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Characteristic fragmentation behavior of some novel anti-calmodulin acridone derivatives studied by electrospray ionization tandem mass spectrometry

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Abstract

The mass spectroscopic behaviors of novel 4-fluoro N^{10}-substituted acridones were studied by using positive ion electrospray ionization (ESI) with tandem mass spectrometry. Protonated molecular ions $[M+H]^{\bullet+}$ were observed for all the compounds studied, and in the case of the parent 4-fluro acridone molecular ion $[M]^{\bullet+}$ peak was obtained. The most interesting feature is that all the novel compounds predominantly gave nitrogen containing fragment ions. Tandem mass spectrometry was performed on these quasimolecular ions, and the product ions formed provided useful fragmentation patterns that were characteristic for the compounds. Fragmentation pathways and possible fragment ion structures are discussed. In order to obtain elemental composition, accurate mass measurements were performed.

Keywords: Acridone; Electrospray ionization (ESI); Mass spectrometry (MS)

1. Introduction

Calmodulin (CaM) is the main protein involved in calcium buffering and in the Ca^{2+}-dependent regulation of specific cell functions. It changes in intracellular calcium levels to modulate a variety of biological and biochemical process, affecting no fewer than two dozen enzymes [1–3]. Higher eukaryotes have several CaM genes which are differentially regulated while encoding identical proteins. Because of the importance of Ca^{2+} in progression of the cell cycle, CaM plays a critical role in the regulation of cell proliferation [4–6]. It has been shown that disease states characterized by unregulated growth such as cancer are correlated with elevated levels of Ca^{2+} bound CaM [7–10]. Thus, CaM is a promising target for the development of drugs that show preferential activity in tumors and as a result, exhibit fewer toxic side effects in normal cells [11].

Various anti-calmodulin acridone derivatives is known to potentiate the uptake of anticancer agents vincristine and vinblastine in multidrug resistant (MDR) GC_3/cl and $KBCh^R$-8-5 cancer cells to a greater extent than a standard modulator verapamil [12].

Subsequently a set of 13 N^{10}-substituted 4-fluoroacridones were prepared and examined for reversing multidrug resistance in cancer cells. The N^{10}-substitution includes propyl and butyl groups attached to different secondary amines.

ESI/MS offers great potential in this area of research since it can generate information about molecular weight and structure. It has been successfully used to determine the molecular weight of small organic molecules at the femtomole level.

Earlier a set of eight anti-MDR 2-trifluro methyl N^{10}-substituted phenoxazines and a series of twenty one 2-chloro N^{10}-substituted phenoxazines were characterized by using electron ionization (EI) and liquid secondary ionization mass spectrometric techniques [13].

Electrospray ionization mass spectrometry (ESI–MS) studies of acridones are previously not reported. This analytical

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Table 1
Mass spectral data of 4-fluro acridone derivatives (*m/z* with relative intensities (%) in parentheses)

Compound	*m/z* with relative intensities (%)
AC-01	213.9 (100), 193.8 (10), 185 (17), 166.1 (45), 166.1 (45), 139 (24)
AC-02	354.4 (25), 336.3 (30), 254.2 (10), 237.9 (6), 226.2 (5), 211.9 (20), 142.2 (21), 127.2 (6), 113.1 (100)
AC-03	339.1 (60), 254 (9), 142.1 (17), 126.0 (100), 98.1 (50)
AC-04	341.2 (8), 144 (10), 128.0 (100), 100.1 (13)
AC-05	325.1 (70), 254.2 (6), 198.0 (13), 128.1 (38), 111.7 (90), 84.2 (100)
AC-06	359.2 (20), 341.3 (16), 245.2 (13), 146.2 (100), 118.1 (80)
AC-07	384.3 (10), 254.2 (30), 226.2 (55), 171.8 (70), 141.1 (81), 100.1 (98), 98.1 (100)
AC-08	368.2 (20), 268.1 (30), 226.2 (28), 154.6 (78), 113.1 (12), 98.1 (100)
AC-09	354.2 (10), 269 (11), 227.2 (12), 156.0 (12), 140.8 (100), 98 (13)
AC-10	355.2 (35), 268.2 (13), 158.0 (15), 141.1 (100), 100.1 (36)
AC-11	339.1 (34), 269 (18), 226 (37), 99.9 (100)
AC-12	398.3 (25), 267.9 (17), 185 (100)
AC-13	373.1 (30), 355.2 (19), 268 (15), 160.1 (100), 116.2 (30)

technique, one of the most important for the analysis of non-volatile and thermally labile compounds, produces higher abundance protonated molecules due to the low residual energy of the ionization process. In the present study the characteristic fragmentation behavior of some novel N^{10}-substituted 4-fluroacridone derivatives under ESI conditions is discussed.

The mass spectral fragmentation characteristics of all the thirteen newly synthesized acridone derivatives under electrospray ionization (ESI) conditions are studied. A common structural feature of (AC#01–AC#13) is substitution of one of the benzene ring of acridone at position C-4 by fluorine. Although the basic structural units in these compounds are same, the difference in their structure arises by the substituents attached to the N^{10}-position are of diverse functionality. They differ from each other because of the terminal hydrogen of an alkyl group is replaced by *N*-methylpiperazine, piperidine, morpholine, pyrrolidine, diethanolamine and (β-hydroxyethyl) piperazine. To obtain detailed and comprehensive data on fragmentation pathways of acridone derivatives, we included thirteen compounds in this study. Mass spectral characterization data of all the novel acridone derivatives studied are given in Table 1.

2. Experimental

2.1. Mass spectrometry

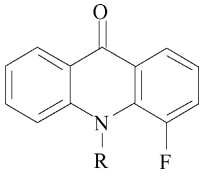
Collision-induced dissociation (CID) spectra were acquired in the positive ion mode on a MDS Sciex (Concord, Ont., Canada) API 4000 triple quadrupole mass spectrometer with direct infusion of each acridone at a concentration of 10 μM in 50% methanol, at flow rate of 25 μl/min. The instrument was operated with a spray voltage of 5.5 kV, a declustering potential of 50 eV a source temperature of 100 °C, a GSI value of and the curtain gas set at 10. Ultra-pure nitrogen was value of 50 and the curtain gas and collision gas. MS/MS spectra of the protonated molecule of each drug were acquired and multiple reactions monitoring (MRM) transition for important fragments was optimised. The data for the fragment ion curves represent an average of five consecutive experiments.

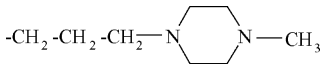
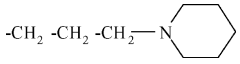
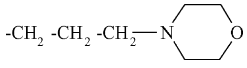
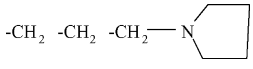
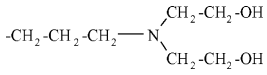
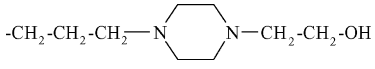
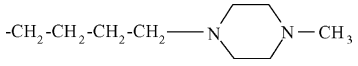
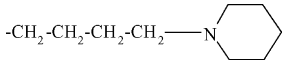
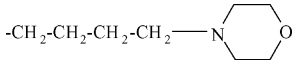
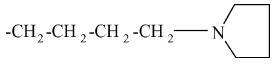
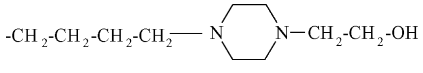
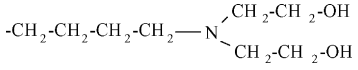
3. Results and discussion

The mass spectra of all the acridone derivatives were analyzed under ESI conditions (Table 2). During ESI, a molecular ion can acquire internal energy leading to extensive fragmentation. Mass spectral features of the acridone derivatives are observed due to cleavage of bonds in the N^{10}-alkyl side chain portion of these compounds, acridone ring system remains intact. This fact is manifested in the mass spectra of all the acridone derivatives.

Molecular ions were observed either in the form of $M^{\bullet+}$ and $M+H$ in the spectra of these 4-fluroacridone derivatives. From

Table 2
Acridone derivatives investigated



Compound	R	MW
AC-01	–H	213.9
AC-02		353.7
AC-03		339.1
AC-04		341.2
AC-05		325.1
AC-06		359.2
AC-07		384.3
AC-08		368.2
AC-09		354.2
AC-10		355.2
AC-11		339.1
AC-12		398.3
AC-13		373.1

the mass spectral data, it is clear that as such there is no difference in fragmentation pattern among the set of acridone series compounds.

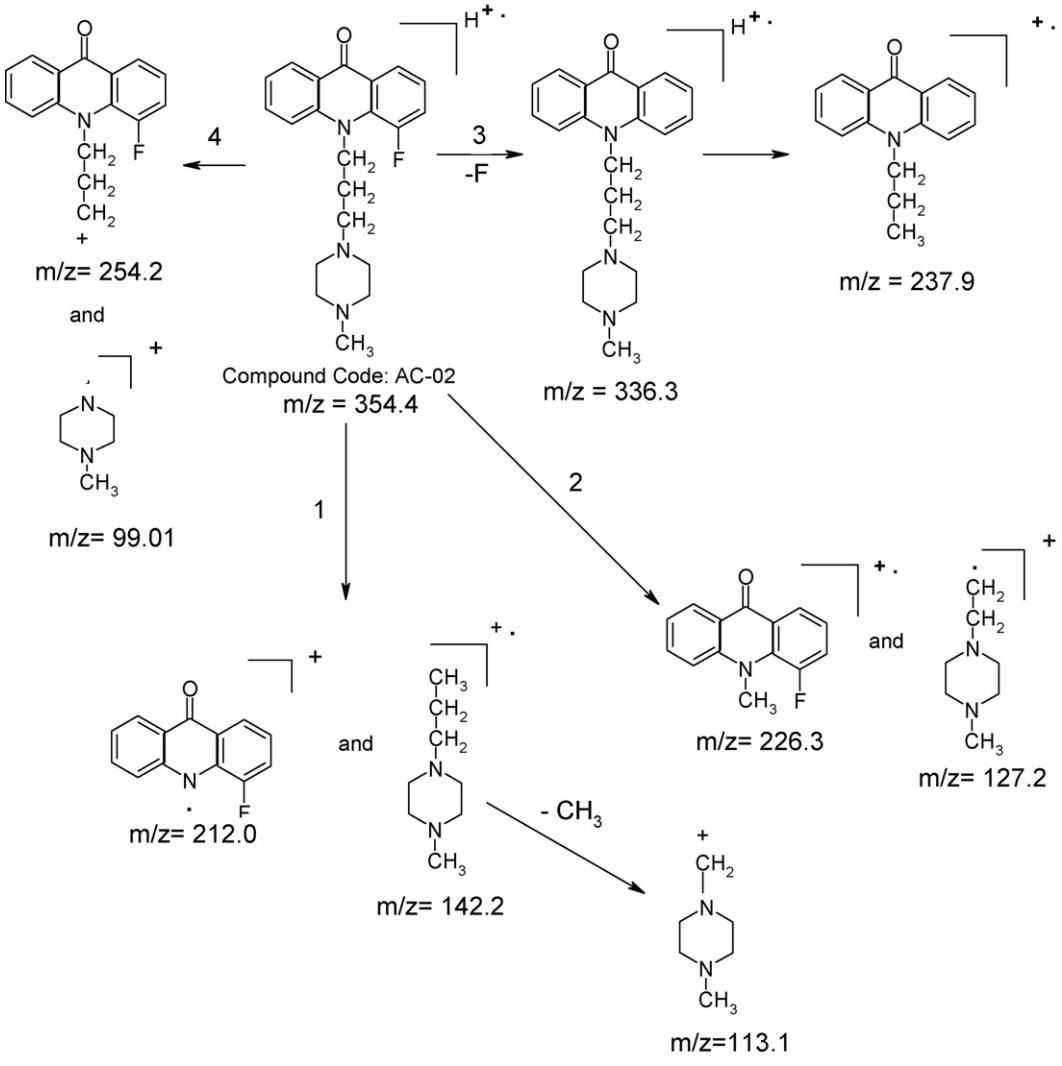
To illustrate the fragmentation pattern of the N^{10}-substituted acridones, fragmentation pathways of compound is given in Scheme 1. A characteristic feature of this compound is the presence of dominant molecular ion and the fission takes place all along the alkyl side chain. Fission of the bond linking the side chain to the acridone ring nucleus occurs (reaction 1), producing peaks at *m/z* 211.9 and 142.2. Fission of the bond 'α' to the ring nitrogen (reaction 2), produces *m/z* 226.3 and 127.2. The prominent fragmentation is the cleavage of the bond to the fluorine-producing ion (reaction 3) at *m/z* 336.3 with the relative abundance of 30%. Fission of the bond linked piperazine ring to the alkyl side chain also occurs (reaction 4), producing peaks at *m/z* 254.2 and 99.0.

In general, mass spectral features of these compounds were similar and straightforward. Most of the compounds yield abundant molecular ions in the form of $M+H$. All bonds in the N^{10}-side chain portion are prone to cleavage. In conclusion, the data presented here demonstrate the usefulness of MS for characterization of acridone derivatives.

4. Protonated acridones

The product ion mass spectrum for 10-[3′-*N*-(methylpiperazino) propyl] 4-fluro acridone (AC-02) *m/z* 354.4, obtained at collision energy of 30 eV. Initially, the collision energy was maintained at 4 eV so as to transmit protonated N^{10}-substituted acridone ions in order to obtain a well-shaped peak for this ion species; then after 1 min, the collision energy was increased to 30 eV for the following 3 min in order to observe the fragment ions of *m/z* 254.2 and 211.9. The parent ion peak (*m/z* 354.4) was used as lock mass for the product ion mass spectrum.

The primary product ions of the fragmentation of protonated acridone (AC-02) are shown in Scheme 1. Five primary product ions were observed and it was proposed that these ions followed



Scheme 1. Primary fragmentation of protonated 10-[3′-*N*-(methylpiperazino) propyl] 4-fluro acridone (AC-02).

Table 3
Formula, observed and calculated mass and mass error of the fragment ions in the product ion mass spectrum of protonated 10-[3′-*N*-(methylpiperazino) propyl] 4-fluro acridone (AC-02)

Predicted formula	Observed mass (Da)	Calculated mass (Da)	Error (mDa)
$C_{21}H_{25}FN_3O^+$	354.4330	354.4330	0.0
$C_{21}H_{25}N_3O^+$	336.3469	336.3436	+3.3
$C_{16}H_{13}FNO^+$	254.2807	254.2836	−2.9
$C_{16}H_{15}NO^+$	237.9502	237.9529	−2.7
$C_{14}H_9FNO^+$	226.2296	226.2260	+3.6
$C_{13}H_7FNO^+$	211.9782[a]	212.0270	[+48.8]
$C_8H_{17}N_2^+$	142.2399	142.2420	−2.1
$C_7H_{16}N_2^+$	128.2165	128.2150	+1.5
$C_5H_{10}N_2^+$	113.1827	113.1810	+1.7
Average			2.4

[a] The mass errors associated with the identification of $C_{13}H_7FNO^+$ have not been included in the calculation of the average error.

four fragmentation pathways. The pathways examined showed in (Table 3) were tabulated as formula, observed mass, calculated mass and mass error for the fragment ions observed in the product ion mass spectra of protonated acridone (AC-02). The error between the observed and calculated masses ranged from 0 to 3.6 mDa with an average value of 2.4 mDa indicating good mass accuracy.

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References

[1] W.Y. Cheung, Harvey Lect. 79 (1985) 173.
[2] H.J. Vogel, Biochem. Cell. Biol. 72 (1994) 357.
[3] A.R. Means, M.F.A. VanBerkum, I. Bagchi, K.P. Lu, C.D. Rasmussen, Pharmacol. Ther. 50 (1991) 225.
[4] C.D. Rasmussen, K.P. Lu, R.L. Means, A.R. Means, J. Physiol. 86 (1992) 83.
[5] K.P. Lu, A.R. Means, Endocrine Rev. 14 (1993) 40.
[6] O. Bachs, N. Agell, E. Carafoli, Biochim. Biophys. Acta 259 (1992) 1113.
[7] J.W. Wei, R.A. Hickie, Biochem. Biophys. Res. Commun. 100 (1981) 1562.
[8] J.W. Wei, R.A. Hickie, Cancer Res. 42 (1982) 2571.
[9] R.A. Hickie, J.W. Wei, L.M. Blyth, D.Y.W. Wong, Can. J. Biochem. Cell. Biol. 61 (1983) 934.
[10] W.N. Hait, J.S. Lazo, J. Clin. Oncol. 4 (1986) 994.
[11] Y.C. Mayur, S. Jagadeesh, K.N. Thimmaiah, Min. Rev. Med. Chem. 6 (2006) 12.
[12] R. Hegde, P. Thimmaiah, M.C. Yerigeri, G. Krishnegowda, K.N. Thimmaiah, P.J. Houghton, Eur. J. Med. Chem. 39 (2004) 161.
[13] C. Dass, K.N. Thimmaiah, B.S. Jayashree, R. Sheshadri, Bio. Mass. Spectrum. 23 (1994) 140.