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Electrospray Ionization Tandem Mass Spectrometric Characteristics And Fragmentation Mechanisms Of Purine Analogues

Corresponding Author

Ling-Bo Qu[a,b]

[a]**The Key Chemical and Biological Laboratory, Department of Chemistry, Zheng Zhou University, Zhengzhou, 450052, (P.R.CHINA)**

[b]**Anyang Normal University, Anyang, 455002, (P.R.CHINA)**

Co-Authors

Xiao-Lan Chen, Juan He, Yu-Fen Zhao

The Key Chemical and Biological Laboratory, Department of Chemistry, Zheng Zhou University, Zhengzhou, 450052, (P.R.CHINA)

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ABSTRACT

The electrospray ionization tandem mass spectrometric (ESI-MS/MS) characteristics and fragmentation pathways of six novel 2,6-disubstituted purines were investigated. A stepwise fragmentation of the $[M+H]^+$ ions was observed. The six novel purine analogues can be distinguished by ESI/MS/MS spectra of protonated molecules and ms/ms/ms spectra of fragment ions.

KEYWORDS

ESI-MS;
Tandem mass spectrometry;
2,6-disubstituted purines;
Fragment passway.

INTRODUCTION

Various modifications of nucleosides on both sugar and hetrocyclic moieties have been extensively studies for antiviral agents. Purine libraries synthesized either in solution or on solid supports have received significant attention due to their potential to target nucleotide-binding proteins[1-5]. Purines are extremely difficult to purify the final product from the excess amine[6]. The purine 2- and 6 can be selectively differentiated to introduce a variety of nucleophiles. Once the products are cleaved from the resin, the 9-position can be further modified. In this paper, a series of novel 2,6-disubstituted purines (SCHEME 1) compounds **(1-6)** were synthesized and determined by electrospray ionization mass spectrometry combined with tandem mass spectrometry (ESI/MS). The six novel purine analogues can be distinguished by MS/MS spectra of protonated molecules and MS/MS/MS spectra of fragment ions.

EXPERIMENTAL

Our approach to generate purine analogs using

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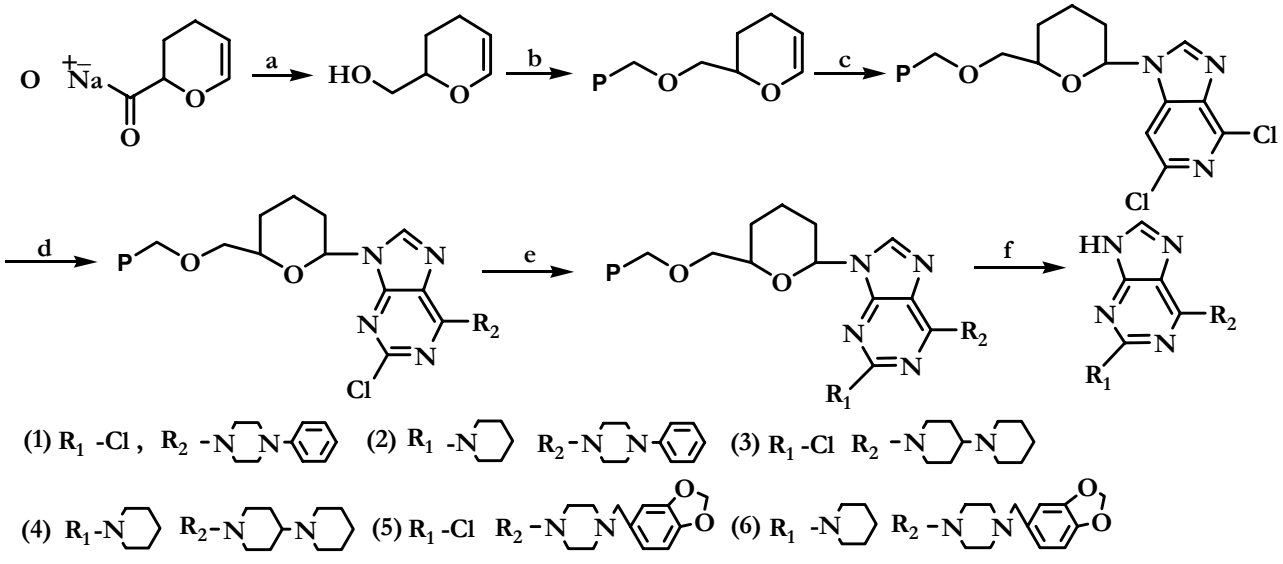

SCHEME 1: Synthetic pathway of six novel 2,6-disubstituted purines

solid-phase chemistry is shown in SCHEME 1.

Reagents and conditions

(a) LAH, THF, rt; (b) NaH, DMF, Merrifield resin, rt; (c) 2,6-dichloripurine, CSA, $ClCH_2CH_2Cl$, 60°C ; (d) phenpyl piperazine, n-BuOH, Et_3N, 85°C ; (e) piperidine, heat, 150°C; (f) TFA, $ClCH_2CH_2Cl$, 65°C Structures of these compounds were checked by UV, ^1H NMR, LC-MS and elemental analysis[7].

Mass spectrometry

ESI tandem mass spectra were obtained using a Bruker Esquire LC-MS ion trap mass spectrometer (Bruker Daltonics, Inc.). The capillary needle voltage was 4000 V and the source temperature was mainted at 300°C. The instrument was operated at unite-mass resolution; calibration of m/z was performed using a standard ES-tuning-mix. Six scans were averaged for each spectrum. Data acquisition and processing were achieved using the Bruker data analysis system.

A methanol solution of each compound with a concentration of approximately 4ul/min was prepared. These solutions were introduced into the electrospray source at a flow rate of 4ul/min.

RESULTS AND DISCUSSION

The MS/MS spectral data of the $[M+H]^+$ ions, and of the most significant fragment ions of the six analogues, are summarized in TABLE 1.

Even though there were six different compounds, only three first-generation fragmentation pathways were found, one for compound **(1-2)** (SCHEME 2), one for compound **(2-3)** (SCHEME 5) and the last for compound **(4-5)** (SCHEME 8). The cleavages of these compounds always occurred first in the 6-substituted groups. It was concluded that the different 2-substituted groups could exert very few influences on the first-generation fragmentation patterns. The differences in 2-substituted groups only could be reflected by the ESI-MS/MS/MS of some special first-generations ions (SCHEME 3, 4, 6, 7, 9,10).

Typical example of ESI positive ion mass spectral fragmentation pathways of compound **(1-2)** were shown in SCHEME 2. The mass spectra of compounds **(1-2)** show the protonated molecules at m/z 315, 364, respectively. It was concluded, that the same first-generation fragmentation patterns were followed by the protonated compounds **(1-2)**. (SCHEME 2). The MS/MS spectra of $[M+H]^+$ ions of compounds **(1-2)** showed characteristic $[M+H-93]^+$, $[M+H-119]^+$ and $[M+H-145]^+$ions, corresponding to $[M+H-s_1]^+$, $[M+H-s_2]^+$ and $[M+H-s_3]^+$, respectively. The protonated s_3 showed its peak ion at m/z 146. The piperazine ring of compounds **(1-2)** showed relatively unstable characteristics and all cleavages happened in this part. The different 2-substituted groups showed no influence on their first generation

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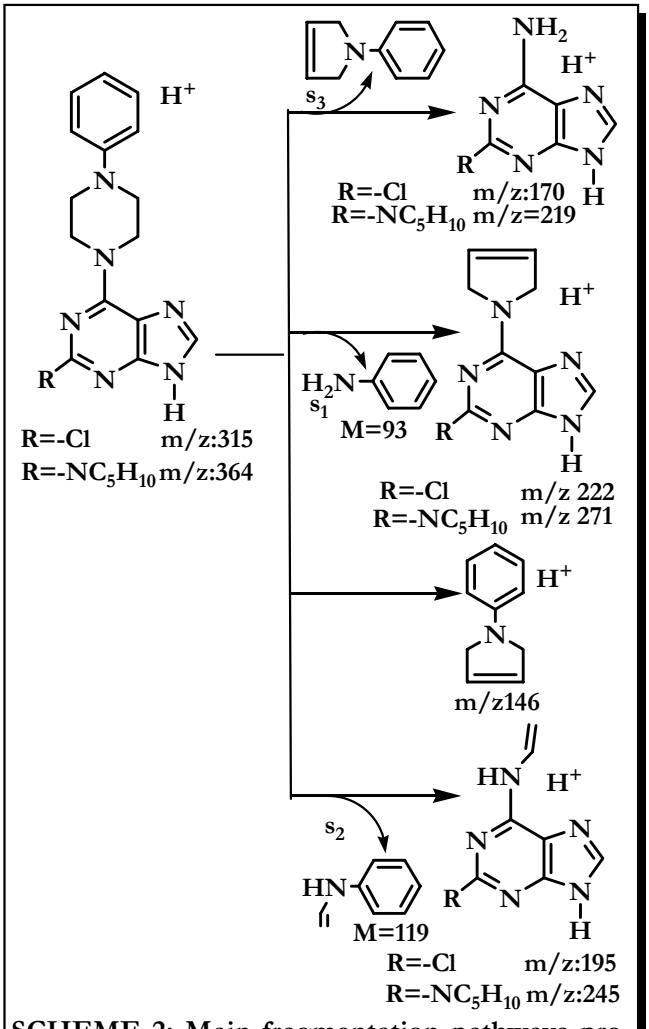
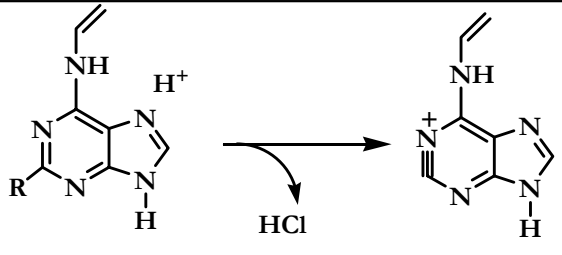
TABLE 1: MS/MS spectral data of $[M+H]^+$ and significant ions of compounds (1-6)

Compound	Precursor ion (m/z)	Fragment ions [m/z (%)]
(1)	315	222(4), 196(100), 170(3), 146(6)
	196	160
(2)	364	271(3), 245(100), 219 (35), 146(7),
	245	243(100), 215(7), 189(2)
(3)	321	236(100), 182(63), 98 (10)
	236	182
	182	155(90), 146(100), 128(20), 119(35)
(4)	370	285(100), 231(24)
	285	231
	231	229(100), 202(16), 175(18), 163(6)
(5)	373	237(6), 135(100)
	237	220(61), 196(59), 182(5), 170(100)
(6)	422	286(100), 245(16), 219(12), 135(50)
	286	245(34), 269(7), 243(100), 231(38), 229(13), 219(85), 217(73), 215(37), 177(17)



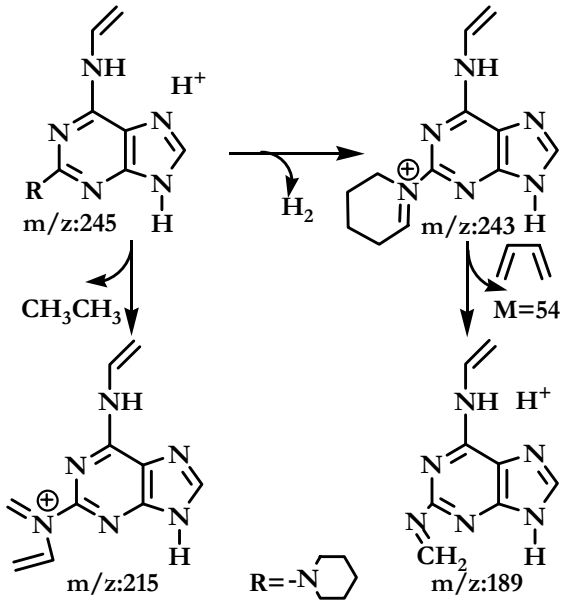
SCHEME 2: Main fragmentation pathways proposed to account for the MS/MS spectra of the ions at m/z 315 and 364 of compounds (1-2)



SCHEME 3: Fragmentation pathways proposed to account for the MS/MS/MS spectra of the ion at m/z 196 for compound (1)

fragmentation pathways. The ESI-MS/MS/MS spectrum of the ion $[M+H-119]^+$, ion m/z 196 for compound **(1)** and ion m/z 245 for compound **(2)** (TABLE 1), implied that the second cleavage patterns (SCHEME 3-4) were not the same. For example, the ion at m/z 196 (SCHEME 3) could produce ion at m/z 160 by expulsion HCl. However the ion at m/z 245 (SCHEME 4) could yield ions at m/z 243 and 189 through losses of molecular H_2 and dibutene C_4H_6. It was also deduced that the difference fragmentation patterns (SCHEME 3-4) corresponding to ESI-MS/MS/MS spectrum of the ion $[M+H-119]^+$ could reflect the structure differences in 2-substituted groups exactly.

ESI positive ion mass spectral fragmentation pathways of compound **(3-4)** are shown in SCHEME 5. The mass spectrum of compounds **(3-4)** showed the protonated molecules at m/z 321, 370 respec-

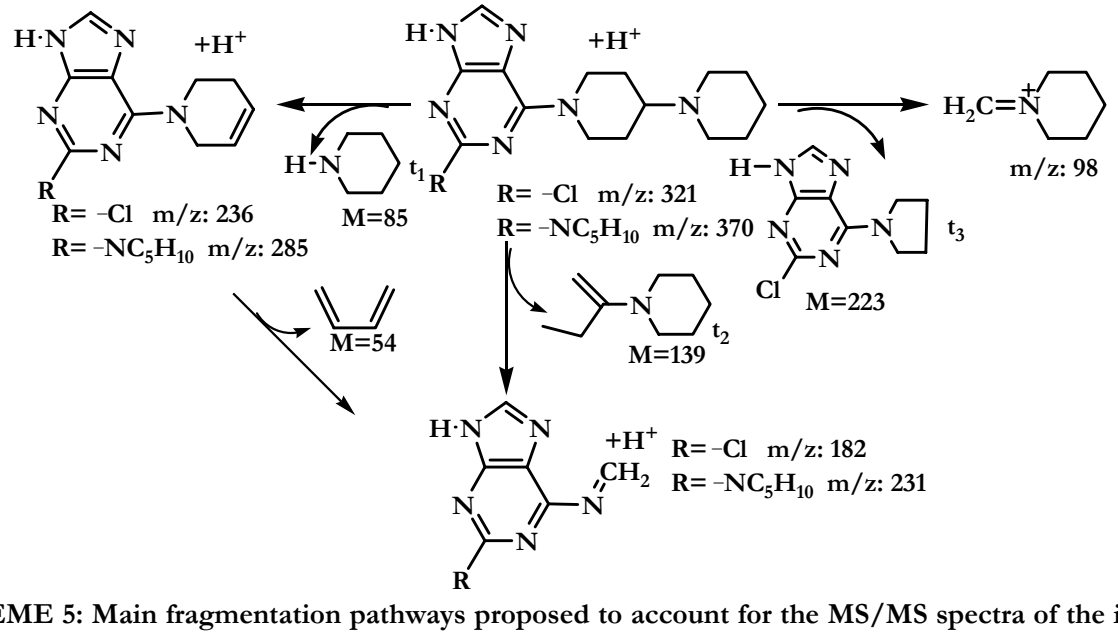


SCHEME 4: Fragmentation pathways proposed to account for the MS/MS/MS spectra of the ion at m/z 245 for compound (2)

tively. The MS/MS spectra of $[M+H]^+$ ions of compounds **(3-4)** showed characteristic $[M+H-85]^+$ $[M+H-139]^+$ ions, corresponding to two significant first-generation fragment ions $[M+H-t_1]^+$ and $[M+H-t_2]^+$, respectively. The $[M+H-139]^+$ ion could also yield ion at m/z 182 or 231 by expulsion of butadiene. An extra weak ion at m/z 98, produced by loss t_3, appeared in the ESI-MS/MS spectrum of compound **(3)**. It was concluded that, even if the 2-sustituted groups were different, all cleavages were only happened firstly in 6-subtituted group and the first-generation fragmentation patterns of compounds **(3-4)** were basically similar while the second-generation fragmentation of the $[M+H-139]^+$ ion for compounds **(3-4)** were different (SCHEME 6, 7). The ESI-MS/MS/MS spectrum of the ion at m/z 23, summarized in SCHEME 6 for compound **(3)**, produced ions at m/z 155, 128 through successively loss two molecular NCH. Other two ions at m/z 146 and 119 were achieved by loss of neutral molecular HCl and then molecular NCH (SCHEME.6).

The ESI-MS/MS/MS spectrum of the ion at m/z 231, summarized in SCHEME 7 for compound **(4)**, produced ions at m/z 229, 202 and 175, through successive losses of molecular hydrogen and two molecular NCH. It was also concluded that the difference fragmentation pathways, corresponding to ESI-MS/MS/MS spectrum of the ion $[M+H-139]^+$ for compounds **(3-4)**, were all triggered off by the structure differences in 2-substituted groups.

The ESI-MS/MS spectra of compounds **(5-6)** provide special structural information (TABLE 1). The mass spectrum of compounds **(5-6)** showed the protonated molecules at m/z 373, 422 respectively.



SCHEME 5: Main fragmentation pathways proposed to account for the MS/MS spectra of the ions at m/z 321 and 370 of compounds (3-4)

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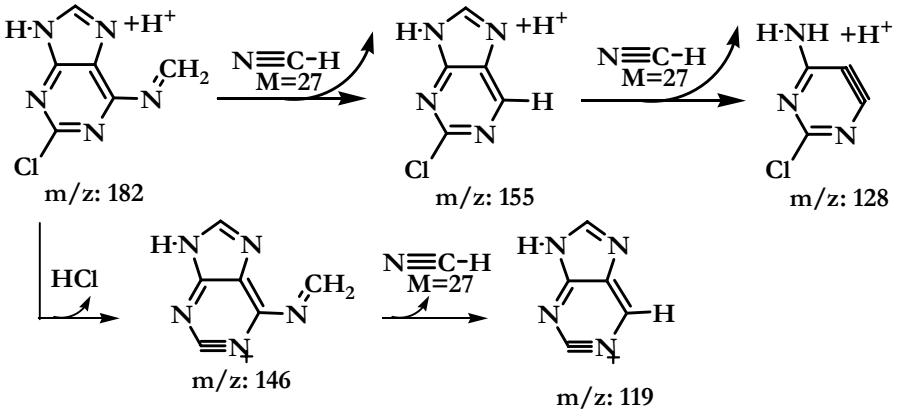
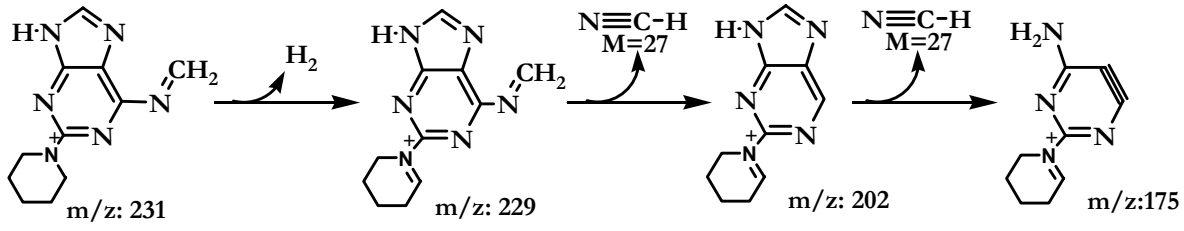
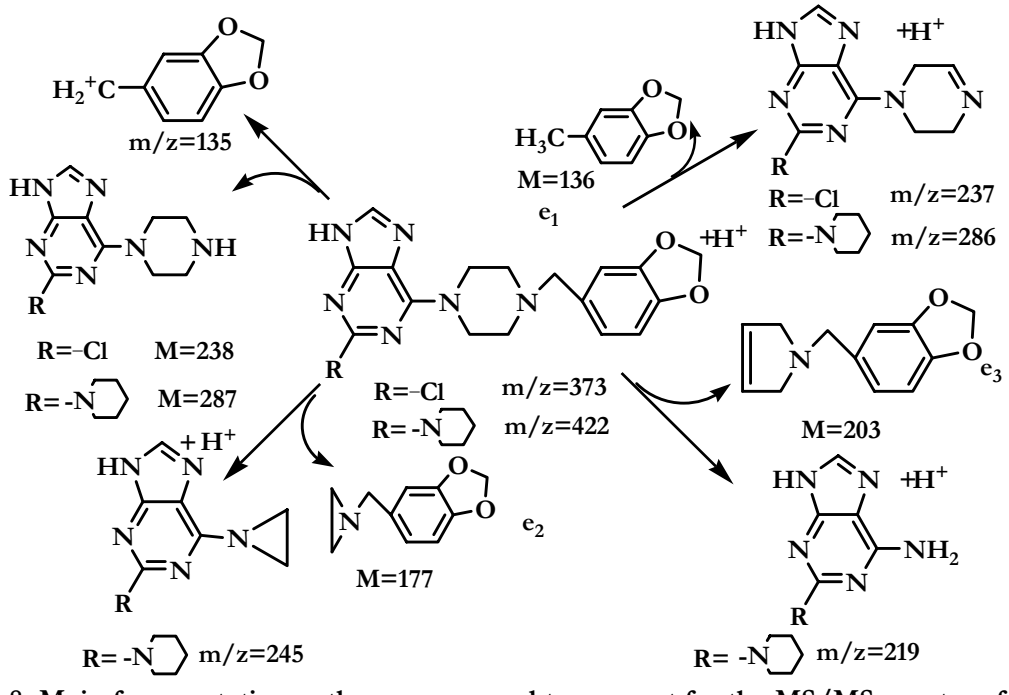


SCHEME 6: Fragmentation pathways proposed to account for the MS/MS/MS spectra of the ion at m/z 182 for compound (3)



SCHEME 7: Fragmentation pathways proposed to account for the MS/MS/MS spectra of the ion at m/z 231 for compound (4)



SCHEME 8: Main fragmentation pathways proposed to account for the MS/MS spectra of the ions at m/z 373 and 422 of compounds (5-6)

The MS/MS spectra of $[M+H]^+$ ions of compounds **(5-6)** showed characteristic $[M-136]^+$ ion, corresponding to one significant first-generation fragment ions $[M+H-e_1]^+$ (SCHEME 8). Through the cleav-

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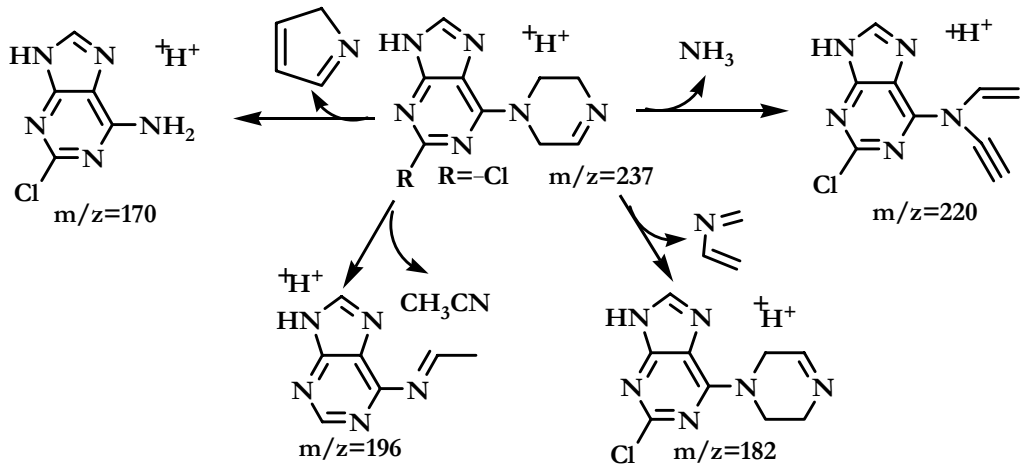
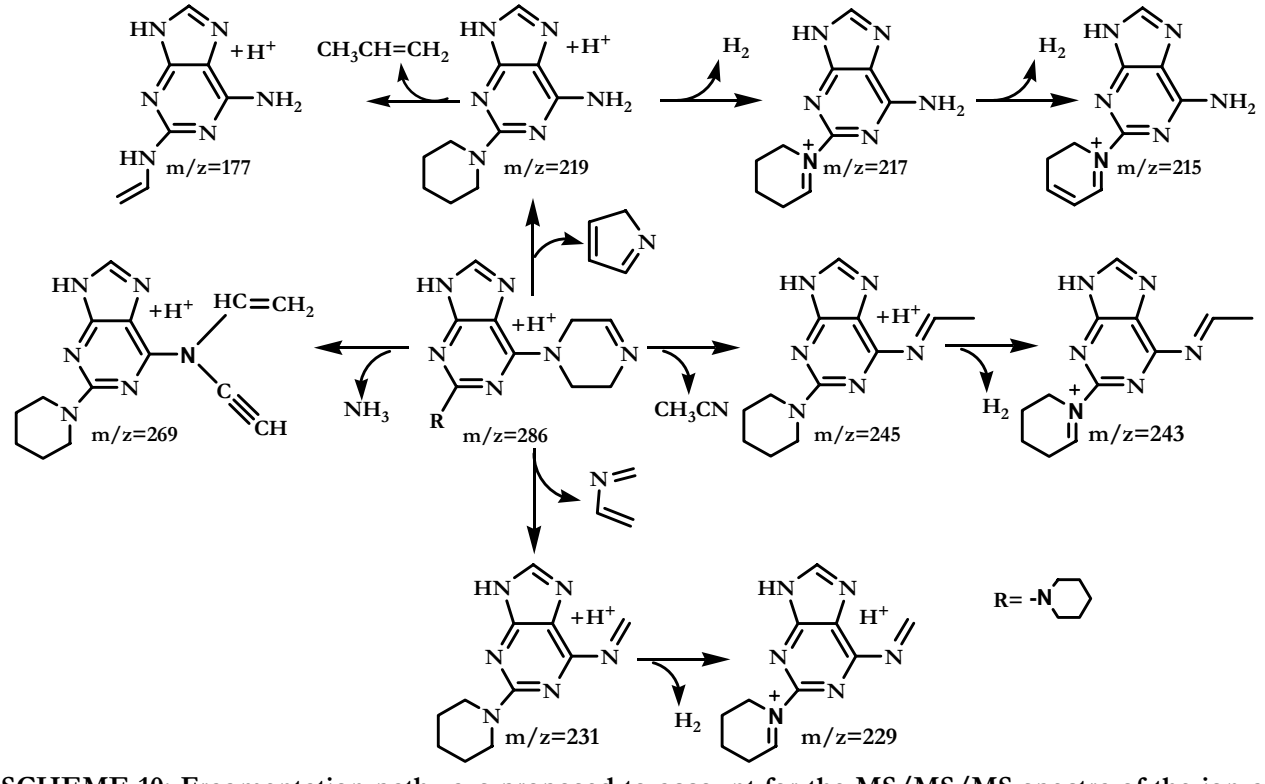


SCHEME 9: Fragmentation pathways proposed to account for the MS/MS/MS spectra of the ion at m/z 237 for compound (5)



SCHEME 10: Fragmentation pathways proposed to account for the MS/MS/MS spectra of the ion at m/z 286 for compound (6)

age of the N-CH_2 bond between piperazine ring and piperonyl group, another characteristic ion at m/z 135 was easily formed owing to its special stability. Two extra weak ions at m/z 245, 219, produced by loss e_2, e_3, respectively, appeared in the ESI-MS/MS spectrum of compound **(6)**. The first cleavage processes and fragmentation patterns for 6-substituted groups of compounds **(5-6)** were the same. The differences in 2-substituted groups could still be reflected by the ESI-MS/MS/MS of the first-generation ions $[M+H^+-136]^+$ and the fragmentation pathways are shown in SCHEME 9 and SCHEME 10. For example, the ion at m/z 245 (SCHEME 10) could yield the base peak ion at m/z 243 by expulsion of molecular hydrogen. The ion at m/z 219 could produce ions at m/z 217 and 215 through successively

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losses of molecular hydrogen. The successively loss of hydrogen was only seen when the 2-substituted group was piperidine ring, as seen in SCHEME 4, 7 and 10. So the second-generation pathways of compound **(5, 6)** show the structural differences in 2-substituted groups again.

CONCLUSION

The ESI-MS/MS spectra of these purine analogues provide special structural information. The first-generation fragmentation pathways only involve cleavages in the 6-substituted groups. It was concluded the first-generation fragmentation pathways were similar if the 6-substituted groups are the same and the different 2-substituted groups could exert very few influences on the first-generation fragmentation patterns. The differences in 2-substituted groups only could be reflected by the ESI-MS/MS/MS of some special first-generations ions. Based on the different fragmentation pathways and the structural information generated in the MS/MS of these compounds, the six novel purine analogues can be distinguished. The ESI-MS/MS technique is thus an excellent tool for the structural characterization and identification of these compounds.

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