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Discovery and optimization of novel phenyldiazepine and pyridodiazepine based Aurora kinase inhibitors

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ABSTRACT

Aurora B plays critical role in the process of chromosome condensation and chromosome orientation during the regulation of mitosis. The overexpression of Aurora B has been observed in several tumor types. As a part of our ongoing effort to develop Aurora B inhibitors, herein, we described the design, synthesis and evaluation of phenyl/pyridine diazepine analogs. The diazepane aniline pyrimidine (**4a**) was identified as an initial hit (Aurora B IC_{50} 6.9 μM). Molecular modeling guided SAR optimization lead to the identification of 8-fluorobenzodiazepine **(6c)** with single digit nM potency (Aurora B IC_{50} 8 nM). In the antiproliferation assay **6c** showed activity across the cell lines with IC_{50} of 0.57, 0.42, and 0.69 μM for MCF-7, MDA-MB 231, and SkoV3 respectively. In the *in vivo* PK profile. **6c** has shown higher bioavailability (73%) along with good exposure (AUC of 1360 ng.h/mL).

1. Introduction

The aurora kinases (A, B and C) belong to the family of serine-threonine kinases that are critical for regulation of cell division. They are overexpressed in a variety of cancers including the lung, breast and colon cancer [1]. Their overexpression levels correlate with the clinical staging of cancers and are also responsible for poor prognosis [2]. The isoforms aurora A, B and C display structural similarity in their kinase domain, however each kinase plays a different role in mitosis. Aurora A has associated with late S phase and enter into the M phase. It is required for many processes including centrosome maturation and separation, chromosome alignment, and mitotic spindle formation. Aurora B (Aur B) plays roles in M phase for chromosome-microtubule alignment and chromosomal cytokinesis [3]. Whereas the function of the Aurora C is not well understood, and it has few overlapping functions with Aur B. Aur B is a member of chromosomal passenger complex (CPC), it required for chromosome condensation and chromosome orientation on the mitotic spindle. CPC is formed by Aur B in the inner centromere protein (INCENP) with Borealin and Survivin [3]. Aur B protein activation requires phosphorylation of INCENP C-terminal IN-box region of TSS motif and it is done by Aur B itself by autophosphorylation of Thr 232 on the activation loop of Aur B [3]. The activity of the Aur B is essential for maintaining the spindle assembly checkpoint (SAC), ensuring the proper chromosome alignment and segregation to enable cytokinesis. Inhibition of Aur B leads to abrogation of the SAC, thereby inducing cells to exit mitosis without dividing [4]. Although both Aurora kinases A & B are being pursued as oncology targets, but more reports focusing on Aur B are found in the literature [5]. Cells appear to be sensitive to Aur B inhibition as the mutations in Aur B found to be enough for high penetrant cell-death phenotype. Suppressing Aur B kinase activity alters alignment of chromosome, cytokinesis and spindle checkpoint function. These features make Aur B an attractive validated drug target [6].

Over the past decades, extensive research has been carried out

Abbreviations: Aur B, Aurora B; DIPEA, *N,N'*-Diisopropylethylamine; T3P, 1-propane phosphonic cyclic anhydride; TFA, Trifluoroacetic acid; LAH, Lithium Aluminium Hydride; TLC, Thin layer chromatography; AcOH, Acetic acid; INCENP, Inner Centromere Protein; PBS, Phosphate buffered saline

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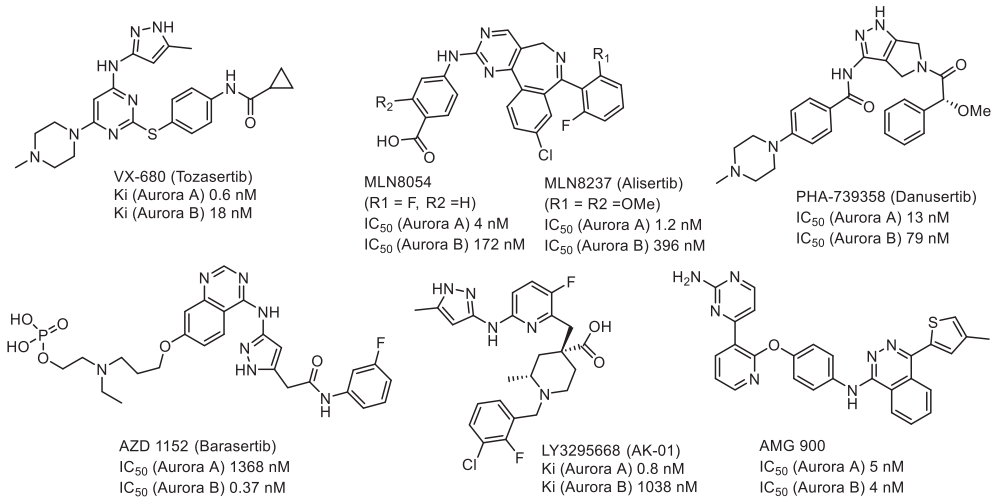


Fig. 1. Structures of known Aurora inhibitors.

towards the identification of small molecules as Aurora kinase inhibitors and a handful of them have entered clinical trials [7,8]. However, most of the earlier designed Aurora kinase inhibitors that reached clinical evaluations lack on the key features which includes pharmacological selectivity, pharmacokinetic profile, and the potential for the clinical assessment of pharmacodynamics biomarkers to correlate clinical outcome and toxicity with target engagement. Continuous research efforts have led to the development of various inhibitors that display selectivity to either Aur A or Aur B. Vertex and Merck has developed inhibitors VX-680 (tozasertib)/MK-0457 [9] for solid tumors. However, its clinical development was halted due to observed safety concerns related to QTC prolongation. Other compounds such as Aur A inhibitor MLN8054 and its close analogue MLN8237 [10] (Fig. 1) taken forward to the clinical trials for solid tumors and leukemia. Furthermore, quinazoline AZD-1152 (barasertib) [11] (Fig. 1) developed by AstraZeneca for solid tumors and Acute Myeloid Leukemia conditions and 3-aminopyrazole PHA-739358 [12] (Fig. 1) developed by Nerviano for solid tumors and Chronic Myelogenous Leukemia are under in phase II trials. Eli Lilly and AurKa Pharma developed Aur A inhibitor LY3295668 (AK-01) [13] for Small cell lung cancer condition, now in Phase I & II clinical trials. Amgen developed pan Aurora inhibitor, oral drug AMG 900, under phase I trial for solid tumor and hematologic cancers [14]. Despite the development of several Aurora inhibitors, currently under clinical development, there is a need for the development of an ideal Aurora inhibitor with better efficacy and safety profile, since many of the clinical trial compounds suffer from one or more other drawbacks. Concerning this, as a part of our ongoing effort to develop an Aur B inhibitor, we designed phenyl/pyridine diazepine analogs as Aur B inhibitor. Similar to the typical kinase inhibitors, our present designed phenyl/pyridine diazepine analogs consist of three parts such as a hinge binder (pyrimidine amine), a solvent accessible group, and DFG-in loop interacting groups. The design idea for compound **4a** is to have the pharmacophores from Pan Aurora inhibitors VX-680 and CCT137690 [15] and hybridize pyrimidine moiety which is expected to have potent anticancer property [16,17], the hinge binder and skeleton with solvent accessible group (Fig. 2). The designed series of phenyl/pyridine diazepine are synthesized and its potential in biological system has been studied and the observed results are rationalized by computational docking studies.

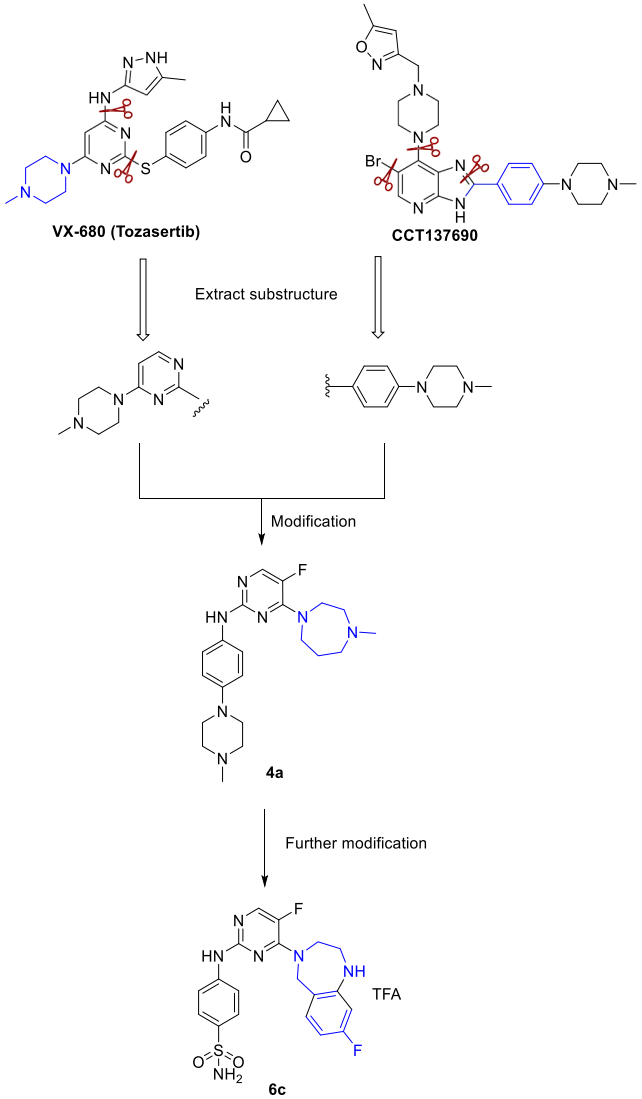
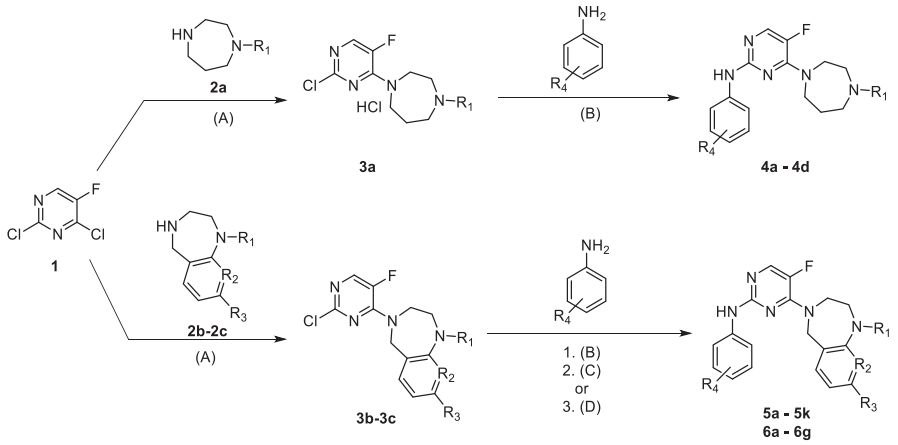


Fig. 2. Rational design idea to prepare Aurora Kinase inhibitors.

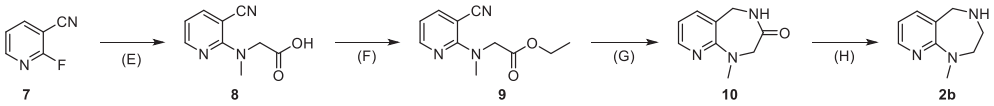
2. Results and discussion

The general synthetic route used for preparation of diazapane compounds **4a-4d** (Scheme 1) was started from commercially available 2,4-dichloro-5-fluoropyrimidine (**1**). Displacement of 4-chloro group [18–21] of 2,4-dichloropyrimidine with 1-methyl-1,4-diazepane gave intermediate **3a** which was coupled with 4-substituted anilines under Buchwald condition [22] to access diazapane analogs **4a-4d**. For



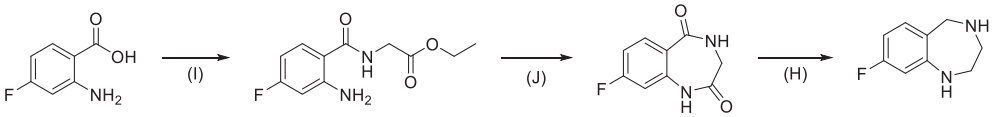
(A) DIPEA, acetonitrile, 80 °C, sealed tube, 12 h. (B) K_2CO_3, X-Phos, $Pd_2(dba)_3$, *tert*-butanol, 100 °C, 12 h, sealed tube, (C) TFA, DCM, rt, 2h. (D) NaOH, EtOH, water, rt, 5 h.

Scheme 1. General scheme for preparation of diazepane/diazepine series.



Scheme 2. Preparation of intermediate 2b.

(E) Sarcosine ethyl ester hydrochloride, DIPEA, EtOH, 80 °C, 12 h, sealed tube. (F) conc. H_2SO_4, EtOH, 80 °C, 12 h. (G) Raney Ni/H_2 (atm), Par shaker (4 kg), 24 h. (H) LAH, THF, 60 °C, 12 h.



Scheme 3. Preparation of intermediate 2c.

(**I**) Glycine ethyl ester HCl salt, T3P, DIPEA, CH_2Cl_2, rt, 12 h (**J**) AcOH, 120 °C, 12 h (**H**) LAH, THF, 60 °C, 12 h

preparing compounds **5a-5k**, first pyridodiazapine intermediate **2b** was synthesized from 2-fluoronicotinonitrile. 2-Fluorine displacement with sarcosine ethyl ester hydrochloride gave intermediate **8**. Further esterification of **8** followed by the reductive cyclization with Raney Ni in the presence of molecular hydrogen gave pyridodiazepinone **10**. The resulting amide bond was reduced with LAH which yield pyridodiazepine intermediate **2b** which was used in the similar fashion to synthesize compounds **5a–5k**. In addition, we also synthesized phenyldiazepine analogs **6a-6g** using intermediate 8-fluorobenzodiazepine which was synthesized from 2-amino-4-fluorobenzoic acid **(11)**. 2-Amino-4-fluorobenzoic acid **(11)** was reacted with glycine ester to form an amide intermediate (**12**). This intermediate **12** was further cyclized with acetic acid followed by the reduction with LAH to give **2c**. 4-Substituted aniline intermediates which were not commercially available, were synthesized starting from 4-fluoronitrobenzene following previously reported methods [23–25] (Schemes 2 and 3).

Scaffold **2a-2c** & **3a-3c**:

2a, R_1 = CH_3

2b, R_1 = CH_3, R_2 = N, R_3 = H

2c, R_1 = H, R_2 = CH, R_3 = F

3a, R_1 = CH_3

3b, R_1 = CH_3, R_2 = N, R_3 = H

3c, R_1 = H, R_2 = CH, R_3 = F

Library-1:

4a, R_1 = CH_3, R_4 = *p-N*-Methylpiperazine (from **3a**)

4b, R_1 = CH_3, R_4 = *p-N*-Acetylpiperazine (from **3a**)

4c, R_1 = CH_3, R_4 = *p*-Morpholine (from **3a**)

4d, R_1 = CH_3, R_4 = *p*-Piperazine (from **3a**)

Library-2:

5a: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *p-N*-Methylpiperazine (from **3b**)

5b: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *p-N*-Acetylpiperazine (from **3b**)

5c: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *p*-Morpholine (from **3b**)

5d: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *p*-$CONH_2$ (from **3b**)

5e: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *N*-Methylpyrazole (from **3b**)

5f: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *p*-CH_2COOH (from **3b**)

5 g: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *p-N,N*-Dimethylpiperidin-4-amine (from **3b**);

5 h: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *p*-Piperidine (from **3b**)

5i: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *p*-Piperazine (from **3b**)

5j: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = *p*-COOH (from **3b**)

5 k: R_1 = CH_3, R_2 = N, R_3 = H, R_4 = 3,4,5-Trimethoxy (from **3b**)

Library-3:

6a: R_1 = H, R_2 = CH, R_3 = F, R_4 = *p-N*-Acetylpiperazine (from **3c**)

6b: R_1 = H, R_2 = CH, R_3 = F, R_4 = *p*-Morpholine (from **3c**)

6c: R_1 = H, R_2 = CH, R_3 = F, R_4 = *p*-SO_2NH_2 (from **3c**)

6d: R_1 = H, R_2 = CH, R_3 = F, R_4 = *p-N*-Methylpiperazine (from **3c**)

6e: R_1 = H, R_2 = CH, R_3 = F, R_4 = 3,4,5-Trimethoxy (from **3c**)

6f: R_1 = H, R_2 = CH, R_3 = F, R_4 = *N*-Methylpyrazole (from **3c**)

6 g: R_1 = H, R_2 = CH, R_3 = F, R_4 = *p-N,N*-Dimethylpiperidin-4-amine (from **3c**)

Enzymatic activity of the prepared compounds against Aurora B kinase was determined using the Lance Ultra kinase assay. The inhibitory activity was represented as the relative percentage inhibition of the positive control PF03814735. The results of the enzymatic activity summarized in Table 1.

The moderate *in vitro* potency (6930 to 1400 nM) observed with compounds **4a-4d** prompted us to investigate their binding modes and

Table 1
Enzymatic assay.

S. No.	Compound number	R_1	R_2	R_3	R_4	*In vitro* IC_{50} (nM)
1	**4a**	CH_3	–	–	*p-N*-Methylpiperazine	6930 ± 1421
2	**4b**	CH_3	–	–	*p-N*-Acetyl piperazine	1800 ± 499
3	**4c**	CH_3	–	–	*p*-Morpholine	1400 ± 810
4	**4d**	CH_3	–	–	*p*-Piperazine	2750 ± 802
5	**5a**	CH_3	N	H	*p-N*-Methylpiperazine	60 ± 2.2
6	**5b**	CH_3	N	H	*p-N*-Acetylpiperazine	36 ± 6.4
7	**5c**	CH_3	N	H	*p*-Morpholine	33 ± 5.6
8	**5d**	CH_3	N	H	*p*-$CONH_2$	42 ± 15.2
9	**5e**	CH_3	N	H	*N*-Methylpyrazole	377 ± 78.4
10	**5f**	CH_3	N	H	*p*-CH_2COOH	31 ± 0.8
11	**5g**	CH_3	N	H	*p-N,N*-Dimethylpiperidin-4-amine	68 ± 16.6
12	**5h**	CH_3	N	H	*p*-Piperidine	96 ± 66.1
13	**5i**	CH_3	N	H	*p*-Piperazine	47 ± 24.5
14	**5j**	CH_3	N	H	*p*-COOH	15 ± 5.2
15	**5k**	CH_3	N	H	3,4,5-Trimethoxy	219 ± 11.1
16	**6a**	H	CH	F	*p-N*-Acetylpiperazine	9 ± 2.8
17	**6b**	H	CH	F	*p*-Morpholine	11 ± 1.9
18	**6c**	H	CH	F	*p*-SO_2NH_2	8 ± 2.6
19	**6d**	H	CH	F	*p-N*-Methylpiperazine	7 ± 0.5
20	**6e**	H	CH	F	3,4,5-Trimethoxy	36 ± 2.4
21	**6f**	H	CH	F	*N*-Methylpyrazole	50 ± 9.7
22	**6g**	H	CH	F	*p-N,N*-Dimethylpiperidin-4-amine	10 ± 8.9
23	**PF-03814735**					4.9 ± 1

IC_{50} values are presented as the means ± SD of triplicate experiments

key interactions in the ATP-binding site of Aur B and accordingly the plan of designs to enhance the potency. Normal rigid docking was performed using the Schrodinger/GLIDE module [26] by docking the dataset compounds in the ATP-binding site of 4AF3.pdb and the results were analyzed to gain insights for future designs. Not all compounds docked in the ATP-binding site, especially, the larger compounds. One of the larger compounds (**4a**), used to run Induced-Fit Docking [27,28], in which conformational changes of residues within the receptor active site as well as those of the ligands being docked are explored combining the Schrodinger modules, Glide and Prime. The output of this routine is an ensemble of receptor-ligand complexes. One of the model selected based on the predicted binding mode of **4a** and the expected interactions. In this routine, the interactions with the kinase hinge were constrained so that all poses will show those interactions and other poses will be discarded by the module. This model then used to perform rigid docking again, in the ATP-binding site. The docking procedure followed; which involves preparation of the ligands using Schrodinger/LigPrep module, conformation analysis using Schrodinger/Macro Model module and finally docking all the conformations [29,30]. The forcefield used in all modules is OPLS2005 [31]. The LigPrep module calculates and fixes charges of all atoms, explores stereoisomers of all chiral centers and generates all possible tautomers. Though the correlation between the experimental potencies and docking scores is not significant for using the model for prediction purposes, the SAR of the series of compounds was studied qualitatively, using this model. All the compounds were docked with a model of Aurora kinase B in complex with compound **4a**, generated using 4AF3.pdb by induced-fit docking (IFD) module of Schrodinger software [3b,32] as mentioned above. Schrodinger/Glide [26] with default settings was used to predict the binding modes. The predicted binding mode of **4a** in the ATP-binding site of Aur B kinase clearly showed that the key interactions and directions of the modifications lead to the improvement of the potency (Fig. 3a). The docking score of **4a** was –9.3.

4a shows a couple of key interactions with residues of the hinge. The pyrimidine nitrogen interacts with the backbone amino group of Ala-157, while the amino linker between the pyrimidine and phenyl rings interacts with the backbone carbonyl of Ala-157. There could be a weak interaction between the proton on the carbon at the 5th position of the pyrimidine ring and the backbone carbonyl of Glu-155. The phenyl ring shows cation-π interactions with the terminal amino group of side chains of Lys-164. The terminal piperazine group is exposed to the solvent, while the diazepane group in compound **4a** sits in a pocket surrounded by the P-loop, the β-strands β_3, β_6, β_7, β_8 and the DFG loop. There appeared to be quite some space in this hydrophobic pocket that can be filled with hydrophobic groups. One of the designs that were considered involved introduction of hydrophobic groups interaction through aromatic conjugation on to diazapane ring. Compound **5a** having a pyridine moiety fused to the diazapane which improved the potency by 116-fold. The docking score of **5a** was –9.4. In the diazepane group of compound **4a**, the protons of carbons present at in the 3rd and 5th positions of the diazepane ring as well as those of the methyl group substituted on the nitrogen in the 4th position of the diazepane ring sterically clashes with the phenyl ring of Phe-88 from the P-loop. Additionally, this methyl substituent on the nitrogen in the 4th position of diazepane ring also sterically clashes with the sidechain of Leu-207 (Fig. 3b). In compound **5a**, since the diazepane ring is fused to the pyridine ring, the carbon in the 5th position of the diazepane ring changes from the sp^3 hybridized carbon to a sp^2 hybridized carbon and hence lack the proton projecting towards the phenyl ring of Phe-88, thus relieving the diazepane ring of clashes with the phenyl ring (Fig. 3c). The pyridine ring of the pyridodiazepine system picks a cation-π interaction with the terminal amino group of Lys-106 from the β_3 strand. Similarly, for compounds **5b**, **5c**, and **5d** potency was improved by 51, 42 and 57-folds respectively. The docking scores for these compounds were –9.8, –9.2 and –9.3 respectively. We explored various *p*-substituted aniline modifications on left hand side keeping pyridodiazapine constant on the right-hand side. Solubilizing groups such as piperidines (**5h** and **5g**), piperazines (**5a**, **5b** and **5i**) and morpholine (**5c**) substituted on 4th position of aniline showed comparable potency in the range of 33–96 nM. Introduction of the carboxylic group at the 4th position on **5j** showed further enhancement in the potency (15 nM). Isostere replacement of acid with amide (**5d**) was also tolerated. The carboxylic group in compounds **5f** and **5j** as well as the carbonyl of amide group in compound **5d** show strong interactions with Lys-164 and Lys-85 leading to a slight boost in potency. Phenyl acetic acid group (**5f**) retained potency similar to benzoic acid (**5j**). Trimethoxy phenyl (**5k**) and *N*-methylpyrazole (**5e**) groups showed potency of 219 nM and 377 nM respectively. Inspired by the above results further, we focused again on the RHS of the compounds to explore design changes to further enhance the potency. The docking scores of all these analogs were in the range of –8.1 to –9.6, while that of **5j** was –10.9.

We examined the predicted binding mode of compound **5a** and observed that the pyridine nitrogen, carrying a partial negative charge is close to the partial negative charge carrying backbone carbonyl of Glu-204, thus leading to a slight repulsion (Fig. 3c). Additionally, we observed that there is still some space beyond the pyridine that can be filled beneficially. Two changes were envisaged: (1) replacement of the pyridine with a fluoro substituted benzene to make the benzodiazepine ring system and (2) removing the methyl substitution from the nitrogen in the diazepine ring. In compound **6d**, the phenyl group of the benzodiazepine ring has a proton in the place of nitrogen, and it exhibits weak interaction with the same backbone carbonyl of Glu-204. Additionally, it is not having the methyl group substituted on the 4th position nitrogen of the diazepine ring, this compound is relieved of steric clashes with the phenyl ring of Phe-88 as well as with the side chain of Leu-207 (Fig. 3d). The fluorine substituted on the phenyl ring of benzodiazepine picks up additional *van der Waals* contacts leading to an improvement in the potency. All these clearly reflect that **6d** enhanced potency by about 8-folds compared to compound **5a**. The

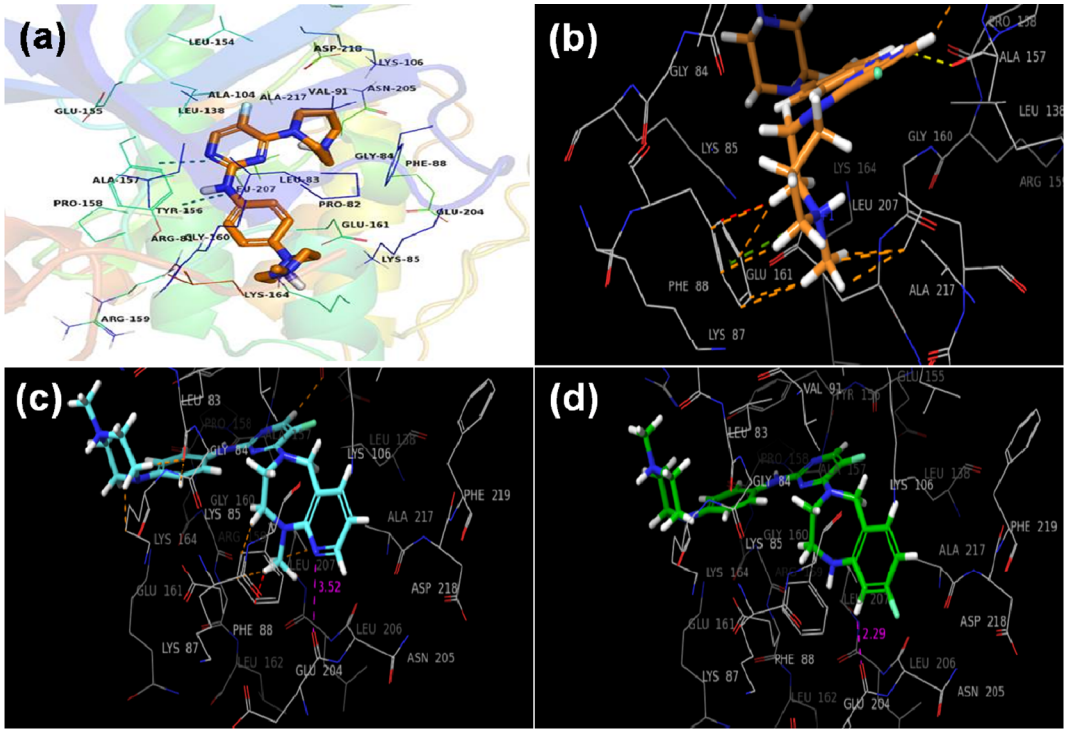


Fig. 3. (a) The predicted binding mode of compound **4a** with Aur kinase B in complex (b) The predicted binding mode of compound **4a** showing the steric clashes (orange and red-colored dashes) of the 4-methyl substituted diazepane ring and residues Phe-88 and Leu-207 (c) The predicted binding mode of compound **5a** showing the steric clashes of the methyl group on the diazepine ring with Phe-88 and Leu-207 as well as the closeness of the pyridine nitrogen to the backbone carbonyl of Glu-204 (d) The predicted binding mode of compound **6d**. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

docking score of **6d** was –9.9.

The 8-fluorobenzodiazepine compounds (**6a, 6b, 6d-6f**) showed better potency compared to their corresponding pyridodiazepine analogs (**5a, 5b, 5c, 5e, 5g** and **5k**). Even the docking scores of the 8-fluorobenzodiazepine series (–8.9 to –10.6) were slightly better than those of the pyridodiazepine series. The milieu of interactions and steric clashes as in the case of compounds **5a** (60 nM) and **6d** (7 nM) also clearly separates the pairs of compounds in terms of their relative potencies: **5k** (219 nM) and **6e** (36 nM), **5e** (377 nM) and **6f** (50 nM) as well as **4d** (2750 nM) and **5i** (47 nM). Similarly, piperidine (**6g**) and morpholine (**6b**) analogs showed potency of 10 nM and 11 nM respectively. The same milieu of interactions and steric clashes that contrast the potencies of compounds **4a** (6930 nM), **5a** (59 nM) and **6d** (7 nM) explain the relative potencies of **4b** (1800 nM), **5b** (36 nM) and **6a** (9 nM) as well as **4c** (1400 nM), **5c** (33 nM) and **6b** (11 nM). Sulfonamide (**6c**) group at 4th position of LHS phenyl showed a potency of 8 nM. The docking score of **6c** was –10.6. Trimethoxyphenyl (**6e**) and *N*-methylpyrazole (**6f**) groups showed improvement in potency with the IC_{50} values of 36 nM and 50 nM respectively.

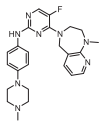
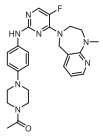
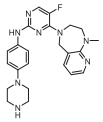
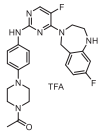
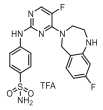
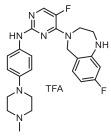
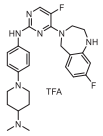
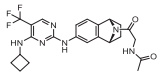
Following the assessment of all the prepared compounds for Aur B enzymatic activity, selected compounds with sufficient activity against Aur B were examined for their antiproliferative activity against human cancer cell lines MCF-7, MDA-MB 231, SkoV3, A375 and A549. The screening results of antiproliferative activity of compounds **5a**, **5b**, **5i**, **6a**, **6c**, **6d**, **6g** are summarized in Table 2. Among the tested compound **6c** showed activity across the cell lines with IC_{50} of 0.57, 0.42, 0.69, 3.97 & 1.53 μM for MCF-7, MDA-MB 231, SkoV3, A375 and A549 respectively. Compounds **5i**, **6a**, **6d** and **6g** showed < 1 μM IC_{50} for MCF7 whereas compounds **6a** and **6d** were more potent against MDA-MB 231 with IC_{50} of 0.23 and 0.16 μM respectively.

Based on the promising biochemical and cellular potency, we selected **5a, 5i, 6a, 6c, 6d** and **6g** for *in vitro* ADME profiling (Table 3). Except for **6a** and **6c**, all the tested compounds showed good solubility (> 50 μM). **6d** and **6g** were quite stable in human liver microsomes compared to the other compounds. In case of mouse liver microsomes, most of the tested compounds showed > 70% disappearance of parent compound. **5i** and **6c** compounds were clean (< 50% @ 20 μM) in CYP inhibition screening for CYP3A4, 2D6 and 2C9 isoforms. Taking into account of potency and ADME data, we selected **5i** and **6c** for pharmacokinetic (PK) profiling in mice by oral and IV administration (Table 4). Compound **6c** showed relatively better PK profile with lower V_d, higher bioavailability (73%) along with good exposure (AUC of 1360 ng h/mL) which correlated well with observed lower clearance. On the other hand, **5i** with high clearance showed moderate exposure (AUC of 635 ng h/mL) and 44.2% bioavailability.

3. Conclusions

A series of novel pyridodiazepine and benzodiazepine analogs were designed and synthesized as Aurora B kinase inhibitors and their structure activity relationship has been investigated. Molecular modeling guided modifications lead to the identification of 8-fluorobenzodiazepine compounds **6a, 6c** and **6d** with single digit nM potency towards Aurora B IC_{50} 9, 8, and 7 nM respectively. Introduction of phenyl or pyridine conjugation with diazepine ring showed the improvement in the potency resulting from enhanced hydrophobic interaction with DFG-in loop. In antiproliferation assay **6c** exhibited activity across the cell lines with IC_{50} of 0.57, 0.42, and 0.69 μM for MCF-7, MDA-MB 231, and SkoV3 respectively. *In vivo* PK profile of **6c** showed higher bioavailability (73%) along with good exposure (AUC of 1360 ng·h/mL). This work provides an attractive lead for further structure optimization in the discovery of potent Aurora B kinase inhibitors. Aurora B inhibitors are under continuous evaluation in clinical trials and a potent inhibitor may soon become a crucial lead in treatment of cancer.

Table 2
Cell proliferation assay.

S. No.	Compound No.	Structure	*In vitro* cellular potency IC_{50} (μM) MCF-7	MDA-MB 231	SkoV3	A375	A549
1	**5a**		1.69 ± 0.70	1.86 ± 0.38	> 10	2.40 ± 0.60	8.39 ± 2.38
2	**5b**		3.09 ± 1.01	> 10	4.53 ± 1.40	4.04 ± 1.19	3.78 ± 1.17
3	**5i**		0.77 ± 0.37	0.71 ± 0.27	> 10	1.30 ± 0.31	3.75 ± 0.83
4	**6a**		0.55 ± 0.24	0.23 ± 0.06	1.12 ± 0.72	1.59 ± 0.23	1.26 ± 0.25
5	**6c**		0.57 ± 0.23	0.42 ± 0.20	0.69 ± 0.30	3.97 ± 0.67	1.53 ± 0.52
6	**6d**		0.95 ± 0.40	0.16 ± 0.06	1.38 ± 0.75	1.19 ± 0.50	> 10
7	**6g**		0.32 ± 0.10	1.12 ± 0.59	> 10	1.37 ± 0.28	1.44 ± 0.60
8	**PF-03814735**[a]		0.44 ± 0.13	0.27 ± 0.06	0.47 ± 0.27	0.58 ± 0.17	0.35 ± 0.05

IC_{50} values are presented as the means ± SD of triplicate experiments
[a] **PF-03814735** suppress expression level of Aur B in cell-based assay [33].

Table 3
ADME properties of selected compounds.

Compounds	5a	5i	6a	6c	6d	6g
Solubility[a] (μM)	92.3	120.2	1.5	3.6	56.5	91
HLM[b] (% disappearance)	65.69	51.96	86.21	64	29.31	32
MLM[c] (% disappearance)	97.92	72.22	93.75	73.41	97.14	98.20
CYP 3A4 IC_{50} (μM)	–	> 20	–	> 20	–	–
CYP2D6 IC_{50} (μM)	–	> 20	–	> 20	–	–
CYP2C9 IC_{50} (μM)	–	> 20	–	> 20	–	–

[a] In PBS pH = 7.4 from DMSO solution.
[b,c] After 30 min.

4. Experimental section

4.1. Lance ultra kinase assay

To determine potencies (IC_{50}) values of the inhibitors against Aurora B kinase, a fluorescence resonance energy transfer (FRET)-based Lance Ultra KinaSelectSer/Thr assay (Perkin-Elmer) was performed under conditions recommended by the manufacturer. In brief, in a half-area 96-well white opaque bottom plate (Perkin Elmer)

5 μL/well of test compound in DMSO was added at a final concentration of 10 μM and serially diluted (3-fold dilution, 10 concentrations). Aurora B kinase inhibitor (PF-03814735) was used as a positive control. 10 μL reaction mixture containing a Ulight-PLK

Table 4
Pharmacokinetic parameters following intravenous and oral administration in mice.

Compounds	Intravenous PK Parameters[a] Dose (mg/Kg)	CL (mL/min/kg)	V_{dss} (L/kg)	$T_{1/2}$ (h)	Oral PK parameters[b] Dose (mg/Kg)	Cmax (nM)	AUC last (ng·h/mL)	F (%)
5i	1	116	6.53	0.80	10	326	635	44.2
6c	1	55.09	1.93	0.41	10	1360	2217	73.3

[a] Formulation for IV dose: 5% DMSO, 5% solutol: absolute alcohol (1:1 v/v) and 90% normal saline.
[b] Formulation for oral dose: 0.025% Tween-80 and 0.5% methyl cellulose in Milli-Q water.

(Ser137) peptide substrate (50 nM) and ATP (15 μM) in assay buffer (50 mM HEPES, pH 7.4, 1 mM EGTA, 10 mM $MgCl_2$, 2 mMDTT and 0.01% Tween 20) was added to each well. The reaction was initiated by adding 5 nM Aurora B enzyme (Invitrogen) at 5 μL per well. After incubation for about 60 min at room temperature, phosphorylation was detected by the addition of a europium-labeled antiphospho-antibody (2 nM). Antibody binding produced a FRET signal that was recorded at using EnVision reader/PheraStar which is excited at 337 nm and emits at 620 and 665 nm. IC_{50} values were determined by plotting % inhibition against concentration of the compound using 4-parameter fit in GraphPad PRISM 5 software.

4.2. Cell proliferation assay

To identify compounds that have inhibitory effect on cancer cell proliferation, the alamar blue cell viability assay was performed as per manufacturer's instructions. Human cancer cells (5000 cells/well) were plated overnight in full-growth medium (containing 10% FBS and 1% penicillin and streptomycin) in 96-well black, flat-bottom polystyrene plate (Corning) at a total volume of 100 μL/well and the plates were incubated in a humidified 37 °C incubator with 5% CO_2. The next day compounds were added to the cells at concentrations ranging from 10 μM to 1.5 nM (final DMSO concentration 0.3%) in the culture medium. After 72 h incubation, effect of compounds on proliferation was determined by the addition of alamar blue (10 μL/well). The plates were then incubated at 37 °C for an additional 3 h and fluorescence was read on a Tecan M200 plate reader (excitation 540 nm; emission 590 nm). A reference compound (Aurora B inhibitor, PF-03814735) was run on each plate as a screening control. GI_{50} values were determined by plotting % inhibition against concentration of the compound using 4-parameter fit in GraphPad PRISM 5 software.

4.3. General information

All reagents were purchased from the commercial suppliers and used without further purification unless otherwise noted. ^{1}H NMR spectra were recorded on Varian 400 MHz spectrometer with $CDCl_3$ or DMSO-d_6 as the solvent. ^{13}C NMR spectra were recorded at 100 MHz. All final compounds were purified to > 95% purity as determined HPLC analysis using a Waters HPLC system.

4.4. General Procedure-A

4.4.1. HCl salt of 1-(2-chloro-5-fluoropyrimidin-4-yl)-4-methyl-1,4-diazepane (3a)

To a stirred solution of 2,4-dichloro-5-fluoropyrimidine (1.4 g, 8.38 mmol) in acetonitrile was added DIPEA (2.16 g, 16.7 mmol) and 1-methyl-1,4-diazepane (0.957 g, 8.38 mmol), then it was stirred at 80 °C for overnight. The reaction mixture was evaporated to give crude. The crude residue was washed with diethyl ether (100 mL) and dried under vacuum to afford the title compound as an off-white solid (1.2 g, 51%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.68 (s, 1H), 8.20 (d, J = 6.4 Hz, 1H), 4.01 (br, 1H), 3.82 (br, 1H), 3.75 (s, 3H), 3.48 (br, 2H), 3.26 (br, 1H), 2.74 (s, 3H), 2.14 (br, 2H).

4.5. General procedure-B

4.5.1. 5-Fluoro-4-(4-methyl-1,4-diazepan-1-yl)-N-(4-(4-methylpiperazin-1-yl)phenyl) pyrimidin-2-amine (4a)

To a solution of HCl salt of 1-(2-chloro-5-fluoropyrimidin-4-yl)-4-methyl-1,4-diazepane (0.1 g, 0.35 mmol) in *tert*-butanol (5 mL) was added 4-(4-methylpiperazin-1-yl)aniline (0.054 g, 0.28 mmol) and potassium carbonate (0.144 g, 1 mmol). The resulting mixture was purged by argon gas for 15 min. *Tris*(dibenzylideneacetone)dipalladium(0) (0.016 g, 0.0175 mmol) and 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (0.016 g, 0.035 mmol) added into reaction mixture and again purged for 5 min and stirred at 100 °C for 12 h. The reaction mixture was concentrated under high vacuum to get crude compound. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as a grey color solid (0.03 g, 21%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.76 (s, 1H), 7.87 (d, J = 6.4 Hz, 1H), 7.46 (d, J = 8.8 Hz, 2H), 6.81 (d, J = 9.6 Hz, 2H), 3.75 – 3.69 (m, 4H), 3.02 (s, 4H), 2.73 (br, 2H), 2.60 (br, 2H), 2.48 (s, 4H), 2.33 (br, 3H), 2.23 (s, 3H), 1.90 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.13, 151.39 (J_{CF} = 5 Hz), 145.96, 143.33 (J_{CF} = 25 Hz), 141.09 (J_{CF} = 242 Hz), 133.99, 119.88, 116.36, 57.82, 56.24, 55.05, 49.30, 48.02, 47.82, 46.05, 27.18; HRMS (ES+) m/z calculated for $C_{21}H_{31}N_7F$, 400.2619; found: 400.2601.

4.5.2. 1-(4-(4-((5-Fluoro-4-(4-methyl-1,4-diazepan-1-yl)pyrimidin-2-yl) amino)phenyl) piperazin-1-yl)ethan-1-one (4b)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as a pale brown color solid (0.217 g, 71.4%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78 (s, 1H), 7.86 (d, J = 7.2 Hz, 1H), 7.49 (d, J = 9.2 Hz, 2H), 6.84 (d, J = 8.8 Hz, 2H), 3.71 – 3.69 (m, 4H), 3.53 (d, J = 4.8 Hz, 4H), 3.01 (t, J = 4.4 Hz, 2H), 2.94 (t, J = 4.8 Hz, 2H), 2.62 (t, J = 4.4 Hz, 2H), 2.48 (s, 2H), 2.25 (s, 3H), 2.00 (s, 3H), 1.85 (t, J = 5.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.63, 156.11, 151.37 (J_{CF} = 5 Hz), 145.77, 143.25 (J_{CF} = 25 Hz), 141.10 (J_{CF} = 241 Hz), 134.57, 119.81, 117.03, 58.03, 56.42, 50.26, 49.84, 48.52 (J_{CF} = 6.2 Hz), 48.18 (J_{CF} = 6.2 Hz), 46.48, 46.08, 27.66. HRMS (ES+) m/z calculated for $C_{22}H_{31}ON_7F$: 428.2569; found: 428.2551.

4.5.3. 5-Fluoro-4-(4-methyl-1,4-diazepan-1-yl)-N-(4-morpholinophenyl) pyrimidin-2-amine (4c)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as a pale pink color solid (0.218 g, 88%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.76 (s, 1H), 7.86 (d, J = 7.2 Hz, 1H), 7.49 (d, J = 9.6 Hz, 2H), 6.82 (d, J = 9.2 Hz, 2H), 3.71 – 3.69 (m, 8H), 2.98 (t, J = 4.8 Hz, 4H), 2.62 (t, J = 4.8 Hz, 2H), 2.25 (s, 3H), 1.85 (t, J = 5.6 Hz, 2H), 1.21 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.13, 151.37 (J_{CF} = 5 Hz), 146.01, 143.25 (J_{CF} = 25 Hz), 141.07 (J_{CF} = 241 Hz), 134.28, 119.85, 116.08, 66.65, 58.03, 56.43, 49.88, 48.35, 48.15, 46.48, 27.67; HRMS (ES+) m/z calculated for $C_{20}H_{28}ON_6F$: 387.2303; found: 387.2300.

4.5.4. tert-Butyl 4-(4-((5-fluoro-4-(4-methyl-1,4-diazepan-1-yl)pyrimidin-2-yl)amino) phenyl)piperazine-1-carboxylate (3d)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as a pale black solid (0.38 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.78 (s, 1H), 7.86 (d, J = 7.2 Hz, 1H), 7.49 (d, J = 8.8 Hz, 2H), 6.83 (d, J = 8.8 Hz, 2H), 3.70 (s, 4H), 3.42 (s, 4H), 2.96 (s, 4H), 2.67 (s, 2H), 2.25 (s, 3H), 1.87 (s, 2H), 1.39 (s, 9H).

4.6. General procedure-C

4.6.1. 5-Fluoro-4-(4-methyl-1,4-diazepan-1-yl)-N-(4-(piperazin-1-yl) phenyl)pyrimidin-2-amine (4d)

To a solution of *tert*-butyl 4-(4-((5-fluoro-4-(4-methyl-1,4-diazepan-1-yl)pyrimidin-2-yl)amino)phenyl)piperazine-1-carboxylate (0.1 g, 0.20 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (0.4 mL) at 0 °C and the resulting mixture was stirred at room temperature for 12 h. The progress of the reaction was monitored by TLC. The reaction mixture was concentrated under reduced pressure to get crude residue. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to give the title compound as a pale yellow solid (0.075 g, 94.9%). ^{1}H NMR (400 MHz,

DMSO-d_6): δ 8.73 (s, 1H), 7.85 (d, J = 7.2 Hz, 1H), 7.46 (d, J = 8.8 Hz, 2H), 6.79 (d, J = 9.2 Hz, 2H), 3.70 (s, 4H), 2.91 (s, 4H), 2.79 (s, 4H), 2.62 (s, 2H), 2.48 (s, 2H), 2.25 (s, 3H), 1.86 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.16, 151.36 (J_{CF} = 5 Hz), 146.78, 143.25 (J_{CF} = 25 Hz), 141.05 (J_{CF} = 242 Hz), 133.89, 119.88, 116.31, 58.05, 56.44, 50.84, 48.56 (J_{CF} = 5.4 Hz), 48.18 (J_{CF} = 5.5 Hz), 46.49, 46.14, 27.69; HRMS (ES-) m/z calculated for $C_{20}H_{27}N_7F$: 384.2306; obtained: 384.2307 (Negative mode).

4.6.2. N-(3-Cyanopyridin-2-yl)-N-methylglycine (8)

To a stirred solution of 2-fluoronicotinonitrile (4 g, 32.7 mmol) in ethanol were added triethyl amine (16.51 g, 163.5 mmol) and sarcosine ethyl ester hydrochloride (7.5 g, 49.1 mmol), then it was stirred at 80 °C for overnight. The reaction mixture was evaporated to give crude product of the title compound as an off-white solid (1.89 g, 87.9%). Crude as such was taken for next step.

4.6.3. Ethyl N-(3-cyanopyridin-2-yl)-N-methylglycinate (9)

To a solution of *N*-(3-cyanopyridin-2-yl)-*N*-methylglycine (6.2 g, 32.4 mmol) in ethanol (150 mL) was added concentrated sulfuric acid (10 mL) at room temperature dropwise for 15 min under nitrogen atmosphere. The resulting mixture was stirred at 80 °C for 12 h. The reaction mixture was concentrated under reduced pressure to remove volatiles. The crude residue was neutralized by aqueous saturated solution of sodium bicarbonate and extracted with ethyl acetate. The organic layer was washed with water, brine, dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure to afford the title compound as a black solid (6.8 g, 95.7%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (d, J = 3.2 Hz, 1H), 7.74 (d, J = 7.2 Hz, 1H), 6.67 (q, J = 6.8 Hz, 1H), 4.32 (s, 2H), 4.20 (q, J = 14 Hz, 2H), 3.46 (s, 3H), 1.26 (t, J = 14.4 Hz, 3H).

4.6.4. 1-Methyl-1,2,4,5-tetrahydro-3H-pyrido[2,3-e][1,4]diazepin-3-one (10)

To a solution of ethyl *N*-(3-cyanopyridin-2-yl)-*N*-methylglycinate (3 g, 13.6 mmol) in ethanol (100 mL) in a clean and dry hydrogenating steel vessel, Raney Ni (0.38 g, 10%) was added, under nitrogen atmosphere. The mixture was hydrogenated at 100 psi for overnight. After TLC showed completion of reaction, the mixture was filtered through a celite bed and the filtrate was concentrated to give title compound as off-white solid (1.6 g, 66%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07 (s, 1H), 7.99 (dd, J = 4.8 Hz, J = 5.2 Hz, 1H), 7.27 (d, J = 6 Hz, 1H), 6.53 (q, J = 7.2 Hz, 1H), 4.27 (d, J = 4.8 Hz, 2H), 4.10 (s, 2H), 3.09 (s, 3H).

4.6.5. 1-Methyl-2,3,4,5-tetrahydro-1H-pyrido[2,3-e][1,4]diazepine (2b)

To a solution of 1-methyl-1,2,4,5-tetrahydro-3*H*-pyrido[2,3-*e*][1,4] diazepin-3-one (2.4 g, 13.5 mmol) in THF (30 mL) was added 2M solution of LAH in THF (33.7 mL, 67.5 mmol) at 0 °C and stirred for 12 h at 60 °C. The progress of the reaction was monitored by TLC. The crude residue was quenched by ice and extracted with dichloromethane. Combined organic layer was dried over anhydrous sodium sulfate, concentrated to get crude residue. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to give the title compound as a yellow liquid (1.9 g, 86.3%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.01 (dd, J = 2 Hz, J = 1.6 Hz, 1H), 7.33 (dd, J = 1.2 Hz, J = 1.6 Hz, 1H), 6.69 (dd, J = 4.8 Hz, J = 4.8 Hz, 1H), 3.64 (s, 2H), 3.05 – 3.02 (m, 2H), 2.89 (s, 5H), 2.38 (br, 1H).

4.6.6. 4-(2-Chloro-5-fluoropyrimidin-4-yl)-1-methyl-2,3,4,5-tetrahydro-1H-pyrido[2,3-e][1,4]diazepine (3b)

The title compound was prepared according to general procedure A. The crude residue was purified by gradient column chromatography using ethyl acetate in hexane to give the title compound as an off-white solid (0.4 g, 94.5%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.12 (d, J = 6.4 Hz, 1H), 8.00 (dd, J = 4.8 Hz, J = 4.8 Hz, 1H), 7.41 (d, J = 7.2 Hz, 1H), 6.67 (q, J = 7.6 Hz, 1H), 4.78 (s, 2H), 3.99 (t, J = 10.4 Hz, 2H), 3.59 (t, J = 10.4 Hz, 2H), 2.97 (s, 3H).

4.6.7. 5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4] diazepin-4-yl)-N-(4-(4-methylpiperazin-1-yl)phenyl)pyrimidin-2-amine (5a)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as a pale yellow solid (0.080 g, 52.6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.75 (s, 1H), 7.98 (d, J = 3.2 Hz, 1H), 7.86 (d, J = 6.4 Hz, 1H), 7.40 – 7.36 (m, 3H), 6.84 (d, J = 8.8 Hz, 2H), 6.64 (t, J = 11.6 Hz, 1H), 4.73 (s, 2H), 3.97 (s, 2H), 3.52 (s, 2H), 3.03 (s, 4H), 2.97 (s, 4H), 2.48 (s, 3H), 2.21 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.54, 156.18, 151.28, 146.30, 143.58 (J_{CF} = 24.8 Hz), 241.7 (J_{CF} = 241.7 Hz), 138.26, 133.62, 120.90, 120.49, 116.33, 114.34, 114.16, 55.14, 52.04, 49.99, 49.92, 49.42, 46.15. HRMS (ES+) m/z calculated for $C_{24}H_{30}FN_8$: 449.2577; found: 449.2575.

4.6.8. 1-(4-(4-((5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4]diazepin-4-yl)pyrimidin-2-yl)amino)phenyl)piperazin-1-yl)ethan-1-one (5b)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as a pale brown solid (0.040 g, 24.6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.79 (s, 1H), 7.98 (d, J = 3.2 Hz, 1H), 7.87 (d, J = 6.8 Hz, 1H), 7.42 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 7.2 Hz, 1H), 6.87 (d, J = 9.2 Hz, 2H), 6.65 (q, J = 7.2 Hz, 1H), 4.74 (s, 2H), 3.97 (s, 2H), 3.54 (t, J = 8.8 Hz, 6H), 3.04 (s, 2H), 2.97 (s, 5H), 2.01 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.64, 160.52, 156.12, 151.34, 146.30, 146.07, 143.72 (J_{CF} = 25 Hz), 141.10 (J_{CF} = 242 Hz), 138.25, 134.21, 120.85, 120.40, 117.04, 114.33, 52.01, 50.26, 49.97, 49.84, 46.10, 21.63; HRMS (ES+) m/z calculated for $C_{25}H_{30}ON_8F$: 477.2521; found: 477.2498.

4.6.9. 5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4] diazepin-4-yl)-N-(4-morpholinophenyl)pyrimidin-2-amine (5c)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as an off-white solid (0.046 g, 31%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.77 (s, 1H), 7.99 (d, J = 3.6 Hz, 1H), 7.87 (d, J = 6.8 Hz, 1H), 7.41 (d, J = 8.8 Hz, 2H), 7.38 (d, J = 7.6 Hz, 1H), 6.85 (d, J = 8.8 Hz, 2H), 6.65 (q, J = 7.2 Hz, 1H), 4.73 (s, 2H), 3.97 (s, 2H), 3.71 (t, J = 9.2 Hz, 4H), 3.52 (s, 2H), 3.00 (t, J = 9.6 Hz, 4H), 2.97 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.52, 156.15, 151.35, 151.30, 146.30, 143.73 (J_{CF} = 25 Hz), 141.09 (J_{CF} = 242 Hz), 138.25, 133.93, 120.87, 120.46, 116.09, 114.33, 66.65, 52.02, 50.09, 49.99, 49.88. HRMS (ES+) m/z calculated for $C_{23}H_{27}ON_7F$: 436.2256; found: 436.2256.

4.6.10. 4-((5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4]diazepin-4-yl)pyrimidin-2-yl)amino)benzamide (5d)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as an off-white solid (0.015 g, 11.1%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.35 (s, 1H), 8.00 – 7.96 (m, 2H), 7.76 (d, J = 8.4 Hz, 2H), 7.72 (s, 1H), 7.65 (d, J = 8.8 Hz, 2H), 7.46 (d, J = 6.8 Hz, 1H), 7.06 (s, 1H), 6.68 (q, J = 7.2 Hz, 1H), 4.80 (s, 2H), 4.01 (s, 2H), 3.58 (s, 2H), 2.98 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.07, 160.41, 155.44, 151.54, 146.39, 144.13, 143.64 (J_{CF} = 25 Hz), 141.56 (J_{CF} = 244 Hz), 138.17, 128.64, 126.60, 120.56, 117.40, 114.28, 51.74, 50.04, 50.03. HRMS (ES+) m/z calculated for $C_{20}H_{21}ON_7F$: 394.1786; found: 394.1778.

4.6.11. 5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4] diazepin-4-yl)-N-(1-methyl-1H-pyrazol-3-yl)pyrimidin-2-amine (5e)

The title compound was prepared according to general procedure B.

The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as an off-white solid (0.050 g, 41.6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (s, 1H), 8.00 (d, J = 4 Hz, 1H), 7.86 (d, J = 6.8 Hz, 1H), 7.46 (s, 2H), 6.66 (t, J = 12 Hz, 1H), 6.36 (s, 1H), 4.72 (s, 2H), 3.97 (s, 2H), 3.96 (s, 3H), 3.49 (s, 2H), 2.96 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.60, 155.60, 151.44, 151.38, 148.95, 146.32, 143.68 (J_{CF} = 25 Hz), 141.29 (J_{CF} = 241 Hz), 138.42, 130.93, 121.00, 114.43, 52.15, 50.15, 49.94, 38.61. HRMS (ES+) *m/z* calculated for $C_{17}H_{20}N_8F$: 355.1789; found: 355.1782.

4.6.12. Ethyl 2-(4-((5-fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido [2,3-e][1,4]diazepin-4-yl)pyrimidin-2-yl)amino)phenyl)acetate (3e)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to afford the title compound as a pale yellow solid (0.060 g, 20%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.59 (s, 1H), 8.11 (s, 1H), 7.68 (d, J = 6.8 Hz, 1H), 7.31 (s, 4H), 7.11 (s, 1H), 6.73 (s, 1H), 4.88 (s, 2H), 4.23 (s, 2H), 4.16 (q, J = 14.4 Hz, 2H), 3.81 (s, 2H), 3.65 (s, 2H), 3.28 (s, 3H), 1.27 (t, J = 14 Hz, 3H).

4.7. General procedure -D

4.7.1. 2-(4-((5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e] [1,4]diazepin-4-yl)pyrimidin-2-yl)amino)phenyl)acetic acid (5f)

A solution of ethyl 2-(4-((5-fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4*H*-pyrido[2,3-*e*][1,4]diazepin-4-yl)pyrimidin-2-yl)amino)phenyl) acetate (0.06 g, 0.13 mmol) in a mixture of ethanol (5 mL) and tetrahydrofuran (5 mL) was added 2 N sodium hydroxide solution (0.13 mL, 0.27 mmol) slowly at room temperature. The resulting mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated to remove volatiles. The aqueous layer was acidified with 1.5 N hydrochloric acid and extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, concentrated to get crude compound. The crude residue was purified by flash column chromatography using methanol in dichloromethane to afford the title compound as an off white solid (0.035 g, 62%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.4 (br, 1H), 9.17 (s, 1H), 7.98 (d, J = 6.8 Hz, 1H), 7.90 (d, J = 5.2 Hz, 1H), 7.62 (s, 1H), 7.46 (d, J = 8.4 Hz, 2H), 7.18 (d, J = 8.8 Hz, 2H), 6.80 (t, J = 12.8 Hz, 1H), 4.91 (s, 2H), 4.03 (s, 2H), 3.88 (s, 2H), 3.49 (s, 2H), 3.10 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 173.37, 158.75, 156.02, 154.41, 152.29, 141.33 (J_{CF} = 25 Hz), 140.83 (J_{CF} = 244 Hz), 139.61, 139.52, 129.91, 128.98, 123.37, 119.93, 113.68, 51.33, 49.61, 49.58, 48.70. HRMS (ES+) *m/z* calculated for $C_{21}H_{22}O_2N_6F$: 409.1783; found: 409.1789.

4.7.2. N-(4-(4-(Dimethylamino)piperidin-1-yl)phenyl)-5-fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4]diazepin-4-yl)pyrimidin-2-amine (5g)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane to afford the title compound as a pale yellow solid (0.1 g, 30.8%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.74 (s, 1H), 7.99 (d, J = 4.4 Hz, 1H), 7.86 (d, J = 6 Hz, 1H), 7.38 (d, J = 8 Hz, 3H), 6.84 (d, J = 8.4 Hz, 2H), 6.64 (t, J = 11.6 Hz, 1H), 4.73 (s, 2H), 3.97 (s, 2H), 3.58 – 3.52 (m, 4H), 2.97 (s, 3H), 2.59 – 2.53 (m, 3H), 2.18 (s, 6H), 1.81 (d, J = 12.4 Hz, 2H), 1.47 (q, J = 21.6 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.54, 156.17, 151.28, 146.50, 146.29, 143.73 (J_{CF} = 25 Hz), 141.06 (J_{CF} = 241 Hz), 138.26, 133.46, 120.9, 120.53, 116.88, 114.33, 61.90, 52.05, 50.00, 49.99, 49.52, 41.87, 28.37. HRMS (ES-) *m/z* calculated for $C_{26}H_{32}N_8F$: 475.2728; found: 475.2736 (Negative mode).

4.7.3. tert-Butyl4-(4-((5-fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido [2,3-e][1,4]diazepin-4-yl)pyrimidin-2-yl)amino)phenyl)piperidine-1-carboxylate (3f)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to give the title compound as a pale-yellow solid (0.1 g, 55.2%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (d, J = 3.6 Hz, 1H), 7.80 (d, J = 6.4 Hz, 1H), 7.39 (d, J = 8.4 Hz, 2H), 7.35 (d, J = 7.2 Hz, 1H), 7.14 (d, J = 8.4 Hz, 2H), 6.68 – 6.65 (m, 2H), 4.72 (s, 2H), 4.23 (br, 2H), 4.07 (s, 2H), 3.42 (br, 2H), 3.09 (s, 3H), 2.79 (t, J = 24.4 Hz, 2H), 2.61 (t, J = 24 Hz, 1H), 1.82 (d, J = 12.8 Hz, 2H), 1.60 (d, J = 12.8 Hz, 2H), 1.48 (s, 9H).

4.7.4. 5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4] diazepin-4-yl)-N-(4-(piperidin-4-yl)phenyl)pyrimidin-2-amine (5h)

The title compound was prepared according to general procedure C. The crude residue was purified by flash column chromatography using methanol in dichloromethane to give the title compound as a white solid (0.025 g, 30.8%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (s, 1H), 7.99 (d, J = 2.8 Hz, 1H), 7.90 (d, J = 6.8 Hz, 1H), 7.47 (d, J = 8.4 Hz, 2H), 7.39 (d, J = 7.2 Hz, 1H), 7.08 (d, J = 8.4 Hz, 2H), 6.64 (t, J = 5.2 Hz, 1H), 4.75 (s, 2H), 3.99 (s, 2H), 3.54 (s, 2H), 3.01 (s, 2H), 2.97 (s, 3H), 2.57 (d, J = 11.6 Hz, 3H), 1.65 (d, J = 12.4 Hz, 2H), 1.50 – 1.42 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.49, 155.96, 151.41, 151.35, 146.32, 143.68 (J_{CF} = 25 Hz), 141.23 (J_{CF} = 245 Hz), 139.21, 138.22, 126.86, 120.77, 119.22, 114.26, 51.95, 50.03, 50.01, 46.99, 42.20, 34.70. HRMS (ES+) *m/z* calculated for $C_{24}H_{29}N_7F$: 434.2463; found: 434.2462.

4.7.5. tert-Butyl 4-(4-((5-fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido [2,3-e][1,4]diazepin-4-yl)pyrimidin-2-yl)amino)phenyl)piperazine-1-carboxylate (3g)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane to give the title compound as a grey color solid (0.1 g, 36.6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 1H), 7.99 (d, J = 3.6 Hz, 1H), 7.88 (d, J = 6.8 Hz, 1H), 7.43 – 7.37 (m, 3H), 6.86 (d, J = 8.8 Hz, 2H), 6.66 (t, J = 12 Hz, 1H), 4.74 (s, 2H), 3.97 (s, 2H), 3.52 (br, 2H), 3.43 (br, 4H), 2.99 – 2.96 (m, 7H), 1.4 (s, 9H).

4.7.6. 5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4] diazepin-4-yl)-N-(4-(piperazin-1-yl)phenyl)pyrimidin-2-amine (5i)

The title compound was prepared according to general procedure C. The crude residue was purified by flash column chromatography using methanol in dichloromethane to give the title compound as a white solid (0.05 g, 61.7%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.74 (s, 1H), 7.99 (d, J = 3.6 Hz, 1H), 7.86 (d, J = 6.8 Hz, 1H), 7.38 (d, J = 8.4 Hz, 3H), 6.82 (d, J = 8.4 Hz, 2H), 6.45 (t, J = 11.6 Hz, 1H), 4.73 (s, 2H), 3.97 (br, 2H), 3.52 (br, 2H), 2.97 (s, 3H), 2.94 (br, 4H), 2.81 (br, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.54, 156.20, 151.28, 147.10, 146.29, 143.72 (J_{CF} = 25 Hz), 141.07 (J_{CF} = 241 Hz), 138.27, 133.52, 120.90, 120.56, 116.31, 114.33, 52.05, 50.82, 50.00, 49.99, 46.13. HRMS (ES-) *m/z* calculated for $C_{23}H_{26}N_8F$: 433.2259; found: 433.2263. (Negative mode)

4.7.7. Ethyl 4-((5-fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e] [1,4]diazepin-4-yl) pyrimidin-2-yl)amino)benzoate (3h)

The title compound was prepared according to general procedure B. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to give the title compound as an off-white solid (0.15 g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (dd, J = 2 Hz, J = 1.6 Hz, 1H), 7.98 (d, J = 9.2 Hz, 2H), 7.85 (d, J = 6.8 Hz, 1H), 7.55 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 6.8 Hz, 1H), 6.99 (s, 1H), 6.71 (q, J = 7.2 Hz,1H), 4.77 (s, 2H), 4.36 (q, J = 12 Hz, 2H), 4.09 (t, J = 10 Hz, 2H), 3.47 (t, J = 9.6 Hz, 2H), 3.10 (s, 3H), 1.39 (t, J = 14 Hz, 3H).

4.7.8. 4-((5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4] diazepin-4-yl) pyrimidin-2-yl)amino)benzoic acid (5j)

The title compound was prepared according to general procedure D. The crude residue was purified by flash column chromatography using methanol in dichloromethane to give the title compound as an off-white solid (0.1 g, 71%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.79 (s, 1H), 8.10 (d, J = 6 Hz, 1H), 7.92 – 7.85 (m, 4H), 7.69 (d, J = 8.4 Hz, 2H), 6.89 (t, J = 6 Hz, 1H), 5.03 (s, 2H), 4.37 (br, 2H), 4.08 (s, 2H), 3.22 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 167.45, 154.09, 153.09, 153.02, 152.78, 143.86 (J_{CF} = 28 Hz), 140.93 (J_{CF} = 245 Hz), 139.12, 138.82, 136.55, 130.83, 124.32, 118.66, 113.27, 50.96, 49.50, 48.34. HRMS (ES+) *m/z* calculated for $C_{20}H_{20}O_2N_6F$: 395.1626; found: 395.1663.

4.7.9. 5-Fluoro-4-(1-methyl-1,2,3,5-tetrahydro-4H-pyrido[2,3-e][1,4] diazepin-4-yl)-N-(3,4,5-trimethoxyphenyl)pyrimidin-2-amine (5k)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane to give the title compound as an off-white solid (0.1 g, 44.4%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.91 (s, 1H), 7.99 (d, J = 3.2 Hz, 1H), 7.91 (d, J = 7.2 Hz, 1H), 7.38 (d, J = 7.2 Hz, 1H), 7.03 (s, 2H), 6.64 (q, J = 6.8 Hz, 1H), 4.75 (s, 2H), 4.02 (br, 2H), 3.73 (s, 6H), 3.59 (s, 3H), 3.55 (d, J = 4.4 Hz, 2H), 2.97 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.45, 155.87, 153.08, 151.39, 146.45, 143.61 (J_{CF} = 26 Hz), 141.24 (J_{CF} = 242.1 Hz), 138.07, 137.56, 132.51, 120.82, 114.35, 97.06, 60.58, 56.22, 51.82, 50.24, 49.78. HRMS (ES +) *m/z* calculated for $C_{22}H_{26}N_6O_3F$: 441.2050; found: 441.2076.

4.8. Ethyl(2-amino-4-fluorobenzoyl)glycinate (12)

A mixture of 2-Amino-4-fluoro benzoic acid (5 g, 32.2 mmol), glycine ethyl ester HCl salt (4.95 g, 35.48 mmol), in dichloromethane (270 mL), DIPEA (19.28 mL, 112.87 mmol) was added at 0 °C then 1-propane phosphonic anhydride (20.51 g, 64.5 mmol) was added and stirred at room temperature overnight. The solution was diluted with dichloromethane, washed with saturated bicarbonate solution and brine, dried over sodium sulfate, filtered and the solvent was removed under vacuum. Purification of the crude product by flash chromatography gave the title compound as an off-white solid (5.12 g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40 (t, J = 2.2 Hz, 1H), 6.48 (br, 1H), 6.39 – 6.35 (m, 2H), 4.26 (q, J = 3.5 Hz, 2H), 4.17 (d, J = 1.2 Hz, 2H), 1.31 (t, J = 3.6 Hz, 3H).

4.9. 8-Fluoro-3,4-dihydro-1H-benzo[e][1,4]diazepine-2,5-dione (13)

To a solution of methyl (2-amino-4-fluorobenzoyl)glycinate (5.12 g, 21.3 mmol) in acetic acid (140 mL) was heated at 120 °C for 12 h. After completion of the reaction as confirmed by TLC, AcOH was removed in vacuo, the residue was dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution and brine. The organic layers were dried over sodium sulfate and the solvent was removed under vacuum. Purification of the crude product by flash chromatography gave the title compound as a yellowish liquid (3.71 g, 89.8%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.41 (s, 1H), 8.49 (t, J = 6 Hz, 1H), 7.79 (q, J = 6.8 Hz, 1H), 7.05 (t, J = 8.4 Hz, 1H), 6.88 (dd, J = 2.8 Hz, J = 2.8 Hz, 1H), 3.60 (d, J = 5.6 Hz, 2H).

4.9.1. 8-Fluoro-2,3,4,5-tetrahydro-1H-benzo[e][1,4]diazepine (2c)

To a solution of 8-fluoro-3,4-dihydro-1*H*-benzo[*e*][1,4]diazepine-2,5-dione (1.6 g, 8.24 mmol) in THF (30 mL) was added 2 M solution of LAH in THF (20.6 mL, 41.2 mmol) at 0 °C and stirred for 12 h at 60 °C. The progress of the reaction was monitored by TLC. The crude residue was quenched with ice and extracted with dichloromethane. The combined organic layers were dried over anhydrous sodium sulfate, concentrated to get crude residue. The crude residue was purified by gradient column chromatography using methanol in dichloromethane to give the title compound as a yellow liquid (1.05 g, 77.2%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.01 (t, J = 7.2 Hz, 1H), 6.51 – 6.44 (m, 2H), 3.84 (s, 2H), 3.11 – 3.09 (m, 2H), 3.04 – 3.02 (m, 2H).

4.9.2. 4-(2-Chloro-5-fluoropyrimidin-4-yl)-1-methyl-2,3,4,5-tetrahydro-1H-pyrido[2,3-e][1,4]diazepine (3c)

The title compound was prepared according to general procedure A. The crude residue was purified by gradient column chromatography using ethyl acetate in hexane to give the title compound as an off-white solid (1.5 g, 80.2%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.13 (d, J = 6.4 Hz, 1H), 7.10 (t, J = 7.2 Hz, 1H), 6.54 (d, J = 11.2 Hz, 1H), 6.47 (t, J = 8.4 Hz, 1H), 5.99 (s, 1H), 4.69 (s, 2H), 3.91 (s, 2H), 3.28 (s, 2H).

4.9.3. TFA salt of 1-(4-(4-((5-fluoro-4-(8-fluoro-1,2,3,5-tetrahydro-4H-benzo[e][1,4]diazepin-4-yl)pyrimidin-2-yl)amino)phenyl)piperazin-1-yl) ethan-1-one (6a)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane. The crude material was once again purified by Prep. HPLC (0.1% TFA in water/ACN) to give the title compound as a pale pink solid (0.049 g, 22.2%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.25 (br, 1H), 7.96 (d, J = 7.6 Hz, 1H), 7.37 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 8.4 Hz, 3H), 6.54 (dd, J = 2.8 Hz, J = 2.4 Hz, 1H), 6.39 (t, J = 6.4 Hz, 1H), 5.97 (br, 1H), 4.68 (s, 2H), 3.94 (s, 2H), 3.56 (s, 4H), 3.28 (s, 2H), 3.11 (s, 2H), 3.04 (s, 2H), 2.02 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 168.70, 162.30 (J_{CF} = 240 Hz), 158.89 (J_{CF} = 34 Hz), 152.90, 152.30 (J_{CF} = 10 Hz), 152.053, 147.13, 140.25 (J_{CF} = 244 Hz), 137.22, 132.072, 122.76, 121.86, 117.07, 104.99 (J_{CF} = 21 Hz), 104.53 (J_{CF} = 23 Hz), 52.46, 51.63, 49.79, 45.65, 21.61. HRMS (ES+) *m/z* calculated for $C_{25}H_{28}ON_7F_2$: 480.2318; found: 480.2320.

4.9.4. TFA salt of 5-fluoro-4-(8-fluoro-1,2,3,5-tetrahydro-4H-benzo[e][1,4]diazepin-4-yl)-N-(4-morpholinophenyl)pyrimidin-2-amine (6b)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane. The crude material was once again purified by Prep. HPLC (0.1% TFA in water/ACN) to give the title compound as a white solid (0.031 g, 16.6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.33 (br, 1H), 7.97 (d, J = 7.6 Hz, 1H), 7.35 (d, J = 8.8 Hz, 2H), 6.96 (d, J = 8.4 Hz, 2H), 6.90 (br, 1H), 6.54 (d, J = 9.2 Hz, 1H), 6.38 (t, J = 7.2 Hz, 1H), 5.98 (br, 1H), 4.69 (s, 2H), 3.95 (s, 2H), 3.73 (s, 4H), 3.29 (s, 2H), 3.08 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.33 (J_{CF} = 241 Hz), 159.11, 158.76, 152.25 (J_{CF} = 10 Hz), 151.98, 139.95 (J_{CF} = 245 Hz), 135.39 (J_{CF} = 21 Hz), 132.12 (J_{CF} = 10 Hz), 130.98, 123.41, 121.46, 116.32, 104.96 (J_{CF} = 21 Hz), 104.48 (J_{CF} = 23 Hz), 66.42, 52.63, 51.65, 49.66, 45.30. HRMS (ES+) *m/z* calculated for $C_{23}H_{25}ON_6F_2$: 439.2052; found: 439.2032.

4.9.5. TFA salt of 4-((5-fluoro-4-(8-fluoro-1,2,3,5-tetrahydro-4H-benzo[e][1,4]diazepin-4-yl)pyrimidin-2-yl)amino)benzenesulfonamide (6c)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane. The crude material was once again purified by Prep. HPLC (0.1% TFA in water/ACN) to give the title compound as a white solid (0.035 g, 19%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.53 (s, 1H), 8.00 (d, J = 6.8 Hz, 1H), 7.75 (d, J = 8.8 Hz, 2H), 7.68 (d, J = 8.8 Hz, 2H), 7.13 (br, 3H), 6.55 (dd, J = 2 Hz, J = 2 Hz, 1H), 6.44 (t, J = 8.4 Hz, 1H), 5.96 (br, 1H), 4.72 (s, 2H), 3.95 (s, 2H), 3.30 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.27 (J_{CF} = 240.2 Hz), 158.81 (J_{CF} = 36.4 Hz), 154.35, 152.30, 151.42, 143.95, 141.79 (J_{CF} = 27.9 Hz), 141.51 (J_{CF} = 244 Hz), 131.9 (J_{CF} = 9 Hz), 126.98, 122.41, 118.26, 105.07 (J_{CF} = 21 Hz), 104.60 (J_{CF} = 23 Hz), 52.19, 51.49, 45.41. HRMS (ES+) *m/z* calculated for

$C_{20}H_{20}N_5O_2F_2S$: 432.1306; found: 432.1297.

4.9.6. TFA salt of 5-fluoro-4-(8-fluoro-1,2,3,5-tetrahydro-4H-benzo[e][1,4]diazepin-4-yl)-N-(4-(4-methylpiperazin-1-yl)phenyl)pyrimidin-2-amine (6d)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane. The crude material was once again purified by Prep. HPLC (0.1% TFA in water/ACN) to afford the title compound as a white solid (0.057 g, 19.9%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.53 (br, 1H), 9.00 (br, 1H), 7.91 (d, J = 7.2 Hz, 1H), 7.44 (d, J = 8.8 Hz, 2H), 6.99 (br, 1H), 6.94 (d, J = 8.8 Hz, 2H), 6.55 (d, J = 9.2 Hz, 1H), 6.40 (t, J = 6.8 Hz, 1H), 5.95 (br, 1H), 4.67 (s, 2H), 3.92 (s, 2H), 3.72 (d, J = 12.4 Hz, 2H), 3.51 (d, J = 12 Hz, 2H), 3.25 (s, 2H), 3.16 (t, J = 10.8 Hz, 2H), 2.90 (s, 5H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.24 (J_{CF} = 240 Hz), 158.71 (J_{CF} = 34 Hz), 154.19, 152.33, 151.58, 145.53, 140.67 (J_{CF} = 244 Hz), 133.35, 131.99, 122.38, 121.77, 117.05, 105.21 (J_{CF} = 21 Hz), 104.60 (J_{CF} = 23 Hz), 52.91, 52.24, 51.53, 46.86, 45.62, 42.56. HRMS (ES+) m/z calculated for $C_{24}H_{28}N_7F_2$: 452.2374; found: 452.2376.

4.9.7. TFA salt of 5-fluoro-4-(8-fluoro-1,2,3,5-tetrahydro-4H-benzo[e][1,4]diazepin-4-yl)-N-(3,4,5-trimethoxyphenyl)pyrimidin-2-amine (6e)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane. The crude material was once again purified by Prep. HPLC (0.1% TFA in water/ACN) to afford the title compound as a white solid (0.07 g, 37.2%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.99 (br, 1H), 7.93 (d, J = 6.8 Hz, 1H), 7.04 (t, J = 7.2 Hz, 1H), 6.98 (s, 2H), 6.54 (dd, J = 2.8 Hz, J = 2 Hz, 1H), 6.40 (t, J = 8 Hz, 1H), 5.93 (br, 1H), 4.68 (s, 2H), 3.96 (s, 2H), 3.72 (s, 6H), 3.59 (s, 3H), 3.27 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.27 (J_{CF} = 241 Hz), 154.85, 153.15, 152.38 (J_{CF} = 10 Hz), 151.33, 141.67, 141.02 (J_{CF} = 244 Hz), 136.81, 133.06, 131.89 (J_{CF} = 10 Hz), 122.72, 105.04 (J_{CF} = 21 Hz), 104.60 (J_{CF} = 23 Hz), 97.90, 60.58, 56.26, 51.82, 51.55, 45.57; HRMS (ES+) m/z calculated for $C_{22}H_{24}O_3N_5F_2$: 444.1842 found: 444.1852.

4.9.8. TFA salt of 5-fluoro-4-(8-fluoro-1,2,3,5-tetrahydro-4H-benzo[e][1,4]diazepin-4-yl)-N-(1-methyl-1H-pyrazol-3-yl)pyrimidin-2-amine (6f)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane. The crude material was once again purified by Prep. HPLC (0.1% TFA in water/ACN) to afford the title compound as a white solid (0.075 g, 47%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.17 (br, 1H), 8.01 (d, J = 8 Hz, 1H), 7.63 (s, 1H), 7.16 (s, 1H), 6.55 (d, J = 9.6 Hz, 1H), 6.45 (t, J = 8 Hz, 1H), 6.20 (s, 1H), 5.98 (s, 1H), 4.76 (s, 2H), 3.99 (s, 2H), 3.76 (s, 3H), 3.31 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.41 (J_{CF} = 240 Hz), 152.37, 152.27, 150.99, 147.17, 140.3 (J_{CF} = 245 Hz), 135.16, 132.18, 132.17, 121.68, 105.11 (J_{CF} = 21 Hz), 104.53 (J_{CF} = 23 Hz), 96.56, 52.56, 51.68, 45.31, 38.90; HRMS (ES+) m/z calculated for $C_{17}H_{18}N_7F_2$: 358.1586 found: 358.1591.

4.9.9. TFA salt of N-(4-(4-(dimethylamino)piperidin-1-yl)phenyl)-5-fluoro-4-(8-fluoro-1,2,3,5-tetrahydro-4H-benzo[e][1,4]diazepin-4-yl)pyrimidin-2-amine (6g)

The title compound was prepared according to general procedure B. The crude residue was purified by flash column chromatography using methanol in dichloromethane. The crude material was once again purified by Prep. HPLC (0.1% TFA in water/ACN) to give the title compound as a pale-yellow solid (0.12 g, 31.4%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.50 (br, 1H), 9.16 (br, 1H), 7.95 (d, J = 7.2 Hz, 1H), 7.38 (d, J = 8 Hz, 2H), 6.95 (d, J = 7.2 Hz, 3H), 6.55 (d, J = 10 Hz, 1H), 6.38 (t, J = 6.8 Hz, 1H), 5.90 (br, 1H), 4.84 (s, 2H), 3.93 (s, 2H), 3.75 (d, J = 12.4 Hz, 2H), 3.27 (s, 3H), 2.77 (s, 6H), 2.67 (s, 2H), 2.05 (d, J = 11.6 Hz, 2H), 1.70 (t, J = 22 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.29 (J_{CF} = 240 Hz), 158.88 (J_{CF} = 34 Hz), 158.36, 152.86, 152.35, 152.11, 146.43, 140.23 (J_{CF} = 245 Hz), 132.06, 122.66, 121.82, 117.32, 104.95 (J_{CF} = 21 Hz), 104.53 (J_{CF} = 23 Hz), 62.76, 52.47, 51.60, 48.71, 45.44, 34.78, 25.98; HRMS (ES-) m/z calculated for $C_{26}H_{30}N_7F_2$: 478.2525 found: 478.2528.(Negative mode).

Author contributions

These authors contributed equally.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.103800.

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