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Synthesis and antimicobial activity of new isoxazolyl-1,3-benzoxazines

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New isoxazolyl-1,3-benzoxazines **4** are synthesized from 4-amino-3,5-dimethyl isoxazole **1** by condensation with different salicylaldehydes, followed by reduction and subsequent smooth ring closure in presence of formaldehyde.

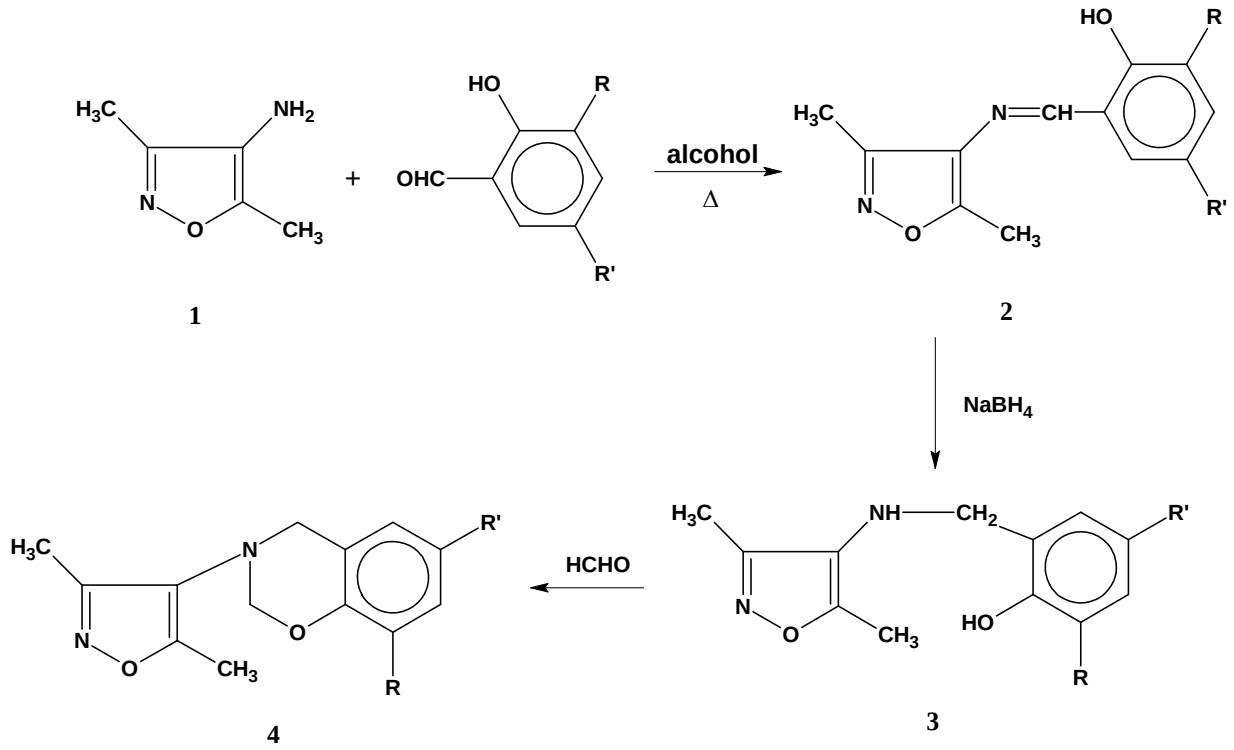
Keywords: isoxazolyl-1,3-benzoxazines, condensation, salicylaldehydes, ring closure, formaldehyde

Among a wide variety of heterocycles that have explored for developing pharmaceutically important molecules, 1,3-benzoxazines constitute an important group due to their wide variety of biological activity[1-5]. Compounds bearing the isoxazole moiety are endowed with various types of pharamacological activitites[6-8]. Literature survey revealed that when one biodynamic heterocyclic system was coupled with another, a molecule with enhanced biological activity[9,10] was produced. The chemistry of these linked biheterocycles has been the fascinating field of investigation in medicinal chemistry as they have been found to exhibit enhanced biological profile[11]. In view of these observations and also in continuation of our work on isoxazole substituted biheterocycles[12-16], it was thought worth-while to synthesize and investigate the activity of the compounds in which isoxazole moiety has been linked with benzoxazine nucleus. We report in this paper, the synthesis and antimicrobial activity of 3-(3,5-dimethyl-isoxazol-4-yl)-3,4-dihydro-2*H*-benzo[*e*][1,3]-oxazines.

4-Amino-3,5-dimethylisoxazole **1** was condensed with substituted salicylaldehydes by refluxing in ethanol to get the desired 2[(3,5-dimethyl-isoxazol-4-ylimino)-methyl]-phenols **2**. The IR spectrum of **2** showed a peak at 1615 cm^{-1} due to C=N stretching frequency and a broad band at 3425 due to hydroxy group. The ^{1}H NMR spectrum of **2** exhibited peak at 8.8 integrating for one proton due to azomethine proton. The structure of **2** was further confirmed by mass spectrum, which exhibited a molecular ion peak at *m/z* 216 and fragment ion peaks at *m/z* 202, 199, 175, 134, 133, 119 and 106. The compounds **2** on reduction with $NaBH_4$ produced 2[(3,5-dimethyl-isoxazol-4-ylamino)-methyl]phenols **3**. Its IR spectrum showed the absorption bands at 3450 and 3300 cm^{-1} due to OH and NH functional groups respectively. The ^{1}H NMR spectrum of **3** showed sharp singlet at δ 4.1 and a broad singlet at 3.8 due to methylene and NH protons respectively confirming reduction. As an additional proof, the mass spectrum of **3** was also recorded, which showed molecular ion peak at *m/z* 218 and fragment ion peaks *m/z* 217, 176 and 113. The compound **3** underwent smooth ring closure in presence of formaldehyde, involving internal Mannich reaction to give 3-(3,5-dimethyl-isoxazol-4-yl)-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazines **4**. The IR spectrum of **4** showed the absence of bands due to OH and NH group which were observed in its precursor. Its ^{1}H NMR spectrum displayed two distinct singlets at δ 4.3 and 5.0 due to newly formed 1,3-oxazine ring methylene protons (CH_2N and CH_2O) respectively confirming cyclization. The mass spectrum of **4** is also in agreement with the cyclized structure (M^+ 230, 170, 169, 132, 117, 104, 90) (**Scheme I**, **Tables I**, **and II**).

Antimicrobial activity

Compounds **5a-e** was evaluated *in vitro* for their antibacterial activity against gram-positive and gram-negative bacteria using acetone as a solvent and MIC was done by broth dilution method[17]. The bacterial strains used for the assays include *B.subtilis* (MTCC 441) *S. aureus* (MTCC 96), *P. aeruginosa* (MTCC 741), *K. aerogenes* (MTCC 39), *B. sphaericus* (MTCC 511), *C. violace* (MTCC 2656). The activity was compared with ciprofloxacin. The results are presented in **Table III**. Antifungal activity of



1 **2** **3** **4**

2, 3 and **4**
a, R = R' = H
b, R = H, R' = Cl
c, R = R' = Cl
d, R = H, R' = Br
e, R = R' = Br

Scheme I

compounds **5a-e** was evaluated against *A. niger* (MTCC 282), *C. tropicum* (MTCC 2821), *R. oryzae* (MTCC 262), *F. moliliforme* (MTCC 1848) and *C. lunata* (MTCC 2030) using acetone as a solvent by cup diffusion method[18]. The activity was compared with clotrimazole. The results are presented in **Table IV**. The zone of inhibition was measured after 24 hr of incubation at 37°C. The zone of inhibition developed if any, was then accurately measured and recorded.

From **Tables III** and **IV** it is clearly evident that isoxazolyl 1,3-benzoxazines are highly active against the bacterial and fungal strains. Among five isoxazolyl [1,3] benzoxazines **5a-e**, unsubstituted benzoxazine **5a** has activity similar to that of standard ciproflaxacin, whereas chloro and bromo **5b** and **5d** benzoxazines exhibited MIC more than that of the standard drug. Dichloro **5c** and dibromo **5e** benzoxazines have high antibacterial activity. The moderate activity of **5b** and **5d** compared to **5c** and **5e** may be due to mono chloro and monobromo benzoxazines and high activity of **5c** and **5e** may be ascribed to dichloro and dibromo benzoxazines. The antibacterial activity of **5c** and **5e** compared to the standard drug ciproflaxacin is promising.

The antifungal activity data indicate that the unsubstituted benzoxazine **5a** has moderate activity, whereas mono chloro and mono bromo benzoxazines **5b** and **5d** exhibited more activity than the standard clotrimazole. Dichloro and dibromo benzoxazines **5c** and **5e** are highly toxic towards the fungi under investigation compared to the standard clotrimazole and can be exploited for formulation of fungicide.

In conclusion, the isoxazolyl benzoxazines have moderate to excellent activity towards the bacteria and fungi under investigation. Some of them, particularly **5c** and **5e** can be exploited for formulation of bactericide and fungicide after detailed study.

In conclusion, new isoxazolyl-1,3-benzoxazines **4** are synthesized in minimum number of steps wherein

Table I — Physical data of compounds **2,3** and **4**

Product	R	R`	m.p. ^{0}C	Yield (%)	Mol.formula (Mol.wt.)	Found (Cald) (%) C	H	N
2a	H	H	56-58	97	$C_{12}H_{12}N_2O_2$ (216.23)	66.60 (66.66	5.57 5.55	12.89 12.96)
2b	H	Cl	93-95	95	$C_{12}H_{11}N_2O_2Cl$ (250.68)	57.63 (57.60	4.32 4.40	11.15 11.20)
2c	Cl	Cl	112-14	93	$C_{12}H_{10}N_2O_2Cl$ (285.12)	48.99 (48.95	3.57 3.52	9.79 9.85)
2d	H	Br	121-23	94	$C_{12}H_{11}N_2O_2Br$ (295.13)	48.99 (48.97	3.70 3.74	9.46 9.52)
2e	Br	Br	128-32	96	$C_{12}H_{10}N_2O_2Br_2$ (374.03)	38.64 (38.70	2.69 2.68	7.45 7.52)
3a	H	H	87-90	96	$C_{12}H_{14}N_2O_2$ (218.25)	66.01 (66.06	6.44 6.42	12.75 12.84)
3b	H	Cl	101-103	94	$C_{12}H_{13}N_2O_2Cl$ (252.69)	57.17 (57.14	5.09 5.15	11.07 11.11)
3c	Cl	Cl	109-13	95	$C_{12}H_{12}N_2O_2Cl_2$ (287.14)	50.28 (50.34	4.24 4.19	9.71 9.79)
3d	H	Br	111-15	93	$C_{12}H_{13}N_2O_2Br$ (297.15)	48.58 (48.04	4.30 4.39	9.38 9.45)
3e	Br	Br	118-21	96	$C_{12}H_{12}N_2O_2Br_2$ (376.04)	38.41 (38.50	3.12 3.20	7.50 7.48)
4a	H	H	106-109	95	$C_{13}H_{14}N_2O_2$ (230.26)	59.12 (59.09	4.91 4.92	10.64 10.60)
4b	H	Cl	115-18	97	$C_{13}H_{13}N_2O_2Cl$ (287.14)	59.12 (59.09	4.91 4.92	10.64 10.60)
4c	Cl	Cl	127-32	94	$C_{13}H_{13}N_2O_2Cl_2$ (299.15)	52.39 (52.34	4.07 4.02	9.30 9.39)
4d	H	Br	129-34	96	$C_{13}H_{13}N_2O_2Br$ (309.16)	50.58 (52.64	4.24 4.22	9.05 9.09)
4e	Br	Br	136-38	95	$C_{13}H_{13}N_2O_2Br_2$ (388.05)	40.36 (40.41	3.05 3.10	7.29 7.25)

Table II — ^{1}H NMR spectral data for compounds **2,3** and **4**

Product	^{1}H NMR (δ)
2a	2.25 (s, 3H, CH_3), 2.40 (s, 3H, CH_3), 6.91-7.82 (m, 4H, Ar-H), 8.81 (s, 1H, N=CH), 12.50 (bs, 1H, OH, D_2O exchangeable)
2b	2.28 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 7.00-7.85 (m, 3H, Ar-H), 8.88 (s, 1H, N=CH), 12.00 (bs, 1H, OH, D_2O exchangeable)
3a	2.20 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.06 (bs, 1H, NH, D_2O exchangeable), 4.15 (s, 2H, CH_2), 6.85-7.26 (m, 4H, Ar-H), 9.06 (bs, 1H, OH, D_2O exchangeable)
3b	2.25 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 3.85 (bs, 1H, NH, D_2O exchangeable), 4.20 (s, 2H, CH_2), 6.92-7.50 (m, 3H, Ar-H), 10.05 (bs, 1H, OH, D_2O exchangeable)
4a	2.22 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 4.33 (s, 2H, CH_2N), 5.01 (s, 2H, CH_2O), 6.81-7.05 (m, 4H, Ar-H)
4b	2.35 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 4.21 (s, 2H, CH_2N), 5.11 (s, 2H, CH_2O), 6.92-7.21 (m, 3H, Ar-H)
4c	2.32 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 4.41 (s, 2H, CH_2N), 5.11 (s, 2H, CH_2O), 7.00-7.50 (m, 2H, Ar-H)
4d	2.26 (s, 3H, CH_3), 2.42 (s, 3H, CH_3), 4.33 (s, 2H, CH_2N), 5.01 (s, 2H, CH_2O), 6.92-7.31 (m, 4H, Ar-H)
4e	2.42 (s, 3H, CH_3), 2.53 (s, 3H, CH_3), 4.42 (s, 2H, CH_2N), 5.14 (s, 2H, CH_2O), 6.93-7.31 (m, 2H, Ar-H)

Table III — Antibacterial activity (MIC)

Compd	Microorganisms					
	Gram-positive			Gram-negative		
	B.subtilis	*B.sphaericus*	*S.aureus*	*P.aeruginosa*	*Kaerogenes*	*C.violaceum*
5a	25	25	20	20	15	15
5b	20	20	18	18	15	15
5c	12.5	12	10.5	15	10.5	11.5
5d	20	20	15	18	15	15
5e	10.5	11	11.5	15	12.5	12.5
Standard : Ciprofloxacin	28	26	22	20	15	15

Table IV — Antifungal activity (Zone of inhibition in mm)

Compd	Zone of inhibition in mm				
	A.niger	*C.tropicum*	*R.oryzae*	*F.moniliforme*	*C.lunata*
5a	25	28	20	25	25
5b	29	35	25	30	30
5c	50	50	45	40	40
5d	30	40	30	35	35
5e	55	50	55	50	50
Standard: Clotrimazole	26	29	23	27	28

isoxazole is coupled with 1,3-benzoxazine. These coupled heterocycles demonstrated some promising antimicrobial activity.

Experimental Section

Melting points were determined on a Cintext melting point apparatus and are uncorrected. The purity of the compounds was checked by TLC. IR spectra was recorded in KBr on a Perkin-Elmer spectrum BX 300 series FT-IR spectrometer, ^{1}H NMR spectra on a Varian Gemini 300 MHz spectrometer using tetramethyl silane as internal standard and mass spectra on a Jeol JMC- 300 spectrometer. The silica gel (0.040 × 0.063 mm) used for column chromatography was purchased from Merck. C, H and N analyses were carried out on Carlo Erba 106 Perkin-Elmer Model 240 analysers.

2[(3,5-Dimethyl-isoxazol-4-ylimino)-methyl]-phenols 2a-e

A mixture of 3,5-dimethyl-4-amino isoxazole (0.01 mole) and salicylaldehydes (0.01 mole) were refluxed in ethanol (10 mL) for 2 hr. The resultant solution was cooled; the solid that separated was filtered and recrystallized from pet. ether (**Table I**).

2[(3,5-Dimethyl-isoxazol-4-ylamino)-methyl]-phenols 3a-e

Sodium borohydride (0.02 mole) was added to a solution of compound **2** (0.01 mole) in methanol (10 mL) and mixture was stirred for 30 min. at room temperature. The solid separated, on pouring the reaction-mixture into ice-cold water, was filtered and recrystallized from pet. ether.

3(3,5-Dimethyl-isoxazol-4-yl)-3,4-dihydro-2*H*-benzo[*e*][1,3]oxazines 4a-e

Compound **3** (0.01 mole) and formalin (37%, 1mL) were refluxed on a water bath for 5 hr in $CHCl_3$ (10 mL). The solvent was removed under vaccum and the crude solid obtained was recrystallized from pet. ether.

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