Bioorganic Chemistry 84 (2019) 493–504

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Structure-activity relationship of pyrazolo pyrimidine derivatives as inhibitors of mitotic kinesin Eg5 and anticancer agents

P. Muthuraja[a], V. Veeramani[a], S. Prakash[a], M. Himesh[b], U. Venkatasubramanian[b], P. Manisankar[a,*]

[a] Department of Industrial Chemistry, Alagappa University, Karaikudi 630006, India
[b] Department of Biotechnology, School of Chemical & Biotechnology, SASTRA Deemed University, Thanjavur 613401, India

ARTICLE INFO

Keywords:
Pyrazaole
Thienopyridines
Kinesin spindle protein
Anticancer agents

ABSTRACT

Human kinesin Eg5 is a potential inhibiting site for cancer chemotherapy. Blocking metaphase by binding foreign inhibitors with Eg5 eventually leads to apoptotic cell death. Here, we report the pyrazolopyrimidine derivates as potent inhibitors of Eg5 that prevents mitotic kinesin progression. IC_{50} values were evaluated against the motor domain of Eg5 using steady-state ATPase assay. To better understanding, we have performed molecular docking simulation. It reveals that the interactions of the proposed inhibitors with both the allosteric sites (helices α2, α3 and loopL5, and helices α4 & α6). Out of fifteen pyrazolopyrimidine derivates, three compounds (12, 25, and 27) have shown significant inhibition of Eg5. The synthesized compounds (12, 25, and 27) were tested for their in-vitro anticancer activity against cervical cancer cell line (HeLa).

1. Introduction

The recent clinical data indicate the extent population affected by cancer directly and indirectly, demands the development new drugs with desired medicinal efficiency that may be synthesized by adopting by simple environmental friendly synthetic methods. Among various mechanisms adopted to control cancer, interfering with the spindle formation has been evolved as a successful mechanism. Spindle formation is one of the important steps in cell multiplication in which spindle formation followed by apoptosis [1–4]. Kinesins a family of molecular motors described as nano-machines convert the chemical energy of ATP into mechanical force used to drive the intracellular cargos towards the plus end of the MT and hence play a critical role in cell cycles [5]. Eg5 known as KSP also a member of kinesin 5 subfamily is an essential factor in mitotic spindle formation hence inhibition of Eg5 will result in monopolar spindles instead of bipolar spindles that may arrest the cell cycle. In recent days several researchers have developed number of Eg5 inhibitors and have established the mechanism of action through SAR studies [6–13]. Various reports reveal that a set of Eg5 inhibitors of biphenyl type such as monastrol, STLC, ispinesib, SB-743921, and ARRY-520 and benzimidazoles bind to the allosteric pocket formed by α2 and α3 helices of L5 at about 10 Å from Eg5 motor domain bound to ATP [5]. It has been observed that these inhibitors established some mutations in various cell culture studies. One of the benzimidazoles, BI8 type of KSP inhibitor were reported to bound to an allosteric pocket formed by helices α4 and α6 in addition to α2 and α3 helices of L5. The identification of α4 and α6 allosteric inhibitor-binding pocket (formed by helix α2&α4) provides the opportunity to develop new series of inhibitors that could be used either alone or in combination with existing Eg5 compounds.

Heterocyclic compounds are more common in biological systems as enzyme cofactors, amino acids and proteins that are playing vital role in the metabolism of living systems. The heterocycles involve in number of intermolecular activities such as hydrogen bonding, coordination to metal atoms, and vander Waals hydrophobic interaction. The possible wide range of shapes and sizes of heterocycles favors different structural range of enzyme binding activities. Thus the extremely common occurrence and role in variety of biological activities make the heterocycles as important medicinal compounds [13]. Sarcoma genes (Src) that potentially induces cell malignant transformation plays significant role in gene/kinase. Thus Src inhibitors may be prospective agents in mitigating pancreatic cancer, breast cancer, stomach cancer etc. A series of pyrazole pyrimidine derivatives have been reported to exhibit significant clinical efficiency as Src kinase inhibitors [14]. Zhang et al has reported that phenyl ethyl substituted pyrazole pyrimidine is a potential agent to treat triple negative breast cancer through their in vitro and in vivo studies [15]. They have also reported that this pyrazole pyrimidine can inhibit Src kinase as well as several other kinase

* Corresponding author.
E-mail addresses: venkatasubramanian@scbt.sastra.edu (U. Venkatasubramanian), manisankarp@alagappauniversity.ac.in (P. Manisankar).

https://doi.org/10.1016/j.bioorg.2018.12.014
Received 18 September 2018; Received in revised form 4 December 2018; Accepted 10 December 2018
Available online 12 December 2018

involve in MAPK signal transduction [16]. Pyrazole pyrimidine derivatives exhibited tumor growth suppression efficiency without any toxic effect as a potential drug for EGFR mutant-driven NSCLC [17,18]. Hela et al has reported that pyrazole pyrimidine derivative act as PDE5A inhibitors [19,20]. Pyrazole pyrimidines were also reported to have higher aqueous solubility with enhanced pharmacokinetic properties and hence a better cytotoxic agent against human glioblastoma U87 cell line [21,22]. Zapf et al has reported that acrylamide substituted pyrazolo pyrimidine acts a nonreceptor tyrosine kinase (Itk) inhibitor [23]. 4-benzyl-1-(7H-pyrrolo[2,3-*d*]pyrimidin-4-yl)piperidin-4-amines was reported to be Akt (PKB) type of inhibitor by preventing deregulation of intracellular signaling pathways [24]. Several pyrazole pyrimidines were reported to have efficient cytotoxic activity even in nanomolar concentrations with promising selectivity against merkinase that may cause childhood acute lymphoblastic leukemia. These pyrazole pyrimidines were also reported to act as chemosensitizers with low toxicities and excellent pharmacokinetic properties [25]. A set thienopyridines have been reported to exhibit superior Bruton's tyrosine kinase (BTK) inhibitory activity with outstanding hydrophilicity and selectivity [26] and also CHK1 inhibition through hydrogen bonding even in single digit nanomolar concentrations [27].

In the present study, we have synthesized several substituted pyrazole pyrimidine pharmacore and thienopyridines pharmacore compounds and evaluated their *Hs*Eg5 inhibitory activities and their specificity. To validate, we have screened the molecules against motor domain of human mitotic kinesin Eg5 using molecular docking (*In silico*), steady-state ATPase assay (*In vitro*). In addition, cell viability assay was performed to determine the nontoxic dose.

2. Results and discussion

2.1. Chemistry

A set of pyrazolopyrimidine derivatives **8–19** were synthesized by direct reductive amination of scaffold **6** with the corresponding aromatic aldehydes **7a-7l** in presence of acetic acid Scheme 1 and another set of derivatives **20–21** obtained from the scaffold **6** and the aldehydes **7m** and **7n** in presence of triethylamine (TEA) Scheme 2. Similarly, pyrazolopyrimidine derivative **25** was obtained from **23** and **24** through *N*-alkylation [28–31] (Scheme 3). The morpholine substituted pyrazolopyrimidine derivative **27** was synthesized from **18** and **26** via Suzuki Miyaura cross-coupling reaction (Scheme 4). The synthetic conditions were optimized by using various palladium catalysts such as $Pd(OAc)_2$, $Pd(dba)_2$, $PdCl_2(PPh_3)_2$ and $Pd(PPh_3)_4$ and different bases like NaOEt, KOtBu and K_3PO_4 Table 1.

The maximum yield was obtained in presence of the catalyst Pd $(dba)_2$ and KOtBu. A different set of novel 3-bromo-7-(5-(morpholinomethyl)thiophen-2-yl)thieno[3,2-*c*]pyridin-4-amine derivatives (**30–32**) were synthesized by adopting Suzuki coupling reaction between 3-bromo-7-(5-(morpholinomethyl)thiophen-2-yl)thieno[3,2-*c*]pyridin-4-amine **28** (1.0 equiv) and different aryl/heteroaryl boronic acids **29a–29c** (1.5 equiv) in presence of various palladium catalyst and different bases to optimize the reaction conditions (Schemes 5, Table 2). The key intermediate **28** was synthesized by adopting the procedure in literatures [32–34].

The structure of a representative compound from each scheme have been established from the FT-IR, NMR, 2D NMR and LC-MS spectroscopic data given in supporting information. HMB correlation is presented in Fig. 1. ^{1}H NMR spectrum of **12**, methylthio protons appear as a singlet at 2.44 ppm (s, 3H) that correlates with C,H-COSY signals at 13.0 ppm corresponding to the methylthio carbon. The signal at 2.44 ppm also shows HMB correlation contour signals at 154 ppm which is assigned to C-7. The singlet at 3.77 ppm is assigned to the methoxy proton (s, 3H). This signal has a C,H-COSY contour with the signal at 56.7 ppm. The signal at 3.77 ppm also shows HMB correlation contour signal at 149 ppm and it is assigned to C-3″. The singlet at 8.14 ppm, which shows C,H-COSY correlation with carbon signal at 159 ppm is assigned to the pyrimidine proton as evident from its HMB correlation with C-1 aromatic carbon at 164.87 ppm and C-9 aromatic carbon at 147 ppm. Phenyl hydroxy protons result in a singlet at 9.84 ppm (s, 1H) and it has C,H-COSY correlation with the signals at 150, 119 and 149 ppm which are assigned to C-2″, C-1″ and C-3″ respectively. The ortho-protons of the phenyl group are observed as doublet at 7.98 ppm (J = 3.87 Hz), two meta-protons as triplet at 7.337 ppm (J = 8.1 Hz), 7.321 ppm (J = 7.8 Hz), and the para-proton as triplet at 7.86 ppm (J = 7.4 Hz). The C,H-COSY correlation of these protons assigned to the carbon signals of *o-,* two-*m-,* and *p*-carbons at 121, 123.3, 116.3 and 120 ppm respectively. The signals at 7.321 and 7.337 ppm are due to *m*-protons of the fluorophenyl ring and are having HMB correlation contour signals with C-1′ at 150 ppm. The ^{1}H NMR signals of *p*-protons at 7.86 ppm and it also shows HMB correlation contour signals at C-2′ at 161.71 ppm and C-6′ at 121 ppm. The doublet at 6.96 ppm (J = 7.7 Hz), triplet at 6.79 ppm (J = 7.8 Hz), doublet at 7.392 ppm (J = 7.7 Hz) are due to C-4″, C-5″ and C-6″ respectively. The C,H-COSY correlation of these protons are assigned to the carbon signals at 120, 116.4, and 123.15 ppm to the *p*-, *m*-, and *o*-carbons respectively. The signal of *p*-protons at 6.96 ppm shows HMB correlation contour signals at 150 ppm, and 123.15 ppm with C-2″ and C-6″. The *m*-proton signal at 6.79 ppm also shows HMB correlation contour signals at 149, and 119 ppm with C-3″ and C-6″ respectively.

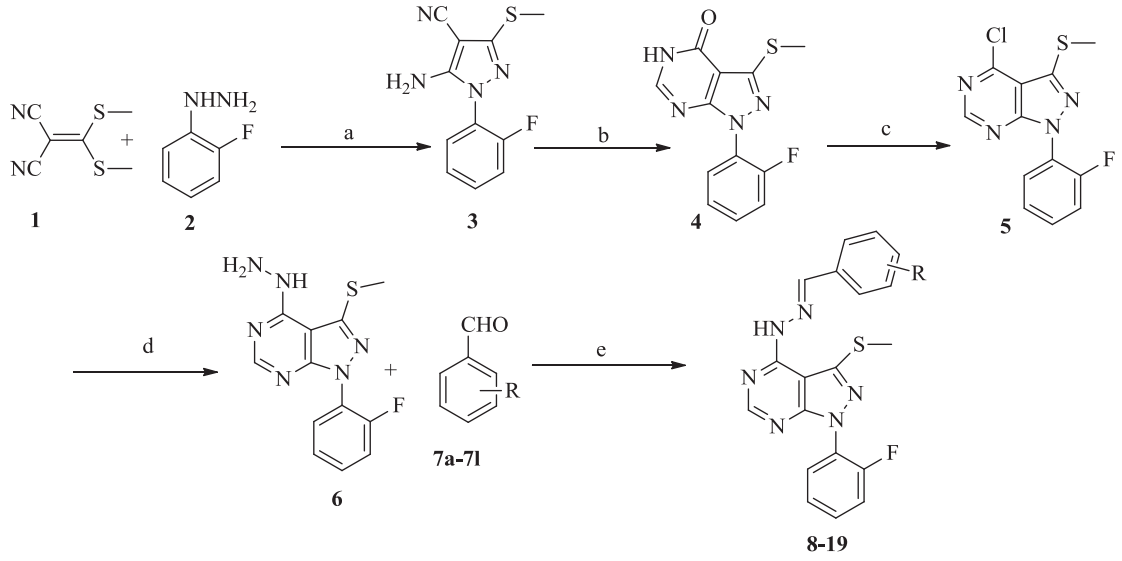
2.2. SAR Studies, inhibition of Eg5 ATPase activity

All the synthesized compounds were tested against the motor domain of the human mitotic kinesin Eg5 [35]. A steady state ATPase activity assay was carried out with STLC as a standard compound. A set of compounds tested 12, 25 and 27 showed activities in micromolar range concentrations against the Eg5 (Fig. 2).

In order to understand the protein-ligand interactions, we have carried out molecular docking studies using maestro software and analyzed the structures of the best docking poses. The results are presented in the Table 3. It has been reported that the KSP contains two allosteric binding sites where the potential inhibitors could bind. The site-I formed by helix α2, helix α3 & the Loop L5 and most of the known compounds bind to this. The site-II which has been identified recently is the helix α4 and helix α6. In order to identify weather, the inhibitor binds to either of the sites or to any one site we have docked the molecules to both positions and analyzed the interactions separately. We have compared the interactions of these molecules with Site 2 to the interactions of two different types of inhibitors Benzimidazole type inhibitor BI8 (PDB ID: 3ZCW) and the biphenyl type inhibitor (PDB ID: 3WPN) PVZB1194 that were well established and for the site 1 we have used the tight binding inhibitor STLC (PDB ID: 2WOG) as the reference.

Unlike for site 2 which has only two protein-inhibitor complex structures, plenty of structures of KSP are available with inhibitor present in site 1. The binding energy of the tested compounds significantly higher at site 1 compared to site 2. In site 1, the ligand interactions are primarily hydrophobic and aromatic in nature. In the crystal structure (PDB ID: 2WOG) the reference compound STLC forms hydrophobic interactions with the side chains of Glu215, Glu116, Arg119 and Pro137 and aromatic π-π interactions through Trp127 and Tyr211. Our compounds also form similar type of interactions Figs. 3–5.

Compound 12 forms hydrophobic interaction with residues Arg221, Thr112 and Thr222 through the 1-(2-fluorophenyl)-1H-pyrazolo[3,4-*d*] pyrimidine and with Gly117, Glu118, Gly217 and Trp127 through the o-cresol group. It also forms weak hydrogen bonds with NH of Arg119 (D···A = 2.97 Å). Compound 11 doesn't form any hydrogen bond but it forms hydrophobic interactions residues Arg221, Thr112 and Thr222 through the 1-(2-fluorophenyl)-1H-pyrazolo[3,4-*d*]pyrimidine and with Gly117, Glu118, Gly217 and Ala218 through the 3-methoxyphenol group. The compound 27 which doesn't form any hydrogen bond and surrounded by amino acid residuesThr112, Phe113, Glu118, Arg221,

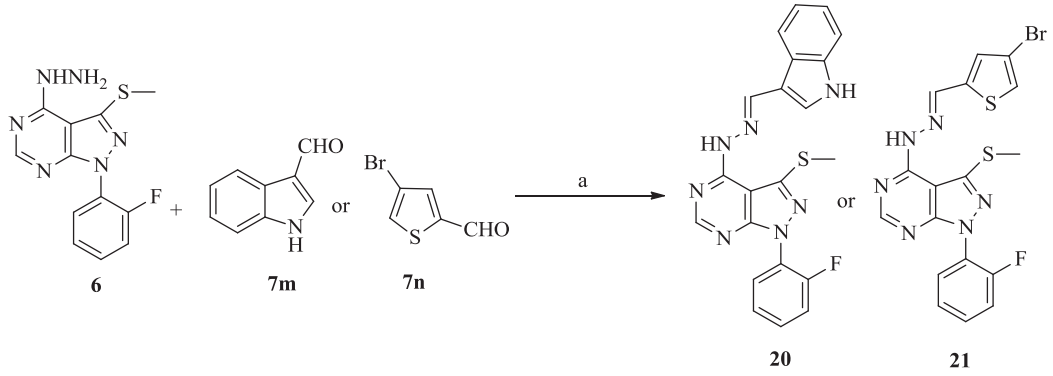


[a]Reagents and conditions: (a) 2-(bis(methylthio)methylene)malononitrile **1** (1.0 equiv), 2-fluorophenylhydrazine **2** (1.0 equiv), DIPEA (2.0 equiv), ethanol, reflux 6 h; (b)1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazole-4,5-dicarbonitrile **3** (1.0 equiv), formic acid (85%, 35 mL), ethanol, reflux, 10 h; (c) 1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one **4**, $POCl_3$(65 mL), methanol, reflux 6 h; (d) 4-chloro-1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidine **5** (1.0 equiv), hydrazine hydrate (4.0 equiv excess), methanol, 60 °C, 3 h; (e) 1-(2-fluorophenyl)-4-hydrazinyl-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidine **6** (1.0 equiv), aromatic aldehyde (**7a-7l**), 60 °C, CH_3COOH (2 mL), methanol, 3 h;

Scheme 1. Synthesis of pyrazole pyrimidine derivatives **8–19**.

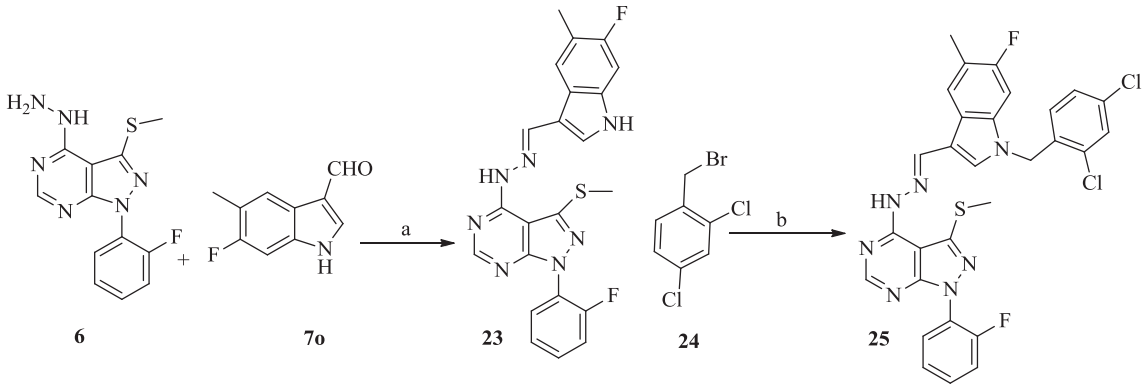
Ser232, Ser233 and Leu266 within the 4 Å region of the site 1.

The newly identified site 2 defined primarily by helix α4 and α6. For this site 2, two types protein inhibitor complex structures have been reported so far which suggest the interactions are primarily hydrophobic and π-π stacking. The aromatic π interaction with residue Tyr352 has been confirmed by mutational studies. In both reference molecules the Tyr352 residue forms face to face π-π interaction with pyrazole pyrimidine group of the ligands. All the three active compounds in our study have similar interactions with those residues Fig. 6. The 7H-pyrrolo[3,4-*d*]pyrimidine group of the compounds 12, 25 forms a hydrogen bond and edge to face π-π interaction with Tyr 352. The amino group attached with the pyrazole pyrimidine of compound 12 and compound 25 forms one hydrogen bond with main chain of oxygen of the Tyr352 (D···A = 2.6 Å). The o-cresol group of compounds 12 and



[a]Reagents and conditions: a) 1-(2-fluorophenyl)-4-hydrazinyl-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidine **6** (1.0 equiv), aromatic aldehyde, 60 ° DIPEA (2.0 equiv), methanol, 3 h;

Scheme 2. Synthesis of pyrazole pyrimidine derivatives **20–21**.



[a]Reagents and conditions: a) 1-(2-fluorophenyl)-4-hydrazinyl-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidine **6** (1.0 equiv), aromatic aldehyde, 60 ° DIPEA (2.0 equiv), methanol, 3 h; b) (4-(2-((6-fluoro-5-methyl-1*H*-indol-3-yl)methylene)hydrazinyl)-1-(2-fluorophenyl)-3-(methylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidine **23** (1.0 equiv), 1-(bromomethyl)-2,4-dichlorobenzene **24** (1.2 equiv), K_2CO_3, (2.0 equiv), DMF,rt , 10 h;

Scheme 3. Synthesis of pyrazole pyrimidine derivatives **23–25**.

27 also forms a hydrogen bond with the main chain oxygen of Tyr352 (D···A = 2.9 Å). All these compounds develop hydrophobic interactions with the residues Ile288, Leu293, Glu344, Glu345, Leu347, Ser348 and Glu351. The BI8 forms a hydrogen bond with Thr300 and also some solvent mediated hydrogen bonds while PVZB1194 doesn't form any hydrogen bond. However, compounds 12 and 25 forms a strong hydrogen bonds with main chain NH_2 of Asn271 (D···A = 2.6 Å). Compounds 27 form Van der Waals interactions similar to the BI8.

2.3. Cytotoxity of MTT assay

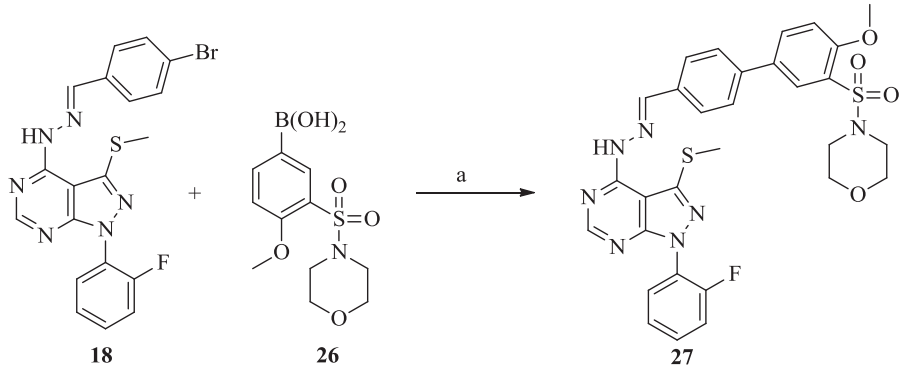
The best compounds (synthesized pyrazole pyrimidine and thienopyridines) obtained from the steady state ATPase assay were screened for antiproliferative activity in vitro against HeLa cells by performing MTT assay (Fig. 7). All the compounds were screened within a range of 0.25–100 μM concentrations, against HeLa cells for 48 h. Among all the tested analogues, compound12, 25 and 27 have shown significantly better inhibition of HeLa cells proliferation in a dose dependent manner. The IC_{50} values for compound 12, 25 and 27 (4.6 μM, 1.43 μM & 2.28 μM respectively) were obtained from a non-linear regression graph plotted between the percentage of cell viability and Log_{10} concentration using Graph Pad Prism software. The results suggest that compounds are effective at low concentration against the human cervical cancer cell line.

3. Conclusion

Both the pyrazole pyrimidine and thienopyridines derivatives were tested for activity against human mitotic kinesin eg5. Three compounds (12, 25 and 27) showed to inhibit the eg5 activity at micromolar concentration. Molecular docking studies suggest that they have higher binding affinity to site 1 of eg5 by interacting with the hotspot residues such as Trp127 and Arg119. These compounds also interact with Tyr352 by forming three hydrogen bonds in the site 2 allosteric binding site of Eg5. The pyrazole pyrimidine derivatives can be used as temple to further structure based drug discovery in order to design more inhibitors with improved potency. The present works provides the basis in designing new and more potent leads and understand the activity of the pyrazole pyrimidine derivatives to develop them further as lead compounds for cancer chemotherapy.

4. Experimental

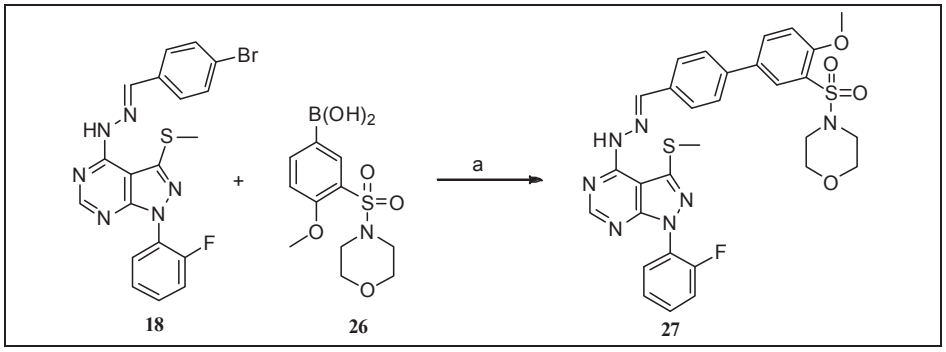
NMR spectra have been taken two different spectrometers n various place at 500, 400 MHz in ^1H and 100, 125 MHz ^{13}C respectively using Bruker (Avance) instrument in $CDCl_3$ using tetramethylsilane (TMS) as internal standard. Chemical shifts are reported as δ values (ppm). All one- and two-dimensional NMR spectra were obtained using standard Bruker software throughout. IR spectra were recorded on a JASCO FT



[a]Reagents and conditions:a) **18** (1.0 equiv), (4-methoxy-3-(morpholinosulfonyl)phenyl)boronic acid,(1.5 equiv), $Pd(dba)_2$, (0.01 equiv), K_2CO_3, (1.5 equiv), 1,4-dioxane:water (4:1), 90 °C in pressure tube 12 h; [b]isolated yield.

Scheme 4. Synthesis of pyrazole pyrimidine derivatives **27**.

Table 1
Screening of catalyst and reaction conditions for pyrazole pyrimidine derivatives.[a]



18 26 27

Entry	Catalyst	Base	Yield[b] (%)
1	$Pd(dba)_2$	NaOEt	52
2	$Pd(dba)_2$	KOtBu	76
3	$Pd(dba)_2$	K_3PO_4	40
4	$Pd(OAc)_2$	NaOEt	22
5	$Pd(OAc)_2$	KOtBu	31
6	$Pd(OAc)_2$	K_3PO_4	36
7	$Pd(PPh_3)_4$	NaOEt	25
8	$Pd(PPh_3)_4$	KOtBu	30
9	$Pd(PPh_3)_4$	K_3PO_4	32
10	$PdCl_2(PPh_3)_2$	NaOEt	20
11	$PdCl_2(PPh_3)_2$	KOtBu	31
12	$PdCl_2(PPh_3)_2$	K_3PO_4	26

[a] Reagents and conditions: (a) **18** (1.0 equiv), 4-methoxy-3-(morpholinosulfonyl)phenyl)boronic acid, **26** (1.5 equiv), $Pd(dba)_2$, (0.01 equiv), KOtBu, (1.5 equiv), toluene: water (8:2) mL, 90 °C in pressure tube 12 h.
[b] Isolated yield. Purity was confirmed by ^{1}H NMR.

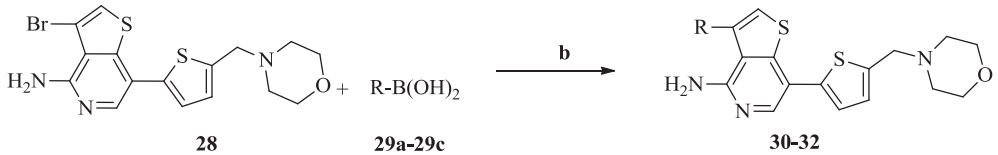
IR instrument (KBr pellet). NMR solvent DMSO-d_6 and CDCl3 were purchased from Sigma Alrich Company

4.1. 5-Amino-1-(2-Fluorophenyl)-3-(methylthio)-1H-pyrazole-4-carbonitrile (3)

A mixture of bis(methylsulphanyl)methylenemalononitrile **1** (3.0 g 0.0130 mmol), and 2-fluorophenylhydrazine **2** (2.14 g, 0.017 mmol) and DIPEA (2.63 g, 0.020 mmol) in absolute ethanol (40 mL) was refluxed for 6 h. The completion of the reaction was monitored by TLC and the mixture was poured into ice cold water. The solid separated was purified by recrystallisation from absolute ethanol. Isolated as ivory solid: Yield: 85%; mp: 132–135 °C; Calculated Mass: 248.2, Observed LC-MS (M + 1): 249.1; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 µm, +ve mode max chromatogram: RT (min) 3.14: Area. 99.53%; FT-IR (cm^{-1}): 3432, 3326, 3224, 3047, 2919, 2857, 2205, 1652, 1594, 1555, 1520, 1450, 1356, 1291, 1019, 978, 924, 764, 699; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.52–7.45 (m, 4H, Ar-H), 4.60 (s, 2H, NH_2), 2.57 (s, 3H, SMe); ^{13}C NMR (100 MHz, DMSO- d_6) δ 157.4, 153.3, 149.2, 145.1, 134.7, 123.4, 115.6, 104.5, 12.7.

4.2. 1-(2-Fluorophenyl)-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one (4)

A mixture of 1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazole-4,5-dicarbonitrile **3** (2.5 g, 0.101 mmol) and formic acid (85%, 35 mL) was heated under reflux for 10 h. After completion of the reaction, monitored by TLC, the mixture was filtered to remove excess of 2-fluorophenylhydrazine and the filtrate was poured into ice cold water. The solid formed was filtered and purified by recrystallisation from formic acid. Isolated as ivory white solid: Yield: 76%; mp: 251–253 °C; Calculated Mass: 276.2, Observed LC-MS (M + 1): 277.1; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min



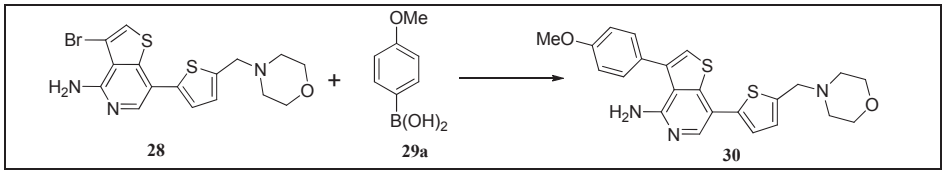
28 29a-29c 30-32

30 = R, 4- OMe-Phenyl
31 = R 4- Pyridine
32 = R, Quinoline

[a]Reagents and conditions: (a) 3-bromo-7-(5-(morpholinomethyl)thiophene-2yl)thieno[3,2-c]pyrimidi-4-amine **28** (1.0 equiv), **29a-29e** Aryl or Heteroaryl boronic acid (1.5 equiv), S-Phos (0.05 equiv), $Pd(OAc)_2$, (0.05 equiv), K_2CO_3, (2.5 equiv), acetonitrile (5 mL), 90 °C in pressure tube 12 h; [b]isolated yield.

Scheme 5. Thienopyridines derivatives **30–32**.

Table 2
Screening of catalyst and reaction conditions for thienopyridine derivatives.[a]



28 29a 30

Entry	Catalyst	Base	Yield[b] (%)
1	$Pd(dba)_2$	Na_2CO_3	< 2
2	$Pd(dba)_2$	K_2CO_3	22
3	$Pd(dba)_2$	K_3PO_4	18
4	$Pd(OAc)_2$	Na_2CO_3	26
5	$Pd(OAc)_2$	K_2CO_3	86
6	$Pd(OAc)_2$	K_3PO_4	49
7	$Pd(PPh_3)_4$	Na_2CO_3	28
8	$Pd(PPh_3)_4$	K_2CO_3	36
9	$Pd(PPh_3)_4$	K_3PO_4	28
10	$PdCl_2(PPh_3)_2$	Na_2CO_3	31
11	$PdCl_2(PPh_3)_2$	K_2CO_3	42
12	$PdCl_2(PPh_3)_2$	K_3PO_4	48

[a] Reagents and conditions: (a) **28** (1.0 equiv), 4-methoxyphenyl boronic acid **29a** (1.5 equiv), S-Phos (0.05 equiv), $Pd(OAc)_2$, (0.05 equiv), K_2CO_3, (2.5 equiv), acetonitrile (5 mL), 90 °C in pressure tube 12 h.
[b] Isolated yield.

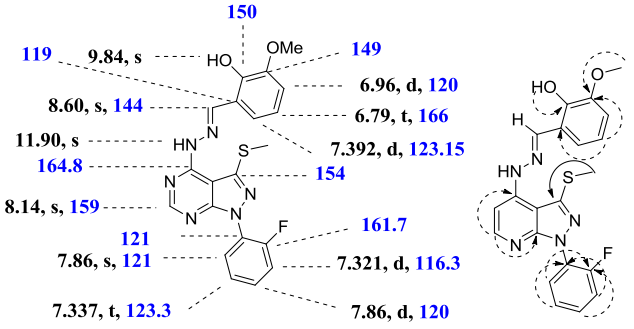


Fig. 1. HMB correlation of 12 and various characteristic ^{1}H NMR and ^{13}C NMR peaks.

column: Atlantis d C18 (50 × 4.6) mm, 5 µm, +ve mode max chromatogram: RT (min) 2.53: Area. 99.11%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.47 (s, 1H), 8.17 (s, 1H), 8.04 (dd, J = 9.1, 4.9 Hz, 2H), 7.40 (t, J = 8.8 Hz, 2H), 2.61 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.1, 159.0, 157.0, 153.3, 145.5, 145.2, 123.2, 115.6, 12.7 FT-IR (cm^{-1}): 3329, 3073, 2991, 2926, 2857, 2272, 1662, 15000, 1437, 1383, 1255, 1150, 1088, 864, 657.

Table 3
Docking scores and Assay results for compounds 5, 11 and 17.

Compound	Gide XP (Kcal/mol)		IC_{50} (µM)
	Site 1	Site 2	
12	−6.93	−5.34	15.39 ± 1.61
25	−7.25	−5.17	16.42 ± 3.84
27	−8.27	−5.39	9.98 ± 0.76
STLC	−7.20	–	1.0 ± 0.2
BI8	–	−10.74	458.8 ± 28.3 nM

4.3. 4-Chloro-1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidine (5)

A suspension of the 1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazolo [3,4-*d*]pyrimidin-4(5H)-one **4** (2.1 g, 0.007 mmol) in phosphorus oxychloride (60 mL) was heated under reflux for 6 h. The reaction was cooled, poured onto ice-cold water (100 mL) and the precipitate was filtered, dried.

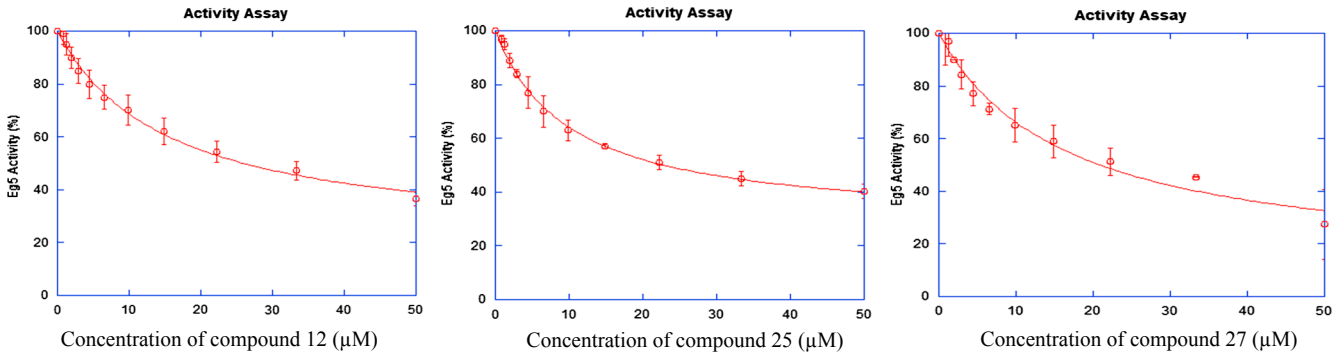


Fig. 2. Inhibition of the basal ATPase activity against Eg5, Compounds 12, 25 and 27. Concentration–response plots for the inhibition of the basal Eg5 ATPase activities [IC_{50} 12 = 15.39 ± 1.61 µM; IC_{50} 25 = 16.42 ± 3.84 µM and IC_{50} 27 = 9.98 ± 0.76 µM].

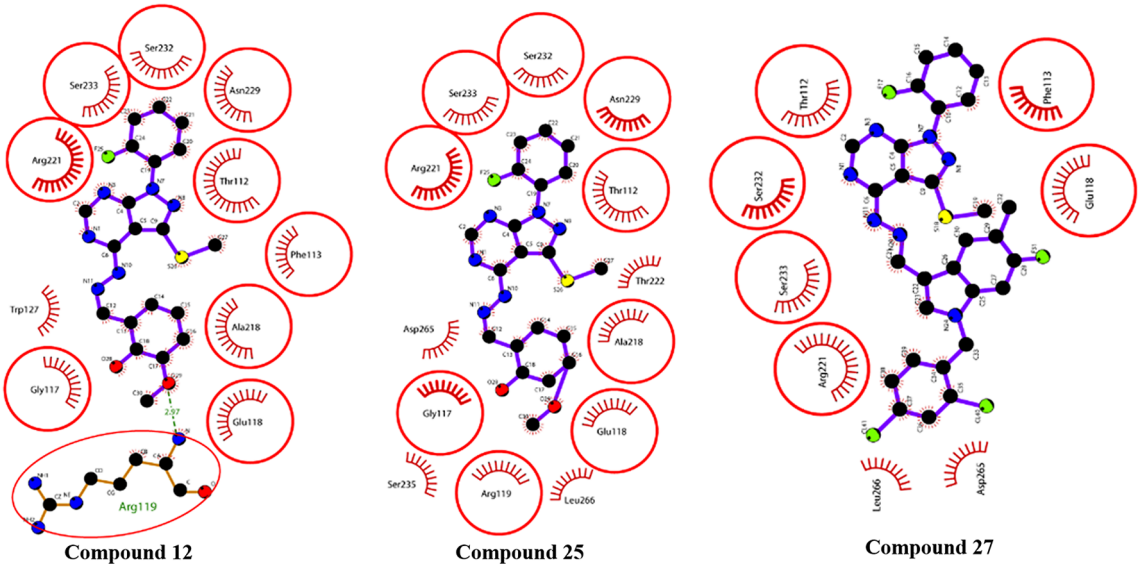


Fig. 3. Ligplot image showing the interactions of the inhibitors with the enzyme KSP at site 1.

4.4. 4-Chloro-1-(2-fluorophenyl-)-4-hydrazinyl-3-(methylthio)-1H-pyrazolo [3,4-d]pyrimidine (6)

A mixture of 4-chloro-1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazolo [3,4-*d*]pyrimidine **5** (1.8 g, 0.0061 mol) and hydrazine hydrate (99%, 3 mL, 0.0183 mmol) in absolute ethanol (40 mL) was heated under reflux for 3 h. The reaction was cooled, and the separated solid was filtered, dried and crystallized from methanol. Isolated as white solid: Yield: 92%; mp: 165–168 °C; Calculated Mass: 290.3, Observed LC-MS: M + 1, 291; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 µm, +ve mode max chromatogram: RT (min) 2.03: Area. 96.54%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.20 (s, 1H, NH), 8.29 (s, 1H), 8.21–8.19 (m, 2H), 8.18–8.13 (m, 2H), 5.09 (s, 2H, NH_2), 2.50 (s, 3H, SMe); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1, 161.0, 148.9, 133.7, 133.5, 126.9, 126.6, 116.2, 113.8, 39.7, 39.4, 39.1, 38.9, 14.4; FT-IR (cm^{-1}): 3328, 2925, 2855, 1501, 1437, 1383, 1255, 1088, 864, 739, 657.

4.5. 4-(2-(4-Chlorobenzylidene)hydrazinyl-1-(2-fluorophenyl)-3(methylthio)-1H-pyrazolo[3,4-d]pyrimidine (8)

A mixture of 4-chloro-1-(2-fluorophenyl-)-4-hydrazinyl-3-(methylthio)-1H-pyrazolo[3,4-*d*]pyrimidine **6** (0.025 g, 0.086 mmol) and the 4-chlorobenzaldehyde **7a** (0.014 g, 0.1033 mmol) in methanol (4 mL) and glacial acetic acid (0.1 mL) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. The crude product was purified by flash column chromatography. (CH_2Cl_2/MeOH) yielded **8** (20 mg, 85%) as a white solid; Calculated Mass: 412.06, Observed LC-MS (M + 1): 413.1; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 µm, +ve mode max chromatogram: RT (min) 3.15: Area. 98.40%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.16 (s, 1H), 8.64 (d, J = 7.7 Hz, 1H), 8.57–8.50 (m, 1H), 8.47 (s, 1H), 8.26–8.15 (m, 1H), 8.02 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 10.9 Hz,

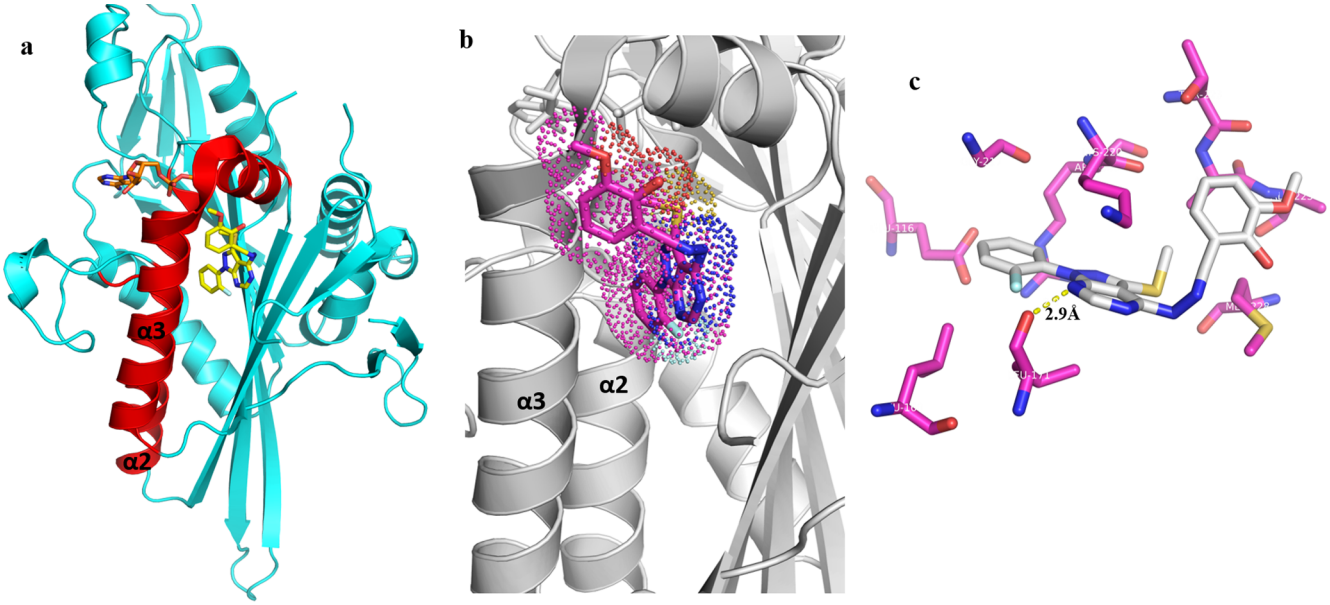


Fig. 4. Structural analysis of first allosteric binding pocket. (a) Eg5 in complex with Mg^{2+}ADP (orange) and compound12 (yellow) bound in the allosteric inhibitor-binding pocket formed by helices α2 and α3 and loops L7 as well as L9. (b) Close view of site-I with the compound 12 (magenta). (c) The magnification of the inhibitor-binding pocket showing residues involved in compound 12 binding.

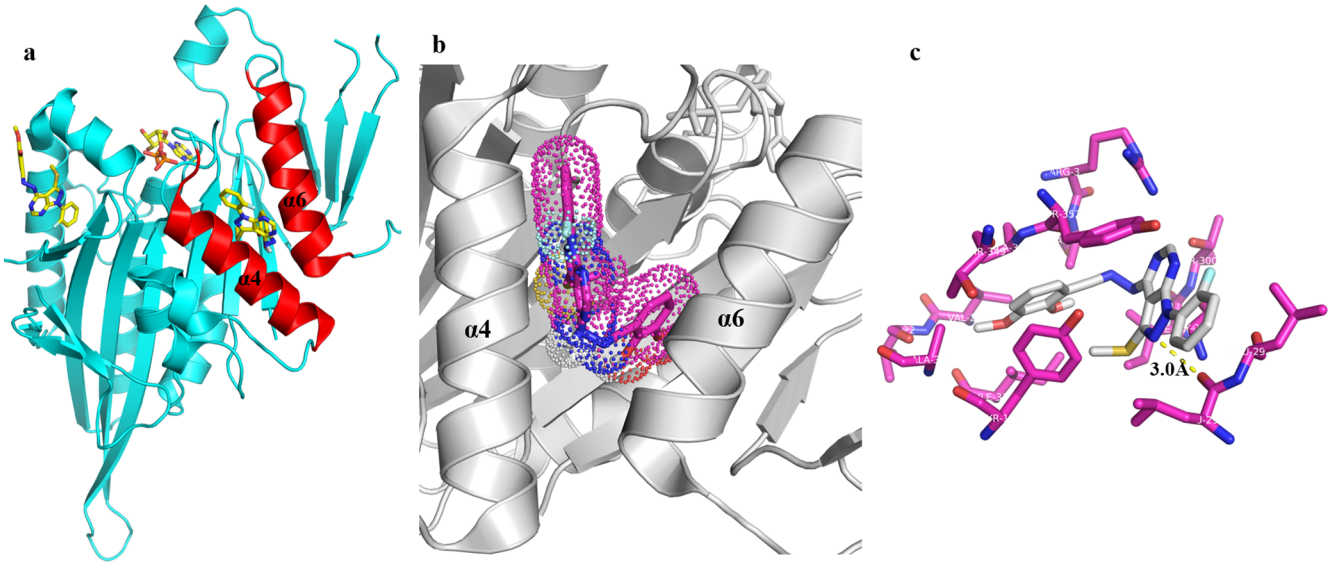
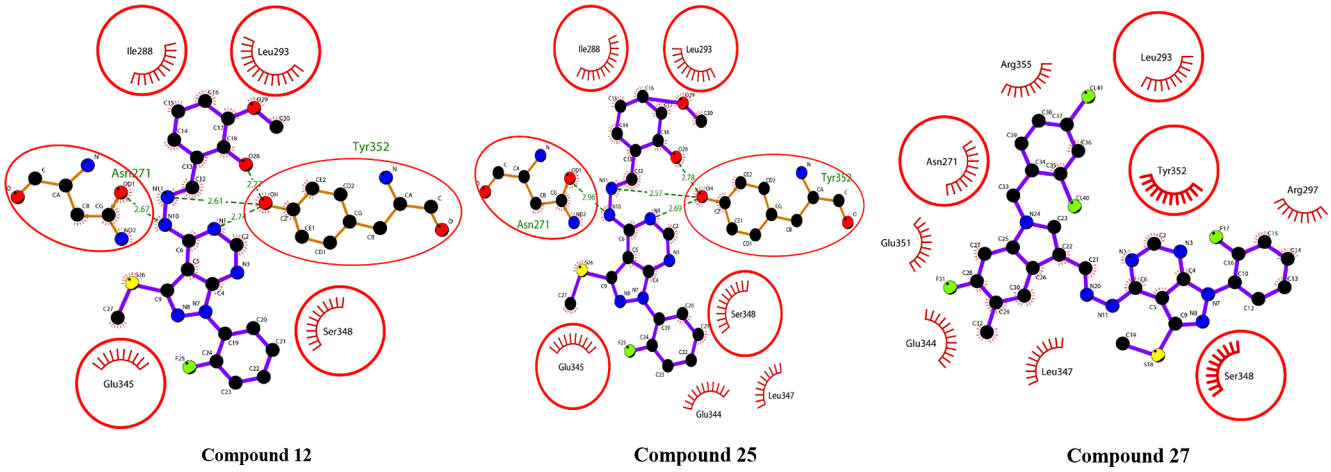


Fig. 5. Structural analysis of second allosteric binding pocket. (a) Eg5 in complex with Mg2 + ADP (orange) and compound12 (yellow) bound in the allosteric inhibitor-binding pocket formed by helices α4 and α6. (b) Close view of site-II with the compound 12 (magenta). (c) The magnification of the inhibitor-binding pocket showing residues involved in compound 12 binding.



Compound 12 **Compound 25** **Compound 27**

Fig. 6. Ligplot image showing the interactions of the inhibitors with the enzyme KSP at site 2.

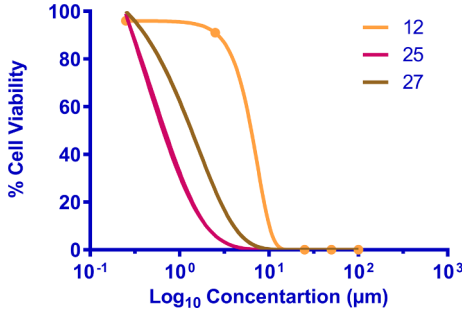


Fig. 7. A non-linear regression graph representing the compound concentration Vs the percentage of cell viability.

1H), 7.44–7.34 (m, 3H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 152.4, 149.8, 148.4, 147, 144.5, 134.1, 131.7, 129.7, 123.2, 116.07, 101.3, 13.05; FT-IR (cm^{-1}); 3525, 3072, 2926, 2857, 1663, 1500, 1437, 1383, 1254, 1151, 1088, 864, 657.

4.6. 4-(2-(2-Chloro-4-fluorobenzylidene)hydrazinyl-1-(2-fluorophenyl)-3(methylthio)-1H-pyrazolo[3,4-d]pyrimidine **(9)**

A mixture of **6** (0.100 g, 0.344 mmol) and the 2-chloro-4-fluorobenzaldehyde (0.054 g, 0.344 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. The crude product was purified by flash chromatography. (CH_2Cl_2/MeOH) yielded **9** (85 mg, 85%) as a white solid; Calculated Mass: 430, Observed LC-MS (M + 1): 431; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 2.21: Area. 94.98%, 220 nm: RT (min) 2.21% Area. 93.63%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 9.91 (s, 1H), 8.67 (d, J = 7.8 Hz, 1H), 8.43 (m, 1H), 8.22 (d, J = 7.0 Hz, 1H), 8.10–7.99 (m, 1H), 7.93 (d, J = 3.8 Hz, 1H), 7.50–7.35 (m, 3H), 7.03 (d, J = 9.9 Hz, 1H), 6.90–6.79 (m, 1H), 2.58 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 154.0, 150.5, 148.5, 147.5, 146.5, 145.7, 144.3, 123.3, 122.7, 120.4, 119.2, 116.0, 115.4, 13.1; FT-IR (cm^{-1}): 3476, 3071, 2929, 2862, 1665, 1497, 1438, 1385, 1254, 1151, 1091, 1062, 658.

4.7. 2-Bromo-4-chloro-6-((2-(1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazono)methyl)phenol **(10)**

A mixture of **6** (0.075 g, 0.258 mmol) and the 3-bromo-5-chloro-2-hydroxybenzaldehyde (0.060 g, 0.344 mmol) in methanol (6 mL) and glacial acetic acid (0.3 mL) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was washed with saturated aqueous $NaHCO_3$ for several times and finally dried over Na_2SO_4. The crude product was purified by flash chromatography. (CH_2Cl_2/MeOH) yielded **10** (66 mg, 89%) as a white solid; Calculated Mass: 505.7, Observed LC-MS (M + 2): 507.0, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 3.47: Area. 87.90%; LC-MS (M − 2): 504, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, −ve mode max chromatogram: RT (min) 3.47 Area. 87.90%;^{1}H NMR (400 MHz, DMSO-d_6) δ 12.15 (s, 1H), 8.60 (s, 1H), 8.54–8.48 (m, 1H), 8.43 (d, J = 7.8 Hz, 1H), 8.21 (d, J = 6.8 Hz, 1H), 8.04 (m, 2H), 7.97 (s, 1H), 7.52 (m, 4H), 7.39 (s, 7H), 2.59 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 162.8, 161.1, 160.1, 152.9, 151.6, 148.9, 133.3, 116.6, 115.4, 113.7, 13.7; FT-IR (cm^{-1}): 3463, 3073, 2930, 2865, 1652, 1496, 1386, 1254, 1093, 1062, 658.

4.8. 1-(2-Fluorophenyl)-4-(2-(4-methoxybenzylidene)hydrazinyl-3-(methylthio)-1H-pyrazolo[3,4-d]pyrimidine) **(11)**

A mixture of **6** (0.075 g, 0.258 mmol) and 4-methoxybenzaldehyde (0.037 g, 0.26 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. The crude product was purified by flash chromatography. (CH_2Cl_2/MeOH) yielded **11** (50 mg, 72%) as a white solid; Calculated Mass: 408.1, Observed LC-MS (M + 1): 409.0, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 1.33 Area. 91.69%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.901 (s, 1H), 8.335 (s, 1H), 7.908 (d, J = 8.72 Hz, 2H), 7.418–7.374 (m, 4H), 7.028 (d, J = 8.64 Hz, 2H), 3.831 (s, 3H, OMe), 2.588 (s, 3H, SMe); FT-IR (cm^{-1}):3328, 3073, 2927, 2857, 1661, 1500, 1438, 1383, 1254, 1150, 1088, 1063, 864, 657; ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.5, 152.8, 150.2, 148.6, 138.6, 132.4, 132.4, 131.4, 129.7, 127.9, 125.8, 125.6, 115.7, 35.4, 10.19.

4.9. 2-((2-(1-(2-Fluorophenyl)-3(methylthio)-1H-pyrazolo[3,4-d]pyrimidin-4-yl)hydrazono)methyl)-6-methoxyphenol **(12)**

A mixture of **6** (0.100 g, 0.258 mmol) and 2-hydroxy-3-methoxybenzaldehyde (0.052 g, 0.344 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. The crude product was purified by flash chromatography. (CH_2Cl_2/MeOH) yielded **12** (60 mg, 86%) as a white solid; Calculated Mass: 424.1, Observed LC-MS (M + 1): 425; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 2.74 Area. 96.34%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.90 (s, 1H, NH), 9.84 (s, 1H,OH), 8.60 (d, J = 8.2 Hz, 1H), 8.14 (s, 1H), 7.98 (d, J = 3.7 Hz, 1H), 7.86 (t, J = 7.4 Hz, 1H), 7.392 (d, J = 7.7 Hz, 1H), 7.337 (t, J = 8.1 Hz, 2H), 7.321 (d, J = 7.8 Hz, 1H), 6.96 (d, J = 7.7 Hz, 1H), 6.79 (t, J = 7.8 Hz, 1H), 3.77 (s, 3H, OCH_3), 2.44 (s, 3H, SMe); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.8, 161.7, 159.7, 154.3, 149.2, 148.3, 147.2, 146, 144, 135, 123.6, 121, 119.3, 116.3, 114.1, 56.42, 13.49; FT-IR (cm^{-1}): 3538, 3330, 3073, 2927, 2858, 1660, 1500, 1437, 1384, 1254, 1151, 1088, 1063, 864, 657.

4.10. 4-2(4-Benzyloxy)-3-methoxybenzylidine)hydrazinyl)-1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazolo[2,4-d]pyrimidine **(13)**

A mixture of **6** (0.75 g, 0.26 mmol) and 4-benzyloxy-3-methoxybenzaldehyde (0.062 g, 0.26 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 4.5 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. The crude product was purified by flash chromatography. (CH_2Cl_2/MeOH) yielded **14** (65 mg, 86%) as a white solid; Calculated Mass: 514.5, Observed LC-MS (M + 1): 515; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 3.14 Area. 94.30%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.89 (s, 1H, NH), 8.38 (s, 1H, ArCH), 8.22 (dd, J = 10.4, 6.5 Hz, 2H,), 8.05 (dd, J = 8.8, 4.9 Hz, 1H, N = CH), 7.96–7.88 (m, 6H), 7.47 (d, J = 7.2 Hz, 2H), 7.39 (dt, J = 18.1, 8.0 Hz, 6H), 7.13 (d, J = 4.1 Hz, 2H), 5.16 (s, 2H, OCH_2), 3.87 (s, 3H, OMe), 2.60 (s, 3H, CH_3) ; ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.7, 161.2, 154.2, 150, 149, 146, 144, 137, 137.27, 128, 128.36, 123, 116, 102, 70, 56, 13.5; FT-IR (cm^{-1}): 3330, 2927, 2857, 1661, 1500, 1437, 1384, 1254, 1150, 1088, 864, 657.

4.11. 4-(2-(2,5-Dichlorobenzylidene)hydrazinyl-1-(2-fluorophenyl)-3(methylthio)-1H-pyrazolo[3,4-d]pyrimidine **(14)**

A mixture of **6** (0.100 g, 0.258 mmol) and 2,5-dichlrobenzaldehyde (0.045 g, 0.26 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 4 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded **14** (60 mg, 82%) as a white solid; Calculated Mass: 446.3, Observed LC-MS (M + 1): 447.7; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 3.39 Area. 80.07%; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.17 (s, 1H), 8.65 (d, J = 6.7 Hz, 5H), 8.58 (m, 1H), 8.48 (m, 1H), 8.27 (m, 2H), 8.04 (m, 3H), 7.55 (d, J = 6.6 Hz, 2H), 7.46 (m, 5H), 2.53 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.3, 153.7, 150.7–150.5, 148.5–148.3, 146.3, 144.5, 136.6, 134.6–134.4, 130.0, 128.9, 128.0, 123.8, 123.2, 123.0, 102.5, 69.7, 15.3, 13.8; FT-IR (cm^{-1}): 3543, 3330, 3073, 2927, 2857, 1660, 1500, 1437, 1383, 1255, 1150, 1088, 1063, 864, 657.

4.12. 1-(2-Fluorophenyl)-3-(methylthio)-4-(2-(4-(phenoxymethyl)benzylidine)hydrazinyl)-1H-pyrazolo[3,4-d]pyrimidine **(15)**

A mixture of **6** (0.075 g, 0.258 mmol) and 4-benzyloxybenzaldehyde (0.055 g, 0.26 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded **15** (66 mg, 90%) as a white solid; Calculated Mass: 484.1, Observed LC-MS (M + 1): 485.2, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 3.20 Area. 77.10%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.5 (s, 1H, NH), 8.55 (d, J = 16.2 Hz, 1H), 8.16 (s, 1H), 8.00 (s, 1H, N = CH), 7.83 (d, J = 8.3 Hz, 1H), 7.73 (s, 1H), 7.39 (m, 6H), 7.19 (d, J = 7.6 Hz, 2H), 7.03 (m, 2H), 5.12 (s, 2H, OCH_2), 2.20 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.3, 157.3, 150.6, 149.1, 146.7, 144.0, 130.01, 128.9, 128.3, 123.7, 116, 115, 102, 69, 13.5; FT-IR (cm^{-1}): 3545, 3329, 3072, 2927, 2857, 1661, 1500, 1437, 1384, 1254, 1151, 1088, 864, 589.

4.13. 2-((2-(1-(2-Fluorophenyl)-3(methylthio)-1H-pyrazolo[3,4-d] pyrimidin-4-yl) hydrazono) methyl)-5-methoxyphenol (16)

A mixture of **6** (0.075 g, 0.258 mmol) and 2-hydroxy-4-methoxybenzaldehyde (0.048 g, 0.26 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 5 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded **16** (65 mg, 85%) as a white solid; Calculated Mass: 424.1, Observed LC-MS (M + 1): 425; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 2.12 Area. 90.21%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.15 (s, 1H, NH), 8.44 (s, 1H, OH), 8.03 (m, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.66 (m, 1H), 7.06 (m, 4H), 6.38 (m, 2H), 3.73 (s, 3H), 2.55 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.49, 144.19, 140.60, 138.55, 127.88, 127.68, 126.51, 126.32, 123.58, 118.38, 114.97, 112.28, 39.50, 33.33, 24.41; FT-IR (cm^{-1}): 3535, 3330, 3073, 2927, 2857, 1661, 1500, 1438, 1384, 1254, 1151, 1088, 864, 748, 657.

4.14. 2-((2-(4-Allyloxy)benzylidene)hydrazinyl-1-(2-fluorophenyl)-3(methylthio)-1H-pyrazolo [3,4-d]pyrimidine (17)

A mixture of **6** (0.075 g, 0.258 mmol) and 4-allyloxybenzaldehyde (0.049 g, 0.26 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 5 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded **17** (64 mg, 86%) as a white solid; Calculated Mass: 434.1, Observed LC-MS (M + 1): 435.1, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 3.17 Area. 95.67%; LC-MS: M − 1, 433.1, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, −ve mode max chromatogram: RT (min): 3.17 Area. 95.67%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.35 (s, 1H, NH), 8.81 (s, 1H, ArCH), 8.40 (m, 1H), 8.26–7.80 (m, 3H), 7.37 (s, 1H), 7.28–7.12 (m, 2H), 7.08–6.88 (m, 2H), 6.10 (m, 1H, =CH), 5.50 (d, J = 16.8 Hz, 1H), 5.35 (s, 1H), 4.64 (s, 2H, OCH_2), 2.69 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 164, 162, 160, 154.7, 151, 149.6, 148.8, 147, 146, 145, 135.8, 135.8, 124.1, 124, 119, 116, 104, 56.83, 13.9; FT-IR (cm^{-1}): 3534, 3329, 3073, 2928, 2858, 1659, 1500, 1438, 1384, 1254, 1088, 1063, 864, 657.

4.15. 4-(2-(4-Bromobenzylidene)hydrazinyl-1-(2-fluorophenyl)-3(methylthio)-1H-pyrazolo [3,4-d]pyrimidine (18)

A mixture of **6** (0.075 g, 0.258 mmol) and 4-bromobenzaldehyde (0.048 g, 0.26 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 4 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded **18** (58 mg, 77%) as a white solid; Calculated Mass: 456, Observed LC-MS (M + 1): 457.0, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 3.22: Area. 90.79%; LC-MS: M − 1, 455, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, −ve mode max chromatogram: RT (min) 3.22 Area: 90.794%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.03 (s, 1H), 8.46 (m, 1H), 8.39–8.33 (m, 1H), 8.30 (m, 1H), 8.08 (m, 1H), 7.85 (m, 3H), 7.36 (d, J = 6.9 Hz, 2H), 7.27 (4, 4H), 2.35 (s, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) 159.5, 152.8, 150.8, 149.08, 147.7, 145.0, 134.9, 132.2, 130.2, 123, 116, 102, 13.5; FT-IR (cm^{-1}): 3542, 3329, 3072, 2927, 2857, 1661, 1500, 1437, 1384, 1254, 1151, 1088, 864, 657.

4.16. 4-(2-(3-Chlorobenzylidene)hydrazinyl-1-(2-fluorophenyl)-3(methylthio)-1H-pyrazolo [3,4-d]pyrimidine (19)

A mixture of **6** (0.075 g, 0.258 mmol) and 3-chlorobenzaldehyde (0.036 g, 0.26 mmol) in methanol (6 mL) and glacial acetic acid (0.4 mL) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated aqueous $NaHCO_3$ and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded **19** (63 mg, 86%) as a white solid; Calculated Mass: 412.0, Observed LC-MS (M + 1): 413.0; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 3.19: Area. 97.31%; M − 1, 411.0; Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, −ve mode max chromatogram: RT (min) 3.21 Area: 97.312%;^{1}H NMR (400 MHz, $CDCl_3$) δ 11.18 (s, 1H, NH), 8.44 (s, 1H, OH), 8.03 (m, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.56 (m, 1H), 7.05 (m, 4H), 6.37 (m, 2H), 3.73 (s, 3H), 2.24 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.9, 159.2, 150.5, 146.1, 145.5, 144.3, 135.1, 134.80, 130.8, 129.1, 124.7, 116.2, 110.9, 11.7; FT-IR (cm^{-1}): 3434, 3199, 3066, 2089, 2918, 1730, 1603, 1578, 1514, 1442, 1213, 1099, 1030, 823.

4.17. 4-(2-((1H-Indol-3-yl)methylene)hydrazinyl)-1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazolo [3,4-d]pyrimidine (20)

A mixture of **6** (0.075 g, 0.258 mmol) and indole-3-carbaldehyde (0.037 g, 0.26 mmol) in methanol (6 mL) and TEA (2.5 equiv) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with saturated water and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded **20** (55 mg, 70%) as a white solid; Calculated Mass: 417.1, Observed LC-MS (M + 1): 418.1, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 2.77 Area: 80.60%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.16 (s, 1H, NH), 10.89 (s, 1H, NH), 8.53 (s, 1H), 8.26 (s, 1H), 8.09 (m, 3H), 7.43 (s, 1H), 7.29 (m, 2H), 7.19 (d, J = 8.5 Hz, 3H), 2.72 (s, 3H, SMe); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.4, 153.8, 150.8, 148.7, 146.5, 144.70, 136.8, 134.8, 130.21, 129.1, 128.1, 123.9, 123.4, 123.1, 102.6, 70.1, 13.9; FT-IR (cm^{-1}): 3550, 3329, 3073, 2927, 2857, 1661, 1500, 1437, 1383, 1254, 1151, 1088, 864, 657.

4.18. 2-(2-(4-Bromothiophen-2-ylmethylene)hydrazinyl-1-(2-fluorophenyl)-3(methylthio)-1H-pyrazolo[3,4-d]pyrimidine (21)

A mixture of **6** (0.075 g, 0.258 mmol) and 4-bromothiophene-2-carbaldehyde (0.049 g, 0.26 mmol) in methanol (6 mL) and TEA (2.5 equiv) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with water and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded **21** (70 mg, 91%) as a white solid; Calculated Mass: 460.9, Observed LC-MS (M + 1): 461, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 5.38:Area. 91.81%, 220 nm: RT (min) 5.38: Area. 91.19%; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.16 (s, 1H), 8.59 (s, 2H), 8.16 (s, 2H), 7.98 (m, 2H), 7.80 (m, 1H), 7.20 (m, 1H), 2.18 (s, 3H, SMe); ^{13}C NMR (125 MHz, DMSO-d_6) δ 160.8, 158.1, 149.4, 145.0, 144.4, 143.3, 134.0, 133.7, 129.7, 128.0, 123.6, 115.1, 109.9, 11.2; FT-IR (cm^{-1}): 3429, 3205, 3063, 2996, 2924, 1606, 1570, 1509, 1445, 1298, 1253, 1168, 1025, 834.

4.19. 4-(2-(6-fluoro-5-methyl-1H-indol-3-yl)methylene)hydrazinyl)-1-(2-fluorophenyl)-3-(methyl thio)-1H-pyrazolo[3,4-d]pyrimidine ***(23)***

A mixture of **6** (0.075 g, 0.258 mmol) and 5-fluoro-6-methyl-1H-indole-3-carbaldehyde (0.098 g, 0.26 mmol) **7o** in methanol (6 mL) and NaOEt (2.5 equiv) was heated under reflux for 3 h. The mixture was subsequently diluted with CH_2Cl_2, and the organic layer was sequentially washed with water and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded **23** (72 mg, 88%) as off white. Calculated Mass: 449.1, Observed LC-MS (M + 1): 450, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 3.57: Area. 98.27%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (m, 6H), 7.74–7.03 (m, 6H), 5.55 (s, 1H), 1.99 (s, 3H, SMe), 1.17 (s, 3H, CH_3).

4.20. 4-(2-(1-(2,4-dichlorobenzyl)-6-fluoro-5-methyl-1H-indol-3-yl) methylene)hydrazinyl-1-(2-fluorophenyl)-3(methylthio)-1H-pyrazolo[3,4-d]pyrimidine ***(25)***

A mixture of **23** (0.075 g, 0.150 mmol) and 1-(bromomethyl)-2,4-dichlorobenzene **24** (1.0 equiv), (0.035 g, 0.150 mmol) in DMF (2 mL) and KOtBu (1.5 equiv) was stirred at room temperature for 10 h. The mixture was concentrated under vacuum and diluted with CH_2Cl_2, and the organic layer was sequentially washed water and finally dried over Na_2SO_4. Purification by flash chromatography (CH_2Cl_2/MeOH) yielded 23 (72 mg, 88%) as a white solid. LC-MS: M + 1, 607.2, Method A: 0.1% Formic acid in water B: Acetonitrile, flow rate: 1.5 mL/min column: Atlantis d C18 (50 × 4.6) mm, 5 μm, +ve mode max chromatogram: RT (min) 4.17 Area:85.8%; ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.43 (d, J = 7.6 Hz, 1H, NH), 8.6 (s, 1H), 8.31 (d, J = 7.6 Hz, 1H), 8.21 (s, 2H), 8.06 (s, 1H), 7.97 (s, 1H), 7.75 (s, 1H), 7.40 (m, 3H), 7.31 (d, J = 7.32 Hz, 1H), 7.03 (s, 1H), 6.28 (d, J = 7.6 Hz, 1H, =CH−), 5.56 (s, 2H, CH_2), 3.34 (s, 3H, Me), 2.50 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.9, 159.5, 152.4, 149.03, 147, 145.06, 138.1, 135.0, 132.8, 131.1, 129.9, 127, 123.7, 123.6, 122.6, 116.6, 102, 34, 31, 29, 24, 13.

4.21. 4-((4′-((2-(1-(2-fluorophenyl)-3-(methylthio)-1H-pyrazolo[3,4-d] pyrimidin-4-yl)hydrazono) methyl)-4-methoxy-[1,1′-biphenyl]-3-yl) sulfonyl)morpholine ***(27)***

The reaction of **18** (0.050 g 0.109 mmol) with 4-methoxy-3-(morpholinosulfonyl)phenyl)boronic acid **26** (0.039 g 0.131 mmol), KOtBu (1.5 equiv), at 90 °C during 12 h in toluene: water (8:2 mL) in the presence $Pd(dba)_2$ (0.007 g, 0.010 mmol) in pressure tube vessel afforded the corresponding adduct. The residue was diluted with CH_2Cl_2 (5 mL), followed by extraction twice (25 mL) with CH_2Cl_2. The organic layer was dried with anhydrous Na_2SO_4, filtered and evaporated under vacuum. The residue was purified by flash chromatography using silica gel 230–400 mesh to afford the desired product yielded **27** (39 mg, 78%);^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.48 (s, 1H), 7.82 (m, 3H), 7.64 (m, 4H), 7.53 (m, 3H), 7.39 (s, 2H), 7.26 (s, 4H), 3.95 (s, 2H), 3.30 (m, 1H), 3.13 (s, 1H), 2.98 (m, 2H), 2.63 (s, 1H), 2.49 (s, 2H), 1.63 (s, 1H), 1.40 (m, 2H), 1.27 (m, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.1, 159.7, 158.3, 152.1, 151.5, 135.7, 130.7, 130.1, 122.2, 116.0, 114.6, 99.6, 68.1, 52.0, 48.7, 46.9, 30.44, 22.11.

4.22. 3-(4-methoxyphenyl)-7-(5-(morpholinomethyl)thiophene-2-yl)thieno [3,2-c]pyridin-4-amine ***(30)***

The reaction of 3-bromo-7-(5-(morpholinomethyl)thiophen-2-yl) thieno[3,2-*c*]pyridin-4-amine **28** (0.050 g 0.122 mmol) with 4-methoxy phenyl boronic acid **29a** (0.027 g 0.183 mmol) K_2CO_3 (0.033 g, 0.244 mmol), at 90 °C during 12 h in acetonitrile in the presence S-Phos (0.008 g, 0.006 mmol), palladium acetate (0.005 g, 0.006 mmol) in pressure tube vessel afforded the corresponding adduct. The reaction mixture was filtered with ceilte pad and washed with CH_2Cl_2. Then the reaction mixture was evaporated under vacuum. The crude solid was purified by column chromatography using silica gel 60–120 mesh to afford the desired product yielded **30** (38 mg, 81%); The product was obtained as white solid; LC-MS: M + 1, 438, Method A: 10 mM NH_4HCO_3, B: ACN; Flow Rate: 1.0 mL/min column: Xbridge C8 (50 × 4.6 mm, 3.5μ), +ve mode, max chromatogram: RT (min) 6.04 Area: 94.68%, 254 nm: RT (min) 6.04% Area 91.89%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44 (m, 4H), 3.59 (m, 4H), 3.71 (s, 2H), 3.81 (s, 3H), 5.61 (s, 2H), 7.03 (d, J = 5.16 Hz, 4H), 7.08 (s, 1H), 7.45 (t, J = 11.8 Hz, 1H), 7.55 (s, 1H), 7.06 (s, 1H).

4.23. 7-(5-(morpholinomethyl)thiophen-2-yl)-3-(pyridin-4-yl)thieno[3,2-c]pyridin-4-amine ***(31)***

The reaction of 3-bromo-7-(5-(morpholinomethyl)thiophen-2-yl) thieno[3,2-*c*]pyridin-4-amine **28** (0.05 g 0.122 mmol) with 4-pyridine boronic acid **29b** (0.022 g 0.183 mmol), K_2CO_3 (0.033 g, 0.244 mmol), at 90 °C during 12 h in acetonitrile (5 mL) in the presence S-Phos (0.008 g, 0.006 mmol), palladium acetate (0.005 g, 0.006 mmol) in pressure tube vessel afforded the corresponding adduct. The reaction mixture was filtered with ceilte pad and washed with CH_2Cl_2. Then the reaction mixture was evaporated under vacuum. The crude solid was purified by column chromatography using silica gel 60–120 mesh to afford the desired product yielded **31** (26 mg, 58%);The product was obtained as white solid; Method A: 0.1% TFA in water B: 0.1% TFA in Acetonitrile, flow rate: 2.0 mL/min column: Xbridge C8 (50 × 4.6 mm, 3.5μ), +ve mode, RT (min) 1.33 Area:91.69%, 254 nm: RT (min) 1.33 Area:94.84%; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.49 (t, J = 2.7 Hz, 4H), 3.59 (t, J = 6.75 Hz, 4H), 3.71 (s, 2H), 5.64 (s, 2H), 7.05 (d, J = 2.7 Hz, 1H), 7.30 (d, J = 2.5 Hz, 1H), 7. 77 (dd, J = 6 Hz, 2.0 Hz, 2H) 7.75 (s, 1H), 8.10 (s, 1H), 8.69 (dd, J = 5.9, 2.0 Hz, 2H); LC-MS: M + 1, 409.

4.24. 7-(5-(morpholinomethyl)thiophen-2-yl)-3-quinolin-2-yl)thieno[3,2-c]pyridin-4-amine ***(32)***

The reaction of 3-bromo-7-(5-(morpholinomethyl)thiophen-2-yl) thieno[3,2-*c*]pyridin-4-amine **28** (0.05 g 0.122 mmol) with 8-quinolinyl boronic acid **29c** (0.031 g 0.183 mmol), K_2CO_3 (0.033 g, 0.244 mmol), at 90 °C during 12 h in acetonitrile (5 mL) in the presence S-Phos (0.008 g, 0.006 mmol), palladium acetate (0.005 g, 0.006 mmol) in pressure tube vessel afforded the corresponding adduct. The reaction mixture was filtered with ceilte pad and washed with CH_2Cl_2. Then the reaction mixture was evaporated under vacuum. The crude solid was purified by column chromatography using silica gel 60–120 mesh to afford the desired product yielded **32** (32 mg, 74%); The product was obtained as white solid; LC-MS: M + 1, 459; Method A: 10 mM NH_4HCO_3, B: ACN; Flow Rate: 1.0 mL/min column: Xbridge C8 (50 × 4.6 nm, 3.5μ), +ve mode RT (min) 5.38 Area: 90.81%, 254 nm: RT (min) 5.38 Area: 91.19%;^{1}H NMR (300 MHz, $CDCl_3$): δ 2.49 (t, J = 3.5 Hz, 4H), 3.60 (t, J = 8.8 Hz, 4H), 3.73 (s, 2H) 5.13 (s, 2H), 7.07 (d, J = 3.52 Hz, 1H), 7.35 (d, J = 3.5 Hz, 1H), 7.26 (dd, J = 12.6 Hz, 4.3 Hz, 1H) 7.72 (d, J = 9.8 Hz, 2H), 7.92–7.84 (m, 2H), 8.09 (s, 1H), 8.19 (d, J = 8.5 Hz, 1H), 8.99 (dd, J = 5.6 Hz, 2.5 Hz, 1H).

Acknowledgments

Prof. P. Manisankar and P. Muthuraja would like to acknowledge the University Grants Commission (UGC), New Delhi, India for providing the BSR Faculty Fellowship (No. F. 18-1/2011(BSR)). One of the authors, S. Prakash is thankful to the University Grants Commission (UGC), New Delhi, India for providing Post Doctoral Research Fellowship. Financial support from Science and Engineering Research Board, Department of Science and Technology, New Delhi, India (SB/YS/LS-51/2014) is acknowledged by Venkatasubramanian.HM thank

Indian Council for Medical Research, India for providing SRF (BIC/11(34)/2015).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.12.014.

References

[1] T.M. Beer, B. Goldman, T.W. Synold, C.W. Ryan, L.S. Vasist, P.J. Van Veldhuizen, S.R. Dakhil, P.N. Lara, A. Drelichman, M.H. Hussain, E.D. Crawford, Clin. Genitourin. Cancer 6 (2008) 103.

[2] H.A. Burris, S.F. Jones, D.D. Williams, S.J. Kathman, J.P. Hodge, L. Pandite, P.T. Ho, S.A. Boerner, P. Lorusso, Invest. New Drugs 29 (2011) 467.

[3] K.W. Wood, L. Lad, L. Luo, X. Qian, S.D. Knight, N. Nevins, K. Brejc, D. Sutton, A.G. Gilmartin, P.R. Chua, R. Desai, S.P. Schauer, D.E. McNulty, R.S. Annan, L.D. Belmont, C. Garcia, Y. Lee, M.A. Diamond, L.F. Faucette, M. Giardiniere, S. Zhang, C.M. Sun, J.D. Vidal, S. Lichtsteiner, W.D. Cornwell, J.D. Greshock, R.F. Wooster, J.T. Finer, R.A. Copeland, P.S. Huang, D.J. Morgans, D. Dhanak, G. Bergnes, R. Sakowicz, J.R. Jackson, Proc. Natl. Acad. Sci. USA 107 (2010) 5839.

[4] E.K. Rowinski, N. Onetto, M.C. Renzo, S.G. Arbuck, Taxol: the first of the taxanes, an important new class of antitumor agents, Semin. Oncol. 19 (1992) 646.

[5] D. Huszar, M. Theoclitou, J. Skolnik, R. Herbst, Canc. Metast. Rev. 28 (2009) 197.

[6] N. Ogo, Y. Ishikawa, S. Jun-ichi, K. Matsuno, A. Hashimoto, A. Asai, A.C.S. Med, Chem. Lett. 6 (2015) 1004.

[7] S. Hotha, J.C. Yarrow, J.G. Yang, S. Garrett, K.V. Renduchintala, T.U. Mayer, T.M. Kapoor, Angew. Chem. Int. Ed. 30 (2003) 2379.

[8] C.D. Cox, P.J. Coleman, M.J. Breslin, D.B. Whitman, R.M. Garbaccio, M.E. Fraley, C.A. Buser, E.S. Walsh, K. Hamilton, M.D. Schaber, R.B. Lobell, W. Tao, J.P. Davide, R.E. Diehl, M.T. Abrams, V.J. South, H.E. Huber, M. Torrent, T. Prueksaritanont, C. Li, D.E. Slaughter, E. Mahan, C. Fernandez-Metzler, Y. Yan, L.C. Kuo, N.E. Kohl, G.D. Hartman, J. Med. Chem. 51 (2008) 4239.

[9] Y. Takeoka, T. Tanino, M. Sekiguchi, S. Yonezawa, M. Sakagami, F. Takahashi, H. Togame, Y. Tanaka, H. Takemoto, S. Ichikawa, A. Matsuda, ACS Med. Chem. Lett. 5 (2014) 566.

[10] C. Carbajales, S. Jun-ichi, G. Marzaro, E. Sotelo, L. Escalante, A.M. Sanchez-Diaz, X. Garcia-Mera, A. Asai, A. Coelho, ACS Comb. Sci. 19 (2017) 153.

[11] T. Takeuchi, S. Oishi, T. Watanabe, H. Ohno, S. Jun-ichi, K. Matsuno, A. Asai, N. Asada, K. Kitaura, N. Fujii, J. Med. Chem. 54 (2011) 4839.

[12] S. Nagarajan, D.A. Skoufias, F. Kozielski, A.N. Pae, J. Med. Chem. 55 (2012) 2561.

[13] (a) C. Geng-Yuan, Y.J. Kang, A.S. Gayek, W. Youyen, E. Tuzel, R. Ohi, W.O. Hancock, ACS Chem. Biol. 12 (2017) 1038;
(b) M.T. El Sayed, N.A. Hamdy, D.A. Osman, K.M. Ahmed, Adv. Mod. Oncol. Res. 1 (2015) 20–25;
(c) N. Shetty, S. Gupta, South Asian J. Cancer 3 (2014) 57–59;
(d) M. Said, H. Elshihawy, Pak. J. Pharm. Sci. 27 (2014) 885–892.

[14] Y. Yan, V. Sardana, B. Xu, C. Homnick, W. Halczenko, C.A. Buser, M. Schaber, G.D. Hartman, H.E. Huber, L.C. Kuo, J. Mol. Biol. 335 (2004) 547.

[15] C. Fraser, J.C. Dawson, R. Dowling, D.R. Houston, J.T. Weiss, A.F. Munro, M. Muir, L. Harrington, S.P. Webster, M.C. Frame, V.G. Brunton, E.E. Patton, N.O. Carragher, A. Unciti-Broceta, J. Med. Chem. 59 (2016) 4697.

[16] C.H. Zhang, M.W. Zheng, Y.-P. Li, X.D. Lin, M. Huang, L. Zhong, G.B. Li, R.J. Zhang, W.T. Lin, Y. Jiao, X.A. Wu, J. Yang, R. Xiang, L.J. Chen, Y.L. Zhao, W. Cheng, Y.Q. Wei, S.Y. Yang, J. Med. Chem. 58 (2015) 3957.

[17] C. Fraser, J.C. Dawson, R. Dowling, D.R. Houston, J.T. Weiss, A.F. Munro, M. Muir, L. Harrington, S.P. Webster, M.C. Frame, V.G. Brunton, E. Elizabeth, N.O. Patton, A. Unciti-Broceta Carragher, J. Med. Chem. 59 (2016) 4697.

[18] A. Wang, X. Li, H. Wu, F. Zou, X.-E. Yan, C. Chen, C. Hu, K. Yu, W. Wang, P. Zhao, J. Wu, Z. Qi, W. Wang, B. Wang, L. Wang, T. Ren, S. Zhang, C.-H. Yun, J. Liu, Q. Liu, J. Med. Chem. 60 (2017) 2944.

[19] M.A. Abdelgawad, R.B. Bakr, O.A. Alkhoja, W.R. Mohamed, Bioorg. Chem. 66 (2016) 88.

[20] O. Rabal, J.A. Saanchez-Arias, M.C. Tejedor, I.D. Miguel, M.P. Gonzaalez, C.G. Barroso, A. Ugarte, A.E. Mendoza, E. Saez, M. Espelosin, S. Ursua, T. Haizhong, W. Wei, X. Musheng, A.G. Osta, J. Oyarzabal, J. Med. Chem. 59 (2016) 8967.

[21] C.J. Helal, E.P. Arnold, T.L. Boyden, C. Chang, T.A. Chappie, K.F. Fennell, M.D. Forman, M. Hajos, J.F. Harms, W.E. Hoffman, J.M. Humphrey, Z. Kang, R.J. Kleiman, B.L. Kormos, C.-W. Lee, J. Lu, N. Maklad, L. McDowell, S. Mente, R.E. O'Connor, J. Pandit, M. Piotrowski, A.W. Schmidt, C.J. Schmidt, H. Ueno, P.R. Verhoest, E.X. Yang, J. Med. Chem. 60 (2017) 5673.

[22] C.W. Zapf, B.S. Gerstenberger, L. Xing, D.C. Limburg, D.R. Anderson, N. Caspers, S. Han, A. Aulabaugh, R. Kurumbail, S. Shakya, X. Li, V. Spaulding, R.M. Czerwinski, N. Seth, Q.G. Medley, J. Med. Chem. 55 (2012) 10047.

[23] G. Vignaroli, G. Iovenitti, C. Zamperini, F. Coniglio, P. Calandro, A. Molinari, A.L. Fallacara, A. Sartucci, A. Calgani, D. Colecchia, A. Mancini, C. Festuccia, E. Dreassi, M. Valoti, F. Musumeci, M. Chiariello, A. Angelucci, M. Botta, S. Schenone, J. Med. Chem. 60 (2017) 6305.

[24] T. McHardy, J.J. Caldwell, K.-M. Cheung, L.J. Hunter, K. Taylor, M. Rowlands, R. Ruddle, A. Henley, A.H. Brandon, M. Valenti, T.G. Davies, L. Fazal, L. Seavers, F.I. Raynaud, S.A. Eccles, G.W. Aherne, M.D. Garrett, I. Collins, J. Med. Chem. 53 (2010) 5.

[25] J. Liu, C. Yang, C. Simpson, D. DeRyckere, A.V. Deusen, M.J. Miley, D. Kireev, J.N. Drouin, S. Sather, D. Hunter, V.K. Korboukh, H.S. Patel, W.P. Janzen, M. Machius, G.L. Johnson, H.S. Earp, D.K. Graham, S.V. Frye, X. Wang, Med. Chem. Lett. 3 (2012) 129.

[26] M. Xin, X. Zhao, W. Huang, Q. Jin, G. Wu, Y. Wang, F. Tang, H. Xiang, Bioorg. Med. Chem. 23 (2015) 6250.

[27] L. Zhao, Y. Zhang, C. Dai, T. Guzi, D. Wiswell, W. Seghezzi, D. Parry, T. Fischmann, M.A. Siddiqui, Bioorg. Med. Chem. Lett. 20 (2010) 7216.

[28] M.K. Abd El Hamid, M.D. Mihovilovic, H.B. El-Nassan, Eur. J. Med. Chem. 57 (2012) 323–328.

[29] A.A. Gaber, A.H. Bayoumi, A.M. El-morsy, F.F. Sherbiny, A.B.M. Mehany, I.H. Eissa, Bioorg. Chem. 80 (2018) 375–395.

[30] G.N. Tageldin, S.M. Fahmy, H.M. Ashour, M.A. Khalil, R.A. Nassra, I.M. Labouta, Bioorg. Chem. 78 (2018) 358–371.

[31] S. Cherukupalli, B. Chandrasekaran, V. Krystof, R.R. Aleti, N. Sayyad, S.R. Merugu, N.D. Kushwaha, R. Karpoormath, Bioorg. Chem. 79 (2018) 46–59.

[32] Y. Loidreau, V. Levacher, T. Besson, Tetrah. Lett. 54 (2013) 1160–1163.

[33] J.M. Hung, H.J. Arabshahi, E. Leung, J. Reynisson, D. Barker, Eur. J. Med. Chem. 86 (2014) 420–437.

[34] L. Zhao, Y. Zhang, C. Dai, T. Guzi, D. Wiswell, W. Seghezzi, David Parry, T. Fischmann, M.A. Siddiqui, Bioorg. Med. Chem. Lett. 20 (2010) 7216–7722.

[35] P. Muthuraja, H. Makala, S. Prakash, U. Venkatasubramanian, P. Manisankar, Eur. J. Med. Chem. 148 (2018) 106.