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Research Article

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In vitro Antimicrobial Evaluation of 3-Aminothiophene-2-carboxylates

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ABSTRACT

*3-Aminothiophene-2-carboxylates **1-12** were screened for antimicrobial activities against gram positive bacteria Staphylococcus aureus (ATCC 29737) and gram negative bacteria Escherichia coli (ATCC 25922) and Candida albicans (MTCC 277) and Aspergillus niger (MCIM 545) fungi. Compounds **4**, **5**, **9**containing chloro, methoxy and amide functionalities showed excellent to moderate antibacterial activities against Gram negative bacteria Escherichia coli and Gram positive bacteria Staphylococcus aureus with MIC 10-20µg/mL compared with standard antibiotic drug Gentamicin (10 µg/mL). Similarly, compounds**5-7,8-12**containing chloro, methoxy and amide groups showed excellent to moderate antifungal activities against Aspergillus niger and Candida albicans with MIC 10-20µg/mL on comparison with standard drug Fluconazole (20 µg/mL).*

Keywords: Thiophene, Aminothiophene carboxylate, Antimicrobial activity

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INTRODUCTION

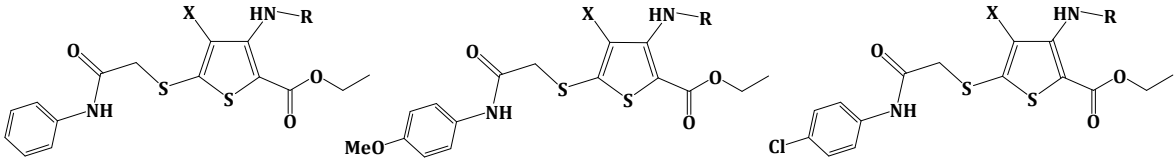
Thiophenes form major class of bioactive heterocycles with broad spectrum of biological activities [1-26]. The substituted thiophenes, polysubstituted 2 and 3-aminothiophenes and fused thiophenes such as thienopyridines, thienopyrimidines, thienopyrazolesetc showed biological activities such as such as antibacterial [1, 2, 4, 5, 13], anti-inflammatory [2, 16], antifungal [3-5, 7],anticancer [4, 5, 17], fungicidal activity [3-5, 7], antiproliferative [6], p53-MDM2 inhibitor [8], anticonvulsant [7, 10], molluscicidal [9], antileishmanial activity [3, 14], antitumor [10, 15], antioxidant [10], kinase inhibitors [11], antiepileptic activity [12],mycolytic [13], analgesic [18], ulcerogenic [18],tubulin inhibitor [18] and anti-tubercular activities [19], antimycobacterial [19].

In this paper we have described the screening of antimicrobial activities of recently synthesized 3-aminothiophene-2-carboxylates [20] in our laboratory. Antimicrobial activities were evaluated against gram positive bacteria *Staphylococcus aureus* (ATCC 29737), gram negative bacteria *Escherichia coli* (ATCC 25922) and *Candida albicans* (MTCC 277) and *Aspergillus niger* (MCIM 545) fungi. This arose from notable biological applications of thiophene and other five member heterocycles. It was observed that amide, chloro and methoxy phenyl functionalities attached to thiophene ring show promising bioactivities. The present work is a continuation of on-going research work on thiophene and here we report antimicrobial activities of 3-aminothiophene-2-carboxylates.

EXPERIMENTAL SECTION

2.1 Antimicrobial assay:

The antimicrobial assay evaluation of the recently synthesized 3-aminothiophene-2-carboxylate derivatives **1-12**[20] was done using agar well plate method. The antibacterial and antifungal assays were performed in Muller-Hinton broth and CrazekDox broth. The standard strains used for the antimicrobial assay was procured from Microbial Culture Collection, Pune, India.



1 X= CN, R= $COCH_3$ **2** X= CN, R= $COCH_3$ **3** X= CN, R= $COCH_3$
4 X= $CONH_2$, R= H **5** X= $CONH_2$, R= H **6** X= $CONH_2$, R= H
7 X= $CONH_2$, R= $COCH_2Cl$ **8** X= $CONH_2$ R= $COCH_2Cl$ **9** X= $CONH_2$ R= $COCH_2Cl$
10 X= $CONH_2$, R= pyrrolyl **11** X= $CONH_2$ R= pyrrolyl **12** X= $CONH_2$ R= pyrrolyl

Fig.1 3-Aminothiophene-2-carboxylates (1-12) were screened for Antimicrobial Activities

Antimicrobial evaluation was performed using the bacteria reseeded in Muller-Hinton broth for 24 hr at 37^{o}C and fungi reseeded in CrazekDox broth for 48 hr at 25^{o}C. The antibacterial activity of tested samples were studied in triplicate against gram positive bacteria *Staphylococcus aureus* (ATCC 29737) and gram negative bacteria *Escherichia coli* (ATCC 25922). The same samples were tested for antifungal activity in triplicate against *Candida albicans* (MTCC 277) and *Aspergillus niger* (MCIM 545). The compounds were dissolved in DMSO at desired concentrations of 40, 20, 10 µg/ mL. DMSO was loaded as negative control. Gentamicin (10 µg/ mL) and Fluconazole (20 µg/ mL) were used as standards for evaluating the antibacterial and antifungal activity. The zone of inhibition (mm) was determined from the diameter of the zone of inhibition using calliper. The lowest concentration that showed invisible growth after spot subculture was considered as Minimum Inhibitory Concentration (MIC µg/mL) value for each sample after 24 hr incubation period at 37^{o}C. (MIC µg/ mL) value for each sample were determined using MH agar plates by pouring the molten agar in unique sized petri dishes as per National Committee for Chemical Laboratory Standards (NCCLS, M7-A5, January 2000).

2.2 Statistical Analysis:

The standard deviation value was calculated using ANOVA method and expressed in terms of ± SD. It has been observed that differences below 0.0001 levels ($p \leq 0.0001$) were considered as statistically significant.

RESULTS AND DISCUSSION

Compounds **3-5**, **8** and **9**exhibited excellent antibacterial activities against gram negative bacteria *Escherichia coli*with MIC 10µg/mL. Compounds**4**, **9** showed excellent activities against gram positive bacteria *Staphylococcus aureus*with MIC 10µg/mL.

Table 1: Antimicrobial screening of compounds 1-12: *Inhibition Zone Diameter (mm) at 40 µg / mL*

Compound	*Escherichia coli (ATCC 25922)*	*Staphylococcus aureus (ATCC 29737)*	*Aspergillus niger (MCIM 545)*	*Candida albicans (MTCC 277)*
Comp 1	16 ± 0.9	12 ± 1.6	13 ± 0.9	15 ± 1.4
Comp 2	16 ± 1.1	14 ± 0.8	15 ± 0.8	14 ± 1.3
Comp 3	**28 ± 0.8**	18 ± 0.7	15 ± 0.9	17 ± 0.6
Comp 4	**27 ± 0.6**	**20 ± 0.6**	16 ± 0.5	15 ± 0.9
Comp 5	**20 ± 1.4**	17 ± 0.8	17 ± 1.2	15 ± 1.3
Comp 6	16 ± 1.4	14 ± 0.5	13 ± 0.7	**24 ± 0.9**
Comp 7	**20 ± 1.4**	**21 ± 0.6**	**20 ± 0.8**	17 ± 1.2
Comp 8	17 ± 0.7	16 ± 0.6	15 ± 1.4	16 ± 0.8
Comp 9	**21 ± 0.9**	19 ± 1.5	14 ± 0.6	18 ± 1.1
Comp 10	18 ± 1.3	17 ± 0.4	17 ± 1.3	19 ± 0.8
Comp 11	19 ± 0.8	15 ± 0.7	17 ± 0.9	16 ± 1.4
Comp 12	14 ± 1.1	14 ± 1.1	15 ± 1.5	17 ± 1.3
DMSO	**12 ± 0.9**	**13 ± 0.8**	**10 ± 0.9**	**11 ± 0.5**
Gentamicin	**21 ± 0.7**	**23 ± 0.4**	NT	NT
Fluconazole	NT	NT	**19 ± 0.4**	**20 ± 0.7**

Gentamicin (10 µg/ mL) and fluconazole (20 µg/ mL)

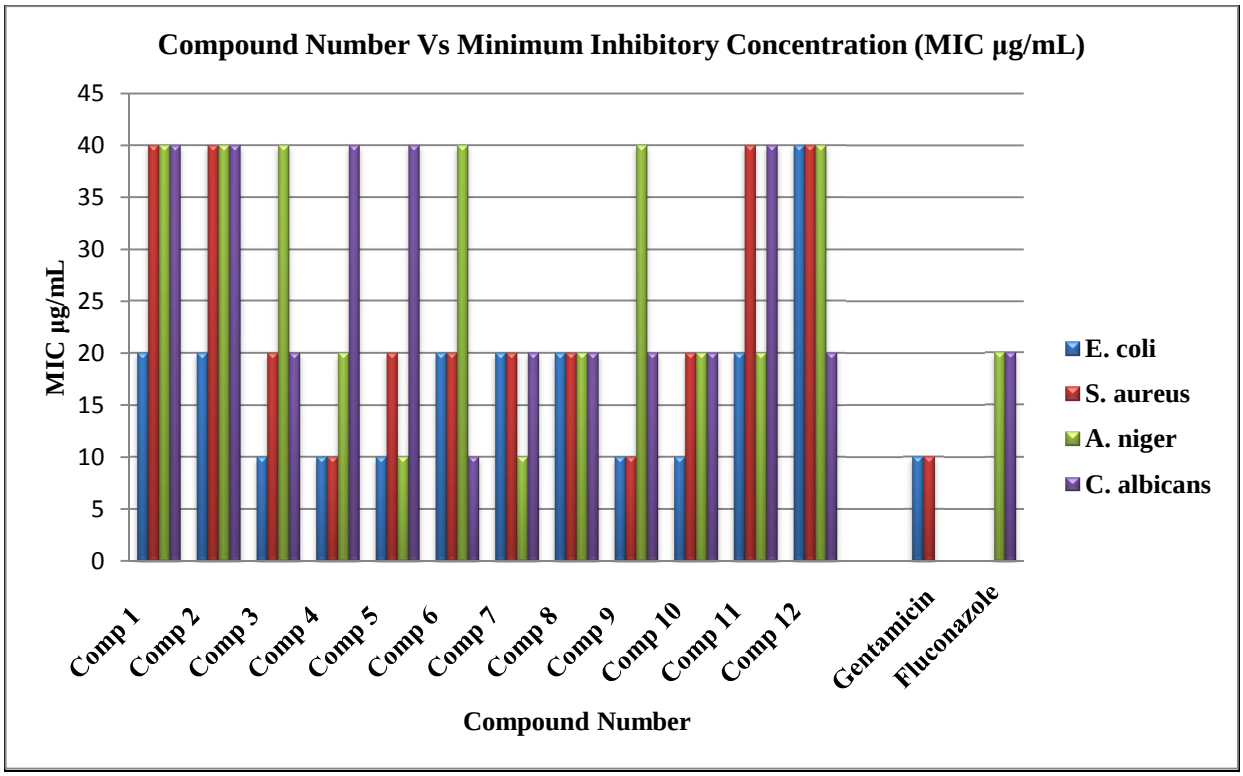
Inhibition Zone= 9-14 mm: slight activity, 15-19 mm: moderate activity, 20 -24 mm : high activity, >25 mm: excellent activity NT: Not Tested

Compounds **3-5**, **9** and **10** exhibited MIC 10 µg/mL when compared with antibiotic drug Gentamicin (10 µg/mL). Compounds **1**, **6-8**,**11** and**3**, **5-8**, **10**showed moderate antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* respectively with MIC 20µg/mL. Compounds **2**, **12** and **1**, **2**, **11**, **12** showed poor antibacterial activities against gram negative bacteria *Escherichia coli* and gram positive bacteria *Staphylococcus aureus* with MIC 40µg/mL when compared with standard antibiotic drug Gentamicin (10µg/mL). (Fig.2)Compounds **5** and **7** exhibited excellent antifungal activity against *Aspergillus niger*. Only compound **6** exhibited excellent antifungal activities against *Candida albicans*. Compounds **4**, **8**, **10**, and **11**showed equivalent antifungal activities against *Aspergillus niger* when compared with standard antifungal drug Fluconazole. Similarly, compounds **3**, **7-10** and**12**showed equivalent antifungal activities against *Candida albicans.* The remaining compounds **1-3**,**6**, **9** and **12**showed poor antifungal activities against *Aspergillus niger*. Similarly compound**1**,**2**, **4**, **5** and **11**showed low antifungal activities against *Candida albicans* when compared with standard antibiotic drug Fluconazole (20µg/mL). The results of antimicrobial activity are shown in Table2.

Table 2Antimicrobial screening of compounds 1-12: MIC in µg / mL values

Compound	*Escherichia coli (ATCC 25922)*	*Staphylococcus aureus (ATCC 29737)*	*Aspergillus niger (MCIM 545)*	*Candida albicans (MTCC 277)*
Comp 1	20	40	40	40
Comp 2	20	40	40	40
Comp 3	**10**	20	40	**20**
Comp 4	**10**	10	**20**	40
Comp 5	**10**	20	**10**	40
Comp 6	20	20	40	**10**
Comp 7	20	20	**10**	**20**
Comp 8	20	20	**20**	**20**
Comp 9	**10**	**10**	40	**20**
Comp 10	**10**	20	**20**	**20**
Comp 11	20	40	**20**	40
Comp 12	40	40	40	**20**
Gentamicin	10	10	NT	NT
Fluconazole	NT	NT	20	20
(MIC in µg / mL)=10 µg / mL: excellent activity, 20 µg / mL: moderate activity, 40 µg / mL: slight activity				

Figure 2Antimicrobial screening of compounds 1-12: MIC in µg / mL values



CONCLUSION

1. Compounds **4**, **5** and **9**containing chloro, methoxy and amide functionality showed excellent to moderate activities against gram negative bacteria *Escherichia coli* and gram positive bacteria *Staphylococcus aureus* with MIC 10-20µg/mL in most of the cases.
2. Compounds **1**, **11**, **12** and**20**containing cyanide and pyrrolyl showed poor activities against gram negative bacteria *Escherichia coli* and positive bacteria *Staphylococcus aureus* with MIC 40µg/mL.
3. Compounds **5-7**, **8-12**containing chloro, methoxy and amide showed excellent to moderate antifungal activities against*Aspergillusniger* and *Candida albicans*with MIC 10 -20µg/mL in most of the cases.
4. Compounds containing cyanide (**1**-**3**), chloro (**6**, **9**, **12**) and pyrrolyl (**11**, **12**) exhibited poor antifungal activities with MIC40µg/mL against*Aspergillus niger* and *Candida albicans*.

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