Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 2117–2120

TETRAHEDRON LETTERS

Fast atom bombardment-promoted reductive ring opening of 1,2-benzisothiazoles

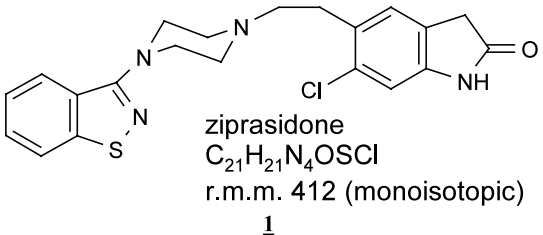
Thomas R. Sharp,[a,*] John F. Lambert[b] and Stanley W. Walinsky[b]

[a]*Analytical R&D Department, Pfizer Global Research & Development, Groton, CT 06340 USA*
[b]*Chemical R&D Department, Pfizer Global Research & Development, Groton, CT 06340 USA*

Received 20 December 2002; revised 15 January 2003; accepted 16 January 2003

Abstract—Fast atom bombardment mass spectral examination of molecules containing a 1,2-benzisothiazole ring, using a thiol reducing agent matrix, promotes reductive ring opening of the benzisothiazole ring, giving an $[M+H]^+$ two daltons higher than expected. Measurements using a non-reducing matrix produce the expected $[M+H]^+$. This is a general phenomenon, observed with a number of molecules containing the benzisothiazole ring. The ring-opened structure has been confirmed by chemical synthesis and observed in metabolic studies.

Fast atom bombardment (FAB) mass spectral characterization of several drug substance candidates and structurally related compounds, which contain the 1,2-benzisothiazole heterocyclic ring system, produce a mass spectrum containing an apparent $[M+H]^+$ which is 2 daltons higher than predicted for the accepted structures of these molecules. Our investigations into the origin of this molecular ion anomaly led to our discovering the following structure-specific FAB-promoted chemical reaction in the mass spectrometer. The compound providing the focal point for this investigation is ziprasidone (**1**),[1,2] a recently approved antipsychotic agent.

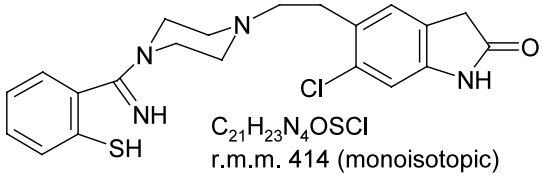


ziprasidone
$C_{21}H_{21}N_4OSCl$
r.m.m. 412 (monoisotopic)

1

Observations were made from either a dithiothreitol–dithioerythritol (reducing) matrix[3] or an *m*-nitrobenzyl alcohol (non-reducing) matrix[4,5] on a VG 70VS magnetic sector mass spectrometer. Samples of the compounds were pre-dissolved in an appropriate volatile organic solvent. Aliquots were mixed with the matrix on the direct insertion FAB probe target. Bombardment with 6 to 8 kV xenon atoms, produced by a saddle field atom gun, generated the spectra and promoted the chemical reactions reported here.

A FAB mass spectrum of ziprasidone from a reducing matrix is shown in Figure 1, in which the abundance of the peak at m/z 415 is higher than would be expected for the simple ^{13}C plus single chlorine isotope pattern expected for this molecule. The phenomenon is more dramatically illustrated by the expanded views of $[M+H]^+$ regions (Fig. 2) of three spectra, taken from early, midway and late in a repetitive scan run. During the run, the relative abundance of m/z 415 grows at the expense of m/z 413. The shifting isotope pattern accompanies the apparent addition of two hydrogen atoms to $[M+H]^+$.



$C_{21}H_{23}N_4OSCl$
r.m.m. 414 (monoisotopic)

2

The m/z 415 species corresponds to **2**, formed by reductive ring opening of the 1,2-benzisothiazole ring of ziprasidone. We cannot currently differentiate between **2** and an isobaric ring-closed isomer in which the C–N double bond has been reduced. However, accompanying the FAB-promoted reduction is the appearance of

* Corresponding author. Tel.: 860-441-5932; fax: 860-441-0437; e-mail: thomas_r_sharp@groton.pfizer.com

0040-4039/03/$ - see front matter
doi:10.1016/S0040-4039(03)00166-7

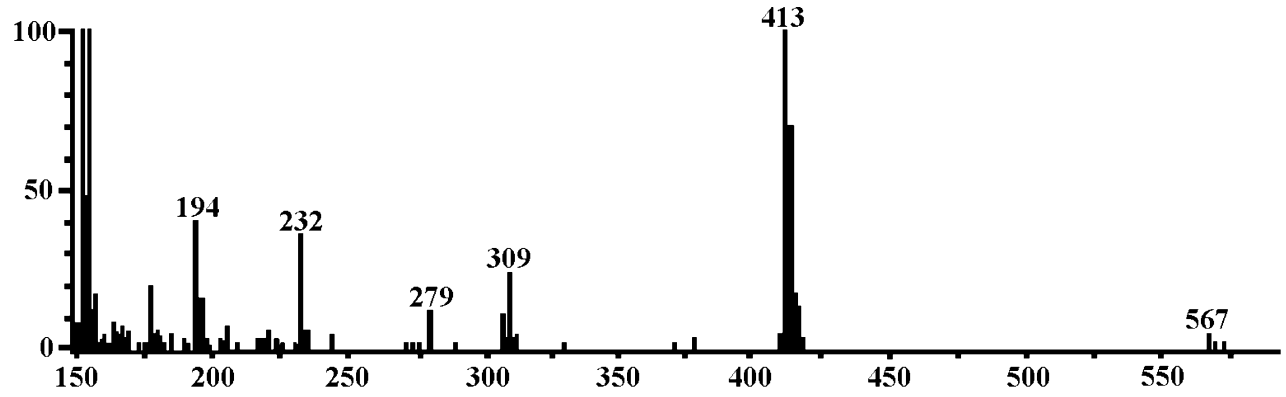
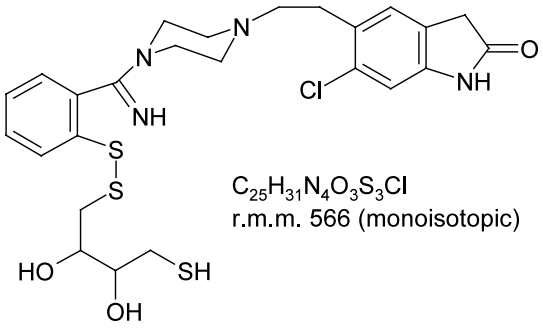


Figure 1. Positive ion FAB mass spectrum of ziprasidone, recorded from a dithiothreitol–dithioerythritol reducing matrix.

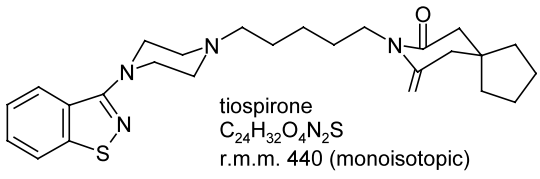
the peak in Figure 1 at m/z 567. This species corresponds to the $[M+H]^+$ of a mixed disulfide formed from **2** and a molecule of the dithiothreitol–dithioerythritol matrix (**3**). This mass is the same as for a proton-bound cluster of ziprasidone and a matrix molecule. However, we assign it as **3** because its abundance grows as the experiment proceeds.



3

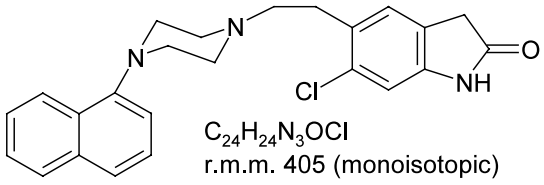
The kinetics of the process can be followed. Disappearance of the m/z 413 $[M+H]^+$, representative of ziprasidone, and appearance of the m/z 415 species, representative of the reductively ring-opened structure, follow pseudo-first order kinetics. This behavior is in contrast to the beam-dependent 'instantaneous' and reversible reduction of oxazine dyes.[6]

Investigation of other structures containing the 1,2-benzisothiazole moiety, including tiospirone (**4**), another antipsychotic drug, reveal that this phenomenon is a general property of the 1,2-benzisothiazole nucleus. Tiospirone[7] and other structural analogs of ziprasidone, which contain the benzisothiazole ring, undergo the reductive ring opening and form the apparent mixed disulfide adduct. A structure similar to that of ziprasidone, in which the benzisothiazole has been replaced by a naphthalene moiety (**5**),[8] does not exhibit this behavior, showing that it is dependent upon the presence of the benzisothiazole.



4

It is unclear whether the benzisothiazole gives rise directly to both the ring-opened compound and the disulfide, or whether the ring-opened compound is a product of the disulfide adduct. Experiments were not carried out to expressly probe this point.



5

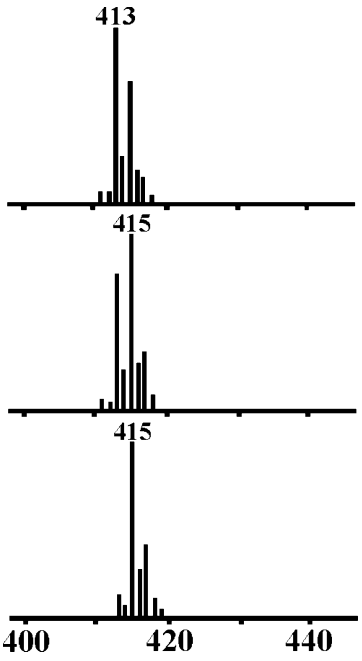


Figure 2. Expanded $[M+H]^+$ region from three spectra taken early (top), midway (middle) and late (bottom) during a FAB mass spectrometric run in which spectra of ziprasidone, placed in a dithiothreitol–dithioerythritol reducing matrix, were repetitively scanned.

Compound **2** has been identified as a metabolite of ziprasidone,[9,10] and an intermediate in formation of other metabolites.[11] Metabolic ring openings of risperidone,[12] iloperidone[13] and zonisamide[14] (all benzisoxazoles) have been reported. However, the nature of the metabolites suggests a different mechanism, namely opening of the benzisoxazole ring to an *ortho*-phenolic imine, followed by hydrolysis to an *ortho*-phenolic ketone.

We refer to this reaction as a FAB-promoted reaction rather than a FAB-induced reaction because we could detect conversion of ziprasidone by dispersing a sample of the material in the dithiothreitol–dithioerythritol matrix prior to introducing it into the mass spectrometer and bombarding it with fast atoms.

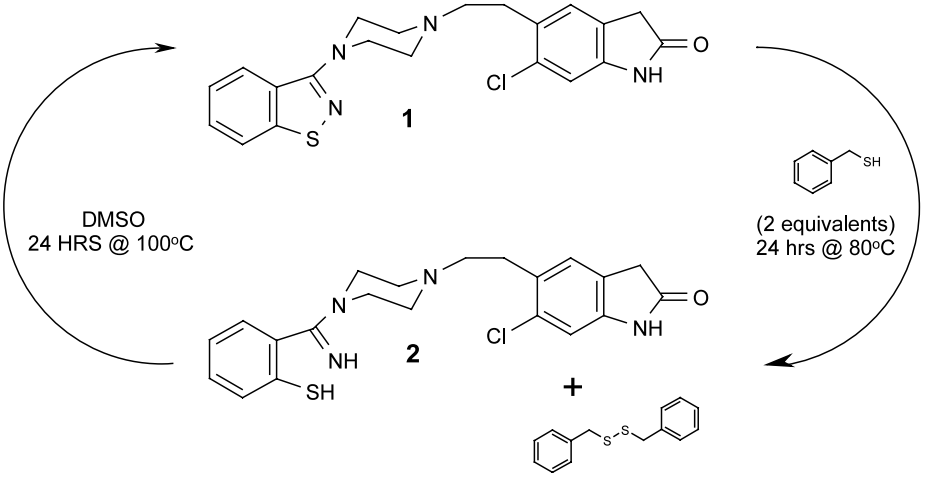
Observation of this ring opening in the mass spectrometer prompted further investigation of the chemistry in solution. Reviews of the chemistry of benzisoxazoles[15] and benzisothiazoles[16] have appeared. The benzisoxazole ring is reported to be opened by a number of treatments. Benzisothiazoles, by contrast are reported to be quite stable. However, hydrazine can open a benzisothiazole. Benzisothiazole quaternary salts can be formed, and produce ring-opened products under acid or alkali reflux treatment.

Böshagen and Geiger, however, have shown[17] that diphenyl disulfides can be induced to disproportionate into the corresponding benzisothiazole and *ortho*-substituted thiophenol, particularly when an amidine is attached *ortho* to the sulfur. The reaction is reversible, and can be driven in one direction or the other by addition of HCl. Compound **2** is an amidine-substituted *ortho*-thiophenol. Ring opening of 3-chloro-1,2-benzisothiazole with thiol reagents has also been reported.[18]

The structure of **2** has been confirmed synthetically.[19] Treatment of ziprasidone dissolved in isopropanol with two equivalents of benzyl mercaptan, and heated to approximately 80°C for 24 h, produced **2** cleanly in 90% isolated yield. See Scheme 1. Thin-layer chromatographic monitoring of the reaction suggests intermediate formation of the mixed disulfide with benzyl mercaptan. This evidence supports the assignment of the m/z 567 species in the FAB mass spectrum as the mixed disulfide **3** with the matrix, and that it is an obligate intermediate. Benzyl disulfide was isolated and identified as a reaction by-product. If less than 2 equiv. of thiol were used, the ring opening reaction was incomplete. **2**, dissolved in methanol, slowly re-oxidizes and closes the ring back to ziprasidone. Ring closure can be driven synthetically by heating in dimethyl sulfoxide for 24 h at 100°C, regenerating ziprasidone in high yield and dimethyl sulfide. Similar conversions could be done with 1,2-benzisothiazole-piperazine.

A sample of synthetically produced **2** was subjected to FAB mass spectral examination, along with a sample of ziprasidone, in both the dithiothreitol–dithioerythritol (reducing) and *m*-nitrobenzyl alcohol (non-reducing) matrices. The expected behavior was observed. While ziprasidone converted upon exposure to the thiol matrix and fast atom bombardment, **2** remained unchanged. Neither compound changed when examined from the non-reducing *m*-nitrobenzyl alcohol matrix.

FAB-induced chemical reactions of analytes in the matrix and on the insertion probe target have been documented a number of times in the literature, and have been reviewed.[20,21] Reduction of disulfides in peptides has been shown to be analytically useful.[22] Apparent oxidations of certain platinum organometallic compounds have been offered[23] as a rationale for the appearance of peaks in the spectrum 16 daltons above molecular ions and certain fragments. Dehydrohalogenation of halogenated nucleosides and nucleotides has been reported.[24] A number of aromatic oximes can be reduced on the probe to their corresponding imines.[25] An investigation of the mechanism and structures produced by the condensation of hydroxyl-containing matrices with carbonyl-containing analytes has been presented.[26]



Scheme 1. Cyclic opening and closing of the benzisothiazole ring.

Acknowledgements

The authors thank J. A. Lowe III and H. R. Howard for discussions during preparation of this manuscript. Part of this paper has been previously presented.[27]

References

1. Seeger, T. F.; Seymour, P. A.; Schmidt, A. W.; Zorn, S. H.; Shulz, D. W.; Lebel, L. A.; McLean, S.; Guanowsky, V.; Howard, H. R.; Lowe, J. A., III; Heym, J. *J. Pharmacol. Exptl. Therapeutics* **1995**, *275*, 101–113.
2. Howard, H. R.; Lowe, J. A., III; Seeger, T. F.; Seymour, P. A.; Zorn, S. H.; Maloney, P. R.; Ewing, F. E.; Newman, M. E.; Schmidt, A. W.; Furman, J. S.; Robinson, G. L.; Jackson, E.; Johnson, C.; Morrone, J. *J. Med. Chem.* **1996**, *39*, 143–148.
3. 'Magic bullet': 1:3 dithioerythritol:dithiothreitol. Witten, J. L.; Schaffer, M. H.; O'Shea, M.; Cook, J. C.; Hemling, M. E.; Rinehart, K. L. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 350.
4. Meili, J.; Seibl, J. *Org. Mass Spectrom.* **1984**, *19*, 581.
5. Sweetman, B. J.; Blair, I. A. *Biomed. Environ. Mass Spectrom.* **1988**, *17*, 337.
6. Brown, S. M.; Busch, K. L. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 256.
7. Jajoo, H. K.; Blair, I. A.; Klunk, L. J.; Mayol, R. F. *Biomed. Environ. Mass Spectrom.* **1990**, *19*, 281.
8. Fouda, H. G.; Navetta, K.; Luther, E.; Schneider, R. P. Presented at the *41st ASMS Conference on Mass Spectrometry and Allied Topics*, San Francisco, CA, 31 May–4 June 1993.
9. Prakash, C.; Kamel, A.; Gummerus, J.; Wilner, K. *Drug Metab. Dispos.* **1997**, *25*, 863.
10. Miao, Z.; Kamel, A.; Prakash, C.; Obach, S.; Fouda H.; Tremaine, L. Presented at the *49th ASMS Conference on Mass Spectrometry and Allied Topics*, Chicago, IL, 27–31 May 2001.
11. Prakash, C.; Kamel, A.; Cui, D. *Drug Metrab. Dispos.* **1997**, *25*, 897.
12. Mannens, G.; Huang, M. L.; Meuldermans, W.; Hendricks, J.; Woestenborghs, R.; Heykants, J. *Drug Metab. Dispos.* **1993**, *21*, 1134.
13. Mutlib, A. E.; Strupczewski, J. T.; Chesson, S. M. *Drug Metab. Dispos.* **1995**, *23*, 951.
14. Stiff, D. D.; Zemaitis, M. A. *Drug Metab. Dispos.* **1990**, *18*, 888.
15. Wünsch, K.-H.; Boulton, A. J. *Adv. Heterocyclic Chem.* **1967**, *8*, 277.
16. Davis, M. *Adv. Heterocyclic Chem.* **1972**, *14*, 43.
17. Böshagen, H.; Geiger, W. *Phosphorus and Sulfur* **1983**, *17*, 325.
18. Crow, W. D.; Leonard, N. J. *J. Org. Chem.* **1965**, *30*, 2660.
19. Walinsky, S. W.; Lambert, J. F. United States Patent 5,935,960, 10 August 1999.
20. Fenselau, C.; Cotter, R. J. *Chem. Rev.* **1987**, *87*, 501.
21. Detter, L. D.; Hand, O. W.; Cooks, R. G.; Walton, R. A. *Mass Spectrom. Rev.* **1988**, *7*, 465.
22. Yazdanparast, R.; Andrews, P. C.; Smith, D. L.; Dixon, J. E. Presented at the *33rd ASMS Conference on Mass Spectrometry and Allied Topics*, San Diego, CA, 26–31 May, 1985.
23. Kowalski, M. H.; Sharp, T. R.; Stang, P. J. *Org. Mass Spectrom.* **1987**, *22*, 642.
24. Sethi, S. K.; Nelson, C. C.; McCloskey, J. A. *Anal. Chem.* **1984**, *56*, 1975.
25. Santana-Marques, M. G. O.; Ferrer-Correia, A. J. V.; Gross, M. L. *Anal. Chem.* **1989**, *61*, 1442.
26. Agnello, A.; DePauw, E.; Natalis, P. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 312.
27. Sharp, T. R. Presented at the *42nd ASMS Conference on Mass Spectrometry and Allied Topics*, Chicago, IL, 29 May–3 June 1994.