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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF 3,4-DIHYDRO-3-(3,5-DIMETHYL-4-ISOXAZOLYL)-2*H*-BENZO[*e*][1,3]-OXAZINES

R. Sanjeev, K. Thirupathaiah and E. Rajanarendar*

Department of Chemistry, Kakatiya University, Warangal-506009(T.S.), India.

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***Corresponding Author**
E. Rajanarendar
Department of Chemistry,
Kakatiya University,
Warangal-506009(T.S.),
India.

ABSTRACT

A new series of 3,4-dihydro-3-(3,5-dimethyl-4-isoxazolyl)-2*H*-benzo[*e*][1,3]-oxazines **(5)** have been synthesized from 4-amino-3,5-dimethylisoxazole **(1)**. Compound **(1)** on condensation with substituted salicylaldehydes **(2)**, followed by reduction with $NaBH_4$, and subsequent ring closure in presence of formaldehyde afforded the title compounds by internal Mannich reaction. All the newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectral data. Compounds **(5a-f)** have been screened for antimicrobial activity. Compounds **5a, 5b,** and **5c** exhibited significant antimicrobial activity when compared to the standard drugs.

KEYWORDS: Isozaxolyl-1,3-benzoxazines, Condensation, Reduction, Ring closure, Internal Mannich reaction, Antimicrobial activity.

INTRODUCTION

The chemistry of 1,3-benzoxazines and their derivatives has received considerable attention due to their wide range of pharmacological activities. These include bactericidal,[1] fungicidal,[2] anticancer,[3] antidiabetic and hypolipidemic,[4] antidepressant,[5] and antiplatlet aggregation activity[6]. A large number of isoxazole derivatives exhibited anticancer,[7] anticonvulsant,[8] analgesic,[9] antibacterial[10] and antifungal activity.[11] Considering pharmacological activities of 1,3-benzoxazine and isoxazole moieties, we have designed and synthesized new compounds consisting of these two units. As a sequel to our work on searching for new biologically active molecules,[12-14] we, herein, report the synthesis and antimicrobial activity of 3,4-dihydro-3-(3,5-dimethyl-4-isoxazolyl)-2*H*-benzo[*e*][1,3]-oxazine derivatives.

MATERIALS AND METHODS

All the melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. IR Spectra (KBr Pellet) were recorded on a Perkin-Elmer BX series FT-IR Spectrometer. ^{1}H NMR spectra were recorded on a Bruker 300 MHz Spectrometer. ^{13}C NMR spectra were recorded on a Bruker 75 MHz Spectrometer. Chemical shift values are given in δ ppm with TMS as internal standard. ESI mass spectra were scanned on an Agilent LC-MSD mass spectrometer. Elemental analyses was performed on a Carlo Erba 106 and Perkin-Elmer model 240 analyzers.

EXPERIMENTAL

General procedure for the synthesis of 3,4-dihydro-3-(3,5-dimethyl-4-isoxazolyl)-2*H*-benzo[*e*][1,3]-oxazines (5a-f)

STEP 1: Synthesis of 2-[(3,5-dimethyl-4-isoxazolyl)imino]methyl phenols (3a-f)

A mixture of 4-amino-3,5-dimethylisoxazole **(1)** (0.01 mol) and substituted salicylaldehydes **(2)** (0.01 mol) were refluxed in methanol (10 mL) for 2 h. The resultant solution was cooled, the solid that separated was filtered and recrystallized from pet. ether.

Spectra data of Compounds (3a-f)

Compound 3a: Yield. 75%, m.p. 140-42ºC, IR (KBr, cm^{-1}): 1611 (C=N), 3448 (OH); ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.4 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 4.0 (s, 3H, OCH_3), 6.9-7.1 (m, 3H, Ar-H), 8.6 (s, 1H, CH=N), 13.2 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 247 $[M + H]^+$. Anal. Calcd. for $C_{13}H_{14}N_2O_3$. C, 63.41; H, 5.69; N, 11.38. Found: C, 63.40; H, 5.67; N, 11.39%.

Compound 3b: Yield. 75%, m.p. 136-38ºC, IR (KBr, cm^{-1}): 1610 (C=N), 3480 (OH); ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.9 (s, 3H, OCH_3), 6.8-7.1 (m, 3H, Ar-H), 8.5 (s, 1H, CH=N), 13.0 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 247 $[M + H]^+$. Anal. Calcd. for $C_{13}H_{14}N_2O_3$. C, 63.41; H, 5.69; N, 11.38. Found: C, 63.42; H, 5.66; N, 11.37%.

Compound 3c: Yield. 80%, m.p. 150-52ºC, IR (KBr, cm^{-1}): 1610 (C=N), 3450 (OH); ^{1}H NMR (300 MHz, $CDCl_3$, ppm) δ: 1.2 (t, 6H, 2 CH_3), 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.4 (m, 4H, 2 CH_2), 6.6 (m, 2H, Ar-H), 7.1 (m, 1H, Ar-H), 8.4 (s, 1H, CH=N), 13.1 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 288 $[M + H]^+$. Anal. Calcd. for $C_{16}H_{21}N_3O_2$. C, 66.89; H, 7.31; N, 14.63. Found: C, 66.90; H, 7.33; N, 14.65%.

Compound 3d: Yield. 70%, m.p. 160-62ºC, IR (KBr, cm^{-1}): 1615 (C=N), 3423 (OH); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.4 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 6.8-7.2 (m, 3H, Ar-H), 8.5 (s, 1H, CH=N), 12.5 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 235 $[M + H]^+$. Anal. Calcd. for $C_{12}H_{11}N_2O_2F$. C, 61.53; H, 4.70; N, 11.96. Found: C, 61.55; H, 4.68; N, 11.96%.

Compound 3e: Yield. 72%, m.p. 168-70ºC, IR (KBr, cm^{-1}): 1615 (C=N), 3430 (OH); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.4 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 6.8-7.1 (m, 3H, Ar-H), 8.6 (s, 1H, CH=N), 12.9 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 262 $[M + H]^+$. Anal. Calcd. for $C_{12}H_{11}N_3O_4$. C, 55.17; H, 4.21; N, 16.09. Found: C, 55.15; H, 4.20; N, 16.11%.

Compound 3f: Yield. 70%, m.p. 180-82ºC, IR (KBr, cm^{-1}): 1617 (C=N), 3446 (OH); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.4 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 7.6 (s, 1H, Ar-H) 8.0 (s, 1H, Ar-H), 8.4 (s, 1H, CH=N), 13.8 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 469 $[M + H]^+$. Anal. Calcd. for $C_{12}H_{10}N_2O_2I_2$. C, 30.76; H, 2.13; N, 5.98. Found: C, 30.77; H, 2.11; N, 5.95%.

STEP 2: Synthesis of 2-[3,5-dimethyl-4-isoxazolyl)amino]methyl phenols (4a-f)

Sodium borohydride (0.02 mol) was added to a solution of the compound **(3)** (0.01 mol) in methanol (10 mL) drop wise with stirring for 30 min. After the addition is completed, the reaction mixture was brought to room temperature, and stirring continued further for another 2 h. The solid separated on pouring the reaction mixture on ice-cold water was filtered, and recrystallized from pet. ether.

Spectra data of Compounds (4a-f)

Compound 4a: Yield. 70%, m.p. 110-12ºC, IR (KBr, cm^{-1}): 3346 (NH), 3447 (OH); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.2 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 3.0 (bs, 1H, NH, D_2O exchangeable), 3.9 (s, 3H, OCH_3), 4.1 (s, 2H, CH_2), 6.7-7.0 (m, 3H, Ar-H), 9.5 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 249 $[M + H]^+$. Anal. Calcd. for $C_{13}H_{16}N_2O_3$. C, 62.90; H, 6.45; N, 11.29. Found: C, 62.91; H, 6.44; N, 11.28%.

Compound 4b: Yield. 70%, m.p. 106-08ºC, IR (KBr, cm^{-1}): 3355 (NH), 3410 (OH); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.1 (bs, 1H, NH, D_2O exchangeable), 3.9 (s, 3H, OCH_3), 4.2 (s, 2H, CH_2), 6.8-7.1 (m, 3H, Ar-H), 10.5 (bs, 1H, OH,

D_2O exchangeable), MS (ESI): *m/z* 249 $[M + H]^+$. Anal. Calcd. for $C_{13}H_{16}N_2O_3$. C, 62.90; H, 6.45; N, 11.29. Found: C, 62.93; H, 6.46; N, 11.27%.

Compound 4c: Yield. 68%, m.p. 136-38ºC, IR (KBr, cm^{-1}): 3310 (NH), 3412 (OH); ^1H NMR (300 MHz, $CDCl_3$, ppm) *δ*: 1.3 (t, 6H, 2 CH_3), 2.4 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.5 (bs, 1H, NH, D_2O exchangeable), 3.7 (m, 4H, 2 CH_2), 4.0 (s, 2H, CH_2), 6.7 (m, 2H, Ar-H), 7.3 (m, 1H, Ar-H), 11.0 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 290 $[M + H]^+$. Anal. Calcd. for $C_{16}H_{23}N_3O_2$. C, 66.43; H, 7.95; N, 14.53. Found: C, 66.40; H, 7.97; N, 14.55%.

Compound 4d: Yield. 60%, m.p. 151-53ºC, IR (KBr, cm^{-1}): 3325 (NH), 3510 (OH); ^1H NMR (300 MHz, $CDCl_3$, ppm) *δ*: 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.2 (bs, 1H, NH, D_2O exchangeable), 4.2 (s, 2H, CH_2), 6.7-7.2 (m, 3H, Ar-H), 12.8 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 237 $[M + H]^+$. Anal. Calcd. for $C_{12}H_{13}N_2O_2F$. C, 61.01; H, 5.50; N, 11.86. Found: C, 61.00; H, 5.51; N, 11.88%.

Compound 4e: Yield. 65%, m.p. 158-60ºC, IR (KBr, cm^{-1}): 3310 (NH), 3500 (OH); ^1H NMR (300 MHz, $CDCl_3$, ppm) *δ*: 2.4 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.2 (bs, 1H, NH, D_2O exchangeable), 4.0 (s, 2H, CH_2), 6.8-7.1 (m, 3H, Ar-H), 12.5 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 264 $[M + H]^+$. Anal. Calcd. for $C_{12}H_{13}N_3O_4$. C, 54.75; H, 4.94; N, 15.96. Found: C, 54.77; H, 4.95; N, 15.96%.

Compound 4f: Yield. 62%, m.p. 165-67ºC, IR (KBr, cm^{-1}): 3271 (NH), 3449 (OH); ^1H NMR (300 MHz, $CDCl_3$, ppm) *δ*: 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.0 (bs, 1H, NH, D_2O exchangeable), 4.1 (s, 2H, CH_2),7.3 (s, 1H, Ar-H), 8.0 (s, 1H, Ar-H), 10.5 (bs, 1H, OH, D_2O exchangeable), MS (ESI): *m/z* 471 $[M + H]^+$. Anal. Calcd. for $C_{12}H_{12}N_2O_2I_2$. C, 30.63; H, 2.55; N, 5.95. Found: C, 30.66; H, 2.53; N, 5.93%.

STEP 3: Synthesis of 3,4-Dihydro-3-(3,5-dimethyl-4-isoxazolyl)-2*H*-benzo[*e*][1,3]-oxazines (5a-f)

Compound **(4)** (0.01 mol) and formalin (37%, 2 mL) were refluxed in ethanol (10 mL) on a hot water bath for 6 h. The solvent was removed under vacuum, and the crude product obtained after usual process was recrystallized from pet. ether.

Spectra data of Compounds (5a-f)

Compound 5a: Yield. 75%, m.p. 160-62ºC, IR (KBr, cm^{-1}): 2937 (CH_2N), 2894 (CH_2O); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.2 (s, 3H, CH_3), 2.3 (s, 3H, CH_3), 3.9 (s, 3H, OCH_3) 4.3 (s, 2H, NCH_2), 5.0 (s, 2H, OCH_2), 6.6 (d, 1H, Ar-H), 6.8 (d, 1H, Ar-H), 6.9 (m, 1H, Ar-H);^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ: 9.99, 11.08, 50.43, 81.76, 93.13, 109.69, 118.20, 120.67, 122.49, 125.81, 143.65, 148.52, 158.03, 161.84. MS (ESI): *m/z* 261 $[M + H]^+$. Anal. Calcd. for $C_{14}H_{16}N_2O_3$. C, 64.61; H, 6.15; N, 10.76. Found: C, 64.65; H, 6.13; N, 10.77%.

Compound 5b: Yield. 75%, m.p. 168-70ºC, IR (KBr, cm^{-1}): 2925 (CH_2N), 2885 (CH_2O); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.9 (s, 3H, OCH_3) 4.3 (s, 2H, NCH_2), 5.1 (s, 2H, OCH_2), 6.7 (d, 1H, Ar-H), 6.8 (d, 1H, Ar-H), 7.0 (m, 1H, Ar-H; MS (ESI): *m/z* 261 $[M + H]^+$. Anal. Calcd. for $C_{14}H_{16}N_2O_3$. C, 64.61; H, 6.15; N, 10.76. Found: C, 64.60; H, 6.14; N, 10.75%.

Compound 5c: Yield. 73%, m.p. 175-77ºC, IR (KBr, cm^{-1}): 2928 (CH_2N), 2875 (CH_2O); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 1.2 (t, 6H, 2 CH_3), 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 3.8 (m, 4H, 2 CH_2) 4.1 (s, 2H, NCH_2), 5.2 (s, 2H, OCH_2), 6.7 (m, 2H, Ar-H), 7.1 (m, 1H, Ar-H); MS (ESI): *m/z* 302 $[M + H]^+$. Anal. Calcd. for $C_{17}H_{23}N_3O_2$. C, 67.77; H, 7.64; N, 13.95. Found: C, 67.80; H, 7.66; N, 13.98%.

Compound 5d: Yield. 65%, m.p. 182-84ºC, IR (KBr, cm^{-1}): 2930 (CH_2N), 2872 (CH_2O); ^1H NMR (300MHz, $CDCl_3$, ppm) δ: 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 4.2 (s, 2H, NCH_2), 5.1 (s, 2H, OCH_2), 6.7-7.1 (m, 3H, Ar-H); MS (ESI): *m/z* 249 $[M + H]^+$. Anal. Calcd. for $C_{13}H_{13}N_3O_2F$. C, 62.90; H, 5.24; N, 11.29. Found: C, 62.91; H, 5.22; N, 11.27%.

Compound 5e: Yield. 68%, m.p. 190-92ºC, IR (KBr, cm^{-1}): 2925 (CH_2N), 2890 (CH_2O); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.3 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 4.2 (s, 2H, NCH_2), 5.1 (s, 2H, OCH_2), 6.8-7.2 (m, 3H, Ar-H); MS (ESI): *m/z* 276 $[M + H]^+$. Anal. Calcd. for $C_{13}H_{13}N_3O_4$. C, 56.72; H, 4.72; N, 15.27. Found: C, 56.75; H, 4.72; N, 15.25%.

Compound 5f: Yield. 70%, m.p. 210-12ºC, IR (KBr, cm^{-1}): 2940 (CH_2N), 2885 (CH_2O); ^1H NMR (300 MHz, $CDCl_3$, ppm) δ: 2.1 (s, 3H, CH_3), 2.2 (s, 3H, CH_3), 4.2 (s, 2H, NCH_2), 5.0 (s, 2H, OCH_2), 7.2 (m, 1H, Ar-H), 7.9 (m, 1H, Ar-H); ^{13}C NMR (75 MHz, $CDCl_3$, ppm) δ:10.35, 11.21, 50.59, 82.65, 124.09, 125.34, 135.15, 136.71, 145.02, 146.08, 153.37, 157.72,

161.78; MS (ESI): *m/z* 483 $[M + H]^+$; Anal. Calcd. for $C_{13}H_{12}N_2O_2I_2$. C, 32.36; H, 2.48; N, 5.80. Found: C, 32.35; H, 2.47; N, 5.82%.

ANTIBACTERIAL ACTIVITY

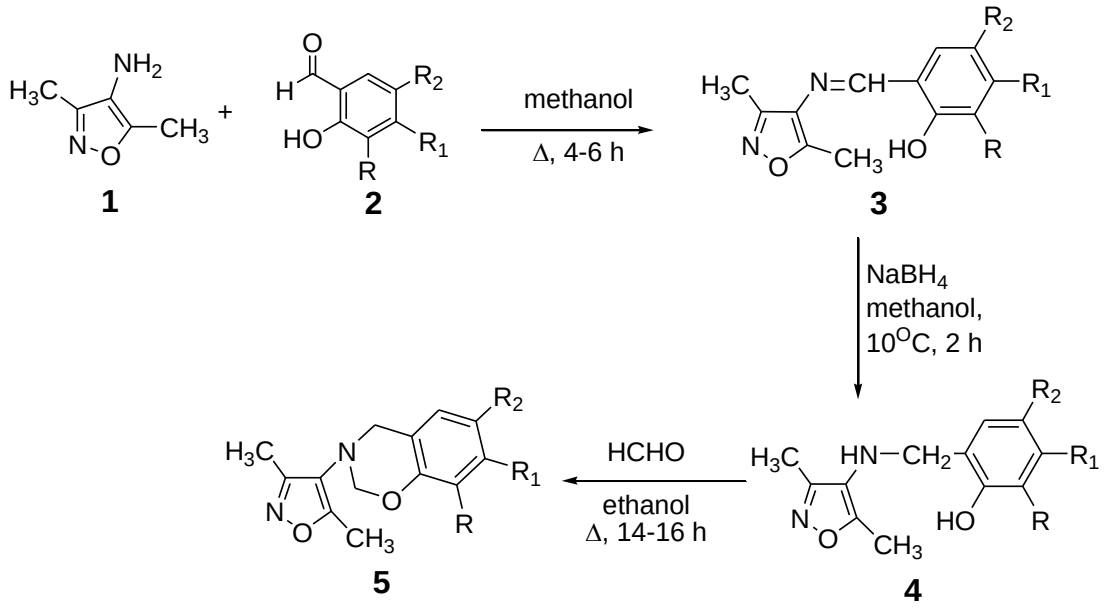
The antibacterial activity was done by broth dilution method[15] and expressed as minimum inhibitory concentration *Ciproflaxacin* was used as reference drug. The ready made nutrient broth medium (Himedia, 24 g) was suspended in distilled water (100 mL) and heated to boiling until it dissolved completely. The medium and test tubes were auto claved at pressure of 15 lb/ inc^2 for 20 min. A set of sterilized test tubes with nutrient broth medium was capped with cotton plugs. The test compound **(5)** is dissolved in suitable solvent (acetone) and concentration of 100 µg/mL of test compound is added in the first test tube, which is serially diluted. A fixed volume of 0.5 mL overnight culture is added in all test tubes and are incubated at 37^{o}C for 24 h. After 24 h, these tubes were measured for turbidity. Bacterial strains used for the present investigation were *Pseudomonas aeruginosa (Pa), Klobsiella aerogenes (Ka), Chromobacterium violaceum (Cv), Bacillus subtilis (Bs), Bacillus sphaericus (Bsp),* and *Staphylococus aureus (Sa).*

ANTIFUNGAL ACIVITY

The antifungal activity was done by using agar cup bioassay method.[16] *Flucanazole* was used as reference drug. The ready made potato dextrose agar (PDA) medium (Himedia, 39g) was suspended in distilled water (1000 mL), and heated to boiling until it dissolved completely. The medium and petri dishes were auto claved at pressure of 15 lb / inc^2 for 20 min. The medium was poured in to sterile petri dishes under aseptic conditions in a laminar flow chamber. When the medium in the plates solidified, 0.5 mL of (Week old) culture of test organism was inoculated and uniformly spread over the agar surface with a sterile L-shaped rod. Solutions were prepared by dissolving the compound **(5)** in acetone (100 µg/ mL). Agar inoculated cups were scooped out with 6 mm sterile cork borer, and the lids of the dishes were replaced. To each cup, 100 µg / mL concentration of test solutions **5a-f** was added. Controls were maintained with acetone and *Flucanazole* (100 µg / mL). The treated and the controls were kept at room temperature for 72-96 h. Inhibition zones were measured and diameter was calculated in millimetre. Three to four replicates were maintained for each treatment. *Aspergillus niger (An), Chysosporium tropicum (Ct), Rhizopus oryzae (Ro), Fusarium moniliforme (Fm)* and *Curvularia lunata (Cl)* were used as fungal strains.

RESULTS AND DISCUSSION

A new series of 3,4-dihydro-3-(3,5-dimethyl-4-isoxazolyl)-2*H*-benzo[*e*][1,3]-oxazines **(5a-f)** were synthesized as outlined in **Sheme 1**. The 4-amino-3,5-dimethylisoxazole **(1)** was treated with substituted salicylaldehydes **(2)**in reluxing methanol to get desired products *viz.*, 2-[(3,5-dimethyl-4-isoxazolyl)imino]methyl phenols **(3a-f),** which on reduction with sodium borohydride produced the corresponding 2-[(3,5-dimethyl-4-isoxazolyl)amino]methyl phenols **(4a-f)** in good yields. The amino phenols **(4a-f)** underwent smooth ring closure on treatment with formaldehyde in ethanol to give title compounds **(5a-f)** by internal Mannich reaction.



Scheme 1: Synthesis of isoxazolyl-1,3-benzoxazines.

3, 4 & 5: a, R = OCH_3, R_1 = H, R_2 = H

3, 4 & 5: b, R = H, R_1 = OCH_3, R_2 = H

3, 4 & 5: c, R = H, R_1 = $N(C_2H_5)_2$, R_2 = H

3, 4 & 5: d, R = H, R_1 = H, R_2 = F

3, 4 & 5: e, R = H, R_1 = H, R_2 = NO_2

3, 4 & 5: f, R = I, R_1 = H, R_2 = I

ANTIBACTERIAL ACTIVITY

The antibacterial activity results showed that compounds **5a-f** exhibited good antibacterial activity compared to standard drug *Ciprofloxacin* **(Table 1).** The activity was expressed in terms of minimum inhibitory concentration (MIC). The compounds **5a, 5b** and **5c** are highly

active, because the activity is considerably affected by the presence of groups like methoxy and *N*-*N*'-diethylamino as substitutes on benzene ring, besides the influence of basic skeleton isoxazolyl benzoxazine. It has been observed that exceptional activity of compound **5c** is due to presence of *N*-*N*'-diethylamino group on phenyl ring. Rest of the compounds **5d, 5e** and **5f** showed moderate activity. However, the degree of inhibition varied both with the test compound as well as with the bacteria used in the present investigation.

Table 1: Antibacterial activity data of 3,4-dihydro-3-(3,5-dimethyl-4-isoxazolyl)-2*H*-benzo[*e*][1,3]oxazines (5a-f).

Compound	Conc. (µg/mL)	Minimum Inhibitory Concentration (MIC)[a]					
		Gram-negeitive			Gram-positive		
		Pa	***Ka***	***Cv***	***Bs***	***Bsp***	***Sa***
5a	100	10	8	9	8	10	12
5b	100	11	7	8	7	8	11
5c	100	9	6	7	6	8	8
5d	100	18	12	14	13	12	16
5e	100	20	15	16	12	15	14
5f	100	16	14	15	14	13	18
Ciprofloxacin		30	25	25	20	20	25

[a]Negative control (acetone) - No activity

ANTIFUNGAL ACTIVITY

The antifungal activity of the compounds **5a-f** showed that they are significantly toxic towards all the five pathogenic fungi and they are lethal even at 100 µg / mL concentration **(Table. 2).** The activity data is indicated as zone of inhibition at 100 µg / mL concentration. Compounds **5a, 5b** and **5c** exhibited high activity and they inhibited the growth of fungi to a remarkable extent, which may be due to the presence of methoxy and *N*-*N*'-diethylamino substituents on benzene ring, besides the influence of isoxazolyl benzoxazine skeleton. These compounds are highly toxic compared to that of standard *Flucanazole*. Compound with *N*-*N*'-diethylamino substituent **5c** showed remarkable toxicity against the fungi used in the present investigation, and it is lethal even at 100 µg/mL concentration in comparison with standard *Flucanazole* at the same concentration, However, the degree of spore germination inhibition varied with the test compound as well as with the fungi under study.

Table 2: Antifungal activity data of 3,4-dihydro-3-(3,5-dimethyl-4-isoxazolyl)-2*H*-benzo[*e*][1,3]oxazines (16a-f).

Compound	Conc. (µg/mL)	Zone of inhibition mm[a]				
		An	***Ct***	***Ro***	***Fm***	***Cl***
5a	100	50	59	56	54	56
5b	100	51	58	60	55	57
5c	100	61	60	62	60	62
5d	100	46	51	52	41	44
5e	100	45	49	35	42	38
5f	100	46	47	55	48	35
Flucanazole		29	30	28	23	20

[a]Negative control (acetone) - No activity

CONCLUSION

In conclusion, we have achieved the synthesis of new isoxazolyl 1,3-benzoxazines by utilizing minimum number of steps, and by easily adoptable method, where in isoxazole is coupled with 1,3-benzoxazine. The results of antimicrobial data indicated that compound **5c** is highly active, hence it can be exploited for formulation of bactericide/fungicide after detailed study.

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