ISSN: 2249 –4820

Chemistry & Biology Interface, 2011, 1, 1, 101-115

Chemistry & Biology Interface

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Research Article

Synthesis and antiamoebic activity of potent γ-butyrolactone and their lactam derivatives

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Received 19 July 2011; Accepted 11 August 2011

Keywords: γ-butyrolactones, pyrrolidone, antiamoebic activity.

Abstract: A series of pyrrolidone derivatives derived from γ-butyrolactones were synthesized and screened for their in vitro biological activity. The structures of synthesized compounds were determined and confirmed with elemental analyses, IR and H^1-NMR spectroscopy. The in vitro antimicrobial activity of compounds were examined and evaluated against the following pathogens: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Aspergillus niger*, and *Candida albicans*. Antiamoebic activity was also carried out with remarkable potency of the synthesized compounds.

Introduction

γ-Lactones are important flavor compounds that occur in a wide range of foods including stone fruit [1], wine [2], milk [3], and dairy products [4]. γ-Lactones are formed by the cyclization of the corresponding γ-hydroxycarboxylic acids [5], with the even-numbered carbon chain lactones predominating in foodstuffs. γ-lactone derivatives have attracted much attention over the years, since the γ-lactone ring unit is an important functional structure in a wide range of natural products [6] and non-racemic γ-lactones are versatile chiral synthons for the synthesis of numerous biologically active natural products or synthetic drugs [7]. To prepare such useful synthetic intermediates, various methods have been developed [8], recently a catalytic enantioselective synthesis of chiral γ-butyrolactones had reported [9].

A much effort has been devoted to the enantioselective synthesis of chiral γ-lactones employing a catalytic amount of a chiral promoter [10].

γ-butyrolactone (GBL) was classified as a Class C drug from 23 December 2009, with a prison term of up to two years for possession and 14 years for dealing, by the end of 2009 [11]. In recent years, combinatorial chemistry has been widely accepted as a powerful concept in organic synthesis, especially in the search for lead compounds to initiate drug discovery research programs. In this way, the development of new methodologies or the adaptation of already existing ones to make them suitable for parallel synthesis has become a dynamic research field [12].

The synthesis of saturated 5-membered nitrogen heterocycles has attracted the attention of chemists for many years. Their

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ISSN: 2249 –4820

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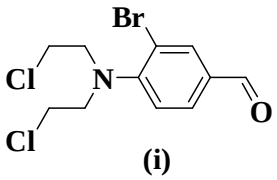
widespread occurrence and diverse range of important biological activities make them particularly attractive synthetic targets [13]. γ-Lactam (pyrrolidin-2-ones) is present in many compounds possessing biological and pharmaceutical activities [14]. The family of 2-pyrrolidones are commercially produced via condensation of GBL (produced via hydrogenation of maleic or succinic anhydride) with ammonia or primary amines at 520 K and 80-90 atm [15]. Parasitic infections constitute one of the most widespread human health problems, and most of them occur through contaminated food or water. The human intestine is a major target of these ingested pathogenic microorganisms, resulting in severe infections, one of which is amoebiasis (potential life threatening dysentery). Amoebiasis is the second leading cause of death from a protozoan parasite, *Entamoeba histolytica* (*E. histolytica*), and remains a major health problem in third world countries [16]. It affects more than 10% of the world's population, and untreated infection may lead to severe complications including hepatic amoebiasis and intestinal tissue destruction [17]. Globally, amoebiasis accounts for 50 million clinical cases and is responsible for approximately 110,000 deaths annually [18, 19]. Only malaria surpasses amoebiasis in mortality of infectious diseases [20].

So, in this present research we report the synthesis of γ- butyrolactones and their pyrrolidone derivatives.

Materials and Methods

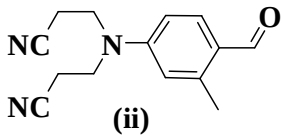
The solvents and reagents used in the synthetic work were of analytical grade obtained from Qualigens India and were purified by distillation or crystallization where necessary and their boiling or melting points were compared with the available literature values. Melting points were determined in open capillaries and are uncorrected. ^{1}H NMR spectra were recorded on a Perkin Elmer FTNMR Cryo-magnet Spectrometer 400 MHz (Bruker) instrument using tetramethylsilane (TMS) as an internal standard and DMSO-*d6* as a solvent. Chemical shifts are given in parts per million (ppm). Infrared spectra were recorded on Shimadzu-IR Prestige 21. The IR spectra were taken on the Perkin Elmer-577 model spectrometer and elemental analysis was carried out by CEST-110 model.

In the present investigation the following two aldehydes (i) & (ii) have been selected for the synthesis of new γ- butyrolactones. The presence of cyano ethyl group and bromo group at m-position can augment antiamoebic activity of γ- lactones



(i)

4-(bis(2-chloroethyl)amino)-3-bromobenzaldehyde



(ii)

4-(bis(2-cyanoethyl)amino)-2-methylbenzaldehyde

γ- lactones of the general Prototype 1 have been obtained by heating an intimate mixture containing 0.05mole of aldehyde, 0.05 mole of 2-aroyl propionic acid, 0.05 mole of freshly fused sodium acetate and 11 ml of acetic anhydride on a low flame till complete solution was obtained and then flask was transferred to a steam bath and heated for two to three hours. The contents were cooled, treated with cold water and the product purified by the recrystallization from ethanol. The yield, colour, melting points and molecular formula have been mentioned in

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Table 1. The ten compounds in Table 1 can generally represent as (I) and (II).

Prototype 1:

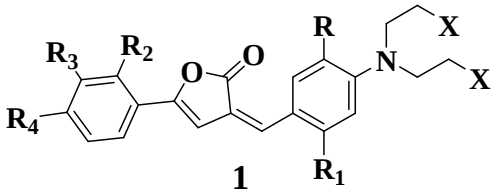

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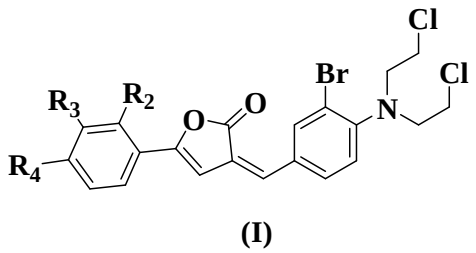
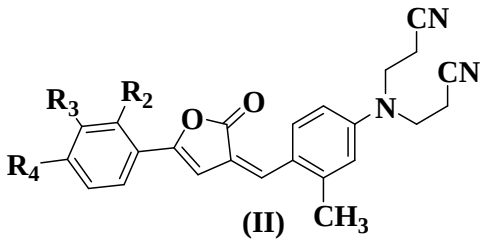
Table 1 : The yield, colour, melting points and molecular formula of synthesized γ-lactones

Compound/ γ-lactones	Molecular Formula	γ-LACTONES						Colour	M. P. ^{0}C	Yield %
		R	R_1	R_2	R_3	R_4	X			
1	$C_{21}H_{18}O_2NCl_2Br$	-Br	-H	-H	-H	-H	-Cl	Yellow	282	80
2	$C_{22}H_{20}O_2NCl_2Br$	-Br	-H	-H	-H	$-CH_3$	-Cl	Orange	285	75
3	$C_{23}H_{22}O_2NCl_2Br$	-Br	-H	-H	$-CH_3$	$-CH_3$	-Cl	Pink	292	81
4	$C_{23}H_{22}O_2NCl_2Br$	-Br	-H	$-CH_3$	-H	$-CH_3$	-Cl	Yellow	295	80
5	$C_{22}H_{20}O_3NCl_2Br$	-Br	-H	-H	-H	$-OCH_3$	-Cl	Red	282	82
6	$C_{24}H_{21}O_2N_3$	-H	$-CH_3$	-H	-H	-H	-CN	Orange	285	79
7	$C_{25}H_{23}O_2N_3$	-H	$-CH_3$	-H	-H	$-CH_3$	-CN	Light Yellow	289	75
8	$C_{26}H_{25}O_2N_3$	-H	$-CH_3$	-H	$-CH_3$	$-CH_3$	-CN	Pink	286	72.1
9	$C_{26}H_{25}O_2N_3$	-H	$-CH_3$	$-CH_3$	-H	$-CH_3$	-CN	Red	272	82
10	$C_{25}H_{23}O_2N_3$	-H	$-CH_3$	-H	-H	$-OCH_3$	-CN	Yellow	291	80



(I)

$R_2 = R_3 = H$ or CH_3



(II)

$R_2 = R_3 = H$

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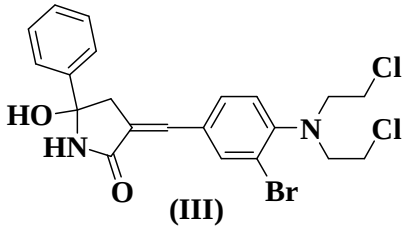
R_4 = H, CH_3 or OCH_3

All these lactones developed deep red colour on treatment with concentrated sulphuric acid. β-Benzoyl [21], β-(p- methyl-Benzoyl) [22], β-(3,4-dimethyl benzoyl)-propionic acid [22], β-(2,4-dimethyl benzoyl) [22] and β-(p-anisoyl) [23] were prepared by using methods described in the literature.

The general procedure for obtaining most of the β-aroyl propionic acids is to condense the succinic anhydride with benzene, toluene, anisole or xylene under the conditions of Friedel-Craft reaction. The solvent was removed by steam distillation and the acids were precipitated by the addition of concentrated hydrochloric acid.

PYRROLIDONE DERIVATIVES

In the present investigation 3-(4-(bis (2-chloroethyl) amino)-3-bromobenzylidene)-5-phenylfuran-2(3H)-one, i.e. compound 1, was refluxed with liquor ammonia for two hours when 3-(4-(bis (2-chloroethyl) amino)-3-bromobenzylidene)-5-hydroxy-5-phenylpyrrolidin-2-one (III) was obtained as yellow powder.



(III)

The behaviour of γ-lactone (I) towards the action of amines is similar to the observation of Walton [24]. The reactions of lactone (I) with fifteen aromatic primary amines have been studied and products of Prototype 2 are found to be coloured having well defined high melting points, as mentioned in **Table 2**. One of the product of this series obtained by the condensation of Lactone of Prototype 1 with sulphanilic acid did not melt up to 291^{0}C (Prototype 2, R= p -SO_3H).

Prototype 2:

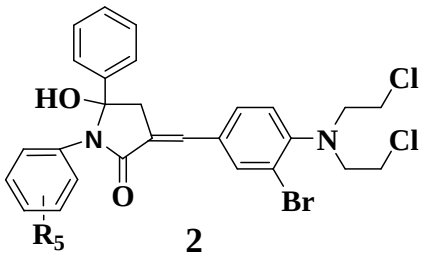


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Table 2: The physical and chemical data for substituted pyrrolidones synthesized from γ-lactones 1.

Compound	Molecular Formula	R_5	Colour	M.P. ^{0}C	Yield %

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11	$C_{27}H_{25}O_2N_2Cl_2Br$	H	Light yellow	289	73.2
12	$C_{28}H_{27}O_2N_2Cl_2Br$	CH_3(o)	Orange	290	75.5
13	$C_{28}H_{27}O_2N_2Cl_2Br$	CH_3(m)	Dark yellow	295	79.2
14	$C_{28}H_{27}O_2N_2Cl_2Br$	CH_3(p)	Yellow	301	80.0
15	$C_{28}H_{27}O_3N_2Cl_2Br$	OCH_3(o)	Light red	291	85.1
16	$C_{28}H_{27}O_3N_2Cl_2Br$	OCH_3(m)	Yellow	297	80.3
17	$C_{28}H_{27}O_3N_2Cl_2Br$	OCH_3(p)	Light Yellow	292	75.2
18	$C_{27}H_{24}O_2N_2Cl_3Br$	Cl (o)	White	299	81.5
19	$C_{27}H_{24}O_2N_2Cl_3Br$	Cl (m)	Red	301	80.9
20	$C_{27}H_{24}O_2N_2Cl_3Br$	Cl (p)	Pale red	310	77.2
21	$C_{29}H_{30}O_2N_3Cl_2Br$	$N(CH_3)_2$	Yellow	305	79.3
22	$C_{31}H_{34}BrCl_2N_3O_2$	$N(C_2H_5)_2$	Light Yellow	300	72.5
23	$C_{29}H_{30}O_4N_2Cl_2Br$	2,5$(OCH_3)_2$	Orange Yellow	310	80.9
24	$C_{28}H_{25}O_4N_2Cl_2Br$	COOH(o)	Brown	300	82.5
25	$C_{27}H_{25}O_5N_2Cl_2BrS$	SO_3H (p)	Dark Yellow	291	75.5

CONDENSATION OF MALONANILIC ACID HYDRAZIDES WITH γ-LACTONES

Malonanilic acid hydrazides were synthesized by reported methods in literature [25]. Biological properties particularly tuberculostatic, antimicrobial activities of Malonanilic acid hydrazides have been tested. Reactions of malonanilic acid hydrazides with **Prototype 3:** azlactones have been studied. Structural similarity of azlactones and lactone and interesting biological activities of the acid hydrazides led the workers to fuse these moieties.

Lactones of **Prototype 1** have condensed smoothly with malonanilic acid hydrazides to yield new products of the **Prototype 3** with yield 78 to 89%.

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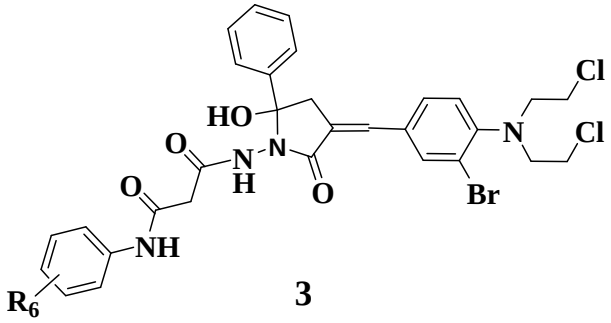

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The reaction conditions leading to the products of the **Prototype 3** involved heating of γ-lactone of the **Prototype 1** with acid hydrazides in an oil bath for about five hours and subsequently treating the mass with aqueous hydrochloric acid. The data of the new products have been presented in the **Table-3**.

Table 3: Pyrrolidones derived from malonanilic acid hydrazides.

Compound	Molecular Formula	R_6	Colour	M.P. ^0C	Yield %
26	$C_{30}H_{29}O_4N_4Cl_2Br$	H	White	290	89.1
27	$C_{31}H_{31}O_4N_4Cl_2Br$	CH_3(o)	Orange	295	89.1
28	$C_{31}H_{31}O_4N_4Cl_2Br$	CH_3(m)	Light red	297	82.0
29	$C_{31}H_{31}O_4N_4Cl_2Br$	CH_3(p)	Brown	299	83.5
30	$C_{30}H_{28}O_4N_4Cl_2Br$	Cl(o)	Light brown	300	85
31	$C_{30}H_{28}O_4N_4Cl_2Br$	Cl(m)	Yellow	302	78.9
32	$C_{30}H_{28}O_4N_4Cl_2Br$	Cl(p)	White	306	85.9
33	$C_{31}H_{31}O_5N_4Cl_2Br$	OCH_3 (o)	Pale Yellow	294	80.1
34	$C_{31}H_{31}O_5N_4Cl_2Br$	OCH_3(m)	Red	298	82
35	$C_{31}H_{31}O_5N_4Cl_2Br$	OCH_3(p)	Yellow	301	79.9

General layout of the synthesis of the present work is given in **Scheme 1,** 4-(bis(2-chloroethyl) amino)-3-bromobenzaldehyde on refluxing with β-benzoyl propionic acid in acidic condition for 2 hours below 100 °C condensed to give 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-phenylfuran-2(3H)-one i.e. γ-lactone 1. This

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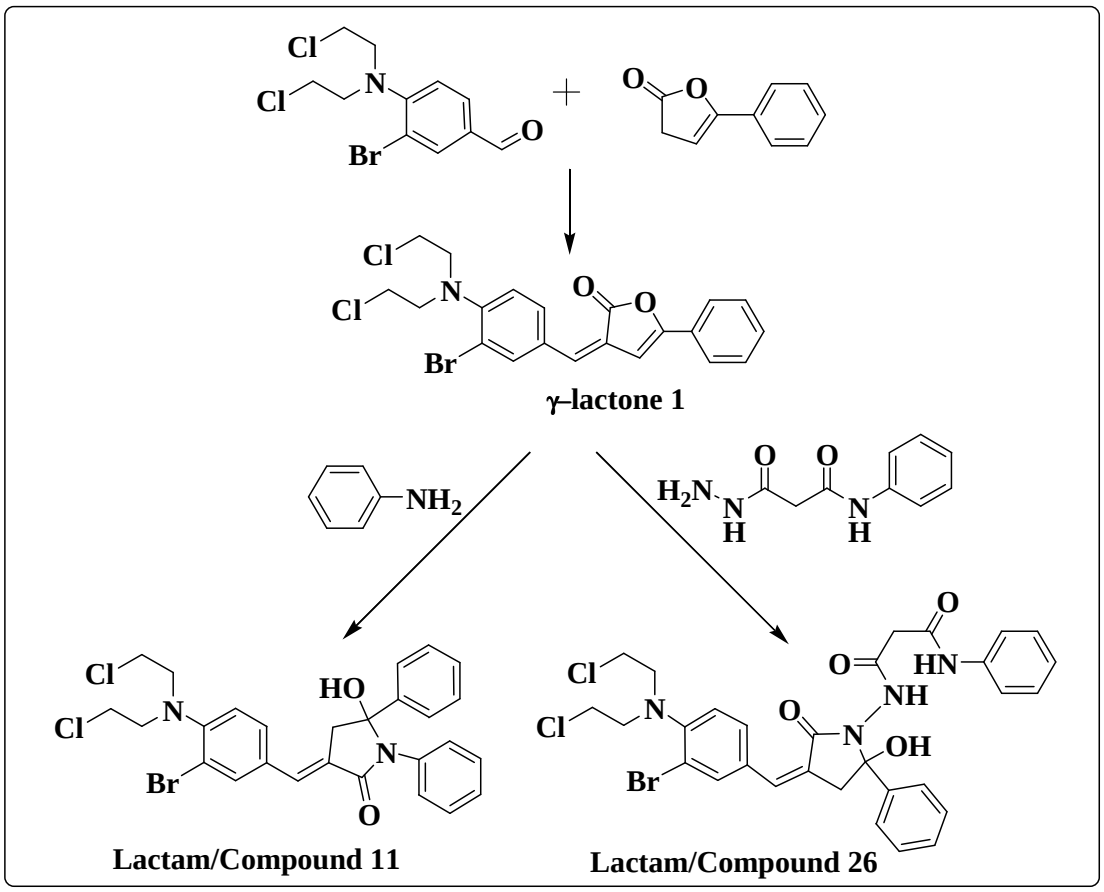
γ-lactone 1on reaction with aniline gives the lactam, 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-hydroxy-1,5-diphenylpyrrolidin-2-one as a product i.e. compound 11 and on reaction with malonanilic acid hydrazide gives lactam, N1-(4-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-2-hydroxy-5-oxo-2-phenylpyrrolidin-1-yl)-N3-phenylmalonamide i.e. compound 26. The detailed procedures for synthesis of the products of **Scheme 1** are mentioned below along with their characterization data.



Scheme-1 : General representation of synthesis of Lactone and their Lactam derivatives

Synthesis of 4-(bis(2-chloroethyl)amino)-3-bromobenzaldehyde

(1) Synthesis of 4-(bis (2-chloroethyl) benzaldehyde

The synthesis of benzaldehyde mustered was carried out according to the literature method [26]. 146 g of DMF was placed in three necked flask equipped with a mechanical stirrer and cooled to 0-5^{0}C and 153 g of phosphorus oxychloride was added slowly during 30min. A solution containing 60.5 g of N,N-bis(hydroxyethyl)benzene-1,4-diamine in 2 mole of DMF was added with stirring and cooling after heating at 85-90^{0}C, for 2.5 hours. The mixture was cooled and poured into ice water mixture. Concentrated ammonium hydroxide was added with vigorous stirring until the solution was strongly basic and the crude yellow product solidified. The solid was collected and recrystallized from aqueous ethanol in cream coloured needles.

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(2) Bromination of 4-(bis (2- formation of chloroethyl)amino) benzaldehyde: 4-(bis(2-chloroethyl)amino)-3-bromo benzaldehyde

Solution of 4-(bis(2-chloroethyl)amino) benzaldehyde (4.9g) in 25ml glacial acid was cooled and solution of 1.8 ml bromine in 7 ml glacial acetic acid was added dropwise with constant stirring. The solution was neutralized by solution of sodium carbonate and kept overnight. The solid was filtered and obtained as cream colour semisolid product. It could not be induced to crystallize and was identified by preparing its DNP and subsequent analysis[21].

Synthesis of 4-(bis(2-cyanoethyl)amino)-2-methyl benzaldehyde

(1) Cyanoethylation of m-toluidine: Formation of N,N-bis(cyanoethyl)-3-methylbenzenamine

Freshly distilled 10 g m-toluidine, 13.3 g acrylonitrile, 14 g glacial acetic acid and freshly prepared dry 1 g cuprous chloride were gently boiled under reflux for 12 hours and then extracted as in the above case of aniline.

(2)Formylation of N,N-bis(cyanoethyl)-3-methylbenzenamine: formation of 4-(bis(2-cyanoethyl)amino)-2-methyl benzaldehyde:

N,N-bis(cyanoethyl)-3-methylbenzenamine (5.8g) was slowly added with stirring to cooled mixture of 4.5 g phosphorus oxy-chloride and 7.5 g dimethyl formamide taken in round bottomed flask provided with a mechanical stirrer and a reflux condenser. The contents were heated on a steam bath for three hours while the mixture was stirred. The brown liquid was cooled, poured over crushed ice and the clear solution was neutralized with sodium acetate. On keeping overnight, the aldehyde separated. It was collected under suction, washed with water and crystallized from ethanol.

Synthesis of β-benzoyl propionic acid

Succinic anhydride (8.5 g) and 44 ml of benzene (sodium dried) were taken in three necked round bottomed flask fitted with a mechanical stirrer and two reflux condensers. The mixture was well stirred and 25 g of anhydrous aluminium chloride was added all at once. The contents were gently refluxed in an oil bath with continued stirring for half an hour. The external temperature was kept at about 90^0C. The flask was cooled in cold water and 18ml of cold water was slowly added from a separatory funnel followed by 12 ml of concentrated hydrochloric acid. The benzene was removed by steam distillation. The hot mixture was transferred to a 500 ml beaker. The β-benzoyl propionic acid separated as colourless oil, which soon solidified. It was cooled in ice and filtered. The precipitate was washed well with 25 ml of cold water. The crude β-benzoyl propionic acid was dissolved in sodium carbonate solution (10 g in 60 ml of water) and filtered hot. The filtrate was cooled in ice and cautiously acidified with conc. HCl (18 ml). The precipitate was filtered and washed well with cold water and dried in vacuum oven.

Synthesis of β-(4-methyl benzoyl)-propionic acid

Succinic anhydride (10 g), nitrobenzene (37.5 g) and dry toluene (9.2 g) were taken in three necked round bottomed flask fitted with reflux condenser and a mechanical stirrer. The mixture was well stirred and anhydrous aluminium chloride (30 g) was slowly added from the separatory funnel from the top of the reflux condenser and then 10 ml conc. HCl was added. The nitrobenzene was removed by steam distillation and the hot mixture was taken in a 500 ml beaker and cooled in ice when β-(4-methyl benzoyl)- propionic acid was obtained as a brown coloured solid. It was dissolved in sodium carbonate solution (8 g in 50 ml of water). Activated charcoal (2 g) was added to the solution and boiled for 10mins and filtered hot. The filtrate was

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cooled in ice bath and acidified with conc. HCl (15 ml). The colourless precipitate was filtered and dried vacuum oven.

Synthesis of β-(p-methoxybenzoyl)-propionic acid

Anisole (11.6 g), succinic anhydride (10 g) and nitrobenzene (37.5 ml) were taken in a three necked flask equipped with a mechanical stirrer and a reflux condenser containing a calcium chloride tube on its top. The mixture was well stirred and anhydrous aluminium chloride (30 g) was added slowly in small portions. After the addition of the aluminium chloride was over stirring was continued for further three hours. The viscous liquid was kept overnight at room temperature. The contents were cooled in ice bath and cold water (12 ml) was slowly added into the flask followed by the addition of conc. HCl (10 ml). Nitrobenzene was removed from the contents by steam distillation. The remaining solution was taken in a 500 ml beaker and cooled in ice bath when the crude β-(p-methoxybenzoyl)-propionic acid separated as colourless solid. The acid was dissolved in sodium carbonate solution (8 g in 50 ml of water). The solution was cooled and β-(p-methoxybenzoyl)-propionic acid was precipitated by addition of concentrated HCl (10 ml). The colourless precipitate was filtered and dried in vacuum oven.

Synthesis of β-(3,4 dimethyl benzoyl)-propionic acid

o-Xylene (11.6 g), succinic anhydride (10 g) and nitrobenzene (37.5 ml) were taken in a three necked flask fitted with a reflux condenser carrying a calcium chloride tube and mechanical stirrer, the contents were stirred and anhydrous aluminium chloride (30 g) was added slowly in small portions. After the addition of the aluminium chloride was over, stirring was continued for further three hours. The flask was cooled in ice bath and cold water (12 ml) was slowly added into the flask followed by the addition of cold conc. HCl (10 ml). The mixture was transferred to a distillation flask and the nitrobenzene was removed by steam distillation. The remaining hot solution was taken in a 500 ml beaker and cooled in ice when the crude β-(3, 4-dimethylbenzoyl)- propionic acid separated as brownish solid. It was filtered under suction and washed well with cold water. The acid was dissolved in sodium carbonate solution (8 g in 50 ml of water), animal charcoal (2 g) was added and contents were boiled for 10minutes. The liquid was filtered hot, the filtrate was cooled in ice and 15ml of concentrated HCl was slowly added when the acid precipitated in colourless crystals. It was filtered under suction and dried in vacuum oven.

Synthesis of β-(2,4 dimethyl benzoyl)-propionic acid

Succinic anhydride (10 g), nitrobenzene (37.5 ml) and m-xylene were taken in a three necked flask equipped with a stirrer and a Liebig condenser having a calcium chloride guard tube. The mixture was stirred and anhydrous aluminium chloride (30 g) was added slowly in small portions. After the addition of the aluminium chloride was over, stirring was continued for further three hours. The contents were left overnight at room temperature. The flask was cooled in ice and 12 ml of cold water was poured, followed by the addition of 10ml of conc. HCl. The mixture was transferred to a distillation flask and nitrobenzene was removed by steam distillation. The hot mixture was taken in a 500 ml beaker and cooled in ice when the crude β-(2,4-dimethylbenzoyl)- propionic acid was obtained as a dirty mass. The solid was filtered under suction and washed well with cold water. The crude acid was dissolved in sodium carbonate solution (8 g in 50 ml of water), animal charcoal (2 g) was added and the solution was gently boiled for 10minutes and filtered hot. The hot filtrate was cooled in ice bath and 15 ml of concentrated HCl was added when colourless leaflets of the acid precipitated out. It was filtered under suction and dried vacuum oven.

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Condensation of 4-(bis(2-chloroethyl)amino)-3-bromobenzaldehyde with β-benzoyl propionic acid:

A mixture of 0.32 g of 4-(bis(2-chloroethyl)amino)-3-bromobenzaldehyde, 0.55 g β-benzoyl propionic acid, 0.25 g fused sodium acetate and 11 ml acetic anhydride was placed in a 50 ml round bottomed flask fitted with an air condenser. The contents were heated on a low flame until the solution was formed. The flask was then transferred to a boiling water bath and heating was continued for two hours when the crystals separated. The flask was cooled and the contents were poured into ice water. The yellow crystals were filtered and washed with cold water, on recrystallization from ethanol and a drop of benzene, shining yellow crystals were obtained as 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-phenylfuran-2(3H)-one i.e. compound 1 with melting 282^0C. The elemental analysis cal. for $C_{21}H_{18}O_2NCl_2Br$: (found) C - 52.2%, H - 3.9%, N - 3.2%, Cl –15.9%, Br – 17.5%; (requires) C - 54.0%, H – 3.6%, N – 3.00%, Cl – 15.2%, Br – 17.1%. The compound shows strong and sharp intensity absorption band characterising γ-Lactone at 1760 cm^{-1}. Bromo group shows absorption band at 554-572 cm^{-1} and strong vibration band of monosubstituted benzene was found at 712-730 cm^{-1}. The compound has a strong band at 1000 cm^{-1} due to stretching of C=O group. The bands at 1620, 1571, 1490 cm^{-1} due to bromo group vibrations. The ^{1}H NMR spectra of the compound in DMSO-*d6* at room temperature using TMS as an internal standard showed the following signals: phenyl as multiplet 7.0 – 8.2 ppm, methylene protons at 3.72 ppm (s, 1H). Methyne proton at 7.33 ppm (s, 1H) and proton on lactone ring at 6.33 ppm (s, 1H). The signal for –OH protons in the compounds showed that cyclocondensation was achieved. There was no appreciable change in all the other signals of the compounds.

Condensation of 4-(bis(2-cyanoethyl)amino)-2-methylbenzaldehyde with β-benzoyl propionic acid:

A mixture of 4-(bis(2-cyanoethyl)amino)-2-methylbenzaldehyde (0.24 g), β-benzoyl propionic acid (0.55 g), fused sodium acetate (0.35 g) and acetic anhydride (11 ml) was placed in a round bottomed flask (50 ml) fitted with an air condenser. The contents were heated on a low flame until the solution was formed. The flask was then transferred to a boiling water bath and heating was continued for two hours when the crystals separated. The flask was cooled and the contents were poured into ice water. The yellow crystals were filtered and washed with cold water, on recrystallization from ethanol and a drop of benzene, shining orange crystals were obtained as 3-(4-(bis(2-cyanoethyl)amino)-2-methylbenzylidene)-5-phenylfuran-2(3H)-one i.e. γ-lactone 6, with melting point 285^0C. The elemental analysis cal. for $C_{24}H_{21}O_2N_3$: found N – 11.2% and requires N – 10.9%. The compound has a strong band at 1000 cm^{-1} due to stretching of C=O group. The characteristic weak –CN stretching absorption band of medium intensity was found at 1090-1340 cm^{-1} and strong vibration band of monosubstituted benzene was found at 712-720 cm^{-1}.

Reaction of 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-phenylfuran-2(3h)-one with ammonia:

A mixture of compound 1 i.e. 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-phenylfuran-2(3h)-one (0.46 g) and liquor ammonia (5 ml, 0.91 g) was taken in a 50 ml round bottomed flask equipped with a reflux condenser. The contents were cooled and diluted with water. The solid obtained was filtered under suction and crystallized from ethanol in light yellow crystals as 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-hydroxy-5-phenylpyrrolidin-2-one i.e. (III) with melting point 285^0C. The elemental analysis cal. for $C_{21}H_{21}BrCl_2N_2O_2$: (Found) N

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- 5.2%, Cl – 12.92%, Br – 14.42%; (Requires) N – 5.00%, Cl – 12.6%, Br – 14.28%.

Reaction of 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-phenylfuran-2(3h)-one with aniline:

A suspension of 0.46 g lactone i.e. compound 1, in 0.9 ml aniline was heated on a steam bath for two hours. The reaction mixture was cooled and acidified with dilute hydrochloric acid to have 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-hydroxy-1,5-diphenylpyrrolidin-2-one as a product (compound 11). The solid obtained was recrystallized from ethanol in light yellow needles with melting point 289^0C. The elemental analysis cal. for $C_{27}H_{25}BrCl_2N_2O_2$: (Found) N-5.3%, Cl – 12.12%, Br – 14.51%; (Requires) N – 5.00%, Cl – 12.6%, Br – 14.28%. The compound shows characteristic I.R. absorption band of -OH group with strong and sharp intensity at 3223 cm^{-1}. Bromo group shows absorption band at 550 cm^{-1} and strong vibration band of monosubstituted benzene was found at 711-730 cm^{-1}. The compound has a strong band at 1000 cm^{-1} due to stretching of C=O group. The ^{1}H NMR spectra of the compound in DMSO-*d6* at room temperature using TMS as an internal standard showed the following signals: phenyl as multiplet 7.0 – 8.2 ppm, methylene protons at 3.60-3.62 ppm (s, 1H). methyne proton at 7.35 ppm (s, 1H), protons on lactone ring at 1.9 and 2.7 ppm (s, 1H). The signal for –OH protons in the compounds was found at 8.20 ppm. There was no appreciable change in all the other signals of the compounds.

Reaction of 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-phenylfuran-2(3h)-one with malonanilic acid hydrazide:

An intimate mixture of the butenolide 1 i.e. compound 1 (0.46 g) and malonanilic acid hydrazide (0.19 g) was taken in a 50 ml round bottomed flask fitted with a condenser. The contents were heated in an oil bath for an hour at 180^0C. The mixture was cooled and treated with aqueous hydrochloric acid when solid separated as N1-(4-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-2-hydroxy-5-oxo-2-phenylpyrrolidin-1-yl)-N3-phenylmalonamide. It was collected under suction and recrystallized from ethanol in white needles with melting point 290°C. The elemental analysis cal. for $C_{30}H_{29}BrCl_2N_4O_4$: (Found) N - 8.98%, Cl – 10.82%, Br – 12.52%; (Requires) N – 8.48%, Cl – 10.75%, Br – 12.12%. The I.R. spectrum of the compound show characteristic medium intensity absorption bands of –CONH group at 1630-1641cm^{-1}. A broad absorption band of reactive methylene group obtained at 1484-1491 cm^{-1}. The compound shows characteristic I.R. absorption band of -OH group with strong and sharp intensity at 3220 cm^{-1}. Bromo group shows absorption band at 552 cm^{-1} and strong vibration band of monosubstituted benzene was found at 712-730 cm^{-1}. The compound has a strong band at 1010 cm^{-1} due to stretching of C=O group.

Results and Discussion:

The mechanism of the reaction of β-aroyl propionic acids with aldehydes under the influence of acetic anhydride to give α-aryl γ-arylidene lactones has been investigated by many workers under these experimental conditions. The enolisation of β-aroyl propionic acid occurs first to give enol form (a). The use of anhydride helps in the formation of inner anhydride of β-aroyl propionic acid (b). Sodium acetate is one of the strongest base which removes proton from the inner anhydride thus helping in carbanion formation (c) followed by the aldol formation (e) which on dehydration gives unsaturated lactone (f). The following reaction path in **Scheme 2** may account for the synthesis of these lactones which is then characterized by ^{1}HNMR of γ-lactone 1 i.e. 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-phenylfuran-2(3H)-one, given in Spectra 1. ^{1}HNMR spectra of its aniline derivative i.e. 3-(4-(bis(2-chloroethyl)amino)-3-bromobenzylidene)-5-hydroxy-1,5-

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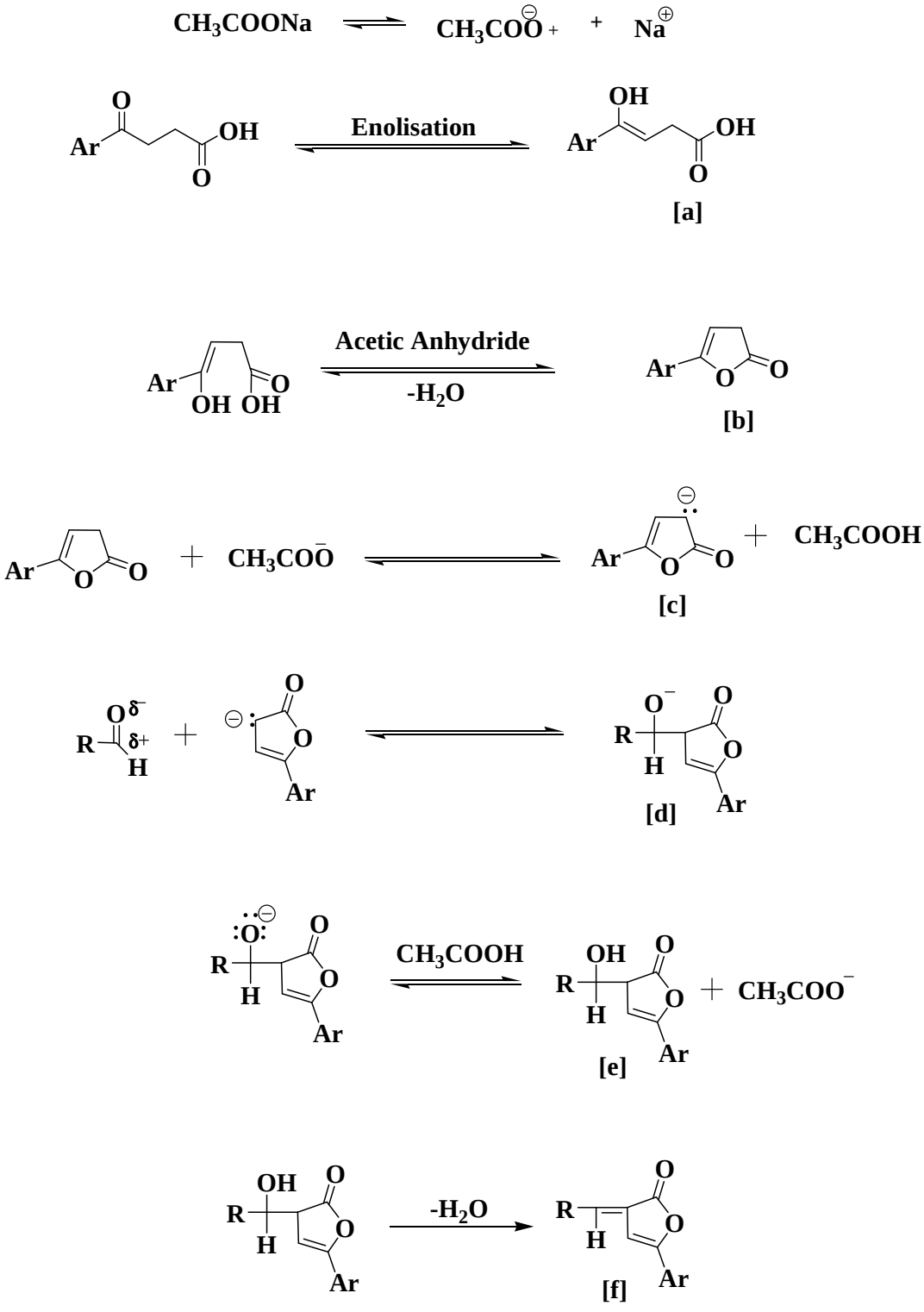
diphenylpyrrolidin-2-one , (compound 11) is given in Spectra 2.



Scheme 2: Proposed mechanism for lactone synthesis

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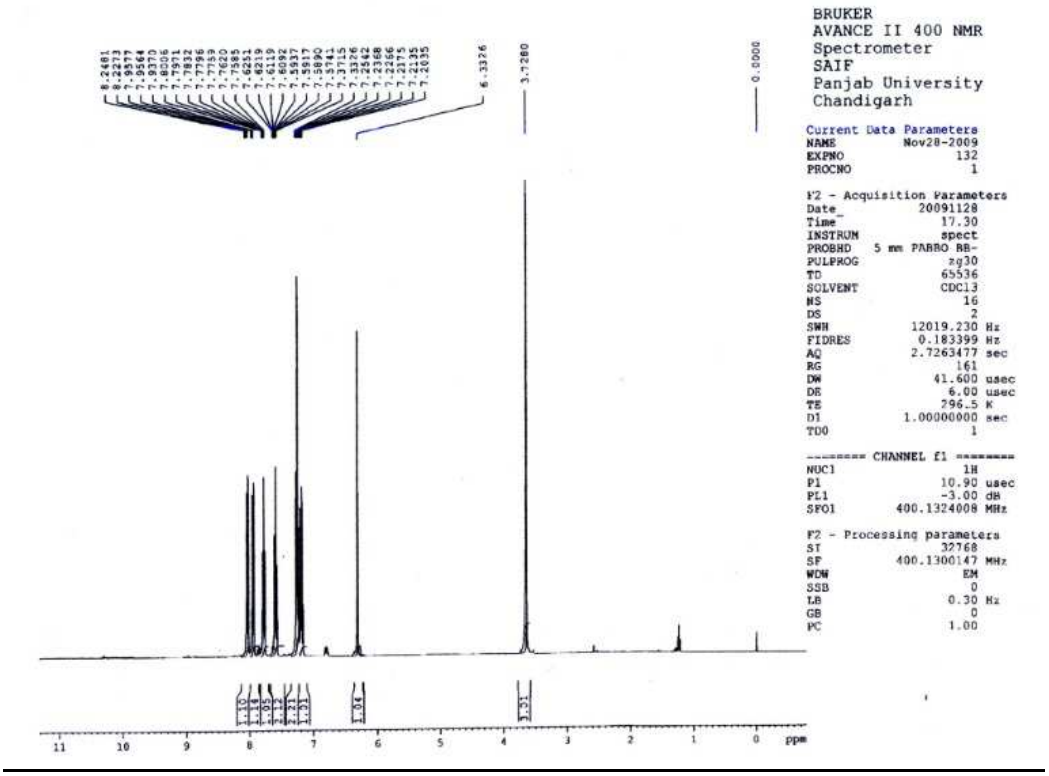
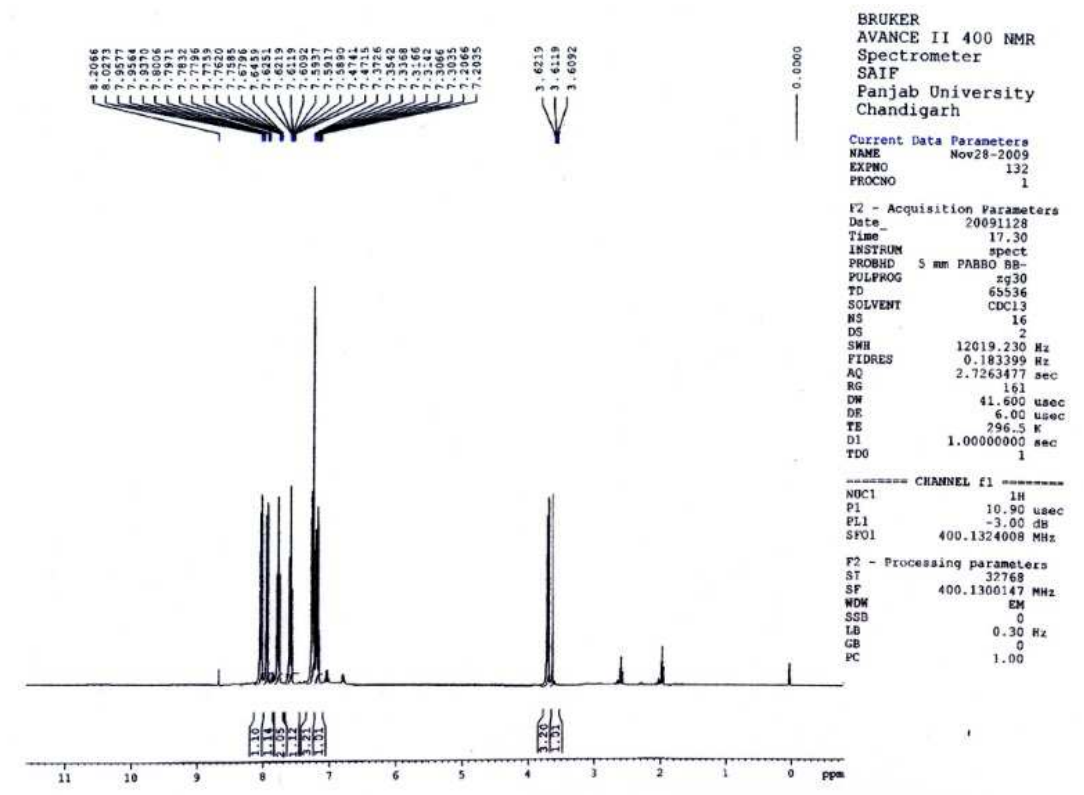


Spectra 1: ^{1}HNMR spectra of compound 1



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Spectra 2: ^{1}HNMR spectra of compound 11

ANTIAMOEBIC ACTIVITY

The newly synthesized γ-lactones of general **Prototype 1** (**Table 1**) pyrrolidones of **Prototype 2** (**Table 2**) and compounds of **Prototype 3** (**Table 3**) were screened for their antiamoebic activity. All compounds have been compared with a standard amoebicide i.e. metronidazole. The synthesized lactones and lactams were tested for amoebiasis in vitro against (HK- 9) strain of *E. histolytica* by microdilution method [27]. The compounds having chloro, bromo, chloroethyl and amino groups have remarkable amoebicidal activity. It has been observed from experimental data that the replacement of the oxygen atom of γ-lactone ring by nitrogen atom (pyrrolidone) augments the antiamoebic activity. The high antiamoebic activity of compounds of **Prototype 3** was due to the presence of malonanilic acid hydrazide group. The amoebicidal activity of these compounds has been presented in **Table 4**. Amoebicidal activity of the compounds **16**, **17, 32, 33** and **35** closely resemble with the known antibiotic **metronidazole** having amoebicidal activity of 7.8 and the compound **15** was found to have amoebicidal activity even less than the metronidazole.

Table 4: Amoebicidal activity of synthesised compounds.

Comp.	AMOEBICIDAL ACTIVITY µg/ml	Comp.	AMOEBICIDAL ACTIVITY µg/ml	Comp.	AMOEBICIDAL ACTIVITY µg/ml
1	20.0	**11**	17.5	**26**	16.0
2	19.5	**12**	16.0	**27**	13.0
3	19.0	**13**	16.0	**28**	14.5
4	20.0	**14**	16.5	**29**	12.0
5	19.0	**15**	7.5	**30**	11.0
6	20.0	**16**	9.0	**31**	10.0
7	19.0	**17**	9.0	**32**	9.0
8	18.5	**18**	10.0	**33**	9.5
9	19.5	**19**	10.5	**34**	11.5
10	19.0	**20**	12.0	**35**	8.5
Metronidazole					7.8

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Conclusion:

Amoebiasis could be eradicated by practicing adequate sanitation worldwide, but it is unlikely that public health interventions will be available to all the world population in the near future; therefore, other approaches need to be considered. The role of amoebiasis in diarrheal illness could be far greater than even suspected because of the high prevalence of *E. histolytica* in developing countries. The study concludes that most of the derivatives showed a reasonable amoebicidal activity as compared with known antibiotic Metronidazole.

The compound **15,** i.e.3-(4-(bis(2-chloroethyl)amino)-3- bromobenzylidene) -5-hydroxy-1-(2-methoxyphenyl)-5-phenylpyrrolidin-2-one, emerged as effective amoebicidal over the standard Metronidazole.

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