Triazolines 34. Structure and Stability Relationships of Novel Δ^2-1,2,3-Triazoline Anticonvulsants

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INTRODUCTION

1-(4-Chlorophenyl)-5-(4-pyridyl)-Δ^2-1,2,3-triazoline (ADD17014; **4**; Fig. 1; Table 1) is a representative member of a novel class of triazoline anticonvulsants in the preclinical developmental stage (1–4). A previous study of the chemical stability and degradation kinetics of 4 in aqueous solutions indicates that it is highly unstable and undergoes rapid degradation with increasing temperature and decreasing pH, yielding aziridine as one of the major products, with loss of nitrogen (5). The observed rapid degradation of 4, particularly at pHs within the acidic and neutral range (~pH 2–7), has posed some intractable problems in the formulation of a stable dosage form for 4 to be employed in preclinical studies, since, regardless of the oral or intravenous route of administration, the compound must be capable of withstanding degradation not only at the gastric and intestinal pHs in the gastrointestinal tract, but also at the physiological pH (7.4) in the blood. The problem of degradation in blood now appears to be less of a concern due to the protection offered by plasma proteins (6); the *in vitro* degradation half-life of 4 in the presence of bovine serum albumin (BSA; 20 and 40 mg/ml) at pH 7 was nearly 5–6 fold longer than in the absence of BSA under similar conditions. However, the formulation hurdle still remains, particularly in the design of an oral preparation which can resist degradation in the gastrointestinal tract. In view of the potential therapeutic utility of this class of compounds (4), we have since started to investigate in more detail, in addition to a stable formulation for 4, alternative analogues of triazolines which are chemically much more stable than 4.

While the nonaromatic Δ^2-1,2,3-triazoline ring system may behave like aliphatic azo compounds, the additional basic ring nitrogen N-1 could give rise to reaction paths not encountered in simpler systems. Thus, the 1,5-disubstituted triazolines are prone to acid catalyzed decomposition as shown in Fig. 2. The first key step in triazoline degradation is protonation of the ring N-1 (7) and is subject to the availability of electrons on this nitrogen. Consequently, the mesomeric resonance of the lone pair of electrons on N-1 with the phenyl substituent assumes significance; the N-phenyl group having a direct effect on the redistribution of these electrons would be expected to exert a marked influence on the chemical stability and degradation kinetics of the triazolines. To investigate this further, a number of triazolines with different para substituents on the phenyl ring have been synthesized and their degradation kinetics at various temperatures at pH 7 studied.

The comparative kinetics studies were conducted selectively at pH 7 for two reasons, *viz:* (a) this pH is close to the physiological pH 7.4, and thus the data will be useful for predicting the rate of degradation of the triazolines in biological fluids; and (b) the degradation at this pH is neither too slow nor too rapid; rapid degradation will render accurate determination of rate constants extremely difficult whereas slow degradation will preclude discrimination of small differences (within a few %) in reaction rates between the triazolines. As observed previously, 4 degrades very rapidly in aqueous medium at acidic pH ($t_{1/2}$ = 0.076 min at pH 2.2), but remains remarkably stable at pH 10.7 (degrades by no more than 2–4% over 24 hr, which is within the errors of measurement) (5). Similar findings (unpublished data) have been obtained with the 1-(4-methylphenyl) (1), 1-(4-fluorophenyl) (3) and 1-(4-trifluoromethylphenyl) (6) triazoline analogues (Fig. 1 and Table 1). Thus it would seem that the various 1-phenyl substituted triazolines behave similarly in terms of their susceptibility to degradation at the various pHs. The objective of the present study was to examine the structure-stability relationships of these triazolines with a view to designing stable triazoline analogues for further pharmacological and toxicological assessments.

MATERIALS AND METHODS

Reagents and Materials

Analytical grade disodium hydrogen phosphate and citric acid (for preparing buffer solutions) were obtained from Merck Chemicals and BDH Chemicals, respectively. Sodium carbonate (anhydrous) was also of analytical grade and supplied by Riedel-de Haën Chemicals. The various 1-phenyl-5-(4-pyridyl)-Δ^2-1,2,3-triazolines with or without substituents on the phenyl group (**1–7**; Fig. 1 and Table 1) and the 1-(4-pyridyl)-1-(4-chloroanilino)-, 1-(4-pyridyl)-1-(4-bromoanilino)-, and 1-(4-pyridyl)-1-(4-fluoroanilino)-ethanes (I–III; Fig. 1) for use as internal standards (i.s.) in the HPLC analysis of the triazolines [i.e. II as i.s. for **1**, **2**, **3**, **6**, **7**; I as i.s. for **4**; and III as i.s. for **5**] were synthesized by methods previously described by Kadaba et al. (8,9). Methanol and acetonitrile were of HPLC grade from Mallinckrodt Chemicals. All water used was double-distilled.

Degradation Kinetics Studies

Freshly prepared McIlvaine citric acid-phosphate buffer was employed to adjust the various solutions to the required pHs (measured at room temperature with a Model 8418 pH meter from Hanna Instruments). The kinetic studies and

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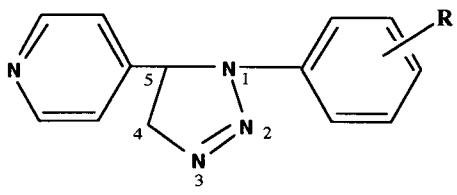
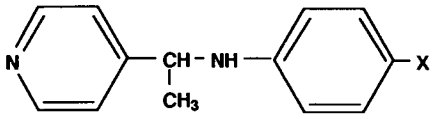


(a)



I: X = Cl
II: X = Br
III: X = F

(b)

Fig. 1. (a) General structure of the 1-phenyl-5-(4-pyridyl) substituted Δ^2-1,2,3-triazolines. [ADD 17014 (4); R = 4-Cl] (b) Chemical structures of 1-(4-pyridyl)-1-(4-chloroanilino)-ethane (I), 1-(4-pyridyl)-1-(4-bromoanilino)-ethane (II) and 1-(4-pyridyl)-1-(4-fluoroanilino)-ethane (III).

HPLC analysis were performed using previously developed procedures with modifications (5). Stock solutions of the various triazolines mixed with a fixed amount of appropriate internal standards in methanol (~0.5 mg/ml) were prepared immediately before the studies. 150 µl aliquot of each solution were added to 1350 µl of McIlvaine citric acid-phosphate buffer (pH 7; μ = 0.54) to give a working concentration of 50 µg/ml. The studies on each triazoline were conducted at 24 ± 0.1, 36 ± 0.1 and 43 ± 0.1 °C at pH 7. For experiments carried out at 24°C, 10 µl of triazoline solution were withdrawn at defined time intervals and immediately injected onto the column, while for those performed at higher temperatures (36 and 43°C), 50 µl aliquots were sampled and mixed with 100 µl sodium carbonate solution (0.2 M) to quench further degradation prior to injection (20 µl) onto the column. Each experiment was carried out in triplicate and student's t test was used for statistical analysis of the acquired data and calculated parameters.

HPLC Analysis

HPLC analysis was conducted using a reversed-phase Hypersil ODS column (0.47 × 10 cm); a mobile phase consisting of methanol (25%), acetonitrile (25%) and McIlvaine's (pH 8) buffer (50%) at a flow rate of 1.5 ml/min; an ISCO Model 2350 liquid chromatograph and an ISCO V^4 UV/visible detector. Detection was by ultraviolet absorption at 290 nm. Peak area integration and retention time determination were manipulated by computer software (ISCO ChemResearch Data System 150). The peak area ratios of the triazoline and the respective internal standard for samples taken at various times were normalized to that at time zero (i.e. at the start of the experiment) which was taken as 100%.

RESULTS AND DISCUSSION

It has been demonstrated previously that **4** decomposes readily in aqueous medium at acidic and neutral pHs, forming aziridine as one of the major products with loss of nitrogen (5; Fig. 2). The aziridine of **4** is more lipophilic and elutes at a longer retention time (R_t) than **4** itself on the reversed phase HPLC column. The aziridine has subsequently been shown to undergo further degradation under strongly acidic conditions to yield the hydrophilic primary and secondary β-amino alcohols which elute at R_t considerably shorter than **4** (10; Fig. 2). Similar degradation reactions have been observed for **3** (6). Indeed, the degradation products of both **3** and **4** have been isolated and their identities confirmed by a variety of chromatographical and spectrometric analytical techniques (5,6). While not having been chemically identified, the unknown peaks observed in the HPLC chromatograms for the other triazolines at pH 7 in the present study most likely correspond to the aziridine and β-amino alcohol degradation products of the respective triazolines, based on chromatographic behaviour on HPLC and UV absorption spectra.

For all the triazolines studied at various temperatures at pH 7, a plot of the logarithm of percentage of drug remaining against time yielded a straight line ($r > 0.99$; $n = 6–9$; $p < 0.05$), suggesting that the decomposition of the compounds

Table I. (a) Arrhenius' Degradation Kinetic Parameters of Various Δ^2-1,2,3-Triazolines

Triazolines with substituent (R) on 1-phenyl	k at 24°C × 10^3, min^{-1} (±s.d.)	k at 36°C × 10^3, min^{-1} (±s.d.)	k at 43°C × 10^3, min^{-1} (±s.d.)	ln A (±s.d.)	E_a, kcal mol^{-1} (±s.d.)
1: R = 4-CH_3	14.04 (0.88)	34.27 (1.50)	51.39 (1.72)	17.5 (0.7)	12.8 (0.4)
2: R = 4-H	15.15 (0.54)	31.82 (0.40)	52.97 (0.51)	17.7 (0.4)	13.0 (0.3)
3: R = 4-F	10.01 (0.21)	25.61 (0.99)	37.26 (2.10)	17.6 (0.8)	13.1 (0.5)
4: R = 4-Cl	10.97 (0.03)	24.98 (0.68)	42.01 (2.78)	17.7 (0.6)	13.1 (0.4)
5: R = 3,4-diCl	8.23 (1.04)	17.06 (1.35)	24.31 (0.57)	13.3 (1.1)	10.7 (0.6)
6: R = 4-CF_3	1.68 (0.08)	5.33 (0.49)	8.83 (1.50)	21.5 (1.4)	16.5 (0.8)
7: R = 4-NO_2	1.15 (0.02)	3.55 (0.04)	6.40 (0.08)	21.8 (0.2)	16.9 (0.1)

Table I. (b) Degradation Half-Lives of Various Δ^2-1,2,3-Triazolines

Triazolines with substituent (R) on 1-phenyl	$t_{1/2}$ at 24°C, min (±s.d.)	$t_{1/2}$ at 36°C, min (±s.d.)	$t_{1/2}$ at 43°C, min (±s.d.)
1: R = 4-CH_3	49.4 (3.1)	20.2 (0.9)	13.5 (0.5)
2: R = 4-H	45.7 (1.6)	21.8 (0.3)	13.1 (0.1)
3: R = 4-F	69.2 (1.5)	27.1 (1.0)	18.6 (1.0)
4: R = 4-Cl	63.2 (0.2)	27.7 (0.8)	16.5 (1.1)
5: R = 3,4-diCl	84.2 (10.6)	40.6 (3.2)	28.5 (0.7)
6: R = 4-CF_3	412.5 (19.6)	130.0 (12.0)	78.5 (13.3)
7: R = 4-NO_2	602.6 (10.5)	195.2 (2.2)	108.3 (1.4)

in aqueous solutions followed pseudo first order kinetics. The degradation rate constants and chemical half-lives at various temperatures calculated from the slope of the plots were shown to be dependent on the triazoline structures (Table 1). The rank order of stability for the triazolines, as determined by the relative magnitude of the rate constants, was 7 > 6 > 5 > 4 ≈ 3 > 2 ≈ 1, which appeared to follow the order of increasing electron releasing or decreasing electron withdrawing effects of the substituents on the N-phenyl ring. As alluded to earlier, the stability of the triazolines depends on the availability of electrons on the N-1 for reactivity (protonation). The presence of electron withdrawing substituents (e.g. trifluoromethyl, nitro) on the phenyl ring (particularly at the para position where mesomeric effect is the strongest) will augment the electron attracting effect of the latter and reduce the availability of electrons on the N-1 for protonation, thus stabilizing the triazoline. Conversely, the presence of electron donating substituents (e.g. methyl) on the phenyl group will abate the electron withdrawing effect of the latter and increase the availability of electrons on N-1 and hence the protonation, thereby destabilizing the triazoline. To verify this argument and to quantitatively establish the relationship of the electronic properties of the substituents to the chemical stability of the triazolines, the logarithms of degradation rate constants determined at each temperature were correlated with the Hammett constants (σ) of the corresponding substituents (11; Fig. 3). In all cases, a good correlation was obtained (r = 0.86, 0.90, 0.91 for k at 24, 36, 43°C, respectively; n = 7; $p < 0.05$; Fig. 3), indicating that the stability of the triazolines is governed predominantly

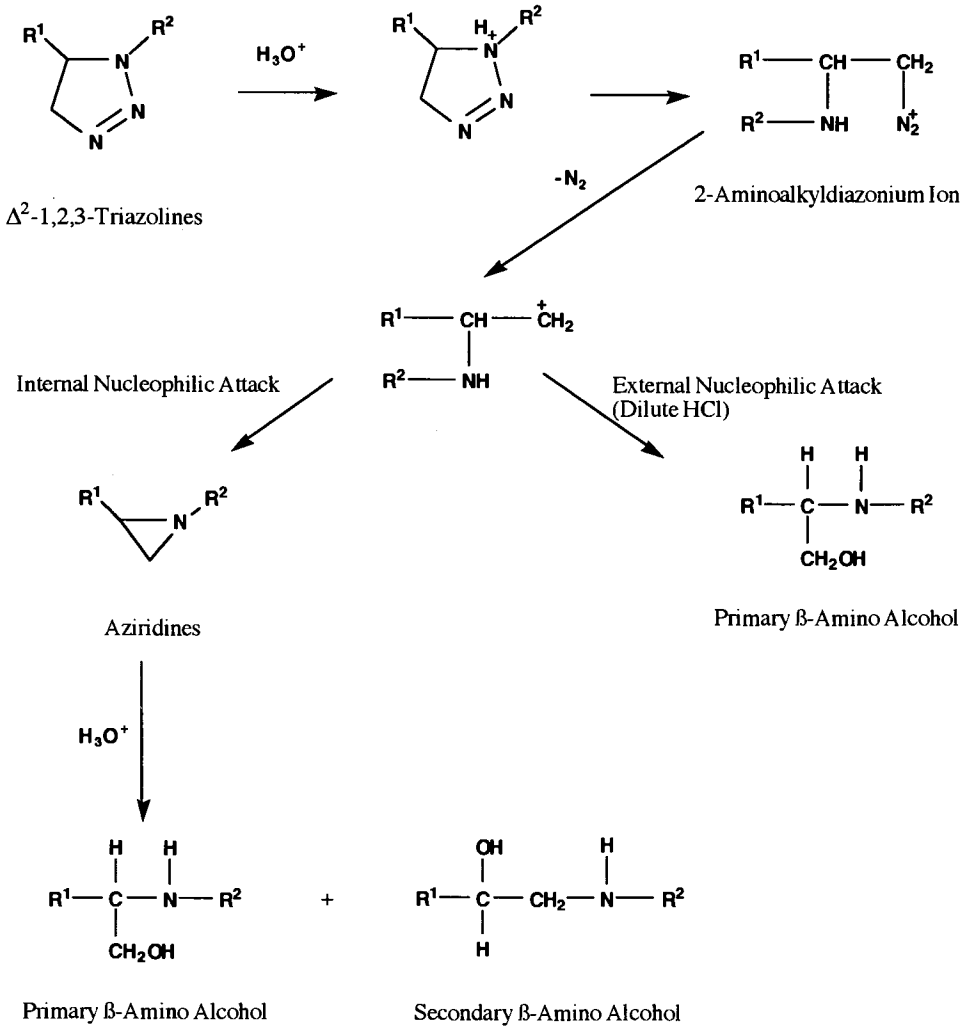


Fig. 2. Chemical degradation of Δ^2-1,2,3-triazolines in aqueous acidic medium.

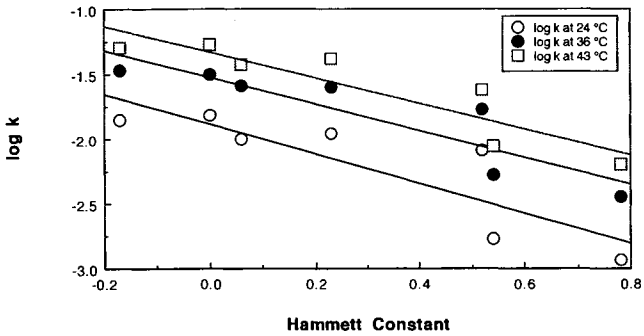


Fig. 3. Linear correlation between logarithms of first-order degradation rate constants (k) of various Δ^2-1,2,3-triazolines and Hammett constants (σ) of corresponding substituents on N-phenyl.

by the electronic effects (i.e. both inductive and mesomeric, as measured by σ) of the substituents, i.e. electron withdrawing groups tend to stabilize the triazolines and vice versa. The Hammett reaction constants (ρ) determined at 24, 36 and 43°C from the slopes of the plots were -1.15 (± 0.30), -1.03 (± 0.22) and -0.99 (± 0.21), respectively, which are statistically identical within the errors of fit. This suggests that the sensitivity of degradation to substituent effects is not significantly affected by a change in temperature. The observed 'constancy' in ρ may also be taken to imply that the mechanisms or rate-limiting steps of degradation of the various triazolines remain invariant with temperature.

The logarithm of the rate constant was further regressed against the reciprocal of absolute temperature to yield the intercept and slope from which the Arrhenius factor (A) and activation energy (E_a), respectively, of degradation at pH 7 were calculated. The Arrhenius plots for all the triazolines displayed excellent linearity with a correlation coefficient of no less than 0.99 ($n = 9$; $p < 0.05$). As can be seen from Table 1, the respective A and E_a of 1–4 appeared to be statistically indistinguishable within experimental errors. The respective A and E_a of 6 and 7 were also similar in magnitude, but higher than those of 1–4. The 1-(3,4-dichlorophenyl) analogue (5) differed from the rest in that A and E_a were both significantly lower than those of the other six triazolines. Thus it would appear that the observed stability of 6 and 7 is largely due to the relatively high E_a required for the molecules to react, whereas that of 5 is mainly attributable to the relatively low frequency of collision between molecules, or more specifically, the relatively low probability of successful collisions leading to a reaction.

CONCLUSIONS

The present study clearly demonstrates the important roles of para substituents on the phenyl group in maintaining the stability of the triazolines. Chemical stability is clearly desirable for oral dosage forms, in order to ensure optimal absorption of intact lipophilic drugs. However, metabolic liability, to afford the putative pharmacologically active primary β-amino alcohol metabolite (10), needs to be designed into these prodrugs; so that the active agent is readily generated metabolically when the prodrug reaches the target tissues. The present chemical stability data, together with efficacy data for these compounds in experimental animal models for epilepsy, will enable the judicious choice of suitable analogues for further preclinical metabolic, pharmacological, and toxicological investigations.

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REFERENCES

1. Kadaba, P. K. Triazolines XIII. Δ^2-1,2,3-Triazolines as a new class of anticonvulsants. *J. Pharm. Sci.* 73:850–852 (1984).
2. Kadaba, P. K. Triazolines 14. 1,2,3-Triazolines and triazoles, a new class of anticonvulsants. Drug design and structure activity relationships. *J. Med. Chem.* 31:196–203 (1988).
3. Kadaba, P. K. and Slevin, J. T. Triazolines 15. Anticonvulsant profile of ADD17014, a potentially unique 1,2,3-triazoline antiepileptic drug in mice and rats. *Epilepsia.* 29:330–337 (1988).
4. Kadaba, P. K. 1,2,3-Triazolines: a new generation of anticonvulsant agents effective in the kindling model of epilepsy, with a unique mechanism for impairing excitatory amino acid neurotransmission. *Drugs of the Future.* 15:1013–1024 (1990).
5. Hamelijnck, M. A. F., Stevenson, P. J., Kadaba, P. K. and Damani, L. A. Triazolines. XXI. Preformulation degradation kinetics and chemical stability of a novel triazoline anticonvulsant. *J. Pharm. Sci.* 81:392–396 (1992).
6. Stevenson, P. J. Preformulation, metabolic and pharmacokinetic studies on 1-(4-chlorophenyl)-5-(4-pyridyl)-Δ^2-1,2,3-triazoline, Ph.D. thesis, University of London, 1994, pp. 169–178.
7. Scheiner, P. Triazoline decomposition. In Thyagarajan, B. S. (ed). *Selective Organic Transformations.* Vol. I, Wiley-Interscience, New York, 1970, pp. 328–362.
8. Kadaba, P. K., Stanovnik, B. and Tisler, M. Δ^2-1,2,3-Triazolines. *Adv. Heterocycl. Chem.* 37:217–349 (1984).
9. Deshmukh, T. R. and Kadaba, P. K. Triazolines 28. Identification of the triazoline pharmacophore and the evolution of the aminoalkylpyridines, a new class of potent orally active anticonvulsant agents. *Med. Chem. Res.* 3:223–232 (1993).
10. Kadaba, P. K., Stevenson, P. J., Nnane, I. P. and Damani, L. A. Triazolines 27. Δ^2-1,2,3-Triazoline anticonvulsants: novel 'built in' heterocyclic prodrugs with a unique 'dual-action' mechanism for impairing excitatory amino acid L-glutamate neurotransmission. *Bioorg. Med. Chem.* (Accepted).
11. Kubinyi, H. The quantitative analysis of structure-activity relationships. In Wolff, M. E. (ed). *Burger's Medicinal Chemistry and Drug Discovery. Vol. I: Principles and Practice.* 5th Ed., John Wiley and Sons, New York, 1995, pp. 497–571.