AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION AND QSAR STUDIES OF THIOPHENE AND ARYL SUBSTITUTED COMPOUNDS

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ABSTRACT

A series of thiophene and aryl substituted compounds 1a-f; 2a-b and 3a-c were synthesized by Erlenmeyer azolactone synthesis. These compounds were screened for antimicrobial activity method *in vitro*. Among the tested compounds 3c showed significant activity and some of the other compounds showed promising activity. The structure of new compounds synthesized during present investigation have been authentically established by their IR, ^{1}H NMR and Mass spectra and some QSAR studies.

Key words: Thiophene; antimicrobial; antibacterial; antifungal.

INTRODUCTION:

Victor Meyer discovered thiophene in 1882 during his ingenious experiment on the investigation of aromatic compounds. Thiophene occurs in coal tar and a large number of thiophene derivatives occur in plants and in animal metabolism[1]. Thiophenes and their derivatives were important class of heterocyclic compounds. Thiophenes and their fused derivatives have shown diverse pharmacological activities including antibacterial, antifungal, immunumodulatory, antidiabetic, antiinflammatory, antiviral and anticancer agents[2]. The chemistry of thiophene is

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Received 22 October 2011, Accepted 14 November 2011

Please cite this article in press as: Mohammad HA *et al.*, Imultaneous HPTLC Determination of Camylofin Dihydrochlroide and Diclofenac Potassium in a Pharmaceutical Formulation. American Journal of PharmTech Research 2012.

well documented in the literature due to their wide spread pharmacological properties when it is fused with different other nucleus such as thienopyrimidine[3], benzothiophene[4], bithienyl[5], thienopyridine[6] etc.

Generally in pharmaceutical field new drugs are discovered by molecular modification of the lead compound of established activity. So an attempt was made to synthesize, new substituted thiophenes as antimicrobial agents by adapting Erlenmeyer azlactone synthesis.

MATERIALS AND METHODS

The chemicals and reagents used in the project work were of AR, LR and synthetic grade, procured from Spectrochem, S.D-Fine, Qualigens, Glaxo, Ranbaxy, Rolex Chem. Ltd and Sigma. Glycine. acetylglycine, benzoyl chloride, thiophen-2-aldehyde, substituted aryl aldehydes, acetic anhydride, sodium acetate, sodium hydroxide etc. were used in this work.

Experimental

The completion of reactions was monitored by TLC technique using Silica gel-G (for TLC) using suitable mobile phase. Determination of melting point was done by open capillary tube method using paraffin bath and are uncorrected. Recrystallization was done by suitable solvent. The ^{1}H NMR of synthesized compounds were recorded in Bruker FT-NMR (400MHz & 200MHz) as TMS as internal standard and IR-spectra were recorded in Bruker alpha FT-IR using KBr pellets. The Mass spectra were recorded on Shimadzu LC-MS with ESI source. Make-Shimadzu at 70 eV. The physical data and characterization data of synthesized thiophene derivatives are tabulated in **Table-1**.

Preparation of 2-(acetyl amino)-3-(thiophen-2-yl) prop-2-enoic acid, 1a

Acetyl glycine (2.9g, 0.025mol) and thiophene-2-aldehyde (2.8g, 0.025mol) was heated on hot plate in the presence of sodium acetate (2.05g, 0.025mol) till the reaction mixture was melted to a clear solution, further it was decomposed in dry ethanol to yield compound **1a**.The solid obtained was filtered, washed with cold ethanol, dried and it was purified by recrystallization from benzene. Similarly compound **1b-f** was prepared.

1a. IR: 3450cm^{-1} (OH), 1736cm^{-1} (C=O), 1490cm^{-1} (C-S-C). **1b** IR: 3432cm^{-1} (OH), 3313cm^{-1} (NH), 1750cm^{-1} (C=O); ^{1}H NMR (DMSO): 7.0δ (t, 1H, Ar-H), 7.3-7.5δ (m, 5H, Ar-H), 7.82δ (s,1H, -NH), 7.87δ (d, 1H, Ar-H), 8.0δ (d, 1H, Ar-H), 8.4δ (s, 1H, -OH), 12.1δ (h, 1H, -COOH); Mass: m/z: 273, 229, 179, 155, 123.

Preparation of 2-methyl-4-(thiophen-2-ylmethylidene)-1,3-oxazol-5(4*H*)one, 2a

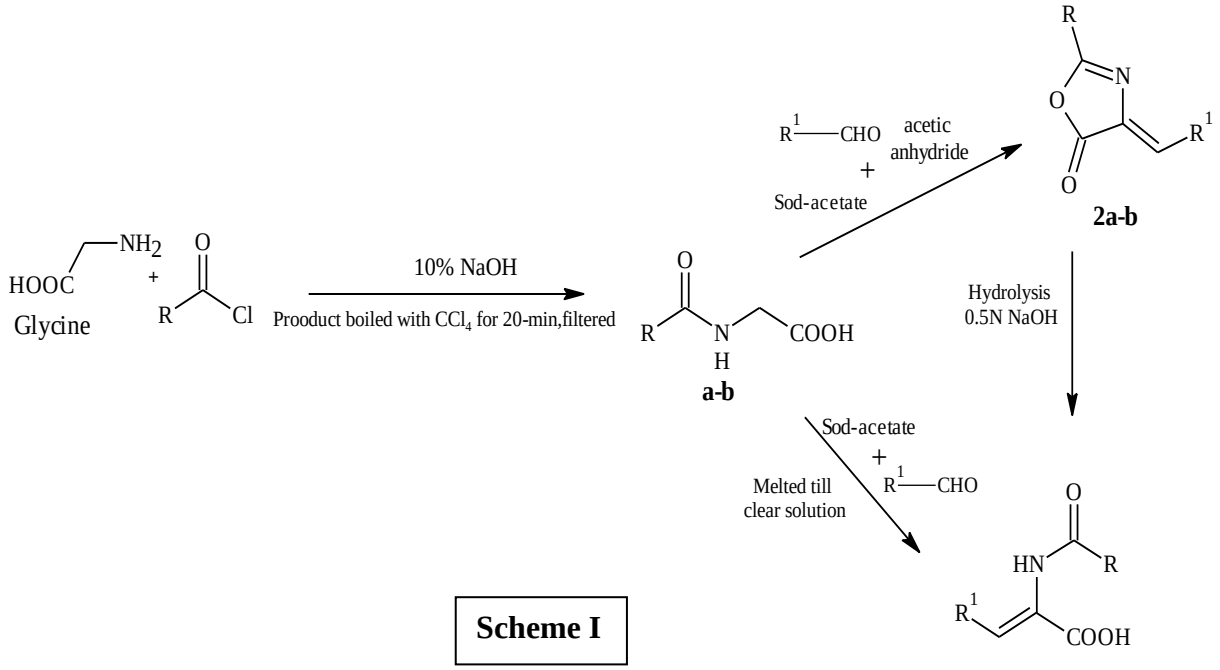
Thiophene-2-carbaldehyde (2.8g, 0.025mol), acetyl glycine (2.9g, 0.025mol) acetic anhydride (7.15ml, 0.075mol) and sodium acetate (2.05g, 0.025mol) were taken in 250ml conical flask and heated on electric hot plate with constant stirring. As soon as the mixture liquefied completely, the flask was transferred to water bath and heated for 2 hours. Then 10ml of ethanol was added slowly to the contents of the flask and allowed the mixture to stand overnight. The solid obtained was filtered, washed with cold ethanol and recrystallized from benzene. Similarly compound **2b** was prepared.

2a. IR: 1785cm^{-1} (O-C=O), 1586cm^{-1} (C=N), 1439cm^{-1} (C-S-C); Mass: m/z 193, 150, 117. **2b.** IR: 1791cm^{-1} (O-C=O), 1638cm^{-1} (C=N), 1490cm^{-1} (C-S-C). ^{1}H NMR ($CDCl_3$): 8.2δ, (d, 1H, thiophenyl); 7.7δ (d, 1H, thiophenyl); 7.1δ (t, 1H, thiophenyl); and 7.6 to 7.1δ (m, 5H, Ar-H).

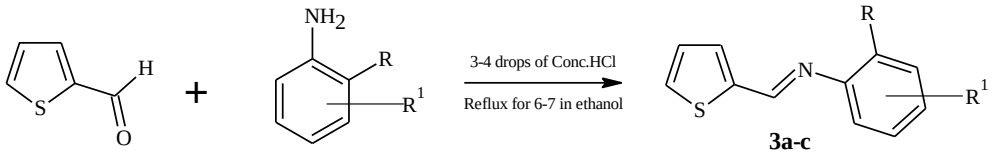
Preparation of 2-thiophen-2-ylmethylidene] amino} benzoic acid, 3a

Refluxing a mixture of thiophene-2-carbaldehyde (1.26g, 0.01mol) and 2-amino benzoic acid (1.37g, 0.01mol) in presence of 2-3 drops of Conc.HCl and dry ethanol for 8-10 hours. The resultant mixture is then they added to ice cold water slowly with constant stirring a precipitate was filtered, washed with cold water and dried in oven, recrystallized by ethanol, to form compound **3a**. Similarly Compounds **3b-c** was prepared.

3a. IR: 3479cm^{-1} (OH), 1671cm^{-1} (C=O), 1609cm^{-1} (C=N), 1498cm^{-1} (C-S-C); **3b**. IR: 3450cm^{-1} (NH), 1622cm^{-1} (C=N), 1452cm^{-1} (C-S-C); **3c.** IR: 3290cm^{-1} (NH), 1608cm^{-1} (C=N), 1487cm^{-1} (C-S-C), 1697cm^{-1} (S=O); ^{1}H NMR (DMSO): 6.5δ (d, 2H, Ar-H), 7.2δ (t, 1H, Ar-H), 7.4δ (d, 2H, Ar-H), 8.0δ (d, 2H, Ar-H), 8.1δ (d,1H,Ar-H), 8.8δ (s, 1H, =CH), 9.9δ (s, 2H, -NH_2).



Scheme I



	R	R^1
3a	2-NH_2	H
3b	2-COOH	H
3c	H	4-SO_2NH_2

Scheme II

Antimicrobial Activity[7-11]:

The *in vitro* antimicrobial activity was carried out against 24 hrs old cultures of bacteria and fungi using cup-plate method (10-11). All the newly synthesized compounds were screened for the antimicrobial activity against gram-positive bacteria *S. arureus* and *B. subtilis* and gram-negative bacteria *E. coli* and *P. aeruginosa* and antifungal activity against *C. albicans* and *A. niger* The compounds were tested at two concentrations namely 50µg/ml and 100µg/ml in DMF against all organisms. The zone of inhibition was compared with penicillin and streptomycin for antibacterial activity after 24 hrs of incubation at 25ºC and Griseofulvin for antifungal activity after 48 h of incubation at 30ºC.

RESULTS AND DISCUSSION

In the quantitative structure activity relationship (QSAR) studies, the biological properties of these molecules were compared with several theoretical parameters such as partition coefficient (*c* LogP), polar surface area (PSA), hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD), calculated using computational softwares. The same QSAR datas for synthesized compounds were tabulated in **Table-1.** Newly synthesized Thiophene derivatives exhibited significant drug likeness when compared with standard drug. Since the compounds were showed considered drug likeness properties when compared to the analysis of Lipinski rule of five.

Among the tested compounds **1b** showed significant activity and other compounds showed promising activity because of their equal HBA, less HBD and PSA, *c* LogP, drug likeness and drug score values were more compared to standard drug.

Table 1: Insilico pharmacological studies, physical data and characterization of synthesized compounds

Comp Codes	R	R^1	Molecular formula	Yield %	M.P. °C	*HBD	*HBA	*PSA (A^2)	**clog *p*	**drug likeness	**drug score
1a	CH_3	Thiophenyl	$C_9H_9NO_3S$	65	166	2	4	66.39	1.6	-2.98	0.49
1b	C_6H_5	Thiophenyl	$C_{14}H_{11}NO_3S$	58	188	**2**	**4**	66.39	3.08	0.26	0.65
1c	CH_3	2-Cl C_6H_4	$C_{11}H_{10}ClNO_3$	49	205	2	4	66.39	2.37	-2.75	0.47
1d	C_6H_5	2-FC_6H_4	$C_{16}H_{12}FNO_3$	54	296	2	4	66.39	3.3	-1.26	0.39
1e	C_6H_5	2-OH C_6H_4	$C_{20}H_{19}NO_6$	50	230	3	5	86.62	2.94	-0.02	0.64
1f	CH_3	3-NO_2 C_6H_4	$C_{11}H_{10}N_2O_5$	65	225	2	4	66.32	1.77	-4.39	0.13
2a	CH_3	Thiophenyl	$C_9H_7NO_2S$	63	192	0	3	38.66	0.98	-0.98	0.59
2b	C_6H_5	Thiophenyl	$C_{14}H_9NO_2S$	63	165	0	3	38.66	2.36	2.59	0.79
3a	NH_2	H	$C_{12}H_9NO_2S$	69	135	1	3	49.66	2.3	1.12	0.78
3b	COOH	H	$C_{11}H_{10}N_2S$	69	145	2	2	38.38	2.06	-1.13	0.16
3c	H	SO_2NH_2	$C_{11}H_{10}N_2O_2S_2$	56	201	2	4	72.52	1.39	0.67	0.75
INH	--	--	--	--	--	3	4	68.01	-0.78	-5.06	0.49
Streptomycine	--	--	--	--	--	16	18	330.4	-7.36	1.09	0.24

*HBD, HBA and PSA was calculated by using www.molinspiration.com

**cLog*p*, drug likeness and drug score was calculated by using www.organic-chemical.org

The formation of **1b** has been confirmed by IR spectrum. The compound **1b** exhibited an absorption band at 3432cm^{-1} characteristic of hydroxyl (COOH), 3313cm^{-1} due to NH stretching and another 1750cm^{-1} which is a characteristic of carbonyl. Further compound **1b** was confirmed by proton ^{1}H NMR spectrum in DMSO which exhibited one triplet and multiplet at δ 7.0 and 7.3-7.5 for thiophene and benzene protons respectively. One singlet and doublet at δ 7.82 and 7.87 for NH and thiophene proton respectively. Appearance of doublet, singlet and one more singlet at δ 8.0, δ 8.4 and δ 12.1 represents thiophene, CH group, and carboxyl group proton respectively. Similarly formation of other compounds **1a** has been confirmed by their IR spectra.

The above said reaction was carried out in presence of acetic anhydride to get substituted thiophenyl methylidene azlactones **2a-b**.

The formation of 2-methyl-4-(thiophen-2-ylmethylidene)-1,3-oxazol-5(4*H*)-one **2a**, has been confirmed by IR spectrum of the compound exhibited an absorption band at 1785cm^{-1} which is a characteristics of lactone carbonyl, another band at 1586cm^{-1} due to C=N, and one more band at 1439cm^{-1} due to C-S-C; Mass: m/z 193, 150, 117. And compound 2-phenyl-4-(thiophen-2-ylmethylene) -1,3-oxazol-5(4*H*)-one **2b,** has been confirmed by IR spectrum of the compound exhibited an absorption band at 1791cm^{-1} which is a characteristics of lactone carbonyl, another

band at 1637cm^{-1} due to C=N and one more band at 1490cm^{-1} due to C-S-C . ^{1}H NMR spectrum of compound **2b** was taken in $CDCl_3$, spectrum exhibited two doublets and one triplet at δ 8.2, 7.7 and 7.1 respectively for the protons of thiophene ring. A multiplet at δ 7.6 to 7.5 for the five aromatic protons and one proton of allyl group was also observed in ^{1}H NMR spectrum.

Further compounds **2a-b** was hydrolyzed in hot alkali to form opened chain Knoevenagel compounds **1a** and **1b.** These compounds were confirmed by correlating the melting point and IR spectra which were synthesized in first step.

Synthesis of thiophene derivatives of some thiophenyl Schiff's bases using substituted aryl amines and thiophene-2-carbaldehyde in presence of 2-3 drops of Conc.HCl and dry ethanol. Then the reaction mixture was added to ice cold water slowly with constant stirring to form compounds **3a-c**.

The above product formed was confirmed by testing for primary amine test, result was fail (except for **3b** which has one more primary NH_2), it confirms that amino group involved in the reaction. The IR (KBr) spectrum of the compound **3c** exhibited an absorption band at 3290cm^{-1} characteristic of NH, 1697cm^{-1} of S=O, 1608cm^{-1} due to C=N and another band at 1487cm^{-1} of C-S-C. ^{1}H NMR spectrum of **3c** in DMSO which exhibited one singlet at δ 9.9 of NH_2 group protons. One triplet and two doublets at δ 7.2, 6.5 and 7.4 for thiophene proton respectively. Appearance of two doublets at δ 8.0, 8.1 represents para substituted benzene ring protons. Appearance of one singlet at δ 8.8 represents vinyl proton.

Table 2: Antibacterial activity of synthesized compounds

Compound Codes	**Zone of inhibition (in mm)**							
	S. Aureus		***B. Subtilis***		***E. Coli***		***P. Aeruginosa***	
	50 µg/ml	**100 µg/ ml**	**50 µg/ ml**	**100 µg/ml**	**50 µg/ml**	**100 µg/ml**	**50 µg/ ml**	**100 µg/ml**
1a	10	15	11	15	14	16	17	19
1b	11	16	12	17	15	18	16	19
1c	11	14	13	16	16	18	18	20
1d	11	14	12	17	16	17	17	19
1e	12	13	13	18	16	18	19	20
1f	10	14	14	17	18	20	15	18
2a	11	15	12	17	16	18	16	18
2b	11	17	14	18	16	19	17	20
3a	10	16	13	16	16	18	15	18
3b	12	17	14	18	16	19	17	20
3c	13	18	15	19	19	24	19	22
Penicillin	14	20	17	21	--	--	--	--
Streptomycin	--	--	--	--	22	25	21	24

Antimicrobial Activity[7-11]:

The antimicrobial activity against gram-positive bacteria *S. arureus* and *B. subtilis* and gram-negative bacteria *E. coli* and *P. aeruginosa* obtained results were tabulated **Table-2**. And antifungal activity against *C. albicans* and *A. niger* and obtained results were tabulated **Table-3**.

Table 3: Antifungal activity of synthesized compounds

Compound Codes	Zone of inhibition (in mm)			
	C. Albicans		*A. Niger*	
	50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
1a	11	12	10	11
1b	10	12	10	11
1c	10	12	10	11
1d	10	13	11	12
1e	10	12	10	11
1f	12	13	12	13
2a	11	13	12	14
2b	11	12	10	11
3a	10	12	12	13
3b	11	12	10	12
3c	12	15	13	14
Griseofulvin	14	16	13	15

CONCLUSION

Compound **3c** showed significant activity and other compounds showed promising activity when compared with the respective activity of standards.

ACKNOWLEDGEMENT

Authors are thankful to Principal SJM College of Pharmacy, Chitradurga. AstraZeneca Pvt. Ltd. Bengaluru, SAIF, Chandigarh, Oxygen Healthcare Research Pvt. Ltd, Ahmedabad and CMCRI, Bhavnagar for providing the Spectral Data.

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