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ORIGINAL ARTICLE

Synthesis of 2-[4-(substituted benzylidene)-5-Oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione Derivatives as Novel Potential Antimicrobial Agents

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ABSTRACT

In the present study, a series of new substituted oxazolone derivatives (4a-4h) were synthesized by the Erlenmeyer condensation of phthaloylglycylglycine with different aldehydes in the presence of anhydrous sodium acetate and acetic anhydride. The structure of newly-synthesized compounds were evaluated by elemental analyses and spectral (UV-Visible, IR, NMR, Mass) studies. All the synthesized derivatives were evaluated for their antimicrobial activity. Preliminary pharmacological evaluation revealed that the compound 4b, 4c, 4d, 4g and 4h showed better performance against *Staphylococcus aureus*, *Staphylococcus epidermidis, Proteus mirabilis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus niger*. The minimum inhibitory concentration (MIC) was determined for the target compounds as well as for standard drugs. Physicochemical similarity of the new derivatives with standard drugs was assessed by calculating from a set of 10 physicochemical parameters using software programs.The result demonstrated the potential and usefulness of developing novel oxazolone derivatives which would be effective against resistant and pathogenic bacterial and fungal strain.

Keywords: *Oxazolone; Substituted benzylidene; Antibacterial activity; Antifungal activity*

Increase in development of multi-drug resistant microbial infections in the past few years have become a serious health hazard. Millions of people were infected and around 20,000 deaths were reported in the tropical regions every year because of bacterial infections [1]. So, there is an urgent need for identification of novel, potent and safe agents which ideally shorten the duration of therapy and are effective against the resistant strain.

Oxazolone is five-membered heterocyclic nucleus having two oxygen and one nitrogen atom in the ring. This compound has been residing an enormous significance in the field of medicinal chemistry due to the number of pharmacological activities such as antimicrobial, anti-inflammatory, anti-HIV [2,3], antiangiogenic [4], anticonvulsant [5], antitumor, antagonistic, sedative [6,7] and cardiotonic activity [8]. Oxazolones are also involved in the synthesis of several organic molecules including amino acids, amino alcohols, thiamine, peptides and polyfunctional compounds [9,10]. Oxazolones also play a key role, as synthons, for the construction of various alkaloid skeletons, immunomodulators and biosensors or photosensitive composition devices for proteins [6,7,11]. Oxazolones are used in semiconductor devices such as electro photographic photoreceptors and in non-linear optical materials. They exhibited promising photophysical, photochemical activities, cyclooxygenase-2 inhibitory property and tyrosinase inhibitory property [2,12-16]. Oxazolones play a vital role for the synthesis of various biologically-active drugs, such as analgesics, anti-inflammatory, antidepressant, anticancer, antimicrobial, antidiabetic, anti obesity, peptides, herbicides, fungicides and pesticides [2,6]. In the present study, a series of new substituted oxazolone derivatives (4a-4h) are

Table 1. Physical data of the compounds (4a-4h)

Compound	R	Molecular Formula	Molecular weight	Yield (%)	m.p. (°C)	λ_{max} (nm)	R_f Value	Calculated (found)		
								C%	H%	N%
4a	H	$C_{19}H_{12}N_2O_4$	332.31	89.92	197-200	298	0.52	68.67 (68.65)	3.64 (3.61)	8.43 (8.40)
4b	4-OH, 3-OCH_3	$C_{20}H_{14}N_2O_6$	378.34	88.75	135-138	328	0.63	63.49 (63.51)	3.73 (3.65)	7.40 (7.42)
4c	4-CH_3	$C_{20}H_{14}N_2O_4$	346.34	80.91	138-141	238	0.52	69.36 (70.23)	4.07 (4.11)	8.09 (8.00)
4d	4-NO_2	$C_{19}H_{11}N_3O_6$	377.31	79. 84	85-89	265	0.50	60.48 (60.42)	2.94 (3.12)	11.14 (11.16)
4e	3-NO_2	$C_{19}H_{11}N_3O_6$	377.31	80.92	60-63	297	0.53	60.48 (60.51)	2.94 (2.82)	11.14 (11.31)
4f	4-OH	$C_{19}H_{12}N_2O_5$	348.31	81.87	146-149	320	0.51	65.52 (66.15)	3.47 (3.34)	8.04 (7.87)
4g	2-OH	$C_{19}H_{12}N_2O_5$	348.31	82.92	120-123	220	0.49	65.52 (65.22)	3.47 (3.40)	8.04 (8.24)
4h	4-Cl	$C_{19}H_{11}ClN_2O_4$	366.75	82.90	160-163	299	0.47	62.22 (61.95)	3.02 (2.94)	7.64 (7.81)

(Mobile phase: chloroform: methanol (9:1, v/v)
(Comd.: compound, Mol.: molecular, m.p.: melting point)

synthesized by the Erlenmeyer condensation of phthaloylglycylglycine with different aldehydes in the presence of anhydrous sodium acetate and acetic anhydride. Then the effects of these new novel oxazolone derivatives against resistant and pathogenic bacterial and fungal strain are examined.

MATERIALS AND METHODS

The melting points were measured, using digital melting point apparatus (Flora; Perfit India) and were found to be uncorrected. The purity of compounds was checked by TLC. The λ_{max} was calculated using double beam UV-Visible 1800 Shimadzu spectrophotometer. IR spectra (υ, cm^{-1}) were recorded on Shimadzu FTIR 1800S spectrometer following nujol method. ^1H NMR (δ, ppm) spectra were recorded by DMSO-D_6 solutions and TMS as an internal standard on a BRUKER AVANCE-II 400 NMR spectrometer. For mass spectra, solutions were made in HPLC grade methanol. Elemental analysis was performed on an ECS 4010 Elemental Combustion System. Structural similarity studies between standard drugs (cefixime, tosufloxacin tosylate) and targeted compounds were performed by Chem3D Ultra, molecular modelling software.

Chemistry

A new series of synthetic oxazolone were prepared from commercially available glycine and phthalic anhydride reaction product phthaloylglycylglycine. The synthetic route is outlined in Scheme 1, the titled compound 2-[4-(substituted benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4a-4h) was synthesized by reacting phthaloylglycylglycine with suitable aldehydes in presence of anhydrous sodium acetate and acetic anhydride in high yields [5,15,17]. The purity of the compounds was checked by TLC, elemental analyses and characterized by spectral data. The physical data and elemental analysis of the synthesized compounds are summarized in Table 1.

Synthesis of Phthaloylglycine [1]

A mixture of phthalic anhydride (9 g, 0.06 mol) and glycine (4.5 g, 0.06 mol) was fused in a boiling tube at 160-190°C (oil bath) for 20-30 min. The product obtained was cooled at room temperature and crystallized from water to get phthaloylglycine [1].

Synthesis of phthaloylglycine chloride [2]

A mixture of phthaloylglycine [1] (8 g, 0.039 mol) and thionyl chloride (16 mL) was refluxed gently for 30 min in a round bottom flask fitted with reflux condenser having a drying tube on the top. Excess thionyl chloride was removed by distillation under reduced pressure. The residual phthaloylglycine chloride [2] was crystallized from petroleum ether.

Synthesis of Phthaloylglycylglycine [3]

A solution of phthaloylglycine chloride (2) (4.2 g, 0.09 mol) in dioxane (25 mL) was added to the stirred suspension of glycine (1.55 g) and magnesium oxide (1.1 g) in water (50 mL). The temperature was kept at 4-5°C during addition, stirring continued for 10-15 min at 25°C and then acidified with hydrochloric acid. The mixture was cooled, the separated product was filtered, washed with cold water and crystallised from hot water to get phthaloylglycylglycine [3].

Synthesis of 2-[4-(substituted benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione [4]

An equimolar mixture of phthaloylglycylglycine and suitable aldehyde (15 mmol) in freshly-distilled acetic anhydride (10 cm^3) containing fused anhydrous sodium acetate (1.2 g) was heated on a steam bath for 4 hours then cooled, yield the formation of yellow solid mass, now filtered off and washed with light petroleum (40-60°C). It was well dried, triturated with cold saturated sodium carbonate solution and again filtered. Then after washing with water, dried and recrystallized from suitable solvent to yield the compounds (4a-4h). All new titled compounds (4a-4h) were synthesized following the same procedure (Scheme I).

Synthesis Protocol

Synthesis of derivatives (1-3) was carried out by following the reported literature procedure [15]

Phthaloylglycine [1]

Yield 98%; m.p. 188–190 °C, λ_{max} 220 nm; IR (υ, cm^{-1}): 3558 (O-H stretch), 3050, 2980 (C-H stretch), 1801,1716 (phthalyl C=O), 1700 (carbonyl stretch),1527(C=C stretch), 671; ^{1}H NMR (400 MHz, DMSO; δ ppm): 10.69 (s, 1H, OH), 7.87 (m, 2H, ArH), 7.80 (m, 2H, ArH), 4.34 (s, 2H, CH_2).

Phthaloylglycine chloride [2]

Yield 97.5%; m.p. 133–135 °C, λ_{max} 224 nm; IR (υ, cm^{-1}): 3045, 2980 (C-H stretch),1782, 1710 (phthalyl C=O), 1705 (carbonyl stretch),1610 (C=C stretch), 790; ^{1}H NMR (400 MHz, DMSO; δ ppm): 8.01 (m, 2H, ArH), 7.74 (m, 2H, ArH), 4.92 (s, 2H, CH_2).

Phthaloylglycylglycine [3]

Yield 93.5%; m.p. 193–195 °C, λ_{max} 248 nm; IR (υ, cm^{-1}): 3464 (O-H stretch), 3280 (N-H stretch), 3103, 2900 (C-H stretch), 1801, 1734 (phthalyl C=O), 1711 (carbonyl stretch), 1642 (peptide stretch), 1622 (N-H bend), 1510 (C=C stretch), 746; ^{1}H NMR (400 MHz, DMSO; δ ppm): 10.27 (s, 1H, OH), 8.13 (m, 2H, ArH), 7.94 (m, 2H, ArH), 4.83 (s, 2H, CH_2), 4.18 (s, 2H, CH_2).

Synthesis of 2-[4-(substituted benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione derivatives (4a-4h)were carried out using the literature procedure (Madkour, 2002) and their spectral data are as given below:

2-(4-Benzylidene-5-oxo-4,5-dihydro-oxazol-2-ylmethyl)-isoindole-1,3-dione (4a)

IR (υ, cm^{-1}): 3021, 2920 (C–H stretch), 1772, 1703 (phthalyl C=O),1767 (lactone C=O stretch),1685 (C=N stretch), 1620 (C=C stretch), 1125 (C–O–C stretch), 719; ^{1}H NMR (400 MHz, DMSO; δ ppm): 7.92 (m, 2H, ArH), 7.87 (m, 2H, ArH), 7.74 (m, 2H, ArH), 7.62 (m, 2H, ArH), 7.51 (m, 1H, ArH), 7.42 (s, 1H,=CH), 4.83 (s,2H, CH_2); MS: m/z 332.08, 333.08 (M+1), 334.09 (M+2).

2-[4-(4-Hydroxy-3-methoxy-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4b)

IR (cm^{-1}): 3500 (O–H stretch), 3066, 2890 (C–H stretch), 1767, 1718 (phthalyl C=O),1761 (lactone C=O stretch),1589 (C=N), 1589, 1517 (C=C stretch),1282, 1120 (C–O–C stretch), 893; ^{1}H NMR (400 MHz, DMSO; δ ppm): 7.93 (m, 2H,ArH), 7.87 (m, 2H,ArH), 7.74 (m, 2H, ArH), 7.62 (m, 2H, ArH), 7.51(m, 1H, ArH) 7.42 (s, 1H,=CH), 5.50 (s, 1H, OH), 4.83 (s, 2H, CH_2), 3.68 (s, 3H, OCH_3); MS: m/z 378.09, 379.09 (M+1), 380.09 (M+2).

2-[4-(4-Methyl-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4c)

IR (cm^{-1}): 3062, 2962 (C–H stretch), 1834, 1701 (phthalyl C=O), 1785 (lactone C=O stretch), 1627 (C=N), 1543 (C=C), 1097 (C–O–C stretch), 734; ^{1}H NMR (400 MHz, DMSO; δ ppm): 7.90 (m, 2H, ArH), 7.86 (m, 2H, ArH), 7.82 (d, 2H, ArH), 7.57 (d, 2H, ArH), 7.24 (s,1H, =CH), 4.85 (s,2H, CH_2), 3.10 (s,3H, CH_3); MS: m/z 346.10, 347.10 (M+1), 348.10 (M+2).

2-[4-(4-Nitro-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4d)

IR (cm^{-1}): 3010, 2974 (C–H stretch), 1834, 1766 (phthalyl C=O), 1790 (lactone C=O stretch), 1627 (C=N stretch), 1548 (C=C stretch), 1458 (asymmetric N=Ostretch), 1375 (symmetric N=O stretch.), 1107 (C–O–C stretch); ^{1}H NMR (400 MHz, DMSO; δ ppm): 8.58 (d, 2H, ArH), 8.32 (d, 2H, ArH), 7.91 (m,2H, ArH), 7.84 (s,1H, =CH), 7.85 (t, 2H, ArH), 4.27 (s,2H, CH_2); MS: m/z 377.06, 378.07 (M+1), 379.07 (M+2).

2-[4-(3-Nitro-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4e)

IR (cm^{-1}): 3057, 2900 (C–H stretch), 1785, 1697 (phthalyl C=O), 1790 (lactone C=O stretch), 1639 (C=N stretch), 1627 (C=C stretch), 1475 (asymmetric N=O stretch), 1300 (symmetric N=O stretch.),1049 (C–O–C stretch); ^{1}H NMR (400 MHz, DMSO; δ ppm): 8.60 (m, 1H, ArH), 8.39 (d, 1H, ArH), 8.10 (d, 1H, ArH), 7.92 (m, 2H, ArH), 7.82 (m, 1H, ArH), 7.75 (m, 1H, ArH), 7.32 (s,1H, =CH), 4.77 (s, 2H, CH_2); MS: m/z 377.06, 378.07 (M+1), 379.07 (M+2).

2-[4-(4-Hydroxy-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4f)

IR (cm^{-1}): 3406 (O–H stretch), 3032 (C–H stretch), 1791, 1714 (phthalyl C=O), 1777 (lactone C=O stretch), 1566 (C=N stretch), 1519 (C=C stretch), 1199 (C–O–C stretch); ^{1}H NMR (400 MHz, DMSO; δ ppm): 7.94 (m, 2H,ArH), 7.81 (m, 2H, ArH), 7.78 (d, 2H, ArH), 7.18 (s,1H,=CH), 6.65 (d, 2H, ArH), 4.87 (s, 1H, OH),3.72 (s,2H, CH_2); MS: m/z 348.07, 349.08 (M+1), 350.08 (M+2).

Table 2. Calculations of various steric and physicochemical parameters of (4a-4h) and standard compounds

Compound	SAS[a] (□^{2})	SA[b] (□^{2})	SEV[c] (□^{3})	Ovality	MR[d] (cm^3/mole)	MTI[e]	WI[f]	BI[g]	MW[h]	Log P
4a	555.633	294.912	248.031	1.5448	1.1904	11658	1579	437983	332.317	2.0355
4b	607.398	329.205	281.211	1.58597	9.9604	15511	2195	755281	378.343	1.5196
4c	589.435	314.998	269.864	1.55977	9.6542	13225	1786	533480	346.344	2.5226
4d	606.299	326.222	284.047	1.56112	9.9793	16039	2254	774797	377.315	0.95
4e	581.733	316.671	288.051	1.50133	9.9793	15670	2197	755959	377.315	0.95
4f	567.592	301.296	255.011	1.54931	9.3435	12809	1786	533480	348.317	1.646
4g	566.137	301.549	255.488	1.54867	9.3435	12601	1748	522587	348.317	1.646
4h	586.94	313.441	264.032	1.57483	9.6818	12601	1786	533480	366.762	2.5937
Cefixime	580.224	331.272	320.889	1.46146	10.691	15459	2353	1046550	437.46	-
Tosufloxacin Tosylate	570.267	318.963	282.947	1.5303	9.792	13519	2039	748946	404.350	2.419

[a]Connolly Solvent Accessible Surface Area
[b]Connolly Molecular Surface Area
[c]Connolly Solvent Excluded Volume
[d]Molar Refractivity
[e]Molecular Topological Index
[f]Wiener Index
[g]Balaben Index
[h]Molecular Weight

2-[4-(2-Hydroxy-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4g)

IR (cm^{-1}):3460 (O–H stretch), 3000 (C–H stretch), 1808, 1728 (phthalyl C=O), 1765 (lactone C=O stretch), 1643 (C=N stretch), 1625 (C=C stretch), 1056 (C–O–C stretch); ^{1}H NMR (400 MHz, DMSO; δ ppm): 7.87 (m, 2H, ArH), 7.80 (m, 2H, ArH), 7.75 (d, 1H, ArH), 7.50 (s,1H, =CH), 7.42 (m, 1H, ArH), 7.17 (m, 1H, ArH), 7.10 (d, 1H, ArH), 5.44 (s, 1H, OH),3.68 (s,2H, CH_2); MS: m/z 348.07, 349.08 (M+1), 350.08 (M+2).

2-[4-(4-Chloro-benzylidene)-5-oxo-4,5-dihydro-oxazol-2-ylmethyl]-isoindole-1,3-dione (4h)

IR (cm^{-1}): 3014 (C–H stretch), 1776, 1716 (phthalyl C=O), 1764 (lactone C=O stretch), 1658 (C=N stretch), 1533 (C=C stretch), 1047 (C–O–C stretch), 582 (C-Cl stretch); ^{1}H NMR (400 MHz, DMSO; δ ppm): 7.95 (m, 2H, ArH), 7.90 (m, 2H, ArH), 7.87 (d, 2H, ArH), 7.82 (d, 2H, ArH), 7.34 (s,1H, =CH), 4.83 (s,2H, CH_2); MS: m/z 366.04, 368.04 (M+1), 367.04 (M+2).

Assessment of structural similarity of test compounds with standards drugs

Assessment of structural similarity of target compounds to standard drugs involves the study of physico-chemical and steric similarity between the standard drugs and new analogues for effective binding with receptors. The usual approach to assess similarity is to examine resemblance between molecular properties of target compounds with standard drugs (16). Therefore, we calculated a number of parameters for test compounds (4a-4h) using Chem3D and compared them to the values obtained for target compounds (Chem3D, version 10). Cefixime and tosufloxacin tosylate were used as the standard drugs for assessment of structural similarity.

Various set of parameters were used for calculations, given in Table 2.The distance d_i of a particular target compound i can presented as:

$$di^2 = \sum_{j=1}^{N} (1-X_{i,j}/X_{i,\,standard})^2/\,n$$

Where,

$X_{i,j}$ is value of molecular parameters i for compound j.

$X_{i,\,standard}$ is the value of the same molecular parameter i for standard drug.

n is the total number of considered molecular parameters.

The similarity of the compounds can be calculated as:

Percentage similarity= (1-R) × 100

Where,

R is quadratic mean also known as the root mean square and R can be calculated as:

$R= \sqrt{d i^2}$

All the synthesized compounds showed good percentage similarity when compared with standard drugs (Table 3).

Table 3. Assessment of structural similarities of tested compounds 4a-4h with standard drugs

S. No.	Compound	Percent similarity	
		Cefixime	Tosufloxacin tosylate
1.	4a	95.74	80.62
2.	4b	96.72	99.96
3.	4c	81.08	96.93
4.	4d	99.7	99.51
5.	4e	98.73	88.41
6.	4f	75.37	87.59
7.	4g	73.96	86.50
8.	4h	80.79	97.09

Pharmacological activity

Procedure for determination of antibacterial activity

The newly-synthesized oxazolone compounds (4a-4h) were screened for their *in vitro* antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Proteus mirabilis* and *Pseudomonas aeruginosa* by cup-plate method. Nutrient agar media was prepared by melting agar on water bath and then cooled it to 45ºC with gentle shaking, to bring about uniform cooling. Nutrient agar media was inoculated with fresh prepared culture media and mixed by gentle shaking before pouring on a sterilized petri dish. Poured the inoculated media into petri dish and allowed to set for some time. Cups were made by punching the agar surface with a sterile cork bore (8 mm) and the punched part of the agar media was removed by scooping. Solutions containing 12.5, 25, 50, 100, 200, 400, 800 and 1600 µg/mL of the test compound, were added to each cup. Dimethyl formamide (DMF) was used as solvent, to prepare the stock solution. Amoxicillin and cefixime were taken as positive control and DMF was taken as blank (did not show any activity against test organism). The plates were incubated at 37ºC for 24 h and the results were recorded. The zones of inhibition of the microbial growth produced by different concentration of test compounds (50 µl/disc) were measured in millimetres (mm) [18].

Procedure for the determination of the antifungal activity

The *in vitro* antifungal activity of test compounds was evaluated using *Candida albicans* and *Aspergillus niger* strains, by cup plate technique, in Saboraud's dextrose broth culture media. The stock solution of test compounds were prepared in dimethyl formamide (DMF) and the serial dilution of test compounds were carried out for obtaining the concentration, ranging from 12.5, 25, 50, 100, 200, 400, 800 and 1600 µg/mL. Fluconazole was taken as positive control and DMF was taken as blank (did not show any activity against test organism). The test compounds at various concentrations were added to the cup made by puncturing the agar dextrose media by sterilised cork bore. The plates were incubated at 37°C for 48 h. The zones of inhibition of the microbial growth (50 µl/disc) produced by different concentration of test compounds were measured in millimetres (mm) [17-19]. The results of minimum inhibitory concentration of the compounds against various pathogenic microorganisms were recorded after incubation at 37°C for 48 h as listed in Table 4. It was determined that solvent had no antimicrobial activity against any of the test microorganisms.

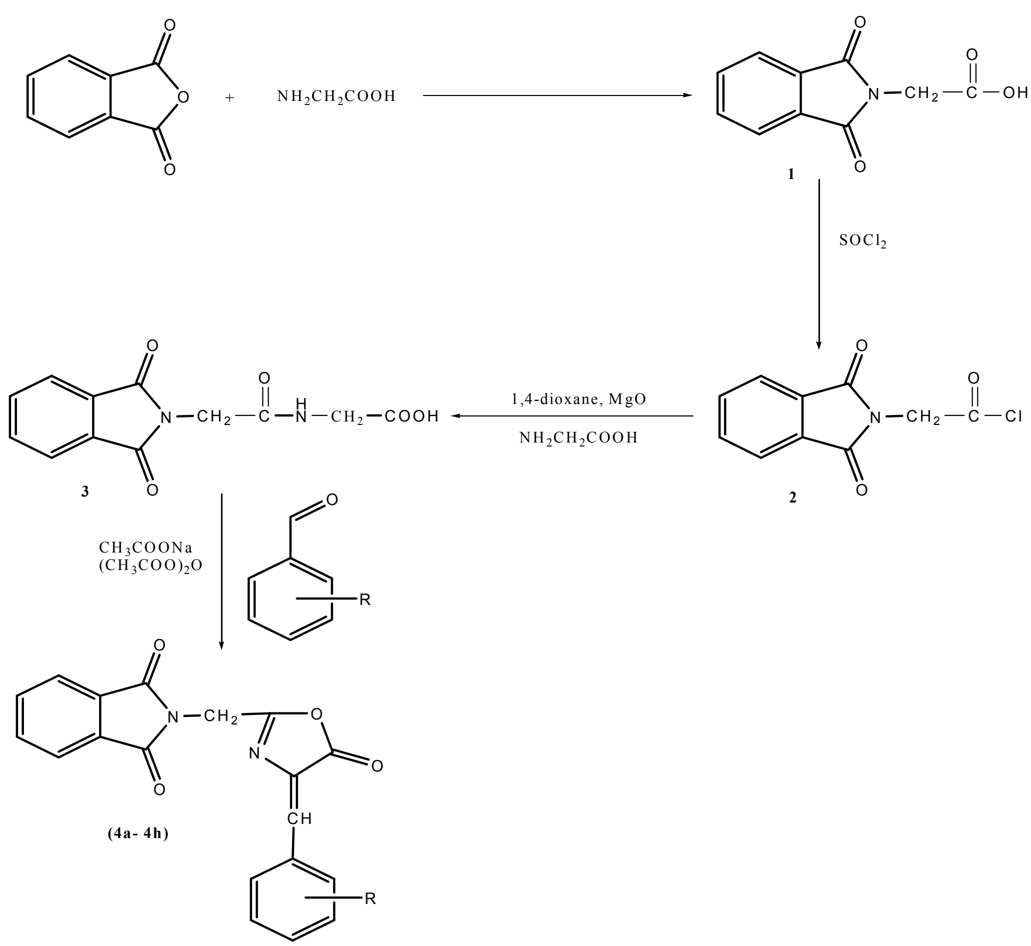
RESULTS AND DISCUSSION

In this work, total eight derivatives of oxazolone containing 4-substituted benzylidene (4a-4h) were prepared by base-induced cycloaddition of phthaloylglycylglycine [2] to aldehyde in a solvent such as acetic anhydride with anhydrous sodium acetate in good yield (Scheme 1). The structures of the synthesized compounds were supported using different spectroscopic methods like UV, IR, ^{1}H NMR, mass and

Table 4. Minimum inhibitory concentration of compounds (4a – 4h)

Compound	*S. aureus*	*S. epidermidis*	*P.mirabilis*	*P.aeruginosa*	*C. albicans*	*A. niger*
4a	<200	100	50	<100	400	<12.5
4b	<50	<50	50	<400	100	12.5
4c	50	<25	<12.5	50	<800	<12.5
4d	<25	50	200	50	800	12.5
4e	<50	200	400	50	<50	12.5
4f	50	-	-	200	200	12.5
4g	100	<50	100	100	1600	<12.5
4h	<50	<50	50	200	50	<12.5
Control	-	-	-	-	-	-
Amoxycillin	25	100	12.5	400	-	-
Cefixime	50	400	50	400	-	-
Fluconazole	-	-	-	-	25	400

Scheme-1 Synthetic route for the target compounds



Scheme 1. Synthetic route for the target compounds

elemental analysis. All the synthesized compounds were also evaluated for their antimicrobial activity. Antibacterial activity of test compounds (4a-4h) were determined using four different strains *Staphylococcus aureus* (Gram positive), *Staphylococcus epidermidis* (Gram positive), *Proteus mirabilis* (Gram negative) and *Pseudomonas aeruginosa* (Gram negative), by cup-plate method. The antifungal activity was evaluated using *Candida albicans* and *Aspergillus niger* strains, by broth dilution method. Stock solutions of test compounds were prepared in dimethyl formamide solution. Antimicrobial activity was carried out at eight different concentrations (12.5, 25, 50, 100, 200, 400, 800 and 1600 µg/mL). Antibacterial activity of test compounds was compared with two different standard compounds (amoxicillin and cefixime) and the antifungal activity were compared with standard (fluconazole). Assessment of structural similarities of all the synthesized compounds showed good percentage similarity when compared with standard drugs (Table 3). The antimicrobial activity has been shown in Table 4 and represented in terms of minimum inhibitory concentrations (MICs, µg/mL). From all the compounds 4b, 4c, 4d, 4g and 4h have shown significant antibacterial activity against *Staphylococcus epidermidis* and compound 4d has shown highest activity against *Staphylococcus aureus* (Fig 1). The entire newly synthesized compound (4a-4h) exhibited considerable activity against *Pseudomonas aeruginosa* and compound 4c showed highest activity against *Proteus mirabilis.* Compound 4c was the most potent antibacterial and antifungal activities among the compounds. The entire screened compound (4a-4h) exhibited excellent antifungal activity against *Aspergillus niger* (Fig 2)*,* when compared to standard drug (fluconazole).

Overall, a series of oxazolone derivatives were synthesized for their antimicrobial activity. Minimum inhibitory concentrations (MICs, µg/mL) of synthesized compounds were determined on different

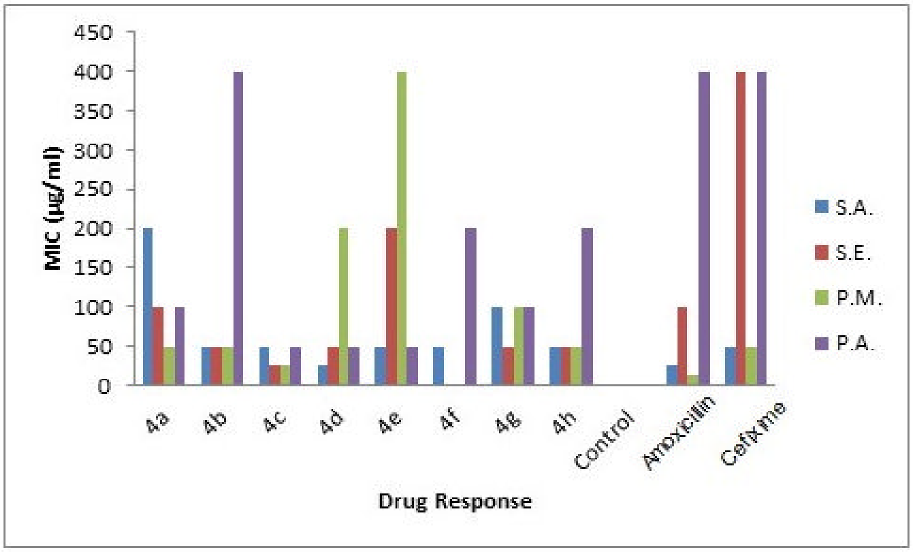


Fig 1. Comparison of MIC of different target compounds and standard drugs against various bacterial strains

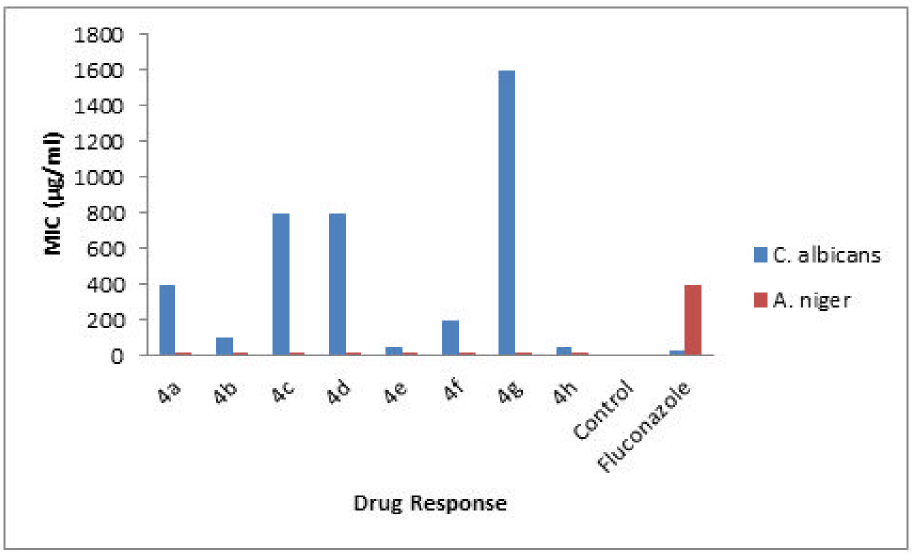


Fig 2. Comparison of MIC of different target compounds and standard drugs against various fungal strains

microorganisms using amoxicillin, cefixime and fluconazole as reference drugs. From the activity (MICs) data it was concluded that all the compounds (4a-4h) showed antimicrobial activity against bacteria including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proteus mirabilis, Pseudomonas aeruginosa* and fungus including *Candida albicans*, *Aspergillus niger*. Amongst all compounds 4b, 4c, 4d, 4g and 4h have shown moderate antibacterial activity against *Staphylococcus epidermidis*. Compound 4c and compound 4d showed highest activity against *Proteus mirabilis* and *Staphylococcus aureus* respectively. All the tested compounds (4a-4h) showed significant antibacterial activity against *Pseudomonas aeruginosa* and potent antifungal activity against *Aspergillus niger* respectively, when compared to the reference drugs. So, the significant antimicrobial activity of compound may be due to the presence of oxazolone moiety in addition to benzylidene nucleus.

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