Synthesis and *in vitro* microbiological evaluation of 5-acetyl-4-aryl-6-methyl-3,4-dihydropyrimidin-2(1H)-thiones using calcium fluoride as catalyst

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Abstract

Seven 5-acetyl-4-aryl-6-methyl-3,4-dihydropyrimidin-2(1H)-thiones **10-16** are prepared by a one-pot cyclocondensation reaction of acetylacetone (**1**), thiourea (**2**) and aldehyde (**3-9**) in ethanol using calcium fluoride as the catalyst is described. All the compounds are screened for their antibacterial activity against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and antifungal activity against *Candida albicans, Aspergillus flavus, Rhizopus* and *Mucor*. Ciprofloxacin is used for the standard for antibacterial and Amphotericin B is used for the standard for antifungal studies. Compounds **12-15** exhibited excellent *in vitro* antibacterial activity against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Whereas the same set of compounds exerted potent *in vitro* antifungal activity against *Candida albicans* and *Aspergillus flavus*.

Keywords: 5-acetyl-4-aryl-6-methyl-3,4-dihydropyrimidin-2(1H)-thiones, synthesis, antibacterial activity, antifungal activity

Introduction

Dihydropyrimidinone derivatives are known to exhibit a wide range of biological activities such as antiviral, antitumour, antibacterial and anti-inflammatory properties [1]. In addition, these compounds have emerged [2] as potential calcium channel blockers, antihypertensive, α-1a-antagonists and neuropeptide antagonists. Dihydropyrimidinones have been used as anticancer drugs capable of inhibiting kinesin motor protein [3]. Recently, several marine alkaloids with interesting biological activities were also found to contain the dihydropyrimidinone-5-carboxylate core [4]. Most notably among them are the batzalladine alkaloids which have been found to be potent human immunodeficiency virus (HIV) gp-120 CD4 inhibitors [5]. Importantly, all the dihydropyrimidin-2(1H)-ones are pharmacologically active as antioxidant agents [6]. Now-a-days sulphur containing compounds possessing diverse type of biological properties [7,8], cardiovascular activity [9]. Dihydropyrimidine derivatives have a wide range of biological [10] and anti-hypertensive activity [11].

These observations places new emphasis on the need of as well as search for alternative new and more effective antimicrobial agents with a broad spectrum.

We wish to report a simple but effective procedure for Biginelli's three component condensation producing high yields of 3,4-dihydropyrimidin-2(1H)-thiones by employing calcium fluoride as a reusable and inexpensive catalyst and evaluated their biological importance **10-16**. In order to extend our knowledge in structure-activity relationship, all the newly synthesized compounds are tested for their *in vitro* antibacterial and antifungal activities and the influence of some structural variations by varying the substituents at the phenyl ring in the synthesized compounds towards their biological activities is evaluated.

To percept structure-activity relationship well, numberings of the target compound is shown Fig. 1.

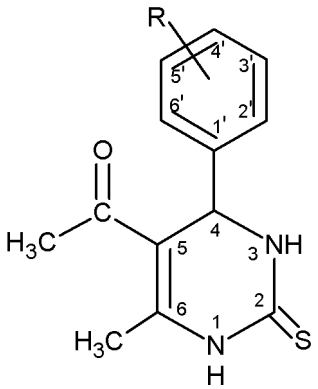


Figure 1

Numbering of 10-16

Materials and Methods

Chemistry

Melting points were determined in open capillaries and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX 400 spectrometer operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C in DMSO-d_6. For recording ^{1}H NMR spectra, solutions were prepared by dissolving about 10 mg of the compound in 0.5 ml of the solvent. For recording ^{13}C NMR spectra, solutions were prepared by dissolving about 50 mg of the compound in 0.5 ml of the solvent. IR spectra were recorded in KBr discs on an Avatar (300 FT-IR) Thermo Nicolet spectrometer.

Preparation and characterization of DHPMs

By adopting the literature procedure [12], the following 3,4-dihydropyrimidin-2(1H)-thiones **10-16** were prepared.

A mixture of acetylacetone (10 mmol), thiourea (15 mmol), aldehyde (10 mmol), CaF_2 (1 mmol, 10 mol %) and EtOH (20 ml), was heated at 40°C. The progress of the reaction was monitored by TLC. The completion of the reaction was inferred by the absence of the spot for the aromatic aldehyde. After completion of the reaction, the reaction mixture was cooled to room temperature and poured into crushed ice. The crude product containing also the catalyst was collected on a Buchner funnel by filtration. The mixture of the product and the catalyst was digested in methanol (40 ml). The undissolved catalyst was removed by filtration. The crude product was obtained by evaporation of methanol and further purified by recrystallization from hot ethanol to afford pure dihydropyrimidin-2(1H)-thiones. The catalyst could be reused in the next run. All the products were characterized by elemental analyses, IR, ^{1}H NMR and ^{13}C NMR spectra. **10** and **11** the observed spectral data were in excellent agreement with those reported [13]. Only for the newly synthesized compounds the spectral data are given below.

5-Acetyl-4-(4-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-thione ***12***. IR (KBr) (cm^{-1}): 3281 and 3179 (N-H str.), 2995 (aromatic C-H str.), 2922 (aliphatic C-H str.), 1621 (C=O str.), 1578 (C=S str.) ^{1}H NMR (δ ppm): 10.25 (s, 1H, H-1), 9.68 (s, 1H, H-3), 7.25-7.42 (m, 4H, aromatic CH), 5.33 (s, 1H, H-4), 2.37 (s, 3H, methyl protons at C-6), 2.17 (s, 3H, methyl protons of the acetyl group). ^{13}C NMR (δ ppm): 194.2 (carbonyl carbon), 174.1 (C = S), 144.5 (C-6), 141.3 (*ipso* carbon of the aryl group), 132.5 (chlorine bearing aromatic carbon), 128.2 and 128.1 (other aromatic carbons), 110.1 (C-5), 53.5 (benzylic carbon at C-4), 30.2 (methyl carbon of the acetyl group), 18.3 (methyl carbon at C-6).

5-Acetyl-4-(2-chlorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-thione ***13***. IR (KBr) (cm^{-1}): 3235 and 3176 (N-H str.), 3002 (aromatic C-H str.), 2931 (aliphatic C-H str.), 1626 (C=O str.), 1567 (C=S str.) ^{1}H NMR (δ ppm): 10.08 (s, 1H, H-1), 8.63 (s, 1H, H-3), 7.15-7.32 (m, 4H, aromatic CH), 5.73 (d, 1H, *J* = 4Hz, H-4), 2.34 (s, 3H, methyl protons at C-6), 1.99 (s, 3H, methyl protons of the acetyl group). ^{13}C NMR (δ ppm): 195.4 (carbonyl carbon), 175.0 (C = S), 145.6 (C-6), 139.6 (*ipso* carbon of the aryl group), 132.8 (chlorine bearing aromatic carbon), 130.3, 130.1, 129.5 and 128.3 (other aromatic carbons), 109.7 (C-5), 52.8 (benzylic carbon at C-4), 30.5 (methyl carbon of the acetyl group), 19.0 (methyl carbon at C-6).

5-Acetyl-4-(4-fluorophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-thione ***14***. IR (KBr) (cm^{-1}): 3232 and 3199 (N-H str.), 3005 (aromatic C-H str.), 2919 (aliphatic C-H str.), 1635 (C=O str.), 1584 (C=S str.) ^{1}H NMR (δ ppm): 9.94 (s, 1H, H-1),

9.40 (s, 1H, H-3), 7.18-7.22 (m, 2H, aromatic CH), 6.88-6.93 (d, 2H, aromatic CH), 5.27 (d, 1H, *J* = 4Hz, H-4), 2.28 (s, 3H, methyl protons at C-6), 2.06 (s, 3H, methyl protons of the acetyl group). ^{13}C NMR (δ ppm): 195.7 (carbonyl carbon), 174.8 (C = S), 162.5 (fluorine bearing aromatic carbon), 145.l (C-6), 139.2 (*ipso* carbon of the aryl group), 129.2, 115.9 (other aromatic carbons), 111.2 (C-5), 54.6 (benzylic carbon at C-4), 30.9 (methyl carbon of the acetyl group), 19.3 (methyl carbon at C-6).

5-Acetyl-4-(4-nitrophenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-thione ***15***. IR (KBr) (cm^{-1}): 3260 and 3178 (N-H str.), 2995 (aromatic C-H str.), 2924 (aliphatic C-H str.), 1618 (C=O str.), 1583 (C=S str.) ^{1}H NMR (δ ppm): 10.32 (s, 1H, H-1), 9.74 (s, 1H, H-3), 8.09 (d, 2H, *J* = 8Hz, aromatic CH), 7.44 (d, 2H, *J* = 8Hz, aromatic CH), 5.40 (d, 1H, *J* = 4Hz, H-4), 2.32 (s, 3H, methyl protons at C-6), 2.17 (s, 3H, methyl protons of the acetyl group). ^{13}C NMR (δ ppm): 194.8 (carbonyl carbon), 175.6 (C = S), 150.6 (nitrogen bearing aromatic carbon), 147.6 (C-6), 146.1 (*ipso* carbon of the aryl group), 128.6, 124.3 (other aromatic carbons), 111.1 (C-5), 54.2 (benzylic carbon at C-4), 31.4 (methyl carbon of the acetyl group), 19.4 (methyl carbon at C-6).

5-Acetyl-4-(4-methoxyphenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-thione ***16***. IR (KBr) (cm^{-1}): 3232 and 3158 (N-H str.), 3004 (aromatic C-H str.), 2930 (aliphatic C-H str.), 1616 (C=O str.), 1579 (C=S str.) ^{1}H NMR (δ ppm): 9.54 (s, 1H, H-1), 8.99 (s, 1H, H-3), 7.13 (d, 2H, *J* = 8Hz, aromatic CH), 6.74 (d, 2H, *J* = 8Hz, aromatic CH), 5.23 (d, 1H, *J* = 4Hz, H-4), 3.68 (s, 3H, methoxy protons at the aryl ring), 2.28 (s, 3H, methyl protons at C-6), 2.01 (s, 3H, methyl protons of the acetyl group). ^{13}C NMR (δ ppm): 195.8 (carbonyl carbon), 174.6 (C = S), 159.6 (methoxy bearing aromatic carbon), 144.6 (C-6), 135.5 (*ipso* carbon of the aryl group), 128.7, 114.4 (other aromatic carbons), 111.0 (C-5), 55.4 (benzylic carbon at C-4), 30.7 (methyl carbon of the acetyl group), 19.2 (methyl carbon at C-6).

In compounds **10**, **11** and **13-16** the benzylic proton appeared as a doublet at around 5.48 ppm. This is due to the coupling with adjacent NH(H-3) proton. In compound **12** the benzylic proton appeared as a broad singlet at 5.33 ppm due to a poor resolution of the coupling with NH(H-3) proton.

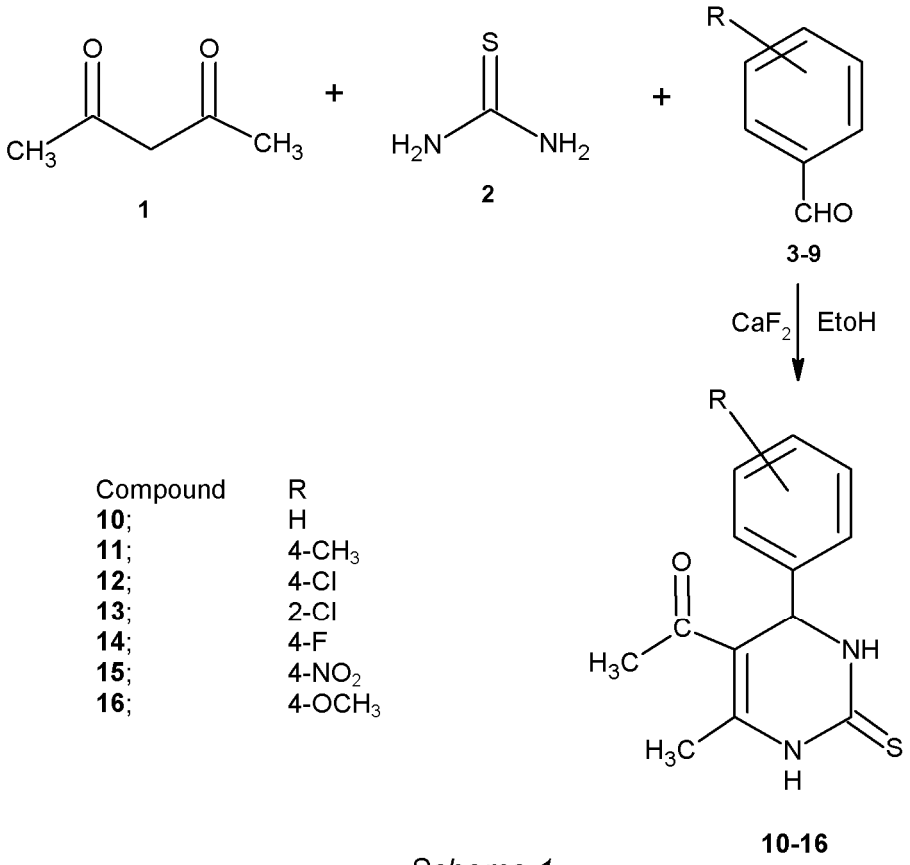
Microbiology

All the bacterial strains namely *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and fungal strains namely *Candida albicans, Aspergillus flavus, Rhizopus* and *Mucor* were obtained from Faculty of Medicine, Annamalai University, Annamalainagar – 608 002, Tamil Nadu, India.

In vitro antibacterial and antifungal activity. The *in vitro* antimicrobial activities of the compounds were tested in Sabouraud's dextrose broth (SDB, Hi-media, Mumbai) for fungi and nutrient broth (NB, Hi-media, Mumbai) for bacteria by the twofold serial dilution method [14]. The test compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain 1 mg/ml stock solutions. Seeded broth (broth containing microbial spores) was prepared in NB from 24 hrs old bacterial cultures on nutrient agar (Hi-media, Mumbai) at 37 ± 1°C while fungal spores from 24 hrs to 7-day-old Sabouraud's agar slant cultures were suspended in SDB. The colony forming units (cfu) of the seeded broth were determined by the plating technique and adjusted in the range of 10^4–10^5 cfu/ml. The final inoculum size was 10^5 cfu/ml for the antibacterial assay and 1.1–1.5 × 10^2 cfu/ml for the antifungal assay. Testing was performed at 7.4 ± 0.2. Exactly 0.2 ml of the solution of test compound was added to 1.8 ml of seeded broth to form the first dilution. One ml of this was diluted with a further 1 ml of the seeded broth to give the second dilution and so on until six such dilutions were obtained. A set of assay tubes containing only seeded broth was kept as control and likewise solvent controls were also run simultaneously. The tubes were incubated in biochemical oxygen demand (BOD) incubators at 37 ± 1°C for bacteria and 28 ± 1°C for fungi. The minimum inhibitory concentrations (MICs) were recorded by visual observations after 24 hrs (for bacteria) and 72–96 hrs (for fungi) of incubation. Ciprofloxacin was used as a standard for the bacterial study while Amphotericin B was used as a standard for the fungal study.

Results and discussion

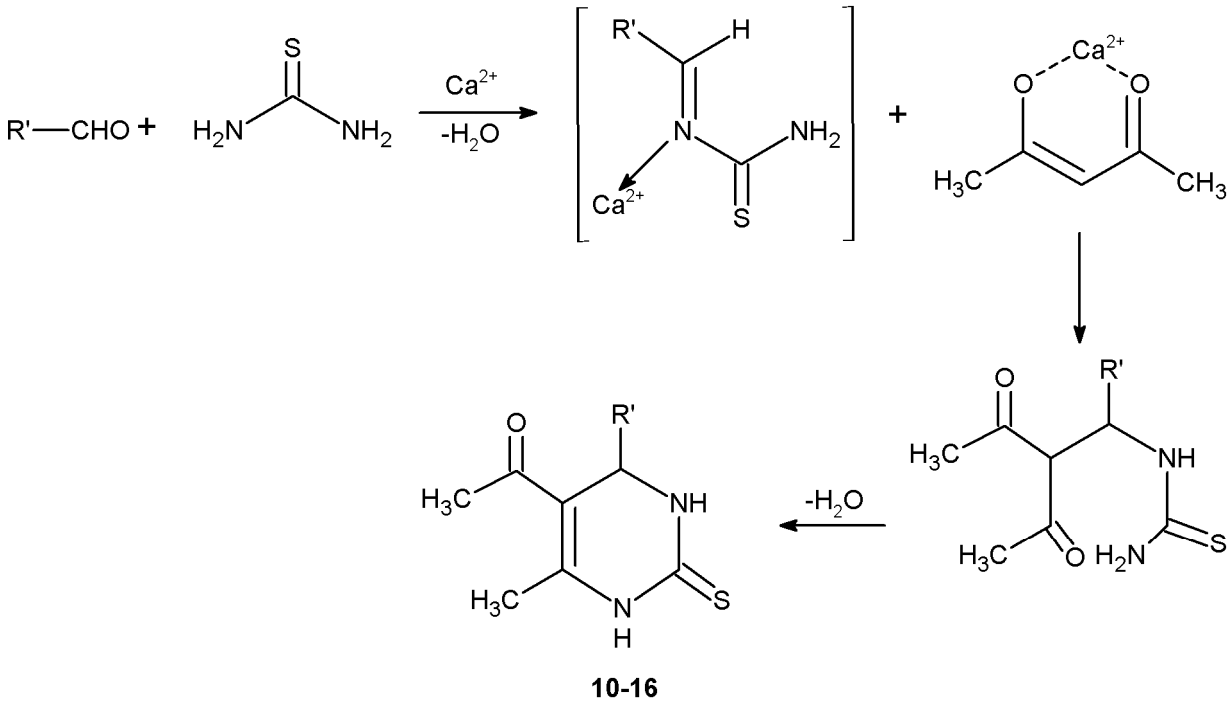
Target molecules 3,4-dihydropyrimidin-2(1H)-thiones **10-16** were synthesized as a result of a one-step synthetic strategy. Synthetic route for the formation of 3,4-dihydropyrimidin-2(1H)-thiones **10-16** is as follows: A mixture of acetylacetone (**1**), thiourea (**2**) and aldehyde **3-9** in the ratio of 1:1.5:1 in ethanol with CaF_2 as a reusable catalyst was heated to reflux for appropriate time (Table 1) to afford 3,4-dihydropyrimidin -2(1H)-thiones **10-16**. The schematic representation and the physical data of compounds **10-16** are given in Scheme 1 and Table 1, respectively and the possible proposed mechanism [15] of formation is shown in Scheme 2. The structure of the newly synthesized compounds **10-16** was confirmed by melting point, FT-IR, one dimensional NMR (^{1}H and ^{13}C) spectroscopic data.



Compound	R
10;	H
11;	4-CH_3
12;	4-Cl
13;	2-Cl
14;	4-F
15;	4-NO_2
16;	4-OCH_3

Scheme 1

Reaction route for the synthesis of 5-acetyl-4-aryl-6-methyl-3, 4-dihydropyrimidin-2(1H)-thions 10-16



Scheme 2

Probable reaction mechanism for the synthesis of target molecules

Table I. Physical and analytical data for compounds 10-16

Compounds	R	Time (h)	Yield (%)[a]	m.p°C
10	H	2	96	250–251
11	4-CH_3	3	94	250–251
12	4-Cl	2	90	256–257
13	2-Cl	1.5	84	211–212
14	4-F	2	90	234–235
15	4-NO_2	3	82	245–246
16	4-OCH_3	2.5	90	196–197

[a]Yields refer to pure solid products, properly characterized by spectral (IR, ^{1}H NMR, ^{13}C NMR) and analytical data

Many of the pharmacologically relevant substitution patterns on the aromatic ring could be introduced with high efficiency. Another important feature of this procedure is the survival of a variety of functional groups such as methyl, methoxy, chloro, nitro, fluoro under the reaction conditions.

A variety of substituted aromatic aldehydes carrying either electron releasing or electron withdrawing substituents in the *ortho* and *para* positions afford high yields of products in high purity.

*Reuse of calcium fluoride catalyst in the synthesis of 5-acetyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-thione(**10**).* Calcium fluoride catalyst can be recovered and reused upto six times (Figure 1) by recrystallized in methanol (40 ml). The undissolved catalyst was removed by filtration and then dried. After, the catalyst could be reused in the next run.

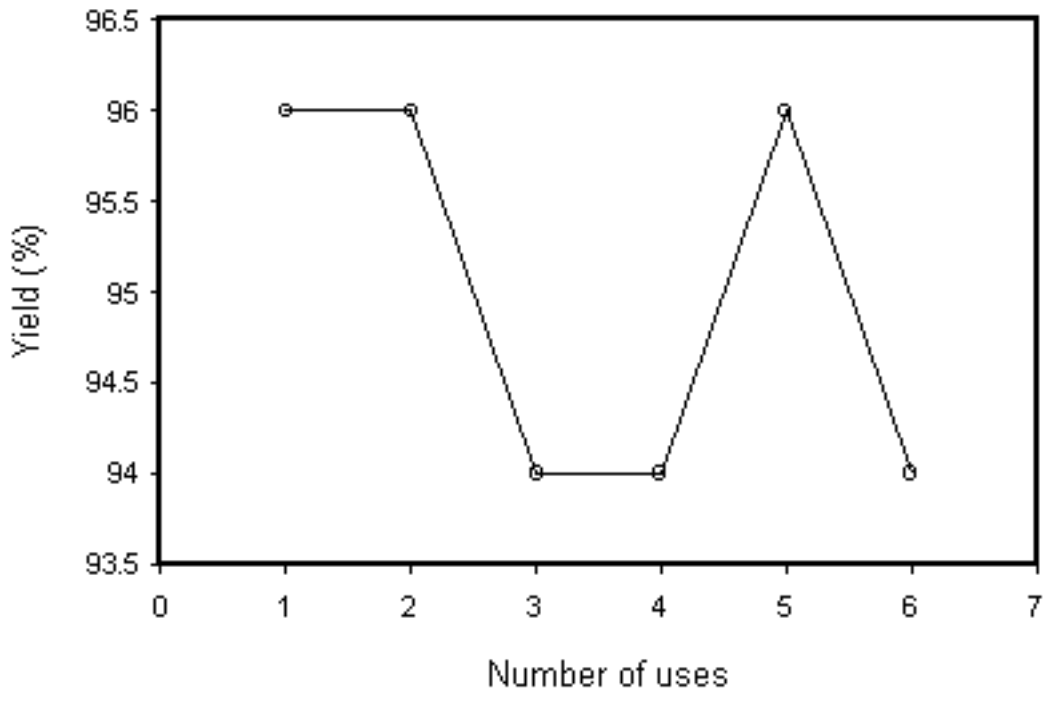


Figure 2

Re-use of CaF_2 in the synthesis of 5-acetyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-thione 10

Antibacterial activity

The synthesized 5-acetyl-4-aryl-6-methyl-3,4-dihydropyrimidin-2(1H)-thiones **10-16** were tested for their antibacterial activity *in vitro* against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhi.* Ciprofloxacin was used as standard drug whose minimum inhibitory concentration (MIC) values were provided in Table 2. In general the dihydropyrimidinones **10-16** exerted a wide range of modest antibacterial activity *in vitro* against the tested organisms.

Table II. *In vitro* antibacterial activities (MIC) values for compounds 10-16

Compounds	Minimum inhibitory concentration (MIC) in μg/ml				
	Staphylococcus aureus	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Salmonella typhi*
10	100	100	200	100	200
11	50	100	100	50	200
12	25	12.5	25	12.5	25
13	12.5	12.5	25	25	25
14	6.25	6.25	12.5	12.5	12.5
15	3.13	3.13	6.25	3.13	6.25
16	50	25	100	50	100
Ciprofloxacin	6.25	12.5	25	12.5	50

Compound **10** without any substituent at *para* position of the aryl moiety at C-4 position of the six membered heterocyclic ring exhibited antibacterial activity *in vitro* at 100 μg/ml against all the tested organisms except. *K. pneumoniae* and *S. typhi.* They inhibit at a MIC of 200 μg/ml.

Due to the introduction of methyl group at the *para* position of the aryl group at C-4 position of the six membered heterocyclic moiety in the place of hydrogen function in **10** (*i.e.,* in **11**) results two fold increase in the activity against all the tested organisms except *E. coli* and *S. typhi.* There is no change in the antibacterial activity against *E. coli* and *S. typhi.*

Due to the introduction of chloro group at the *para* position of the aryl moiety at C-4 position of the six membered heterocyclic ring in the place of methyl function in **11** (*i.e.,* in **12**) showed increase in activity against all the tested organisms.

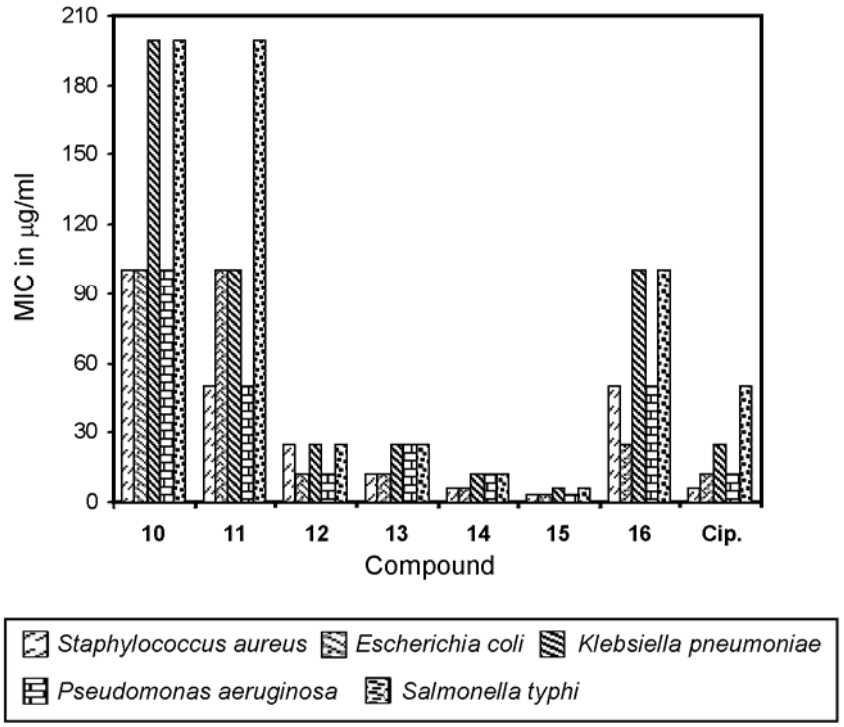
Replacement of hydrogen present at the *ortho* position of the aryl moiety at C-4 position of the six membered heterocyclic ring by a chloro function in **10** (*i.e.,* in **13**) results activity in the range of 12.5 to 25 μg/ml against all the tested organisms.

Instead of chloro functionality, substitution of fluoro group in **12** (i.e., in **14**) showed excellent activity against all the tested organisms.

Due to the introduction of nitro group at the *para* position of the aryl group at C-4 position of the six membered heterocyclic moiety in the place of fluoro function in **14** (*i.e.,* in **15**) results amazing antibacterial activity against all the tested organisms.

Replacement of hydrogen present at the *para* position of the aryl moiety at C-4 position of the six membered heterocyclic ring by a methoxy function in **15** (*i.e.,* in **16**) the activity was suppressed against all the tested organisms.

A comparative studies of minimum inhibitory concentration for the compounds **10-16** using standard Ciprofloxacin versus bacterial strains given in Fig. 3.



Cip. – Ciprofloxacin

Figure 3

Comparison of minimum inhibitory concentration of compounds **10-16** with Ciprofloxacin (as standard) against bacterial strains from serial dilution method

Antifungal activity

The *in vitro* antifungal activity of the 5-acetyl-4-aryl-6-methyl-3,4-dihydropyrimidin-2(1H)-thiones **10-16** was studied against the fungal strains *viz.*, *Candida albicans*, *Aspergillus flavus*, *Rhizopus* and *Mucor*. Amphotericin B was used as a standard drug whose minimum inhibitory concentration (MIC) values were furnished in Table 3.

Table III. *In vitro* antifungal activities (MIC) values for compounds 10-16

Compounds	Minimum inhibitory concentration (MIC) in μg/ml			
	Candida albicans	*Aspergillus flavus*	*Rhizopus*	*Mucor*
10	100	100	200	200
11	50	100	100	100
12	25	25	50	50
13	25	25	50	50
14	12.5	12.5	25	25
15	6.25	6.25	6.25	25
16	50	50	50	100
Amphotericin B	25	50	12.5	12.5

'—' no inhibition even at a higher concentration of 200 μg/ml

The antifungal profile of compound **10** without any substituent at the *para* position of the aryl group at C-4 position of the six membered heterocyclic moiety falls in the region of 100-200 μg/ml against all the tested organisms.

Due to the introduction of methyl function at the *para* position of the aryl group at C-4 position of the six membered heterocyclic moiety in the place of hydrogen function in **10** (*i.e.,* in **11**) results increase in antifungal activity against all the tested organisms except *A. flavus.* There is no change in the activity against *A. flavus.*

Replacement of hydrogen present at the *para* position of the aryl moiety at C-4 position of the six membered heterocyclic ring by a chloro function in **11** (i.e., in **12**) showed activity in the range of 25 to 50 μg/ml against all the tested organisms.

Due to the introduction of chloro function at the *ortho* position of the aryl group at C-4 position of the six membered heterocyclic moiety in the place of hydrogen function in **10** (*i.e.,* in **13**) results highly increase in antifungal activity against all the tested organisms.

Replacement of hydrogen present at the *para* position of the aryl moiety at C-4 position of the six membered heterocyclic ring by a fluoro function in **12** (*i.e.,* in **14**) showed two fold increase in activity against all the tested organisms.

Instead of fluoro functionality substitution of nitro group in **14** (i.e., in **15**) results excellent activity against all the tested organisms except *Mucor*. They inhibit at a MIC of 25 μg/ml.

Due to the introduction of methoxy function at the *para* position of the aryl group at C-4 position of the six memebred heterocyclic moiety in the place of nitro function in **15** (*i.e.,* in **16**) showed highly decrease in antifungal activity against all the tested organisms.

Minimum inhibitory concentration of compounds **10-16** was compared with standard Amphotericin B against fungal strains shown in Fig. 4.

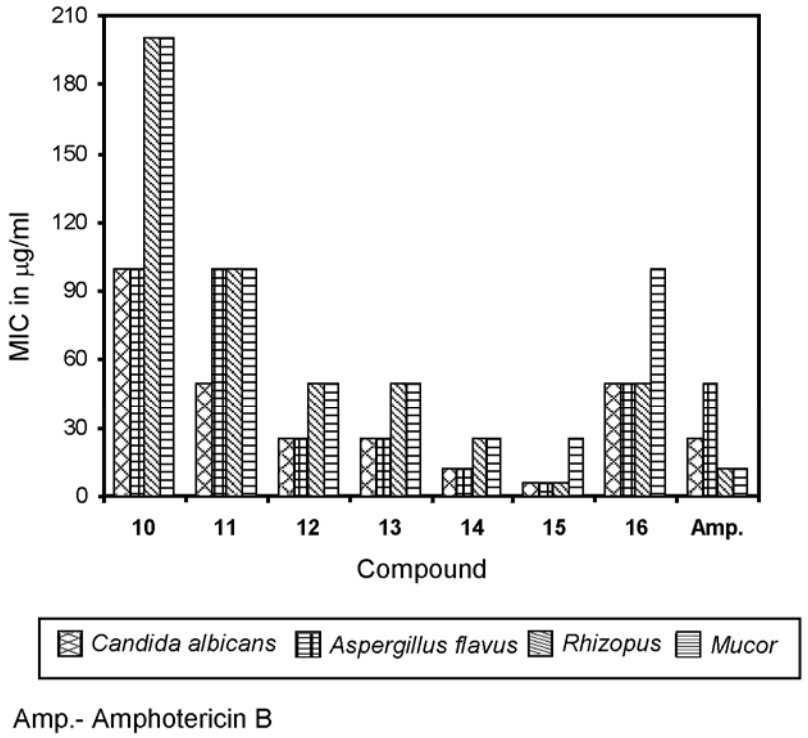


Figure 4

Comparison of minimum inhibitory concentration of compounds **10**-**16** with Amphotericin B (as standard) against fungal strains from serial dilution method

Conclusion

A close examination of the *in vitro* antibacterial and antifungal activity profile in differently substituted novel 5-acetyl-4-aryl-6-methyl-3,4-dihydropyrimidin-2(1H)-thiones **10-16** against the tested bacterial strains *viz., S. aureus, E. coli, K. pneumoniae, P. aeruginosa* and *S. typhi* and the fungal strains *viz., C. albicans, A. flavus, Rhizopus* and *Mucor* respectively, provides a better structure activity relationship correlation. This may be summarized as follows: the results of this study show that the presence of both electron-donating substituent (methyl, methoxy) and electron-withdrawing substituent (chloro, fluoro, nitro) at the *ortho*, *para* positions on the phenyl ring in compounds **10-16** are responsible for the activity against all the tested organisms.

Specifically of the some 5-acetyl-4-aryl-6-methyl-3,4-dihydropyrimidin-2(1H)-thiones tested, the compounds with nitro function at the *para* position of the aryl moiety exhibited amazing antibacterial activity against all the tested organisms and the 5-acetyl-4-aryl-6-methyl-3,4-dihydropyrimidin-2(1H)-thiones with fluoro moieties at the *para* position of the aryl group at C-4 position of the six membered heterocyclic ring showed excellent antibacterial activity against all the tested organisms.

The novel-5-acetyl-4-aryl-6-methyl-3,4-dihydropyrimidin-2(1H)-thiones with nitro moieties at the *para* position of the aryl group at C-4 position of the six membered heterocyclic ring exerted excellent antifungal activity against all the tested organisms.

These observations may promote a development of our research in this field. Further development of this group of compounds may lead to compounds with better pharmacological profile than standard drugs and serve as templates for the construction of better drugs to combat bacterial and fungal infection.

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