Supporting Information

Fast-forwarding Hit to Lead: Aurora and Epidermal Growth Factor Receptor Kinase Inhibitor Lead Identification[∝]

Mohane Selvaraj Coumar,[†,◊] Chang-Ying Chu,[†,◊] Cheng-Wei Lin,[†,¶] Hui-Yi Shiao,[†] Yun-Lung Ho,[†] Randheer Reddy,[†] Wen-Hsing Lin,[†] Chun-Hwa Chen,[†] Yi-Hui Peng,[†] Jiun-Shyang Leou,[†] Tzu-Wen Lien,[†] Chin-Ting Huang,[†] Ming-Yu Fang,[†] Szu-Huei Wu,[†] Jian-Sung Wu,[†] Santhosh Kumar Chittimalla,[†] Jen-Shin Song,[†] John T.-A. Hsu,[†,‡] Su-Ying Wu,[†] Chun-Chen Liao,[¶,∏] Yu-Sheng Chao,[†] and Hsing-Pang Hsieh[†,]*

Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, 35 Keyan Rd., Zhunan, Miaoli County 350, Taiwan, ROC, Department of Chemistry, National Tsing Hua University, 101 Guangfu Road Sec. 2, Hsinchu 300, Taiwan, ROC, Department of Biological Science and Technology, National Chiao Tung University, 1001 University Road, Hsinchu 300, Taiwan, ROC, Department of Chemistry, Chung Yuan Christian University, 200 Zhongbei Rd., Zhongli 320, Taiwan, ROC.

[∝] The atomic coordinates and crystal structure have been deposited in the Protein Data Bank as entry 3M11.

[†] National Health Research Institutes

[◊] These authors contributed equally to this work

[¶] National Tsing Hua University

[‡] National Chiao Tung University

∏ Chung Yuan Christian University

* Corresponding author: Phone: +886-37-246-166 ext. 35708, Fax: +886-37-586-456, E-mail: hphsieh@nhri.org.tw

[a]Abbreviations: ATP, adenosine triphosphate; DM, double mutant; EGFR, epidermal growth factor receptor; LC-MS, liquid chromatography coupled mass spectroscopy; NSCLC, non-small cell lung cancer; PDB ID, protein data bank identification number; SBDD, structure-based drug design; WT, wild type.

Contents

1. Chart

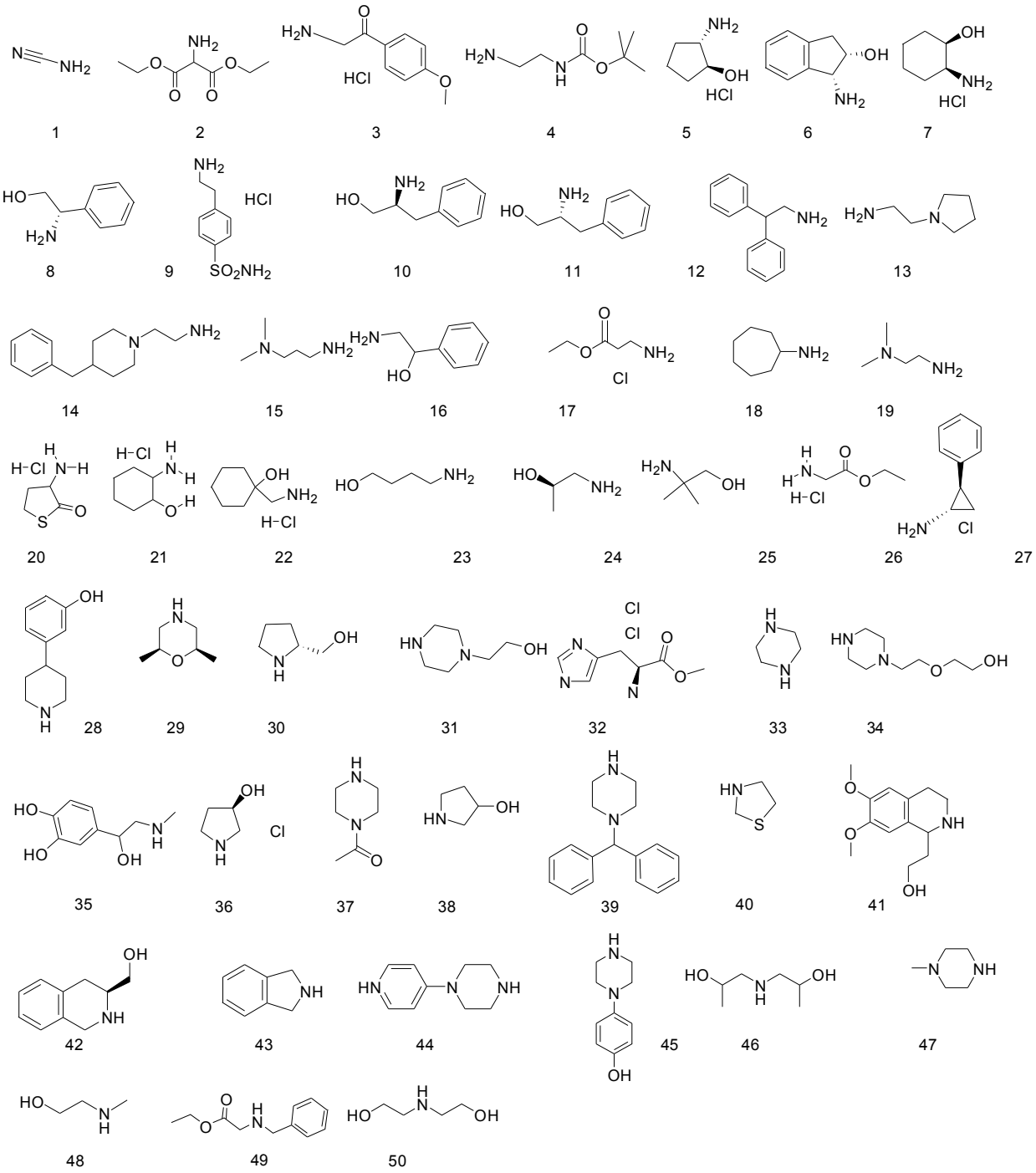


Chart 1s: Amines used for parallel synthesis of furano-pyrimidine **1** (Batch 1)

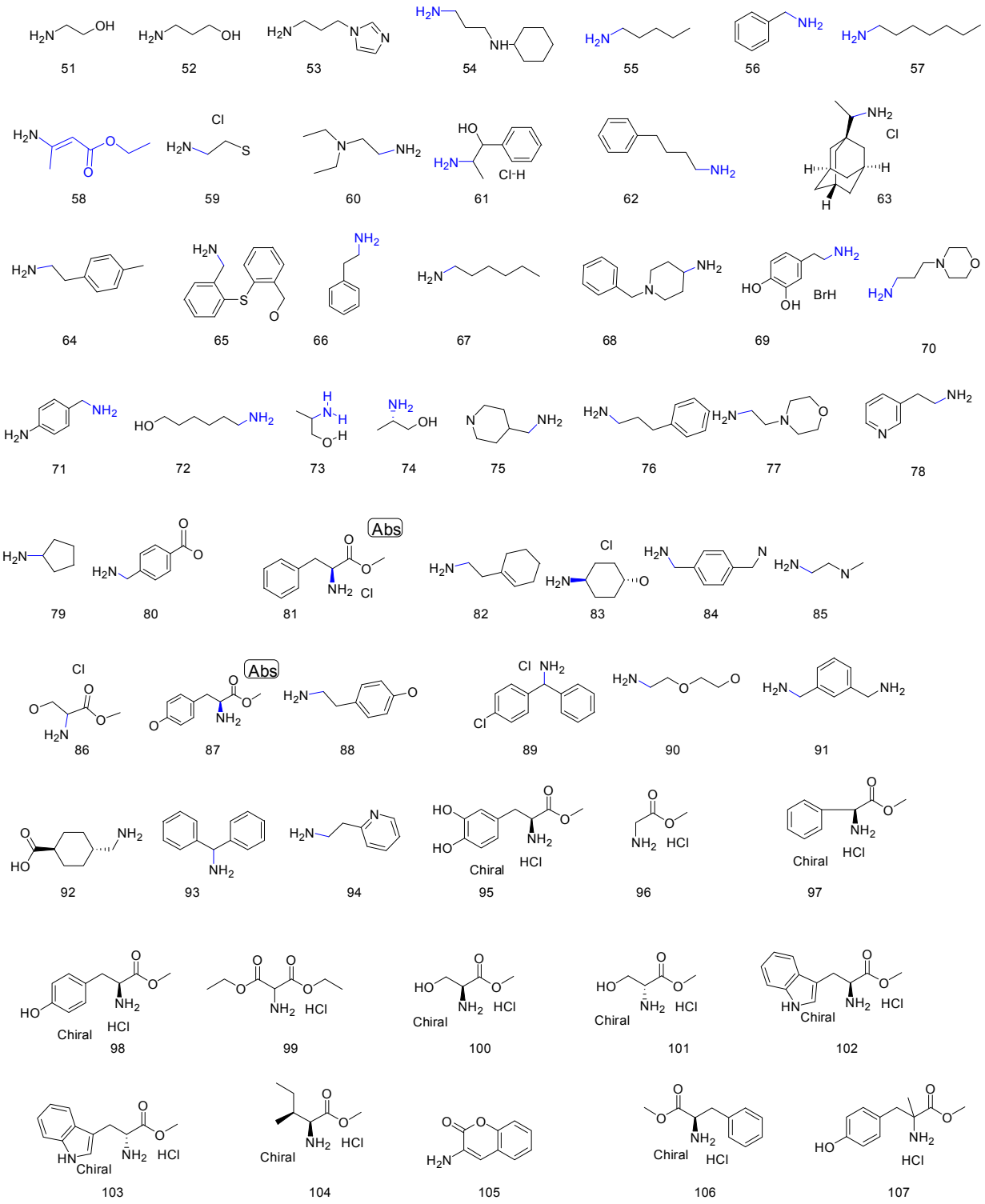


Chart 1s: Amines used for parallel synthesis of furano-pyrimidine **1** (Batch 2)

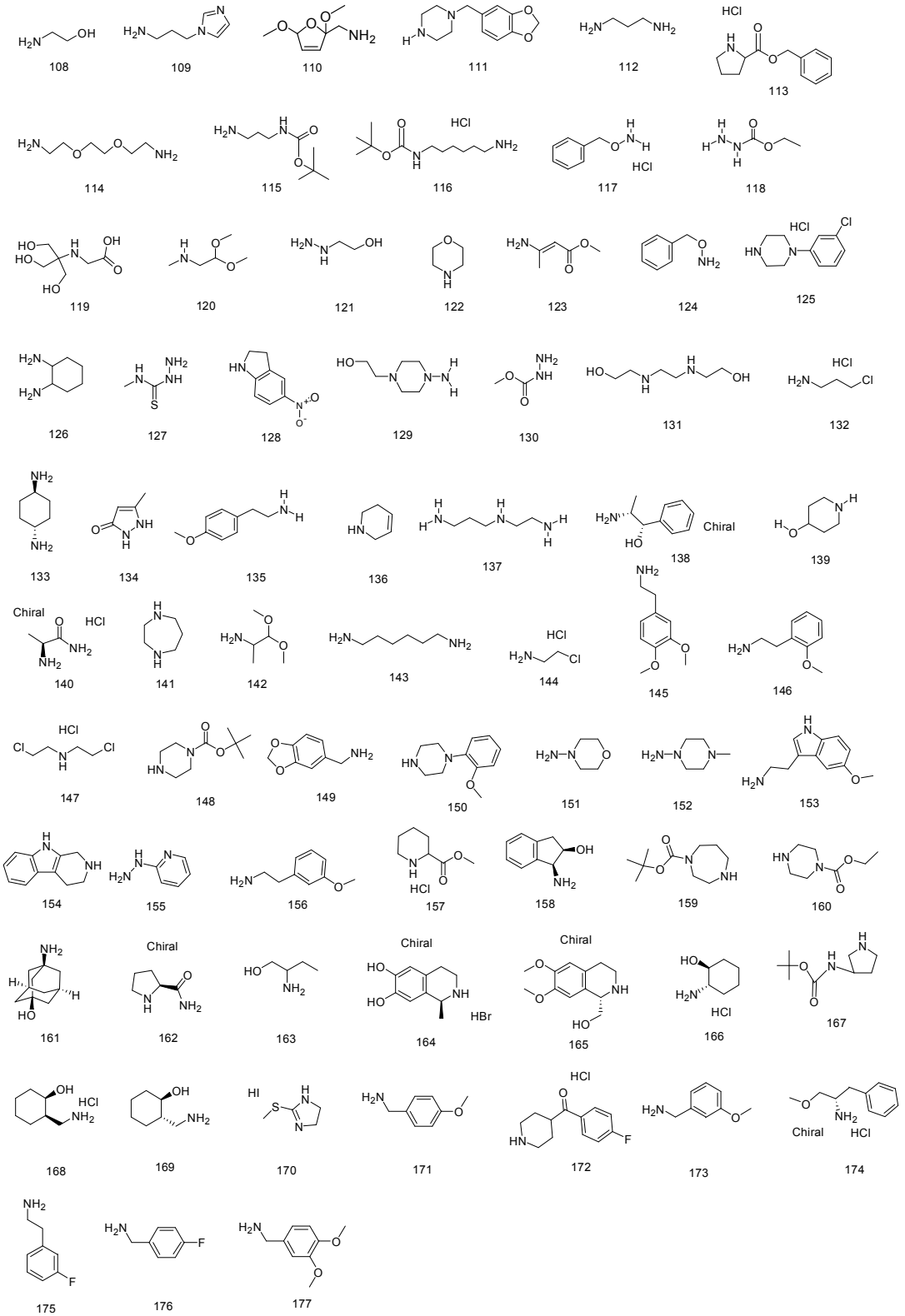


Chart 1s: Amines used for parallel synthesis of furano-pyrimidine **1** (Batch 3)

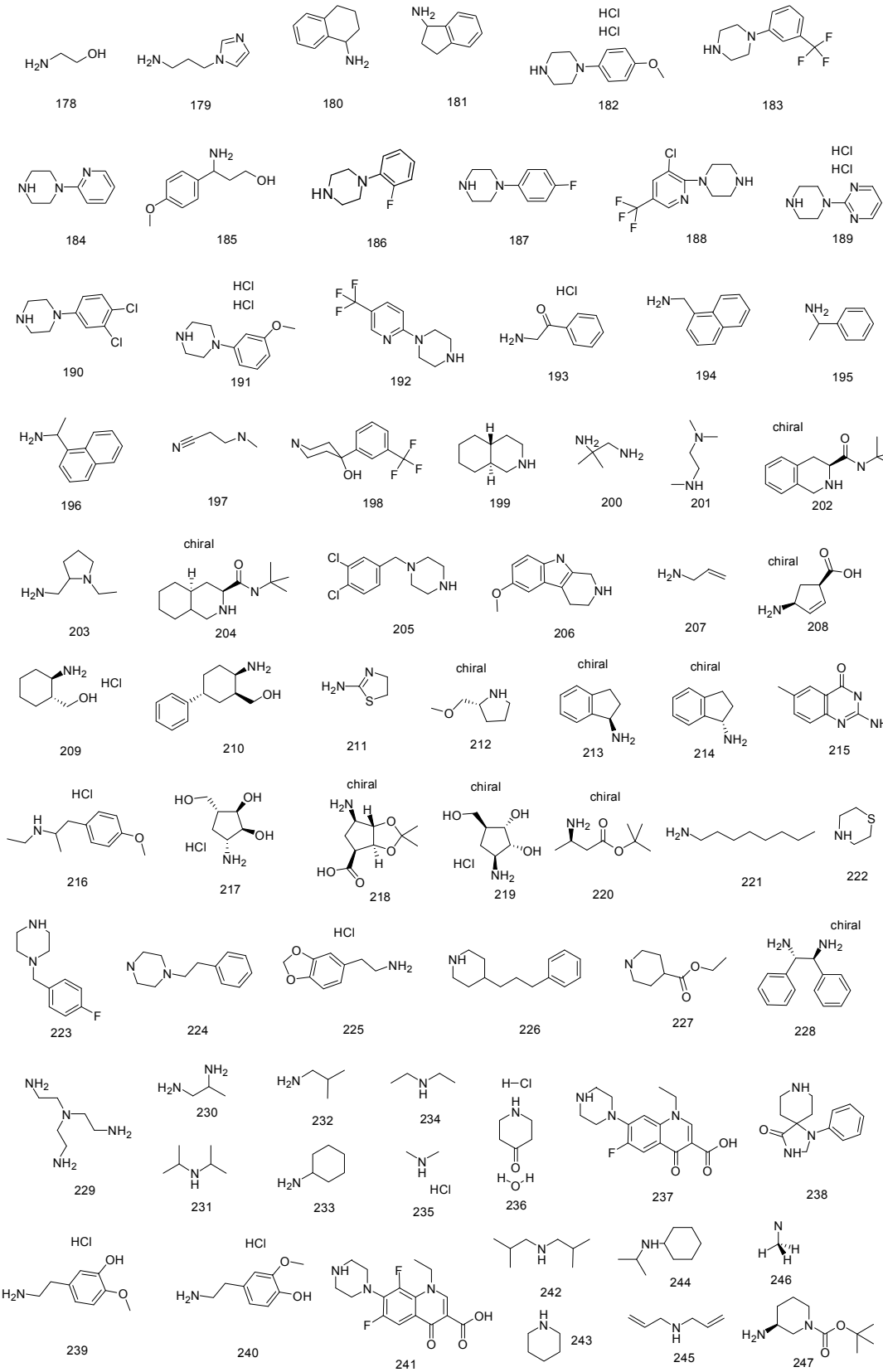


Chart 1s: Amines used for parallel synthesis of furano-pyrimidine **1** (Batch 4)

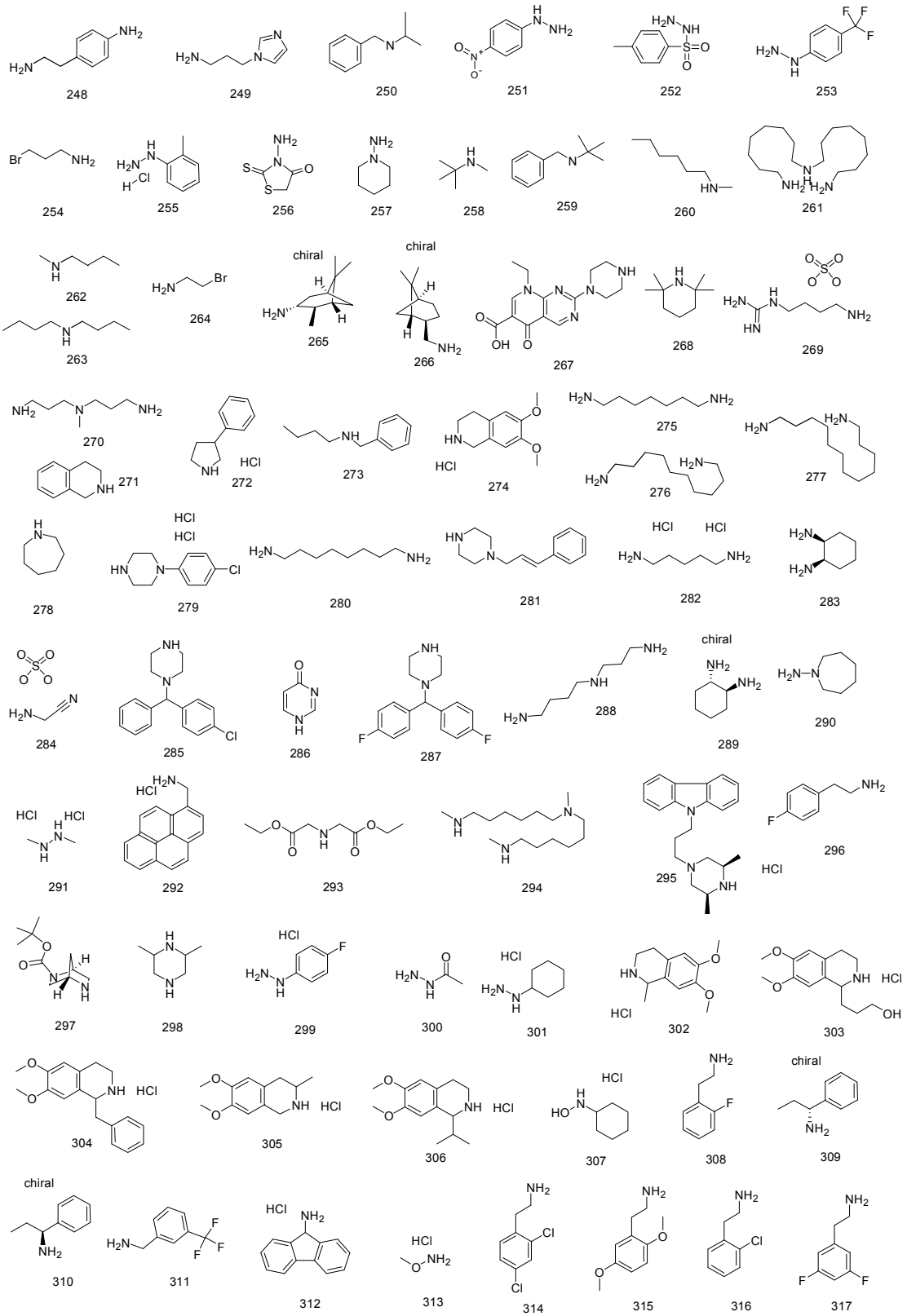


Chart 1s: Amines used for parallel synthesis of furano-pyrimidine **1** (Batch 5)

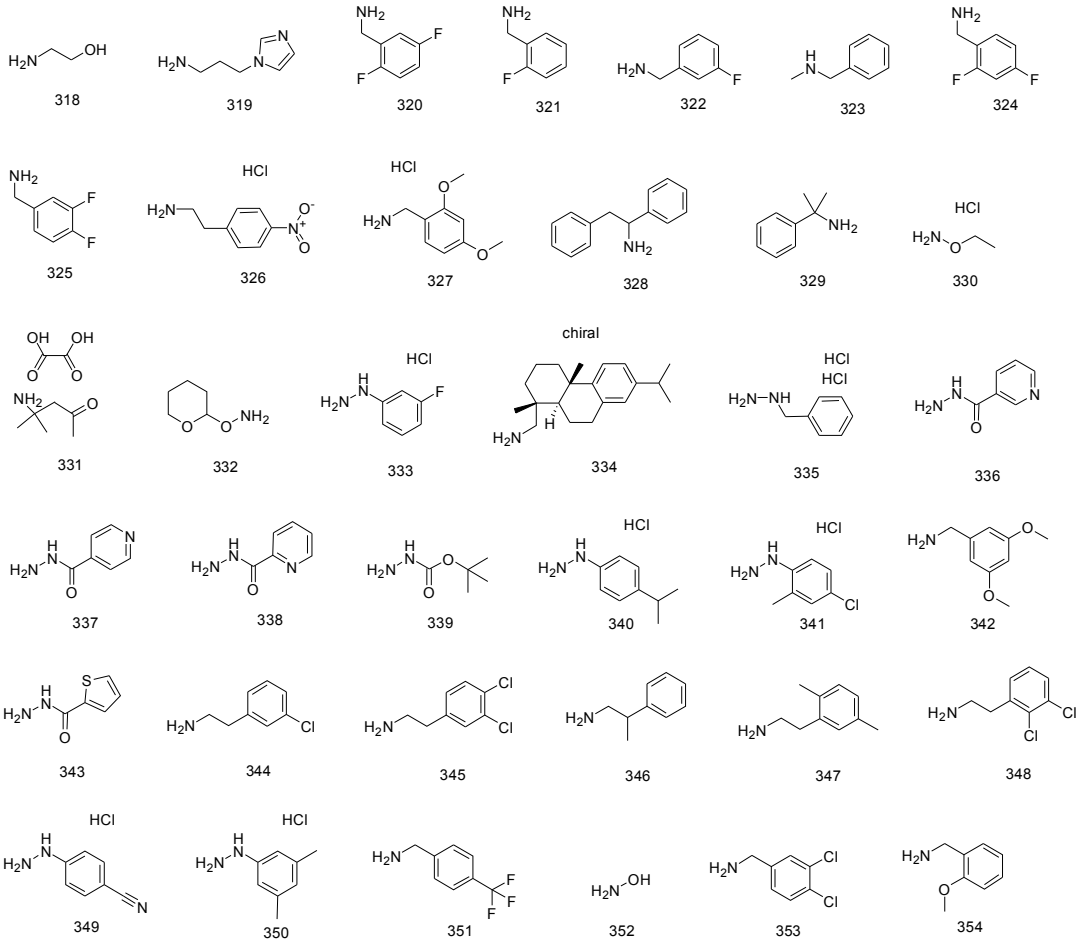


Chart 1s: Amines used for parallel synthesis of furano-pyrimidine **1** (Batch 6)

2. Figures

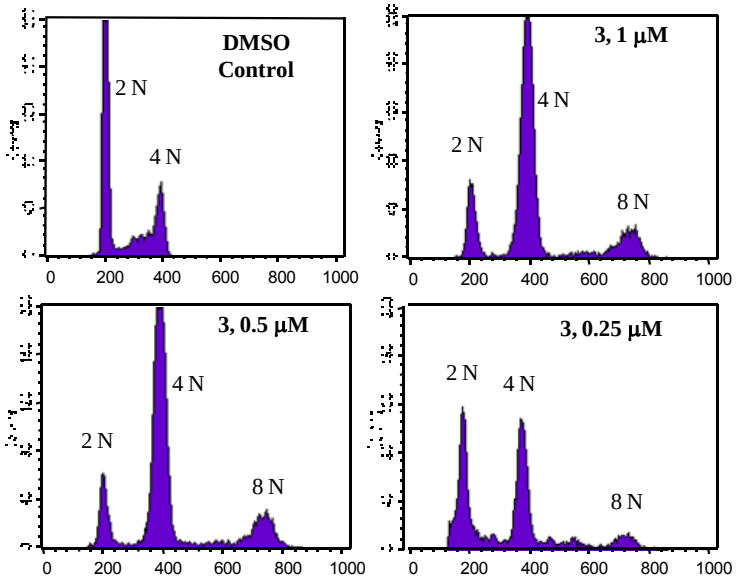


Figure 1s. Flow cytometry analysis for DNA contents of HCT-116 cells after treatment with inhibitor **3** at different concentration. Treatment from 250 nM onwards led to higher percentage of cells with DNA content > 2N (polyploidy), resulting from failure of cytokinesis.

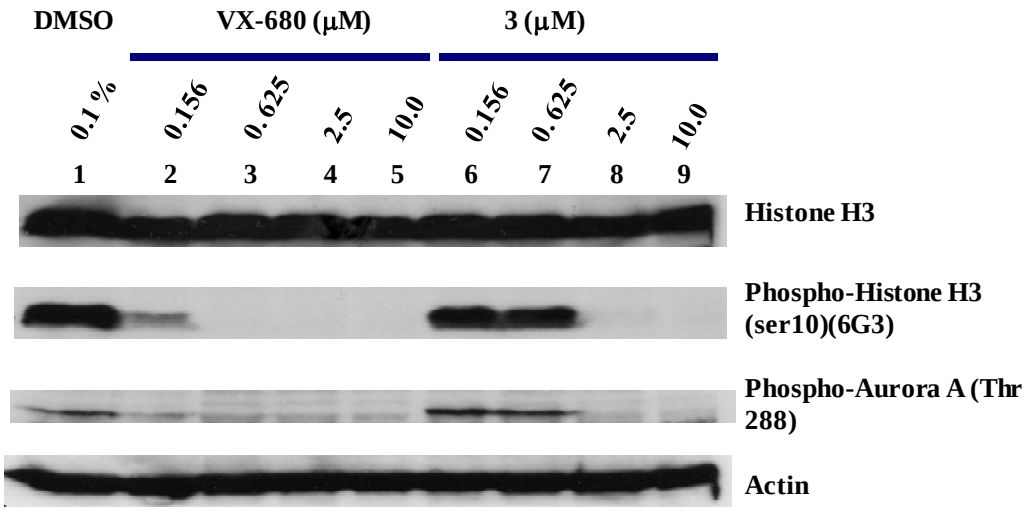


Figure 2s. Western blotting analysis for cellular target modulation in HCT-116 cell line by inhibitor **3**. Compound **3** inhibits both phospho Aurora A (T288) and phospho Histone H3 (Ser10) formation in HCT-116 cells in a dose dependant manner, suggesting both Aurora A and B inhibition inside the HCT-116 cells.

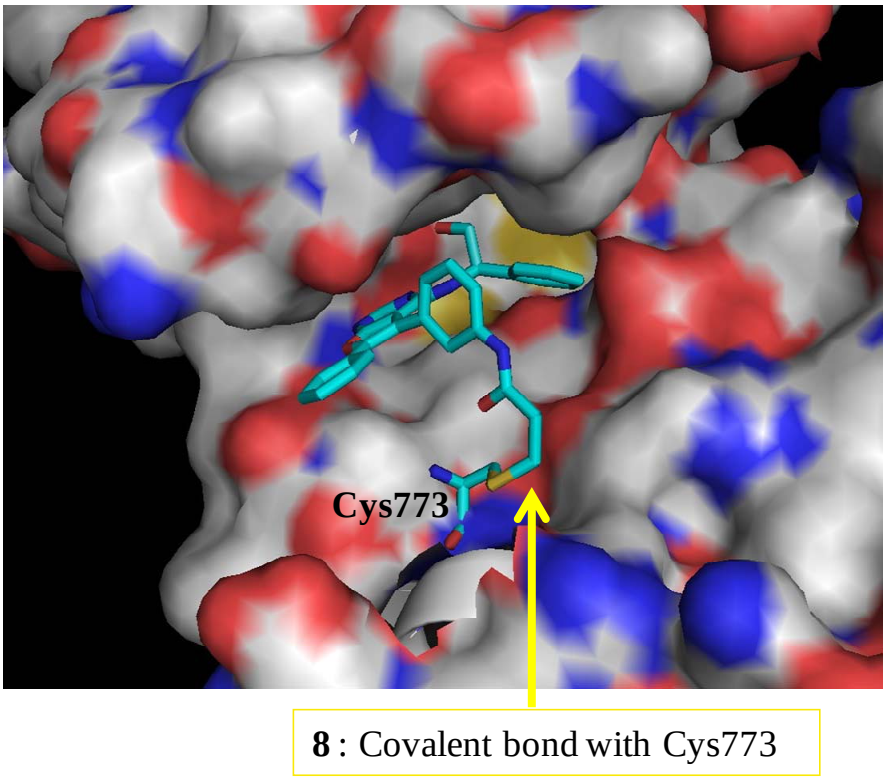
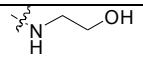
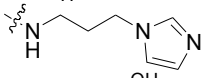
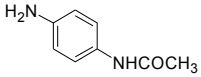
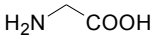
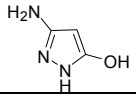


Figure 3s. X-ray cocrystal structure of EGFR protein in complex with inhibitor **8**. Inhibitor **8** binds irreversibly to EGFR through a covalent bond to Cys773 residue. Moreover, a hydrogen bond from the furan O to the hinge Met769 residue is observed. (However it should be noted that some parts of the ligand (phenyl group) were not clear in the density map, indicating they might be flexible in the EGFR pocket, precluding deposition of the coordinates to PDB).

3. Tables

Table 1s. Optimization of reaction condition for library synthesis.

Compounds	-NR^1R^2	Nucleophile type	Note[a]
1a		Primary amine	Reaction complete
1b		Primary amine	Reaction complete
1c		Secondary amine	Reaction complete
1d		Aniline	~50% reaction complete
1e		Amino acid	Required product not formed
1f		Heterocyclic amine	Required product not formed

[a] Reaction accessed by LCMS for the absence of starting material **2** and presence of products **1**.

Table 2s. X-ray data collection and structure refinement statistics of compound **3** and **8**.

Parameter	3	8
resolution (Å) Unit cell	30-2.75	30-2.59
P6122 (α=β=90°,γ=120°)		I23 (α=β=γ=*90*°)
a, Å	82.27	146.435
b, Å	82.27	146.435
C, Å	170.28	146.435
total reflections observed	708938	279529
unique reflections	8896	16372
multiplicity	79.69	17.07
*R*merge, % (outer shell)	4.6 (34.4)	5.6 (31.9)
<I/σ(I)>	33.50	21.41
(outer shell)	(8.19)	(4.4)
completeness, %	95.1	96.5
(outer shell)	91.8	99.6
*R*work, %	21.75	22.56
*R*free, %	29.92	27.822
RMS bonds, Å	0.012	0.014
RMS angles, deg	1.431	1.578

4. HPLC purity determination

Instrument & column: HPLC purity were determined using an Hitachi 2000 series HPLC system using C-18 column (Agilent ZORBAX Eclipse XDB-C18 5 μm. 4.6 mm × 150 mm).

Solvent system: Elution conditions: Mobile phase A-Acetonitrile; Mobile phase B-Water containing 0.1% formic acid + 10 mmol NH_4OAc. The flow-rate was 0.5 ml/min and the injection volume was 5 μl. The system operated at 25 °C. Peaks were detected at 210 nm.

Elution condition:

Time (min)	Mobile Phase A (ratio)	Mobile Phase B (ratio)
0	10	90
45	90	10
50	10	90
60	10	90