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SYNTHESIS, CHARACTERIZATION AND *IN-VITRO* OXIDOREDUCTASE INHIBITORS OF SUBSTITUTED IMIDAZOLE DERIVATIVES

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ABSTRACT:

Imidazole is an organic compound with the formula $C_3N_2H_4$. It is a white or colourless solid that is soluble in water, producing a mildly alkaline solution. Imidazole is a highly polar compound, as evidenced by its electric dipole moment of 3.67 and is highly soluble in water. All the chemicals and glassware procured from our college, physicochemical characterization like melting point determination by open capillary method and TLC has been done according to solubility of compounds or derivatives in specific solvent alone or polarity ratio. Nine compoundshave been selected for synthesis and characterized by FTIR, NMR (^{1}H, Proton analysis) Mass spectral analysis and further oxidoreductase inhibitorevaluation.Compound AP4 is also moderately active but less than the AP2, reason can be seen as AP4 is having a nitro group but also have electron acceptor group chlorine which is making it less potent than other only nitro substituted compounds.

Keywords: Imidazole, *In-vitro*oxidoreductase inhibitors, Anti-bacterial, Anti-infective agents.

1. INTRODUCTION:

The human body exists in a state of dynamic equilibrium with microorganism. In a healthy individual this balance is maintained as peaceful co-existence and lack of disease. But sometimes, micro-organisms cause an infection or a disease.

A microorganism or microbe is an organism that is microscopic (usually too small to be seen by the naked human eye). Microorganisms are very diverse; they include bacteria, fungi, archaea, and protists; microscopic plants (green algae) and animals such as plankton the planarian and the amoeba.

Imidazole is an organic compound with the formula $C_3N_2H_4$. It is a white or colourless solid that is soluble in water, producing a mildly alkaline solution. In chemistry, it is an aromatic heterocycle, classified as a diazole, and has non-adjacent nitrogen atoms in meta-substitution.

Many natural products, especially alkaloids, contain the imidazole ring. This ring system is present in important biological building blocks, such as histidine and the related hormone histamine. Many drugs contain an imidazole ring, such as certain antifungal drugs, the nitroimidazole series of antibiotics, and the sedative midazolam.[4-6]When fused to a pyrimidine ring, it forms a purine, which is the most widely occurring nitrogen-containing heterocycle in nature.[7]The name "imidazole" was coined in 1887 by the German chemist Arthur Rudolf Hantzsch (1857–1935) [8].

Imidazole is a planar 5-membered ring, that exists in two equivalent tautomeric forms because hydrogen can be bound to one or another nitrogen atom. Imidazole is a highly polar compound, as evidenced by its electric dipole moment of 3.67 D,[11] and is highly soluble in water. The compound is classified as aromatic due to the presence of a planar ring containing 6 π-electrons (a pair of electrons from the protonated nitrogen atom and one from each of the remaining four atoms of the ring). [9]

2. MATERIALS AND METHODS:

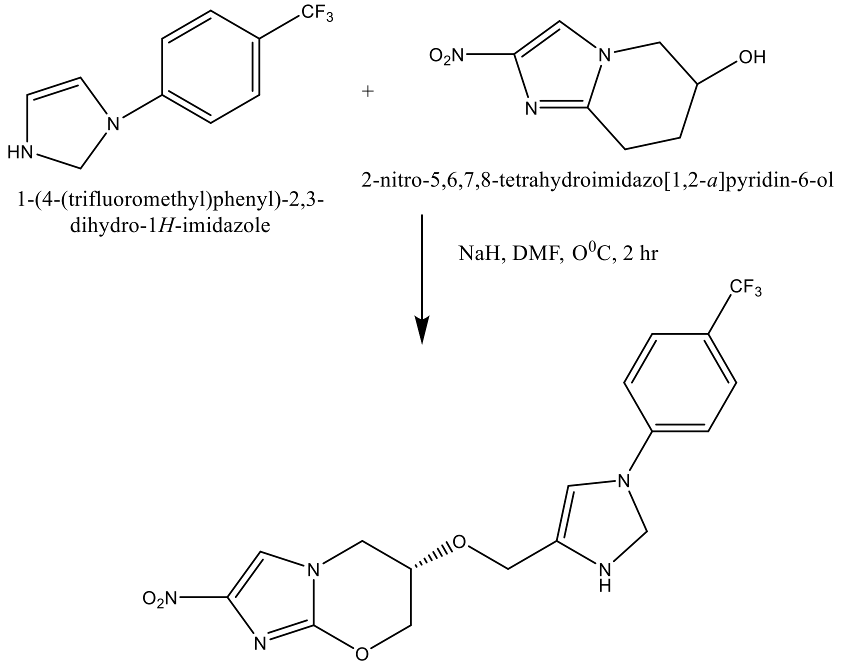
All the chemicals and glassware procured from our college, physicochemical characterization like melting point determination by open capillary method and TLC has been done according to solubility of compounds or derivatives in specific solvent alone or polarity ratio.

Spectral characteristic has been done by the FTIR, NMR, and Mass spectroscopy to conformation for the structure of synthesized compounds.

Synthesis of compounds:

2.1 Synthesis of *Synthesis of (S)-2-nitro-6-((1-(4-fluorometyl)-phenyl)-2,3-dihydro-1H-imidazole-4-yl-4-yl)methoxy)-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (*AP 1-Compound 1a*):*

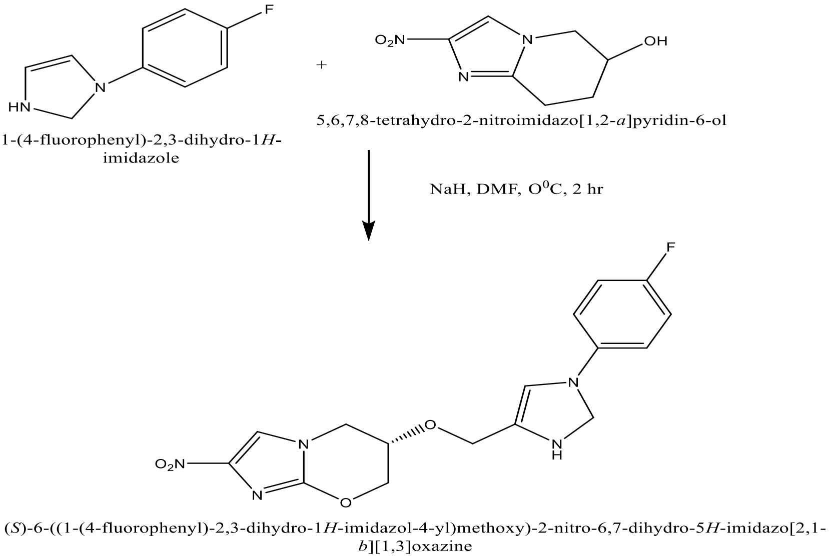
Synthetic Scheme IA:



(*S*)-2-nitro-6-((1-(4-(trifluoromethyl)phenyl)-2,3-dihydro-1*H*-imidazol-4-yl)methoxy)-6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]oxazine

2.2 Synthesis of (S)-6-((1-(4-fluorophenyl)-2,3-dihydro-IH-imidazole-4-yl)methoxy)- 2-nitro-6,7-dihydro-5H-imidazo[2,1-b][1,3]oxazine (AP 2-Compound 2c):

Scheme IB:

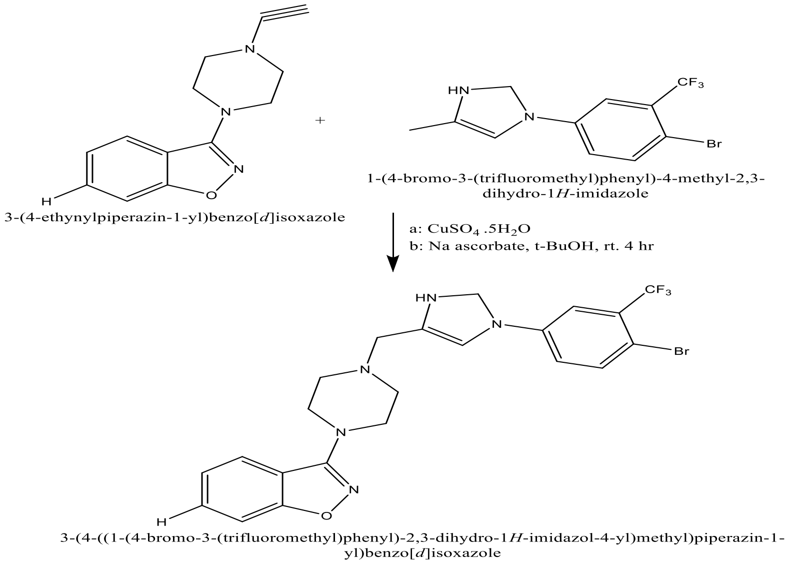


Synthetic Procedure:

The compounds were prepared by base-catalyzed alkylation of the known chiral alcohol with the 4-halo heterocycles followed by Suzuki coupling of the products with appropriate (Scheme IA and IB). Similar base-catalyzed alkylation of alcohol with 1-trifluloro and 1-fluro reacted with 5,6,7,8-tetrahydro-2-nitroimidazo[1,2-a]pyridin-6-ol in the presence of sodium hydride and dimethyl formamide reflux at O^0c up to 2 hrs gave the 1-aryl-1,2,3-imidazole derivative [10] (1a and 2c), crude products were recrystallized with ethanol and perform the TLC and melting point to find out the purity of the product and report the % yield.

2.3 Synthesis of 3-(4-((1-(4-bromo-3-(trifluoromethyl)phenyl)- 2,3-dihydro-IH-imidazole -4-yl) methyl)piperazin-1-yl)benzo[d]isoxazole (AP 3-Compound 5a):

Scheme II:



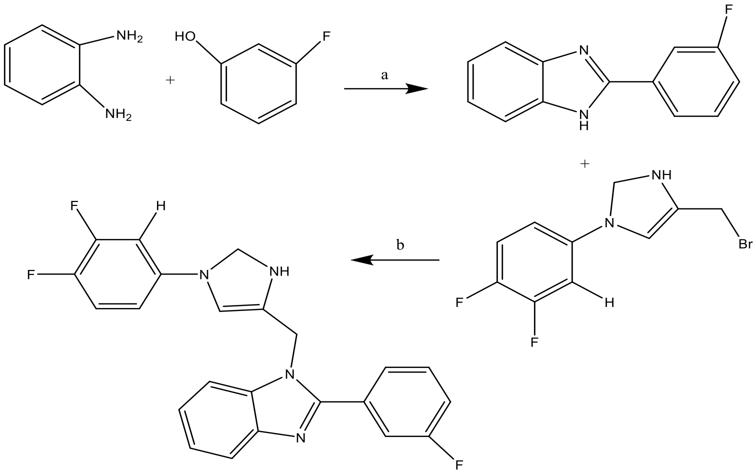
Synthetic Procedure:

In this work, we synthesized 3-(4-((1-(4-bromo-3-(trifluoromethyl)phenyl)-1H-2,3-imidazol-4-yl)methyl)piperazin-1-yl)benzo[d] isoxazole as depicted in Schemes II. title compounds 5A were synthesized by reacting 3-(4-ethynylpiperazin-1-yl)benzo[d]isoxazole with 1-(4-bromo-3-

(trifluoromethyl)phenyl)-4-methyl-1H-1,2,3-imidazole as aryl azides, in the presence of $CuSO_4$. $5H_2O$, Na ascorbate, t-BuOH and H_2O at room temperature for 4hours and raw products were recrystallized with ethanol and perform the TLC [11] and melting point to find out the purity of the product and report the % yield.

2.4 Synthesis of 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1-H-[2,3-dihydro-1*H*-imidazole -4-yl-methyl)-1H benzo[d] imidazole (AP 4-compound 7b):

Scheme III:



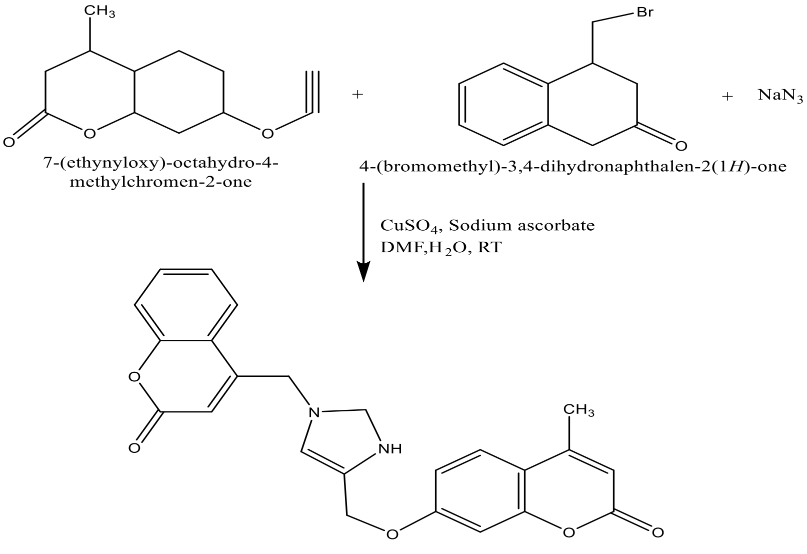
(a) Toluene, 110 C, 30–60 min.; (b) NaH, 4-bromomethyl-1-phenyl-1H-[1,2,3]-triazole, DMF, rt.

Synthetic Procedure:

We have synthesized the target molecule, 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1-H-[2,3]-dihydro-imidazol-4-yl-methyl)-1Hbenzo[d]imidazole. Condensation of o-phenylenediamine with 3-fluoro benzaldehyde in toluene or xylene at 110^{o}C for 1 h by air oxidation without using oxidizing agents or catalysts, furnished the 2-(3-fluoro-phenyl)-1H benz [d] imidazole. Then the subsequent reaction of 3-fluoro benzimidazole with substituted 4-(bromomethyl)-1-phenyl-1H-[2,3]- dihydro-imidazol- using NaH as a base proceeded at room temperature to furnish 2-(3-fluoro-phenyl)-1-[1-(substituted-phenyl)-1H-[1,2,3]-triazol4-yl methyl)-1H-benzo[d]imidazole **[12]** and raw products were recrystallized with ethanol and perform the TLC and melting point to find out the purity of the product and report the % yield.

2.5 Synthesis of coumarin 2, 3-imidazole hybrids (AP-5 compound 10):

Scheme IV:

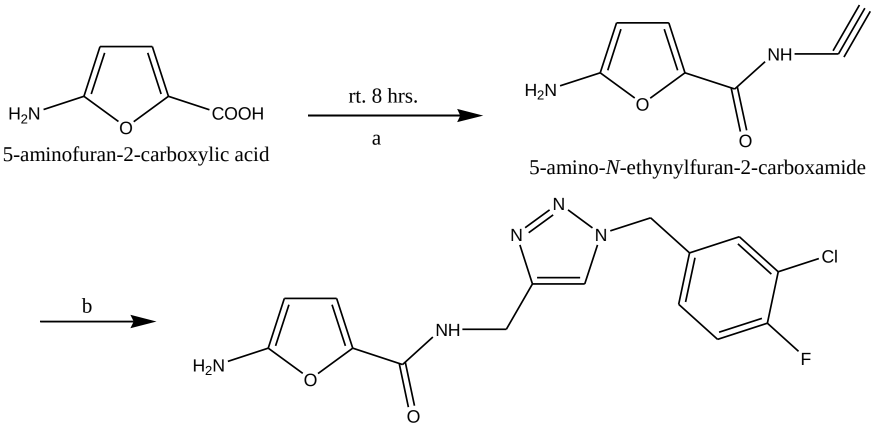


Synthetic Procedure:

Three component one pot in situ azide-alkyne cycloaddition of 2-propargyl thiobenzimidazole with 2/3 in presence of sodium azide were carried out using copper ascorbate in DMF/water at room temperature to obtain exclusively the 1,4 substituted-1,3- imidazole hybrids (compound 10), and raw products were recrystallized with ethanol and perform the TLC [13] and melting point to find out the purity of the product and report the % yield.

2.6: Synthesis of furan 2,3-imidazole hybrids (AP 6- Compound 13):

Scheme V:



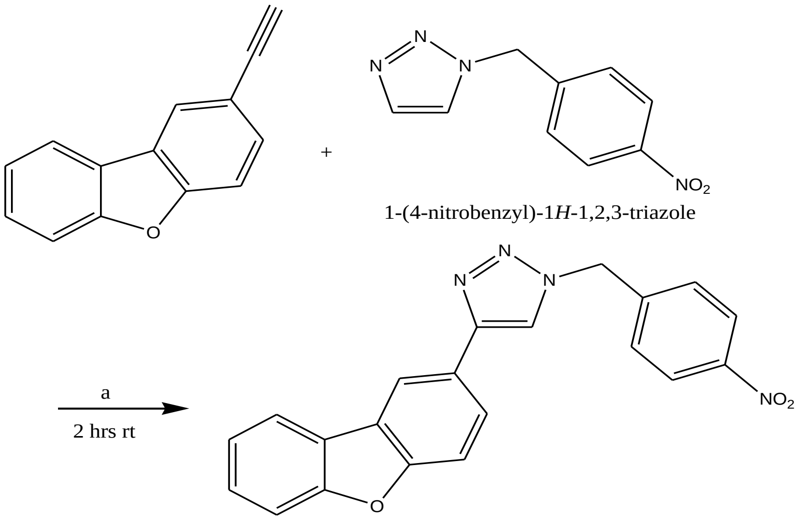
a: propargyl amine, EDC, BtOH, CH_2Cl
b: $CuSO_4$, sodium ascorbate, t-BuOH/ H_2O (1:3)

Synthetic Procedure:

The synthetic route to achieve the title compounds 13 is outlined in Scheme V. As shown, 5-amino-2-furoic acid was reacted with propargyl amine in presence of EDC and HOBt in CH_2Cl_2. The resultant amide containing terminal alkyne functionality was reacted with substituted benzyl azides to obtain the final compound was recrystallized [14] with ethanol and perform the TLC and melting point to find out the purity of the product and report the % yield.

2.7: Synthesis of isatin 2,3-imidazole hybrids (CS 7-17f):

Scheme VI:



a: CuSO4, sodium ascorbate,t-butanol, water (1:1 v/v)

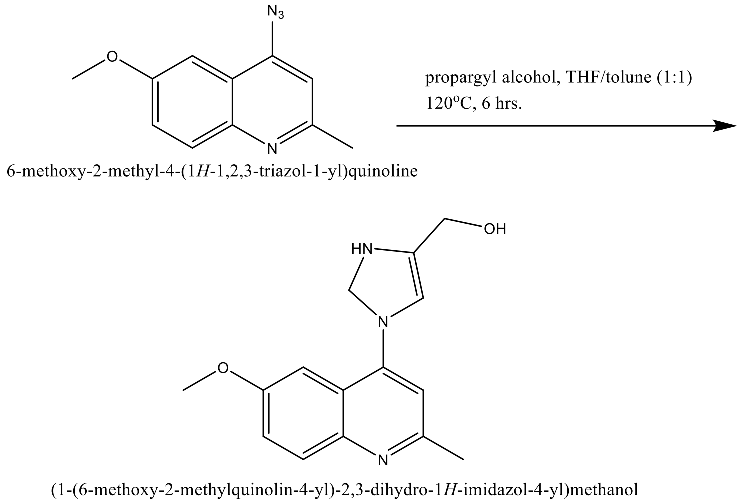
Synthetic Procedure:

Having both alkyne andazides in hand, we employed Huisgen'scycloaddition in the presence of $CuSO_4$ catalyst, sodium ascorbate in t-butanol and water (1:1, v/v). All the azides reacted well with

2-ethynyldibenzo[b,d]furan to give isatin 1,2,3-imidazole hybrids [15-16] and the final compound was recrystallized with ethanol and perform the TLC and melting point to find out the purity of the product and report the % yield.

2.8: Synthesis of quinoline 2, 3-imidazole hybrids (AP 8- compound 25):

Scheme VII:

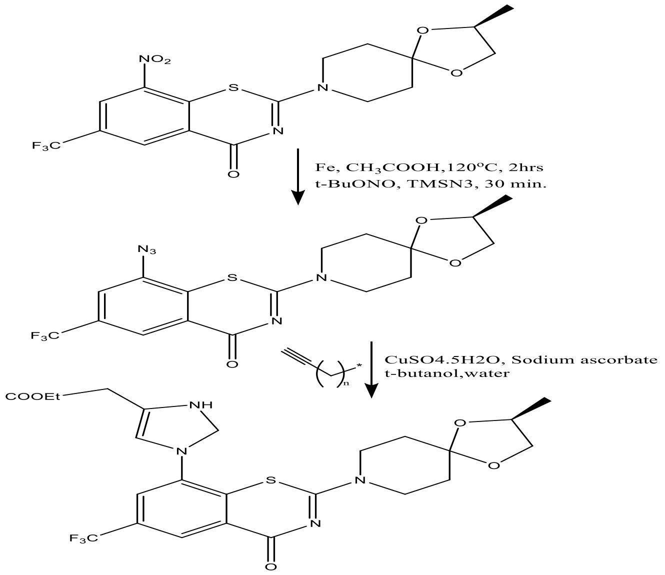


Synthetic Procedure:

The compound (1-(6-methoxy-2-methylquinolin-4-yl)-1H-2,3-imidazol-4-yl)methanol was obtained by heating a mixture of 4-azido-6-methoxy-2-methylquinoline and propargyl alcohol in dry THF/toluene (1:1) at 120 C for 6 hours, [17-20] and the final compound was recrystallized with ethanol and perform the TLC and melting point to find out the purity of the product and report the % yield.

2.9: Synthesis of miscellaneous 2,3-imidazole hybrid (AP 9- compound 36c):

Scheme VIII:

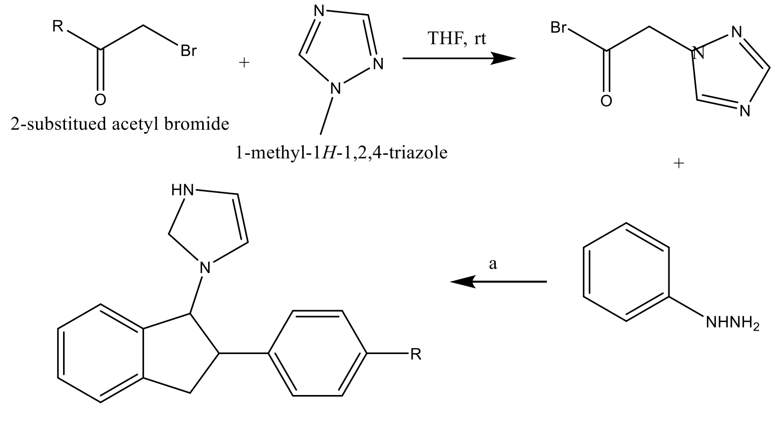


Synthetic Procedure:

Azide was synthesized in two steps from 1 by first reducing the nitro group of the latter using Fe/CH_3COOH under reflux conditions (120°C) for 2 h to give the reduced amine. The amino group of intermediate was subsequently treated with tert-butyl nitrite (t-BuONO) followed by azidotrimethylsilane (TMSN3) to afford in good yield over two steps. Therefore, was treated with ethylpropiolate as representative alkynes under standard reaction conditionsto give imidazoles derivative and final compound was recrystallized with ethanol and perform the TLC and melting point to find out the purity of the product and report the % yield.

2.10 SYNTHESIS OF 2,4-IMIDAZOLE DERIVATIVES (AP 10-compound 45b and AP 11-Compound 45c):

Scheme IX:



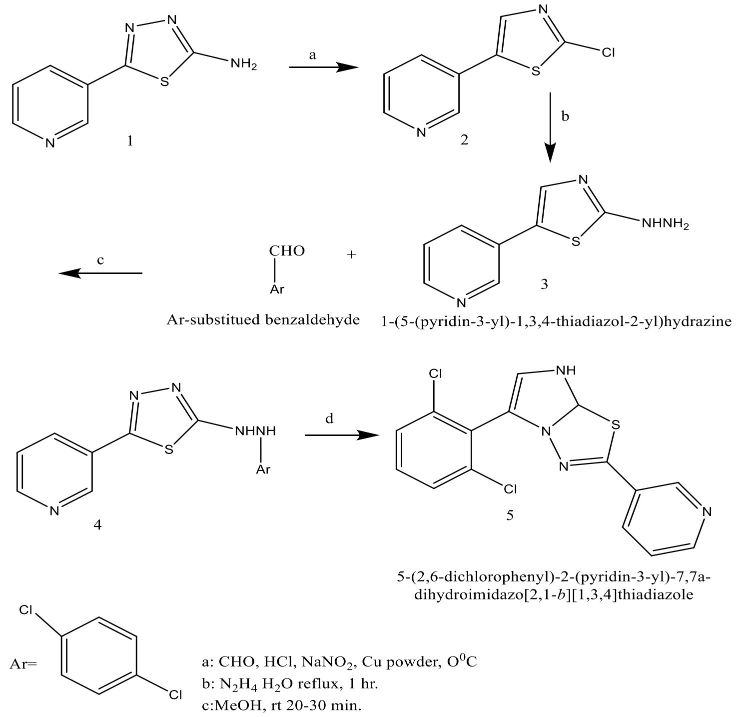
a: EtOH, reflux HCl, b: MW(140^{o}C, 90 minutes) R=Br, Cl

Procedure:

The synthesis of compounds, was carried out via a two-steps reaction which involved a N-alkylation of imidazole or 2,4-imidazole with para-substituted 2-bromoacetophenones to afford the corresponding 1-aryl-2-(1H-imidazol-1-yl) or (1H-1,2,4-triazol-1-yl)-ethanonesin accordance with the literature procedure. The indole derivatives were obtained through the classical Fischer cyclization and final compound was recrystallized with ethanol and performs the TLC and melting point to find out the purity of the product and report the % yield.

2.11 Synthesis of 2,4-Imidazole derivatives (AP 12- Compound 51):

Scheme X:



a: CHO, HCl, $NaNO_2$, Cu powder, $O^{0}C$
b: N_2H_4 H_2O reflux, 1 hr.
c:MeOH, rt 20-30 min.
d: chloramine, t-trihydrate,ethanol, rt 5-6 min

Procedure:

The starting compound 1 was prepared by direct cyclization of nicotinic acid and thiosemicarbazide in polyphosphoric acid, which was diazotized in hydrochloric acid and glacial acetic acid with Cu powder catalysis, to get the 2-chloro compound 2. Then the compound 2 was treated with hydrazine hydrate in ethanol under reflux condition to get 1-(5-(pyridine-3-yl)-1,3,4-thiadiazol-2-yl) hydrazine 3, which served as a good precursor for new fused heterocycles. The structure of the compound 3 was confirmed by its spectral data (see Supporting Information). The required aldehyde N-1-(5- (pyridine-3-yl)-3,4-thiadiazol-2-yl) hydrazones 5 were prepared in good yields by the condensation of the hydrazine derivative 3 with the appropriate aldehydes 4. The final compound was recrystallized with ethanol and performs the TLC and melting point to find out the purity of the product and report the % yield.

3. PHYSICOCHEMICAL CHARACTERIZATION

3.1 Melting Point: Melting points were determined by Thiele's tube method and were uncorrected.

3.2 Thin layer chromatography: Thin layer chromatography was performed on precoated silica gel-G 0.2mm thick using different solvent system to ascertain the purity of the synthesized compounds. UV and iodine vapours were used as the detecting agents.

3.3 Infrared spectrum: Infrared spectrum was taken on FTIR-8400S Shimadzu at School of Pharmacy, Devi Ahilya Vishwavidyalaya, Indore.

3.4 Nuclear Magnetic Resonance: - ^{1}HNMR and ^{13}CNMR spectrums were obtained on Bruker DRX-400 (400 MHz FT NMR) at Sophisticated Analytical Instrument Facility, Panjab University, Chandigarh.

3.5 Mass spectral analysis studies: Mass spectrum was obtained on WATERS, Q-TOF MICROMASS (LC-MS), CIL, Punjab University, Chandigarh.

4. *INVITRO*OXIDOREDUCTASE INHIBITION ASSAY

Initially, enzyme solution was prepared by dissolving 0.5mg of enzyme to 10ml Ethanol. Along with this in a separate beaker a 0.1M Phosphate buffer was prepared and 5mM solution of substrate (enoyl-reductaseInhA) was prepared by adding 15mg substrate to 100ml Ethanol. The control sample was prepared by taking 200µl enzyme, 1200 µl buffer and substrate. 5ml ethanol was added to this test tube. This was then kept for incubation for 30 minutes. Its absorbance was taken using Schimadzo UV Spectrophotometer at 400nm. The test samples of different compounds were made in six different concentrations (50 µl, 100 µl, 150µl, 200 µl, 250µl and 300µl) by adding to the enzyme-substrate solution in a test tube. This was kept for incubation for 30minutes and their absorbance was taken at 400nm.Acarbose was taken as standard in this assay. The enzyme inhibitory rates of samples were calculated as follows:

Inhibition % = [(Control absorbance – test sample absorbance) / control absorbance)] * 100

5. RESULTS AND DISCUSSION:

5.1 STRUCTURE ELUCIDATION OF DESIGNED AND SYNTHESIZED COMPOUNDS:

Table 1: Physicochemical parameter of synthesized derivatives

Comp. code	Melting point range in ⁰C	Rf value
AP 1	212-213	0.57*
AP 2	254-255	0.65*
AP 3	219-220	0.74*
AP 4	316-317	0.39#
AP 5	236-224	0.59#
AP 6	265-267	060$^{@}$

AP 7	198-199	0.81@
AP 8	287-288	0.77#
AP 9	256-257	0.86*
AP 10	241-242	0.46@
AP 11	263-264	0.55#
AP 12	301-303	0.89*

Solvent System for TLC
* Ethyl acetate: Ethanol (7:3), #Toluene: methanol (6:4), @ n-hexane: chloroform (5:5)

5.2 FT-IR SPECTRAL ANALYSIS:

The FTIR absorption values of imidazole derivatives have been recognised on the basis of FTIR and were reported in the experimental section. All the synthesized derivatives exhibited identical peaks of N-H of hydrazine in the region of 3200-3300 cm^{-1}, this indicates the presence of the hydrazine moiety in all the synthesized compounds. All the compounds shows stretching in the ranges of 1640-1670 cm^{-1} and denotes the carbonyl group (C=O) present in the compounds. Apart from that common peak of C=N hydrazine stretching is observed in the region of 1400-1600 cm^{-1} of frequency. A strong band is observes in the region of 1640-1720 cm^{-1}shows the C=C presence in the compound.

AP-1: 1627 (Ar), 3286, 3109 (N-H, Str), 1627, 1542 (N-O, Bend), 1697, 1627 (C=N), 1697, 1542 (C=C), 1627 & 1442 (Ar) 1573 & 1350 (NO2). **AP-2:** 1596 & 1350 (Ar), 3471, 2985 (N-H, Str), 1666, 1573 (N-H, Bend), 1704, 1666 (C=N), 1704, 1596 (C=C) 1350 (NO2) 516 (C-F). **AP-3:** 1596 & 1350 (Ar), 3471, 2985 (N-H, Str), 1666, 1573 (N-H, Bend), 1704, 1666 (C=N), 1704, 1596 (C=C). **AP-4:** 1589 & 1357 (Ar), 3456, 3116 (N-H,Str), 1666, 1558 (N-H, Bend), 1712, 1656 (C=N), 1586 & 1359 (Ar), 1558 & 1357 (CH3) 516, 527 & 612 (C-F). **AP-5:** 151542 & 1450 (Ar), 3496, 3062 (N-H, Str), 1643, 1542 (N-H, Bend), 1728,1643 (C=N), 1643, 1542 (C=O). **AP-6:** 1627 & 1442 (Ar), 3394, 2977 (N-H, Str), 1627, 1496 (N-H, Bend), 1735, 1627 (C=N), 1735, 1591 (C=C), 1442 & 1375 (CH$_3$). **AP-7:** 1593 (NO$_2$), 1598 & 1400 (Ar), 3417, 3116 (N-H, Str), 1620, 1560 (N-H, Bend), 1712, 1620 (C=N), 1596 & 1435 (Ar), 1596 & 1249 (CH$_3$). **AP-8:** 1612 & 1508 (Ar), 3248 (-OH) 3116 (N-H, Str), 1612, 1573 (N-H, Bend), 1666, 1612 (C=N), 1712, 1612 (C=C), 1504 & 1380 (CH$_3$, Bend). **AP-9:** 1573 & 1512 (Ar), 3479, 3101 (N-H, Str), 1426 (C-S), 1550 (N-H, Bend), 1743, 1660 (C=N), 1572 & 1512 (Ar), 1330 (NO$_2$) 1710 (C=O).**AP-10:** 1573 & 1512 (Ar), 1743, 1660 (C=N), 1572 & 1512 (Ar), 715 (C-Br).**AP-11:** 1573 & 1512 (Ar), 1666, 1550 (N-H, Bend), 1743, 1660 (C=N), 1572 & 1512 (Ar) C-Cl (678).**AP-12:** 1573 & 1512 (Ar), 3479, 3101 (N-H, Str), 1666, 1550 (N-H, Bend), 1743, 1660 (C=N), 1572 & 1512 (Ar), 674 & 658 (C-Cl).

5.3 NUCLEAR MAGNETIC RESONANCE STUDIES

AP-1:^{1}H NMR revealed that compound (S)-6-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-6,7-dihydro-2-nitro-5H-imidazo[2,1-b][1,3]oxazineshows the characteristiAP peaks in benzene ring (A) of imidazole ring, imidazole ring (B) of imidazole, benzene ring (C) attached to imidazole and ring attached oxazine group (D) in the range of δ 7.0- 8.10 for11 hydrogen. In ring E the peaks of amine group and azo group was observed at δ 8.98 and δ 8.18 respectively. **AP-2:^{1}H NMR** revealed that compound (S)-6-((1-(4-fluorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-6,7-dihydro-2-nitro-5H-imidazo[2,1-b][1,3]oxazineshows the characteristiAP peaks in benzene ring (A) of imidazole ring, imidazole ring (B) of imidazole, benzene ring (C) attached to imidazole and ring attached hydrazine group (D) in the range of δ 6 – 8 for11 hydrogen. In ring E the peaks of amine group and azo group was observed at δ 8.13 and δ 8.11 respectively. **AP-3:^{1}H NMR** revealed that compound 3-(4-((1-(4-bromo-3-(trifluoromethyl)phenyl)-1H-1,2,3-triazol-4-yl)methyl)piperazin-1-yl) benzo [d] is oxazole shows the characteristic AP peaks in benzene ring (A) of imidazole ring, thiazole ring (B) of imidazole, benzene ring (C) attached to imidazole and ring attached isoxazole group (D) in the range of δ 6.5- 8.20 for11 hydrogen. In ring E the peaks of amine group and bromo group was observed at δ 8.8 and δ 8.3 respectively. **AP-4:^{1}H NMR** revealed that compound 2-(3-fluoro-phenyl)-1-[1-

(substituted-phenyl)-1-H-[1,2,3]-triazol-4-yl-methyl)-1H benzo[d] imidazole shows the characteristic AP peaks in benzene ring (A) of imidazole ring, imidazole ring (B) of imidazole, benzene ring (C) attached to imidazole and ring attached fluro group (D) in the range of δ 6.7- 8.3 for11 hydrogen. In ring E the peaks of amine group and aza group was observed at δ 8.60 and δ 8.44 respectively. **AP-5: ^{1}H NMR** revealed that coumarin 1, 2, 3-imidazole derivatives shows the characteristiAP peaks in benzene ring (A) of imidazole ring, coumarin ring (B) of imidazole, benzene ring (C) attached to methyl coumarin and ring attached oxa group (D) in the range of δ 6.7- 8.50 for11 hydrogen. In ring E the peaks of amine group and azo group was observed at δ 10.2 and δ 8.5 respectively. **AP-6:^{1}H NMR** revealed that furan 1,2,3-imidazole derivativesshows the characteristiAP peaks in benzene ring (A) of imidazole ring, furan ring (B) of imidazole, benzene ring (C) attached to imidazole and ring attached Chloro-substituted group (D) in the range of δ 6.7- 8.10 for11 hydrogen. In ring E the peaks of amine group and amine group was observed at δ 9.9 and δ 8.2 respectively. **AP-7:^{1}H NMR** revealed that isatin 1,2,3-imidazole hybrids shows the characteristiAP peaks in benzene ring (A) of imidazole ring, isatin ring (B) of imidazole, benzene ring (C) attached to imidazole and ring attached nitro group (D) in the range of δ 7.0 - 8.5 for11 hydrogen. In ring E the peaks of amine group and azo group was observed at δ 8.6 and δ 8.40 respectively. **AP-8: ^{1}H NMR** revealed that compound quinoline 1, 2, 3-imidazole hybrids shows the characteristiAP peaks in benzene ring (A) of imidazole ring, quinoline ring (B) of imidazole, benzene ring (C) attached to imidazole and ring attached hydroxyl group (D) in the range of δ 6.7- 8.0 for11 hydrogen. In ring E the peaks of OH group group was observed at δ 8.5 and δ 8.1 respectively. **AP-9: ^{1}H NMR** revealed that compound Azide-1,2,3-imidazole hybrid shows the characteristiAP peaks in benzene ring (A) of imidazole ring, azide ring (B) of imidazole, benzene ring (C) attached to imidazole and ring attached carboxyl group (D) in the range of δ 6.7- 8.0 for11 hydrogen. In ring E the peaks of amine group and sulfa group was observed at δ 8.3 and δ 8.1 respectively.**AP-10:^{1}H NMR** revealed that compound 1,2,4-imidazole derivatives shows the characteristiAP peaks in benzene ring (A) of imidazole ring, indene ring (B) of imidazole, benzene ring (C) attached to imidazole and ring attached bromo group (D) in the range of δ 6.6- 8.0 for11 hydrogen.**AP-11:^{1}H NMR** revealed that compound 1,2,4-imidazole-fluro substituted derivatives shows the characteristiAP peaks in benzene ring (A) of imidazole ring, phenyl ring (B) of imidazole, benzene ring (C) attached to indene and ring attached aza group (D) in the range of δ 7.4- 8.0 for11 hydrogen.**AP-12: ^{1}H NMR** revealed that compound 1, 2, 4-imidazolethiadizol derivatives shows the characteristiAP peaks in benzene ring (A) of imidazole ring, thiadizole ring (B) of imidazole, pyridine ring (C) attached to imidazole and ring attached chloro group (D) in the range of δ 6.5- 8.2 for11 hydrogen. In ring E the peaks of dichloro group of benzene was observed at δ 8.3 and δ 8.1 respectively.

5.4 MASS SPECTRAL STUDIES:

In **AP-1** compound, molecular ion peak should be at 461, but because of removal of chlorine group from the benzene ring, and complete removal of imidazole ring with trifluoro group it is seen at 277 (M+1). In **AP-2** compound, molecular ion peak should be at 472, but because of removal of substituted 4 nitro benzene ring and trifluoro group attached to imidazole ring it is seen at 258 (M+2). In **AP-3** compound, molecular ion peak should be at 459, but because of removal of 2,4 dimethoxy substituted benzene ring, azo group and trifluoro group attached to imidazole ring it is seen at 272 (M+2). In **AP-4** compound, molecular ion peak should be at 506, but because of removal of 2-chloro 5-nitro substituted benzene ring and trifluoro group attached to imidazole ring it is seen at 256 (M+2). In **AP-5** compound, molecular ion peak should be at 461, but because of removal of 3-chloro substituted benzene ring and trifluoro group attached to imidazole ring it is seen at 322 (M+2). In **AP-6** compound, molecular ion peak should be at 473, but because of removal of 3-ethoxy 4-hydroxy substituted benzene ring and trifluoro group attached to imidazole ring it is seen at 262 (M+2). In **AP-7** compound, molecular ion peak should be at 506, but because of removal of 3-bromo substituted benzene ring and trifluoro group attached to imidazole ring it is seen at 287 (M+2). In **AP-8** compound, molecular ion peak should be at 443, but because of removal of 4-hydroxy substituted benzene ring and trifluoro group attached to imidazole ring it is seen at 273 (M+2). In **AP-9** compound, molecular ion peak should be at 443, but because of removal of 3-hydroxy substituted benzene ring and trifluorogroup attached to imidazole ring it is seen at 285 (M+2).In **AP-10** compound, molecular ion peak should be at 487, but because of removal of 3,4 dimethoxy substituted benzene ring and trifluoro group attached to imidazole ring it is seen at 278 (M+2).In **AP-11** compound, molecular ion peak should be at 445, but because of removal of 4-chloro substituted benzene ring and trifluoro group attached to imidazole ring it is seen at 258 (M+2).In **AP-12** compound, molecular ion peak should be at 470, but because of removal of 4-dimethyamino substituted benzene ring and trifluoro group attached to imidazole ring it is seen at 320 (M+2).

6. *IN-VITRO*OXIDOREDUCTASE INHIBITION ASSAY:

The oxidoreductase inhibition activity was carried out at Instrument PBIR LAB Bhopal, M.P. and the results were expressed in IC_{50} µg/ml (Table 5.1). All the synthesized Imidazole derivatives were found to be moderately active in *in-vitro*oxidoreductase inhibition activity ranges from 290µg/mL to 523µg/mL. Compound AP2, AP8, and AP9 showed moderate *in-vitro*oxidoreductase inhibition as compared to standard Ampicillin (110 µg/mL).

Table 2: Minimum inhibitory concentration of compounds and standards

S no	Compound	IC_{50} µg/ml
1	AP 1	484.03±3.3
2	AP 2	290.55±0.36
3	AP 3	512.2±0.14
4	AP 4	477.3±2.26
5	AP 5	452.49±3.47
6	AP 6	523.5±1.7
7	AP 7	406.12±9.5
8	AP 8	332.3±2.65
9	AP 9	344.2±3.85
10	AP 10	454.9±5.8
11	AP 11	427.5±5.20
12	AP 12	335.7±1.4
13	Ampicillin (Standard)	110.9±0.04

CONCLUSION:

Physicochemical characterization like melting point determination by open capillary method and TLC has been done according to solubility of compounds or derivatives in specific solvent alone or polarity ratio. Nine compoundshave been selected for synthesis and characterized by FTIR, NMR (^{1}H, Proton analysis) Mass spectral analysis and further oxidoreductase inhibitorevaluation.Compound AP2, AP8, AP9 and AP12 showed moderate *in-vitro*oxidoreductase inhibition as compared to standard ampicilline(110 µg/mL). Compound AP4 is also moderately active but less than the AP2, reason can be seen as AP4 is having a nitro group but also have electron acceptor group chlorine which is making it less potent than other only nitro substituted compounds. AP7 compound is also moderately potent as it is having a bromo group attached to it and this shows that bromo group which is a bulky halogen is required for activity. AP1, AP 5, AP11 are less active and have an electron accepting chlorine group which is not much giving much potency to the compounds.

CONFLICT OF INTREST: NIL

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