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SYNTHESIS OF MANNICH BASES OF DESLORATADINE USING DIFFERENT TYPES OF ACTIVATED METHYLENE AROMATIC COMPOUNDS AS CYTOTOXIC AGENT

Sumaiya Khan[1*], Md. Mahtabur Rahman[2], Md. Rabiul Islam[2]

[1]Lecturer, Department of Chemistry, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh.
[2]Active Fine Chemicals Ltd, West Muktarpur, Munsiganj, Bangladesh.

ABSTRACT

The aim of this research is to synthesize Mannich bases of Desloratadine, a tricyclic H_1-antihistamine that is used to treat allergies and an active metabolite of loratadine, with activated methylene aromatic compounds. Pharmacological activity of the Mannich bases are tested by brine shrimp lethality bioassay. All of the synthesized compounds were characterized by the use of IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data analysis. LD_{50} values have been determined to establish SAR of the series. Mannich bases of Desloratadine 2a, 2b, 2c, 3 showed moderate to weak cytotoxic activity. Among the synthesized compounds, Mannich base (3) of Desloratadine with 2'-fluroacetophenone showed the highest cytotoxic activity with LD_{50}=1.2, whereas Mannich base (2a), with 4'-chloroacetophenone; LD_{50}=2.3 showed less cytotoxicity.

Keywords: Mannich base, Desloratadine, Antihistamine drug, Cytotoxicity, Structure-Activity Relationship.

Corresponding Author: - **Sumaiya Khan** Email: sumaiyapariza311@gmail.com

INTRODUCTION

The Mannich reaction is an organic reaction which consists of an amino alkylation of an acidic proton placed next to a carbonylfunctional group by formaldehyde and a primary or secondary amine or ammonia [1].

The final product is a β-amino-carbonyl compound also known as a Mannich base [2].Reactions between aldimines and α-methylene carbonyls are also considered Mannich reactions because these imines form between amines and aldehydes [3] [4].Mannich bases can be synthesized by the reaction of activated methylene compounds with amide acetals to form enaminones and further conversion via reduction [5]. Aminomethylation of Schiff bases with secondary amines and substituted primary amines give Mannich bases [6]. The literature studies enlighten the fact that the Mannich reaction is also used in the synthesis of medicinal compounds which are very reactive and recognized to anthelmintic, antitubercular, analgesic, anti-HIV, antimalarial, antipsychotic, antiviral activities [7] [8]. Mannich bases are reported as potent as toxic agent and against human cancer cell lines. The biological activity of Mannich bases is mainly attributed to α, β-unsaturated ketone which can be generated by deamination of hydrogen atom of the amine group [4]. In continuation of our work on Mannich bases as cytotoxic agent, we planned to convert Desloratadine (1) into Mannich bases 2a, 2b, 2c & 3 [Scheme 2] with activated methylene aromatic compounds or substituted acetophenones.

In our present research work we prepared some new Mannich bases 2a, 2b, 2c & 3 of Desloratadine (1) with activated methylene aromatic compounds to investigate their cytotoxic activity by brine shrimp

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lethality bioassay in order to establish Structure-Activity Relationship (SAR).

MATERIALS AND METHODS

Synthesis of Mannich base (2a) from Desloratadine(8-chloro-6, 11-dihydro-11-(4-piperidilydene)-5H-benzo [5, 6] cyclohepta [1, 2-b] pyridine) viareaction with 4'-chloroacetophenone

A mixture of Desloratadine 0.3227g (1mmol) in methanol & with an excess amount of formaline (40% HCHO) maintaining the pH= 4-5 (using conc. HCl acid) was refluxed at 110˚C. After two hours of refluxing the mixture turned into light red color solution. Then 0.1546 g (1mmol) 4'-chloroacetophenone was dissolved in sufficient methanol and the solution was added to the reaction mixture slowly. The pH of the reaction mixture was set at 4-5 using conc. HCl. The reaction mixture was refluxed for 42 hrs at 90˚C. Orange –red color crystalline compound (2a) was found after usual work up of the reaction mixture; yield; 45% ; m.p 135-140 ˚C; TLC R_f = 0.38; Product was identified spectroscopically ;

UV; λmax (nm): 284;IR; ν max KBr (cm^{-1}): 1683 cm^{-1}(sh. νC=O ketone), 3021 cm^{-1} (m νC-H aromatic).2926 cm^{-1}, 2862 cm^{-1} (sh. νC-H aliphatic), 1653 cm^{-1} (m νC=C alkene), 1226 cm^{-1}(sh. νC-O ester), 1116 cm^{-1}(sh. νC-N), 619cm-1(sh.νC-Cl); ^{1}H-NMR ($CDCl_3$/TMS)(δ^1_H)8.52 (d, 1H, H-2), 7.56 (m, 1H, H-3), 7.98 (d, 1H, H-4), 7.26-7.34 (m, 3H, H-7, H-9, H-10), 2.89-3.05 (m, 2H, H-5, H-6), 2.56-2.61 (m, 2H, H-17, H-21). 3.49-3.68(m, 2H,H-18, H-20), 3.49-3.68 (m,4H,H-22, H-23), 8.03(d, 2H, H-26, H-30), 7.53 (d, 2H, H-27, H-29)

^{13}C-NMR ($CDCl_3$):^{13}C (δ_{13_C})195.4 (C-24), 143(C-11),124.1 (C-12), 51.6 (C-22),32.9 (C-23), 30.4 (C-17),30.5 (C-21), 52.5(C-18), δ 52.6 (C-20),30.2 (C-5),27.3 (C-6),134.4(C-15),136.6 (C-16),142.8(C-2), 142.3(C-3),135.4 (C-4), 137.61 (C-12): See Table:1

MS; m/z (% of Relative Intensities): $[M+1]^{+\bullet}$477 (25): 479(8) (^{35}Cl and ^{37}Cl; 3:1), 323 (100): 325(33), 294 (15), 239 (5), 225 (20).

Synthesis of Mannich base (2b) from Desloratadine(8-chloro-6, 11-dihydro-11-(4-piperidilydene)-5H-benzo [5, 6] cyclohepta [1, 2-b] pyridine) viareaction with 4'-bromoacetophenone

According to the above reaction procedure Mannich base; 2b was prepared using Desloratadine (0.3119; ~1 mmol), formaline and 4'-bromoacetophenone (0.1987; ~1 mmol) by refluxing at 120˚C for 11 hrs. Deep reddish brown colored crystalline compound; 2bwas found after recrystallization from methanol. Yield; 52%, m.p.165-168ºC;

UV; λ_{max} (nm): 284, 170, 206.IR; ν_{max}KBr (cm^{-1}): 3060 (weak, ν C-H Aromatic), 1682 (strong, νC=O), 2926, 2711, 2897(sh, νC-H aliphatic), 1584, 1478 (m, νC=C aromatic), 1654(sh, νC=C alkene), 1118 (stng, νC-N).^{1}H-NMR (MeOD/TMS); δ_H (ppm)(δ_{1_H}): 8.40 (d, 1H, H-2), 7.75 (dd, 1H, H-3), 7.92 (d, 1H, H-4),7.68- 7.75 (m, 3H, H-7, H-9, H-10), 2.84-3.97 (m, 4H, H-5 and H-6), 2.46-2.71 (m, 4H, H-17, H-21), 3.36-3.46 (m, 4H, H-18, H-20), 3.36-3.46 (m, 2H, H-22), 3.16-3.23(m, 2H, H-23), 7.27-7.35 (d, 2H, H-26, H-30), 7.16-7.24 (d 2H, H-27, H-29)

MS; m/z (% of Relative Intensities): $[M+1]^{+\bullet}$383 (10), 311 (100), 323 (60), 239 (90), 282 (75), 223 (65). See Scheme-3

Synthesis of Mannich base (2c) from Desloratadine(8-chloro-6, 11-dihydro-11-(4-piperidilydene)-5H-benzo [5, 6] cyclohepta [1, 2-b] pyridine) *via* reaction with 4'-nitroacetophenone

Desloratadine 0.3100g (1mmol) in methanol with an excess amount of formaline (40% HCHO) maintaining the pH= 4-5 (using conc. HCl acid) was refluxed at 130˚C. (TLC; Chloroform: Methanol = 4: 1; R_f value = 0.70). In the second step 4'-nitroacetophenone(0.1656 g; 1mmol) in sufficient methanol was added to the reaction mixture slowly maintaining the pH of the reaction 4-5. The reaction was continued for 15 hrs. under refluxing condition at 100˚C. A red colored crystalline compound; **2c** was found after recrystallization in methanol. yield; (0.1657 g; 38%); m.p.145-150˚C;

UV; λ_{max} (nm): 284, 179, 265.IR; ν_{max}KBr (cm^{-1}): 3063 (weak, νC-H Aromatic), 1693 (strong, νC=O), 2961, 2803(sh, νC-H aliphatic), 1522 (m, νC=C aromatic), 1607 (sh, νC=C alkene), 1116 (stng, νC-N).^{1}H-NMR (MeOD/TMS); δ_{1_H} (ppm): 8.37 (d, 1H, H-2), 7.31 (m, 1H, H-3), 7.84 (d, 1H, H-4),7.20- 7.26 (m, 3H, H-7, H-9, H-10), 2.86-2.99 (m, 4H, H-5 and H-6), 2.44-2.49 (m, 4H, H-17, H-21), 3.33-3.49 (m, 4H, H-18, H-20), 3.32-3.34 (m, 2H, H-22), 3.16-3.18 (m, 2H, H-23), 7.43 (d, 2H, H-26, H-30), 8.44 (d, 2H,H-29,H-27).^{13}C-NMR (MeOD): δ_C (ppm); ^{13}C (δ_{13_C}): C-2 (143.8), C-3 (126.1), C-4 (135.9), C-5 (27.5), C-6 (27.0), C-7 (133.6), C-8 (133.7), C-9 (129.3), C-10 (129.2), C-11 (153.1), C-12 (123.1), C-13 (136.0), C-14 (139.9), C-15 (136.1), C-16 (134.9), C-17 (30.6), C-18 (53.9), C-20 (53.3), C-21 (30.5), C-22 (44.2), C-23 (42.3), C-24 (197.8), C-25 (141.6), C-26 (130.0) ,C-27 (123.9) ,C-28 (151.1) ,C-29 (123.8) ,C-30 (129.9). See Table-2

MS; m/z (% of Relative Intensities): $[M+1]^{+\bullet}$488 (15):490(5) (^{35}Cl and ^{37}Cl; 3:1), 282 (100), 294 (75), 311 (65), 325 (45), 239(65), 225(50).

Synthesis of Mannich base (3) from Desloratadine(8-chloro-6, 11-dihydro-11-(4-piperidilydene)-5H-benzo [5, 6] cyclohepta [1, 2-b] pyridine) viareaction with 2'-flouroacetophenone

Desloratadine (0.31451 mmol) in methanol, with an excess amount of formaline (40% HCHO) was

refluxed at 130˚C maintaining the pH= 4-5 (using conc. HCl acid) (TLC; Chloroform: Methanol = 4: 1; R_f value = 0.70). After hours later the mixture gives a light red color solution forming an intermediate then methanolic solution of 2'-flouroacetophenone (0.1387; 1 mmol**)** was added to the reaction mixture slowly, keeping the pH 4-5. The reaction was refluxed at 110˚C for 15 hrs. A light orange colored crystalline compound; **3** was found after recrystallization. Yield ;0.6788 g; 52%; m.p.175-180ºC; $R_f$value 0.70;

UV; λ_{max} (nm): 284, 179.IR; ν_{max}KBr (cm^{-1}): 3060 (weak, νC-H Aromatic), 1693 (strong, νC=O), 2952, 2925, 2897 (sh, νC-H aliphatic), 1534, 1479(m, νC=C aromatic), 1610 (sh, νC=C alkene), 1114 (stng, νC-N).^{1}H-NMR (MeOD/TMS); δ_H (ppm)δ_{1_H}: 8.47 (d, 1H, H-2), 7.50 (dd, 1H, H-3), 7.93 (d, 1H, H-4),7.22- 7.33(m, 3H, H-7, H-9, H-10), 2.87-3.19 (m, 4H, H-5 and H-6), 2.45-2.76 (m, 4H, H-17, H-21), 3.32-3.49 (m, 4H, H-18, H-20),3.66-4.10 (dt, 4H, H-22, H-23),7.60-7.70 (m, 3H, H-27, H-28, H-29), 8.23 (dd, 1H,H-30).

MS; m/z (% of Relative Intensities): $[M+1]^{+\bullet}$461(10), 323 (50), 282 (100), 294 (75), 259 (65), 239 (95), 223(75). See Scheme-4

CYTOTOXICITY SCREENING TEST

Cell cytotoxicity refers to the ability of certain chemicals or mediator cells to destroy living cells. By using a cytotoxic compound, healthy living cells can either be induced to undergo necrosis (accidental cell death) or apoptosis (programmed cell death) [9]. The cytotoxicity of the synthesized compounds 2a, 2b, 2c & 3 were carried out by "Brine Shrimp Lethality" [10][11] test by finding 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. Chemotherapy as a treatment of cancer often relies on the ability of cytotoxic agents to kill or damage cells which are reproducing; this preferentially targets rapidly dividing cancer cells [12].

Brine shrimps scientifically known as *Artemia Salina*, as test animal for the investigation of cytotoxicity activity were hatched in a beaker with some salt water, provided a temperature between 28-30 ºC, salinity of 30-35 ppt and a pH of 8-9 and strong aeration. After mixing salts in water, about one-third of a teaspoon eggs were added into the beaker and waited 24 hours. An aquarium pump was dipped into the beaker with supplying oxygen constantly and to ascertain the presence of light even in night, a table lamp was set above the beaker. The aggregated brine shrimps nauplii were then collected in another beaker, rinsed with fresh water, and applied for testing.

Test samples of different concentrations like 1, 10, 100 & 150 ppm were prepared in DMSO. To each test tube containing 5 mL sample solution, about 15 brine shrimp nauplii were released. For control test, 5mL DMSO was taken without sample solution and same number of nauplii were placed in.

After 2, 4, 6 & 8 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 then logarithm of concentration was plotted against % of mortality and the value of LD_{50} was calculated for each sample as shown in Table-3.

Table 1. Assignment of ^{1}H and ^{13}C signals of compound 2a

Position	Experimental value ^{1}H		Experimental value ^{13}C	Reference value Value ^{1}H		Reference value ^{13}C
	δ_H (ppm)	Splitting Type	δ^{13}C (ppm)	δ_H (ppm)	Splitting Type	δ^{13}C (ppm)
2	8.52	d	142.8	8.61	d	146.2
3	7.56	m	142.3	7.18	m	122.3
4	7.98	d	135.4	7.55	d	135.4
5	2.89-3.05	m	30.2	2.7-3.5	m	33.0
6	2.89-3.05	m	27.3	2.7-3.5	m	32.5
7	7.26-7.34	m	128.5	7.0-7.3	m	127.5
8	*	-	133.9	-	-	133.4
9	7.26-7.34	m	126.3	7.0-7.3	m	126.0
10	7.26-7.34	m	129.0	7.0-7.3	m	127.7
11	*	-	143.3	-	-	155.3
12	*	-	124.1	-	-	116.4
13	*	-	135.7	-	-	135.5
14	*	-	142.4	-	-	139.5
15	*	-	134.4	-	-	134.0
16	*	-	136.6	-	-	135.9

17	2.56-2.61	m	30.4	2.2-2.6	m	33.9
18	3.49-3.68	m	52.5	3.1-3.8	m	57.3
20	3.49-3.68	m	52.6	3.1-3.8	m	57.3
21	2.56-2.61	m	30.5	2.2-2.6	m	33.9
22	3.49-3.68	m	51.6	2.85	m	49.4
23	3.49-3.68	m	32.9	2.65	m	37.7
24	*	-	195.4	-	-	200.1
25	*	-	135.3	-	-	134.9
26	8.03	d	133.8	7.83	d	130.2
27	7.53	d	129.6	7.35	d	128.8
28	*	-	139.9	-	-	138.7
29	7.53	d	129.7	7.35	d	128.8
30	8.03	d	130.0	7.83	d	130.2

Table 2. Assignment of ^{1}H and ^{13}C signals of compound 2c

Position	Experimental value ^{1}H		Experimental value ^{13}C	Reference value ^{1}H		Reference value ^{13}C
	δ_H (ppm)	Splitting Type	$\delta^{13}C$ (ppm)	δ_H (ppm)	Splitting Type	$\delta^{13}C$ (ppm)
2	8.37	d	143.8	8.61	d	146.2
3	7.31	m	126.1	7.18	m	122.3
4	7.84	d	135.9	7.55	d	135.4
5	2.86-2.99	m	27.5	2.88	m	33.0
6	2.86-2.99	m	27.0	2.88	m	32.5
7	7.20-7.26	m	133.6	7.08	m	127.5
8	-	-	133.7	-	-	133.4
9	7.20-7.26	m	129.3	7.04	m	126.0
10	7.20-7.26	m	129.2	7.19	m	127.7
11	-	-	153.1	-	-	155.3
12	-	-	123.1	-	-	116.4
13	-	-	136.0	-	-	135.7
14	-	-	139.9	-	-	139.7
15	-	-	136.1	-	-	135.4
16	-	-	134.9	-	-	135.9
17	2.44-2.49	m	30.6	2.06	m	33.9
18	3.33-3.49	m	53.9	2.40	m	57.3
20	3.33-3.49	m	53.3	2.40	m	57.3
21	2.44-2.49	m	30.5	2.06	m	33.9
22	3.32-3.34	m	44.2	2.55	m	49.4
23	3.16-3.18	m	42.3	2.65	m	37.7
24	-	-	197.8	-	-	200.1
25	-	-	141.6	-	-	142.9
26	7.43	d	130.0	8.15	d	129.7
27	8.44	d	123.9	8.27	d	121.0
28	-	-	151.1	-	-	152.8
29	8.44	d	123.8	8.27	d	121.0
30	7.43	d	129.9	8.15	d	129.7

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

		Total no. of shrimp	After 2 hr.		After 4 hr.		After 6 hr.		After 8 hr.				
	Conc. Of solution (µg/mL)		**Alive**	**Dead**	**Alive**	**Dead**	**Alive**	**Dead**	**Alive**	**Dead**	**% of Mortality**	**LD_{50}**	**Toxicity**
Sample-2a	150	8	8	0	6	2	4	4	2	6	75	**1.7**	Toxic
	100	9	9	0	8	1	8	1	8	1	11.11		
	10	7	7	0	6	1	6	1	6	1	14.29		
	1	12	12	0	11	1	11	1	11	1	8.33		
Sample-2b	150	9	7	2	7	2	7	2	4	5	55.56	**2.0**	Weakly Toxic
	100	9	8	1	8	1	6	3	5	4	44.44		
	10	8	8	0	8	0	8	0	8	0	0		
	1	7	7	0	7	0	7	0	7	0	0		
Sample-2c	150	10	8	2	5	3	4	4	2	5	50	**2.3**	Weakly Toxic
	100	7	7	0	6	1	6	1	5	2	28.57		
	10	9	9	0	9	0	9	0	9	0	0		
	1	10	10	0	10	0	10	0	10	0	0		
Sample-3	150	7	6	1	3	4	1	6	0	7	100	**1.2**	Moderately Toxic
	100	14	11	3	4	10	4	10	2	12	85.71		
	10	8	8	0	8	0	8	0	8	0	0		
	1	7	7	0	7	0	7	0	7	0	0		

Fig 1. Mannich base (2a)

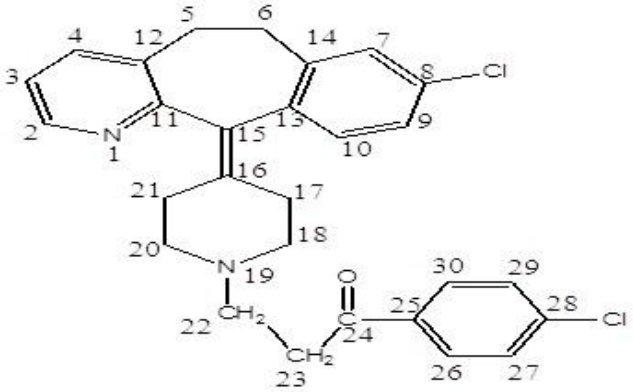


Fig 2. Mannich base (2b)

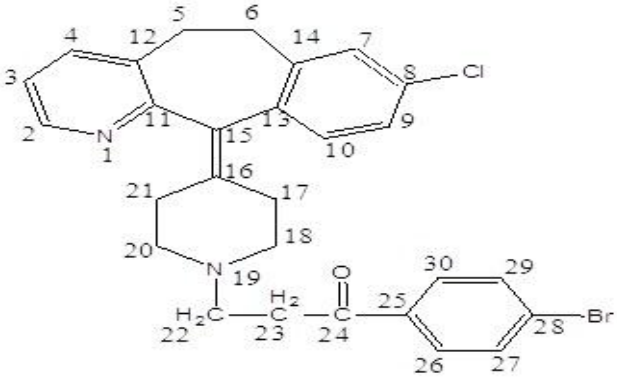


Fig 3. Mannich base (2c)

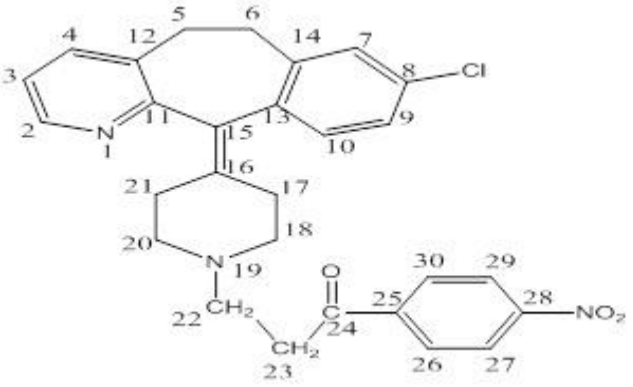
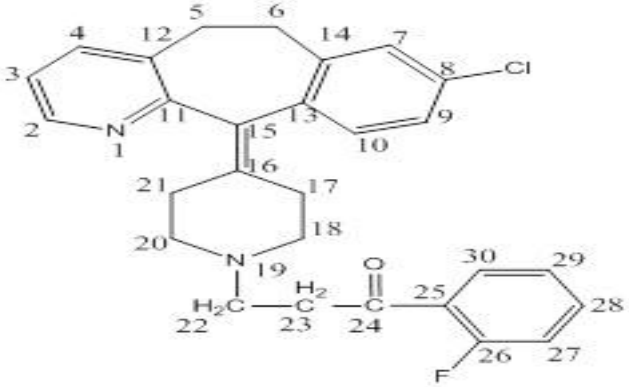
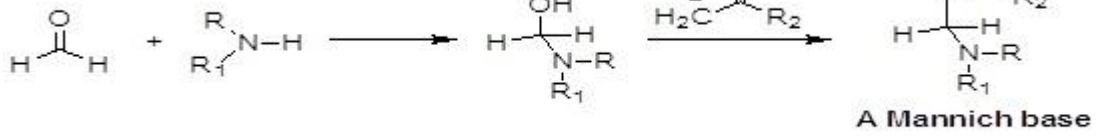
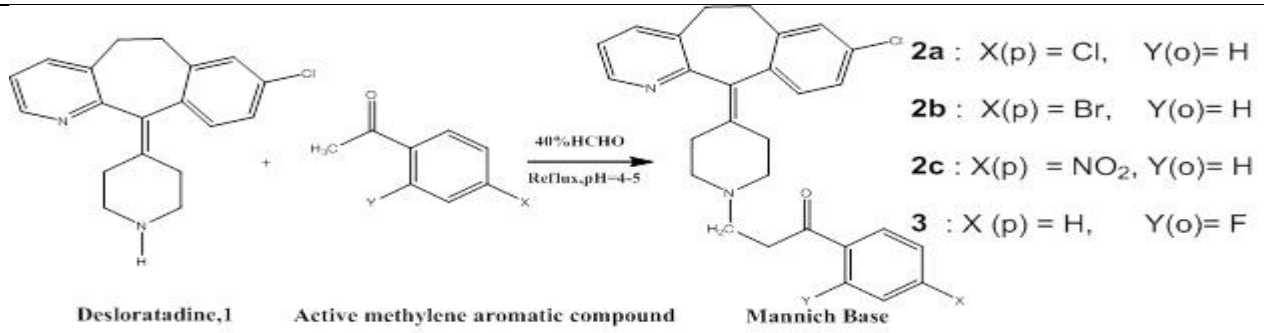


Fig 4. Mannich base (3)



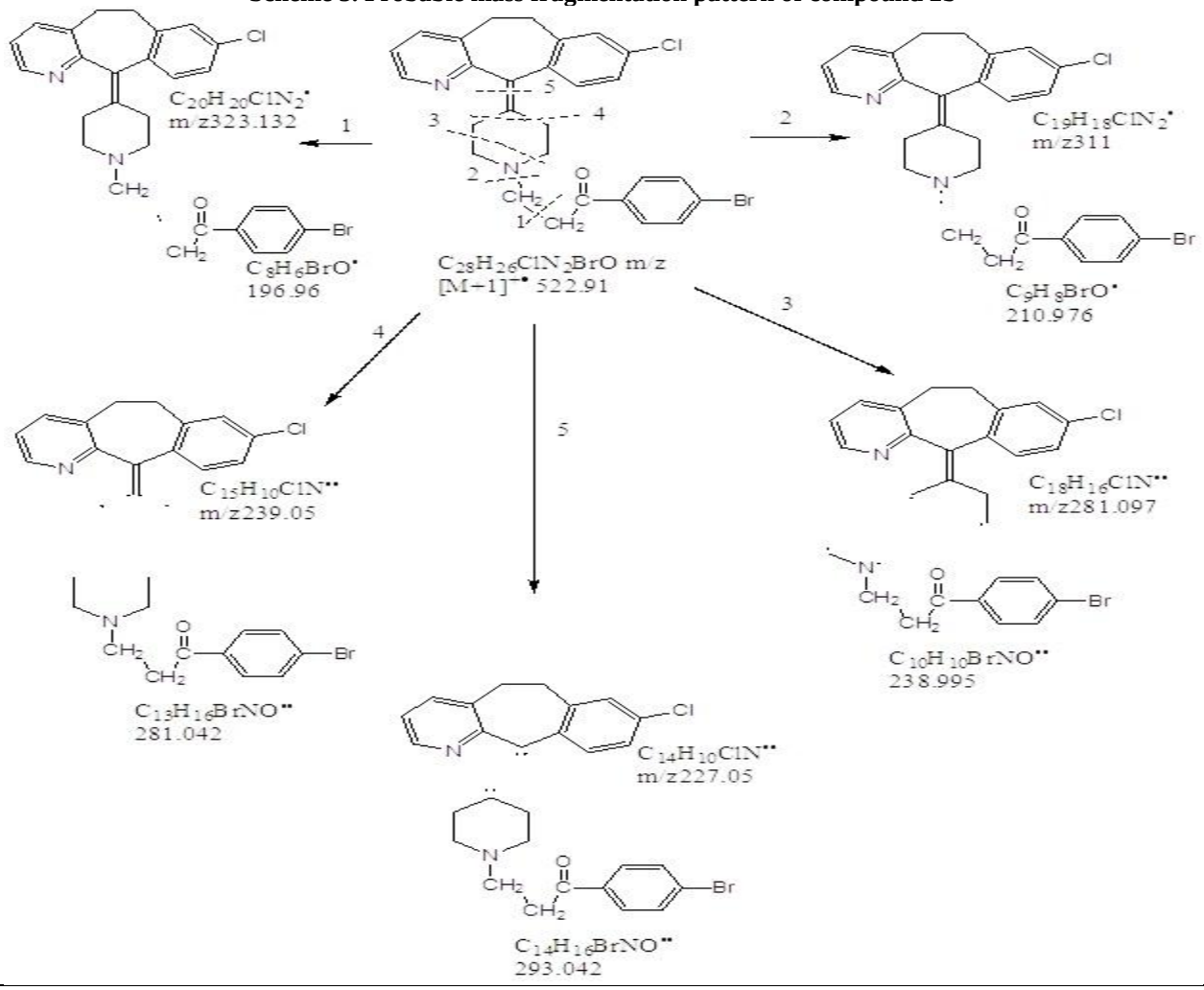


Scheme: 1

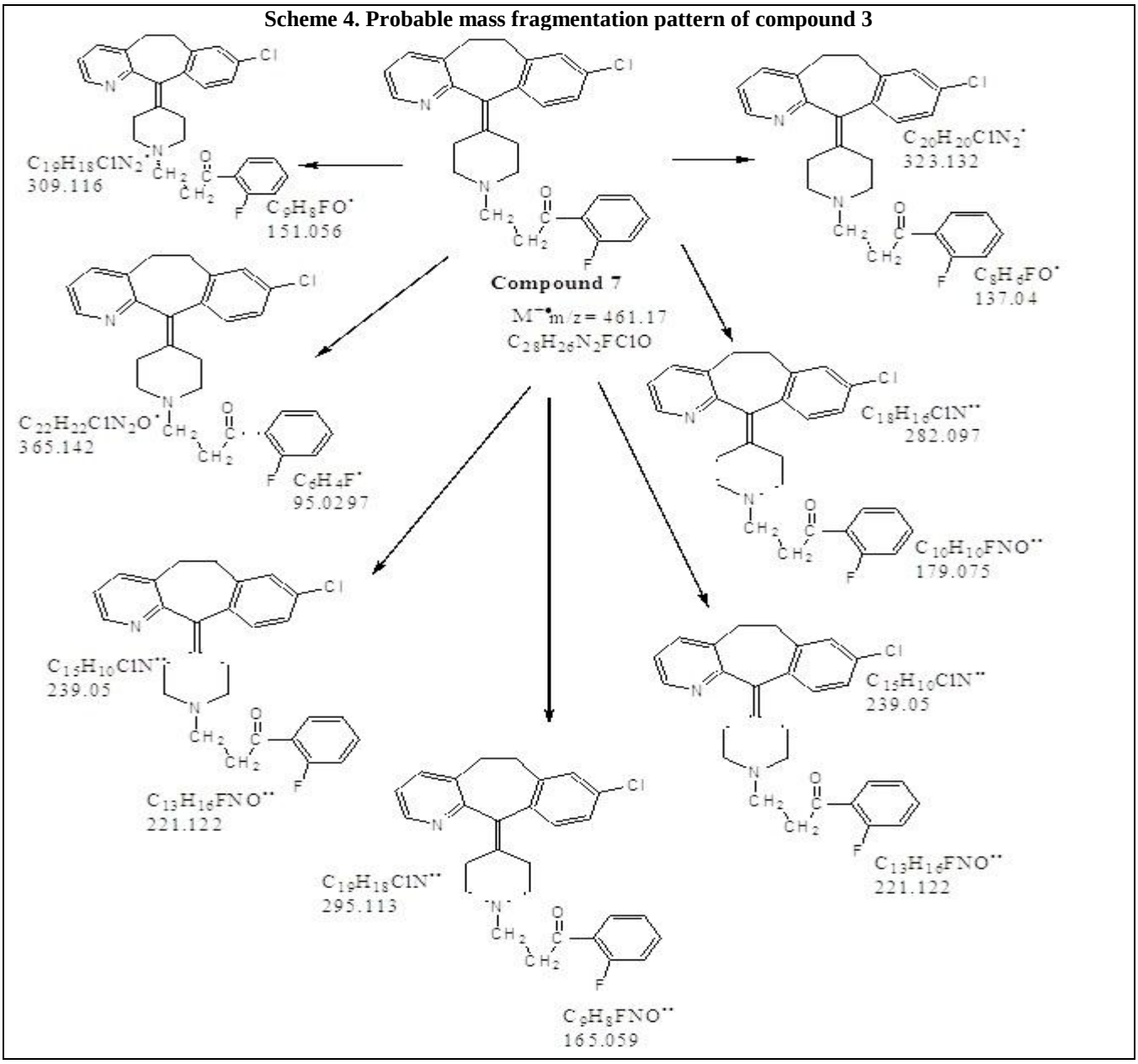


Scheme - 2: Mannich bases of desloratadine

Scheme 3. Probable mass fragmentation pattern of compound 2b



Scheme 4. Probable mass fragmentation pattern of compound 3



RESULT AND DISCUSSION

Mannich bases 2a, 2b, 2c & 3 were synthesized from desloratadine (1), formaline& substituted acetophenones at refluxing condition. Desloratadine, a secondary amine reacts with formaline (40% formaldehyde), while refluxed to form an intermediate in an acidic condition, which then again refluxed with methanloic solution of the acetophenone derivatives maintaining the pH= 4-5. Activated methylene aromatic compounds with electron withdrawing group at ortho or para position form Mannich base with Desloratadine more swiftly than electron donating group in the aromatic region.

thesized compounds were identified with the aid of IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectral data analysis. The probable mass fragmentation of compound 2b is shown in scheme- 3. The two chlorine atoms show three peaks in the ratio of 9:6:1 due to isotopic pattern of ^{35}Cl and ^{37}Cl. The mass fragmentation pattern of compound 3 is shown in scheme-4.

The LD_{50} values indicate that compound 3 have F atom in the ortho-position of the benzene ring is the most potent cytotoxic agent among the synthesized compounds as F is a highly electronegative atom.

CONCLUSION

All of the synthesized Mannich bases of Desloratadine2a, 2b, 2c & 3 were screened for their cytotoxic activity against brine shrimps nauplii. The LD_{50} values of 2a, 2b, 2c & 3 are 1.70, 2.00, 2.30 and 1.20 respectively. This indicates that the Flouro derivative (3) is most toxic than other synthesized compounds because of the presence of highly electronegative atom F.

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CONFLICT OF INTEREST

No interest

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