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Colon Targeting Drug - Synthesis and evaluation of prodrugs of Quiniodochlor by β -Glucosidase enzyme with HPLC methodology

Gera Parul*, Sharma S.K., Budhiraja Rajat
SDPC, Ghaziabad,INDIA

Abstract

Quiniodochlor has well established antimicrobial properties. It is used for treatment of amoebiasis caused by *Entamoeba histolytica*. The drug is to be delivered to the colon for its effective action against *Entamoeba histolytica*. But the pharmacokinetic profile of Quiniodochlor indicates that the drug is not completely and promptly absorbed after oral administration. Quiniodochlor is specifically targeted to colon by making its prodrugs. Chemical structure of Quiniodochlor posses a hydroxyl group which can be exploited to make glycosides through glycosidic conjugation. Prodrugs of Quiniodochlor were synthesized and their colon targeting evaluation by HPLC was done in order to deliver it specifically to colon for treatment of amoebiasis and other colonic diseases. Three prodrugs of quiniodochlor QG_1, QG_2, QG_5 are synthesized and *In vitro* drug release studies of the synthesized glucosides in simulated GIT fluids containing β-glucosidase was done. The studies showed that these prodrugs hydrolyzed most rapidly in simulated ceacal fluid (0.1 M HCl, pH 1.2), rapidly in simulated intestinal fluid (phosphate buffer, pH 7.4) and slowly in simulated gastric fluid (acetate buffer, pH 5.0).By comparing all the results with time period, it may be concluded that - Quiniodochlor Acetylated Glucoside (QG_1) > Quiniodochlor Acetylated Galactoside (QG_2) > Quiniodochlor Acetylated Xyloside (QG_5) .The antiprotozoal activity is best for acetylated glucoside of quiniodochlor as compared to acetylated galactoside and xyloside.

*Corresponding author, Mailing address:
Gera Parul
E-mail: Parul.gera2004@gmail.com

Key words:
Prodrugs, Glycosides, Quinidochlor, Antiprotozoal activity, Glycosidase enzyme, Koenings and Knorr

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Introduction

Prodrug is defined as pharmacologically inert chemical derivatives that can be converted in vivo to the active drug molecules, enzymatically or non-enzymatically to exert a therapeutic effect. Ideally the prodrug should be converted to the original drug as soon as the goal is achieved, followed by the subsequent rapid elimination of the released

derivatizing group[1]. It implies a covalent link between drug and chemical moiety.. This promoiety essentially has same pharmacological features as the lead drug but posses different Pharmacokinetic properties. There are three factors that should be optimized to achieve the site specific delivering of drugs through promoiety approach:

1) The promoiety must be rapidly transported to the site of action and uptake to the site must be rapid and preferably via perfusion process.

2) After reaching the site of action promoiety should be selectively dissociated to active drug relative to its cleavage at other body sites.

3) Once selectively cleaved at the site of action and the active drug must be retained at site.[3]

Colon targeted drug delivery system is to protect the drug from absorption and/ or to the environment of the upper GI tract and enable selective release of drugs in the proximal colon. It is naturally of value for the tropical treatment of diseases of colon such as colon's disease, colorectal cancer and amoebiasis[2]. Colon specific delivery of bioactive compounds is known to occur naturally in man through the liberation of aglycones from poorly absorbed plant glycosides following ingestion. Release takes place in the colon, mediated by glycosidases produced by colonic bacteria.

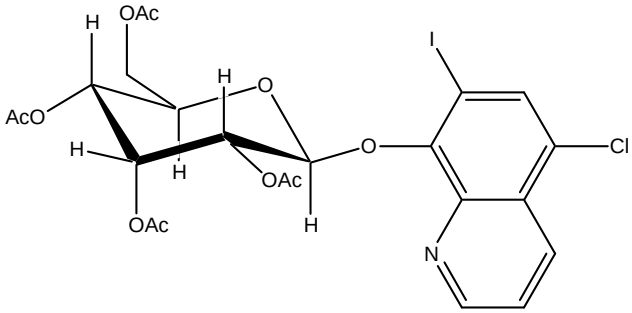
A glycoside/glycosidase-based delivery system should derive its site specificity from the colonic location of intestinal microflora and their unique glycosidases. Glycosides of drugs are larger and usually more hydrophilic than the drugs themselves. These properties tend to reduce penetration across biological membranes. If an orally administered drug glycoside is not cleaved by digestive enzymes of the upper intestine, it should pass unabsorbed into the large intestine (i.e. the colon), where bacterial glycosidases can hydrolyze the glycoside prodrug [7,8]

Chemical structure of Quiniodochlor posses a hydroxyl group which can be exploited to make glycosides through glycosidic conjugation. The synthesized prodrugs of Quiniodochlor (QG_1,QG_2,QG_5)and their colon targeting evaluation by HPLC in order to deliver it specifically to colon for treatment of amoebiasis and other colonic diseases.

Material and Methods

Melting points were determined in open capillary . The purity of compounds was established single spot on silica gel G plates using **:** Benzene:Methanol (50:50) as mobile phase. IR spectra in KBr pellets (cm^{-1}) were recorded on Perkin Elmer1600 series FTIR spectrometer by Bruker Avance 400 MHz NMR ultra shield spectrometer using TMS as an internal standard (Chemical shifts are expressed in δ ppm).

QG_1 : Quiniodochlor acetylated glucoside



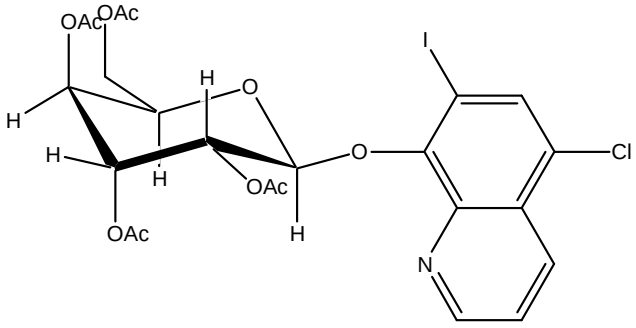
^{1}H-NMR ($CHCl_3$) δ :

8.82-8.83 (d, 1H, C_2 of quinoline ring), 8.49-8.52 (d, 1H, C_4 of quinoline ring), 7.88 (s, 1H, C_6 of quinoline ring), 7.59-7.62 (t, 1H, C_3 of quinoline ring), 2.09-2.10 (d, 2H, CH_2 of CH_2OCH_3), 2.02-2.05 (s, 12H, CH_3 of $COCH_3$)

IR (KBr Pellets) cm^{-1} :

2968.5 (CH str., aliphatic), 1652.8 (C=C str., aromatic), 703.4 (C-Cl str., Ar-Cl), 910.4 (CH bending, out of plane in quinoline ring), 1374.1 (CH bend., $COCH_3$), 1745.9 (CO str., ester $OCOCH_3$), 545.7 (C-I str., Ar-I).

QG_2 : Quiniodochlor acetylated galactoside



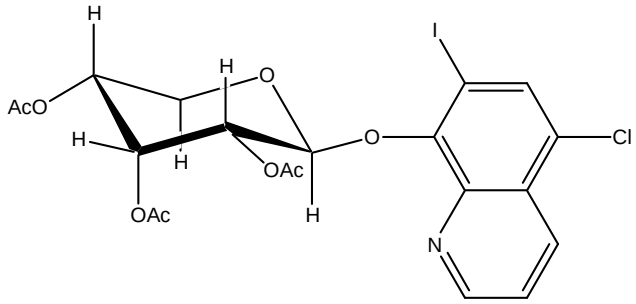
^{1}H-NMR ($CHCl_3$) δ :

8.81-8.83 (d, 1H, C_2 of quinoline ring), 8.50-8.52 (Cd, 1H, C_4 of quinoline ring), 7.88 (s, 1H, C_6 of quinoline ring), 7.59-7.62 (t, 1H, C_3 of quinoline ring), 7.59-7.62 (t, 1H, C_3 of quinoline ring), 2.17-2.21 (d, 2H, CH_2 of quinoline ring), 2.17-2.21 (d, 2H, CH_2 of CH_2OCH_3), 1.83-2.16 (s, 12H, CH_3 of $COCH_3$)

IR (KBr Pellets) cm^{-1} :

2969 (CH str., aliphatic), 3080 (C=C str., aromatic), 1575 (C=C str., aromatic), 703.4 (C-Cl str., Ar-Cl), 905.3 (CH bending, out of plane in quinoline ring), 1375.2 (CH bend., $COCH_3$), 1746.6 (CO str., ester $OCOCH_3$), 545.7 (C-I str., Ar-I).

QG_5 : Quiniodochlor acetylated xyloside



^{1}H-NMR ($CHCl_3$) δ

8.81-8.83 (d, 1H, C_2 of quinoline ring), 8.49-8.52 (d, 1H, C_4 of quinoline ring), 7.88 (s, 1H, C_6 of quinoline ring), 7.88 (s, 1H, C6 of quinoline ring), 7.58-7.61 (t, 1H, C_3 of quinoline ring), 1.89-2.09 (s, 9H, CH of $COCH_3$).

IR (KBr Pellets) cm^{-1} :

2967.1 (CH str., aliphatic), 3057.0 (CH str., aromatic), 1575.0 (C=C str., aromatic), 706.1 (C-Cl str., Ar-Cl), 909.8 (CH bending, out of plane in quinoline ring), 1378.2 (CH bend., $COCH_3$), 1747.2 (CO str., ester $OCOCH_3$), 565.5 (C-I str., Ar-I).

Table 1.1: Percent Drug release from QG_1 with 5 unit enzyme concentration

Time (min)	% Cumulative Release in SGF	% Cumulative Release in SIF	% Cumulative Release in SCF
0	0.00	0.00	0.00
5	8.12	16.22	48.96
10	9.32	19.38	61.64
15	9.61	29.10	69.20
20	9.98	44.31	71.02
25	17.32	68.98	77.21
30	19.72	71.17	89.20
35	20.14	89.10	–
40	33.56	–	–
50	54.81	–	–
60	74.88	–	–

Table 1.2: Percent Drug release from QG_2 with 5 unit enzyme concentration

Time (min)	% Cumulative Release in SGF	% Cumulative Release in SIF	% Cumulative Release in SCF
0	0.00	0.00	0.00
5	7.19	8.38	48.70
10	9.43	9.30	57.33
15	18.21	29.85	54.33
20	28.10	30.12	66.21
25	49.00	57.26	81.12
30	54.89	69.81	–
35	56.10	72.82	–
40	62.90	–	–

Table 1.3: Percent Drug release from QG_5 with 5 unit enzyme concentration

Time (min)	% Cumulative Release in SGF	% Cumulative Release in SIF	% Cumulative Release in SCF
0	0.00	0.00	0.00
5	7.01	17.12	54.60
10	11.66	17.64	59.21
15	18.00	19.21	60.11
20	29.10	28.04	73.42
25	49.86	34.12	79.00
30	61.01	49.82	–
35	69.24	61.10	–
40	70.18	79.41	–
50	73.28	–	–

Table 1.4: Percent Drug release from QG_1 with 10 unit enzyme concentration

Time (min)	% Cumulative Release in SGF	% Cumulative Release in SIF	% Cumulative Release in SCF
0	0.00	0.00	0.00
5	8.11	17.04	50.02
10	10.08	24.48	59.74
15	19.10	46.64	84.00
20	39.60	60.10	91.10
25	51.21	82.18	–
30	60.12	–	–
35	79.90	–	–

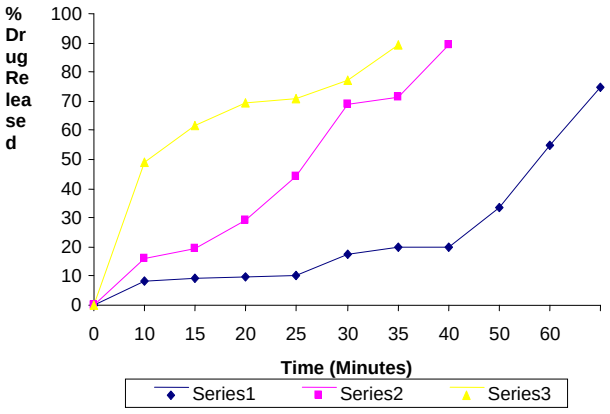
Table 1.5: Percent Drug release from QG_2 with 10 unit enzyme concentration

Time (min)	% Cumulative Release in SGF	% Cumulative Release in SIF	% Cumulative Release in SCF
0	0.00	0.00	0.00
5	3.21	24.18	26.68
10	7.14	39.09	42.68
15	18.14	66.42	59.20
20	21.96	74.41	79.68
25	54.90	77.80	83.20
30	69.86	–	–

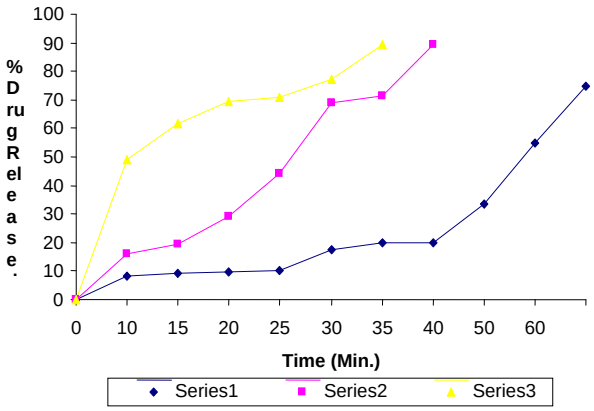
Table 1.6: Percent Drug release from QG_5 with 10 unit enzyme concentration

Time (min)	% Cumulative Release in SGF	% Cumulative Release in SIF	% Cumulative Release in SCF
0	0.00	0.00	0.00
5	7.01	17.12	54.60
10	11.66	17.64	59.21
15	18.00	19.21	60.11
20	29.10	28.04	73.42
25	49.86	34.12	81.00
30	61.01	49.82	–
35	69.24	61.10	–
40	70.18	80.01	–
50	71.00	–	–

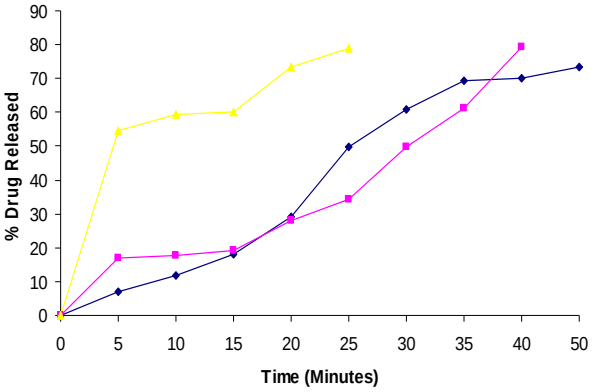
Graph 1.1



Graph 1.2



Graph 1.3



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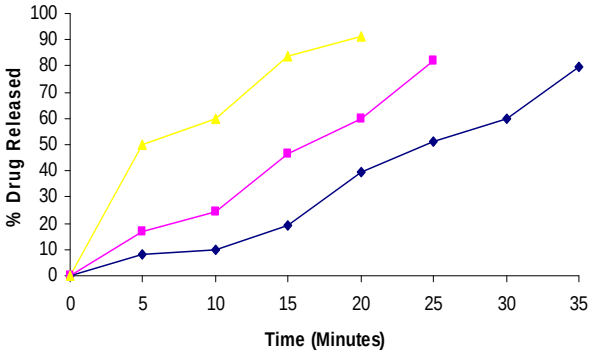
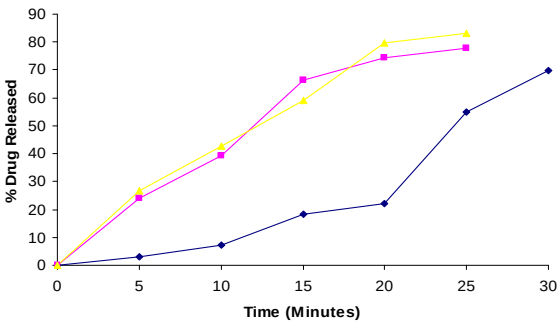


Graph 1.5



Graph 1.6

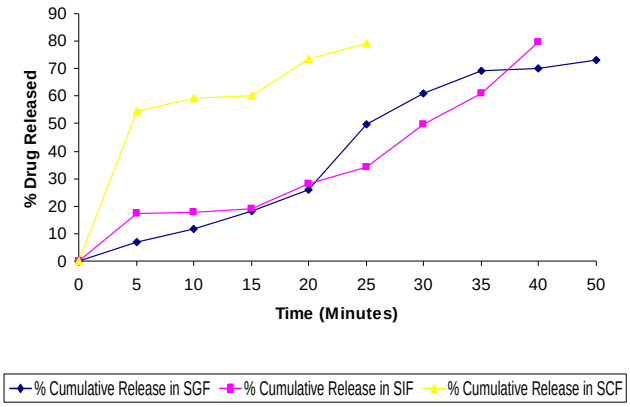


Table 4.2: Physicochemical characteristics of different quiniodochlor (QG_1-QG_5)

S. No.	Compd.	Mol. Form.	Mol. Wt.	m. p. (°C)	Rf value	% Yield
1.	QG_1	$C_{23}H_{23}ClINO_{10}$	635.01	105-109	0.71	60.2
2.	QG_2	$C_{23}H_{23}ClINO_{10}$	635.01	90-95	0.42	46.6
3.	QG_5	$C_{20}H_{19}ClINO_8$	563.72	154-156	0.57	54.2
TLC Mobile Phase: Chloroform:Benzene (50:50)						

Results and Discussion

The synthesized prodrugs of quiniodochlor showed marked activity against *Entamoeba histolytica*.

The tetra (QG_5) and penta(QG_1,QG_2)) acetylated of quiniodochlor were synthesized and Evaluated by HPLC for colon targarting as antiprotozoal.

The testing for antiprotozoal activity was doneby *In vitro* drug release studies of the synthesized glucosides in simulated GIT fluids i.e. in simulated ceacal fluid (0.1 M HCl, pH 1.2), simulated intestinal fluid (phosphate buffer, pH 7.4) and simulated gastric fluid (acetate buffer, pH 5.0). containing β-glucosidase.

QG_1 was found to be hydrolyzed about 90% (30 min) in SCF, about 90% in SIF (35 min) and about 20% in SGF (35 min) with **5 unit enzyme** concentration(graph 1.1). In case of **10 unit enzyme concentration** it was found to be hydrolyzed more than 90% (20 min) in SCF,more than 80% (25 min) in SIF and about 50% (25 min) in SGF (graph 1.4). **QG_2** hydrolyzed more than 80% (25 min) in SCF, about 70% (30 min) in SIF and less than 60% (30 min) in SGF with **5 unit enzyme** concentration (graph 1.2). In **10 unit enzyme** concentration QG_2 was found to be hydrolyzed about 90% (25 min) in SCF, about75% (25 min) in SIF and about 50% (25 min) in SGF (graph 1.5). **QG_5** hydrolyzed about 80% (25 min) in SCF, about 80% (40 min) in SIF and about 70% (40 min) in SGF with **5 unit enzyme** concentration (graph 1.3). In **10 unit enzyme** concentration QG_5 was found to be hydrolyzed about 80% (25 min) in SCF, about 75% (40 min) in SIF and about 70% (40 min) in SCF(graph 1.6).

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