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Synthesis of Some Novel fused pyrazolo[4,5-e]pyrimidine derivatives bearing oxa/thiadiazole Nucleus as Potential Antimicrobial Agent

Ashutosh Singh[a], Abhishek Singh[b], Akhilesh Singh[a] & Priti Singh[a] and Vivek Singh[c]

[a]*Department of Chemistry, K.S. Saket PG College, Ayodhya, India*

[b]*Department of Chemistry, U.P. College, Varanasi, India*

[c]*Department of Botany, U.P. College, Varanasi, India*

[b]*Corresponding Author Email: abhupc@gmail.com*

Abstract

A series of some novel fused 1-(substituted phenoxymethyl)-7-(4-tolyloxymethyl)-4-oxopyrazolo [4,5-e][1,3,4]oxadiazolo[2,3-b]pyrimidine **(2)** & 1-(2-chlorophenoxymethyl)-7-(4-tolyloxymethyl)-4-oxopyrazolo-[4,5-e][1,3,4]thiadiazolo[2,3-b]pyrimidine **(3)** derivatives have been synthesized from Michael adduct 1-(2-methylphenoxymethyl)-5-amino-4-carboethoxypyrazoles **(1)** respectively which in turn have been prepared via intermolecular Michael addition, followed by cyclisation reaction between tolyloxyacetohydrazine and ethyl-2-cyano-3-ethoxyacrylate in methanol and glacial acetic acid as catalyst. The Michael adduct **(1)** afforded fused system **(2)** & **(3)** via nucleophilic addition followed by cyclisation with 2-(4-tolyloxymethyl)-5-mercapto-1,3,4-oxadiazole and 3-(4-chlorolphenyl)-5-mercapto-1,3,4-thiadiazole, respectively. The structures of these compounds have been established on the basis of spectral data IR, ^{1}H NMR, ^{13}C NMR and elemental analysis. All the synthesized compounds have been screened for antibacterial activity against Escherichia coli, Pseudomonas aeruginosa Streptococcus pneumonia and Bacillus subtilis and antifungal activity against fungi viz. Candida albicans, Aspergillus fumigatus, Aspergillus havue and Aspergillus niger. The results showed that these compounds are better antibacterial and antifungal agent as compared to the standard drug ciprofloxacin and fluconazole respectively.

Keywords: pyrazolopyrimidine**,** 1,3,4-oxadiazole, thiadiazole, Antifungal, Antibacterial activity

Introduction

In the hierarchy of man's need, the provision of food & control of disease come at the top of priority. Food is the ultimate outcome of agricultural crops. To have nutritional and pure forms of food, the crops must be free from various diseases. The first objective of biocides research must therefore be to control the harms, caused by micro-organisms, pests and weeds, which destroy our crops, stored grains, fruits, vegetables, fabrics, leather, plastic etc. But the problems have not been solved completely. It is because the number and kind of pests and microorganisms are numerous and mode of living of one set differs widely from others. Hence it is impossible to control almost every pests and microorganisms with a limited number of compounds in hand. Therefore, it is necessary to have a large number of compounds so that selection of compounds of versatile activities can be made. Thus the basis of selecting heterocycles as the subject of this investigation was realization of the fact that heterocyclic compounds are in clinical use since a long time derived from natural source such as vitamins, hormones, and antibiotics [1-2]. From the above observation we selecting the fused heterocyclic nucleus is prazolo[4,5-e]pyrimidine. Prazolo[4,5-e]pyrimidine is one of the most important class of fused heterocyclic nucleuses which possess a wide variety of biocidal activities. Pyrazolo [4,5-e]pyrimidine are reported to

encompass pharmacological potential as antiviral[4-7], anticoccidials[8-9], antimicrobial [10-13], antitumor [14-17], pesticides [18], inhibitory activity against c-Src Kinase and good CNS penetration agents[19], herbicidal [20] inflammatory [20-21], CNS depressant [22] agents in veterinary medicinal**.** 1,3,4-thia/oxadiazole heterocyclic rings were shows as promising biocidal and pharmaceutical activities such as anti-inflammatory [23-25], hypoglycemic [26], antifungal [27-30] and antibacterial [30-32]. By considering the above literature facts it was of interest to construct a system which may couple these three active pharmacophore nucleuses together in a single molecular framework. The investigation further appeared interesting due to planarity and compactness of such ring system is an additional factor for enhancing biological activities. Therefore, it was thought thiadiazole and oxadiazole nuclei were fused with prazolo[4,3-b]pyrimidine nucleus to yield the afforded fused targeted compound. These compounds were screened as better biocidal agents.

Experimental section:

Apparatus and Chemicals:

All reagents were purchased from Aldrich, solvents used were extra dried. Procedure for one typical case for each step has been described. All melting points were determined in open glass capillaries and are uncorrected. IR spectra were recorded in KBr disc on a Perkin-Elmer-157 spectrophotometer (cm^{-1}), ^{1}H NMR and ^{13}CNMR spectra in DMSO-d_6 on a Varian EM-360 (200 MHz) spectrometer using TMS as internal reference (chemical shift in δ ppm). Elemental (C, H, N) analysis indicated that calculated and observed values were within acceptable limit. The purity of compounds checked by this layer chromatography on silica gel plate using ether and ethyl acetate as solvent system. Iodine chamber was used as developing chamber.

General procedure for the preparation of 1-(2-methylphenoxymethyl)-5-amino-4-carboethoxypyrazoles (I)

A mixture of 2-tolyloxyacetohydrazine (1.80g. 0.01M) and ethyl-2-cyano-3-ethoxyacrylate (1.69g. .01M) was refluxed in methanol for four hours using 2-3 drops of glacial acetic acid as catalyst. The reaction mixture was cooled and poured into water. The solid mass re-crystallised out which was filtered, washed with water, dried and re-crystallised from aqueous ethanol.

All other compounds of (I) were prepared similarly [33].

General procedure for the preparation of 1-(substitutedphenoxymethyl)-7-(4-tolyloxymethyl)-4-oxopyrazolo [4,5-e][1,3,4]oxadiazolo[2,3-b]pyrimidines (2)

1-(Substitutedphenoxymethyl)-5-amino-4-carboethoxypyrazoles (I) (3.03g, 0.01M) and 2-(4-tolyloxymethyl)-5-mercapto-1, 3, 4-oxadiazole (2.20g, 0.01M) was refluxed in dioxane for six to seven hours. The reaction mixture was left for overnight and then poured into water. The solid mass separated out which was filtered, washed with water, dried and re-crystallised from aqueous ethanol.

Other compounds of the type (2a-2f) were prepared similarly.

1-(phenoxymethyl)-7-(4-tolyloxymethyl)-4-oxopyrazolo[4,5e][1,3,4]oxadiazolo[2,3-b]pyrimidines (2a): m. p. 145-146^{0}C, yield (59%). IR (KBr): 2995(C-H), 1690(C=O), 1634(C=N), 1500,1490,1430 (aromatic ring);1224, 1188(C-O-C); ^{1}H NMR (DMSO-d_6): 7.9(s, 1H, -N=C-H), 6.5-7.34 (m, 9H, Ar-H), 4.8 & 4.58(d,

4H,CH_2), 2.23(s, 3H, CH_3),^{13}C NMR (DMSO-d_6) :167.6, 162.1, 157.9, 156, 155, 133.2, 146, 130, 113, 71.3, 70; Calcd. $C_{22}H_{17}N_5O_5$: C, 61.25; H, 3.94; N, 16.24 Found: C, 61.40; H, 3.80; N, 16.04

1-(2-chlorophenoxymethyl)-7-(4-tolyloxymethyl)-4-oxopyrazolo[4,5e][1,3,4]oxadiazolo[2,3-b]pyrimidines (2b) : m. p. 173^{0}C, yield (64%). IR (KBr): 3005(C-H),1705(C=O), 1635(C=N), 1505,1495,1450 (aromatic ring);1224, 1188(C-O-C); ^{1}H NMR (DMSO-d_6): 7.9(s, 1H, -N=C-H), 6.5-7.7 (m, 9H, Ar-H), 4.8 & 4.75(d, 4H,CH_2), 2.23(s, 3H, CH_3), 2.04(s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) :168.6, 162.1, 157.9, 157, 155, 133.2, 146,3, 130, 114, 72.3, 71; Calcd. $C_{22}H_{16}N_5O_5Cl$: C, 56.71; H, 3.44; N, 15.04 Found: C, 56.61; H, 3.60; N, 14.94

1-(4-chlorophenoxymethyl)-7-(4-tolyloxymethyl)-4-oxopyrazolo[4,5e][1,3,4]oxadiazolo[2,3-b]pyrimidines (2c): m. p. 168^{0}C, yield (60%). IR (KBr): 3005(C-H atom),1705(C=O), 1638(C=N), 1505,1495,1450 (aromatic ring);1235, 1190(C-O-C); ^{1}H NMR (DMSO-d_6): 7.9(s, 1H, -N=C-H), 6.8-7.8 (m, 8H, Ar-H), 4.8 & 4.95(d, 4H,CH_2), 2.25(s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) :168.6, 162.1, 157.9, 157, 155, 133.2, 146,3, 130, 114, 72.3, 71; Calcd. $C_{22}H_{16}N_5O_5Cl$: C, 56.71; H, 3.44; N, 15.04 Found: C, 56.61; H, 3.24; N, 14.89

1-(2,4-diclorohylphenoxymethyl)-7-(4-tolyloxymethyl)-4-oxopyrazolo[4,5e][1,3,4]oxadiazolo[2,3-b]pyrimidines (2d): m. p. 195-196^{0}C, yield (65%). IR (KBr): 3015(C-H), 1720(C=O), 1640(C=N), 1515,1495,1460 (aromatic ring);1240, 1195(C-S-C); ^{1}H NMR (DMSO-d_6): 7.9(s, 1H, -N=C-H), 6.8-7.9 (m, 7H, Ar-H), 4.8 & 5.0(d, 4H,CH_2), 2.28(s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) :170, 166, 159, 158, 156, 135.2, 147,3, 132, 115, 72.3, 71; Calcd. $C_{22}H_{15}N_5O_5Cl_2$: C, 56.80; H, 3.00; N, 14.00 Found: C, 56.60; H, 2.89; N, 16.78

1-(4-methylphenoxymethyl)-7-(4-tolyloxymethyl)-4-oxopyrazolo[4,5e][1,3,4]oxadiazolo[2,3-b]pyrimidines (2e): m. p. 150^{0}C, yield (54%). IR (KBr): 2900(C-H), 1685(C=O), 1634(C=N), 1495,1490,1433 (aromatic ring);1224, 1188(C-O-C); ^{1}H NMR (DMSO-d_6): 7.9(s, 1H, -N=C-H), 6.5-7.7 (m, 8H, Ar-H), 4.8 & 4.75(d, 4H,CH_2), 2.21(s, 3H, CH_3), 2.04(s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) :167, 161.1, 157.3, 156, 155, 133.2, 146,3, 130, 113, 72.3, 71, 21.; Calcd. $C_{23}H_{19}N_5O_5$: C, 62.02; H, 4.26; N, 16.73 Found: C, 62.02; H, 4.06; N, 15.63

1-(2-methylphenoxymethyl)-7-(4-tolyloxymethyl)-4-oxopyrazolo-[4,5-e][1,3,4]oxadiazolo[2,3-b]pyrimidines (2f) : m. p. 165^{0}C, yield (62%). IR (KBr): 2900(C-H), 1685(C=O), 1630(C=N), 1495,1490,1432 (aromatic ring);1224, 1188(C-O-C); ^{1}H NMR (DMSO-d_6): 7.9(s, 1H, -N=C-H), 6.5-7.7 (m, 8H, Ar-H), 4.8 & 4.75(d, 4H,CH_2), 2.23(s, 3H, CH_3), 2.04(s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) :167, 161.1, 157.3, 156, 155, 133.2, 146,3, 130, 113, 72.3 70, 21; Calcd. $C_{23}H_{19}N_5O_5$: C, 63.61; H, 4.10; N, 16.87 Found: C, 63.74; H, 4.21; N, 16.78

General procedure for the preparation of 1-(substitutedphenoxymethyl)-7-(4-chlorolphenyl)-4-oxopyrazolo [4,5-e][1,3,4]thiadiazolo[2,3-b]pyrimidines (3):

The 1-(Substitutedphenoxymethyl)-5-amino-4-carboethoxypyrazoles (1) (3.03g, 0.01M) and 3-(4-chlorolphenyl)-5-mercapto-1,3,4-thiadiazole (2.29g, 0.01M) was refluxed in dioxane for six to seven

hours. The reaction mixture was left for overnight and then poured into water. The solid mass separated out which was filtered, washed with water, dried and re-crystallised from aqueous ethanol.
Other compounds of the type (3a-3f) were prepared similarly.

1-(phenoxymethyl)-7-(4-chlorolphenyl))-4-oxopyrazolo[4,5-e][1,3,4]thiadiazolo[2,3-b]pyrimidines (3a): m. p. 142^{0}C, yield (58%). 2995(C-H), IR (KBr): 2995(C-H),1690(C=O), 1610(C=N), 1495,1494,1433 (aromatic ring),1238(C-S-C); ^{1}H NMR (DMSO-d_6): 7.7(s, 1H, -N=C-H), 6.84-7.95 (m, 8H, Ar-H), 4.3(d, 2H,OCH_2), ^{13}C NMR (DMSO-d_6) :168, 162.1, 157, 156, 155, 142, 134.3, 130.2,125, 115, 117, 71; Calcd. $C_{21}H_{14}N_5O_3SCl$; C, 54.85; H, 2.74; N, 16.00 Found: C, 54.91; H, 2.63; N, 16.07

1-(2-chlorophenoxymethyl)-7-(4-chlorolphenyl))-4-oxopyrazolo[4,5-e][1,3,4]thiadiazolo[2,3-b]pyrimidines (3b): m. p. 123^{0}C, yield (63%). 2995(C-H), IR (KBr): 3005(C-H) 1705(C=O), 1630(C=N), 1510,1500,1495 (aromatic ring),1238(C-S-C); ^{1}H NMR (DMSO-d_6): 7.8(s, 1H, -N=C-H), 6.84-7.95 (m, 8H, Ar-H), 4.35(d, 2H,OCH_2), ^{13}C NMR (DMSO-d_6) :168, 162.1, 157, 156, 155, 142, 134.3, 130.2,125, 115, 117, 71, ; Calcd. $C_{20}H_{11}N_5O_3SCl_2$; C, 50.85; H, 2.33; N, 14.83 Found: C, 50.90; H, 2.42; N, 14.75

1-(4-clorohylphenoxymethyl)-7-(4-chlorolphenyl))-4-oxopyrazolo[4,5-e][1,3,4]thiadiazolo-[2,3-b]pyrimidines (3c): m. p. 130-131^{0}C, yield (60%). 2995(C-H), IR (KBr): 3007(C-H), 1705(C=O), 1630(C=N), 1510,1500,1495 (aromatic ring),1240(C-S-C); ^{1}H NMR (DMSO-d_6): 7.8(s, 1H, -N=C-H), 6.84-5.1 (m, 8H, Ar-H), 4.35(d, 2H,OCH_2); ^{13}C NMR (DMSO-d_6) :168, 162.1, 157, 156, 155, 142, 134.3, 130.2,125, 115, 117, 71; Calcd. . $C_{20}H_{11}N_5O_3SCl_2$; C, 50.85; H, 2.33; N, 14.83 Found: C, 50.963; H, 2.40; N, 14.74

1-(2,4-dichlorophenoxymethyl)-7-(4-chlorolphenyl))-4-oxopyrazolo[4,5-e][1,3,4]thiadiazolo[2,3-b]pyrimidines (3d)m. p. 145^{0}C, yield (65%). 2995(C-H), IR (KBr): 3015(C-H), 1715(C=O), 1634(C=N), 1520,1505,1500 (aromatic ring),1242(C-S-C); ^{1}H NMR (DMSO-d_6): 7.9(s, 1H, -N=C-H), 6.91-8.0 (m, 8H, Ar-H), 4.64(d, 2H,OCH_2); ^{13}C NMR (DMSO-d_6) :168.3, 162.1, 157, 156, 155, 143, 134.3, 132,127, 116, 117, 71; Calcd. $C_{20}H_{10}N_5O_3SCl_3$; C, 47.38; H, 1.93; N, 13.82 Found: C, 47.28; H, 2.00; N, 13.93

1-(4-methylphenoxymethyl)-7-(4-chlorolphenyl))-4-oxopyrazolo[4,5-e][1,3,4]thiadiazolo[2,3-b]pyrimidines (3e): m. p. 142^{0}C, yield (58%). 2995(C-H), IR (KBr): 2995(C-H), 1695(C=O), 1610 (C=N), 1490,1492,1435 (aromatic ring),1236(C-S-C); ^{1}H NMR (DMSO-d_6): 7.6(s, 1H, -N=C-H), 6.75-7.61 (m, 8H, Ar-H), 4.20(d, 2H,OCH_2), 2.23(s, 3H, CH_3), ^{13}C NMR (DMSO-d_6) :168, 162.1, 157, 156, 155, 142, 132.3, 130.,125, 115, 113, 71, 21; Calcd. $C_{21}H_{14}N_5O_3SCl$; C, 55.81; H, 3.10; N, 15.50 Found: C, 55.85; H, 3.05; N, 15.64

1-(2-methylphenoxymethyl)-7-(4-chlorolphenyl)-4-oxopyrazolo[4,5-e][1,3,4]thiadiazolo[2,3-b]pyrimidines (3f): m. p. 126^{0}C, yield (64%). 2995(C-H), IR (KBr): 2995(C-H), 1695(C=O), 1610(C=N), 1490,1492,1435 (aromatic ring),1236(C-S-C); ^{1}H NMR (DMSO-d_6): 7.6(s, 1H, -N=C-H), 6.75-7.62 (m, 8H, Ar-H), 4.20(d, 2H,OCH_2), 2.21(s, 3H, CH_3), ^{13}C NMR (DMSO-d_6) :168, 162.1, 157, 156, 155, 142, 134.3, 130.2,125, 115, 117, 71, 21; Calcd. $C_{21}H_{14}N_5O_3SCl$; C, 55.81; H, 3.10; N, 15.50 Found: C, 55.81; H, 3.00; N, 15.65

Antibacterial activity

All the newly synthesized fused heterocyclic compounds (2a-2f and 3a-3f) were screened for their antibacterial activity in vitro against S. pneumoniae, B. subtilis, E. coli and P. aeruginosa by disc diffusion method [29]. The bacterial strains were sub-cultured in both agar and incubated for 18 h at 37^0C and freshly prepared bacterial cells were spread onto nutrient agar plate in laminar flow cabinet. Sterilized paper disc (6.0 mm in diameter) were placed on nutrient agar plate. 5mg was dissolved in 1mL of dimethylsulfoxide (DMSO) separately to prepare stock solution. From stock solution different concentration 50 μg /mL of each compound were prepared. Thus, proper amount of different concentrations of compounds were piped on blank disc, which were placed on the plates. The plate were incubated at 37^0C 24 h. The minimum inhibitory concentration (MICs), the lowest concentration (μg/mL) of the test compound that result no visible growth on plates was recorded. DMSO was used as a solvent control to insure that solvent had no effect on bacterial growth. Ciprofloxacin was designated in our experiment as control drug.

Antifungal Activity

The antifungal activity of all newly synthesized fused hetrocyclic compounds (2a-2f and 3a-3f) were determined against C. albicans, A. fumigates, A. flavus and A. niger (recultured) in DMSO by serial plate dilution method [33], Test compound 15 mg were dissolved in 1 mL of dimethylsulfoxide (DMSO) and solution was diluted with water (9 mL). Further progressive dilution with melted Mutler-Hinton agar were performed to obtained required concentrations of 50 μg/mL, 25 μg/mL , 12.5μg/mL, 6.25 μg/mL, 3.12 μg/mL and 1.56 μg/mL. Petri-dishes were incubated at 37^0C for 26h. The minimum inhibitory concentration (MIC in μg/mL) was noted. To insure that solvent has no effect on fungal growth a control test was performed with use medium supplemented with DMSO at the same dilution as used in experiment Ketoconazole and Fluconazole were used as a standard drug. The results are incorporated in Table-2.

Results and discussion

All the final compounds (**2a-42f and 3a-3f**) were prepared in good yields. The physical parameters of these compounds are mentioned in section and synthetic route is given in scheme 1. The structure of intermediates and final compounds were evidenced by their elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and D_2O exchange studies.

Pharmacology:

Antibacterial studies

All the synthesized compounds **2a-2f** and**-3a-3f** were screened for antibacterial activity in vitro, against Streptococcus pneumoniae, Bacillus subtilis, E. coli and Pseudomonas aeruginosa by disc diffusion method [34].

The activity of antibacterial screening data (table-1) revealed that the entire tested compound, showed moderate to very good inhibitory activity. The compounds **2a**, **3b**, **3c** and **3d** showed comparatively very good inhibitory activity against all tested bacteria with MIC 3.12 μg/mL, 6.25 μg/mL. Whereas the rest of the compounds showed moderate activity with MICs 12.5 μg/mL, 25 μg/mL and 50 μg/mL as compared

to standard drug Ciprofloxacin (MIC= 1.56 μg/mL, 3.12 μg/mL and 6.25 μg/mL). Compounds **3b** and **3c** were equipotent as Ciprofloxacin against P. aeruginsa with MIC 3.12 μg/mL whereas compound **3d** was more potent against pathogenic bacteria E. coli. The good activity is attributed to the presence of pharmacologically active group like 2-chloro, 4-chloro and 2,4-dichloro, the tested newly synthesized compounds. Therefore it can be inferred that presences of polar substituent like Cl played noticeable antibacterial activity while two chlorine atoms together work well of these compounds. The presence of alkyl group (-CH_3) in the phenyl ring does not important activity. The fusion of three heterocyclic at ring C-2 and N-3 of main pyrimidine viz pyrazole, 1,3,4-thiadiazole, and 1,3,4 oxadiazole ring exert a significance influence on the antibacterial activity. The fusion of 1,3,4 thiadiazole ring with pyrazolo[4,5-e]pyrimidine showed greater activity than that of 1,3,4 oxadiazole fused other systems. This reveals that, more activity of thiadiazole fused system than other oxadiazole fused system towards antibacterial activity probably by virtue of incorporating a toxophoric N=C-S linkage.

Antifungal Studies:

All the twelve compounds were screened for their antifungal activity against Candida albicans, Aspergillus famigatus, Aspergillus flavous and Aspergellus niger, (re-cultured) in DMSO by serial plate dilution methods. The Fluconazole, a commercial fungicide was also tested for comparison.

The screening data of antifungal activity of these series of compounds showed moderate to good antifungal activity.

It is of interest that compounds **2b, 3b, 3c** and **3d** showed pronounced antifungal activity against several tested pathogenic fungi with MIC 3.12 μg/mL and 6.25 μg/mL while compound of **3b, 3c and 3d** showed promising activity against A. niger and A. fumigatus with MIC 3.12 μg/mL.

It is interesting to note that a minor alteration in the molecular configuration of investigated compounds may have pronounced effect on antimicrobial studies. Compounds **2a**, **3a** having no substituent in phenyl ring are less active while compound having 2-chloro, 4-chloro, 2,4-chloro groups in **3b, 3c** and **3d** are more active than all other compounds. Therefore it can be inferred that the number of polar substituent like Cl increases, the antifungal activity increases The compounds a fused1,3,4-triadiazole ring at C-2 and N-3 of parent compound pyrazolo[4,5-e]pyrimidine, were found more potent than those having fused oxadiazole ring.

Conclusion

The antibacterial and antifungal activity results of this investigation revealed that, 1,3,4 thiadiazole ring fused with pyrazolo[4,5-e]pyrimidine nucleus are more potent than 1,3,4 oxadiazole fused other systems. The presence of 2-chloro, 4-chloro and 2,4-dichloro moiety with pyrazolo [4,5-e]pyrimidine bearing thiadiazole fused nucleus, import much antibacterial activity towards all tested bacteria. The fused compound **4d** has highest activity against antibacterial screening. Most of the titled compounds were found to have strong antifungal activities on A. niger than other species of fungi. Therefore, it is inferred that the fusion of 1,3,4-thiadiazole ring with pyrazolo[4,5-e]pyrimidine showed better antimicrobial activity.

The importance of such work lies in the possibility that the new compounds might be a more efficacious drug against bacteria and fungi for which a thorough investigation regarding the structure activity relationship, toxicity and its biological effects is essential, which could be helpful in designing more antimicrobial agents for therapeutic use.

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Table: 1

Antibacterial activities of compounds (2a-3f)

MICs (μg/mL)

Compound No.	R	E. coli	P. aeruginsa	S.pneumoniae	B. subtilies
2a	**H**	>25	>50	>25	>50

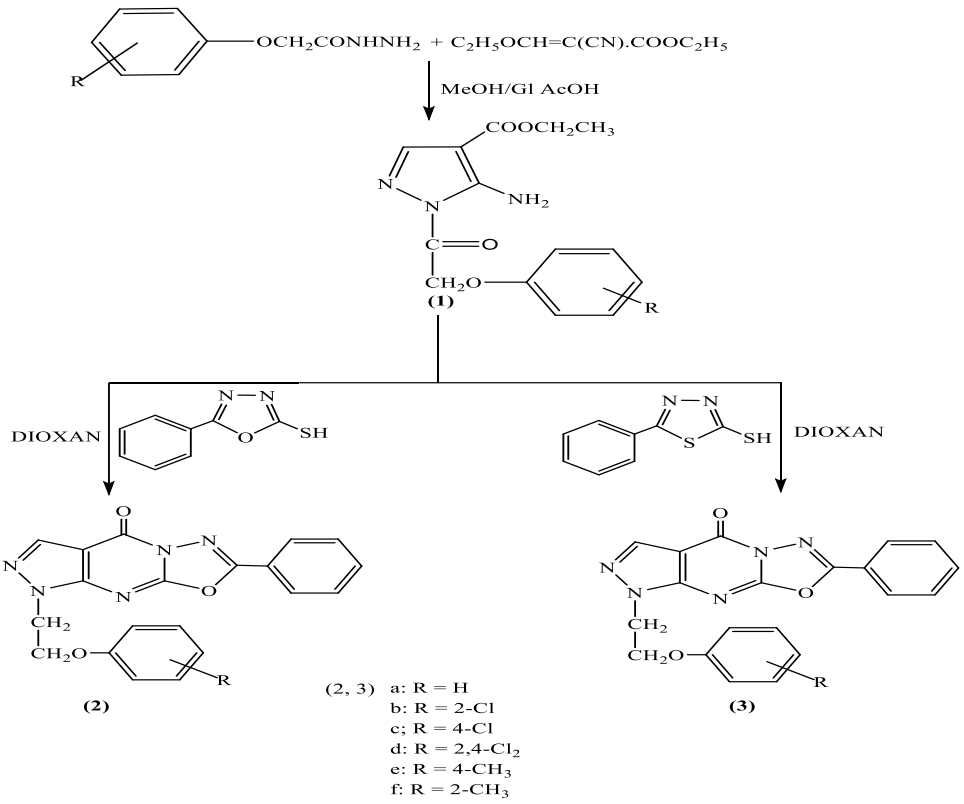
2b	**2-Cl**	3.12	12.5	12.5	6.25
2c	**4-Cl**	12.5	6.25	3.12	12.5
2d	**2,4-Cl_2**	12.5	6.25	12.5	25
2e	**4-CH_3**	12.5	25	12.5	50
2f	**2-CH_3**	25	12.5	25	25
3a	**H**	50	25	25	50
3b	**2-Cl**	25	12.5	25	3.12
3c	**4-Cl**	6.25	12.5	6.25	3.12
3d	**2,4-Cl_2**	6.25	3.12	3.12	6.25
3e	**4-CH_3**	25	12.5	25	12.5
3f	**2-CH_3**	12.5	25	25	12.5
Fluconazole	**-**	1.56	6.25	3.12	1.56

Table: 2

Antifungal activities of compounds (2a-3f)

MICs (μg/mL)

Compound No.	**R**	**C.albicans**	**A. fumigatus**	**A. flavus**	**A. niger**
2a	**H**	>50	>25	>50	>25
2b	**2-Cl**	12.5	3.12	12.5	6.25
2c	**4-Cl**	6.25	12.5	12.5	25
2d	**2,4-Cl_2**	12.5	6.25	12.5	3.12
2e	**4-CH_3**	25	50	12.5	25
2f	**2-CH_3**	12.5	25	50	12.5
3a	**H**	>25	>50	>25	>50
3b	**2-Cl**	6.25	12.5	3.12	12.5
3c	**4-Cl**	3.12	6.25	25	6.25
3d	**2,4-Cl_2**	6.25	3.12	3.12	12.5
3e	**4-CH_3**	12.5	25	50	12.5
3f	**2-CH_3**	25	50	12.5	25
Fluconazole	**-**	**1.56**	**3.12**	**6.25**	**1.56**


OCH2CONHNH2 + C2H5OCH=C(CN).COOC2H5
R
MeOH/Gl AcOH
COOCH2CH3
NH2
C=O
CH2O
(1)
DIOXAN
SH
DIOXAN
SH
(2)
(3)
(2, 3) a: R = H
b: R = 2-Cl
c; R = 4-Cl
d: R = 2,4-Cl2
e: R = 4-CH3
f: R = 2-CH3

Scheme 1