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Synthesis, and anti-proliferative, Pim-1 kinase inhibitors and molecular docking of thiophenes derived from estrone

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ABSTRACT

Heterocyclization of steroids were reported to give biologically active products where ring D modification occured. Estrone (**1**) was used as a template to develop new heterocyclic compounds. Ring D modification of **1** through its reaction with cyanoacetylhydrazine and elemental sulfur gave the thiophene derivative **3**. The latter compound reacted with acetophenone derivatives **4a-c** to give the hydrazide-hydrazone derivatives **5a-c**, respectively. In addition, compound **3** formed thiazole derivatives through its first reaction with phenylisothiocyanate to give the thiourea derivative **9** followed by the reaction of the later with α-halocarbonyl compounds. In the present work a series of novel estrone derivatives were designed, synthesized and evaluated for their in vitro biological activities against c-Met kinase, and six typical cancer cell lines (A549, H460, HT-29, MKN-45, U87MG and SMMC-7721). The most promising compounds **5b**, **5c**, **11a**, **13c**, **15b**, **15c**, **15d**, **17a** and **17b** were further investigated against the five tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR. Compounds **5b**, **15d**, **17a** and **17b** were selected to examine their Pim-1 kinase inhibition activity where compounds **15d** and **17b** showed high activities. Molecular docking of some of the most potent compounds was demonstrated.

1. Introduction

Several D-ring alkylated estrone analogues showed exceptionally high affinity for estrogen receptors [1–3]. In particular, compounds in which an E-ring is formed are known to be involved in the inhibition of steroidogenic enzymes [4,5]. Such compounds also have an effect on steroid dehydrogenase activity and the ability to inhibit the detrimental action of the steroid sulfatase enzyme [6–8]. Generally, E-ring extended steroids have been obtained by modification of the C17-ketone in the D-ring by either arylimine or oximino formation [9–11], addition of a carbon nucleophile [12] or hydrazone formation [13-15]. Other reports have included ketone reduction, silyl enol ether formation or ring-closing metathesis (giving five- or six membered E-rings) [16,17]. Chemical modification of the steroid D-ring provides a way to change the functional groups, sizes and stereochemistry of the D-ring, and numerous structure–activity relationships have been established by such synthetic modifications. Steroid molecules for which heterocycles fused to the D-ring of the steroid nucleus have been of pharmaceutical interest [18–21]. Moreover, anticancer drugs administered for the treatment of estrogen dependent cancers are often analogs of natural estrogenic compounds. The development of estrone-based anticancer agents [22–25] lacking estrogenic activity is one of the major challenges in the medicinal chemistry of steroids. Various types of substituted estrone analogs or homoestrones have been synthesized as cytotoxic or cytostatic (anti-proliferative) anti-cancer agents [25–28]. One of the major requirements for the pharmacological use of anti-tumor estrone derivatives is that they must be devoid of estrogenic activity [29].

On the other hand, the c-Met receptor tyrosine kinase plays a vital role in signal transduction pathways [30]. The pleiotropic effects of c-Met receptor tyrosine kinase are strictly regulated under normal physiological conditions. However, numerous experimental data supported that c-Met kinase was aberrantly activated in various cancers, such as bladder, breast, colorectal, gastric, and lung cancers [31–33]. In terms of such linkage between inhibition of c-Met kinase and cancer treatment, the aim of the present work was to synthesize a series of estrone heterocyclic compounds through ring E-extension followed by studying their inhibition towards c-Met kinase and studying their anti-proliferative activities towards some cancer cell lines. Thus, we present the reaction of cyanoacetylhydrazine and elemental sulfur with estrone followed by heterocyclization of the reaction product through its reactions with different chemical reagents. The biological activities of the

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newly synthesized products were studied against c-Met kinase, antiproliferative activities against six typical cancer cell lines and the Pim-1 kinase inhibition activity of some selected compounds were also studied.

2. Results and discussion

2.1. Chemistry

In order to circumvent problems associated with classical combinations of cytotoxic drugs, protein kinase and Pim-1 kinase inhibitors we developed a novel approach, termed 'combi-targeting', that sought to design compounds designated as 'combi-molecules' to not only possess anti-tumor activities but also kinase inhibition. Here, given that synthetic lethality is a selective tumor targeting approach, we sought to design new molecules with cytotoxic properties that are targeted to Kinase inhibition and some of them being potential Pim-1 kinase inhibitors. To achieve this goal, our strategy was to synthesis a series of steroidal heterocyclic compounds using estrone nucleus. Recently, we studied the reaction of pregnenolone with cyanoacetylhydrazine followed by heterocyclization of the product [34] through ring E-extension in the steroid nucleus. In continuation of this work, we investigate the Gewald's reaction [35-38] of estrone (**1**) with cyanoacetylhydrazine (**2**) in ethanol and triethylamine which gave the thiophene derivative **3**. The analytical and spectral data of the product were consistent with its structure. Thus, the ^{1}H NMR spectrum showed two singlets at δ 4.68 and 5.22 ppm equivalent to the two NH_2 groups, a multiplet at δ 7.27–7.40 ppm for the aromatic protons and two singlets at δ 8.20, 10.04 ppm corresponding for the two NH & OH groups. In addition the ^{13}C NMR spectrum showed signals at δ 119.8, 120.4, 122.3, 125.0, 127.9, 128.2, 129.4, 132.1, 138.5, 144.2 ppm for the phenolic and thiophene carbons and a signal at δ 167.3 ppm for the C═O group. The hydrazide group present in compound **3** reacted with any of acetophenone (**4a**), 4-chloroacetophenone (**4b**) or 4-methylacetophenone (**4c**) to give the hydrazide-hydrazone derivatives **5a-c**, respectively (Scheme 1).

The 2-amino group present in compound **3** is capable of amide formation. Thus, compound **3** reacted with ethyl cyanoacetate in dimethylformamide to afford the 2-cyanoacetamide derivative **7**. The analytical and spectral data of compound **7** were consistent with its structure. Thus, the ^{1}H NMR spectrum of compound **7** showed a singlet at δ 4.82 ppm corresponding to the NH_2 group, a singlet at δ 5.48 ppm for the acetyl CH_2 group. In addition, the ^{13}C NMR spectrum revealed the presence of a signal at δ 52.4 ppm corresponding to the acetyl CH_2 carbon, a signal at δ 116.8 ppm equivalent to the cyano group, signals at δ 120.8, 122.9, 123.6, 124.0, 126.5, 127.8, 128.3, 132.5, 138.6, 144.6 ppm for the phenolic and thiophene carbons and two signals at δ 164.2 and 166.8 ppm for the two C═O groups. Compound **7** reacted with phenylisothiocyanate (**8**) to give the N-phenylthiosemicarbazide derivative **9**. Compound **9** reacted with either of ethyl chloroacetate (**10a**) or α-chloroacetone (**10b**) to give the thiazole derivatives **11a** and **11b**, respectively (Scheme 2).

The reaction of compound **9** with any of the ω-bromoacetophenone (**12a**), 4-methyl-ω-bromoacetophenone (**12b**) or 4-chloro-ω-bromoacetophenone (**13c**) gave the thiazole derivatives **13a-c**, respectively. Compound **7** reacted with any of benzenediazonium chloride (**14a**) 4-methylbenzenediazonium chloride (**14b**), 4-chlorobenzenediazonium chloride (**14c**) or 4-methoxybenzenediazonium chloride (**14d**) to give the arylhydrazone derivatives **15a-d**, respectively. The analytical and spectral data of the latter products were in agreement with their respective structures. On the other hand, the reaction of compound **7** with either of malononitrile (**16**) or ethyl cyanoacetate (**6**) in refluxing ethanol containing triethylamine gave the pyridine derivatives **17a** and **17b**, respectively (Scheme 3).

2.2. Biology

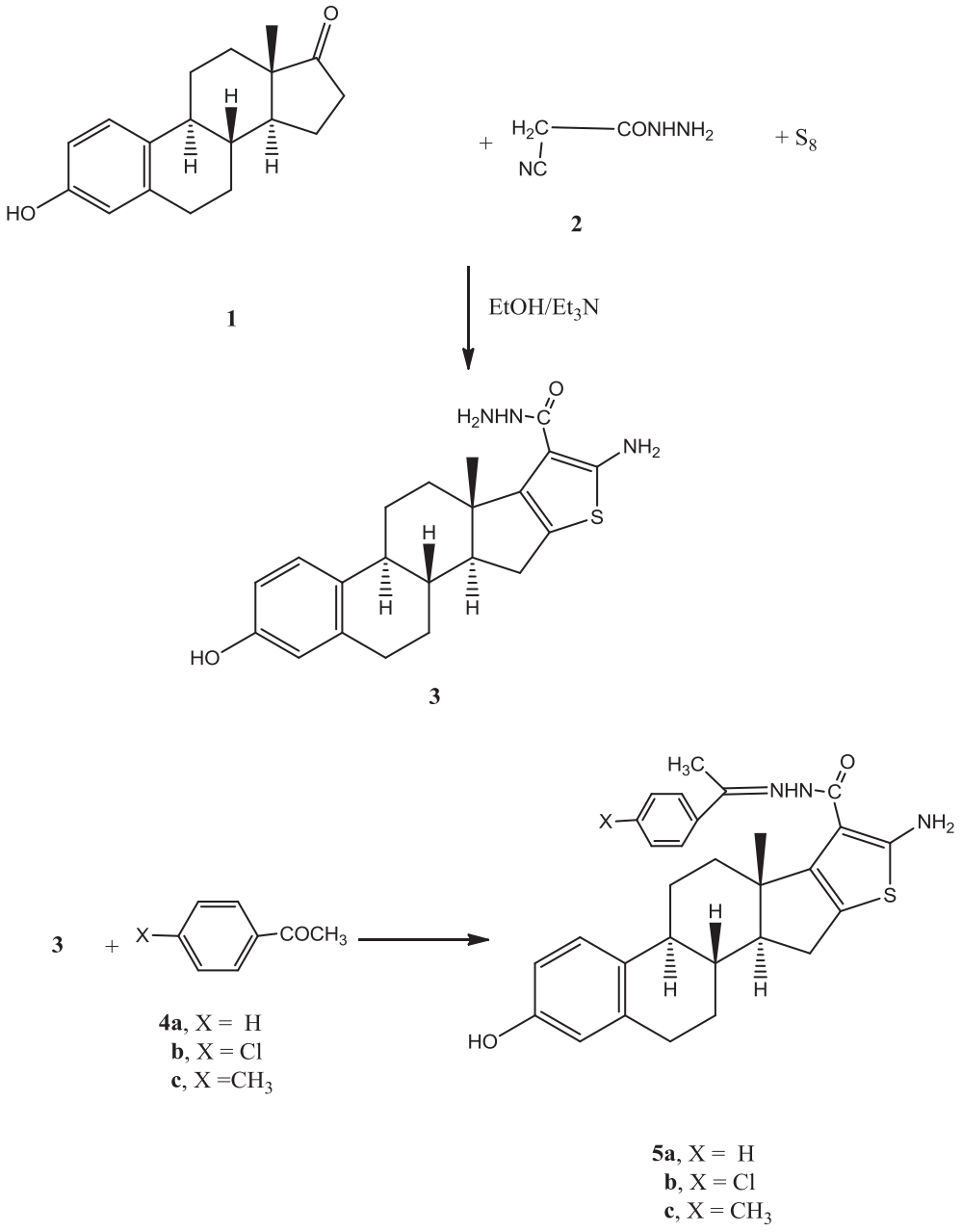
2.2.1. HTRF kinase assay

The c-Met kinase activity of all compounds were evaluated using homogeneous time-resolved fluorescence (HTRF) assay as previously reported [39,40]. Taking foretinib as the positive control, the results expressed as IC_{50} were shown in Table 1. In addition, the most promising compounds **5b**, **5c**, **11a**, **13c**, **15b**, **15c**, **15d**, **17a** and **17b** were further evaluated against other five tyrosine kinase (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) (Table 2) using the same screening method. Briefly, 20 μg/mL poly (Glu, Tyr) 4:1 (Sigma) was percolated as a substrate in 384-well plates. Then 50 μL of 10 mM ATP (Invitrogen) solution diluted in kinase reaction buffer (50 mM 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. Different 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: % 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.

As illustrated in Table 1, all the tested compounds displayed potent c-Met enzymatic activity with IC_{50} values ranging from 0.02 to 12.36 nM. Compared with foretinib (IC_{50} = 1.16 nM), seven of them (**5b**, **11a**, **13c**, **15c**, **15d**, **17a** and **17b**) exhibited equivalent or higher potency with IC_{50} values less than 2 nM. For the hydrazide-hydrazone derivatives **5a-c**, compound **5b** with the Cl group showed IC_{50} 0.02 nM while the CH_3 group of compound **5c** is responsible for the low potency with IC_{50} 6.58 nM. For compounds **9** and **11a**, the presence of the thiosemicarbazide moiety in compound **9** and the thiazole moiety in compound **11a** are responsible for their high potency. Considering the 4-arylthiazole derivatives **13a-c**, it is clear that the presence of the 4-chlorophenyl moiety is responsible for its high potency. For the arylhydrazone derivatives **15a-d**, the presence of the 4-Cl and 4-OCH_3 groups in **15c** and **15d** are responsible for their activities. For the pyridine derivatives **17a,b** both of them showed high potency with IC_{50}'s 0.12 and 0.02 nM, respectively. It is obvious that the presence of 6-hydroxypyridin-1-yl group in **17b** showed more potency than **17a** with the 6-aminopyridin-1-yl group.

2.2.2. Inhibitory effect of compounds 5b, 5c, 11a, 13c, 15b, 15c, 15d, 17a and 17b towards tyrosine kinases

An evaluation of the inhibitory activities for nine of the synthesized compounds against the five tyrosine kinases was carried out. The results of this SAR exploration, focusing on the effects of X substituents through the phenyl nucleus in some compounds and the nature of the heterocyclic ring together with the R substituent through the rest of compounds are given in Table 2. The findings showed that compound **17b** with the 6-hydroxypyridin-1-yl moiety had the highest inhibitory effect among the eight test compounds. Compounds **5b** and **15c** (X = Cl) exhibited high inhibitory effect towards c-Kit tyrosine kinase with IC_{50}'s 4.23 and 6.49 nM, respectively. This is attributed to the presence of the electronegative substituent in aryl moiety. Compound **13c** was the most active compound against EGFR with IC_{50} 2.04 nM while compound **17b** was the most active towards VEGFR-2 tyrosine kinase with IC_{50} 1.63 nM. Moreover, compounds **11a**, **13c**, **15c** and **15d** showed moderate inhibitory effect.

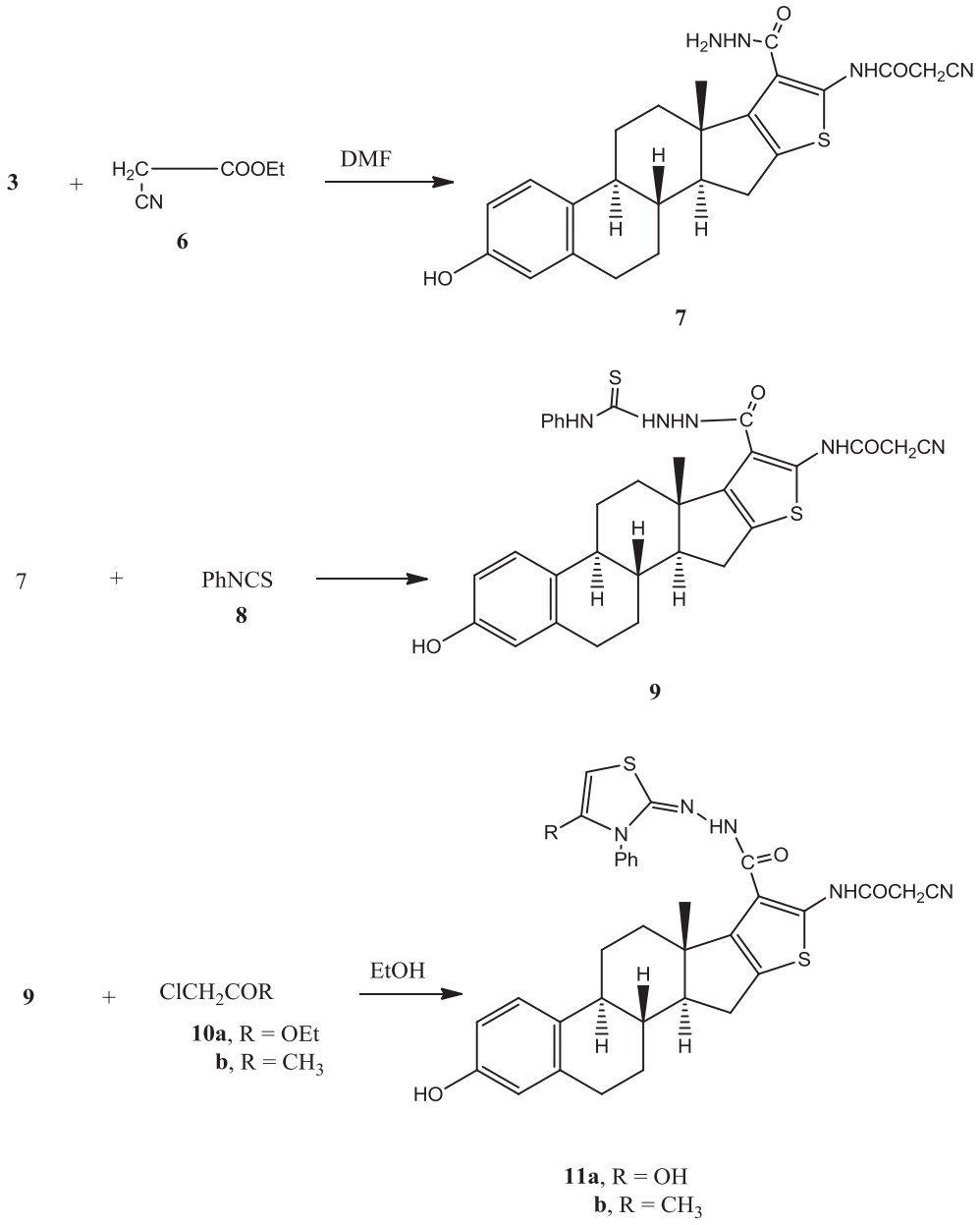


Scheme 1. Synthesis of compounds **3** and **5a-c**.

2.2.3. Cell proliferation assay

The anti-proliferative activities of all the target compounds were evaluated against five c-Met-dependent [41] cancer cell lines namely A549 (non-small cell lung cancer), HT-29 (human colon cancer), MKN-45 (human gastric cancer), U87MG (human glioblastoma) , SMMC-7721 (human liver cancer) cell lines, and one c-Met-independent cancer cell line H460 (human lung cancer) using the standard MTT assay in vitro (Table 3) with foretinib as the positive control [42–44]. The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). Approximate 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 left for further 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, in each case, with an ELISA reader. All compounds were tested three times in each cell line and the results expressed as IC_{50} (inhibitory concentration 50%). The IC_{50} values are the averages of at least three independent experiments and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

The data listed in Table 3 revealed that all compounds possessed moderate to strong cytotoxicity against the six tested cell lines in the single-digit μM range, and high selectivity for inhibition A549, H460, HT-29 and MKN-45 cells. The promising compounds **5b**, **5c**, **11a**, **13c**, **15b**, **15c**, **15d**, **17a** and **17b** were the most active compounds against A549 cell line with IC_{50} values of 0.14, 1.49, 1.63, 1.93, 3.29, 0.32, 0.09, 0.26 and 0.08 μM, respectively. The study of structure–activity relationships (SARs) indicated that these analogs showed similar SARs as summarized in the c-Met kinase level.



Scheme 2. Synthesis of compounds **7**, **9** and **11a,b**.

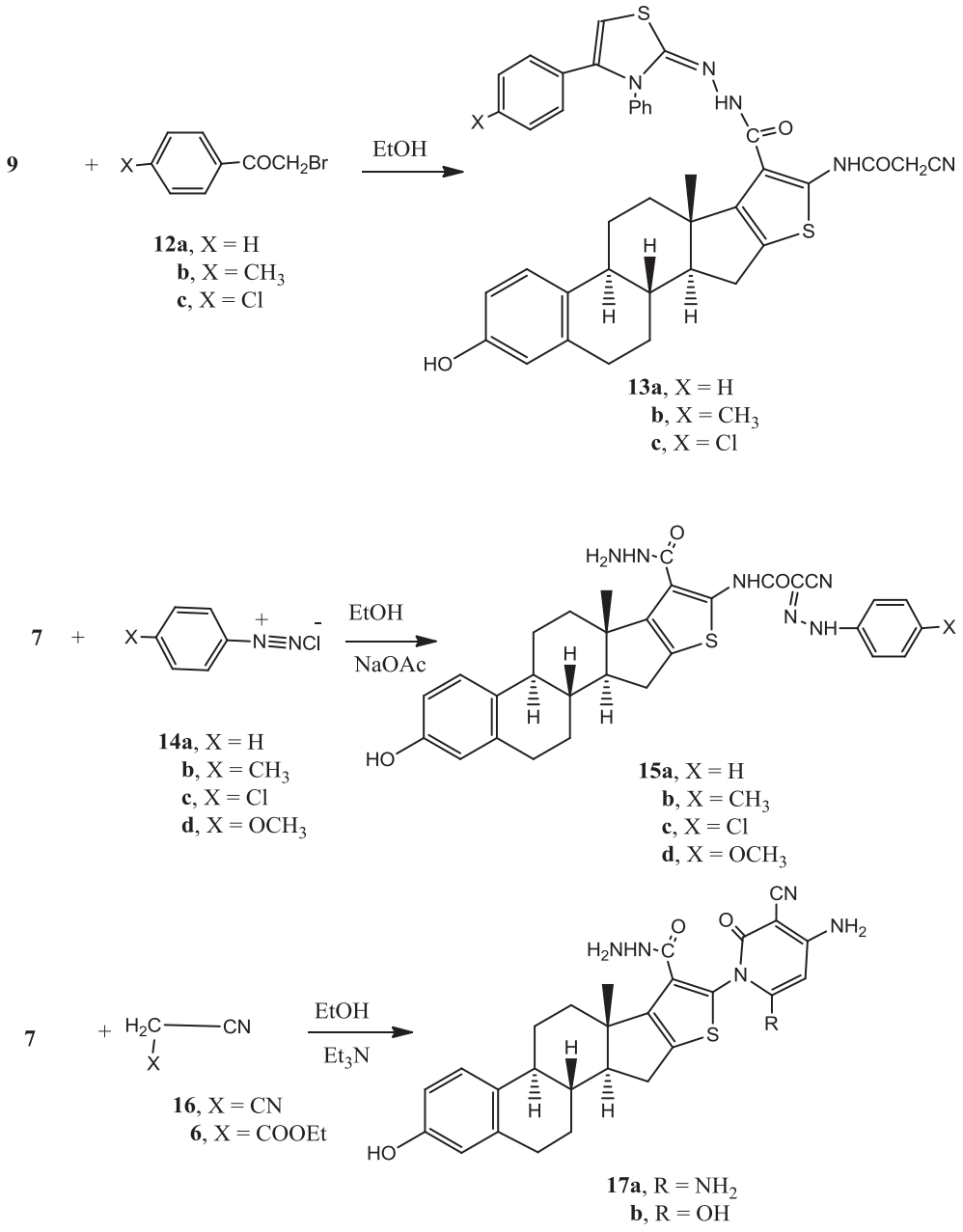
*2.2.4. Inhibitory effect of compounds **5b, 15d, 17a** and **17b** towards Pim-1 kinase*

Compounds **5b**, **15d**, **17a** and **17b** were selected to examine their Pim-1 kinase inhibition activity at a range of 10 concentrations and the IC_{50} values were calculated (Table 4). Compounds **15d** and **17b** were the most potent to inhibit Pim-1 activity with IC_{50} value of 0.68 and 0.49 μM, respectively while **5b** and **17a** 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 **5b**, **15d** and **17b** listed in Table 3 indicated that Pim-1 kinase was a potential target of these compounds.

2.2.5. Toxicity

Bioactive compounds are often toxic to shrimp larvae. Thus, in order to monitor these chemicals in vivo lethality to shrimp larvae (*Artemia salina*), Brine-Shrimp Lethality Assay [45,46] was used. Results were analyzed with LC_{50} program to determine LC_{50} values and 95% confidence intervals [47]. Results are given in Table 5 for the compounds which exhibited optimal cytotoxic effect against cancer cell lines which are the following nine compounds**:** **5b**, **5c**, **11a**, **13c**, **15b**, **15c**, **15d**, **17a** and **17b**. The shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials, natural and synthetic organic compounds [48]. It has also been shown that *A. salina* toxicity test results have a correlation with rodent and human acute oral toxicity data. Generally, a good correlation was obtained between *A. salina* toxicity test and the rodent data. Likewise, the predictive screening potential of the aquatic invertebrate tests for acute oral toxicity in man, including *A. salina* toxicity test, was slightly better than the rat test for test compounds [49,50].

In order to prevent the toxicity results from possible false effects originated from solubility of compounds and DMSO's possible toxicity effect, solutions of the test compounds were prepared in the suggested DMSO volume ranges. It is clear from Table 5 that compounds **5b**, **13c**, **15c**, **15d** and **17a** showed no toxicity against the tested organisms.



Scheme 3. Synthesis of compounds **13a-c**, **15a-d** and **17a,b**.

2.3. Docking study

2.3.1. Experimental protocol of docking study

Docking studies were performed by Molecular Operating Environment (MOE 2008.10; Chemical Computing Group, Canada). The X-ray crystal structure of c-Met kinase in complex with XL880 (Foretinib) was obtained from RCSB Protein Data Bank (https://www.rcsb.org/pdb/explore.do) (PDB ID: 3LQ8) with a 2.02 Å resolution. Deleting all water of crystallization away from the active site except the one involved in interaction with the ligand. Hydrogens and partial charges were added to the system using protonate 3D application. Isolation of the active site, recognition of the amino acids and the backbone was hidden. The docking algorithm was validated via docking of the native ligand (Foretinib) into its c-Met kinase active site where the docking procedure was able to retrieve the co-crystallized pose with RMSD value of 0.55 Å. The three-dimensional structures of the most active compounds **5b**, **13c**, **15c**, **15d**, **17a** and **17b** were built using MOE molecular builder, then they were energy minimized by Merk Molecular Force Field (MMFF94x). Hydrogens and partial charges were added to the system using protonate 3D application.

For each docked compound, only one pose was selected based on number of binding interactions, superposition with the original ligand, docking score and the formed H-bonds were measured. The docking results obtained from the docking study were summarized in Table 6.

2.3.2. Discussion of molecular modeling

The X-ray crystal structure of XL880 (Foretinib) in complex with c-Met showed that the inhibitor forms two hydrogen bonds between quinoline N and Met^{1160}, CO of malonamide moiety and Lys^{1110}. Phe^{1223} of the activation loop has relocated from the position in the active conformation to stack underneath the fluorophenyl ring (π–π interaction), placing the kinase in a pseudo-unactivated conformation [51] (Fig. 1). Docking study revealed that the most potent compounds **5b**, **13c**, **15c**, **15d**, **17a** & **17b** were capable of occupying the active binding site of c-Met while maintaining the essential key interactions

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

Compound No	IC_{50} (nM) c-Met
3	6.25 ± 1.58
5a	10.26 ± 2.38
5b	0.02 ± 0.001
5c	6.58 ± 1.82
7	12.36 ± 2.41
9	2.05 ± 0.53
11a	1.33 ± 0.89
11b	3.29 ± 0.83
13a	11.26 ± 2.51
13b	10.49 ± 1.53
13c	1.61 ± 0.38
15a	8.22 ± 1.39
15b	4.29 ± 1.21
15c	0.05 ± 0.002
15d	0.83 ± 0.06
17a	0.12 ± 0.09
17b	0.02 ± 0.001
Foretinib	1.16 ± 0.17

Table 2
Inhibition of tyrosine kinases Enzyme IC_{50} (nM) by compounds **5b**, **5c**, **11a**, **13c**, **15b**, **15c**, **15d**, **17a** and **17b**.

Compound	c-Kit	Flt-3	VEGFR-2	PDGFR	EGFR
5b	4.23	7.32	15.28	28.04	22.52
5c	18.91	20.32	4.86	12.50	6.29
11a	6.39	12.77	10.39	4.99	4.79
13c	12.53	8.39	6.49	5.82	2.04
15b	22.73	2.84	3.93	4.75	3.78
15c	6.49	14.72	2.80	3.75	6.79
15d	10.48	10.11	12.47	18.90	6.83
17a	12.73	18.90	10.92	12.59	14.59
17b	3.18	5.37	1.63	8.93	4.65

with higher binding energy scores than foretinib. Thiophene ring is essential in all synthesized compounds as it is responsible for the π–π interaction with Phe[1223] (Figs. 2–7). Compounds **5b**, **15c** & **17b** made more hydrogen bonds than foretinib with the amino acid residues which declare their higher potency.

Table 4
The inhibitor activity of compounds **5b**, **15d**, **17a** and **17b** on Pim-1 Kinase.

Compound	Inhibition ratio At 10 μM	IC_{50} (μM)
5b	23	> 10
15d	89	0.68
17a	48	> 10
17b	93	0.49
SGI-1776	–	0.048

Table 5
Toxicity of the most potent compounds.

Compound No.	Conc. (μg/ml)	Mortality[a]	Toxicity	LC_{50}	Upper 95% lim.	Lower 95% lim.
5b	10	0	Non toxic	977.18	–	–
	100	0				
	1000	3				
5c	10	1	Harmful	248.42	98.28	65.81
	100	2				
	1000	8				
11a	10	1	Harmful	239.26	110.20	77.40
	100	3				
	1000	9				
13c	10	0	Non toxic	980.28	–	–
	100	2				
	1000	5				
15b	10	1	Harmful	162.19	130.29	68.37
	100	4				
	1000	9				
15c	10	0	Non toxic	922.59	–	–
	100	1				
	1000	4				
15d	10	0	Non toxic	977.48	187.37	96.42
	100	1				
	1000	6				
17a	10	0	Non toxic	880.93	–	–
	100	2				
	1000	4				
17b	10	3	Very toxic	269.36	183.13	120.73
	100	6				
	1000	10				

[a] Ten organisms (*A. salina*) tested for each concentration.

Table 3
Anti-proliferative activities of the newly synthesized compounds.

Compound No	IC_{50} ± SEM (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
3	9.28 ± 1.66	8.30 ± 2.41	9.36 ± 1.69	9.08 ± 2.49	7.26 ± 1.80	8.08 ± 1.29
5a	8.41 ± 1.44	9.04 ± 1.22	8.04 ± 1.59	7.28 ± 1.53	4.69 ± 1.39	6.03 ± 1.53
5b	0.14 ± 0.03	0.06 ± 0.01	0.05 ± 0.03	0.25 ± 0.01	0.18 ± 0.08	0.31 ± 0.02
5c	1.49 ± 0.36	2.49 ± 0.53	2.59 ± 0.63	1.39 ± 0.79	2.39 ± 0.88	3.63 ± 0.93
7	9.31 ± 2.27	8.14 ± 2.58	6.30 ± 1.59	7.38 ± 1.38	10.16 ± 2.62	9.58 ± 2.41
9	4.48 ± 1.59	3.29 ± 0.38	6.69 ± 1.39	2.31 ± 0.48	2.08 ± 0.69	2.59 ± 0.49
11a	1.63 ± 0.88	1.08 ± 0.48	3.39 ± 0.38	1.09 ± 0.28	2.38 ± 1.08	3.55 ± 0.93
11b	8.49 ± 2.68	6.31 ± 1.59	4.25 ± 0.88	6.29 ± 1.60	2.59 ± 0.38	4.29 ± 0.38
13a	9.18 ± 2.26	8.27 ± 2.39	6.49 ± 1.69	9.27 ± 2.28	8.27 ± 1.65	7.39 ± 1.32
13b	8.26 ± 2.41	6.73 ± 1.69	8.39 ± 2.58	7.41 ± 1.63	9.38 ± 2.44	6.49 ± 2.58
13c	1.93 ± 0.39	1.24 ± 0.55	0.69 ± 0.27	2.39 ± 0.70	1.66 ± 0.59	0.89 ± 0.05
15a	7.37 ± 1.47	6.38 ± 1.41	8.62 ± 2.69	6.28 ± 1.04	7.73 ± 1.69	5.90 ± 1.52
15b	3.29 ± 0.80	2.06 ± 0.41	4.59 ± 0.93	2.04 ± 1.08	4.08 ± 1.39	2.51 ± 0.25
15c	0.32 ± 0.08	0.29 ± 0.04	0.38 ± 0.07	0.28 ± 0.03	0.80 ± 0.05	0.29 ± 0.06
15d	0.09 ± 0.02	0.11 ± 0.03	0.42 ± 0.59	1.34 ± 0.06	1.49 ± 0.39	0.89 ± 0.18
17a	0.26 ± 0.08	0.39 ± 0.08	0.19 ± 0.08	0.93 ± 0.27	1.24 ± 0.09	2.08 ± 0.27
17b	0.08 ± 0.03	0.11 ± 0.02	0.40 ± 0.02	0.29 ± 0.05	0.06 ± 0.02	0.29 ± 0.02
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 6
Docking study data showing amino acid interactions and the hydrogren bond lengths of target compounds and foretinib on c-Met kinase enzyme.

Compound number	Number of H-bonds	Number of π–π interactions with Phe^{1223}	Atoms of compound forming H-bond	Amino acid residues forming H-bonds (H-bond length in Å)	Binding energy score (Kcal/mol)
Ligand (Foretinib)	2	1	Quinoline N (H-acceptor) CO (H-acceptor)	Met^{1160} (3.05) Lys^{1110} (2.89)	−16.37
5b	3	1	NH_2 (H-donor) NH_2 (H-donor) CONH (H-donor)	Phe^{1223} (1.95) Asp^{1222} (2.5) Asp^{1222} (1.73)	−17.23
13c	2	–	$COCH_2$ (H-acceptor) CN (H-acceptor)	Met^{1160} (2.85) Tyr^{1159} (3.16)	−16.38
15c	3	1	$CONHNH_2$ (H-donor) $CONHNH_2$ (H-acceptor) CN (H-acceptor)	Phe^{1223} (1.6) Lys^{1110} (3.13) Lys^{1110} (3.12)	−17.51
15d	2	–	$CONHNH_2$ (H-donor) CN (H-acceptor)	Phe^{1223} (2.05) Met^{1160} (2.72)	−16.50
17a	2	1	CO pyridone (H-acceptor) NH_2 at C4 pyridone (H-donor)	Lys^{1110} (2.44) Glu^{1127} (1.61)	−18.03
17b	6	1	CO pyridone (H-acceptor) NH_2 at C4 pyridone (H-donor) $CONHNH_2$ (H-donor) $CONHNH_2$ (H-donor) $CONHNH_2$ (H-acceptor) $CONHNH_2$ (H-donor)	Asp^{1222} (3.08) Glu^{1127} (1.49) Asp^{1222} (1.71) Phe^{1223} (1.95) Lys^{1110} (2.92) Phe^{1223} (1.67)	−18.00

3. Conclusions

A series of heterocyclic estrone derivatives were synthesized, their inhibition towards c-Met kinase, and six typical cancer cell lines (A549, H460, HT-29, MKN-45, U87MG and SMMC-7721) was measured. The nine compounds **5b**, **5c**, **11a**, **13c**, **15b**, **15c**, **15d**, **17a** and **17b** showed the highest potency against c-Met kinase and the six cancer cell lines. These compounds were further investigated against the five tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR where compound **17b** showed the highest inhibitory effect. Compounds **5b**, **15d**, **17a** and **17b** were selected to examine their Pim-1 kinase inhibition activity where compounds **15d** and **17b** were the most active compounds. The toxicity against shrimp larvae revealed that compounds **5b**, **13c**, **15c**, **15d** and **17a** were non-toxic against the tested organisms.

4. Experimental

4.1. Synthetic methods, analytical and spectral data

The solvents used through this work were dried by distillation prior to their use. All melting points of the synthesized compounds 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 for protons and solvent signals as internal standard for carbon spectra where chemical shift values 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 progress 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).

For the nomenclature of steroid derivatives, we used the definitive

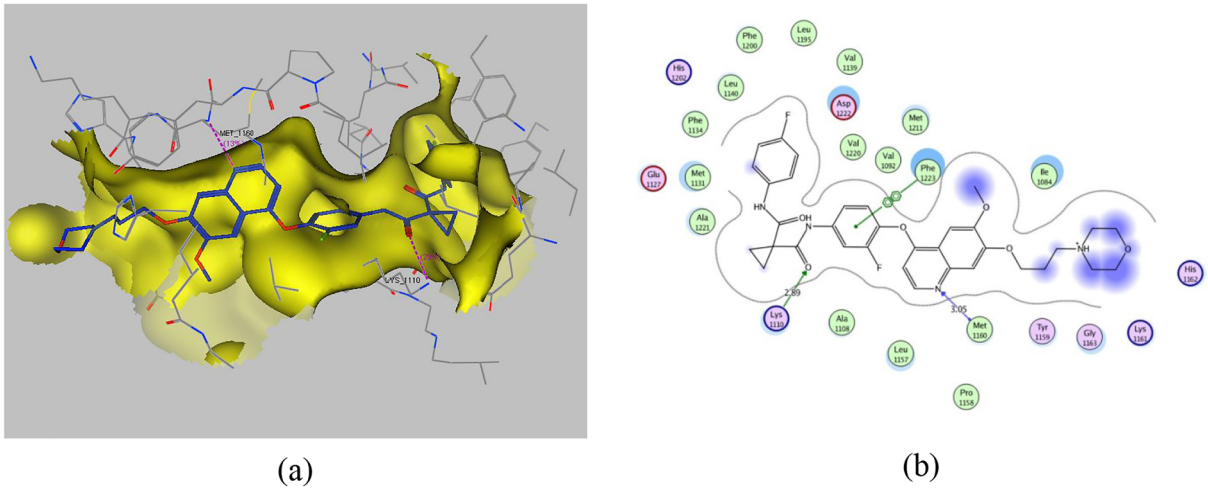


Fig. 1. Interactions of XL880 (Foretinib) with the amino acid residues of the active site of c-Met (a) 3D and (b) 2D.

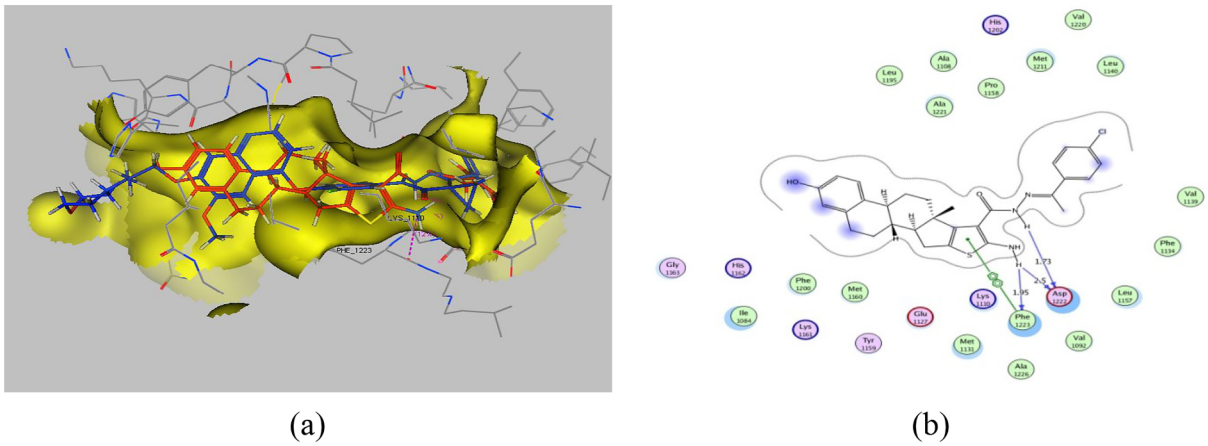


Fig. 2. (a) The superposition of Foretinib (Blue) and compound **5b** (red) docked in the binding site of c-Met, the dotted lines represent H-bonding interactions. (b) 2D ligand interaction of **5b** in binding site of c-Met. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

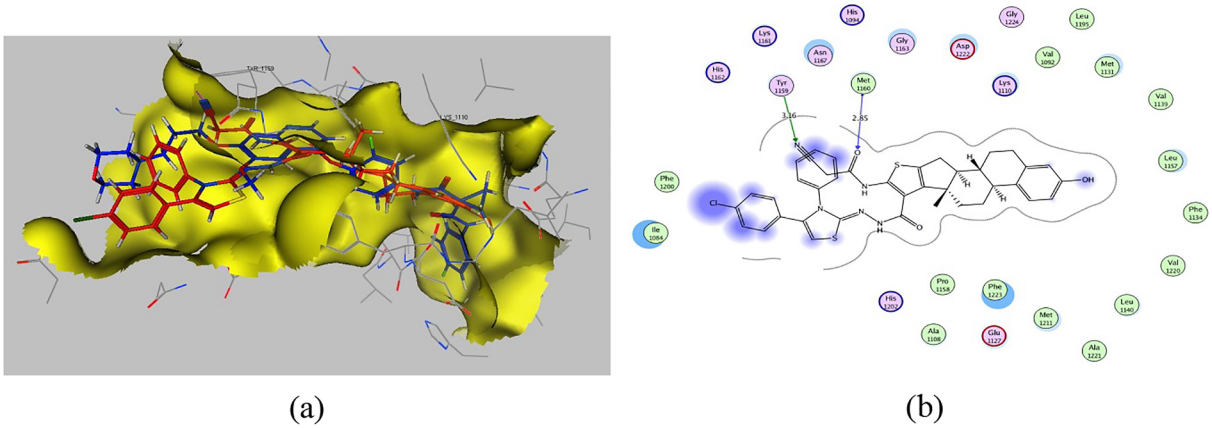


Fig. 3. (a) The superposition of Foretinib (Blue) and compound **13c** (red) docked in the binding site of c-Met, the dotted lines represent H-bonding interactions. (b) 2D ligand interaction of **13c** in binding site of c-Met. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

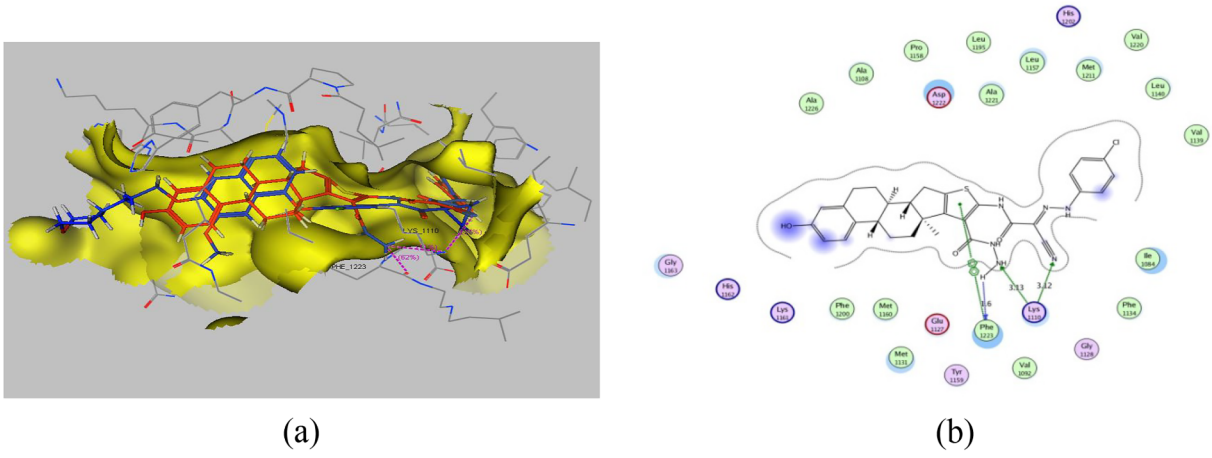


Fig. 4. (a) The superposition of Foretinib (Blue) and compound **15c** (red) docked in the binding site of c-Met, the dotted lines represent H-bonding interactions. (b) 2D ligand interaction of **15c** in binding site of c-Met. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

rules for the nomenclature of steroids published by the Joint Commission on the Biochemical Nomenclature (JCBN) of IUPAC [52,53].

*4.1.1. (6bS,8aS,12aS,12bS)-10-Amino-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b] thiophene-9-carbohydrazide (**3**)*

To a solution of estrone (**1**) (0.27 g, 0.001 mol), in absolute ethanol

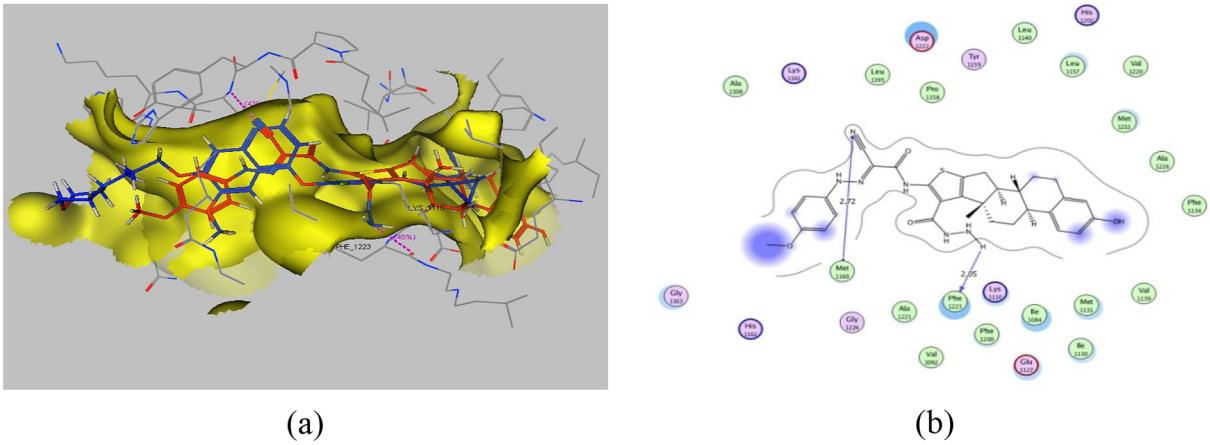


Fig. 5. (a) The superposition of Foretinib (Blue) and compound **15d** (red) docked in the binding site of c-Met, the dotted lines represent H-bonding interactions. (b) 2D ligand interaction of **15d** in binding site of c-Met. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

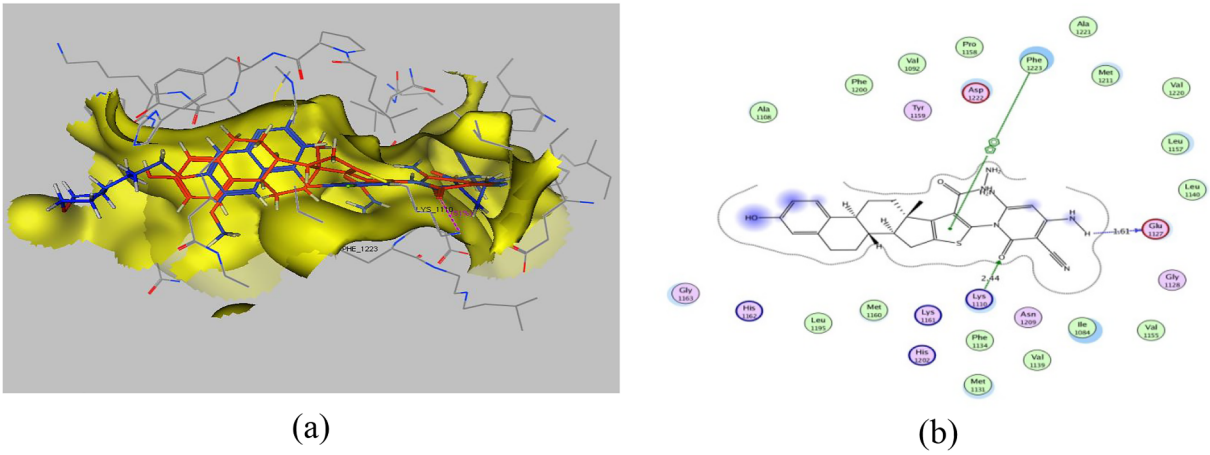


Fig. 6. (a) The superposition of Foretinib (Blue) and compound **17a** (red) docked in the binding site of c-Met, the dotted lines represent H-bonding interactions. (b) 2D ligand interaction of **17a** in binding site of c-Met. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

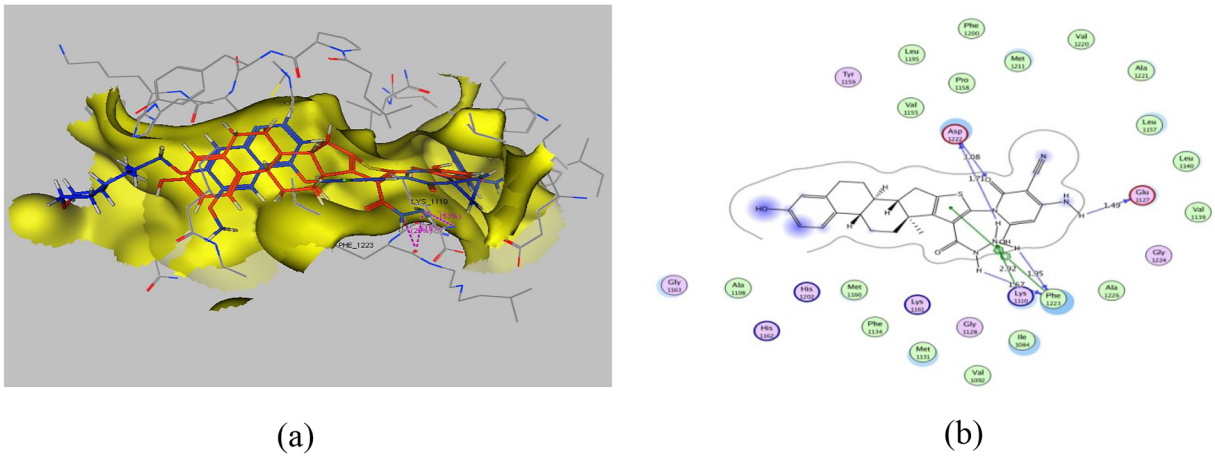


Fig. 7. (a) The superposition of Foretinib (Blue) and compound **17b** (red) docked in the binding site of c-Met, the dotted lines represent H-bonding interactions. (b) 2D ligand interaction of **17b** in binding site of c-Met. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(40 mL) containing triethylamine (0.5 mL) 2-cyanoacetylhydrazine (0.1 g, 0.001 mol) and elemental sulfur (0.032 g, 0.001 mol) were added. The whole reaction mixture was heated under reflux for 3 h then left to cool. The solid product formed upon pouring onto ice/water containing a few drops of hydrochloric acid was collected by filtration.

Pale yellow crystals from ethanol, yield 78%, M.P. 211–214 °C. IR

(KBr) cm^{-1}: 3644–3427, 3055, 2948, 1688, 1634, 1563; ^1H NMR (200 MHz, DMSO-d_6): δ 0.86 (s, 3H), 1.30–2.83 (m, 10H), 3.22 (s, 2H), 4.68, 5.22 (2 s, 4H, D_2O exchangeable), 5.73 (s, 1H), 7.27–7.40 (m, 3H), 8.20, 10.04 (2 s, 2H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.5, 19.4, 22.5, 24.7, 31.2, 31.8, 37.2, 38.9, 43.9, 48.2, 119.8, 120.4, 122.3, 125.0, 127.9, 128.2, 129.4, 132.1, 138.5, 144.2, 167.3; ESI-MS: m/z = 383 ($[M]^+$, 33%). Anal. Calcd. for $C_{21}H_{25}N_3O_2S$: C, 65.77; H, 6.57; N, 10.96; S, 8.36. Found: C, 65.58; H, 6.66; N, 10.63; S, 8.04.

*4.1.2. General procedure the synthesis of the benzylidine derivatives **5a-c***

To a solution of compound **3** (3.83 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.5 mL) any of acetophenone (1.2 g, 0.01 mol), 4-chloroacetophenone (1.54 g, 0.01 mol) or 4-methylacetophenone (1.34 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.

*4.1.2.1. (6bS,8aS,12aS,12bS)-10-Amino-4-hydroxy-8a-methyl-N′-(1-phenylethylidene)-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophene-9-carbohydrazide (**5a**)*.. Pale yellow crystals from ethanol, yield 75%, M.P. 180–183 °C. IR (KBr) cm^{-1}: 3580–3432, 3054, 2949, 1688, 1634, 1560; ^1H NMR (200 MHz, DMSO-d_6): δ 0.84 (s, 3H), 1.32–2.83 (m, 10H), 2.97 (s, 3H), 3.24 (s, 2H), 4.68 (s, 2H, D_2O exchangeable), 5.70 (s, 1H), 7.26–7.42 (m, 8H), 8.22, 10.12 (2 s, 2H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.8, 19.6, 21.3, 22.5, 24.9, 31.2, 31.8, 37.6, 38.6, 48.2, 48.5, 120.3, 121.6, 122.8, 123.3, 124.0, 125.1, 125.7, 126.1, 126.8, 127.9, 128.0, 129.2, 132.3, 138.5, 144.6, 167.0; ESI-MS: m/z = 485 ($[M]^+$, 26%). Anal. Calcd. for $C_{29}H_{31}N_3O_2S$: C, 71.72; H, 6.43; N, 8.65; S, 6.60. Found: C, 71.90; H, 6.63; N, 8.80; S, 6.73.

*4.1.2.2. (6bS,8aS,12aS,12bS)-10-Amino-N′-(1-(4-chlorophenyl)ethylidene)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophene-9-carbohydrazide (**5b**)*. Orange crystals from ethanol, yield 70%, M.P. 212–215 °C. IR (KBr) cm^{-1}: 3573–3448, 3052, 2933, 1686, 1632, 1560; ^1H NMR (200 MHz, DMSO-d_6): δ 0.85 (s, 3H), 1.34–2.85 (m, 10H), 2.99 (s, 3H), 3.23 (s, 2H), 4.64 (s, 2H, D_2O exchangeable), 5.71 (s, 1H), 7.24–7.39 (m, 7H), 8.25, 10.14 (2 s, 2H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.5, 19.7, 21.3, 22.2, 24.9, 31.3, 31.8, 37.8, 38.6, 48.3, 48.5, 120.8, 121.8, 122.3, 122.6, 123.1, 123.5, 123.8, 124.5, 125.9, 126.3, 128.5, 129.0, 134.6, 136.2, 145.8, 167.3; ESI-MS: m/z = 520 ($[M]^+$, 22%). Anal. Calcd. for $C_{29}H_{30}ClN_3O_2S$: C, 66.97; H, 5.81; N, 8.08; S, 6.17. Found: C, 67.77; H, 6.03; N, 8.21; S, 5.93.

*4.1.2.3. (6bS,8aS,12aS,-12bS)-10-Amino-4-hydroxy-8a-methyl-N′-(1-(p-tolyl)ethylidene)-2,6b,7,8,8a,12,-12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophene-9-carbohydrazide (**5c**)*. Pale yellow crystals from ethanol, yield 83%, M.P. 180–183 °C. IR (KBr) cm^{-1}: 3548–3434, 3056, 2973, 1689, 1630, 1560; ^1H NMR (200 MHz, DMSO-d_6): δ 0.84 (s, 3H), 1.36–2.83 (m, 10H), 2.97 (s, 3H), 3.09 (s, 3H), 3.24 (s, 2H), 4.65 (s, 2H, D_2O exchangeable), 5.70 (s, 1H), 7.28–7.36 (m, 7H), 8.25, 10.14 (2 s, 2H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.5, 19.7, 21.3, 22.2, 24.9, 31.3, 31.8, 37.8, 38.6, 48.3, 48.5, 120.8, 121.8, 122.0, 122.8, 123.1, 123.5, 123.8, 124.5, 125.9, 126.3, 128.5, 129.0, 134.6, 136.2, 145.8, 167.3. ESI-MS: m/z = 499 ($[M]^+$, 36%). Anal. Calcd. for $C_{30}H_{33}N_3O_2S$: C, 72.11; H, 6.66; N, 8.41; S, 6.42. Found: C, 72.30; H, 6.49; N, 8.39; S, 6.80.

*4.1.3. 2-Cyano-N-((6bS,8aS,12aS,12bS)-9-(hydrazinecarbonyl)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)acetamide (**7**)*

To a solution of compound **3** (3.83 g, 0.01 mol) in dimethylformamide (40 mL) ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 4 h then poured onto ice/water and the formed solid product was collected by filtration. White crystals from 1,4-dioxane, yield 70%, M.P. 266–269 °C. IR (KBr) cm^{-1}: 3569–3337, 3054, 2937, 2260, 1703, 1688, 1642, 1565; ^1H NMR (200 MHz, DMSO-d_6): δ 0.82 (s, 3H), 1.30–2.86 (m, 10H), 3.24 (s, 3H), 4.82 (s, 2H, D_2O exchangeable), 5.48 (s, 2H), 7.25–7.39 (m, 3H), 8.26, 8.30, 10.16 (3 s, 3H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.5, 19.7, 21.6, 22.6, 24.3, 31.3, 31.5, 37.9, 38.2, 48.6, 52.4, 116.8, 120.8, 122.9, 123.6, 124.0, 126.5, 127.8, 128.3, 132.5, 138.6, 144.6, 164.2, 166.8; ESI-MS: m/z = 450 ($[M^+$, 28%). Anal. Calcd. for $C_{24}H_{26}N_4O_3S$: C, 63.98; H, 5.82; N, 12.44; S, 7.12. Found: C, 64.24; H, 5.79; N, 12.29, S, 7.30.

*4.1.4. 2-Cyano-N-((6bS,8aS,12aS,12bS)-4-hydroxy-8a-methyl-9-(2-(phenylcarbamothioyl)hydrazinecarbonyl)-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)acetamide (**9**)*

To a solution of compound **7** (4.50 g, 0.01 mol) in 1,4-dioxane (40 mL) phenylisothiocyanate (1.35 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 1.5 h then left to cool and the formed solid product was collected by filtration. White crystals from 1,4-dioxane, yield 64%, M.P. 180–183 °C. IR (KBr) cm^{-1}: 3573–3349, 3056, 2938, 2260, 1701, 1689, 1642, 1565; ^1H NMR (200 MHz, DMSO-d_6): δ 0.83 (s, 3H), 1.30–2.88 (m, 10H), 3.26 (s, 2H), 5.22 (s, 2H), 5.49 (s, 1H), 7.27–7.37 (m, 8H), 8.23–8.27, 10.19 (5 s, 5H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.7, 19.9, 22.6, 24.3, 31.6, 31.8, 37.9, 38.0, 48.3, 48.5, 52.6, 116.5, 120.4, 121.8, 122.4, 122.6, 123.9, 124.2, 125.2, 125.8, 126.7, 127.3, 128.3, 132.8, 138.9, 143.8, 164.2, 166.8, 179.3; ESI-MS: m/z = 585 ($[M]^+$, 26%). Anal. Calcd. for $C_{31}H_{31}N_5O_3S_2$: C, 63.57; H, 5.33; N, 11.96; S, 10.95. Found: C, 63.83; H, 5.62; N, 11.73; S, 11.08.

*4.1.5. General procedure for the synthesis of the thiazole derivatives **11a,b***

To a solution of compound **9** (5.85 g, 0.01 mol) in absolute ethanol (40 mL) either ethyl chloroacetate (1.22 g, 0.01 mol) or α-chloroacetone (0.92 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then left to cool. The formed solid product, in each case, was collected by filtration.

*4.1.5.1. 2-Cyano-N-((6bS,8aS,12aS,12bS)-4-hydroxy-9-((E)-2-(4-hydroxy-3-phenylthiazol-2(3H)-ylidene)hydrazinecarbonyl)-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)acetamide (**11a**)*. Yellow crystals from ethanol, yield 70%, M.P. 166–168 °C. IR (KBr) cm^{-1}: 3529–3328, 3053, 2972, 2263, 1706, 1688, 1646, 1568; ^1H NMR (200 MHz, DMSO-d_6): δ 0.85 (s, 3H), 1.37–2.89 (m, 10H), 3.28 (s, 2H), 5.30 (s, 2H), 5.49 (s, 1H), 6.09 (s, 1H), 7.27–7.37 (m, 8H), 8.23, 8.26, 10.02, 10.19 (4 s, 4H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.4, 19.7, 24.3, 31.3, 31.8, 37.8, 38.1, 48.5, 48.7, 52.8, 116.9, 120.3, 122.4, 124.0, 124.2, 126.9, 127.3, 127.6, 128.0, 128.8, 130.7, 131.4, 132.8, 138.9, 140.6, 142.7, 143.8, 164.6, 166.4, 171.3; ESI-MS: m/z = 625 ($[M]^+$, 33%). Anal. Calcd. for $C_{33}H_{31}N_5O_4S_2$: C, 63.34; H, 4.99; N, 11.19; S, 10.25. Found: C, 63.74; H, 5.06; N, 11.28; S, 10.59.

*4.1.5.2. 2-Cyano-N-((6bS,8aS,12aS,12bS)-4-hydroxy-8a-methyl-9-((E)-2-(4-methyl-3-phenylthiazol-2(3H)-ylidene)hydrazinecarbonyl)-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)acetamide (**11b**)*. Orange crystals from ethanol, yield 83%, M.P. 190–193 °C. IR (KBr) cm^{-1}: 3539–3338, 3056, 2973, 2262, 1690, 1689, 1633, 1572; ^1H NMR (200 MHz, DMSO-d_6): δ 0.83 (s, 3H), 1.34–2.86 (m, 10H), 3.08 (s 3H), 3.29 (s, 2H), 5.33 (s, 2H), 5.46 (s, 1H), 6.12 (s, 1H), 7.23–7.39 (m, 8H), 8.22, 8.39, 10.21 (3 s, 3H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.1, 19.8, 22.1, 24.3, 31.6, 31.8, 37.4, 38.0, 48.5, 48.6, 52.9, 116.7, 120.6, 121.6, 122.6, 123.6, 124.2, 126.3, 127.0, 128.3, 128.8, 129.9, 130.6, 131.6, 133.6, 140.8, 142.9, 143.6, 164.8, 166.2, 170.2; ESI-MS: m/z = 623 ($[M]^+$,

41%). Anal. Calcd. for $C_{34}H_{33}N_5O_3S_2$: C, 65.47; H, 5.33; N, 11.23; S, 10.28. Found: C, 65.19; H, 5.27; N, 11.33; S, 10.42.

*4.1.6. General procedure for the synthesis of the thiazole derivatives **13a-c***

To a solution of compound **9** (5.85 g, 0.01 mol) in absolute ethanol (40 mL) any of ω-bromoacetophenone (1.99 g, 0.01 mol), 4-methyl-ω-bromoacetophenone (2.13 g, 0.01 mol), or 4-chloro-ω-bromoacetophenone (2.33 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then left to cool. The formed solid product, in each case, was collected by filtration.

*4.1.6.1. 2-Cyano-N-((6bS,8aS,12aS,12bS)-9-((E)-2-(3,4-diphenylthiazol-2(3H)-ylidene)hydrazinecarbonyl)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b] thiophen-10-yl)acetamide (**13a**)*. Orange crystals from ethanol, yield 69%, M.P. 133–136 °C. IR (KBr) cm^{-1}: 3548–3337, 3057, 2970, 2260, 1693, 1688, 1650, 1637, 1587; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.84 (s, 3H), 1.32–2.88 (m, 10H), 3.26 (s, 1H), 5.21 (s, 2H), 5.32 (s, 2H), 6.11 (s, 1H), 7.24–7.38 (m, 13H), 8.23, 8.26, 10.24 (3 s, 3H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.1, 19.8, 24.3, 31.8, 31.9, 37.6, 38.0, 48.8, 48.7, 52.9, 116.9, 120.8, 121.6, 121.9, 122.8, 123.6, 124.5, 124.9, 125.0, 125.7, 126.9, 127.2, 128.3, 129.2, 129.6, 130.9, 131.2, 133.9, 140.2, 142.4, 144.5, 164.3, 166.6, 171.0; ESI-MS: m/z = 685 ([M]$^+$, 38%). Anal. Calcd. for $C_{39}H_{35}N_5O_3S_2$: C, 68.30; H, 5.14; N, 10.21; S, 9.35. Found: C, 68.59; H, 5.42; N, 10.31; S, 9.53.

*4.1.6.2. 2-Cyano-N-((6bS,8aS,12aS,12bS)-4-hydroxy-8a-methyl-9-((E)-2-(3-phenyl-4-(p-tolyl)thiazol-2(3H)-ylidene)hydrazinecarbonyl)-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b] thiophen-10-yl)acetamide (**13b**)*. Yellow crystals from ethanol, yield 79%, M.P. 180–183 °C. IR (KBr) cm^{-1}: 3532–3312, 3055, 2972, 2256, 1691, 1689, 1639, 1583; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.83 (s, 3H), 1.30–2.89 (m, 10H), 3.09 (s, 3H), 3.28 (s, 1H), 5.24 (s, 2H), 5.30 (s, 2H), 6.10 (s, 1H), 7.26–7.39 (m, 12H), 8.21, 8.58, 10.23 (3 s, 3H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.2, 19.8, 21.9, 24.6, 31.3, 31.9, 37.6, 38.0, 48.6, 48.8, 52.3, 116.5, 120.7, 122.7, 123.0, 123.6, 123.8, 124.3, 124.9, 125.3, 126.2, 126.9, 127.0, 128.5, 129.1, 129.6, 130.3, 131.0, 133.4, 140.6, 142.8, 144.5, 164.2, 166.9; ESI-MS: m/z = 699 ([M]$^+$, 22%). Anal. Calcd. for $C_{40}H_{37}N_5O_3S_2$: C, 68.64; H, 5.33; N, 10.01; S, 9.16. Found: C, 68.81; H, 5.50; N, 10.31; S, 9.41.

*4.1.6.3. N-((6bS,8aS,12aS,12bS)-9-((E)-2-(4-(4-Chlorophenyl)-3-phenylthiazol-2(3H)-ylidene)hydrazinecarbonyl)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b] thiophen-10-yl)-2-cyanoacetamide (**13c**)*. Yellow crystals from ethanol, yield 58%, M.P. 221–223 °C; IR (KBr) cm^{-1}: 3548–3331, 3053, 2978, 2252, 1690, 1687, 1632, 1580; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.83 (s, 3H), 1.32–2.89 (m, 10H), 3.24 (s, 1H), 5.26 (s, 2H), 5.31 (s, 2H), 6.10 (s, 1H), 7.28–7.42 (m, 12H), 8.23, 8.28, 10.20 (3 s, 3H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.4, 19.3, 24.3, 31.6, 31.8, 37.8, 38.3, 48.4, 48.9, 52.5, 116.8, 120.3, 122.8, 123.8, 124.5, 124.9, 125.3, 125.6, 126.0, 126.3, 126.8, 127.3, 127.5, 128.0, 128.6, 129.9, 130.8, 132.8, 133.9, 140.3, 143.1, 144.0, 164.8, 166.4. ESI-MS: m/z = 719 ([M]$^+$, 18%). Anal. Calcd. for $C_{39}H_{34}ClN_5O_3S_2$: C, 65.03; H, 4.76; N, 9.72; S, 8.90. Found: C, 64.93; H, 4.43; N, 9.83; S, 9.18.

*4.1.7. General procedure for the synthesis of the hydrazonoyl derivatives **15a-d***

To a solution of compound **7** (4.5 g, 0.01 mol) in ethanol (50 mL) containing sodium acetate (3.5 g) any of the diazonium salts namely benzenediazonium chloride (0.01 mol), p-tolyldiazonium chloride (0.01 mol), 4-chlorobenzenediazonium chloride or 4-methoxybenzenediazonium chloride (0.01 mol) [prepared via the addition of sodium nitrite solution (0.69 g, 0.01 mol) in water (10 mL) to a cold solution (0–5 °C) of the appropriate aromatic amine dissolved in concentrated hydrochloric acid (10 mL, 18 mol) with continuous stirring] was added with continuous stirring. The whole reaction, in each case, was stirred at room temperature for 2 h and the formed solid product was collected by filtration.

*4.1.7.1. 2-(((6bS,8aS,12aS,12bS)-9-(Hydrazinecarbonyl)-4-hydroxy-8a-methyl-2,6b,7,8,8a,-12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)amino)-2-oxo-N′-phenylacetohydrazonoyl cyanide (**15a**)*. Red crystals from ethanol, yield 78%, M.P. 210–213 °C. IR (KBr) cm^{-1}: 3569–3348, 3054, 2978, 2243, 1690, 1689, 1630, 1582; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.85 (s, 3H), 1.33–2.86 (m, 10H), 3.28 (s, 2H), 4.39 (s, 2H, D_2O exchangeable), 6.18 (s, 1H), 7.22–7.36 (m, 8H), 8.23, 8.29, 8.30, 10.24 (4 s, 4H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.3, 19.2, 24.6, 31.7, 31.8, 37.8, 38.0, 48.8, 48.9, 52.3, 117.2, 119.5, 120.3, 121.4, 122.8, 123.6, 126.9, 128.3, 129.7, 133.4, 134.6, 136.2, 140.2, 142.4, 143.3, 144.5, 164.3, 166.6; ESI-MS: m/z = 554 ([M]$^+$, 28%). Anal. Calcd. for $C_{30}H_{30}N_6O_3S$: C, 64.96; H, 5.45; N, 15.15; S, 5.78. Found: C, 64.72; H, 5.80; N, 15.28; S, 5.57.

*4.1.7.2. 2-(((6bS,8aS,12aS,-12bS)-9-(Hydrazinecarbonyl)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)amino)-2-oxo-N′-(p-tolyl)aceto-hydrazonoyl cyanide (**15b**)*. Red crystals from ethanol, yield 68%, M.P. 166–169 °C. IR (KBr) cm^{-1}: 3580–3327, 3055, 2966, 2240, 1693, 1689, 1624, 1572; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.83 (s, 3H), 1.32–2.89 (m, 10H), 2.99 (s, 3H), 4.33 (s, 2H, D_2O exchangeable), 5.28 (s, 2H), 6.13 (s, 1H), 7.20–7.38 (m, 7H), 8.20, 8.26, 8.32, 10.26 (4 s, 4H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.3, 19.2, 24.6, 31.7, 31.8, 37.8, 38.0, 48.8, 48.9, 52.3, 117.2, 119.9, 120.3, 121.8, 123.6, 126.9, 128.2, 130.7, 133.4, 134.3, 136.8, 140.1, 142.2, 143.8, 144.2, 164.0, 166.8, 172.6; ESI-MS: m/z = 568 ([M]$^+$, 33%). Anal. Calcd. for $C_{31}H_{32}N_6O_3S$: C, 65.47; H, 5.67; N, 14.78; S, 5.64. Found: C, 65.72; H, 5.83; N, 14.90; S, 5.48.

*4.1.7.3. N′-(4-Chlorophenyl)-2-(((6bS,8aS,12aS,12bS)-9-(hydrazinecarbonyl)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)amino)-2-oxoacetohydrazonoyl cyanide (**15c**)*. Orange crystals from ethanol, yield 73%, M.P. 188–190 °C. IR (KBr) cm^{-1}: 3565–3347, 3055, 2980, 2253, 1690, 1689, 1628, 1561. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.84 (s, 3H), 1.33–2.87 (m, 10H), 4.35 (s, 2H, D_2O exchangeable), 5.29 (s, 2H), 6.14 (s, 1H), 7.24–7.40 (m, 7H), 8.26, 8.28, 8.32, 10.41 (4 s, 4H, D_2O exchangeable). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.2, 19.8, 24.6, 31.6, 31.7, 37.8, 38.3, 48.8, 48.9, 52.3, 117.2, 120.0, 121.3, 121.9, 123.6, 127.4, 129.6, 130.7, 133.6, 134.3, 136.8, 140.3, 142.2, 143.8, 144.2, 164.2, 166.3, 172.8. ESI-MS: m/z = 589 ([M]$^+$, 20%). Anal. Calcd. for $C_{30}H_{29}ClN_6O_3S$: C, 61.16; H, 4.96; N, 14.27; S, 5.44. Found: C, 61.29; H, 5.04; N, 14.30; S, 5.62.

*4.1.7.4. 2-(((6bS,8aS,12aS,12bS)-9-(Hydrazinecarbonyl)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12, 12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophen-10-yl)amino)-N′-(4-methoxyphenyl)-2-oxoacetohydrazonoyl cyanide (**15d**)*. Orange crystals from ethanol, yield 68%, M.P. 142–145 °C. IR (KBr) cm^{-1}: 3529–3317, 3056, 2983, 2256, 1693, 1683, 1620, 1543. ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.82 (s, 3H), 1.31–2.89 (m, 10H), 2.89 (s, 3H), 3.21 (s, 1H), 4.37 (s, 2H, D_2O exchangeable), 5.23 (s, 2H), 7.26–7.38 (m, 7H), 8.23, 8.25, 8.30, 10.41 (4 s, 4H, D_2O exchangeable).^{13}C NMR (75 MHz, DMSO-d_6): δ 13.4, 19.8, 21.7, 24.6, 31.7, 31.8, 37.8, 38.3, 48.2, 48.9, 52.0, 116.9, 120.1, 122.3, 122.4, 126.0, 127.8, 128.3, 129.5, 133.8, 134.6, 138.2, 140.1, 142.4, 143.6, 144.2, 164.2, 166.6, 172.4; ESI-MS: m/z = 584 ([M]$^+$, 38%). Anal. Calcd. for $C_{31}H_{32}N_6O_4S$: C, 63.68; H, 5.52; N, 14.37; S, 5.48. Found: C, 63.49; H, 5.39; N, 14.24; S, 5.70.

*4.1.8. General procedure for the synthesis of the pyridine derivatives **17a,b***

To a solution of compound **7** (4.5 g, 0.01 mol) in ethanol (50 mL) containing triethylamine (0.5 mL) 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 6 h then poured onto ice/water mixture containing few drops of hydrochloric acid and the formed solid product was collected.

*4.1.8.1. 6bS,8aS,12aS,12bS)-10-(4,6-Diamino-3-cyano-2-oxopyridin-1(2H)-yl)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]indeno[2,1-b]thiophene-9-carbohydrazide **(17a)***. Yellow crystals from ethanol, yield 83%, M.P. 188–192 °C. IR (KBr) cm^{-1}: 3542–3348, 3056, 2972, 2223, 1690, 1688, 1639, 1580; ^{1}H NMR (DMSO-d_6): δ 0.83 (s, 3H), 1.32–2.89 (m, 10H), 3.06 (s, 2H), 3.23 (s, 1H), 4.25, 4.68, 5.13 (3 s, 6H, D_2O exchangeable), 6.33 (s, 1H), 7.28–7.36 (m, 3H), 8.19, 10.22 (2 s, 2H, D_2O exchangeable); ^{13}C NMR (DMSO-d_6): δ 13.2, 19.8, 21.9, 24.6, 31.9, 37.6, 38.0, 48.6, 48.8, 52.3, 116.8, 120.7, 122.7, 123.0, 124.3, 124.9, 127.0, 128.2, 128.7, 129.4, 130.3, 132.7, 136.5, 140.6, 144.5, 164.3, 165.6; ESI-MS: m/z = 516 ($[M]^+$, 18%). Anal. Calcd. for $C_{27}H_{28}N_6O_3S$: C, 62.77; H, 5.46; N, 16.27; S, 6.21. Found: C, 62.51; H, 5.38; N, 16.08; S, 6.33.

*4.1.8.2. (6bS,8aS,12aS,12bS)-10-(4-Amino-3-cyano-6-hydroxy-2-oxopyridin-1(2H)-yl)-4-hydroxy-8a-methyl-2,6b,7,8,8a,12,12a,12b-octahydro-1H-naphtho[2′,1′:4,5]-indeno[2,1-b]thiophene-9-carbohydrazide **(17b)***. Yellow crystals from ethanol, yield 77%, M.P. 144–146 °C. IR (KBr) cm^{-1}: 3563–3325, 3054, 2970, 2220, 1691, 1689, 1636, 1582; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.82 (s, 3H), 1.31–2.87 (m, 10H), 3.04 (s, 2H), 3.22 (s, 1H), 4.23, 5.16 (2 s, 4H, D_2O exchangeable), 6.30 (s, 1H), 7.26–7.38 (m, 3H), 8.23, 10.03, 10.22 (3 s, 3H, D_2O exchangeable); ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.2, 19.8, 21.9, 24.6, 31.9, 37.6, 38.0, 48.6, 48.8, 52.3, 116.8, 120.7, 122.1, 122.9, 123.0, 124.5, 127.3, 129.4, 130.2, 134.6, 138.2, 139.8, 140.2, 142.5, 144.3, 164.2, 164.9; ESI-MS: m/z = 517 ($[M]^+$, 40%). Anal. Calcd. for $C_{27}H_{27}N_5O_4S$: C, 62.65; H, 5.26; N, 13.53; S, 6.19. Found: C, 62.73; H, 5.41; N, 13.63; S, 6.24.

Conflict of interest

The author(s) confirm that this article content has no conflict of interest.

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