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Design, Synthesis, Antibacterial And Antifungal Activity Of Some Substituted Thiophene Derivatives

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Abstract: In the present study a new series of thiophene derivatives have been synthesized and screened for their *in-vitro* antifungal and antibacterial activity. 2-cyano-N-(4-fluorophenyl) acetamide was synthesized by reacting a mixture of 4-fluoro benzenamine and ethyl 2- cyanoacetate, which is further, reacts with 1-(4-fluorophenyl) methanone using ammonium acetate and glacial acetic acid as an acidic catalyst to give intermediate, 2-cyano-N, 3-bis (4-fluorophenyl) but-2-enamide, was directly taken up for the next step, where it was reacted with elemental sulfur in the presence of basic catalyst, diethyl amine in ethanol to form 2-amino -3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene which was further derivatised into the desired title compounds (4a-4l) by reacting with various substituted aromatic aldehydes. The newly synthesized compounds were characterized by M.P, TLC, UV, IR, NMR spectra. The title compounds were screened for their *in-vitro* antifungal activity using ketoconazole as standard and antibacterial activity using ampicillin as standard.
Keywords: Thiophene, *In-vitro* antifungal, *In-vitro* antibacterial activity.

Introduction

Thiophene and its derivatives are an important class of heterocyclic compound[1]. Thiophene has exhibited an array of biological activities ranging from anti-microbial, anti-tumour and anti-inflammatory activity. Among the antimicrobial agents thiophene derivatives are known to have a promising activity[2]. Based upon the fact, the present investigation was planned and substantial interest has been shown in the synthesis and characterization of various substituted thiophene derivatives in search of potential drugs.

Experimental

The progress of the reaction was monitored by TLC analysis. Melting points were determined in open capillary tubes on an electrical apparatus and are uncorrected, the IR spectra were recorded using KBr pellets in range of 4000-400 cm^{-1} on a Fourier Transform IR Spectrometer (Shimadzu 8700) and the frequencies are recorded in wave numbers. ^{1}H-NMR (400 MHz) spectra were recorded in $CDCl_3$-d_6 in Amx-400 liquid state PMR

spectrometer. Chemical shifts (δ) are reported in parts per million downfield from internal reference tetramethylsilane (TMS).

Synthesis of 2-cyano-N-(4-fluorophenyl)acetamide [1]

A mixture of 4-fluoro benzenamine (0.1 M, 9.74 ml) and ethyl 2- cyanoacetate (0.177M, 10.66 ml) was heated at 160-170^{0}C for 6 hrs on an oilbath or microwave irradiation at 900 watt for 150 seconds. The reaction mixture was left at room temperature over night. The solid obtained was washed with ethanol, dried and recrystallized from acetone water mixture (5:1).

Synthesis of 2-cyano-N,3-bis(4-fluorophenyl)but-2-enamide[2]

The compound was synthesized by following the reported procedure[3]

Synthesis of 2-amino -3-(p-fluoro phenyl carboxamido)-4-(p-fluorophenyl) thiophene [3]

The compound was synthesized by following the reported procedure[4]

General method for syntheses of (-2-Subsitituted benzylidine imino-3-(p-fluro phenyl carboxamido)-4-(p-fluoro phenyl) thiophene) [4a-l]

The compounds were synthesized by following the reported procedure[4]

2-Amino -3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene [3]

M. P: 140^{0}C, λ_{max} (278nm), IR (KBr)cm^{-1}:3427(NH_2 str), 3125 (NH str), 3101(Ar CH str), 2939 (Ali-CH), 1654 (C=0),1508 (NH-bend),1213 (C-O), 837(C-N), ^{1}H NMR ($CDCl_3$) δ (ppm): δ 6.85-7.76 (*m*, 8H, Ar-H), 8.0 (*s*, 1H, NH), 4.01 (*s*, 2H, NH_2).

2-(4-Chlorobenzylideneimino)-3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene [4a] : M.P:151^{0}C, λ_{max} (369nm), IR (KBr)cm^{-1}: 3402 (NH-str); 3227(Ar-CH str); 2922(Ali-CH); 1653 (C=0); 1506 (HC=N); 1213 (C-O), ^{1}H NMR($CDCl_3$) δ (ppm): δ 6.95-7.82 (*m*, 12H, Ar-H), 8.0 (*s*, 1H, NH), 8.1 (*s*, 1H, CH=N).

2-(4-Nitrobenzylideneimino)-3-(*p*-fluorophenylcarboxamido)-4-(*p*-fluorophenyl) thiophene [4b] : M.P:220^{0}C, λ_{max} (336nm), IR (KBr)cm^{-1}: 3450 (NH-str); 3055(Ar-CH str); 2920 (Ali-CH); 1690 (C=O); 1410 (C=N); 1211(C-O); 1519 (NO_2); 825 (C-N).

2-(2-Chlorobenzylideneimino)-3-(*p*-fluorophenylcarboxamido)-4(*p*-fluorophenyl)thiophene[4c]: M.P:178^{0}C, λ_{max} (339nm).

2-[(4-Dimethylamino)benzylideneimino]-3-(*p*-fluorophenylcarboxamido)-4(*p*-fluorophenyl) thiophene [4d] : M.P:130^{0}C, λ_{max} (369nm).

2-(3,4-Dimethoxybenzylideneimino)-3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene [4e] :M.P:90^{0}C, λ_{max} (341nm), IR (KBr) cm^{-1}: 3227(N-H); 2941(Ar CH); 2847(Al- CH); 1730 (C=0);1587(C=N) 1255(C-O).

2-(3,4,5-Trimethoxybenzylideneimino)-3(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene [4f] : M.P:110^{0}C, λ_{max} (331nm), IR (KBr)cm^{-1}: 3257(NH str);2945(Ar-CH str); 2846 (Al-CH);1666 (C=0);1521(C=N) 1238(C-O).

2-(2-Nitrobenzylideneimino)-3(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene [4g] : M.P:235^{0}C, λ_{max} (359nm).

2-(4-Hydroxy-3-methoxybenzylideneimino)-3(*p*-fluorophenylcarboxamido)-4-(*p*-fluorophenyl) thiophene [4h]: M.P: 115 ^{0}C, λ_{max} (310nm), IR (KBr) cm^{-1}: 3427(OH); 3153(NH); 3045. (Ar-CH); 2926 (Ali-CH); 1730 (C=O); 1521(C=N) 1255(C-O).

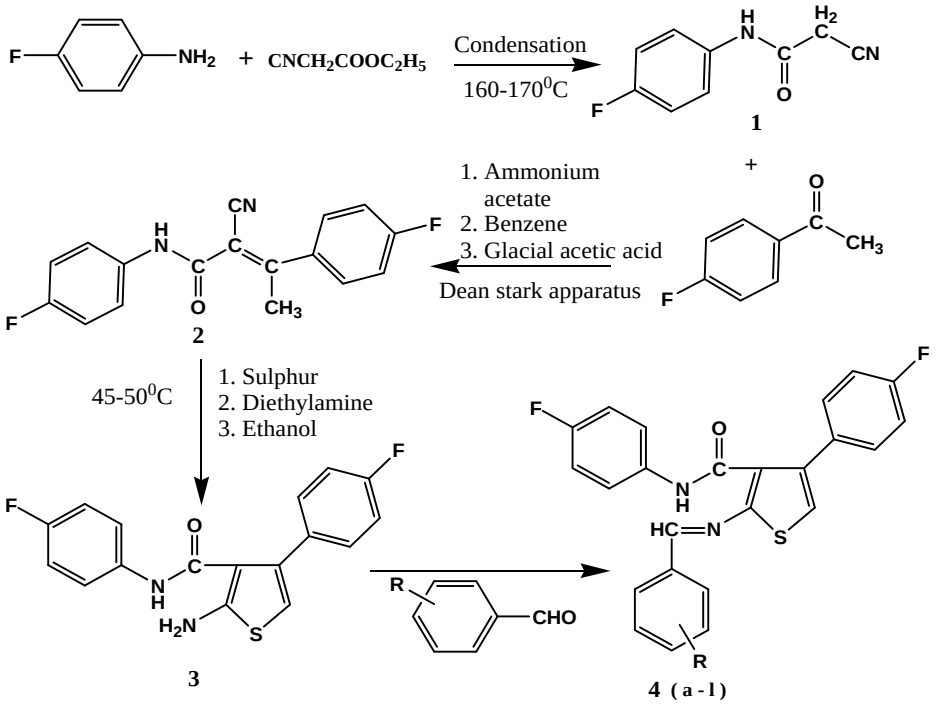
2-(4-Methyl benzylideneimino)-3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene [4i] : M.P:166 ^{0}C, λ_{max} (349nm), IR (KBr)cm^{-1}: 3295 (NH) ; 2918 (Al- CH);1702 (C=O); 1577 (C=N); 1290(C-O); 854 (C-N).

2-(4-hydroxy benzylideneimino)-3-(p-fluoro phenyl carboxamido)-4-(p-fluorophenyl) thiophene [4j] : M.P:186 ^{0}C, λ_{max} (335nm), IR (KBr)cm^{-1}: 3427(OH); 3153 (NH); 3045 (Ar-CH) 2926 (Al-CH); 1612 (C=O); 1548 (C=N); 1035(C-N), ^{1}H NMR ($CDCl_3$) δ (ppm): δ 6.53-7.89 (*m*, 12H, Ar-H), 8.85 (*s*, 1H, NH), 8.51 (*s*, 1H, CH=N), 5.37 (*s*, 1H, OH).

2-(3-nitrobenzylideneimino)-3-(p-fluoro phenyl carboxamido)-4-(p-fluorophenyl) thiophene [4k] : M.P.196^{0}C, λ_{max} (361nm), IR (KBr)cm^{-1}: 3427 (NH-str); 3093(Ar-CH str); 2918 (Ali-CH); 1633(C=O); 1410.01(C=N);1521(HC=N) 1375(NO_2); 1199(C-O); 810(C-N).

2-(4-methoxybenzylideneimino)-3-(p-fluoro phenyl carboxamido)-4-(p-fluorophenyl) thiophene [4l] : M.P.173^{0}C, λ_{max} (352nm), IR (KBr)cm^{-1}: 3427(NH); 2940 (Ar-CH str); 2847(Al-CH); 1730(C=O); 1255(C-S);1145 (C-O methoxy); 813(C-N).

Scheme1: Reaction scheme for synthesis of thiophene derivatives **4(a-l).**



Compound code	R
4a	4-Chloro
4b	4-Nitro
4c	2-Chloro
4d	4-Dimethyl amino
4e	3,4-Dimethoxy
4f	3,4,5-Trimethoxy
4g	2-Nitro
4h	4-Hydroxy, 3-Methoxy
4i	4-Methyl
4j	4-Hydroxy
4k	3- Nitro
4l	4-Methoxy

Biological Activity

Antibacterial activity

All the synthesized compounds were tested for their antibacterial activity against both gram positive and gram negative organisms viz., *K. pneumoniae, E. coli, S. aureus and B. subtilus.* The activity was performed by following the procedure of cup plate agar diffusion method[2]. By measuring the zone of inhibition the responses of organisms to the synthesized compounds were compared with the response of the standard reference drug. The standard reference drug was ampicillin.

Antifungal activity

All the synthesized compounds were tested for their antifungal activity against *Aspergillus nigar, Candida albicans, Aspergillus flavus and Chrysosporium keratinophilum.* The activity was performed by following the reported procedure[2]. The diameter of the zone of inhibition was measured and recorded in Table 1.

Table 1: In vitro antibacterial and antifungal activity of the synthesized compounds **(4a-4l).**

Compound No	Zone of inhibition in (mm)							
	Antibacterial activity				Antifungal activity			
	K. *pneumoniae*	E. *coli*	S. *aureus*	B. *subtilus*	Aspergillus *nigar*	Candida *albicans*	Aspergillus *flavus*	Chrysosporium *keratinophilum*
4a	10	06	16	18	16	18	14	11
4b	07	04	11	13	08	05	07	06
4c	08	05	12	16	15	12	14	11
4d	NA	03	07	NA	NA	04	NA	08
4e	06	NA	06	12	07	NA	NA	06
4f	09	04	08	15	NA	NA	09	05
4g	09	05	10	12	06	05	07	06
4h	02	NA	06	NA	03	NA	NA	04
4i	NA	05	NA	08	NA	07	NA	05
4j	04	06	05	08	10	09	08	12
4k	08	05	12	16	06	06	04	07
4l	NA	06	04	NA	09	NA	NA	07
Ampicillin	14	10	18	20	-	-	-	-
Ketoconazole	-	-	-	-	22	24	16	18

*NA= No activity.

Results And Discussion

The synthetic method involves the condenasation of 4-flourobenzenamine and ethyl 2-cyanoacetate to give 2-cyano-N-(4-fluorophenyl) acetamide. The melting point of 2-cyano-N-(4-fluorophenyl) acetamide was fnound to be 188^0C which is different from that of 4-fluorobenzenamine (210^0C). The difference in TLC spots also confirmed the formation of 2-cyano-N-(4-fluorophenyl)acetamide. The R_f values obtained for 4-fluorobenzenamine and 2-cyano-N-(4fluorophenyl) acetamide were 0.72 and 0.44 respectively. Further, condensation of the 1-(4-fluorophenyl) methanone with 2-cyano-N-(4-fluorophenyl) acetamide using ammonium acetate and glacial acetic acid as an acidic catalyst was carried out by refluxation in benzene with the arrangement for the continuous separation of water involving Dean Stark apparatus. The intermediate, 2-cyano-N,3-bis(4-fluorophenyl)but-2-enamide obtained was directly taken up in the next step, where it was reacted with elemental sulfur in the presence of basic catalyst, diethyl amine in ethanol to form 2-amino -3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene. The melting point of 2-amino -3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene was found to be 140^0C on the other hand the melting point of 2-cyano-N-(4-fluorophenyl) acetamide was found to be 188^0C. The difference in the TLC spots also confirmed the formation of 2-cyano-N-(4-fluorophenyl)acetamide. The R_f values obtained for 2-cyano-N-(4-fluorophenyl)

acetamide and 2-amino –3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene were 0.44 and 0.54 respectively.

After synthesis and characterization of the intermediate 2-amino –3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene the title compounds were synthesized. 2-amino -3-(*p*-fluoro phenyl carboxamido)-4-(*p*-fluorophenyl) thiophene and various substituted aromatic aldehydes in 20 ml of isopropyl alcohol or ethanol along with catalytic amount of glacial acetic acid (2 ml) was irradiated on a microwave for 2-4 min at 900W. The reaction mixture was allowed to cool. Solid obtained was filtered and washed with isopropyl alcohol. These derivatives could also be synthesized by reflux on a mantle for two hours. Their specific IR peaks confirm the formations of new Schiff bases. The IR spectra of compounds are given in the spectral section.

IR spectra of the NH_2 peak of the intermediate (3) was found at 3427 cm^{-1} whereas the HC=N peak of all the derivatives was found in the region of 1800-1500 cm^{1}.

The λ_{max} of the intermediate (3) was found to be 278 nm whereas the the λ_{max} of the compounds (4a-4l) were found to be in the range of 300-370 nm. The Bathochromic shift observed in the compounds is attributed to the attachment of aldehydic phenyl ring. On the other hand, the structures of newly synthesized compounds have also been ascertained based on the NMR spectrum of the representative compounds (3), (4a) and (4j). The NMR spectrum of the representative compounds of the series indicated the formation of the new compounds.

Both antibacterial and antifungal activity of the newly synthesized compounds has been carried out by agar diffusion method at concentration of 100µg/0.1ml using DMF as a solvent. The zones of inhibition were measured in mm and are reported in related Table 1.

It was observed that (4a) with 4-chloro benzylidene substitution showed almost comparable activity against gram positive organisms and gram negative organisms. 2-chloro, 2-nitro, 2-hydroxy and compounds containing methoxy substitution showed moderate activity with respect to the standards. Most of the compounds showed negative antifungal activity except those with chloro, hydroxy and nitro benzylidene substitution,which gave minimal activity, compared to that of standard ketoconazole.

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