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CODEN: IAASCA **ORIGINAL ARTICLE**

Synthesis and Antimicrobial Evaluation of Novel Thiophene Derivatives

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ABSTRACT
Thiophene nucleus has been established as the potential entityinthe largely rising chemical world of heterocyclic compounds possessing promising pharmacological character. A series of tetrahydrobenzothiophene derivatives was synthesized with an purpose to build up 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 multi component condensation between sulphur,cyclohexanoneandethylcyanoacetate adopting Gewald Reaction. The Compound1 was changed in to respective Schiff bases(KM_1-KM_4)by refluxing it with various aromatic aldehydes in dioxanefor 15 hours. The Schiff bases were further processed into the final compounds i.e. thiazolidinonederivatives (KM_1-KM_4) by treating them with thioglycollicacidinpresenceofanhydrous $ZnCl_2$ 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.
Keyword: *antibacterial activity, antifungal activity, Schiff base and thiazolidinone*

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INTRODUCTION

Heterocyclic compounds are widely distributed in nature and are essential for life. There are vast numbers of pharmacologically active heterocyclic compounds many of which are in regular clinical use[1]. The investigational approaches towards Structure- Activity Relationship focusing the search of optimized candidates have become immensely important. Literature survey reveals that thiophene is parent of a series of compounds that are important in medicinal and industrial chemistry. Thiophene is one of the most important classes of heterocyclic compounds with variety of biological activities. Substituted thiophene and their biheterocycles have received considerable attention during last two decades as they are endowed with wide range of therapeutic properties such as analgesic[2], antibacterial[3], antioxidant & anti-inflammatory[4], antifungal[5], anticancer[6] and local anaesthetic activity[7]. 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 exhibit antiallergic[8], antibacterial[9], antidepressant[10], antidiabetic[11], analgesic and anti-inflammatory[12] 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 thiazolidinone derivatives and evaluate them for their antimicrobial potential.

METHOD AND MATERIAL

The melting points of synthesized compounds were determined in open capillary tubes using Kshitij Innovations melting point apparatus, expressed in °C and are uncorrected. The IR spectra of compounds were recorded on Shimadzu Affinity-1 FTIR in KBr disc and absorption bands are expressed in cm-1. 1H NMR spectra were recorded on Bruker Advance 400.13 MHz NMR Spectrometer (Chemical shift if δ ppm) using TMS as internal standard. The purity of the compounds was checked by TLC on silica gel G plates using ethyl acetate: nhexane (1:2) solvent system and iodine vapours as a visualizing agent.

Synthesisofethyl-2-amino-4,5,6,7-tetrahydro-1-benzothiophene 3-arboxylate(1)

Sulphur(0.06mole)was added toa 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°Cfor2 hours.Completionof 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 (KM_1-KM_4)

Equimolar quantities of Compound 1 (0.1mole) and suitably chosen substituted aldehydes(0.1mole)were suspended in100ml dioxane and the mixture was refluxed for14-15hours.Reaction was monitore dy TLC and the mixture was cooled and poured into crushed ice. Solid thusobtained was filtered, washed with water, dried and recrystallized from ethanol.

Synthesis of thiazolidinones(KM_1-KM_4)

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 4hours.Mixture was cooled and poured into crushed ice.Solid thus obtained was filtered,washed,dried and recrystallized from ethanol.

Chemistry

IR,^{1}H-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 (KM_1):

Yield: 68.75%; Melting Point: 115°C; IR (KBr, cm^{-1}): 2854(C-H str.), 1501(C=C str.),1282(C-Ostr.),3072(Ar-Hstr.),1358(C-Nstr.),791(C-Sstr.),1686(C=Ostr.),3297(O-Hstr.);^{1} HNMR ($CDCl_3$, δppm) :1.408-1.444(t,3H,OCH_2CH_3),1.754-1.803(m,4H,C_5 and C_6),2.503 - 2.518 (d,2H,C_4), 2.765 - 2.734 (d,2H,C_7),3.470 (s,2H,CH_2 of thiazolidine), 4.249 -4.302(q,2H,OCH_2CH_3), 5.421(s,1H,OH),5.900(s,1H,N-CH),6.920-7.021(m,4H,Ar H);Anal.Calcd. for $C_{20}H_{21}NO_4S_2$: C(59.23), H(5.78), N(3.40), O(15.81),S(15.82);found: C(58.62),H(6.36),N(4.00),O(15.72),S(15.12);Mol.Wt.:409.

Ethyl2-[2-(4-methoxyphenyl)-4- oxo-1,3-thiazolidin-3-yl]-4,5,6,7-tetrahydro-1-benzothiophene-3- carboxylate(KM_2):

Yield:70.5%;MeltingPoint:120°C;IR (KBr,cm^{-1}):2846(C-Hstr.), 1282(C-Ostr.),3024(Ar- CHstr.),1391(C-Nstr.),780(C-Sstr.),1614(C=Ostr.),1125(C-Ostr.inC-O-C);^{1}HNMR($CDCl_3$,δppm):1.316-1.392(t,3H,OCH_2 CH_3),1.790-1.798 (m,4H,C_5 and C_6),2.481-2.499 (d,2H,C_4), 2.682-2.700(d,2H,C_7), 3.416(s,2H,$CH_2$of thiazolidine),3.739(s,3H,OCH_3),4.222-4.275(q,2H,OCH_2CH_3),5.939(s,1H,N-CH), 6.442-7.248(m,4H, Ar-H); Anal. Calcd. for $C_{21}H_{23}NO_4S_2$: C(60.56), H(5.42), N(3.09), O(15.66), S(15.83); found: C(59.95), H(5.22), N(4.09), O(15.31), S(15.35); Mol.Wt.:444.

Ethyl2-[2-(3,4-dimethoxyphenyl)-4-oxo-1,3-thiazolidin-3-yl]-4,5,6,7-tetrahydro1benzothiophene-3- carboxylate (KM3):

Yield: 68.4%; MeltingPoint: 130°C; IR (KBr, cm^{-1}): 2856(C-H str.), 1262(C-O str.),3043(Ar-CHstr.), 1278(C-Nstr.),759(C-Sstr.),1627(C=Ostr.),1136(C-Ostr.inC-O-C); ^{1}HNMR ($CDCl_3$, δppm):1.324-1.362 (t,3H,OCH_2CH_3), 1.749-1.756 (m,4H,C_5 and C_6), 2.667-2.731 (m,4H,C and C_7),3.442(s,2H,$CH_2$of thiazolidine), 3.945 (s,6H, OCH_3), 4.228-4.281(q,2H,OCH_2CH_3),5.964(s,1H,N-CH),6.452-7.042(m,3H,Ar-H); Anal.Calcd.for $C_{22}H_{25}NO_5S_2$: C(59.14), H(5.12) ,N(3.17) ,O(17.78), S(14.34) ;found:C(58.63), H(6.14),N(3.60), O(17.58),S(14.17);Mol.Wt.:444.

Ethyl2-[2-(4-dimethylaminophenyl)-4-oxo-1,3-thiazolidin-3-yl]-4,5,6,7tetrahydro-1-benzothi-ophene-3- carboxylate (KM4):

Yield:56.2%;Melti ngPoint:135°C;IR(KBr,cm^{-1}):2842(C-Hstr.),1532(C=Cstr.),1281(C- O str.),3076(Ar-CHstr.),1364(C-Nstr.),759(C-Sstr.),1662(C=Ostr.);^{1}HNMR ($CDCl_3$,δppm): 1.324-1.359 (t,3H, OCH_2CH_3), 1.750-2.689(m,8H,C_4,C_5,C_6,andC_7),3.050(s,6H,$(CH_3)_2$),3.875(s,2H,$CH_2$of thiazolidine), 4.241-4.387 (q,2H,OCH_2CH_3),5.940(s,1H, N-CH),6.679-7.723(m,4H,Ar-H);Anal.Calcd.For $C_{22}H_{26}N_2O_3S_2$:C(61.30), H(6.01),N(6.50),O(11.17),S(14.80);found:C(60.93),H(6.51),N(6.75),O(10.86),S(14.94);Mol.Wt.:431.

Biological Screening

All the synthesized compounds were subjected to antimicrobial screening at a concentration of 100μg/ml involving three Gram-ve bacteria (*Eschericha coli*, *Staphylococcus aureus* and *Klebsiella pneumoniae*); three Gram+ ve(*Seratiareticulata*,*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 [13].The response of organisms to the synthesized compounds were measured in terms of zone of inhibition and compared with that obtained with standard.

Preparation of Mueller Hinton agar media

Mueller Hinton Agar Media was used for antimicrobial screening and its composition is as: Casein Acid Hydrolysate17.50gm,Beef Heart Infusion2.00gm,Starch soluble 1.50gm and Agar 17.00gm. For preparing Mueller Hinton Agar (MHA) Media,38gmof Mueller Hinton AgarNo.2was dissolved in 1000ml distilled water.It was mixed properly and heated to boil to dissolve the medium completely.It was autoclaved at15lbs pressure(121°C) for15 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 incubatorat37°C.

Preparation of Standard antibiotic solution

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

Preparation of Test solution

10mg of the synthesized compound(s) was dissolved in 10ml of DMF.1ml of this solution was taken and diluted to10ml(withDMF) so that the concentration of the test solution became100μg/ml.

Preparation of inoculums

For the preparation of inoculums, 5g of nutrient agar was dissolved in100ml 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 24hour old culture was used for the preparation of bacterial suspension. Likewise suspension of all the organisms were prepared as per standard procedure.

Preparation of Discs

Discs of 6-7mm in diameter were punched from No.1Whattmann filter paper with sterile corkborer of same size. These discs were sterilized by keeping in oven at140°C one hour.Standard and test solutions were added separately to these discs which were air dried lateron.

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-hourdiffusion at room temperature and then for incubation at 37°C for24hours in an incubator.The zones of inhibition after24 hours weremeasured in millimeters.The results obtained are shown inTable1andTable2.

RESULT AND DISCUSSION

According to Gewald [14], heating under stirring of a mixture of ethylcyanoacetate, sulphur and cyclohexanone in diethylaminefor 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(KM1-KM4) has been provided by differences in melting points and yield value from that of parent compound. Compound1 on reaction with various substituted aldehydes yielded various Schiff bases which on cyclization withthioglycollicacid in catalytic amount ofZnCl2 yielded novel thiophene derivatives (KM1- KM4). 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, ethylacetate, benzene,DMSO and DMF but in soluble in hexane and toluene.With the help of analytical techniques such as meltingpoint, IRand1 HNMR, synthesized derivatives were characterized . These compounds showed abandat1646cm-1for cyclic>C=O group[15]. All the compounds showed NMR signals for differentkind of protons at their respective positions.

Table 1: Antibacterial activity of synthesized thiazolidinones (KM1- KM4)

	Zone of Inhibition in mm					
Sample	***E.coli***	***S.reticulata***	***S.aureus***	***B.subtilis***	***S. pneumoniae***	***K.pneumoniae***
KM1	-	14	15	16	10	11
KM2	13	20	14	14	15	15
KM3	14	18	-	-	13	13
KM4	10	17	16	16	13	9
Ampicillin	22	30	24	25	22	20
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 KM2and

KM4 exhibited promising antibacterial activity while compound KM3 exhibited promising antifungal activity.

Table 2: Antifungal activity of synthesized thiazolidiones

	Zone of Inhibition in mm	
Sample	***P.aeruginosa***	***C. albican***
KM1	10	14
KM2	9	14
KM3	13	13
KM4	12	11
Ampicillin	21	24
DMF	-	-

However other compounds of the series also exhibited moderate to significant activity against the microorganisms as mentioned above. Therefore compound KM2, KM3 and KM4 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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