Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 10, pp. 1105-1110, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00181-3

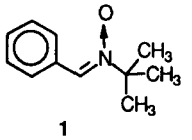
SYNTHESIS AND RADICAL SCAVENGING ACTIVITY OF 3,3-DIALKYL-3,4-DIHYDRO-ISOQUINOLINE 2-OXIDES

Ronald C. Bernotas,* Craig E. Thomas, Albert A. Carr, Thaddeus R. Nieduzak, Ginette Adams, David F. Ohlweiler, and David A. Hay

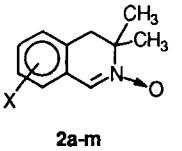
Hoechst Marion Roussel, 2110 East Galbraith Road, Cincinnati, Ohio 45215

Abstract: The syntheses and antioxidant activities of several cyclic nitrones related to phenyl t-butyl nitrone (PBN) are described. These nitrones may act as radical scavengers and have potential uses in the treatment of stroke and septic shock.

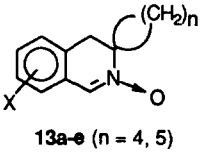
In vivo production of excess free radicals plays a role in several human diseases. Septic shock, triggered by bacterial endotoxins, is linked to radical-induced tissue injury which can lead to pulmonary and cardiac collapse and even death.[1] Reperfusion of hypoxic tissue following stroke generates high concentrations of oxygen radicals which can lead to irreversible destruction of cells.[2] In stroke, septic shock, and related diseases radical scavengers might be used to trap high energy radicals. In doing so, scavengers generate less reactive species which would thereby minimize tissue damage. One molecule under investigation as a therapeutic agent is phenyl t-butyl nitrone **1** (PBN). PBN has been demonstrated to reduce lethality associated with septic shock in rats[3] and to reduce neuronal cell injury in a gerbil model of stroke.[4] Our interest in developing treatments for both these diseases led to our investigation of 3,4-dihydro-3,3-dimethyl-isoquinoline 2-oxide **2a** (X = H), a cyclized version of PBN. We speculated **2a** might be a more potent radical trap due to the constrained and



1



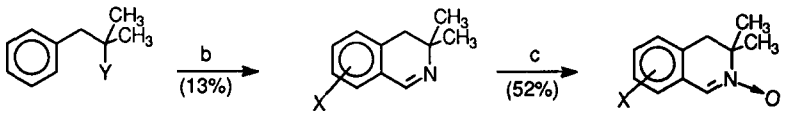
2a-m



13a-e (n = 4, 5)

potentially more coplanar orientation of the nitrone and aromatic ring.[5] Tying the t-butyl group back may also expose the nitrone carbon making radical trapping easier on steric grounds. In an earlier communication, we reported that **2a** is indeed more potent than PBN in several tests of antioxidant and radical trapping activity[6] and in preventing endotoxin-induced mortality.[7] Here we describe the syntheses of aryl-substituted nitrones **2a–m** as well as related spiro compounds **13a–e** and their activity as inhibitors of peroxyl radical-dependent lipid peroxidation.

SCHEME 1

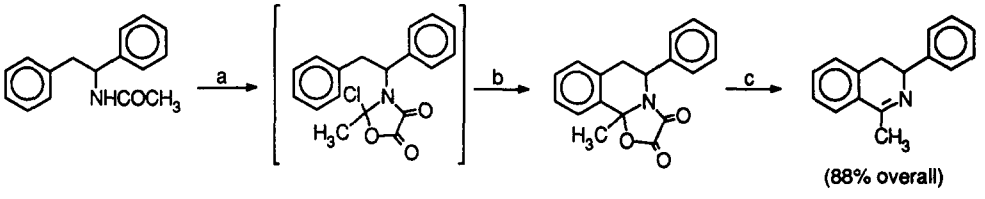


(a) $NaCN/AcOH/H_2SO_4$ (b) PPA (c) $Na_2WO_4/H_2O_2/H_2O/EtOH$

While numerous 3,4-dihydro-isoquinolines have been synthesized, few 3,3-dialkyl-3,4-dihydro-isoquinolines or their corresponding nitrones have been described.[8] To synthesize **2a**, commercially available **3** was subjected to a Ritter reaction[9] affording **4a** which was cyclized to **5a** with polyphosphoric acid in a low-yielding Bischler-Napieralski reaction[10] (Scheme 1). The imine was oxidized to **2a** using aqueous hydrogen peroxide in ethanol catalyzed by sodium tungstate.[11] The oxidation of imines typically required long reaction times (several days versus 2-3 hours) and multiple additions of hydrogen peroxide. Nonetheless, a good yield of **2a** was obtained.[12] In vitro testing of **2a** showed over a 100-fold increase in hydroxyl radical trapping ability relative to PBN and a ten-fold increase in its lipid antioxidant activity.[6] These promising results led us to synthesize substituted analogs for further testing.

The initial target was **2b**, the 7-chloro analog of **2a**. Formamide **4b** was readily prepared[13] but gave virtually no 3,4-dihydro-isoquinoline using PPA or other reagents such as H_2SO_4, P_2O_5, or $POCl_3$; only alkene-containing compounds were found. To overcome this facile elimination, we turned to a modification of the Bischler-Napieralski reaction developed by Merck chemists to synthesize 3,4-dihydro-3-phenyl-isoquinolines

SCHEME 2

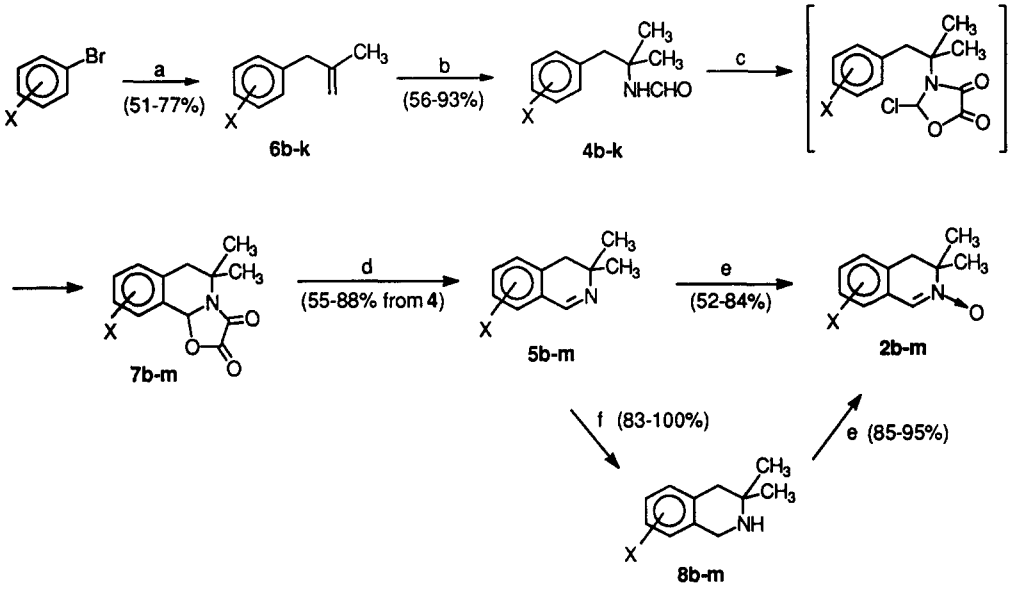


(a) $ClCOCOCl/CH_2Cl_2$ (b) $FeCl_3$ (anhydrous) (c) H_2SO_4/CH_3OH

(Scheme 2).[14] This method involved treating amides with oxalyl chloride followed by anhydrous ferric chloride to generate oxazolidine-4,5-diones which upon heating in acidic methanol produced 3,4-dihydro-isoquinolines. Thus, when **4b** was subjected to these conditions, oxazolidine-2,3-dione **7b** was formed in 75% crude yield (Scheme 3). Heating **7b** in acidic alcohol provided imine **5b** which was pure by ^1H and ^{13}C NMR.[15] Oxidation of imine **5b** as described above gave nitrone **2b** but the reaction suffered the same problems of long reaction times (ca. 7 days) and required multiple additions of oxidant. This problem was overcome by first reducing imine **5b** to amine **8b** and then oxidizing the crude product to **2b**. The oxidation time was decreased to three hours and side reactions were minimized.

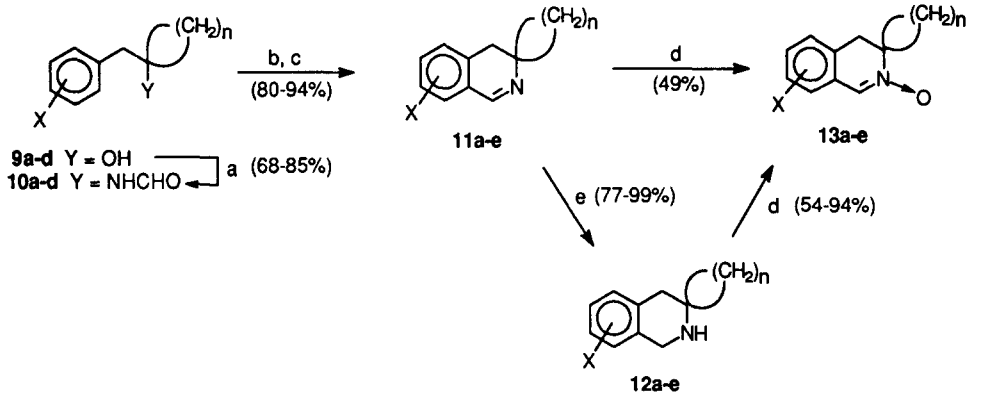
Using this route, a series of 3,4-dihydro-3,3-dimethyl-isoquinoline 2-oxides with one or more substituents on the aromatic ring were targeted (**2c–m**, see Table). Since alkenes can also serve as substrates for the Ritter

SCHEME 3



(a) Mg^0/THF, then $CuBrS(CH_3)_2$ (cat.)/3-chloro-2-methyl-propene (1-2 equiv) (b) NaCN (1.1 equiv)/AcOH/H_2SO_4 (c) oxalyl chloride (1.1 equiv)/CH_2Cl_2, then $FeCl_3$ (1.2 equiv) (d) H_2SO_4/CH_3OH (e) $Na_2WO_4{\cdot}2H_2O$ (0.05 equiv)/30% H_2O_2 (xs)/H_2O/EtOH (f) $NaBH_4$ (1-2 equiv)/CH_3OH

SCHEME 4



(a) NaCN (~1.1 equiv)/AcOH/H_2SO_4 (b) oxalyl chloride (1.1 equiv)/CH_2Cl_2, then $FeCl_3$ (1.2 equiv) (c) 5% H_2SO_4/CH_3OH (d) $Na_2WO_4 \cdot 2H_2O$ (0.05 equiv)/30% aqueous H_2O_2 (xs)/H_2O/EtOH (e) $NaBH_4$ (1-2 equiv)/CH_3OH

reaction[16] and many aryl Grignards are readily available via their respective aryl bromides, alkenes **6c–m** were synthesized by alkylation of aryl Grignards with 3-chloro-2-methyl-propene (Scheme 3 and Table).[17] These alkenes were then converted into formamides **4c–k**.[18] We were pleased to find that application of Merck's

modified Bischler-Napieralski reaction to **4c–k** gave good to excellent yields in every case. Unsymmetrical formamides **4d** and **4h** each gave two 3,4-dihydro-isoquinolines, readily separable by flash chromatography. The major isomer in both cases was the 6-substituted product. Direct oxidation or two step reduction/oxidation gave nitrones **2c–m**. A series of spirocyclic nitrones **13a–e** was prepared using essentially identical chemistry (Scheme 4). As in the syntheses of nitrones **2**, alcohols **9**[19] were converted into formamides **10** and these in turn were cyclized using Merck's two step procedure to give imines **11**. Direct oxidation of these imines or, more efficiently, reduction to amines **12** followed by oxidation afforded spirocyclic nitrones **13a–e** (see Table).

TABLE

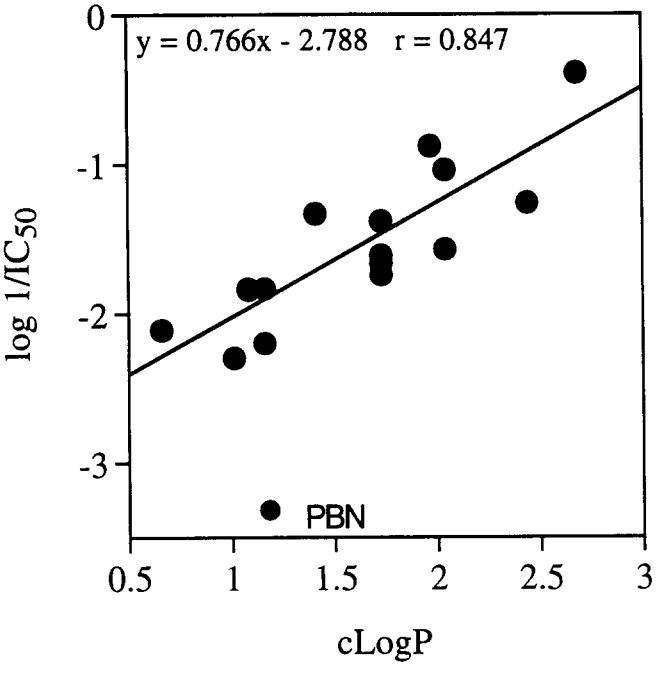
Formamide	X	Nitrone	X	n	IC_{50} (μ m) antioxidant
		PBN			2,170
4a	H	**2a**	H	--	200
4b	4-Cl	**2b**	7-Cl	--	41
4c	2-Cl	**2c**	5-Cl	--	24
4d	3-Cl	**2d**	6-Cl	--	55
		2e	8-Cl	--	45
4e	3,5-Cl_2	**2f**	6,8-Cl_2	--	18
4f	3-F	**2g**	6-F	--	160
4g	4-F	**2h**	7-F	--	67
4h	3-OCH_3	**2i**	6-OCH_3	--	69
		2j	8-OCH_3	--	nd
4i	4-OCH_3	**2k**	7-OCH_3	--	67
4j	3,4-$(OCH_3)_2$	**2l**	6,7-$(OCH_3)_2$	--	130
4k	3-F, 6-OCH_3	**2m**	5-OCH_3, 8-F	--	56
10a	H	**13a**	H	4	21
10b	H	**13b**	H	5	8
10c	4-Cl	**13c**	7-Cl	5	3
10d	3-OCH_3	**13d**	6-OCH_3	5	11
		13e	8-OCH_3	5	38

The compounds (**2a–m**, **13a–e**) were evaluated for their ability to inhibit peroxyl radical-dependent oxidation of liposomes. Briefly, liposomes were prepared from soybean phosphatidylcholine by ethanol injection. Oxidation was initiated using the peroxyl radical precursor 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) which undergoes thermolysis and addition of dioxygen to produce the initiating peroxyl radicals. Reactions were conducted at 37 °C with 50 mM AAPH and aliquots of the reaction mixtures were then analyzed for peroxidation at 0, 15, and 30 minutes using the thiobarbituric acid reactive substances (TBARS) test.[20] The nitrone concentration required to inhibit peroxidation by 50% (IC_{50}) was determined using the 30 min time point.

From the Table, it is apparent that the cyclic nitrones are all superior to PBN as inhibitors of lipid peroxidation. The activity of the compounds in this model system is an overall reflection of their ability to form adducts with AAPH-derived radicals, as well as carbon and oxygen centered radicals derived from the polyunsaturated fatty acids. Computer generated 1-octanol/water partition coefficients (cLogP) were determined via a fragment-based approach[21] using the program PC MODELS®.[22] When the cLogP values were plotted

against the log $1/IC_{50}$ values, a reasonable correlation was obtained (see Figure). Variances from the correlation are likely due to the differences among the compounds with respect to which radical species they react with most readily. For example, the more hydrophilic members of the group (i.e. **2a**, **2i–l**) likely react to a greater extent with soluble AAPH-derived radicals than do the more hydrophobic compounds (i.e. **2f**, **13c–e**) which would react preferentially with lipid-derived radicals. Nonetheless, it is obvious that PBN falls considerably off this line indicating that the cyclic nitrones are inherently more active as radical traps than PBN.

FIGURE



In conclusion, a series of cyclic nitrones has been prepared with a core 3,3-dialkyl-3,4-dihydroisoquinoline motif. In a peroxyl radical-dependent liposome oxidation assay, these compounds were all at least 10-fold better inhibitors of liposome oxidation compared to phenyl t-butyl nitrone (PBN). The best and most lipophilic compound (**13c**) was over 500-fold more potent than PBN at blocking oxidation. A plot of lipophilicity versus the inhibition demonstrates a strong correlation within the cyclic nitrone series but also suggests the cyclic nitrones are inherently more active as radical trapping agents. The nitrones are currently undergoing evaluation as potential therapeutic agents for stroke, septic shock, and other therapeutic targets in which free radicals play an important role.

Acknowledgments: We thank Dr. Roy Vaz for calculating cLogP values and Dr. John M. Kane for helpful discussions.

REFERENCES AND NOTES

1. Biasi, F.; Chiarpotto, E.; Lanfranco, G.; Capra, A.; Zummo, U.; Chiappino, I.; Scavazza, A.; Albano, E.; Poli, G. *Free Rad. Biol. Med.* **1994**, *17*, 225.
2. Kontos, H. A. *Chem.-Biol. Interactions* **1989**, *72*, 229.
3. Hamburger, S. A.; McCay, P. B. *Circ. Shock* **1989**, *29*, 329.
4. Oliver, C. N.; Starke-Reed, P. E.; Stadtman, E. R.; Liu, G. J.; Carney, J. M.; Floyd, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5144.
5. More recent studies indicate PBN is more planar than **2a**. Thomas, C. E.; Ohlweiler, D. F.; Carr, A. A.; Nieduzak, T. R.; Hay, D. A.; Adams, G.; Vaz, R.; Bernotas, R. C. *J. Biol. Chem.* **1996**, *271*, 3097.
6. Thomas, C. E.; Carney, J. M.; Bernotas, R. C.; Hay, D. A.; Carr, A. A. *Annals NY Acad. Sci.* **1994**, *234*, 243.
7. French, J. F.; Thomas, C. E.; Downs, T. R.; Ohlweiler, D. F.; Carr, A. A.; Dage, R. C. *Circ. Shock* **1994**, *43*, 130.
8. Seeger, E.; Engel, W.; Teufel, H.; Machleidt, H. *Chem. Ber.* **1970**, *103*, 1674. Nordlander, J. E.; Payne, M. J.; Njoroge, F. G.; Balk, M. A.; Laikos, G. D.; Vishwanath, V. M. *J. Org. Chem.* **1984**, *49*, 4107.
9. Ritter, J. J.; Kalish, J. *Organic Synthesis*, **1964**, *44*, 44.
10. Review: Whaley, W. M.; Govindachari, T. R. In *Organic Reactions*; Adams, R., Ed.; Wiley and Sons: New York, 1951; Vol. 6, pp 74–150.
11. Murahashi, S.; Shiota, T.; Imada, Y. *Org. Synth.* **1991**, *70*, 265.
12. All nitrones gave satisfactory elemental analyses (C, H, and N $\pm$ 0.4) and spectral data (^{1}H, ^{13}C NMR, CIMS or EIMS, and IR).
13. Baker, G. B.; Coutts, R. T.; Benderly, A.; Cristofoli, W. A.; Dewhurst, W. G. *Can. J. Pharm. Sci.* **1981**, *15*, 71.
14. Larsen, R. D.; Reamer, R. A.; Corley, E. G.; Davis, P.; Grabowski, E. J. J.; Reider, P. J.; Shinkai, I. *J. Org. Chem.* **1991**, *56*, 6034.
15. Satisfactory elemental analyses were difficult to obtain so the crude imines were generally used without further purification.
16. See Krimen, L. I.; Cota, D. J. In *Organic Reactions*; Dauben, W. G., Ed.; Wiley and Sons: New York, 1969; Vol. 17, pp 213–325.
17. This reaction could be run with or without addition of the copper catalyst. For examples, see Najera, C.; Sansano, J. M. *Tetrahedron Lett.* **1993**, *34*, 3781. Rahman, M. T.; Nahar, S. K. *J. Organomet. Chem.* **1987**, *329*, 133. Residual biaryl contaminants were readily removed after the Ritter reaction.
18. Aryl substituted alcohols of type **3** were also used if readily available.
19. The alcohols were synthesized by addition of a benzyl Grignard reagent to cyclopentanone or cyclohexanone. Pallos, L.; Zolyomi, G.; Budai, Z; Komlos, E.; Petocz, L. E. Hungarian patent 151 865, 1965; *Chem. Abstr.* **1965**, *62*, 16125b.
20. Thomas, C. E.; McLean, L. R.; Parker, R. A.; Ohlweiler, D. F. *Lipids* **1992**, *27*, 543.
21. Leo, A. J. *Chem. Rev.* **1993**, *93*, 1281.
22. Available from Daylight Chemical Information Systems, Inc., Irvine, CA, USA.

(*Received in USA* 9 *February* 1996; *accepted* 17 *April* 1996)