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Synthesis and Antimicrobial screening of some Novel Substituted *Thiophenes*

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Abstract

Plan: *To synthesize some novel 2-amino thiophenes with various substitutions at 2-amino position for antibacterial and antifungal activity.*
Prologue*: Thiophenes containing organic compounds forms a significant group of drugs which exhibit an array of biological activities like anti-inflammatory, antibacterial, antifungal, anti-neoplastic, antiarthritic etc. Thus a series of new thiophenes have synthesized with various substituents at 2-amino position and screened for antimicrobial activity.*
Methodology*: The parent compound 2-amino-3-(N-furfuryl amido)-4, 5-dimethyl thiophene was synthesized by condensing butan-2-one with furfurylcyano acetamide in presence of sulphur and diethylamine. It was then derivatized to various Schiff bases by reacting with various substituted aromatic aldehydes. The synthesized new compounds were characterized by MP, TLC, IR, NMR and Mass spectra and were screened for their antibacterial and antifungal activity by using Ampicillin and Miconazole nitrate as standard respectively.*
Outcome*: The compounds MAI-2e, MAI-2k, & MAI-2l showed potent antibacterial and MAI-2a, MAI-2c, MAI-2e, & MAI-2f showed potent antifungal activity.*
Keywords*: 2-amino thiophenes, Schiff bases, Antimicrobial activity, S.aureus, B.subtilis, E.coli, K. pneumoniae*

1. Introduction:

A large number of medicinal compounds which have been discovered belong to a major class of heterocycles containing Nitrogen and Sulphur. The versatile synthetic applicability and biological activity of these heterocycles has helped the medicinal chemist to plan, organize and implement new approaches towards the discovery of novel drugs. Thiophenes and its derivatives are an important class of heterocyclic compound, specifically, 2-amino substituted thiophenes reported to possess a wide spectrum of biological properties such as antibacterial, antifungal, analgesic, anti-inflammatory, antioxidant and antitumor and local anesthetic activity. For example thiophene containing (1) β-lactam antibiotics like Ticarcillin, Cefoxitin, Cephalothin and Cephalorodine have shown good antibacterial activity.

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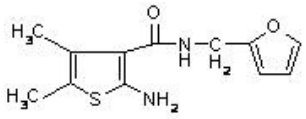
Antifungal agents like Ticonazole and Sertaconazole also contain the thiophene. Generally, in Pharmaceutical field new drugs are discovered by molecular modification of the lead compound of established activity. So far, a range of new thiophenes have been synthesized and screened for their biological activity. The encouraging results prompted us to continue the investigation.

Hence, we have attempted the synthesis of some new compounds containing thiophene nucleus with furan in one framework. We have synthesized 2-substituted amino-3-(N- furfuryl amido)-4, 5-dimethyl thiophene as the starting compound derivatized the starting compound to various 2-substituted amino-3-(N- furfuryl amido)-4,5-dimethyl thiophenes (2-9). The newly synthesized compounds were characterized by spectral data and screened for their in-vitro antimicrobial (antibacterial, antifungal) activity (10-11) by agar diffusion method.



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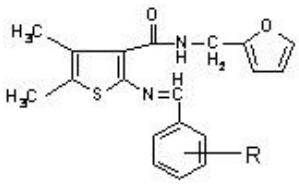

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2. Experimental

Melting points were determined in open glass capillary tubes and uncorrected. The Thin layer chromatography was carried out using silica gel G to find the purity of synthesized compounds. The NMR spectra were recorded in $CDCl_3$ using tetra methyl silane (TMS) as an internal standard. The IR spectra of the synthesized compounds were recorded using KBr pallet on a FTIR spectrophotometer and the frequencies are recorded in wave numbers. The parent compound and derivatives were prepared as per scheme-I

2.1. *Scheme of synthesis*

Step 1. Synthesis of Furfurylcyanoacetamide:

A mixture of furfuryl amine (46.29 ml; 0.50 M) and ethyl cyano acetate (53.35 ml; 0.50 M) was taken in a conical flask and heated in microwave oven at 750 watt for 180 sec. The reaction mixture was left at room temperature for overnight. The solid obtained was filtered, washed with water and dried. Recrystallization was done by ethanol: water mixture (5:1). The same product was obtained by conventional heating on an oil bath at 160-170 ºC for 5-6 hours. The yield was better with microwave assisted method. Yield 61.80%, M Pt. 60^0C, Rf value: 0.25 [Mobile phase- chloroform and ethyl acetate (1:1)].

Step 2. Synthesis of 2-Cyano-2-(isobutyl-1-ylidene) furfurylamide:

A mixture of furfurylcyanoacetamide (6.56 g; 0.04 M), ethylmethylketone (3.58 ml; 0.04 M), ammonium acetate (2 g) and glacial acetic acid (2 ml) in benzene (80 ml) was refluxed with an arrangement for continuous separation of water involving dean stark apparatus. After 10 hours the reaction mixture was cooled, diluted with 10 ml benzene and washed with sodium carbonate solution (10% w/v in water) and water successively and dried over anhydrous sodium sulphate. The solvent was removed under vacuum and the intermediate crude product obtained was immediately processed for next step.

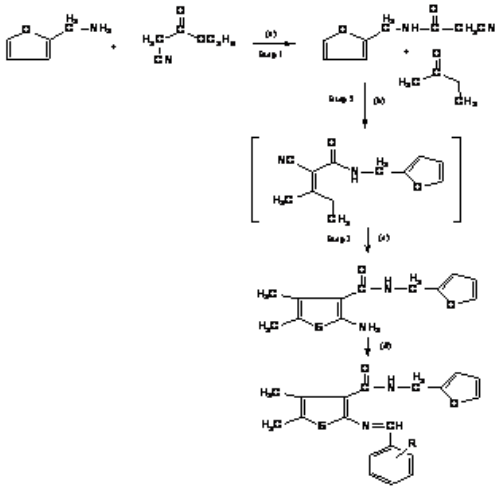
Step 3. Synthesis of 2-amino-3-(N-furfuryl amido)-4, 5-dimethyl thiophene:

To a mixture of 2-cyano-2-(isobutyl-1-ylidene)-furfurylamide in alcohol (30 ml) was added sulphur (1.28 g; 0.04 M) in portions followed by the addition of, diethyl amine (6.0 ml) drop wise with stirring. The reaction mixture was stirred for 3 hours at 40-45 ºC and chilled over night. The solid obtained was filtered, washed with ethanol and crystallized from ethanol: water mixture (9:1). Yield 47.44%, M Pt. 138^0C, Rf value: 0.42 [Mobile phase- chloroform and ethanol (9:1)].

General method for the synthesis of 2-[(substituted benzylidene) imino]-3-(N-furfuryl amido)-4,5-dimethyl thiophenes (Schiff bases):

A mixture of the starting compound (MAI-2) (0.005 M) and the required aryl aldehydes (Substituted benzaldehydes, 0.005 M) in ethanol (20 ml) and catalytic amount of glacial acetic acid (2 ml) was heated in microwave oven at 750 watt for 120 sec (2 min). The mixture was cooled to room temperature; the solid separated was filtered, washed with ethyl alcohol and crystallized with suitable solvent.

Scheme-I



R=2-Cl, 4-N(CH_3)$_2$, 3,4-(OCH_3)$_2$, 4-OH, 4-OCH_3, 3-NO_2, 3,4,5-(OCH_3)$_3$, 3-OCH_3-4-OH, 2- OH, 4-CH_3, 4-Cl , 2-NO_2. Refer Table-1 and 3

(a) Microwave irradiation, at 750 watt for 180 seconds (b) CH_3COOH/ CH_3COONH_4, Benzene. (c) S, EtOH, C_2H_5-NH-C_2H_5 , 45-50^{o}C. (d) C_2H_5OH, CH_3COOH, Substituted Aromatic Aldehyde

2.2. *Antibacterial activity* [12]*:*

The antibacterial activity of the synthesized compounds was determined by cup plate method against Gram +ve (*S. aureus, B. subtalis*) and Gram –ve (*E. coli, k. pneumonia*) bacteria.

Preparation of Nutrient agar media:

A mixture of known quantities of peptone, meat extract, sodium chloride, dextrose and agar was dissolved in 1000 ml of distilled water by heating. The pH was adjusted to 7.4. Finally the medium was sterilized by autoclaving at 121ºC for 15 minutes at 15 lb pressure per square inch. Afterwards the mixture was cooled to 45ºC and then inoculums were added to the above cooled medium, mixed properly and poured into the sterile petridishes, spread uniformly with sterile cotton swabs. The plates were then allowed to dry in the inverted position in an incubator for 30min for solidifying. Bores were made on the medium using sterile borer. 0.1 ml of test and standard solutions at a concentration of 50 g/0.1ml were taken. Standards (Ampicillin) were maintained with same concentration in each plate and a control having only DMSO in one plate. Then the petridishes were incubated at 37ºC for 24 hours and zones of inhibition were observed and measured.

2.3. *Antifungal activity* [13]*:*

The antifungal activity was carried out by the cup plate method against *A. niger* and *C. albicans.* Here responses of organisms to the synthesized compounds were measured (Zone of inhibition) and compared with the response of the standard reference drug. The standard reference drugs used in the present work were Miconazole nitrate. Stock solution of newly synthesized compounds and standard Miconazole nitrate were prepared in DMSO to get a concentration of 50 µg/0.1ml. 5ml of subculture was added to 300ml of sterile assay media at 40-45^{0}c and mix well. 30ml of media pour into each petri dish by spreading uniformly with sterile cotton swab and allow standing for 5min. A sterile bore was used to make wells; 0.1ml of test solution of each derivative was added, in wells. The petridishes covered and set aside for one hour and then incubated at 28^{0}C for 48 hrs. The zones of inhibition were measured.

2.4. *Spectral data*

IR spectra (cm^{-2}) were recorded in KBr on a Shimadzu FTIR-8700 spectrometer.^{1}H NMR (ppm) in $CDCl_3$ using TMS as reference on Bruker 400 AMX. Mass spectra of the compound coded MAI-2e was carried out.

IR (KBr) cm^{-1}

Compound MAI-2a-l: 3460 (-NH-); 2925 (Ar-CH); 3115(Ali-CH); 1650 (C=O); 1584 (C=N); 1052 (C-O); 815(C-N); 759(C-S); 1366(Ar-C=C); 1071(Ar-Cl); 1350 (C-N of N-CH_3); 3659 (O-H); 1076 (Ar C-O); 1518 (N=O of NO_2); 1230 (Ar-C-O of Ar-OCH_3); 1086 (C-N amine).

^{1}NMR ($CDCl_3$) δ (ppm)

*Compound MAI-2a***:** 9.05 (s, 1H, N-H), 7.72 (s, 1H, N=CH), 2.41 (s, 3H, -CH_3), 2.36 (s, 3H, -CH_3), 4.65 (d,2H, -CH_2-), 6.32 (s, 1H, Ar-CH(a)of furan), 6.38 (s, 1H, Ar-CH(b)of furan), 7.37-7.42 (m, 7H, Ar-CH(c,d,e,f,g) of benzene ring).

Compound MAI-2e: 9.36 (s, 1H, N-H), 8.27 (s, 1H, N=CH), 2.40 (s, 3H, -CH_3), 2.32 (s, 3H, -CH_3), 3.86 (s, 3H, -OCH_3), 4.63 (d,2H, -CH_2-), 6.31 (t, 1H, Ar-CH(b)of furan), 6.88 (d, 2H, Ar-CH(d,g) of benzene ring), 7.40 (d,1H, Ar-CH(c)of furan), 7.55 (d,2H, Ar-CH (e,f,) of benzene).

2.5. *Physical data*

Melting points were determined in open capillaries and are uncorrected. Purity of the compounds was checked by TLC on silica gel plates. The solvent system used to carry out the TLC is Benzene: Chloroform (7:3). Table 3

Table-1: antibacterial activity of synthesized compounds

Comp Code	*R*	*Zone of inhibition (mm).**			
		S.aureus	*B.subtilis*	*E.coli*	*K. pneumoniae*
MAI-2a	2'-chloro	08	04	01	NA
MAI-2b	4'-dimethyl amino	03	04	02	03
MAI-2c	3', 4'-dimethoxy	05	04	05	06
MAI-2d	4'-hydroxy	06	05	06	04
MAI-2e	4'-methoxy	06	06	05	02
MAI-2f	3'-nitro	08	09	05	01
MAI-2g	3', 4', 5'-trimethoxy	04	02	NA	NA
MAI-2h	3'-methoxy-4-hydroxy	05	04	02	06
MAI-2i	2'-hydroxy	05	06	NA	NA
MAI-2j	4'-methyl	04	02	04	03
MAI-2k	4'-chloro	11	09	08	11
MAI-2l	2'-nitro	10	08	11	09
Ampicillin	----------	11	12	19	12

Dose concentration: 50 µg / 0.1 ml ,NA: No Activity, Control : DMSO (Dimethyl sulfoxide) Medium: Nutrient agar media. , Method: Agar diffusion method (cup plate method)

Table-2: Antifungal activity data for MAI 2a-2l

Comp Code	*Zone of inhibition (mm).**	
	Aspergillus niger	*Candida albicans*
MAI-2a,	09	07
MAI-2b	02	NA
MAI-2c	08	01
MAI-2d,	05	02
MAI-2e,	06	03
MAI-2f,	10	04
MAI-2g,	05	03
MAI-2h	02	NA
MAI-2i	05	NA
MAI-2j	04	NA
MAI-2k,	09	06
MAI-2l,	05	02
Miconazole nitrate	20	15

Dose concentration : 50 µg/0.1 ml , NA : No activity, Control : DMSO (Dimethyl sulfoxide) ,
**Note:-Zone of inhibition excludes bore size (4mm) and zone of inhibition of control (8mm).*

Table-3: Physical data of compounds prepared

Compound	*Molecular formula*	*M.W. (gm)*	*M.P. (^oC)*	*R_f Value*	*Yield (%)*
MAI-1	$C_8H_8O_2N_2$	164	60	0.55	61.77
MAI-2	$C_{12}H_{14}N_2O_2S$	250	138	0.61	27.19
MAI-2a	$C_{19}H_{17}N_2O_2SCl$	373	165	0.50	72.41
MAI-2b	$C_{21}H_{23}N_3O_2S$	381	124	0.59	28.66
MAI-2c	$C_{21}H_{22}N_2O_4S$	398	138	0.80	31.00
MAI-2d	$C_{19}H_{18}N_2O_3S$	354	235	0.75	20.25
MAI-2e	$C_{20}H_{20}N_2O_3S$	368	138	0.60	47.44
MAI-2f	$C_{19}H_{17}N_3O_4S$	383	168	0.50	55.00
MAI-2g	$C_{22}H_{24}N_2O_5S$	428	172	0.77	61.33
MAI-2h	$C_{20}H_{20}N_2O_3S$	368	180	0.80	30.00
MAI-2i	$C_{19}H_{18}N_2O_3S$	354	218	0.90	66.03
MAI-2j	$C_{20}H_{20}N_2O_2S$	352	174	0.50	64.63
MAI-2k	$C_{19}H_{17}N_2O_2SCl$	373	165	0.70	25.5
MAI-2l	$C_{19}H_{17}N_3O_4S$	383	185	0.70	55.00

3. Results and Discussion

All the compounds were screened for antimicrobial (antibacterial, antifungal) activity. Many of the synthesized compounds showed mild to moderate antimicrobial activity and some were equipotent to the standard employed. Finally it was observed that the synthesized compounds possessing 'electron withdrawing groups' on the aldehydic phenyl ring exhibited better antibacterial and antifungal activity compared to the compounds possessing 'electron donating groups'. The synthesized compounds were analyzed mainly by IR spectral data, physical and chromatography readings, NMR and Mass spectroscopy.

4. Conclusion

Among all the compounds tested for antibacterial activity, MAI-2e, MAI-2k, MAI-2l with 4'-methoxy, 4'-methoxy, 2'-nitro substitution at R showed very good antibacterial activity and the compounds MAI-2a, MAI-2c, MAI-2f and MAI-2i exhibited moderate to mild antibacterial activity against both gram negative and against gram positive organisms, compared to Ampicillin.

And among all the compounds tested for antifungal activity, MAI-2a, MAI-2c, MAI-2e, and MAI-2f, with 2'-chloro, 3',4'-dimethoxy, 4'-methoxy, and 3'nitro, substitution at R, showed comparable antifungal activity with Miconazol nitrate. Whereas, the other compounds MAI-2g and MAI-2l, with 3',4',5'-trimethoxy and 2'-nitro substitution at R shown mild to moderate antifungal activity.

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