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Molecular fragmentation of some novel cytotoxic dichloro acridones by electrospray ionization tandem mass spectrometry

Yarlagadda Rajesh Babu* and Mantripragada Bhagavanraju

Department of Pharmaceutical chemistry, Gland Institute of Pharmaceutical Sciences, Kothapet, Medak India

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ABSTRACT

The mass spectroscopic behaviors of novel Dichloro N^{10}-substituted acridones were studied by using positive ion electrospray ionization (ESI) with tandem mass spectrometry. Collision-induced dissociation (CID) spectra were acquiredin the positive ion mode on a MDS Sciex API 4000 triple quadrupole mass spectrometer withdirect infusion of each acridone.MS/MS spectra of theprotonated molecule of each drug were acquired and multiplereactions monitoring (MRM) transition for important fragments was optimised. Protonated molecular ions [M+H]•+ were observed for all the compounds studied, and in the case of the parent4-fluro acridone molecular ion [M]•+ peak was obtained. The most interesting feature is that all the novel compounds predominantly gave nitrogen containing fragment ions. The pathways examined showedin were tabulated as formula, observed mass, calculatedmass and mass error for the fragment ions observed in the product ion mass spectra of protonated acridone.

Key Words: Acridone, Electrospray ionization (ESI); Mass Spectrometry (MS)

INTRODUCTION

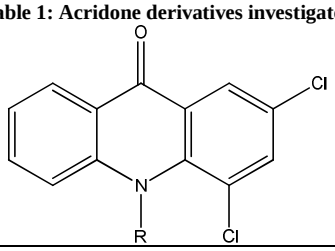
Cancer is mainly a disease of aging. The management of cancer in the older age group is going to become the most common practice of oncology [1]. The interactions of cancer and age are multiple and complex.They includes carcinogenesis, tumor biology, as well as cancer prevention and treatment [2.3]. The unique properties of cancer-initiating cells capable with a high self-renewal and abnormal differentiation potential including their elevated expression levels of anti-apoptotic factors, multidrug transporters, and DNA repair and detoxifying enzymes might be associated with their resistance to current clinical cancer therapies and disease recurrence. Major progress toward the identification of new therapeutic targets in cancer cells in recent years has led to the discovery and development of new classes of anti-cancer drugs.

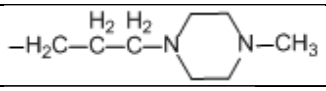
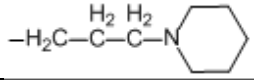
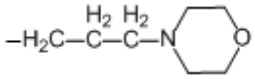
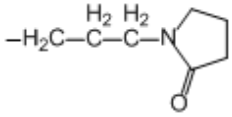
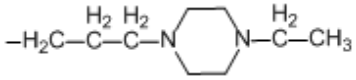
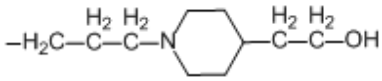
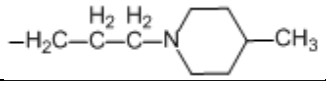
From decades researchers identified numerous planar tricyclic molecules with different side chains possessing useful cytotoxicand/or cytostatic potencies. These tricyclic systems include anthraquinone, acridine/acridone, Phenoxazine, and xanthenes [4,5]. A number of acridone alkaloids have been isolated from plants of the Rutaceae family. Acronycine possesses significant antitumor activity [6, 7]. Glyfoline, another natural acridone alkaloid, was found to be the most potent compound for inhibition of cellular growth of human leukemia HL-60 cells in vitro [8]. 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 [9 13]. 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. [10]. Electrospray

ionization mass spectrometry (ESI–MS) studies of dichloro acridones are previously not reported. This analytical technique is one of the most important for the analysis of non-volatile and thermally labile compounds. In the present study the characteristic fragmentation behavior of some novel N^{10}-substituteddichloroacridone derivatives under ESI conditions is discussed.

The mass spectral fragmentation characteristics of all seven newly synthesized acridone derivatives under electrospray ionization (ESI) conditions are studied. A common structural feature of (01–09) is substitution of one of the benzene ring of acridone at position C-2 and C-4 by chlorine. 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, and (β-hydroxyethyl) piperazine. To obtain detailed and comprehensive data on fragmentation pathways of acridone derivatives, we included seven compounds in this study. Mass spectral characterization data of all the novel acridone derivatives studied are given in Table 1.

Table 1: Acridone derivatives investigated



Compound Code.	R	Mol. Weight
1	H	264.11
3		404.33
4		389.32
5		391.29
6		389.28
7		418.36
8		434.36
9		403.34

MATERIALS AND METHODS

Mass spectrometry

Collision-induced dissociation (CID) spectra were acquiredin the positive ion mode on a MDS Sciex (Concord, Ont.,Canada) API 4000 triple quadrupole mass spectrometer withdirect infusion of each acridone at a concentration of 10 µM in50% 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 ofand the curtain gas set at 10. Ultra-pure nitrogen was value of50 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.

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•+ and M+H in the spectra of these 2,4-dichloroacridone derivatives. From the mass spectral data, it is clear that as such there is no difference in fragmentation pattern among the set of acridone series compounds.

Table 2: Mass spectral data of 2, 4-dichloro derivatives (m/z with relative intensities (%) in parentheses)

Compound	m /z with relative intensities (%)
3	403.12, 336.3,304.03,261.98,237.9,142.15
4	388.11, 321.20,304.33, 261, 237,142
5	390.09,304.03,322.17, 261.9, 237.1, 129.12,
6	388.07, 320.15, 304.03, 127.10,84.04
7	417.14, 349.22, 304.03, 262.9, 238.12, 156.16, 113.11
8	433.13, 365.21,304, 261.9,238.1, 172.16, 129.1
9	402.13, 334.2, 262.9,238.12, 141.15,98.1

Scheme 1: Primary fragmentation of protonated 2,4-dichloro-10-(3-(4-methylpiperazin-1-yl)propyl)acridin-9(10H)-one

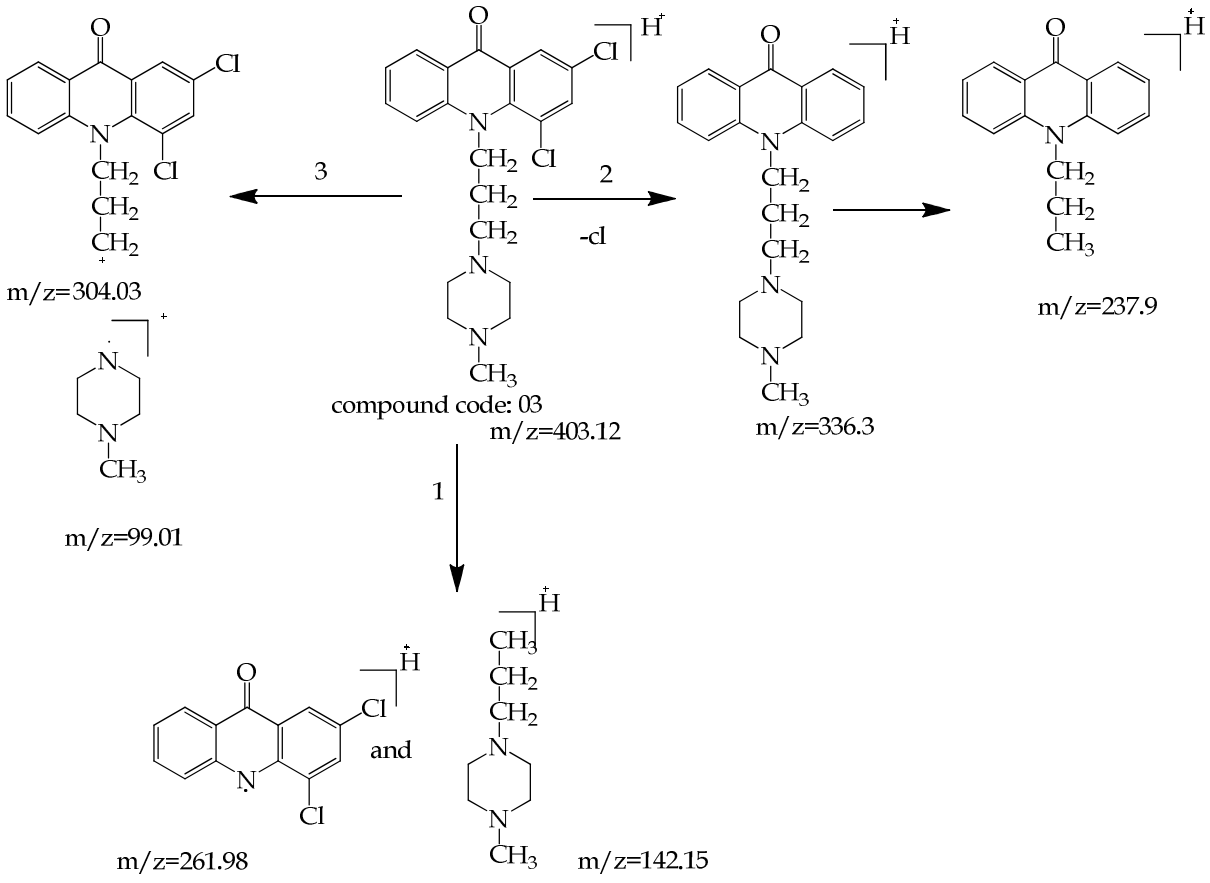


Table 3 : Formula, observed and calculated mass and mass error of the fragment ions in the product ion mass spectrum of protonated 2,4-dichloro-10-(3-(4-methylpiperazin-1-yl)propyl)acridin-9(10H)-one (03)

Predicted formula	Observed mass (Da)	Calculated mass (Da)	Error (mDa)
$C_{21}H_{24}Cl_2N_3O^+$	404.1291	404.1291	0.0
$C_{21}H_{26}N_3O^+$	336.3469	336.3436	+3.3
$C_{16}H_{12}Cl_2NO^+$	304.0291	304.0320	-2.9
$C_{16}H_{15}NO^+$	237.9502	237.9529	-2.7
$C_{13}H_6Cl_2NO^+$	261.9826	261.9862	+3.6
$C_8H_{17}N_2^+$	142.2399	142.2420	-2.1
Average			2.7

To illustrate the fragmentation pattern of the N^{10}-substitutedacridones, fragmentation pathways of compound is given in Scheme 1. A characteristic feature of this compound is the presenceof dominant molecular ion and the fission takes place allalong the alkyl side chain. Fission of the bond linking the sidechain to the acridone ring nucleus occurs (reaction 1), producing peaks at m/z 261.9 and 142.15.The prominent fragmentation is the cleavage of the bond to the chlorine-producing ion (reaction 2) at m/z 336.3 with the relativeabundance of 30%. Fission of the bond linked piperazine ring to the alkyl side chain also occurs (reaction 3), producing peaks at m/z 304.03 and 99.0. Initially, the collision energy was maintained at 4 eV so as to transmit protonated N^{10}-substituted acridone ions in order to obtain awell-shaped peak for this ion species; then after 1 min, the collision energy was increased to 30 eV for the following 3 mins in order to observe the fragment ions of m/z 304.03 and 261.9.The parent ion peak (m/z 403.12) was used as lock mass for the product ion mass spectrum. In general, mass spectral features of these compounds were similar and straight forward. Most of the compounds yield abundant molecular ions in the form of M+H. All bonds in theN^{10}-side chain portion are prone to cleavage.

CONCLUSION

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 (03). The error between the observed and calculated masses ranged from 0 to 3.6 mDa with an average value of 2.7 mDa indicating good mass accuracy. In conclusion, the data presented here demonstrate the usefulness of MS for characterization of acridone derivatives.

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