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Design and synthesis of some novel imidazole derivatives as potent antimicrobial & antimalarial agents

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ABSTRACT

Some new 2-substituted aryl-4-fluoro phenyl-5-(2-Chloropyridinyl)-1H-imidazole derivatives have been synthesized by the reaction of 2-(2-chloropyridin-4-yl)-1-(4-fluorophenyl) ethanone [obtained by the reaction of ethyl -4- fluoro benzoate with 2-chloro-4-methylpyridine] with selenium dioxide in dioxane followed by cyclisation with substituted aryl(or hetero aryl)aldehyde in presence of acetic acid and ammonium acetate. All the synthesized compound were characterized by elemental analysis, ¹H NMR and LCMS and also screened for their in- vitro antimicrobial activity against two gram positive (*Streptococcus pyogenes* and *Staphylococcus aureus*) and two gram negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) along with antifungal and antimalarial activity.

Keywords: Imidazoles, antimicrobial and antimalarial activity.

INTRODUCTION

Imidazole is a planar five-membered heterocyclic ring, highly polar and ionisable aromatic compound. The physiological and pharmacological significance of imidazole and its derivatives has interested many investigators since imidazole compounds were first discovered. This five-membered ring is a component of many important natural products, such as purine and nucleic acid [1-3]. The imidazole scaffold is an important heterocyclic nucleus due to its wide spectrum of applications in the field of biology, chemistry as well as in pharmaceutical products. It is found in a large number of pharmacologically active compounds such as Omeprazole [4]. Cimetidine and lansoprazole [5, 6]. The substituted imidazole derivatives have been reported to have a wide range of applications in diverse therapeutic areas including anti-inflammatory, antiviral, antibacterial, anti-allergic, and antitumor [7-10]. Imidazoles and their salts in particular comprise a boundless and emerging field. The polar imidazole ring, which contains two nitrogens separated with a methylene, hydrogen bonds through the amino hydrogen as the donor and the imino nitrogen as the acceptor [11]. The importance of these heterocyclic nuclei, it is thought of interest to devote some attention for the synthesis of new substituted imidazoles derivatives and to evaluate these derivatives for antimicrobial and antimalarial activity.

MATERIALS AND METHODS

3.1 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. ¹H NMR spectral was recorded in CDCl₃ /DMSO with tetramethylsilane (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.

3.2 Chemistry:

We have prepared the novel imidazoles in four steps. Using ester compound with active methyl group and formed ethanone. Oxidize with SeO_2 formed ethane-1, 2-dione. Cyclise with substituted aldehyde with intermediate ethane-1, 2-dione and formed new imidazoles. The clear procedure for the preparation of desired imidazoles was given below.

4. Preparation of new substituted imidazoles

4.1. General procedure for the Synthesis of ethyl -4- fluoro benzoate

To the stirred solution of compound 4-fluorobenzoic acid (13 gm, 1mmole) in T.H.F, (250 ml) .Added thionyl chloride solution (25 ml) at 0°C of reaction mass .Heated the reaction mass at 80°C for 6 hr. Evaporated the reaction mass under reduced pressure under nitrogen atmosphere. Maintain 0°C of residue and added ethanol (25 ml) and stirred at R.T. Progress of reaction mass was monitored through Thin layer chromatography (T.L.C). Reaction mass was evaporated under reduced pressure. Further this diluted with water and extracted with three times ethyl acetate (500 ml).Ethyl acetate layer was dried over anhydrous sod sulphate. Filtered and evaporated under reduced pressure. Product (Viscous liquid) 13.5 gm. (Yield: 86.5%) was obtained and characterized by its NMR spectroscopy.

$^1\text{HNMR}$: (400MHz, CDCl_3): δ 8.04-8.07 (m, 2H), 7.08-7.12 (m, 2H), 4.368 (q, $J = 7.2$ Hz, 2H), 1.399 (t, $J = 7.2$ Hz, 3H).

4.2. General procedure for the Synthesis of 2-(2-chloropyridin-4-yl)-1-(4-fluorophenyl) ethanone –

To the stirred solution of compound 2-chloro-4-methylpyridine (6.5 gm, 50.95 m.mole) in 300 ml T.H.F and added Lithium hexamethyldisilazane (2.0 M) solution (38.2 ml, 76.42 m.mole) in reaction mass under nitrogen atmosphere. Maintained 0°C of reaction mass for 1 hr. Added synthesized compound of ethyl -4- fluoro benzoate (8.5 gm, 50.95 mmole) and stirred at R.T for 6 hr. Progress of reaction mass was monitored through thin layer chromatography (T.L.C). Reaction mass was diluted with saturated solution of ammonium chloride and extracted with three times ethyl acetate (300 ml).Total ethyl acetate layer was dried over anhydrous sod sulphate. Filtered and evaporated under reduced pressure. Product (Yellow solid) 9.7 gm. (Yield: 76.3%) was obtained and characterized by its NMR spectroscopy.

$^1\text{HNMR}$: (400MHz, CDCl_3): δ 8.357 (s, $J = 5.2$ Hz, 1H), 8.00-8.04 (m, 2H), 7.12-7.26 (m, 4H), 4.26 (s, 2H).

4.3. General procedure for the Synthesis of 1-(2-chloropyridin-4-yl)-2-(4-fluorophenyl) ethane -1, 2- dione –

To the stirred solution of synthesized compound 2-(2-chloropyridin-4-yl)-1-(4-fluorophenyl) ethanone (9.7 gm, 38.85 m.mole) in 15 dioxane solution, 150 ml and added selenium di oxide (9.4 gm, 93.24 m.mole) at 0°C of reaction mass. Refluxed the reaction mass for 5 hr. Progress of reaction mass was monitored through thin layer chromatography (T.L.C). Reaction mass was filtered through celite bed. Filtrated solution diluted with water and extracted with three times ethyl acetate (300 ml).Total ethyl acetate layer was dried over anhydrous sod sulphate. Filtered and evaporated under reduced pressure. Product (Yellow solid) 9.5 gm. (Yield: 93.1%) was obtained and characterized by its NMR spectroscopy.

$^1\text{HNMR}$: (400MHz, CDCl_3): δ 8.645 (d, $J = 5.2$ Hz, 1H), 8.02-8.04 (m, 2H), 7.82 (s, 1H), 7.71-7.72 (m, 1H), 7.21-7.26 (m, 2H).

4.4. General procedure for the Synthesis of substituted imidazoles derivative-

To the stirred solution of synthesized compound 1-(2-chloropyridin-4-yl)-2-(4-fluorophenyl) ethane -1, 2- dione (0.973 m.mole) in 5.0 ml acetic acid, added substituted aldehyde (0.97m,mole) at 0°C of reaction mass. Furthur this added ammonium acetate (2.91 m.mole). Refluxed the reaction mass for 5 hr. Progress of reaction mass was monitored through thin later chromatography (T.L.C). Reaction mass was diluted with water and basify with saturated solution of sod bicarbonate and maintain the pH about 8.0. Extracted the aquous layer with three times ethyl acetate (30 ml).Total ethyl acetate layer was dried over anhydrous sod sulphate. Filtered and evaporated under reduced pressure. Crude compound was obtained and purify by using column silica and characterized by its NMR

spectroscopy and by mass spectroscopy... Same procedure was followed for the compound H1 (a), H1 (b), H1(c).....H1 (j).

Spectral data of Imidazoles :

2 -Chloro-4-(2-(2, 6-difluorophenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl) pyridine. H1 (a)

¹HNMR: (400MHz, DMSO-d₆): δ 13.25 (bs, 1H), 8.33 (d, *J* = 5.2Hz, 1H), 7.56-7.65 (m, 4H), 7.23-7.40 (m, 5H). LCMS: 386.05 (M⁺), 388.03 (M+2). Purity: 95.84 %, Anal. calcd. For C₂₀H₁₁C₁F₃N₃: C, 62.27; H, 2.87; N, 10.89.

2- Chloro-4-(4-(4-fluorophenyl)-2-phenyl-1H-imidazol-5-yl) pyridine. H1 (b) –

¹HNMR: (400MHz, DMSO-d₆): δ 13.10 (bs, 1H), 8.28 (s, 1H), 8.08 (d, *J* = 7.2Hz, 2H), 7.58-7.61 (m, 3H), 7.48-7.52 (m, 2H), 7.40-7.44 (m, 4H).LCMS; 350.04 (M⁺), 352.02 (M+2).Purity; 99.97%, Anal. calcd. For C₂₀H₁₃C₁FN₃: C, 68.67; H, 3.75; N, 12.01.

2 -Chloro-4-(2-(2, 4, 6-trifluorophenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl) pyridine. H1 (c) –

¹HNMR: (400MHz, DMSO-d₆): δ 13.26 (bs, 1H), 8.28 (d, *J* = 5.2Hz, 1H), 7.55-7.60 (m, 2H), 7.23-7.47(m, 6H).LCMS; 404.3(M⁺), 406.2(M+2).Purity; 98.3%Anal. calcd. For C₂₀H₁₀C₁F₄N₃: C, 59.49; H, 2.50; N, 10.41.

4 - (5-(2-Chloropyridin-4-yl)-4-(4-fluorophenyl)-1H-imidazol-2-yl) benzonitrile. H1 (d) –

¹HNMR: (400MHz, DMSO-d₆): δ 13.24 (bs, 1H), 8.24-8.38 (m, 3H), 7.98 (d, *J* = 8.0Hz, 2H), 7.58-7.65 (m, 3H), 7.39-7.44 (m, 3H).LCMS; 375.3 (M⁺), Purity; 95.9% Anal. calcd. For C₂₁H₁₂C₁FN₄: C, 67.30; H, 3.23; N, 14.95.

2 -Chloro-4-(4-(4-fluorophenyl)-2-(thiophen-3-yl)-1H-imidazol-5-yl) pyridine. H1 (e) –

¹HNMR: (400MHz, DMSO-d₆): δ 12.93 (bs, 1H), 8.30 (d, *J* = 5.2Hz, 1H), 8.07-8.11 (m, 1H), 7.62-7.69 (m, 2H), 7.55-7.61 (m, 3H), 7.25-7.40 (m, 3H).LCMS; 355.98 (M⁺), Purity: 99.5% Anal. calcd. For C₁₈H₁₁C₁FN₃S: C, 60.76; H, 3.12; N, 11.81.

2- Chloro-4-(4-(4-fluorophenyl)-2-(4-methoxyphenyl) - 1H-imidazol-5-yl) pyridine. H1 (f) –

¹HNMR: (400MHz, DMSO-d₆): δ 12.90 (bs, 1H), 8.27 (s, 1H), 8.01 (d, *J* = 8.8Hz, 2H), 7.57-7.60 (m, 3H), 7.38-7.39 (m, 3H), 7.06 (d, *J* = 8.4Hz, 2H).3.82 (s, 3H).LCMS; 380.04(M⁺), 382.03(M+2).Purity;98.1%Anal. calcd. For C₂₁H₁₅C₁FN₃O: C, 66.41; H, 3.98; N, 11.06.

4- (2-(2, 4-bis (trifluoromethyl) phenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl)-2 chloropyridine. H1 (g) –

¹HNMR: (400MHz, DMSO-d₆): δ 13.35 (bs, 1H), 8.12-8.39 (m, 4H), 7.63 (d, *J* = 5.2Hz, 2H), 7.53 (s, 1H), 7.22-7.43 (m, 3H).LCMS; 486.24(M⁺), 488.23(M+2).Purity; 99.89%Anal. calcd. For C₂₂H₁₁C₁F₇N₃: C, 54.39; H, 2.28; N, 8.65

2- Chloro-4-(2-(2-fluoro-5-(trifluoromethyl) phenyl)-4-(4 fluorophenyl)-1H-imidazol-5-yl) pyridine. H1 (h) –

¹HNMR: (400MHz, DMSO-d₆): δ 13.15 (bs, 1H), 8.38 (d, *J* = 5.6Hz, 1H), 8.28-8.31 (m, 1H), 7.89 (s, 1H), 7.55-7.68 (m, 4H), 7.23-7.42 (m, 3H).LCMS; 435.99 (M⁺).Purity; 99.89% Anal. calcd. For C₂₁H₁₁C₁F₅N₃: C, 57.88; H, 2.54; N, 9.64.

2- Chloro-4-(2-(2, 4-difluorophenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl) pyridine. H1 (i) –

¹HNMR: (400MHz, DMSO-d₆): δ 12.95 (bs, 1H), 8.28 (s, 1H), 8.01-8.07 (m, 1H), 7.54-7.59 (m, 3H), 7.51 (m, 1H), 7.38-7.39 (m, 3H), 7.25-7.29 (m, 1H).LCMS; 386.15(M⁺), 388.14 (M+2) Purity; 98.45%.Anal. calcd. For C₂₀H₁₁C₁F₃N₃: C, 62.27; H, 2.87; N, 10.89.

2-Chloro-4-(2-(2-fluoro-4-(tri fluoro methyl) phenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl) pyridine H1 (j) –

¹HNMR: (400MHz, DMSO-d₆): δ 13.15 (bs, 1H), 8.25-8.38 (m, 2H), 7.91 (d, *J* = 10.4Hz, 1H), 7.75 (d, *J* = 7.6Hz, 1H), 7.55-7.63 (m, 3H), 7.23-7.42 (m, 3H).LCMS; 436.3 (M⁺), Purity; 98.3%Anal. calcd. For C₂₁H₁₁C₁F₅N₃: C, 57.88; H, 2.54; N, 9.64.

5-Antimicrobial studies-

5.1- Antibacterial and antifungal studies-

Antibacterial and antifungal activity of newly synthesized compounds H1 (a), H1 (b), H1(c).....H1 (j) were determined by 'Broth Dilution Method'. Main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC as well. All the synthesized compounds were tested

against two gram positive bacteria (*Staphylococcus aureus*, *Streptococcus Pyogenes*) and two gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*)

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37°C overnight. The tubes are then incubated overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The lowest concentration inhibiting growth of the organism is recorded as the MIC.
5. The amount of growth from the control tube before incubation [which represents the original inoculum] is compared. [12-16].

6- Antimalarial studies-

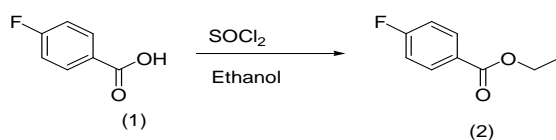
All the synthesized compounds were screened for antimalarial activity in the Microcare laboratory & TRC, Surat, Gujarat. In vitro antimalarial assay was carried out in 96 well microliter plates according to the micro assay protocol of Rieckmann and co-workers with minor modifications. The culture of *P. falciparum* strain medium RPMI1640 supplemented with 25 mm HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat 1% inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, an initial ring stage parasitaemia of 0.8 to 1.5% at 3% haematocrit in a total volume of 200 µl of medium RPMI-1640 was determined by Jaswant Singh Bhattacharya (JSB) staining to assess the percent parasitaemia (rings) and uniformly 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 µl volume were added to the test wells so as to obtain final concentrations (at fivefold dilutions) ranging between 0.4 µg/ml to 100 µg/ml in duplicate well containing parasitized cell preparation. The culture plates were incubated at 37°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 parasites into 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). Chloroquine was used as the reference drug. [17-22].

RESULTS AND DISCUSSION

7.1. Chemistry

In this present investigation a novel series of substituted Imidazoles compounds were synthesized as per Schemes (1– 2). Scheme 1 illustrates the pathway used for the synthesis of *Ethyl -4- fluoro benzoate* which was used as an intermediate in scheme 2. Scheme 2 illustrates the pathway used for the synthesis of *2-(2-chloropyridin-4-yl)-1-(4-fluorophenyl) ethanone*, Synthesis of *1-(2-chloropyridin-4-yl)-2-(4-fluorophenyl) ethane -1, 2- dione* and synthesis of novel active imidazoles derivatives.

SCHEME: 1- Synthesis of *ethyl -4- fluoro benzoate*



SCHEME: 2- Synthesis of novel Imidazole derivatives

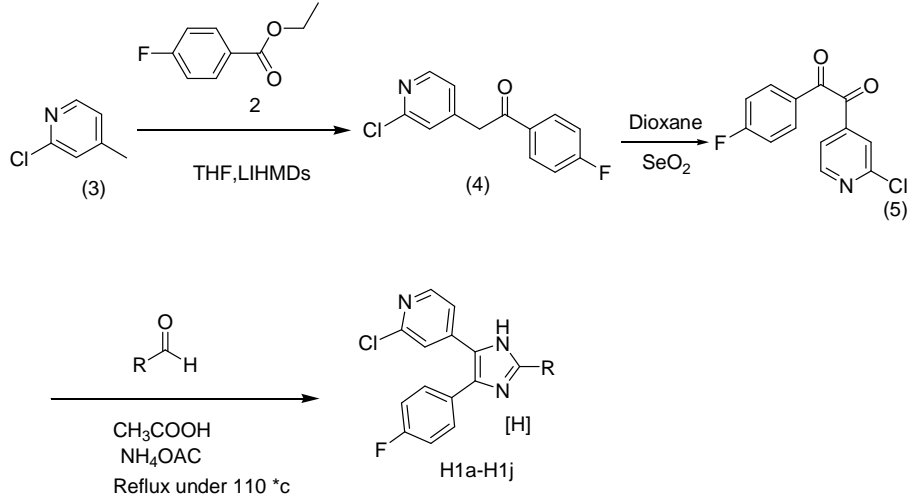


Table-1 List of synthesized compound

Compound	R	M.P	Yield
H1(a)	2,6-difluoro phenyl	(228–229 0 ^o C)	85.2%
H1(b)	Phenyl	(295–296 0 ^o C)	85.9%
H1(c)	2,4,6-trifluoro phenyl	(238–239 0 ^o C)	87.1%
H1(d)	4-Cyano phenyl	(225–226 0 ^o C)	83.1%
H1(e)	3-Thiophene	(283–284 0 ^o C)	87.5%
H1(f)	4-methoxy phenyl	(240–241 0 ^o C)	80.5%
H1(g)	2,4-Bis(trifluoro methyl)phenyl	(239–240 0 ^o C)	82.9%
H1(h)	2-F,5-CF ₃ phenyl	(239–240 0 ^o C)	82.9%
H1(i)	2,4-difluoro phenyl	(213–214 0 ^o C)	82.2%
H1(j)	2-F,4-CF ₃ phenyl	(241 -242 0 ^o C)	83.3%

Antibacterial activity (In Minimum inhibitory concentration)

The antibacterial activity of all the synthesized compounds were tested in-vitro against pathogenic *E. coli* MTCC 443, *P.aeruginosa* MTCC 1688, *S.aureus* MTCC96 and *S.pyogenus* MTCC 442 and the results were compared with standard drugs (Ampicillin and Chloramphenicol). In case of *S.aureus* MTCC96 compounds H1(e), H1(a), H1(c) and H1(h), exhibit good activity while H1(b), H1(d), H1(i) and H1(j) show moderate activity. In case of *S.pyogenus* MTCC 442 compounds H1(c) exhibit higher activity while H1(a), H1(e), H1(h) and H1(j) shows moderate activity. In case of *E. coli* MTCC 443 Compound H1(a), H1(b), H1(f) and H1(i) shows higher activity and H1(e), H1(g) and H1(j) shows moderate activity while rest of the compounds possess less activity. In case of *P.aeruginosa* MTCC 1688 compounds H1(b), H1(g) and H1(h) and shows good activity while H1(f), H1(i) and H1(j) shows moderate activity while rest of the compounds possess less activity. The results are given in Table-2

Compound	<i>E.COLI</i> MTCC 443	<i>P.AERUGINOSA</i> MTCC 1688	<i>S.AUREUS</i> MTCC96	<i>S.PYOGENUS</i> MTCC 442
H1(a)	200	200	100	200
H1(b)	200	100	250	500
H1(c)	500	250	100	125
H1(d)	500	500	250	250
H1(e)	250	500	62.5	200
H1(f)	200	125	200	500
H1(g)	250	100	200	250
H1(h)	250	100	125	200
H1(i)	200	250	200	250
H1(j)	250	250	250	200
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. The results are given in Table-3.

Compound	<i>C.Albicans</i> MTCC 227	<i>A.Niger</i> MTCC 282	<i>A.Clavatus</i> MTCC 1323
H 1(a)	1000	500	1000
H1(b)	500	250	500
H1(c)	>1000	>1000	>1000
H1(d)	1000	500	500
H1(e)	1000	>1000	>1000
H1(f)	500	500	500
H1(g)	500	500	1000
H1(h)	1000	1000	1000
H1(i)	1000	1000	1000
H1(j)	1000	1000	>1000
Nystatin	100	100	100
Greseofulvin	500	100	100

Antimalarial activity:

Similarly for antimalarial all the newly synthesized compounds were screened for antimalarial activity and the results were compared with standard drugs Quinine. The results are given in Table-4.

Compound	Mean IC50 (microgram/ml)
H 1(a)	0.54
H1(b)	0.56
H1(c)	0.64
H1(d)	0.94
H1(e)	1.23
H1(f)	1.98
H1(g)	1.05
H1(h)	1.22
H1(i)	1.89
H1(j)	1.13
Quinine	0.268

CONCLUSION

The series of novel substituted imidazole derivatives were synthesized in reasonably good yields. They were characterized by ¹H NMR, Liquid chromatography mass spectrometry and elemental analyses. All the newly synthesized compounds were screened for antimicrobial activity. Among the screened samples compound **H1(e)** has showed good anti-bacterial activity at 62.5 microgram/mL concentrations against particular tested microbial strains *S.AUREUS* MTCC96 as compared to the standard drug. While H1(a) has showed excellent anti-bacterial activity at 100 microgram/mL concentrations against *S.AUREUS* MTCC96 and 200 microgram/mL concentrations against *E.COLI* MTCC 443, *P.AERUGINOSA* MTCC 1688 and *S.PYOGENUS* MTCC 442 as compared to the standard drug. For antifungal all the newly synthesized compounds were screened for antifungal activity. Among the screened samples compound H1 (b) has showed excellent antifungal activity against the fungus strains of *C.ALBICANS* MTCC 227, *A.NIGER* MTCC 282 and *A.CLAVATUS* MTCC 1323, H1(f) and H1(g) has showed good antifungal activity against the fungus strains of *C. ALBICANS* MTCC 227, *A.NIGER* MTCC 282 and *A.CLAVATUS* MTCC 1323. Similarly for antimalarial all the newly synthesized compounds were screened for antimalarial activity. Among the screened samples compound H1 (a) and H1 (b) showed good antimalarial activity against *Plasmodium falciparum*. While H1(c) and H1 (d) showed moderate antimalarial activity against *Plasmodium falciparum*.

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