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Der Pharma Chemica, 2012, 4 (1):489-496
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Facile synthesis of thiazolidinones bearing thiophene nucleus as antimicrobial agents

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ABSTRACT

A series of thiophene 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 reacted with various substituted aldehydes to synthesize Schiff bases which on cyclization with thioglycollic acid in catalytic amount of $ZnCl_2$ yielded the final products (R_1-R_9). Synthesized compounds were purified, characterized and evaluated for their antimicrobial activity. Most of the compounds exhibited moderate to significant activities.

Keywords: Benzothiophene, Gewald Reaction, Thiazolidinone, Antibacterial Activity, Antifungal Activity.

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.

MATERIALS AND METHODS

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: n-hexane (1:2) solvent system and iodine vapours as a visualizing agent.

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 to give Compound 1.

Synthesis of Schiff bases (B_1- B_7)
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. Physical and analytical data of synthesized Schiff bases (B_1- B_9) is summarized in Table 1.

Synthesis of thiazolidinones (R_1- R_9)
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. Physical and analytical data of synthesized compounds (R_1- R_9) is summarized in Table 2.

Compound R_1: IR (KBr, cm^{-1}): 2847(C-H str.), 1590(C=C str.), 1279(C-O str.), 3117(Ar-H str.), 1363(C-N str.), 780(C-S str.), 1646(C=O str.), 632(C-Cl str.); ^{1}HNMR($CDCl_3$, δppm): 1.3(t, 3H, OCH_2CH_3), 4.2(q, 2H, OCH_2CH_3), 1.7(m, 4H, C_5 and C_6), 2.5(m, 4H, C_4 and C_7), 3.2(s, 2H, CH_2 of thiazolidine), 5.9(s, 1H, N-CH), 6.9-7.4(m, 4H, Ar-H); Anal. Calcd. for $C_{20}H_{20}NO_3S_2Cl$: C(56.93), H(4.78), Cl(8.40); N(3.32), O(11.38), S(15.20); found: C(57.20), H(4.61), Cl(8.22), N(3.70), O(11.57), S(14.70); Mol. Wt.: 421.

Compound R_2: IR (KBr, cm^{-1}): 2847(C-H str.), 1594(C=C str.), 1282(C-O str.), 3171(Ar-H str.), 1414(C-N str.), 780(C-S str.), 1644(C=O str.), 690(Monosubsti. ring); ^{1}HNMR($CDCl_3$, δppm): 1.3(t, 3H, OCH_2CH_3), 4.2(q, 2H, OCH_2CH_3), 1.7(m, 4H, C_5 and C_6), 2.5(m, 4H, C_4 and C_7), 3.4(s, 2H, CH_2 of thiazolidine), 5.8(s, 1H, N-CH), 7.1-7.8(m, 5H, Ar-H); Anal. Calcd. for $C_{20}H_{21}NO_3S_2$: C(61.99), H(5.46), N(3.61), O(12.39), S(16.55); found: C(62.21), H(5.72), N(3.24), O(11.92), S(16.91); Mol. Wt.: 387.

Compound R_3: IR (KBr, cm^{-1}): 2847(C-H str.), 1589(C=C str.), 1279(C-O str.), 3084(Ar-H str.), 1365(C-N str.), 780(C-S str.), 1647(C=O str.), 3297(O-H str.); ^{1}HNMR($CDCl_3$, δppm): 1.3(t, 3H, OCH_2CH_3), 4.2(q, 2H, OCH_2CH_3), 1.7(m, 4H, C_5 and C_6), 2.7(m, 4H, C_4 and C_7), 3.2(s, 2H, CH_2 of thiazolidine), 5.9(s, 1H, N-CH), 5.4(s, 1H, OH), 6.8-7.2(m, 4H, Ar-H); Anal. Calcd. for $C_{20}H_{21}NO_4S_2$: C(59.53), H(5.25), N(3.47), O(15.86), S(15.89); found: C(60.44), H(6.02), N(3.79), O(14.52), S(15.23); Mol. Wt.: 403.

Compound R_4: IR (KBr, cm^{-1}): 2847(C-H str.), 1586(C=C str.), 1278(C-O str.), 1365(C-N str.), 780(C-S str.), 1647(C=O str.), 2931(C-H str. of methyl gp.); ^{1}HNMR($CDCl_3$, δppm): 1.3(t, 3H, OCH_2CH_3), 4.2(q, 2H, OCH_2CH_3), 1.7(m, 4H, C_5 and C_6), 2.5-2.7(m, 4H, C_4 and C_7), 3.3(s, 2H, CH_2 of thiazolidine), 4.8(q, 1H, N-CH), 1.3(d, 3H, CH_3); Anal. Calcd. for $C_{15}H_{19}NO_3S_2$: C(55.36), H(5.88), N(4.30), O(14.75), S(19.71); found: C(56.50), H(6.25), N(3.89), O(14.04), S(19.32); Mol. Wt.: 325.

Compound R_5: IR (KBr, cm^{-1}): 2848(C-H str.), 1587(C=C str.), 1287(C-O str.), 1342(C-N str.), 778(C-S str.), 1648(C=O str.); ^{1}HNMR($CDCl_3$, δppm): 1.3(t, 3H, OCH_2CH_3), 4.2(q, 2H, OCH_2CH_3), 1.7(m, 4H, C_5 and C_6), 2.7(m, 4H, C_4 and C_7), 3.3(s, 2H, CH_2 of thiazolidine), 4.3(s, 2H, N-CH_2); Anal. Calcd. for $C_{14}H_{17}NO_3S_2$: C(55.99), H(5.50), N(4.50), O(15.41), S(20.59); found: C(54.70), H(6.80), N(4.35), O(14.75), S(19.40); Mol. Wt.: 311.

Compound R_6: IR (KBr, cm^{-1}): 2846(C-H str.), 1593(C=C str.), 3170(Ar-H str.), 1280(C-O str.), 1376(C-N str.), 780(C-S str.), 1647(C=O str.), 2935(C-H str. of methyl gp.); ^{1}HNMR($CDCl_3$, δppm): 1.3(t, 3H, OCH_2CH_3), 4.2(q, 2H, OCH_2CH_3), 1.7(m, 4H, C_5 and C_6), 2.5-2.7(m, 4H, C_4 and C_7), 3.3(s, 2H, CH_2 of thiazolidine), 5.8(s, 1H, N-CH), 2.4(s, 3H, CH_3), 6.9(m, 4H, Ar-H); Anal. Calcd. for $C_{21}H_{23}NO_3S_2$: C(62.81), H(5.77), N(3.49), O(11.95), S(15.97); found: C(63.75), H(6.33), N(3.71), O(11.11), S(15.10); Mol. Wt.: 401.

Compound R_7: IR (KBr, cm^{-1}): 2847(C-H str.), 1592(C=C str.), 3171(Ar-H str.), 1283(C-O str.), 1377(C-N str.), 777(C-S str.), 1646(C=O str.), 2934(C-H str. of methyl gp.); ^{1}HNMR($CDCl_3$, δppm): 1.3(t, 3H, OCH_2CH_3), 4.2(q, 2H, OCH_2CH_3), 1.7(m, 4H, C_5 and C_6), 2.4(m, 4H, C_4 and C_7), 3.4(s, 2H, CH_2 of thiazolidine), 5.7(s, 1H, N-CH), 2.5(s, 3H, CH_3), 6.8(m, 4H, Ar-H); Anal. Calcd. for $C_{21}H_{23}NO_3S_2$: C(62.81), H(5.77), N(3.49), O(11.95), S(15.97); found: C(63.50), H(6.33), N(3.82), O(11.00), S(15.35); Mol. Wt.: 401.

Compound R_8: IR (KBr, cm^{-1}): 2846(C-H str.), 1598(C=C str.), 1284(C-O str.), 1339(C-N str.), 778(C-S str.), 1645(C=O str.), 1148(C-O str. in C-O-C); ^{1}HNMR($CDCl_3$, δppm): 1.3(t, 3H, OCH_2CH_3), 4.2(q, 2H, OCH_2CH_3), 1.7(m, 4H, C_5 and C_6), 2.4(m, 4H, C_4 and C_7), 3.4(s, 2H, CH_2 of thiazolidine), 5.8(s, 1H, N-CH), 3.9(s, 3H, OCH_3), 6.5(m, 4H, Ar-H); Anal. Calcd. for

$C_{21}H_{23}NO_4S_2$: C(60.41), H(5.55), N(3.35), O(15.33), S(15.36); found: C(59.55), H(5.65), N(4.20), O(15.15), S(15.00); Mol. Wt.: 417.

Compound R_9: IR (KBr, cm^{-1}): 2846(C-H str.), 1594(C=C str.), 1271(C-O str.), 1341(C-N str.), 788(C-S str.), 1644(C=O str.), 2935 (C-H str. of methyl gp.); ^{1}HNMR($CDCl_3$, δppm): 1.3(t, 3H, OCH_2CH_3), 4.2(q, 2H, OCH_2CH_3), 1.7(m, 4H, C_5 and C_6), 2.4-2.7(m, 4H, C_4 and C_7), 3.5(s, 2H, CH_2 of thiazolidine), 4.3(t, 1H, N-CH), 1.9(m, 2H, CH_2); 0.9(t, 3H, CH_3) Anal. Calcd. for $C_{16}H_{21}NO_3S_2$: C(56.61), H(6.24), N(4.13), O(14.14), S(18.89); found: C(57.20), H(7.55), N(3.72), O(13.69), S(17.84); Mol. Wt.: 339.

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 (*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[13]. 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
Distilled water	1lt.

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 for 15 minutes i.e. 121°C.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 100µg/ml.

D) Preparation of inoculum

For the preparation of inoculum, 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 3 and Table 4.

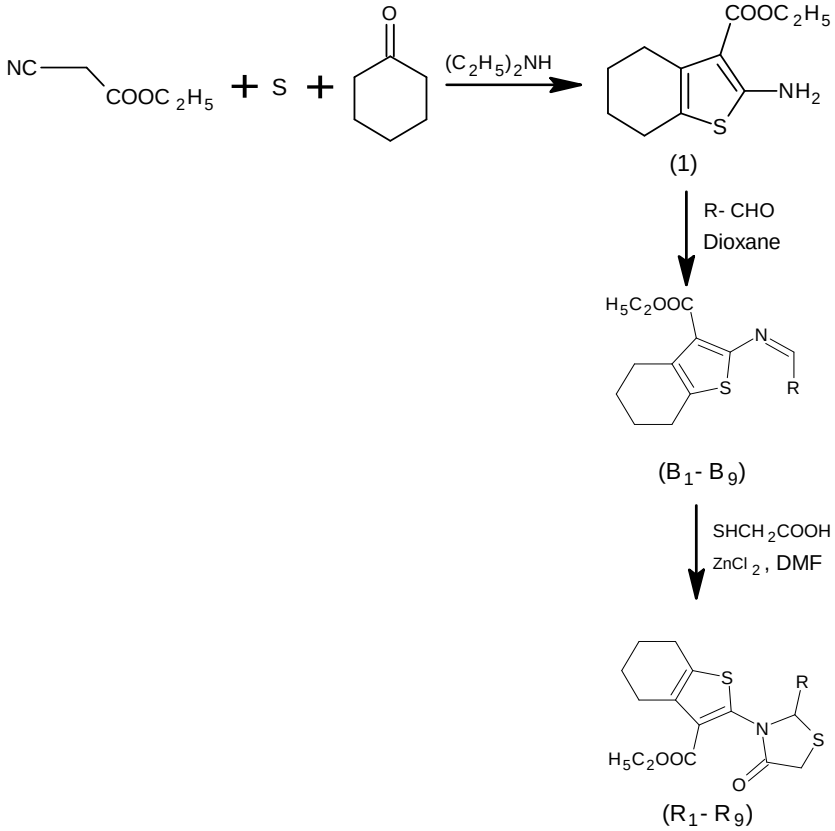
RESULTS AND DISCUSSION

According to Gewald[14], 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). The IR Spectrum of the Compound (1) shows a distinct peak at 3370 cm^{-1}(NH_2). Substantial proof for the formation of Schiff base (B_1- B_9) 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 $ZnCl_2$ yielded novel thiophene derivatives (R_1- R_9). 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 ^{1}H-NMR, synthesized derivatives were characterized. These compounds showed a band at 1646 cm^{-1} for cyclic >C=O group[15]. All the compounds showed NMR signals for different kinds of protons at their respective positions. All of them were found to be in full consignment with assigned structures.

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. Compounds R_6 and R_1 have shown best antibacterial activity while compound R_6 and R_2 have shown best antifungal activity. Compound R_6 which is having a methyl group at ortho position in benzene ring has shown best antimicrobial activity as when compared to other derivatives. However other compounds of the series also exhibited moderate to significant activity against the microorganisms as mentioned above. Therefore compounds R_6, R_1 and R_2 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.



R= 4- Cl C_6H_4; C_6H_5; 3- OH C_6H_4; CH_3; H; 2- CH_3 C_6H_4; 3- CH_3 C_6H_4; 3- OCH_3 C_6H_4; C_2H_5

Scheme

Table 1: Physical data of Schiff Bases (B_1- B_9)

S. No.	Code. No.	R	Mol. Formula	Melting Pt.	Mol. Wt.	Yield(%)
1	B_1	4- Cl C_6H_4	$C_{18}H_{18}ClNO_2S$	110°C	347	64
2	B_2	C_6H_5	$C_{18}H_{18}NO_2S$	118°C	313	61
3	B_3	3- OH C_6H_4	$C_{18}H_{19}NO_3S$	135°C	329	68
4	B_4	CH_3	$C_{13}H_{17}NO_2S$	124°C	251	73
5	B_5	H	$C_{12}H_{15}NO_2S$	116°C	237	78
6	B_6	2- CH_3 C_6H_4	$C_{19}H_{21}NO_2S$	100°C	327	63
7	B_7	3- CH_3 C_6H_4	$C_{19}H_{21}NO_2S$	98°C	327	66
8	B_8	3- OCH_3 C_6H_4	$C_{19}H_{21}NO_3S$	95°C	343	41
9	B_9	C_2H_5	$C_{14}H_{19}NO_2S$	120°C	265	57

Table 2: Physical data of synthesized thiazolidinones (R_1- R_9)

S. No.	Code. No.	R	Mol. Formula	Melting Pt.	Yield(%)
1	R_1	4- Cl C_6H_4	$C_{20}H_{20}NO_3S_2Cl$	125°C	64.7
2	R_2	C_6H_5	$C_{20}H_{21}NO_3S_2$	128°C	62.5
3	R_3	3- OH C_6H_4	$C_{20}H_{21}NO_4S_2$	119°C	75
4	R_4	CH_3	$C_{15}H_{19}NO_3S_2$	117°C	76.9
5	R_5	H	$C_{14}H_{17}NO_3S_2$	118°C	76.4
6	R_6	2- CH_3 C_6H_4	$C_{21}H_{23}NO_3S_2$	127°C	68.7
7	R_7	3- CH_3 C_6H_4	$C_{21}H_{23}NO_3S_2$	114°C	81.25
8	R_8	3- OCH_3 C_6H_4	$C_{21}H_{23}NO_4S_2$	115°C	76.4
9	R_9	C_2H_5	$C_{16}H_{21}NO_3S_2$	129°C	61.5

Table 3: Antibacterial activity data of synthesized thiazolidinones (R_1- R_9)

Sample	Zone of inhibition in mm					
	E. coli	*S. reticulata*	*S. aureus*	*B. subtilis*	*S. pneumoniae*	*K. pneumoniae*
R_1	10	19	15	16	14	12
R_2	11	15	13	12	14	9
R_3	15	20	10	-	14	10
R_4	12	13	14	15	13	11
R_5	11	16	17	13	11	13
R_6	13	19	15	17	15	12
R_7	12	17	12	11	11	10
R_8	9	12	9	11	15	11
R_9	15	19	10	15	12	12
Standard	22	30	22	25	22	20
DMF	-	-	-	-	-	-

Table 4: Antifungal activity data of synthesized thiazolidinones (R_1- R_9)

Sample	Zone of inhibition in mm	
	P. aeruginosa	*C. albicans*
R_1	10	11
R_2	11	15
R_3	10	14
R_4	12	11
R_5	8	9
R_6	13	13
R_7	10	10
R_8	12	12
R_9	9	14
Standard	19	21
DMF	-	-

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.

Acknowledgement
The authors are thankful to librarians of various Institutions & libraries like Jamia Hamdard New Delhi, National Medical Library New Delhi, NISCAIR New Delhi, CDRI Lucknow, IVRI Bareilly and to the Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad for providing literature survey facility to carry out the work.

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