SYNTHESIS AND EVALUATION OF OXAZOLIDINONES HAVING BENZO THIAZINEN MOIETIES AS ANTIMICROBIAL AND ANTI-INFLAMMATORY AGENTS

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Article Received on: 30/07/13 Revised on: 21/07/13 Approved for publication: 01/08/13

DOI: 10.7897/2230-8407.04853

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ABSTRACT

Phenyl oxazolidinones were prepared from R-glycidyl butarate and Para bromo aniline, further (3-(4-bromophenyl) methylene oxazolidine-5yl) and substituted benzothiazinen was refluxed resulted in oxazolidinones benzothiazinen moieties. Treatment of these oxazolidinones benzo thiazinen moieties with methane sulfonyl gives its sulphonates on further treatment with sodium azide and tri phenyl phosphine in acetic anhydride gives acetamide derivatives. Further synthesized compounds were characterized by IR, ¹H NMR and Mass spectral studies. The synthesized compounds were evaluated for antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and antifungal activity against, *Candida albicans* and *Aspergillus niger*. The synthesized compound was screened for their anti-inflammatory activity by carrageenan induced paw-edema method.

Keywords: Oxazolidinones, Benzothiazinen, Antibacterial, Antifungal, Antimicrobial, Anti-inflammatory.

INTRODUCTION

Many heterocyclic compounds due to their specific activity are employed in the treatment of many infectious diseases. Their use in the treatment is attributed to their inherent toxicity to various pathogens. Though the heterocyclic compounds were recognized lately their biological activities attracted the researchers. Generally five or six membered ring compounds have occupied the first place among various classes of organic compounds for their diverse biological activities. Oxazolidinone are well known five membered nitrogen and oxygen containing compounds. These have been reported to possess biological activities such as antibacterial activity¹. The emergence of bacterial resistance to the antibiotics poses a serious concern for medical professionals during the last decade². In particular multi-drug-resistant Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA)³ and *Staphylococcus epidermidis* (MRSE) and vancomycin-resistant *Enterococci* (VRE) are of major concern⁴. Many oxazolidinone derivatives are in clinical use such as linezolid, eperezolid as antimicrobial agent⁵. Linezolid is the first oxazolidinone approved for the treatment of Gram-positive bacterial infections in humans⁶. Since Linezolid, the many attractive traits of oxazolidinone series have encouraged further work in this area, and also the literature reveals extensive chemical programs exist⁷ at present, most efforts are focused on substituted phenyl oxazolidinones. Benzothiazinen are associated with diverse biological and pharmacological activities like antimicrobial⁸, anti-inflammatory.⁹ By considering the above facts and their increasing importance in pharmaceutical and biological field, it was considered of interest to synthesize some new chemical entities incorporating the two active pharmacophores in a single molecular frame work and to evaluate their biological activities. The incorporation of two moieties increases biological activity of both and thus it was of value to synthesize some new heterocyclic derivatives having two moieties in the same molecules. The synthesized (PKSN2A-PKSN2I) compounds were screened for anti-inflammatory, antibacterial and antifungal activities.

MATERIAL AND METHODS

All the chemicals were analytical grade; Tetrahydrofuran, Butyl lithium, R- glycidylbutarate Bezo thiazine , Triethylamine, Methane sulfonate, Dichloro methane, Hydrochloric acid, Glacial acetic acid ,Tri phenyl phosphine, Sodiumazide.

STEP 1: Synthesis of (3-(4-fluorophenyl) methylene oxazolidine-5yl) methanol.

Synthesis of (3-(4-fluorophenyl) methylene oxazolidine-5yl) methanol by reacting with R-glycidylbutarate with Para halo aniline in the presence of tetrahydrofuran and butyl lithium for 22 h at room temperature.¹⁰

STEP 2: Synthesis of oxazolidinones having benzo thiazinen and its derivatives.

- Synthesis of 5-(hydroxymethyl)-3-(4-(4-methyl-3, 4 dihydro-2H-benzo[b][1,4]thiazin-3-ylamino) phenyl) oxazolidin-2-one derivatives from benzthiazine amines derivatives and (3-(4- fluorophenyl) methylene oxazolidine-5yl) by simple reflux for three hours using acetonitrile solvent.¹¹
- Conversion of 5-(hydroxymethyl)-3-(4-(4-methyl-3,4dihydro-2H-benzo[b][1,4]thiazin- 3-yl amino) phenyl) oxazolidin-2-one derivatives to its methane sulfonate derivatives by using triethylamine in DCM later methanesulfonyl chloride added drop wise under vigorous stirring. Stirring for an additional 10–15 minutes completed the reaction.¹²
- 3-(4-(3,4dihydro-2h-benzo[b] [1,4] thiazin-3-ylamino) phenyl)-oxoxazolidin-5-yl) methyl methane sulfonate derivatives was converted to azido derivatives by treating with sodiumazide in N,N-dimethyl formamide (DMF).¹²
- 5-(Azidomethyl)-3-(4-(4-Methyl-3,4dihydro-2h-Benzo[B][1,4]Thiazin3-ylamino) Phenyl) oxazolidin-2-one was converted to its acetamide derivatives by treating with tri phenyl phosphine and hydrochloric acid later extracted with AcOEt.¹²

General Scheme of Synthesis

STEP 1

STEP 2

All reactions were carried out under prescribed laboratory conditions. All the reactions requiring anhydrous conditions were conducted in flame dried apparatus. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques wherever necessary and their melting points were checked with the available literature. Melting points of newly synthesized compounds were determined by open capillary method and were uncorrected. The final products were purified by re crystallization. The entire synthesised compound was purified by TLC method and characterised by IR, H NMR¹ and mass spectral method. IR was recorded in bruker alpha model using ATR. H NMR¹ data were recorded in (DMSO) on an Avance 400MHZ spectrophotometer using TMS as an internal standard. Mass spectra were recorded using LC-MS (SHIMADZU 2010-AT) under electro spray ionisation (ESI) technique.

N-((3-(4-(ethyl(4-methyl-3,4-dihydro-2h-benzo[b][1,4]thiazin-3-yl)amino)phenyl)-2-oxooxazolidin-5-yl) methyl) acetamide (PKSN2B)

IR (KBr) cm⁻¹: 3389(N-H), 3110 (aromatic C-H stretching), 2500 (S-H stretching) 2310 (aromatic C=C stretching), 1612 (aromatic C=C stretching), 1466 (C-N stretching) 824 (aromatic C-H deformation), 670 (C-Cl stretching), 1442 (C-N stretching), 1670 (C=O stretching in Oxoazolidine),
H NMR¹ (δ) in ppm: 8.03 (1H, s, NH), 6.58-7.33 (9H, d, Ar-H), 6.66-7.21 (4H, d, Ar-H in Benzothiazine ring), 3.31(1H, d, N-C-H in Benzothiazine ring), 4.03 (1H, d, S-C-H in Benzothiazine), 3.33 (1H, d, Oxazolidine ring), 3.03 (1H,d,N-CH₃ in Benzothiazine ring)
MS m/z (M⁺): 441.

Table 1: Physical Data of Synthesized Compounds

Comp. Code	Mol. Formula	Mol. Wt	M. P ⁰ C	Rf value (solvent system)	Physical Nature	% Yield
PKSN2A	C22H26N4O3S	426	160-163	0.26 C2H5COO:C6H6 20:80	White Crystal	80
PKSN2B	C23H28N4O3S	440	162-165	0.32 C2H5COO:C6H6 20:80	White Crystal	80
PKSN2C	C24H30N4O3S	454	170-172	0.38 C2H5COO:C6H6 20:80	White Crystal	75
PKSN2D	C24H30N4O3S	454	168-171	0.37 C6H5CH3:CH3OH (95:5)	White Crystal	71
PKSN2 E	C24H30ClN4O3S	488	180-183	0.33 C6H5CH3:CH3OH (95:5)	Greenish Crystal	70
PKSN2 F	C24H30BrN4O3S	533	186-189	0.38 C6H5CH3:CH3OH (95:5)	Brown Crystal	75
PKSN2 G	C25H31ClN4O3S	502	182-185	0.33 C2H5COO:C6H6 20:80	Greenish Crystal	70
PKSN2 H	C25H31BrN4O3S	547	192-195	0.42 C2H5COO:C6H6 20:80	Brown Crystal	75
PKSN2 I	C27H35N4O3S	495	184-187	0.31 C2H5COO:C6H6 20:80	White Crystal	65

Table 2: Antimicrobial Data Activity of Oxazolidinones having Benzo Thiazinen Moieties

Compound Number	Diameter of zone of inhibition (mm)					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. niger</i>
PKSN2 A	19	18	20	19	21	18
PKSN2 B	17	19	19	20	18	16
PKSN2 C	20	23	22	24	18	15
PKSN2 D	14	16	21	21	16	16
PKSN2 E	20	24	23	24	17	17
PKSN2 F	17	20	19	18	18	14
PKSN2 G	16	20	16	19	22	19
PKSN2 H	17	16	11	11	12	12
PKSN2 I	18	18	15	12	19	15
Amoxicillin	23	28	29	28	-	-
Fluconazole	-	-	-	-	25	21
CONTROL	-	-	-	-	-	-

Table 3: Anti-inflammatory Effect of Oxazolidinones having Benzo Thiazinen using Carrageen in induced Paw Oedema in Rats

Treatment	Dose mg/kg	Increase in paw volume (in ml)			
		1 h	2 h	3 h	4 h
Control		0.36 ± 0.06	0.68 ± 0.05	0.77 ± 0.03	0.81 ± 0.03
Diclofenac Sodium	13.5	0.18 ± 0.03* (50)	0.35 ± 0.05* (48.52)	0.41 ± 0.04* (46.75)	0.45 ± 0.05* (43.20)
PKSN2 A	200	0.20 ± 0.04* (44.41)	0.37 ± 0.03* (45.62)	0.41 ± 0.03* (45.47)	0.46 ± 0.03* (43.20)
PKSN2 B	200	0.23 ± 0.04 (36.11)	0.41 ± 0.04* (39.70)	0.47 ± 0.04* (38.96)	0.52 ± 0.04* (35.80)
PKSN2 C	200	0.24 ± 0.03* (33.33)	0.42 ± 0.02* (38.23)	0.46 ± 0.03* (40.25)	0.52 ± 0.03* (35.80)
PKSN2 D	200	0.19 ± 0.04* (47.22)	0.37 ± 0.03* (45.58)	0.42 ± 0.03* (45.45)	0.46 ± 0.04* (43.20)
PKSN2 E	200	0.19 ± 0.04* (47.22)	0.36 ± 0.03* (47.11)	0.41 ± 0.03* (46.75)	0.48 ± 0.02* (40.74)
PKSN2 F	200	0.20 ± 0.03* (44.44)	0.37 ± 0.03* (45.58)	0.42 ± 0.03* (45.45)	0.46 ± 0.03* (43.20)
PKSN2 G	200	0.19 ± 0.04* (47.22)	0.36 ± 0.03* (47.05)	0.41 ± 0.03* (46.75)	0.48 ± 0.03* (40.74)
PKSN2 H	200	0.16 ± 0.04* (17.77)	0.43 ± 0.03* (36.76)	0.50 ± 0.04* (35.06)	0.57 ± 0.03* (30.86)
PKSN2 I	200	0.23 ± 0.03* (36.11)	0.40 ± 0.03* (41.17)	0.46 ± 0.03* (40.96)	0.50 ± 0.04* (37.03)

All values are expressed as Mean ± SEM (n = 6). *P < 0.05 significant compared to control

N-((3-(4-((3-chloropropyl)(4-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)amino)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (PKSN2E)

IR (KBr) cm^{-1} : 3386 (N-H stretching), 3009 (aromatic C-H stretching), 2510 (S-H stretching) 2350 (aromatic C=C stretching), 1612 (aromatic C=C stretching), 825 (aromatic C-H deformation), 2550 (S-H stretching) 1189 (C-N stretching), 1670 (C=O stretching in Oxazolidine ring)

$^1\text{H NMR}$ (δ) in ppm: 8.03 (1H, s, NH), 6.58-7.27 (8H, d, Ar-H), 6.66-7.21 (4H, d, Ar-H in Benzothiazine ring), 3.31 (1H, d, N-C-H in Benzothiazine ring), 4.03 (1H, d, S-C-H in Benzothiazine), 3.33 (1H, d, Oxazolidine ring), 3.03 (1H, d, N-CH₃ in Benzothiazine ring)

MS m/z (M^+): 489.

N-((3-(4-((4-chlorobutyl)(4-methyl-3,4-dihydro-2H-benzo[b][1,4]thiazin-3-yl)amino)phenyl)-2-oxooxazolidin-5-yl)methyl)acetamide (PKSN2G)

IR (KBr) cm^{-1} : 3381 (N-H stretching), 3050 (aromatic C-H stretching), 2550 (S-H stretching), 1612 (aromatic C=C stretching), 824 (C-H deformation), 1189 (C-N stretching), 1671 (C=O stretching in Oxazolidine ring).

$^1\text{H NMR}$ (δ) in ppm: 8.03 (1H, s, NH), 6.58-7.21 (8H, d, Ar-H), 6.66-7.21 (4H, d, Ar-H in Benzothiazine ring), 3.31 (1H, d, N-C-H in Benzothiazine ring), 4.03 (1H, d, S-C-H in Benzothiazine), 3.33 (1H, d, Oxazolidine ring), 3.03 (1H, d, N-CH₃ in Benzothiazine ring).

MS m/z (M^+): 503.

Anti-microbiological Evaluation**Antibacterial Activity and Antifungal Activity Studies**

All the synthesized compounds were evaluated for the antimicrobial activity by cup-plate method. The following micro organisms were used to study the antibacterial activity of synthesized compound *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa* where as antifungal activities of synthesized compounds were studied against *Candida albicans* and *A. niger*. Amoxicillin and Fluconazole were taken as standard drug for the comparison of the activity of the synthesized compound for antibacterial and anti-fungal activity respectively.

Pharmacological Screening**Acute Toxicity Studies**

The preliminary pharmacological studies were conducted to assess the acute pharmacological effects and LD₅₀ of the drug. The acute toxicity study was carried out in adult female albino rats by "up and down" method (OECD guidelines 425).¹³

Selection of Doses

For the assessment of anti-inflammatory activity, three dose levels were chosen in such a way that, middle dose was approximately one tenth of the maximum dose during acute toxicity studies, and a low dose which was 50 % of the one tenth dose, and a high dose, which was twice that of one tenth dose (200 mg/kg, 400 mg/kg, 100 mg/kg).

Anti-inflammatory Activity

All the synthesized compounds were screened by Carrageenan induced rat paw oedema model (acute-inflammatory model) for the screening of anti-inflammatory activity. Diclofenac was taken as standard drug.

RESULTS AND DISCUSSION

In our study, new series of compounds namely Oxazolidinones having benzo thiazinen moieties (PKSN2A-PKSN2I) showed significant anti-inflammatory activity ($p < 0.05$) when compared with respective control groups. The effect of synthesized oxazolidinones having benzo thiazinen moieties has shown antibacterial and antifungal activity to certain extent. The results of these synthesized compounds are summarized in Table 2. Among the screened compounds, PKSN2C and PKSN2E have shown good antibacterial activity against gram +ve and gram -ve bacteria compared to the standard drug amoxicillin. Whereas PKSN2A and PKSN2G have shown significant antifungal activity against both *C. albicans* and *A. niger* compared to the standard drug Fluconazole. The anti-inflammatory activity studies of synthesized compounds by carrageenan induced paw oedema in rats are summarized in Table 3. Compounds, PKSN2A, PKSN2D and PKSN2F showed good anti-inflammatory activity whereas PKSN1E and PKSN1F showed moderate anti-inflammatory activity.

CONCLUSION

Results of present study demonstrate that a new class of different Oxazolidinones having benzothiazinen moieties were synthesized and evaluated for anti-inflammatory and anti-microbial activities. Among tested compounds PKSN2A, PKSN2D and PKSN2F moiety showed better anti-inflammatory activity, whereas PKSN2C, PKSN2E, PKSN2A, and PKSN2G moiety showed better anti-microbial activity. It can be concluded that Oxazolidinones having benzothiazinen moieties class of compounds certainly holds great promise towards the good activity leads in medicinal chemistry. A further study requires more information concerning pharmacological activity is in progress.

ACKNOWLEDGEMENT

The authors are thankful to Nitte University for providing the necessary facilities to carry out this work. The authors are also grateful to the director RSIC Chandigarh and CDRI Lucknow, India for providing IR, NMR and Mass Spectra Data.

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Cite this article as:

Jennifer Fernandes, Pankaj Kumar and Abhishek Kumar. Synthesis and evaluation of oxazolidinones having benzo thiazinen moieties as antimicrobial and anti-inflammatory agents. *Int. Res. J. Pharm.* 2013; 4(8):260-264 <http://dx.doi.org/10.7897/2230-8407.04853>

Source of support: Nil, Conflict of interest: None Declared