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Design, Synthesis and Pharmacological Evaluation of Some Novel Methylpyrimidine Derivatives as an Antimicrobial Agents

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The goal of the current work was to create, describe, and test derivatives of methyl pyrimidines for antibacterial activity. Guanidine hydrochloride has been used to create a new class of 2-amino-4-(substituted phenylamino)-6-methylpyrimidine derivatives. IR, NMR, mass spectra, and elemental analysis were used to characterise the structures of the synthesised compounds. Using ciprofloxacin as a standard for antibacterial activity and clotrimazole as a standard for antifungal activity, all of the prepared derivatives were tested for antibacterial and antifungal activity using the disc diffusion method using nutrient agar media against Bacillus subtilis, Bacillus pumilus, Escherichia coli, and Pseudomonas aeruginosa, and potato dextrose agar medium for activity against Aspergillus niger and Candida albicans. Compounds derived from 5c have demonstrated encouraging action against P. aeruginosa and other gram-ve bacteria. Some compounds have shown moderate antifungal and antibacterial activity.

Keywords: Pyrimidine, Antibacterial activity, Antifungal activity.

1. Introduction

Antibiotic-resistant bacterial strains pose a significant challenge to the treatment of bacterial infections (1); for pathogens like Pseudomonas aeruginosa, the situation can be extremely severe (2, 3, 4). The composition of antibiotics (5) or the use of matching inhibitors (6) to shield them from deactivation enzymes is recognised to be one potential solution to this issue. Another possible tactic to enhance treatment is to choose more stable molecules from the current classes of antibiotics; meropenem (9) and plazomicin (7), dalbavancin, and oritavancin (8) are excellent examples of such molecules.

Making inhibitors of bacterial protein targets is another approach to developing antimicrobial drugs, but it has drawbacks and restrictions (7). It may even lead to the creation of a new family of antibiotics or antibacterial agents. Bacterial TrmD is one of these alluring targets, and it differs significantly from its homolog in eukaryotes and archaea (8). At the ribosomal translation stage of protein synthesis, +1 frameshifting occurs when this enzyme is blocked (9). The production of bacterial membrane proteins, such as efflux drug pumps (8), is adversely affected by disruption of protein synthesis, which in turn causes the outer membrane of Gram-negative bacterial cells to malfunction (10). This can encourage the accumulation of antibiotics and increase their activity. (11)

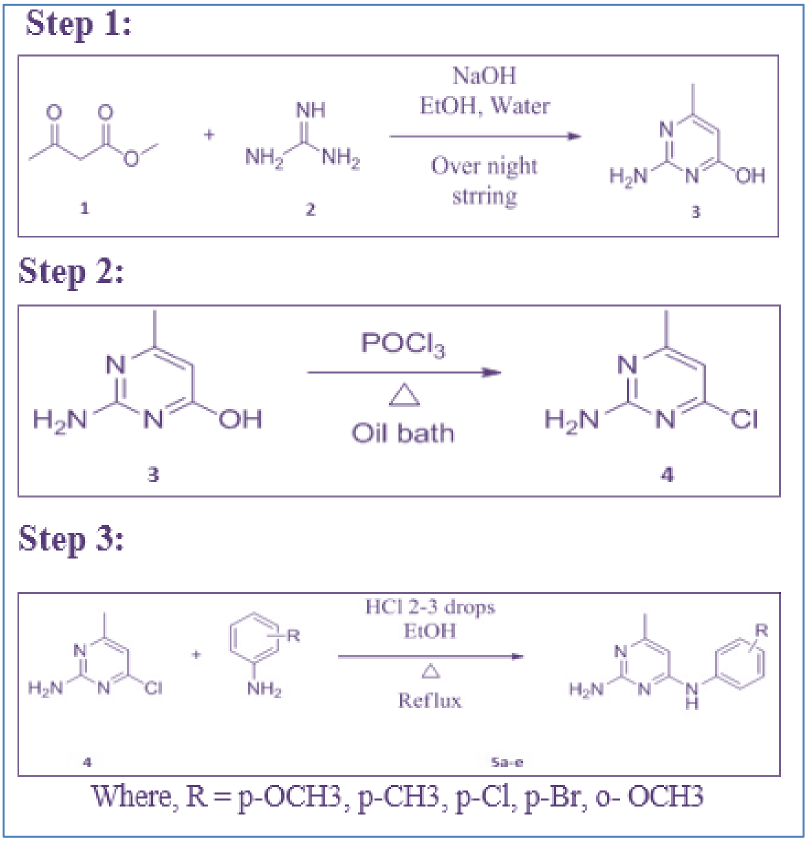
Many compounds of biological or pharmacological relevance contain the pyrimidine ring, an aza-aromatic scaffold. There are many applications for spiro heterocycles in the pharmaceutical sector. One characteristic that sets apart a variety of synthetic and natural substances with remarkable biological activity is their spiro heterocyclic structure. (12–14) Because of their favourable pharmacological properties and geochemistry, spiro heterocycles have been extensively documented to have prospective uses in pharmaceutical chemistry. But because of their many chemical and biological applications, pyrimidines and compounds related to them are a significant family of heterocycles. Both material science and medicine make extensive use of them. They have anti-inflammatory (15), antipyretic (16), antihypotensive, anticonvulsant, antiviral, antibacterial, and antidiabetic (18, 19) pharmacological activities. We have already created and manufactured a few unique antioxidant and anti-inflammatory compounds. (20) Our study's primary goal was to carry on with this strategy by creating and describing a fresh batch of heterocyclic compounds for antibacterial drugs.

2. Materials and Methods

Chemicals and Instruments: All compounds' melting points were measured in uncorrected open glass capillaries. Microscopic slides coated with Silica Gel-GF were used for thin-layer chromatography of the synthesised chemicals. Spots were visible when exposed to iodine vapour and UV light. All compounds' UV spectra were captured using the Shimadzu UV-Visible spectrophotometer UV-160A. Using KBr as an internal reference, the FT-IR 8400S Shimadzu spectrophotometer was used to record the IR spectra of the compounds. The LCMS

2010EV Shimadzu Mass Spectrometer was used to record the mass spectra of the substances. Chemical shifts were determined as parts per million (δ ppm) downfield from tetramethylsilane as an internal reference after 1H NMR spectra were acquired in CDCl3 using a Bruker Advance-II 400 MHz instrument. 13C NMR spectra of all synthesized compounds were taken in DMSO using Bruker Advance-II 400 MHz instrument, and chemical shifts were measured as parts per million (δ ppm).

Synthesis and Scheme: 2-amino-4-hydroxy-6-methylpyrimidine 3 was produced by treating methyl acetoacetate 1 with guanidine hydrochloride 2, which had first been neutralised with sodium hydroxide. After refluxing compound 3 with phosphorous oxychloride, 2-amino-4-chloro-6-methyl-pyri-midine 4 was produced. 2-amino-4-(substituted phenylamino) is produced by refluxing compound 4 with various reagents, such as p-anisidine, p-toluene, p-chloro-aniline, p-bromoaniline, and o-anisidine, in the presence of ethanol and hydrochloric acid.2-amino-4-(4-methoxyphenyl-amino)-6-methylpyrimidine 5a and 2-amino-4- (4-methyl phenylamino) are examples of 6-methyl-pyrimidine derivatives.2-amino-4-(4-chloro phenyl amino)-6-methylpyrimidine 5c, 2-amino-4- (2-methoxy-phenylamino)-6-methylpyrimidine 5e, 2-amino-4-(4-bromo phenylamino)-6-methylpyrimidine 5d, and 6-methyl-pyrimidine 5b, respectively. Using spectrum analysis, the structures of every synthesised molecule were verified. Scheme 1 introduced the synthesis scheme. (21-25)



Where, R = p-OCH3, p-CH3, p-Cl, p-Br, o- OCH3

Scheme 1: Synthetic Scheme for the Methyl- pyrimidine Derivatives:

Characterization of Methyl-pyrimidine Derivatives

Synthesis of 2 - amino - 4 - hydroxyl - 6 -methylpyrimidine (Compound 3): A solution of sodium hydroxide (0.022 mol) in ethanol was used to neutralise guanidine hydrochloride (0.018 mol). After 15 minutes of stirring at room temperature, the mixture was filtered. To the aforementioned filtrate, methyl acetoacetate (0.022 mol) was added dropwise and swirled all night. Hot water was used to filter, separate, and recrystallise a crude 2-amino-4-hydroxy-6-methylpyrimidine. Yellowish white solid; Yield 92%; M.P. 267°C; Rf value 0.41 (Hexane: ethyl acetate -7:3); Mol. formula: $C_5H_7N_3O$; Mol. Wt.: 125.13 g/mol.

Synthesis of 2 - amino - 4 - chloro - 6 - methyl -pyrimidine (Compound 4): Phosphorous oxychloride (7.0 mL, 0.78 mol) and 2-amino-4-hydroxy-6-methylpyrimidine (3.0 gm, 0.024 mol) were refluxed for 30 minutes. The sticky residue left behind after the surplus phosphorous oxychloride was distilled off was put into ice-cold water and neutralised with a saturated sodium bicarbonate solution. 2-amino-4-chloro-6-methylpyrimidine was filtered, separated, and recrystallised using methanol to produce a crude solid. Yellowish white solid; Yield 62%; M.P. 211 °C; Rf value 0.67 (Hexane: ethyl acetate -7:3); Mol. formula: $C_5H_6ClN_3$; Mol. Wt.: 143.57 g/mol.

Synthesis of 2 - amino - 4 - (4 -methoxyphenylamino) - 6 - methylpyrimidine (Compound 5a): Hydrochloric acid (0.3 mL) was added to a solution containing 2-amino-4-chloro-6-methylpyrimidine (1.0 gm, 0.007 mol) and p-anisidine (1.0 gm, 0.0084 mol) in ethanol (10 mL). Refluxing the reaction mixture was done till it was finished. TLC tracked the course of the reaction. Methanol was used to filter and recrystallise the crude material, yielding the 2-amino-4- (4-methoxy-phenylamino)-6-methylpyrimidine. Yellowish white solid; Yield 61%; M.P. 223 °C; Rf value 0.68 (n-hexane: ethyl acetate-6:4); Mol. formula: $C_{12}H_{14}N_4O$; Mol. Wt.: 230.27 g/mol. IR: 3345, 3212 (-NH_2), 1238, 1045 (-O-) cm^{-1}; Mass (m/e): 233(M^{+1}); ^1H NMR (DMSO-d6, 400 MHz, δ ppm): 2.32(s, 3H, CH_3); 3.55(s, 3H, OCH_3); 7.2-7.3(m, 1H, Ar-NH); 7.66-7.72(m, 2H, Ar-H); 8.30 (s, 1H, Ar-NH); 10.36(s, 2H, Ar- NH2). ^{13}C NMR (DMSO-d6, 100 MHz, δ ppm): 22; 52.6; 63.3; 98.67; 114.6; 121.6; 129.4; 149.8; 158.6; 163.3.

Synthesis of 2-amino-4-(4-methyl phenylamino)-6-methylpyrimidine (Compound 5b): Hydrochloric acid (0.3 mL) was added to a solution containing 2-amino-4-chloro-6-methylpyrimidine (1.0 gm, 0.007 mol), p-toluene (1.0 gm, 0.0084 mol) in ethanol (10 mL). Refluxing the reaction mixture was done till it was finished. TLC tracked the course of the reaction. Methanol was used to filter the crude solid and recrystallise it, yielding 2-amino-4-(4-methyl phenylamino)-6-methylpyrimidine. Yellow solid; Yield 56 %; M.P. 199°C; Rf value 0.59 (n-hexane: ethyl acetate-6:4); Mol. formula: $C_{12}H_{14}N_4$; Mol. Wt.: 214.27 g/mol. IR: 3298, 3276 (-NH_2), 3087(-NH-) cm^{-1}; ^1H-NMR (DMSO-d6, 400 MHz, δ ppm): 2.51(s, 3H, CH_3); 2.5(s 3H, CH_3); 5.7(s, 1H, Ar-h); 7.24-7.3(d, 2H, Ar-H); 7.43-7.62(d, 2H, Ar-H); 8.6(s, 1H, Ar-NH), 10.34(s, 2H, Ar-NH_2); ^{13}C NMR (DMSO-d6,100 MHz, δ ppm): 21.5; 27.6; 87.4; 118.4; 132.6, 139.4; 163.4; Mass (m/e): 217(M^{+1}).

Synthesis of 2-amino-4-(4-chlorophenylamino)-6-methylpyrimidine (Compound 5c): Hydrochloric acid (0.3 mL) was added to a solution containing 2-amino-4-chloro-6-methylpyrimidine (1.0 gm, 0.007 mol) and p-chloroaniline (1.0 gm, 0.0084 mol) in ethanol (10 mL). Refluxing the reaction mixture was done till it was finished. TLC tracked the course of the reaction. Methanol was used to filter and recrystallise the crude material, yielding 2-

amino-4-chlorophenylamino-6-methylpyrimidine. Yellow solid; Yield 72%; M.P. 215 °C; Rf value 0.75 (n-hexane: ethyl acetate-6:4); Mol. formula: $C_{11}H_{11}ClN_4$; Mol. Wt.: 234.68 g/mol. IR: 3456, 3256(NH_2), 3143(-NH-) cm^{-1}; 1H NMR (DMSO-d6, 400 MHz, δ ppm): δ2.4(s, 3H, CH_3); 7.24-7.6(m, 1H, Ar-H); 7.45-7.59(d, 2H, Ar-H); 8.45(s, 1H, Ar-NH), 10.6(s, 2H, Ar-NH2); ^{13}C NMR (DMSO-d6,100 MHz, δ ppm): 21.3; 93.5; 119.7; 128.2; 133.2; 163.6; Mass (m/e): 232(M^{+1}), 235(M^{+2}).

Synthesis of 2-amino-4-(4-bromophenylamino)-6-methylpyrimidine (Compound 5d): Hydrochloric acid (0.3 mL) was added to a solution containing 2-amino-4-chloro-6-methylpyrimidine (1.0 gm, 0.007 mol) and p-bromoaniline (1.0 gm, 0.0084 mol) in ethanol (10 mL). Refluxing the reaction mixture was done till it was finished. TLC tracked the course of the reaction. Methanol was used to filter and recrystallise the crude material, yielding 2-amino-4- (4-bromophenylamino)-6-methylpyrimidine. Yellow solid; Yield 78%; M.P. 234°C; Rf value 0.65 (n-hexane: ethyl acetate-6:4); Mol. formula: $C_{11}H_{11}BrN_4$; Mol. Wt.: 279.14 g/mol. IR: 3449, 3278(NH_2), 3126(-NH-) cm^{-1}; ^1H NMR (DMSO-d6, 400 MHz, δ ppm): 2.65(s, 3H, CH_3); 7.01(d, 2H, Ar-H); 7.36-7.42(d, 2H, Ar-H); 8.30(s,1H, Ar-NH); 10.36(s, 2H, Ar-NH_2); ^{13}C NMR (DMSO-d6,100 MHz, δ ppm): 22.6; 89.9; 115.3; 121.3; 132.6; 142.3; 166.3; Mass (m/e): 279(M^{+1}), 282(M^{+2}).

Synthesis of 2 - amino - 4 - (2 –methoxy-phenylamino)-6-methylpyrimidine (Compound 5e): Hydrochloric acid (0.3 mL) was added to a solution containing 2-amino-4-chloro-6-methylpyrimidine (1.0 gm, 0.007 mol), o-anisidine (1.0 gm, 0.0084 mol) in ethanol (10 mL). Refluxing the reaction mixture was done till it was finished. TLC tracked the course of the reaction. Methanol was used to filter and recrystallise the crude solid, yielding 2-amino-4-(2-methoxy-phenyl-amino)-6-methylpyrimidine. Yellow solid; Yield 71%; M.P. 239°C; Rf value 0.72 (n-hexane: ethyl acetate-6:4); Mol. formula: $C_{12}H_{14}N_4O$; Mol. Wt.: 230.27 g/mol. IR: 3465,3293 (-NH_2) 3134(-NH-) cm^{-1}; ^1H NMR (DMSO-d6, 400 MHz, δ ppm): δ2.33(s, 3H, CH_3); 3.67(s 3H, OCH_3); 7.25-7.35(m, 2H, Ar-H); 7.66-7.62(m, 2H, Ar-H); 8.50(s, 1H, Ar-NH), 10.6(s, 2H, Ar-NH_2); ^{13}C-NMR (DMSO-d6,100 MHz, δ ppm): 23.9; 55.7; 93.8; 116.3; 119.4; 123.2; 125.2; 132.6; 148.4; 166.5. Mass (m/e): 231(M+1);

Antibacterial And Antifungal Activity: The antibacterial activity of the compounds created during the current study was assessed. Four common microorganisms—Bacillus subtilis, Bacillus pumilus, Escherichia coli, and Pseudomonas aeruginosa—that are representative of gram-positive and gram-negative organisms, respectively, were used in the antibacterial testing. Disc diffusion was used to evaluate the compounds' antibacterial efficacy. (26-29) Using DMF as a control and Clotrimazole 100 µg/ml as a standard, the antifungal activity of each compound was assessed against Aspergillus niger and Candida albicans on potato dextrose agar medium. After being cooled to 45 °C and carefully mixed with test organisms, the sterile molten potato dextrose medium was aseptically transferred into sterile Petri plates. (30-33) All the inoculated Petri dishes were incubated at 28 °C for 4 days, and the extent diameter of inhibition was measured as the zone of inhibition in millimeters the results are shown in Table 1 and Table 2.

Table 1: Antibacterial Activity of the Compounds

Derivatives	Inhibition zone diameter in mm							
	B. subtilis		P. aeruginosa		B. pumilus		E. coli	
	50µg	100 µg	50 µg	100 µg	50 µg	100 µg	50 µg	100 µg
5a	7	14	4	26	6	12	5	8
5b	4	7	5	12	4	9	7	10
5c	5	12	7	11	7	14	6	9
5d	4	6	8	13	5	10	6	10
5e	5	10	6	14	5	13	5	11
DMF	-	-	-	-	-	-	-	-
Ciprofloxacin	20	32	22	31	20	33	21	30

Table 2: Antifungal Activity of the Compounds

Derivatives	Inhibition zone diameter in mm			
	A. niger		C. albicans	
	50µg	100 µg	50 µg	100 µg
5a	4	9	4	9
5b	5	9	5	10
5c	5	11	6	13
5d	5	15	4	9
5e	4	9	6	10
DMF	-	-	-	-
Clotrimazole	24	26	22	27

3. Results and Discussion

The methyl acetoacetate 1 was treated with guanidine hydrochloride 2 which was previously neutralized with sodium hydroxide to afford 2-amino-4-hydroxy-6-methylpyrimidine 3. Compound 3 was refluxed with phosphorous oxy-chloride to yield 2-amino-4-chloro-6-methyl-pyri-midine 4. Refluxing of compound 4 with different reagents like p-anisidine, p-toluene, p-chloro-aniline, p-bromoaniline, o-anisidine in presence of hydrochloric acid and ethanol to afford 2-amino-4-(substituted phenylamino)- 6- methyl- pyrimidine derivatives like 2-amino-4-(4-methoxyphenyl-amino)-6-methylpyrimidine 5a, 2-amino - 4 - (4 - methyl phenylamino)-6-methyl-pyrimidine 5b, 2-amino-4-(4-chlorophenylamino)-6- methyl pyrimidine 5c, 2 - amino - 4 - (4 –bromo phenylamino)-6-methylpyrimidine 5d, 2-amino - 4 - (2 - methoxy-phenylamino) - 6 -methylpyrimidine 5e respectively. Disc diffusion was used to evaluate the compounds' antibacterial efficacy. Using DMF as a control and Clotrimazole 100 µg/ml as a standard, the antifungal activity of each compound was assessed against Aspergillus niger and Candida albicans on potato dextrose agar medium. After being cooled to 45 °C and carefully mixed with test organisms, the sterile molten potato dextrose medium was aseptically transferred into sterile Petri plates. For four days, all of the inoculated Petri dishes were incubated at 28 °C. The zone of inhibition, or the diameter of inhibition, was measured in millimetres.

4. Conclusion

In this work, methylpyrimidine derivatives were synthesised and their structures were determined by IR, 1HNMR, and mass spectroscopy. Using standard technique, the compounds were evaluated for antibacterial and antifungal activity; a small number of them had considerable antifungal and moderate antibacterial activity against Candida albicans. Perhaps molecules with enhanced activity will result from the molecular change. Therefore, it would be beneficial to conduct a thorough investigation into these kinds of compounds and explain the molecule to boost the efficacy in the search for newer generation antibiotics.

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