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Efficient Synthesis, Spectral Analysis, Antimicrobial Studies and Molecular Docking Studies of Some Novel 2-Aminopyrimidine Derivatives

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Abstract

Background/Objectives: An important aspect of medicinal chemistry has been to establish a relationship between chemical structure and pharmacological activity. Nitrogen containing heterocycles widely used as key building blocks for pharmaceutical agents. **Method/Statistical analysis:** 2,4,6-trisubstituted aminopyrimidine derivatives (9–16) have been synthesized by the cyclization of 3-phenyl-1-(pyridin-2-yl)prop-2-en-1-one (1–8) with guanidine nitrate in ethanolic NaOH solution and were characterized by elemental analysis, IR, 1H NMR and 13C NMR spectral analysis. All the synthesized compounds were screened for antibacterial and antifungal activities using serial dilution method. **Findings:** The microbiological analysis showed that the electron withdrawing function substituted phenyl group at C-4 exposed significant antimicrobial activity *against S .aureus, V. cholerae, S. typhi, K. pneumoniae, A. flavus, C. albicans, Mucor and Candida 6* at MIC of 6.25 µg/ml. *In silico* docking studies indicate that *minor pseudopilin EpsH* from *Vibrio cholera* (PDB 2QV8) and *M. tuberculosis DHFR* (PDB IDF7) is the possible target of these compounds.

Keywords: Antimicrobial Activity, Chalcones, Molecular Docking, 2-aminopyrimidine, DHFR

1. Introduction

Nitrogen containing heterocyclic compounds have been received considerable attention due to their wide range of chemical and biological significance to medicinal chemistry[1–4]. Pyrimidines showed application in agricultural and industrial chemicals. Some of the pyrimidine derivatives are pesticides[5], herbicides and plant magnification regulators[6].

Aminopyrimidine represent one of the most biologically active classes of compounds, possessing a wide spectrum of pharmacological and biological activities such as anticancer[7,8], antiviral[9], antibacterial[10], antifungal[11], antiprotozoal[12,13], antihypertensive[14], anti-inflammatory[15], central nervous activities[16], antitumour agents[17], analgesic activities[18]. Many drugs containing aminopyrimidine nucleus are available in the market as Sulfadiazine, Glivec and Rosuvastatin[19,20]. The necessity to design new compounds to overcome this resistance has become one of the most important areas of research today.

The aim of this work is to investigate the antibacterial and antifungal activities of the target compounds by the modification of phenyl group substituents in pyrimidine ring. The synthesized compounds (9–16) are characterized by analytical and spectral techniques.

The reaction pathways of different aminopyrimidine modifications are sketched in scheme 1. The 3-aryl-1-(pyridin-2-yl) prop-2-en-1-ones (1–8) were prepared according to the precedent literature by the condensation of 2-acetylpyridine, benzaldehyde and NaOH in ethanol (1:1). In the present work, the key intermediate (E)-3-phenyl-1-(pyridin-2-yl)prop-2-en-1-ones (1–8) react with guanidine nitrate in ethanolic solution of NaOH under reflux condition (60°C) afforded the corresponding cyclised aminopyrimidine derivatives (9–16). Then the synthesized compounds were purified by column chromatography using benzene - ethyl acetate (2:1) as eluent on neutral alumina.

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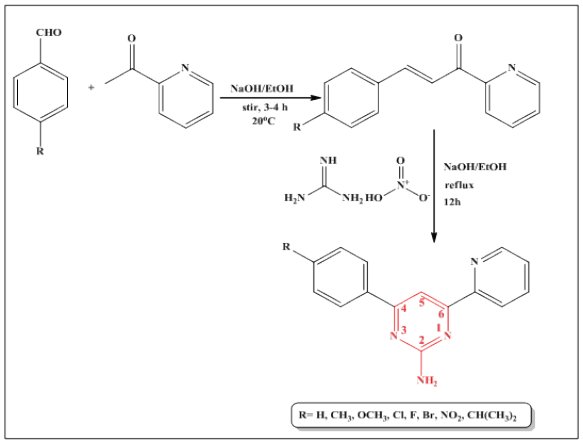

4-phenyl-6-(pyridin-2-yl)pyrimidin-2-amine(9)
4-(pyridin-2-yl)-6-(p-tolyl)pyrimidin-2-amine (10)
4-(4-methoxyphenyl)-6-(pyridin-2-yl)pyrimidin-2-amine (11)
4-(4-chlorophenyl)-6-(pyridin-2-yl)pyrimidin-2-amine (12)
4-(4-fluorophenyl)-6-(pyridin-2-yl)pyrimidin-2-amine (13)
4-(4-bromophenyl)-6-(pyridin-2-yl)pyrimidin-2-amine (14)
4-(4-nitrophenyl)-6-(pyridin-2-yl)pyrimidin-2-amine (15)
4-(4-isopropylphenyl)-6-(pyridin-2-yl)pyrimidin-2-amine (16)

2. Results and Discussion

IR spectra of 2,4,6-trisubstituted aminopyrimidines (9–16) in Figure 1 showed weak absorption band in the region of 3057– 2923 cm^{-1} and a sharp and intense absorption band around 3337 cm^{-1} which is due to aromatic C-H stretching. and NH stretching frequency. The compounds in the present series also exhibit similar absorptions around 1644 cm^{-1} and 1539 cm^{-1} are characteristic for C = N and N-C bond stretching vibrations. In addition, the appearance of N-C-N bending vibration around 1359 cm^{-1} is the confirmatory evidence for six membered ring closures. Figure 2 shows mass spectrum of compound 9 was correlation with proposed molecular structure of compound 9.

In order to assign the ring proton and carbon signals, compound 9 has been chosen as representative compound. H NMR[1] spectrum of (9) in Figure 3 gives a singlet at 5.17 ppm with two protons integral which is assignable to amine protons. A multiplet in the region of 7.40–8.73 ppm is assigned to aromatic protons of (9). The aminopyrimidine (9) formation was confirmed by the presence of carbon signals at 165.01, 163.47, 104.09 ppm which are assigned to C-4, C-2 and C-5 respectively. The aromatic carbon signals appeared in the region of 121.65–131.91 ppm showed in Figure 4.

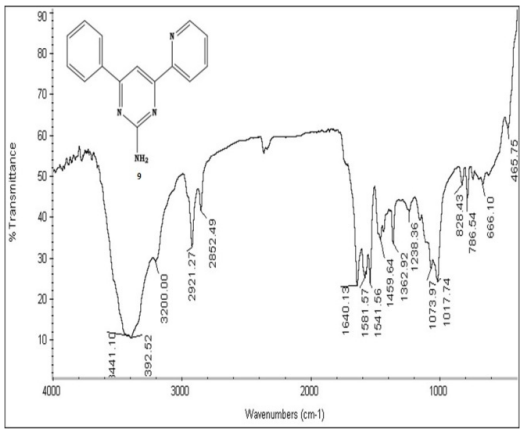

Figure 1. FT-IR spectrum of compound 9.

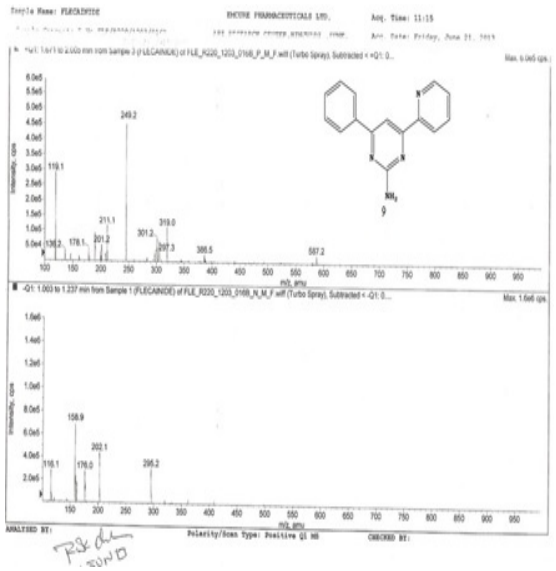

Figure 2. Mass spectrum of compound 9.

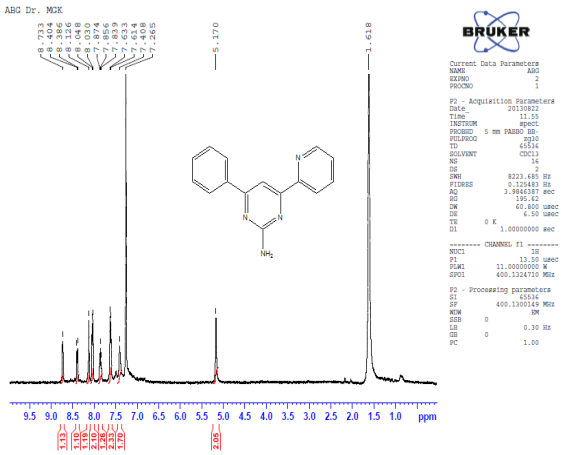

Figure 3. [1]H NMR spectrum of compound 9.

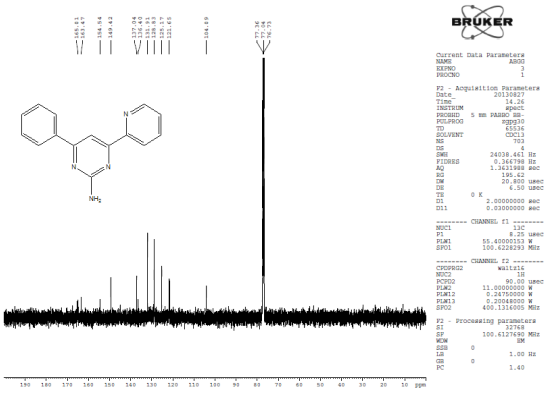

Figure 4. ^{13}C NMR spectrum of compound 9.

3. Antimicrobial Activity

All the synthesized compounds were screened for their antibacterial efficacy *in vitro* against a spectrum of Gram-positive pathogenic bacteria including resistant strains viz. Ciprofloxacin-resistant and sensitive *S. aureus, B. subtilis, S. typhi, E. coli, V. cholerae* and *K. pneumonia* using the literature precedent by Dhar et al., and their MIC values are depicted in Table 2. A glance at the MIC values in Table 1 indicates that among the 2-amino pyrimidine derivatives (9–16), compounds without substitution at C–4 phenyl (compound 9) showed moderate activity against all the bacterial strains which were used for this study. Among the compounds (9–16), the *para* substituted methyl, methoxy, isopropyl analogue compounds (10, 11 and 16) exerted good antibacterial activity (6.25 µg/mL) against *K. Pneumonia*, but the halogen substituted compounds (12, 13, 14 and 15) exhibited two, three fold decreased activity against the same strain. However, the replacement of methyl analogue by methoxy analogue in compound 10 (11) registered one fold decreased activity against *S. typhi, V. cholerea and E. coli*.

The introduction of chloro analogue in compound 9 (12) showed maximum inhibition activity against *S. typhi, S. aureus and V. cholerae,* registered excellent antibacterial activity (MIC at 6.25–12.5 µg/mL). The replacement of chlorine in compound 12 by fluorine, bromine analogues (compound 13, 14) showed better antibacterial activity against all the strains but compound 13 against *S. aureus, B. subtilis and E. coli* and compound 14 against *S. typhi, V. Cholerae and E. coli* registered maximum activity (MIC at 6.25–12.5 µg/mL).

The MIC values of compound 15 showed maximum inhibition activity (12.5 µg/mL) against *S. aureus, S. typhi and E. coli.* Among the various substituted compounds,

Table 1. Antibacterial activity of compounds 9–16 against some bacterial strains (MIC in µg/mL)

Compounds	Minimum inhibitory concentration (MIC) in µg/ml					
	S. aureus	B. Subtils	S. typhi	V. cholerae	E.coli	K. pneumonia
9	50	50	100	100	50	25
10	200	200	100	50	50	6.25
11	100	100	200	100	100	6.25
12	6.25	6.25	12.5	6.25	50	25
13	6.25	6.25	50	25	6.25	25
14	6.25	6.25	6.25	12.5	12.5	200
15	12.5	12.5	12.5	25	12.5	25
16	50	50	50	100	50	6.25
Ciprofloxacin	25	12.5	12.5	25	25	12.5

Table 2. Antifungal activity of compounds 9–16 against some fungal strains (MIC in µg/mL)

Compounds	Minimum inhibitory concentration (MIC) in µg/ml					
	A. flavus	A. niger	C. albicans	Mucor	Candida 6	Rhizopus
9	50	200	6.25	100	6.25	100
10	50	50	100	100	200	12.5
11	100	12.5	200	200	50	100
12	6.25	25	25	6.25	6.25	25
13	12.5	25	6.25	25	6.25	50
14	200	100	200	100	6.25	100
15	12.5	25	25	100	25	25
16	50	50	100	100	50	12.5
Fluconazole	12.5	12.5	25	25	25	25

compound 10 against *S. aureus, B. subtilis,* compound 11 against *S. typhi,* did not show any activity even at maximum concentration (200 µg/mL). In a overall view, halogen substituted compounds showed better antibacterial activity whose inhibitory potency falls in the order 13 > 14 > 12.

In order to extend the antimicrobial evaluation, the synthesized compounds (9–16) were also screened for *in vitro* antifungal activity with five fungal strains viz. *A. flavus, A. niger, C. albicans, Mucor, Candida 6* and *Rhizopus.* Here, Fluconazole was used as standard drug. The obtained MIC values are depicted in Table 2. Unsubstituted phenyl groups in compound 9 recorded minimum to moderate

activity (100–200 µg/mL) against all the tested organisms except *Candida* and *Candida 6*, for which inhibition potency was shown at minimum concentration at 6.25 µg/mL. However, the introduction of chlorine functionality at *para* position of phenyl groups in compound 9 (12) registered moderate inhibition potency against all the tested fungal organisms with MIC ranging from 6.25 – 50 µg/mL. Besides, compound 12 against *A. flavus, Mucor and Candida 6* showed superior inhibition potency at minimum concentration (6.25 µg/mL). By the substitution of fluorine in compound 12 (13) exhibited well pronounced activity against *C. albicans* and *Candida 6* (MIC at 6.25 – 12.5 µg/mL), but the replacement of fluorine by bromine analogue (Compound 14) showed minimum activity against all the tested fungal strains except *Candida 6*.

Instead of halogens, the nitro substituted compound (15) showed maximum antifungal potency against *A. flavus*. A modification of *para* proton by methyl, methoxy and isopropyl group in compound 9 (compounds 10, 11 and 16) showed moderate activity against the entire tested fungal strains but registered high inhibition against *Rhizopus and A. niger* respectively. Among the compounds under the antifungal study compound 9 against *A. niger* compound 11 against *C. albicans* and *Mucor* compound 14 against *A. flavus* and *C. albicans*, seldom showed inhibition even at maximum concentration (200 µg/mL).

4. Molecular Docking Studies

Molecular docking study is a well-established technique to determine the interaction of two molecules and find the best orientation of ligand would form a complex with overall minimum energy. All the newly synthesized compounds (9–16) were docked with *minor pseudopilin EpsH* from the Type 2 secretion system of *V. cholera* at ten different orientations. The protein structure file (PDB ID: 2QV8) taken from PDB (www.rcsb.org/pdb) and the ligands molecules were drawn and analysed using Chem Draw Ultra 8.0. 3D, coordinates were prepared using docking server. The acting force of this binding mode is mainly hydrogen bonding, electrostatic forces, van-der waals forces and hydrophobic interaction due to non-polar residue interaction and water structure effect alteration. Based on the *in vitro* antimicrobial studies, it is worthwhile to do *in silico* studies; it supports the *in vitro* activity. The best orientation of hydrogen bonds and hydrophobic interaction of docked molecules are given in the Table 3. The binding energy and docking energy of the docked ligand molecules ranging from –6.20 to –6.73 kcal/mol and –6.73 to –7.09 kcal/mol. The docking results revealed that compounds 12, 13 and 14 showed minimum binding and docking energies –6.73, –6.28, –6.47 kcal/mol is due to dipole–dipole and hydrogen bond interaction with amino acids of targeted protein.

Table 3. Molecular docking results of the target molecules with *minor pseudopilin EpsH* from *Vibrio cholera* (PDB ID 2QV8)

Compound	Binding Energy	Docking Energy	Inhibition Constant	Intermol Energy	Residues involving interactions
9	–6.49	–6.98	17.52	–7.06	GLN41, ARG42, LEU45, LEU46, PHE70, TRP82,
10	–6.37	–6.87	21.54	–6.93	ARG42, LEU45, PHE70, TRP82,
11	–6.30	–6.91	23.97	–7.17	GLN41, ARG42, LEU45, PHE70, TRP82,
12	–6.73	–7.08	26.94	–7.30	GLN41, ARG42, LEU45, LEU46, PHE70, TRP82,
13	–6.28	–6.76	25.13	–6.85	GLN41, ARG42, LEU45, PHE70, TRP82, ASN84
14	–6.47	–6.96	18.02	–7.04	GLN41, ARG42, LEU45, PHE70,
15	–6.50	–7.20	17.0	–7.33	LEU45, PHE70, TRP82, ASN84, THR88
16	–6.37	–7.09	21.52	–7.24	GLN41, ARG42, LEU45, LEU46, PHE70,

The *in vitro* antimicrobial MIC values are correlated well with binding energies and docking energies obtained through molecular modeling with *minor pseudopilin EpsH* from the Type 2 secretion system of *V. cholera*. Docked ligand molecule 13 with the secondary structure of *M. tuberculosis DHFR* in solid and ribbon model is depicted in Figure 5. The minimum bacterial inhibition potency *V. cholera* of compounds 12 (6.25 µg/ml), 13 (25 µg/ml) and 14 (12.5 µg/ml) showed excellent total docking energies. Its total docking energies are –13.81, –13.04, –13.0 kcal/mol respectivly. From the comparative analysis, compound 12 showed minimum docking energy with targeted protein and the *in vitro* studies also support compound 12 has emerged active against all the tested microorganisms. So, we suggested compound 12 with chlorine substituted phenyl group at the pyrimidine ring to exhibit better inhibition.

The most potent compounds from docking studies compounds 12, 13 and 14 were further analyzed for their interaction with *M. tuberculosis DHFR* (PDB IDF7) *in silico*. The above mentioned compounds utilize their amino head group to interact with the crucial amino acid residues such as LEU65, SER66, ILE14, ASP19, THR46, ILE94, SER49 through hydrogen bonds. Docking score of ligand molecules 12, 13 and 14 with minimum binding energy –7.91, –7.53, –7.56 kcal/mol respectively. From the resulting compound 12 showed the minimum binding energy which may be a potential lead as *M. tuberculosis DHFR*. Compound 13, 14 showed the comparable docking energy with compound 12. The active binding sites NAP, MTX were occupied with target ligand molecules at active site of the protein. The surface cavity with target molecule 13 at the active pocket of the protein structure is depicted in Figure 6. Interestingly, the compound 12 showed minimum binding energy but number of hydrogen bond is quite low compared with compound 13.

Table 4 showed counting of hydrogen bond, hydrogen bonding and hydrophobic interacting residues of modeled *M. tuberculosis DHFR* (PDB ID 1DF7) and ligand molecules 12, 13, 14 based on this, the fluorinated pyrimidine compound was most potential lead molecule against *M. tuberculosis*. 2D plot of hydrogen bond forming amino acids with target ligand and HB plot of interacted residues in protein of *M. tuberculosis* with compound 13 is depicted in Figure 7. Finally, we conclude that these synthesized compounds of aminopyrimidine can act as a good antitubercular agent. Therefore, it is pleasing to state that the docking studies have widened the scope of developing a new class of antitubercular agents.

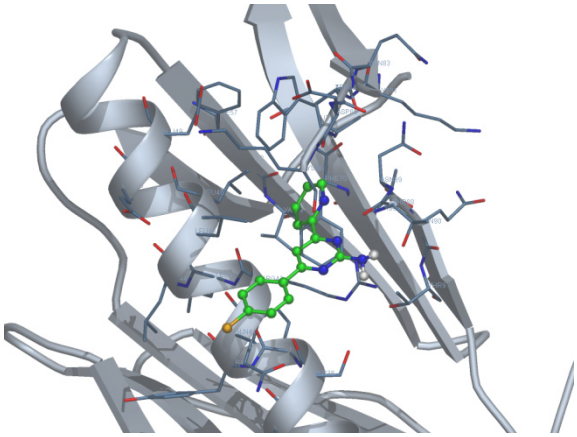

Figure 5. Docked ligand molecule 13 with the secondary structure of *M. tuberculosis* DHFR in solid and ribbon model.

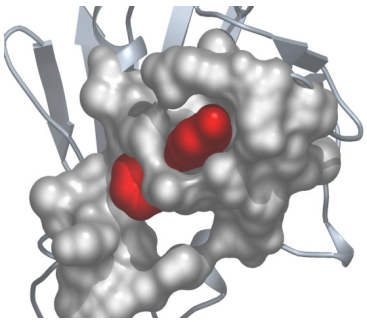

Figure 6. The surface cavity with target molecule 13 at the active pocket of the protein.

Table 4. Docking features (counting of hydrogen bond, hydrogen bonding and hydrophobic interacting residues of modeled M. tuberculosis DHFR and ligand molecules 12, 13, 14 (PDB ID 1DF7)

Compound	Docking Energy	No. of hydrogen bonds	Interacting residues forming hydrogen bonds
12	–8.41	5	THR46, ASP19, GLY18
13	–8.10	9	LEU65, SER66, ILE14, ASP19, GLN98, THR46, ILE94, SER49
14	–8.14	6	THR46, GLY18, ARG45

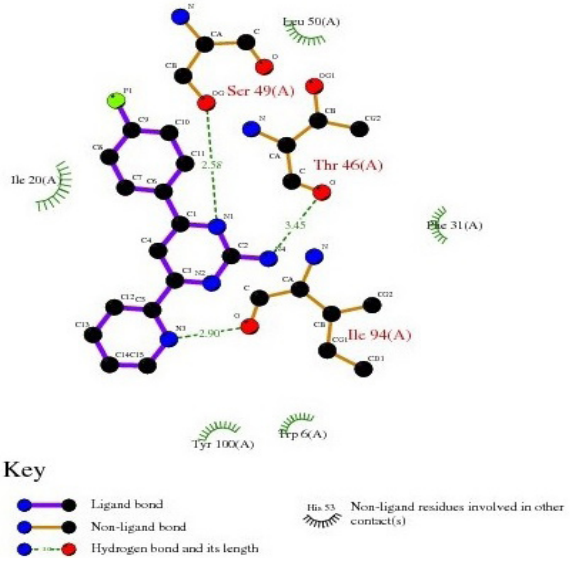

Figure 7. 2D plot of hydrogen bond forming amino acids with target ligand Dashed lines.

5. Conclusion

In conclusion, some biologically potent 2-amino pyrimidine derivatives (9–16) were synthesised from 3-phenyl-1-(pyridin-2-yl) prop-2-en-1-one with guanidine nitrate. Biological potencies of the compounds 12, 13, 14 and 15 showed excellent activity (6.25–12.5 µg/mL) against the microbial strains used. The biological results revealed that halogen substituted compounds showed significant activity than alkyl substituted compounds and it follows the order of activity being: 13>14>12. Hence, enhancement of activity can be ascribed for the synthesised pharmacophores through electron withdrawing effects exerted by the substituents. The docking analysis performed on a series of 4-phenyl-6-(pyridin-2-yl) pyrimidin-2-amine**.** The inhibitory activity depends on the substitution pattern on the aromatic rings. All the synthesized compounds are found to exhibit binding affinity with different parts of nucleosides to define and optimize the 4–(4-chlorophenyl)-6-(pyridin-2-yl)pyrimidin-2-amine as good antitubercular and antibacterial agent and 4-(4-fluorophenyl)-6-(pyridin-2-yl)pyrimidin-2-amine as strong antitubercular agent.

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