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Research Article

SYNTHESIS, CHARACTERIZATION AND ANTI-FUNGAL ACTIVITY OF SOME NOVEL THIOSEMICARBAZIDES

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ABSTRACT

2-amino-3-N-(substituted carboxanilido)-4, 5-disubstituted thiophene JSR 1A – 1L were synthesized using versatile Gewald reaction. First step was preparation of substituted cyanoacetanilides (Comp. No JSR a, b & c) which were carried out by condensation of substituted anilines and Ethyl cyano acetate which was then reacted with Methylenic ketone, ammonium acetate, glacial acetic acid, benzene, sulphur to produce 2-amino-3-N-(substituted carboxanilido)-4, 5-disubstituted thiophenes (JSR 1A – 1L). Later the compound 2-amino-3-N-(substituted carboxanilido)-4,5-disubstituted thiophenes in DMF were treated with NaOH & carbon di sulfide further the mixture was stirred with hydrazine hydrate to yield twelve substituted new thiosemicarbazides (JSR 2A-2L). The compounds were characterized IR, 1H NMR spectral data and screened for anti-fungal activity.

Keywords: Thiophenes, Thiosemicarbazides, Spectral analysis, Antifungal activity.

INTRODUCTION

Most of the therapeutic agents are heterocyclic compounds; hence heterocyclic chemistry has been the most fruitful area for drug discovery. Among the heterocyclic compounds sulphur containing moieties have attracted maximum attention as they have several pharmacological activities as antimicrobial[1,2,5,7,8], antifungal[6,9,11], antibacterial[4], anticonvulsant[10], antitumor[13], anti-inflammatory[14], antioxidant[12] activity and so on. Similarly thiosemicarbazide[6,7] derivatives also have been reported to possess various biological activities as antifungal[6,9,11]. The therapeutic importance of these rings promoted us synthesize novel benzo (b)thiophenes by Gewald[3] reaction and their thiosemicarbazide derivatives. Characterize the compound by IR and 1H NMR spectroscopic techniques and evaluate them for their antifungal activity.

MATERIAL AND METHODS

Chemicals

Substituted anilines (o-anisidine, p-anisidine and o-toluene), ethyl cyanoacetate, glacial acetic acid, methylinic ketones (ethyl methyl ketone, cyclopentanone, cyclohexanone, cycloheptanone), benzene, ammonium acetate, sodium sulfate, sulphur, diethyl amine, ethanol, dimethyl formamide, sodium hydroxide, carbon di sulfide and Hydrazine hydrate were obtained from local dealer. All other chemical used were of laboratory grade.

Preparation of substituted cyanoacetanilides(Comp. No JSR a, b & c)

A mixture of substituted anilines (0.50 M) and ethyl cyano acetate (56.5 ml; 0.50 M) were taken in a conical flask, mixed well and heated on a heating mantle at 160-170 ºC for 5-6 hrs. Then the reaction mixture was left at room temperature for overnight. The solid obtained was collected, washed with ethanol and dried. Recrystallization was done by acetone: water mixture (5:1)

Preparation of 2-cyano-2-(methylidene)-N-substituted carboxanilides.

A mixture of substituted cyano acetanilide (0.04 M), appropriate methylenic ketone (4.6 ml; 0.04 M), ammonium acetate (1 g) and glacial acetic acid (2 ml) in benzene (100 ml) was refluxed with an arrangement for continuous separation of water involving dean stark apparatus. After 8 hrs 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. The intermediate crude product obtained was immediately processed for the next step.

Preparation of 2- amino-3-N- substituted carboxanilido - 4,5- disubstitutedthiophenes (Comp. no. JSR 1A- 1L)

To a mixture of 2-Cyano –2- (methylidene) - N-substituted carboxanilides in alcohol (30 ml) was added sulphur (1.28 g; 0.04 M) with stirring maintaining the temperature between 40-45ºC during addition. Then to the reaction mixture, diethyl amine (4.0 ml) was added drop wise with stirring. The reaction mixture was stirred for 1 hr at 40-45ºC and chilled overnight. The solid obtained was filtered, washed with ethanol and recrystallized from isopropyl alcohol.

Preparation of synthesis of 2-(3' thiosemicarbazide) -3-Substituted carboxanilido-4,5-disubstituted thiophenes (JSR 2A- 2L)

To a solution of 2-amino-3-N-(substituted carboxanilido)-4,5-disubstituted thiophenes (0.005M) in DMF (10 ml) was added NaOH (0.01 M) & carbon di sulfide (0.75 ml). The mixture was stirred at 15 – 20^0C for 1 hr. While stirring the reaction mixture was added hydrazine hydrate (0.01M) & stirring continued at 60^0C for 1hr more. On adding water a pale yellow solid separated out which is recrystallized from DMF: water (5:1).

ANTI-FUNGAL STUDIES

All the synthesized compounds were screened for their antifungal activity by agar diffusion method[15] at a conc of 50µg/ml against Aspergillus niger and candida albicans. After 24 hours of drug addition, Zone of inhibition was measured and recorded. Miconazole Nitrate at 50µg/ml was used as standard in the experiment.

RESULTS

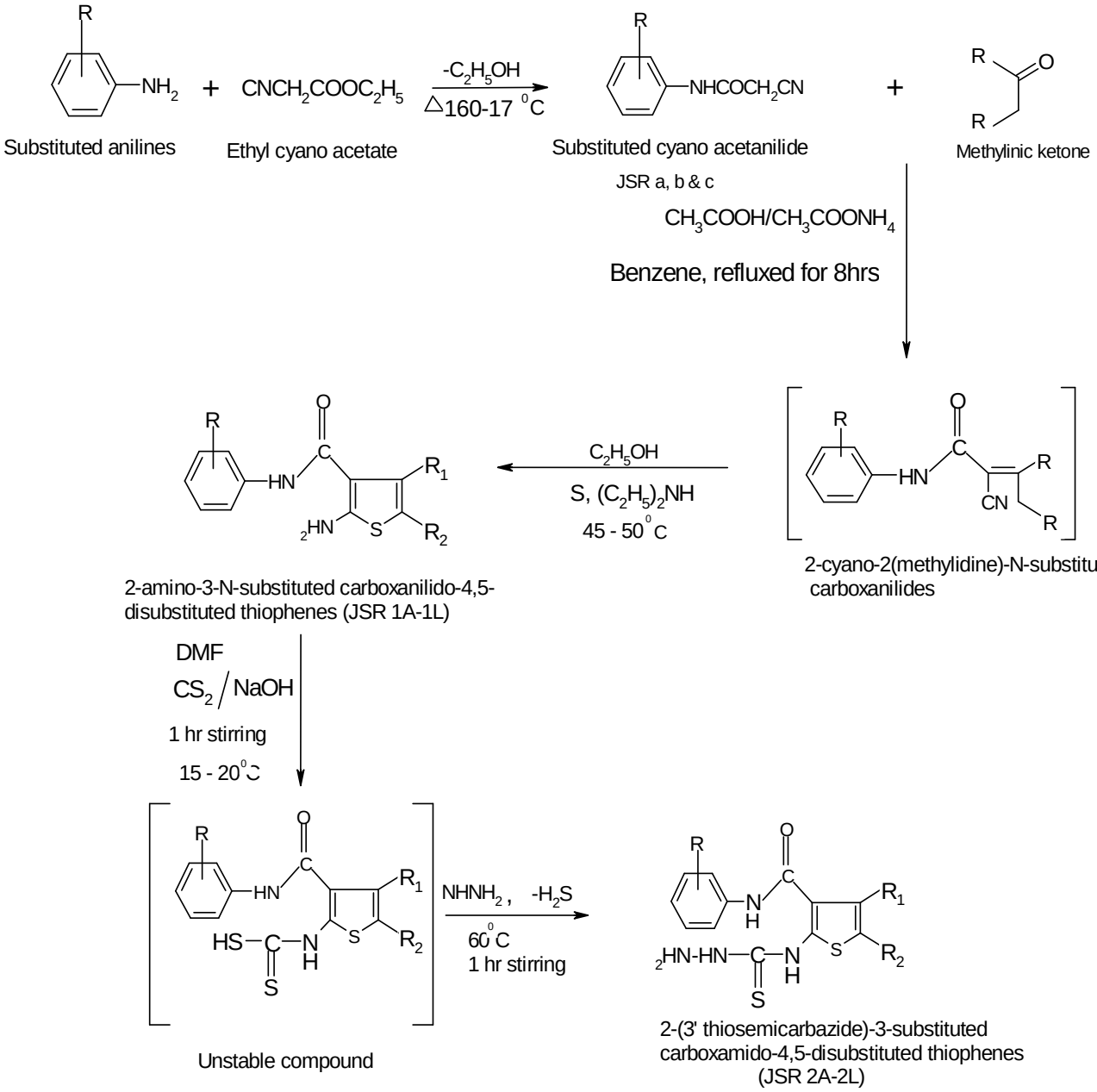
From the antifungal activity results it was observed that all the compounds influenced the activity. Among the drugs tested for antifungal activities Table 5 showed that compounds JSR-2A, JSR-2C, JSR-2E, and JSR-2I exhibited potent activity by showing zone of inhibition ranging from 15mm-18mm. All these drugs showed potent activity against *Candida albicans and Aspergillus niger* with high zone of inhibition. All other drugs showed moderate inhibititory properties against the test organism. Miconazole nitrate exhibited potent inhibitory properties against the entire test organism.

DISCUSSION

From the IR, 1H NMR, and Mass spectrum obtained, characterization of data has been done and given in table 2, 3 and 4. The formation of the new series of thiosemicarbazides were confirmed by the shift of IR peaks between 1627 – 1640 cm^{-1} as seen in the starting materials to 1690 – 1700 cm^{-1} in the final compounds indicating the aryl cyclization due to the cyclic keto group and the presence of prominent peaks between 3217 - 3460$cm^{-1}$of NH-NH_2 in thiosemicarbazides compared to their starting compounds is sufficient to explain the formation of the new thiosemicarbazides.

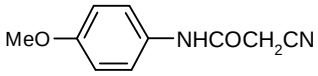
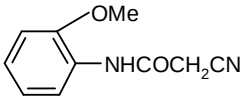
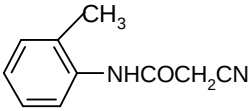
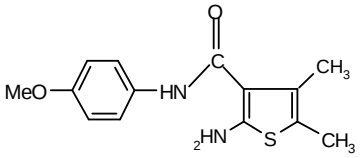
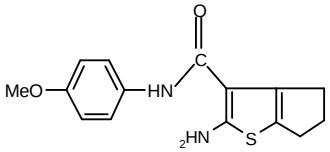
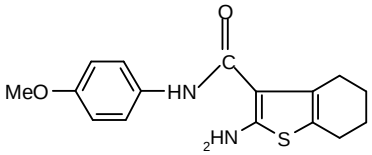
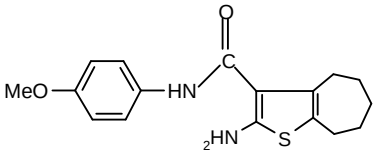
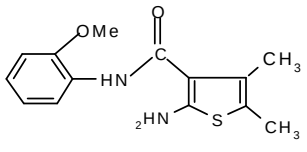
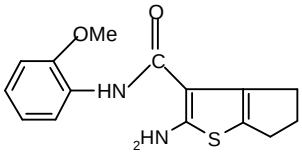
The NMR spectra of JSR-2D and JSR-2E, were indicated the formation of new compounds. The sharp singlet peaks at δ (ppm) = 9.35-9.0(JSR-2D), δ = 9.42-9.10(JSR-2E), indicated the presence of NH-CS-NH group in the compounds. It will indicate the formation of new compounds.

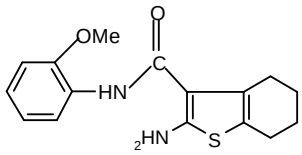
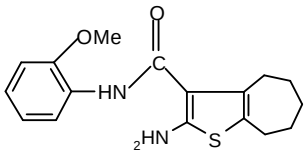
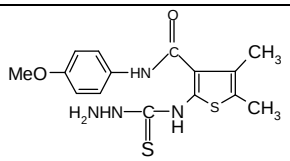
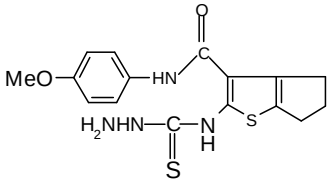
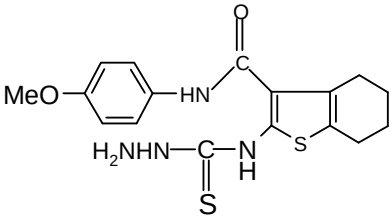
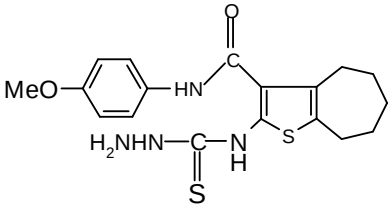
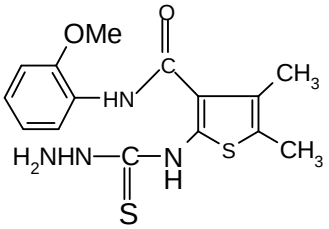
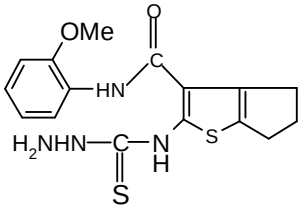
SCHEME



Where: **R =** p-OCH_3, o-OCH_3, o-CH_3
R_1, R_2 = -CH_3, - $(CH_2)_3$, - $(CH_2)_4$, - $(CH_2)_5$.

Table 1: Physical data of 2-Cyano-2-(methylidene) -N-substituted carboxanilides (JSR a, b & c), 2- amino-3-N-substituted carboxanilido-4,5-disubstituted thiophenes (JSR 1A – 1L) and 2-(3' thiosemicarbazide) -3-Substituted carboxanilido-4,5- disubstituted thiophenes (JSR 2A-2L)

Sr. No.	Comp. No.	Structure	Recrystalization Solvent	M.W. (g)	M.P. (OC)	% Yield	TLC	
							Solvent System	Rf
1	JSR-a		acetone: water (5:1)	190	132	66.50	Chloroform: Ethyl acetate(8:2)	0.28
2	JSR-b		acetone: water (5:1)	190	122	64.33	Chloroform: Ethyl acetate(8:2)	0.34
3	JSR-C		acetone: water (5:1)	174	115	58.85	Chloroform: Ethyl acetate(9:1)	0.30
4	JSR-1A		Isopropyl alcohol	276	130	85.18	Chloroform: Ethyl acetate(9:1)	0.24
5	JSR-1B		Isopropyl alcohol	288	142	82.07	Chloroform: Ethyl acetate (9:1)	0.78
6	JSR-1C		Isopropyl alcohol	302	150	73.25	Chloroform: Ethyl acetate (9:1)	0.70
7	JSR-1D		Isopropyl alcohol	316	180	67.84	Chloroform: Ethyl acetate (9:1)	0.32
8	JSR-1E		Isopropyl alcohol	276	124	66.96	Chloroform: Ethyl acetate(9:1)	0.35
9	JSR-1F		Isopropyl alcohol	288	149	80.16	Chloroform: Ethyl acetate(9:1)	0.56

10	JSR-1G		Isopropyl alcohol	302	131	69.84	Chloroform: Ethyl acetate(9:1)	0.74
11	JSR-1H		Isopropyl alcohol	316	178	55.36	Chloroform: Ethyl cetate(9:1)	0.50
12	JSR-2A		DMF:Water (5:1)	350	102	60.27	Benzene: Ethanol (8:2)	0.72
13	JSR-2B		DMF:Water (5:1)	362	122	45.87	Benzene: Ethanol (9:1)	0.55
14	JSR- 2C		DMF: Water (5:1)	376	110	69.25	Benzene: Ethanol (9:1)	0.64
15	JSR-2D		DMF: Water (5:1)	390	190	64.18	Benzene: Ethanol (9.5:0.5)	0.57
16	JSR-2E		DMF: Water (5:1)	350	146	76.35	Benzene: Ethanol (9:1)	0.38
17	JSR-2F		DMF: Water (5:1)	362	118	60.16	Benzene: Ethanol (9:1)	0.51

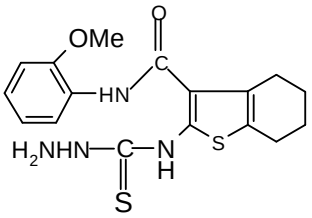
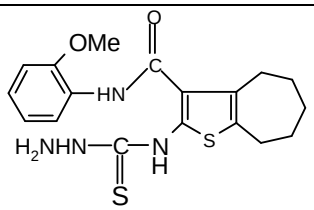
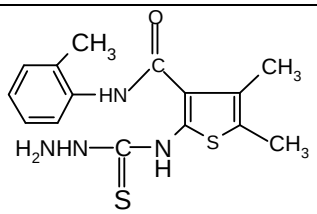
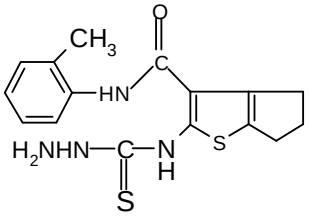
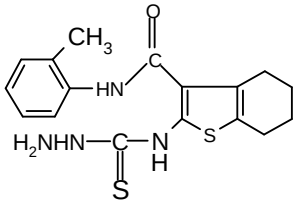
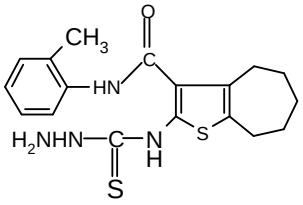
18	JSR-2G		DMF: Water (5:1)	376	98	48.35	Benzene: Ethanol (8:2)	0.36
19	JSR-2H		DMF: Water (5:1)	390	116	69.84	Benzene: Ethanol (9:1)	0.65
20	JSR-2I		DMF: Water (5:1)	334	96	45.36	Benzene: Ethanol (9:1)	0.34
21	JSR-2J		DMF: Water (5:1)	346	135	55.66	Benzene: Ethanol (9:1)	0.52
22	JSR-2K		DMF:Water (5:1)	360	148	49.04	Benzene: Ethanol (9.2:0.8)	0.50
23	JSR-2L		DMF:Water (5:1)	374	120	80.66	Benzene: Ethanol (9:1)	0.28

Table 2: (Spectral data) of 2-Cyano-2-(methylidene) -N-substituted carboxanilides (JSR a, b and c)

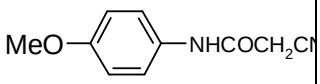
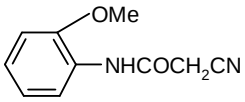
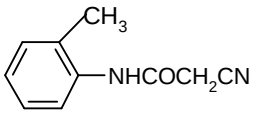
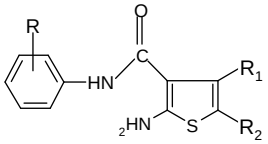
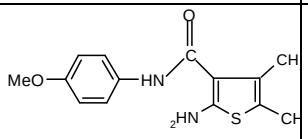
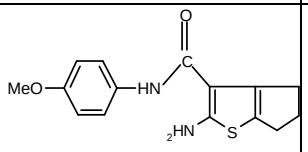
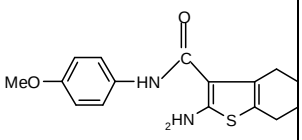
Comp. No.	Structure	λ_{max} (nm)	IR (KBr) cm^{-1}	^1H NMR (DMSO)
JSR-a		279	3287.12 (-NH-); 3015.54(Ar-CH); 2987.78 (Ali-CH); 2235.30 (-CN); 1643.8 (C=O); 1564.32(C=C);1552.44(NH-bend); 1047.06 (C-O); 710.34(S-C).	–
JSR-b		272	3272 (-NH-); 3020.15(Ar-CH);2979.65(Ali-CH); 2250 (CN); 1697 (C=O); 1541 (NH-bend); 1543(C=C); 1123(C-O); 824(C-N). 705.86(S-C).	–
JSR-c		290	3293.73 (-NH-); 3098.12(Ar-CH);2982.66(Ali-CH); 2263,24 (CN); 1652.95 (C=O); 1591.98 (NH-bend); 1253.34(C-O); 877(C-N); 723.4(S-C).	–

Table 3: (Spectral data) 2- amino-3-N-substituted carboxanilido-4,5-disubstituted thiophenes (JSR 1A – 1L)

General structure



Comp. No.	Structure	λ_{max} (nm)	IR (KBr) cm^{-1}	^1H NMR (DMSO)
JSR-1A		342	3328.7 & 3222.28(-NH_2); 3205.61 (-NH-); 3048.22 (Ar-CH); 2924.36(Ali-CH); 1641.48(C=O); 1539.1 (C=C); 1113.08 (C-O).	–
JSR-1B		349	3342.12 & 3312.08(-NH2); 3294 (-NH-); 3024 (Ar-CH); 2929 (Ali-CH); 1656 (C=O); 1521 (C=C); 1242 (C-O).	–
JSR-1C		353	3301.33 & 3296.24(-NH_2); 3270 (-NH-); 3043 (Ar-CH); 2941 (Ali-CH); 1658 (C=O); 1522 (C=C); 1239 (C-O); 824(C-N).	–

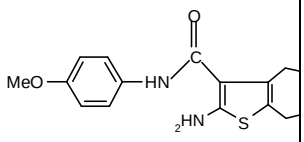
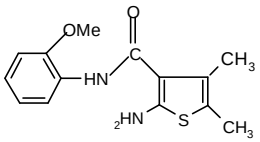
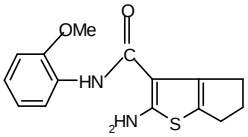
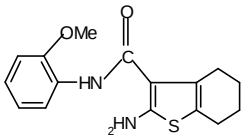
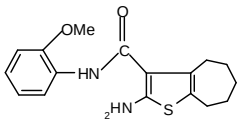
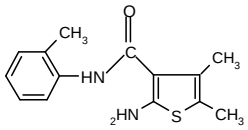
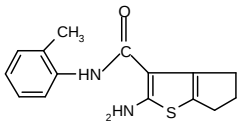
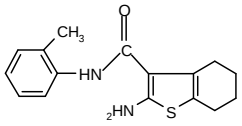
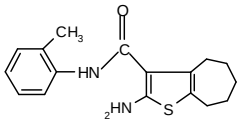
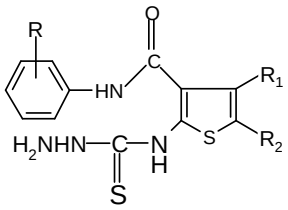
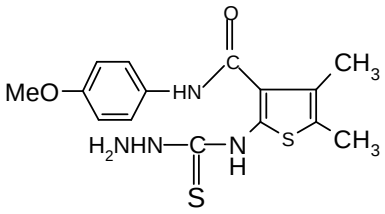
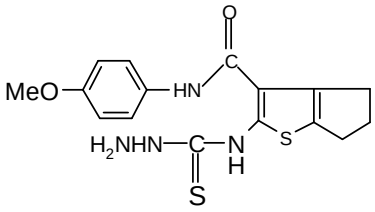
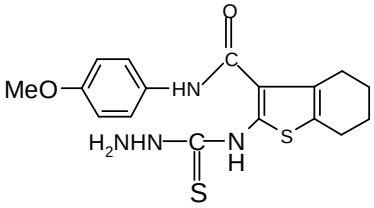
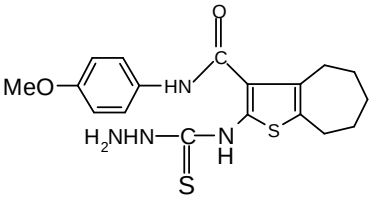
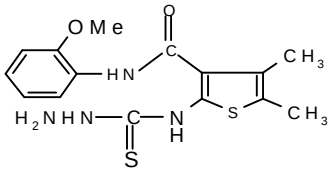
JSR-1D		354	3312.11 & 3293.44(-NH_2); 3267 (-NH-); 3143 (Ar-CH); 2938.24 (Ali-CH); 1652 (C=O); 1527 (C=C); 1239 (C-O).	8.52(s, 1H, -NH, g); 7.53 (d, 2H, Ar-H, h,k); 6.84 (d, 2H, Ar-H, i,j); 5.36 (s, 2H, NH_2, f); 3.78 (s, 3H, -OCH_3,); 2.81(t, 2H, -CH_2-, e); 2.61 (t, 2H, -CH_2-, a); 1.83 (s, 2H, -CH_2-, d); 1.65 (t, 4H,-CH_2,b,c).
JSR-1E		338	3332.44 & 3220.05(-NH_2); 3211.41 (-NH-); 3065.8 (Ar-CH); 2932.26(Ali-CH); 1650.5(C=O); 1542.23 (C=C); 1132.80 (C-O).	8.44(d, 1H, Ar-H,h); 8.29 (s, 1H,-NH, d); 7.02(m, 2H, Ar-H, f,g); 6.89(d, 1H, Ar-H,e)5.95 (br, 2H, NH_2, c); 3.89 (s, 3H, -OCH_3,); 2.33(s, 3H, -CH_3, b); 2.20 (s, 3H, -CH_3, a).
JSR-1F		341	3346.12&3302.04(-NH_2); 3263.53(-NH-); 3068.05(Ar-CH); 2976.14 (Ali-CH); 1652.93 (C=O); 1591.95(C=C); 1140.82 (C-O).	–
JSR-1G		346	3315.9 & 3239.8(-NH_2); 3212.68(-NH-); 3065.28 (Ar-CH); 2945.86(Ali-CH); 1655.42 (C=O); 1572.3 (C=C); 1170 (C-O); 836 (C-S).	–
JSR-1H		349	3254 (-NH-); 3011 (Ar-CH); 2972 (Ali-CH); 1647 (C=O); 1540 (C=C); 1292 (C-O); 824 (C-S).	–
JSR-1I		323	3324.10 & 3255.66(-NH_2); 3245.11 (-NH-); 3125.12 (Ar-CH); 2939.24 (Ali-CH); 1656.87 (C=O); 1540.66 (C=C); 826 (C-S).	–
JSR-1J		326	3349.55 & 3328(-NH_2); 3239 (-NH-); 3078.34(Ar-CH); 2927 (Ali -CH); 1659 (C=O); 1540 (C=C); 823 (C-S).	–
JSR-1K		332	3322.55 & 3289.66(-NH_2); 3245.98(-NH); 23042.9(Ar-CH); 2924 (Ali-CH); 1643 (C=O); 1540 (C=C); 827 (C-S).	–
JSR-1L		334	3339 (-NH-); 2927 (Ali-CH); 1669 (C=O); 1566 (C=C); 1220(C-O); 1086 (C-N amine); 832 (C-S).	–

Table 4: 2-(3' thiosemicarbazide) -3-Substituted carboxamido-4,5- substituted thiophenes(JSR 2A-2L)
General structure



JSR 2A-2L

Comp. No.	Structure	λ_{max} (nm)	IR (KBr) cm^{-1}	^1H NMR ($CDCl_3$)
JSR-2A		360	3344.57& 3316.52.38(-NH_2); 3288 (-NH-); 3040 (Ar-CH); 2952.38 (Ali-CH); 1641.75 (C=O); 1518 (C=C); 1253 (C-O); 799.6(C-S).	-
JSR-2B		363	3453.22 & 3308.87 (-NH_2); 3294 (-NH-); 3024 (Ar-CH); 2929 (Ali-CH); 1656 (C=O); 1521 (C=C); 1242 (C-O).	-
JSR-2C		366	3324.99 & 3298(-NH_2); 3250(-NH-); 3034 (Ar-CH); 2924.87 (Ali-CH); 1668(C=O); 1522 (C=C); 1239 (C-O); 809(C-S).	–
JSR-2D		370	3321.32 & 3276.56 (-NH_2),3267 (-NH-); 3143 (Ar-CH); 2941 (Ali-CH); 1652 (C=O); 1527 (C=C); 1239 (C-0); 820 (C-S).	9.35 (s, 1H, -CS-NH,g); 9.0(s, 1H, -NH, f); 8.53 (s, 1H, -NH, 5); 7.53 (d, 2H, Ar-H, 1,4); 6.84 (d, 2H, Ar-H, 2,3); 5.36 (s, 2H, NH_2, h); 3.78 (s, 3H, -OCH_3,); 2.81(t, 2H, -CH_2-, e); 2.61 (t, 2H, -CH_2-, a); 1.83 (s, 2H, -CH_2-, d); 1.65 (t, 4H,-CH_2,b,c).
JSR-2E		350	3451 & 3256.04 (-NH_2) 3242.34 (-NH-); 3026.23 (Ar-CH); 2886.25 (Ali-CH); 1634.04 (C=O); 1553 (C=C); 1259 (C-0); 816 (C-S).	9.42 (s, 1H, -CS-NH,d); 9.10(s, 1H, -NH, c); 8.44(d, 1H, Ar-H,4); 8.29 (s, 1H,-NH,5); 7.02(m, 2H, Ar-H, 2,3); 6.89(d, 1H, Ar-H,1); 5.95 (br, 2H, NH_2, e); 3.89 (s, 3H, -OCH_3,); 2.33 (s, 3H, -CH_3, b); 2.20 (s, 3H, -CH_3, a).

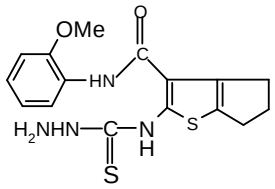
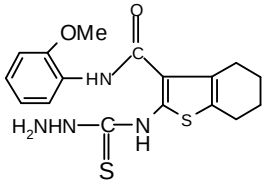
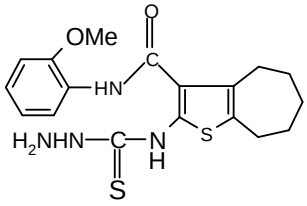
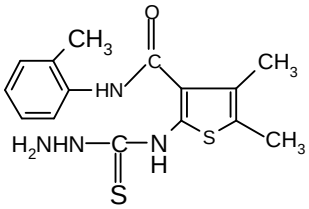
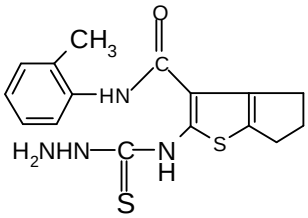
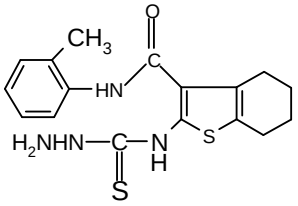
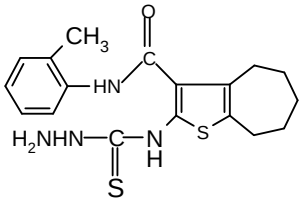
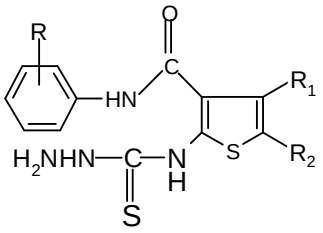
JSR-2F		354	3451.12 & 3342.64 (-NH_2); 3326 (-NH-); 3027(Ar-CH);2924(Ali-CH); 1650 (C=O); 1532 (C=C); 1245.6 (C-O); 814 (C-S).	–
JSR-2G		357	3425 & 3245(-NH_2); 3239 (-NH-); 3026 (Ar-CH); 1656 (C=O); 1539 (C=C); 1246 (C-O); 831 (C-S). .	–
JSR-2H		362	3395 & 3362.56(-NH_2); 3254 (-NH-); 3011 (Ar-CH); 2972 (Ali-CH); 1647 (C=O); 1540 (C=C); 1292 (C-O); 822 (C-S).	–
JSR-2I		330	3423.45 & 3402.22(-NH_2); 3289.66(-NH); 3098.6(Ar-CH);2998(Ali-CH); 1656(C=O); 1556.6(C=C); 1234.23 (C-O); 823.3 (C-S).	–
JSR-2J		337	3433.54 & 3387.66(-NH_2); 3279 (-NH-); 3098(Ar-CH); 2943.23 (Ali-CH); 1659 (C=O); 1540 (C=C); 1246 (C-O); 824 (C-S). .	–
JSR-2K		341	3452.65&3398.66 (-NH_2);3322(-NH-); 3125.89(Ar-CH); 2924 (Ali-CH); 1643 (C=O); 1540 (C=C); 1220 (C-O); 815 (C-S).	–
JSR-2L		343	3315.93 & 3212.64(-NH_2); 3209.6(-NH-); 3065.25(Ar-CH); 2945.28 (Ali-CH); 1655.42 (C=O); 1527.3 (C= C); 1220(C-O); 1086 (C-N amine); 768.2 (C-S).	–

Table 5: Antifungal activity data of 2-(3' thiosemicarbazide) -3-Substituted carboxamido-4,5- substituted thiophenes (JSR 2A-2L)



JSR 2A-2L

Compound Code	R	R_1& R_2	Zone of inhibition (mm)	
			Aspergillusniger	*Candida albicans*
JSR 2A	p-OCH_3	$(CH_3)_2$	16	18
JSR 2B	p-OCH_3	$(CH_2)_3$	13	10
JSR 2C	p-OCH_3	$(CH_2)_4$	15	16
JSR 2D	p-OCH_3	$(CH_2)_5$	11	13
JSR 2E	o-OCH_3	$(CH_3)_2$	16	18
JSR 2F	o-OCH_3	$(CH_2)_3$	14	09
JSR 2G	o-OCH_3	$(CH_2)_4$	13	14
JSR 2H	o-OCH_3	$(CH_2)_5$	11	12
JSR 2I	o-CH_3	$(CH_3)_2$	18	18
JSR 2J	o-CH_3	$(CH_2)_3$	14	11
JSR 2K	o-CH_3	$(CH_2)_4$	12	14
JSR 2L	o-CH_3	$(CH_2)_5$	11	12
Miconazole nitrate	----------	---------	25	23

Dose concentration: 50 µg/0.1 ml
NA : No activity
Control : DMF (Dimethyl formamide)

CONCLUSION

From the antifungal results, it was observed that the presence of methyl group on heterocyclic nucleus as in JSR-2A, 2C, 2E and 2I exhibited considerable activity against Aspergillus niger and Candida albicans employed on par with the standards used.

ACKNOWLEDGEMENT

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thieno[2,3-d][1,2,3]-triazin-4(3H)- ones as potential antimicrobial agents. Eur J Med Chem. 2010;45:4365-4369.
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