of 6. Recrystallization from EtOH afforded an analytical sample, mp 186–187 °C.

Method B. *N*-[2-Cyano-2-(1*H*-tetrazol-5-yl)ethenyl]-benzothiazol-2-amine Hydrate (12). 2-[(Benzothiazol-2-ylamino)methylene]propanedinitrile (6; 7.5 g, 0.033 mol), NaN_3 (2.3 g, 0.033 mol), and NH_4Cl (1.8 g, 0.033 mol) were stirred at 80 °C in 40 mL of DMF for 16 h. The reaction mixture was poured into 150 mL of H_2O and acidified with 10 mL of HOAc to produce a yellow precipitate, which was collected and afforded 8.0 g of 12. Recrystallization from *i*-PrOH gave pure 12, mp 263–265 °C.

Method C. 3-(1*H*-Tetrazol-5-yl)-4*H*-pyrimido[2,1-*b*]-benzothiazol-4-one (18). A solution of *N*-[2-cyano-2-(1*H*-tetrazol-5-yl)ethenyl]benzothiazol-2-amine (12; 7.29 g, 0.027 mol) in 18 mL of H_2SO_4 was heated with stirring at 95 °C for 1 h. It was cooled and then carefully diluted with 7 mL of H_2O, followed by heating again at 95 °C for 2 h. This solution was cooled and diluted with 18 mL of H_2O, precipitating the crude product. This solid was dissolved in 7.5 mL of 40% NaOH and then acidified to precipitate 4.59 g (63%) of 18, mp 320 °C. Recrystallization from DMF gave analytically pure material, mp 329–330 °C.

Method D. *N*-[2-Cyano-2-(1*H*-tetrazol-5-yl)ethenyl]benzothiazol-2-amine (0.35 g, 0.0013 mol) was heated on a steam bath with 5 mL of CF_3CO_2H and 10 mL of 48% HBr for 1 h. CF_3CO_2H was removed under reduced pressure to afford 0.27 g (77%) of 18.

Method E. 3-(1*H*-Tetrazol-5-yl)-4*H*-pyrimido[2,1-*b*]-benzothiazol-4-imine Hydrochloride (20). *N*-[2-Cyano-2-(1*H*-tetrazol-5-yl)ethenyl]benzothiazol-2-amine (12; 3.0 g, 0.011 mol) was refluxed in 50 mL of 2 *N* ethanolic HCl for 4 h. The resulting yellow precipitate was collected. Crystallization from DMF afforded 0.5 g of 20, mp 274–275 °C.

Method F. 3-(1*H*-Tetrazol-5-yl)-4*H*-pyrimido[2,1-*b*]-benzothiazol-4-one Potassium Salt (19). 3-(1*H*-Tetrazol-5-yl)-4*H*-pyrimido[2,1-*b*]benzothiazol-4-one (3.5 g, 0.013 mol) was treated with 85% KOH (0.83 g, 0.013 mol) in 5 mL of water with stirring. The solution was stirred with Darco at 80–90 °C for 0.5 h and filtered through Celite, and the filtrate was diluted with 35 mL of EtOH. After the solution was left standing for 16 h, the solid was collected by filtration and air-dried to provide 3.08 g (71%) of 19, mp 326–328 °C. Recrystallization from MeOH raised the melting point to 336–339 °C.

Biological Test Methods. The tests employing rat passive cutaneous anaphylaxis, inhibition of methacholine bronchospasm in rats, and inhibition of allergic bronchospasm in sensitized rats were performed as previously described.[7]

Acknowledgment. The X-ray analyses of compound 29 were performed by the Molecular Structure Corp., College Station, TX. Other spectral and analytical data were obtained under the supervision of Charles M. Combs.

Notes

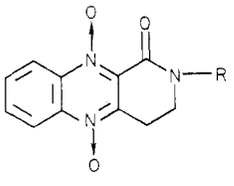
Pyridoquinoxaline *N*-Oxides. 2. Synthesis and Antibacterial Activity of Tricyclic Lactams[1]

Edward A. Glazer* and Joseph E. Presslitz

Pfizer Central Research, Groton, Connecticut 06340. *Received December 28, 1981*

A series of novel 3,4-dihydropyrido[3,4-*b*]quinoxalin-1(2*H*)-one 5,10-dioxides was synthesized using an intramolecular amidation reaction. The lactams were screened in vitro and in vivo against *Salmonella choleraesuis*, *Pasteurella multocida*, and *Escherichia coli*. An *N*-methyl analogue was the most potent member of this series, with antibacterial activity comparable to that of the commercially important quinoxaline 1,4-dioxide carbadox.

Quinoxaline 1,4-dioxides (QNO's) featuring carboxamide side chains,[2a] including fused five-ring lactams,[2b] are known to have good antibacterial activity. Based on our interest in the pyridoquinoxalines,[1] we chose to investigate the synthesis and antibacterial activity of the tricyclic 3,4-dihydropyrido[3,4-*b*]quinoxalin-1(2*H*)-one 5,10-dioxides 1.



1

Synthesis. We envisioned the desired lactams 1 as arising from the corresponding aminoethyl esters 2 by intramolecular amidation (Scheme I). A likely starting material for the synthesis of 2 was methyl 3-[2-(phenylsulfonyl)ethyl]quinoxaline-2-carboxylate (3). The phenylsulfonylethyl side chain in 3 was viewed as a latent vinyl group which could be unmasked by elimination of benzenesulfinic acid under basic conditions. The resulting unsaturated QNO 4 could then undergo Michael addition with amines to give the intermediate amino esters 2.

Preparation of precursor 3 involved condensation[3] of benzofurazan 1-oxide (BFO) with methyl 3-oxo-5-(phenylthio)pentanoate in the presence of catalytic amounts of calcium hydroxide to give the sulfide 5. The crude sulfide was treated with *m*-chloroperbenzoic acid to afford sulfone 3 in 40% overall yield. Reaction of 3 with primary amines in acetonitrile gave the targeted lactams **1a–h** in good yield (Table I). None of the proposed aminoethyl ester intermediates 2 could be detected under the reaction conditions. However, evidence for the intermediacy of 2 was obtained by treatment of sulfone 3 with diethylamine in acetonitrile to give the open-chained amino ester 6.

Biology. The 3,4-dihydropyrido[3,4-*b*]quinoxalin-1-ones (**1a–h**) were screened in vitro and in vivo against Gram-

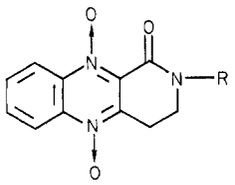
(1) For paper 1 in this series, see E. A. Glazer and L. R. Chappel *J. Med. Chem.*, under Articles in this issue.

(2) (a) F. Seng, C. Buchheim, K. Ley, O. Gloebusch, K. G. Metzer, and W. Elberfeld, U.S. Patent 3 682 906 (1972); K. Ley, O. Globusch, U. Eholzer, C. Stammheim, K. Nast, C. Buchheim, and K. G. Metzer, U.S. Patent 3 600 391 (1972). (b) T. H. Cronin and P. D. Hammen, U.S. Patent 3 753 387 (1973).

(3) (a) M. J. Haddadin and C. H. Issidorides, *Tetrahedron Lett.*, 3253 (1965). (b) *Heterocycles*, 4, 767 (1976).

0022-2623/82/1825-0868$01.25/0

Table I. Pyrido[3,4-*b*]quinoxalin-1-one 5,10-Dioxides (1)



no.	R	mp, °C	yield,[a,b] %	formula	anal.
1a	H	177-180	64	$C_{11}H_9N_3O_3$	C, H, N
1b	CH_3	155-156	85	$C_{12}H_{11}N_3O_3$	C, H, N
1c	CH_3CH_2	152-153	51	$C_{13}H_{13}N_3O_3$	C, H, N
1d	CH_2CH_2OH	175-176	53	$C_{13}H_{13}N_3O_4$	C, H, N
1e	$CH_2CH{=}CH_2$	158-159	50	$C_{14}H_{13}N_3O_3$	C, H, N
1f	CH_2CH_2N-piperidyl	135-137	73	$C_{18}H_{22}N_4O_3 \cdot 0.25H_2O$	C, H, N
1g	cyclohexyl	188-190	59	$C_{17}H_{19}N_3O_3$	C, H, N
1h	$CH_2CH_2OCH_3$	161-162	46	$C_{14}H_{15}N_3O_4 \cdot 0.33H_2O$	C, H, N

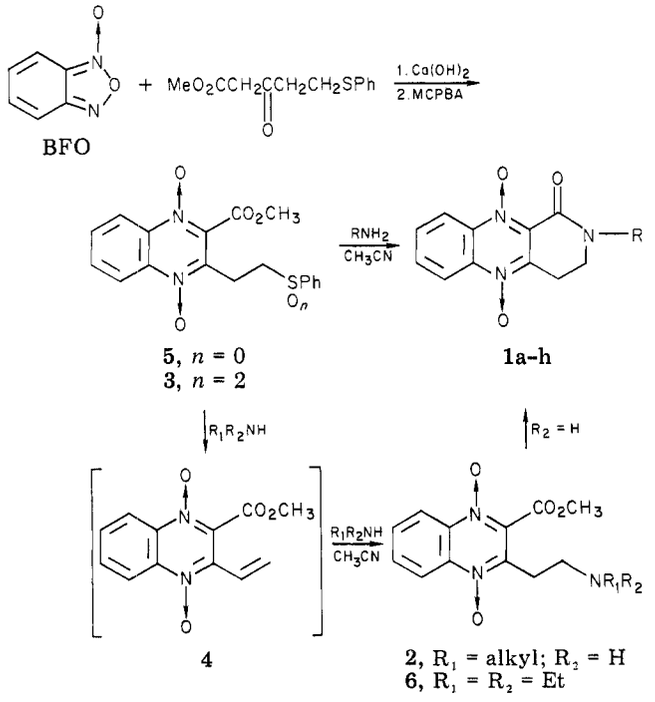
[a] Yield after purification. [b] Reference 11.

Table II. In Vitro Antibacterial Activity

	MIC,[a] μg/mL			
compd	*Strep. pyogenes*	*E. coli*	*S. choleraesuis*	*P. multocida*
1a	1.56	1.56	1.56	≤0.39
1b	1.56	0.78	1.56	1.56
1c	1.56	3.12	1.56	6.25
1d	6.25	12.5	12.5	12.5
1e	0.78	3.12	1.56	6.25
1f	≤0.39	3.12	1.56	25.0
1g	3.12	50.0	50.0	25.0
1h	0.78	1.56	3.12	3.12
carbadox	0.19	0.39	0.19	0.78

[a] Minimum inhibitory concentration; determined under anaerobic conditions as described under Experimental Section.

Scheme I



negative and Gram-positive bacteria. As shown in Table II, compounds **1a–c,e,h** had the best in vitro activity of the tricyclic QNO's. When evaluated in vivo against *S. choleraesuis* at 25 mg/kg, the *N*-methyl analogue **1b** was clearly the most potent lactam, with efficacy comparable to that of the commercially important QNO carbadox[4] (Table III). A more detailed comparison of the relative potencies of **1b** and carbadox against several important veterinary pathogens is shown in Table IV. As indicated by the PD_{50} values, **1b** had comparable activity against *E. coli*, *S. choleraesuis*, and *P. multocida*. In addition, **1b** was equivalent to carbadox when evaluated in vitro (MIC's = 0.09 μg/mL) against *T. hyodysenteriae*, the causative agent in swine dysentery.[5]

Table III. In Vivo Activity against *Salmonella choleraesuis*

	% protection at 25 mg/kg[a]	
compd	po	sc
1a	0	80
1b	100	100
1c	50	70
1d	0	0
1e	0	30
1f	0	30
1g	0	0
1h	60	90
carbadox	100	100

[a] Determined as described under Experimental Section.

Table IV. Comparative in Vivo Antibacterial Activity of **1b** and Carbadox

		PD_{50},[a] mg/kg	
microorganism		1b	carbadox
E. coli	po	8.53 ± 1.06	5.30 ± 1.65
	sc	6.39 ± 0.75	6.44 ± 1.57
S. choleraesuis	po	5.10 ± 0.62	4.47 ± 1.17
	sc	2.96 ± 0.81	5.44 ± 2.26
P. multocida	po	4.70 ± 0.7	6.14 ± 1.72
	sc	3.80 ± 0.60	4.05 ± 1.51

[a] Determined as described under Experimental Section; 95% confidence limits; average of two determinations.

Experimental Section

Biology. Infections in Mice. Male and female mice weighing 11–13 g obtained from Blue Spruce Farms, Alamont, NY, were used in all experiments. Acute systemic infections were produced by intraperitoneal inoculation of one to ten times the number of

(4) Carbadox (Mecadox; Pfizer Inc., New York, NY) is highly effective as a growth promotant for swine: G. W. Thrasher, J. E. Shrively, C. E. Askelson, W. E. Babcock, and R. R. Chalquest, *J. Anim. Sci.*, **31**, 333 (1970).

(5) (a) D. J. Taylor and T. J. L. Alexander, *Br. Vet. J.*, **127**, 58 (1971). (b) D. L. Harris, J. M. Kinyon, M. T. Mullin, and R. D. Glock, *Can. J. Comp. Med.*, **36**, 74 (1972). (c) R. D. Glock and D. L. Harris, *Vet. Med. Small Anim. Clin.*, **67**, 65 (1972).

organisms necessary to kill 100% of the nonmedicated mice in 4 days. Standardized bacterial cultures of *Escherichia coli* and *Salmonella choleraesuis* were suspended in 5% hog gastric mucin, and *Pasteurella multocida* was suspended in brain–heart infusion broth. Treatment (10 mice per group) was initiated 0.5 h after infection. A second treatment was administered at 4.0 h and a third at 24 h. A 50% protective dose value (PD_{50}) was calculated by the probit method.[6]

Antimicrobial Susceptibility Tests. Minimum inhibitory concentrations were determined anaerobically as previously described.[7]

Chemistry. General. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded on a Varian T-60 spectrometer with Me_4Si as internal standard. IR spectra were determined with a Perkin-Elmer Model 21 spectrophotometer. UV spectra were recorded on a Cary Model 14 spectrophotometer. Mass spectra were obtained with a Perkin-Elmer RMU-6E mass spectrometer. All compounds gave spectral data consistent with the proposed structure. Microanalyses were performed by the Pfizer Analytical Department.

Methyl 3-[2-(Phenylsulfonyl)ethyl]quinoxaline-2-carboxylate 1,4-Dioxide (3). To a solution of methyl 3-oxo-5-(phenylthio)pentanoate[8] (5.10 g, 0.021 mol) in 115 mL of 2-propanol/chloroform (8:1) was added benzofurazan 1-oxide (2.90 g, 0.021 mol) and calcium hydroxide (700 mg, 9.40 mmol). The reaction mixture was heated at 60 °C for 2 h, washed with water, dried (magnesium sulfate), and evaporated to give methyl 3-[2-(phenylthio)ethyl]quinoxaline-2-carboxylate 1,4-dioxide (**5**) as an oily yellow solid. Without further purification,[9] the sulfide was dissolved in 500 mL of chloroform, and a solution of *m*-chloroperbenzoic acid (7.4 g, 0.043 mol) in 50 mL of chloroform was added dropwise. The resulting solution was stirred for 0.5 h at room temperature, washed with 10% sodium bicarbonate, and evaporated to give the sulfone as a yellow solid. The crude product was purified by trituration with ether/acetone (20:1) to afford 3.3 g (40%) of **3**, mp 180–183 °C. Anal. ($C_{18}H_{16}N_2O_6S$) C, H, N.

2-Methyl-3,4-dihydropyrido[3,4-*b*]quinoxalin-1(2*H*)-one 5,10-Dioxide (1b). A solution of **3**[10] (1.0 g, 2.58 mmol) in 400 mL of acetonitrile was perfused with anhydrous methylamine gas for 20 min at room temperature. The reaction mixture was stirred for an additional 2 h and then evaporated to dryness.[11] The crude product was chromatographed (silica gel, 10:1 chloroform/MeOH as eluent) to give 540 mg (85%) of **1b**, mp 155–156 °C. The NMR spectrum ($CDCl_3$) had characteristic absorption at δ 3.15 (s, 3 H, N-CH_3), 3.60 (m, 4 H, CH_2CH_2), 7.90 (m, 2 H, H-8 and H-7), 8.60 (m, 2 H, H-6 and H-9). The infrared spectrum (KBr) had a strong lactam carbonyl band at 1666 cm^{-1}. Anal. ($C_{18}H_{16}N_2O_6S$) C, H, N.

Methyl 3-[2-(Diethylamino)ethyl]quinoxaline-2-carboxylate 1,4-Dioxide (6). Compound **6** was prepared by treating sulfone **3** with excess diethylamine in acetonitrile under conditions identical with those for the synthesis of the pyrido-[3,4-*b*]quinoxalines. The crude product was purified by chromatography (silica gel, 5% methanol/chloroform as eluent) to give **6** (40%) as a light-sensitive yellow solid, mp 100–103 °C. Anal. ($C_{16}H_{21}N_3O_4 \cdot 0.5H_2O$) C, H, N.

Acknowledgment. We thank Larry Pisko and Jan Watrous for technical assistance.

(6) H. E. Batson, "An Introduction to Statistics in the Medical Sciences", Burgess Publishing Co., Minneapolis, MN, 1957, pp 64–69.
(7) John P. Dirlam, Leonard J. Czuba, Beryl W. Dominy, Richard B. James, Richard M. Pezzullo, Joseph E. Presslitz, and Wendell W. Windisch, *J. Med. Chem.*, **22**, 1118 (1979).
(8) B. M. Trost and R. A. Fung, *J. Org. Chem.*, **39**, 2648 (1974).
(9) The crude sulfide **5** could be purified by recrystallization from methanol, mp 96–98 °C. Anal. ($C_{18}H_{16}N_2O_6S$) C, H, N.
(10) The pyrido[3,4-*b*]quinoxalines could also be obtained from sulfide **5**. However, the cyclization proceeds more rapidly and in higher yield from the sulfone (**3**).
(11) The nongaseous amines employed in the synthesis of **1d–h** were used in a tenfold molar excess. Reaction times in these cases were 24–36 h.

Synthesis and Tissue Distribution Study of Iodine-Labeled Benzyl- and Xylylamines

Kenneth M. Tramposch,*,† Hank F. Kung,‡ and Monte Blau‡

Medical Research Department, V.A. Medical Center, and Department of Nuclear Medicine, State University of New York at Buffalo, Buffalo, New York 14215. *Received June 29, 1981*

Four iodine-125 labeled mono- and diamines were prepared and evaluated as potential brain-imaging agents. The diamines are analogues of the previously reported selenium-75 labeled diamines, which show high brain uptake and retention. All of the radioiodinated amines display high initial brain uptake in rats after intravenous injection (1.7–2.4% dose/organ). The xylylenediamines show prolonged brain retention ($t_{1/2} \approx 18$ h), which is desirable for brain imaging. In contrast, the benzylamine is rapidly cleared from brain tissue ($t_{1/2} \approx 15$ min).

Conventional single-photon radiopharmaceuticals currently available for clinical brain scanning are chelation compexes of ^{99m}Tc. These water-soluble complexes are excluded from normal brain tissue by the presence of an intact blood–brain barrier. Measurement of the state of the blood–brain barrier is useful in some cases. However, present emphasis in nuclear medicine is directed at the regional determination of cerebral blood flow, metabolism, and pH. The development of new lipid-soluble single-photon radiopharmaceuticals which are able to penetrate the blood–brain barrier to reflect regional blood flow would have a significant impact on the management of neurological diseases.[1]

Recently, we reported the synthesis and brain localization of a series of ^{75}Se-labeled diamines **1**.[2,3]

$$(R)_2N(CH_2)_nSe(CH_2)_nN(R)_2$$
1

The high energy γ emission of ^{75}Se (120 days, 465 keV) and the relatively high patient radiation dose limits the

*Address correspondence to Westwood Pharmaceuticals, Buffalo, NY 14213.
†V.A. Medical Center.
‡SUNY at Buffalo.

(1) T. C. Hill, *J. Nucl. Med.*, **21**, 1197 (1980).
(2) H. F. Kung and M. Blau, *J. Nucl. Med.*, **21**, 147 (1980).
(3) H. F. Kung and M. Blau, *J. Med. Chem.*, **23**, 1127 (1980).