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ARTICLE

Design, synthesis, and anticancer evaluation of acetamide and hydrazine analogues of pyrimidine

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Abstract

A library of acetamide and hydrazine analogues were generated on the pyrimidine ring through a multistep reaction starting from 5-nitro-pyrimidine-4,6-diol and pyrimidine-4,6-diol, respectively. The synthesized analogues were screened for in vitro cytotoxic activity against various human cancer cell lines like HCT-1 and HT-15 (colon), MCF-7(breast), PC-3 (prostrate), SF268 (CNS) using MTT method. From the bioassay results, it was observed that even though many of the synthesized derivatives exhibited a good potency against various screened cancer cell lines, compound **14a** from the acetamide series was found to show potent anticancer activity on all the tested cancer cell lines with IC_{50} value of 0.36μM on CNS cell line and 1.6μM on HT-21 cell line, and compound **19xxi** from hydrazine series of pyrimidine showed potent activity against three tested cancer cell lines with IC_{50} value of 0.76μM on HT-29 cell line, 2.6μM on HCT-15, and 3.2μM on MCF-7 cell line.

1 | INTRODUCTION

With the continuous increase in mortality rate due to cancer, it has gradually become one of the most complicated diseases that threatens the human life and is responsible for an estimated 9.6 million deaths in 2018.[1] Globally, about one in six deaths is due to cancer. Although there are many effective therapeutic methods in clinical use for the control of cancer,[2] chemotherapy alone or in combination with surgery is commonly the most efficient method for the treatment of cancer. However, the use of currently available chemotherapeutic drugs is limited because of their toxic side effects and drug resistance.[3] Therefore, over the years, the main effort in the field of medicinal chemistry has been the identification of new chemical entities with special characteristics as effective anticancer molecules from both natural and synthetic sources. Out of diverse chemical entities with proven biological properties, nitrogen-containing heterocyclic compounds are of great importance as their structural subunits exist in many natural products such as vitamins, hormones, and antibiotics because of which they have engrossed significant attention in the field of medicinal chemistry.[4–6]

Heterocyclic compound pyrimidine occupies a significant position in the medicinal world because of its diverse biological properties. It has been identified as an important pharmacophore interacting with the synthesis and functions of nucleic acids.[7] Pyrimidine ring is the building component of DNA and RNA because derivatives of pyrimidine exhibit diverse pharmacological activities such as antiviral, antimalarial, antimicrobial, anti-inflammatory, and anticancer.[8–13] Among its diverse medicinal attributes, anticancer activity is most extensively reported. From the last few decades, several chemotherapeutic agents have been developed from the reprivatization of the

pyrimidine ring and have found wide clinical applications. Various nucleoside drugs containing pyrimidine nucleus were synthesized and developed as highly effective anticancer agents. These drugs like cytarabine (**1**), gemcitabine (**2**) are analogues of cytosine and drugs like fluorouracil (**3**), floxuridine (**4**) are analogues of uracil (Figure 1) and these have been found to demonstrate a range of antineoplastic activity in cell and animal models.[14–19] These agents induce cytotoxicity by acting as antimetabolites, competing with physiologic pyrimidine nucleosides and, consequently, interacting with a large number of intracellular targets.[20,21] Their antiproliferative activities is achieved through incorporation into DNA which leads to chain termination and finally inhibits the synthesis of DNA. They can also interfere with enzymes involved in nucleic acid synthesis, such as DNA polymerases and ribonucleotide reductase.

Other drugs containing fused pyrimidine ring in the moiety include epidermal growth factor receptor—tyrosine kinase inhibitors Gefitinib (**5**) and Erlotinib (**6**) as shown in Figure 1, which are approved for the treatment of nonsmall-cell lung cancer.[22,23] Protein tyrosine kinase inhibitor like Imatinib (**7**) (Figure 1) is approved for the treatment of chronic myeloid leukemia, gastrointestinal stromal tumors, and a number of other malignancies.[24] Tegafur (**8**; Figure 1) is an oral anticancer drug containing 5-fluorouracil widely used for adjuvant chemotherapy of colorectal cancer.[25]

Because of the wide clinical applications, interest in pyrimidine derivatives has led to the preparation and evaluation of hundreds of such type of molecules as potent anticancer agents revealing that pyrimidine is an important pharmacophore in the discovery of novel active molecules. In view of the above facts and in continuation of our previous efforts in the field of synthesis of novel anticancer therapeutics,[26,27] the target in this study was to synthesize analogues having pyrimidine moiety conjugated through acetamide and hydrazinyl linkage with different aromatic/heterocyclic groups of documented cytotoxic potency against several cancer cell lines.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The acetamide derivatives of pyrimidine given in Table 1 are synthesized as described in Scheme 1. Synthesis was started with the regioselective chlorination of hydroxyl group at C-6 of 5-nitro-pyrimidine-4,6-diol (**9**) with phosphoxoxyl chloride leading to the formation of 6-chloro-5-nitro-pyrimidin-4-ol (**10**). The nitro group of (**10**) was reduced to amino group forming 5-amino-6-chloro-pyrimidin-4-ol (**11**).The SNAr on C-6 of (**11**) was efficiently achieved using benzylamine as a nucleophile, 1-butanol as solvent and disopropylethylamine as base

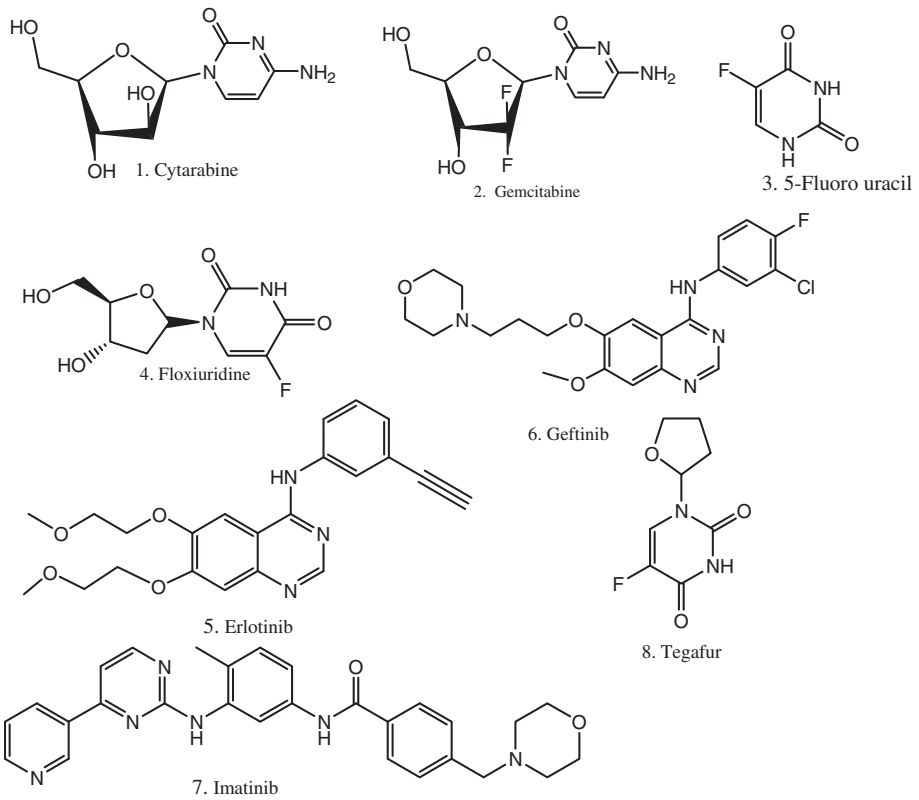
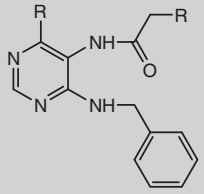
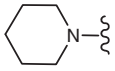
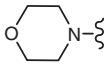
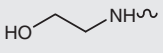
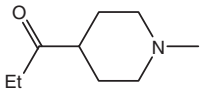
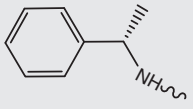
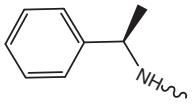


FIGURE 1 Structures of anticancer drugs containing pyrimidine nucleus

followed by refluxing for 12 hours to form 5-amino-6-benzylamino-pyrimidin-4-ol (**12**), which was treated with chloroacetylchloride leading to the substitution of amino group with chloro acetyl group and the hydroxyl group at position C-2 was also replaced with a chloro group to form the N-(4-benzylamino-6-chloro-pyrimidin-5-yl)-2-chloro-acetamide (**13**). Finally, the SNAr on C-2 of (**13**) was achieved using the cyclic secondary amine in the presence of 1-butanol as solvent to form the required acetamide compounds **14**(**a-g**). The compounds were identified by ^{1}HNMR, ^{13}C, and mass spectroscopy (MS). Figure 2 represents the X-ray crystallographic structure of compound (**14a**).

TABLE 1 Acetamide analogues of pyrimidine



S.NO	R	Yield
14a		85%
14b		90%
14c		92%
14d		80%
14e		90%
14f		87%
14g		85%

The hydrazinyl derivatives of pyrimidine given in Table 2 are synthesized as described in Scheme 2. Pyrimidine-4,6-diol (**15**) was used as a starting material. The chlorination of two hydroxyl groups located at C-4 and C-6 of starting substrate (**15**) was done with chlorinating agent phosphoxoxyl chloride forming 4,6-dichloro-pyrimidine (**16**). The SNAr on C-6 of (**16**) was regioselectively achieved using hydrazine hydrochloride as a nucleophile and 1-Butanol as solvent followed by refluxing for 4 hours to form 6-chloro-(pyrimidin-4-yl)-hydrazine (**17**). Condensation reaction on the amino group at C-6 of (**17**) was carried out with different substituted aldehydes to form analogues of the type (**18**). Finally, the SNAr on C-2 of (18) was achieved using the different cyclic secondary amine in the presence of triethylamine and THF as solvent to form the required compounds **19**(**i-xxviii**). The compounds were identified by ^{1}HNMR, ^{13}C, and MS.

2.2 | Biological activity

Acetamide analogues (**14a-g**) and hydrazine analogues (**19i-xxviii**) of pyrimidine were evaluated for in vitro anticancer activity against a panel of human cancer cell

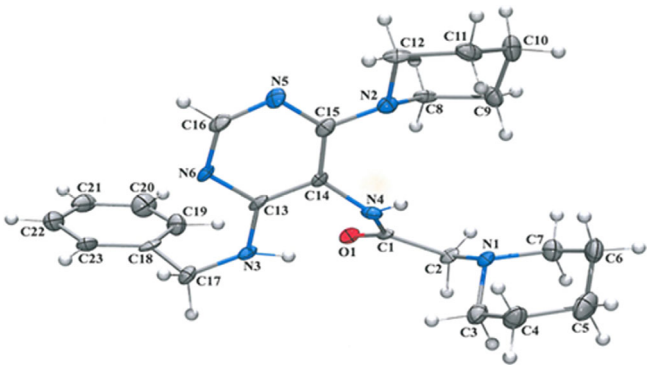
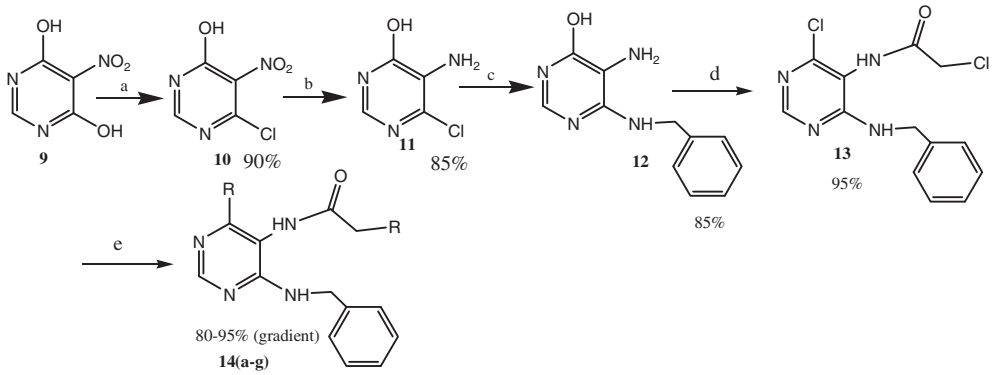
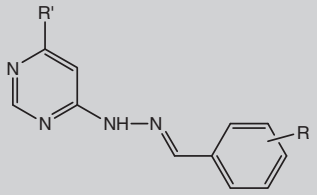


FIGURE 2 X-ray crystal structure of compound **14a**



SCHEME 1 Synthesis of target compounds **14**(**a-g**). Reagents and conditions: (A) $POCl_3$ (B) Zn, HCOOH, rt, 2 hours; (C) benzyl amine, N-BuOH, DIPEA, reflux 6 hours; (D) chloroacetyl chloride, DMF, reflux, 8 hours; (E) substituted amine, Et_3N, reflux, 12 hours

TABLE 2 Hydrazinyl analogues of pyrimidine

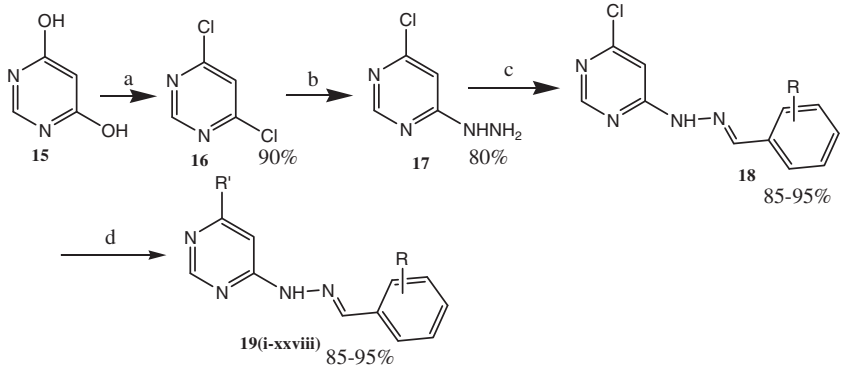


S.No	R	R′	Yield
19i	Piperidine	H	90%
19ii	”	2-OMe	92%
19iii	”	4-OMe	95%
19iv	”	3,4 dimethoxy	90%
19v	”	2 Br	98%
19vi	”	4 Br	97%
19vii	”	2 OH	95%
19viii	Pyrolidine	H	96%
19ix	”	2-OMe	93%
19x	”	4-OMe	90%
19xi	”	3,4 dimethoxy	94%
19xii	”	2 Br	95%
19xiii	”	4 Br	92%
19xiv	”	2 OH	90%
19xv	Morpholine	H	85%
19xvi	”	2-OMe	88%
19xvii	”	4-OMe	90%
19xviii	”	3,4 dimethoxy	95%
19xix	”	2 Br	91%
19xx	”	4 Br	90%
19xxi	”	2 OH	87%
19xxii	(Piperidine-4-yl)-propan-l-one	H	86%
19xxiii	”	2-OMe	98%
19xxiv	”	4-OMe	90%
19xxv	”	3,4 dimethoxy	95%
19xxvi	”	2 Br	92%
19xxvii	”	4 Br	87%
19xxviii	”	2 OH	90%

lines such as HCT-1 and HT-15 (colon), MCF-7(breast), PC-3(prostrate), and SF268 (CNS).Tables 3 and 4 summarizes the inhibitory activity (IC_{50}) values of the analogues synthesized and the well-known anticancer drugs paclitaxel, doxorubicin, and 5-fluorouracil were used as standard drugs. It was observed from the results of screening that some of the acetamide and hydrazine analogues of pyrimidine showed good anticancer activities in lower μM range against all the human cancer cell lines tested. Further, it was also observed that while many of the synthesized analogues exhibited potency against colon cancer cell lines, compound **14a** from acetamide series was found to show potent anticancer activity on all the tested cancer cell lines with IC_{50} value of 0.36μM on CNS cell line and 1.6μM on HT-21 cell line (Table 3) and compound **19xxi** from hydrazine series of pyrimidine showed potent activity against three tested cancer cell lines with IC_{50} value of 0.76μM on HT-29 cell line, 2.6μM on HCT-15, and 3.2μM on MCF-7 cell line (Table 4).

3 | CONCLUSION

Commercially available 5-nitro-pyrimidine-4,6-diol was used as starting substrate for the synthesis of acetamide analogues of pyrimidine and pyrimidine-4,6-diol was used as starting substrate for the synthesis of hydrazine analogues of pyrimidine. C-4 position of pyrimidine was substituted with cyclic secondary amines like pyrolidine, piperidine, and morpholine and (piperidine-4-yl)-propane-1-one. All the analogues were tested for their cytotoxic activity against a panel of four human cancer cell lines. Many of the synthesized derivatives exhibited potent cytotoxicity. However, compound **14a** from the acetamide series was found to show potent anticancer activity on all the tested cancer cell lines with IC_{50} value of 0.36μM on CNS cell line and 1.6μM on HT-21 cell line while compound **19xxi** from the hydrazine series of pyrimidine showed potent activity against three tested cancer cell lines with IC_{50} value of 0.76μM on HT-29 cell line, 2.6μM on HCT-15, and 3.2 μM on MCF-7 cell line.



SCHEME 2 Synthesis of compounds 19(**i-xxviii**). Reagents and conditions: (A) POCl3, rt, 2 hours; (B) NH2NH2.HCl, n-BuOH, reflux at 90°C, 2 hours; (c) substituted aromatic aldehydes, EtOH, THF, reflux at 90°C, 4 hours; (D) cyclic amines, Et3N, THF, reflux, 12 hours

TABLE 3 IC50 values of acetamide analogues of pyrimidine against panel of human cancer cell lines

	Cancer cell lines				
	Colon		Breast	Prostrate	CNS
Compound No.	[HCT-15	HT-29]	MCF-7	PC-3	SF268
14a	10.2 ± 0.25	1.6 ± 0.2	12.9 ± 0.3	13.9 ± 0.22	0.36 ± 0.25
14b	65.0 ± 0.2	44.0 ± 0.25	15.6 ± 0.3	12.0 ± 0.25	10.5 ± 0.22
14c	>23.5 ± 0.25	35.6 ± 0.30	41.0 ± 0.25	19.0 ± 0.30	85.0 ± 0.30
14d	>34.9 ± 0.22	54.5 ± 0.36	26.0 ± 0.22	56.0 ± 0.25	>100
14e	66.7 ± 0.2	32.4 ± 0.3	21.2 ± 0.2	24.5 ± 0.30	69.8 ± 0.25
14f	>100	>100	>100	54.0 ± 0.23	>100
14g	10.2 ± 0.25	18.0 ± 0.25	34.5 ± 0.2	>100	88.8 ± 0.22

Note: Results are Mean ± SD of three independent experiments.

Finally, it was observed that analogues from the acetamide series containing the piperidine and pyrolidine secondary amine base showed better results compared with the analogues with other amine bases. Analogues from the hydrazine series with piperidine, pyrolidine, and morpholine amine bases and with 2-OH, 2-OMe, and 4-OMe substitutions on the benzene ring showed much better activity on all cancer cell lines than compounds with other substitution.

4 | EXPERIMENTAL

Buchi melting point apparatus D-545 was used to record the melting points of the synthesized compounds. Bruker DPX400 instrument was used to record the NMR spectra with samples taken in $CDCl_3$. δ (ppm) was used to report chemical shift values with coupling constants taken in Hertz. Electrospray ionization MS (ESI-MS) was used to record mass spectra. Monitoring of reactions was done by thin-layer chromatography (TLC) on a 2- to 5-cm percolated silica gel $60F_{254}$ plates of thickness 0.25 mm (Merck). Ultraviolet (UV) 254 to 366 nm and iodine were used to visualize the chromatograms.

4.1 | Procedure for synthesis of 6-chloro-5-nitro-pyrimidin-4-ol (10)

5-Nitro-pyrimidine-4,6-diol (2 g) was taken in a round bottom flask and DMF was added as a solvent, to it was added phosphoxychloride (1 equiv, 2.07 g) and kept for stirring at room temperature (rt) for 2 hours. The progress of reaction was checked with TLC and after the completion of reaction, the reaction mixture was quenched with water and transferred to a separatory funnel and finally extracted with diethyl ether. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated on rotavapor. The residue was purified by flash chromatography eluted with hexane/ethyl acetate (8:2 v/v), and compound (**10**) with (90%) yield was obtained as yellowish crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.81 (s, 1H), 5.02 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 139.5, 150.0, 153.2, 170.0; ESI MS: 174.0.

4.2 | Procedure for synthesis of compound 5-Amino-6-chloro-pyrimidin-4-ol (11)

6-Chloro-5-nitro-pyrimidin-4-ol (1.6 g), taken in a round bottom flask, was added formic acid in excess as solvent and then zinc (1 equiv, 4.4 g) was added to it in small portions with continuous stirring. The reaction mixture was left for stirring at rt for 2 hours. The progress of the reaction was monitored with TLC and after the completion of reaction, the reaction mixture was quenched with saturated solution of Na_2CO_3 and transferred to a separatory funnel and finally extracted with diethyl ether. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated on rotavapor. The residue was purified by flash chromatography eluted with hexane/ethyl acetate, and compound (**11**) with (85%) yield was obtained as white crystals; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (s, 1H), 5.02 (s, 1H), 4.0(s, 2H); ^{13}C NMR (100 MHz,$CDCl_3$): 139.5, 149.0.0, 150.2, 163.0; ESI MS: 145.0.

4.3 | Procedure for synthesis of compound 5-Amino-6-benzylamino-pyrimidin-4-ol (12)

5-Amino-6-chloro-pyrimidin-4-ol (9) (0.5 g) was taken in a two-necked, round bottom flask, to which n-Butanol (10 mL) was added. To the above solution, benzyl amine (1.2 equiv, 0.56 g) and diisopropylamine (1.5 equiv, 0.37 g) was added, and

TABLE 4 IC50 values of hydrazine analogues of pyrimidine against panel of human cancer cell lines

	Cancer cell lines				
	Colon		Breast	Prostrate	CNS
Compound No.	[HCT-15	HT-29]	MCF-7	PC-3	SF268
19i	53.2 ± 0.3	>100	>100	>100	97.5 ± 0.3
19ii	85.0 ± 0.25	68.3 ± 0.2	>100	>100	>100
19iii	15.2 ± 0.23	>100	65.0 ± 0.3	76 ± 0.20	25.5 ± 0.25
19iv	>100	24.0 ± 0.3	37.5 ± 0.2	45.6 ± 0.35	>100
19v	>100	>100	>100	>100	>100
19vi	19.8 ± 0.23	38.6 ± 0.3	22.1 ± 0.2	49.7 ± 0.75	23.0 ± 0.35
19vii	16.5 ± 0.26	5.6 ± 0.3	16.5 ± 0.2	14.2 ± 0.3	12.0 ± 0.3
19viii	8.8 ± 0.4	68.6 ± 0.2	>100	>100	>100
19ix	3.9 ± 0.2	5.4 ± 0.18	11.9 ± 0.11	14.6 ± 0.75	21.3 ± 0.21
19x	2.5 ± 0.2	8.4 ± 0.3	12.4 ± 0.23	>100	>100
19xi	>100	>100	>100	>100	>100
19xii	35.0 ± 0.2	26.0 ± 0.3	28.0 ± 0.30	56.0 ± 0.30	67.0 ± 0.3
19xiii	>100	>!00	>100	>100	>100
18xiv	>100	>!00	>100	>100	>100
19xvv	13.2 ± 0.23	25.6 ± 0.24	43.5 ± 0.23	>100	10.9 ± 0.3
19xvi	37.6 ± 0.4	37.5 ± 0.22	87.8 ± 0.21	63.7 ± 0.3	23.4 ± 0.2
19xvii	47.1 ± 0.1	18 ± 0.5	18.6 ± 0.4	19.3 ± 0.33	12.6 ± 0.2
19xviii	>100	4.6 ± 0.4	>100	>100	>100
19xix	50.6 ± 1	32 ± 0.3	>100	>100	>100
19xx	8.2 ± 0.23	12.3 ± 0.2	15.3 ± 0.23	>100	>100
19xxi	2.6 ± 0.13	0.76 ± 0.75	3.2 ± 0.2	7.3 ± 0.13	11.8 ± 0.2
19xxii	>100	>100	>100	>100	>100
19xxiii	>100	>100	>!00	>100	>100
19xxiv	12.6 ± 0.3	13.2 ± 0.2	12.5 ± 0.25	49.5 ± 0.32	>100
19xxv	>100	>100	>100	>100	58.5 ± 0.3
19xxvi	80.7 ± 0.2	61.4 ± 0.3	>100	44.8 ± 0.2	>100
19xxvii	>100	>100	>100	>100	>100
19xxviii	67.5 ± 0.2	45.3 ± 0.75	>100	>100	>100
Paclitaxel(1μM)	—	—	—	72	—
Doxorubicin(1μM)	—	—	62	—	—
5-FU (20μM)	—	58	—	—	—

the reaction mixture was allowed to reflux at 90°C for 12 hours. The progress of the reaction was monitored with TLC and after the completion of reaction; the reaction mixture was quenched with water, transferred to a separatory funnel and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated on rotavapor. The residue was purified by flash chromatography eluted with hexane/ethyl acetate, and compound (**12**) was obtained with 95% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.1(s, 1H), 7.05 (d, 2H), 7.00 (d, 2H), 6.96(t, 1H), 5.00 (s, 1H), 4.31(s, 2H); ^{13}C NMR (100 MHz,$CDCl_3$): 57.6, 123.4, 126.0, 127.2, 127.3, 128.3, 128.5, 137.5, 142.4, 152.0, 163.9; ESI MS: 216.0.

4.4 | Procedure for synthesis of N-(4-Benzylamino-6-chloro-pyrimidin-5-yl)-2-chloro-acetamide (13)

5-Amino-6-benzylamino-pyrimidin-4-ol (0.5 g) was taken in a round bottom flask and dissolved in DMF, chloro acetamide (1.5 equiv, 0.77 g) was added to it in small

portions at 0°C. The reaction was then left overnight at rt. The progress of reaction was monitored with TLC. After completion of reaction, water was added to the reaction mixture, transferred to a separatory funnel, and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated on rotavapor. The residue was purified by flash chromatography eluted with hexane/ethyl acetate, and compound (**13**) was obtained with 85% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.43 (s, 1H), 7.15 (dd, 2H), 7.10 (t, 1H), 7.00 (d, 2H), 4.31(s, 2H), 4.25(s, 2H); ^{13}C NMR (100 MHz,$CDCl_3$): 46.7, 57.6, 114.5, 126.5127.1, 127.1, 129.3, 129.3, 142.4, 152.0, 163.0,163.9; ESI MS: 310.0.

4.5 | General reaction procedure for synthesis of compounds 14(a-g)

N-(4-benzylamino-6-chloro-pyrimidin-5-yl)-2-chloro-acetamide (0.5 g) was taken in a 100 mL two-necked RBF. THF (5 mL) was added to it under inert atmosphere as solvent. Triethylamine (0.5 equiv, 3.06 g) was added to the above solution, followed by the addition of cyclic secondary amine piperidine/pyrolidine (2 equiv) and the reaction mixture was allowed to reflux at 90°C for 12 hours. The progress of reaction was monitored with TLC. After completion of reaction, the reaction mixture was concentrated on rotavapor; water was added to the reaction mixture, transferred to a separatory funnel and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated on rotavapor. The residue was purified by flash chromatography eluted with hexane/ethyl acetate, and compound **14**(**a**-**g**) were obtained with varying yield.

4.6 | Procedure for synthesis of compound 4,6-Dichloro-pyrimidine (16)

Pyrimidine-4,6-diol (2 g) was taken in a round bottom flask and DMF was added as a solvent. Phosphoxychloride (1 equiv) was added to it and kept for stirring at rt for 2 hours. The progress of reaction was checked with TLC and after the completion of reaction, the reaction mixture was quenched with water and transferred to a separatory funnel and finally extracted with diethyl ether. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated on rotavapor. The residue was purified by flash chromatography eluted with hexane/ethyl acetate, and compound (**16**) with (90%) yield was obtained as crystalline solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.31 (s, 1H), 8.02 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 122.0, 159.0, 163.0, 163.2; ESI MS: 147.0.

4.7 | Procedure for synthesis of compound 6-Chloro-pyrimidin-4-yl)-hydrazine (17)

4, 6-dichloro-pyrimidine (16) (1.5 g) was taken in a 100-mL round bottom flask to which n-BuOH was added as a solvent. To this solution, hydrazine hydrochloride (1 equiv) was added at 0 to 5°C and the reaction mixture was allowed to reflux at 90°C for 2 hours. The progress of reaction was checked with TLC and after the completion of reaction, the reaction mixture was quenched with water and transferred to a separatory funnel and finally extracted with diethyl ether. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated on rotavapor. The residue was purified by flash chromatography eluted with hexane/ethyl acetate, and compound (**17**) with (80%) yield was obtained as crystalline solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (s, 1H), 6.78 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): 105.0, 159.0, 161.0, 173.2; ESI MS: 144.0.

4.8 | General procedure for synthesis of compounds of type 18

6-Chloro-pyrimidin-4-yl-hydrazine (1.5 g) was taken in a 100-mL round bottom flask to which EtOH was added as a solvent. To the solution, various substituted aromatic aldehydes were added, and the reaction mixture was allowed to reflux at 90°C for 4 hours. The progress of reaction was checked with TLC and after the completion of reaction, the reaction mixture was concentrated on rotavapor and transferred to a separatory funnel and finally extracted with ethyl acetate. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated on rotavapor. The residue was purified by flash chromatography eluted with hexane/ethyl acetate, and compounds of type (**18**) were obtained with varying yields.

4.9 | General procedure for synthesis of hydrazine analogues of pyrimidine (19i-xxviii)

Compounds (**18**) (1.2 g) was taken in a 100-mL two-necked RBF. THF (5 mL) was added to it under inert atmosphere as solvent. Triethylamine (0.5 equiv) was added to the above solution, followed by the addition of cyclic secondary amine piperidine/pyrolidine (2 equiv), and the reaction mixture was allowed to reflux at 90°C for 12 hours. The progress of reaction was monitored

with TLC. After completion of reaction, the reaction mixture was concentrated on rotavapor; water was added to it, transferred to a separatory funnel, and extracted with EtOAc. The combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated on rotavapor. The residue was purified by flash chromatography eluted with hexane/ethyl acetate, and compound **19(i-xxviii)** were obtained with varying yields.

4.10 | Cell culture and growth conditions

A panel of human cancer cell lines—HCT-116 (colon), HT-29 (colon), MCF-7 (breast), PC-3 (prostate), SF-268 (CNS)—were procured from the US National Cancer Institute (NCI). Cell lines were grown in tissue culture flasks in complete growth medium (RPMI-1640) supplemented with 10% fetal bovine serum, 100 μg/mL streptomycin, and 100 units/mL penicillin in carbon dioxide incubator (New Brunswick, Galaxy 170R, Eppendorf) at 37°C, 5% CO_2, and 98% RH.

4.11 | Method for sulforhodamine B assay

For cytotoxicity evaluation, cell suspension of optimum cell density was seeded in 96 well flat bottom plates (NUNC). Cell lines were seeded at respective inoculation densities per well as HCT-116 (7500), HT-29 (7500), MCF-7 (8000), Prostate (7500), SF-268 (10000). Cell suspension of 100 μL was plated. The cells were then exposed to different concentrations of test materials containing complete growth medium for 24 hours. The plates were again incubated under the same conditions for another 48 hours at 37°C. Further, cells were fixed with ice cold TCA (trichloroacetic acid) for 1 hour at 4°C. After 1 hour, the plates were rinsed three times with water and allowed to air dry. After drying, 100 μL of 0.4% sulforhodamine B assay (SRB) dye was added for half an hour at room temperature. Plates were then washed three times with 1% vol/vol acetic acid to remove the unbound SRB. After drying at room temperature, the bound dye was solubilized by adding 100 μL of 10 mM TRIS (tris [hydroxymethyl]aminomethane) buffer (pH −10.4) to each well. The plates were kept on the shaker for 5 minutes to solubilize the protein-bound dye. Finally, OD was taken at 540 nm in a microplate reader (Thermo Scientific). IC_{50} was determined by plotting OD against concentration using graph pad prism software.

5 | COMPOUND CHARACTERIZATION

5.1 | N-(4-Benzylamino-6-piperidin-1-yl-pyrimidin-5-yl)-2-piperidin-1-yl-acetamide (14a)

Crystalline white solid; mp: 149-151°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H), 7.15 (dd, 2H, J = 6.7 Hz, 7.3 Hz), 7.05-7.00 (m, 3H), 4.30 (s, 2H), 3.25(s, 2H), 2.7-2.65(m, 4H), 2.24-2.21(m, 4H), 1.60-1.53(m, 12H);^{13}C (100 MHz, $CDCl_3$): 23.1,24.9, 25.0, 25.2, 26.2, 26.5, 51.5, 51.9, 54.3, 57.1, 97.0, 126.5, 126.8, 127.5, 128.5, 128.7, 143.2, 151.5, 162.0, 162.5, 169.0; ESI MS: 409 (M^+); Anal. Calcd for $C_{23}H_{32}N_6O$: C,67.65; H, 7.58; Found: C, 66.45; H, 5.54.

5.2 | N-(4-Benzylamino-6-pyrrolidin-1-yl-pyrimidin-5-yl)-2-pyrrolidin-1-yl-acetamide (14b)

White solid; mp: 143-145°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H), 7.15 (dd, 2H, J = 6.7 Hz, 7.3 Hz), 7.05-7.00 (m, 3H), 4.30 (s, 2H), 3.25(s, 2H), 2.8-2.75(m, 4H), 2.24-2.21(m, 4H), 1.55 (m, 8H);^{13}C (100 MHz, $CDCl_3$): 22.0, 23.1, 24.0, 51.0, 53.9, 57.1, 57.5, 97.0, 126.5, 126.8, 127.5, 128.0, 128.3, 142.2, 150.5, 162.0, 162.5, 169.0; ESI MS: 381 (M^+);Anal. Calcd for $C_{21}H_{28}N_6O$: C, 66.25; H, 7.42; Found: C, 64.25; H, 6.24.

5.3 | N-(4-Benzylamino-6-morpholin-4-yl-pyrimidin-5-yl)-2-morpholin-4-yl-acetamide (14c)

White solid; mp: 145-146°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H), 7.15 (dd, 2H, J = 6.7 Hz, 7.3 Hz), 7.05-7.00 (m, 3H), 4.32 (s, 2H), 3.65-3.50 (m, 8H), 3.25 (s, 2H), 2.9-2.85 (m, 4H), 2.34-2.29 (m, 4H);^{13}C (100 MHz, $CDCl_3$): 55.9, 57.1, 57.5, 60.0, 70.1, 71.0, 71.5, 97.0, 126.5, 127.5, 128.0, 142.2, 150.5, 162.0, 162.5, 169.0; ESI MS: 413 (M^+);Anal. Calcd for $C_{21}H_{28}N_6O_3$: C, 61.25; H, 6.82; Found: C, 60.25; H, 6.24.

5.4 | N-[4-Benzylamino-6-(2-hydroxy-ethylamino)-pyrimidin-5-yl]-2-(2-hydroxy-ethylamino)-acetamide (14d)

White solid; mp: 152-154°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H), 7.15 (dd, 2H, J = 6.7 Hz, 7.3 Hz), 7.05-7.00 (m, 3H), 4.32 (s, 2H), 3.70 (t, 2H), 3.65(t, 2H),

3.25(t, 2H), 2.70 (t, 2H); ^{13}C (100 MHz, $CDCl_3$): 51.0, 53.5, 54.0, 57.1, 64.0, 64.5, 70.1, 97.0, 126.5, 127.0, 128.0, 142.2, 150.5, 162.0, 162.5; ESI MS: 361 (M^+);Anal. Calcd for $C_{17}H_{24}N_6O_3$: C,56.25; H, 6.72; Found: C, 55.25; H, 6.24.

5.5 | N-[4-Benzylamino-6-(4-propionyl-piperidin-1-yl)-pyrimidin-5-yl]-2-(4-propionyl-piperidin-1-yl)-acetamide (14e)

White solid; mp: 155-156°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H), 7.15 (dd, 2H, *J* = 6.7 Hz, 7.3 Hz), 7.05-7.00 (m, 3H), 4.32 (s, 2H), 3.25 (s, 2H), 2.70 (m, 4H), 2.55-2.49 (m, 4H), 2.40-2.35 (m, 2H), 2.26 (m, 4H), 1.80-1.75 (m, 8H), 1.06 (t, 6H);^{13}C (100 MHz, $CDCl_3$): 8.0, 8.5, 23.0, 23.5, 23.9, 30.9, 31.5, 45.3, 45.5, 48.5, 48.9, 52.0, 55.0, 57.1, 97.0, 126.5, 127.0, 128.0, 128.3, 150.5, 162.0, 162.5. 168.2, 210.5; ESI MS: 521 (M^+);Anal. Calcd for $C_{29}H_{40}N_6O_3$: C,66.90; H, 7.74; Found: C, 65.25; H, 7.24.

5.6 | N-[4-Benzylamino-6-(1-phenyl-ethylamino)-pyrimidin-5-yl]-2-(1-phenyl-ethylamino)-acetamide (14f)

White solid; mp: 143-145°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H), 7.20-7.17 (m, 4H), 7.15 (dd, 2H, *J* = 6.7 Hz, 7.3 Hz), 7.12 (m, 4H), 7.08 (t, 2H), 7.07 (t, 2H), 7.06 (d, 2H), 4.32 (s, 2H), 4.00 (m, 2H), 1.35 (d, 6H); ^{13}C (100 MHz, $CDCl_3$): 23.0, 23.5,52.0, 56.0, 57.1, 97.0, 126.0, 126.5, 127.0, 127.5, 128.0, 128.3, 137.5, 142.6, 150.5, 162.0, 162.5. 168.2, 210.5; ESI MS: 482.0 (M^+); Anal. Calcd for $C_{29}H_{32}N_6O$: C,72.47; H, 6.74; Found: C, 70.25; H, 5.34.

5.7 | N-[4-Benzylamino-6-(1-phenyl-ethylamino)-pyrimidin-5-yl]-2-(1-phenyl-ethylamino)-acetamide (14g)

White solid; mp: 148-149°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H), 7.20-7.17 (m, 4H), 7.15 (dd, 2H, *J* = 6.7 Hz, 7.3 Hz), 7.12 (m, 4H), 7.08 (t, 2H), 7.07 (t, 2H), 7.06 (d, 2H, *J* = 6.4 Hz), 4.32 (s, 2H), 4.00 (m, 2H), 1.35 (d, 6H, *J* = 3.4 Hz); ^{13}C (100 MHz, $CDCl_3$): 23.0, 23.5, 52.0, 56.0, 57.1, 97.0, 126.0, 126.5, 127.0, 127.5, 128.0, 128.3, 137.5, 142.6, 150.5, 162.0, 162.5. 168.2, 210.5; ESI MS: 482.0 (M^+);Anal. Calcd for $C_{29}H_{32}N_6O$: C, 72.47; H, 6.74; Found: C, 70.25; H, 5.34.

5.8 | N-Benzylidene-N′-(6-piperidin-1-yl-pyrimidin-4-yl)-hydrazine (19i)

White solid; mp: 126-127°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.60 (d, 2H, *J* = 6.8 Hz), 7.55 (s, 1H), 7.32-7.30(m, 3H), 5.55(s, 1H), 2.80-2.75(m, 4H), 1.50-1.47 (m, 6H); ^{13}C (100 MHz, $CDCl_3$): 25.0, 25.2, 25.7, 53.7, 54.5, 92.0, 127.9, 128.2, 129.0, 130.3, 131.2, 154.5, 155.9, 170.0; ESI MS: 282.0 (M^+);Anal. Calcd for $C_{16}H_{19}N_5$: C, 68.30; H, 6.81; Found: C, 67.25; H, 6.34.

5.9 | N-(2-methoxy-benzylidene)-N′-(6-piperidin-1-yl-pyrimidin-4-yl)-hydrazine (19ii)

White solid; mp: 116-118°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.60 (d, 2H, *J* = 7.8 Hz), 7.55 (s, 1H), 7.50 (d, 1H, *J* = 7.7 Hz), 7.2 (dd, 2H, *J* = 6.7 Hz, 5.4 Hz), 6.80 (m, 2H), 5.55 (s, 1H), 3.75(s, 3H), 2.80-2.75(m, 4H), 1.50-1.47 (m, 6H); ^{13}C (100 MHz, $CDCl_3$): 25.0, 25.2, 25.7, 53.7, 54.5, 56.0, 92.0, 114.5, 122.0, 130.3, 131.2, 154.5, 155.9, 162.5, 170.0; ESI MS: 313.0 (M^+);Anal. Calcd for $C_{17}H_{21}N_5O$: C,65.57; H, 6.81; Found: C, 65.25; H, 6.34.

5.10 | N-(4-methoxy-benzylidene)-N′-(6-piperidin-1-yl-pyrimidin-4-yl)-hydrazine (19iii)

White solid; mp: 122-124°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.55 (s, 1H), 7.50 (d, 2H, *J* = 7.7 Hz), 6.80 (d, 2H, *J* = 5.7 Hz), 5.55 (s, 1H), 3.75 (s, 3H), 2.80-2.75 (m, 4H), 1.50-1.47 (m, 6H); ^{13}C (100 MHz, $CDCl_3$): 25.0, 25.2, 25.7, 53.7, 54.5, 56.0, 92.0, 114.5, 122.0, 130.3, 131.2, 154.5, 155.9, 164.5, 170.0; ESI MS: 313.0 (M^+);Anal. Calcd for $C_{17}H_{21}N_5O$: C, 65.57; H, 6.81; Found: C, 65.25; H, 6.34.

5.11 | N-(3,4-Dimethoxy-benzylidene)-N′-(6-piperidin-1-yl-pyrimidin-4-yl)-hydrazine (19iv)

White solid; mp: 118-120°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.55 (s, 1H), 7.10 (d, 1H, *J* = 7.1 Hz), 7.00 (s, 1H), 6.80 (d, 1H, *J* = 5.7 Hz), 5.55 (s, 1H), 3.73(s, 6H), 2.80-2.75 (m, 4H), 1.50-1.47 (m, 6H); ^{13}C (100 MHz, $CDCl_3$): 25.0, 25.2, 25.7, 53.7, 54.5, 56.0, 56.1, 92.0, 115.5, 122.0, 124.5, 147.5, 149.0, 154.5, 170.0; ESI MS: 342.0 (M^+);Anal. Calcd for $C_{18}H_{23}N_5O_2$: C, 63.57; H, 6.79; Found: C, 62.25; H, 6.14.

5.12 | N-(2-Bromo-benzylidene)-N′-(6-piperidin-1-yl-pyrimidin-4-yl)-hydrazine (19v)

White solid; mp: 120-122°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.55 (s, 1H), 7.50 (d, 2H, J = 7.8 Hz), 7.20 (d, 2H, J = 7.0 Hz), 5.55 (s, 1H), 2.80-2.75 (m, 4H), 1.50-1.47 (m, 8H); ^{13}C (100 MHz, $CDCl_3$): 25.0, 25.2, 25.7, 53.7, 54.5, 92.0, 123.0, 127.6131.2, 131.9133.0, 134.5, 154.5156.0, 170.0; ESI MS: 360.0 (M^+);Anal. Calcd for $C_{16}H_{18}BrN_5$: C, 53.34; H, 5.04; Found: C, 52.25; H, 4.54.

5.13 | N-(4-Bromo-benzylidene)-N′-(6-piperidin-1-yl-pyrimidin-4-yl)-hydrazine (19vi)

White solid; mp: 123-124°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.55 (s, 1H), 7.50-7.45 (m, 4H), 5.55 (s, 1H), 2.80-2.75(m, 4H), 1.50-1.47 (m, 8H); ^{13}C (100 MHz, $CDCl_3$): 25.0, 25.2, 25.7, 53.7, 54.5, 92.0, 123.0,130.2, 131.5, 131.7, 131.9, 156.2, 170.0; ESI MS: 360.0 (M^+);Anal. Calcd for $C_{16}H_{18}BrN_5$: C, 53.34; H, 5.04; Found: C, 52.25; H, 4.54.

5.14 | 2-[(6-Piperidin-1-yl-pyrimidin-4-yl)-hydrazonomethyl]-phenol (19vii)

White solid; mp: 128-129°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.55 (s, 1H), 7.40 (d, 1H, J = 7.7 Hz), 7.10 (dd, 1H, J = 7.5 Hz, 6.8 Hz), 6.80 (m, 2H), 5.55 (s, 1H), 2.80-2.75 (m, 4H), 1.50-1.47 (m, 8H); ^{13}C (100 MHz, $CDCl_3$): 25.0, 25.2, 25.7, 53.7, 54.5, 92.0, 115.5, 117.9, 121.5, 130.2, 133.0, 154.5156.0, 157.8, 170.0; ESI MS: 298.0 (M^+);Anal. Calcd for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.44; Found: C, 62.25; H, 5.74.

5.15 | N-Benzylidene-N′-(6-pyrrolidin-1-yl-pyrimidin-4-yl)-hydrazine (19viii)

White solid; mp: 124-125°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.60 (d, 2H, J = 6.7 Hz), 7.52 (s,1H), 7.3 (m, 3H), 5.52 (s, 1H), 2.80-2.75 (t, 4H), 1.56 (t, 4H); ^{13}C (100 MHz, $CDCl_3$): 23.0, 52.5, 92.0, 127.5, 129.0, 130.0, 131.0, 154.7156.0, 169.5, 170.0; ESI MS: 268.0 (M^+);Anal. Calcd for $C_{15}H_{17}N_5$: C, 67.39; H, 6.42; Found: C, 65.12; H, 5.74.

5.16 | N-(2-Methoxy-benzylidene)-N′-(6-pyrrolidin-1-yl-pyrimidin-4-yl)-hydrazine (19ix)

White solid; mp: 128-130°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.50 (d, 1H, J = 6.7 Hz), 7.25(t, 1H), 6.8 (d, 1H J = 5.7 Hz), 5.52 (s, 1H), 3.73(s, 3H), 2.80-2.75(t, 4H), 1.56(t, 4H); ^{13}C (100 MHz, $CDCl_3$): 23.0, 52.5, 56.5, 92.0, 113.5, 117.5, 120.0,130.0, 131.5, 154.7156.0, 162.5, 170.0; ESI MS: 298.0 (M^+);Anal. Calcd for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.42; Found: C, 62.76; H, 5.92.

5.17 | N-(4-Methoxy-benzylidene)-N′-(6-pyrrolidin-1-yl-pyrimidin-4-yl)-hydrazine (19x)

White solid; mp: 114-115°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.50 (d, 2H, J = 7.8 Hz), 6.8(d, 2H, J = 5.7 Hz), 5.52 (s, 1H), 3.73(s, 3H), 2.80-2.75 (t, 4H), 1.56 (t, 4H); ^{13}C (100 MHz, $CDCl_3$): 23.0, 52.5, 56.5, 92.0, 113.5, 123.5, 130.0, 154.7156.0, 164.5, 170.0; ESI MS: 298.0 (M^+); Anal. Calcd for $C_{16}H_{19}N_5O$: C, 64.63; H, 6.42; Found: C, 62.76; H, 5.92.

5.18 | N-(3,4-dimethoxy-benzylidene)-N′-(6-pyrrolidin-1-yl-pyrimidin-4-yl)-hydrazine (19xi)

White solid; mp: 131-133°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.15 (d, 1H, J = 6.7 Hz), 7.00 (s, 1H), 6.73(d, 1H, J = 5.7 Hz), 5.52 (s, 1H), 3.73 (s, 6H), 2.80-2.75 (t, 4H), 1.56 (t, 4H); ^{13}C (100 MHz, $CDCl_3$): 23.0, 52.5, 56.5, 92.0, 115.5, 115.8, 121.5, 124.5, 154.7156.0, 170.0; ESI MS: 328.0 (M^+);Anal. Calcd for $C_{17}H_{21}N_5O_2$: C,62.37; H, 6.47; Found: C, 62.06; H, 5.63.

5.19 | N-(2-bromo-benzylidene)-N′-(6-pyrrolidin-1-yl-pyrimidin-4-yl)-hydrazine (19xii)

White solid; mp: 113-115°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.50 (d, 2H, J = 7.7 Hz), 7.20 (dd, 2H, J = 5.4 Hz, 6.2 Hz), 5.52 (s, 1H), 2.80-2.75 (t, 4H), 1.56 (t, 4H); ^{13}C (100 MHz, $CDCl_3$): 23.0, 52.5, 92.0, 123.5, 126.0, 130.0, 131.9, 133.2, 134.5, 154.7156.0, 170.0; ESI MS: 347(M^+); Anal. Calcd for $C_{15}H_{16}BrN_5$: C,52.05; H, 4.67; Found: C, 51.06; H, 4.63.

5.20 | N-(4-bromo-benzylidene)-N′-(6-pyrrolidin-1-yl-pyrimidin-4-yl)-hydrazine (19xiii)

White solid; mp: 128-129°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (m, 4H), 5.52 (s, 1H), 2.80-2.75 (t, 4H),

1.56 (t, 4H); ^{13}C (100 MHz, $CDCl_3$): 23.0, 52.5, 92.0, 125.5, 130.0, 131.2, 131.9, 133.2, 134.5, 154.7156.0, 170.0; ESI MS: 347 (M^+);Anal. Calcd for $C_{15}H_{16}BrN_5$: C, 52.05; H, 4.67; Found: C, 51.06; H, 4.63.

5.21 | 2-[(6-Pyrrolidin-1-yl-pyrimidin-4-yl)-hydrazonomethyl]-phenol (19xiv)

White solid; mp: 126-127°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.40 (d, 1H, J = 6.7 Hz), 6.75 (dd, 2H, J = 6.7 Hz, 5.3 Hz), 7.10 (dd, 1H, J = 6.8 Hz, 6.0 Hz), 5.52 (s, 1H), 2.80-2.75(t, 4H), 1.56 (t, 4H); ^{13}C (100 MHz, $CDCl_3$): 23.0, 52.5, 92.0, 115.5, 118.5121.0, 130.0, 132.2, 131.9, 154.7156.0, 170.0; ESI MS: 284(M^+); Anal. Calcd for $C_{15}H_{17}N_5O$: C,63.59; H, 6.05; Found: C, 62.15; H, 5.73.

5.22 | N-Benzylidene-N′-(6-morpholin-4-yl-pyrimidin-4-yl)-hydrazine (19xv)

White solid; mp: 132-133°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.65 (d, 2H, J = 5.7 Hz), 7.52 (s, 1H), 7.30 (m, 3H), 5.55 (s, 1H), 3.67(m, 4H), 2.80-2.75(m, 4H); ^{13}C (100 MHz, $CDCl_3$): 38.4, 57.6, 71.0, 109.0, 112.5, 118.5, 121.7, 126.0, 127.0, 128.0, 129.3, 134.5, 138.5, 162.0, 170.0; ESI MS: 280.0 (M^+);Anal. Calcd for $C_{19}H_{21}NO$: C, 81.68; H, 7.58; Found: C, 80.25; H, 7.04.

5.23 | N-(2-Methoxy-benzylidene)-N′-(6-morpholin-4-yl-pyrimidin-4-yl)-hydrazine (19xvi)

White solid; mp: 121-123°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.50 (d, 1H, J = 6.3 Hz), 7.20 (dd, 1H, J = 5.6 Hz, 6.4 Hz), 6.80 (m, 2H), 5.52 (s, 1H), 3.67(m, 4H),2.80-2.75 (m, 4H); ^{13}C (100 MHz, $CDCl_3$): 55.5, 58.9, 71.0, 92.0, 114.5, 116.0, 120.0, 130.0, 131.5, 154.5, 156.5, 162.0, 170.0; ESI MS: 315.0 (M^+);Anal. Calcd for $C_{16}H_{19}N5O_2$: C, 61.33; H, 6.11; Found: C, 60.12; H, 6.04.

5.24 | N-(4-Methoxy-benzylidene)-N′-(6-morpholin-4-yl-pyrimidin-4-yl)-hydrazine (19xvii)

White solid; mp: 118-120°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.50 (d, 1H, J = 7.3 Hz), 6.80 (d, 2H, J = 5.4 Hz), 5.52 (s, 1H), 3.67 (m, 4H), 2.80-2.75 (m, 4H); ^{13}C (100 MHz, $CDCl_3$): 55.5, 58.9, 71.0, 92.0, 114.5, 116.0, 123.0, 130.0, 131.5, 154.5, 156.5, 162.0, 170.0; ESI MS: 315.0 (M^+);Anal. Calcd for $C_{16}H_{19}N5O_2$: C, 61.33; H, 6.11; Found: C, 60.12; H, 6.04.

5.25 | N-(3,4-Dimethoxy-benzylidene)-N′-(6-morpholin-4-yl-pyrimidin-4-yl)-hydrazine (19xviii)

White solid; mp: 116-118°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.10 (d, 1H, J = 6.3 Hz), 7.00 (s, 1H), 6.80 (d, 1H, J = 5.7 Hz), 5.52 (s, 1H), 3.70 (s, 6H), 3.67 (m, 4H), 2.80-2.75 (m, 4H); ^{13}C (100 MHz, $CDCl_3$): 55.5, 58.9, 71.0, 92.0, 114.5, 116.0, 123.0, 124.5, 147.5, 149.0, 154.5, 156.5170.0; ESI MS: 344.0 (M^+); Anal. Calcd for $C_{17}H_{21}N_5O_3$: C, 59.96; H, 6.16; Found: C, 57.12; H, 6.04.

5.26 | N-(2-Bromo-benzylidene)-N′-(6-morpholin-4-yl-pyrimidin-4-yl)-hydrazine (19xix)

White solid; mp: 122-123°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.50 (d, 2H, J = 6.3 Hz), 7.20 (dd, 2H, J = 5.7 H z, 7.8 Hz), 5.52 (s, 1H), 3.67 (m, 4H), 2.80-2.75(m, 4H); ^{13}C (100 MHz, $CDCl_3$): 58.9, 71.0, 92.0, 123.0, 127.5, 131.5, 133.0, 134.5, 154.5, 156.5170.0; ESI MS: 362.0 (M^+);Anal. Calcd for $C_{15}H_{16}BrN_5O$: C, 49.74; H, 4.45; Found: C, 47.72; H, 4.04.

5.27 | N-(4-Bromo-benzylidene)-N′-(6-morpholin-4-yl-pyrimidin-4-yl)-hydrazine (19xx)

White solid; mp: 116-118°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.50 (m, 4H), 5.52 (s, 1H), 3.67 (m, 4H),2.80-2.75 (m, 4H); ^{13}C (100 MHz, $CDCl_3$): 58.9, 71.0, 92.0, 125.0, 130.0, 131.5, 154.5, 156.5170.0; ESI MS: 362.0 (M^+);Anal. Calcd for $C_{15}H_{16}BrN_5O$: C, 49.74; H, 4.45; Found: C, 47.72; H, 4.04.

5.28 | 2-[(6-Morpholin-4-yl-pyrimidin-4-yl)-hydrazonomethyl]-phenol (19xxi)

White solid; mp: 110-111°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.40 (d, 1H, J = 7.8 Hz), 7.1(t, 1H), 6.8 (dd, 1H, J = 3.4 Hz, 5.2 Hz), 5.52 (s, 1H), 3.67(m, 4H), 2.80-2.75(m, 4H); ^{13}C (100 MHz, $CDCl_3$): 58.9, 71.0, 92.0, 115.8, 118.0, 121.0, 130.0, 132.2, 154.5, 156.5, 157.5, 170.0; ESI MS: 300.0 (M^+);Anal. Calcd for $C_{15}H_{17}N_5O_2$: C, 60.19; H, 5.72; Found: C, 58.42; H, 5.04.

5.29 | 1-{1-[6-(N′-Benzylidene-hydrazino)-pyrimidin-4-yl]-piperidin-4-yl}-propan-1-one (19xxii)

White crystalline solid; mp: 112-114°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.60 (d, 2H, J = 6.3 Hz), 7.52 (s,1H), 7.30 (m, 3H), 5.52 (s, 1H), 2.70 (t, 4H), 2.45 (q, 2H), 2.36-2.30 (m, 1H), 1.00 (t, 3H); ^{13}C (100 MHz, $CDCl_3$): 7.1, 23.0, 23.5, 30.5, 45.0, 52.1, 52.7, 92.0, 128.0, 128.3, 129.0, 130.5, 131.2, 154.0, 156.0, 170.0, 170.3, 211.0; ESI MS: 338(M^+);Anal. Calcd for $C_{19}H_{23}N_5O$: C, 67.63; H, 6.87; Found: C, 65.15; H, 6.13.

5.30 | 1-(1-{6-[N′-(2-Methoxy-benzylidene)-hydrazino]-pyrimidin-4-yl}-piperidin-4-yl)-propan-1-one (19xxiii)

Yellow solid; mp: 124-126°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.55 (d, 1H, J = 5.7 Hz), 7.52 (s,1H), 7.20 (dd, 1H, J = 5.4 Hz, 6.0 Hz), 5.52 (s, 1H), 3.72 (s, 3H), 2.70 (t, 4H), 2.45 (q, 2H), 2.36-2.30 (m, 1H), 1.00 (t, 3H); ^{13}C (100 MHz, $CDCl_3$): 7.1, 23.0, 23.5, 30.5, 45.0, 52.1, 52.7, 56.5, 92.0, 114.5, 115.9, 120.5, 130.5, 131.2, 154.0, 156.0, 162.5, 170.0, 170.3, 211.0; ESI MS: 368(M^+);Anal. Calcd for $C_{20}H_{25}N_5O_2$: C, 65.37; H, 6.87; Found: C, 65.09; H, 5.73.

5.31 | 1-(1-{6-[N′-(4-Methoxy-benzylidene)-hydrazino]-pyrimidin-4-yl}-piperidin-4-yl)-propan-1-one (19xxiv)

Pale yellow solid; mp: 118-120°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.55 (d, 1H, J = 6.3 Hz), 7.52 (s,1H), 6.75 (d, 2H, J = 5.8 Hz), 5.52 (s, 1H), 3.72(s, 3H), 2.70 (t, 4H), 2.45 (q, 2H), 2.36-2.30 (m, 1H), 1.00 (t, 3H); ^{13}C (100 MHz, $CDCl_3$): 7.1, 23.0, 23.5, 30.5, 45.0, 52.1, 56.5, 92.0, 114.5, 124.5, 130.5, 154.0, 156.0, 170.0, 170.3, 211.0; ESI MS: 368(M^+);Anal. Calcd for $C_{20}H_{25}N_5O_2$: C, 65.37; H, 6.87; Found: C, 65.09; H, 5.73.

5.32 | 1-(1-{6-[N′-(3,4-Dimethoxy-benzylidene)-hydrazino]-pyrimidin-4-yl}-piperidin-4-yl)-propan-1-one (19xxv)

Pale yellow solid; mp: 123-124°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.10 (d, 1H, J = 6.8 Hz), 7.00 (s, 1H), 6.75 (d, 1H, J = 5.7 Hz), 5.52 (s, 1H), 3.72 (s, 6H), 2.70 (t, 4H), 2.45 (q, 2H), 2.36-2.30 (m, 1H), 1.00 (t, 3H); ^{13}C (100 MHz, $CDCl_3$): 7.1, 23.0, 23.5, 30.5, 45.0, 52.1, 56.5, 92.0, 114.5, 115.0, 122.0, 124.5, 147.0, 148.0, 154.0, 156.0, 170.0, 170.3, 211.0; ESI MS: 398(M^+);Anal. Calcd for $C_{21}H_{27}N_5O_3$: C, 63.47; H, 6.87; Found: C, 62.09; H, 6.83.

5.33 | 1-(1-{6-[N′-(2-Bromo-benzylidene)-hydrazino]-pyrimidin-4-yl}-piperidin-4-yl)-propan-1-one (19xxvi)

White crystalline solid; mp: 108-110°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.50 (d, 2H, J = 7.7 Hz), 7.20 (dd, 2H, J = 4.5 Hz, 5.8 Hz), 5.52 (s, 1H), 2.70 (t, 4H), 2.45 (q, 2H), 2.36-2.30 (m, 1H), 1.00 (t, 3H); ^{13}C (100 MHz, $CDCl_3$): 7.1, 23.0, 23.5, 30.5, 45.0, 52.1, 92.0, 123.5, 127.0, 130.5, 131.5, 133.0, 154.0, 156.0, 170.0, 170.3, 211.0; ESI MS: 416(M^+);Anal. Calcd for $C_{19}H_{22}BrN_5O$: C, 54.82; H, 5.33; Found: C, 53.09; H, 5.13.

5.34 | 1-(1-{6-[N′-(4-Bromo-benzylidene)-hydrazino]-pyrimidin-4-yl}-piperidin-4-yl)-propan-1-one (19xxvii)

White solid; mp: 114-115°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.50-7.43 (m, 4H), 5.52 (s, 1H), 2.70 (t, 4H), 2.45 (q, 2H), 2.36-2.30 (m, 1H), 1.00 (t, 3H); ^{13}C (100 MHz, $CDCl_3$): 7.1, 23.0, 23.5, 30.5, 45.0, 52.1, 92.0, 123.5, 127.0, 130.5, 131.5, 131.9, 133.0, 154.0, 156.0, 170.0, 170.3, 211.0; ESI MS: 416(M^+); Anal. Calcd for $C_{19}H_{22}BrN_5O$: C, 54.82; H, 5.33; Found: C, 53.09; H, 5.13.

5.35 | 1-(1-{6-[N′-(2-Hydroxy-benzylidene)-hydrazino]-pyrimidin-4-yl}-piperidin-4-yl)-propan-1-one (19xxviii)

White crystalline solid; mp: 123-124°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.52 (s,1H), 7.43 (d, 1H, J = 8.3 Hz), 7.10 (dd, 1H, 6.8 Hz, 5.3 Hz), 6.73(dd, 2H, 7.8 Hz, 6.3 Hz), 5.52 (s, 1H), 2.70 (t, 4H), 2.45 (q, 2H), 2.36-2.30 (m, 1H), 1.00 (t, 3H); ^{13}C (100 MHz, $CDCl_3$): 7.1, 23.0, 23.5, 45.0, 52.0, 52.1, 92.0, 115.0, 117.5, 120.5, 130.5, 131.5, 132.9, 154.0, 156.0, 170.0, 170.3, 211.0; ESI MS: 354(M^+);Anal. Calcd for $C_{19}H_{23}N_5O_2$: C, 64.82; H, 6.56; Found: C, 63.39; H, 6.19.

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