ANNEXURE - III

SUMMARY OF THE FINDINGS

Synthesis of Some Heterocyclic Compounds and their Screening Against *Entamoeba histolytica*

MAJOR RESEARCH PROJECT-FINAL REPORT

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Introduction

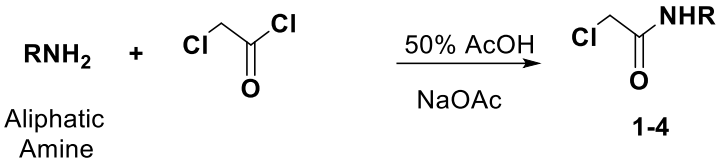
Amoebiasis , caused by the anaerobic protozoan parasite *Entamoeba histolytica.*, is the most common infection of the human gastrointestinal tract. This desease is caused by ingestion of contaminated food and water. The parasite invades the intestinal mucosa causing amoebic dysentery. At times, they migrate towards the liver, causing an amoebic liver abscess. *Infection of this parasite is common and occurs in a number of developing countries with poor sanitation facilities, resulting in 50 million cases of invasive disease and up to 100,000 fatalities per year. heterocyclic compounds have significant role in drug synthesis against* Amoebiasis. The heterocycles bearing nitrogen such as imidazole and indole have attracted considerable attention owing to their extensive biological spectrum such as antifungal, antimicrobial, anticancer, antiviral, anti-inflammatory, analgesic. Nitroimidazole ring is common in various drugs used for the treatment of amoebiasis such as metronidazole, tinidazole, and ornidazole etc. Hybrid molecules based chemotherapy for various diseases is an emerging field of Medicinal chemistry.

Work Plan

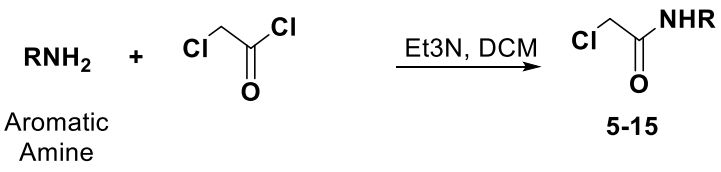
Scheme 1

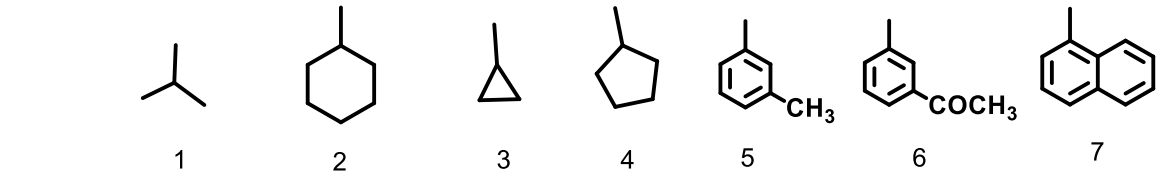
Step1

(a)



(b)





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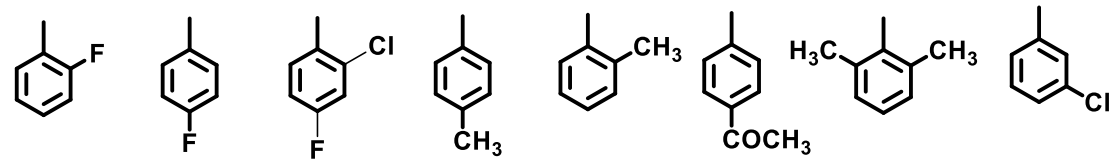
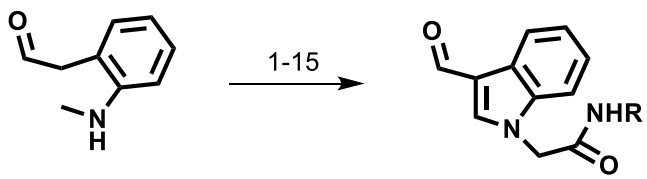
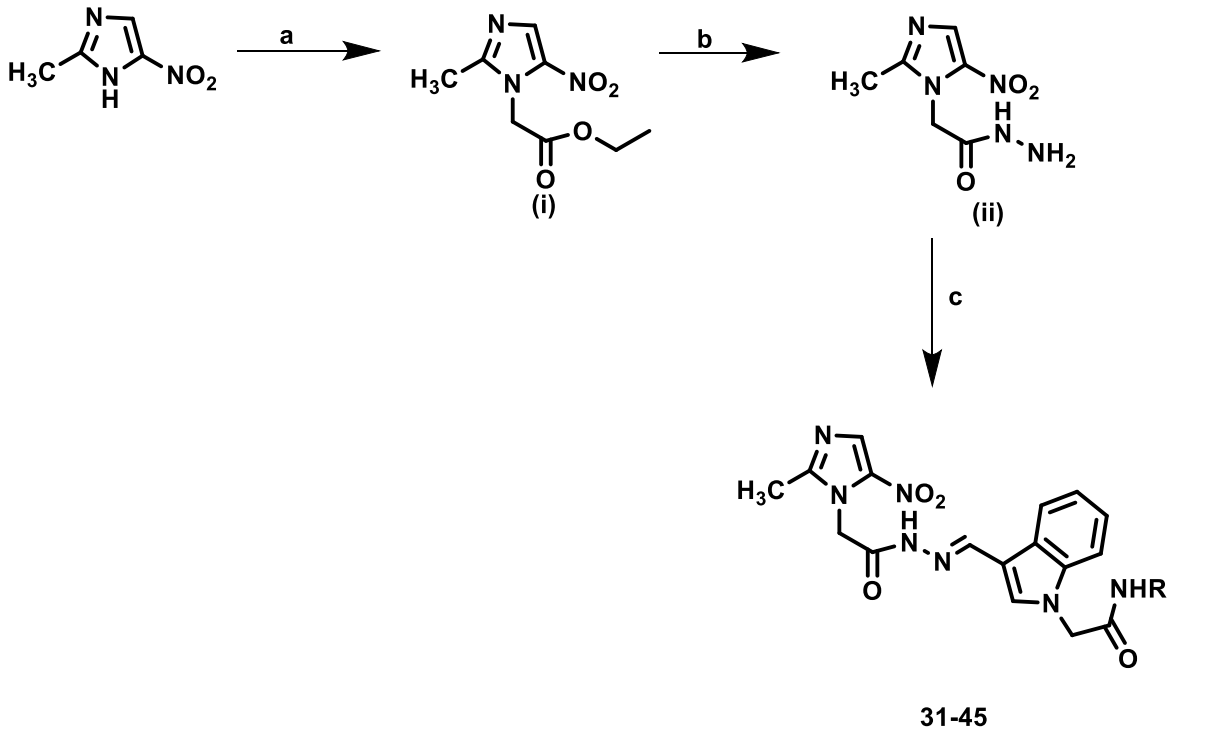


Step -2



Step -3



Methodology

Step 1: (a) To a stirring solution of aniline in glacial acetic acid 50% aqueous sodium acetate, chloro acetyl chloride was added dropwise. The reaction mixture was stirred at room temperature for 4 hr ; the product was precipitated by adding reaction mixture to crushed ice and then filtered on a Buckner funnel. The products(1-4) formed were recrystallized from ethanol.

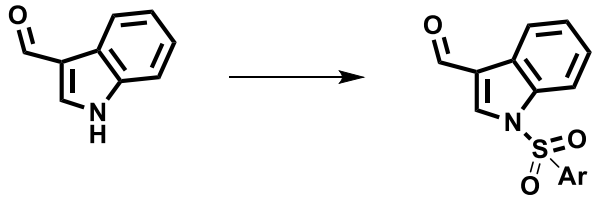
(b) To a stirring solution of amine in 10 ml dichloromethane, triethylamine was added. The reaction mixture was cooled to 0°C (under ice bath) and chloroacetyl chloride was added. Then, the reaction mixture was stirred for 3-4 h and partitioned between water and dichloromethane. Organic extract was separated and dried on sodium sulphate and concentrated to yield acetylated products (5-15).

Step 2: To a stirring solution of indole -3-carbaldehyde in dimethyl formamide, K_2CO_3, catalytic amount of KI and the amides(1-15) synthesized in step 1 were added. The reaction mixture was heated to 90°C till the completion of reaction which was monitored by thin layer chromatography. The reaction mixture was poured on crushed ice. The final products(16-30) precipitated were filtered over Buckner funnel.

Step 3: To a suspension of 2-methyl-5-nitroimidazole in acetonitrile, ethylchloroacetate and anhydrous potassium carbonate were added and refluxed overnight. The mixture was then filtered and the solvent was removed *in vaccuo*. The resulting solid was crystallized from ethanol to afford **A.** The mixture of **A** and hydrazine hydrate in ethanol was refluxed overnight. The solvent was removed *in vaccuo* and the solid was washed with water and then hexane which was dried and recrystallized from ethanol to obtain hydrazide **B**.Finally, condensation of compound (**B**) with different substituted aldehydes (16-19) in the presence of catalytic amount of conc.sulphuric acid, under reflux in ethanol for 2 h furnished the title compounds (31-45) in reasonable yields (60–90%).

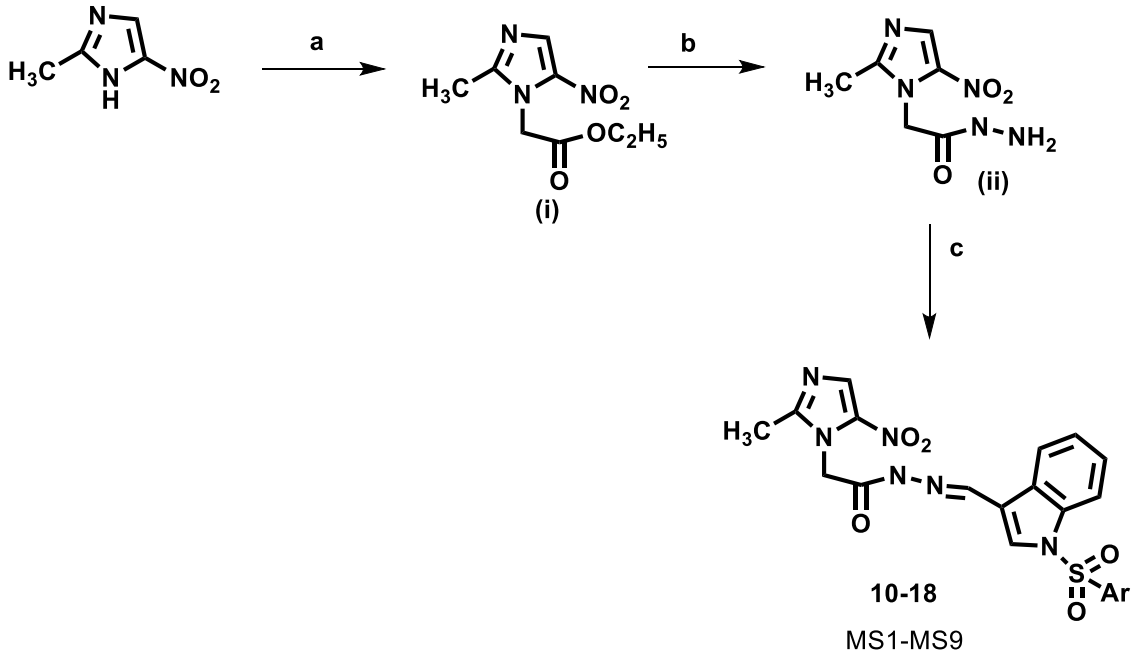
Scheme 2

Step1



S1-S9

Step 2



Methodology

Step 1: The indole-3-carboxaldeyde and sodium carbonate were stirred in 50%THF: water for 15 minutes. Then different substituted sulphonyl chlorides were slowly added. The reaction was kept at room temperature overnight .The solid product was extracted using water/DCM and DCM was concentrated to get the solid product.

Step 2: Synthesis of nitroimidazoles based sulfonamides (10-18)

The condensation of compound (**ii**) with various synthesized substituted aldehydes (1-9) in the presence of catalytic amount of conc.sulphuric acid, under reflux in ethanol for 2 h furnished the title compounds (10-18) in reasonable yields (60–90%).

In **scheme-1** all the synthesized compounds were screened *in vitro* for antiamoebic activity by micro-dilution method using HM1:IMSS strain of *E. histolytica*. Metronidazole (Mtz) is used as a reference drug having IC_{50} value 1.80µM in our experiment. The *N*-substituted-2-(3-formyl-1H-indol-1-yl) acetamide (C1-C15)were found IC_{50} values in the range 0.5 to 23.7 µM. Out of the fifteen derivatives of indole-3-carboxaldehyde (C1-C15) only two (C4 and C8) showed IC_{50} values less than Mtz. However, the hydrazone formation enhanced the antiamoebic activity. Though no trend has been followed, the IC_{50} values for C4 (IC_{50} 0.83µM ±0.012) was lower than its hydrazone RC4 (IC_{50} 1.60µM ±0.031) whereas for C8 (IC_{50} 0.76µM ±0.008) was higher than RC8 (IC_{50} 0.44µM ±0.028). The IC_{50} values for the hydrazones

(RC1-RC15) lies in the range 0.2 to 11.6 µM. Among all the fifteen compounds eight compounds (RC2, RC3, RC4, RC6, RC8, RC9, RC10, RC11) showed promising results while three compounds exhibited considerable activity (RC1, IC_{50} 4.20µM ±0.010, RC7, IC_{50} 3.48µM ±0.032, RC14 IC_{50} 2.30µM ±0.010). None of the alicyclic compound (C13-15, RC13-15) was found with significant activity.

In **scheme-2** according to present study the compound consisting of 2-methyl-5-nitroimidazole and indole sulfonyl acetohydrazides screened against *Entamoeba histolytica* were significant antiamoebic agents. The hydrazones turned out to be better amoebicidals than their respective aldehydes. None of the aldehydes (S1-S9) was active against the protozoan whereas five compounds (M2, MS3, MS4, MS6, MS8) were found efficacious against *E. histolytica*. The cytotoxicity profile illustrated the active compounds were non toxic on HeLA cervical cancer cell line. *In vitro* and *In vivo* Inhibition kinetics showed MS3 could be the most effective inhibitor against *E. histolytica,* which could be further optimization in the development of novel antiamoebic agents. Hemolytic assay was also performed to check the toxicity effect of both the selected compounds and only 5-8% of hemolysis occurred as compared to standard drug Ciprofloxacin. Molecular docking of compounds (**MS1-MS9)** with *EhOASS* was done to examine the binding pattern that can help us to understand the mechanism of inhibition. Analysis of docking results revealed a strong binding affinity of **MS-3** with *EhOASS*. The interaction analysis suggests that **MS-3** occupies the same position of *EhOASS* and mimicking the binding pose. The *EhOASS*-**MS-3** complexes stabilized by 4 hydrogen bonds as well as several other significant interactions. Overall, the study suggested **MS6** and **MS8** have potential inhibitory effect of selective bacterial strains and can be further evaluated for their potential as antibacterial agents.