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SYNTHESIS OF LORATADINE AND SOME OF ITS AMIDE DERIVATIVES AS CYTOTOXIC AGENTS

Lipiar Khan Mohammad Osman Goni[1], Nahida Akter[1], Md Rabiul Islam[1]*, Anisur Rahman[2], Mohammad Karim[2]

[1]Department of Chemistry, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh.
[2]Department of Chemistry, Tennessee State University, Nashville, TN 37209, USA.

ABSTRACT

A widely used antihistamine drug loratadine (**2**) and some of its amide derivatives (3, 4 & 5) have been synthesized for the interest of studying the biological activity-specially, for cytotoxicity by brine shrimp lethality bioassay. All of the synthesized compounds were characterized by the extensive use of IR, ^{1}H-NMR, ^{13}C-NMR & Mass spectral analysis and LD_{50} values have been determined to establish SAR of the series. The synthesized antihistamine drug loratadine (2) and its amide derivatives (3-5) showed noteworthy cytotoxic activity. Among the synthesized compounds, diethylamine derivative (3) showed least cytotoxic activity, whereas ethylenediamine derivative (4) and propylenediamine derivative (5) showed more cytotoxic activity than their precursor drug loratadine (2) with the propylenediamine derivative being the most toxic of all. From this investigation we concluded that increased presence of electronegative atoms, such as Oxygen (O), Nitrogen (N) etc. in a particular compound enhances its chance of being biologically more active.

Keywords: Loratadine, Amides, Cytotoxicity, Structure-Activity Relationship.

INTRODUCTION

The term antihistamine historically has referred to drugs that antagonize the actions of histamine at H_1-receptors rather than H_2-receptors. Loratadine is a second-generation [1] H_1 histamine antagonist drug used to treat scores of diseases from ordinary sneezing and rhinorrhea associated with the common cold, to allergic rhinitis, vasomotor rhinitis, and allergic conjunctivitis due to inhalant allergens and foods, to mild atopic asthma [2].

Because of the zwitterionic property of the second-generation antihistamine drugs at physiological $_{pH}$, they are much more selective for peripheral H_1 receptors and this selectivity significantly reduces the occurrence of adverse drug reactions, such as sedation, while still providing effective relief of allergic conditions. In structure, loratadine (**2**) is closely related to tri-cyclic antidepressants, such as imipramine, and is distantly related to the atypical antipsychotic quetiapine [3]. Loratadine, being one of the well-known antihistamine drugs, could cause some side effects including headaches, drowsiness, fatigue, nervousness, hyperactivity etc. to a very minor extent, which in most cases, require no treatment or are easily treated by one's healthcare provider [4]. In continuation of our work on cytotoxicity of various classes of organic molecules [5], in our present investigation, we synthesized loratadine, 2 and some of its amide derivatives 3, 4 & 5 (scheme 1) and studied their cytotoxic activity by brine shrimp lethality bioassay so as to establish Structure-Activity Relationship (SAR) to see whether any of the derivatives had more activity than the precursor drug itself.

MATERIALS AND METHODS

Fisher-John's electrothermal melting point apparatus was used for recording the melting temperature (T_m) of all the synthesized compounds by thin disc method. The infrared (IR) spectra were recorded from NICOLET iS10 IR spectrophotometer in KBr disc from the Department of Chemistry, Jahangirnagar University,

Corresponding Author: - **Md Rabiul Islam** Email: rabiulju@gmail.com

Savar, Dhaka. ^{1}H and ^{13}C-NMR spectra were recorded on 400 MHz and 100 MHz spectrometer respectively in Department of Chemistry, Tennessee State University, Nashville, USA, using TMS as internal standard and mass spectra were recorded through GC Mass Spectrometer, Bangladesh Council of Scientific & Industrial Research (BCSIR), Dhaka. Elemental analysis of the synthesized compounds were carried out in Wazed Miah Science Research Centre, Jahangirnagar University, Savar, Dhaka through the help of the machine named Elementar, Model No. Vario El Cube.

Preparation of Ethyl-4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene)-1-pipeiridnecarboxylate (2)

Compound **1** (5g ; 15.35 mmol) was dissolved in toluene (50 mL) and to this solution, triethylamine (2.45 mL ; 17.52 mmol) and ethyl chloroformate (7.15 mL ; 76.86 mmol) were added at room temperature. Then the reaction mixture was heated with magnetic stirring for 4 hours and 15 mins at 78° C to give a solid mass. This solid mass was further recrystallized from methanol to give white crystalline solid [6]; yield 1.79g, 30.52%; m.p. 132-137° C; HPLC purity 96.42%; TLC R_f 0.78; UV; λ_{max} (nm): 245; IR; ν_{max} KBr (cm^{-1}): 1702 (sh, νC=O ester), 3050 (sh, νC-H aromatic), 2924,2981 (m, νC-H aliphatic), 1578, 1560 (sh, νC=C aromatic), 1641 (sh, νC=C alkene), 1300 (sh, δC-O ester), 1322 (sh, δC-N). MS; m/z: 383/385 (3:1; $[M+1]^+$ / $[M+3]^+$), 325/327, 341/343, 86, 130. ^{1}H-NMR ($CDCl_3$/TMS) and ^{13}C-NMR ($CDCl_3$): See table 1.

Preparation of diethylamine derivative (3) of Ethyl-4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene)-1-pipeiridnecarboxylate (2)

Loratadine (0.2039g; 0.5325 mmol) was dissolved in 5 mL of methanol. Then the solution was stirred at RT for 15 mins. Then diethylamine (0.05 mL; 0.53 mmol) was added to the mixture and the reaction was continued at room temperature (RT) for 2 hours and then at elevated temperature (50 °C) for 2 hours to give a solid mass. The yield of the product was 0.0939 g (43.85%) after the solid mass was recrystallized from methanol as light brown solid; m.p. 150-152 °C; TLC R_f 0.64; UV; λ_{max} (nm): 255.IR; ν_{max} KBr (cm^{-1}): 1654 (m, νC=O amide), 3100 (sh, νC-H aromatic), 2981, 2924, 2904 (m, νC-H aliphatic), 1579 (sh, νC=C alkene), 1560 (sh, νC=C aromatic), 1323 (sh, δC-N). MS; m/z: 411/413 (3:1; $[M+1]^+$/$M+3]^+$), 384/386, 349/351, 337/339, 217, 74. ^{1}H-NMR ($CDCl_3$/TMS); δ_H: 1.2 (t, 3H, H-24), 4.1(q, 2H, H-23), 8.48 (m, 3H, H-7, H-9 and H-10), 7.20 (m, 3H, H-2, H-3 and H-4), 2.3-2.6 (m, 2H, H-21), 2.3-2.6 (m, 2H, H-17), 3.1-3.8 (m, 2H, H-18), 3.1-3.8 (m, 2H, H-20), 2.7-2.9 and 3.2-3.5 (m, 4H, H-5, H-6).

Preparation of ethylenediamine derivative (4) of Ethyl-4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11-ylidene)-1-pipeiridnecarboxylate (2)

Loratadine (0.40g ; 1.044 mmol) was dissolved in 8 mL of methanol and the solution was stirred at RT for 20 mins. to dissolve the loratadine in methanol very well. Then ethylenediamine (0.062 mL ; 0.933 mmol) was added to the reaction mixture and the mixture was stirred at 50 °C for 3 hours. The yield of the product was 0.23 g (56.09%) after it was recrystallized from methanol as light brown solid; m.p. 124-126 °C; TLC R_f 0.72; UV; λ_{max} (nm): 254.

IR; ν_{max} KBr (cm^{-1}): 3442 (sh, νN-H asymmetric), 3321 (sh, νN-H symmetric) 1656 (m, νC=O amide), 2984, 2924 (m, νC-H aliphatic), 1630 (m, νC=C alkene), 1556 (sh, νC=C aromatic), 3042 (w, νC-H aromatic). MS; m/z: 397/399 (3:1; $[M+1]^+$ / $[M+3]^+$), 383/385, 384/386, 353/355, 345/347, 74, 88. ^{1}H-NMR ($CDCl_3$/TMS); δ_H: 2.0 (br,s, 2H, $-NH_2$), 3.8 (s, 1H, -NH), 8.4 (d, 1H, H-2), 7.4 (d, 1H, H-4), 7.1 (m, 1H, H-3), 7.0-7.3 (m, 3H, H-7, H-9, H-10), 2.7-2.9 (m, 4H, H-5, H-6), 3.2-3.5 (m, 4H, H-5, H-6), 2.1-2.6 (m, 4H, H-17, H-21), 3.1-3.8 (m, 4H, H-18, H-20), 3.4 (t, 3H, H-23), 2.89 (t, 3H, H-24). ^{13}C-NMR ($CDCl_3$): δ_C (ppm); ^{13}C (δ_C): C-2 (146.4), C-3 (122.3), C-4 (137.6), C-5 (44.7), C-6 (44.7), C-7 (130.6), C-8 (133.6), C-9 (129.0), C-10 (126.2), C-11 (155.4), C-12 (137.6), C-13 (133.6), C-14 (133.0), C-15 (137.8), C-16 (139.4), C-17 (30.5), C-18 (31.4), C-20 (31.6), C-21 (30.6), C-22 (156.8), C-23 (90.6), C-24 (61.3).

Preparation of propylenediamine derivative (5) of Ethyl-4-(8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridine-11ylidene)1pipeiridnecarboxylate (2)

Loratadine (0.50 g ; 1.305 mmol) was dissolved in 8 mL of methanol and the solution was stirred for 15 mins. at RT. Then propylenediamine (0.11 mL ; 1.29 mmol) was added to the reaction mixture and the mixture was stirred at 50 °C for 4 hours. The resulting solid was then recrystallized from methanol to give yellowish crystalline solid; yield 0.40g, 80.00%; m.p. 110-113° C; TLC R_f 0.58; UV; λ_{max} (nm): 258.

IR; ν_{max} KBr (cm^{-1}): 3423 (s, br, νN-H symmetric), 3550 (m, sh, νN-H asymmetric) 1653 (m, νC=O amide), 2981, 2924 (m, νC-H aliphatic), 1640 (m, νC=C alkene), 1617 (m, νC=C aromatic), 1323 (sh, δC-N). MS; m/z: 413/415 (3:1; $[M+1]^+$ / $[M+3]^+$), 383/385, 349/351, 341/343, 311, 325, 88.

Microanalysis Report

All of the synthesized compounds were analysed for acquiring the data on elemental compositions. The report is given in Table-2.

CYTOTOXICITY SCREENING TEST

Cytotoxicity is the quality of being toxic to cells. It is measured as functions of fundamental biochemical pathways leading to cell death. The cytotoxic activity test of the synthesized compounds 2, 3, 4 & 5 were carried out

by "Brine Shrimp Lethality" [8] [9] test by finding out their corresponding LD_{50} values. In toxicology, the median lethal dose, LD_{50} (abbreviation for "lethal dose, 50%") of a toxin, radiation, or pathogen is the dose required to kill half the members of a tested population after a specified test duration.

The test animals, brine shrimps, known scientifically as *Artemia Salina*, had to be hatched first before they were used for cytotoxic study. Brine shrimps, in order to be hatched, prefer a temperature between 28-30° C, a pH of 8-9 and strong aeration. After mixing appropriate amount of salts with water in a 1000 mL beaker, about one-third of a teaspoon brine shrimp eggs were added into the beaker. An aquarium pump was dipped into the beaker with a view to supplying oxygen constantly and to ascertain the presence of light even in night, a table lamp was set above the beaker. Brine shrimps nauplii, hatched after a day were collected in another beaker and rinsed with fresh water in order to be applied for testing.

Test samples of different concentrations like 50, 100 & 150 ppm were prepared in methanol. To each test tube containing 5 mL of sample solution, about 15 brine shrimp nauplii were released. For control test, one test tube containing 5 mL methanol was taken without sample solution and same number of nauplii were placed in.

After 1, 2 & 3 hours, the test tubes were observed using a magnifying glass and the number of alive nauplii in each test tube was counted to get a representative LD_{50}. From the percentage of mortality of brine shrimp nauplii against each concentration, an approximate linear correlation was observed when logarithm of concentration was plotted against % of mortality and the value of LD_{50} was calculated for each sample as shown in Table-3.

RESULTS AND DISCUSSION

Loratadine (2) was synthesized from 8-Chloro-11-(1-methyl-4-piperidine)-6,11-dihydro-5H-benzo[5,6]-cyclohepta-[1,2-b]pyridine (1) with the help of ethyl chlorofromate in presence of triethylamine. The antihistamine drug was characterized with the help of spectral analysis. The band at 1702 cm^{-1} corresponds to the stretching frequency of C=O (ester) group, whereas, the bands at 1578,1560 cm^{-1} and 1641 cm^{-1} are due to νC=C (aromatic) and νC=C (alkene) accordingly. The quartet at δ 4.1 for $–OCH_2$ group and the triplet at δ 1.2 for $–CH_3$ group declare the formation of an ester functional group in loratadine, given that a sharp band for the stretching frequency of C=O (ester) group was found in compound's IR spectrum. The formation of an ester functional group is even more evident from the presence of a peak at δ 156.89 at ^{13}C-NMR spectrum due to the carbonyl carbon of the ester group. Other ^{1}H and ^{13}C-NMR peaks were in accordance with the structure of loratadine.

The structure is further supported by mass spectrum of the synthesized drug. In the mass spectrum, the molecular ion peak (M⁺) appears at m/z 383/385 as $[M+1]^+/[M+3]^+$ ion having the molecular formula $C_{22}H_{23}ClN_2O_2$ and the molecular ion peak is the base peak in the mass spectrum. Other prominent peaks at m/z 325/327, 130, 86, 341/343 are consistent with the proposed structure as shown in scheme 2. It is to be mentioned that because of the different isotopic composition of the Chlorine atom (^{35}Cl and ^{37}Cl; 3:1), some peaks appear at two mass units apart in the mass spectrum of loratadine (**2**) and its amide derivatives (3, 4 & 5).

In IR spectrum of compound **3**, the band at 1654 cm^{-1} is due to νC=O (amide). The bands at 2981, 2924 and 2904 cm^{-1} are due to νC-H (aliphatic) and the band at 3100 cm^{-1} is due to νC-H (aromatic). The quartet at δ 4.2 and the triplet at δ 1.2 in ^{1}H-NMR spectrum are due to the amine moiety of the amide functional group that came from diethylamine. In mass spectrum, molecular ion peak appears at m/z 411/413 and the base peak appears at m/z 384/386 due to loss of C_2H_4. Other important peaks at m/z 349/351, 337/339, 217/219 and 74 are consistent with the proposed structure as shown in the scheme 3.

Similarly, the formation of compound 4 and 5 were confirmed with the aid of IR, ^{1}H, ^{13}C-NMR and MS spectral data analysis.

Table 1. Assignments of H and C signals of compound 2

Position	^{1}H		^{13}C	Reference Value [7] ^{1}H		Reference Value [7] ^{13}C
	δ_H (ppm)	Splitting Type	$\delta 13_C$ (ppm)	δ_H (ppm)	Splitting Type	$\delta 13_C$ (ppm)
2	8.4	d	146.4	8.4	d	146.4
3	7.1	m	122.3	7.1	m	121.9
4	7.4	d	137.6	7.4	d	137.1
5	2.7-3.5	m	44.7	2.7-3.5	m	31.4
6	2.7-3.5	m	44.7	2.7-3.5	m	31.1
7	7.0-7.3	m	129.0	7.0-7.3	m	125.8
8	-	-	130.6	-	-	133.9
9	7.0-7.3	m	126.2	7.0-7.3	m	130.2

10	7.0-7.3	m	122.3	7.0-7.3	m	128.7
11	-	-	155.4	-	-	155.1
12	-	-	137.6	-	-	137.2
13	-	-	133.5	-	-	132.5
14	-	-	132.9	-	-	133.0
15	-	-	137.8	-	-	137.4
16	-	-	139.4	-	-	139.2
17	2.1-2.6	m	30.5	2.2-2.6	m	30.4
18	3.1-3.8	m	31.4	3.1-3.8	m	44.5
20	3.1-3.8	m	31.6	3.1-3.8	m	44.5
21	2.1-2.6	m	30.7	2.2-2.6	m	30.2
22	-	-	156.8	-	-	156.7
23	4.1	q	61.3	4.1	q	60.9
24	1.2	t	14.7	1.2	t	14.4

Table 2. Elemental analysis of the synthesized compounds

Lab ID No.	Sample ID	Molecular Formula		% N	% C	% H
JU_CHEM_ 260114	2	$C_{22}H_{23}ClN_2O_2$	Exp. Value	7.47	64.77	6.09
			Calc. Value	7.31	69.01	6.05
JU_CHEM_ 260114	3	$C_{24}H_{28}ClN_3O$	Exp. Value	10.32	67.45	7.50
			Calc. Value	10.25	70.31	6.88
JU_CHEM_ 260114	4	$C_{22}H_{25}ClN_4O$	Exp. Value	13.22	65.14	5.65
			Calc. Value	14.11	66.57	6.34
JU_CHEM_ 260114	5	$C_{23}H_{27}ClN_4O$	Exp. Value	12.67	70.46	4.87
			Calc. Value	13.63	67.22	6.62

Table 3. % of mortality due to sample and LD_{50} from the graph

Sample No.	Conc. Of the solution (µg/mL)	Total No. of Brine Shrimps	After 1 Hour		After 2Hours		After 3Hours		Percentage of Mortality After 3 Hrs.	LD_{50} Value
			Dead	Alive	Dead	Alive	Dead	Alive		
2	50	15	2	13	5	10	6	9	40.00	1.80
	100	12	3	9	5	7	7	5	58.33	
	150	14	3	11	6	8	9	5	64.28	
3	50	13	1	12	4	9	5	8	38.46	1.90
	100	11	2	9	4	7	6	5	54.54	
	150	14	5	9	7	7	12	2	85.71	
4	50	14	3	11	5	9	6	8	42.85	1.30
	100	15	4	11	6	9	8	7	53.33	
	150	13	5	8	8	5	10	3	76.92	
5	50	12	4	8	5	7	7	5	58.33	1.10
	100	14	6	8	7	7	9	5	64.28	
	150	11	7	4	9	2	11	0	100.00	

Fig 1. Compound 2 for ^{1}H and ^{13}C-NMR

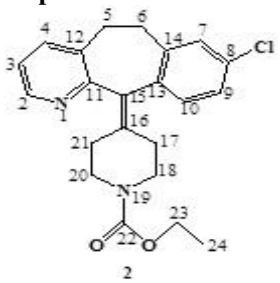


Fig 2. Compound 3 for ^{1}H-NMR

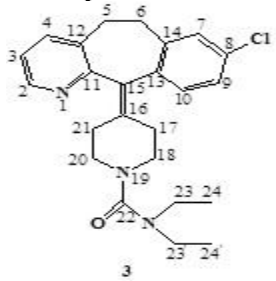


Fig 3. Compound 4 for ^{1}H and ^{13}C-NMR

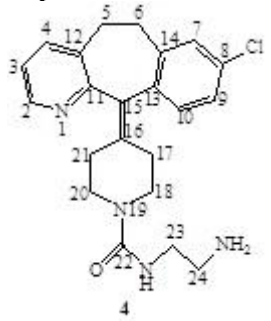
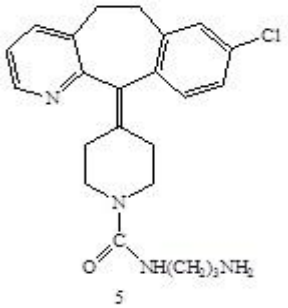
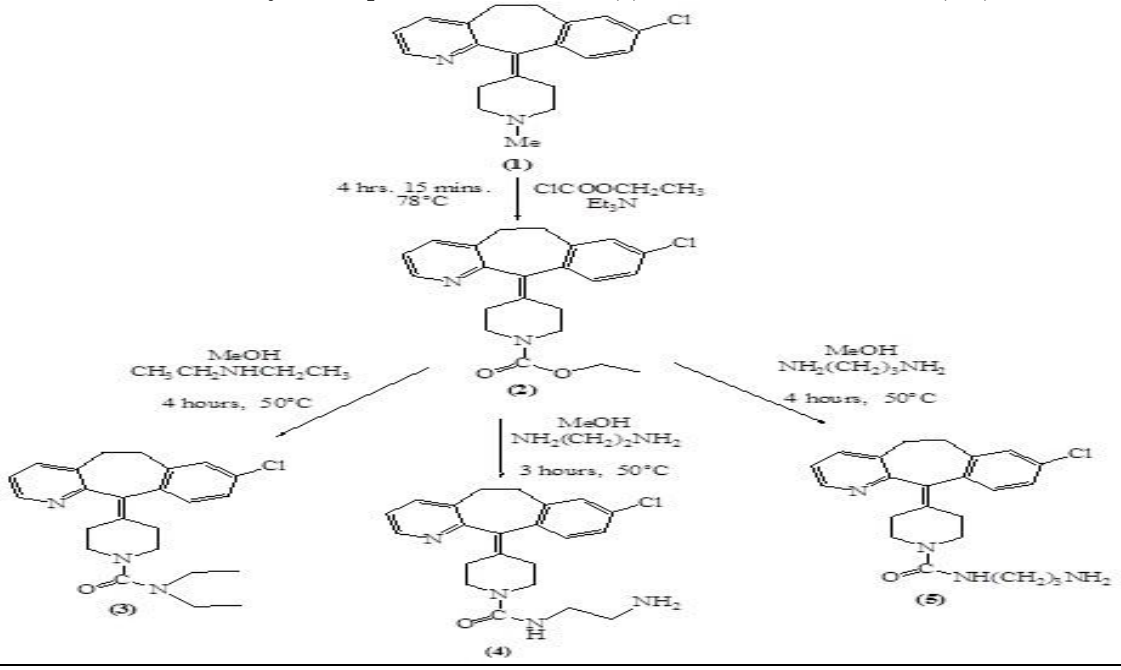


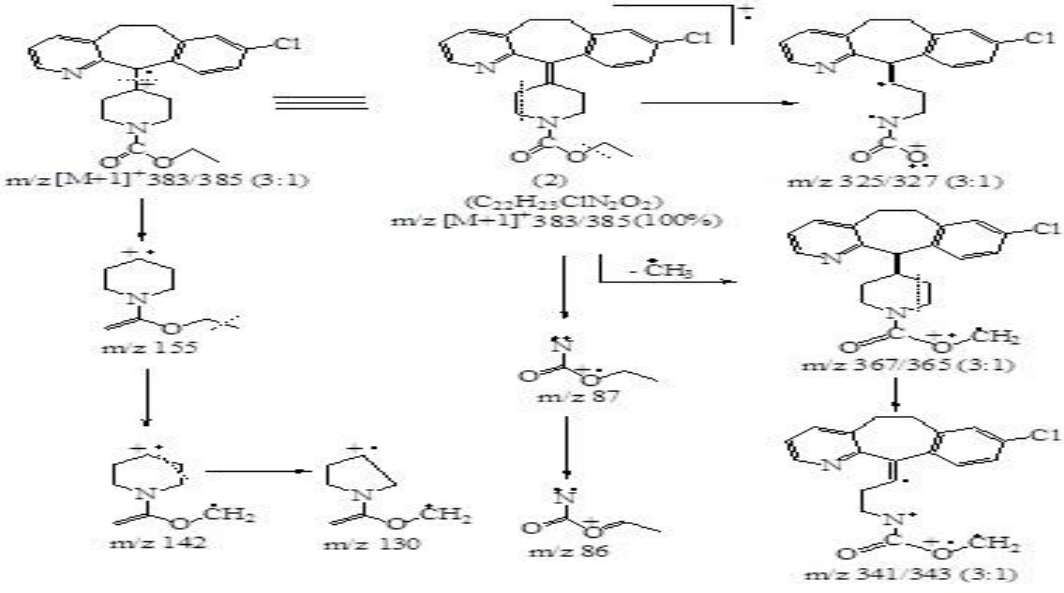
Fig 4. Compound 5



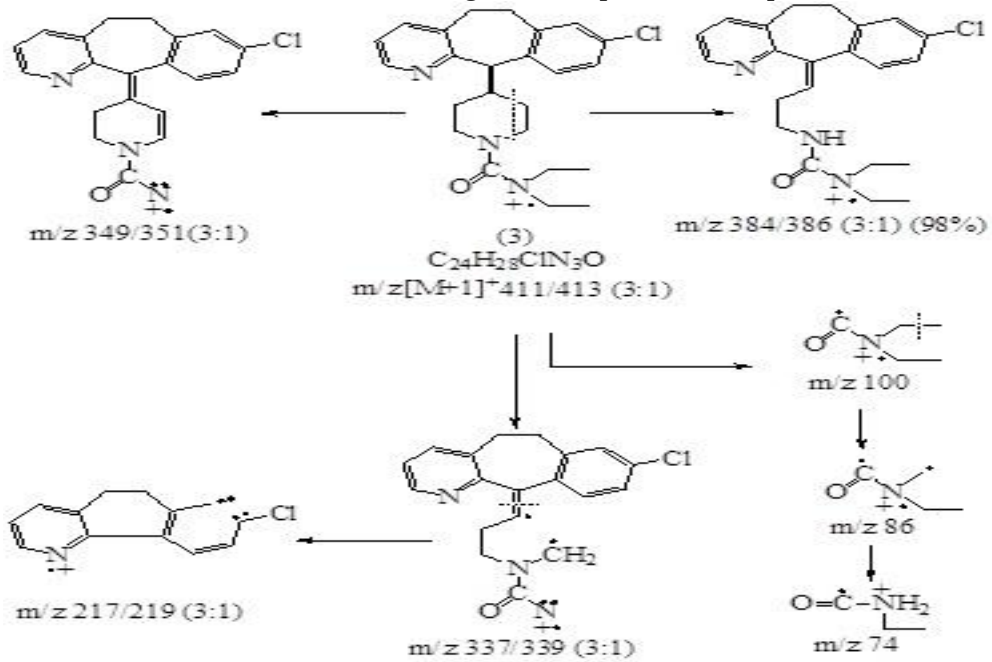
Scheme 1. Synthesis pattern of loratadine (2) and its amide derivatives (3-5)



Scheme 2. Probable mass fragmentation pattern of compound 2



Scheme 3. Probable mass fragmentation pattern of compound 3



CONCLUSION

All of the synthesized compounds 2-5 were screened for their cytotoxic activity against brine shrimps nauplii. The LD_{50} values of 2, 3, 4 and 5 are 1.80, 1.90, 1.30 and 1.10 respectively. This idicates that the diethylamine derivative (3) is less toxic than the precursor drug, loratadine (2), the reason of which might be attributed to the replacement of an Oxygen atom by a Nitrogen atom in the original drug. Ethylenediamine (4) and propylenediamine (5) derivatives are more toxic than loratadine (2), with the propylenediamine derivative being the most toxic of all, which is understandably because of the increased presence of Nitrogen atoms in its molecular structure.

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REFERENCES

1. Holdcroft C. Terfenadine, astemizole and loratadine: Second generation antihistamines. *The Nurse Practitioner,* 18(11), 1993, 13-14.
2. Retrieved from http://pubchem.ncbi.nlm.nih.gov/compound/loratadine#section=Drug-Information
3. Harris AG, Kay GG. Loratadine: a nonsedating antihistamine. Review of its effects on cognition, psychomotor performance, mood and sedation. *Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology*, 29(Suppl 3), 1999, 147–50.
4. Retrieved from http://drugs.emedtv.com/loratadine/loratadine.html
5. Al-Amin M, Bagum H, Hossain MA, Islam MR. *J. Bangladesh Chem. Soc.*, 26(1), 2013, 93-99.
6. Khunt et al. US patent. US 2008/0194823 A1, 2008.
7. Vyas K *et al*., Impurity profile study of loratadine. *Journal of Pharmaceutical and Biomedical Analysis,* 32(1), 2003, 29-39.
8. Anderson JE, Goetz CM, McLaughlin JL, Suffens M. A blind comparison of simple bench-top bioassays and human tumor cell cytotoxicities as antitumor prescreens. *Phytochemical Analysis*, 2, 1991, 107-111.
9. Ferrigni NR, Jacobsen LB, McLaughlin JL, Meyer BN, Nichols DE, Putnam JE. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica*, 45(5), 1982, 31-34.