Int J Pharm Bio Sci 2013 Apr; 4(2): (P) 296 - 308

Research Article **Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

SYNTHESIS OF NEW ALKYL-4-(4-(DIALKYLAMINO) PHENYL)-1, 6-DIMETHYL-2-OXO/THIOXO-1, 2, 3, 4-TETRAHYDROPYRIMIDINE-5-CARBOXYLATES AND THEIR ANTI-MICROBIAL AND ANTI-MYCOBACTERIAL STUDIES

YEDDULA HARANADHA REDDY,[1] CHINTHAPARTHI RADHA RANI,[1] BHATNAGAR IRA,[2] GANGIREDDY CHANDRA SEKHAR REDDY[3] AND KALLURU SUBRAMANYAM REDDY[1]*

[1]Department of Chemistry, Sri Venkateswara University, Tirupati – 517502, India
[2]Laboratory of Infectious Diseases & Molecular Virology, Centre for Cellular and Molecular Biology, Hyderabad – 500007, India
[3]The WCU Centre for Synthetic Polymer Bio-conjugate Hybrid materials,Department of Polymer Science and Engineering, Pusan National University,Busan - 609-735, Republic of Korea

ABSTRACT

A series of novelalkyl-4-(4-(dialkylamino) phenyl)-1, 6-dimethyl-2-oxo/thioxo-1, 2, 3,4-tetrahydropyrimidine-5-carboxylates (4a-r) have been synthesized by Biginelli condensation with an efficient chloroindate(III) ionic liquid under mild reaction conditions in high yields. All the chemical structures of the title compounds were confirmed by elemental analysis and spectral data analysis. The newly synthesized compounds were evaluated their anti-microbial activity against *Escherichia coli*, *Staphylococcus aurous* and *Candida albicans* and the *in vitro* anti-tuberculosis activity also evaluated against *Mycobacterium tuberculosis* $H_{37}Rv$ (MTB). Among these compounds, 4m, 4o, 4n and 4pwere found to be the potent anti-tubercular compounds with minimum inhibition concentration (MIC).In addition, comparatively other compounds these compounds are also shown efficient biological activity against bacteria as well as fungi in order of 4m>4o>4n>4p.From the results it is indicative that these analogues were found to possess a significant broad spectrum of anti-microbial and anti-tubercular potentiality.

KEYWORDS:4-(Dialkyamino)benzaldehydes, Methyl/ethyl/propyl-3-oxobutanoate, 1-Methyl urea/thiourea, BMI.InCl$_4$ ionic liquid, Biginelli condensation, Anti-microbial activity, anti-tubercular activity

KALLURU SUBRAMANYAM REDDY
Department of Chemistry, Sri Venkateswara University, Tirupati – 517502, India

Corresponding author

INTRODUCTION

The 4-aryl-3,4-dihydropyrimidin-2(1H) ones/thiones are an important class of compounds exhibiting broad spectra of pharmacological activities such as anti-microbial and anti-hypertensive activities[1] and hence, in recent years these compounds gain a huge interest in the medicinal chemistry field. Moreover, this class of heterocycles revealed other pharmacological events such as anti-inflammatory,[2] anti-fungal, anti-bacterial activity,[3] calcium channel modulators,[4] as an antagonist to melanin concentrating hormone receptor (MCH1-R),[5] chemical modulators of heat shock protein 70 (Hsp 70),[6] hepatitis-B replication inhibitors and inhibitors of the fatty acid transporters.[7] This set of potentialities linked to the possibility of chemical modulation in all positions of the dihydropyrimidineone/thione rings make dihydropyrimidines a privileged structure, justifying the great interest in their synthesis Designing chemical products and processes that reduce or eliminate the use and generation of hazardous substances has become an ultimate goal in present-day chemistry.[8] Ionic liquids (ILs) are designed with large organic cations such as imidazolium, pyridinium or quaternary ammonium, with alkyl-chain substituents. Common IL anions include hexafluorophosphate (PF_6), tetrafluoroborate (BF_4), chloride (Cl) and bromide (Br). Ionic liquids, especially those derived from the dialkylimidazolium cation, because of their unique features, have efficiently been used in biphasic catalysis, where the product can easily be isolated by extraction and the remaining ionic liquid phase containing the catalyst can be reused. In recent years, metal-ion containing task-specific ionic liquids have attracted extensive interest, as they can combine the advantages of both homogeneous catalysis (e.g., high reactivity) and heterogeneous catalysis such as facile recovery and reusability of the catalyst.[9,10] However, the major drawbacks associated with these compounds are their difficult handling, including reactivity toward air and water. On the other hand, organoindate ionic liquids, which have similar physicochemical properties, are more stable in air, water, and organic molecules and, hence, can easily be handled. This ionic liquid has been successfully used in some reactions.[11-13] In this paper, we report the synthesis of alkyl-4-(4-(dialkylamino) phenyl)-1,6-dimethyl-2-oxo/thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates (4a-r) using chloroindate(III) ionic liquid ($BMI.InCl_4$) medium as an efficient catalyst through Biginelli condensation under mild reaction conditions (Scheme 1), and also evaluate their anti-microbial and anti-tubercular activities.

MATERIALS AND METHODS

GENERAL

All the chemicals and reagents used in the synthesis were of standard analytical grade and obtained from Merck and Sigma-Aldrich and were used without further purification. The other commercial grade solvents were distilled and stored over molecular sieves. The using drying agents were anhydrous Na_2SO_4 and $MgSO_4$. Thin-layer chromatography (TLC) was performed on Merck 60F254 plates. Reactions were monitored by TLC using ethylacetate and hexane (1:3) by detecting with UV light (254 nm) andiodine charged silica. Melting points were determined in open capillary tubes on a Mel-temp apparatus and were uncorrected. Microanalysis was performed at the University of Hyderabad, Hyderabad. IR Spectra were recorded as KBr discs on a Nicolet-380 FT-IR spectrophotometer. ^1H and ^{13}C-NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C-NMR in DMSO-d_6 and chemical shifts were referenced to tetramethylsilane (TMS). Mass spectra were recorded on Jeol SX 102 DA/ 600 mass spectrometer using Argon/ Xenon (6 keV, 10 mA) as the FAB gas.

(i) Synthesis of 1-n-Butyl-3-methylimidazolium tetrachloroindate($BMI.InCl_4$)ionic liquid

The ionic liquid 1-*n*-Butyl-3-methylimidazolium tetrachloroindate ($BMI.InCl_4$) was readily prepared from 1-*n*-butyl-3-methylimidazoium

chloride and indium trichloride by following the literature procedure.[11]

(ii) General procedure for the synthesis of methyl-4-(4-(dimethylamino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a)

A 25 mL round bottomed flask was charged with 4-(dimethylamino)benzaldehyde (1, 0.223 g, 1.5 mmol), methyl acetoacetate (2, 0.16 mL, 1.5 mmol), 1-methylurea (3, 0.148 g, 2.0 mmol) and BMI.$InCl_4$ ionic liquid (0.5 mL). The resulting mixture was stirred at 50 °C for 2 h. The progress of the reaction was monitored by TLC analysis by ethylacetate and hexane (1:3). After completion of the reaction, cold and the solidified reaction mixture was added to methanol (5 mL). The crude reaction product thus obtained was collected by filtration and further purified by recrystallization with hot ethanol to afford pure yellow solid, methyl-4-(4-(dimethylamino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a), 0.36 g, (80%), mp 136-138 °C. The filtrate so obtained was concentrated under reduced pressure to recover ionic liquid BMI.$InCl_4$, which could be reused in subsequent experiments.[14]The same procedure was adopted for the preparation of other compounds 4b-r and the spectral data of all the compounds are given under supplemental data.

(iii) Anti-microbial activity

All the title compounds (4a-r) were diluted in dimethylsulfoxide (DMSO) with required concentrations (100 mg/disc) for bioassay. Anti-microbial activity was evaluated by screening the compounds by standard agar disc diffusion method against a panel of human pathogenic microorganisms: one Gram positive bacteria, *Staphylococcus aurous* (*S. aureus*) and one Gram negative bacteria, *Escherichia coli* (*E. coli*) were used for the anti-bacterial assay, while for the anti-fungal assay, *Candida albicans* (*C. albicans*) was used. Microorganisms were maintained at 37 °C on Mueller Hinton (MH) agar slants. MH agar and Sabouraud's broth were used to evaluate anti-bacterial and anti-fungal activity respectively. To make a judgment of anti-bacterial and anti-fungal potency of the synthesized compounds, commercial antibiotics such as ciprofloxacin (10 mg/disc) and clotrimazole (10 mg/disc) in DMSO served as reference standards to compare inhibition of growth. The plates containing bacterial organism were incubated at 37 °C for 24 h and the plates containing fungal organism were incubated at 28 °C for 48 h. The zone of inhibition was calculated by measuring the diameter of zone of inhibition (ZI) for bacterial and fungal growth around the disc. Averages of three independent determinations were recorded. The minimum inhibitory concentrations (MIC) of the samples were determined by agar dilution method. MH broth was moulted and poured in sterile.[15] Overnight culture were grown at 37 °C by Kirby- Bauer procedure and diluted to the Muller Hinton Broth. 100 µL of culture was added to all the test tubes containing serial double dilution of drugs as well as the test compounds. The MIC values were evaluated by considering the concentration range 1.56-50 µg/mL of each test compound. All the tubes were incubated at 37 °C for 18-24 h. After incubation, the optical density (OD) values were observed by spectrophotometric method. The minimum inhibitory concentration (MIC) was noted by seeing the lowest concentration of the drug at which there was no visible growth of the microbe.

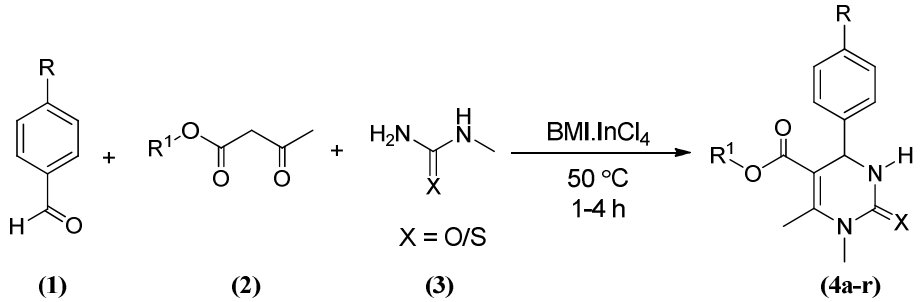
(iv) Anti-tubercular activity

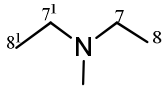
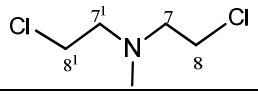
Ten-fold serial dilutions of each test compounds 4a-r were incorporated into Middle brook 7H11 agar medium with OADC as Growth Supplement. Inoculum of M. tuberculosis $H_{37}Rv$ inoculums were prepared about 10^7cfu/mL concentration from fresh Middle brook 7H11 agar slants by taking 1 mg/mL (wet weight) of OADC in Tween 80 (0.05%) saline diluted to 10^{-2}. The 7H11 agar tubes containing 10-fold serial dilutions of drug per mL were incubated at 37 ºC which are previously spotted with 5 µL amounts of bacterial suspension, and after 28 days the activity was measured. Minimum inhibitory concentration (MIC) which is the minimum concentration of compound required to inhibit the growth of bacterial completely.

RESULTS AND DISCUSSION

(i) Chemistry

Preparation of alkyl-4-(4-(dialkylamino) phenyl)-1, 6-dimethyl-2-oxo/thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates (4a-r) was accomplished in one-pot reaction (Scheme 1). The synthetic route involves the condensation of 4-(substitutedamino)benzaldehydes (1), methyl/ethyl/ propyl acetoacetate (2) and methyl urea/thiourea (3) in the presence of 1-*n*-butyl-3-methylimidazolium tetrachloroindate ($BMI.InCl_4$) ionic liquid at 50 °C about 1-4 h to get the title compoundsin high yields (Table1).



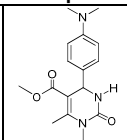
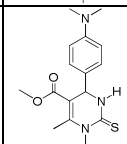
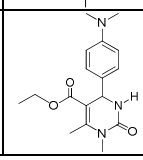
Compounds	R	Compounds	R^1
4a-f		4a, 4b, 4g, 4h, 4m, 4n	CH_3
		4c, 4d, 4i, 4j, 4o, 4p	C_2H_5
		4e, 4f, 4k, 4l, 4q, 4r	n-C_3H_7
4g-l		Compounds	X
4m-r		4a, 4c, 4e, 4g, 4i, 4k, 4m, 4o, 4q	O
		4b, 4d, 4f, 4h, 4j, 4l, 4n, 4p, 4r	S

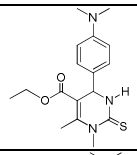
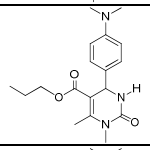
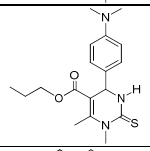
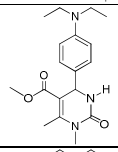
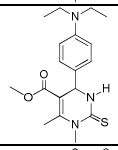
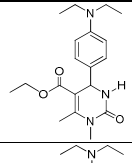
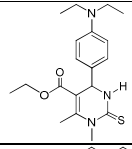
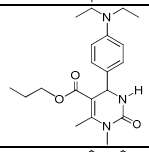
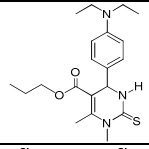
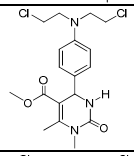
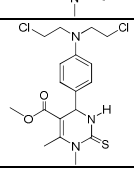
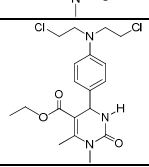
Scheme 1

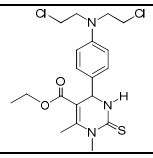
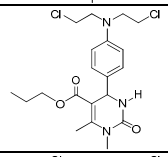
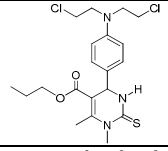
Synthesis of the alkyl-4-(4-(dialkylamino) phenyl)-1, 6-dimethyl-2-oxo/ thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates (4a-r)

Table1

Synthesis of the alkyl-4-(4-(dialkylamino) phenyl)-1, 6-dimethyl-2-oxo/thioxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates[a] (4a-r)

S.No.	Compound		Reaction time (h)	% of yield	Melting point (°C)
1	4a		2	80	136-138
2	4b		2	78	114-116
3	4c		2	82	123-125

4	4d		2	81	165-167
5	4e		2	85	147-149
6	4f		2	85	133-135
7	4g		3	84	123-125
8	4h		3	83	142-144
9	4i		2	85	129-131
10	4j		2	83	122-124
11	4k		1	86	117-119
12	4l		1	85	138-140
13	4m		4	85	119-121
14	4n		4	82	141-143
15	4o		3	86	147-149

16	4p		4	84	133-134
17	4q		2	86	134-136
18	4r		2	85	121-123

[a]*All the compounds are synthesized at 50 °C reaction temperature.*

(ii) Pharmacology

(a) Biological activities of synthesized compounds for antimicrobial potential

Gram-positive bacteria *S. aurous* (ATCC 11632) and a Gram-negative bacteria *E. coli* (ATCC 25922) and for their anti-fungal activity against *C. albicans* (ATCC 90028) by an agar disc diffusion method using ciprofloxacin and clotrimazole as reference standards, respectively. The minimum inhibition concentrations (MIC) and zone of inhibition (ZI) were determined for compounds 4a-r and the results are summarized along with that of ciprofloxacin and clotrimazole (Table 2). The All the synthesized compounds were evaluated for their *in vitro* anti-bacterial activity against a results showed that most of the designed compounds have moderate to good anti-bacterial and anti-fungal activities (MIC from 10.2 to >25.0 µg/mL). Compounds 4m, 4n**,** 4o and 4p showed significant anti-bacterial activities with a ZI of 29, 26, 28 and 28 mm, respectively. These results are comparable to that of the standard drugciprofloxacin (MIC 6.25 µg/mLand 32 mm) against *E. coli*. On the other hand, compounds 4a-f showed moderate activity of 14**,** 19**,** 17, 17, 18 and 19 mm of ZI, respectively against *S. aureus*. Similar to the anti-bacterial results, compounds 4a-r, showed moderate anti-fungal activity, whereas the compounds 4m, 4n, 4o, 4p, 4q and 4r showed promising anti-fungal activity against *C. albicans*. Among all the compounds, the test compound 4m showed highest MIC of 14.4 µg/mL, which is comparable to the standard anti-fungal drug, clotrimazole (2.5 µg/mL). The compounds from 4g to 4l does not possess significant anti-microbial activity as their MIC values and ZI values are > 25 mg/mL and < 10 mm, respectively. It was observed that, the alky chain increases on nitrogen atom of the benzene ring at para-position as well as in ester moiety which is linked to pyrimidine ring the activity gradually decreased. But when chlorines placed as substitutions at end of ethyl groups which linked to a nitrogen atom, at para-position of benzene ring the activity tremendously increased. It's may be due to the enhancement binding nature of the molecule by the presence of non-bonding electrons and more electronegativity of chlorine. And also observed that the C=O group containing compounds showed better activity than C=S compounds in pyrimidine ring.

Table 2
In vitroanti-bacterial, anti-fungal and anti-mycobacterial activities of the test compounds (4a-r)

Compounds	MIC[a](ZI)[b]			
	Anti-bacterial		Anti-fungal	Anti-mycobacterial
	E. coli 25922	*S.aureus* 11632	*C.albicans* 90028	MTB
4a	20.3 (11)	24.5 (14)	22.9 (15)	19.9
4b	24.5 (10)	21.4 (19)	24.3 (17)	17.4
4c	19.5 (13)	19.4 (17)	20.1 (15)	18.3
4d	22.6 (11)	23.9 (17)	21.4 (19)	14.8
4e	21.5 (11)	22.5 (18)	23.0 (20)	16.3
4f	24.9 (12)	22.4 (19)	19.2 (16)	18.9
4g	>25.0 (<10)	>25.0 (<10)	>25.0 (<10)	24.2
4h	>25.0 (<10)	>25.0 (<10)	>25.0 (<10)	22.4
4i	>25.0 (<10)	>25.0 (<10)	>25.0 (<10)	24.4
4j	>25.0 (<10)	>25.0 (<10)	>25.0 (<10)	23.6
4k	>25.0 (<10)	>25.0 (<10)	>25.0 (<10)	20.2
4l	>25.0 (<10)	>25.0 (<10)	>25.0 (<10)	24.1
4m	10.2 (29)	11.3 (26)	14.4 (27)	5.6
4n	12.1 (26)	12.9 (24)	19.5 (22)	7.4
4o	11.3 (28)	11.7 (22)	17.4 (26)	8.2
4p	13.2 (25)	12.5 (26)	19.9 (24)	9.5
4q	11.4 (22)	12.4 (25)	16.2 (23)	15.6
4r	13.6 (21)	12.8 (22)	18.6 (22)	12.8
Ciprofloxacin	6.5 (32)	4.5 (28)	-	-
Clotrimazole	-	-	2.5 (29)	-
Isoniazid	-	-	-	0.20
Rifampicin	-	-	-	0.25
Ethambutol	-	-	-	3.53

[a]***Minimum inhibitory concentration (MIC) in µg/mL.***
[b]***Zone of inhibition (ZI) in mm for 100 mg/disc of each compound.***

The analytical data of all the compounds are given by referencing as Figure S1.

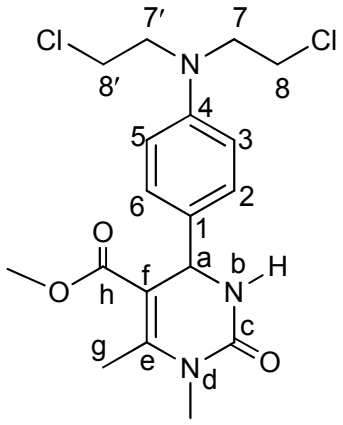


Figure S1
Schematic presentation of numbering for reprehensive compound 4m

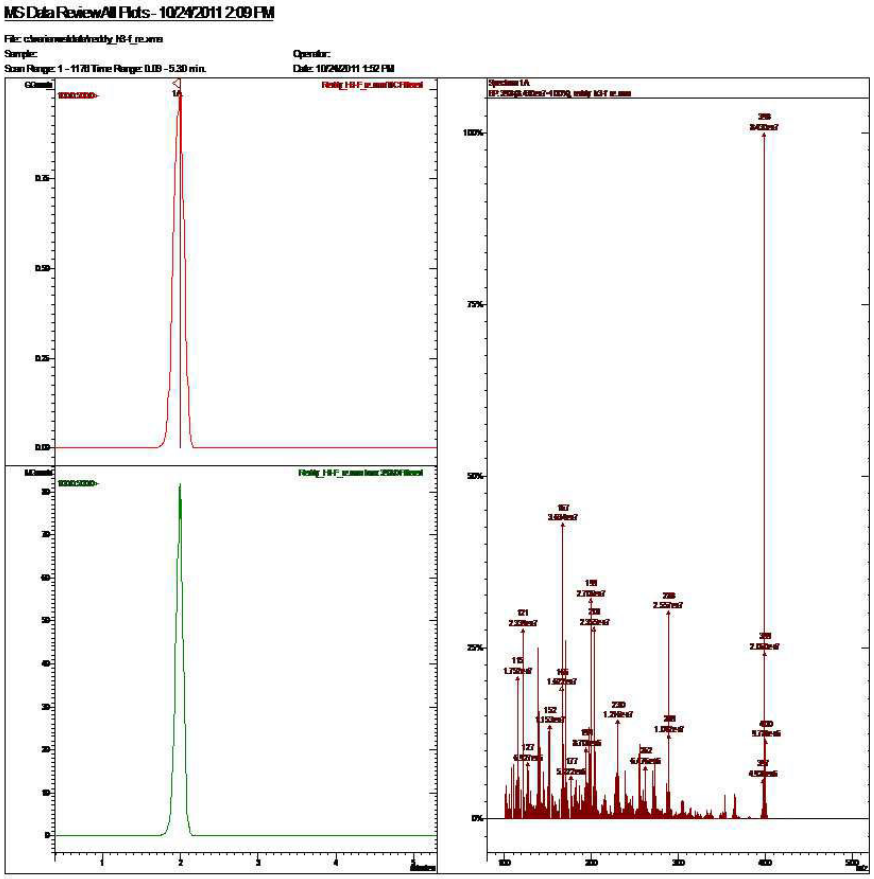


Figure S2
Mass spectrum of compounds 4m.

Spectral Characterization

i).Methyl-4-(4-(dimethylamino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4a)

Yellow solid; IR ($v_{max}cm^{-1}$): 3410 (N-H), 1742 (C=O), 1656 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.06 (brs, 1H, NH), 6.96 (d, *J* = 7.21 Hz, 2H, H-2 & 6), 6.26 (d, *J* = 5.1 Hz, 2H, H-3 & 5), 5.62 (d, *J* = 6.46 Hz, 1H, H-a), 4.32 (s,3H, O-CH_3), 3.42 (s, 3H, N-CH_3), 3.52 (s, 6H, H-7 & H-7^1), 2.42 (s, 3H, H-g); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 49.3 C-a, 151.6 C-c, 149.8 C-e, 108.8 C-f, 18.9 C-g, 169.8 C-h, 30.2 N-$\underline{C}H_3$, 62.4 $\underline{C}H_3$, 113.5 C-1, 156.6 C-2 & 6, 99.8 C-3 & 5, 150.2 C-4, 48.8 C-7 & C-7^1; MS *m/z* (%): 303.18 (29.20%), 248.19 (18.90 %), 169.19 (100.00 %), 108.18 (61.12%), 45.18 (56.12%), 29.36 (31.14%), 15.18 (21.13%); Anal. Calcd for $C_{16}H_{21}N_3O_3$: C, 63.35; H, 6.98; N, 13.85; Found: C, 63.30; H, 6.99; N, 13.82.

ii).Methyl-4-(4-(dimethylamino)phenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4b)

Brown solid; IR (v_{max} cm^{-1}) 3485 (N-H), 1746 (C=O), 1255 (C=S); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.01 (br s, 1H, H-b), 6.92 (d, *J* = 7.28 Hz, 2H, H-2 & 6), 6.27 (d, *J* = 5.1 Hz, 2H, H-3 & 5), 5.60 (d,*J* = 6.50 Hz, 1H, H-a),3.53 (s,6H, H-7 & H-7^1),4.30 (s,3H, O-CH_3), 3.37 (s, 3H, N-CH_3),2.41 (s, 3H, H-g); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 49.3 C-a, 151.6 C-c, 149.8 C-e, 108.8 C-f, 18.9 C-g, 169.8 C-h, 39.5 N-$\underline{C}H_3$,62.4 $\underline{C}H_3$, 113.5 C-1, 156.6 C-2& 6, 99.8 C-3& 5, 150.2 C-4, 48.8 C-7 & C-7^1; MS *m/z* (%): 319.14 (100.0 %), 347.18 (47.22%), 248.36 (19.90%), 201.46(47.99 %), 169.12 (36.87%), 102.76 (19.14%), 31.18 (33.12%), 15.18 (28.86%); Anal. Calcd for $C_{16}H_{21}N_3O_2S$: C, 60.16; H, 6.63; N, 13.16; Found: C, 60.19; H, 6.61; N, 13.13.

iii).Ethyl-4-(4-(dimethylamino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4c)

Reddish brown solid; IR (v_{max} cm^{-1}) 3454 (N-H), 1749 (C=O), 1668 (C=O); ^{1}H NMR (400 MHz, DMSO-*d_6*) δ: 10.02 (brs, 1H, H-b), 6.98 (d, *J* = 7.28 Hz, 2H, H-2 & 6),6.24 (d, *J* = 5.16 Hz, 2H, H-3 & 5),5.61 (d,*J* = 6.48 Hz, 1H, H-a),4.34 (q,*J* = 6.80 Hz, 2H, O-CH_2),3.51 (s, 6H, H-7 & H-7^1), 2.45 (s, 3H, H-g),3.40 (s, 3H, N-CH_3), 1.27 (t,*J* = 7.18 Hz, 3H,-CH_3),^{13}C NMR (100 MHz, DMSO-*d_6*) δ: 49.3 C-a, 151.6 C-c, 149.8 C-e, 108.8 C-f, 18.9 C-g, 169.8 C-h, 30.5 N-$\underline{C}H_3$, 62.4 $\underline{C}H_2$, 15.3 $\underline{C}H_3$, 113.5 C-1, 156.6 C-2& 6 , 99.8 C-3& 5, 150.2 C-4, 48.8 C-7 & C-7^1;MS *m/z* (%): 317.17 (32.03%), 345.10 (23.80%), 237.79 (100.00 %), 169.19 (49.69%), 108.38 (64.28%), 31.16 (38.76%), 15.10 (22.17%); Anal. Calcd for $C_{17}H_{23}N_3O_3$:C, 64.33; H, 7.30; N, 13.24; Found: C, 64.37; H, 7.31; N, 13.20.

iv).Ethyl-4-(4-(dimethylamino)phenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4d)

Yellow solid; IR (v_{max} cm^{-1}) 3449 (N-H), 1740 (C=O), 1247 (C=S); ^{1}H NMR (400 MHz, DMSO-*d_6*) δ: 10.09 (br s, 1H, H-b), 6.93 (d, *J* = 7.28 Hz, 2H, H-2 & 6), 6.24 (d, *J*= 5.16 Hz, 2H, H-3 & 5), 5.66 (d,*J* = 6.52 Hz, 1H, H-a),3.54 (s,6H, H-7 & H-7^1), 4.36 (q,*J* = 6.64 Hz, 2H, O-CH_2),3.42 (s, 3H, N-CH_3), 2.40 (s, 3H, H-g), 1.29 (t,*J* = 7.20 Hz, 3H,-CH_3); MS *m/z* (%): 333.17 (32.03 %), 345.10 (23.80%), 237.79 (100.0%), 169.19 (49.69%), 108.38 (64.28%), 31.16 (38.76%), 15.10 (22.17%); Anal. Calcd for $C_{17}H_{23}N_3O_2S$:C, 61.23; H, 6.95; N, 12.60; Found: C, 61.19; H, 6.97; N, 12.56.

v).Propyl-4-(4-(dimethylamino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4e)

Yellow solid; IR (v_{max} cm^{-1}) 3487 (N-H), 1752 (C=O), 1659 (C=O), ^{1}H NMR (400 MHz, DMSO-*d_6*) δ:10.03 (brs, 1H, H-b), 6.98 (d, *J* = 7.28 Hz, 2H, H-2 & 6), 6.29 (d, *J* = 5.18 Hz, 2H, H-3 & 5),5.61 (d,*J* = 6.48 Hz, 1H, H-a),3.51 (s, 6H, H-7 & H-7^1), 3.43 (s, 3H, N-CH_3),2.43 (s, 3H, H-g), 4.36 (t,*J* = 6.64 Hz, 2H, O-CH_2), 1.73 (m, 2H,-CH_2), 1.29 (t,*J* = 7.20 Hz, 3H,-CH_3); ^{13}C NMR (100 MHz, DMSO-*d_6*) δ:49.3 C-a, 151.6 C-c, 149.8 C-e, 108.8 C-f, 18.9 C-g, 169.8 C-h, 39.9 N-$\underline{C}H_3$, 55.3 $\underline{C}H_2$, 35.1 $\underline{C}H_2$, 15.5 $\underline{C}H_3$, 113.5 C-1, 156.6 C-2 & 6, 99.8 C-3 & 5, 150.2 C-4,54.8 C-7 &7^1; Anal. Calcd for $C_{18}H_{25}N_3O_3$:C, 65.23; H, 7.60; N, 12.68; Found: C, 65.25; H, 7.59; N, 12.61.

vi). Propyl-4-(4-(dimethylamino)phenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4f)

Faint yellow solid; IR (v_{max} cm^{-1}) 3456 (N-H), 1743 (C=O), 1250 (C=S);^{1}H NMR (400 MHz, DMSO-*d_6*) δ: 10.09 (brs, 1H, H-b), 6.93 (d, *J* = 7.76 Hz, 2H, H-2 & 6), 6.64 (d, *J* = 5.81 Hz, 2H, H-3 & 5), 5.63 (d,*J* = 6.68 Hz, 1H, H-a), 3.54 (s,6H, H-7 & H-7^1), 3.42 (s, 3H, N-CH_3),2.45 (s, 3H, H-g),4.33 (t,*J* = 6.60 Hz, 2H, O-CH_2), 1.76 (m, 2H,-CH_3), 1.28 (t,*J* = 7.22 Hz, 3H,-CH_3); MS *m/z* (%):347.17 (20.00 %), 347.18 (19.28%), 267.45 (19.93%), 169.19 (100.00%), 108.29 (61.70%), 45.11 (36.62%), 29.16 (21.84%), 15.10 (30.33%); Anal. Calcd for $C_{18}H_{25}N_3O_2S$: C, 62.22; H, 7.25; N, 12.09; Found: C, 62.14; H, 7.59; N, 12.61.

vii).Methyl-4-(4-(diethylamino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4g)

Yellow solid, mp; IR (v_{max} cm^{-1}) 3481 (N-H), 1740 (C=O), 1669 (C=O);^{1}H NMR (400 MHz, DMSO-*d_6*) δ:10.03 (brs, 1H, H-b), 6.93 (d, *J* = 7.89 Hz, 2H, H-2 & 6), 6.67 (d, *J* = 5.71 Hz, 2H, H-3 & 5),5.60 (d,*J* = 6.48 Hz, 1H, H-a), 4.31 (s,3H, O-CH_3), 3.52 (q, *J* = 6.48 Hz, 4H, H-7 & H-7^1),3.41 (s, 3H, N-CH_3),2.38 (s, 3H, H-g), 1.25 (t, *J* = 6.82 Hz, 6H, H-8 & 8^1); ^{13}C NMR (100 MHz, DMSO-*d_6*) δ:49.7 C-a, 151.9 C-c, 148.9 C-e, 108.7 C-f, 18.7 C-g, 169.7 C-h, 32.3 N-$\underline{C}H_3$,55.5 -O$\underline{C}H_3$,113.9 C-1,155.9 C-2 & 6,99.9 C-3 & 5, 45.5 C-7 & 7^1,14.9 C-8 & 8^1; MS *m/z* (%): 331.19 (21.00 %), 316.15 (21.46%), 287.15 (54.97%), 147.16 (100.00%) 31.11 (23.52%), 15.23 (34.67%); Anal. Calcd for $C_{18}H_{25}N_3O_3$: C, 65.23; H, 7.60; N, 12.68; Found: C, 62.28; H, 7.52; N, 12.65.

viii).Methyl-4-(4-(diethylamino)phenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4h)

Reddish brown solid; IR (v_{max} cm^{-1}) 3481 (N-H), 1749 (C=O), 1259 (C=S);^{1}H NMR (400 MHz, DMSO-*d_6*) δ: 10.15 (brs, 1H, H-b), 6.93 (d, *J* = 7.89 Hz, 2H, H-2 &6), 6.67 (d, *J* = 5.71 Hz, 2H, H-3 & 5),5.60 (d,*J* = 6.48 Hz, 1H, H-a),4.32

(s,3H, O-CH_3), 3.52 (q, *J* = 6.48 Hz, 4H, H-7 & H-7^1), 3.42 (s, 3H, N-CH_3),2.38 (s, 3H, H-g), 1.25 (t, *J* = 6.82 Hz, 6H, H-8 & 8^1);MS *m/z* (%): 347.17 (21.00 %), 316.15 (21.46%), 287.15 (54.97%), 147.16 (100.00%) 31.11 (23.52%), 15.23 (34.67%); Anal. Calcd for $C_{18}H_{25}N_3O_2S$: C, 62.22; H, 7.25; N, 12.09; Found: C, 62.24; H, 7.22; N, 12.05.

ix).Ethyl-4-(4-(diethylamino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4i)

Pale yellow solid; IR (v_{max} cm^{-1}) 3481 (N-H), 1743 (C=O), 1676 (C=O);^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ:10.03 (brs, 1H, H-b), 6.93 (d, *J* = 7.89 Hz, 2H, H-2 & 6), 6.67 (d, *J* = 5.71 Hz, 2H, H-3 & 5), 5.60 (d,*J* = 6.48 Hz, 1H, H-a),3.52 (q, *J* = 6.48 Hz, 4H, H-7 & H-7^1), 4.35 (q,*J* = 6.86 Hz, 2H, O-CH_2),3.45 (s, 3H, N-CH_3),2.38 (s, 3H, H-g),1.25 (t, *J* = 6.82 Hz, 6H, H-8 & 8^1), 1.30 (t,*J* = 7.18 Hz, 3H,-CH_3); MS *m/z* (%): 345.21 (21.00 %), 316.15 (21.46%), 287.15 (54.97%), 147.16 (100.00%) 31.11 (23.52%), 15.23 (34.67%); Anal. Calcd for $C_{19}H_{27}N_3O_3$: C, 66.06; H, 7.88; N, 12.16; Found: C, 66.12; H, 7.82; N, 12.15.

x).Ethyl-4-(4-(diethylamino)phenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4j)

Brown solid; IR (v_{max} cm^{-1}) 3483 (N-H), 1734 (C=O), 1245 (C=S); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 10.01 (brs, 1H, H-b), 6.83 (d, *J* = 7.29 Hz, 2H, H-2 & 6), 6.22 (d, *J* = 5.1 Hz, 2H, H-3 & 5), 5.65 (d,*J* = 6.81 Hz, 1H, H-a), 3.51 (q, *J* = 6.57 Hz, 4H, H-7 & H-7^1),1.25 (t, *J* = 6.82 Hz, 6H, H-8 & 8^1), 4.34 (q,*J* = 6.83 Hz, 2H, O-CH_2),3.42 (s, 3H, N-CH_3),2.45 (s, 3H, H-g),1.27 (t,*J* = 7.18 Hz, 3H,-CH_3); MS *m/z* (%): 361.18, 316.15 (21.46%), 287.15 (54.97%), 147.16 (100.00%) 31.11 (23.52%), 15.23 (34.67%);Anal. Calcd for $C_{19}H_{27}N_3O_2S$: C, 63.13; H, 7.53; N, 11.62; Found: C, 63.16; H, 7.49; N, 11.60.

xi).Propyl-4-(4-(diethylamino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4k)

Reddish brown solid; IR (v_{max} cm^{-1}) 3489 (N-H), 1751 (C=O), 1654 (C=O);^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 9.34 (brs, 1H, H-b), 6.87 (d, *J* = 8.02 Hz, 2H, H-2 & 6), 6.34 (d, *J* = 5.94 Hz, 2H, H-3 & 5),5.88 (d,*J* = 7.68 Hz, 1H, H-a),3.63 (q, *J* = 6.43 Hz, 4H, H-7 & H-7^1), 3.45 (s, 3H, N-CH_3),2.43 (s, 3H, H-g),4.38 (t,*J* = 6.66 Hz, 2H, O-CH_2), 1.74 (m, 2H,-CH_2), 1.27 (t,*J* = 7.20 Hz, 3H,-CH_3); 1.35 (t, *J* = 6.19 Hz, 6H, H-8 & 8^1); Anal. Calcd for $C_{20}H_{29}N_3O_3$:C, 66.83; H, 8.13; N, 11.69; Found: C, 66.87; H, 8.10; N, 11.63.

xii).Propyl-4-(4-(diethylamino)phenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4l)

Yellow solid; IR (v_{max} cm^{-1}) 3489 (N-H), 1748 (C=O), 1250 (C=S); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 9.34 (brs, 1H, H-b), 6.87 (d, *J* = 8.02 Hz, 2H, H-2 & 6), 6.34 (d, *J* = 5.94 Hz, 2H, H-3 & 5),5.88 (d,*J* = 7.68 Hz, 1H, H-a), 3.63 (q, *J* = 6.43 Hz, 4H, H-7 & H-7^1), 3.40 (s, 3H, N-CH_3), 2.43 (s, 3H, H-g),4.38 (t,*J* = 6.65 Hz, 2H, O-CH_2), 1.75 (m, 2H,-CH_2), 1.29 (t,*J* = 7.22 Hz, 3H,-CH_3); 1.35 (t, *J* = 6.19 Hz, 6H, H-8 & 8^1);Anal. Calcd for $C_{20}H_{29}N_3O_2S$:C, 63.97; H, 7.78; N, 11.19; Found: C, 63.94; H, 7.80; N, 11.13.

xiii).Methyl-4-(4-(bis(2-chloroethyl)amino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4m)

Pale yellow solid; IR (v_{max} cm^{-1})3483 (N-H), 1743 (C=O), 1653 (C=O);^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 9.84 (br s, 1H, H-b), 6.42 (d, *J* = 6.38 Hz, 2H, H-2 & 6), 6.26 (d, *J* = 5.88 Hz, 2H, H-3 & 5),5.73 (d,*J* = 6.87 Hz, 1H, H-a), 4.35 (s,3H, O-CH_3),3.42 (s, 3H, N-CH_3), 3.33 (t, *J* = 6.18 Hz, 4H, H-8 & 8^1), 3.18 (t, *J* = 6.64 Hz, 4H, H-7& 7^1),2.42 (s, 3H, H-g); ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ:49.3 C-a, 151.6 C-c, 149.8 C-e, 108.8 C-f, 18.9 C-g, 169.8 C-h, 31.9 N-$\underline{C}H_3$,55.3 $\underline{C}H_3$,113.5 C-1,156.6 C-2& 6,99.8 C-3 &5, 45.7 C-7& 7^1,54.7 C-8 & 8^1, 150.2 C-4; MS *m/z* (%): 399 (20.50%), 242.18 (51.08%), 148.27 (100.00%), 45.18 (46.69%), 29.25 (41.34%), 15.13 (22.56%); Anal. Calcd for $C_{18}H_{23}Cl_2N_3O_3$: C, 54.01; H, 5.79; N, 10.50;Found: C, 54.09; H, 5.75; N, 10.51.

xiv).Methyl-4-(4-(bis(2-chloroethyl)amino)phenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4n)

Brown solid; IR (v_{max} cm^{-1}) 3477 (N-H), 1735 (C=O), 1258 (C=S); ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ: 10.07 (brs, 1H, H-b), 6.87 (d, *J* = 7.84 Hz,

2H, H-2 & 6), 6.26 (d, *J* = 5.54 Hz, 2H, H-3 & 5),5.61 (d,*J* = 7.15 Hz, 1H, H-a), 4.32 (s,3H, O-CH_3),3.47 (s, 3H, N-CH_3),3.38 (t, *J* = 6.32 Hz, 4H, H-8 & 8^1), 3.10 (t, *J* = 6.19 Hz, 4H, H-7 & 7^1),2.48 (s, 3H, H-g); MS*m/z* (%): 415.09 (20.43%), 268.15 (22.72%), 162.64 (14.28%), 106.16 (100.00%) 31.10 (42.27%), 15.13 (18.76%); Anal. Calcd for $C_{18}H_{23}Cl_2N_3O_2S$: C, 51.92; H, 5.57; N, 10.09;Found: C, 51.96; H, 5.53; N, 10.11.

xv).Ethyl-4-(4-(bis(2-chloroethyl)amino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4o)

Deep red solid; IR (v_{max} cm^{-1})3464 (N-H), 1743 (C=O), 1661 (C=O);^{1}H NMR (400 MHz, DMSO-*d_6*) δ:10.09 (brs, 1H, H-b), 6.98 (d, *J* = 7.82 Hz, 2H, H-2 & 6), 6.34 (d, *J* = 6.14 Hz, 2H, H-3 & 5),5.68 (d,*J* = 7.43 Hz, 1H, H-a),4.38 (q,*J* = 6.88 Hz, 2H, O-CH_2),3.37 (t, *J* = 6.18 Hz, 4H, H-8 & 8^1), 3.26 (t, *J* = 6.58 Hz, 4H, H-7 & 7^1),2.86 (s, 3H, H-g),3.43 (s, 3H, N-CH_3),1.24 (t,*J* = 7.24 Hz, 3H,-CH_3); ^{13}C NMR (100 MHz, DMSO-*d_6*) δ: 49.3 C-a, 151.6 C-c, 149.8 C-e, 108.8 C-f, 18.9 C-g, 169.8 C-h,30.7 N-$\underline{C}H_3$,62.4 $\underline{C}H_2$, 55.3 $\underline{C}H_3$, 113.5 C-1,156.6 C-2& 6,99.8 C-3& 5,150.2 C-4,45.7 C-7& 7^1,54.8 C-8 & 8^1;Anal. Calcd for $C_{19}H_{25}Cl_2N_3O_3$: C, 55.08; H, 6.08; N, 10.14; Found: C, 55.12; H, 6.07; N, 10.09.

xvi).Ethyl-4-(4-(bis(2-chloroethyl)amino)phenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4p)

Yellow solid; IR (v_{max} cm^{-1})3473 (N-H), 1724 (C=O), 1255 (C=S); ^{1}H NMR (400 MHz, DMSO-*d_6*) δ: 10.09 (brs, 1H, H-b), 6.98 (d, *J* = 7.82 Hz, 2H, H-2 & 6), 6.34 (d, *J* = 6.14 Hz, 2H, H-3 & 5),5.68 (d,*J* = 7.43 Hz, 1H, H-a), 4.38 (q,*J* = 6.78 Hz, 2H, O-CH_2),3.42 (s, 3H, N-CH_3),3.35 (t, *J* = 6.30 Hz, 4H, H-8 & 8^1), 3.26 (t, *J* = 6.58 Hz, 4H, H-7 & 7^1),2.86 (s, 3H, H-g),1.30 (t,*J* = 7.18 Hz, 3H,-CH_3); Anal. Calcd for $C_{19}H_{25}Cl_2N_3O_2S$: C, 53.02; H, 5.85; N, 9.76; Found: C, 53.05; H, 5.87; N, 9.73.

xvii).Propyl-4-(4-(bis(2-chloroethyl)amino)phenyl)-1,6-dimethyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4q)

Pale yellow solid; IR (v_{max} cm^{-1}) 3486 (N-H), 1742 (C=O), 1663 (C=O);^{1}H NMR (400 MHz, DMSO-*d_6*) δ: 9.02 (brs, 1H, H-b), 6.74 (d, *J* = 7.83 Hz, 2H, H-2 & 6), 6.27 (d, *J* = 5.34 Hz, 2H, H-3 & 5),5.67 (d,*J* = 6.46 Hz, 1H, H-a),3.28 (s, 3H, N-CH_3),3.36 (t, *J* = 6.16 Hz, 4H, H-8 & 8^1),3.09 (t, *J* = 6.56 Hz, 4H, H-7 & 7^1), 2.44 (s, 3H, H-g),4.36 (t,*J* = 6.64 Hz, 2H, O-CH_2), 1.73 (m, 4H,-CH_3), 1.29 (t,*J* = 7.20 Hz, 3H,-CH_3); ^{13}C NMR (100 MHz, DMSO-*d_6*) δ: 49.3 C-a, 151.6 C-c, 149.8 C-e, 108.8 C-f, 18.9 C-g, 169.8 C-h, 32.9 N-$\underline{C}H_3$,62.4 $\underline{C}H_2$, 55.3 $\underline{C}H_2$, 43.2 $\underline{C}H_3$,113.5 C-1, 156.6 C-2 & 6,99.8 C-3 & 5,150.2 C-4,45.7 C-7 & 7^1, 54.8 C-8 & 8^1;Anal. Calcd for$C_{20}H_{27}Cl_2N_3O_3$:C, 56.08; H, 6.35; N, 9.81; Found: C, 56.10; H, 6.31; N, 9.83.

Xviii).Propyl-4-(4-(bis(2-chloroethyl)amino)phenyl)-1,6-dimethyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4r)

Yellow solid; IR (v_{max} cm^{-1}) 3477 (N-H), 1743 (C=O), 1251 (C=S); ^{1}H NMR (400 MHz, DMSO-*d_6*) δ:9.02 (brs, 1H, H-b), 6.74 (d, *J* = 7.83 Hz, 2H, H-2 & 6), 6.27 (d,*J* = 5.34 Hz, 2H, H-3 & 5),5.67 (d,*J* = 6.46 Hz, 1H, H-a),3.43 (s, 3H, N-CH_3),3.38 (t, *J* = 6.28 Hz, 4H, H-8 & 8^1),3.09 (t, *J* = 6.56 Hz, 6H, H-7 & 7^1),2.44 (s, 3H, H-g),4.39 (t,*J* = 6.64 Hz, 2H, O-CH_2), 1.76 (m, 2H,-CH_2), 1.25 (t,*J* = 7.26 Hz, 3H,-CH_3); Anal. Calcd for $C_{20}H_{27}Cl_2N_3O_2S$: C, 54.05; H, 6.12; N, 9.46; Found: C, 54.01; H, 6.14; N, 9.43.

(b) Biological activities of synthesized compounds for anti-tubercular activity

All the synthesized compounds were also screened for their *in vitro* anti-mycobacterial activity against MTB - *Mycobacterium tuberculosis* H_{37}Rv (*M. tuberculosis*, ATCC 25618) in Middle brook 7H11 agar medium supplemented with oleic acid-albumin-dextrose-catalase (OADC) by agar dilution method similar to that recommended by the national committee for clinical laboratory standards[15] for the determination of MIC in duplicate. The MTB clinical isolate was resistant to isoniazid, rifampicin, and ethambutol. The minimum concentration of compound required to hinder 99% of bacterial growth in the culture is referred as MIC and the corresponding values for the synthesized compounds at pH 7.4 are given in Table 2. All the titled compounds are exhibiting excellent

in vitro activity against MTB in the first phase of screening ranging from 5.6 to 24.4 µg/ mL. However, four compounds (4m, 4n, 4o and 4p) inhibited MTB with MIC less than 10 µg/ mL and were compared with the first line anti-TB drug, ethambutol (MIC 3.53 µg/ mL). It was observed that, as like as anti-microbial activity the alky chain increases the activity gradually decreased and when chlorines placed as substitutions activity increased. But interestingly, the C=S group containing compounds showed better activity than C=O compounds in pyrimidine ring.

CONCLUSION

In conclusion, we have described an efficient synthesis of 3,4- tetrahydropyrimidinones with chloroindate ionic liquid (BMI.$InCl_4$) under mild reaction conditions. The corresponding dihydropyrimidinones/thiones were obtained in good to excellent yields in short reaction times, easy workups.The newly synthesized compounds were evaluated for their anti-microbial activity against *E. coli* (ATCC 25922), *S.aureus* (ATCC 11632) and *C.albicans* (ATCC 90028) and anti-tuberculosis activity against *M. tuberculosis* H_{37}Rv (MTB) *invitro*. Four compounds were found to be the potent anti-tubercular compounds.In addition, these compounds shown to be biologically efficient against bacteria as well as fungi than other compounds.

ACKNOWLEDGMENTS

The authors express their thanks to Prof. C. Suresh Reddy, Department of Chemistry, Sri Venkateswara University, Tirupati, India for encouragement and helpful discussion.

REFERENCES

1. a). Kappe CO, 100 years of the biginelli dihydropyrimidine synthesis, Tetrahydron, 49(32):6937-6963, (1993). b). Grover GI, Dzwonczyk S, McMullen DE, Parham CS, Sleph PG, Moreland S, Pharmacologic Profile of the Dihydropyrimidine Calcium Channel Blockers SQ 32, 547 and SQ 32,946, J. Cardiovasc, Pharmacol., 26(2): 289-294 (1995).
2. Bahekar SS, Shinde DB, Synthesis and anti-inflammatory activity of some [4,6-(4-substituted ary)-2-thioxo-1,2,3,4-tetrahydro-pyrimidin-5-yl]-acetic acid derivatives, Bioorg. Med. Chem. Lett.,14(7): 1733-1736 (2004).
3. Ashok M, Holla BS, Kumari NS, Convenient one pot synthesis of some novelderivatives of thiazolo [2, 3-b]dihydropyrimidinone possessing 4-methyl thiophenyl moiety and evaluation of their antibacterial and antifungal activities, Eur. J. Med. Chem., 42(3): 380-385 (2007).
4. Sujatha K, Shanmugam P, Perumal PT, Muralidharan D, Rajendran M, Synthesis and cardiac effects of 3, 4-dihydropyrimidin-2(1*H*)-one-5 carboxylates, Bioorg. Med.Chem. Lett., 16(18): 4893-4897 (2006).
5. Basso AM., Bratcher NA, Gallagher KB, Cowart MD, Zhao C, Sun M, EsbenshadeTA, Brune ME, Fox GB, Schmidt M, Collins CA, Souers AJ,Iyengar R, Vasudevan A, Kym PR, HancockAA, Rueter LE, Lack of efficacy of melanin-concentrating hormone-1 receptor antagonists in model of depressionand anxiety, Eur. J. Pharmacol., 540(1-3): 115-120 (2006).
6. Wisen S, Androsavich J, Evans CG, Chang L, Gestwicki JE, Chemicalmodulators of heat shock protein 70 (Hsp70) by sequential, microwave-accelerated reactions on solid phase, Bioorg. Med. Chem. Lett., 18(1): 60-65 (2008).

7. Blackburn C, Guan B, Brown J, Cullis C, Condon SM, Jenkins TJ, Peluso S, Ye Y, Gimeno RE, Punreddy S, Sun Y, Wu H, Hubbard B, KaushikV, Tummino P, SanchettiP, Sun DY, Daniels T, Tozzo E, Balani SK, Raman P, Identification and characterization of 4-aryl-3,4-dihydropyrimidin-2(1H)-ones as inhibitors of the fatty acid transporter FATP4, Bioorg. Med. Chem. Lett., 16(13): 3504-3509 (2006).
8. Anastas PT, Kirchhoff MM, Origins, Current Status, and Future Challenges of Green Chemistry, Acc. Chem. Res., 35: 686–694 (2002).
9. Welton T, Room-Temperature Ionic Liquids. Solvents for Synthesis and Catalysis, Chem. Rev., 99: 2071–2083 (1999).
10. Lin IJB, Vasam CS, Metal Containing Ionic Liquids and Ionic Liquid Crystals Based on Imidazolium Moiety, J. Organomet. Chem., 690: 3498–3512 (2005).
11. da Silveira Neto BA, Ebeling G, Goncalves RS, Gozzo FC, Eberlin MN, Dupont J, Organoindate room temperature ionic liquid: synthesis, physicochemical properties and application, Synthesis, 8:1155–1158 (2004).
12. Pilli, R. A.; Robello, L. G.; Camilo, N. S.; Dupont, J.; Lapis, A. A. M.; da Silveira Neto, B. A. Addition of Activated Olefins to Cyclic N-Acyliminium Ions in Ionic Liquids. Tetrahedron Lett., 47: 1669–1672 (2006).
13. da Silveira Neto BA, Alves MB, Lapis AAM, Nachtigall FM, Eberlin MN, Dupont J, Suarez PAZ, 1-n-Butyl-3-methylimidazolium Tetrachloro-Indate ($BMI.InCl_4$) as a Media for the Synthesis of Biodiesel from Vegetable Oils, J. Catal., 249: 154–161 (2007).
14. Jomy KJ, Suman LJ, Sweety S, Bir S, Efficient Synthesis of 3,4-dihydropyrimidinones in 1-n-butyl-3-methylimidazoliumtetrachloroindate ($BMI.InCl_4$), Ind. Eng. Chem. Res., 50:11463–11466 (2011).
15. Wayne Pa, 385 Methodology for the Serum Bactericidal Test. Approved guideline, National Committee for Clinical Laboratory Standards (NCCLS), (1999).