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Synthesis and antimicrobial evaluation of novel thiophene derivatives

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ABSTRACT

Thiophene nucleus has been established as the potential entity in the largely growing chemical world of heterocyclic compounds possessing promising pharmacological characteristics. A series of tetrahydrobenzothiophene derivatives was synthesized with an objective to develop novel and potent antimicrobial agents of synthetic origin. The required starting material ethyl-2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate(1) was synthesized via a multicomponent condensation between sulphur, cyclohexanone and ethylcyanoacetate adopting Gewald Reaction. The Compound 1 was converted into respective Schiff bases (RBA_1 - RBA_4) by refluxing it with various aromatic aldehydes in dioxane for 15 hours. The Schiff bases were further processed into the final compounds i.e. thiazolidinone derivatives (RSB_1 - RSB_4) by treating them with thioglycollic acid in presence of anhydrous $ZnCl_2$ in DMF and refluxing the reaction mixture for 4- 5 hours. Synthesized compounds were purified, characterized and evaluated for their antimicrobial activity. Most of the compounds exhibited moderate to significant activities.

Keywords: Antibacterial Activity, Antifungal Activity, Gewald Reaction, Schiff bases, Thiazolidinone.

INTRODUCTION

Substituted thiophenes and their biheterocycles have received considerable attention during last two decades as they are endowed with wide range of therapeutic properties. A number of thiophene derivatives and Schiff bases have been reported to possess significant and diverse biological activities such as antimicrobial [1], analgesic [2], antiinflammatory [3], antioxidant [4], antitumor [5] and local anesthetic [6] activities. Thiophene can be fused with various heterocyclic nuclei giving rise to newer compounds having enhanced biological activities. Thienopyrimidines occupy special position among these compounds. Many of these derivatives exhibited antiallergic [7], antibacterial [8], antidepressant [9], antidiabetic [10], analgesic and anti-inflammatory [11] activities. In continuation to these efforts and with an objective to develop novel and potent therapeutic agents of synthetic origin, it was decided to synthesize certain tetrahydrobenzothiophene derivatives and evaluate them for their antimicrobial potential.

MATERIALS AND METHODS

Melting points were measured using Kshitij Innovations apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using ethyl acetate: n- hexane (1:2) solvent system and iodine vapours as a visualizing agent. IR spectra were recorded using KBr pellets on a Shimadzu

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Affinity-1 FTIR spectrophotometer. ¹H- NMR spectra were recorded on a Bruker Advance 400 spectrometer at 400.13 MHz using CDCl₃ solvent and TMS as internal standard. Chemical shifts were expressed in δ ppm unit.

Synthesis of ethyl-2-amino-4,5,6,7-tetrahydro-1-benzothiophene-3-carboxylate (1)

Sulphur (0.06mole) was added to a mixture of ethylcyanoacetate (0.05mole) and cyclohexanone (0.05mole) at room temperature with stirring. Diethylamine (0.05mole) was added to this heterogeneous mixture and the reaction mixture was stirred at 45°C for 2 hours. Completion of reaction was monitored using TLC and mixture was kept overnight at room temperature. The precipitate was filtered, washed, dried and recrystallized from ethanol.

Synthesis of Schiff bases (RBA₁-RBA₄)

Equimolar quantities of Compound 1 (0.1mole) and suitably chosen substituted aldehydes (0.1mole) were suspended in 100ml dioxane and the mixture was refluxed for 14-15 hours. Reaction was monitored by TLC and the mixture was cooled and poured into crushed ice. Solid thus obtained was filtered, washed with water, dried and recrystallized from ethanol.

Synthesis of thiazolidinones (RSB₁- RSB₄)

Equimolar mixture of Schiff base (0.1mole) and thioglycollic acid (0.1mole) were suspended in DMF (60ml). Catalytic amount of zinc chloride (1g) was added to it and the mixture was refluxed for 4 hours. Mixture was cooled and poured into crushed ice. Solid thus obtained was filtered, washed, dried and recrystallized from ethanol.

Chemistry

IR, ¹H-NMR and elemental analysis were consistent with the assigned structure.

Ethyl 2-[2-(2-hydroxyphenyl)- 4- oxo- 1,3- thiazolidin-3-yl]- 4,5,6,7- tetrahydro- 1-benzothiophene-3-carboxylate (RSB₁): Yield: 68.75%; Melting Point: 115° C; IR (KBr, cm⁻¹): 2846(C-H str.), 1590(C=C str.), 1282(C-O str.), 3083(Ar-H str.), 1378(C-N str.), 778(C-S str.), 1646(C=O str.), 3297(O-H str.); ¹HNMR(CDCl₃, δ ppm): 1.408-1.444(t, 3H, OCH₂CH₃), 1.754-1.803(m, 4H, C₅ and C₆), 2.503-2.518(d, 2H, C₄), 2.719-2.734(d, 2H, C₇), 3.424(s, 2H, CH₂ of thiazolidine), 4.249-4.302(q, 2H, OCH₂CH₃), 5.455(s, 1H, OH), 5.957(s, 1H, N-CH), 6.920-7.049(m, 4H, Ar-H); Anal. Calcd. for C₂₀H₂₁NO₄S₂: C(59.53), H(5.25), N(3.47), O(15.86), S(15.89); found: C(58.90), H(6.30), N(4.00), O(15.60), S(15.20); Mol. Wt.: 403.

Ethyl 2-[2-(4-methoxyphenyl)- 4- oxo- 1,3- thiazolidin- 3-yl]- 4,5,6,7- tetrahydro- 1-benzothiophene-3-carboxylate (RSB₂): Yield: 70.5%; Melting Point: 120°C; IR (KBr, cm⁻¹): 2846(C-H str.), 1282(C-O str.), 3084(Ar-CH str.), 1349(C-N str.), 778(C-S str.), 1646(C=O str.), 1145(C-O str. in C-O-C); ¹HNMR(CDCl₃, δ ppm): 1.316-1.352(t, 3H, OCH₂CH₃), 1.756-1.764(m, 4H, C₅ and C₆), 2.486-2.501(d, 2H, C₄), 2.699-2.714(d, 2H, C₇), 3.416(s, 2H, CH₂ of thiazolidine), 3.734(s, 3H, OCH₃), 4.230-4.283(q, 2H, OCH₂CH₃), 5.934(s, 1H, N-CH), 6.456-7.249(m, 4H, Ar-H); Anal. Calcd. for C₂₁H₂₃NO₄S₂: C(60.41), H(5.55), N(3.35), O(15.33), S(15.36); found: C(59.75), H(5.55), N(4.00), O(15.15), S(15.10); Mol. Wt.: 417.

Ethyl 2-[2-(3,4- dimethoxyphenyl)- 4- oxo-1,3- thiazolidin-3-yl]- 4,5,6,7- tetrahydro- 1-benzothiophene-3-carboxylate (**RSB**₃): Yield: 68.4%; Melting Point: 130° C; IR (KBr, cm⁻¹): 2846(C-H str.), 1283(C-O str.), 3081(Ar-CH str.), 1283(C-N str.), 778(C-S str.), 1647(C=O str.), 1145(C-O str. in C-O-C); ¹HNMR(CDCl₃, δ ppm): 1.314-1.350(t, 3H, OCH₂CH₃), 1.754-1.762(m, 4H, C₅ and C₆), 2.698-2.711(m, 4H, C₄ and C₇), 3.413(s, 2H, CH₂ of thiazolidine), 3.930(s, 6H, OCH₃), 4.228-4.281(q, 2H, OCH₂CH₃), 5.945(s, 1H, N-CH), 6.455-7.049(m, 3H, Ar-H); Anal. Calcd. for C₂₂H₂₅NO₅S₂: C(59.04), H(5.63), N(3.13), O(17.87), S(14.33); found: C(58.62), H(6.10), N(3.67), O(17.51), S(14.10); Mol. Wt.: 447.

Ethyl 2-[2-(4- dimethylaminophenyl)- 4-oxo- 1,3- thiazolidin-3-yl]- 4,5,6,7- tetrahydro- 1-benzothiophene-3-carboxylate (RSB₄): Yield: 56.2%; Melting Point: 135°C; IR (KBr, cm⁻¹): 2847(C-H str.), 1509(C=C str.), 1281(C-O str.), 3083(Ar-CH str.), 1347(C-N str.), 780(C-S str.), 1647(C=O str.); ¹HNMR(CDCl₃, δ ppm): 1.315-1.350(t, 3H, OCH₂CH₃), 1.754-2.697(m, 8H, C₄, C₅, C₆, and C₇), 3.058(s, 6H, (CH₃)₂), 3.890(s, 2H, CH₂ of thiazolidine),4.213-4.363(q, 2H, OCH₂CH₃), 5.954(s, 1H, N-CH), 6.694-7.753(m, 4H, Ar-H); Anal. Calcd. for C₂₂H₂₆N₂O₃S₂: C(61.37), H(6.09), N(6.51), O(11.15), S(14.89); found: C(60.90), H(6.55), N(6.75), O(10.86), S(14.94); Mol. Wt.: 430.

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Biological Screening

All the synthesized compounds were subjected to antimicrobial screening at a concentration of 100μ g/ml involving three Gram -ve bacteria (*Eschericha Coli, Staphylococcus aureus* and *Klebsiella pneumoniae*); three Gram +ve (*Seratia reticulata, Bacillus subtilis* and *Streptococcus pneumoniae*) and two fungal strains (*P. aeruginosa* and *C. albicans*) using Ampicillin as standard at the same concentration. The work, in reference, was carried out by Agar disc diffusion method [12]. The response of organisms to the synthesized compounds were measured in terms of zone of inhibition and compared with that obtained with standard.

A) Preparation of Mueller Hinton Agar (MHA) Media

Mueller Hinton Agar Media was used for antimicrobial screening and its composition is as:

Casein Acid Hydrolysate	17.50gm
Beef Heart Infusion	2.00gm
Starch, soluble	1.50gm
Agar	17.00gm

For preparing Mueller Hinton Agar (MHA) Media, 38gm of Mueller Hinton Agar No. 2 was dissolved in 1000ml distilled water. It was mixed properly and heated to boil to dissolve the medium completely. It was autoclaved at 15lbs pressure (121°C) for 15 minutes. It was than cooled and poured into sterilized plates. All the plates were kept for 4-5 hours in laminar airflow until the media got solidified. The plates were than kept in an incubator at 37°C.

B) Preparation of standard antibiotic solution

A solution (100µg/ml) of standard drug (Ampicillin) was prepared in sterile water.

C) Preparation of Test solution

10 mg of the synthesized compound(s) was dissolved in 10 ml of DMF. 1 ml of this solution was taken and diluted to 10 ml (with DMF) so that the concentration of the test solution became 100μ g/ml.

D) Preparation of inoculums

For the preparation of inoculums, 5g of nutrient agar was dissolved in 100 ml of distilled water and the pH was adjusted at 7.2 ± 0.2 . It was poured in test-tubes as per requirement and then sterilized by autoclaving at 121°C. A 24 hour old culture was used for the preparation of bacterial suspension. Likewise suspensions of all the organisms were prepared as per standard procedure.

E) Preparation of discs

Discs of 6-7 mm in diameter were punched from No. 1 Whattmann filter paper with sterile cork borer of same size. These discs were sterilized by keeping in oven at 140°C one hour. Standard and test solutions were added separately to these discs which were air dried later on.

F) Method of testing

Inoculums were added to the prepared media plates and allowed to solidify. The previously prepared discs were carefully kept on the solidified media by using sterilized forceps. These petridishes were kept for one- hour diffusion at room temperature and then for incubation at 37° C for 24 hours in an incubator. The zones of inhibition after 24 hours were measured in millimeters. The results obtained are shown in Table 1 and Table 2.

RESULTS AND DISCUSSION

According to Gewald [13], heating under stirring of a mixture of ethylcyanoacetate, sulphur and cyclohexanone in diethylamine for 2-3 hours afforded ethyl-2-amino-4,5,6,7-tetrahydro-1- benzothiophene-3-carboxylate (1). Substantial proof for the formation of Schiff base (RBA₁- RBA₄) has been provided by differences in melting points and yield value from that of parent compound. Compound 1 on reaction with various substituted aldehydes yielded various Schiff bases which on cyclization with thioglycollic acid in catalytic amount of ZnCl2 yielded novel thiophene derivatives (RSB₁-RSB₄). The primary structural difference within this series involves the nature of various substituted aldehydes.

Synthesized compounds were found to be crystalline in nature and easily soluble in chloroform, ethyl acetate, benzene, DMSO and DMF but insoluble in hexane and toluene. With the help of analytical techniques such as melting point, IR and 1H-NMR, synthesized derivatives were characterized. These compounds showed a band at 1646 cm-1 for cyclic >C=O group [14]. All the compounds showed NMR signals for different kinds of protons at their respective positions.



Sampla	Zone of inhibition in mm					
Sample	E. coli	S. reticulata	S. aureus	B. subtilis	S. pneumoniae	K. pneumoniae
RSB ₁	-	14	15	15	10	11
RSB ₂	13	20	14	12	15	15
RSB ₃	14	18	-	-	13	13
RSB ₄	10	17	15	16	13	9
Ampicillin	22	30	22	25	22	20
DMF	-	-	-	-	-	-

Table 1: Antibacterial Activity of synthesized thiazolidinones (RSB₁- RSB₄)

Table 2: Antifungal	Activity of sy	nthesized thiazo	olidinones (R	SB ₁ - RSB ₄)
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Sampla	Zone of inhibition in mm		
Sample	P. aeruginosa	C. albicans	
RSB_1	9	13	
RSB_2	8	13	
RSB ₃	13	13	
RSB_4	11	11	
Ampicillin	19	21	
DMF	-	-	

All compounds have been screened for their antimicrobial activity. From the screening results it was observed that the presence of electron withdrawing group and ester linkage made the compounds to exhibit moderate to significant activity in comparison to standard drug Ampicillin. Compound RSB₂ and RSB₄ exhibited promising antibacterial

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activity while compound RSB_3 exhibited promising antifungal activity. However other compounds of the series also exhibited moderate to significant activity against the microorganisms as mentioned above. Therefore compound RSB_2 , RSB_3 and RSB_4 can be recommended for further studies. The above results established the fact that thiophene substituted with various aldehydes (substituted) can be studied further to explore out newer antimicrobial compounds.

CONCLUSION

The analytical and other informational data, available in literature so far, have rendered thiophene significantly important class of heterocyclic compounds and their applications in ever challenging chemotherapy of various ailments/ infections etc. since last two decades immensely hiked interests of medicinal chemist and biochemist.

This particular research study, in reference, would extend great deal of help to researchers in reckoning and determining the best and most productive, economical, suggestive and conclusive access to various thiophenes of clinical importance superseding other compounds of their class.

Further combinatorial libraries of these compounds can be generated which can be screened for optimal pharmacological activities by optimization techniques using 2D and 3D QSAR investigation.

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