# CHEMICAL INVESTIGATION OF NEW ANTIPROTOZOAL AGENTS

THESIS SUBMITTED

TO

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# Title of the Ph. D. Thesis

**"Chemical Investigation of New Antiprotozoal Agents"**

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# ABSTRACT

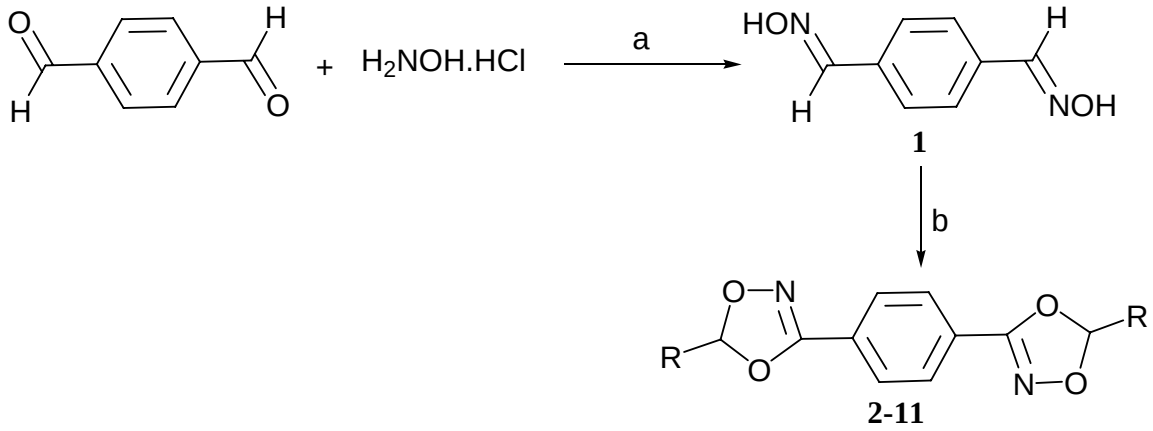
Amoebiasis, a disease caused by *Entamoeba histolytica*, remains one of the major threats to public health in most parts of the globe and is considered to be the second or third leading cause of death amongst the parasitic diseases. More than 50 million people worldwide are infected and up to 110,000 die every year due to amoebiasis. Metronidazole is known to be highly effective amoebicide and is considered to be the drug of choice for the treatment of amoebiasis, but this drug has been shown to be mutagenic in a microbiological system and carcinogenic to rodents. In addition, this drug has several adverse effects for which the most common are gastrointestinal disturbances, especially nausea, vomiting and diarrhea or constipation may also occur. Due to its adverse effects and the emergence of drug resistance, it is desirable to search new lead molecules, which are safer than metronidazole. Thus in the present work attempts have been made to isolate and derivatize some chemical constituents from two medicinal plants and to synthesize some new bisdioxazoles and carbamates.

The thesis comprises of general introduction and four chapters. General introduction includes the literature of the work done in the area of amoebiasis and defines the objectives of the investigation.

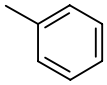
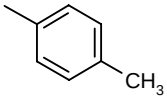
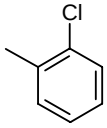
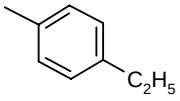
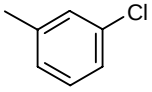
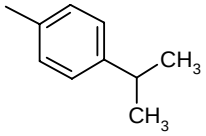
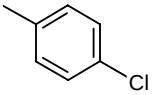
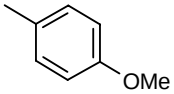
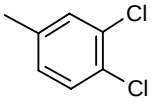
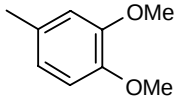
The **First Chapter** deals with the synthesis, characterization and antiamoebic activity of **10** new bisdioxazoles**.** Azoles have long been targeted for their biological properties like cognitive-enhancing and anxiolytic-like activity. Oxazoles, an important member of the azole family contains a number of biologically active molecules which play an important role in the drug chemistry. A number of compounds had been screened for anti-tuberculosis activity.

The bisdioxazoles were prepared by treating benzene-1, 4-dicarbaldehyde dioxime with different aldehydes using the following reaction sequence (scheme-1).

**Synthesis of bisdioxazoles:**



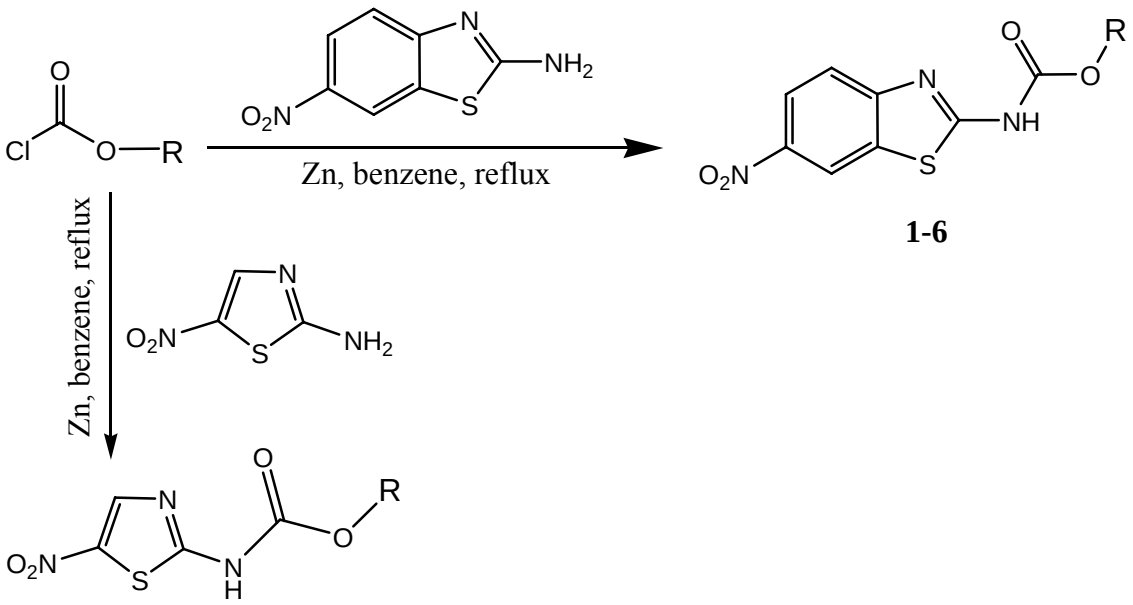

**Scheme-1.** a = Pyridine, $C_2H_5OH$, reflux 24 h, b = aq. NaOCl, $Et_3N$, EtOAC, different aldehydes.
**R =** aryl group of different aldehydes:

| Compound | R | Compound | R |
|---|---|---|---|
| **2** |  | **7** |  |
| **3** |  | **8** |  |
| **4** |  | **9** |  |
| **5** |  | **10** |  |
| **6** |  | **11** |  |

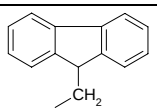
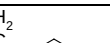
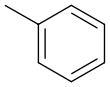
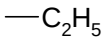
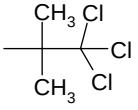
The structures of the bisdioxazole derivatives were confirmed by spectroscopic studies (UV, IR, $^{1}H$ and $^{13}C$ NMR & Mass spectra). Biological results showed that out of eleven compounds screened *in vitro* for antiamoebic activity, four compounds (**3-4**, **7**, **10**) with $IC_{50}$ values of 1.22 μM, 1.41 μM, 1.05 μM, and 1.01 μM respectively, exhibited higher antiamoebic activity than the standard drug metronidazole ($IC_{50}$ = 1.80 μM).

The **Second Chapter** deals with the synthesis, characterization and antiamoebic activity of **12** carbamate derivatives. Carbamates ($H_2NCOOR$) also called urethans are esters of carbamic acid ($H_2NCOOH$). Carbamates are well known for their activity in the field of medicine and agriculture. Benzimidazole 2-carbamates, such as albendazole and mebendazole are used for the treatment of helmintic infections. Apart from the use of polyurethanes in plastics they are also used as drugs for the treatment of Alzheimer's disease. Organic carbamates are valuable synthetic intermediates. They are also useful as protecting groups in organic synthesis, particularly, in peptide synthesis. The carbamates (**1-12**) were prepared by refluxing 2-amino-6-nitrobenzothiazole and 2-amino-5-nitrothiazole with 9-fluorenyl methyl chloroformate, phenyl chloroformate, ethyl chloroformate, methyl chloroformate, 4-nitrobenzyl chloroformate, and 1,1,1-trichloro-2-methyl propyl chloroformate in anhydrous benzene using activated zinc powder as a catalyst as shown in the scheme-2.

**Synthesis of carbamates:**



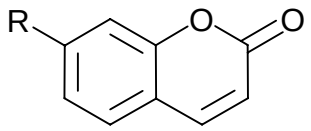

**Scheme-2.** Where **R**:

| Compound | R | Compound | R |
|---|---|---|---|
| **1,7** |  | **4,10** |  |
| **2,8** |  | **5,11** |  |
| **3,9** |  | **6,12** |  |

The structures of the carbamate derivatives were confirmed by spectroscopic data. The results of the bioassay showed that out of twelve compounds screened *in vitro* for antiamoebic activity, two compounds (**3, 7**) with $IC_{50}$ values of 1.31 μM and 1.58 μM,

respectively, exhibited higher antiamoebic activity than the standard drug metronidazole ($IC_{50}$ = 1.80 μM).

The **Third Chapter** deals with the isolation, characterization, derivatization and antiamoebic activity of the biologically active constituents from the root bark of *Adina cordifolia*. The plant has been used in oriental medicine since ancient times as an essential component of various antiseptic and febrifuge prescriptions. The root bark is traditionally used in folklore medicine for the treatment of dysentery in different parts of India, especially in Pauri-Garhwal region of Uttrakhand. The root bark of *Adina cordifolia* was extracted with methanol and fractionated with different solvents according to increasing polarity. These fractions were screened against *HM1:IMSS* strain of *E. histolytica.* The biologically active (benzene and ethyl acetate) fractions were subjected to column chromatography, which gave two compounds umbelliferone (**1**) and skimmin (**2**). These compounds were assessed *in vitro* for antiamoebic activity and it was found that umbelliferone ($IC_{50}$ = 1.04 μg/ml) and skimmin ($IC_{50}$ = 1.35 μg/ml) exhibited better activity than their corresponding extracts (benzene, $IC_{50}$ = 2.92 μg/ml and ethyl acetate, $IC_{50}$ = 2.50 μg/ml).

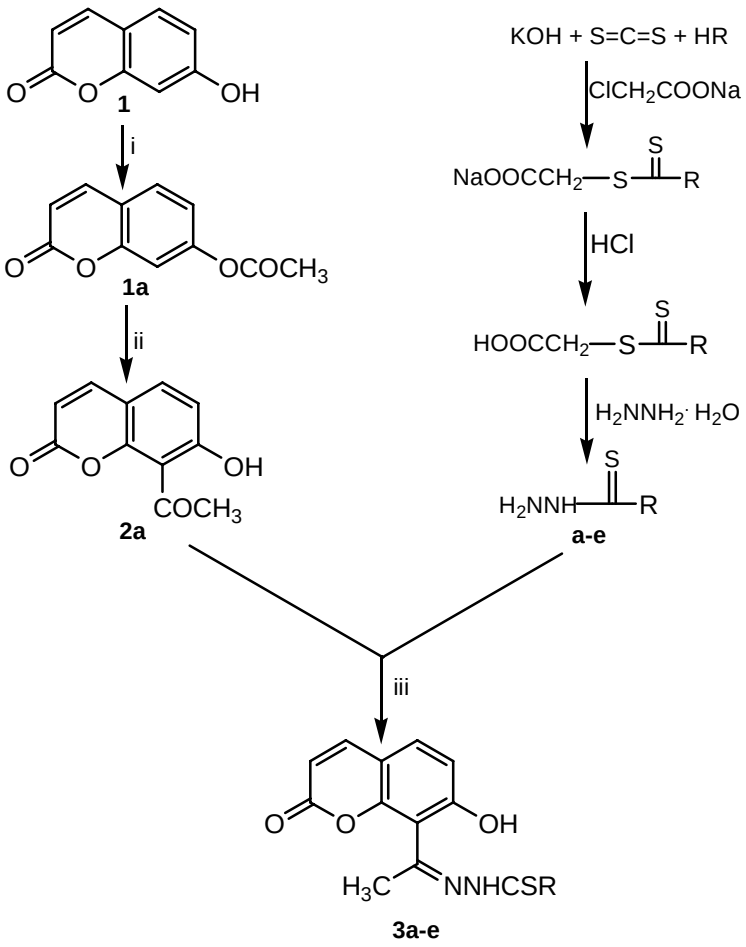



**1.** R = OH (Umbelliferone)

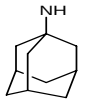
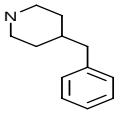
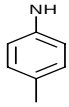
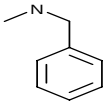
**2.** R = OGlc (Skimmin)

In view of the pharmacological significances of coumarin derivatives, a new series of umbelliferone thiosemicarbazones were synthesized and evaluated against *HM1:IMSS* strain of *E. histolytica*. Umbelliferone (7-hydroxycoumarin) (**1**) was initially reacted with acetic anhydride to give 7-acetoxycoumarin (**1a**), which on treatment with aluminum chloride afforded 7-hydroxy-8-acetylcoumarin (**2b**). Different thiosemicarbazides (**a-e**) were prepared according to the literature procedure. A series of thiosemicarbazones (**3a-e**) of 7-hydroxy-8-acetylcoumarin with various thiosemicarbazides was synthesized. Methyl derivative (**4**) of umbelliferone was synthesized by refluxing umbelliferone in dry acetone with dimethyl sulphate and potassium carbonate for 72 hrs.

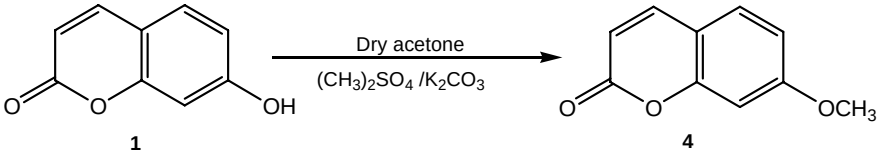
**Synthesis of umbelliferone thiosemicarbazones:**




i: $Ac_2O$ reflux/5 h, ii: $AlCl_3$/125-127$^oC$/2 h, iii: Propan-1-ol/reflux/24 h

Where **R** = **3a** $NH_2$ , **3b**  , **3c**  , **3d**  , **3e** 

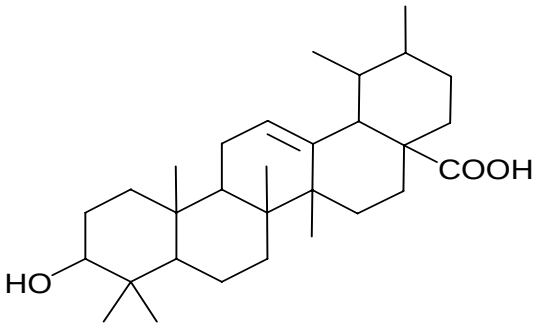
**Methylation of umbelliferone:**



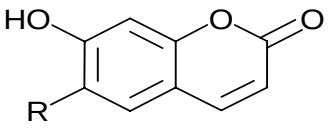
The antiamoebic activity of compound (**1a-2a**) with $IC_{50}$ values of 2.12 μg/ml and 1.33 μg/ml respectively, was lower than that of umbelliferone (**1**) ($IC_{50}$ = 1.04 μg/ml). The activity drastically increases by converting compound (**2a**) into its thiosemicarbazone derivatives (**3a-e**) with $IC_{50}$ values ranging between 0.39-0.91 μg/ml. Compound (**3b**, **3e**) with $IC_{50}$ values of 0.39 μg/ml and 0.41 μg/ml respectively, exhibited even higher antiamoebic activity than the standard drug metronidazole. The activity of methoxy derivative (**4**) ($IC_{50}$ = 1.57 μg/ml) was less than umbelliferone (**1**).

The **Fourth Chapter** deals with the isolation, characterization, derivatization and antiamoebic activity of the biologically active constituents from the leaves of *Fraxinus micrantha*. The *Fraxinus* species have been used in folklore medicine for their diuretic and mild purgative effects as well as for the treatment of constipation, dropsy, arthritis, rheumatic pain, cystitis and itching scalp. The leaves of *Fraxinus micrantha* are traditionally used for the treatment of dysentery in different parts of India especially in Pauri-Garhwal region of Uttrakhand. The leaves of *Fraxinus micrantha* were extracted by refluxing with methanol. The methanol extract was fractionated with different solvents according to increasing polarity. These fractions were subjected to *in vitro* antiamoebic activity against *HM1:IMSS* strain of *E. histolytica*. It was found that the benzene, ethyl acetate and n-butanol extracts ($IC_{50}$ = 0.39 μg/ml, 0.41 μg/ml and 0.43 μg/ml, respectively) exhibited higher antiamoebic activity than the standard drug metronidazole ($IC_{50}$ = 0.45 μg/ml). These active fractions were subjected to column chromatography, which gave ursolic acid (**1**), esculetin (**2**) and esculin (**3**). These compounds were then assessed *in vitro* for antiamoebic activity and it was found that ursolic acid ($IC_{50}$ = 2.20 μg/ml), esculetin ($IC_{50}$ = 2.25 μg/ml) and esculin ($IC_{50}$ =

2.97 μg/ml) exhibited moderate antiamoebic activity but less than that of their corresponding extracts and metronidazole.



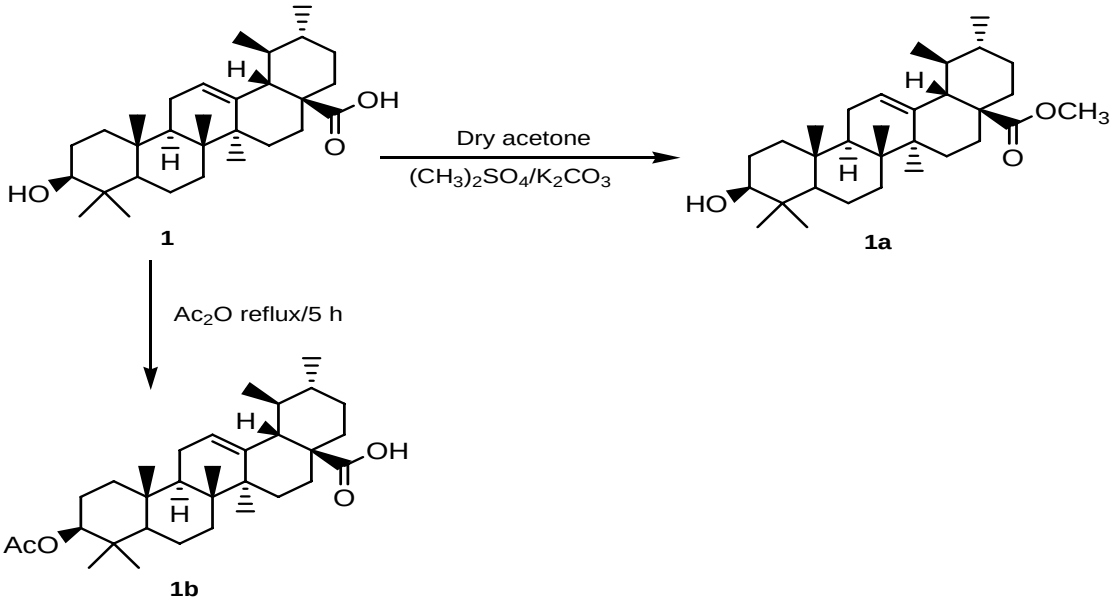

**1.** Ursolic acid




**2.** R = OH (Esculetin)
**3.** R = OGlc (Esculin)

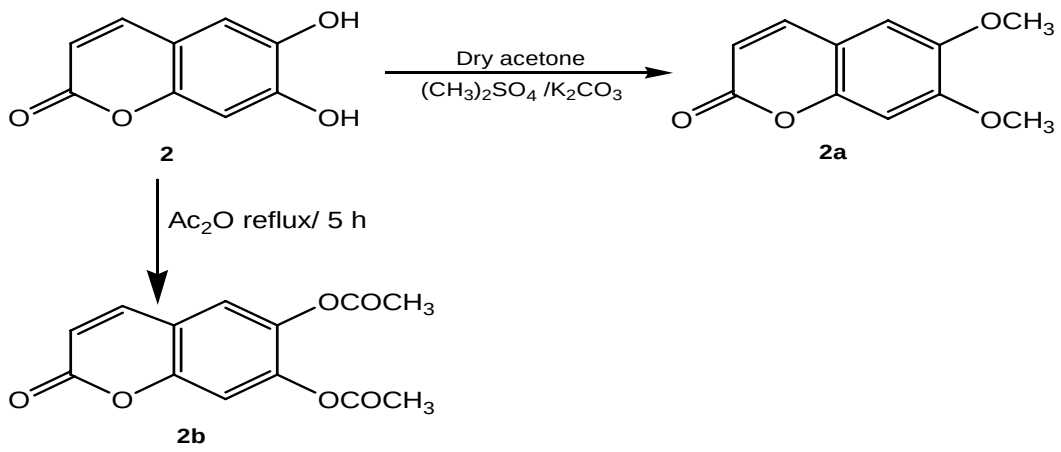
Methyl ursolate (**1a**) was prepared by treating ursolic acid (**1**) with dimethyl sulphate and potassium carbonate in dry acetone. Ursolic acid was refluxed with acetic anhydride to prepare acetyl ursolic acid (**1b**). Esculetin (**2**) was converted to its methyl derivative (**2a**) by treating with dimethyl sulphate and potassium carbonate in dry acetone and acetyl derivative (**2b**) by refluxing with acetic anhydride.

**Esterification and acetylation of ursolic acid:**




**1** **1a** **1b**

**Methylation and acetylation of esculetin:**




Results indicated that the methyl ursolate (**1a**) ($IC_{50}$ = 3.11 µg/ml) and acetyl ursolic acid (**1b**) ($IC_{50}$ = 2.87 µg/ml) showed lower activity than the parent compound ursolic acid (**1**) ($IC_{50}$ = 2.20µg/ml). Methoxy derivative (**2a**) ($IC_{50}$ = 1.95 µg/ml) showed higher antiamoebic activity whereas acetoxy derivative (**2b**) ($IC_{50}$ = 2.74 µg/ml) showed lower activity than esculetin (**2**).