10b)

Pale yellow crystals from 1,4-dioxane; m.p. 98–100 °C; yield 67%. IR (KBr) cm^{-1}: 2984, 1688, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 7.26–7.38 (m, 5H, C_6H_5), 6.74 (s, 1H, pyrazole H-3), 2.37, 2.23 (2s, 4H, $2CH_2$), 1.09, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.5$, 172.8 (2C=N), 165.5 (C-4), 136.3, 132.5 (pyrazole C-4, C-5), 128.4, 126.2, 125.3, 123.6, 121.2 (C_6H_5), 56.7 (C-2), 36.6 (C-6), 28.3 (C-7), 24.8 ($2CH_3$); EIMS: m/z 240 $[M]^+$ (24%); analysis Calcd for $C_{15}H_{16}N_2O$ (240.30): C, 74.97; H, 6.71; N, 11.66%. Found: C, 74.83; H, 6.48; N, 11.80%.

6,6-Dimethyl-6,7-dihydrobenzo[c]isoxazol-4(5H)-one (11)

Hydroxylamine hydrochloride (1.40 g, 0.01 mol) was added to a solution of compound **3** (1.93 g, 0.01 mol) in 1,4-dioxane (40 mL) containing sodium acetate (1.00 g). The reaction mixture was heated under reflux for 2 h then poured onto ice/water and the formed solid product was collected by filtration.

Pale yellow crystals from 1,4-dioxane; m.p. 207–210 °C; yield 68%. IR (KBr) cm^{-1}: 3054, 2985, 1688, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 7.03 (s, 1H, isoxazole H-5), 2.37, 2.25 (2s, 4H, $2CH_2$), 1.09, 1.03 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta =$ 176.3 (C=N), 166.4 (C-4), 138.3, 133.1 (isoxazole C-4, C-5), 56.8 (C-2), 36.3 (C-6), 32.5 (C-5), 28.6 (C-7), 24.8 ($2CH_3$); EIMS: m/z 165 $[M]^+$ (28%); analysis Calcd for $C_9H_{11}NO_2$ (165.19): C, 65.44; H, 6.71; N, 8.48%. Found: C, 65.73; H, 6.54; N, 8.69%.

General procedure for the synthesis of the thieno [3,2-e]indazole derivatives 12a–d

Each of elemental sulfur and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) were added to a solution of either **10a** (1.49 g, 0.01 mol) or **10b** (2.40 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture, in each case, was heated under reflux for 2 h then left to cool. The formed solid product was collected by filtration, dried and crystallized from ethanol to give **12a–d**, respectively.

7-Amino-5,5-dimethyl-4,5-dihydro-2H-thieno[3,2-e] indazole-8-carbonitrile (12a)

Orange crystals from 1,4-dioxane; m.p. 200–204 °C; yield 72%. IR (KBr) cm^{-1}: 3489–3337, 3054, 2985, 2220, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 8.29 (s, 1H, D_2O exchangeable, NH), 6.65 (s, 1H, pyrazole H-3), 5.21 (s, 2H, D_2O exchangeable, NH_2), 2.36 (s, 2H, CH_2), 1.09, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.8$ (C=N), 118.2 (CN), 136.9, 132.6 (pyrazole C-4, C-5), 138.4, 135.2, 132.8, 130.2 (thiophene C), 36.6 (C-4), 28.6 (C-7), 24.8 ($2CH_3$); EIMS: m/z 244 $[M]^+$ (48%); analysis Calcd for $C_{12}H_{12}N_4S$ (244.32): C, 58.99; H, 4.95; N, 22.93; S, 13.12%. Found: C, 59.76; H, 5.19; N, 23.26; S, 13.26%.

Ethyl 7-amino-5,5-dimethyl-4,5-dihydro-2*H*-thieno [3,2-*e*]indazole-8-carboxylate (12b)

Orange crystals from 1,4-dioxane; m.p. > 300 °C; yield 78%. IR (KBr) cm^{-1}: 3474–3348, 3054, 2985, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 8.32 (s, 1H, D_2O exchangeable, NH), 6.68 (s, 1H, pyrazole H-3), 4.82 (s, 2H, D_2O exchangeable, NH_2), 4.22 (q, 2H, $J = 7.26$ Hz, $O\underline{CH_2}CH_3$), 2.38 (s, 2H, CH_2), 1.12 (t, 3H, $J = 7.26$ Hz, $OCH_2\underline{CH_3}$), 1.09, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 174.3$ (C=N), 136.6, 132.4 (pyrazole C-4, C-5), 138.4, 135.2, 132.8, 130.2 (thiophene C), 52.3 ($O\underline{CH_2}CH_3$), 36.6 (C-4), 28.6 (C-7), 24.8 ($2CH_3$), 16.2 ($OCH_2\underline{CH_3}$); EIMS: m/z 291 $[M]^+$ (28%); analysis Calcd for $C_{14}H_{17}N_3O_2S$ (291.37): C, 57.71; H, 5.88; N, 14.42; S, 11.00%. Found: C, 57.98; H, 5.73; N, 14.59; S, 10.83%.

7-Amino-5,5-dimethyl-2-phenyl-4,5-dihydro-2H-thieno[3,2-e]indazole-8-carbonitrile (12c)

Orange crystals from 1,4-dioxane; m.p. 76–78 °C; yield 75%. IR (KBr) cm^{-1}: 3492-3332, 3054, 2985, 2220, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 7.43–7.26 (m, 5H, C_6H_5), 6.68 (s, 1H, pyrazole H-3), 4.82 (s, 2H, D_2O exchangeable, NH_2), 2.38 (s, 2H, CH_2), 1.09, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 174.3$ (C=N), 118.3 (CN), 136.6, 132.4 (pyrazole C-4, C-5), 138.4, 135.2, 132.8, 130.2 (thiophene C), 128.3, 127.4, 125.4, 122.1 (C_6H_5), 36.8 (C-4), 28.3 (C-7), 24.5 ($2CH_3$); EIMS: m/z 320 $[M]^+$ (38%); analysis Calcd for $C_{18}H_{16}N_4S$ (320.41): C, 67.47; H, 5.03; N, 17.49; S, 10.01%. Found: C, 67.68; H, 5.25; N, 17.68; S, 10.25%.

Ethyl 7-amino-5,5-dimethyl-2-phenyl-4,5-dihydro-2H-thieno[3,2-e]indazole-8-carboxylate (12d)

Orange crystals from 1,4-dioxane; m.p. 86–88 °C; yield 66%. IR (KBr) cm^{-1}: 3483–3326, 3053, 2985, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 7.42–7.25 (m, 5H, C_6H_5), 6.66 (s, 1H, pyrazole H-3), 4.80 (s, 2H, D_2O exchangeable, NH_2), 4.23 (q, 2H, $J = 6.56$ Hz, $O\underline{CH_2}CH_3$), 2.37 (s, 2H, CH_2), 1.12 (t, 3H, $J = 6.56$ Hz, $OCH_2\underline{CH_3}$), 1.09, 1.07 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 174.6$ (C=N), 136.9, 132.4 (pyrazole C-4, C-5), 138.8, 135.6, 132.2, 130.5 (thiophene C), 127.3, 125.6, 124.1, 122.0 (C_6H_5), 52.6 ($O\underline{CH_2}CH_3$), 36.9 (C-4), 28.4 (C-7), 24.6 ($2CH_3$), 16.2 ($OCH_2\underline{CH_3}$); EIMS: m/z 367 $[M]^+$ (48%); analysis Calcd for $C_{20}H_{21}N_3O_2S$ (367.46): C, 65.37; H, 5.76; N, 11.44; S, 8.73%. Found: C, 65.48; H, 5.49; N, 11.59; S, 9.02%.

General procedure for the synthesis of the dihydrothieno[3′,2′:3,4] benzo[1,2-*c*]isoxazol-derivatives 13a, b

To a solution of compound **11** (1.65 g, 0.01 mol) in 1,4-dioxan (40 mL) containing triethylamine (0.50 mL) each of elemental sulfur and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) were added. The reaction mixture was heated under reflux for 2 h then left to cool. The formed solid product was collected by filtration, dried and crystallized from ethanol to give **13a**, **b**, respectively.

7-Amino-5,5-dimethyl-4,5-dihydrothieno[3′,2′:3,4] benzo[1,2-*c*]isoxazole-8-carbonitrile (13a)

Pale yellow crystals from 1,4-dioxane; m.p. 170–173 °C; yield 70%. IR (KBr) cm^{-1}: 3447–3328, 3054, 2985, 2220, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 6.67 (s, 1H, isoxazole H-5), 4.90 (s, 1H, D_2O exchangeable, NH_2), 2.38 (s, 2H, CH_2), 1.09, 1.07 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.5$ (C=N), 116.4 (CN), 138.3, 133.2 (isoxazole C-4, C-5), 140.1, 138.1, 136.4, 134.2, 131.6, 130.0 (thiophene, isoxazole C), 36.6 (C-4), 28.4 (C-7), 24.8 ($2CH_3$); EIMS: m/z 245 $[M]^+$ (36%); analysis Calcd for $C_{12}H_{11}N_3OS$ (245.30): C, 58.76; H, 4.52; N, 17.13; S, 13.07%. Found: C, 58.93; H, 4.66; N, 17.29; S, 13.38%.

Ethyl 7-amino-5,5-dimethyl-4,5-dihydrothieno [3′,2′:3,4]benzo[1,2-*c*]isoxazole-8-carboxylate (13b)

Orange crystals from 1,4-dioxane; m.p.180–182 °C; yield 60%. IR (KBr) cm^{-1}: 3474-3352, 3055, 2985, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 6.68 (s, 1H, isoxazole H-5), 4.85 (s, 2H, D_2O exchangeable, NH_2), 4.21 (q, 2H, $J = 6.30$ Hz, $O\underline{CH_2}CH_3$), 2.39 (s, 2H, CH_2), 1.13 (t, 3H, $J = 6.30$ Hz, $OCH_2\underline{CH_3}$), 1.09, 1.08 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 174.8$ (C=N), 136.5, 132.6 (pyrazole C-4, C-5), 138.9, 137.3, 134.5, 130.2 (thiophene C), 52.3 ($O\underline{CH_2}CH_3$), 36.7 (C-4), 28.6 (C-7), 24.7 ($2CH_3$), 16.1 ($OCH_2\underline{CH_3}$); EIMS: m/z 292 $[M]^+$ (28%); analysis Calcd for $C_{14}H_{16}N_2O_3S$ (292.35): C, 57.52; H, 5.52; N, 9.58; S, 10.97%. Found: C, 57.47; H, 5.63; N, 9.80; S, 9.68%.

General procedure for the synthesis of the 6, 7-dihydrobenzo[*b*]thiophene derivatives 14a, b

Each of elemental sulfur and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) were added to a solution of compound **3** (1.96 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 2 h then left to cool. The formed solid product was collected by filtration, dried and crystallized from ethanol to give **14a**, **b**, respectively.

2-Amino-4-(ethoxymethylene)-7,7-dimethyl-5-oxo-4,5,6,7-tetrahydrobenzo-[*b*]-thiophene-3-carbonitrile (14a)

Yellow crystals from 1,4-dioxane; m.p. 166–168 °C; yield 65%. IR (KBr) cm^{-1}: 3473–3341, 3054, 2985, 2220, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ6.59 (s, 1H, CH=C), 4.92 (s, 2H, D_2O exchangeable, NH_2), 3.89 (q, 2H, $J = 7.01$ Hz, CH_2), 2.36 (s, 2H, CH_2), 1.13 (t, 3H, $J = 7.01$ Hz, CH_3), 1.09, 1.07 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 116.8$ (CN), 138.1,134.2, 131.6, 130.0 (thiophene C), 52.6 ($O\underline{CH_2}CH_3$), 36.9 (C-4), 28.4 (C-7), 24.8 ($2CH_3$), 16.0 ($OCH_2\underline{CH_3}$); EIMS: m/z 276 $[M]^+$ (24%); analysis Calcd for $C_{14}H_{16}N_2O_2S$ (276.35): C, 60.85; H, 5.84; N, 10.14; S, 11.60%. Found: C, 60.94; H, 5.71; N, 10.26; S, 11.83%.

Ethyl 2-amino-4-(ethoxymethylene)-7,7-dimethyl-5-oxo-4,5,6,7-tetrahydro-benzo[*b*]-thiophene-3-carboxylate (14b)

Orange crystals from 1,4-dioxan; m.p. 225–228 °C; yield 77%. IR (KBr) cm^{-1}: 3496–3331, 3054, 2985, 1630. ^1H NMR (300 MHz, DMSO-d_6) δ 6.65 (s, 1H, CH=C), 4.87 (s, 2H, D_2O exchangeable, NH_2), 4.21, 3.87 (q, 4H, $J = 6.93$, 5.42 Hz, two $O\underline{CH_2}CH_3$), 2.34 (s, 2H, CH_2), 1.11, 1.14 (2t, 6H, $J = 6.93$, 5.42 Hz, two $OCH_2\underline{CH_3}$), 1.09, 1.08 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 138.9$,136.7, 133.2, 130.4 (thiophene C), 51.6, 52.3 (two OCH_2CH_3), 36.7 (C-4), 28.8 (C-7), 24.8 ($2CH_3$), 16.2, 16.3 (two OCH_2CH_3); EIMS: m/z 323 $[M]^+$ (40%); analysis Calcd for $C_{16}H_{21}NO_4S$ (323.41): C, 59.42; H, 6.54; N, 4.33; S, 9.91%. Found: C, 59.58; H, 6.80; N, 4.52; S, 10.16%.

General procedure of the 2-((phenylamino) methylene)cyclohexane derivatives 16a–c

To a solution of compound **3** in 1,4-dioxane (40 mL) either aniline (0.93 g, 0.01 mol), 4-chloroaniline (1.27 g, 0.01 mol) or 4-methylaniline (1.07 g, 0.01 mol) was added. The reaction was heated under reflux for 3 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the precipitated solid product was collected by filtration.

5,5-Dimethyl-2-((phenylamino)methylene) cyclohexane-1,3-dione (16a)

Orange crystals from ethanol; m.p. 165–167 °C; yield 78%. IR (KBr) cm^{-1}: 3480–3226, 3055, 2986, 1689, 1688, 1632. ^1H NMR (200 MHz, DMSO-d_6) δ 8.40 (s, 1H, D_2O exchangeable, NH), 7.28–7.39 (m, 5H, C_6H_5), 6.65 (s, 1H, CH), 2.36, 2.28 (2s, 4H, $2CH_2$), 1.09, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 166.8$, 164.5 (C-1, C-3), 128.6, 127.3, 125.0, 122.3 (C_6H_5), 108.4, 103.5 (CH=C), 50.6 (C-4), 36.2 (C-5), 28.8 (C-4), 24.5 ($2CH_3$); EIMS: m/z 243 $[M]^+$ (38%); analysis Calcd for $C_{15}H_{17}NO_2$ (243.30): C, 74.05; H, 7.04; N, 5.76%. Found: C, 73.84; H, 7.32; N, 5.90%.

2-(((4-Chlorophenyl)amino)methylene)-5, 5-dimethylcyclohexane-1,3-dione (16b)

Orange crystals from ethanol; m.p. 185–188 °C; yield 65%. IR (KBr) cm^{-1}: 3472–3346, 3055, 2986, 1689, 1688, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.35 (s, 1H, D_2O exchangeable, NH), 7.49–7.25 (m, 4H, C_6H_4), 6.65 (s, 1H, CH), 2.36, 2.28 (2s, 4H, $2CH_2$), 1.09, 1.07 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 167.3$, 164.8 (C-1, C-3), 128.8, 126.1, 124.2, 122.3 (C_6H_4), 108.6, 104.1 (CH=C), 50.4 (C-4), 36.6 (C-5), 28.3 (C-4), 24.6 ($2CH_3$); EIMS: m/z 277 $[M]^+$ (24%); analysis Calcd for $C_{15}H_{16}ClNO_2$ (277.75): C, 64.87; H, 5.81; N, 5.04%. Found: C, 64.64; H, 5.79; N, 5.19%.

5,5-Dimethyl-2-((p-tolylamino)methylene) cyclohexane-1,3-dione (16c)

Orange crystals from ethanol; m.p. 190–194 °C; yield 74%. IR (KBr) cm^{-1}: 3484–3353, 3055, 2985, 1689, 1687, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.38 (s, 1H, D_2O exchangeable, NH), 7.48–7.23 (m, 4H, C_6H_4), 6.68 (s, 1H,

CH), 2.78 (s, 3H, CH_3), 2.38, 2.26 (2s, 4H, $2CH_2$), 1.09, 1.07 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta =$ 167.7, 164.6 (C-1, C-3), 127.3, 125.6, 123.8, 121.7 (C_6H_4), 108.8, 104.5 (CH=C), 50.6 (C-4), 36.8 (C-5), 30.2 (CH_3), 28.4 (C-4), 24.8 ($2CH_3$); EIMS: m/z 257 $[M]^+$ (36%); analysis Calcd for $C_{15}H_{19}NO_2$ (257.33): C, 74.68; H, 7.44; N, 5.55%. Found: C, 74.51; H, 7.67; N, 5.63%.

General procedure for the synthesis of the 6, 7-dihydrobenzo[*b*] thiophene derivatives 17a–f

Each of elemental sulfur and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) were added to a solution of either **16a** (2.43 g, 0.01 mol), **16b** (2.77 g, 0.01 mol), or **16c** (2.42 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 4 h then left to cool. The formed solid product was collected by filtration, dried, and crystallized from ethanol to give **17a–f**, respectively.

2-Amino-7,7-dimethyl-5-oxo-4-((phenylamino) methylene)-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carbonitrile (17a)

Orange crystals from 1,4-dioxane; m.p.127–129 °C; yield 75%. IR (KBr) cm^{-1}: 3487–3326, 3054, 2985, 2220, 1688, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.40 (s, 1H, D_2O exchangeable, NH), 7.46–7.26 (m, 5H, C_6H_5), 6.80 (s, 1H, C=CH), 4.88 (s, 2H, D_2O exchangeable, NH_2), 2.35 (s, 2H, CH_2), 1.09, 1.08 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 136.6$, 132.4 (pyrazole C-4, C-5), 138.1, 136.7, 132.4, 130.8 (thiophene C), 128.3, 126.1, 123.8, 122.1 (C_6H_5), 116.5 (CN), 36.8 (C-4), 28.6 (C-7), 24.5 ($2CH_3$); EIMS: m/z 323 $[M]^+$ (40%); analysis Calcd for $C_{18}H_{17}N_3OS$ (323.41): C, 66.85; H, 5.30; N, 12.99; S, 9.91%. Found: C, 66.73; H, 5.28; N, 13.30; S, 10.16%.

Ethyl 2-amino-7,7-dimethyl-5-oxo-4-((phenylamino) methylene)-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carboxylate (17b)

Orange crystals from 1,4-dioxane; m.p. 90–92 °C; yield 70%. IR (KBr) cm^{-1}: 3472–3341, 3054, 2985, 2220, 1702, 1688, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.38 (s, 1H, D_2O exchangeable, NH), 7.44–7.25 (m, 5H, C_6H_5), 6.82 (s, 1H, C=CH), 4.85 (s, 2H, D_2O exchangeable, NH_2), 4.22 (q, 2H, $J = 6.55$ Hz, CH_2), 2.37 (s, 2H, CH_2), 1.12 (t, 3H, $J =$ 6.55 Hz, CH_2), 1.09, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 138.3$, 136.5, 133.8, 130.3 (thiophene C), 127.4, 125.6, 123.3, 121.5 (C_6H_5), 52.1 ($O\underline{CH_2}CH_3$), 36.8 (C-4), 28.4 (C-7), 24.7 ($2CH_3$), 16.2 ($OCH_2\underline{CH_3}$); EIMS: m/z 370 $[M]^+$ (24%); analysis Calcd for $C_{20}H_{22}N_2O_3S$ (370.47): C, 64.84; H, 5.99; N, 7.56; S, 8.66%. Found: C, 64.69; H, 5.74; N, 7.69; S, 8.73%.

2-Amino-4-(((4-chlorophenyl)amino)methylene)-7, 7-dimethyl-5-oxo-4,5,6,7-tetrahydro-benzo[*b*] thiophene-3-carbonitrile (17c)

Orange crystals from 1,4-dioxane; m.p. 123–125 °C; yield 78%. IR (KBr) cm^{-1}: 3469–3346, 3054, 2985, 2220, 1688, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.36 (s, 1H, D_2O exchangeable, NH), 7.49–7.21 (m, 4H, C_6H_4), 6.82 (s, 1H, C=CH), 4.84 (s, 2H, D_2O exchangeable, NH_2), 2.38 (s, 2H, CH_2), 1.09, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 138.5$, 135.2, 133.1, 130.4 (thiophene C), 127.5, 125.2, 123.3, 122.1 (C_6H_4), 116.8 (CN), 36.6 (C-4), 28.3 (C-7), 24.8 ($2CH_3$); EIMS: m/z 357 $[M]^+$ (26%); analysis Calcd for $C_{18}H_{16}ClN_3OS$ (357.86): C, 60.41; H, 4.51; N, 11.74; S, 8.96%. Found: C, 60.58; H, 4.73; N, 11.90; S, 9.25%.

Ethyl 2-amino-4-(((4-chlorophenyl)amino) methylene)-7,7-dimethyl-5-oxo-4,5,6, 7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (17d)

Pale brown crystals from 1,4-dioxane; m.p. 118–121 °C; yield 62%. IR (KBr) cm^{-1}: 3492–3329, 3054, 2984, 2220, 1701, 1687, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.40 (s, 1H, D_2O exchangeable, NH), 7.49–7.23 (m, 4H, C_6H_4), 6.83 (s, 1H, C=CH), 4.83 (s, 2H, D_2O exchangeable, NH_2), 4.23 (q, 2H, $J = 7.11$ Hz, CH_2), 2.37 (s, 2H, CH_2), 1.12 (t, 3H, $J = 7.11$ Hz, CH_2), 1.09, 1.08 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 138.1$, 136.8, 135.6, 130.1 (thiophene C), 129.8, 124.8, 122.6, 120.1 (C_6H_4), 52.3 ($O\underline{CH_2}CH_3$), 36.6 (C-4), 28.1 (C-7), 24.9 ($2CH_3$), 16.2 ($OCH_2\underline{CH_3}$); EIMS: m/z 404 $[M]^+$ (32%); analysis Calcd for $C_{18}H_{21}ClN_2O_3S$ (404.91): C, 59.33; H, 4.23; N, 6.92; S, 7.92%. Found: C, 59.28; H, 4.41; N, 7.36; S, 8.25%.

2-Amino-7,7-dimethyl-5-oxo-4-((p-tolylamino) methylene)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carbonitrile (17e)

Orange crystals from 1,4-dioxane; m.p. 120–123 °C; yield 58%. IR (KBr) cm^{-1}: 3496–3352, 3054, 2985, 2220, 1688, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.34 (s, 1H, D_2O exchangeable, NH), 7.46–7.26 (m, 4H, C_6H_4), 6.80 (s, 1H, C=CH), 4.87 (s, 2H, D_2O exchangeable, NH_2), 2.87 (s, 3H, CH_3), 2.36 (s, 2H, CH_2), 1.09, 1.07 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 137.3$, 136.1, 132.8, 130.9 (thiophene C), 127.5, 123.9, 122.6, 120.8 (C_6H_4), 116.6 (CN), 36.6 (C-4), 28.6 (C-7), 24.7 ($2CH_3$); EIMS: m/z 337 $[M]^+$ (30%); analysis Calcd for $C_{19}H_{19}N_3OS$ (337.44):

C, 67.63; H, 5.68; N, 12.45; S, 9.50%. Found: C, 67.58; H, 5.73; N, 12.60; S, 9.25%.

Ethyl 2-amino-7,7-dimethyl-5-oxo-4-((p-tolylamino) methylene)-4,5,6,7-tetrahydro-benzo[*b*]thiophene-3-carboxylate(17f)

Pale brown crystals from 1,4-dioxane; m.p. 115–117 °C; yield 70%. IR (KBr) cm^{-1}: 3472–3331, 3054, 2984, 2220, 1705, 1687, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.43 (s, 1H, D_2O exchangeable, NH), 7.52–7.21 (m, 4H, C_6H_4), 6.85 (s, 1H, C=CH), 4.82 (s, 2H, D_2O exchangeable, NH_2), 4.21 (q, 2H, $J = 7.38$ Hz, CH_2), 2.78 (s, 3H, CH_3), 2.37 (s, 2H, CH_2), 1.12 (t, 3H, $J = 7.38$ Hz, CH_2), 1.08, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 137.8$, 136.6, 134.9, 131.6 (thiophene C), 127.1, 125.6, 123.2, 120.7 (C_6H_4), 52.6 ($O\underline{CH_2}CH_3$), 36.8 (C-4), 30.3 (CH_3), 28.0 (C-7), 24.8 ($2CH_3$), 16.1 ($OCH_2\underline{CH_3}$); EIMS: m/z 384 $[M]^+$ (24%); analysis Calcd for $C_{21}H_{24}N_2O_3S$ (384.49): C, 65.60; H, 6.29; N, 7.29; S, 8.34%. Found: C, 65.42; H, 6.09; N, 7.41; S, 8.50%.

General procedure for the synthesis of the 4,5,6, 7-tetrahydro-2H-indazole derivatives 18a–f

To a solution of either **16a** (2.43 g, 0.01 mol), **16b** (2.77 g, 0.01 mol), or **16c** (2.42 g, 0.01 mol) in 1,4-dioxane (40 mL) either of hydrazine hydrate (0.1 mL, 0.02 mol) or phenylhydrazine (3.16 g, 0.02 mol) was added. The reaction mixture, in each case, was heated under reflux for 3 h then was left to cool and the formed solid product was collected by filtration upon pouring onto ice/water mixture containing a few drops of hydrochloric acid.

4-Hydrazono-6,6-dimethyl-N-phenyl-4,5,6, 7-tetrahydro-2H-indazol-3-amine (18a)

Pale yellow crystals from 1,4-dioxane; m.p. 225–228 °C; yield 68%. IR (KBr) cm^{-1}: 3484–3331, 3052, 2985, 1655, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.29, 8.31 (2s, 2H, D_2O exchangeable, 2NH), 7.26–7.39 (m, 5H, C_6H_5), 4.86 (s, 2H, D_2O exchangeable, NH_2), 2.42, 2.29 (2s, 4H, $2CH_2$), 1.07, 1.03 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.3$, 172.8 (2C=N), 136.6, 132.8 (pyrazole C-4, C-5), 120.3, 122.5, 124.6, 125.1 (C_6H_5), 56.6 (C-5), 36.8 (C-6), 28.3 (C-7), 24.8 ($2CH_3$); EIMS: m/z 269 $[M]^+$ (33%); analysis Calcd for $C_{15}H_{19}N_5$ (269.34): C, 66.89; H, 7.11; N, 26.00%. Found: C, 66.74; H, 6.94; N, 25.80%.

6,6-Dimethyl-N,2-diphenyl-4-(2-phenylhydrazono)-4,5,6,7-tetrahydro-2H-indazol-3-amine (18b)

Pale yellow crystals from 1,4-dioxane; m.p. 110–112 °C; yield 73%. IR (KBr) cm^{-1}: 3476–3353, 3050, 2985, 1653, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.28, 8.34 (2s, 2H, D_2O exchangeable, 2NH), 7.24–7.42 (m, 15H, $3C_6H_5$), 2.43, 2.25 (2s, 4H, $2CH_2$), 1.09, 1.02 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.6$, 172.4 (2C=N), 136.2, 132.5 (pyrazole C-4, C-5), 120.0, 121.3, 122.7, 123.4, 123.6, 124.1, 124.5, 124.8, 125.0, 125.6, 126.4, 127.1 ($3C_6H_5$), 56.4 (C-5), 36.3 (C-6), 28.9 (C-7), 24.6 ($2CH_3$); EIMS: m/z 421 $[M]^+$ (26%); analysis Calcd for $C_{27}H_{27}N_5$ (421.54): C, 76.93; H, 6.46; N, 16.61%. Found: C, 76.83; H, 6.70; N, 16.84%.

N-(4-Chlorophenyl)-4-hydrazono-6,6-dimethyl-4,5,6,7-tetrahydro-2H-indazol-3-amine (18c)

Pale yellow crystals from 1,4-dioxane; m.p. 240–244 °C; yield 68%. IR (KBr) cm^{-1}: 3474–3358, 3052, 2985, 1650, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.27, 8.30 (2s, 2H, D_2O exchangeable, 2NH), 7.23–7.40 (m, 4H, C_6H_4), 4.84 (s, 2H, D_2O exchangeable, NH_2), 2.41, 2.27 (2s, 4H, $2CH_2$), 1.07, 1.04 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.1$, 172.5 (2C=N), 136.8, 132.5 (pyrazole C-4, C-5), 120.1, 123.8, 124.3, 125.6 (C_6H_4), 56.4 (C-5), 36.9 (C-6), 28.6 (C-7), 24.9 ($2CH_3$); EIMS: m/z 303 $[M]^+$ (42%); analysis Calcd for $C_{15}H_{18}ClN_5$ (303.13): C, 59.30; H, 5.97; N, 23.05%. Found: C, 59.53; H, 6.15; N, 22.92%.

N-(4-Chlorophenyl)-6,6-dimethyl-2-phenyl-4-(2-phenylhydrazono)-4,5,6,7-tetrahydro-2H-indazol-3-amine (18d)

Yellow crystals from 1,4-dioxane; m.p. 107–110 °C; yield 78%. IR (KBr) cm^{-1}: 3486–3348, 3050, 2985, 1655, 1632. ^1H NMR (200 MHz, DMSO-d_6) δ 8.26, 8.32 (2s, 2H, D_2O exchangeable, 2NH), 7.26–7.48 (m, 14H, $2C_6H_5$, C_6H_4), 2.40, 2.28 (2s, 4H, $2CH_2$), 1.08, 1.02 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.8$, 172.5 (2C=N), 136.5, 132.3 (pyrazole C-4, C-5), 120.1, 120.8, 121.6, 123.7, 123.9, 124.2, 124.6, 125.2, 125.4, 125.9, 126.6, 127.3 ($2C_6H_5$, C_6H_4), 56.6 (C-5), 36.2 (C-6), 28.5 (C-7), 24.6 ($2CH_3$); EIMS: m/z 455 $[M]^+$ (36%); analysis Calcd for $C_{27}H_{26}ClN_5$ (455.98): C, 71.12; H, 5.75; N, 15.36%. Found: C, 71.30; H, 5.93; N, 15.41%.

4-Hydrazono-6,6-dimethyl-N-(p-tolyl)-4,5,6, 7-tetrahydro-2H-indazol-3-amine (18e)

Pale yellow crystals from 1,4-dioxane; m.p. 235–236 °C; yield 70%. IR (KBr) cm^{-1}: 3474–3358, 3052, 2985, 1650, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.29, 8.33 (2s, 2H, D_2O exchangeable, 2NH), 7.25–7.46 (m, 4H, C_6H_4), 4.86 (s, 2H, D_2O exchangeable, NH_2), 2.80 (s, 3H, CH_3), 2.43, 2.29 (2s, 4H, $2CH_2$), 1.09, 1.04 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.3$, 172.6 (2C=N), 136.5,

132.8 (pyrazole C-4, C-5), 120.9, 123.2, 125.1, 126.8 (C_6H_4), 56.4 (C-5), 38.1 (CH_3), 36.9 (C-6), 28.8 (C-7), 24.9 ($2CH_3$); EIMS: m/z 283 $[M]^+$ (36%); analysis Calcd for $C_{16}H_{21}N_5$ (283.37): C, 67.82; H, 7.47; N, 24.71%. Found: C, 67.59; H, 7.53; N, 24.65%.

6,6-Dimethyl-2-phenyl-4-(2-phenylhydrazono)-N-(p-tolyl)-4,5,6,7-tetrahydro-2H-indazol-3-amine (18f)

Yellow crystals from 1,4-dioxane; m.p. 87–90 °C; yield 65%. IR (KBr) cm^{-1}: 3492–3326, 3050, 2985, 1653, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 8.28, 8.31 (2s, 2H, D_2O exchangeable, 2NH), 7.28–7.53 (m, 14H, $2C_6H_5$, C_6H_4), 2.84 (s, 3H, CH_3), 2.40, 2.28 (2s, 4H, $2CH_2$), 1.08, 1.03 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 173.8$, 172.5 (2C=N), 136.5, 132.3 (pyrazole C-4, C-5), 120.1, 120.8, 120.9, 121.7, 121.9, 122.2, 122.4, 123.6, 124.4, 124.9, 125.6, 128.8 ($2C_6H_5$, C_6H_4), 56.8 (C-5), 38.3 (CH_3), 36.4 (C-6), 28.3 (C-7), 24.8 ($2CH_3$); EIMS: m/z 435 $[M]^+$ (28%); analysis Calcd for $C_{28}H_{29}N_5$ (435.56): C, 77.21; H, 6.71; N, 16.08%. Found: C, 77.40; H, 6.52; N, 16.36%.

General procedure for the synthesis of the isoxazole derivatives 19a–c

Hydroxylamine hydrochloride (0.78 g, 0.01 mol) was added to a solution of compound either **16a** (2.43 g, 0.01 mol), **16b** (2.77 g, 0.01 mol), or **16c** (2.42 g, 0.01 mol) in 1,4-dioxane (40 mL) containing sodium acetate (1.00 g). The reaction mixture, in each case, was heated under reflux for 2 h then poured onto ice/water and the formed solid product was collected by filtration.

6,6-Dimethyl-3-(phenylamino)-6,7-dihydrobenzo[c]isoxazol-4(5H)-one oxime (19a)

Pale yellow crystals from 1,4-dioxane; m.p. 252–255 °C; yield 63%. IR (KBr) cm^{-1}: 3580–3328, 3054, 2985, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 10.22 (s, 1H, D_2O exchangeable, OH), 8.25 (s, 1H, NH, D_2O exchangeable, NH), 7.22–7.39 (m, 5H, C_6H_5), 2.39, 2.23 (2s, 4H, $2CH_2$), 1.06, 1.05 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta =$ 176.6 (C=N), 166.2 (C-4), 138.6, 133.3 (isoxazole C-4, C-5), 119.3, 121.6, 123.6, 124.8 (C_6H_5), 56.8 (C-2), 36.7 (C-6), 32.2 (C-5), 28.4 (C-7), 24.8 ($2CH_3$); EIMS: m/z 271 $[M]^+$ (34%); analysis Calcd for $C_{15}H_{17}N_3O_2$ (271.31): C, 66.40; H, 6.32; N, 15.49%. Found: C, 66.27; H, 6.48; N, 15.27%.

3-((4-Chlorophenyl)amino)-6,6-dimethyl-6,7-dihydrobenzo[c]isoxazol-4(5*H*)-one oxime (19b)

Pale yellow crystals from 1,4-dioxane; m.p. 230–233 °C; yield 72%. IR (KBr) cm^{-1}: 3559–3341, 3056, 2983, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 10.23 (s, 1H, D_2O exchangeable, OH), 8.28 (s, 1H, NH, D_2O exchangeable, NH), 7.21–7.47 (m, 4H, C_6H_4), 2.36, 2.27 (2s, 4H, $2CH_2$), 1.08, 1.06 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 176.8$ (C=N), 166.1 (C-4), 138.8, 133.1 (isoxazole C-4, C-5), 120.8, 121.9, 124.2, 125.6 (C_6H_4), 56.5 (C-2), 36.3 (C-6), 32.3 (C-5), 28.6 (C-7), 24.7 ($2CH_3$); EIMS: m/z 305 $[M]^+$ (23%); analysis Calcd for $C_{15}H_{16}ClN_3O_2$ (305.76): C, 58.92; H, 5.27; N, 13.74%. Found: C, 59.21; H, 5.39; N, 13.53%.

6,6-Dimethyl-3-(p-tolylamino)-6,7-dihydrobenzo[c]isoxazol-4(5H)-one oxime (19c)

Pale yellow crystals from 1,4-dioxane; m.p. 180–183 °C; yield 70%. IR (KBr) cm^{-1}: 3559–3348, 3055, 2983, 1630. ^1H NMR (200 MHz, DMSO-d_6) δ 10.26 (s, 1H, D_2O exchangeable, OH), 8.28 (s, 1H, NH, D_2O exchangeable, NH), 7.25–7.44 (m, 4H, C_6H_4), 2.79 (s, 3H, CH_3), 2.39, 2.23 (2s, 4H, $2CH_2$), 1.06, 1.03 (2s, 6H, $2CH_3$); ^{13}C NMR (DMSO-d_6, 75 MHz): $\delta = 176.8$ (C=N), 166.2 (C-4), 138.8, 133.4 (isoxazole C-4, C-5), 120.1, 121.4, 123.8, 124.6 (C_6H_4), 56.3 (C-2), 36.5 (C-6), 32.6 (C-5), 30.3 (CH_3), 28.2 (C-7), 24.6 ($2CH_3$); EIMS: m/z 285 $[M]^+$ (42%); analysis Calcd for $C_{16}H_{19}N_3O_2$ (285.34): C, 67.35; H, 6.71; N, 14.73%. Found: C, 67.40; H, 6.39; N, 14.62%.

Conclusion

In summary, in order to develop potent antitumor agents, we have designed and synthesized a series of new heterocyclic compounds using dimedone. The produced compounds were screened through six cancer cell lines where compounds **7b**, **7c**, **7d**, **9b**, **11**, **12c**, **12d**, **14b**, **16b**, **17c**, **17d**, **18c**, **18d**, and **18e** were the most cytotoxic compounds. Their further tests toward the five tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR and Pim-1 kinase showed that compounds **7b**, **7d**, **14b**, **16b**, and **18e** were the most potent of the tested compounds toward the five tyrosin kinases and compounds **7b**, **7d**, **14b**, **16b**, and **18e** were of the highest inhibitions toward Pim-1 kinase. PAINS the most cytotoxic compounds showed zero PAINS alert thus these compounds are considered anticancer agents without restrictions. Therefore, the results laid a foundation for further improving the potency and the selectivity of this series of compounds.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Ahmad M, King TA, Ko DK, Cha BH, Lee J (2002) Performance and photostability of xanthene and pyrromethene laser dyes in sol-gel phases. J Phys D Appl Phys 35:1473

Al-Majid AM, Barakat A, Al-Najjar HJ, Mabkhot YN, Ghabbour HA, Fun HK (2013) Tandem Aldol–Michael reactions in aqueous diethylamine medium: a greener and efficient approach to bis-pyrimidine derivatives. Int J Mol Sci 14:23762–23773

Al-Majid AM, Islam MS, Barakat A, Al-Qahtani NJ, Yousuf S, Choudhary MI (2017) Tandem Knoevenagel–Michael reactions in aqueous diethylamine medium: a greener and efficient approach toward bis-dimedone derivatives. Arab J Chem 10:185–193

Al-Najjar HJ, Barakat A, Al-Majid AM, Mabkhot YN, Ghabbour HA, Fun HK (2014) A greener, efficient approach to Michael addition of barbituric acid to nitroalkene in aqueous diethylamine medium. Molecules 19:1150–1162

Ashry ES, Awad LF, El Kilany Y, Ibrahim EI (2009) Dimedone: a versatile precursor for annulated heterocycles. Adv Heterocycl Chem 98:1–141

Banerjee A, Mukherjee AK (1981) Chemical aspects of santalin as a histological stain. Stain Technol 56:83–85

Barakat A, Al-Majid AM, AL-Najjar HJ, Mabkhot YN, Ghabbour HA, Fun HK (2014) An efficient and green procedure for synthesis of rhodanine derivatives by aldol-thia-Michael protocol using aqueous diethylamine medium. RSC Adv 10:4909–4916

Barakat A, Al-Majid AM, Al-Ghamdi AM, Mabkhot YN, Siddiqui MR, Ghabbour HA, Fun HK (2014) Tandem Aldol–Michael reactions in aqueous diethylamine medium: a greener and efficient approach to dimedone-barbituric acid derivatives. Chem Cent J 8:9

Barakat A, Islam MS, Al-Majid AM, Al-Othman ZA (2013) Highly enantioselective Friedel-Crafts alkylation of indoles with a, b-unsaturated ketones with simple Cu(II)-oxazoline-imidazoline catalysts. Tetrahedron 69:5185–5192

Benitez LV, Allison WS (1974) The inactivation of the acyl phosphatase activity catalyzed by the sulfenic acid form of glyceraldehyde 3-phosphate dehydrogenase by dimedone and olefins. J Biol Chem 249:6234–6243

Bodini ME, Pardo J, Arancibia V (1990) Spectrophotometric determination of selenium(IV) with 5,5-dimethyl-1,3-cyclohexanedione. Talanta 37:439–442

Bonsignore L, Loy G, Secci D, Calignano A (1993) Synthesis and pharmacological activity of 2-oxo-(2H) 1-benzopyran-3-carboxamide derivatives. Eur J Med Chem 28:517–520

Cheng F, Li AW (2012) SAR: a comprehensive source and free tool for assessmentof chemical ADMET properties. J Chem Inf Model 52:3099–3105

Cremlyn RJ, Saunders D (1993) Chlorosulfonation of 9-aryl 3,3,6,6-tetramethyloctahydro-xanthen-1,8-diones. Phosphorus Sulfur Silicon 81:73–82

Cui Y, Dang Y, Yang Y, Ji R (2005) Synthesis of novel oxazolidinone derivatives for antibacterial investigation. Curr Sci 83:531–534

Deng JZ, Sun DA, Starck SR, Hecht SM, Cerny RL, Engen JR (1999) Chrysochlamic acid, a new diterpenoid-substituted quinol from Chrysochlamysulei that inhibits DNA polymerase. J ChemSoc Perkin Trans 1:1147–1150

Dewse CD, Potter CG (1975) Inhibitory effect of phenylbutazone and oxyphenbutazone on DNA synthesis in normal human bone marrow cells in vitro. J Pharm Pharmcol 27:523–526

DiSalvo J, Bayne ML, Conn G, Kwok PW, Trivedi PG, Soderman DD, Palisi TM, Sullivan KA, Thomas KA (1995) Purification and characterization of a naturaly occurring vascular endothelial growth factor-placetna growth factor. J Biol Chem 270:7717–7723

Feng BY, Shelat A, Doman TN, Guy RK, Shoichet BK (2005) High-throughput assays for promiscuous inhibitors. Nat Chem Biol 1:146–148

Feng BY, Shoichet BK (2006) Synergy and antagonism of promiscuous inhibition in multiple-compound libraries. J Med Chem 49:2151–2154

Ferrara N (2002) VEGF and the quest for tumour angiogenesis factors. Nat Rev Cancer 2:795–803

Greenhill JV (1977) Enaminones. Chem Soc Rev 6:277–294

Guan-Wu W, Chun-Bao M (2006) Environmentally benign one-pot multi-component approaches to the synthesis of novel unsymmetrical 4-arylacridinediones. Green Chem 8:1080–1085

Hideo T, Teruomi J (1981) (Sankyo Co.) Benzopyrano[2,3-b]xanthene derivatives and its preparation. Jp Patent 56005480

Huth JR, Song D, Mendoza RR, Black-Schaefer CL, Mack JC, Dorwin SA, Ladror US, Severin JM, Walter KA, Bartley DM, Hajduk P (2007) Toxicological evaluation of thiol-reactive compounds identified using a La assay to detect reactive molecules by nuclear magnetic resonance. Chem Res Toxicol 20:1752–1759

Ilkhanizadeh S, Khalafy J, Dekamin MG (2019) Sodium alginate: a biopolymeric catalyst for the synthesis of novel and known polysubstituted pyrano[3,2-*c*]chromenes. Intern J Biol Macromol 140:605–613

Jae-Min H, Sung-Ho Y, Kang-Yeoun J (1997) Synthesis of oxazolidinone phosphonate derivatives, part II. Bull Korean Chem Soc 28:821–826

James LD, Richard AG, Surya KD (2006) An efficient one-pot synthesis of polyhydroquinoline derivatives through the Hantzsch four component condensation. J Mol Catal A: Chem 256:309–311

Jayme LD, Michael AW (2014) The essential roles of chemistry in high-throughput screening triage. Future Med Chem 6:1265–1290

Jo YW, Im WB, Rhee JK, Shim MJ, Kim WB, Choi EC (2004) Synthesis and antibacterial activity of oxazolidinones containing pyridine substituted with heteroaromatic ring. Bioorg Med Chem 12:5909–5915

Jonathan BB, Georgina AH (2010) New substructure filters for removal of Pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. J Med-Chem 53:2719–2740

Jonathan BB, Michael AW (2014) Chemistry: chemical con artists foil drug discovery. Natur 513:481–483

Knight CG, Stephens T (1989) Xanthene-dye-labelled phosphatidylethanol-amines as probes of interfacial pH. Studies in phospholipid vesicles. Biochem J 258:683–687

Lambert RW, Martin JA, Merrett JH, Parkes KEB, Thomas GJ (1997) Pyrimidine nucleosides, PCT Int Appl WO 9706178

Majumdar KC, Samanta SK (2002) Sulfoxide-claisein rearrangement: synthesis of dimedone-annaleted unusual heterocycles. Tetrahedron 58:4551–4554

Majumdar KC, Samanta SK (2001) Aza-claisein rearrangement: synthesis of dimedone-annaleted unusual heterocycles. Tetrahedron 57:4955–4958

Martin P, Stephane J(2016) Pan assay interference compounds (PAINS) and other promiscuous compounds in antifungal research J Med Chem 59:497–503

McGovern SL, Caselli E, Grigorieff N, Shoichet BK (2002) A common mechanism underlying promiscuous inhibitors from virtual and high-throughput screening. J Med Chem 45:1712–1722

McGovern SL, Shoichet BK (2003) Kinase inhibitors: not just for kinases anymore. J Med Chem 46:1478–1483

Menchen SM, Benson SC, Lam JYL, Zhen W, Sun D, Rosenblum BB, Khan SH, Taing M (2003) US patent 6, 168, 6583168

Metz JT, Huth JR, Hajduk PJ (2007) Enhancement of chemical rules for predicting compound reactivity towards protein thiol groups. J Comput-Aided Mol Des 21:139–144

Mohareb RM, Abdo NY, EL-Sharkawy KA (2018) New approaches for the uses of cyclohexan-1,4-dione for the synthesis of 5,6,7,8-tetrahydrobenzo[4,5]thieno[2,3-*b*]pyridine derivatives used as potential anti-prostate cancer agents and Pim-1 kinase inhibitors. Anticancer Agent Med Chem 18:1736–1749

Mohareb RM, Samir EM, Halim PA (2019) Synthesis, and antiproliferative, Pim-1 kinase inhibitors and molecular docking of thiophenes derived from estrone. Bioorg Chem 83:402–413

Mohareb RM, El-Sharkawy KA, El-Farouk FO (2019) Synthesis, cytotoxicity against cancer and normal cell lines of novel hydrazide–hydrazone derivatives bearing 5*H*-chromen-5-one. Med Chem Res 28:1885–1900

Pae AN, Kim HY, Joo HJ, Kim BH, Cho YS, Choi KI, Choi JH, Koh HY (1999) Synthesis and in vitro activity of new oxazolidinone antibacterial agents having substituted isoxazoles. Bioorg Med Chem Lett 18:2679–2684

Poupelin JP, Saint-Ruf G, Foussard-Blanpin O, Marcisse G, Uchida-Ernouf G, Lacroix R (1978) Synthesis and antiinflammatory properties of bis(2-hydroxy-1-naphthyl)methane derivatives. I. monosubstituted derivatives. Eur J Med Chem 13:67–71

Ramazani A, Karimi Z, Souldozi A, Ahmadi Y (2012) Four-component synthesis of 1,3,4-oxadiazole derivatives from N-isocyaniminotriphenyl-phosphorane, aromatic carboxylic acids, aromatic bisaldehydes, and secondary amines. Turk J Chem 36:81–91

Ramazani A, Rezaei A (2010) Novel one-pot, four-component condensation reaction: an efficient approach for the synthesis of 2,5-disubstituted 1,3,4-oxadiazole derivatives by a Ugi-4CR/aza-wittig sequence. Org Lett 12:2852–2855

Ramazani A, Rouhani M, Rezaei A, Shajari N, Souldozi A (2011) The reaction of (N-Isocyanimino)triphenylphosphorane with biacetyl in the presence of aromatic carboxylic acids: efficient one pot three-component reaction for the synthesis of 3-(5-Aryl-1,3,4-oxadiazol-2-yl)-3-hydroxybutan-2-one derivatives. Helv Chim Acta 94:282–289

Saitoh T, Taguchi K, Hiraide M (2002) Derivatization of formaldehyde with dimedone and subsequent sorption on sodium dodecylsulfate/alumina admicelles for fluorometric analysis. Anal Sci 18:1267–1268

Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF (1983) Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219:983–985

Sonderegger OJ, Burgi T, Baiker A (2003) Asymmetric hydrogenation of cyclohexane-1,2-dione over cinchonidine-modified platinum. J Catal 215:116–121