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Kinetics of rearrangement and hydrolysis of amino acid derivatives of prazosin

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Abstract

Amino acid amides of prazosin have been synthesized as potential prodrugs to increase the water solubility of the parent compound and target peptidase enzymes for cleavage of the prodrug in vivo (bioreversion). The α-amino acid derivatives degraded rapidly in aqueous solution at pH values > 5 with half-lives on the order of 10–50 min. The rapid degradation of these derivatives was attributed to intramolecular nucleophilic attack of the α-amine of the amino acid resulting in a rearranged product, not prazosin. In the absence of a free α-amino group, greater stabilization was achieved and the primary route of degradation at all pH values was hydrolysis of the amide bond to give prazosin.

Key words: Prodrug; Prazosin; Stability; Amino acid; Intramolecular rearrangement; Aromatic amine

1. Introduction

Prazosin, I, is an α_1-antagonist which has been used in the treatment of hypertension. Its salts are sparingly water-soluble and therefore prazosin was used as a model drug to investigate the applicability of *N*-amino acid derivatives as soluble prodrugs of heterocyclic aromatic amines. Amino acids have been used in the past as promoieties to enhance the aqueous solubility of various alcohols (Cho and Haynes, 1985; Johnson et al., 1985), carboxylic acids (Persico et al., 1988; Springer et al., 1990), and aliphatic amines (Amidon et al., 1980; Upshall et al., 1990) but information is limited regarding amino acid prodrugs of primary aromatic amines (Slojkowska et al., 1982). Advantages of amino acids as promoieties for amines include good water solubility and enzymatic reversion in vivo by the action of peptidases. Previous studies of amino acid ester prodrugs have shown facile in vitro enzymatic cleavage of the ester bond, but relatively rapid chemical hydrolysis precluded development of solution formulations (Bundgaard et al., 1984; Cho and Haynes, 1985). Theoretically, amide bonds should exhibit greater chemical stability than ester linkages yet still maintain enzymatic susceptibility. This paper discusses the pH-dependent degradation of various amino acid derivatives of prazosin

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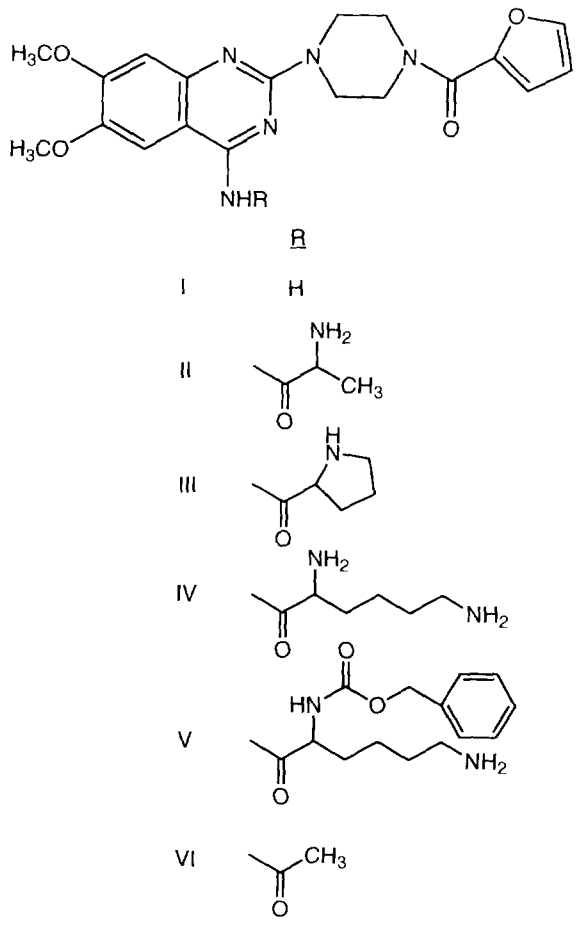


Fig. 1. Structures of prazosin and its derivatives.

and the contribution of the promoiety (amino acid) to the instability.

2. Materials and methods

2.1. Materials

Prazosin HCl was generously supplied by Chelsea Laboratories, West Hempstead, NY. The free base was obtained by suspending the HCl salt in water and titrating with 1 N NaOH to a constant pH. The solid was collected by filtration and dried.

The amino acids, ninhydrin, dicyclohexylcarbodiimide (DCC), and formic acid (99%) were obtained from Sigma Chemical, St. Louis, MO. Sodium acetate, triethylamine, *N,N*-dimethylaminopyridine (DMAP), octanesulfonic acid and tetrabutylammonium hydrogen phosphate were obtained from Aldrich Chemical, Milwaukee, WI. Dimethylformamide (DMF) was obtained from Mallinckrodt and was distilled over ninhydrin before use. All other solvents were HPLC grade and were obtained from Fisher Scientific.

The HPLC system consisted of a Waters HPLC pump or Shimadzu Model LC-6A chromatographic dual pump system operated isocratically equipped with a Rheodyne injector (20 μl loop), a Waters Model 440 ultraviolet detector operated at 254 nm, a Shimadzu C-R3A integrator, and a C-18 reversed-phase column (150 × 4 mm) with a 60 × 4 mm guard column (ODS Hypersil, 5 μm particle size).

^{1}H-NMR were recorded at ambient temperature on a Varian XL-300 spectrometer using standard pulse sequences. Chemical shifts were referenced to TMS (tetramethylsilane) in $CDCl_3$ or DMSO (dimethylsulfoxide) in DMSO-d_6. Mass spectra were obtained with a Varian CH-5 or a Ribermag R-10-10 quadropole mass spectrometer operated in either the electron impact (EI) or chemical ionization (CI) mode. Stability studies were carried out at controlled temperature at either 25 or 37°C (±0.1°C) in circulating water baths. pH values were determined using a standardized Corning Model 155 pH meter. Ionic strength was maintained constant at 0.2 M using NaCl.

2.2. Synthesis of prodrugs

The synthesis was a standard amide coupling reaction between an *N*-protected amino acid and the prazosin free base using the coupling agent DCC with DMAP as a base catalyst (Sheehan and Hess, 1955). The appropriate *t*-butyloxycarbonyl (t-BOC) *N*-protected amino acid (17 mmol) was dissolved in 100 ml freshly distilled CH_2Cl_2, 3.52 g (17 mmol) DCC and 10 mg DMAP were added, and the mixture stirred under nitrogen in an ice bath for 30 min. The solution was filtered to remove the dicyclohexylurea (DCU) formed, and 4.5 g (12 mmol) prazosin free base in

200 ml DMF was added and stirred at room temperature under nitrogen. After 48 h, the solvent was evaporated to dryness under vacuum to yield a light brown residue which was stirred in 150 ml of CH_2Cl_2 and filtered to remove insoluble materials. The methylene chloride was washed with 50 ml of water, 2×20 ml of 5% Na_2HCO_3, 2×20 ml dilute HCl, and 50 ml saturated NaCl, then dried over Na_2SO_4. The solvent was concentrated to 20 ml and chromatographed on 80 g silica with ethyl acetate as the eluent. Those fractions containing product were combined and evaporated to dryness. After crystallization from ethyl acetate/methylene chloride, the products were characterized by NMR and mass spectral analysis. The results were consistent with the expected products. The *N*-protected L-aminoacyl amide of prazosin was then dissolved in 99% formic acid or trifluoroacetic acid and stirred at room temperature for 2–4 h until TLC (silica/ethyl acetate) indicated total disappearance of starting material. The solvent was then removed under vacuum. The residue was washed with CH_2Cl_2 and a light yellow solid collected as either the formate or trifluoroacetate salt. All of the deprotected products had an $R_f = 0$ on TLC. Products were characterized by NMR and mass spectrometry and the results are summarized in Table 1. Due to the poor chemical stability of these compounds the final products contained small amounts of prazosin ($\sim 1\%$) as determined from relative peak areas by HPLC. Attempts to further purify the amides by crystallization or chromatographic methods resulted in greater degradation. Therefore, elemental analyses were not obtained for the end products and subsequent experiments were performed with the products containing approx. 1% prazosin.

N-Acetylprazosin was synthesized by adding 3 ml of 99% acetic anhydride to 266 mg of prazosin free base in 20 ml DMF/CH_2Cl_2 1:1 and stirring at room temperature overnight. The solvent was evaporated to dryness under vacuum to yield a rose-colored residue which was stirred in CH_2Cl_2 and the insoluble material removed by filtration. The solid was dissolved in 250 ml ethyl acetate, any insoluble material removed by filtration, the ethyl acetate concentrated to 100 ml, and washed with 3×20 ml of 1 N acetic acid. The ethyl acetate fraction was dried over Na_2SO_4 and evaporated to dryness. The results of the product characterization are summarized in Table 1.

2.3. HPLC assay

HPLC assays were carried out using UV detection at 254 nm. The mobile phase consisted of acetonitrile and 0.05 M acetate buffer at pH 3.50 containing 10 mM tetrabutylammonium phosphate (TBA) and 10 mM octanesulfonic acid (OSA) at a flow of 1.5 ml/min. The ratio of organic:aqueous was 25:75 for analysis of II–IV and 35:65 for analysis of V and VI. Retention

Table 1
Properties of amino acid amides of prazosin

Compound	Salt	Mass spectral data	^{1}H-NMR chemical shifts
Alanylprazosin (II)	TFA	M^+ at 454 (electron impact)	$CDCl_3$: 1.54 (d,3); 3.8–4.0 (m,14); 4.80 (m,1); 6.58 (m,1); 7.20 (s,1); 7.23 (d,1); 7.38 (s,1); 7.61 (d,1)
Prolylprazosin (III)	formate		$CDCl_3$: 1.5–2.3 (m,4); 1.9–3.2 (m,2); 3.8–4.0 (m,14); 4.2(m,1); 6.4 (m,1); 6.8–7.0 (m,3); 7.45 (m,1)
Lysylprazosin (IV)	formate	M^+ at 511 (electron impact)	DMSO-d_6: 1.4–2.0 (m,6); 3.35 (m,2); 3.8–4.0 (m,14); 4.90 (m,1); 6.57 (m,1); 7.10 (s,1); 7.31 (d,1); 7.55 (s,1); 7.71 (d,1)CC
CBZ-lysylprazosin [a] (V)	formate	M + 1 at 646 (chemical ionization)	$CDCl_3$: 1.2–2.1 (m,6); 3.0 (m,2); 3.8–4.0 (m,14); 4.3 (m,1); 5.1 (s,2); 6.45 (m,1); 6.75 (s,1); 6.85 (m,1); 6.9 (s,1); 7.3 (s, 5); 7.45 (m,1)
N-Acetylprazosin (VI)		M^+ at 425 (electron impact)	$CDCl_3$: 2.6 (s,3); 3.8–4.0 (m,14); 6.4 (m,1); 6.85 (s,1); 6.95 (m,1); 7.05 (s,1); 7.4 (m,1)

[a] CBZ: α-*N*-carbobenzoxy-.

Table 2
HPLC retention volumes for compounds I–VI and the observed degradation peaks

Compound	Mobile phase [a]	Retention volume (ml)
Prazosin	A	6.3
Prazosin	B	2.6
II	A	10
Degradation product from II	A	4.7
III	A	7.5
Degradation product from III	A	3.6
IV	A	15
Degradation product from IV	A	5.2
V	B	8
VI	B	4.3

[a] Acetonitrile/0.05 M acetate buffer, pH 4, 10 mM octanesulfonic acid, 10 mM tetrabutylammonium phosphate; 25:75 for mobile phase A and 35:65 for mobile phase B.

volumes of the various derivatives are summarized in Table 2. Standard curves were reproducible and linear from 1 to 80 μg/ml prazosin free base. Within and between day coefficients of variation were $< 5\%$.

2.4. Chemical stability of prodrugs

The chemical stability of II–VI was studied in aqueous buffers at varying pH values at a buffer concentration of 0.05 M at 37°C. Solutions of the prodrugs at 20–30 μg/ml were prepared in buffer, maintained at constant temperature, and samples were removed periodically and analyzed by HPLC for both prodrug and degradation products. The reaction was followed for at least four half-lives. The observed rate constants were determined from pseudo-first-order plots of loss of starting material as a function of time. A complete pH-rate profile was prepared for IV at 25°C, including varying buffer concentrations (0.01, 0.025, and 0.05 M) at each pH to assess the possibility of buffer catalysis. All rate constants for IV at 25°C were determined in triplicate except for pH 11.0 and 12.5 ($n = 1$).

2.5. Isolation of degradation product of II

A sample of II (7 mg) was stirred in 10 ml of 0.05 M phosphate buffer at pH 8 overnight. The solution was extracted with 2×10 ml portions of dichloromethane, the organic fractions dried over $MgSO_4$ and evaporated to dryness. On TLC in acetone prazosin had an $R_f = 0.31$, degradation product $R_f = 0.18$, and II $R_f = 0.0$. On crystallization from acetone, a single compound was isolated (single spot on TLC and single peak on HPLC) that corresponded to the observed degradation product. The off-white globular crystals had a melting point of 236–237°C. Electron impact mass spectral analysis showed peaks at $m/e = 454$ (M^+), 410, 304, 259, 95. High-resolution mass spectrometry indicated M^+ at 454.198 and M + 1 isotope peak at 455.2018. ^{1}H-NMR in DMSO-d_6/$CDCl_3$ yielded: δ 1.557 (d, 3, $J = 7.05$; $CHCH_3$); δ 3.8–4.0 (m, 14; piperazine CH_2s and OCH_3); δ 4.68 (m, 1; chiral CH); δ 6.5 (s, 1; amide NH); δ 6.541 (m, 1, $J = 3.24$, 1.75; furan CH at C-4); δ 6.831 (s, 1; quinazoline CH); δ 6.974 (d, 1, $J = 3.42$; furan CH at C-3); δ 7.046 (s, 1; quinazoline CH); δ 7.386 (s, 1; amide NH); δ

Table 3
Half-lives for degradation of II–VI in 0.05 M acetate buffer (pH 4.0) and 0.05 M phosphate buffer (pH 7.4) at 37°C and major routes of degradation

Compound	pH 4.0		pH 7.4	
	$t_{1/2}$ (min)	Major route of degradation	$t_{1/2}$ (min)	Major route of degradation
Alanylprazosin (II)	n.d.	hydrolysis	11	rearrangement
Prolylprazosin (III)	373	hydrolysis	43	rearrangement
Lysylprazosin (IV)	269 [a]	hydrolysis	9	rearrangement
CBZ-lysylprazosin (V)	1100	hydrolysis	1317	hydrolysis
*N*Acetylprazosin (VI)	8319	hydrolysis	44710	hydrolysis

[a] Catalysis evident due to acetate buffer. Half-life was extrapolated to zero buffer concentration. n.d., half-life not determined.

7.601 (m, 1, $J = 1.64$; furan CH at C-5); δ 7.791 (s, 1; amine NH).

3. Results and discussion

The kinetics of degradation of II–VI were determined as a function of pH at 37°C and the results are summarized in Table 3. Fig. 2 summarizes data for degradation of IV at 25 and 37°C. At pH 4.0 and 5.0, catalysis was evident due to the acetate buffer but at all other pH values the rate constants were independent of buffer concentration. The values plotted in Fig. 2 represent the observed rate constants extrapolated to zero buffer concentration for pH 4.0 and 5.0, and mean values for all other data points. Fig. 3 summarizes the data on the stability of V and VI at 37°C. These rate constants were all determined at 0.05 M buffer concentration.

Compounds V and VI followed the expected route of degradation and quantitatively produced prazosin via hydrolysis of the amide bond. However, the simple aminoacyl amides (II–IV) exhibited two distinct pathways of degradation, with

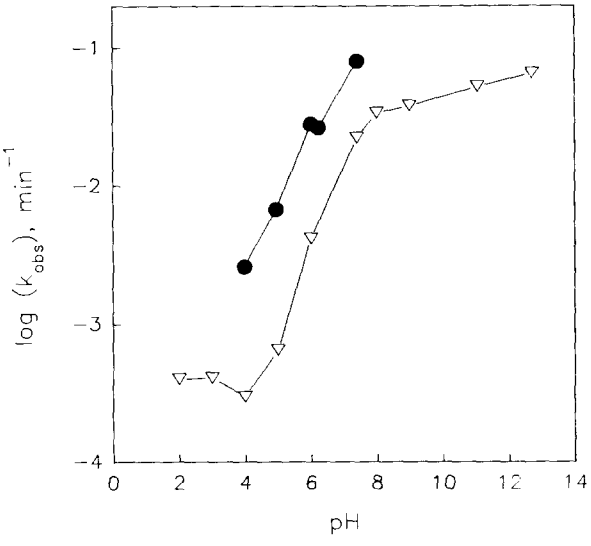


Fig. 2. pH-rate profile for degradation of L-lysyl prazosin (IV) at 25°C (▽) and 37°C (●). At pH 4.0 and 5.0 catalysis was observed due to acetate buffer and these values were extrapolated to zero buffer concentration.

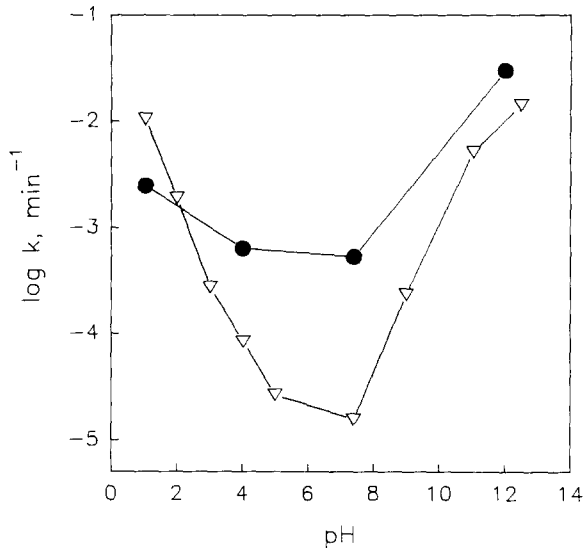


Fig. 3. pH-rate profiles for degradation of α-*N*-carbobenzoxy-L-lysyl prazosin (V, ●) and *N*-acetylprazosin (VI, ▽) at 37°C. Rate constants were determined in 0.05 M buffer.

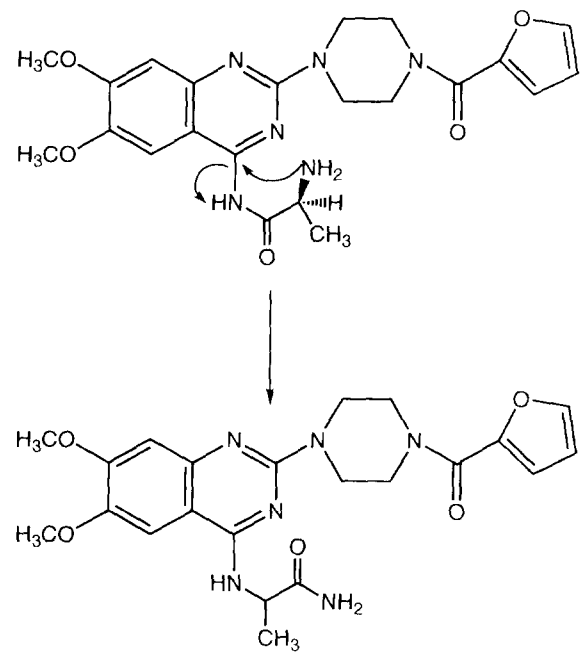
the extent of each pathway dependent on the pH. At pH values less than 4, prazosin was the only observed degradation product. Above pH 4, another product appeared which eluted on the chromatogram close to prazosin, but was distinct for each amino acid derivative. The relative magnitude of this alternative reaction increased rapidly as pH increased. The proportions of this pathway for IV at pH 5, 7.4 and 11 were 66, 89 and 100%, respectively. The half-lives for II–IV at pH 7.4 ranged from 9 to 43 min (Table 3). This marked instability was noted only for those compounds with a free α-amino group on the amino acid.

In order to elucidate the route of degradation of these compounds, the degradation product from II was isolated and characterized. Mass spectral analysis showed the molecular ion at *m/e* 454, which corresponds to the molecular weight of the starting material. This suggests that the degradation involves an intramolecular process resulting in no net loss of mass. The exact mass was confirmed by high-resolution mass spectrometry, with M^+ at 454.198 and M + 1 isotope at 455.2018. The ^{1}H-NMR spectrum was compared to that of the parent amide and the only

differences noted in the spectrum of the isolated degradation product were three additional upfield peaks (6.5–8 ppm). The solubility of the product was limited in acetone, dichloromethane, water and dilute HCl; it was soluble only in DMSO. The insolubility in water and acid was consistent with the absence of a free aliphatic amino group. The fluorescence spectra of the product were similar to that of prazosin, with excitation and emission maxima at 340 and 385 nm in contrast to the prodrug which excites and emits at higher wavelengths (365 and 450 nm, respectively).

The involvement of the amino group was implicated in the degradation process since only those species with a free α-amino group followed the non-hydrolytic route of degradation. Derivatization of this functionality (V) or absence of an amine (VI) completely prevented the alternative reaction. The rate of this reaction was also a function of the nature of the amino group. Compound III, which contains a secondary amine, was approx. 4-times more stable than compounds with a primary α-amine (II and IV). This suggests a steric contribution to the reaction. Variation among the pK_a values of the various amino acids is not likely to be a major factor. The pK_a of alanine is approximately midway between lysine and proline (Weast, 1982), but the alanyl (II) and lysyl (IV) derivatives exhibit similar kinetics. The possibility of an intermolecular process was explored by studying the kinetics as a function of concentration. No differences in rates of reaction were observed up to 80 μg/ml, suggesting that the degradation was not occurring intermolecularly.

A mechanism consistent with the results is proposed in Scheme 1. The reaction involves nucleophilic attack of the free α-amino group of the amino acid at position C-4 of the quinazoline ring of prazosin, resulting in displacement of the amide nitrogen. The proposed product contains a secondary amine attached to the aromatic ring and a primary amide. The additional peaks which were observed in the ^{1}H-NMR spectrum of the isolated degradation product are consistent with this product. The two slightly broadened peaks at δ 6.5 and 7.4 ppm are in the expected range for the primary amide protons and the third at 7.91 ppm corresponds to the proton on the aromatic amine (Kostek, 1989). The pH dependency of the reaction is also consistent with the proposed mechanism. An unionized amine would act as a better nucleophile than the respective ionized species. The relatively pH-independent region in the basic pH range is kinetically consistent with an uncatalyzed reaction of the fully non-ionized species. The difference in rates between the primary and secondary amines would also be explained by this process. The differences between the fluorescence spectra of the starting materials and degradation products can be explained by a bathochromic shift due to an electronic effect of the carbonyl upon acylation of an amine (Zimmerman et al., 1976, 1977), consistent with the fluorescence shift observed for the prazosin amides. The degradation product proposed in Scheme 1 contains an amine on the aromatic ring and therefore would be expected to fluoresce at lower wavelengths than the amide.



Scheme 1. Proposed route of degradation of aminoacyl amides of prazosin in aqueous solution at pH > 6, illustrated here for alanyl prazosin (II).

Intramolecular rearrangements involving the α-amino group of amino acids have been reported previously. For example, the α-amino group of glycineimide has been shown to undergo a similar reaction, where nucleophilic attack by the α-amine results in displacement of an imide nitrogen (De Mayo, 1964). This is consistent with formation of the five-membered ring intermediate postulated in Scheme 1. Reactive amino acids have also been reported for dipeptidyl derivatives of ara-C, although the products of the intramolecular degradation were a diketopiperazine and the parent drug (Wipf et al., 1991).

The susceptibility of the prazosin amino acid amides to the intramolecular rearrangement proposed in Scheme 1 is most likely due to the chemistry of the heteroaromatic ring, since the presence of the nitrogens in the aromatic ring increases the potential for nucleophilic substitution or addition. With quinazolines, preferential nucleophilic substitution has been shown to occur at the 4-position due to activation by both a *para*- and *ortho*-ring nitrogen (Gilchrist, 1985), further supporting the proposed degradation mechanism.

The results from the stability studies of the prazosin derivatives suggest that the only effective way of stabilizing the amino acid amides of prazosin is through derivatization of the α-amino group of the amino acid to prevent the intramolecular rearrangement. However, compounds such as V and VI, which undergo pH-dependent amide hydrolysis (Fig. 3), exhibit enhanced hydrolytic lability of the amide bonds as compared to amino acid amides of other aromatic amine compounds (Bundgaard, 1985; Hirai et al., 1991). This suggests a possible contributing factor to the enhanced reactivity of the amide bonds in the prazosin may relate to the electron-withdrawing and stabilizing effects of the heteroaromatic ring compared to benzene, resulting in activation of these compounds relative to aniline derivatives.

4. Conclusions

Because of their facile degradation, the alanyl, prolyl and lysyl derivatives of prazosin are not acceptable candidates for prodrugs. The intramolecular degradation process may be attributed to the nature of the heterocyclic nucleus of prazosin. Carbamylation of the α-amino group of the lysine promoiety resulted in a derivative with greater chemical stability toward intramolecular rearrangement but with an unacceptable degree of hydrolytic lability of the amide bond for utility as a prodrug.

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