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Synthesis Of New Tetra Substituted Thiophene Derivatives And Its Antibacterial And Antifungal Activity

Surwase Santosh M.

Shri Chhatrapati Shivaji College, Omerga. Dist. Osmanabda (M.S.)

Email: smsurwase79@gmail.com

Abstract:-

A number of tetra substituted thiophene species have been prepared .Most of these synthesized compounds shows good antibacterial and antifungal activity among these , compounds MMS-135,137,139,96, 149, 151, 153, 152,163 shows comparative good activity towards antibacterial and antifungal

1.Introduction:-

Thiophenes are important class of heterocyclic compounds that are widely used as building blocks in many agrochemicals and pharmaceuticals.A variety of molecules containing the thiophene ring system display biological activity and find application as pharmaceuticals[2], fragrance compounds, or pharmacophores[2]. Thiophene is scaffold in heterocyclic chemistry a major, found in many compounds. Moreover some of them have interesting properties. With the dual aim of developing potential therapeutic agents and studying their chemistry, we undertook the synthesis of biologically active tetra substituted Thiophene as antimicrobial and antifungal[3] An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoan's. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbistatic). Disinfectants are antimicrobial substances used on non-living objects.

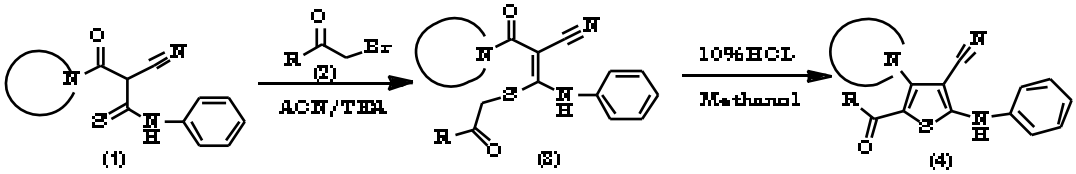
The history of antimicrobials begins with the observations of Pasteur and Joubert, who discovered that one type of bacteria could prevent the growth of another. They did not know at that time that the reason one bacterium failed to grow was that the other bacterium was producing an antibiotic. Technically, antibiotics are only those substances that are produced by one microorganism that kill, or prevent the growth, of another microorganism. Of course, in today's common usage, the term antibiotic is used to refer to almost any drug that attempts to rid our body of a bacterial infection. Antimicrobials include not just antibiotics, but synthetically formed compounds as well. The discovery of antimicrobials like penicillin and tetracycline paved the way for better health for millions around the world. Before penicillin became a viable medical treatment in the early 1940's, no true cure for gonorrhea, strep throat, or pneumonia existed. Patients with infected wounds often had to have a wounded limb removed, or face death from infection. Now, most of these infections can be cured easily with a short course of antimicrobials. However, with the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents.

The old antimicrobial technology was based either on poisons or heavy metals, which may not have killed the microbe completely, allowing the microbe survive, change, and become resistant to the poisons and/or heavy metals. An antifungal drug is medication used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others. Antifungal work by exploiting differences between mammalian and fungal cells to kill off the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus, fungal and human cells are similar at the molecular level, making it more difficult to find a target for an antifungal drug to attack that does not also exist in the infected organism. Consequently, there are often side effects to some of these drugs. Some of these side effects can be life-threatening if the drug is not used properly[4].In our search for better antimicrobial agents having optimum requirements at the second and fifth position of Thiophene, in the present work we report design and synthesis of some novel tetra substituted Thiophene analogs.

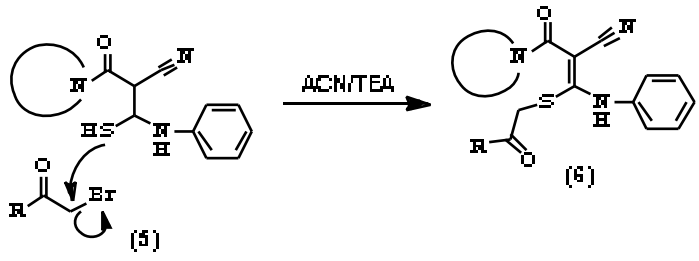
2. EXPERIMENTAL:-

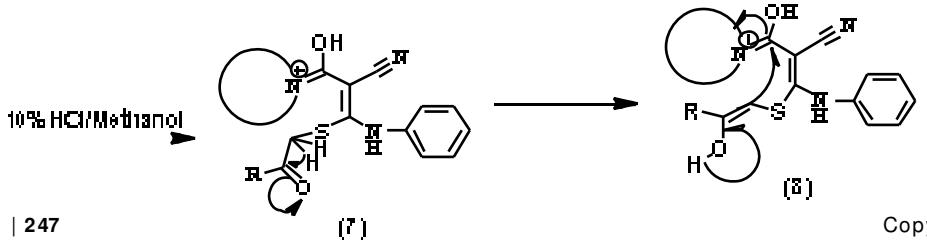
All chemicals were purchased from Sigma Aldrich, SD Fine, Spectrochem, Merck and Himedia. Yields refer to purified products and are not optimized. Melting points were determined on VEEGO - VMP I melting point apparatus and are uncorrected. IR spectrums were recorded on JASCO-FTIR 4100 spectrophotometer. ^{1}H NMR were recorded at Pune University on MERCURY VARIAN 300 MHz instrument and chemical shifts (δ) were reported in parts per million (ppm) with $CDCl_3$ 7.26 ppm as solvent. TMS was used as internal standard for NMR. Multiplates are represented by s (singlet), d (doublet), t (triplet), q (quartate), m (multiplate).Thin layer chromatography (TLC) was performed on precoatedaluminium plate.

Scheme: 2.1



Mechanism



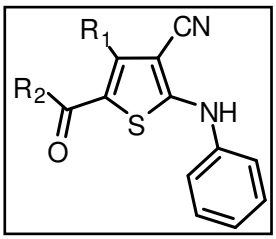


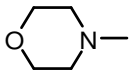
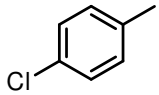
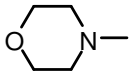
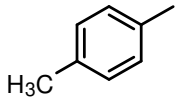
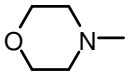
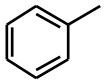
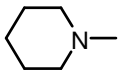
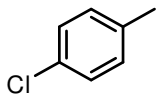
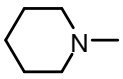
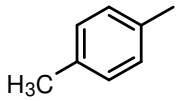
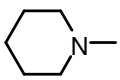
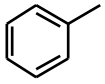
2.1 General procedure for the preparation of Thiophene derivatives:

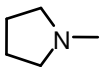
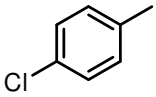
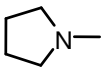
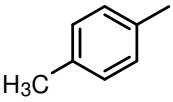
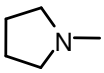
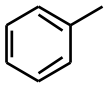
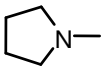
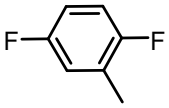
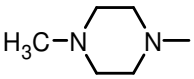
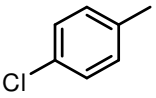
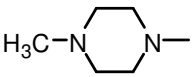
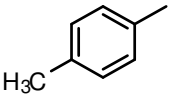
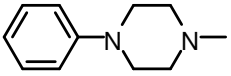
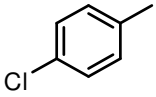
General procedure:

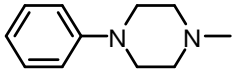
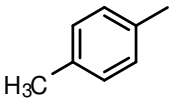
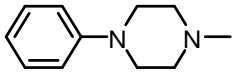
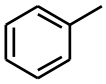
A round bottom flask equipped with magnetic stirrer is charged with (1mol, 1eq) Ketene N-S acetal, (1 mol, 1eq.) Phenacyl bromide Triethylamine, Acetonitrile (10V) was stirred at Room Temprature for 1hr. An uncyclized intermediate was acidified by using 10% MethanolicHCl and refluxed at 80-90^{0}C for 2-3hr. The solid crystals were filtered and washed with water. To get corresponding pure product it was recrystalized by ethyl acetate.

Table: 2.2: Synthesized Tetra Substituted Thiophene derivatives:

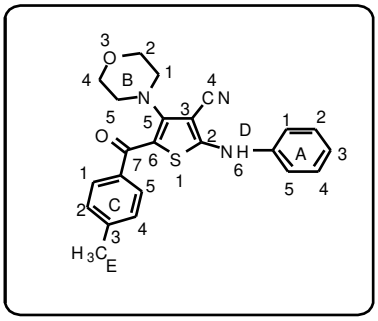


Sr.No.	Comp. Code	R_1	R_2	% yield	Melting point (°C)
1.	MMS-137			71.12	280-282
2.	MMS-150			88.23	278-280
3.	MMS-149			77.86	278-280
4.	MMS-134			67.79	250-252
5.	MMS-139			75.75	240-242
6.	MMS-163			71.72	260-262

7.	MMS-135			78.94	258-260
8.	MMS-138			74.46	250-252
9.	MMS-140			78.94	260-262
10.	MMS-141			70	238-240
11.	MMS-153			78.94	262-264
12.	MMS-147			66.66	288-290
13.	MMS-96			71.42	252-254

14.	MMS-151			70.17	276-278
15.	MMS-152			59.70	258-260

2.3 Characterization:



5-(4-chlorobenzoyl)-4-morpholino-2-(phenylamino)thiophene-3-carbonitrile:(MMS-137).%yield: 71.12 ,Molecular Formula: $C_{22}H_{18}ClN_3O_2S$ Molecular Weight: 423.92, Melting Point: 280-282°C Rf: 0.63,I.R.1598.7 (C=O-str.), 2176.27(CN-str.), 3119.3(NH-str.), (KBr, cm^{-1}) ^1H NMR (200.13 MHz, $CDCl_3$) δ [ppm]: 6.68 (s, 1H, D_6), 3.54 - 3.58 (t, 2H, $B_{1,5}$), 3.65 - 3.69 (T,2H,$B_{2,4}$), 6.96 - 7.0 (d, 2H, $C_{2,4}$), 7.43 - 7.45 (d, 2H, $C_{1,5}$), 7.27 (d, 2H, $A_{1,5}$), 7.17-7.2 (m, 3H, $A_{2,3,4}$), HRMS (ESI) (M+Na): Found - 446.0738 Calculated - 446.0706.

5-(4-methylbenzoyl)-4-morpholino-2-(phenylamino)thiophene-3-carbonitrile(MMS-150)% yield: 88.23Molecular Formula: $C_{23}H_{21}N_3O_2S$ Molecular Weight: 403.50 Melting Point: 278-280°C Rf: 0.50,I.R. 1592.91(C=O-str.), 2165.67(CN-str.), 3125.08(NH-str.), (KBr, cm^{-1}) ^1H NMR (200.13 MHz, $CDCl_3$) δ [ppm]: 6.63 (s, 1H, D6), 2.27 (s, 3H,E3), 3.53-3.58 (T,2H,B1,5), 3.64-3.69 (T,2H,B2,4), 7.42-7.43 (d,2H, $C_{1,5}$), 7.26 (d,2H,C2,4), 6.89-7.02 (m,5H,A,3,2,4,1,5)

5-benzoyl- 4 – morpholino (phenylamino) thiophene-3-carbonitrile(MMS- 149)% yield: 77.86 Molecular Formula: $C_{22}H_{19}N_3O_2S$, Molecular Weight: 389.47, Melting Point: 278-280Rf: 0.52,I.R. 1582.31(C=O-str.), 2171.45(CN-str.), 3114.47(NH-str.), (KBr, cm^{-1}) ^1H NMR (200.13 MHz, $CDCl_3$) δ [ppm]: 6.67 (s, 1H, D6), 3.54-3.58 (t, 2H,B1,5), 3.65-3.69 (t,2H,B2,4), 7.41-7.42 (d,2H, $C_{1,5}$), 7.26-7.23 (d,2H,C2,4), 7.20 (t,1H,C3) 7.02-7.06 (m, 5H ,A,$3_{,2,4,1,5}$) .

5-(4-chlorobenzoyl)-2-(phenylamino)-4-(piperidin-1-yl)thiophene-3-carbonitrile(MMS-134)% yield: 67.79,Molecular Formula: $C_{23}H_{20}ClN_3OS$, Molecular Weight: 421.94, Melting Point: 288-2°C Rf: 0.64,I.R.1598 C=Ostr, 2171.45 CNstr, 3103.87 NHstr (KBr, cm^{-1}) ^1H NMR (200.13 MHz, $CDCl_3$) δ [ppm]: 6.62 (s, 1H, D6), 3.48 - 3.51 (t, 2H, $B_{1,5}$), 1.58 (m, 3H, $B_{3,2,4}$), 7.44 - 7.45 (d, 2H, $C_{1,5}$), 7.27 (d, 2H, $C_{2,4}$), 6.95-7.20(m ,5H , $A_{3,2,4,1,5}$) HRMS (ESI) (M+Na): Found – 444.0899 Calculated-444.0913.

5-(4-methylbenzoyl)-2-(phenylamino)-4-(piperidin-1-yl)thiophene-3-carbonitrile(MMS-139)% yield: 75.75, Molecular Formula: $C_{24}H_{23}N_3OS$ Molecular Weight: 401.52, Melting point: 240 - 242°CRf: 0.60,I.R. 1592.91(C=O-str.), 2171.42(CN-str.), 3114.47(NH-str.), (KBr, cm^{-1}) HRMS (ESI) (M+Na): Found – 424.1498 Calculated - 424.1460.

5-benzoyl-2-(phenylamino)-4-(piperidin-1-yl)thiophene-3-carbonitrile(MMS-163)%yield: 71.72 Molecular Formula: $C_{23}H_{21}N_3OS$ Molecular Weight: 387.50 Melting point: 260 - 262°CRf: 0.40, I.R. 1640.16(C=O-str.), 2249.56 (CN-str.), 2858.95 (NH-str.), (KBr, cm^{-1})

5-(4-chlorobenzoyl)-2-(phenylamino)-4-(pyrrolidin-1-yl)thiophene-3-carbonitrile% (MMS-135).yield: 78.94 Molecular Formula: $C_{22}H_{18}ClN_3OS$, Molecular Weight: 407.92 Melting point: 258 - 260°CRf: 0.60,I.R. 1572.66(C=0-str), 2176.27(CN-str.), 3108.69(NH-str.), (KBr, cm^{-1}) NMR (200.13 MHz, $CDCl_3$) **δ [ppm]:** 6.62 (s, 1H, D6), 1.79 - 1.86 (t, 2H, $B_{2,4}$), 3.5-3.56(t, 2H, $B_{1,5}$), 7.42 -7.43 (d,2H, $C_{1,5}$), 7.26 (d,2H,$C_{2,4}$), 6.94 - 7.18(m, 5H, $A_{,3,2,4,1,5}$) HRMS (ESI) (M+Na): Found - 430.0785 Calculated - 430.0757.

5-(4-methylbenzoyl)-2-(phenylamino)-4-(pyrrolidin-1-yl)thiophene-3-carbonitrile% (MMS-138) yield: 74.46 Molecular Formula: $C_{23}H_{21}N_3OS$ Molecular Weight: 387.50 Melting point: 250-252°CRf: 0.56,I.R.1592.91(C=O-str.), 2165.67(CN-str.), 3099.05(NH-str.), (KBr, cm^{-1}) HRMS (ESI) (M+Na): Found – 410.1316 Calculated - 410.1303.

5-benzoyl-2-(phenylamino)-4-(pyrrolidin-1-yl)thiophene-3-carbonitrile(MMS-140) %yield: 78.94 Molecular Formula: $C_{22}H_{19}N_3OS$Molecular Weight: 373.47 Melting point: 260-262°CRf: 0.52, I.R. 1592.91(C=O-str.), 2171.45(CN-str.), 2880.17(NH-str.), (KBr, cm^{-1}) HRMS (ESI) (M+Na): Found –396.1125 Calculated - 396.1147.

5-(2,4-difluorobenzoyl)-2-(phenylamino)-4-(pyrrolidin-1-yl)thiophene-3-carbonitrile(MMS-141)%yield: 70.00 Molecular Formula: $C_{22}H_{17}F_2N_3OS$ Molecular Weight: 409.45 Melting point: 238 - 240°CRf: 0.52,I.R. 1577.49(C=Ostr.), 2182.06(CN-str.), (KBr, cm^{-1}) HRMS (ESI) (M+Na): Found – 432.0984 Calculated - 396.1147.

5-(4-chlorobenzoyl)-4-(4-methylpiperazin-1-yl)-2-(phenylamino)thiophene-3-carbonitrile(MMS- 153) %yield: 78.94 Molecular Formula: $C_{23}H_{21}ClN_4OS$ Molecular Weight: 436.96 Melting point: 238-240°CRf: 0.52,I.R.1592.91 (C=O-str.), 2176.27(CN-str.), 3099.05(NH-str.), (KBr, cm^{-1}) NMR (200.13 MHz, $CDCl_3$) δ [ppm]: 6.76 (s, 1H, D_6), 2.76 (s, 3H, B_3) 3.13 (s, 2H, $B_{2,4}$) 3.96 (s, 2H, $B_{1,5}$) 7.48 (d, 2H, $C_{1,5}$), 7.27 (d,2H, $C_{2,4}$), 6.97 - 7.22 (m,5H,$A_{,3,2,4,1,5}$)

5-(4-methylbenzoyl)-4-(4-methylpiperazin-1-yl)-2-(phenylamino)thiophene-3-carbonitrile(MMS-147) %yield: 66.66Molecular Formula: $C_{24}H_{24}N_4OS$ Molecular Weight: 416.54 Molecular Weight: 409.45 Melting point: 288 -290°CRf: 0.46,I.R. 1598.7(C=O-str.), 2348.87 (CN-str.), 3077.83(NH-str), (KBr, cm^{-1}) NMR (200.13 MHz, $CDCl_3$) δ [ppm]: 13.06 (s, 1H, D6), 2.28 (s, 3H,C3) 2.75 (s, 2H, B2,4) 3.40-4.23 (t, 2H, B1,5) 7.45 (d,2H, $C_{1,5}$), 7.27 (d,2H,C2,4), 6.71-7.04 (m,5H,A,3,2,4,1,5)

5-(4-chlorobenzoyl)-2-(phenylamino)-4-(4-phenylpiperazin-1-yl)thiophene-3-carbonitrile(MMS-96). %yield: 71.42Molecular Formula: $C_{28}H_{23}C_lN_4OS$ Molecular Weight: 499.03, Melting point: 252 - 254°CRf: 0.40,I.R. 1588.09 C=O str,2182.06 CN str, 3431.71 NH str (KBr, cm^{-1})

5-(4-methylbenzoyl)-2-(phenylamino)-4-(4-phenylpiperazin-1-yl)thiophene-3-carbonitrile(MMS-151) %yield:70.17 Molecular Formula: $C_{29}H_{26}N_4OS$ Molecular Weight: 478.61 Melting point: 276-278°CRf: 0.40,I.R. 1599.7(C=O –str.), 2165.67(CN-str.), 2848.35(NH –str.), (KBr, cm^{-1})

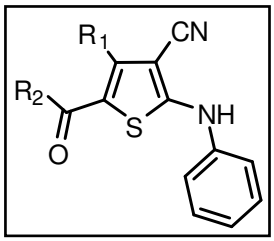
5-benzoyl-2-(phenylamino)-4-(4-phenylpiperazin-1-yl)thiophene-3-carbonitrile(MMS-152)%yield: 59.70 Molecular Formula: $C_{28}H_{24}N_4OS$ Molecular Weight: 464.58 Melting point: 258-260°CRf: 0.43,I.R. 1598.7(C=O-str.), 2171.45(CN-str.), 3114.47 (NH-str.), (KBr, cm^{-1}) NMR (200.13 MHz, $CDCl_3$) δ [ppm]: 6.70 (s, 1H, D_6), 3.30 (s, 2H,$B_{1,5}$) 3.90 (s, 2H, $B_{2,4}$) 7.44-7.43 (d,2H, $C_{1,5}$), 7.41 (s,1H,C_3), 7.26(d, 2H,$C_{2,4}$) 7.21-7.24 (m, 5H, B_3) 7.03-7.07 (m, 5H, $A_{3,2,4,1,5}$)

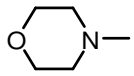
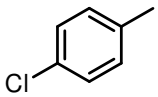
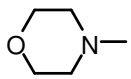
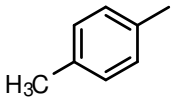
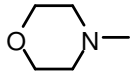
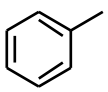
Biological activity

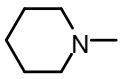
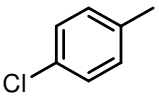
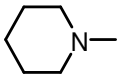
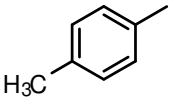
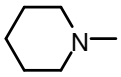
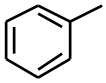
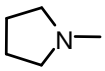
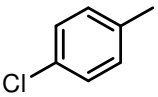
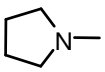
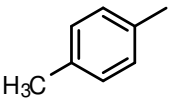
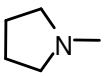
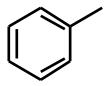
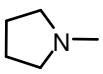
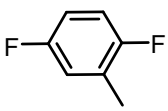
The synthesized compounds were evaluated for their antibacterial and antifungal activity[5,6] against different micro-organisms such as Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeuroginosa and fungal strains such as Candida albicans by Cup – plate method at 100 µg/ml concentration.

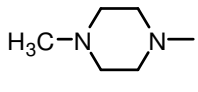
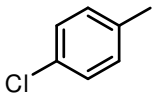
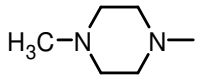
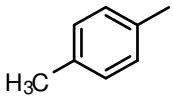
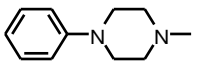
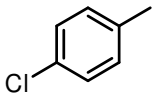
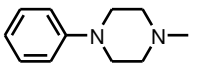
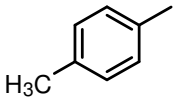
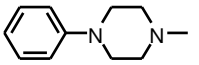
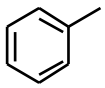
The activity of all the compounds were determined by observing the zones of inhibition formed around the cup after 24h of incubation for antibacterial and 48h for antifungal activities. Azithromycin and Fluconazole were used as a standard for comparison of antibacterial and antifungal activity respectively. Dimethyl sulphoxide was used as a solvent control. All the organisms were obtained from NCL Pune as a slant and were subcultured and used.

Antibacterial activity of synthesized MMS series.



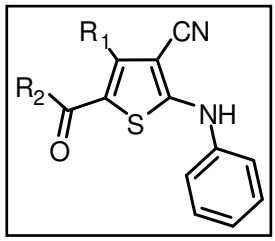
Sr.no.	Compound code	Compound.conc. (100 µg/ml)		Zone of inhibition (Avg.diameter in mm)			
				S. Aureus	*B. subtilis*	*E.coli*	*P. aeuroginosa*
		R_1	R_2	Avg.	Avg.	Avg.	Avg.
1	MMS-137			9.00 (8-9)	8.33 (8-9)	10.33 (10-11)	10.33 (10-11)
2	MMS-150			9.00 (8-10)	8.33 (8-9)	7.00 (6-8)	6.66 (6-7)
3	MMS-149			8.00 (8)	4.00 (4)	12.00 (12)	11.66 (11-12)

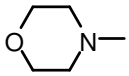
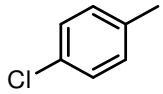
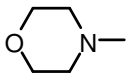
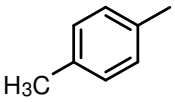
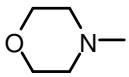
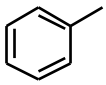
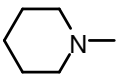
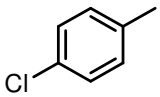
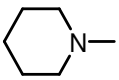
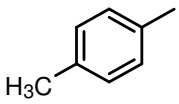
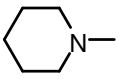
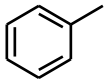
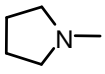
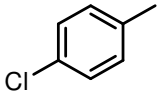
4	MMS-134			**9.33 (9-10)**	**6.66 (6-7)**	**6.33 (6-7)**	**8.33 (8-9)**
5	MMS-139			**16.33 (16-17)**	**8.33 (8-9)**	**10.00 (10)**	**10.00 (10)**
6	MMS-163			**6.00 (6)**	**14.00 (14)**	**16.00 (16)**	**12.00 (12)**
7	MMS-135			**7.66 (7-8)**	**10.66 (10-11)**	**8.66 (8-9)**	**10.00 (10)**
8	MMS-138			**11.00 (10-12)**	**8.33 (8-9)**	**10.66 (10-12)**	**8.00 (8)**
9	MMS-140			**11.00 (10-11)**	**10.33 (10-11)**	**12.00 (11-13)**	**10.33 (10-11)**
10	MMS-141			**14.00 (14)**	**8.33 (8-9)**	**11.00 (10-11)**	**8.33 (8-9)**

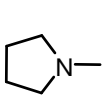
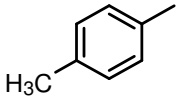
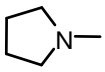
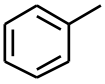
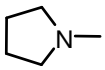
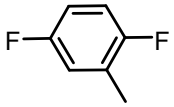
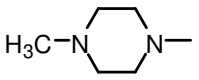
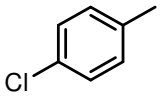
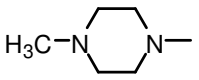
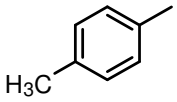
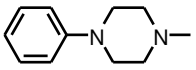
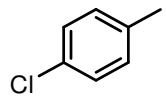
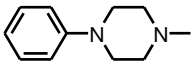
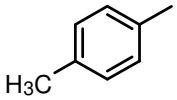
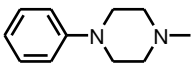
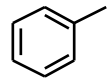
11	MMS-153			**6.33 (6-7)**	**12.00 (12)**	**14.66 (14-15)**	**14.33 (14-15)**
12	MMS-147			**10.66 (10-11)**	**6.00 (6)**	**13.66 (13-14)**	**10.00 (10)**
13	MMS-96			**4.33 (4-5)**	**11.66 (11-12)**	**16.33 (16-17)**	**14.33 (14-15)**
14	MMS-151			**6.66 (6-7)**	**7.66 (7-8)**	**10.00 (10)**	**10.33 (10-11)**
15	MMS-152			**8.33 (8-9)**	**2.33 (2-3)**	**11.00 (10-11)**	**10.00 (10)**
16	Standard	Azithromycin		**10.66 (10-12)**	**10.00 (10)**	**24.00 (22-24)**	**14.66 (14-16)**

Antifungal activity of synthesized Thiophene derivatives against *Candida* albicans:

Table: 3.10: Antifungal activity of synthesized MMS series against *Candida* albicans



Sr.no.	Compound code	Comp.conc. (100 µg/ml)		Zone of inhibition (Avg.diameter in mm)
				Fluconazole
		R_1	R_2	Avg.
1	MMS-137			**8.00 (8)**
2	MMS-150			**12.33 (12-13)**
3	MMS-149			**10.66 (10-11)**
4	MMS-134			**10.00 (10)**
5	MMS-139			**10.33 (10-11)**
6	MMS-163			**4.00 (4)**
7	MMS-135			**8.00 (8)**

8	MMS-138			**6.66 (6-7)**
9	MMS-140			**10.33 (10-11)**
10	MMS-141			**6.00 (6)**
11	MMS-153			**4.33 (4-5)**
12	MMS-147			**10.00 (10)**
13	MMS-96			**2.00 (2)**
14	MMS-151			**7.66 (7-8)**
15	MMS-152			**12.00 (12)**
16	Standard	Fluconazole		**23.66**

Conclusion:

Finally we conclude that these tetra substituted Thiophene derivatives have better antimicrobial and antifungal activity in comparison with other heterocycles.

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