SYNTHESIS AND BIOACTIVITIES OF FUSED PYRIMIDOBENZOTHIAZOL-4-ONE AND ITS CHARATERIZATION BY HNMR, IR, MASS SPECTROMETRY AND UV

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ABSTRACT

In the present work 8-hydroxy-2-methyl-4H-pyrimido[2,1-b]- benzothiazol-4-one is synthesized by reacting 2-amino-6-hydroxybenzotiazole with ethylacetoacetate in presence of polyphosphoric acid. The structural characterization of the synthesized compound is done by H^1NMR, IR and mass spectrometry. Synthesized compound has shown biological activity against E.Coli and antifungal activity against A.Fumigates.

Keywords: 2-amino-6-hydroxybenzothiazole, 8-hydroxy-2-methyl-4H-pyrimido[2,1-b]-benzothiazol-4one, ethylacetoacetate, FTIR, etc.

1. Introduction

Heterocycles have constituted one of the largest areas of research in organic chemistry. The presence of heterocycles in all kinds of organic compounds of interest in biology, pharmacology, optics, electronics, materials science and so on is very well known. Among them, sulfur and nitrogen containing heterocyclic compounds have maintained the interest of researchers and their unique structures led to several applications in different areas. Pyrimidobenzothiazoles are nitrogen-sulphur containing compounds that have been reported to exhibit a wide spectrum of activities like GABA receptor binding agents, antiviral, antitumour, anti-inflammatory, etc. During past few decades, interest has been rapidly growing in the properties and transformations of these heterocycles. In the present work we had synthesized a derivative of pyrimidobenzothiazole from derivative of 2-aminobenzothiazole and screened for bioactivity.[1-5]

The purity of the synthesized compound was checked by thin layer chromatography using mixtures of different polar and non polar solvents. The infra red spectrum was recorded in KBr on SHIMADZU-8400S FTIR, ^{1}H NMR spectra were recorded on an AV500 FT spectrometer operating in DMSO/$CDCl_3$ mixture with TMS as an internal reference. The melting point is uncorrected.

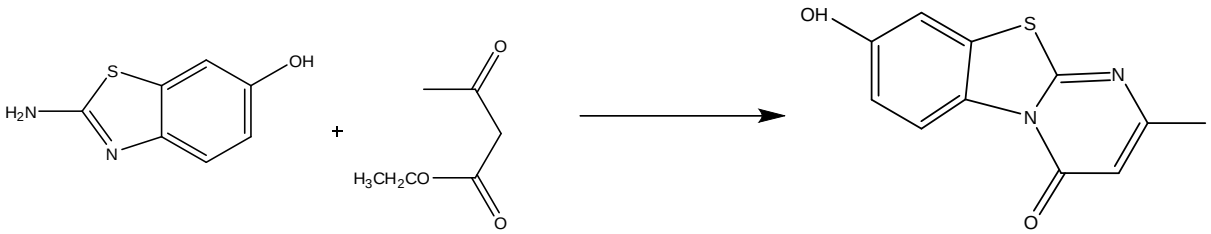
2. Scope and Purpose

For more than a century, the nitrogen and sulfur containing heterocycles have constituted one of the emerging branches of chemical sciences in the fields of pharmacology, pathology, anticancer, anti-inflammatory, etc. In view of the structural changes with the presence of heteroatoms and the relationship of structures with the biological / pharmacological activities, we have synthesized nitrogen and sulfur containing heterocycles of biological and pharmacological importance incorporating diverse structural features due to diversity in substituents, heterocyclic systems and appended pharmacologically active functional groups for making them available for biological evaluation and SAR (structural activity relationship) studies. In the present investigation, we have synthesized nitrogen and sulphur containing heterocycles of potential therapeutic interest especially with thiazole, and pyrimidine heterosystems; Pyrimidobenzothiazoles mainly due to their unique structural features, which enable them to exhibit a number of pharmacological and biological activities. The pharmacological / biological activities of heterocyclic compounds mainly depend on the structural specificity and the strength of interaction between a drug and receptors present in biological system.[6-9]

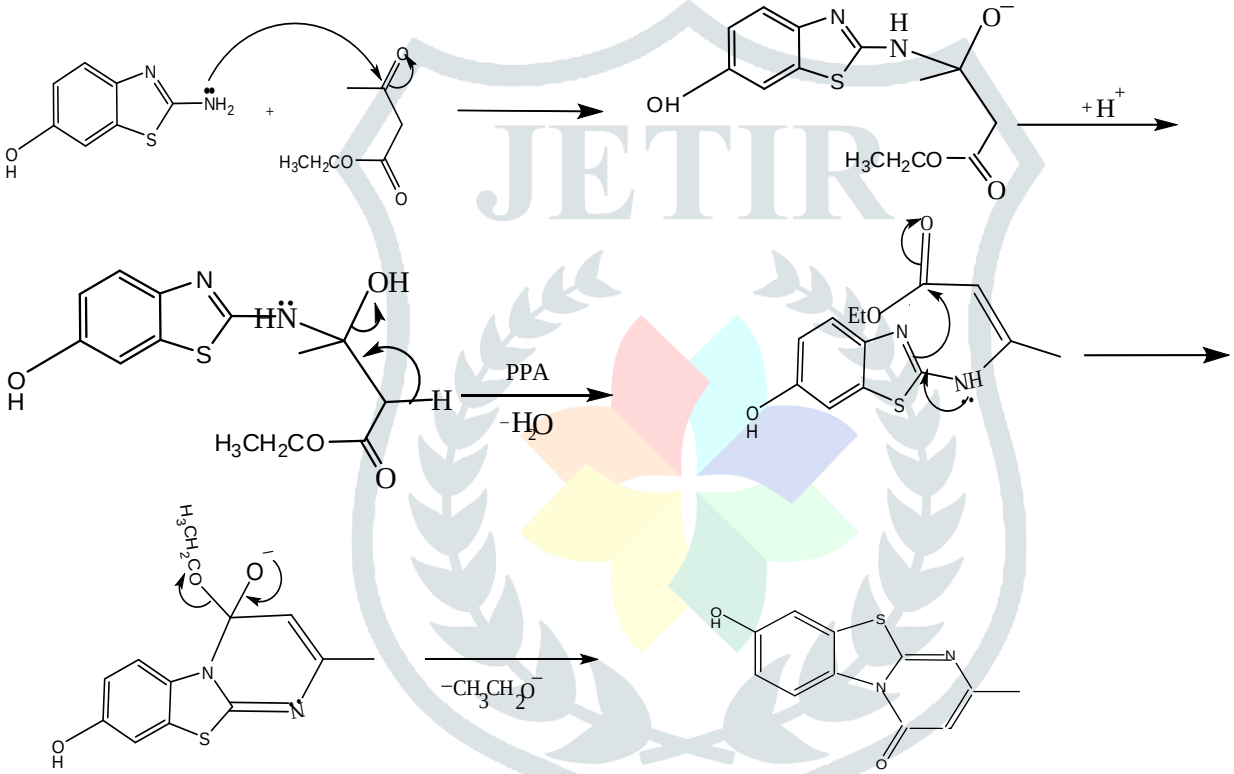
3. Chemistry

3.1 Synthesis of 8-hydroxy-2-methyl-4H-pyrimido[2,1-b]- benzothiazol-4-one

In the present work we have synthesized 8-hydroxy-2-methyl-4H-pyrimido[2,1-*b*]benzothiazol-4-one by a simple and convenient regioselective one-pot synthesis by the reaction of 2-amino-6-hydroxybenzothiazole with ethylacetoacetate.



MECHANISM



3.2 Experimental Work

2-amino-6-hydroxybenzothiazole required for the synthesis of 8-hydroxy-2-methyl-4H-pyrimido-[2,1-b]benzothiazol-4-one have been purchased from the market, the name of the company is SPECTROCHEM PVT. LTD. MUMBAI (INDIA). All the errors or changes are subjected to the company.

To synthesise 8-hydroxy-2-methyl-4H-pyrimido[2,1-b]benzothiazol-4-one we refluxed 0.5g (0.0033389 moles) of 2-amino-6-hydroxybenzothiazole with 2ml of ethylacetoacetate in presence of polyphosphoric acid (4g) for 5 hours. The reaction was analysed instantly by TLC. In TLC we get clear Rf- value differences for different solvents front. The TLC for this reaction is best obtained in DCM (Dicholoromethane) as a solvent. When we observed the greater differences in the Rf-values, the reaction was stopped and the refluxing was over.

The reaction mixture was cooled and neutralized by Sodium Bicarbonate ($NaHCO_3$). After neutralization was over, then ethanol was distilled out from the mixture using Water Rotor till precipitation was observed in the mixture. When the distillation was done, then the remaining solution was filtered to the crystals. These crystals were then re-crystallized in DCM. The pure crystals were then dried at up to 50 ^{o} C in the oven and kept in butter paper to avoid water sensitivity. The TLC was then again performed to assure the product formation. The melting point was found to be 149-157^{o} C with percentage yield of 69 %.

4 Results

4.1 Solubility:-

The solubility of the synthesized compound was tested in various solvents. The solubility characters are listed below:

Table-01 Solubility of Synthesized compounds in various Solvents:

S.No.	Solvent	Compound
01	Water	Insoluble
02	Chloroform	Soluble
03	DCM	Soluble
04	Butanol	Soluble
05	Ethanol	Soluble
06	Methanol	Soluble
07	Hexane	Insoluble

4.2 Thin Layer Chromatography:-

Chromatography is an important technique to identify the formation of an organic compound and also to identify the purity of such compounds. The Retardation Factor (R_f) value of these compounds is characteristic for each of them.

(a) Preparation of Chromatoplate:-

Clean and dry glass slides were taken. A thin silica gel in water is taken and applied on the slides in the ratio of 1:2. The slurry was then poured into the chamber of the applicator, which was then fixed and the thickness was set to 0.5mm. The plates were then dried to 110^{o}C.

(b) Preparation of solvent system:-

The solvent mixture is prepared by taking one solvent or by proper mixing of different solvents in proper ratios.

(c) Application of the sample:-

The solution of the parent compounds and its derivatives were taken in small bored capillaries and spotted at 2 cm from the base of the slide.

(d) Development of chromatogram:-

Plates were developed by ascending technique when solvent front had reached a distance of 10-12 cm, they were taken out an dried at room temperature.

(e) Detection of spots:-

The spots were detected in iodine chambers.

(f) Calculation of Rf values:-

The Rf values were calculated using the following formula;

$$RF = \frac{\text{Distance Travelled by the sample}}{\text{Distance Travelled by the solvent}}$$

Table-02 Thin Layer Chromatography Data for the Synthesised Compounds

COMPOUND	SOLVENT SYSTEM	PROPORTION	Rf- VALUE
4H-pyrimido[2,1-b]-benzothiazol-4-one	DCM	100%	0.5

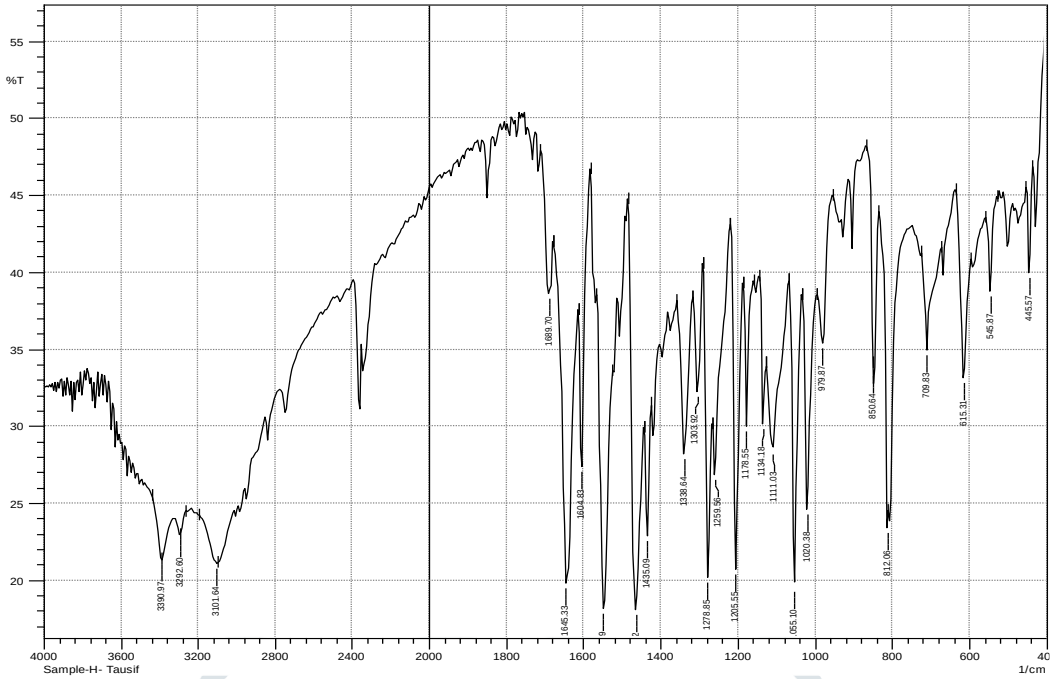
4.3 Spectral Data:-

8-hydroxy-2-methyl-4H-pyrimido[2,1-*b*]benzothiazol-4-ones was confirmed mainly by spectroscopic studies. I have performed FTIR (model name of the FTIR instrument is SHIMADZU 8400s) to characterize my products and match them with the literature spectra of the synthesized compounds.

I have confirmed my product by determining the melting point of the sample and characterizing various peaks by spectrometric techniques. I have correlated my product FTIR spectrum with the literature spectrum of reactant and found the differences. Furthermore the compound was correlated with the literature available and confirmed the peaks.

On the FTIR bases the compound was confirmed by the presence of following peaks:

S. No	Group	Wave No.(cm^{-1})
1	C=O(Str.)	1645
2	Alkenic SP^2C-H(Str.)	3063
3	Alkenic C=C(Str.)	1492
4	Aromatic C=C(Str.)	1589



The presence of SP^2C-H (Str.) peak at 3063 cm^{-1} confirms the formation of c=c in the product. The reactant was lacking alkenic part. Furthermore the presence of SP^2 c=c peak at 1492 cm^{-1} confirms the formation of product.

Mass Spectrometry And ^{1}HNMR

The m/z peak at 282 (M^+) confirms the synthesis of the compound. Number of shielded and deshielded protons were confirmed with the literature available.

4.4 Antimicrobial Activity

The synthesised compounds were analysed for their antibacterial activities against *Escherichia Coli* and *Bacillus subtilis* species.

The nutrient agar media prepared by using following ingredients:-

→Nutrient agar 16 g

→Distilled water 500 ml

Weighed-quantity of nutrient agar mix in the 500 ml distilled water to dissolve the agar solution. For complete dissolution heated the solution in hot oven. Then the prepared nutrient agar media is sterilized by autoclave at 120˚C for 20 minutes at 15lbs/ in2 pressure. 10 mg test compound dissolved in 10 ml ethanol. From this 1ml solution was taken a dilute to 10 ml with ethanol. Now the concentration of test compound is 100 ppm or µg/ml. These sample solution made in suitably labelled sterilized test tubes.

Method of testing:-

The sterilized media was cooled at 45˚C with gentle shaking to bring about uniform cooling. This was poured into sterilized Petri dishes and allows the media to set. After solidification all media dishes transferred to the laminar air flow unit. Then the discs which are previously prepared carefully kept on the solidified media by using sterilized forceps. These Petri dishes kept at room temperature for 1 hr and then for incubation at 37˚C for 24 hrs in incubator. The extent diameter of inhibition after 24 hrs was measured as the zone of inhibition in centimetre.

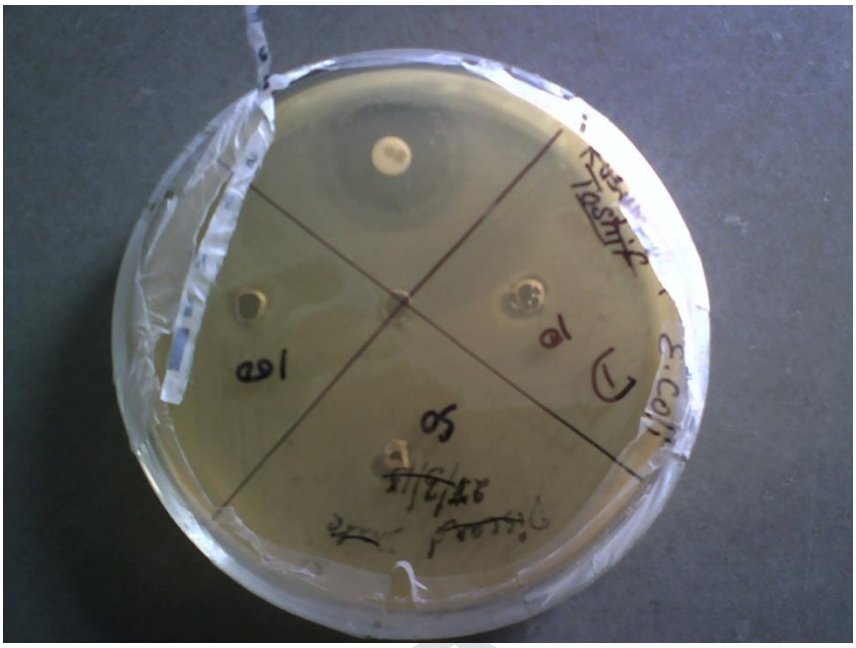


Figure-01:-Photograph showing effect of compound-1on *Bacillus subtilis*

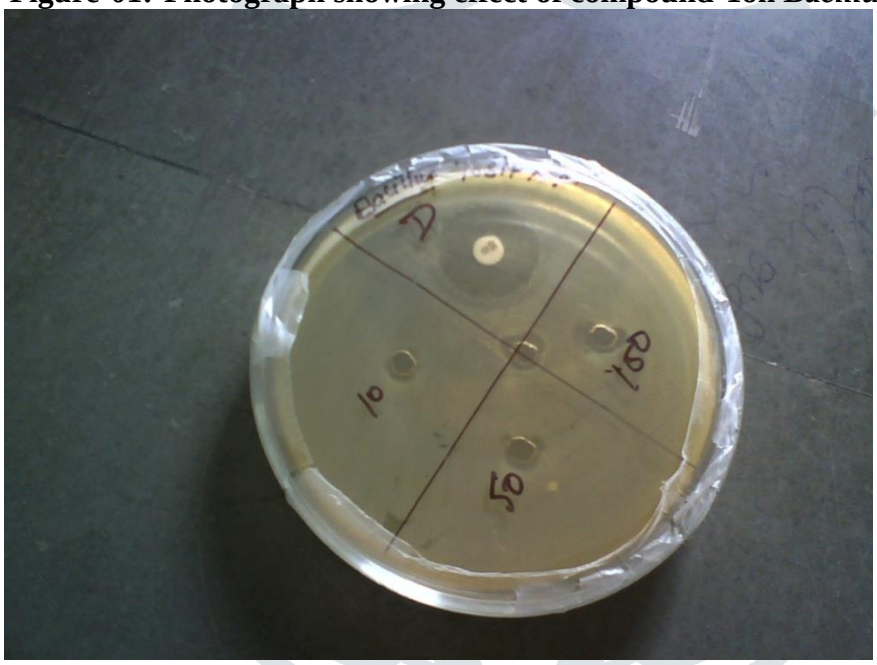


Figure-02:- Photograph showing effect of compound-2 on *Bacillus subtilis*

Conclusion

In the present research work two different schemes were established for the synthesis of substituted pyrimidobenzothiazol-4-one according to literature bases. The compound was synthesized and the structure was confirmed by physical data and chemical analysis using literature available. The compounds synthesized were screened to antimicrobial where it showed extensive behavior. The extensive modifications to our synthesis may have better results in future.

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