Study of Heterocyclic Ring Systems: Biopharmaceutical Applications of Substituted 4H-1,4-Benzothiazine and Piperazine

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Abstract: In present work study of biologically important heterocycles such as 4H-1,4-benzothiazine and substituted piperazine was carried out. Reaction mixture of substituted 4H-1,4-Benzothiazine and piperazine was examined for their importance as biopharmaceutical material such as antimicrobial and antioxidant agents. Current research article based on fundamental concept of green chemistry best out of waste.

Keywords: Benzothiazine, Piperazine, Antioxidant, Antimicrobial activities.

Introduction

Among the publications of the organic chemistry, Heterocyclic chemistry is one of the most important parts of organic chemistry. Out of all the organic compounds up to two thirds are heterocyclic compounds. Heterocyclic compounds are the dominant structure of numerous biologically active compounds. Indole has an important place in several heterocyclic systems because it’s existing as a component in a number of compounds possessing a wide-ranging field of biological activities. In Natural products and Pharmaceutical products heterocyclic spiro-oxindole structure is a vital structure in biological compounds. Indole ring which involves itself with sulphur and nitrogen containing heterocyclic compounds is the chemistry of spiro-indoles. Because of the biological and physiological activities, spiro carbon atoms are of great interest [1-10]. Heterocyclic compounds are divided into two classes, aliphatic and aromatic. Aliphatic heterocyclic compounds are the cyclic analogues of amines, ethers, tho-ethers, and amides. Literature confirmed that heterocycles are rich in nature and mainly concerned in the manufacturing of major medicinal used products. Piperazine-Benzothiazine holding heterocycles play a dynamic part in chemistry; especially heterocyclic chemistry. In present days researchers give more focus on bio-dynamic heterosystem; piperazine-1,4-benzothiazine integrating physically as well as chemically. Such grouping will mark them important to connect more efficiently with biological receptors [11-21].

Methodology

Preparation of substituted 4H-1,4-benzothiazine and substituted piperazine mixture.

Substituted Piperazine(0.009mol) in isopropanol(7.5ml) and methanol(3ml) mix with the solution of substituted 4H-1,4-benzothiazine (0.003mol) in equimolar amount of isopropanol and methanol and stirred followed by refluxing for 58 hours. Solution was dried and washed with pet ether [22-23]. Possibilities of chemical interaction is also examined with TLC (Thin Layer Chromatography) and sophisticated instruments such as IR(figure-1), NMR. 4H-1,4-benzothiazine and substituted piperazine mixture were also examined for biological activities such as Anti-oxidant, Anti-bacterial, Anti-fungal.
Figure 1: IR spectra of 4H-1,4-benzothiazine and substituted piperazine mixture

Biopharmaceutical Applications

4H-1,4-benzothiazine and substituted piperazine mixture was examined for antimicrobial and antioxidant activities.

(A) Antimicrobial activities:-

Antimicrobial activities of 4H-1,4-benzothiazine and substituted piperazine mixture was carried out by disk diffusion method.

Preparation of Broth- Dissolve 13g nutrient broth in 1000ml of water and place that nutrient broth and other glassware like petri plate, conical flask in autoclave for sterilization of media and glassware for 25 minutes. Remove from autoclave and place in laminar air flow which creates an aseptic condition where inoculation of bacterial culture is added in broth then remove and put it into incubator for 24hours for incubation of broth takes place.

Preparation of Nutrient Agar- Take 30g of Mueller Hinton agar in 250ml water and autoclave is done. After autoclave we placed nutrient agar in laminar air flow and pour in petri plates and let them solidify. After solidify spread the inoculation of culture from broth and then put the test disc on bacteria lawn with the test solution of different concentration and incubation it in incubator at 37degree and check the zone inhibition after 24hours.

(a) Anti-bacterial Activities

Table 1: Antibacterial activities of 4H-1,4-benzothiazine and substituted piperazine mixture with ethanol as control at different concentrations.
| Name of microbial species (Bacteria) | Zone of inhibition in various concentration in (cm) |
|-------------------------------------|-----------------------------------------------|
|                                     | 125ppm | 250ppm | 500ppm | 1000ppm |
| **Clostridium perfringens (MTCC NO. 450)** | 1.0 | 1.0 | 1.2 | 1.5 |
| **Bacillus amyloliquefaciens (MTCC NO.10456)** | 1.1 | 1.1 | 1.2 | 1.3 |
| Ofloxacin 200 | 1.6 | 1.9 | 2.1 | 2.1 |

Figure 2-5: Represent the Antibacterial activities of 4H-1,4-benzothiazine and substituted piperazine mixture in *Clostridium perfringens*(MTCCNO.450)

(b) Anti-fungal Activities
Preparation of Broth- Dissolve 13g Yeast Extract- Peptone-Dextrose (YPD) broth in 1000ml of water and place that nutrient broth and other glassware like petri plate, conical flask in autoclave for sterilization of media and glassware for 25 minutes. Then remove from autoclave and place in laminar air flow which creates an aseptic condition where we add inoculation of fungal culture in broth then remove and put it into incubator for 24hours for incubation of broth takes place.

Preparation of Nutrient Agar- Take 30g of Sabouraud Dextrose Agar (SDA) in 250ml water and autoclave is done. After autoclave we placed nutrient agar in laminar air flow and pour in petri plates and let them solidify. After solidify spread the inoculation of culture from broth and then put the test disc on fungal lawn with the test solution of different concentration and incubation it in incubator at 37degree and check the zone inhibition after 7days.

**Table-2: Antifungal activities of 4H-1,4-benzothiazine and substituted piperazine mixture with ethanol as control at different concentrations.**

| Name of microbes (Fungus) | Zone of inhibition in different concentration (in cm) |
|---------------------------|------------------------------------------------------|
|                           | 125ppm | 250ppm | 500ppm | 1000ppm |
| *Candida parapsilosis*    | 0.6    | 0.9    | 1.0    | 0.5     |
| *Candida tropicalis*      | 0.7    | 0.5    | 0.4    | 0.5     |

**Figure 6**

**FIGURE 6: Represent the Antifungal activities of 4H-1,4-benzothiazine and substituted piperazine mixture in *Candida tropicalis* **
Anti-Oxidant Activities

A DPPH solution were prepared by the addition of 0.0197 milligram of DPPH to 100 ml of CH₃OH (Methyl Alcohol) and place in dark for 30 minutes. Main solution prepared by the addition of 1.0 milligram of piperaine-benzothiaiaine mixture to 5 ml of C₂H₅OH (ethanol). Dilution were finished by addition of 0.1, 0.09, 0.08, 0.07, 0.06, 0.05, 0.04, 0.03, 0.02, 0.01/0.1 solution from main solution. After addition of 3.9 ml of DPPH solution to all mentioned concentration, measure the wavelength of all the sample solutions distinctly by UV spectrophotometer.

Determination of percentage inhibition:

Percentage (% ) of inhibition = \{ ( control - sample ) / sample\} *100

Absorption of control at 517 nm = 0.16904

Absorption of sample (max) at same wavelength = 0.08424

Percentage (% ) of inhibition =50.16%

Absorption of sample (mean) at same wavelength = 0.139006

Percentage (% ) of inhibition = 17.76%

Table 3: Anti-oxidant activities of 4H-1,4-benzothiazine and substitututed piperazine mixture.

| sample (ml) | Absorbance at 517nm | % of inhibition |
|-------------|----------------------|-----------------|
| 0.10 ml     | 0.08424              | 50.16%          |
| 0.09 ml     | 0.10596              | 37.73%          |
| 0.08 ml     | 0.14212              | 15.92%          |
| 0.07 ml     | 0.12256              | 27.49%          |
| 0.06 ml     | 0.13524              | 19.99%          |
| 0.05 ml     | 0.15532              | 8.11%           |
| 0.04 ml     | 0.14305              | 15.37%          |
| 0.03 ml     | 0.14434              | 14.61%          |
| 0.02 ml     | 0.18861              | 11.57%          |
| 0.01 ml     | 0.16862              | 2.48%           |
Figure 7: Shows the percentage of inhibition of 4H-1,4-benzothiazine and substituted piperazine mixture by anti-oxidant agent i.e. DPPH at different concentration.

Result and Discussion

4H-1,4-benzothiazine and substituted piperazine mixture was prepared from the substituted 4H,1-4, Benzothiazine with piperazine and possibilities of chemical interaction with each other is also examined with TLC (Thin Layer Chromatography) and sophisticated instruments such as IR(figure-1), NMR. Presence of peaks (3275.24cm$^{-1}$ singlet peak of NH, 2941.54cm$^{-1}$=CH peak, 2800.73cm$^{-1}$-CH peak, 1647.26 cm$^{-1}$ C=O peak)in IR spectrum and no confirmative signal in NMR spectra confirmed that no chemical interaction done between benzothiazine and pipeerazine.4H-1,4-benzothiazine and substituted piperazine mixture exhibits biological activities such as antifungal, antibacterial and antioxidant. 4H-1,4-benzothiazine and substituted piperazine mixture shows antibacterial activities against *Clostridium perfringens* (MTCC NO. 450) and *Bacillus amyloliquefaciens*(MTCC NO.10456) and antifungal activities against *Candida parapsilosis* and *Candida tropicalis*. On the basis of behaviour of 4H-1,4-benzothiazine and substituted piperazine mixture against DPPH, this mixture can be used as potential antioxidant material in different biopharmaceutical applications.

Conclusion

As per literature survey and above mentioned work present work it has been confirmed that nitrogen containing heterocyclic compounds are biological active such as antifungal, anti-bacterial, anti-oxidant and consider as potential pharmaceutical agents.

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