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Note

Conventional as well as microwave assisted synthesis of some new N^9-[hydrazinoacetyl-(2-oxo-3-chloro-4-substituted aryl azetidine)]-carbazoles: Antifungal and antibacterial studies

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Carbazole on reaction with chloroacetyl chloride afford N^9-(chloroacetyl)-carbazole, **1** which on treatment with hydrazine hydrate has yielded N^9-(hydrazinoacetyl)-carbazole, **2**. Condensation of **2** with various aromatic aldehydes afforded N^9-(arylidene hydrazinoacetyl)-carbazoles, **3** which on cycloaddition with chloroacetyl chloride in the presence of triethylamine yielded N^9-[hydrazinoacetyl-(2-oxo-3-chloro-4-substituted aryl azeitidine)]-carbazoles **4**. All the above reactions are also performed by microwave assisted synthetic method. The structures of all the products are characterized by microanalytical data and spectroscopic techniques. All the synthesized products are screened for their antibacterial activity against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and antifungal activity against *Aspergillus nigar, Aspergillus flavus, Fusarium oxisporium* and *Trichoderma viride*.

Keywords : Azetidinones, carbazoles, conventional, microwave assisted synthesis, antifungal activity, antibacterial activity

Carbazole and their derivatives posses potent biological activities such as anti-inflammatory, analgesic,[1] antibiotic,[2] insecticidal,[3] fungicidal, bactericidal, trypanocidal,[4] larvicidal,[5] diuretic,[6] anticonvulsant, antiallergic and neuroleptic[7]. Azetidine and their derivatives have been extensively studied for their application in the field of medicine. Azetidin-2-ones are of great importance because of the use of β-lactam derivatives as an antibacterial agents[8]. 2-Azetidinones displayed with diverse type of pharmacological activity such as hypnotic, antimicrobial, antiviral, anaesthetic and anticonvulsant[9,10]. In view of these some new 2-oxo-azetidines linked with carbazole at N^9 position through an acetylhydrazino bridge (**Scheme I**) are synthesized by conventional as well as microwave assisted synthesis for comparison purposes particularly the reaction time and yields. The products are evaluated for their antibacterial and antifungal activities.

Results and Discussion

Synthetic Procedure. Conventional. carbazole on treatment with chloroacetyl chloride afforded N^9-(chloroacetyl)-carbazole **1** which on reaction with hydrazine hydrate yielded (hydrazinoacetyl)-carbazole **2**. Condensation of **2** with various aromatic aldehydes gave N^9-(arylidene hydrazinoacetyl)-carbazoles **3** which on cycloaddition with chloroacetyl chloride in the presence of $(C_2H_5)_3N$ yielded N^9-[hydrazinoacetyl-(2-oxo-3-chloro-4-substituted aryl azetidine)]-carbazoles **4**.

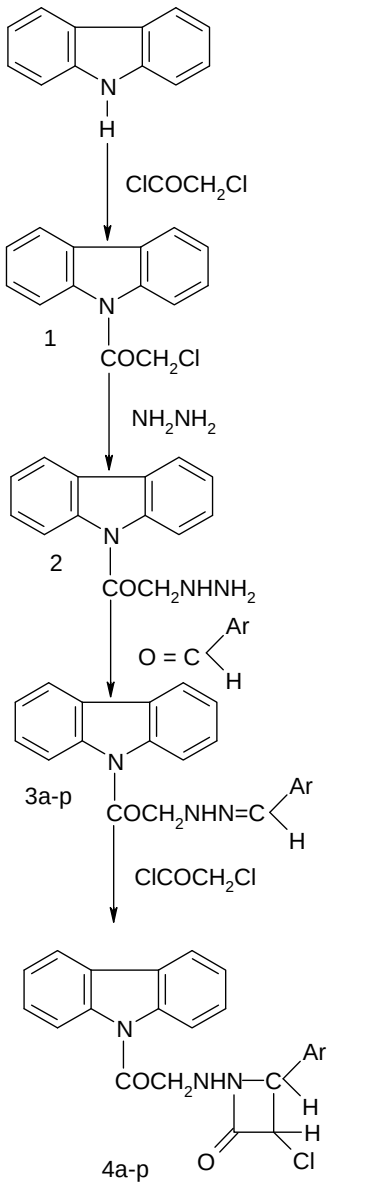
In conventional method the yield of all the products is slightly lower as compared to the synthesis by microwave irradiation technique. Microwave irradiation method facilitates the polarization of the reacting molecule under the irradiation causing fast reaction to occur. A comparative study in terms of yield and reaction time is shown in **Table I**.

Antimicrobial activity. The compounds were screened for their antibacterial activity against *B.substilis. E.coli, K. pneumonia* and *S. aureus* at two concentrations (50 and 100 ppm) and antifungal activity against *A. nigar, A. flavous, F. oxisporium* and *T. viride* at two concentrations (100 and 500 ppm) by filter paper disc technique[11]. Standard antibacterial streptomycin and antifungal griseofulvin were also screened under the similar conditions for comparison. Result are present in **Tables II** and **III** respectively.

Experimental Section

Melting points were taken in open capillary tube. IR spectra were recorded on a Shimadzu 8201 PC spectrophotometer and ^1H NMR spectra on a Bruker DRX-300 in $CDCl_3$ at 300 MHz using TMS as an internal standard. The mass spectrum was recorded on a Jeol SX 102 (FAB) spectrometer. The purity of the compounds was monitored by silica gel G coated TLC plates. Microwave assisted reactions were carried out in a Q Pro-M-modified microwave oven.

N^9-(chloroacetyl)-carbazole 1 (Conventional method). Chloroacetyl chloride (3.81 mL, 0.04 mole) was added to a solution of carbazole (8.00 g,



Ar = Substituted aromatic aldehydes

Scheme I

0.04 mole) in acetone (60 mL) and the reaction-mixture was refluxed on a water-bath for about 2 hr. The solvent was removed *in vacuo* and the residue was purified over the column of silica gel and eluted with chloroform. The eluate was concentrated and the product was recrystallized with ethanol to give compound **1**. yield 84%, m.p. 220-22°C; (Found. C, 68.95; H, 4.06; N, 5.70. $C_{14}H_{10}NOCl$ required C, 68.99; H, 4.10; N 5.74%); IR: 3419, 1774, 1598, 1487, 857, 748 (carbazole), 2270, 2242, 1785 (-N-CO), 2879 (C-CH_2), 767 (C-Cl) cm^{-1}; ^1H NMR : 4.41 (s, 2H, CH_2), 7.06 - 8.07 (m, 8H, ArH).

Microwave method. Chloroacetyl chloride (3.81 mL, 0.04 mole) was added to a solution of carbazole (8 g, 0.04 mole) in acetone (60 mL) taken in a round bottommed flask and iradiated in microwave oven for 2 min. The completion of the reaction was monitored by TLC. The solvent was removed and the product was recrystallised from ethanol to give compound **1**, yield 89%. Spectral and analytical data were found to similar as reported for conventional method.

N^9-(hydrazinoacetyl)-carbazole 2 (conventional method). A mixture of compound **1** (6.7 g, 0.027 mole) and hydrazine hydrate (1.33 mL, 0.027 mole) in ethanol : dioxan (9:1 v/v) was refluxed on a water-bath for about 4 hr. It was cooled, filtered and concentrated to get a solid compound which was passed through a column of silica gel and eluted with chloroform. The elute was concentrated and the product was crystallized from chloroform to give compound **2**, yield 65%, m.p. 253-55°C; (Found C, 70.25; H, 5.40; N, 17.49. $C_{14}H_{13}N_3O$ requires C, 70.29; H, 5.43; N, 17.57%. IR: 3419, 1770, 1595, 1493, 857, 747 (carbazole), 2279, 2240, 1787 (-N-CO), 3370, 3320 ($NHNH_2$), 2875 (C-CH_2) cm^{-1}; ^1H NMR : 4.37 (s, 2H, CH_2), 4.51 (s, 2H, -NH_2), 8.83 (s, 1H, -NH), 7.10 - 8.05 (m, 8H, ArH).

Microwave method

Hydrazine hydrate (1.33 mL, 0.027 mole) was add to a solution of N^9-(chloroacetyl)-carbazole compound **1** (6.7 g, 0.027 mole) in ethanol : dioxon (9:1v/v) taken in a round bottommed flask and iradiated in microwave oven for 1.30 min. The completion of the reaction was monitored by TLC. The solvent was removed and the product was recrystallised from ethanol to give compound **2**, yield 70%. Spectral and analytical data were found to similar as reported for conventional method.

N^9-(Arylidene acetylhydrazino)-carbazole 3a (conventional method). A mixture of **2** (1.80 g, 0.007 mole) with benzaldehyde (0.760 mL, 0.007 mole) in ethanol : dioxan (9:1 v/v) was refluxed on a water-bath for about 8 hr. It was cooled, filtered and concentrated to get a solid compound which was passed through a column of silica gel and eluted with methanol. The eluate was concentrated and the product was crystallized from methanol to give compound **3a**, yield 61%, m.p. 218-19ºC; (Found: C, 77.02; H, 5.15; N, 12.78, $C_{21}H_{17}N_3O$ requires C, 77.06; H, 5.19; N, 12.84%).; IR : 3417, 1772, 1590, 1490, 854, 745 (carbazole), 2275, 2238, 1789 (-N-CO), 2880 (C-CH_2), 1604 (-N = CH) cm^{-1}; ^1H NMR : 4.38 (s, 2H,

Table I —— Characterization data of the compounds **3b-p** and **4b-p**

Compd	Ar group	m. p. (°C)	Yield		Mol. formula	Calcd (Found)		
			MWT(min)	Conventional method (hr)		C	H	N
3b	2-ClC_6H_4	224-25	68, (3)	63, (6)	$C_{21}H_{16}N_3OCl$	69.70 (69.68	4.42 4.40	11.61 11.58)
3c	3-ClC_6H_4	225-26	69, (3)	64, (7)	$C_{21}H_{16}N_3OCl$	69.70 (69.65	4.42 4.39	11.61 11.58)
3d	4-ClC_6H_4	223-25	68, (2)	65, (6)	$C_{21}H_{16}N_3OCl$	69.70 (69.66	4.42 4.38	11.61 11.55)
3e	2-BrC_6H_4	253-55	69, (1)	66, (8)	$C_{21}H_{16}N_3OBr$	62.06 (62.04	3.94 3.91	10.34 10.28)
3f	3-BrC_6H_4	248-50	68, (2)	64, (8)	$C_{21}H_{16}N_3OBr$	62.06 (62.03	3.94 3.92	10.34 10.30)
3g	4-BrC_6H_4	250-52	70, (2)	65, (7)	$C_{21}H_{16}N_3OBr$	62.06 (62.02	3.94 3.90	10.34 10.29)
3h	2-$NO_2C_6H_4$	230-32	66, (1)	62, (7)	$C_{21}H_{16}N_4O_3$	67.74 (67.71	4.30 4.28	15.05 15.01)
3i	3-$NO_2C_6H_4$	229-30	68, (3)	64, (5)	$C_{21}H_{16}N_4O_3$	67.74 (67.69	4.30 4.25	15.05 15.00)
3j	4-$NO_2C_6H_4$	227-29	65, (3)	61, (6)	$C_{21}H_{16}N_4O_3$	67.74 (67.68	4.30 4.24	15.05 14.98)
3k	2-$OCH_3C_6H_4$	260-62	75, (4)	70, (5)	$C_{22}H_{19}N_3O_2$	73.94 (73.89	5.32 5.25	11.76 11.71)
3l	3-$OCH_3C_6H_4$	263-65	72, (4)	68, (8)	$C_{22}H_{19}N_3O_2$	73.94 (73.87	5.32 5.26	11.76 11.70)
3m	4-$OCH_3C_6H_4$	262-64	76, (5)	71, (8)	$C_{22}H_{19}N_3O_2$	73.94 (73.90	5.32 5.28	11.76 11.71)
3n	2-$CH_3C_6H_4$	232-34	75, (2)	69, (7)	$C_{22}H_{19}N_3O$	77.41 (77.35	5.57 5.50	12.31 12.28)
3o	3-$CH_3C_6H_4$	232-33	72, (2)	67, (6)	$C_{22}H_{19}N_3O$	77.41 (77.37	5.57 5.49	12.31 12.25)
3p	4-$CH_3C_6H_4$	235-37	74, (2)	70, (6)	$C_{22}H_{19}N_3O$	77.41 (77.36	5.57 5.51	12.31 12.27)
4b	2-ClC_6H_4	218-20	75, (3)	70, (9)	$C_{23}H_{17}N_3O_2Cl_2$	63.01 (62.97	3.88 3.80	9.58 9.50)
4c	3-ClC_6H_4	219-21	73, (3)	68, (8)	$C_{23}H_{17}N_3O_2Cl_2$	63.01 (62.95	3.88 3.79	9.58 9.50)
4d	4-ClC_6H_4	225-27	74, (2)	69, (9)	$C_{23}H_{17}N_3O_2Cl_2$	63.01 (62.93	3.88 3.81	9.58 9.51)
4e	2-BrC_6H_4	240-42	72, (2)	68, (5)	$C_{23}H_{17}N_3O_2ClBr$	57.20 (57.15	3.52 3.48	8.70 8.65)
4f	3-BrC_6H_4	244-45	76, (1)	72, (4)	$C_{23}H_{17}N_3O_2ClBr$	57.20 (57.13	3.52 3.46	8.70 8.63)
4g	4-BrC_6H_4	241-43	76, (2)	73, (4)	$C_{23}H_{17}N_3O_2ClBr$	57.20 (57.16	3.52 3.45	8.70 8.64)
4h	2-$NO_2C_6H_4$	198-200	74,(2)	69, (6)	$C_{23}H_{17}N_4O_4Cl$	61.53 (61.48	3.79 3.71	12.48 12.40)
4i	3-$NO_2C_6H_4$	196-97	70, (1)	65, (6)	$C_{23}H_{17}N_4O_4Cl$	61.53 (61.46	3.79 3.73	12.48 12.42)
4j	4-$NO_2C_6H_4$	199-01	71,(1)	66,(7)	$C_{23}H_{17}N_4O_4Cl$	61.53 (61.47	3.79 3.75	12.48 12.43)
4k	2-$OCH_3C_6H_4$	245-46	77, (3)	72, (5)	$C_{24}H_{20}N_3O_3Cl$	66.43 (66.40	4.61 4.55	9.68 9.60)
4l	3-$OCH_3C_6H_4$	247-48	73, (3)	69, (5)	$C_{24}H_{20}N_3O_3Cl$	66.43 (66.38	4.61 4.56	9.68 9.62)
4m	4-$OCH_3C_6H_4$	245-47	76, (2)	71, (4)	$C_{24}H_{20}N_3O_3Cl$	66.43 (66.37	4.61 4.57	9.68 9.64)

—*Contd*

Table I — Characterization data of the compounds **3b-p** and **4b-p** — *Contd*

Compd	Ar group	m. p. (°C)	Yield		Mol. formula	Calcd (Found)		
			MWT(min)	Conventional method (hr)		C	H	N
4n	2-$CH_3C_6H_4$	220-22	72,(3)	68, (8)	$C_{24}H_{20}N_3O_2Cl$	68.98 (68.90	4.79 4.70	10.05 9.97)
4o	3-$CH_3C_6H_4$	224-26	69, (4)	65, (9)	$C_{24}H_{20}N_3O_2Cl$	68.98 (68.92	4.79 4.72	10.05 10.00)
4p	4-$CH_3C_6H_4$	220-21	70, (4)	67, (9)	$C_{24}H_{20}N_3O_2Cl$	68.98 (68.94	4.79 4.75	10.05 9.98)

MWT = Microwave assisted technique

3b; ^{1}H NMR : 4.40 (s, 2H, CH_2), 8.81 (s, 1H, -NH), 4.44 (s, 1H,-N=CH), 7.05-8.06 (m, 12H, Ar-H).

3e; ^{1}H NMR : 4.36 (s, 2H, CH_2), 8.78 (s, 1H, -NH), 4.41(s, 1H, -N=CH), 7.09-8.04 (m, 12H, Ar-H).

3h; ^{1}H NMR : 4.38 (s, 2H, CH_2), 8.83 (s, 1H, -NH), 4.45 (s, 1H, -N=CH), 7.06-8.05 (m, 12H, Ar-H).

3k; ^{1}H NMR : 4.39 (s, 2H, CH_2), 8.76 (s, 1H, -NH), 4.46 (s, 1H, -N=CH), 3.92 (s, 3H, Ar-OCH_3), 7.06-8.02 (m, 12H, Ar-H).

3n; ^{1}H NMR : 4.41 (s, 2H, CH_2), 3.81 (s, 1H, -NH), 4.43 (s, 1H, -N=CH), 1.92 (s, 3H, Ar-CH_3) 7.08-8.02 (m, 12H, Ar-H).

4b; ^{1}H NMR : 4.37 (s, 2H, CH_2), 8.81 (s, 1H, -NH), 4.19 (d, 1H, *J* = 5Hz, -N-CH), 5.15 (d, 1H, *J* = 5Hz, CHCl), 7.11-8.06 (m, 12 H, Ar-H).

4e; ^{1}H NMR : 4.34 (s, 2H, CH_2), 8.79 (s, 1H, -NH), 4.16 (d, 1H, *J* = 5Hz, -N-CH), 5.18 (d, 1H, *J* = 5Hz, CHCl), 7.09- 8.08 (m, 12H, Ar-H).

4h; ^{1}H NMR : 4.36 (s, 2H, CH_2), 8.80 (s, 1H, -NH), 4.18 (d, 1H, *J* = 5Hz, -N-CH), 5.20 (d, 1H, *J* = 5Hz, -CHCl), 7.07-8.07 (m, 12H, Ar-H).

4k; ^{1}H NMR : 4.33 (s, 2H, CH_2), 8.77 (s, 1H, -NH), 4.15 (d, 1H, *J* = 5Hz, -N-CH), 5.22 (d, 1H, *J* = 5Hz, -CHCl), 3.91 (s, 3H, Ar-OCH_3), 7.05-8.03 (m, 12H, Ar-H).

4n; ^{1}H NMR : 4.35 (s, 2H, CH_2), 8.79 (s, 1H, -NH), 4.19 (d, 1H, *J* = 5Hz, -N-CH), 5.18 (d, 1H, *J* = 5Hz, CHCl), 1.92 (s, 3H, Ar-CH_3), 7.08-8.05 (m, 12H, Ar-H).

CH_2), 8.80 (s, 1H, -NH), 4.42 (s, 1H, -N = CH), 7.06 - 8.06 (m, 13H, Ar-H).

Microwave method. Benzaldehyde (0.760 mL, 0.007 mole) was add to a solution of compound **2** (1.80 g, 0.007 mole) in ethanol:dioxan (9:1v/v) taken in a round bottomed flask and irradiated in microwave oven for 3 min. The completion of the reaction was monitored by TLC. The solvent was removed and the product was recrystallised from methanol to give compound **3a**, yield 66%. Spectral and analytical data were found to similar as reported for conventional method.

Other compounds **3b-p** were synthesized similarly by both the methods using different aldehydes. Characterization data are presented in **Table I**.

N^9-[Hydrazine acetyl-(2-oxo-3–chloro-4- substituted aryl azetidine)]-carbazoles 4a (conventional method). A mixture of compound **3a** (1 g, 0.003 mole) in acetone (50 mL) and chloroacetyl chloride (0.268 mL, 0.003 mole) with Et_3N (0.003 mole) was stirred from about 2 hr followed by refluxing on a water-bath for about 3 hr. It was cooled, filtered and concentrated to get a solid compound which was passed through a column of silica gel and eluted with ethanol. The eluate was concentrated and the product was recrystallized from chloroform to give compound **4a**, yield 65%; m.p. 220-21ºC; (Found: C, 68.36; H, 4.42; N, 10.36. $C_{23}H_{18}N_3O_2Cl$ requires C, 68.40; H, 4.46; N, 10.40%); IR: 3419, 1776, 1601, 1492, 850, 751 (carbazole), 2278, 2245, 1788 (N-CO), 2882 (C-CH_2), 758 (C-Cl), 1765 (β-lactam) cm^{-1}; ^{1}H NMR: 4.36 (s, 2H, CH_2), 8.79 (s, 1H, -NH), 4.17 (d, 1H, *J* = 5Hz, -N-CH), 5.16 (d, *J* = 5Hz, CHCl), 7.09 - 8.07 (m, 13H, ArH). The mass spectra showed fragments at *m/z* : 403 (M^+). 375, 223, 209, 208, 195, 194, 181, 180, 167, 166, 152 (**Chart I**).

Microwave Method. A mixture of compound **3a** (1 g, 0.003 mole) in acetone (45 mL) was taken in a round bottomed flask, chloroacetyl chloride (0.268 mL, 0.003 mole) and (Et_3 N; 0.003 mole) were added followed by stirring for about 2 hr and irradiated in microwave oven for 3 min. The completion of the reaction was monitored by TLC. The solvent was removed and the product was recrystallized from chloroform to give compound **4a**, yield 68%. spectral and analytical data were found to similar as reported for conventional method.

Other compounds **4b-p** were synthesized similarly by both the methods using **3b-p**. Characterization data are presented in **Table I**.

Table II — Antibacterial data of the compounds **1**, **2**, **3a-p** and **4a-p**

Compds	*E.coli*		*K.pneumoniae*		*B.subtilis*		*S.aureus*	
	50ppm	100ppm	50ppm	100ppm	50ppm	100ppm	50ppm	100ppm
1	+	+	+	+	+	++	-	-
2	-	+	+	+	-	+	-	+
3a	+	++	+	+	+	+	+	++
3b	++	++	+	++	+	++	+	+
3c	+	++	+	++	+	++	+	+
3d	+	++	++	++	++	++	+	++
3e	++	+++	++	+++	++	++	+++	+++
3f	+++	+++	++	+++	+	++	+++	+++
3g	++	+++	+++	+++	++	++	+++	+++
3h	+	++	++	++	+	+	+	++
3i	+	+	+	++	+	++	+	+
3j	+	+	-	+	+	++	++	++
3k	-	-	-	+	+	+	-	-
3l	+	+	+	-	-	-	+	+
3m	-	+	+	+	-	+	-	+
3n	-	-	+	+	-	+	++	+
3o	-	+	-	-	-	-	-	+
3p	+	+	-	+	-	-	+	+
4a	+	+	+	+	-	+	++	++
4b	++	++	+	++	++	++	+	++
4c	++	++	+	++	+	++	++	++
4d	+	++	+	+	++	++	++	++
4e	++	++	+++	+++	+++	+++	+	++
4f	+++	+++	++	+++	++	+++	+++	+++
4g	++	+++	++	++	++	+++	+	++
4h	+	+	++	+	+	++	+	+
4i	+	++	+	+	++	++	+	+
4j	+	++	++	++	+	++	++	++
4k	+	+	+	+	-	+	-	-
4l	++	++	-	-	+	+	+	++
4m	+	+	+	+	-	+	+	+
4n	+	+	-	-	+	++	-	-
4o	-	+	+	+	-	-	+	+
4p	-	+	-	+	-	-	+	+
SM	+++	++++	+++	++++	+++	++++	+++	++++

SM : Streptomycin, inhibition diameter in mm; (-) 7 mm; (+) 7 - 15 mm; (++) 15-23 mm; (+++) 23-28 mm and (++++) 28-35 mm.

Table III — Antifungal data of the compounds **1**, **2**, **3a-p** and **4a-p**

Compd	*A.nigar*		*F.oxisporium*		*T.viride*		*A.flavus*	
	100ppm	500ppm	100ppm	500ppm	100ppm	500ppm	100ppm	500ppm
1	+	+	-	+	+	+	+	+
2	-	+	-	+	-	-	+	+
3a	+	+	+	+	-	+	-	-
3b	+	++	+	+	++	++	+	++
3c	+	+	++	++	+	++	+	+
3d	+	++	+	+	+	++	++	++
3e	++	++	+	++	+	++	++	+++
3f	++	+++	+++	+++	+	++	+++	+++
3g	+++	+++	++	++	+++	+++	++	++
3h	+	++	+	+	++	++	+	+
3i	+	++	+	++	+	+	-	+
3j	+	+	+	+	+	++	+	+
3k	+	++	+	+	-	+	-	-
3l	-	+	-	+	++	++	+	+
3m	+	+	+	++	-	+	-	+
3n	+	+	-	-	+	++	-	-
3o	-	+	+	+	-	+	-	+
3p	-	+	+	+	-	-	+	+
4a	+	+	+	+	-	+	+	+
4b	+	++	++	+	-	+	+	+
4c	+	++	++	++	++	+++	++	+++
4d	++	++	+	++	+	++	+	-
4e	+++	+++	+++	+++	++	+++	++	+++
4f	++	+++	++	+++	+++	+++	+++	+++
4g	++	++	++	+++	+	++	+++	+++
4h	+	++	-	+	++	++	+	+
4i	+	+	+	++	+	+	+	+
4j	++	++	+	+	-	+	+	++
4k	+	+	-	+	+	+	-	-
4l	+	+	-	+	+	+	+	+
4m	-	+	+	+	-	+	-	-
4n	-	-	-	+	-	-	-	-
4o	-	+	+	+	-	-	+	+
4p	-	+	-	-	-	+	-	+
GF	+++	++++	+++	++++	+++	++++	+++	+++

GF : Griseofulvin inhibition diameter in mm; (-) 4 min; (+) 4-10 mm; (++) 10-16 mm; (+++) 16-24 mm.

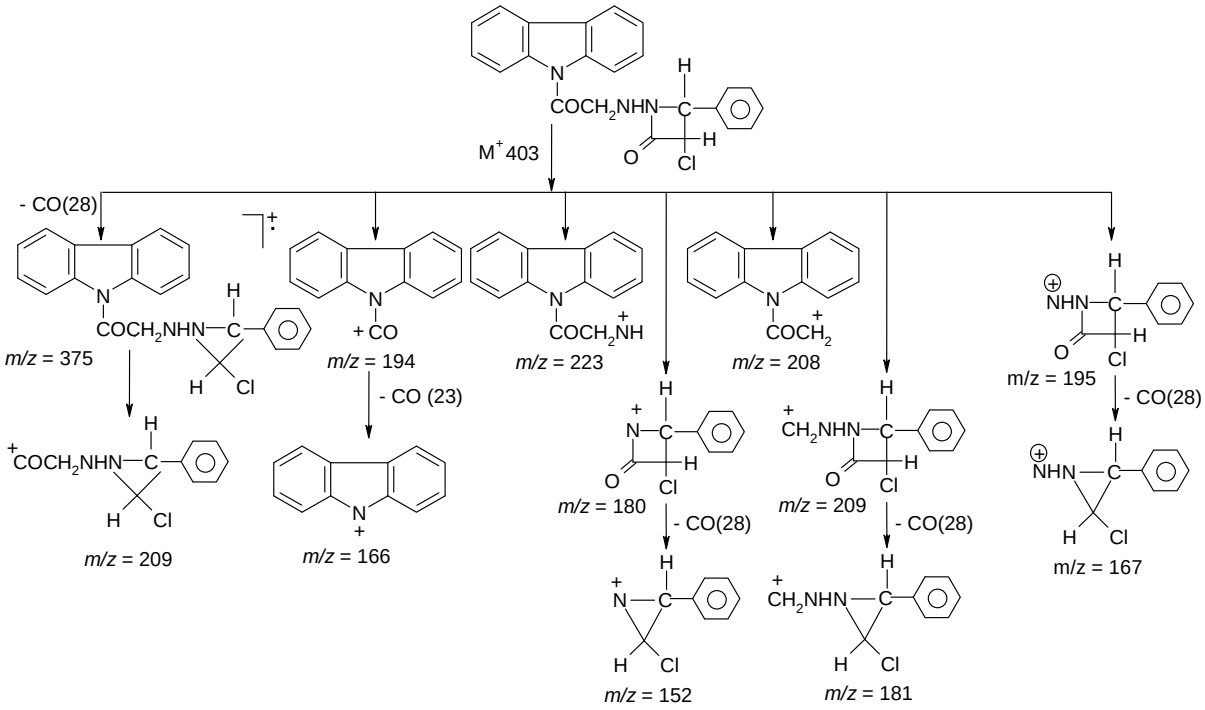


Chart 1

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