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Synthesis and Antimicrobial Evaluation of 6-(4-(4-Chlorophenylamino)piperidine-1-yl) pyrimidin-4-amino Analogues

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Abstract: A series of compounds based on 6-(4-chlorophenylamino) piperidin-1-yl)pyrimidin-4-amino derivatives were synthesized and its anti-microbial potential was explored. All the synthesized compounds (**5a-5m**) were screened for their antibacterial and antifungal activity. Among all the compounds **5e** (MIC (µg/mL)/Inhibition (mm): 6.25/19-23; cLogP: 4.66) and **5f** (MIC (µg/mL)/Inhibition (mm): 6.25/22-25; cLogP: 5.21) found to have good activity in antibacterial and antifungal.

Keywords: Pyrimidine, Piperidine, Antimicrobial activity, Anti-bacterial, Anti-fungal, Microwave.

Introduction

In this research, we have synthesized 4,6-disubstituted pyrimidine derivatives and explored antibacterial and antifungal activities, because the multiple diverse functionalization and significant importance of heterocycles in the preparation of drugs. Among all the heterocyclic derivatives substituted pyrimidine derivatives have a wide range pharmacological profile lies in their similarity of cytosine, thymine and uracil which are the building blocks of DNA and RNA. The pyrimidine derivatives are highly effective in antimicrobial [1], anti-inflammatory [2], anti-infective [3], analgesic [4], anticancer [5] etc. and HIV effect. Apart from these, pyrimidines are key compounds in antiviral [6-8], antiallergic [9], cardiovascular [10] and central nervous system [11]. Since the pyrimidines had like these good properties, from past century research is going on pyrimidines and its derivatives.

Antimicrobial resistance happens when microorganisms such as fungi, parasites, bacteria and viruses change in ways that cause to be the medications used to heal the infections. The superbugs resistant to many antimicrobials and cause to resistant infection, which can explore with others, may kill, and inflict huge costs to individuals and society. The reduction in the effectiveness of a drug against to disease and the drug is not intended to kill or inhibit a pathogenis called drug resistance.

The challenge in now days, an antimicrobial resistance and antineoplastic resistance becoming clinical care and requires to synthesize small hetero cyclic molecules. The development of antibiotic resistance in particular stems from the drugs targeting only proteins. These molecules will interfere with or negate its destructive effect, because of the drug mutation and leads to antibiotic resistance. Henceforth, we have taken an opportunity to synthesis good antimicrobial compounds.

The major concept of the research work done in this paper is to explore new pyrimidine derivatives as

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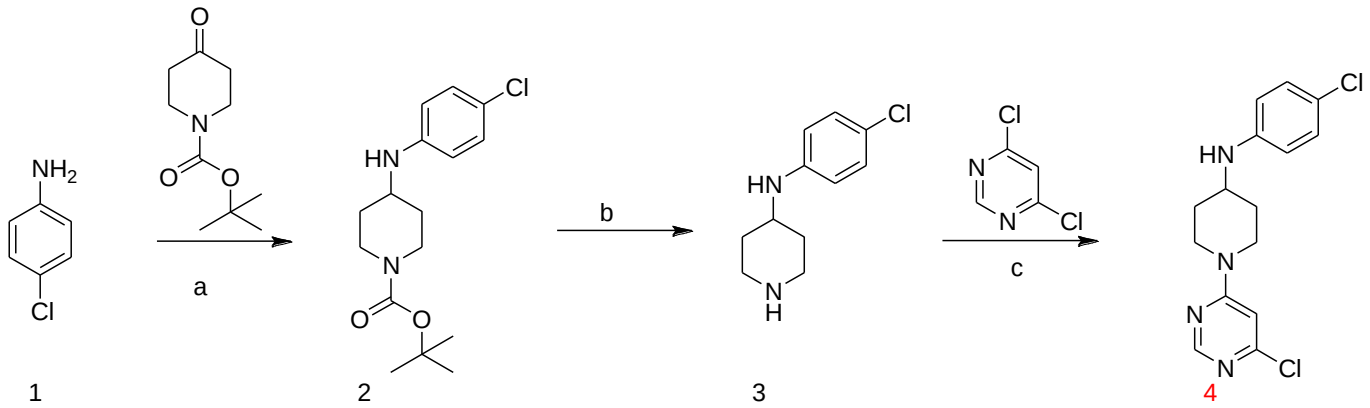
antimicrobial agents by maintaining good cLogP values (4-6). cLogP is one of the important physiochemical property in terms of drug absorption. This may allow the discovery of some novel analogues of 4,6-disubstituted pyrimidine core with improved antimicrobial activities. Accordingly, we have synthesized 6-(4-chlorophenylamino) piperidin-1-yl) -4-amino/benzylamino/phenylamino-pyrimidine derivatives, which were very useful in medicinal chemistry for new drugs, to support that we have screened antimicrobial activity to all synthesized compounds.

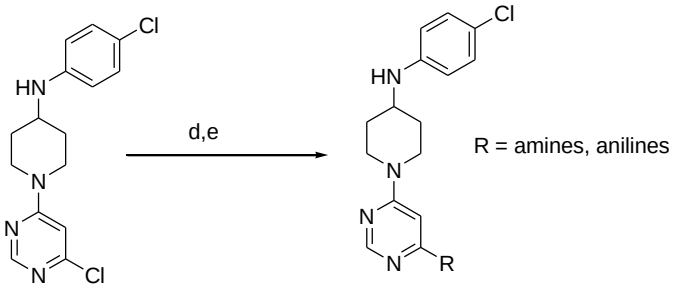
A major diversity of 4,6-disubstituted pyrimidine analogues employing (Scheme **2**) the synthesis of the key intermediate (**4**) was accomplished as shown Scheme **1**. The chloro intermediate (**4**) being highly activated; we envisioned that displacing the functionality with divers set of aliphatic, cyclic, benzyl amines and anilines would result in terms of pyrimidine analogues which can have better biological properties. Therefore, we synthesized novel analogues based on pyrimidine core and explored both antifungal and antibacterial studies.

Results and discussion



Reagents and conditions: (a) $NaBH(OAc)_3$, AcOH, 1,2-DCE, rt, 3 h, 52% ; (b) Dioxane-HCl, 1,4-dioxane, rt, 4 h, 88% ; (c) DIPEA, 1,4-dioxnae, 110 ^oC, 14 h, 76%.

Scheme 1: Synthesis of *N*-(4-Chlorophenyl)-1-(6-chloropyrimidin-4-yl)piperidin-4-amine.



Reagents and conditions: (d) If R = Amine, DIPEA, n-BuOH, 140 ^oC, 14 h; (e) If R = Aniline, p-TSA, DMF, 140 ^oC, MW, 1.5 h.

Scheme 2: Synthesis of 6-(4-(4-Chlorophenylamino)piperidine-1-yl)pyrimidin-4-amino analogues.

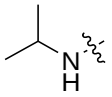
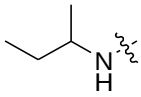
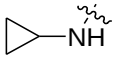
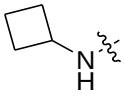
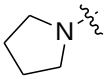
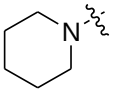
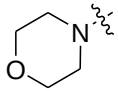
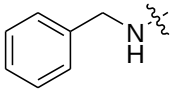
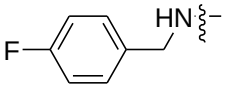
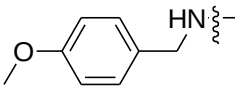
The first step involves the reductive amination of *tert*-butyl 4-oxopiperidine-1-carboxylate with 4-chloro aniline (**1**) in presence of triacetoxy sodiumborohydride to access the tert-butyl 4-[(4-chlorophenyl)amino]piperidine-1-carboxylate (**2**). Compound (**2**) was then treated with 4M HCl in 1,4-dioxane to obtain *N*-(4-chlorophenyl) piperidin-4-amine hydrochloride (**3**). Subjecting the intermediate (**3**) to displacement reaction with 4,6-dichloropyrimidine and DIPEA (diisopropylethyl amine) to get main scaffold *N*-(4-Chlorophenyl)-1-(6-chloropyrimidin-4-yl)piperidin-4-amine (**4**) which was used for further late stage diversification. Among the solvents explored for optimizing the condition for final

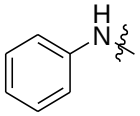
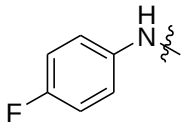
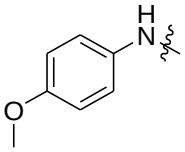
coupling, use of polar protic solvent and higher reaction temperatures resulted in excellent yields of the product. For aniline analogues microwave assisted condition was used.

Table 1: Effect of solvent and temperature of amine coupling reaction.

Entry	Temperature	Solvent	Yield (%)
1	110 ^{o}C	Acetonitrile	3
2	110 ^{o}C	1,4-dioxane	2
3	110 ^{o}C	Toluene	4
4	140 ^{o}C	Toluene	10
5	140 ^{o}C	n-Butanol	33-58

Table 2. Lipophilicity **(**cLogP) values for the final compounds **5a-5m.**

Entry	**Compound code**	**R**	**cLogP**
1	5a		4.76
2	5b		5.29
3	5c		4.28
4	5d		4.84
5	5e		4.66
6	5f		5.21
7	5g		3.68
8	5h		5.37
9	5i		5.52
10	5j		5.29

11	5k		5.91
12	5l		6.09
13	5m		5.85

Structural-Activity Relationship:

All the synthesized compounds were tested for their antimicrobial activity and results were summarized in Table **3** and **4**. All the compounds showed moderate potency in both antibacterial and antifungal activity. We have screened aliphatic amines, cyclic amines, benzyl amines and anilines coupled derivatives. Among all the compounds 5e (MIC (µg/mL)/Inhibition (mm): 6.25/19-22; cLogP = 4.66) and 5f (MIC (µg/mL)/Inhibition (mm): 6.25/19-25; cLogP = 5.21) we found to have a better inhibition profile with respect to standards. Among the amines explored, aliphatic amines showed moderate potency. Moving on to cyclic amines resulted in compounds with good inhibition profile. But in case of morpholine we had seen only moderate potency which having cLogP around 3.9. It's clear that lipophilicity is playing a vital role in the study of biological activity. Benzyl amine explored, also showed only moderate potency. By this SAR we learnt that cyclic amines (5e & 5f) and with cLogP values between 4.5-5.5 had a good activity in overall antimicrobial study.

Antibacterial Activity:

All the synthesized compounds (**5a-5m**) were screened for both antibacterial and antifungal activity. For antibacterial activity all the compounds were screened against the follow strains of bacteria *viz. Escherichia coli* (ATTC-25922), *Staphylococcus aureus* (ATTC-25923), *Pseudomonasaeruginosa* (ATTC-27853) and *Klebsiella pneumonia* by serial plate dilution method [12]. The pH up to 5.0 was adjusted by using phosphate buffer and at 37 ^{o}C all test bacterium were incubated for 20-22 h.

All the antibacterial discs were placed on the agar, and each Petri dish was filled with 20 mL of agar media. By using incubator all the plates were dried for one hour at 37 ^{o}C. The test compounds were dissolved in dimethyl sulfoxide (DMSO) and added to Petri dishes. All the Petri dishes were prepared in triplicate method and kept for 3-4 days by maintaining temperature 37 ^{o}C. Inhibition zone of antibacterial activity was determined by measuring the diameter. The activity of each compound was measured by comparing with Ampicillin as standard [13, 14], in different concentration ranges (1.56 µg/mL, 6.25 µg/mL, 12.5 µg/mL and 25 µg/mL) and the average reading of each was taken. Zone of inhibition values and the MIC values in µg/mL was determined for **5a-5m** and results are summarized in Table **3**.

Table 3: Antibacterial activity data of compounds **5a-5m**.

Compound No.	**MIC [*µ*g/mL] and zone of inhibition (mm) in parentheses**			
	S. aureus	E. coli	P. aeruginosa	K. pneumonia
5a	12.5 (15)	12.5 (14)	12.5 (13)	12.5 (13.5)
5b	12.5 (13)	12.5 (13.5)	12.5 (15)	12.5 (14)
5c	12.5 (14)	12.5 (14.5)	12.5 (13)	12.5 (13)

5d	12.5 (15)	12.5 (15.5)	12.5 (16)	12.5 (15)
5e	6.25 (22)	6.25 (21)	6.25 (23)	6.25 (19.5)
5f	6.25 (23)	6.25 (24)	6.25 (25)	6.25 (24)
5g	12.5 (15)	12.5 (14.5)	12.5 (14)	12.5 (14.5)
5h	12.5 (16)	12.5 (13.5)	12.5 (15)	12.5 (14.5)
5i	12.5 (12.5)	12.5 (13)	12.5 (14)	12.5 (13.5)
5j	12.5 (15)	12.5 (14)	12.5 (14)	12.5 (14.5)
5k	12.5 (13.5)	12.5 (15)	12.5 (14.5)	12.5 (16)
5l	12.5 (15)	12.5 (14.5)	12.5 (15)	12.5 (13.5)
5m	12.5 (16)	12.5 (16.5)	12.5 (15)	12.5 (14)
Standard (Ampicillin)	1.56 (22-30)	6.25 (30-40)	6.25 (25-33)	6.25 (23-27)

Antifungal Activity:

All the newly synthesized compounds were also screened for antifungal activity in the above manner, against the follow four strains of fungi, *viz. Aspergilusflavus* (NCIM No. 524), *Aspergilusfumigatus* (NCIM No. 902), *Penicilliummaneffei* (recultured) and *Trichophytonmentagrophytes* (recultured) by serial plate dilution method[15]. By using peptone (1g), D-glucose (4g) and agar (2g) in distilled water (100mL); Sabourauds agar media was prepared and pH was adjusted to 5.7. 3mL of saline and a loopful of particular fungal strain were mixed to get a suspension of corresponding species. 20 mL of agar media was filled in each Petri dish and plates were dried by placing in an incubator for 1 h at 37 ^{o} C. In all seeded agar plates wells were made by using a punch and these wells were filled with the test compounds which dissolved in DMSO. All the Petri dishes were maintained at 37 ^{o}C for 3-4 days and prepared in triplicate method. Antifungal activity was determined by measuring the diameter of inhibition zone with respect to Itraconozole as standard. Zones of inhibition were determined for 5a-5m, the results of which are summarized in Table **4**. The MIC values were evaluated at concentrations of 6.25 µg/mL, 12.5 µg/mL and 25 µg/mL and the average reading of each was taken.

Table 4. Antifungal activity data of compounds **5a-5m**.

Compound No.	**MIC [*µ*g/mL] and zone of inhibition (mm) in parentheses**			
	P. marneffei	**T. mentagrophytes**	**A. flavus**	**A. fumigates**
5a	12.5 (11)	12.5 (13)	12.5 (11.5)	12.5 (12)
5b	12.5 (13)	12.5 (14)	12.5 (12)	12.5(11.5)
5c	12.5 (14)	12.5 (13)	12.5 (13)	12.5 (14.5)
5d	12.5 (12)	12.5 (12.5)	12.5 (11)	12.5 (15)
5e	6.25 (22)	6.25 (21)	6.25 (20.5)	6.25 (19)

5f	6.25 (25)	6.25 (22)	6.25 (23)	6.25 (24)
5g	12.5 (11)	12.5 (14)	12.5 (12.5)	12.5 (13)
5h	12.5 (15)	12.5 (16)	12.5 (14.5)	12.5 (13)
5i	12.5 (13.5)	12.5 (12)	12.5 (13)	12.5 (15)
5j	12.5 (14.5)	12.5 (15)	12.5 (16)	12.5 (15.5)
5k	12.5 (13.5)	12.5 (12.5)	12.5 (15)	12.5 (14)
5l	12.5 (14)	12.5 (12.5)	12.5 (13)	12.5 (13.5)
5m	12.5 (15)	12.5 (14.5)	12.5 (12.5)	12.5 (13)
Standard (Itraconozole)	1.56 (22-30)	6.25 (30-40)	6.25 (25-33)	6.25 (23-27)

Conclusion

A series of compounds based on 6-(4-chlorophenylamino) piperidin-1-yl)-4-amino/benzylamino/phenylamino-pyrimidine were synthesized. Over all 13 compounds were synthesized by varying different aliphatic, alicyclic, cyclic, benzyl amines and anilines substitutions. All the compounds were screened for antibacterial and antifungal activity. All the 6-(4-chlorophenylamino) piperidin-1-yl)-4-amino/benzylamino/phenylamino-pyrimidine derivatives showed moderate activity, among all **5e** and **5f** shows good inhibition profile.

Experimental

All chemicals and solvents were procured from Sigma Aldrich, Alfa and Spectrochem Chemicals Pvt. Ltd. All the solvents were distilled and dried before usage. The progress of the reactions was monitored by TLC. All the synthesized compounds were purified by recrystallization and flash column chromatography by using silica gel (60-120 mesh). Mass spectra were recorded on an Agilent 1100 MSD/TRAP/XCT system. The melting point of the synthesized compounds was recorded by a Stuart SMP3 melting point apparatus. The ^{1}H and ^{13}C NMR spectra were recorded on bruker advance 300 MHz and 400 MHz NMR spectrometer. All spectra were obtained in $CDCl_3$ and DMSO-d_6 as a solvent. Chemical shifts values are reported as values in ppm relative to TMS as internal standard.

Synthesis:

Tert-butyl 4-[(4-chlorophenyl)amino]piperidine-1-carboxylate ***(2):***

To a stirred solution of 4-chloroaniline (2.0 g, 15.70 mmol) in 1,2-dichloroethane (40 mL) and acetic acid (3 mL) was added *tert*-butyl 4-oxopiperidine-1-carboxylate (3.44 g, 17.2 mmol). The reaction mixture was stirred at RT for 30 minutes then was added triacetoxy sodiumborohydride (5.00 g, 23.5 mmol) in three portions over a period of 10 minutes. The reaction mixture was stirred at RT for 3 h[16]. The reaction mixture was concentrated under reduced pressure and diluted with ethyl acetate (50 mL). The organic layer was washed with water (2 x 50 mL) and brine (1 x 50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude compound was purified by flash column chromatography using silica gel, the product was eluted with 5% MeOH in DCM to get *tert*-butyl 4-[(4-chlorophenyl)amino] piperidine-1-carboxylate (2.5 g, 8.04 mmol, 52% yield).^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.45 (s, 9H), 1.59-1.62 (m, 2H), 1.86-1.89 (m, 2H), 2.74-2.77 (m, 1H), 3.42-3.53 (m, 4H), 6.21 (s, br, 1H), 6.45 (d, J = 8.00 Hz, 2H), 7.22 (d, J = 8.00 Hz, 2H). ms: m/z 311 (M+1).).^{13}C NMR (100 MHz, DMSO-d_6): δ 143.57, 125.67, 121.43, 112.48, 49.64, 41.36, 31.73. Anal.Calcd. For $C_{16}H_{23}ClN_2O_2$: C, 61.83; H, 7.46; N, 9.01. Found: C, 61.96; H, 7.53; N, 9.45.

N-(4-Chlorophenyl)piperidin-4-amine ***(3):***

To a stirred solution of *tert*-butyl 4-[(4-chlorophenyl)amino] piperidine-1-carboxylate (1.0 g, 3.22 mmol) in 1,4-dioxane (10 mL) was added 4M HCl in 1,4-dioxane (10 mL) and stirred at RT for 4 h

[17]. Reaction mixture was concentrated under reduced pressure and co-evaporated with toluene (2 x 15 mL). The residue was washed with ethyl acetate (2 x 15 mL) and dried under reduced pressure to afford *N*-(4-chlorophenyl)piperidin-4-amine hydrochloride (0.7 g, 2.8 mmol, 88% yield).^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.68-1.71 (m, 2H), 1.92-1.95 (m, 2H), 2.74-2.79 (m, 5H), 6.49 (s, br, 1H), 6.39 (d, *J* = 8.20 Hz, 2H), 7.31 (d, *J* = 8.20 Hz, 2H). ms: m/z 211 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ 143.57, 125.67, 121.43, 112.48, 49.64, 41.36, 31.73. Anal.Calcd. For $C_{11}H_{15}ClN_2$: C, 62.70; H, 7.18; N, 13.30. Found: C, 62.84; H, 7.36; N, 13.71.

N-(4-Chlorophenyl)-1-(6-chloropyrimidin-4-yl) piperidin-4-amine ***(4):***

To a stirred solution of *N*-(4-chlorophenyl)piperidin-4-amine hydrochloride (0.5 g, 2.02 mmo) in 1,4-dioxane (10 mL) was added diisopropylamine (1.05 mL, 6.06 mmol) followed by 4,6-dichloropyrimidine (0.303 g, 2.02 mmol) and then heated to 110 ^{o} C for 14 h[18]. Reaction mixture was cooled to RT concentrated under reduced pressure; to that residue water (50 mL) was added and extracted with ethyl acetate (2 x 50 mL). The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure to get crude was purified by flash column chromatography by using silica gel (eluted with 20% EtOAc:Hexane) to afford *N*-(4-chlorophenyl)-1-(6-chloropyrimidin -4-yl)piperidin-4-amine (0.5 g, 1.54 mmol, 76% yield).^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.72-1.74 (m, 2H), 1.94-1.99 (m, 2H), 2.76-2.82 (m, 5H), 6.56 (s, br, 1H), 6.39 (d, *J* = 8.00 Hz, 2H), 6.93 (s, 1H), 7.31 (d, *J* = 8.00 Hz, 2H), 8.3 (s, 1H). ms: m/z 323 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ 162.46, 160,73, 154.67, 143.24, 127.49, 121.61, 114.72, 49.94, 43.45, 29.87. Anal.Calcd. For $C_{15}H_{16}Cl_2N_4$: C, 55.74; H, 4.99; N, 17.33. Found: C, 55.96; H, 4.89; N, 17.46.

Synthesis and characterization of final molecules ***(5a-5j):***

6-{4-[(4-Chlorophenyl)amino]piperidin-1-yl}-N-(propan-2-yl)pyrimidin-4-amine ***(5a):***

To a stirred solution of *N*-(4-chlorophenyl)-1-(6-chloropyrimidin-4-yl)piperidin-4-amine (0.1 g, 0.303 mmol) in n-butanol (2 mL) was added DIPEA (0.19 mL, 1.55 mmol) followed by isopropylamine (0.092 g, 1.55 mmol) and heated to 140 ^{o}C for 14 h[19]. Reaction mixture was cooled to RT concentrated under reduced pressure; to this residue water (5 mL) was added and extracted with ethyl acetate (2 x 10 mL). The combined organic layer was dried over sodium sulfate and concentrated under reduced pressure to get crude was purified by flash column chromatography by using silica gel (eluted with 60-70% Ethylacetate:Hexane) to afford 6-{4-[(4-chlorophenyl)amino]piperidin-1-yl}-*N*-cyclopropylpyrimidin-4-amine (35 mg, 0.102 mmol, 33% yield) as brown solid, mp = 296-298 ^{o}C, ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.10 (d, *J* = 6.40 Hz, 6H), 1.25-1.30 (m, 2H), 1.85 (d, *J* = 10.00 Hz, 2H), 2.94-3.00 (m, 2H), 3.46 (t, *J* = 8.00 Hz, 1H), 3.95 (d, *J* = 6.80 Hz, 1H), 4.12 (d, *J* = 13.20 Hz, 2H), 5.60 (s, 1H), 5.67 (d, *J* = 8.00 Hz, 1H), 6.45 (d, *J* = 8.00 Hz, 1H), 6.58-6.61 (m, 2H), 7.05-7.08 (m, 2H), 7.98 (s, 1H). ms: m/z 346 (M+1). ^{13}C NMR (100 MHz, DMSO-d_6): δ162.35, 161.27, 157.62, 147.29, 129.32, 119.34, 114.63, 81.71, 49.82, 43.28, 39.51, 31.62, 22.92.Anal.Calcd. For $C_{18}H_{24}ClN_5$: C, 62.51; H, 6.99; N, 20.25. Found: C, 62.67; H, 6.73; N, 20.12.

6-(4-(4-Chlorophenylamino)piperidin-1-yl)-N-sec-butylpyrimidin-4-amine ***(5b):***

Brown thick mass, 49% yield. ^{1}H-NMR (300 MHz, DMSO-d_6):δ 0.88 (d, *J* = 6.00 Hz, 6H), 1.24-1.37 (m, 2H), 1.73-1.82 (m, 1H), 1.90 (d, *J* = 9.90 Hz, 2H), 2.93-3.03 (m, 4H), 3.41-3.48 (m, 1H), 4.13 (d, *J* = 12.90 Hz, 2H), 5.63 (s, 1H), 5.69 (d, *J* = 8.10 Hz, 1H), 6.60 (d, *J* = 9.00 Hz, 2H), 6.67 (t, *J* = 5.40 Hz, 1H), 7.07 (d, *J* = 8.70 Hz, 2H), 7.97 (s, 1H). ms: m/z 360 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ162.41, 161.34, 157.79, 147.42, 129.53, 119.51, 114.78, 81.92, 49.95, 47.35, 43.48, 31.73, 21.47, 10.41. Anal.Calcd. For $C_{19}H_{26}ClN_5$: C, 63.41; H, 7.28; N, 19.46. Found: C, 63.62; H, 7.16; N, 19.34.

6-{4-[(4-Chlorophenyl)amino]piperidin-1-yl}-N-cyclopropylpyrimidin-4-amine ***(5c):***

Brown thick mass**,** 47% yield. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 0.35-0.42 (m, 2H), 0.54-0.61 (m, 2H), 1.26-1.29 (m, 2H), 1.91-1.95 (m, 2H), 2.45-2.50 (m, 1H), 3.01-3.32 (m, 2H), 3.47-3.52 (m, 1H), 4.18 (d, *J* = 13.80 Hz, 2H), 5.70 (d, *J* = 8.10 Hz, 1H), 5.77 (s, 1H), 6.60 (d, *J* = 7.20 Hz, 2H), 6.94 (d, *J* = 2.10 Hz, 1H), 7.07 (d, *J* = 8.70 Hz, 2H), 7.97 (s, 1H). ms: m/z 344 (M+1). ^{13}C NMR (100 MHz, DMSO-d_6): δ162.11, 161.18, 157.53, 147.21, 129.22, 119.29, 114.54, 81.66, 49.69, 43.15, 31.41, 25.62, 9.47.Anal.Calcd. For $C_{18}H_{22}ClN_5$: C, 62.87; H, 6.45; N, 20.37. Found: C, 63.45; H, 6.32; N, 20.23.

6-{4-[(4-Chlorophenyl)amino]piperidin-1-yl}-N-cyclobutylpyrimidin-4-amine ***(5d):***

Brown semi solid,45 % yield. ^{1}H-NMR (400 MHz, DMSO-d_6):δ 1.27-1.30 (m, 2H), 1.60-1.67 (m, 2H), 1.83-1.92 (m, 4H), 2.21-2.27 (m, 2H), 2.94-3.00 (m, 2H), 3.45-3.47 (m, 1H), 4.14 (m, 3H), 5.54 (s, 1H), 5.67 (d, *J* = 8.00 Hz, 1H), 6.59-6.61 (m, 2H), 6.89 (d, *J* = 7.20 Hz, 1H), 7.06-7.09 (m, 2H), 7.96 (s, 1H). ms: m/z 358 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ162.27, 161.29, 157.66, 147.37, 129.41, 119.34, 114.63, 81.59, 49.74, 49.87, 43.37, 31.56, 29.86, 15.43.Anal.Calcd. For $C_{19}H_{24}ClN_5$: C, 63.77; H, 6.76; N, 19.57. Found: C, 63.52; H, 6.68; N, 19.47.

N-(4-chlorophenyl)-1-[6-(pyrrolidin-1-yl)pyrimidin-4-yl]piperidin-4-amine ***(5e):***

Yellow thick mass, 55% yield. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.22-1.32 (m, 2H), 1.88-1.92 (m, 6H), 2.96-3.03 (m, 2H), 3.43-3.50 (m, 5H), 4.22 (d, *J* = 13.60 Hz, 2H), 5.55 (s, 1H), 5.68 (d, *J* = 8.00 Hz, 1H), 6.58-6.62 (m, 2H), 7.05-7.09 (m, 2H), 8.02 (d, *J* = 0.80 Hz, 1H). ms: m/z 358 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ162.52, 161.16, 157.32, 147.42, 129.31, 119.28, 114.47, 81.74, 49.74, 46.69, 43.52, 32.14. Anal.Calcd. For $C_{19}H_{24}ClN_5$: C, 63.77; H, 6.76; N, 19.57. Found: C, 63.93; H, 6.86; N, 19.41.

N-(4-chlorophenyl)-1-[6-(piperidin-1-yl)pyrimidin-4-yl]piperidin-4-amine ***(5f):***

Brown thick mass, 58% yield. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.23-1.31 (m, 2H), 1.45-1.51 (m, 4H), 1.57-1.61 (m, 2H), 1.89-1.92 (m, 2H), 2.96-3.03 (m, 2H), 3.46-3.54 (m, 5H), 4.25(d, *J* = 13.60, 2H), 5.68 (d, *J* = 8.40 Hz, 1H), 5.89 (s, 1H), 6.59-6.61 (m, 2H), 7.06-7.08 (m, 2H), 8.04 (s, 1H). ms: m/z 372 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ162.29, 161.21, 157.48, 147.14, 129.09, 119.06, 114.25, 81.51, 49.51, 46.41, 43.15, 31.41, 25.26.Anal.Calcd. For $C_{20}H_{26}ClN_5$: C, 64.59; H, 7.05; N, 18.83. Found: C, 64.71; H, 7.13; N, 18.94.

N-(4-Chlorophenyl)-1-(6-morpholinopyrimidin-4-yl)piperidin-4-amine ***(5g):***

Yellow semi solid, 44% yield. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.21-1.31 (m, 2H), 1.91 (d, *J* = 11.10 Hz, 2H), 3.02 (t, *J* = 12.00 Hz, 2H), 3.49-3.64 (m, 9H), 4.27 (d, *J* = 13.80 Hz, 2H), 5.69 (d, *J* = 8.10 Hz, 1H), 5.94 (s, 1H), 6.60 (d, *J* = 8.40 Hz, 2H), 7.07 (d, *J* = 8.40 Hz, 2H), 8.08 (s, 1H). ms: m/z 374 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ162.34, 161.62, 157.58, 147.36, 129.46, 119.11, 114.39, 81.63, 64.57, 49.49, 45.74, 43.29, 31.67. Anal.Calcd. For C19H24ClN5O: C, 61.04; H, 6.47; N, 18.73. Found: C, 61.24; H, 6.62; N, 18.81.

N-Benzyl-6-(4-(4-chlorophenylamino)piperidin-1-yl)pyrimidin-4-amine ***(5h):***

Brown semi solid, 43% yield. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.18-1.29 (m, 2H), 1.89 (d, *J* = 10.50 Hz, 2H), 2.96 (t, *J* = 11.40 Hz, 2H), 3.44-3.50 (m, 1H), 4.11 (d, *J* = 13.20 Hz, 2H), 4.44 (d, *J* = 6.30 Hz, 2H), 5.68 (d, *J* = 6.60 Hz, 2H), 6.59 (d, *J* = 9.00 Hz, 2H), 7.07 (d, *J* = 8.70 Hz, 2H), 7.17-7.31 (m, 6H), 7.99 (s, 1H). ms: m/z 394 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ163.72, 162.22, 157.86, 147.13, 140.69, 129.09, 128.69, 127.61, 127.07, 119.07, 114.27, 49.43, 44.11, 43.05, 31.31.Anal.Calcd. For C22H24ClN5: C, 67.08; H, 6.14; N, 17.78. Found: C, 67.23; H, 6.31; N, 17.86.

6-(4-(4-Chlorophenylamino)piperidin-1-yl)-N-(4-fluorobenzyl)pyrimidin-4-amine ***(5i):***

Brown semi solid, 44% yield. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.19-1.29 (m, 2H), 1.90 (d, *J* = 8.70 Hz, 2H), 2.97 (t, *J* = 11.10 Hz, 2H), 3.45 (t, *J* = 8.10 Hz, 1H), 4.11 (d, *J* = 13.50 Hz, 2H), 4.42 (d, *J* = 6.30 Hz, 2H), 5.68 (d, *J* = 8.10 Hz, 2H), 6.59 (d, *J* = 9.00 Hz, 2H), 7.05-7.23 (m, 5H), 7.31-7.36 (m, 2H), 7.99 (s, 1H). ms: m/z 412 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ163.62, 162.74, 162.22, 160.33, 157.87, 147.12, 136.86, 129.56, 129.48, 129.09, 119.07, 115.49, 115.28, 114.27, 88.65, 49.42, 43.36, 43.04, 31.32. Anal.Calcd. For $C_{22}H_{23}ClFN_5$: C, 64.15; H, 5.63; N, 17.00. Found: C, 64.27; H, 5.72; N, 17.13.

6-(4-(4-Chlorophenylamino)piperidin-1-yl)-N-(4-methoxybenzyl)pyrimidin-4-amine ***(5j):***

Brown semi solid, 44% yield. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.19-1.29 (m, 2H), 1.84-1.91 (m, 2H), 2.96 (t, *J* = 11.40 Hz, 2H), 3.45-3-47 (m, 1H), 3.71 (s, 3H), 4.11 (d, *J* = 13.20 Hz, 2H), 4.36 (d, *J* = 6.00 Hz, 2H), 5.65-5.69 (m, 2H), 6.59 (d, *J* = 9.00 Hz, 2H), 6.86 (d, *J* = 8.70 Hz, 2H), 7.05-7.11 (m, 3H), 7.23 (d, *J* = 8.70 Hz, 2H), 7.99 (s, 1H). ms: m/z 424 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ163.78, 162.81, 162.42, 160.41, 155.26, 147.62, 136.73, 129.72, 129.56, 129.33, 119.24, 115.82, 115.63, 114.73, 88.47, 58.43, 49.36, 43.45, 43.13, 31.69. Anal.Calcd. For $C_{23}H_{26}ClN_5O$: C, 65.16; H, 6.18; N, 16.52. Found: C, 65.09; H, 6.07; N, 16.46.

Synthesis and characterization of final molecules ***(5k-5m):***

6-(4-(4-Chlorophenylamino)piperidin-1-yl)-N-phenylpyrimidin-4-amine ***(5k):***

To a stirred solution of N-(4-chlorophenyl)-1-(6-chloropyrimidin-4-yl)piperidin-4-amine (0.1 g, 0.309 mmol) in DMF (2 mL) was added p-TSA (0.106 g, 0.618 mmol) followed by aniline (0.086 g, 0.928 mmol) and heated to 140 ^{o}C in micro wave for 1.5 h[20]. Reaction mixture was diluted with ethyl acetate (20 mL) and washed with sodiumbicarbonate (10%) solution (2 x 15 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure to get crude and was purified by flash column chromatography using silica gel column (eluted with 70% Ethylacetate:Hexane) to afford 6-(4-(4-Chlorophenylamino)piperidin-1-yl)-*N*-phenylpyrimidin-4-amine (40 mg, 0.105 mmol, 34% yield) as brown semi solid.^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.24-1.36 (m, 2H), 1.94 (d, *J* = 10.50 Hz, 2H), 3.06 (t, *J* = 11.10 Hz, 2H), 3.45-3.55 (m, 1H), 4.15 (d, *J* = 13.50 Hz, 2H), 5.71 (d, *J* = 8.40 Hz, 1H), 6.00 (s, 1H), 6.61 (d, *J* = 9.00 Hz, 2H), 6.93 (t, *J* = 7.40 Hz, 1H), 7.08 (d, *J* = 8.70 Hz, 2H), 7.26 (t, *J* = 8.10 Hz, 2H), 7.58 (d, *J* = 7.50 Hz, 2H), 8.19 (s, 1H), 9.02 (s, 1H). ms: m/z 380 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ162.31, 161.55, 157.92, 147.12, 141.29, 129.12, 121.81, 119.12, 114.29, 84.53, 49.36, 43.07, 31.30. Anal.Calcd. For $C_{21}H_{22}ClN_5$: C, 66.39; H, 5.84; N, 18.44. Found: C, 66.51; H, 5.97; N, 18.58.

6-(4-(4-Chlorophenylamino)piperidin-1-yl)-N-(4-fluorophenyl)phenylpyrimidin-4-amine ***(5l):***

Brown thick mass, 36% yield, ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.24-1.34 (m, 2H), 1.92-1.99 (m, 2H), 2.99-3.09 (m, 2H), 3.49-3.52 (m, 1H), 4.15 (d, *J* = 13.50 Hz, 2H), 5,71 (d, *J* = 8.40 Hz, 1H), 5.94 (s, 1H), 6.61 (d, *J* = 9.00 Hz, 2H), 7.06-7.13 (m, 4H), 7.56-7.60 (m, 2H), 8.18 (s, 1H), 9.04 (s, 1H). ms: m/z 398 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ162.74, 161.72, 160.74, 158.12, 147.31, 141.17, 129.37, 121.64, 119.76, 119.25, 114.72, 84.82, 49.19, 43.23, 31.42.Anal.Calcd. For $C_{21}H_{21}ClFN_5$: C, 63.39; H, 5.32; N, 17.60. Found: C, 63.26; H, 5.21; N, 17.56.

6-(4-(4-Chlorophenylamino)piperidin-1-yl)-N-(4-methoxyphenyl)phenylpyrimidin-4-amine ***(5m):***

Brown semi solid, 41% yield, ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.15-1.33 (m, 2H), 1.91-1.99 (m, 2H), 2.99-3.06 (m, 2H), 3.47-3.50 (m, 1H), 3.72 (s, 3H), 4.04-4.14 (m, 2H), 5.70 (d, *J* = 8.10 Hz, 1H), 5.87 (s, 1H), 6.60 (d, *J* = 8.70 Hz, 2H), 6.83 (d, *J* = 8.70 Hz, 2H),7.07 (d, *J* = 8.70 Hz, 2H), 7.42 (d, *J* = 8.70 Hz, 2H), 8.13 (s, 1H), 8.80 (s, 1H). ms: m/z 410 (M+1).^{13}C NMR (100 MHz, DMSO-d_6): δ162.21,161.05, 152.17, 157.41, 147.18, 139.72, 129.43, 121.47, 118.92, 115.34, 114.39, 84.92, 54.39, 49.24, 43.37, 31.38. Anal.Calcd. For $C_{22}H_{24}ClN_5O$: C, 64.46; H, 5.90; N, 17.09. Found: C, 64.62; H, 5.98; N, 17.21.

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