Research Article

5-(2-AMINOPHENYL)-1,3,4-OXADIAZOLE-2(3H)-THIONE DERIVATIVES: SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION

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ABSTRACT

A series of 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione derivatives have been synthesized by mannich reaction. The reaction progress of the synthesized compounds was checked by TLC. The different in melting points of the synthesized compounds indicated the formation of new chemical analogues. The structures of the newly synthesized compounds were confirmed by IR and ^{1}H NMR spectral data. *Invitro* anti-microbial activity was evaluated by disc diffusion method for all the newly synthesized compounds against gram +ve organisms such as *Staphylococcus aureus, Streptococcus pyogenes,* gram –ve organisms such as *Escherichia coli, Klebsiella aerogenes* and fungus such as *Candida albicans*. Amikacin and ketoconazole (10 µg/ml) were used as reference standard for antibacterial and antifungal activity respectively. Compounds 1a, 1b, 1c and 1d showed moderate antibacterial and antifungal activities at a concentration of 100 µg/ml.

Keywords: 1, 3, 4-oxadiazole, mannich reaction, antibacterial, antifungal.

INTRODUCTION

Nitrogen containing heterocycles with an oxygen atom are considered as an important class of compounds in medicinal chemistry because of their interesting diversified biological applications[1]. During the past years considerable evidences have also accumulated to demonstrate the efficacy of 1,3,4-oxadiazoles including antibacterial[1], antifungal[2], anthelmintic[3], antitubercular[4], anticancer[5], antiHIV[6], antioxidant[7], analgesic[8], anti-inflammatory[9] and anticonvulsant[10] activities.

A large number of oxadiazole derivatives have been prepared and many of these compounds have shown a wide spectrum of antimicrobial activity. Some oxadiazoles with different substituent at different location on the heterocyclic ring resulted in fungicidal and bactericidal agents of various potencies[11]. These observations prompted us to synthesis 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione followed by a novel series of mannich bases of 5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione.

MATERIALS AND METHODS

Melting points were determined on electrical melting point apparatus by open-ended capillary tube and were uncorrected. TLC using Silica Gel as stationary phase and chloroform-methanol (8:2) as eluent was used to check the purity of the compounds and the spots were visually detected in an Iodine chamber. The structure of the synthesized compounds was elucidated by IR spectra in υ_{max} (cm^{-1}) on FT-IR (Shizmadu-8400 series) using KBr disc technique and ^{1}H NMR spectra in δ units (ppm) relative to an internal standard of tetramethylsilane on ^{1}H NMR (Brucker 400 MHz) in DMSO-d_6. The synthetic method is depicted in Scheme 1.

Synthesis of substituted 1,3,4-oxadiazole (1)

Anthranilic acid (0.01 mol) was dissolved in 20 ml ethanol. To this, concentrated sulphuric acid was added by drop wise until the white precipitate was formed and dried. Then the residue was dissolved in ethanol and to this solution, hydrazine hydrate (0.5 mol) was added with constant shaking for 10 min. The white precipitate obtained was collected by filtration and dissolved in ethanol. Potassium hydroxide (0.56 mol) was added into the above solution followed by carbon disulphide solution (0.76 mol) drop wise by constant shaking until the formation of yellow precipitate of substituted oxadiazole **(1)** and recrystallized from ethanol[12].

Synthesis of Mannish base substituted 1,3,4-oxadiazoles (1a-f)

A same experimental condition was followed for the synthesis of titled compounds. Equimolar quantities (0.01mol) of substituted oxadiazole **(1)** and respective compound containing secondary amine such as N-(4-hydroxyphenyl) acetamide, N-(2,3-xylyl) anthranilic acid and potassium 2-(2-(2,6-dichloro anilino) phenyl) acetate were dissolved in ethanol (30 mL). To this the corresponding aldehyde (0.01 mol) such as formaldehyde and acetaldehyde was added and reflux for 3-5 h. The content was kept overnight in the freezer. The respective compound obtained **(1a-f)** was recrystallised from ethanol[13].

5-(2-aminophenyl)-1,3,4-oxadiazole-2(3H)-thione (1)

$C_8H_7N_3OS$; Yield: 85.5%;; mp: 155-157^{0}C; R_f: 0.45; IR (KBr, υ_{max} cm^{-1}): 3482.24 (NH_2), 3154 (NH), 1624.32 (N –N), 1224.21 (C=S), 1224.21 (C=S); ^{1}H NMR (δ ppm): 3.8 (s, NH_2, 2H), 6.0 (s, NH, 1H), 6.6 – 7.8 (m, ArH, 4H).

N-((5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methyl)-N-(4-hydroxyphenyl) acetamide (1a)

$C_{17}H_{16}N_4O_3S$; Yield: 71.78%; mp: 178-180^{0}C; R_f 0.581.IR (KBr, υ_{max} cm^{-1}): 3426 (OH), 3343 (NH_2), 1651 (N –N), 1457(CH_3), 1426 (CH_2) 1120 (C=S); ^{1}H NMR (δ ppm): 2.2(s, 3H, CH_3), 3.5 (s, 2H, NH_2), 4.7(s, 2H, CH_2), 6.6 – 7.4 (m, 8H, ArH), 9.8 (s, 1H, OH).

N-(1-(5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)ethyl)-N-(4-hydroxyphenyl) acetamide (1b)

$C_{18}H_{18}N_4O_3S$; Yield: 70.76%; m.p.198-200 ^{o}C; R_f 0.641. IR (KBr, υ_{max} cm^{-1}): 3386 (OH), 3286 (NH_2), 2084 (CH), 1605(N-N), 1454 (CH_3), 1422 (CH_2), 1122 (C=S); ^{1}H NMR (δ ppm): 1.28 (d, 3H, CH_3), 1.98 (s, 3H, CH_3), 3.42 (s, 2H, NH_2), 4.64(q, 1H, CH), 6.46 – 7.44 (m, 8H, ArH), 9.62 (s, 1H, OH).

Potassium-2-(2-(((5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl) methyl) (2,6-dichloro phenyl) amino) phenyl) acetate (1c)

$C_{23}H_{17}N_4O_3SCl_2K$; Yield: 69.19%; m.p. 210-212 ^{o}C; R_f 0.612. IR (KBr, υ_{max} cm^{-1}):. 3257 (NH_2), 1650 (C=O), 1615 (N-N), 1436(CH_2), 1120(C=S); ^{1}H NMR (δ ppm) : ^{1}H NMR (δ ppm): 3.44 (s, 2H, CH_2), 4.62 (s, 2H, CH), 4.26 (s, 2H, NH_2), 6.52 – 7.48 (m, 11H, ArH),

Potassium-2-(2-((1-(5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl) ethyl) (2,6-dichloro phenyl) amino) phenyl) acetate (1d)

$C_{24}H_{19}N_4O_3SCl_2K$; Yield: 59.18%; m.p. 186-188 ^{o}C; R_f 0.812. IR (KBr, υ_{max} cm^{-1}): 3272 (NH_2), 1649 (C=O), 1622 (N-N), 1456 (CH_3), 1449 (CH_2), 1121(C=S); ^{1}H NMR (δ ppm): 1.26 (d, 3H, CH_3), 3.52 (s, 2H, CH_2), 3.98 (q, 1H, CH), 4.12 (s, 2H, NH_2), 6.36 – 7.24 (m, 11H, ArH), 9.62 (s, 1H, OH).

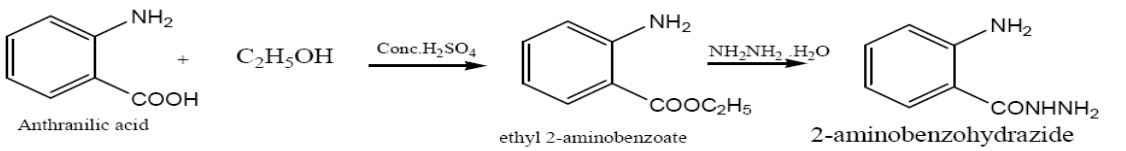
2-(((5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)methyl) (2,3-dimethylphenyl) amino benzoic acid (1e)

$C_{24}H_{22}N_4O_3S$; Yield: 68.70 %; m.p 254-256 ^{o}C; R_f 0.840 . IR (KBr, υ_{max} cm^{-1}):. 3335 (OH). 3226 (NH_2), 1675(C=O), 1615 (N-N), 1459 (CH_3),1439 (CH_2), 1190 (C=S); ^{1}H NMR (δ ppm) : 2.28 (s, 3H, CH_3), 4.46(s, 2H, CH_2), 3.82 (s, 2H, NH_2), 6.16 – 7.64 (m, 11H, ArH), 10.46 (br, 1H, OH).

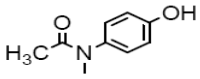
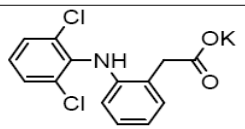
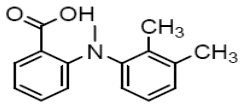
2-((1-(5-(2-aminophenyl)-2-thioxo-1,3,4-oxadiazol-3(2H)-yl)ethyl) (2,3- dimethylphenyl) amino) benzoic acid (1f)

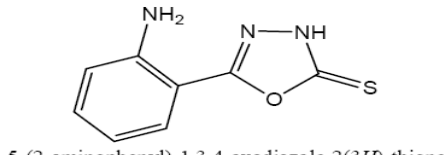
$C_{25}H_{24}N_4O_3S$; Yield: 70.76 %; m.p. 244-246 ^{o}C; R_f 0.770. IR (KBr, υ_{max} cm^{-1}):. 3343 (OH), 3286 (NH_2), 1650 (C=O), 1628 (N-N), 1449 (CH_3), 1428 (CH_2), 1123 (C=S); ^{1}H NMR (δ ppm) 1.24 (d, 3H, CH_3), 2.26 (s, 3H, CH_3), 3.72 (s, 2H, NH_2), 6.08 – 8.24 (m, 11H, ArH), 10.64 (br, 1H, OH).

Scheme 1



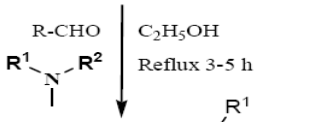
Where,

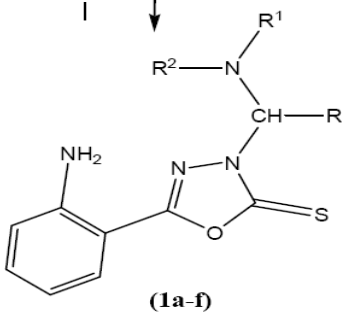
Compound Code	R	R^1, R^2
1a	-H	
1b	-CH_3	
1c	-H	
1d	-CH_3	
1e	-H	
1f	-CH_3	



5-(2-aminophenyl)-1,3,4-oxadiazole-2(3*H*)-thione

(1)





(1a-f)

Table 1: Antimicrobial activity-sensitivity testing of compounds

Compound code	Zone of inhibition (mm)				
	Antibacterial Activity				*Antifungal Activity*
	S. aureus	*S. pyogenes*	*E. coli*	*K. aerogenes*	*C. albicans*
1a	10	13	12	8	14
1b	13	11	14	9	12
1c	12	13	15	9	14
1d	12	11	13	10	13
1e	9	9	10	7	11
1f	8	9	9	6	10
Amikacin	16	15	17	18	-
Ketoconazole	-	-	-	-	18

ANTIMICROBIAL ACTIVITY

Collection of Microorganisms

Gram positive organisms such as *Staphylococcus aureus*, *Streptococcus pyogenes*, gram negative organisms such as *Escherchia coli*, *Klebsiella aerogenes* and fungus *Candida albicans* were used for this study. The cultures of microorganisms were obtained from microbiology lab, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamilnadu, India.

Disc Diffusion Method

The disc diffusion method[14] was used to evaluate antimicrobial activities. For susceptibility testing, 100 µg/ml of synthesized compound was prepared in 2% CMC. Sterile antibiotic assay discs (Whatman, 6 mm) were impregnated with 100 µL of the test compounds and were dried completely. The discs were placed on the surface of agar dispersion plates inoculated with microbes. 2%CMC saturated assay discs and blank assay discs were used as negative controls. Standard antibiotics disc such as Amikacin (10 µg/disc), and ketoconazole (10 µg/disc) were used as positive controls. Plates of Gram +ve organisms such as *Staphylococcus aureus, Streptococcus pyogenes* and Gram –ve organisms such as *Escherichia coli, Klebsiella aerogenes* and fungus such as *Candida albicans* were incubated at 35^0C in an incubator for 24 h and 48 h respectively. Inhibition zones were recorded as the diameter of growth-free zones, including the diameter of the disc, at the end of the incubation period. All the compounds were tested in triplicate and the average reading was taken.

RESULTS AND DISCUSSION

A series of six novel mannich bases of 1,3,4-oxadiazole derivatives were synthesized using mannich reaction by the reaction between compounds having secondary amine and respective aldehydes. The formation of new chemical analogues was accused by the melting point and R_f value. The structure of the synthesized compounds was established by spectral data such as IR and ^{1}HNMR spectrums. *Invitro* anti-microbial activity was evaluated by disc diffusion method for all the newly synthesized compounds against gram +ve organisms such as *S. aureus, S. pyogenes*, gram –ve organisms such as *E. coli, K. aerogenes* and fungus such as *C. albicans*.

Compound 1a, 1b, 1c and 1d at a concentration of 100 µg/ml showed good antibacterial and antifungal activities against all the tested organisms followed by 1e and 1f. All the compounds at a concentration of 100 µg/ml showed mild antibacterial activity against *K. aerogenes.* Amikacin and ketoconazole (10 µg/ml) were used as reference standard for antibacterial and antifungal activity respectively.

CONCLUSION

All the newly synthesized compounds showed moderate to mild antimicrobial activity. These findings concluded that the titled compounds have the property to kill the microbes in some extent when compared with standard drug; it gives a future scope to study the mechanism of action and would be worthy of further investigation.

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