

Synthesis, Characterization and Biological Screening of Azepine Derivative: 2-hydroxy-1,3-di(naphthalene-2-yl)-1H-benzo[b]azepine-5(4H)one

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Abstract

The synthesis of unsaturated heterocyclic compounds containing nitrogen atoms in the ring is very important due to its various biological application in the pharmaceutical industry. Azepine derivatives find numerous application almost every field in medicinal chemistry and some of its are commercially available as drugs. The two-component of azepine derivatives were synthesized by using the aniline and maleic anhydride as a starting material followed by condensation with sodium borohydride in presence of dry benzene, subsequently cyclization by polyphosphoric acid then, finally by an addition reaction with naphthalene-2-ol to form the desired derivative. The formation of the synthesized azepine derivative was confirmed by spectral techniques such as IR, ¹H-NMR, and ¹³C-NMR. The antibacterial assay shows that the synthesized compound (2A) possesses the most highly potent activity in the *Bacillus subtilis* and moderate activity against other different strains of bacteria and fungi.

Keywords: Azepine derivatives, heterocyclic, pharmacological, antibacterial, antifungal

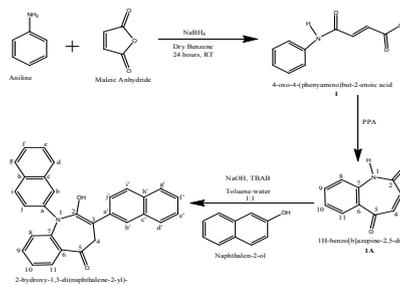
Introduction

Azepine is an unsaturated heterocyclic organic compound of seven atoms, with nitrogen replacing a carbon at one position. Its chemical formula is C₆H₇N and its molar mass is 93.13 gmol⁻¹ [1]. Azepine is a seven-membered nitrogen heterocycle with a biologically active epitope and a useful building block in the construction of various organic molecules. When a group or group of atoms is attached to the azepine ring, where there are profound novel influences in the biological activity of these molecules [2].

Azepine and its derivatives are imperative types of compounds that have been widely explored for the biological activities in the pharmaceutical industry. Pharmacological activities of azepine include antibacterial [3], antiviral, antioxidant, anticancer and antitumor [4,5], antiparasitic [6], antiemetic, antihistaminic, spasmolytic, antagonistic [7], anticonvulsive, anti-inflammatory, and anti-fungicidal activities [8]. These derivatives are also used in antimalarial drug therapy, anti-HIV, and other various pharmaceutical applications [9]. Even azepine based drugs are used for the treatment of Parkinson's and Alzheimer's diseases since they exhibit anticonvulsant activity and may be used as minor tranquilizers. Some azepine derivatives (Triazepine) affects the central

nervous system and exhibit pesticidal and herbicidal activities [10,11].

Due to the unique flexibility of the seven-membered ring, it has received much more attention in both materials and the medicinal industry. An effective anticonvulsant drug, 5H-dibenzo[b,f]azepine-5-carboxamide, was the first synthesis by Schindler in 1960, and since, then it has become the most frequently prescribed the first-line drug for the treatment of epilepsy [12,13]. Because of extensive biological activities of azepine analogous, a facial synthesis of 2-hydroxy-1,3-di(naphthalene-2-yl)-1H-benzo[b]azepine-5(4H)one (2A) and 1H-dibenzo[b]azepine-2,5-dione (1A) using PPA has been undertaken and tested for antimicrobial activity against different pathogens.



Scheme 1: Scheme for the synthesis of azepine derivatives

Materials and Methods

A typical procedure for the synthesis of 4-oxo-4-(phenylamino)but-2-enoic acid (1)

Sodium borohydride (0.38 g) in dry benzene (15 mL) was stirred for 15 min. Then, aniline (0.95 mL) was added to it and stirred for an hour. The powdered maleic anhydride (1.48 g) was added to the reaction mixture and stirred for 24 hours.

The reaction mixture was diluted with an equal volume of water. The benzene layer along with precipitate was separated from the aqueous layer. The benzene layer was extracted with 5% of NaHCO₃ and acidified with 2N HCl. The precipitate was filtered and the crude solid was recrystallized from ethanol to achieve (1) as white solid [8].

A typical procedure for the synthesis of 1H-dibenzo[b]azepine-2,5-dione (1A)

The compound (1) obtained from the above procedure i.e. 4-oxo-4-(phenylamino)but-2-enoic acid (1.91 g) in PPA (20 mL) was refluxed for 3 hours at 80 °C and then stirred at room temperature for 12 hours.

The reaction mixture was diluted with cold water and extracted with chloroform (3×20 mL). The organic layer was washed with 5% NaHCO₃ (3×20 mL) and washed with water (3×20 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness. The crude solid was recrystallized with ethanol to achieve (1A) as a brownish white solid [8].

Physical properties

Molecular formula = C₁₀H₉NO₂, Molecular mass = 175.18 gmol⁻¹, Color = Brownish white, Yield = 74 %
Melting point = 167-169 °C

IR ν_{\max} (selected bands) cm⁻¹ = 3344(s), 3084(m), 1675(w), 1573(m), 1379(s), and 994(s). ¹H-NMR (500 MHz, DMSO-d₆) = δ ppm: 7.8614-7.7962 (1H, d, H-11), 7.7962-7.7679 (1H, t, H-9), 7.7763-7.6749 (1H, d, H-8), 7.6074-7.5776 (1H, d, t-10), 5.4206 (1H, H-3), and 5.4206 (1H, H-4).

¹³C-NMR (125 MHz, DMSO-d₆) = δ ppm: 170.6355 (C-5), 147.3104 (C-2), 134.1688 (C-7), 128.9088 (C-9), 128.9088 (C-11), 124.8834 (C-8), 122.9313 (C-6), 122.9313 (C-10), 69.8616 (C-3), and 69.8616 (C-4).

A typical procedure for the synthesis of 2-hydroxy-1,3-di(naphthalene-2-yl)-1H-benzo[b]azepine-5(4H)-one (2A)

The compound (1A) which was obtained from the above step i.e. 1H-dibenzo[b]Azepine-2,5-dione was taken in a mixture of toluene and water in the ratio of 1:1 and sodium hydroxide was added followed by the tetra-n-butylammonium bromide at room temperature. After 15 minutes, naphthalen-2-ol was

added to a reaction mixture, which was heated at 60 °C for 5 hours. After the completion of the reaction, the reaction mixture was diluted with water 50 mL. The aqueous layer was extracted with ethyl acetate (3×20 mL), and the combined ethyl acetate layer was washed with 0.1N hydrochloric acid (2×25 mL). Finally, the organic layer was dried over anhydrous sodium sulfate. Then, the compound was recrystallized in ethyl acetate and hexane, to yield (2A) as a light greenish solid [14].

Physical properties

Molecular formula = C₃₀H₂₁NO₂, Molecular mass = 427.49 gmol⁻¹, Color = Light greenish, Yield = 69 %
Melting point = 212 °C

IR ν_{\max} (selected bands) cm⁻¹ = 3479(w), 3017-2914(m), 1771(s), 1437(m), 1078(s), and 718(s).

¹H-NMR (500 MHz, DMSO-d₆) = δ ppm: 13.406 (1H, s, HO-), 8.100 (1H, H-d'), 8.093 (1H, H-g'), 8.087 (1H, H-g), 8.081 (1H, H-i), 8.076 (1H, H-b'), 8.068 (1H, H-d), 8.025 (1H, H-i'), 8.022 (1H, s, H-b), 8.015 (1H, H-e'), 8.009 (1H, H-f'), 8.004 (1H, d, H-11), 7.998 (1H, H-e), 7.990 (1H, H-j), 7.987 (1H, H-j'), 7.853-7.778 (1H, d, H-8), 7.763-7.603 (1H, dd, H-f), 7.588-7.567 (1H, t, H-10), 7.562-7.55 (1H, dd, H-9), and 5.415 (2H, s, H-4).

¹³C-NMR (125 MHz, DMSO-d₆) = δ ppm: 171.116 (C-5), 168.998 (C-2), 163.720 (C-7), 163.72 (C-a), 147.787 (C-9), 136.659 (C-11), 136.659 (C-c), 136.659 (C-d), 136.659 (C-h), 136.659 (C-c'), 136.659 (C-h'), 134.654 (C-g'), 134.654 (C-i'), 131.722 (C-8), 131.722 (C-i), 129.396 (C-d'), 129.396 (C-e'), 129.396 (C-f'), 125.825 (C-j'), 125.371 (C-e), 125.371 (C-f), 125.371 (C-g), 125.339 (C-6), 125.339 (C-10), 123.417 (C-b), 123.41 (C-j), 123.41 (C-a'), 123.41 (C-b'), and 70.346 (C-3).

Results and Discussion

The characteristics of the two azepine derivatives, that are 1H-dibenzo[b]azepine-2,5-dione (1A) and 2-hydroxy-1,3-di(naphthalene-2-yl)-1H-benzo[b]azepine-5(4H)-one (2A) are examined by the various spectroscopic parameters such as Fourier-transform infrared spectroscopy and Nuclear magnetic resonance spectroscopy (¹H-NMR and ¹³C-NMR). The biological activities of the compounds are also analyzed.

FT-IR analysis

The FTIR spectra of the studied compound 2A show a weak band for stretching hydroxyl group (-OH) at the range of 3479 cm⁻¹ due to the properties that exhibit excellent hydrogen bonding and also shows a -OH bending vibration at 1437 cm⁻¹. Furthermore,

medium bands in the range of 3017-2914 cm^{-1} are observed for compound 2A are attributed to the stretching vibration of aromatic hydrogen. However, a strong band in the region of 1771 cm^{-1} is observed in the spectra due to stretching vibration of the carbonyl group ($\text{C}=\text{O}$) and a strong band at 1078 cm^{-1} due to the C-H bend vibration.

Meanwhile, the spectra of compound 1A show a sharp and strong band at 3344 cm^{-1} is due to the presence of stretching vibration of the N-H bond. The compound exhibit Amide I and Amide II bands at 1675 cm^{-1} 1573 cm^{-1} respectively. These two bands are due to the $\text{C}=\text{O}$ stretching vibration and N-H bending vibration respectively. Also, a weak band at the range of 3084 cm^{-1} is observed which are the bands of aromatic hydrogen.

¹H-NMR analysis

The signal of most deshielded proton of -OH is observed most downfield at around 13 ppm because

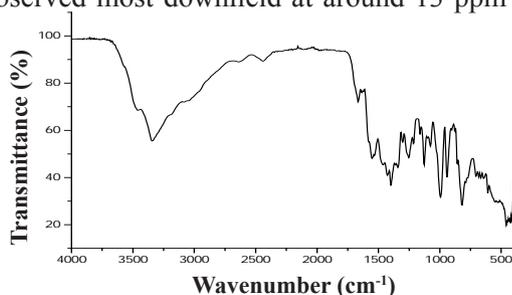


Figure 1: IR spectra of synthesized compound (1A)

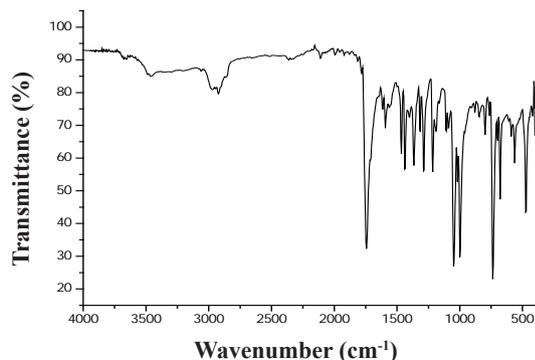


Figure 2: IR spectra of synthesized compound (2A)

this hydrogen is bonded with highly electronegative atom oxygen and α -position is occupied by more electronegative atom nitrogen. On the other hand, the H-4 proton is shielded and is observed upfield at around 5 ppm, it is because of the resonance effects of an oxygen atom and double bond present at the β -position of the H-4 atom and also shows an integration of two protons. The H-9 proton shows a signal at around 7.562-7.55 ppm and the H-f proton shows the signal at around 7.763-7.603, whereas both the proton shows a duplet of duplet. Similarly, the

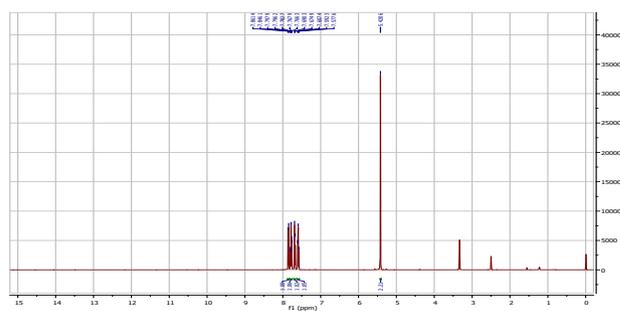


Figure 3: ¹H-NMR spectra of synthesized compound (1A)

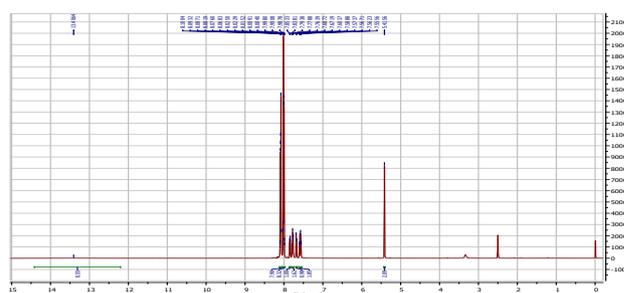


Figure 4: ¹H-NMR spectra of synthesized compound (2A)

signals for all the naphthyl protons are deshielded which are observed downfield at around 7.990 - 8.100 ppm.

However, for compound 1A, the proton of H-3 and H-4 are shielded and are observed upfield at around 5.5 ppm, it is because that the proton of double-bonded carbon is a resonance with the two carbonyl group present neighbor to the double bond. Whereas the signal for aromatic proton i.e. H-8, H-9, H-10, and H-11 appears at 7.5776 - 7.8614 ppm.

¹³C-NMR analysis

The signal of ¹³C-NMR depends upon the carbon atom bonded with the substitution group or atom. The signal of the C-5 position of carbon is more deshielded due to the presence of an oxygen atom. ¹³C-NMR signals of C-5 in two different compounds 1A and 2A appear at 170.6355 ppm and 171.116 ppm respectively. Similarly, the signal of the C-2 position of carbon is also deshielded due to the presence of a hydroxyl group directly attached to the C-2 Carbon and one nitrogen atom at the α -position of the azepine ring. The ¹³C-NMR signals of C-2 in compound 2A appeared at 168.998 ppm. Likewise the ¹³C-NMR signals of C-8, C-a, and C-a' are also deshielded and appear at 163.70 ppm. It is because of the presence of nitrogen atom directly to that carbon. On the other hand, the signal for C-3 is more shielded and appears upfield with a 70.346 ppm. The signal is upfield due to the presence of double bond and electronegative nitrogen as well as hydroxyl group directly attached to the α -position of C-3, which undergo resonance

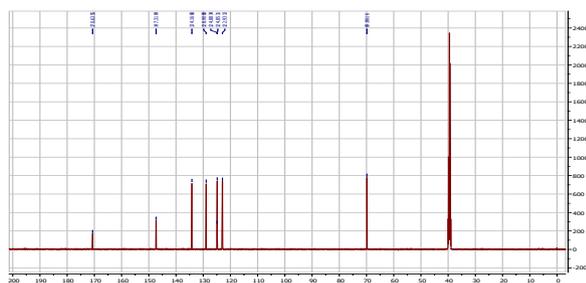


Figure 5: $^{13}\text{C-NMR}$ of synthesized compound (1A)

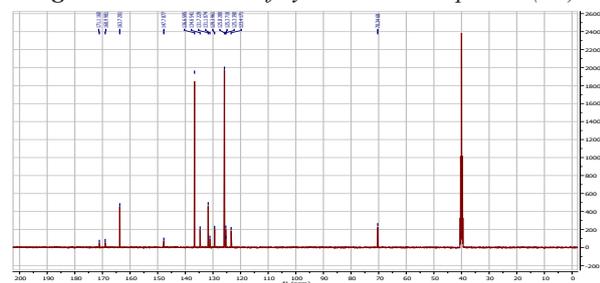


Figure 6: $^{13}\text{C-NMR}$ of synthesized compound (2A)

hybrid. Similarly, the signal for C-4 is overlapped with the signal of the solvent. In a compound 2A, the peaks for the naphthyl carbon are recorded at a range of 123.417 – 136.659 ppm.

Although the C-2 Carbon has directly attached more electronegative nitrogen atom and an oxygen atom, it is less deshielded than the C-5 Carbon in compound 1A is due to the presence of a double bond at the α -position of C-2 carbon which undergoes resonance with the lone pair of nitrogen electron and an oxygen atom. The signal for the C-2 carbon atom of compound 1A is recorded at 147.3104 ppm. However, the signal for C-3 carbon is observed at more shielded, with a 69.8616 ppm due to the resonance of double bond with the lone pair of electron of two oxygen atoms. Finally, the signals for the aromatic carbon are recorded at the range of 122.9313 – 134.1668 ppm.

Antimicrobial activity

The antimicrobial activity of all the synthesized azepine derivatives was studied against two different bacterial strains. One gram-positive i.e. *Bacillus subtilis* and another is gram-negative i.e. *Enterobacter cloacae*, using Ofloxacin as the positive control, whereas fungal strain *Aspergillus spp.* using methanol as the solvent control.

Table 1: Antibacterial activity of synthesized compounds

| Bacterial Strain | The diameter of the Zone of Inhibition (mm) | | | | |
|-----------------------------|---|---------|---------------|---------|-----------|
| | Compound (1A) | | Compound (2A) | | Ofloxacin |
| | 1 mg/mL | 5 mg/mL | 1 mg/mL | 5 mg/mL | |
| <i>Bacillus subtilis</i> | 12 | 13 | 22 | 24 | 42 |
| <i>Enterobacter cloacae</i> | 10 | 12 | 0 | 06 | 39 |

Table 2: Antifungal activity of synthesized compounds

| Fungal strain | The diameter of the zone of inhibition (mm) | | | | |
|-------------------------|---|-------------------|-------------------|-------------------|--------------------|
| | Compound (1A) | | Compound (2A) | | Control (Methanol) |
| <i>Aspergillus spp.</i> | 400 μg | 500 μg | 400 μg | 500 μg | |
| | 3 mm | 4 mm | 9 mm | 11 mm | No activity |

Antibacterial activity

The zone of inhibition shows by the two synthesized compounds have been tabulated in table 1. The antibacterial activity of the two synthesized compounds viz. 1A and 2A show good activity against the *Bacillus subtilis*. Compound 2A was found to exhibit the most highly potent activity than the compound 1A in the *Bacillus subtilis*. Against *Enterobacter cloacae*, compound 2A was found to be inactive at a concentration of 1 mg/mL. The other compound 1A shows the moderated activity at both the concentration.

Antifungal activity

Against *Aspergillus spp.* the compound 1A was found to be least effective whereas compound 2A exhibit moderate activity.

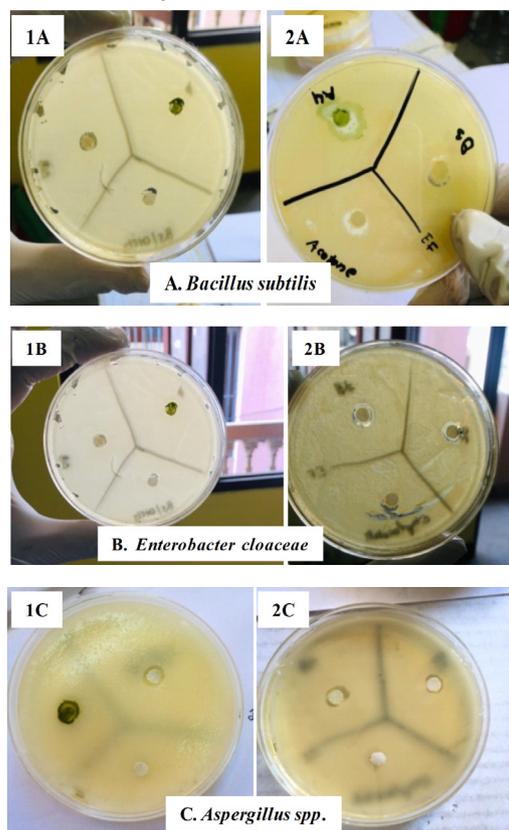


Figure 7: Antimicrobial activities of synthesized compounds against (A) *Bacillus subtilis* (B) *Enterobacter cloacae*, and (C) *Aspergillus spp.*

Conclusion

This study concludes that the two pieces of azepine derivatives were synthesized i.e.

1H-dibenzo[b]azepine-2,5-dione and 2-hydroxy-1,3-di(naphthalene-2-yl)-1H-benzo[b]azepine-5(4H)one. Both the compounds are synthesized from aniline and maleic anhydride as a starting material. However, a former one is a two-step process and the latter one is synthesized from a former one by addition reaction. The formation of azepine derivatives was supported by IR, ¹H-NMR, and ¹³C-NMR spectroscopy. In the present study, it has found that compound 2A was found to be exhibit the most highly potent activity than the compound 1A in *Bacillus subtilis*. And, against *Aspergillus spp*, the compound 1A was found to be the least activity whereas compound 2A exhibits moderate activity.

On the other hand, these research results have pointed out further possibilities of works on these virgin compounds creating an opportunity for scientific research to give birth to potent lead molecules as in pharmaceutical and also for the welfare of humanity.

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