SYNTHESIS, CHARACTERIZATION AND IN VITRO STUDY OF ANTIBACTERIAL, ANTIFUNGAL ACTIVITIES OF SOME NOVEL DIHYDROPYRIMIDINTHIONE DERIVATIVES

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***Abstract*— Dihydropyrimidinthione is an important class of heterocyclic compounds which exhibits wide spectrum of biological activities. In the present study, some bio-active dihydropyrimidinthione derivatives have been synthesized and their characterization was done by IR, NMR and mass spectral data. The antibacterial and antifungal activities of synthesized compounds have also been studied in N,N-dimethyl formamide and Dimethyl sulfoxide.**

***Keywords*— Dihydropyrimidinthione derivatives, N, N-dimethylformamide, Dimethyl sulfoxide, Antibacterial activity, Antifungal activity.**

I. INTRODUCTION

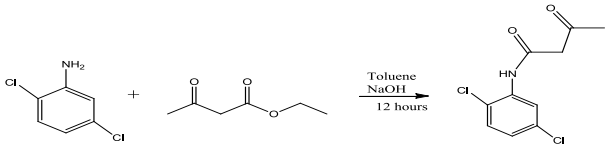
Multicomponent reactions are efficient tools in modern synthetic organic chemistry due to their significant features such as atom economy and straightforward reaction design. Biginelli reaction is an important multicomponent reaction that allows one pot three component synthesis of 3, 4-dihydroxypyrimidi-2-(1H)-ones and their analogues (Holden and Crouch, 2001; Bose *et al*., 2005; Zhang and Piqani, 2011; Heravi *et al.* 2013). The chemistry of dihydropyrimidinthione derivatives is well known for their wide range of bioactivities and their application in medicinal chemistry (Kappe *et al*., 1997; Kappe 2000). Many dihydropyrimidinone and dihydropyrimidinthione derivatives are known to exhibit a wide spectrum of pharmacological activities such as antitumor and antiviral (Klein *et al*., 2007; Li *et al*; 2009; Zabihollahi *et al*., 2012), antimicrobial (Borse *et al*., 2012; Sedaghati *et al*., 2012; Reem Al-Harbi and Adel Abdel-Rahman, 2013; Barot and Patel, 2013), anti-inflammatory (Brook *et al*., 1997; Bahekar and Shinde, 2004) , antibacterial (Sheng *et al*., 1984; Deguchi *et al*., 1993), antifungal (Joshi *et al*., 1999; Vernekar *et al*., 2001), as calcium channel blockers (Fabian *et al*., 1998; Kappe 1998; Debache *et al*., 2012), etc. Hence, synthesis of such compounds is of considerable interest. Thus, in the present study, some new derivatives of N-(2,5-dichlorophenyl)-3-oxobutanamide with thiourea and different aryl aldehydes have been synthesized. Their characterization was done by spectroscopic methods. Further, antibacterial and antifungal activities of these derivatives have been studied in dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO).

II. METHODS

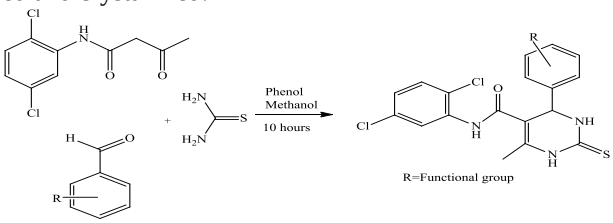
Reagent grade chemicals were used without further purification. All the melting points were taken by open capillaries. The purity of the synthesized compounds was checked by Thin Layer Chromatography.

Synthesis of RAT-1 to RAT-10

Synthesis of N-(2,5-dichlorophenyl)-3-oxobutanamide: A mixture of 2,5-dichloro aniline and ethyl acetoacetate in toluene was refluxed for 12 hours in presence of sodium hydroxide. The excess of toluene was distilled out and the reaction mixture was poured in hexane with stirring. The resulting product was isolated in hexane and filtered. It was dried and dissolved in aqueous NaOH solution. On neutralization with dilute hydrochloride acid, the resulting product was precipitated and crystallized.



Synthesis of N-(2,5-dichlorophenyl)-4-(4-methoxy phenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimi dine -5-carboxamide (RAT-1): A solution of substituted benzaldehyde,N-(2,5-dichlorophenyl)-3-oxobutanamide and thiourea in methanol was refluxed for 10 hours in presence of phenol (as catalyst). The product was isolated and crystallized.



Similarly, other compounds were also synthesized.

Spectroscopic study

The characterization of synthesized compounds was done by IR, NMR and mass spectral data. IR spectra were scanned on SHIMADZU FTIR-8400 Spectrophotometer in frequency range of 4000-400 cm^{-1} by KBr–DRS Method. ^1H NMR spectral was recorded in $CDCl_3$/DMSO with tetramethylsilane (TMS) as the internal standard at 400 MHz on a BRUKER spectrophotometer. The chemical shifts are reported as parts per million (ppm). The mass spectra of synthesized compounds were recorded by GCMS-SHIMADZU-QP2010.

Biological activity

All the synthesized compounds have been screened for *in-vitro* antibacterial activity against two gram positive bacteria viz. *Bacillus cereus* ATCC11778 (BC), *Micrococcus flavus* ATCC10240 (MF) and two Gram negative bacteria viz. *Proteus mirabilis* NCIM2241 (PM) and *Escherichia coli* NCIM2241 (EC) by using Agar well diffusion method. The antifungal activity was determined against viz. *Cryptococcus Iuteolus* ATCC 32044 (CL), *Candida tropicalis* ATCC 4563 (CT) fungal strains.

All the compounds were recrystallized prior to use. The solvents, DMF and DMSO were also purified before use by standard methods (Riddick *et al*., 1986). The solutions were prepared at a concentration of 1 mg/µl for all these compounds.

Preparation of the plates and microbiological assay:

The antibacterial and antifungal evaluation was done by the agar well diffusion method (Perez *et al*., 1990; Parekh *et al*., 2005) using Mueller Hinton Agar No.2 as the nutrient medium. The agar well diffusion method was preferred in this study because it was found to be better than the disc diffusion method as suggested by Parekh et al (Parekh *et al*., 2005). The bacterial strains were activated by inoculating a loop full of test strain in 25 ml of N-broth and the same was incubated for 24 h in an incubator at 37^{o}C. Next, 28-30 ml of the molten Mueller Hinton Agar No.2 with 0.2 ml inoculum were poured in a 100 mm diameter Petri plate. To maintain an sterile condition, these procedures were done under laminar air flow. The media was allowed to solidify. After solidification, 0.85 cm ditches were made in the plates using a sterile cork borer and these were completely filled with the test solutions. The plates were incubated for 24 h at 37^{o}C. The mean value obtained for the three wells was used to calculate the zone of growth inhibition of each sample. The controls were maintained for each bacterial strain and each solvent. The inhibition zone formed by these compounds against the particular test bacterial and fungal strain determined the antibacterial activities of these synthesized compounds.

III. RESULTS AND DISCUSSION

Table 1 shows the physical properties and substituted groups (R) of synthesized compounds. The general structural formula of synthesized dihydropyrimidinthione derivatives is given in Figure 1.

Spectral data :

RAT-1: *N*-(2,5-dichlorophenyl)-4-(4-methoxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 673(C-Cl str.), 1028(C-O-C sym.), 1180(C=S str.), 1247(C-O-C asym.), 1465, 2964(C-H asym.), 1551(C=C str.), 1554(N-H sym.), 1674 (C=O str.), 2835, 3007(C-H str.), 3281(N-H asym.), **^{1}H NMR; δ ppm:** 2.35(s, 3H, CH_3), 3.82(s, 3H, Ar-OCH_3), 5.29(s, 1H, CH), 6.89-6.91(d, 2H, Ar-H, J=8 H_z), 6.97-6.98(d, 2H, Ar-H, J=8 H_z), 7.22-7.24(d, 1H, Ar-H, J=8 H_z), 7.34-7.36(d, 1H, Ar-H, J=8 H_z), 7.90(s, 1H, Ar-H), 8.15(s, 1H, NH), 9.19(s, 1H, NH), 9.86(s, 1H, NH), **Mass (m/z):**422.

RAT-2: *N*-(2,5-dichlorophenyl)-6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 683(C-Cl str), 1172(C=S str.), 1559(C=C str.), 1691 (C=O str.), 1563(N-H sym.), 1449, 2981(C-H asym.), 2829, 3017(C-H str.), 3289(N-H asym.), 2.39(s, 3H, CH_3), 5.25(s, 1H, CH), 6.95-7.12(m, 5H, **^{1}H NMR; δ ppm:** Ar-H), 7.25-7.28(d, 1H, Ar-H, J=12 H_z), 7.38-7.40(d, 1H, Ar-H, J=8 H_z), 7.98(s, 1H, Ar-H), 8.25(s, 1H, NH), 9.29(s, 1H, NH), 9.93(s, 1H, NH), **Mass (m/z):**392.

RAT-3: *N*-(2,5-dichlorophenyl)-6-methyl-2-thioxo-4-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 691 (C-Cl str.), 1169(C=S str.), 1481, 2973(C-H asym.), 1571(C=C str.), 1563(N-H sym.), 1681 (C=O str.), 2812, 3014(C-H str.), 3295(N-H asym.), **^{1}H NMR; δ ppm:** 2.01(s, 3H, CH_3), 2.37(s, 3H, Ar-CH_3), 5.39(s, 1H, CH), 6.92-6.95(d, 2H, Ar-H, J=12 H_z), 6.97-6.99(d, 2H, Ar-H, J=8 H_z), 7.13-7.15(d, 1H, Ar-H, J=8 H_z), 7.19-7.21(d, 1H, Ar-H, J=8 H_z), 7.89(s, 1H, Ar-H), 8.11(s, 1H, NH), 9.09(s, 1H, NH), 9.81(s, 1H, NH), **Mass (m/z):**406.

RAT-4: *N*-(2,5-dichlorophenyl)-4-(4-fluorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 669 (C-Cl str.), 701(C-F str.), 1180(C=S str.), 1465, 2964(C-H asym.), 1555(C=C str.), 1568(N-H sym.), 1671 (C=O str.), 2844, 3009(C-H str.), 3299(N-H asym.), **^{1}H NMR; δ ppm:** 2.18(s, 3H, CH_3), 5.21(s, 1H, CH), 6.96-6.99(d, 2H, Ar-H, J=12 H_z), 7.03-7.05(d, 2H, Ar-H, J=8 H_z), 7.24-7.26(d, 1H, Ar-H, J=8 H_z), 7.29-7.32(d, 1H, Ar-H, J=12 H_z), 7.96(s, 1H, Ar-H), 8.29(s, 1H, NH), 9.33(s, 1H, NH), 10.01(s, 1H, NH), **Mass (m/z):**410.

RAT-5: *N*-(2,5-dichlorophenyl)-4-(4-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 677(C-Cl str.), 1158(C=S str.), 1457, 2969(C-H asym.), 1546(C=C str.), 1563(N-H sym.), 1688 (C=O str.), 2847, 3024(C-H str.), 3299(N-H asym.), 3545(O-H str.), **^{1}H NMR; δ ppm:**2.27(s, 3H, CH_3), 5.07(s, 1H, CH), 6.89-6.91(d, 2H, Ar-H, J=8 H_z), 6.96-6.99(d, 2H, Ar-H, J=12 H_z), 7.15-7.17(d, 2H, Ar-H, J=8 H_z), 7.26-7.28(d, 1H, Ar-H, J=8 H_z), 7.29-7.32(d, 1H, Ar-H, J=12 H_z), 7.83(s, 1H, Ar-H), 8.41(s, 1H, NH), 9.09(s, 1H, OH), 9.32(s, 1H, NH), 10.21(s, 1H, NH), **Mass (m/z):**408.

RAT-6: 4-(3-chlorophenyl)-*N*-(2,5-dichlorophenyl)- 6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 669(C-Cl str.), 1138(C=S str.), 1462, 2998(C-H asym.), 1578(C=C str.), 1561(N-H sym.), 1692 (C=O str.), 2812, 3017(C-H str.), 3309(N-H asym.), **^{1}H NMR; δ ppm:** 2.33(s, 3H, CH_3), 5.08(s, 1H, CH), 6.88(s, 1H, Ar-H), 6.91-6.93(d, 1H, Ar-H, J=8 H_z), 6.95-6.97(d, 1H, Ar-H, J=8 H_z), 7.03-7.05(t, 1H, Ar-H, J=8 H_z), 7.24-7.26(d, 1H, Ar-H, J=8 H_z), 7.29-7.32(d, 1H, Ar-H, J=12 H_z), 7.91(s, 1H, Ar-H), 8.41(s, 1H,

NH), 9.56(s, 1H, NH), 10.11(s, 1H, NH), **Mass (m/z):**426.

RAT-7: *N*-(2,5-dichlorophenyl)-6-methyl-4-(3-nitro phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 653 (C-Cl str.), 1147(C=S str.), 1485, 3014 (C-H asym.), 1589(C=C str.), 1583(N-H sym.), 1681 (C=O str.), 2834, 3039(C-H str.), 3323(N-H asym.), **^{1}H NMR; δ ppm:** 2.09(s, 3H, CH_3), 4.12(s, 1H, CH), 6.25(s, 1H, Ar-H), 6.28-6.30(d, 1H, Ar-H, J=8 H_z), 6.32-6.35t, 1H, Ar-H, J=12 H_z), 6.41-6.43(d, 1H, Ar-H, J=8 H_z), 7.13-7.16(t, 1H, Ar-H, J=12 H_z), 7.41-7.43(d, 1H, Ar-H, J=8 H_z), 7.45-7.47(d, 1H, Ar-H, J=8 H_z), 7.81(s, 1H, Ar-H), 8.69(s, 1H, NH), 9.76(s, 1H, NH), 10.32(s, 1H, NH), **Mass (m/z):**437.

RAT-8: 4-(2-chlorophenyl)-*N*-(2,5-dichlorophenyl)- 6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 659 (C-Cl str.), 1161(C=S str.), 1455, 2995 (C-H asym.), 1579(C=C str.), 1567(N-H sym.), 1692 (C=O str.), 2849, 3065(C-H str.), 3346(N-H asym.), **^{1}H NMR; δ ppm:** 2.26(s, 3H, CH_3), 5.22(s, 1H, CH), 6.81-7.08(m, 4H, Ar-H), 7.34-7.37(d, 1H, Ar-H, J=12 H_z), 7.42-7.44(d, 1H, Ar-H, J=8 H_z), 7.45-7.47(d, 1H, Ar-H, J=8 H_z), 7.78(s, 1H, Ar-H), 8.46(s, 1H, NH), 9.63(s, 1H, NH), 10.16(s, 1H, NH), **Mass (m/z):**426.

RAT-9: *N*-(2,5-dichlorophenyl)-6-methyl-4-(2-nitro phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 674(C-Cl str.), 1172(C=S str.), 1463, 2989 (C-H asym.), 1586(C=C str.), 1594(N-H sym.), 1672 (C=O str.), 2857, 3081(C-H str.), 3353(N-H asym.), **^{1}H NMR; δ ppm:** 2.07(s, 3H, CH_3), 5.11(s, 1H, CH), 6.73-7.04(m, 4H, Ar-H), 7.14-7.16(d, 1H, Ar-H, J=8 H_z), 7.20-7.23(d, 1H, Ar-H, J=12 H_z), 7.27-7.29(d, 1H, Ar-H, J=8 H_z), 7.69(s, 1H, Ar-H), 8.76(s, 1H, NH), 9.77(s, 1H, NH), 10.21(s, 1H, NH), **Mass (m/z):**437.

RAT-10: *N*-(2,5-dichlorophenyl)-4-(2-hydroxyphenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxamide.

IR (KBR) cm^{-1}: 688(C-Cl str.), 1159(C=S str.), 1438, 2999 (C-H asym.), 1567(C=C str.), 1587(N-H sym.), 1689 (C=O str.), 2852, 3058(C-H str.), 3391(N-H asym.), 3567(O-H str.), **^{1}H NMR; δ ppm:** 2.43(s, 3H, CH_3), 5.04(s, 1H, CH), 6.64-7.09(m, 4H, Ar-H), 7.32-7.35(d, 1H, Ar-H, J=12 H_z), 7.39-7.41(d, 1H, Ar-H, J=8 H_z), 7.94(s, 1H, Ar-H), 8.44(s, 1H, NH), 9.38(s, 1H, Ar-OH), 9.63(s, 1H, NH), 10.06(s, 1H, NH), **Mass (m/z):**408.

Figures 2 and 3 show the inhibition zones of synthesized compounds against the Gram positive and Gram negative bacterial strains in DMF and DMSO.

It is observed from Fig. 2 that in DMF, all the compounds exhibited inhibition against *B. cereus*, whereas in DMSO, compound RAT-10 showed no inhibition at all. However, for *M. flavus*, RAT-10 showed maximum inhibition in both the solvents. However, against *M. flavus*, only RAT-2 had no effect at all in DMF whereas in DMSO, three compounds; RAT-2, RAT-3 and RAT-8 had no effect at all.

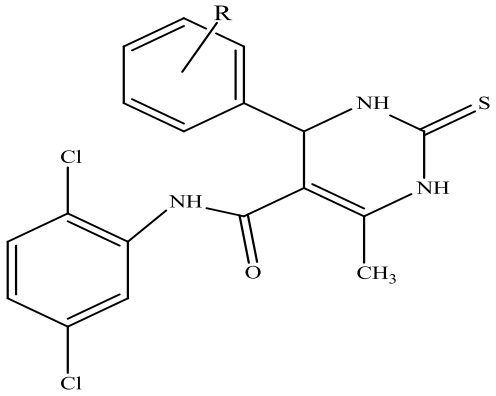


Figure 1: General structural formula of synthesized compounds.

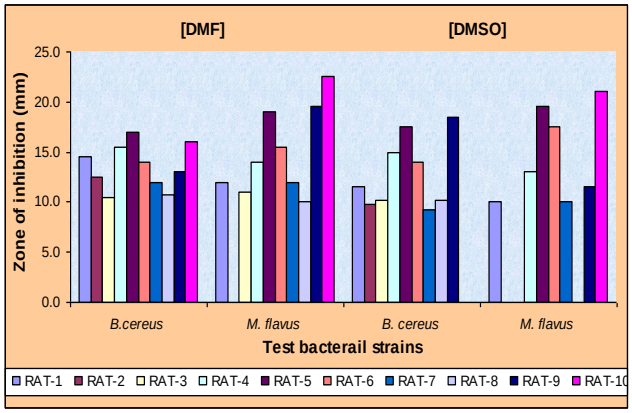


Figure 2: Antibacterial activity of dihydropyrimidinthione derivatives against Gram positive bacteria in DMF and DMSO.

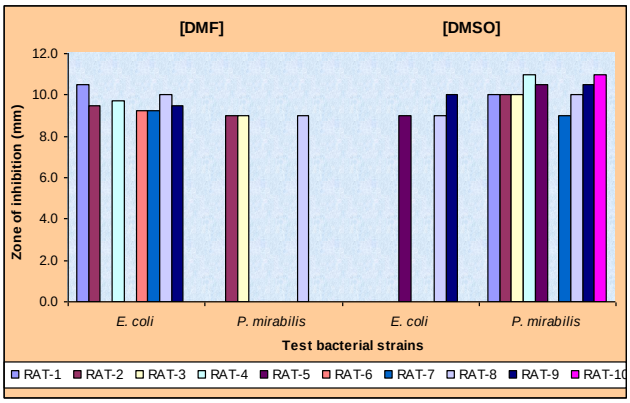


Figure 3: Antibacterial activity of dihydropyrimidinthione derivatives against Gram negative bacteria in DMF and DMSO.

The inhibition depends on the three factors: solvent, strain and substitution. All the compounds contain the same central moiety with different side chains. Thus, compound RAT-10 which contains o-hydroxy substitution showed maximum inhibition in DMF for both Gram positive bacteria. However, in DMSO, RAT-10 showed maximum inhibition only for *M. flavus* and had no effect at all against *B. cereus* where o-nitro substitution (as in RAT-9) was found to be most effective. When there was no substitution in aryl ring as in RAT-2, no inhibition was observed against *M. flavus* in both the solvents. Further, p-methyl (as in RAT-3) and o-

chloro (as in RAT-8) substitution had no effect against *M. flavus* in DMSO.

Table-1:- Physical data of synthesized compounds

Code	R	M.Wt. g/mol	R_f* Value	M.P. ^oC	Yield %
RAT-1	4-OCH_3	422	0.51	180	56
RAT-2	-H	392	0.62	194	61
RAT-3	4-CH_3	406	0.42	211	53
RAT-4	4-F	410	0.46	254	62
RAT-5	4-OH	408	0.43	194	57
RAT-6	3-Cl	426	0.46	167	60
RAT-7	3-NO_2	437	0.57	235	48
RAT-8	2-Cl	426	0.61	247	41
RAT-9	2-NO_2	437	0.58	238	63
RAT-10	2-OH	408	0.53	208	60

***** Methanol: Chloroform 2.5:7.5

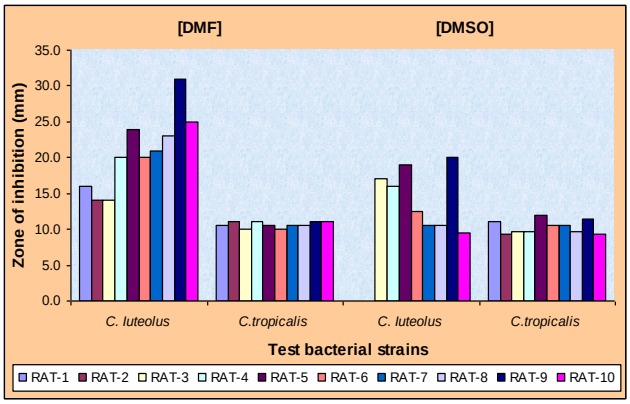


Figure 4: Antifungal activity of dihydropyrimidinthione derivatives in DMF and DMSO.

Figure 3 shows the inhibition zone of compounds against gram negative bacteria viz. *P. mirabilis* and *E. coli* in DMF and DMSO. It is observed that against *E. coli*, maximum inhibition is exhibited by RAT-1 and RAT-9 in DMF and DMSO respectively. In DMSO, most of the studied compounds had no effect against *E. Coli.* Thus, for *E. Coli*, p-methoxy substitution (as in RAT-1) and o-nitro substitution (as in RAT- 9) are most effective in DMF and DMSO respectively.

In DMF, against *P. mirabills*, only RAT-2, RAT-3 and RAT-8 showed inhibition and that inhibition is almost to the same extent. Rest of the compounds had no effect at all. Whereas in DMSO, only RAT-6 showed no inhibition while p-fluoro substitution (as in RAT-4) and o-hydroxyl substitution (as in RAT-10) exhibited maximum inhibition against *P. mirabills*.

Thus, in DMF, *P. mirabills* is most resistant bacteria whereas in DMSO, *E. Coli* and *M. flavus* are resistant bacteria.

Figure 4 shows inhibition against fungal strains. For *C. luteolus*, inhibition is maximum in both DMF and DMSO. RAT-9 containing o-nitro substitution exhibited maximum inhibition in both the solvents whereas RAT-1 containing p-methoxy substitution showed no inhibition in DMSO. In both the solvents, all compounds exhibited more or less similar inhibition against *C. tropicalis*. The inhibition against this fungal strain is almost half than that for *C. luteolus*. Thus, *C. tropicalis* is resistant strain for the studied compounds and there is not much effect of solvent for this strain.

III. CONCLUSIONS

A novel series of dihydropyrimidinthione derivatives have been synthesized and characterized by IR, ^1H NMR and mass spectral data. All the compounds were screened for antibacterial and antifungal activity in DMF and DMSO. DMF is found to be a better solvent for both antibacterial and antifungal activity. Antibacterial activity is found to be higher for RAT-10.

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Received: August 30, 2013
Accepted: February 10, 2014
Recommended by Subject Editor: Marcelo Seckler