Study of Microwave Assisted Synthesis and Biological Activities of Some Pyrimidine Linked Oxadiazole Pharmacophores

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Abstract

In present work microwave assisted synthesis of (5-aryl/alkylamino-[1,3,4]-oxadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines have been carried out. Initially ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate was prepared by reacting 2-amino-4,6-dimethyl-pyrimidine with ethyl chloroacetate. It was further reacted with hydrazine hydrate to afford (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide. This hydrazide was then reacted with N-aryl/alkyl isothiocyanates followed by oxidative cyclization using alkaline ethanolic solution of iodine in presence of potassium iodide to afford the respective title compounds with differently substituted pharmacophores. The constitutions of synthesized compounds were delineated on the basis of chemical transformation, elemental analysis, equivalent weight determination and IR, ^{1}H-NMR, mass spectral studies. Progress of the reactions was monitored by TLC. Title compounds were screened for their biological activities.

***Keywords**: Microwave, pyrimidine linked oxadiazoles, biological activities*.

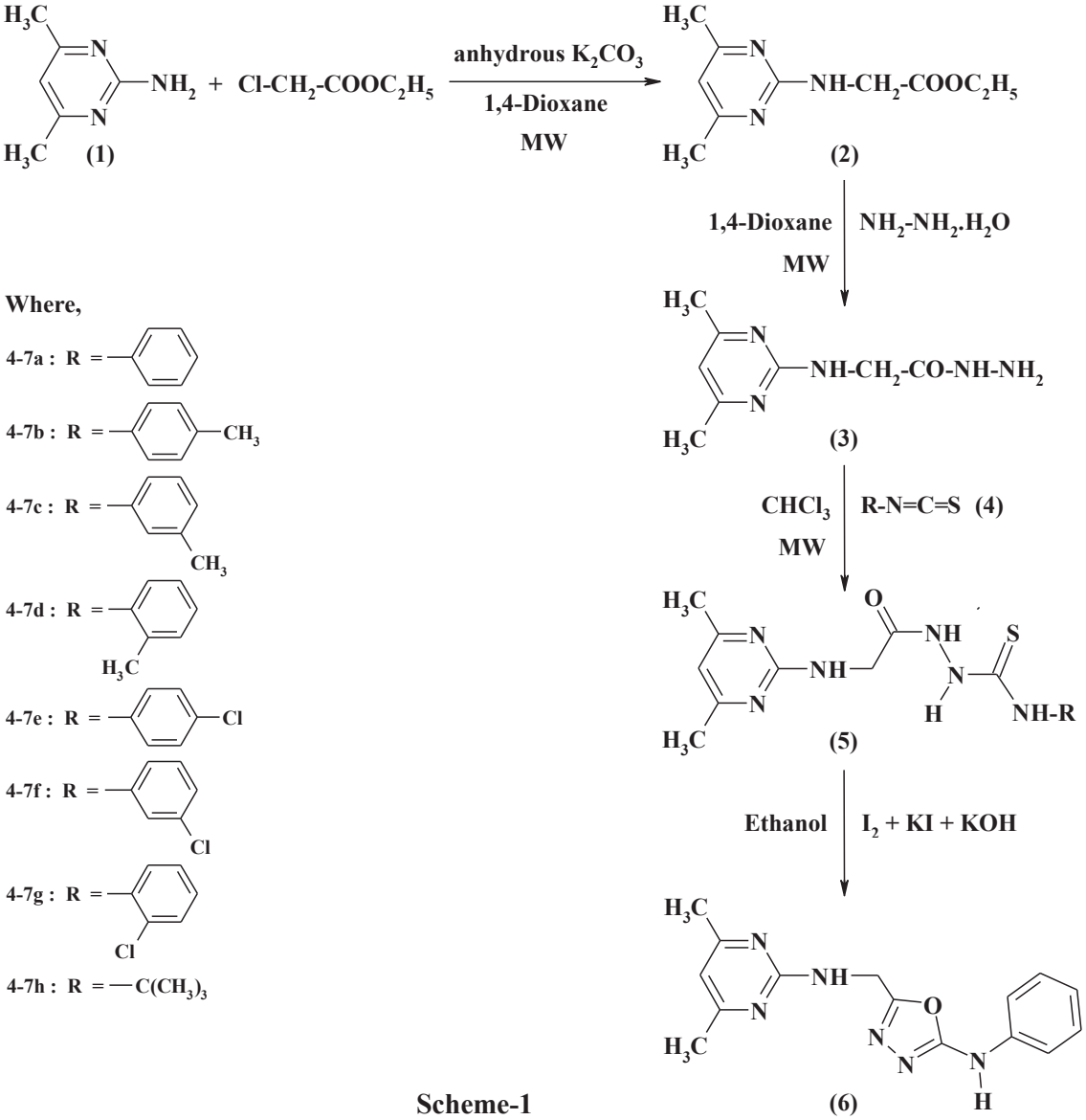
Introduction

The heterocyclic compounds especially with distinguished pharmacological activities have proved to be excellent and versatile drugs in the field of medicinal chemistry[1]. Pyrimidine as a heterocyclic compound is an excellent core structure with diversified therapeutic applications[2]. Its fascinating use as a medicinally important compound is evidential from its varied biological properties[3]. Similar to pyrimidine[4-5]; 1,3,4-oxadiazoles derivatives are introduced in medicinal substances as antibacterial, anti-inflammatory, antifungal, anti-tubercle, antiviral agents[6-8]. Oxadiazole ring is also used as a substantial part of the pharmacophore, which have anti-proliferative activity[9.] The fusion of pyrimidine nucleus with oxadiazole derivatives proved to be excellent biological compounds[10-12].

In the present work efforts are made for microwave assisted synthesis[13-14] and characterization of series of (5-aryl/alkylamino-[1,3,4]-oxadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines.

Results and Discussion

The starting product ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate (2) was prepared by the reaction of 2-amino-4,6-dimethyl pyrimidine (1) and ethyl chloroacetate in 1,4-dioxane medium using anhydrous potassium carbonate as a catalyst[2]. The product ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate (2) was reacted with hydrazine hydrate in 1,4-dioxane to give (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide (3) and further reacted with N-phenyl isothiocyanate (4a) to afford (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide (5a). The compound (5a) was transformed into (4,6-dimethyl-pyrimidin-2-yl)-(5-phenyl-amino-[1,3,4]-oxadiazol-2-yl-methyl)-amine (6a) by oxidative cyclization using alkaline ethanolic solution of iodine in presence of potassium iodide with evaluation of hydrogen sulphide gas. Most of these reactions were carried by microwave irradiation. These reactions were extended to synthesize (5-aryl/alkylamino-[1,3,4]-oxadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines (6b-h) (Scheme-1).



Scheme-1

The products obtained were characterized by spectral method[15-17]. The elemental analysis[18] satisfied the structural properties of the synthesized compounds. To conclude, the chemistry of the reactions employed together with their chemical behaviour was discussed exhibiting the importance of novel molecular templates.

Antimicrobial activity

Various methods have been proposed and adopted for the measurement of antimicrobial activity[19]. In present antimicrobial study the newly synthesized compounds were screened for their antibacterial activity using disc diffusion method[20-22]. 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 the nutrient media loaded in petri plates. The zones of inhibition were recorded after incubation for 24 hr at 37ºC, using Vernier caliper. The bacterial organisms used included both gram-positive as well as gram-negative strains. The medium used for the study of antibacterial activity of newly synthesized compounds was Muller-Hinton agar. It was of bacteristatic grade and found to be suitable for the growth of all bacterial strains used in the present study.

The antibacterial activity and inhibition effect of the compounds (6a-h) on the growth of various bacterial organisms is summarised in table given below along with the inhibition effect of standard drug ofloxacin for comparison purpose. The compounds (6b) and (6e) were found to be highly sensitive (bactericidal) against the microorganisms *E. coli* and *P. vulgaris* whereas moderately sensitive against *B. subtilis*. Majority of the compounds were found to be moderately sensitive against *S. aureus* and slightly sensitive against *B. subtilis*. Compound (6h) was resistant against almost all the microorganisms. To determine the minimum inhibitory concentration (MIC), the serial dilution technique was followed using nutrient broth medium. The MIC[23] values of compounds (6e) against *E. coli* and *P. vulgaris* were found to be 60 and 64 µg/mL respectively.

The synthesized compounds (6a) and (6b) were screened for their antifungal activity[24] against *C. albicans*. The potato dextrose agar (PDA) was used as a nutrient media. Discs of 5 mm size impregnated with test compound solutions of 100 µg/mL were placed in the fungal culture of *C. albicans* in petri plates and allowed to incubate at 26^0C for 4 days. The zones of inhibition were measured and compared with the action of fluconazole. The compounds (6a) and (6b) showed prominent inhibitory activity against *C. albicans*.

Antibacterial and antifungal activity of (5-aryl/alkylamino-[1,3,4]-oxadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines (6a-h)

Compounds	Microorganisms					
	E. coli	***S. aureus***	***S. typhi***	***B. subtilis***	***P. vulgaris***	**C. *albicans***
6a	R	S 14	R	S 14	S 11	S 20
6b	S 30	R	S 13	S 11	R	S 24
6c	S 15	S 17	R	S 12	S 12	-
6d	S 18	S 19	R	S 15	R	-
6e	S 22	S 15	S 11	S 17	S 23	-
6f	S 19	S 14	S 17	S 12	S 15	-
6g	R	S 16	S 19	S 14	S 14	-
6h	R	S 13	R	R	R	-
Standard	S 35	S 35	S 40	S 28	S 18	S 18
Standard drugs for antibacterial activity - ofloxacin and antifungal activity - fluconazole						

(Concentration 100 µg/ml) (Diameter of inhibition zone in mm)
R (Resistant) : (11 mm and below)
S (Sensitive) (Bactericidal) : (11 mm above)
Slightly Sensitive : (11 mm above to 15 mm)
Moderately Sensitive : (15 mm above to 20 mm)
Highly Sensitive : (20 mm above)

Experimental

Melting points of all the synthesized compounds were determined on a digital melting point apparatus (Veego, VMP-D) and are uncorrected. All chemicals used were of AR grade. The C, H and S analysis was carried out on Carlo-Erba analyser, N estimation was carried out on Colman-N-analyser-29. Purity of the title compounds were checked by TLC. All the reactions carried out in GMG20E-08-SLGX microwave oven at 800 W. IR spectra were recorded on Perkin-Elmer spectrophotometer using KBr disc. ^{1}H-NMR spectra were obtained on a Bruker-DRX-600 spectrophotometer in $CDCl_3$ with TMS as internal standard using $CDCl_3$ and DMSO-d_6 as solvents. Mass spectral measurements were carried out by EI method on a Jeol-JMC-300 spectrometer at 70 eV. The reagents used in the synthesis of (5-aryl/alkylamino-[1,3,4]-oxadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines (6a-h) have been prepared as follows.

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

The parent compound ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate (2) was prepared by irradiating the mixture of 2-amino-4,6-dimethyl-pyrimidine (1) (0.01 mole) and ethyl chloroacetate (0.01 mole) in 1,4-dioxane under microwave for 4 min. 10 sec. using anhydrous potassium carbonate as a catalyst. When 1,4-dioxane was evaporated, crude solid mass was obtained, it was crystallised from absolute ethanol and identified as ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate (2), yield 88%, m.p. 142^0C (Found: C, 55.11; H, 6.98; N, 20.10. 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)

The compound (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide (3) was prepared by irradiating the mixture of ethyl (4,6-dimethyl-pyrimidin-2-yl-amino)-acetate (2) (0.01 mole) and hydrazine hydrate (0.01 mole) in 1,4-dioxane under microwave for 1 min. 30 sec., progress of the reaction was monitored by TLC. The crude solid mass obtained was crystallised from absolute ethanol in cold condition (3), yield 88%, m.p. 138^0C (Found: C, 48.17; H, 6.38; N, 35.01. Calcd. for $C_8H_{13}N_5O$: C, 49.22; H, 6.71; N, 35.87%); IR: 3401, 3310 (NH), 1705 (C=O), 1628 (C=N), 1336 (C-N), 1156 cm^{-1} (N-N); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 7.38 (1H, s, CO-NH), 6.46 (1H, s, Pyrm-NH), 6.32 (1H, s, Pyrm-H), 3.57 (2H, s, CO-CH_2), 2.52 (2H, s, NH_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)

The mixture of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid hydrazide (3) (0.01 mole) and N-phenyl isothiocyanate (4a) (0.01 mole) in chloroform was irradiated in a microwave oven for 1 min. 30 sec., progress of the reaction was monitored by TLC. The crude solid mass obtained was crystallized from ethanol in cold condition and identified as (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide (5a), yield 79%, m.p. 134^{0}C (Found: C, 53.77; H, 5.05; N, 25.40; S, 9.08. 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): 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): (5b) (84%), m.p. 118^{0}C; (5c) (87%), m.p. 112^{0}C; (5d) (88%), m.p. 111^{0}C; (5e) (79%), m.p. 64^{0}C; (5f) (91%), m.p. 149^{0}C; (5g) (88%), m.p. 210^{0}C; (5h) (90%), m.p. 94^{0}C. The reactions were monitored on silica gel-G plates by TLC.

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

A paste of (4,6-dimethyl-pyrimidin-2-yl-amino)-acetic acid N-(N'-phenyl-thioamido)-hydrazide (5a) (0.01 mole) was prepared in ethanol. To this, iodine solution in ethanolic potassium hydroxide containing potassium iodide was added drop by drop with constant stirring, the colour of iodine initially disappeared and addition was continued till the violet colour of iodine persisted. The mixture was allowed to stand for 2 hr. The separated solid (4,6-dimethyl-pyrimidin-2-yl)-(5-phenyl-amino-[1,3,4]-oxadiazol-2-yl-methyl)-amine (6a) was crystallized from ethanol, yield 85%, m.p. 111^{0}C (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); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 6.88-7.91 (6H, m, Ar-H, Pyrm-H), 6.36 (1H, s, Ar-NH), 6.32 (1H, s, Pyrm-NH), 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): (6b) (80%), m.p. 97^{0}C (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); ^{1}H NMR ($CDCl_3$+DMSO-d_6): 6.95-7.62 (5H, m, Ar-H, Pyrm-H), 6.39 (1H, s, Ar-NH), 6.33 (1H, s, Pyrm-NH), 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^{0}C; (6d) (90%), m.p. 162^{0}C; (6e) (84%), m.p. 119^{0}C; (6f) (85%), m.p. 122^{0}C; (6g) (80%), m.p. 186^{0}C; (6h) (86%), m.p. 141^{0}C. The reactions were monitored on silica gel-G plates by TLC.

Conclusion

In present work microwave irradiative synthesis of (5-aryl/alkylamino-[1,3,4]-oxadiazol-2-yl-methyl)-(4,6-dimethyl-pyrimidin-2-yl)-amines has been reported. The compounds obtained were of good quality and purity with high % yield. Microwave assisted method applied for the synthesis is simple, efficient, fast, clean, economic and eco-friendly.

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