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ANTIMICROBIAL AND ANTIMALARIAL EVALUATION OF SOME NOVEL 2,3-DIHYDRO-4-METHYL-2-THIOXO-1H-IMIDAZOL-5-YL)ETHANONE

Ram Kishore[a]* and Monika Kamboj[a]

[a]Department of Applied Chemistry, Amity School of Applied Sciences, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, U. P., India.

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***Corresponding Author**
Ram Kishore
Department of Applied Chemistry, Amity School of Applied Sciences, Amity University Uttar Pradesh, Lucknow Campus, Lucknow-226028, U. P., India.

ABSTRACT

Novel cyclic thio-urea(aryl or hetero aryl) substituted 2,3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone derivatives have been synthesized by the amine, CS_2 and aqueous NH_3, Triton-B and 3-chloro-2,4-pentanedione. These were screened for in-vitro antimicrobial activity against two gram positive (*Streptococcus pyogenes* and *Staphylococcus aureus*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) as well as for antifungal and antimalarial activity against *Plasmodium falciparum strain*. Compound 3(d) and 3(g) exhibited good antimicrobial and antimalarial activity.

KEYWORDS: 3-chloro-2,4-pentanedione, amine, CS_2, aqueous NH_3, catalyst (trimethyl benzyl ammonium hydroxide)Triton-B, Antimicrobial and antimalarial activity.

INTRODUCTION

Imidazole are a class of heterocyclic compounds that are believed to occur in nature from post- translational modification of serine and threonine residues in peptides.[1,2] They are key building blocks of natural products, pharmaceuticals and synthetic intermediates.[3-5] Imidazole have not only attracted great interests due to their appearance as subunits of various biologically active natural products but also because of their utilities as valuable precursors in many useful synthetic transformations.[6]

Imidazoles play a vital role in the manufacture of various biologically active drugs as brainderived neurotrophic factor inducers[7], analgesic[8], trypanocidal activity[9], antimitotic agents with pro-apoptotic activity.[10] Over the years, a number of methods have been devised for the synthesis of imidazoles.[11] Classically, the cyclic thiourea synthesis was the most common route to imidazole, which involves cyclisation of 2,3-dihydro-4-methyl-2-thioxo-1H-imidazolyl) ethanone.[12]

In recent decades, microbial diseases are more prevalent than they were during the first half of the last century and are still difficult to be diagnosed clinically. To combat them, various synthetic and semi-synthetic antimicrobial drugs have been used in clinical practice.[13,14] In literature, a number of research paper are available describing the antimicrobial behavior of aromatic and heterocyclic compound.[15-18] But, in the treatment of microbial infections only limited numbers of efficacious antimicrobial drugs are used even after availability of a number of antimicrobial agents. Many of the currently available drugs are toxic, enable recurrence because they are bacteriostatic/fungistatic and not bactericidal/fungicidal or lead to the development of resistance due in part to the prolonged periods of administration. The impact is more acute in developing countries due to non-availability of desired medicines.[19,20] There is a real perceived need for the discovery of new compounds that are endowed with antibacterial and antifungal activities, possibly acting through mechanism of actions, which are distinct from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant.[21-23]

The derivatives of Imidazole have become increasingly important in the past few years because of their use in intermediates for the preparation of new biological materials. The imidazole ring is present in numerous pharmacologically important compounds, including those used as antibiotics[24] and antiproliferative.[25] The wide range of biological activities of imidazoles includes anti-inflammatory[26], analgesic[27], antibacterial, antifungal[28], hypoglycaemic[29], antiproliferative[30], anti-tuberculosis[31], muscle relaxant[32] and HIV inhibitor activity.[33] In addition, imidazole derivatives are useful synthetic intermediates and can be used as diversity scaffolds in combinatorial chemistry[34] and also as peptidomimetics.[35] Standard drugs used in some of the medicinally important derivatives containing imidazole are Trimethadione etc. which possess antiepileptic[36] properties. The imidazole derivatives have raised considerable attention to medicinal research, and a large

number of investigations on their synthesis and biological activities have been reported during the last ten years.[37-39]

Looking at the importance of these heterocyclic nuclei, it is thought of interest to devote some attention for the synthesis of phenyl substituted imidazole derivatives and to evaluate these derivatives for antimicrobial and antimalarial activity against *plasmodium falciparum* strain.

Antimicrobial activity

All the synthesized compounds were tested against two gram positive bacteria (*Staphylococcus aureus*, *Streptococcus Pyogenes*) and two gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) using micro broth dilution method[40-43] for the determination of minimal inhibition concentration. For the antifungal activity the common standard strains that were used, are *C. Albicans*, *A, Niger* and *A. Clavatus*. Muller Hinton broth (Microcare laboratory & Tuberculosis Research Centre, Surat-3, India) was used as nutrient medium to grow and dilute the drug suspension for the test bacteria. Inoculum Size for Test Strain was adjust to 10^8 Cfu[Colony Forming Unit] per milliliter by comparing the turbidity. DMSO was used as diluents / vehicle to get desired concentration of drugs to test upon Standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. In primary screening 1000 µg/ml, 500 µg/ml, and 250 µg/ml concentrations of the synthesized compounds were taken. The active synthesized compounds found in this primary screening were further tested in a second set of dilution against all microorganisms. The highest dilution showing at least 99 % inhibition zone is taken as MIC. The test mixture should contain 10^8 organism/ml. Standard drugs Ampicillin and Chloramphenicol were used as antibacterial for comparison. Standard drugs Nystatin and Greseofulvin were used as antifungal for comparison.

Antimalarial activity

The in vitro antimalarial assay was carried out in 96 well microtitre plates according to the micro assay protocol reference. The cultures of *Plasmodium falciparum* strain were maintained in medium RPMI 1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous]parasites of *Plasmodium falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For arrying out the assay, an initial ring] stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µ l of medium RPMI-1640 was

determined by Jaswant Singh Bhattacharya (JSB staining to assess the percent parasitaemia (rings) and uniformally maintained with 50% RBCs (O+). A stock solution of 5mg/ml of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The diluted samples in 20 µ l volume were added to the test wells so as to obtain final concentrations (at fivefold dilutions) ranging between 0.4 µ g/ml to 100 µ g/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37^{o}C in a candle jar. After 36 to 40 h incubation, thin blood smears from each well were prepared and stained with JSB stain. The slides were microscopically observed to record maturation of ring stage parasite sinto trophozoites and schizonts in presence of different concentrations of the test agents. The test concentration which inhibited the complete maturation into schizonts was recorded as the minimum inhibitory concentrations (MIC). Quinine was taken as the reference drug.

MATERIALS AND METHODS

General Procedures

Reagent grade chemicals were used without further purification. All the melting points were taken in open capillaries and are uncorrected. The purity and mass of the synthesized compounds was checked by MS. 1H NMR spectral was recorded in CDCl3 /DMSO with tetra methyl silane (TMS) as the internal standard at 400 MHz on a Bruker DRTX-400 spectrophotometer. The chemical shifts are reported as parts per million (ppm). Elemental analysis was performed using a (EURO EA 3000 instrument). Acme silica gel-G and Merck silica gel (100 to 200, 60 to 120 meshes) were used for analytical TLC and Column chromatography respectively.

Chemistry

We have prepared the novel cyclic thiourea (aryl or hetero aryl) substituted aniline-2, 3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone derivatives derivatives in one steps, using 4-tertbutyl aniline, CS_2 and substituted aryl or heteroaryl amine as the starting materials. 4-Tertbutyl aniline, on CS_2 reaction with aqueous NH_3, catalyst trimethyl benzyl ammonium hydroxide results 4-tertbutyl phenyl urea which on cyclisation reaction with 3-chloro-2,4-pentanedione results 1-(1-(4-tert-butylphenyl)-2,3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone.

Preparation of desired cyclic thiourea (aryl or hetero aryl) substituted aniline-2-thioxo-1H-imidazole derivatives

To A solution of amine (1.0 mmol), CS_2 (1.5 mmol) in ammonium hydroxide solution 28% in NH_3 (2.0 mmol), was stirred at room temperature for 15 min. Then Triton-B (1.5 mmol) was added and again stirred for 15 more minutes, then reaction mass was reflux at 80^oC for 2h. Then 3-chloro-2,4-pentanedione (1.0 mmol) was added at rt. The reaction mixture was than stirred for 2h. The progress of reaction was monitored by TLC. After completion, water (50 mL) was added and the product was extracted with ethyl acetate (3x20 mL). The combined organic layer was washed with brine, dried over Na_2CO_3 and concentrated under reduced pressure to afford crude product. This crude product was further purified by silica gel column chromatography with 100-200 silica-gel by using eluent ethyl acetate:hexane (1:5) to afford pure compound 3(a-j) Scheme 1.

Preparation of 1-(1-(4-tert-butylphenyl)-2,3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone 3(a)

M.P. 135-140, Pale yellow solid, ^1H NMR 400 MHz ($CDCl_3$): δ 7.881 (bs, 1H), 7.422 (d, *J* = 6.8 Hz, 2H), 7.240 (s, 2H), 2.588 (s, 3H), 2.445 (s, 3H), 1.326 (s, 9H). MS (ESI+) m/z: 289.13 (M^+). Anal. Calcd for $C_{16}H_{20}N_2OS$: C- 66.63%; H, 6.99%; N, 9.71%; O, 5.55%; S, 11.12%.

1-(1-(2,4-difluorophenyl)-2,3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone 3(b)

M.P. 132-135^oC, Pale yellow solid, ^1H NMR 400 MHz ($CDCl_3$): δ 7.881 (bs, 1H), 7.896-7.816 (m, 2H), 6.848 (s, 1H), 2.589 (s, 3H), 2.455 (s,3H). MS (ESI+): m/z: 267.10 (M^-1). Anal. Calcd for $C_{12}H_{10}F_2N_2OS$: C, 53.72%; H, 3.76%; F, 14.16%; N, 10.44%; O, 5.96%; S, 11.95%.

1-(2,3-dihydro-4-methyl-1-(2,3-dimethylphenyl)-2-thioxo-1H-imidazol-5-yl)ethanone(3c)

M.P. 133-134^oC, Pale yellow solid, ^1H NMR 400 MHz ($CDCl_3$): δ 8.282 (bs, 1H), 7.291 (d, *J* = 7.2 Hz, 1H), 7.179 (t, *J* = 7.6 Hz, 1H), 7.133 (d, *J* = 7.2 Hz, 1H), 2.503 (s, 3H), 2.373 (s,3H), 2.337 (s, 3H), 2.23 (s, 3H). MS (ESI): m/z $(M)^-$ 259.05. Anal. Calcd for $C_{14}H_{16}N_2OS$: C, 64.58; H, 6.19; N, 10.76; O, 6.15; S, 12.32%.

1-(2,3-dihydro-1-(4-isopropylphenyl)-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone (d)

M.P. 131-133^oC, off white solid, ^1H NMR 400 MHz ($CDCl_3$): δ 8.282 (bs, 1H), 7.272 (d, *J* = 8.0 Hz, 1H), 6.885 (d, *J* = 4.4 Hz, 1H), 6.846 (d, *J* = 6.4 Hz, 1H), 6.689 (d, *J* = 6.4 Hz, 1H),

4.598-4.507 (m, 1H), 2.580 (s, 3H), 2.452 (s,3H), 1.354 (d, J = 7.6 Hz, 6H). MS (ESI): m/z $(M)^-$ 273.05. Anal. Calcd $C_{15}H_{18}N_2OS$: C, 65.66; H, 6.61; N, 10.21; O, 5.83; S, 11.69%.

1-(1-(3,5-bis(trifluoromethyl)phenyl)-2,3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone (e)

M.P. 115-117^oC, pale yellow solid, ^1H NMR 400 MHz ($CDCl_3$): δ 8.282 (bs, 1H), 7.988 (s, 1H), 7.818 (s, 1H), 7.587 (s, 1H), 2.567 (s, 3H), 2.511 (s,3H). MS (ESI): m/z 267.05 $(M)^-$. Anal calcd for $C_{14}H_{10}F_6N_2OS$: C, 45.66; H, 2.74; F, 30.95; N, 7.61; O, 4.34; S, 8.71%.

1-(1-(3,4-difluorophenyl)-2,3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone (f)

M.P. 120-122^oC, pale yellow solid, ^1H NMR 400 MHz ($CDCl_3$): δ 7.976 (bs, 1H), 7.407-7.354 (m, 1H), 7.216-7.148 (m, 1H), 7.074-7.032 (m, 1H), 2.590 (s, 3H), 2.463 (s,3H). MS (ESI): m/z: 267.01 $(M)^-$. Anal calcd for $C_{12}H_{10}F_2N_2OS$: C, 53.72; H, 3.76; F, 14.16; N, 10.44; O, 5.96; S, 11.95%.

1-(1-(3-CHLORO-4-METHYLPHENYL)-2,3-DIHYDRO-4-METHYL-2-THIOXO-1H-IMIDAZOL-5-YL)ETHANONE (G)

M.P. 134-136^oC, off white solid, ^1H NMR 400 MHz ($CDCl_3$): δ 8.690 (bs, 1H), 7.374 (s, 1H), 7.253 (d, *J* = 8.4 Hz, 1H), 7.151 (d, J = 6.0 Hz, 1H), 2.559 (s, 3H), 2.450 (s,3H), 2.364 (s, 1H). MS (ESI): m/z: 279.05 $(M)^-$. Anal calcd for $C_{13}H_{13}ClN_2OS$: C, 55.61; H, 4.67; Cl, 12.63; N, 9.98; O, 5.70; S, 11.42%.

1-(1-(2-fluorophenyl)-2,3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone (h)

M.P. 135-137^oC, off white solid, ^1H NMR 400 MHz ($CDCl_3$): δ 7.886 (t, *J* = 8.4 Hz, 1H), 7.675 (bs, 1H), 7.225-7.133 (m, 2H), 7.144-7.063 (m, 1H), 2.631 (s, 3H), 2.480 (s, 3H). MS (ESI): m/z: 249.05 $(M)^-$. Anal calcd for $C_{12}H_{11}FN_2OS$: C, 57.58; H, 4.43; F, 7.59; N, 11.19; O, 6.39; S, 12.81%.

1-(2,3-dihydro-4-methyl-1-(2,4-dimethylphenyl)-2-thioxo-1H-imidazol-5-yl)ethanone (i)

M.P. 130-132^oC, off white solid, ^1H NMR 400 MHz ($CDCl_3$): δ 8.454 (bs, 1H), 7.317 (d, *J* = 8.0 Hz, 1H), 7.113 (s, 1H), 7.083 (d, *J* = 8.0 Hz, 1H), 2.477 (s, 3H), 2.366 (s, 3H), 2.346 (s, 3H), 2.277 (s, 3H). MS (ESI) m/z: 259.05 $(M)^-$. Anal. calcd for $C_{14}H_{16}N_2OS$: C, 64.58; H, 6.19; N, 10.76; O, 6.15; S, 12.32%.

1-(2,3-dihydro-4-methyl-1-(4-(methylthio)phenyl)-2-thioxo-1H-imidazol-5-yl)ethanone (j)

M.P. 129-132^{o}C, off white solid, ^{1}H NMR 400 MHz ($CDCl_3$): δ 8.454 (bs, 1H), 7.355 (d, *J* = 6.8 Hz, 2H), 7.147 (d, *J* = 6.8 Hz, 2H), 2.543 (s, 3H), 2.141 (s, 3H), 1.993 (s, 3H). MS (ESI) m/z: 278.05 $(M)^{-}$. Anal. calcd for $C_{13}H_{14}N_2OS_2$: C, 56.09; H, 5.07; N, 10.06; O, 5.75; S, 23.04%.

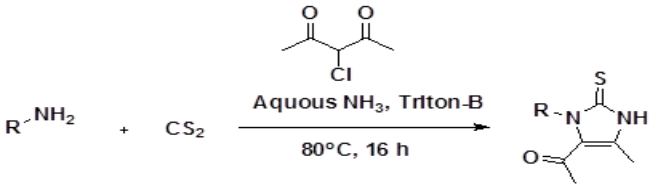
1-(1-(3-(trifluoromethyl)phenyl)-2,3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone (k)

M.P. 116-118^{o}C, pale yellow solid, ^{1}H NMR 400 MHz ($CDCl_3$): δ 7.933 (bs, 1H), 7.649 (s, 1H), 7.604 (d, *J* = 8.0 Hz, 1H), 7.519 (t, J = 8.0 Hz, 1H), 7.384 (d, *J* = 7.6 Hz, 1H), 2.620 (s, 3H), 2.484 (s, 3H). MS (ESI) m/z: 299.05 $(M)^{-}$. Anal. Calcd. for $C_{13}H_{11}F_3N_2OS:)^{-}$: C, 51.99; H, 3.69; F, 18.98; N, 9.33; O, 5.33; S, 10.68%.

RESULT AND DISCUSSION

Cyclic thiourea was prepared by substituted amine, on CS_2 reaction with aqueous NH_3, catalyst trimethyl benzyl ammonium hydroxide results substituted thio urea which on cyclisation reaction with 3-chloro-2,4-pentanedione results 2,3-dihydro-4-methyl-2-thioxo-1H-imidazol-5-yl)ethanone. **Table-1.**

Scheme



List of synthesized compound Table 1.

Compound	R	M.P(^{0}C)	Yield(%)
3a	4-Tert-butyl phenyl	135-140	61.1
3b	2,4-difluoro phenyl	132-135	63.3
3c	2,3-dimethyl phenyl	133-134	50.1
3d	4-isopropyl phenyl	131-133	60.2
3e	3,5-Trifluoromethyl phenyl	115-117	51.2
3f	3,4-difluoro phenyl	120-122	49.1
3g	3-chloro-4-methyl phenyl	134-136	54.8
3h	2-Fluoro phenyl	135-137	62.7
3i	2,4-dimethyl phenyl	130-132	61.3
3j	4-(methyl thio) phenyl	129-131	49.3
3k	3-Trifluoromethyl phenyl	116-118	55.2

Antibacterial activity

The antibacterial activity of all the synthesized compounds were tested in-vitro against pathogenic *E. coli*, *P.aeruginosa*, *S. aureus* and *S.pyogenus* and the results were compared with standard drugs (Ampicillin and Chloramphenicol). In case of *S.aureus* compounds 3(g) exhibit higher activity while 3(a) and 3(d) 3(k) exhibit good activity while rest of the compounds show moderate activity. In case of *S.pyogenus* compounds 3(d) and 3(i) exhibit good activity while rest of the compounds show moderate activity. In case of *E. coli* Compound 3(d) shows higher activity while 3(g), 3(i) and 3(j) 3(k) show good activity while rest of the compounds possess less activity. In case of *P.aeruginosa* compound 3(f) and 3(d) show good activity while rest of the compounds possess less activity.

Table-2 Antibacterial activity (minimum inhibitory concentration in µg/ml).

Compound	E.COLI	P.AERUGINOSA	S.AUREUS	S.PYOGENUS
3(a)	600	400	100	100
3(b)	200	400	250	500
3(c)	200	250	150	500
3(d)	60	125	100	125
3(e)	500	500	200	200
3(f)	300	100	200	500
3(g)	100	250	62.5	250
3(h)	500	200	500	500
3(i)	100	250	500	100
3(j)	100	500	500	250
3(k)	600	500	500	250
Ampicillin	100	100	250	100
Chloramphenicol	50	50	50	50

Antifungal activity

The antifungal activity of all the synthesized compounds were tested in-vitro against fungi *C.Albicans*, *A.Niger* and *A.Clavatus* and the results were compared with standard drugs (Nystatin and Greseofulvin). In case of *C.Albicans* compound 3(b) and 3(g) exhibit higher activity while 3(e), 3(f) and 3(h) show good activity and rest of the compounds possess less activity. In case of *A, Niger* and *A.Clavatus* all the compounds possess less activity. The results are given in Table-3.

Antimalarial activity

For antimalarial activity, Compounds 3(d) 3(g) and 3(h) exhibit good activity closer to reference compound Quinine against *plasmodium falciparum* strain while rest of the compounds possess less activity. The results are given in Table-4.

Table 3: Antifungal Activity (In MIC).

Compound	C.Albicans	A.Niger	A.Clavatus
3(a)	900	900	900
3(b)	230	400	>1000
3(c)	1000	>1000	1000
3(d)	>1000	500	>1000
3(e)	600	>1000	>1000
3(f)	500	850	1000
3(g)	250	500	500
3(h)	500	>1000	>1000
3(i)	1000	>1000	1000
3(j)	1000	1000	>1000
3(k)	1000	1000	>1000
Nystatin	100	100	100
Greseofulvin	500	100	100

Table 4: Antimalarial Activity.

Compound	Mean IC50 (µg/ml)
3(a)	1.80
3(b)	1.68
3(c)	1.85
3(d)	0.70
3(e)	1.0
3(f)	0.79
3(g)	0.52
3(h)	0.76
3(i)	1.31
3(j)	0.95
3(k)	1.67
Quinine	0.268

CONCLUSION

All the newly synthesized compounds were screened for antibacterial, antifungal and antimalarial activity. The data in the Table-2 indicate that among the synthesized compounds, compounds 3(d) and 3(g) exhibit excellent antibacterial activity. However, the activities of the tested compounds are much less than those of standard agents used. Including 3(d) and 3(g), Compound 3(h) also exhibit good antimalarial activity. From the results of various biological activities it is clear that these compounds would be of better use in drug development to combat bacterial infections and as antimalarial agents in the future.

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