Bioorg. Med. Chem. Lett. 41 (2021) 128011

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Selective formation of a phenathridine derivative by photodegradation of azilsartan

Takahiro Yoshikawa [a,b,*], Naoto Hayashi [a], Naoya Hatta [b], Masayuki Yokota [b]

[a] Graduate School of Science and Engineering, University of Toyama, Gofuku, Toyama 9308555, Japan
[b] Research & Development, KONGO CHEMICAL CO., LTD, Himata, Toyama 9300912, Japan

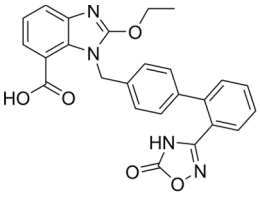
ARTICLE INFO

Keywords:
Azilsartan
Photolysis
Phenanthridine
Imidoylnitrene
Intramolecular cyclization

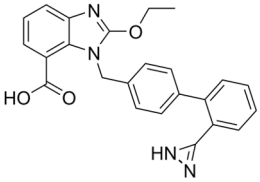
ABSTRACT

Photodegradation of azilsartan produces a phenanthridine derivative, with its molecular structure determined by ^{1}H and ^{13}C NMR spectroscopy. This structure is confirmed by single-crystal X-ray diffraction and alternative synthesis. The phenanthridine ring formation is explained through the ring closure of an imidoylnitrene intermediate produced by decarboxylation of the 5-oxo-1,2,4-oxadiazole ring (oxadiazolone).

The non-peptide antagonists of angiotensin II type 1 (AT1) receptors[1–3] are useful for treating hypertension and related medical conditions. Among these AT1 receptor blockers (ARBs) also termed sartans, azilsartan (AZL)[4,5] has attracted particular attention because of its relatively higher hypotensive potential.[6–8] Its chemical structure includes a benzimidazole core with a carboxy group at position 7 and a biphenylmethyl moiety involving an acidic group at position 2′. Although most sartans contain a tetrazole ring, AZL involves a 5-oxo-1,2,4-oxadiazole (or oxadiazolone) moiety as an acidic functional group on the biphenyl moiety.



azilsartan (AZL)



expected AZL photodegradation product (1*H*-diadirine derivative)

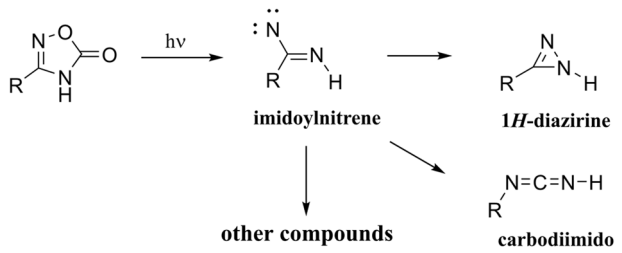
According to guidelines[9,10] from the International Conference on Harmonization (ICH), studying forced decomposition including light irradiation is important for establishing the stability characteristics of pharmaceutical products. In addition, to evaluate the toxicity of impurities retained in pharmaceuticals or impurities that may increase during storage, chemical structure information is required. In fact, even if this involves an in silico method,[11,12] experimentally identifying the structure of any impurity is vital for drug safety assurance.

In previous studies, AZL medoxomil (prodrug) was reported to photohydrolyze to the free acid,[13] while AZL itself yield 1*H*-diazirine derivative when irradiated with ultraviolent (UV) rays.[14] In addition to 1*H*-diaziridine, carbodiimide is also produced, these products being assumed to form through decarboxylation of the oxadiazolone moiety and generation of imideylnitrene (Scheme 1).[15–17] However, little experimental evidence is provided for these reactions by previous studies, especially, considering that except in special cases, 1*H*-diazilines are unstable and challenging to isolate because of their cyclic 4π electron systems.[18] Consequently, in AZL photolysis, the isolated product from imidoylnitrene is considered different from 1*H*-diazirine. The purpose of this study is to clarify the structure of this product.

When a solid sample of AZL was irradiated by UV light at the same condition as described in ref. 14, no observable change was found (Fig. S1). In contrast, when the AZL photolysis was conducted by irradiating (265 nm) a dilute AZL–methanol solution for 64 h, the solution changed gradually from colorless to brown and a brown powder precipitated. Analysis of the suspension by HPLC/ESI-MS revealed a single decomposition product (**4**), with a molecular ion peak at *m/z* of 413.17 ($M+H^{+}$) (Fig. 1). The mass difference compared with AZL (exact mass *m/z* 456.14) suggests that **4** is formed by AZL decarboxylation, consistent with previous studies on AZL photolysis.[14] Notably, ^{1}H NMR measurements reveal that signals for several aromatic hydrogens in **4** are considerably low-field shifted (δ = 8.5–7.0) compared to those in

* Corresponding authors at: Research & Development, KONGO CHEMICAL CO., LTD, Toyama, Japan.
E-mail address: yoshikawa@kongo-chemical.co.jp (T. Yoshikawa).

https://doi.org/10.1016/j.bmcl.2021.128011
Received 17 January 2021; Received in revised form 22 March 2021; Accepted 28 March 2021
Available online 1 April 2021



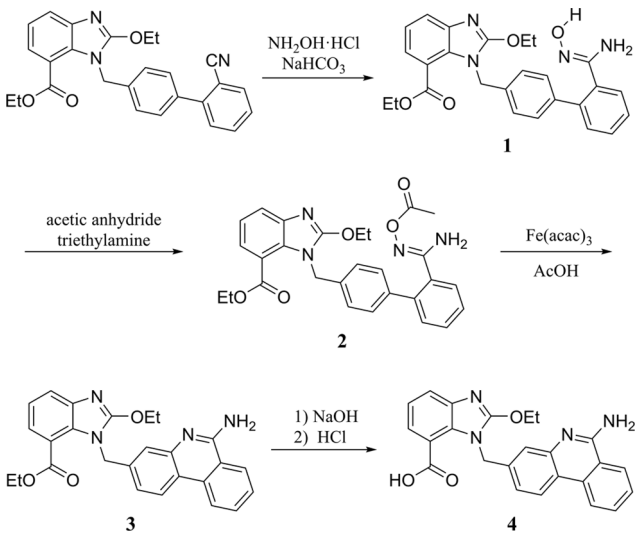
Scheme 1. Illustration of the photolysis of oxadiazolone.

AZL (δ = 7.5–7.0). These results suggest that **4** is a phenanthridine instead of 1*H*-diazirine. To confirm this hypothesis, we set to synthesize **4** using another method. Establishing the synthetic pathway of **4** is also important for understanding the decomposition of AZL and related substances as well as the biological activities of their decomposition products.

We synthesized **4** as shown in Scheme 2. First, *N*-hydroxyarenecarboximidamide **1,** which was prepared from the corresponding nitrile, was acetylated with acetic anhydride to **2**. Next, **2** was converted to phenanthridine **3** using iron (III) as the catalyst. The phenanthridine ring is generally synthesized from cyclization by creating a C–C or C–N bond with a radical[19–21] or transition metal.[22,23] However, the method involving the O-acetyl oxime group[24–26] is preferred because it produces an intramolecular C–N bond on the *ortho*-functionalized biphenyl. In preparation of **3**, although the LC–MS analysis of the crude product showed a molecular ion peak corresponding to a decarboxylation product, isolating **3** was unsuccessful. Therefore, the ester moiety was hydrolyzed with sodium hydroxide to obtain the target carboxylic acid **4**. Both ^{1}H and ^{13}C NMR results supported that the obtained **4** was a putative structure of the photolysis product.

Fortunately, we obtained a high-quality single crystal of the salt of the photodegraded product with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). X-ray structural analysis of the crystal revealed a compound containing the phenanthridine ring (Fig. 2), supporting the putative structure obtained from synthesis. Note that formation of phenanthridine ring was not reported on phododegradation of AZL medoxomil, where only photohydrolysis of the ester group occurred.[13] The discrepancy of the photodegradation products could be due to different reaction conditions including solvent system.

The compound **4** (photodegradation product of AZL) exhibits a phenanthridine structure. The phenanthridine ring is attributed to the formation of a central ring through the intramolecular C–N bond of the *ortho*-functionalized biphenyl precursor. Probably, the reactive intermediate of this reaction involves imidoylnitrene. Owing to intensive



Scheme 2. Steps and compounds involved in the synthesis of the photodegradation product of azilsartan.

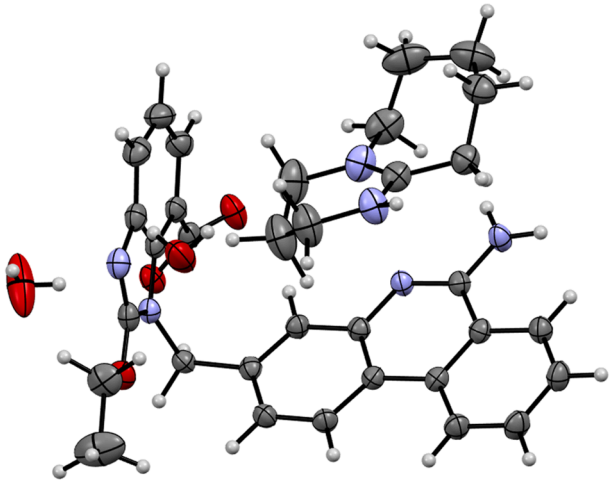


Fig. 2. X-ray structure of the DBU salt of **4**. Oxygen atoms are shown in red, while nitrogens are purple.

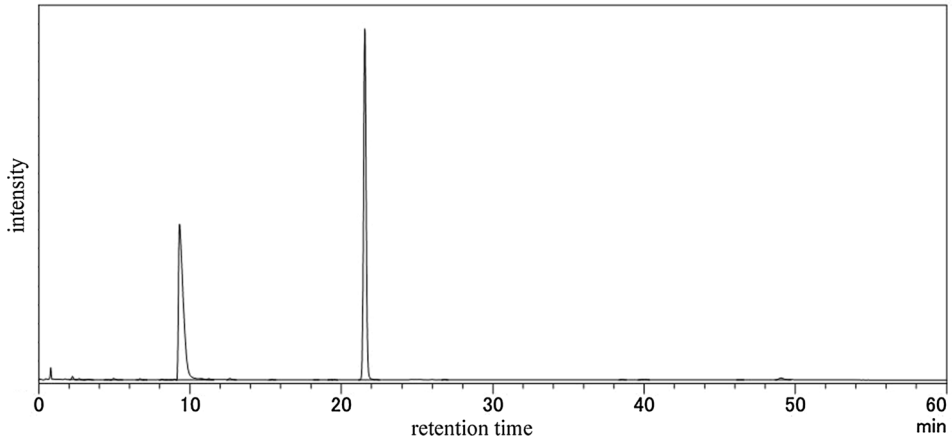
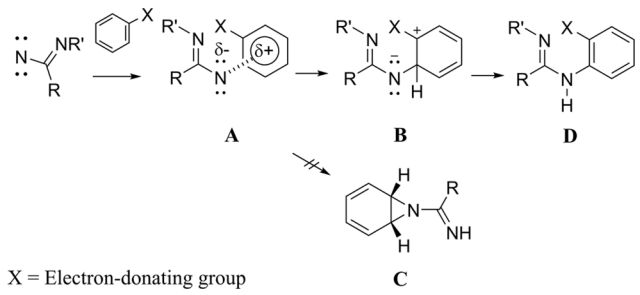
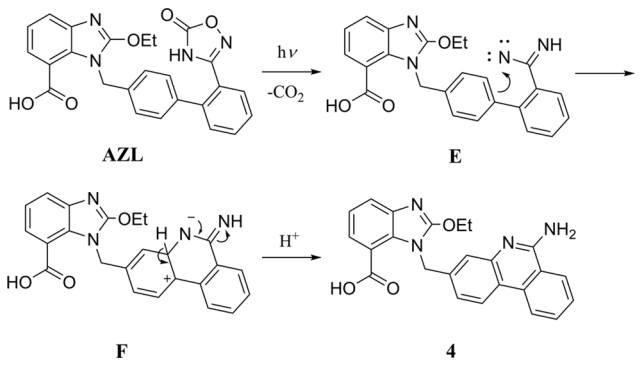


Fig. 1. HPLC chromatogram for the reaction solution from azilsartan photolysis showing the azilsartan photo-product (retention time = 9 min) and azilsartan (retention time = 21 min).



Scheme 3. Reactions of imidoylnitrene with mono-substituted benzene bearing electron-donating group.



Scheme 4. Proposed mechanism for azilsartan photolysis.

studies on five-membered *N*-heterocyclic compounds containing N–O bonds such as oxadiazolone as nitrene precursors[27–29] and the 3-phenyl-2*H*-1,2,4-oxadiazol-5-one showing a structure similar to AZL, imidoylnitrene-derived fragments have been obtained through UV light irradiation.[29]

Generally, imidoylnitrenes react with mono-substituted benzene (PhX) as shown in Scheme 3. First, the imidoylnitrene (in a singlet state) and PhX form a complex (**A**), such that **A** can afford either or both the benzenium ion (**B**) through the C–H insertion reaction and/or aziridine (**C**) from a cheletropic reaction, depending on the character of the X group. Especially, when X is an electron-donating group, the benzenium ion is stabilized by a resonance effect, so that **B** formation proceeds preferentially.[30]

The AZL photolysis is explained similarly (Scheme 4). First, imidoylnitrene **E** is produced by a photoreaction, with position 2 of the biphenyl as the most suitable reaction point for the nitrate nitrogen, considering the bond distance. The reaction at this position likely occurs before the phenanthridine ring forms because the resonance effect of the phenyl group at position 1 stabilizes the benzenium ion **F** produced. Considering that because of the C–N bond the two 6-membered rings are coplanar, the resonance stabilizing effect of carbocations in **F** is expected to be high. Finally, unlike in general reactions between imidylnitrene and benzene, further proton transfer occurred, producing **4** through aromatization of the central ring of the phenanthridine moiety.

In summary, we have shown that photodegradation of AZL was not observed in the solid state, while it occurred significantly in the solution. We demonstrated using X-ray structural analysis that a phenanthridine compound is produced as the photodegradation product, instead of a conventionally assumed 1*H*-diazirine derivative. In the photoreaction, we proposed a thermodynamically stable aromatization reaction mechanism involving the closure of a central ring through an intramolecular C–N bond of the reactive intermediate imideylnitrene. The findings of this study are important for properly managing azilsartan. Studies on the toxicity of the photodegradant are ongoing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by KONGO CHEMICAL CO., LTD.

We appreciate Professor Y. Igarashi and Assistant Professor E. Harunari (Toyama Prefectural University) for the HRMS analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128011.

References

1 Imaizumi S, Miura S, Yahiro E, Uehara Y, Komuro I, Saku K. Class- and molecule-specific differential effects of angiotensin II type 1 receptor blockers. *Curr Pharm Des*. 2013;19:3002–3008.
2 Timmermans PB. Angiotensin II receptor antagonists: an emerging new class of cardiovascular therapeutics. *Hypertens Res*. 1999;22:147–153.
3 van Kesteren CA, Saris JJ, Dekkers DH, et al. Cultured neonatal rat cardiac myocytes and fibroblasts do not synthesize renin or angiotensinogen: evidence for stretch-induced cardiomyocyte hypertrophy independent of angiotensin II. *Cardiovasc Res*. 1999;43:148–156.
4 Kohara Y, Imamiya E, Kubo K, Wada T, Inada Y, Naka T. A new class of angiotensin II receptor antagonists with a novel acidic bioisostere. *Bioorg Med Chem Lett*. 1995;5:1903–1908.
5 Kohara Y, Kubo K, Imamiya E, Wada T, Inada Y, Naka T. Synthesis and angiotensin II receptor antagonistic activities of benzimidazole derivatives bearing acidic heterocycles as novel tetrazole bioisosteres. *J Med Chem*. 1996;39:5228–5235.
6 Si M, Okabe A, Matsuo Y, Karnik SS, Saku K. Unique binding behavior of the recently approved angiotensin II receptor blocker azilsartan compared with that of candesartan. *Hypertens Res*. 2013;36:134–139.
7 Rakugi H, Kario K, Enya K, Igeta M, Ikeda Y. Effect of azilsartan versus candesartan on nocturnal blood pressure variation in Japanese patients with essential hypertension. *Blood Press*. 2013;22(S1):22–28.
8 Bakris GL, Sica D, Weber M, et al. The comparative effects of azilsartan medoxomil and olmesartan on ambulatory and clinic blood pressure. *J Clin Hypertens (Greenwich)*. 2011;13:81–88.
9 I.C.H. Q1A(R2). *Stability testing of new drug substances and products. International Conference on Harmonization*. Geneva: IFPMA; 2003.
10 I.C.H. Q1B. *Stability testing: photostability testing of new drug substances and products. International Conference on Harmonization*. Geneva: IFPMA; 1998.
11 Stanton K, Kruszewski FH. Quantifying the benefits of using read-across and in silico techniques to fulfill hazard data requirements for chemical categories. *Regul Toxicol Pharm*. 2016;81:250–259.
12 Amberg A, Beilke L, Bercu J, et al. Principles and procedures for implementation of ICH M7 recommended (Q)SAR analyses. *Regul Toxicol Pharm*. 2016;77:13–24.
13 Swain D, Patel PN, Palaniappan I, Sahu G, Samanthula G. Liquid chromatography/tandem mass spectrometry study of forced degradation of azilsartan medoxomil potassium. *Rapid Commun Mass Spectrosc*. 2015;29:1437–1447.
14 Kaushik K, Kaur J, Paul Kaur V, Saini B, Bansal Y, Bansal G. Forced degradation, LC-UV, MSn and LC-MS-TOF studies on azilsartan: identification of a known and three new degradation impurities. *J Pharm Biomed Anal*. 2016;120:202–211.
15 Boyer JH, Ellis PS. Fragmentation-rearrangement of Δ3-oxadiazolin-5- and 3-ones. *J Chem Soc Perkin Trans*. 1979;1:483–487.
16 Shimbayashi T, Sasakura K, Eguchi A, Okamoto K, Ohe K. Recent progress on cyclic nitrenoid precursors in transition-metal-catalyzed nitrene-transfer reactions. *Chem Eur J*. 2019;25:3156–3180.
17 Boyer JH, Frints PJA. The azomethine nitrene. II. Photolysis of 3-phenyl-Δ^2-oxadiazol-5-one. *J Heterocycl Chem*. 1970;7(1):71–74.
18 Dubai-Assibat N, Baceiredo A, Bertrand G. Synthesis and reactivity of the first spectroscopically observed 1*H*-diazirine. *J Am Chem Soc*. 1996;118:5216–5220.
19 Katritzky AR, Du W, Matsukawa Y, Ghiviriga I, Denisenko SN. Polycyclic fused phenanthridines: an alternative approach from benzotriazoles. *J Heterocycl Chem*. 1999;36:927–932.
20 Budén ME, Dorn VB, Gamba M, Pierini AB, Rossi RA. Electron-transfer-mediated synthesis of phenanthridines by intramolecular arylation of anions from *N*-(*ortho*-halobenzyl)arylamines: regiochemical and mechanistic analysis. *J Org Chem*. 2010;75:2206–2218.
21 Wang Y-F, Lonca GH, Le Runigo M, Chiba S. Synthesis of polyfluoroalkyl aza-polycyclic aromatic hydrocarbons enabled by addition of perfluoroalkyl radicals onto vinyl azides. *Org Lett*. 2014;16:4272–4275.
22 Pearson R, Zhang S, He G, Edwards N, Chen G. Synthesis of phenanthridines via palladium-catalyzed picolinamide directed sequential C–H functionalization. *Beilstein J Org Chem*. 2013;9:891–899.

23 Ghosh M, Ahmed A, Dhara S, Ray JK. Synthesis of phenanthridine and its analogues via aerobic ligand-free domino Suzuki coupling-Michael addition reaction catalyzed by in situ generated palladium-nanoparticles in water. *Tetrahedron Lett.* 2013;54:4837–4840.

24 Deb I, Yoshikai N. Phenanthridine synthesis through iron-catalyzed intramolecular *N*-arylation of *O*-acetyl oxime. *Org Lett.* 2013;15:4254–4257.

25 McBurney RT, Walton JC. Interplay of ortho- with spiro cyclisation during iminyl radical closures onto arenes and heteroarenes. *Beilstein J Org Chem.* 2013;9:1083–1092.

26 McBurney RT, Slawin AM, Smart LA, Yu Y, Walton JC. UV promoted phenanthridine syntheses from oxime carbonate derived iminyl radicals. *Chem Commun.* 2011;47:7974–7976.

27 Ullman EF, Singh B. Photochemical transposition of ring atoms in five-membered heterocycles. The photorearrangement of 3,5-diphenylisoxazole. *J Am Chem Soc.* 1966;88:1844–1845.

28 Sauer J, Mayer KK. Thermolyse und photolyse von 3.4-diphenyl-Δ^2-1.2.4-oxdiazolinon-(5) und 2.4-diphenyl-Δ^2-1.3.4-oxdiazolinon-(5). *Tetrahedron Lett.* 1968;9:325–330.

29 Boyer JH, Frints PJA. The azomethine nitrene. I. Pyrolysis and photolysis of Δ^2-1,2,4-oxadiazoline-5-ones. *J Heterocycl Chem.* 1970;7:59–70.

30 Dabbagh HA, Ghaelee S. Functionalization of phenyl rings by imidoylnitrene 2. Cycloaddition or electrophilic aromatic substitution? *J Org Chem.* 1996;61:3439–3445.