ACTA SCIENTIFIC MEDICAL SCIENCES (ISSN: 2582-0931)

Volume 4 Issue 5 May 2020

Research Protocol

Investigation of Anti-Microbial Activity of 5-(6-(2,4-Dichlorophenyl)-[1,2,4] Triazolo[3,4-b][1,3,4] Thiadiazol-3-yl)Benzene-1,2,3-Triol

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Received: April 09, 2020

Published: May 04, 2020

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Abstract

At present drug discovery includes the identification of screening hits, medicinal chemistry and optimization of these hits to increase the affinity, selectivity (to reduce the potential of side effects), efficacy/potency, metabolic stability (to increase the half-life) and oral bioavailability.

Antimicrobial agents are any variety of c compounds that can destroy or prevent the growth of microorganisms. In the present paper antimicrobial activity of 5-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)benzene-1,2,3-triol was screened for antimicrobial activity against two representatives of Gram-positive bacteria viz. *S. aureus, B. subtilis*, two Gram-negative bacteria viz. *E. coli, P. aeruginosa* and two fungi viz. *C. albicans, A. niger* by the broth microdilution MIC method. Results suggest that the compound possess optimum activity when compared with standard drug.

Keywords: Anti-Microbial; Triazolo-Thiadiazole Derivative; Standard Drug

Introduction

5-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)benzene-1,2,3-triol; Yield: 72%; m.p.: 124 - 126° C; R_f: 0.67; FT-IR υ_{max} (KBr, cm^{-1}): 3451 (NH stretching), 3089 (Ar- CH stretching), 2930 (aliphatic-CH stretching), 1796 (C=O stretching), 1531 (C=C bending), 1148 (C-O-C stretching), 1317 (CH bending), 1234 (C-N stretching), 1208 (C=N-N stretching), 1067 (O-CH_3 stretching), 782 (C-Cl stretching), 748 (substituted benzene), 678 (C-S bending); ^{1}H NMR δ ($CDCl_3$, ppm): 6.5 - 8.2 (m, 11H, aromatic), 5.2 (s, 2H, -$COOCH_2$-), 4.4 (s, 1H, NH), 3.45 (s, 2H, -CH_2COO-), 3.25 (s, 3H, -OCH_3); MS: 540.54 (M^+, 40%), 470 (30%), 405 (30%), 320 (28%), 285 (48%), 240 (48%), 215 (80%), 180 (36%), 132 (100%), 107 (28%), 77 (50%). In the present paper anti-microbial activity of 5-(6-(2,4-dichlorophenyl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-3-yl)benzene-1,2,3-triol was evaluated against bacterial and fungal species.

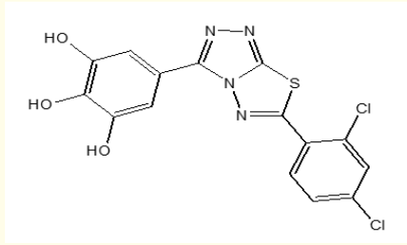

Figure 1: Structure of 5-(6-(2,4-dichlorophenyl)-[1,2,4] triazolo[3,4-b][1,3,4] thiadiazol-3-yl)benzene-1,2,3-triol.

Methodology

Antimicrobial activity of the compounds and standard drugs were assessed against two representatives of Gram-positive bacteria viz. *S. aureus*, *B. subtilis*, two Gram-negative bacteria viz. *E. coli*, *P. aeruginosa* and two fungi viz. *C. albicans*, *A. niger* by the broth microdilution MIC method. Mueller Hinton broth and Sabouraud dextrose broth were used as a nutrient medium to grow and dilute the compound suspension for the test bacteria and fungi, respectively. Ampicillin, Norfloxacin was used as standard antibacterial drugs, whereas fluconazole was used as standard antifungal drug [1-7]. Primary inoculation of bacteria was done into Mueller- Hinton agar for overnight growth to produce a number of colonies, which were then directly suspended in saline solution until the turbidity matched the turbidity of the McFarland standard (10 CFU ml), i.e. inoculum size for test strain was adjusted to 10^8 colony forming unit (CFU)/ml per well by comparing the turbidity (turbidimetric method). Similar procedure was adopted for fungi with Sabouraud dextrose broth. Dimethyl sulfoxide (DMSO) was used as diluents to get desired concentration of the compounds and standard drugs. Compound and standard drugs were diluted to obtain 500 µg/ml concentrations, as a stock solution. Stock solution was further progressively diluted with the test medium and required concentrations were obtained for primary and secondary screening. In primary screening 500, 250 and 125 µg/ml concentrations of the compounds were tested. The active compounds found in this primary screening were further diluted and tested against the corresponding microorganism. Each test tube was then put for incu-

bation at 37° for 24h for bacteria and 48 for fungi. Growth or a lack of growth in the tubes containing the antimicrobial agent was determined by comparison with the growth control, indicated by turbidity. The lowest concentration that completely inhibited visible growth of the organism was recorded.

Results, Discussion and Conclusion

Antimicrobial activity of 5-(6-(2,4-dichlorophenyl)-[1,2,4] triazolo[3,4-b][1,3,4] thiadiazol-3-yl)benzene-1,2,3-triol and standard drugs were assessed against two representatives of Gram-positive bacteria viz. *S. aureus*, *B. subtilis*, two Gram-negative bacteria viz. *E. coli*, *P. aeruginosa* and two fungi viz. *C. albicans*, *A. niger* by the broth micro-dilution MIC method. Ampicillin was used as standard antibacterial drugs, whereas fluconazole was used as standard antifungal drug. The results obtained as mentioned in table 1 revealed that compounds exhibit antibacterial activities against both Gram-positive strains. The obtained results for antifungal activities as depicted in table 1 revealed that compounds could inhibit the growth of the tested fungal strains.

Compound	MIC, μg/ml					
	S. aureus	***B. subtilis***	***P. aeruginosa***	***E. coli***	***A. niger***	***C. albicans***
Compound	24.2	20.2	18.6	18.0	24.8	30.2
Ampicillin	10.6	10.8	-	-	-	-
Norfloxacin	11.8	14.2	10.4	7.8	-	-
Fluconazole	-	-	-	-	9.6	10.4

Table 1: Minimum inhibitory concentration (MIC, μg/ml) of compounds.

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