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FACILE SYNTHESIS, CHARACTERIZATION AND IN VITRO ANTI-TUBERCULAR SCREENING OF NOVEL IMIDAZOLE INCORPORATED HETEROCYCLIC 2-SUBSTITUTED ARYL-4-FLUORO PHENYL-5-(2-CHLOROPYRIDINYL)-1H-IMIDAZOLE DERIVATIVES

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ABSTRACT

The valuable treatment of tuberculosis is difficult, due to the complicated or unusual structure and chemical composition of the mycobacterial cell wall. These complex properties of cell wall make many antibiotics and currently used drugs ineffective and hinder the entry of drugs in to the mycobacterium cells. Tuberculosis is still the one of the most crucial infectious disease worldwide. This paper describes the facile synthesis, characterization and in vitro anti-tubercular screening of novel imidazole incorporated heterocyclic 2-substituted aryl-4-fluoro phenyl-5-(2-Chloropyridinyl)-1H-imidazole derivatives which were 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. The entire synthesized compounds were characterized by elemental analysis, 1H NMR and LCMS and also screened for their in- vitro antitubercular activity against Mycobacterium tuberculosis.

KEYWORDS: Imidazole, selenium dioxide, ammonium acetate and anti tubercular activity.

INTRODUCTION

Naturally occurring substituted imidazoles as well as synthetic derivatives thereof exhibit a wide range of biological activities, making them attractive compounds for biochemists and organic chemists. Imidazole ring is one of the important motifs which have been found in a large number of natural products and pharmacologically active compounds. Different imidazole derivatives show many biological activities such as fungicidal[1], anti-bacterial[2], anti-tumoral[3], anti-inflammatory[4], and antithrombotic.[5] Also various substituted imidazoles act as plant growth regulators[6], inhibitors of p38 MAP[7] and B-Raf kinase[8], and glucagon receptors.[9] Omeprazole[10], Pimobendan[11], Losartan, Olmesartan, Eprosartan, and Trifenagrel[12] are some of the drugs with diverse functionalization around the imidazole ring. Thus, the development of novel synthetic strategies of imidazole units is an interesting topic in modern organic chemistry.

MATERIALS AND METHODS

2.1 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. ^{1}H 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.

2.2 Chemistry

We have prepared the novel 2-substituted aryl-4-fluoro phenyl-5-(2-Chloropyridinyl)-1H-imidazole in four steps. Using ester compound with active methyl group and formed ethanone. Oxidize with SeO2 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.

3. Preparation of new substituted Imidazole

3.1. Synthesis of ethyl -4- fluoro benzoate (1)

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 0^0C of reaction mass. Heated the reaction

mass at 80 0 ^{0}C for 6 hr. Evaporated the reaction mass under reduced pressure under nitrogen atmosphere. Maintain 0^{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.

1HNMR: (400MHz, CDCl3): 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* = 7.2Hz, 3H).

3.2 Synthesis of 2-(2-chloropyridin-4-yl)-1-(4-fluorophenyl) ethanone (2)

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 ^{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.

1HNMR: (400MHz, CDCl3): δ 8.357 (s, *J* = 5.2Hz, 1H), 8.00-8.04 (m, 2H), 7.12-7.26 (m, 4H), 4.26 (s, 2H).

3.3 Synthesis of 1-(2-chloropyridin-4-yl)-2-(4-fluorophenyl) ethane -1, 2- dione (3)

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 ^{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.

1HNMR: (400MHz, CDCl3): δ 8.645 (d, *J* = 5.2Hz, 1H), 8.02-8.04 (m, 2H), 7.82 (s, 1H), 7.71-7.72 (m, 1H), 7.21- 7.26 (m, 2H).

3.4 General procedure for the synthesis of desired 2-substituted aryl-4-fluoro phenyl-5-(2-Chloropyridinyl)-1H-imidazole derivatives

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 0C of reaction mass. Further 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 5(a), 5(b), 5(c)…….5(j).

2 -Chloro-4-(2-(2, 6-difluorophenyl)-4-(4-fluorophenyl)-1H imidazol-5-yl) pyridine. 5 (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+), Purity: 95.84%, Anal. cacld. For C20H11ClF3N3: C, 62.27; H, 2.87; N, 10.89.

2- Chloro-4-(4-(4-fluorophenyl)-2-phenyl-1H-imidazol-5-yl) pyridine. 5 (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+). Purity; 99.97%, Anal. cacld. For C20H13ClFN3: 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. 5 (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. cacld. For C20H10ClF4N3: C, 59.49; H, 2.50; N, 10.41.

4 - (5-(2-Chloropyridin-4-yl)-4-(4-fluorophenyl)-1H-imidazol-2-yl) benzonitrile. 5 (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. cacld. For C21H12ClFN4: C, 67.30; H, 3.23; N, 14.95.

2 -Chloro-4-(4-(4-fluorophenyl)-2-(thiophen-3-yl)-1H-imidazol-5-yl) pyridine. 5 (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. cacld. For C18H11ClFN3S: C, 60.76; H, 3.12; N, 11.81.

2- Chloro-4-(4-(4-fluorophenyl)-2-(4-methoxyphenyl) - 1H-imidazol-5-yl) pyridine. 5 (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. cacld. For C21H15ClFN3O: C, 66.41; H, 3.98; N, 11.06.

4-(2-(2, 4-bis (trifluoromethyl) phenyl) -4-(4-fluorophenyl)-1H-imidazol-5-yl)-2chloro pyridine.5 (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. cacld. For C22H11ClF7N3: 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.5 (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. cacld. For C21H11ClF5N3: C, 57.88; H, 2.54; N, 9.64.

2- Chloro-4-(2-(2, 4-difluorophenyl)-4-(4-fluorophenyl)-1H-imidazol-5-yl) pyridine. 5 (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. cacld. For C20H11ClF3N3: 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 5 (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. cacld. For C21H11ClF5N3: C, 57.88; H, 2.54; N, 9.64.

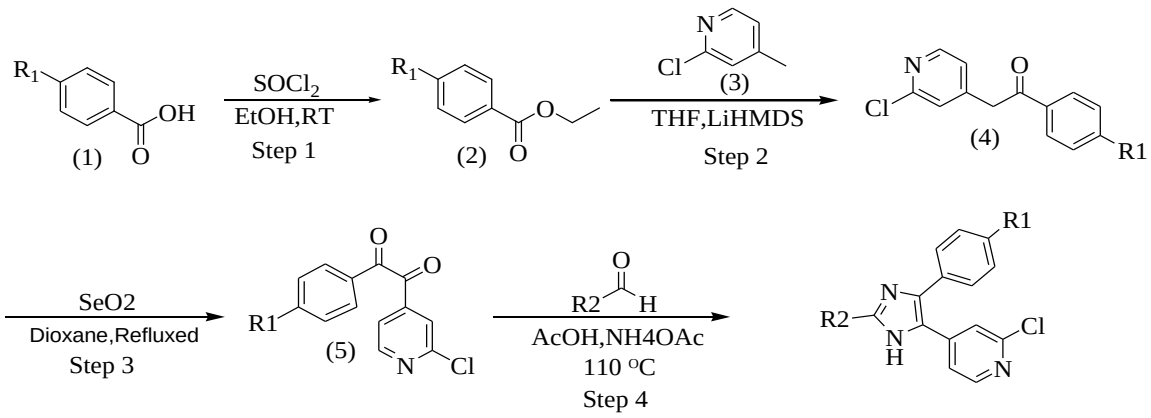
4. ANTI TUBERCULAR STUDIES

All the compounds were screened for their in vitro antimycobacterial activity against mycobacterium tuberculosis (MTB) H37Rv. The primary screening was carried out by agar dilution method using double dilution technique recommended by the National committee for clinical laboratory standards.[12] Isoniazid and thiacetazone were used as standard drugs. MTB H37Hv was grown in Middle brook 7H11 broth medium supplemented with 10% OADC(Oleic acid, albumin, dextrose and catalase,1,10,100 mg/L) In brief 10^3 and 10^4 colony forming unit (CFU) were inculcated in to 7H11 medium. The minimum inhibitory concentration (MIC) was defined as the minimum concentration of compound required to 90% inhibition of bacterial growth.

5. RESULT AND DISCUSSION

5.1 Chemistry

In this present investigation a Scheme 1 illustrates the pathway used for the synthesis of novel imidazole's derivatives. In step1 obtained substituted acid to substituted ethyl ester. 2-Chloro-4-methyl pyridine is used as another reactant for step 2. *2-(2-chloropyridin-4-yl)-1-(4- fluoro phenyl) ethanone* formed through oxidation process in step 3. Cyclization of oxidized product with substituted phenyl aldehyde at heating condition formed some novel imidazole derivative in step 4.



Scheme 1

Table 1.

List of synthesized compound				
S. No	**Compound**	**R**	**M.P(0 C)**	**Yield (%)**
1	5a	2,6-difluoro phenyl	228-229	85.2
2	5b	Phenyl	295-296	85.9
3	5c	2,4,6-trifluoro phenyl	238239	87.1
4	5d	4-cyano phenyl	225-226	83.1
5	5e	3-thiophene	283-284	87.5
6	5f	4-methoxy phenyl	240-241	80.5
7	5g	2,4-Bis(trifluoro methyl)phenyl	239-240	82.9
8	5h	2-fluoro-5-trifluoromethyl phenyl	239-240	82.9
9	5i	2,4-difluoro phenyl	213-214	82.2
10	5j	2-fluoro-4-trifluoromethyl phenyl	241-242	83.3

5.2 In vitro anti mycobacterial activity

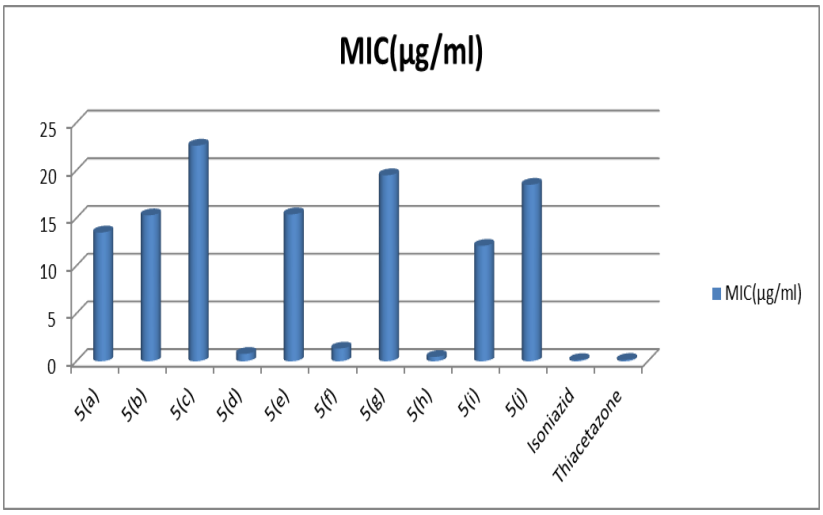
All the compounds were screened for their in vitro anti mycobacterial activity against mycobacterium tuberculosis (MTB) H37Rv.

Table 2: Anti mycobacterial activity.

S.NO	**Compound**	**MIC(µg/ml)**
1	5(a)	13.5
2	5(b)	15.3
3	5(c)	22.6
4	5(d)	0.77
5	5(e)	15.4
6	5(f)	1.33
7	5(g)	19.5
8	5(h)	0.45
9	5(i)	12.1
10	5(j)	18.5
11	**Isoniazid**	0.125
12	**Thiacetazone**	0.138

MIC=Minimum inhibitory concentration that is the lowest concentration to inhibit 90% of mycobacterium tuberculosis H37Rv growth: Isoniazid and thiacetazone were used as standard.

5.3 Graphical representation for anti mycobacterial activity



6. 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 anti tubercular activity. Some of the compound shows moderate to weak activity. Compound ***2-Chloro-4-(2-(2-fluoro-5-(trifluoromethyl)phenyl)-4-(4fluorophenyl)-1H-imidazol-5-yl)pyridine*** **5(h)** exhibit very good type anti tubercular activity with MIC of 0.45 µg/ml and compound ***4 - (5-(2-Chloropyridin-4-yl)-4-(4-fluorophenyl)-1H-imidazol-2-yl)benzonitrile*** **5(d)** exhibit good type anti tubercular activity with MIC of 0.77 µg/ml.

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REFERENCES

1. A.F. Pozherskii, A.T. Soldatenkov, A.Y. Katritzky, Heterocycles in Life and Society, Wiley, New York, 1997; 179–180.
2. M. Antolini, A. Bozzoli, C. Ghiron, G. Kennedy, T. Rossi, A. Ursini, Analogues of 4,5-bis(3,5-dichlorophenyl)-2- trifluoromethyl-1H-imidazole as potential antibacterial agents, Bioorg. Med. Chem. Lett., 1999; 9: 1023–1028.

3. L. Wang, K.W. Woods, Q. Li, K.J. Barr, R.W. McCroskey, S. M. Hannick, L. Gherke, R.B. Credo, Y.-H. Hui, K. Marsh, R. Warner, J.Y. Lee, N. Zielinsky Mozng, D. Frost, S.H. Rosenberg, H.L. Sham, Potent, orally active heterocycle-based combretastatin A-4 Analogues: synthesis, structure-activity relationship, pharmacokinetics, and in vivo antitumor activity evaluation, J. Med. Chem., 2002; 45: 1697–1711.
4. J.G. Lombardino, E.H. Wiseman, Preparation and antiinflammatory activity of some nonacidic trisubstituted imidazoles, J. Med. Chem., 1974; 17: 1182–1188.
5. A.P. Phillips, H.L. White, S. Rosen, Eur Patppl. EP 58, 890, Sep. 1, 1982. Chem. Abstr, 1983; 98: 53894z.
6. R. Schmierer, H. Mildenberger, H. Buerstell, German Patent, 1987, Chem. Abstr, 108: 37838.
7. J.C. Lee, J.T. Laydon, P.C. McDonnell, T.F. Gallagher, S. Kumar, D. Green, D. McNulty, M.J. Blumenthal, J.R. Keys, S. W.L. Vatter, J.E. Strickler, M.M. McLaughlin, I.R. Siemens, S. M. Fisher, G.P. Livi, J.R. White, J.L. Adams, P.R. Young, A protein kinase involved in the regulation of inflammatory cytokine biosynthesis, Nature, 1994; 372: 739–746.
8. A.K. Takle, M.J.B. Brown, S. Davies, D.K. Dean, G. Francis, A. Gaiba, A.W. Hird, F.D. King, P.J. Lovell, A. Naylor, A.D. Reith, J.G. Steadman, D.M. Wilson, The identification of potent and selective imidazole-based inhibitors of B-Raf kinase, Bioorg. Med. Chem. Lett., 2006; 16: 378–381.
9. S.E. de Laszlo, C. Hacker, B. Li, D. Kim, M. MacCoss, N. Mantalo, J.V. Pivnichny, L. Colwell, G.E. Koch, M.A. Cascieri, W.K. Hagmenn, Potent, orally absorbed glucagon receptor antagonists, Bioorg. Med. Chem. Lett., 1999; 9: 641–646.
10. P. Lindberg, P. Nordberg, T. Alminger, A. Brandstorm, B. Wallmark, The mechanism of action of the antisecretory agent omeprazole, J. Med. Chem., 1986; 29: 1327–1329.
11. S.G. Gordon, M.W. Miller, A.B. Saunders, Pimobendan in heart failure therapy-a silver bullet?, J Am. Anim. Hosp. Assoc, 2006; 42: 90–93.
12. S.L. Abrahams, R.J. Hazen, A.G. Batson, A.P. Phillips, Trifenagrel: a chemically novel platelet aggregation inhibitor, J. Pharmacol. Exp. Ther., 1989; 249: 359–365.