

Electrospray ionization mass and tandem mass spectra of a series of *N*-pyrazolylmethyl and *N*-triazolylmethyl *N*-phenylpiperazines: new dopaminergic ligands with potential antipsychotic properties

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Recently, two analogous series of *N*-pyrazolylmethyl and *N*-triazolylmethyl *N*-phenylpiperazines have been prepared and found to be potential antipsychotic drugs acting as new selective ligands of the dopamine D2 receptor. Herein we report a systematic study of their high-resolution electrospray ionization mass and tandem mass spectra in which the main dissociation routes of their protonated molecules are determined and rationalized. The ESI-MS/MS data is very characteristic for both series allowing straightforward isomeric differentiation. A single and dominant fragment ion for the pyrazole series and four major fragment ions for the triazole series are useful for selective reaction MS monitoring of these potential drugs in biological fluids. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: tandem mass spectrometry; antipsychotic drugs; dopamine receptor ligands; ion chemistry

INTRODUCTION

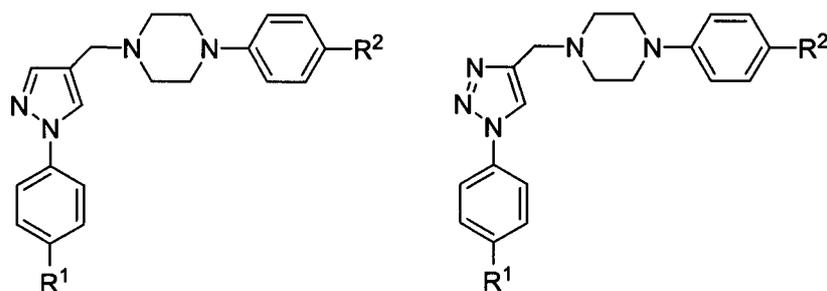
Schizophrenia is a serious disorder of the central nervous system affecting as many as 1 to 2% of the worldwide population.¹ The causes of this important psychotic syndrome are still unclear, but both genetic and environmental factors are likely involved.² It is believed that decreased dopaminergic tonus in prefrontal cortical areas accounts for the negative symptoms of schizophrenia such as apathy and social withdrawal, whereas increased dopaminergic activity in the striatum is associated with the positive symptoms of the disease such as delusions and hallucinations.²

The positive symptoms of schizophrenia respond well to classical antipsychotic drug treatment such as that using haloperidol, a prototype of the butyrophenone's group that acts primarily as a D2 receptor antagonist. Clozapine is an atypical antipsychotic agent binding to dopaminergic receptors controlling both the positive and some of the negative symptoms of schizophrenia.³ In spite

of their therapeutic profile, when used clinically, classical antipsychotic drugs produce a series of Parkinsonian-like motor dysfunctions and other side effects, and these drawbacks have stimulated the search for new drugs with better therapeutic efficacy and fewer side effects.²

With this goal in mind, we have synthesized two analogous nine-component series of *N*-pyrazolylmethyl- (1) and *N*-triazolylmethyl- (2) *N*-phenyl piperazine derivatives.⁴ These heterocyclic compounds, which have been designed as dopamine D2 receptor ligands by using bioisosterism and molecular simplification strategies,² have been found to be potential antischizophrenia drugs since they act as effective and selective ligands of the D2 receptor. They also display important hypothermic properties *in vivo*, probably because of their interference with the dopaminergic system.⁵ Here we report detailed mass and tandem mass spectrometric characterization of 1 and 2. The gentle^{6–8} electrospray ionization technique was used and found to efficiently transfer these relatively labile compounds to the gas phase as their intact protonated molecules. ESI tandem mass spectrometry (ESI-MS/MS), which is the most selective and sensitive technique used to monitor drugs in biological fluids,⁹ was then used to study the dissociation chemistry of the gaseous protonated molecules, and to determine the best

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1a, R¹ = R² = H
1b, R¹ = R² = F
1c, R¹ = R² = Cl
1d, R¹ = Cl, R² = OCH₃
1e, R¹ = H, R² = Cl
1f, R¹ = H, R² = F
1g, R¹ = F, R² = H
1h, R¹ = Cl, R² = H
1i, R¹ = NO₂, R² = H

2a, R¹ = R² = H
2b, R¹ = R² = F
2c, R¹ = R² = Cl
2d, R¹ = Cl, R² = OCH₃
2e, R¹ = H, R² = Cl
2f, R¹ = H, R² = F
2g, R¹ = F, R² = H
2h, R¹ = Cl, R² = H
2i, R¹ = NO₂, R² = H

parameters for selective reaction monitoring (SRM) in future *in vivo* pharmacokinetic studies of these drug candidates.

EXPERIMENTAL

The two analogous series of nine *N*-pyrazolylmethyl- (**1a-i**) and nine *N*-triazolylmethyl- (**2a-i**) *N*-phenylpiperazine derivatives were available from previous studies.¹⁰ ESI mass and tandem mass spectra in the positive ion mode were acquired using a Micromass (Manchester—UK) Q-Tof instrument with 7.000 mass resolution in the ToF mass analyzer. Typical operating conditions, which are described in detail elsewhere,¹¹ were 3 kV capillary voltage, 40 V cone voltage, and desolvation gas temperature of 100 °C. ESI tandem mass spectra were collected after 10 to 25 eV collision-induced dissociation (CID) of mass-selected protonated molecules with argon. Mass selection was performed by Q1 using a unitary *m/z* window and collisions were performed in the rf-only quadrupole collision

cell, followed by mass analysis of product ions by the high-resolution orthogonal-reflectron TOF analyzer.

RESULTS AND DISCUSSION

ESI-MS

All the ESI mass spectra (not shown) of the 18 molecules of **1** and **2** are dominated by their protonated molecules, which are detected as their respective set of isotopologue ions with matching isotopic patterns and masses.

ESI-MS/MS of the pyrazole derivatives

When mass-selected, and subjected to low-energy CID, the ESI tandem mass spectra of the protonated molecules of the pyrazole derivatives **1** (Table 1) display simple and very characteristic dissociation chemistry, exemplified by the spectra of the two isomeric fluoro-substituted protonated molecules of **1f** and **1g** (Fig. 1). Dissociation proceeds mainly by a major dissociation route (Scheme 1) that involves likely

Table 1. Major fragment ions from ESI tandem mass spectra of **1a-i** resulting from low-energy CID of their protonated molecules

Compound	[M + H] ⁺ <i>m/z</i>	Fragment Ions (<i>m/z</i> , % relative abundance)		
		3	4	5
1a	319	3a (157, 100)	4a (161, 3)	5a (175, 1)
1b	355	3b (175, 100)	4b (179, 3)	5b (193, 1)
1c	387 (³⁵ Cl)	3c (191, 100)	4c (195, 3)	5c (209, 1)
1d	385 (³⁷ Cl)	3c (193, 100)	4d (191, 4)	5d (205, 1)
1e	353 (³⁵ Cl)	3a (157, 100)	4c (195, 2)	5c (209, nd ^a)
1f	337	3a (157, 100)	4b (179, 3)	5b (193, nd)
1g	337	3b (175 ^b , 100)	4a (161, 6)	5a (175, 1 ^b)
1h	353 (³⁵ Cl)	3c (191, 100)	4a (161, 9)	5a (171, nd)
1i	364	3d (202, 100)	4a (161, 12)	5a (175, 1)

^a nd = not detected.

^b The high resolution and accuracy of the MS/MS measurements performed on the Q-Tof hybrid mass spectrometer allows easy separation and quantitation of both isobaric fragment ions **5a** and **3b**.

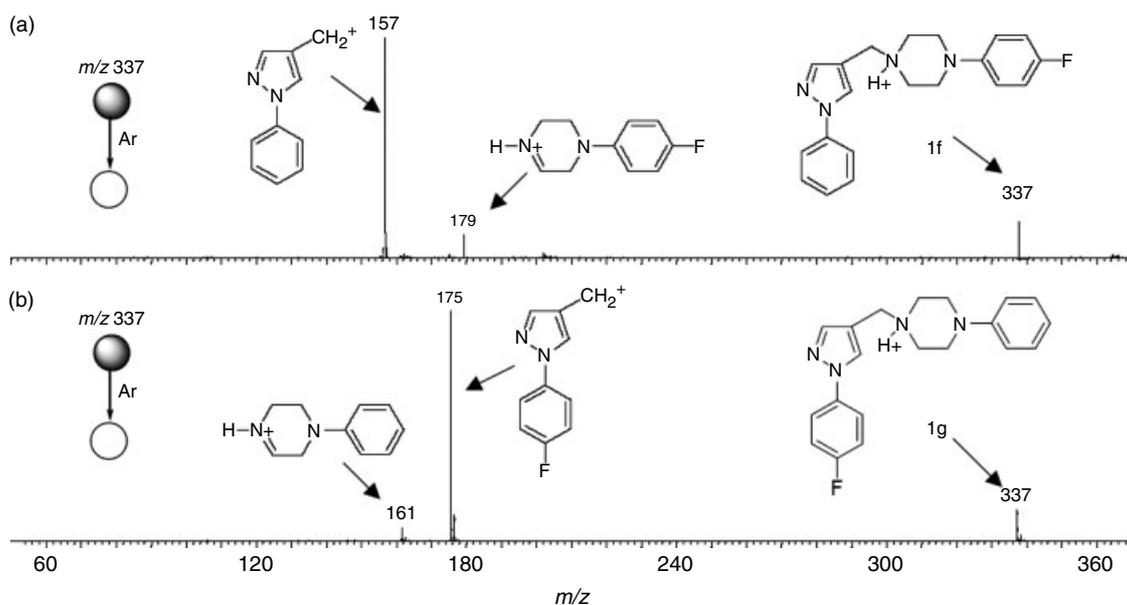
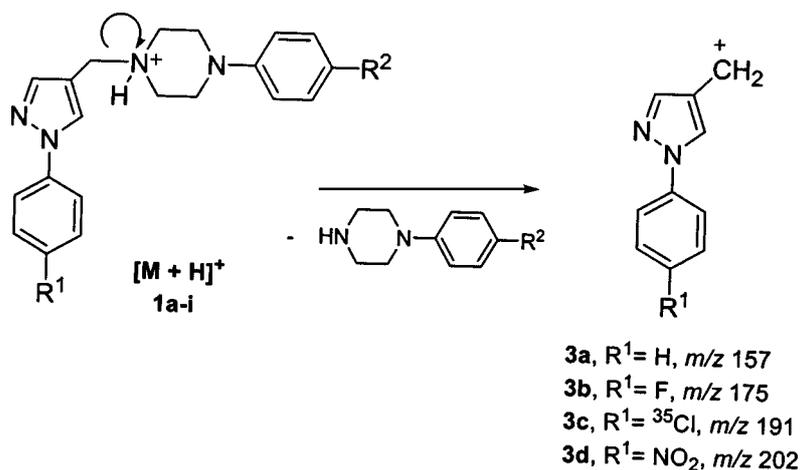


Figure 1. ESI tandem mass spectra of the protonated molecules of the isomeric pyrazole derivatives **1f** and **1g**.



Scheme 1

N-protonation and cleavage of the *N*-pyrazolymethyl bond, which releases the respective neutral molecule of *N*-aryl piperazine and forms the respective pyrazolymethyl cations **3a–d** as the nascent fragment ions. Note that for the isomeric **1f** and **1g**, the location of the F substituent at either benzene ring is clearly perceived by the characteristic m/z ratios of both major fragment ions **3a** of m/z 157 and **3b** of m/z 175.

A second but generally minor dissociation route for the protonated **1a–i** is that rationalized in Scheme 2 yielding ions **4a–d** (Table 1). Such a process also involves the breaking of the *N*-pyrazolymethyl bond, but it forms the protonated molecules of the respective *N*-aryl piperazines (**4a–d**) via the release of the respective neutral molecules of methyl pyrazoles. This route becomes more pronounced for electron-withdrawing R^1 groups such as F (**1g**), Cl (**1h**), and NO_2 (**1i**) forming **4a** with 6%, 9% and 12% relative abundances, respectively (Table 1). The effect of such R^1 groups is likely indirect, that is, the F, Cl, and NO_2 ortho substituents likely de-stabilize cations **3b**, **3c**

and **3d** (Scheme 1) thereby indirectly favoring formation of **4a**.

A third very minor, but not general, dissociation route also operating for the protonated molecules of **1a–i** (Table 1) is that rationalized in Scheme 3. It likely requires protonation at the pyrazole ring followed by cleavage of the pyrazole-methylene bond with the release of neutral pyrazole molecules thus yielding the iminium ions **5a–d**.

ESI-MS/MS of the 1,2,3-triazole derivatives

Although it follows some routes similar to those of **1a–i**, the dissociation chemistry of the protonated molecules of the triazole derivatives **2a–i** (Table 2) is much richer and strongly influenced by the triazole ring, exemplified by the tandem mass spectra of protonated **2f** and **2g** shown in Fig. 2. The dissociation route rationalized in Scheme 4 is analogous to the dominant dissociation route of **1a–i** (Scheme 2). For protonated **2a–i**, however, this process is major, but not dominant. It requires a similar protonation at the piperazine ring, but bond cleavage is preceded by

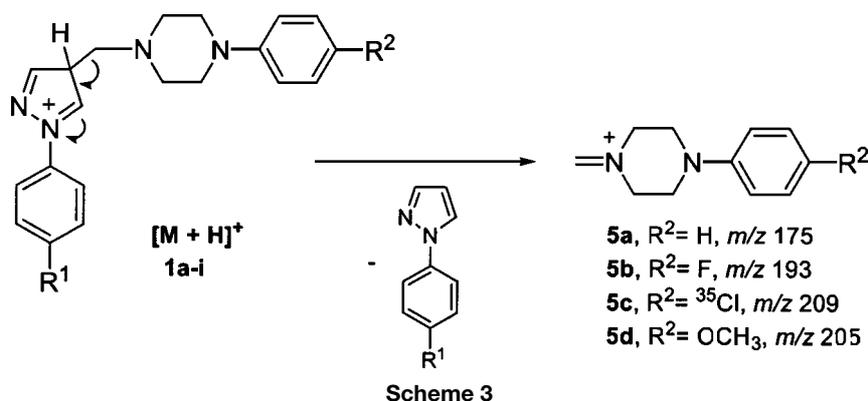
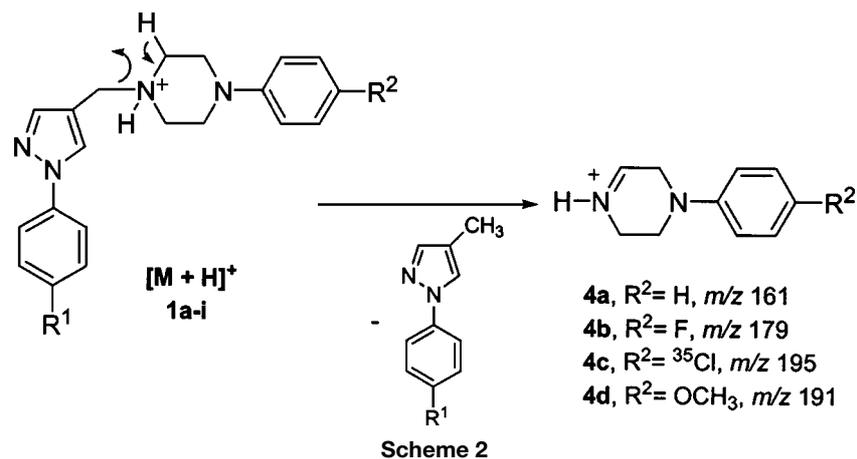


Table 2. Major fragment ions from ESI tandem mass spectra of **2a-i** resulting from low-energy CID of their protonated molecules

compound	[M + H] ⁺ <i>m/z</i>	Fragment Ions (<i>m/z</i> , % relative abundance)			
		5	6	7	8
2a	320	5a (175, 100)	6a (292, 22)	7a (130, 62)	8a (132, 57)
2b	356	5b (193, 100)	6b (328, 33)	7b (148, 72)	8b (150, 53)
2c	388 (³⁵ Cl)	5c (209, 100)	6c (360, 33)	7c (164, 79)	8c (166, 61)
2d	384 (³⁵ Cl)	5d (205, 100)	6d (356, 11)	7c (164, 22)	8d (162, 28)
2e	354 (³⁵ Cl)	5c (209, 79)	6e (326, 48)	7a (130, 100)	8c (166, 61)
2f	338	5b (193, 100)	6f (310, 31)	7a (130, 88)	8b (150, 62)
2g	338	5a (175, 100)	6g (310, 18)	7b (148, 54)	8a (132, 39)
2h	354 (³⁵ Cl)	5a (175, 100)	6h (326, 15)	7c (164, 35)	8a (132, 32)
2i	365	5a (175 ^a , 100)	6i (337, 5)	7d (175 ^a , nd ^b)	8a (132, 20)

^a The high resolution and accuracy of the MS/MS measurements performed on the QTOF hybrid mass spectrometer allows easy separation and quantitation of both the isobaric fragment ions **5a** and **7d**.

^b nd = not detected.

the release of the stable N₂ neutral from the triazole ring, forming two major fragment ions: **6**, and then, analogous to **3**, (Scheme 1) methylazirine cations **7** (Scheme 4).

The dissociation route of Scheme 3, which is minor and not even general for protonated **1a-i**, has become one of the major processes for protonated **2a-i**. This great difference in the dissociation chemistry between the pyrazole and triazole derivatives can be rationalized, at least in part, by considering that protonation at the more basic triazole ring is more favored than at the pyrazole ring, and hence formation of ions **5a-d** is favored (Scheme 5).

Scheme 6 rationalizes another unique and major dissociation route for the protonated molecule of the triazole derivatives **2a-i**. For such species, rupture of the piperazine ring occurs favorably, likely initiated by N-protonation, and leads to the relatively stable^{12,13} *N*-aryl 2-azabutadienyl cations **8**.

Note therefore, that, whereas the pyrazole series is characterized by the dominance of fragment ion **3**, the triazole series is characterized by a set of four major fragment ions **5**, **6**, **7**, and **8**. Fragments **5**, **7** and **8** are the structure diagnostic ones; for instance, the isomeric pair of protonated molecules

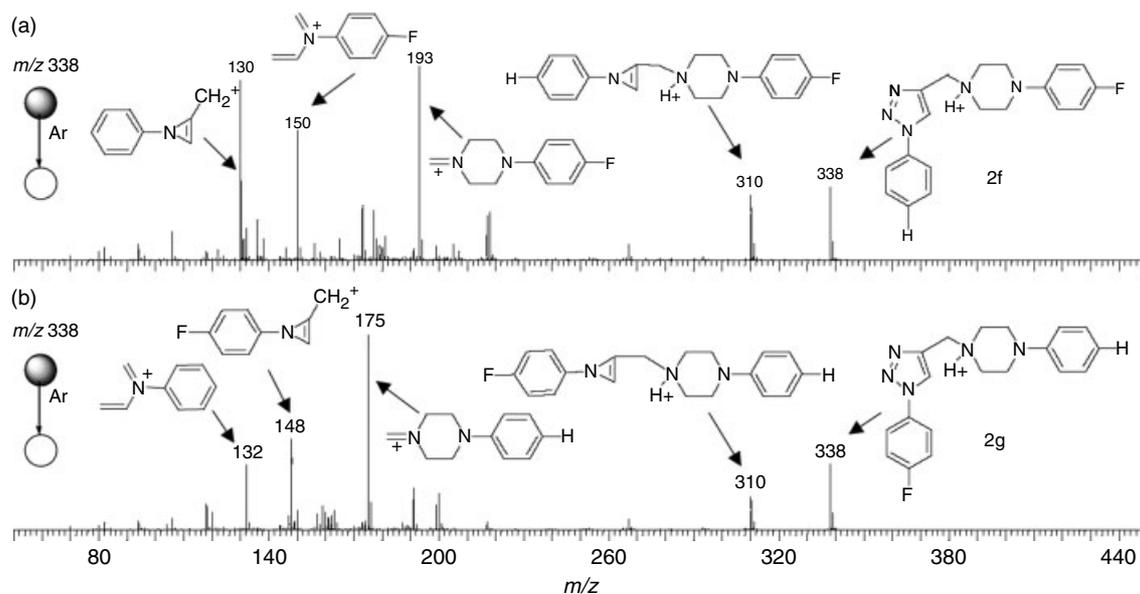
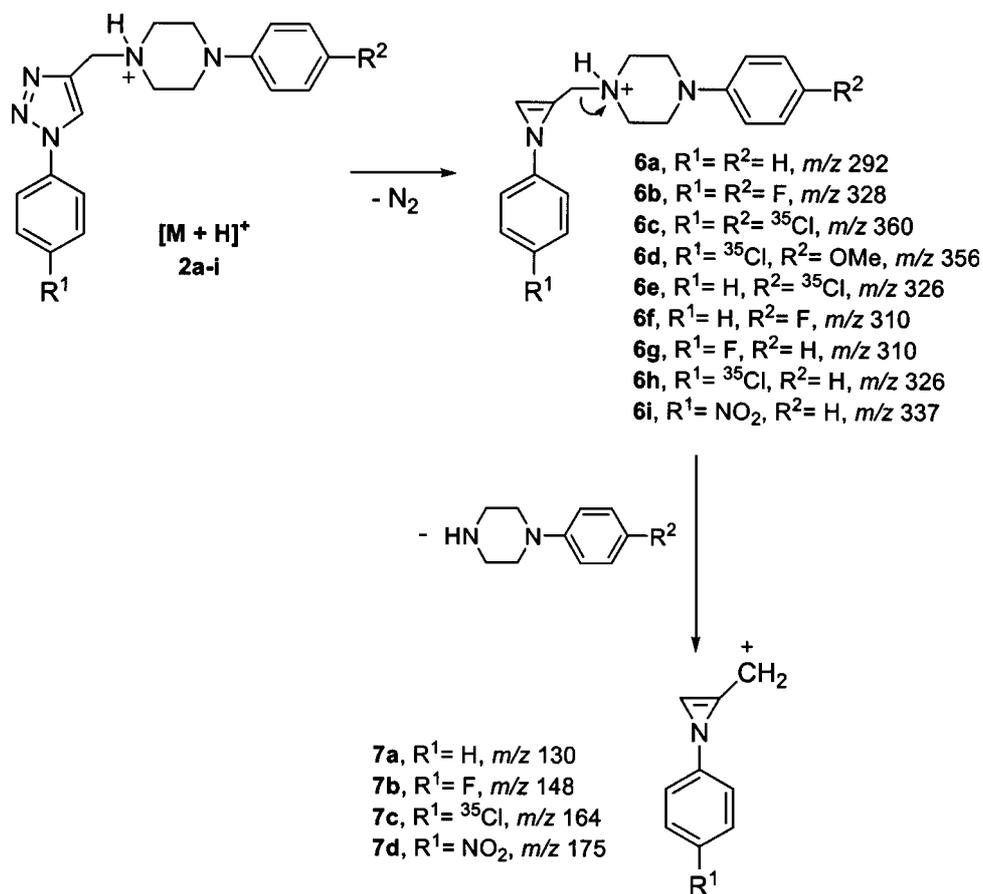


Figure 2. ESI tandem mass spectra of the protonated molecules of the isomeric triazole derivatives 2f and 2g.

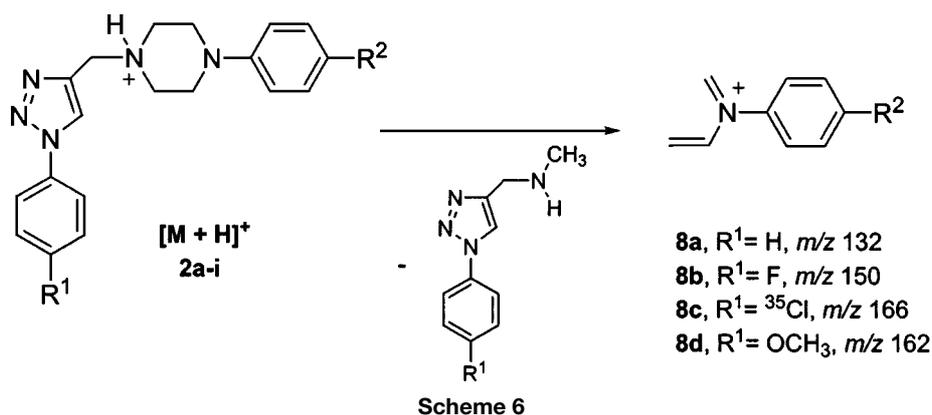
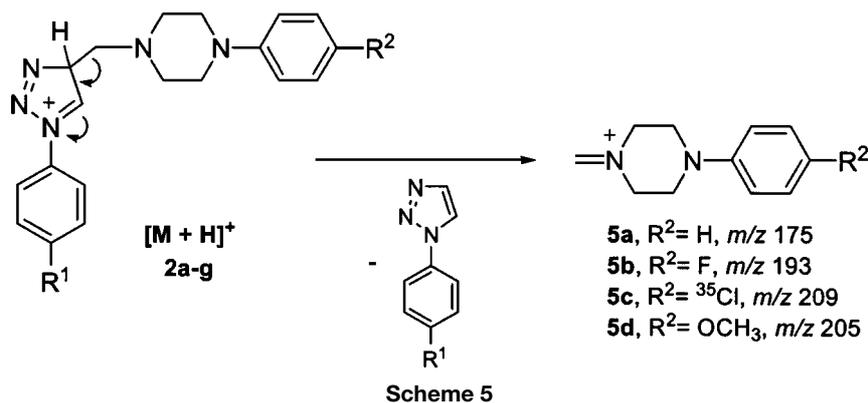


Scheme 4

2f and 2g can be easily distinguished by their respective sets of fragment ions 5, 7 and 8: protonated 2f forms, to a great extent, 5b of *m/z* 193, 7a of *m/z* 130, and 8b of *m/z* 150, whereas protonated 2g forms 5a of *m/z* 193, 7b of *m/z* 148, and 8a of *m/z* 132 (Fig. 2). Likewise, protonated 2e yields 5c of *m/z* 209, 7a of *m/z* 130, and 8c of *m/z* 166, whereas protonated 2h yields 5a of *m/z* 175, 7c of *m/z* 164 and 8a of *m/z* 132.

CONCLUSION

A systematic study of the high-resolution electrospray ionization mass and tandem mass spectra of two analogous series of nine *N*-pyrazolylmethyl- (1) and *N*-triazolylmethyl- (2) *N*-phenylpiperazines has been performed. Main dissociation routes of the protonated molecules of these new



antischizophrenia drug candidates have been determined and rationalized. ESI-MS/MS data has been found to be very characteristic for both series allowing straightforward isomeric differentiation. A single and dominant fragment ion is formed for the protonated pyrazole molecules **3**, whereas the analogous protonated triazole molecules yield four major fragment ions **5**, **6**, **7** and **8**. These contrasting and characteristic dissociation routes can be used therefore, for selective-reaction MS-monitoring of these potential drugs in biological fluids.

Acknowledgments

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