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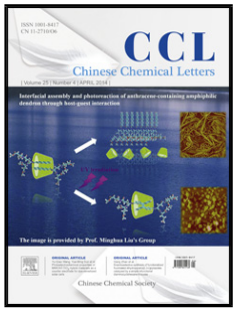

Title: An efficient one-pot three-component synthesis and antimicrobial evaluation of tetra substituted thiophene derivatives

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Graphical Abstract

An efficient one-pot three-component synthesis and antimicrobial evaluation of tetra substituted thiophene derivatives

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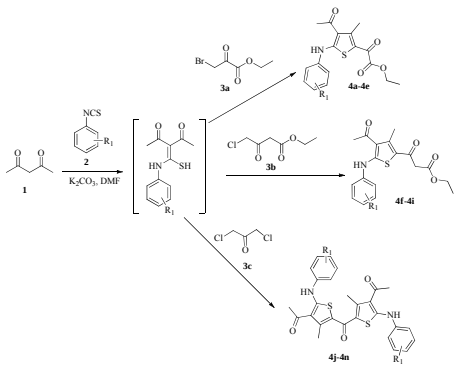

A simple and efficient method has been developed for preparation of tetra-substituted thiophene derivatives. The synthesized compounds were characterized by infrared spectroscopy, ^{1}H NMR, ^{13}C NMR and MS. Antimicrobial activities of synthesized compounds were reported

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Original article

An efficient one-pot three-component synthesis and antimicrobial evaluation of tetra substituted thiophene derivatives

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ABSTRACT

A convenient one-pot three-component method for the preparation of tetra-substituted thiophene derivatives has been developed. Reaction of acetyl acetone **1**, phenyl isothiocynate **2** and 2-chloromethyl derivatives **3a-3c** in the presence of potassium carbonate afforded the target compounds, namely ethyl 2-(4-acetyl-3-methyl-5-(phenylamino)thiophen-2-yl)-2-oxoacetate derivatives **4a-4e**, ethyl 3-(4-acetyl-3-methyl-5-(phenylamino)thiophen-2-yl)-3-oxopropanoate derivatives **4f-4i**, di((4-acetyl-3-methyl-5-phenylamino)thiophen-2-yl)ketone derivatives **4j-4n** in reasonable overall yields. The synthesized compounds were screened for antimicrobial activity. The detailed synthesis, spectroscopic data and antimicrobial activities of synthesized compounds were reported.

1. Introduction

Multicomponent reactions (MCRs) have emerged as a valuable tool in the preparation of structurally diverse chemical libraries of heterocyclic compounds [1]. They are inherently atom economical processes in which relatively complex products can be obtained in a one-pot reaction from simple starting materials, and thus they exemplify many of the desired features of an ideal synthesis. MCRs are generally much more environmentally friendly and offer access to large compound libraries with diverse functionalities with the avoidance of protection and deprotection steps for possible combinatorial surveying of structural variations. In view of the increasing interest in the preparation of a large variety of heterocyclic compound libraries, the development of new synthetically valuable MCRs with several diversity points remains a challenge for both academic and industrial institutions [2].

Thiophene and its derivatives are an important class of heterocyclic compounds possessing broad biological activities, such as anti-inflammatory [3], analgesic [3], antioxidant [4], antitubercular [5], antidepressant [6], sedative [6], antiamoebic [7], oral analgesic [8], anti-metabolite [9], and antineoplastic properties [10]. From the aforementioned reports, it seemed that the development of an efficient, rapid, and clean synthetic route toward focused libraries of such compounds is of great importance to both medicinal and synthetic chemists. Hence in this paper, we report a one-pot, three-component reaction for the synthesis of 4-acetyl tetra-substituted thiophene derivatives and antimicrobial evaluation.

All the synthesized compounds were characterized using FT-IR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry and were subjected to minimum inhibitory concentration (MIC) antimicrobial screening against two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), two Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and two fungi (*Candida albicans*, *Aspergillus niger*) using the serial plate dilution method.

2. Experimental

All chemicals were purchased from Sigma Aldrich, SD Fine, Spectrochem, Merck, and Himedia. Yields refer to purified products and are not optimized. Melting points were determined on a VEEGO-VMP I melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO-FTIR 4100 spectrophotometer. ^{1}H NMR were recorded on a MERCURY VARIAN 300 MHz instrument and chemical shifts (δ) were reported in parts per million (ppm) with $CDCl_3$ (7.26 ppm) as the solvent. TMS was used as the internal standard for NMR. MS analyses were done on an Applied Biosystem API 2000. Thin layer chromatography (TLC) was performed on precoated aluminium plates with silica gel 60 F_{254}.

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General procedure for synthesis of compounds **4a-4n**: Acetyl acetone **1** (1.0 mmol, 1 equiv.) and dried potassium carbonate (1.0 mmol, 1 equiv.) were added in dimethyl formamide (3 mL), and the mixture was stirred for 1 h at room temperature. Aryl isothiocyanate **2a-2e** (1.0 mmol, 1 equiv.) was then added drop wise, and the mixture was stirred for 1 h at room temperature. Then, bromoethylpyruvate **3a** (1.0 mmol, 1 equiv.) or chloroethylacetoacetate **3b** (1.0 mmol, 1 equiv.) or dichloroacetone **3c** (0.5 mmol, 0.5 equiv.) was added and the reaction mixture was heated for 1 h. The reaction was quenched with 10 mL water. The crude product **4a-4n** precipitated and was purified by filtration followed by crystallization in methanol.

Physical, analytical and spectroscopic characterization data of compounds **4a-4n** are given in Supporting information.

3. Results and discussion

3.1 *Chemistry*

We hypothesized that the *N,S*-ketene acetal obtained by condensation of acetyl acetone **1** with substituted phenyl isothiocynates **2a-e** would react *in situ* with 2-chloromethyl derivatives **3a-3c** in the presence of potassium carbonate to give the target compounds **4a-4n** in one step. Our initial investigations were mainly aimed at finding a suitable base and solvent for the one-pot preparation of target compounds **4a-4n**.

After careful experimentation, we discovered that 1.0 equiv. of acetyl acetone **1**, reacts with 1.0 equiv. of phenyl isothiocynates **2a-e** in the presence of 1.0 equiv. of potassium carbonate in DMF at room temperature to give *N,S*-ketene acetals. To this unisolated intermediate, 1.0 equiv. of **3a-3b** or 0.5 equiv. of **3c** was added and the resultant reaction mixture when stirred at room temperature for 1.0 h gave **4a-4n** in moderate to good yields (Scheme 1). The main attractions of this protocol are short reaction time and elimination of the intermittent workup procedures necessary to isolate the intermediates, thus directly leading to the formation of the target compounds. Encouraged by the successful results, we synthesized target compounds **4a-4n** from the reactions of the acetyl acetone **1**, phenyl isothiocynates **2a-2e,** and 2-chloromethyl derivatives **3a-3c** by using the same strategy and the different substituents.

During the reaction, a proton from the active methylene of the acetylacetone **1** gets abstracted by the base added, *i.e.* K_2CO_3, to give a nucleophile. This nucleophile then attacks the electron deficient carbon of the phenyl isothiocynates **2a-2e** to give the intermediate *i.e. N,S*-ketene acetals. After the addition of chloromethyl derivatives **3a-3b**, the electronegative sulfur of the intermediate adduct attacks the electron deficient carbon atom of chloromethyl derivatives **3a-3b**. The keto-enol tautomerism takes place, and then intramolecular cyclisation takes place to give 4-acetyl tetra-substituted thiophene derivatives **4a-4i** with the removal of water as shown in Scheme 2. In the case of dichloro acetone **3c** the same mechanism takes place at both ends as shown in Scheme 3 to give target compounds **4j-4n**.

The title compounds **4a**–**4n** were characterized by ^{1}H NMR, FT-IR, and mass spectra. The IR spectra of title compounds **4a**–**4e** show a peak at 3441.35 cm^{-1} for an amino group, at 1634.38 cm^{-1} for a ketone group, at 2990.09 cm^{-1} for an aromatic group, and at 1726.94 cm^{-1} for an ester group. The ^{1}H NMR spectrum of ethyl 2-(4-acetyl-3-methyl-5-(phenylamino)thiophen-2-yl)-2-oxoacetate **4a** shows a triplet at δ1.40 for the 3 protons of a methyl group, singlet for the 3 protons of a methyl group at δ2.62, a multiplet for the 2 protons of a methylene group at δ4.42, a multiplet for an aromatic region proton at δ7.3-7.5, a singlet for a –NH proton at δ12.04. ^{13}C NMR spectral data of the title compound **4a**: The signals at around δ 9.70, 13.80 stand for methyl and δ 28.80 stands for methoxy, while signals around δ 117.80–195.60 are attributed to all the aromatic carbons of compound **4a**. Also, a distinctive signal at δ 60.80 stands for methylene carbon. The mass spectra of compound **4a** show a molecular ion peak at 332.2 and calculated mass of compound found to be 331.09. The IR spectrum of title compounds **4f**–**4i** shows at 3427.85 cm^{-1} a peak for an amino group, at 1636.3 cm^{-1} a peak for a ketone group, at 2922.59 cm^{-1} for an aromatic group, and at 1744.3 cm^{-1} for an ester group. The ^{1}H NMR spectrum for ethyl 3-(4-acetyl-3-methyl-5-(phenylamino)thiophen-2-yl)-3-oxopropanoate **4f** shows at δ 1.29 a triplet for the 3 protons of a methyl group, at δ 2.6 a singlet for the 3 protons of a methyl group, at δ 2.82 a singlet for the 3 protons of a methyl group, at δ 3.8 a singlet for the 2 protons of a methylene group, at δ 4.25 a multiplet for the 2 protons of a methylene group, at δ 7.3-7.5 a multiplet for aromatic region proton, and at δ 11.96 shows singlet for –NH proton. ^{13}C NMR spectral data of the title compound **4f**: The signals at around δ 9.78, 14.10 stand for methyl and δ 28.85 stands for methoxy, while signals around δ 117.80–195.60 are attributed to all the aromatic carbon of compound **4f**. Also, the distinctive signals δ 49.71 & 60.80 stand for methylene carbon. Mass spectra of compound **4f** shows molecular ion peak at 346.3 and calculated mass of compound found to be 345.10. The IR spectra of title compounds **4j**–**4n** show peak at 3436.53 cm^{-1} for the an amino group, at 1632.45 cm^{-1} for ketone group, and at 2927.41cm^{-1} for aromatic group. The ^{1}H NMR spectrum of di((4-acetyl-3-methyl-5-phenylamino)thiophen-2-yl)ketone **4j** shows at δ 2.58 a singlet for the 6 protons of two methyl groups, at δ 2.6 a singlet for the 6 protons of two methyl groups, at δ 7.2-7.4 a multiplet for an aromatic region proton, and at δ 12.11 a singlet for two –NH protons. ^{13}C NMR spectral data of the title compound **4j**: The signal at around δ 9.72 stands for methyl and δ 28.82 stands for methoxy, while signals around δ 117.89–195.60 are attributed to all the aromatic carbons of compound **4j**. Mass spectra of compound **4j** show a molecular ion peak at 489.3, and the calculated mass of the compound found to be 488.12.

3.2 Antimicrobial studies

All the synthesized compounds were analyzed for antimicrobial activity. The *in vitro* antibacterial and antifungal activity of the title compounds was determined by the serial dilution method [11-14]. The minimum inhibitory concentration (MIC) is given in µg/mL.

Samples of different concentrations (0-50 µg/mL) were prepared using dilutions of a 100 µg/mL stock solution, prepared by dissolving the compounds in dimethyl sulfoxide and adjusting the volume to 100 mL. Under identical conditions, Azithromycin and Fluconazole were tested as the reference standard drugs for bacteria and fungi respectively. Synthesized compounds were tested for activity against two Gram-positive bacteria (*Bacillus substilis*, *Staphylococcus aureus*), two Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherchia coli*), and two fungal strains (*Candida albicans*, *Aspergillus niger*). Specifications of the microorganisms are given in Table 1. The synthesized series showed excellent to good activity against Gram-negative bacteria (*P. aeruginosa and E. coli*) and the least activity against Gram-positive bacteria (*S. aureus and B. subtilis*). All compounds of the series exhibited excellent to moderate antifungal activity against *Aspergillus niger* and *Candida albicans*. Examination of the antimicrobial data (Table 2) revealed that against Gram-positive bacteria *Staphylococcus aureus*, compounds **4a**, **4c**, and **4j** were found to be equipotent to azithromycin. Compounds **4d-4h** and **4k** have shown MIC values (1 µg/mL), indicating good antibacterial activity. Against the species *Bacillus subtilis*, compound **4f** shows more potency than standard azithromycin. The compounds **4d** and **4e** are equipotent to azithromycin. Compounds **4a-c** and **4g-k** show moderate to good activity. Towards *E. coli*, compounds **4a-4n** shows more potency than the standard. Against *Pseudomonas aeruginosa*, compounds **4a-4d** and **4f-4m** shows more activity than standard azithromycin. All compounds show moderate to minimal activity against fungi *Candida Albicans* and *Aspergillus niger.*

The structure–activity relationship study (SAR) indicates that a change in the substituent might also affect the antibacterial activity of title compounds **4a**–**4n**. Compounds having R=H/Cl appeared to have more potential against Gram-positive bacteria *Staphylococcus aureus*, Gram-negative bacteria *E. coli* and *Pseudomonas aeruginosa*; and moderate potential against fungal pathogens *Candida albicans* and *Aspergillus niger*. Compounds having R=CH_3/OCH_3 were found to be more active against Gram-positive *Bacillus subtilis*, Gram-negative bacteria *Escherichia coli*, and *Pseudomonas aeruginosa* .

4. Conclusion

We have reported a one-pot synthesis of ethyl 2-(4-acetyl-3-methyl-5-(phenylamino)thiophen-2-yl)-2-oxoacetate derivatives **4a-4e**, ethyl 3-(4-acetyl-3-methyl-5-(phenylamino)thiophen-2-yl)-3-oxopropanoate derivatives **4f-4i**, and di((4-acetyl-3-methyl-5-phenylamino)thiophen-2-yl)ketone derivatives **4j-4n** from readily available acetyl acetone **1**, phenyl isothiocynates **2a-2e**, and 2-chloromethyl derivatives **3a-3c** under mild conditions. The reaction is applicable to a wide range of starting materials. All the synthesized compounds were evaluated for their antibacterial activities against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *Candida albicans*, and *Aspergillus* niger microorganisms by the serial dilution method. The synthesized series showed excellent to good activity against Gram-negative micro-organisms (*P. aeruginosa and E. coli*) and the least activity against Gram-positive bacteria (*S. aureus and B. subtilis*). All compounds of the series exhibited moderate to less antifungal activity against *Aspergillus niger* and *Candida albicans*.

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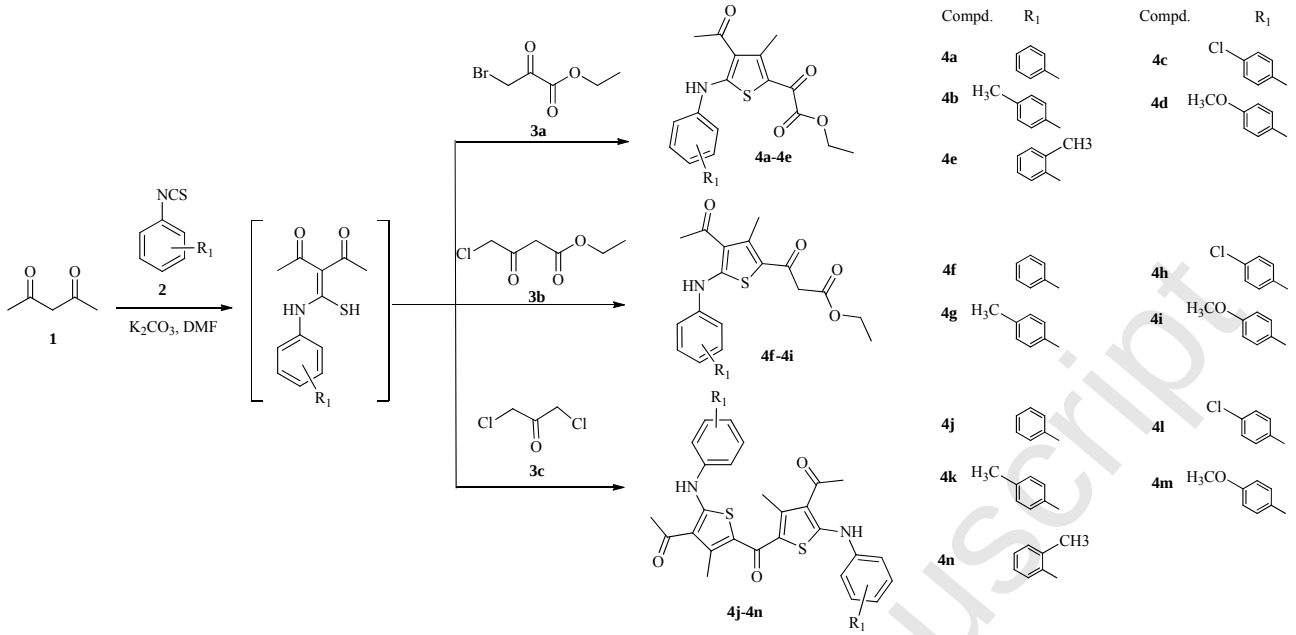
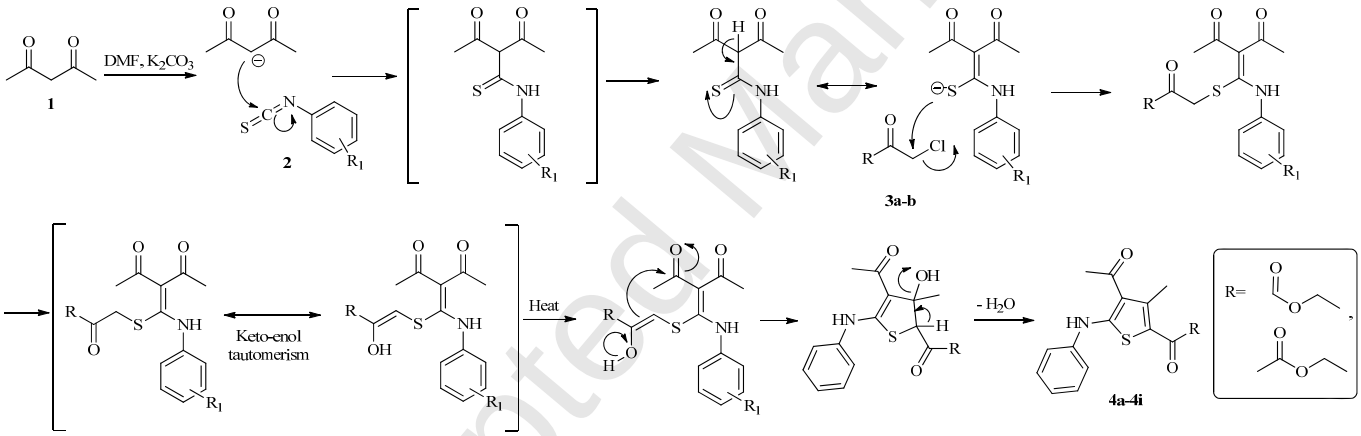
References

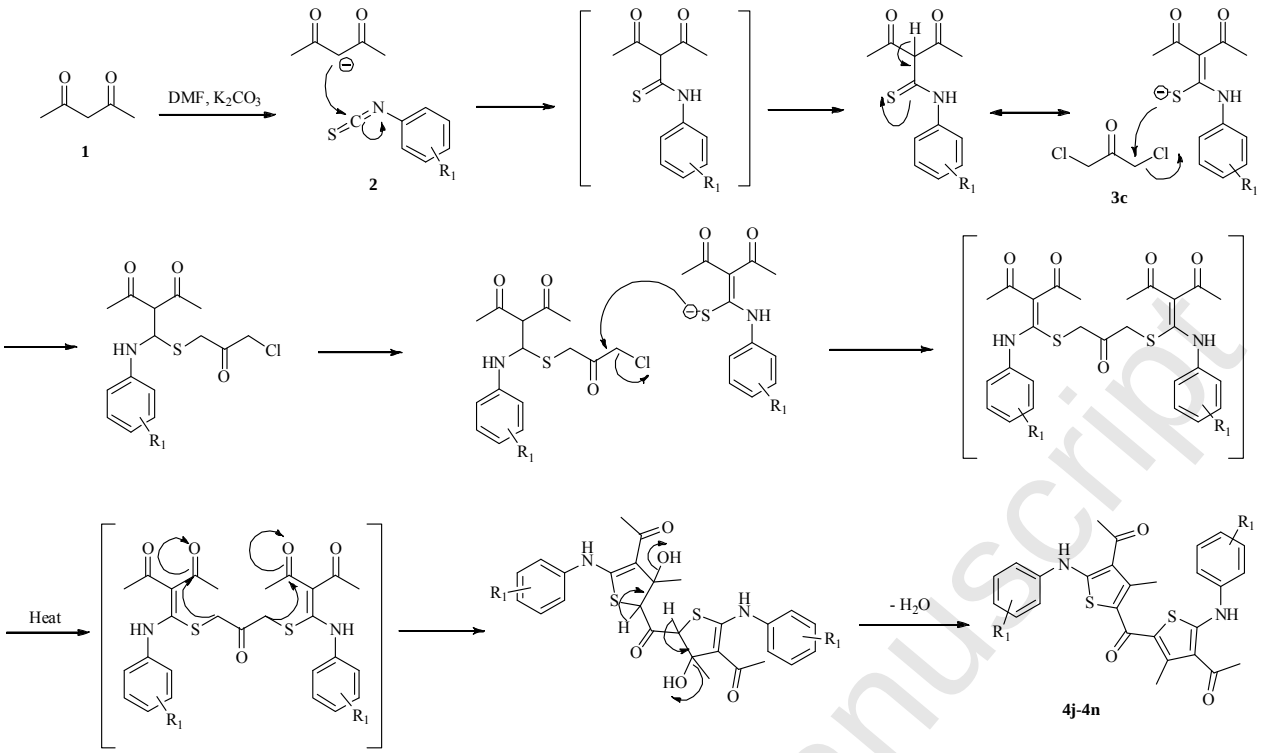
[1] A. Dömling, W. Wang, K. Wang, Chemistry and biology of multicomponent reactions, Chem. Rev. 112 (2012) 3083-3135.
[2] A. Dömling, Recent developments in isocyanide based multicomponent reactions in applied chemistry, Chem. Rev. 106 (2006) 17-89.
[3] F.M. Moghaddam, H.Z. Boinee, An efficient and facile one-step synthesis of highly substituted thiophenes, Tetrahedron 60 (2004) 6085-6089.
[4] K.I. Molvi, M. Mansuri, V. Sudarsanam, et al., Synthesis, anti-inflammatory, analgesic and antioxidant activities of some tetrasubstituted thiophenes, J. Enzyme. Inhib. Med. Chem. 23 (2008) 829-838.
[5] M.K. Parai, G. Panda, V. Chaturvedi, Y.K. Manju, S. Sinha, Thiophene containing triarylmethanes as antitubercular agents, Bioorg. Med. Chem. Let. 18 (2008) 289-292.
[6] W. Wardakhan, O. Abdel-Salam, G. Elmegeed, Screening for antidepressant, sedative and analgesic activities of novel fused thiophene derivatives, Acta. Pharm. 58 (2008) 1-14.
[7] S. Sharma, F. Athar, M.R. Maurya, A. Azam, Copper(II) complexes with substituted thiosemicarbazones of thiophene-2-carboxaldehyde: synthesis, characterization and antiamoebic activity against *E. histolytica*, Eur. J. Med. Chem. 40 (2005) 1414-1419.
[8] O.F. William, Principles of Medicinal Chemistry, 3rd. ed., Lippincott Williams & Wilkins Publication, Philadelphia, 1989.
[9] A.A. Sagardoy, M.J. Gil, R. Villar, et al., Benzo[b]thiophene-6-carboxamide 1,1-dioxides: Inhibitors of human cancer cell growth at nanomolar concentrations, Bioorg. Med. Chem. 18 (2010) 5701-5707.
[10] A.A. Fadda, E. Abdel-Latif, R.E. El-Mekawy, Synthesis and molluscicidal activity of some new thiophene, thiadiazole and pyrazole derivatives, Eur. J. Med. Chem. 44 (2009) 1250-1256.
[11] Indian Pharmacopoeia, Microbiological assay of Antibiotics, vol. I, 2007, pp. 45-52.
[12] C.R. Kokare, Pharmaceutical Microbiology and Biotechnology, 3rd. ed., Nirali Prakashan, Pune, 2006, pp. 21.1-21.12.
[13] Ananthanarayan and Paniker, Textbook of Microbiology, 7th. ed., Orient Blackswan, 2005, pp. 628-630.
[14] Y.J. Huang, A. Dömling, The Gewald multicomponent reaction, Mol. Divers. 15 (2011) 3-33.



Scheme 1. The synthesis route for compounds **4a-4n**.



Scheme 2. Possible reaction mechanism involved in formation of compounds **4a-4i**.



Scheme 3. Possible reaction mechanism involved in formation of compounds **4j-4n**.

Table 1
Specification of microorganisms.

Sr. no.	Microorganism	Nature	NCIM no.	ATCC no.
1	*Staphylococcus aureus*	Gram-positive	2079	6538P
2	*Bacillus subtilis*	Gram-positive	2063	6633
3	*Escherichia coli*	Gram-negative	2065	8739
4	*Pseudomonas aeruginosa*	Gram-negative	2200	9027
5	*Candida Albicans*	Fungus	3471	10231
6	*Aspergillus niger*	Fungus	545	9029

Table 2
Minimum inhibitory concentration.

Compd.	Minimum inhibitory concentration (MIC) (μg/mL)					
	Gram-positive bacteria		Gram-negative bacteria		Fungi	
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Candida albicans*	*Aspergillus niger*
4a	0.1	4.5	8.5	4	1	*
4b	9.5	5	0.8	4	2.5	*
4c	0.5	4	0.5	3.5	*	*
4d	1	1	1	0.6	*	4.5
4e	1	1	7.5	7.5	*	*
4f	1	0.2	0.3	0.6	2.5	1
4g	1	3	1	2	*	*
4h	1	7.5	0.3	0.3	2.5	7.5
4i	9	3	4	2.5	1	*
4j	0.1	4	8	4.5	*	*
4k	1	10	1	0.3	*	1
4l	4.5	*	9.5	0.3	8	9.5
4m	4.5	*	1	0.2	1	9
4n	*	*	7.5	7.5	1	*
Azithromycin	0.1	1	12.5	6.25	-----	-----
Fluconazole	-----	-----	-----	-----	0.25	0.5

* Indicates microorganisms are resistant to the compounds up to 50 μg/mL