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Synthesis of pyrimidine linked heterocyclic scaffolds by intramolecular cyclization and study of biological potential

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Synthesis of some interesting pyrimidine linked heterocyclic scaffolds by intramolecular cyclization has been worked out. Initially compound (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide has been prepared by reacting 2-amino-4,6-dimethyl pyrimidine with ethyl chloroacetate, followed by condensation with hydrazine hydrate. It has then been treated with N-aryl/alkyl isothiocyanates, followed by intramolecular cyclization using alkaline ethanolic solution of I_2 with KI, *o*-phosphoric acid and aqueous KOH to afford respective heterocyclic compounds with differently substituted pharmacophores *viz*. (5-aryl/alkyl-amino-[1,3,4]-oxadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines, (5-aryl/alkyl-amino-[1,3,4]-thiadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines and (4-aryl/alkyl-5-mercapto-[1,2,4]-triazol-3-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines. Developments during the synthesis have been monitored by TLC. Constitution of synthesized compounds have been delineated in accordance with equivalent weight, elemental assay, chemical transformation and IR, ^{1}H NMR and mass spectral investigations. Title compounds have been tested for their biological potential.

Keywords: Synthesis, pyrimidine linked heterocyclic scaffolds, biological potential

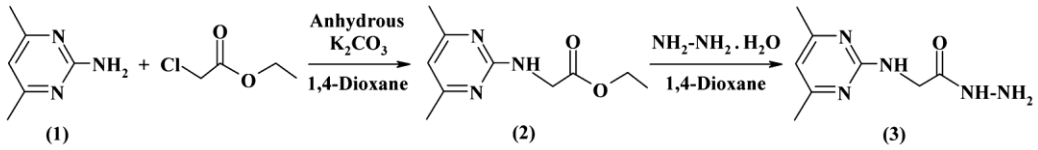
As a heterocyclic compound, pyrimidine is supreme core structure with variegated therapeutic usage[1]. Its utilization as a pharmaceutically dominant compound is evincive from its diversified biological characteristics. Like pyrimidine[2,3]; derivatives of oxadiazole[4,5], thiadiazole[6,7] and triazole[8,9] are incorporated in pharmaceutical stuffs as anti-inflammatory, antitubercular, antiviral, antibacterial, antifungal agents[10,11], *etc*. These heterocyclic rings are also used as fundamental part of pharmacophores which have anticonvulsants[12-14], antiproliferative[15,16], analgesics[17,18] and other biological properties[19,20]. Fusion of pyrimidine nucleus with these heterocycles proved to be excellent biological compounds[21,22]. These diverse attributes of oxadiazole[23], thiadiazole[24] and triazole[25] nuclei have driven the interest to develope some interesting heterocyclic molecules with promising biological activities. As the presence of two or more bioactive rings within a single molecule enhances biological activity profile[26-28], herein synthesis of pyrimidine linked heterocyclic scaffolds have been reported. To establish structure-activity relationship, synthesized compounds were evaluated for their antitubercular, antimicrobial potential and screened for insecticidal activity.

Results and Discussion

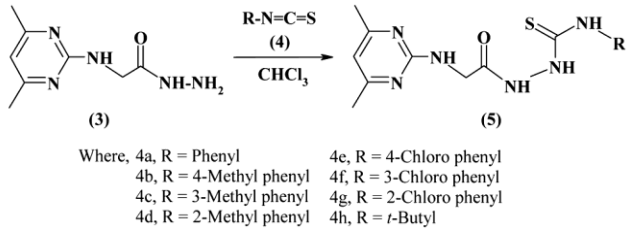
To begin with, compound ethyl-(4,6-dimethyl-pyrimidin-2-yl-amino)-acetate **2** was synthesized by reacting 2-amino-4,6-dimethyl-pyrimidine **1** (0.01 mol) with ethyl chloroacetate (0.01 mol) in 1,4-dioxane using anhydrous K_2CO_3 as catalyst[1] for 6 hr, followed by its condensation with hydrazine hydrate (0.01 mol) in 1,4-dioxane for 5 hr to afford (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide **3** (Scheme I).

Further, compound **3** (0.01 mol) was reacted with N-aryl/alkyl isothiocyanates **4a-h** (0.01 mol) in chloroform medium for 2 to 3 hrs to give (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-aryl/alkyl-thioamido)-hydrazides **5a-h** (Scheme II).

Substituted hydrazides **5a-h** were separately reacted with alkaline ethanolic solution of I_2 with KI, *o*-phosphoric acid and aqueous KOH by dropwise addition of these reagents with constant stirring and allowing to stand at RT for specified time to undergo intramolecular cyclization and produce (5-aryl/alkyl-amino-[1,3,4]-oxadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines **6a-h**, (5-aryl/alkyl-amino-[1,3,4]-thiadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines **7a-h** and (4-aryl/alkyl-5-mercapto-



Scheme I



Scheme II

[1,2,4]-triazol-3-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines **8a-h** respectively (Scheme III).

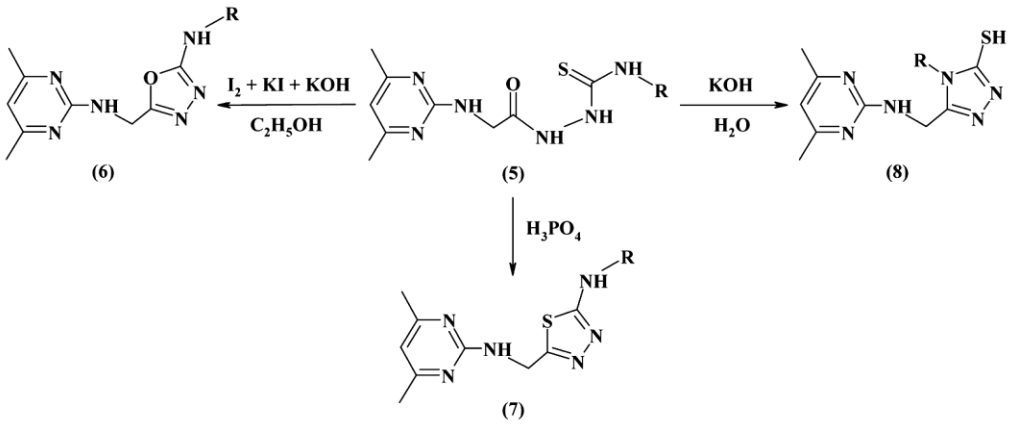
Synthesized compounds were characterized by spectral investigations[29,30] and their structural properties were confirmed by elemental analysis[31]. These compounds showed single spots on silica gel-G plates in TLC.

Antitubercular activity

Synthesized pyridine linked oxadiazoles, thiadiazoles and triazoles were assessed for their *in-vitro* antitubercular potency[32] by BACTEC-TB and MABA techniques to determine MIC against *M. tuberculosis*. Compounds to be tested were dissolved in DMSO (10%, v/v) at a concentration of 10 mM. For BACTEC-TB analysis, test vial sjnhjn of 7H12 medium with ^{14}C labelled palmitic acid has been inoculated with mycobacterium and incubated at 37°C. Amount of $^{14}CO_2$ reflects the rate and amount of growth, which is expressed in term of growth index. When compound is added to test vial, suppression of growth of *M. tuberculosis* could be detected by routine observation of output of growth index in comparison to standard drug Rifampin (2 µg/mL). For MABA analysis, two fold serial dilutions of compounds to be tested were made in Middle brook 7H9 medium supplemented with ADC (10%, v/v), in well plates (Nunc) in duplicate. Inoculum of 10^5 CFU/mL was prepared and 200 µL was added per well. For each analysis, growth controls having no drug and a sterile control lacking bacteria were also prepared. Plates were incubated at 37°C for 5 days before adding 20 µL of sterile 0.01% resazurin to wells and incubating for further 24 hr at 37°C. Colour change from blue to pink (oxidized to reduced state) indicated the growth of bacteria. Compounds having MIC at 50 µM were again tested to determine CFU using agar dilution method. Serial dilutions of compounds prepared in 0.1 mL DMSO (10%, v/v) were added to each well of well plates (Nunc). Subsequently 1.9 mL MB7H10 agar medium supplemented with OADC (10%, v/v) were poured to respective wells and allowed to solidify at RT. For positive control, rifampin was dissolved in water, filtered, sterilized and used in 2 µg/mL concentration. Solution 10 µL was inoculated in each well on solidified agar medium, incubated at 37°C for four weeks and growth was recorded. Compounds **6g**, **7e**, **7f**, **7g** and **8g** showed promising activity against *M. tuberculosis*. MIC values of compounds **7e** and **7f** were found to be 6.25 µM and of compounds **6g**, **7g** and **8g** were found to be 25, 12.5 and 50 µM respectively (Table I).

Antimicrobial activity

Synthesized pyridine linked oxadiazoles, thiadiazoles and triazoles were analyzed for their antibacterial potential by disc diffusion method[32,33]. Both gram-positive and gram-negative bacterial strains *E. coli*, *S. aureus*, *S. typhi*, *B. subtilis* and *P. vulgaris* were used. Drug ofloxacin was used as standard for comparative reason. Nutrient media used was Muller-Hinton agar of bacteristatic grade. Sensitivity plates were seeded with a bacterial inoculum of 1×10^6 CIU/mL and 5 mm discs impregnated with test solution were placed on nutrient media loaded in Petri plates. Concentration of each test compound solution was 100 µg/mL. Zones of inhibition were recorded after incubation for 24 hr at 37°C. It was observed that, compounds **6b** and **6e** were highly active against *E. coli* and *P. vulgaris* and moderately active against *B. subtilis*. Compounds **7b**, **8b** and **8e** were found to be highly active against *E. coli*, *S. typhi* and *S. aureus* and moderately active against *P. vulgaris*. Most of other compounds were found to be weakly active against all of these bacteria whereas some were moderately active. Compound **6h**, **7h** and **8h** were inactive against almost all microorganisms (Table II). To determine MIC, serial



Where, 5-8a, R = Phenyl
5-8b, R = 4-Methyl phenyl
5-8c, R = 3-Methyl phenyl
5-8d, R = 2-Methyl phenyl
5-8e, R = 4-Chloro phenyl
5-8f, R = 3-Chloro phenyl
5-8g, R = 2-Chloro phenyl
5-8h, R = *t*-Butyl

Scheme III

Table I — Antitubercular activity

Compd	BACTEC-TB					MABA				
(Conc. µM)	50	25	12.5	6.25	3.125	50	25	12.5	6.25	3.125
6g	+	+	–	–	–	+	+	–	–	–
7e	+	+	+	+	–	+	+	+	+	–
7f	+	+	+	+	–	+	+	+	+	–
7g	+	+	+	–	–	+	+	+	–	–
8g	+	–	–	–	–	+	–	–	–	–
Standard	+	+	+	+	+	+	+	+	+	+

(+) : Active, (–) : Inactive

Table II — Antibacterial and antifungal activity

Compd	Microorganisms					
	E. coli	*S. aureus*	*S. typhi*	*B. subtilis*	*P. vulgaris*	*C. albicans*
6b	+++	+	+	++	+++	+++
6e	+++	+	+	++	+++	+
7b	+++	+++	+++	+	++	+++
7e	+	+	+	++	–	+++
8b	+++	+++	+++	+	++	+++
8e	+++	+++	+++	–	++	+++
Standard	+++	+++	+++	++	+++	+++

(–) : Inactive (10 mm and less), (+) : Weakly active (11-15 mm),
(++) : Moderately active (16-20 mm), (+++) : Highly active (21 mm and above)

dilution technique[34] was followed using nutrient broth medium. MIC values of compounds **6b**, **6e**, **7b**, **8b** and **8e** against *E. coli* were found to be 65, 60, 80, 60 and 55 µg/mL respectively.

Evaluation of antifungal activity of pyridine linked oxadiazoles, thiadiazoles and triazoles was done by disc diffusion method[35] against fungal strain *C. albicans*. Drug fluconazole was used as standard to compare the results. Nutrient media used was potato dextrose agar of fungistatic grade. Concentration of each test compound solution was 100 µg/mL. Zones of inhibition were recorded after incubation for 48 hr at 37°C. It showed that, compounds **6b**, **7b**, **7e**, **8b** and **8e** were highly active / prominent inhibitory activity against *C. albicans*, whereas other compounds showed low to moderate activity (Table II).

Insecticidal activity

To study insecticidal property of pyridine linked oxadiazoles, thiadiazoles and triazoles, insect affected

plant surface having species Pseudococcidae[36] *i.e*. mealy bug was selected. Insecticidal study was done by direct contact application[36,37]. Heavy infested plant parts affected by insect pests were selected for application. Aqueous solutions of 2,4,6 ppm of test compounds were applied by direct spray method on differently labelled affected plant parts under similar conditions of temperature and sunlight. For single application[36], amount of solution sprayed was 2 mL. Mortality results of insects were monitored time to time for about 1 to 48 hrs with simultaneous checking of any movement of body parts of insects using simple microscope. In most of cases, it was observed that aqueous solutions of 2 ppm were sufficiently active against insect pests and no plant parts were affected due to toxicity of compounds. Activity of test solutions was compared with that of ethanol and hexane solutions of same concentrations and it was found to be good enough.

Experimental Section

Melting points of synthesized compounds were determined using Veego, VMP-D digital melting point apparatus and are uncorrected. Chemicals used were of AR grade. C, H and S analysis was carried out on Carlo-Erba analyser, N estimation was performed on Colman-N 29 analyser. IR spectra were recorded on Perkin-Elmer spectrophotometer using KBr disc. ^{1}H NMR spectra were obtained from Bruker-DRX 600 spectrophotometer using TMS as internal standard and $CDCl_3$, DMSO-d_6 as solvents. Mass spectral measurements were done by EI method at 70 eV on Jeol-JMC 300 spectrometer. Purity of synthesized compounds was checked on silica gel-G plates by TLC and spots were visualized by iodine vapours.

Synthesis of ethyl-(4,6-dimethyl-pyrimidin-2-yl-amino)-acetate, 2

Compound ethyl-(4,6-dimethyl-pyrimidin-2-yl-amino)-acetate **2** was synthesized by refluxing mixture of 2-amino-4,6-dimethyl-pyrimidine **1** (0.01 mol) and ethyl chloroacetate (0.01 mol) in 1,4-dioxane (15 mL) using anhydrous K_2CO_3 as catalyst for 6 hr. On distilling off solvent, crude solid mass obtained was crystallized from ethanol. Completion of reaction was confirmed by TLC.

2: (80%), m.p. 142°C. Anal. Found: C, 55.21; H, 7.05; N, 19.98. Calcd. for $C_{10}H_{15}N_3O_2$: C, 57.41; H, 7.17; N, 20.09%.

Synthesis of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide, 3

Synthesis of compound (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide **3** was performed by condensation of ethyl-(4,6-dimethyl-pyrimidin-2-yl-amino)-acetate **2** (0.01 mol) with hydrazine hydrate (0.01 mol) by refluxing mixture in 1,4-dioxane (15 mL) for 5 hr. On distilling of solvent, crude solid mass obtained was crystallized from ethanol in cold condition. Reaction was monitored by TLC.

3: (75%), m.p. 138°C. Anal. Found: C, 48.02; H, 6.69; N, 35.66. Calcd. for $C_8H_{13}N_5O$: C, 49.22; H, 6.71; N, 35.87%. IR: 3401, 3310 (NH), 1729 (C=O), 1628 (C=N), 1336 (C-N), 1156 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): δ 7.38 (3H, bs, NH-NH_2), 6.46 (1H, s, Pyrm-NH), 6.32 (1H, s, Pyrm-H), 3.57 (2H, s, CO-CH_2), 2.17 (6H, s, Pyrm-CH_3).

Synthesis of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide, 5a

Compound (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide **5a** was synthesized by refluxing mixture of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide **3** (0.01 mol) and N-phenyl isothiocyanate **4a** (0.01 mol) in chloroform (15 mL) for 2 hr. Reaction mixture was cooled and crude solid mass obtained was crystallized from ethanol in cold condition.

5a: (76%), m.p. 133°C. Anal. Found: C, 53.98; H, 5.33; N, 25.31; S, 9.45. Calcd. for $C_{15}H_{18}N_6OS$: C, 54.54; H, 5.45; N, 25.45; S, 9.69%. IR: 3402, 3311 (NH), 1764 (C=O), 1649 (C=N), 1311 (C-N), 1246 (C=S), 1170 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): δ 7.99 (1H, s, CO-NH), 7.75 (1H, s, Ar-NH), 7.73 (1H, s, CS-NH), 7.09-7.58 (5H, m, Ar-H), 6.41 (1H, s, Pyrm-NH), 6.29 (1H, s, Pyrm-H), 3.64 (2H, s, CO-CH_2), 2.21 (6H, s, Pyrm-CH_3).

This reaction was extended to synthesize other compounds **5b-h** using different N-aryl/alkyl isothiocyanates **4a-h**. Developments during reactions were checked by TLC.

5b: (78%), m.p. 119°C. Anal. Found: C, 55.62; H, 5.78; N, 24.23; S, 9.27. Calcd. for $C_{16}H_{20}N_6OS$: C, 55.81; H, 5.81; N, 24.41; S, 9.30%.

5c: (80%), m.p. 112°C. Anal. Found: C, 55.46; H, 5.56; N, 24.20; S, 9.18. Calcd. for $C_{16}H_{20}N_6OS$: C, 55.81; H, 5.81; N, 24.41; S, 9.30%.

5d: (76%), m.p. 110°C. Anal. Found: C, 55.33; H, 5.49; N, 24.38; S, 9.05. Calcd. for $C_{16}H_{20}N_6OS$: C, 55.81; H, 5.81; N, 24.41; S, 9.30%.

5e: (70%), m.p. 65°C. Anal. Found: C, 49.28; H, 4.52; N, 22.92; S, 8.61. Calcd. for $C_{15}H_{17}N_6OSCl$: C, 49.38; H, 4.66; N, 23.04; S, 8.77%.

5f: (82%), m.p. 150°C. Anal. Found: C, 48.94; H, 4.63; N, 22.84; S, 8.47. Calcd. for $C_{15}H_{17}N_6OSCl$: C, 49.38; H, 4.66; N, 23.04; S, 8.77%.

5g: (78%), m.p. 210°C. Anal. Found: C, 49.30; H, 4.59; N, 23.01; S, 8.70. Calcd. for $C_{15}H_{17}N_6OSCl$: C, 49.38; H, 4.66; N, 23.04; S, 8.77%.

5h: (80%), m.p. 95°C. Anal. Found: C, 50.23; H, 7.03; N, 26.97; S, 10.26. Calcd. for $C_{13}H_{22}N_6OS$: C, 50.32; H, 7.09; N, 27.09; S, 10.32%.

Synthesis of (4,6-dimethyl-pyrimidin-2-yl)-(5-phenyl-amino-[1,3,4]-oxadiazol-2-yl-methyl)-amine, 6a

For synthesis of compound (4,6-dimethyl-pyrimidin-2-yl)-(5-phenyl-amino-[1,3,4]-oxadiazol-2-yl-methyl)-amine **6a**, paste of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide **5a** (0.01 mol) was prepared in ethanol. To this, alkaline ethanolic solution of I_2 with KI containing KOH was added drop by drop with constant stirring till there was no decolourisation of violet colour of iodine. Reaction mixture was allowed to stand overnight at RT and separated solid was crystallized from ethanol.

6a: (85%), m.p. 111°C. Anal. Found: C, 59.37; H, 5.31; N, 26.89. Calcd. for $C_{15}H_{16}N_6O$: C, 60.81; H, 5.40; N, 28.37%. IR: 3393, 3189 (NH), 1628 (C=N), 1313 (C-N), 1243 (C-O), 1163 cm^{-1} (N-N); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 6.88-7.91 (5H, m, Ar-H), 6.36 (2H, s, Pyrm-NH, Ar-NH), 6.32 (1H, s, Pyrm-H), 3.38 (2H, s, NH-CH_2), 2.16 (6H, s, Pyrm-CH_3); MS: m/z 295 (M^+-H), 281 (M^+-CH_3), 204 (M^+-NH.C_6H_5), 160 (M^+-$(CH_3)_2$.C_4HN_2.NH.CH_2), 122 $(CH_3)_2$.C_4HN_2.NH^+), 92 (C_6H_5.NH^+).

This reaction was extended to synthesize other compounds **6b-h**. Progress of reactions was monitored by TLC.

6b: (80%), m.p. 97°C. Anal. Found: C, 60.11; H, 5.49; N, 27.10. Calcd. for $C_{16}H_{18}N_6O$: C, 61.93; H, 5.80; N, 27.09%. IR: 3396, 3181 (NH), 1632 (C=N), 1315 (C-N), 1240 (C-O), 1165 cm^{-1} (N-N); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 6.95-7.62 (4H, m, Ar-H), 6.39 (2H, s, Pyrm-NH, Ar-NH), 6.33 (1H, s, Pyrm-H), 3.47 (2H, s, NH-CH_2), 2.40 (3H, s, Ar-CH_3), 2.16 (6H, s, Pyrm-CH_3); MS: m/z 310 (M^+), 295 (M^+-CH_3), 188 (M^+-$(CH_3)_2$.C_4HN_2.NH), 174 (M^+-$(CH_3)_2$.C_4HN_2.NH.CH_2), 122 $(CH_3)_2$.C_4HN_2.NH^+), 106 (CH_3.C_6H_4.NH^+).

6c: (82%), m.p. 155°C. Anal. Found: C, 61.88; H, 5.71; N, 26.88. Calcd. for $C_{16}H_{18}N_6O$: C, 61.93; H, 5.80; N, 27.09%.

6d: (90%), m.p. 162°C. Anal. Found: C, 59.99; H, 5.67; N, 26.62. Calcd. for $C_{16}H_{18}N_6O$: C, 61.93; H, 5.80; N, 27.09%.

6e: (84%), m.p. 119°C. Anal. Found: C, 53.12; H, 4.31; N, 25.19. Calcd. for $C_{15}H_{15}N_6OCl$: C, 54.46; H, 4.53; N, 25.41%.

6f: (85%), m.p. 122°C. Anal. Found: C, 54.22; H, 4.52; N, 25.10. Calcd. for $C_{15}H_{15}N_6OCl$: C, 54.46; H, 4.53; N, 25.41%.

6g: (80%), m.p. 186°C. Anal. Found: C, 53.88; H, 4.44; N, 25.33. Calcd. for $C_{15}H_{15}N_6OCl$: C, 54.46; H, 4.53; N, 25.41%.

6h: (86%), m.p. 141°C. Anal. Found: C, 54.73; H, 7.12; N, 30.29. Calcd. for $C_{13}H_{20}N_6O$: C, 56.52; H, 7.24; N, 30.43%.

Synthesis of (4,6-dimethyl-pyrimidin-2-yl)-(5-phenyl-amino-[1,3,4]-thiadiazol-2-yl-methyl)-amine, 7a

Compound (4,6-dimethyl-pyrimidin-2-yl)-(5-phenyl-amino-[1,3,4]-thiadiazol-2-yl-methyl)-amine **7a** was synthesized by adding *o*-phosphoric acid (10 mL) to (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide **5a** (0.01 mol) dropwise with constant stirring for 30 min. Reaction mixture was left for 3 hr at RT, poured in distilled water and separated solid was crystallized from ethanol.

7a: (94%), m.p. 107°C. Anal. Found: C, 56.60; H, 5.07; N, 26.83; S, 10.20. Calcd. for $C_{15}H_{16}N_6S$: C, 57.69; H, 5.12; N, 26.92; S, 10.25%. IR: 3396, 3186 (NH), 1628 (C=N), 1312 (C-N), 1165 (N-N), 749 cm^{-1} (C-S); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 6.87-7.99 (5H, m, Ar-H), 6.40 (1H, s, Ar-NH), 6.32 (1H, s, Pyrm-H), 6.30 (1H, s, Pyrm-NH), 2.51 (2H, s, CH_2), 2.16 (6H, s, CH_3); MS: m/z 312 (M^+), 297 (M^+-CH_3), 220 (M^+-C_6H_5.NH), 176 (M^+-$(CH_3)_2$.C_4HN_2.NH.CH_2), 136 $(CH_3)_2$.C_4HN_2.NH.CH_2^+), 122 $(CH_3)_2$.C_4HN_2. NH^+).

This reaction was extended to synthesize other compounds **7b-h**. Formation of products was verified by TLC.

7b: (88%), m.p. 111°C. Anal. Found: C, 57.44; H, 5.48; N, 24.91; S, 9.70. Calcd. for $C_{16}H_{18}N_6S$: C, 58.89; H, 5.52; N, 25.76; S, 9.81%. IR: 3394, 3193 (NH), 1631 (C=N), 1310 (C-N), 1162 (N-N), 746 cm^{-1} (C-S); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 6.79-7.95 (4H, m, Ar-H), 6.56 (3H, s, Pyrm-NH, Ar-NH, Pyrm-H), 2.53 (2H, s, CH_2), 2.42 (3H, s, Ar-CH_3),

2.28 (6H, s, CH_3); MS: m/z 325 (M^+-H), 311 (M^+-CH_3), 235 (M^+-$C_6H_4.CH_3$), 204 (M^+-$(CH_3)_2.C_4HN_2.NH$), 122 $(CH_3)_2.C_4HN_2.NH^+$), 106 ($CH_3.C_6H_4.NH^+$), 91 ($CH_3.C_6H_4^+$).

7c: (84%), m.p. 128°C. Anal. Found: C, 58.66; H, 5.26; N, 24.96; S, 9.79. Calcd. for $C_{16}H_{18}N_6S$: C, 58.89; H, 5.52; N, 25.76; S, 9.81%.

7d: (82%), m.p. 179°C. Anal. Found: C, 57.88; H, 5.33; N, 25.68; S, 9.44. Calcd. for $C_{16}H_{18}N_6S$: C, 58.89; H, 5.52; N, 25.76; S, 9.81%.

7e: (84%), m.p. 120°C. Anal. Found: C, 51.43; H, 4.13; N, 24.21; S, 9.12. Calcd. for $C_{15}H_{15}N_6SCl$: C, 51.94; H, 4.32; N, 24.24; S, 9.24%.

7f: (90%), m.p. 188°C. Anal. Found: C, 51.67; H, 4.28; N, 24.19; S, 8.97. Calcd. for $C_{15}H_{15}N_6SCl$: C, 51.94; H, 4.32; N, 24.24; S, 9.24%.

7g: (95%), m.p. 162°C. Anal. Found: C, 51.35; H, 4.36; N, 24.28; S, 9.07. Calcd. for $C_{15}H_{15}N_6SCl$: C, 51.94; H, 4.32; N, 24.24; S, 9.24%.

7h: (90%), m.p. 132°C. Anal. Found: C, 52.27; H, 6.42; N, 27.77; S, 10.81. Calcd. for $C_{13}H_{20}N_6S$: C, 53.42; H, 6.84; N, 28.76; S, 10.95%.

Synthesis of (4,6-dimethyl-pyrimidin-2-yl)-(4-phenyl-5-mercapto-[1,2,4]-triazol-3-yl-methyl)-amine, 8a

Synthesis of compound (4,6-dimethyl-pyrimidin-2-yl)-(4-phenyl-5-mercapto-[1,2,4]-triazol-3-yl-methyl)-amine **8a** was carried out by adding 5% aqueous KOH (10 mL) to (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide **5a** (0.01 mol) dropwise with constant stirring for 30 min. Reaction mixture was left for 3 hr at RT, poured in distilled water and separated solid was crystallized from ethanol.

8a: (80%), m.p. 73°C. Anal. Found: C, 57.60; H, 5.07; N, 26.87; S, 10.11. Calcd. for $C_{15}H_{16}N_6S$: C, 57.69; H, 5.12; N, 26.92; S, 10.25%. IR: 3160 (NH), 2650 (SH), 1635 (C=N), 1310 (C-N), 1162 (N-N), 754 cm^{-1} (C-S); ^1H NMR ($CDCl_3$+DMSO-d_6): δ 6.86-7.61 (5H, m, Ar-H), 6.53 (2H, s, Pyrm-H, Pyrm-NH), 2.53 (2H, s, CH_2), 2.40 (1H, s, Triz-SH), 2.20 (6H, s, CH_3); MS: m/z 311 (M^+-H), 297 (M^+-CH_3), 235 (M^+-C_6H_5), 136 ($(CH_3)_2.C_4HN_2.NH.CH_2^+$), 122 ($(CH_3)_2.C_4HN_2.NH^+$), 107 ($(CH_3)_2.C_4HN_2^+$), 77 ($C_6H_5^+$).

This reaction was extended to synthesize other compounds (8b-h). Formation of compounds was checked by TLC.

8b: (79%), m.p. 103°C. Anal. Found: C, 58.70; H, 5.53; N, 25.74; S, 9.77. Calcd. for $C_{16}H_{18}N_6S$: C, 58.89; H, 5.52; N, 25.76; S, 9.81%.

8c: (86%), m.p. 177°C. Anal. Found: C, 57.94; H, 5.31; N, 24.79; S, 9.56. Calcd. for $C_{16}H_{18}N_6S$: C, 58.89; H, 5.52; N, 25.76; S, 9.81%.

8d: (84%), m.p. 144°C. Anal. Found: C, 58.80; H, 5.47; N, 25.24; S, 9.26. Calcd. for $C_{16}H_{18}N_6S$: C, 58.89; H, 5.52; N, 25.76; S, 9.81%.

8e: (82%), m.p. 131°C. Anal. Found: C, 51.50; H, 4.16; N, 24.03; S, 8.98. Calcd. for $C_{15}H_{15}N_6SCl$: C, 51.94; H, 4.32; N, 24.24; S, 9.24%.

8f: (86%), m.p. 182°C. Anal. Found: C, 51.90; H, 4.30; N, 24.26; S, 9.18. Calcd. for $C_{15}H_{15}N_6SCl$: C, 51.94; H, 4.32; N, 24.24; S, 9.24%.

8g: (90%), m.p. 120°C. Anal. Found: C, 50.88; H, 4.33; N, 23.93; S, 9.29. Calcd. for $C_{15}H_{15}N_6SCl$: C, 51.94; H, 4.32; N, 24.24; S, 9.24%.

8h: (82%), m.p. 189°C. Anal. Found: C, 53.40; H, 6.60; N, 28.70; S, 10.65. Calcd. for $C_{13}H_{20}N_6S$: C, 53.42; H, 6.84; N, 28.76; S, 10.95%.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/58776.

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References

1 Joule J A & Mills K, *Heterocyclic Chemistry*, 5th Edn (John Wiley and Sons, UK) (2010).
2 Babu K S, Prabhakar V, Ravindranath L K & Latha J, *Int J Pharma Res Reviews*, 4 (2015) 23.
3 Tolam H E M, El-Saeyed A, Tawfek N, Abdul Megied F & Kutkat O M, *Nucleosides, Nucleotides and Nucleic Acids*, 39(5) (2019) 649.
4 Liu Z, Yand G & Qin X, *J Chem Tech*, 76 (2001) 1154.
5 Sekhar M M, Nagarjuna U, Padmavati V, Padmaja A, Reddy N V & Vijaya T, *Eur J Med Chem*, 145 (2018) 1.
6 Serban G, Stanasel O, Serban E & Bota S, *Drug Des Dev Therap*, 12 (2018) 1545.
7 Juszczak M, Matysiak J, Szeliga M, Zarowski P & Albrecht N, *Bioorg Med Chem Lett*, 22 (2012) 5466.
8 Mabasa T F, Awe B, Laming D & Knife H H, *Med Chem*, 15(6) (2019) 685.
9 Mohamed M A A, Abd-Allah O A, Bekhit A A, Kadry A M & El-Saghier A M M, *J Heterocycl Chem*, 57(6) (2020) 2365.
10 Deohate P P, *Indian J Heterocycl Chem*, 27(4) (2017) 377.
11 Deohate P P, *J Indian Chem Soc*, 94 (2017) 1221.

12 Shetgiri N P & Nayak B K, *Indian J Chem*, 44B (2005) 1267.
13 Sharma B, Verma A, Prajapati S & Sharma U K, *Int J Med Chem*, 2013 (2013) 348948.
14 Amir M & Shiksha K, *Eur J Med Chem*, 39 (2004) 535.
15 Dalunge S M, Skoezny P, Roth B & Raukman B S, *US Patent*, 4, 590 (1986) 271.
16 El-Shereif H A M, Youssif B G M, Abdelazeem A H, Abdel-Aziz M & Abdel-Rahman H M, *Anticancer Agents Med Chem*, 19(5) (2019) 697.
17 Amis M & Kumar S, *Indian J Heterocycl Chem*, 14 (2003) 51.
18 Khanage S G, Raju A, Mohite P B & Pandhare R B, *Adv Pharm Bull*, 3(1) (2013) 13.
19 Berad B N & Deohate P P, *J Indian Chem Soc*, 85 (2008) 1153.
20 Deohate P P, *J Indian Chem Soc*, 89 (2012) 253.
21 Coates A R M & Hu Y, *British J Pharm*, 152 (2007) 1147.
22 Poole K, *J Pharm Pharmacol*, 53 (2001) 283.
23 Bostrom J, Hogner A, Llinas A, Wellner E & Plowright T A, *J Med Chem*, 55 (2012) 1817.
24 Wood T C, Johnson K L, Naylor S & Weinshilboum R M, *Drug Metab Disp*, 30(10) (2002) 1123.
25 Holla B S, Poojary K N, Rao B S & Shivanda M K, *Eur J Med Chem*, 37 (2002) 511.
26 Palaspagar K A & Deohate P P, *Indian J Chem*, 60B (2021) 611.
27 Mulani R S & Deohate P P, *Indian J Chem*, 59B (2020) 1707.
28 Mulani R S & Deohate P P, *Asian J Chem*, 31(5) (2019) 1087.
29 Silverstein R M, Bassler G C & Morril T C, *Spectrometric Identification of Organic Compounds*, 4th Edn (John Wiley and Sons, New York) (1981).
30 Pavia D L, Lampman G M, Kriz G S & Vyvyan J R, *Introduction to Spectroscopy*, 4th Edn (Cengage Learning, Canada) (2010).
31 Furniss B S, Hannaford A J, Smith P W G & Tatchell A R, *Vogel's Text Book of Practical Organic Chemistry*, 5th Edn (Longman, UK) (1989).
32 Babalola I B, Adelakun E A, Wang Y & Shode F O, *J Pharm Phytochem*, 1 (2012) 17.
33 Barry A L, *The Antimicrobial Susceptibility Test*: *Principle and Practices* (Lea and Fibiger, Philadelphia, USA) (1976).
34 Pelczar M J, Reid R D & Chan E C S, *Microbiology* (Tata McGraw Hill, India) (1977).
35 Ananthanarayan R & Jagram-Pancka G K, *Text Book of Microbiology*, 4th Edn (Orient Longmans) (1990).
36 Dhaliwal G S, Singh R & Chhillar B S, *Essentials of Agricultural Entomology* (Kalyani Publishers, India) (2014).
37 Evans J W, *Insect Pests and their Control* (Laurier Books Ltd.) (2005).