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Communications to the Editor

N-2,3-Butadienyl-1,4-butanediamine Derivatives: Potent Irreversible Inactivators of Mammalian Polyamine Oxidase

Sir:

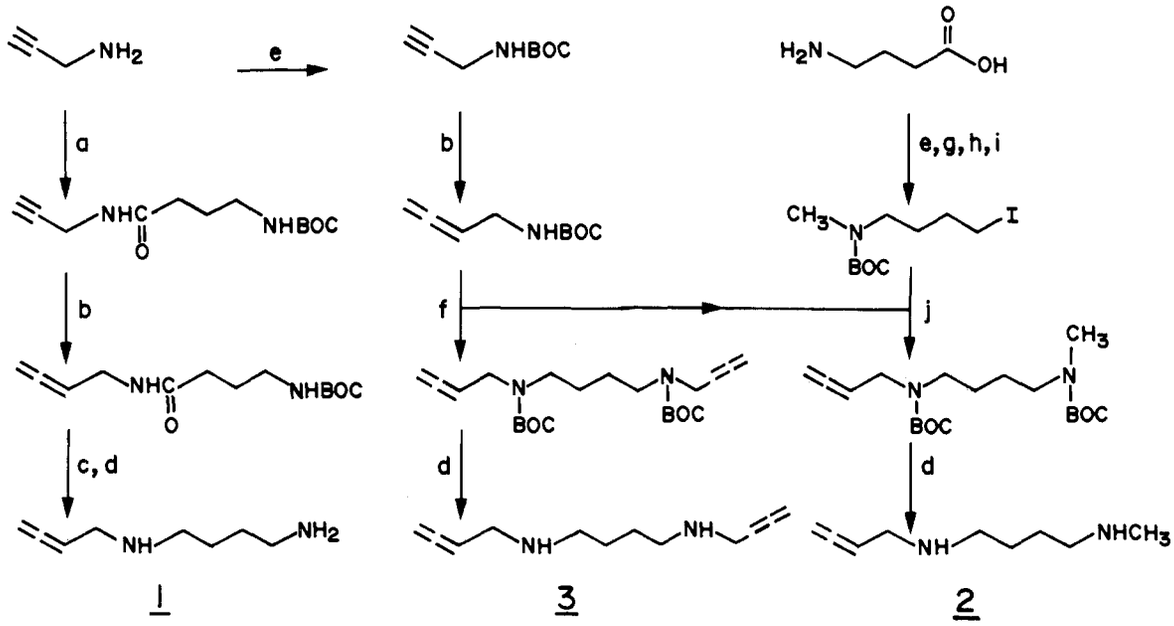
The ubiquitous polyamines putrescine, spermidine, and spermine are implicated in cellular growth and/or differentiation.[1] Spermidine and spermine have been shown to be transformed back to their respective bioprecursors putrescine and spermidine[2] by the action of acetyl CoA: polyamine N^1-acetyltransferase and polyamine oxidase (PAO). This interconversion pathway of polyamines occurs apparently in all mammalian tissues.

PAO is a flavin-dependent enzyme[3] that catalyzes a reaction closely akin to that performed by monoamine oxidase (MAO, EC 1.4.3.4, flavin dependent). In analogy with the irreversible inhibition of MAO by propargyl-,[4] allyl-,[5] and 2,3-butadienylamines,[6] we have prepared a series of putrescine derivatives substituted on nitrogen with similar unsaturated groups as potential inactivators of PAO. Of most interest are the *N*-2,3-butadienyl derivatives of putrescine **1–3**. They display in vitro kinetic properties of enzyme-activated irreversible inhibitors and in vivo high potency and selectivity for PAO.

The butadienyl putrescine derivatives **1–3**[7] were synthesized as depicted in Scheme I. The allylic and propargylic derivatives **4**, **5** and **6**, **7**[7] were obtained in a straightforward manner by reduction of the amides derived from allyl- or propargylamine and appropriate derivatives of N-protected γ-aminobutyric acids, followed by removal of the protecting group(s).

Incubation of a partially purified preparation of PAO from pig liver with varying concentration of **1–3** resulted in a time- and dose-dependent loss of enzyme activity that followed pseudo-first-order kinetics for at least 2 half-lives. Exhaustive dialysis or incubation with the substrate N^1,N^{12}-diacetylspermine of the inactivated enzyme did not restore activity. Preincubation of PAO with **1–3** in the

Scheme I[a]



[a] (a) *N*,*N*′-Dicyclohexylcarbodiimide, $HO_2C(CH_2)_3NHBOC$, CH_3CN; (b) $(i\text{-Pr})_2NH$, CuBr, HCHO in dioxane, reflux, 1 h;[12] (c) $LiAlH_4$ in Et_2O, room temperature, 2 days; (d) HCl gas in Et_2O; (e) $(t\text{-BuOCO})_2O$; (f) NaH in DMF, 1,4-diiodobutane, 3 h, room temperature; (g) NaH in THF, CH_3I; (h) BH_3 in THF, 20 h, room temperature; (i) mesyl chloride, NEt_3, −10 °C in CH_2Cl_2, then MgI_2 in Et_2O; (j) NaH in DMF, 0 °C, 2 h.

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Table I. Kinetic Constants for the Inhibition of Pig Liver PAO by Putrescine Derivatives $R_1NH(CH_2)_4NHR_2 \cdot 2HCl$[a]

substituents					
R_1	R_2	no.	mp, °C	K_I,[b] μM	τ_{50},[b] min
$CH_2=C=CHCH_2$	H	1	197	0.7	1
$CH_2=C=CHCH_2$	CH_3	2	226	0.3	0.5
$CH_2=C=CHCH_2$	$CH_2=C=CHCH_2$	3	242	0.09	2.2
$CH_2=CHCH_2$	H	4	205	$t_{1/2}$ = 35 min at 50 μM[c]	
$CH_2=CHCH_2$	CH_3	5	230	$t_{1/2}$ = 12 min at 12 μM[c]	
$CH\equiv CCH_2$	H	6	196	425[d]	260[d]
$CH\equiv CCH_2$	CH_3	7	200	310[d]	100[d]

[a] The specific activity of the stock solution of PAO was 2.2 nmol of N^1-acetylspermidine h^{-1} mg^{-1} of protein with use of N^1,N^{12}-diacetylspermine as substrate. For a typical experiment, 91 units of pig liver PAO (1 unit is defined as the amount of protein that produces 1 nmol of N^1-acetylspermidine/h from N^1,N^{12}-diacetylspermine under saturating conditions) were mixed at time zero with 250 μL of a solution of inhibitor in a borate buffer (pH 9) containing DTT (1 mmol) and the solution incubated at 30 °C. At different time intervals, 10-μL aliquots were transferred into 200 μL of assay medium containing borate buffer (pH 9), 1 mg of horseradish peroxidase, and 4 mg of homovanillic acid per 50 mL. A solution (10 μL) of N^1,N^{12}-diacetylspermine in borate buffer, pH 9 (10 mg/mL), was added. The reaction was allowed to proceed for 1 h at 37 °C and then was terminated by cooling in an ice bath. The residual enzyme activity was calculated from the measurement of fluorescence intensity at 425 nm after addition of 0.15 N NaOH and excitation at 315 nm according to the method of Snyder and Hendley.[11] [b] Apparent dissociation constant (K_I) and $t_{1/2}$ under saturating condition (τ_{50}) were determined according to the method of Kitz and Wilson.[8] [c] Inactivation of enzyme did not follow first-order kinetics. [d] Values determined by using $t_{1/2}$ measured from extrapolation of the inhibition curve.

presence of N^1,N^{12}-diacetylspermine provided protection against inactivation. For each inhibitor, a minimum half-life (τ_{50}) and an apparent dissociation constant (K_I) (Table I) could be determined by plotting $t_{1/2}$ as a function of $1/I$ according to Kitz and Wilson.[8] Taken together with the lack of intrinsic chemical reactivity of 2,3-butadienylamines,[6] these results strongly suggest **1–3** to be enzyme-activated irreversible inhibitors of PAO.

The allylic derivatives **4** and **5** of putrescine led also to a dose- and time-dependent inhibition of PAO, but in contrast with the butadienyl analogues, the inactivation did not follow pseudo-first-order kinetics and could be reversed upon incubation with the substrate N^1,N^{12}-diacetylspermine. In fact, **4** and **5** were extensively oxidized by PAO as indicated by the formation of putrescine and *N*-methylputrescine[9] in the incubation medium. Interestingly, the propagylic derivatives **6** and **7** of putrescine displayed only marginal activity toward PAO. Thus, it appears that the relationship between potency and the nature of the unsaturation differs markedly in the inactivation of PAO or MAO. Butadienylamines that are not very potent inhibitors of MAO display high inhibitory activity toward PAO whereas propargylamines that are very effective inhibitors of MAO are practically devoid of activity toward PAO.

In accordance with their postulated mode of action, **1–3** show high selectivity for PAO. They neither inhibit the amine oxidases MAO and DAO nor any enzyme other than PAO involved in the metabolism of polyamines. In vivo, they are exquisitely potent. The ID_{50} for the inhibition of mouse brain, kidney, and liver PAO is 5, 0.1, and 0.04 mg/kg, respectively.[10] In conclusion **1–3** are the first reported irreversible inhibitors of PAO. Their in vivo potency and selectivity make them ideal tools for assessing the physiological importance of the polyamine interconversion pathway in different biological systems.

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