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Novel approach to the synthesis of 3-substituted (1,2,4)triazolo(3,4-*b*)1,2-benzisoxazole and their antimicrobial activity

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ABSTRACT

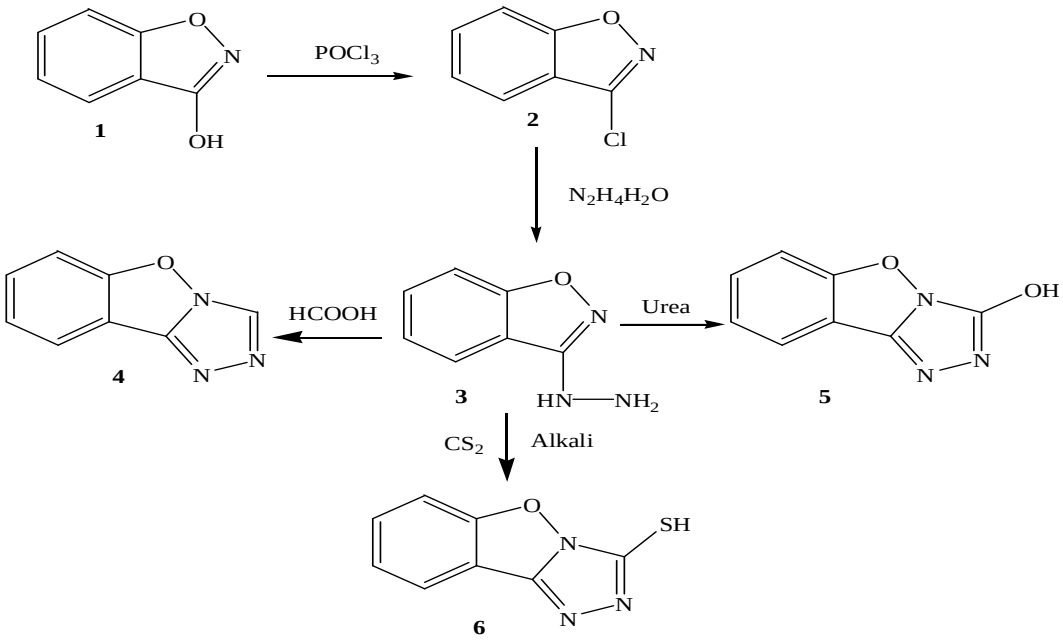
*Hydroxy benzisoxazole **1** was converted into chlorobenzisoxazole **2** and then to hydrazinobenzisoxazole **3**. New tricyclic compounds, triazolobenzisoxazole **4** was obtained from hydrazinobenzisoxazole when treated with formic acid. Similarly 3-hydroxytriazolo benzisoxazole **5** and 3-mercaptotriazolobenzisoxazole were prepared by the action of urea and CS_2 in alkali respectively. The compounds have been characterized by elemental analysis and spectral data. Newly synthesized compounds have been screened for their antimicrobial activities against several microbes.*

Keywords: Triazolobenzisoxazole, 3-hydroxytriazolobenzisoxazole, 3-mercapto triazolobenz - isoxazole, antimicrobial activity.

INTRODUCTION

While the chemistry including biological activity of some triazolobenzoxazole [1, 2] has been extensively studied. Little is known about triazolobenzisoxazole [3, 5] references are mostly available in the patent form only. In view of recent reports for structure, activity relationship of oxaza heterocycles, it was considered of much interest to device convenient rout for the synthesis of isoxazolo compounds [6, 7]. In this communication we wish to report the synthesis of novel heterocycles triazolobenzisoxazole and their antimicrobial activity (**scheme-1**).

Scheme-1



EXPERIMENTAL SECTION

Melting points were determined in open capillary tubes and are uncorrected. ir spectra were recorded in potassium bromide discs (ע max in cm^{-1}) on Perkin-Elmer FT-IR (spectrum ONE) spectrometer, ^1H nmr spectra on Gemini 200 MHz spectrometer and Mass spectra on FT.VG-7070 mass spectrometer. The progress of reaction was monitored by TLC.

General Procedures.

1) 3-Chloro-1, 2-benzisoxazole (2):- A mixture of (6.70 g, 0.049 mole) 3-hydroxy benzisoxazole, (16.4 mL) phosphorous oxychloride and (7 mL) triethylamine was refluxed for 16 hours in oil bath at temperature 150-160 °C. The contents were cooled and ice cold water (10 mL) was added. Then reaction mass was evaporated to dryness. Yield 4.342 g (57%), ir (potassium bromide): 1612 (C=N), 1250 (C-N), 1200 (C-O), 757 (C-Cl), 3170 (Ar-H) cm^{-1}; ^1H nmr (D_2O): δ 7.5 (m, 4H, Ar-H); ms: m/z 153(M^+). Anal. Calcd for C_7H_4ClNO: C, 54.72; H, 2.60; N, 9.12. Found: C, 54.15; H, 2.32; N, 8.91.

2) 3-Hydrazino 1,2-benzisoxazole (3):- A mixture of compound **2** (5.33 g, 0.034 mole), ethanol (35 mL) and hydrazine hydrate (35 ml 80%) refluxed for half an hour. Ethanol was evaporated to dryness. Compound obtained was hygroscopic in nature. Yield 3.20 g (62%), mp 350 °C; ir (potassium bromide): 1642 (C=N); 1269 (C-N); 1173 (C-O); 3163 (Ar-H); 3417 (N-H) cm^{-1}; ^1H nmr (D_2O): δ 7.3 (m, 4H, Ar-H), 4.2 (s, 1H, NH), 2.0 (s, 2H, NH_2); ms: m/z 149(M^+). Anal. Calcd for $C_7H_7N_3O$: C, 56.37; H, 4.71; N, 28.18. Found: C, 56.03; H, 4.13; N, 27.83.

3) 1,2,4-Triazolo-(3,4-*b*)1,2-benzisoxazole (4):- A mixture of compound **3** (2.98 g, 0.02 mole) and formic acid (30 mL) refluxed for 90 minutes in oil bath at 150 °C. The contents were

cooled and poured with constant stirring into ice water. The resulting solution was neutralized with sodium carbonate solution (5%) and extracted with petroleum ether. Yield 1.876 g (59%), mp 382 °C; ir (potassium bromide): 1650 (C=N); 1296 (C-N); 1219 (C-O); 3173 (Ar-H) cm^{-1}; ^1H nmr (D_2O): δ 8.2 (m, 4H, Ar-H), 8.5 (s, 1H, heteryl proton CH); ms: m/z 159(M^+). Anal. Calcd for $C_8H_5N_3O$: C, 60.37; H, 3.14; N, 26.41. Found: C, 60.13; H, 2.96; N, 26.38.

4) 3-Hydroxy-1,2,4-triazolo-(3,4-*b*)1,2-benzisoxazole (5):- Compound **3** (0.447 g, 0.003 mole) was heated with pre-dried urea (0.4 g) at 180-190 °C for six hours on oil bath. Then the contents were cooled and sodium hydroxide (30 mL, 5%) was added to it. The solution was filtered and acidified with dilute hydrochloric acid. Thus obtained solid was filtered, dried and purified by recrystalization from absolute ethanol to yield colorless crystalline compound **5**. Yield 0.341 g (65%), mp 360 °C; ir (potassium bromide): 1637 (C=N); 1400 (C-N); 1078 (C-O); 3205 (Ar-H); 3416 (broad, -O-H) cm^{-1}; ^1H nmr (D_2O): δ 5.2 (s, 1H, OH), 7.26 (m, 4H, Ar-H); ms: m/z 175(M^+). Anal. Calcd. for $C_8H_5N_3O_2$: C, 54.85; H, 2.85; N, 24.00. Found: C, 54.35; H, 2.54; N, 23.78.

5) 3-Mercapto1,2,4-triazolo-(3,4-*b*) 1,2-benzisoxazole (6):- A mixture of 1.52 g carbon disulphide, ethanol (60 mL), 3-hydrazino benzisoxazole (2.682 g, 0.018 mole) and 1.12 g of potassium hydroxide in (5 mL) water was refluxed on water bath for two hours. Ethanol was removed and the residue was dissolved in aqueous (20 mL, 5%) potassium hydroxide. The solution was filtered and the filtrate was acidified with dilute hydrochloric acid and again filtered. The solid thus obtained was recrystalized with ethanol. Yield 1.890 g (55%), mp 105 °C; ir (potassium bromide): 1600 (C=N); 1431 (C-N); 1108 (C-O); 2890 (SH); 3205 (Ar-H) cm^{-1}; ^1H nmr (D_2O): δ 7.6 (m, 4H, Ar-H), 3.0 (s, 1H, SH); ms: m/z 191(M^+). Anal. Calcd for $C_8H_5N_3OS$: C, 50.26; H, 2.61; N, 21.98. Found: C, 50.01; H, 2.38; N, 21.78.

6) Attempted isomerization of 1,2,4-triazolo-(3,4-*b*)1,2-benzisoxazole:-

a) With hydrochloric acid :- Compound **4** (1.2 g) was refluxed with (25 mL) of 2N hydrochloric acid over wire gauze for six hours. On cooling, contents of the flask neutralized with sodium carbonate. The precipitate obtained was filtered and crystallized from ethanol, melting point 382 °C. Mixed melting point with starting material was unchanged.

b) With sodium hydroxide: Compound **4** (0.5 g) was refluxed with (25 mL) of 20% sodium hydroxide for six hours on wire gauze. On cooling s-triazolo-(3,4-*b*)-1,2-benzisoxazole was recovered unchanged. The product obtained had high melting point 382 °C. Mixed melting point remained unchanged.

c) Heating with formic acid: Compound **4** (0.7 g) was refluxed with (25mL) 98% formic acid for six hours over an oil bath. After cooling contents were poured with stirring into ice cold water, neutralized it by sodium carbonate. The precipitate was filtered, dried and purified by recrystallization with absolute ethanol. The product obtained had high melting point mixed melting point with starting material unchanged.

This shows that the 1,2,4-triazolo-(3,4-*b*)1,2-benzisoxazole **4** is a stable ring towards acid, alkali and heat treatments.

Antimicrobial activity:

Compounds were tested for their antibacterial activity by paper disc method against bacterial species *Eschershia coli*, *Staphylococcus aureus* and *Bacillius subtilis* and the fungal species *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum* and *Fusarium moneli forme.* DMSO was used as a control solvent and *Penicillin* was taken as standard antibacterial. Similarly

for antifungal studies *Grysofulvin* was selected as standard antifungia, the solvent was DMSO and hence used as a control. The test bacterium was seeded in the medium and its sensitivity towards synthesized compound was determined by measuring the zones of growth inhibitors. Nutrient medium was sterilized at 120 ^{0}C and at 15 lbs/sq inch pressure for 20 minutes. A suitable dilution growth culture of the test bacteria was spread over media with the help of micropipette and allowed to diffuse for 1.00 hr. These plates were incubated at 35 ^{0}C for 24 hrs for antibacterial and antifungal activity. The degree of sensitivity was determined by measuring growth inhibition zone around the disc Table 1 and 2.

Table 1. Evaluation of antibacterial activity of synthesized compounds (zone of inhibition in mm)

Sr. No	Compound	*Eschershia Coli*	*Staphylococcus aureus*	*Bacillus subtilis*
1	1,2,4-Triazolo(3,4-*b*)1,2-benzisoxazole	-ve	17 mm	-ve
2	3-Hydroxys-1,2,4-triazolo(3,4-*b*)1,2-benzisoxazole	18 mm	19 mm	20 mm
3	Standard (*Penicillin*)	11 mm	40 mm	28 mm
4	Control (DMSO)	-ve	-ve	-ve

Legends - -ve = no antibacterial activity, Zone of inhibition - -- mm

Table 2. Evaluation of antifungal activity of synthesized compounds (zone of inhibition in mm)

Sr. No	Compound	*Aspergillus niger*	*Aspergillus flavus*	*Penicillium chrysogenum*	*Fusarium moneliforme*
1	1,2,4-triazolo(3,4-*b*)1,2-benzisoxazole	+ve	+ve	+ve	+ve
2	3-Hydroxy-1,2,4-triazolo(3,4-*b*)1,2-benzisoxazole	+ve	+ve	+ve	+ve
3	Control (DMSO)	+ve	+ve	+ve	+ve
4	Standard (Grysofulvin)	-ve	-ve	-ve	-ve

Legends - +ve –Growth- No Antifungal activity, -ve–Growth- Antifungal activity observed

RESULT AND DISCUSSION

The antibacterial studies show that 3-hydroxytriazolobenzisoxazole shows some activities towards *Eschershia coli*, *Staphylococcus aureus* and *Bacillius subtilis*, where as simple 1,2,4-triazolobenzisoxazole is not active against any bacterial species except staphylococcus aureus. This indicates the presence of hydroxyl group at 3-position induces some sort of antibacterial activity. As regards antifungal activity both the compounds triazolobenzisoxa -zole and 3-hydroxytriazolobenzisoxazole are found to be inactive.

As far as stability studies of new heterocycles is concerned it is reported that simple oxazole are stable to alkali but under drastic conditions isoxazole rings open up, much depends upon substituent present. In present investigation we found triazolobenzisoxazole ring is very stable to acid, alkali and heat treatment. Even ring isomerisation is not possible as was observed in triazolopyridines. Sandwiching of isoxazole ring between benzene and triazole prevents ring opening and imparts stability.

Perhaps we are the first to report the synthesis of simple 1,2,4-triazolo(3,4-*b*)1,2-benzisoxazole a novel oxaza heterocycles which may in future prove to be of versatile utility for medicinal chemistry, pharmaceutical formulation and in agro industries also.

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