Available online at www.elixirpublishers.com (Elixir International Journal)

Pharmacy

Elixir Pharmacy 54 (2013) 12315-12318

ISSN: 2229-712X

Evaluation of antimicrobial activity of 4-arylidene-2-phenyl oxazol-5-one derivatives against selected skin microorganisms

Sudheer Moorkoth, Srinivasan K.K and Divyashree Bukka

Department of Pharmaceutical Quality Assurance, MCOPS, Manipal University, Manipal.576104.

ARTICLE INFO

Article history:

Received: 5 November 2012;

Received in revised form:

27 December 2012;

Accepted: 1 January 2013;

Keywords

Oxazolone,

Azlactone,

MIC,

Growth inhibition

ABSTRACT

Five derivatives of oxazolone were synthesized by using Erlenmeyer azlactone synthesis. Antibacterial and antifungal activity of these oxazolones was evaluated on selected skin microorganisms. Preliminary screening was performed to identify growth inhibition, and MIC (Minimum Inhibitory Concentration) was determined for the active compounds. Results obtained were very promising and the most active compound in our screen was the dimethoxy substituted oxazolone, which demonstrated a significant enhancement of antimicrobial activity both against selected bacterial and fungal strains. The MIC value of the highly active compound was found to be 12.5µg/ml.

© 2013 Elixir All rights reserved.

Introduction

The resistance of pathogenic microorganism to existing antimicrobial agents is a serious concern. In spite of the wide range of antibiotics available to us, microbial infections are still a major cause for death. Toxicity of these compounds is another problem. In this situation it is very essential to identify a molecule that have a broad spectrum of activity against microorganism and at the same time less toxic. 4-arylidene-2-phenyl oxazol-5-one is a well-known heterocyclic compound and is an essential part of several biologically active molecules. There are several reports on the antimicrobial activity of this compound. This class of heterocycle exhibited moderate antibacterial activity against S. aureus, B. subtilis, P. aeruginosa and E. coli and significant antifungal activity against C. albicans, C. pannical and A. niger[1]. These oxazolones are also used as intermediates in the synthesis of imidazolidinones which is an established pharmacophore[2]. These compounds exhibit a variety of biological activities such as analgesic[3], anti-inflammatory[4], antimicrobial[5,6], neuroleptic[7], anticancer[8] etc.

Considering the above activities and in recognition of the potential of oxazolones as antimicrobial agents, we have attempted to synthesize various derivatives of 4-arylidene-2-phenyl oxazol-5-one, and screen these compounds against skin microorganisms. Synthesis was carried out by employing the Erlenmeyer azlactone synthesis[9] as described under scheme 1 (Figure 2). Microbial strains used were the ones recommended by NCCLS including some clinical isolates.

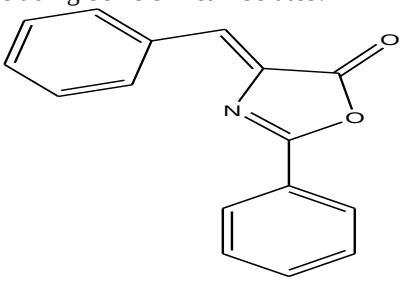
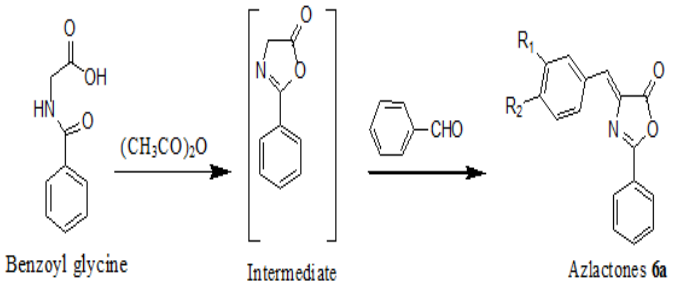


Figure 1: Structure of oxazolone

Materials and Methods

In our experiments various chemicals, reagents, solvents and instruments were used. Acetic anhydride and anhydrous sodium acetate were obtained from Qualigens, India. Hippuric acid was obtained from Sd fine chemicals, India. Various aldehydes such as Benzaldehyde, 3,4-dimethoxy benzaldehyde, 3,4-dichlorobenzaldehyde, 4-methoxybenzaldehyde, and 4-hydroxybenzaldehyde were obtained from Sigma Aldrich Chemical Company, USA. Melting points of all derivatives were recorded in glass capillaries using liquid paraffin. Purity of the compounds was identified by performing Thin Layer Chromatography and visualized under UV chamber. ^{1}H NMR spectra obtained using a Bruker NMR 400MHz instrument having TMS as an internal standard and $CDCl_3$ as solvent. IR spectrum of compounds in KBr pellets were recorded on a FTIR Spectrophotometer, Model 8310, Shimadzu, Japan. Ultraviolet (UV) spectra were recorded on a UV-Visible Spectrophotometer Model UV-2400PC, Shimadzu, Japan. Mass spectra were recorded using a GCMS instrument, Model GC-MS QP5050, Shimadzu Japan.

General procedure for synthesis of oxazolone : (4-benzylidene-2-phenyloxazol-5-one)



	a	b	c	d	e
R_1	H	H	Cl	OCH_3	H
R_2	H	OCH_3	Cl	OCH_3	OH

Figure 2: (Scheme 1) Synthesis of oxazolones

Tele:

E-mail addresses: shmoorkoth@yahoo.com

© 2013 Elixir All rights reserved

A mixture of benzaldehyde (27 g), benzoyl glycine (45 g), acetic anhydride (77 g) and anhydrous sodium acetate (20.5 g) was placed in a 500 ml conical flask on an electric hot plate with constant shaking. As soon as the mixture liquefied completely, the flask was transferred to a water bath and heated for 2 hours. 95.5% ethanol (100 ml) was added slowly to the contents of the flask, and the mixture was allowed to stand overnight. The crystalline product obtained was filtered under suction, washed with two 25 ml portions of ice-cold ethanol, followed by washing with two 25 ml portions of boiling water and then dried in an oven at 100°C. Recrystallization from toluene afforded yellow needle shaped crystals; Yield: 40 g (65%); m.p.:167°C. Various other azlactones 6(a-e), were synthesized in the same way by using the corresponding aromatic aldehydes. Specific reaction conditions for compounds 6 (a-e) are shown in Table 1. Azlactones 6(a-e) synthesized above were characterized by their physical and spectral data.

Physical and spectral data of various oxazolones (azlactones) synthesized

Compound 6a: 4-benzylidene-2-phenyl oxazol-5-one: Molecular weight: 249; yield: 40 g (65%); m.p.:167°C; Rf value: 0.694 (mobile phase, Hexane: Acetone, 6:5); UV λmax (nm): 282,360; mass spectrum (EI-MS) m/z values: 249(M^+) 18%, 116 (C_6H_5-CH=CH-N) 10%, 105 (C_6H_5-CO) 100%, 77 (C_6H_5) 62%. FTIR (KBr) ν: 3081.06 (aromatic C-H str.), 1796.69 (-C=O str.), 1650.81 (-C=N str.), 1551.63 (C=C str.), 1290.28 (C-N str.), 1160.19 (-C-O str.), 691.43 cm-1 (Ar-H def. of monosubstituted benzene). ^{1}HNMR ($CDCl_3$) δ: 7.157 (s, 1H, =CH-), 7.230-7.925 ppm (m, 10H, Ar-H).

Compound 6b: 4-(4-methoxybenzylidene)-2-phenyl oxazol-5-one: Molecular weight: 279; yield: 54 g (54%); m.p.:158-160°C; Rf value: 0.673 (mobile phase, Hexane: Acetone, 6:5); UV λmax (nm): 260, 384; mass spectrum (EI-MS) m/z values: 279 (M^+) 38%, 146 (OCH_3-C_6H_5-CH=CH-N) 8%, 105 (C_6H_5-CO) 100%, 77 (C_6H_5) 34%. FTIR (KBr) ν: 3039.60 (aromatic C-H str.), 1780.17 (-C=O str.), 1650.95 (-C=N str.), 1512.03 (C=C str.), 1263.29 (C-N str.), 1159.14 (-C-O str.), 1105.14 (-C-O str.) Ar-OCH3, 830.13 cm-1 (Ar-H def. of meta disubstituted benzene). ^{1}HNMR($CDCl_3$) δ:3.755 (s, 3H, -OCH3), 6.914 (s, 1H, =CH-), 7.161-7.649 ppm (m, 9H, Ar-H).

Compound 6c: 4-(3,4-dichlorobenzylidene)-2-phenyl oxazol-5-one: Molecular weight: 317; yield: 59 g (61%); m.p.:172°C; Rf value: 0.633 (mobile phase, Hexane: Acetone, 6:5); UV λmax (nm): 292,364; mass spectrum (EI-MS) m/z values: 317(M^+) 13%, 184 (Cl_2-C_6H_5-CH=CH-N) 5%, 105 (C_6H_5-CO) 100%, 77 (C_6H_5) 49%. FTIR (KBr) ν: 3030.13 (aromatic C-H str.), 1793.68 (-C=O str.), 1652.88 (-C=N str.), 1552.59 (C=C str.), 1301.86 (C-N str.), 1159.14 (-C-O str.), 827.41 (Ar-H def. of para disubstituted benzene), 692.40 cm-1 (C-Cl str.). ^{1}HNMR($CDCl_3$) δ : 7.061 (s, 1H, =CH-), 7.260-7.892 ppm (m, 9H, Ar-H).

Compound 6d: 4-(3,4-dimethoxybenzylidene)-2-phenyl oxazol-5-one: Molecular weight: 309; yield 60 g (64%); m.p.:150°C; Rf value: 0.653 (mobile phase, Hexane: Acetone, 6:5); UV λmax (nm): 264, 402; mass spectrum (EI-MS) m/z values: 309 (M^+) 6%, 176 [$(OCH3)_2$-C_6H_5-CH=CH-N] 2%, 105 (C_6H_5-CO) 100%, 77 (C_6H_5) 45%. FTIR (KBr) ν: 3031.89 (aromatic C-H str.), 1799.46 (-C=O str.), 1652.88 (-C=N str.), 1485.09 (C=C str.), 1282.57 (C-N str.), 1166.85 (-C-O str.), 1041.49 (-C-O str.) of Ar-OCH3, 773.40 cm-1 (Ar-H def. of meta disubstituted benzene). ^{1}HNMR($CDCl_3$) δ:3.462 (s, 3H, -OCH3), 6.841 (s, 1H, =CH-), 7.247-7.681 ppm (m, 9H, Ar-H).

Compound 6e: 4-(4-hydroxybenzylidene)-2-phenyl oxazol-5-one: Molecular weight: 265.07; yield: 62 g (64%); m.p.:182°C; Rf value: 0.643 (mobile phase, Hexane: Acetone, 6:5); UV λmax (nm): 290, 364; mass spectrum (EI-MS) m/z values: 265 (M^+) 30%, 132 (OH-C_6H_5-CH=CH-N) 2%, 105 (C_6H_5-CO) 100%, 77 (C_6H_5) 40%. FTIR (KBr) ν: 3560.05 (O-H str.), 3045.39 (aromatic C-H str.), 1793.68 (-C=O str.), 1654.81 (-C=N str.), 1562.25 (C=C str.), 1363.58 (C-O str.) of Ar-OH, 1325.01 (C-N str.), 1164.92 (-C-O str.), 808.12 cm-1 (Ar-H def. of para disubstituted benzene). ^{1}HNMR ($CDCl_3$) δ : 7.081 (s, 1H, =CH-), 7.249-7.681 (m, 9H, Ar-H), 8.851 ppm (s, 1H, OH) D2O exchangeable.

Antimicrobial evaluation:

All the synthesized compounds were screened for antibacterial and antifungal activities. Microbial strains recommended by NCCLS and clinical isolates were used for the study. The bacterial species selected for the study were Bacillus subtilus MTCC 441 and Staphylococcus aureus ATCC 25923. The two fungal strains were Trichophyton rubrum and Trichophyton mentagrophytes PSSF 66/01. The opportunistic fungi Aspergilus niger MTCC 1344 and the yeast Candida albicans MTCC227 were also screened. Isolates were maintained in nutrient agar slants at 4°C and fungal cultures were maintained in Sabouraud Dextrose agar (SDA) slants.

Preparation of inoculum: Mueller Hinton Broth (MHB) was used and incubated overnight at 37°C at 159 rpm. The final dilution was 5×10^5 cfu/ml for both bacteria and Candida. Viable counts were performed to confirm the inoculum size for the assay[10]. Fungal cultures were grown on SDA slants and incubated at 27°C for ten days or until the medium got covered with mycelium. From the agar surface spores were collected using sterile cold water. After vortexing heavy metals were allowed to settle for 3-5 minutes. The spores were counted using haemocytometer and density adjusted to 1×10^4 spores/ml[11].

Antibacterial study

Agar dilution method: Agar plates were prepared by adding the following concentration of newly synthesized compounds 6(a-e) at concentrations of 100, 50, 25, and 12.5 µg/ml to 10 ml of molten MHA. Control plates were maintained with respective solvents. Standardized bacterial inoculum 5µl was spotted in triplicates on the medium. Growth was noted after 24h incubation at 37°C[12].

Broth micro dilution method: Minimum Inhibitory Concentration (MIC) was studied by broth microdilution method using 96 well microtitre plate; the compounds 6(a-e) were diluted into MHB and tested in a doubling dilution series (100 to 1.56 µg/ml). The bacterial cultures were inoculated in the respective wells and incubated at 37°C for 16h[10,12]. At the end of the incubation period, 5µl of the test broth was taken from the wells and inoculated onto MHA plates. The plates were incubated under aerobic conditions; the MIC was determined as the lowest concentration of the compound inhibiting the growth of the organism.

Antifungal study

Antifungal study was performed using agar dilution technique[13]. The required concentration of the oxazolones 6(a-e), from 100, 50, 25, and 12.5 µg/ml were mixed with the molten MHA and allowed to solidify. The fungal spores, 5µl (1×10^4 spores/ml) were placed on the media and incubated at 27°C for 7-10 days. The percent of growth inhibition was calculated based on the extent of mycelia growth in comparison with the control.

Table 1. Reaction conditions of various azlactones 6 (a-e) synthesized

Compound synthesised	Aldehyde used	Amount taken	Reflux temp. (°C)	Duration of reflux (hours)
6a	Benzladehyde	26 ml	110	2
6b	4-methoxybenzaldehyde	30.4 ml	120	2.5
6c	3,4-dichlorobenzaldehyde	44 g	100	2
6d	3,4-dimethoxybenzaldehyde	46g	110	2
6e	4-hydroxybenzaldehyde	30.5 g	100	2

Table 2: Antibacterial activity of oxazolones (6a-e) at 100µg/ml concentration

Microorganisms	Compounds screened				
	6a	6b	6c	6d	6e
Bacteria					
Bacillus subtilus MTCC 441	+	+*	+	+**	+
Staphylococcus aureus ATCC 25923	+	+*	+	+**	+
Yeast					
Candida albicans MTCC227	-	-	-	-	-

MTCC: Microbial Type Culture Collection; ATCC: American Type Culture Collection; + = growth inhibition, - = no growth inhibition; * = MIC 25µg/ml; **=MIC 12.5 µg/ml.

Table 3: Antifungal activity of oxazolones (6a-e) at 100µg/ml concentration

Microorganisms	Compounds screened				
	6a	6b	6c	6d	6e
Dermatophytes	Growth inhibition (%)^m				
Trichophyton rubrum MTCC 296	24.1*	67.8*	6.94	100*	44.2*
Trichophyton mentagrophytes PSSF 66/01	19.7*	52.5*	6.17	100*	42.7*
Opportunistic fungi					
Aspergilus niger MTCC 1344	3.9	7.7	3.1	9.7	5.6

MTCC: Microbial Type Culture Collection PSSF: Patholab Spic Science Foundation clinical isolates; (%)^m = mean % of growth inhibition (triplicate); * = MIC 50µg/ml.

Results and Discussion

The results of the antibacterial and antifungal screening together with the data on MIC are presented in tables 2 and 3. Initial screening for antibacterial and antifungal activity was performed using a concentration of 100µg/ml. All the newly synthesized oxazolones exhibited antibacterial activity against Bacillus subtilus MTCC 441 and Staphylococcus aureus ATCC 25923. None of the oxazolone was able to show any activity against Candida albicans MTCC227. The % growth inhibition determined for the fungal species Trichophyton rubrum MTCC 296 and Trichophyton mentagrophytes PSSF 66/01 were also promising. The compounds were showing varying degrees of growth inhibition. The compound 6d; 4-(3,4-dimethoxybenzylidene)-2-phenyl oxazol-5-one exhibited a very prominent antidermatophytic activity of 100% growth inhibition against both Trichophyton rubrum MTCC 296 and Trichophyton mentagrophytes PSSF 66/01. Compound 6b; 4-(4-methoxybenzylidene)-2-phenyl oxazol-5-one exhibited 67.8 and 52.5% inhibition respectively against the above mentioned fungal species. The least activity among the series was for the compound 6c; 4-(3,4-dichlorobenzylidene)-2-phenyl oxazol-5-one. The compounds were unable to elicit any response against the opportunistic fungi Aspergilus niger MTCC 1344. MIC was determined for those compounds that were active against the selected bacterial and fungal strains at 100µg/ml. For the compound 6d, the MIC was found to be 12.5 µg/ml against Bacillus subtilus MTCC 441 and Staphylococcus aureus ATCC 25923. For compound 6b, it was 25 µg/ml against the above two bacterial species. For compounds 6a, 6c, and 6e, the MIC was more than 25 µg/ml against the two bacterial strains selected. The MIC value for the compounds 6a, b, d, and e was found to be 50 µg/ml against Trichophyton rubrum MTCC 296 and Trichophyton mentagrophytes PSSF 66/01. For compound 6c, the MIC was more than 50 µg/ml.

The results indicate that the synthesized oxazolones are active against the bacterial strains such as Bacillus subtilus MTCC 441 and Staphylococcus aureus ATCC 25923. It also reveals that the methoxy groups on the oxazolones enhances the antimicrobial activity, with the highly active compound in this screen being compound 6d, containing two methoxy groups and have the MIC value of 12.5 µg/ml. It was also observed that the electron withdrawing groups such as chloride present on the oxazolone have resulted in a reduction of the antibacterial activity, which was evidenced by the activity of compound 6c, for which the MIC value is more than 25 µg/ml. The antifungal activity was also in accordance with the above observation, that the compound containing the methoxy groups demonstrated the highest activity and the compound having dichloro substitution being the least active. The compound 6e, that contain the hydroxyl substitution demonstarated a moderate activity in comparison to that contain a methoxy substitution.

Conclusion

Five oxazolone derivatives were synthesized and characterized completely. These oxazolones were active against the skin pathogens such as Bacillus subtilus MTCC 441, Staphylococcus aureus ATCC 25923, Trichophyton rubrum MTCC 296 and Trichophyton mentagrophytes PSSF 66/01. Compound 6d was found to be the highly active one against these organisms. The compounds were not active against the yeast strain Candida albicans MTCC227, and the opportunistic fungi Aspergilus niger MTCC 1344.

Acknowledgements

The authors acknowledge Dr. Kannan, SPIC Science foundation, Chennai, for antimicrobial screening.

References

1. Gadada N., Patchanita T., and Amorn P. Synthesis and Antimicrobial Activity of Oxazolone, Imidazolone and Triazine Derivatives Containing Benzothiophene. Journal of the Korean Chemical Society. 2011. Vol. 55: No. 5, 794-804.

2. Mistry R.N., Desai K.R. Studies on synthesis of some novel heterocyclic azlactone derivative and imidazolinone derivatives and their antimicrobial activity. J Med Chem. 2005; Vol 2, No.6, 42-51.

3. Jakeman D.L., Farrell S., Young N., Doucet R.J., Timmons S.C. Novel jadomycins incorporation of non-natural and natural amino acids. Bioorg Med Chem Lett. 2005; Vol. 15, No. 5, pp. 1447-1449.

4. Crespo M.I., et al, Synthesis and biological evaluation of 3,4-diaryloxazolonnes: A new class of orally active cyclooxygenase-2 inhibitors. J Med Chem. 2000; Vol. 43, No. 2, 214-223.

5. Shinde D.B., Aaglawe M.J., Dhule S.S., Bahekar S.S., Wakte P.S. Synthesis and antibacterial activity of some oxazolone derivatives. J Korean Chem Soc. 2003; Vol. 47, No. 2, 133-136.

6. Sah P., Nair S., Garg S.P. Synthesis and antimicrobial activity of some new oxazolone derivatives of 4,5-disubstituted -2-aminothiazole. J Indian Chem Soc. 2006; Vol. 83, No. 2, 205-207.

7. Cascio G., Manghisi E., Fregnan G. 5-Piperazinylalkyl-2(3H)-oxazolones with neuroleptic activity. J Med Chem. 1989; Vol. 32, No. 10, 2241-2247.

8. Jat L.R., Mishra R., and Pathak, D. Synthesis and anticancer activity of 4-benzylidene-2- phenyloxazol-5(4h)-one derivatives. International Journal of Pharmacy and Pharmaceutical Sciences. Vol 4: Issue 1, 2012, 378-380.

9. Brian, S.F., Antony, J. Hannaford., Peter, W.G. Smith., Austin, R.Tatchell Vogel's Textbook of practical organic chemistry. 5 ed. 2005: p. 747. Pearson Education.

10. Goodson,B., Ehrhardt,A., Simon,N.G., Nuss, J., Johnson, K., Giedlin,M., Yamamoto, R., Moos,W.H., and Winter J. Characterization of novel antimicrobial peptides. Antimicrob. Agents Chemother. 1999: 43: 1429-1434.

11. Manavathu, E.K., Alangadem,G.J. and Lerner,S.A. A comparative study of the broth micro-and macro-dilution techniques for the determination of in vitro susceptibility of Aspergilus fumigates. Can. J. Microbiol. 1996:42: 960-964.

12. NCCLS (national Committee for Clinical Laboratory Standards) Document M 31.A. performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, approved standard. 1999: NCCLS, Villanova, PA.

13. Hammer, K.A., Carson,C.F., and Riely, T.V. In vitro activity of essential ios, in particular Melaleuca alternifolia (tea tree) and tea tree oil products, against Candida spp. J. Antimicrob. Chemother. 1998: 42:591-595.