3. E. R. Smith and J. M. Davidson, *Endocrinology* **80**, 725 (1967).
4. L. W. Christensen and L. G. Clemens, *Endocrinology* **95**, 984 (1974).
5. J-A. Gustaffson, S. A. Gustaffson, M. Ingleman-Sundberg, A. Pousette, A. Stenberg and Ö. Wrange, *J. Steroid. Biochem.* **5**, 855 (1974).
6. J. Bitman, H. C. Cecil and J. J. Harris, *Environ. Hlth Perspect.* **1**, 145 (1972).
7. B. S. McEwen, *Scient. Am.* **235**, 48 (1976).
8. J. Bitman and H. C. Cecil, *J. agric. Fd Chem.* **18**, 1108 (1970).
9. R. J. Lutz, R. L. Dedrick, H. B. Matthews, T. E. Eling and M. W. Anderson, *Drug Metab. Dispos.* **5**, 386 (1977).
10. G. W. Lucier and O. S. McDaniel, *Ann. N.Y. Acad. Sci.*, in press.
11. P. Skett, P. Eneroth, J. A. Gustaffson and C. Sonnenschein, *Endocrinology* **100**, 1090 (1977).
12. H. B. Matthews and M. W. Anderson, *Drug Metab. Dispos.* **3**, 371 (1975).
13. G. W. Lucier, O. S. McDaniel, C. M. Schiller and H. B. Matthews, *Drug Metab. Dispos.* **6**, 584 (1978).
14. G. W. Lucier, G. J. Davis and J. A. McLachlan, in *Developmental Toxicology of Energy-Related Pollutants*, (eds. D. Mahlum, M. Sikov, P. L. Hackett and F. Andrew), U.S. Dept of Energy Technical Information Center, pp. 188 (1978).
15. J. A. Goldstein, P. Hickman, H. Bergman, J. D. McKinney and M. P. Walker, *Chem. Biol. Interact.* **17**, 69 (1977).
16. A. Poland and E. Glover, *Molec. Pharmac.* **13**, 924 (1977).
17. R. M. Welch, W. Levin and A. H. Conney, *J. Pharmac. exp. Ther.* **155**, 167 (1967).
18. G. W. Lucier, *International Congress of Pharmacology*, Paris, France, July 1978, Abstr. 2555.

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2-Chloro-11-(1-piperazinyl)dibenz[b, f][1, 4]oxazepine (Amoxapine), an antidepressant with antipsychotic properties—A possible role for 7-hydroxyamoxapine

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Amoxapine, 2-chloro-11-(1-piperazinyl)dibenz[b,f][1,4]-oxazepine (Anamox), is the desmethyl derivative of loxapine, a potent neuroleptic of the dibenzoxazepine class [1–3]. Various clinical studies have shown that amoxapine, unlike loxapine, is a potent antidepressant [4–7]. Moreover, other investigators have reported that amoxapine possesses both antidepressant and neuroleptic activities in laboratory animals and in man [8, 9]. Since amoxapine is metabolized in mammals to 7-hydroxyamoxapine (7-OH-Amox) and to 8-hydroxyamoxapine (8-OH-Amox) [10], it was of interest to study not only amoxapine, but also its hydroxylated metabolites for their effects in neurochemical tests suggestive of antipsychotic and antidepressant actions. Three *in vitro* tests were chosen: (a) synaptosomal uptake of the labeled biogenic amines [^{3}H]-L-norepinephrine ([^{3}H]NE) and [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT); (b) dopamine-sensitive adenylate cyclase (DSAC); and (c) membrane [^{3}H]spiroperidol bindings.

Table 1. Calculated IC_{50} values for amoxapine and its analogs in blocking the accumulation of monoamines by crude synaptosomal fractions of rat diencephalon midbrain*

Drug	Inhibition of norepinephrine uptake (2×10^{-8} M)	Inhibition of serotonin uptake (2×10^{-8} M)
	IC_{50} (nM ± S.E.M.)	IC_{50} (nM ± S.E.M.)
Amoxapine	22.5 ± 2.0	566.5 ± 51.0
7-OH-Amox	16.6 ± 1.6	424.2 ± 14.8
8-OH-Amox	33.8 ± 7.5	323.2 ± 25.5
Imipramine	16.8 ± 2.3	55.8 ± 1.2

*Each drug was studied at four to five levels of concentration ranging from 0.005 to 1.0 μM. Each determination was done in duplicate and in three different tissue pools. The IC_{50} values were determined by log probit analysis of the data and represent the concentration of each drug required to inhibit the uptake of either [^{3}H]NE or [^{3}H]-5-HT at 2×10^{-8} M by 50 per cent.

Loxapine, amoxapine, 7-OH-Amox and 8-OH-Amox were synthesized by Dr. C. F. Howell of Lederle Laboratories (Pearl River, NY). Imipramine·HCl (Ciba-Geigy Corp., Summit, NJ) and spiroperidol (Janssen Pharmaceutical, Belgium) were generously donated to us. Radiolabeled [^{3}H]-L-norepinephrine, [^{3}H]-5-hydroxytryptamine and [^{3}H]spiroperidol were all purchased from New England Nuclear, Boston, MA. Brian tissues were from 200 to 300 g male Wistar rats obtained from Royalhart Farms (New Hampton, NY).

The preparations of synaptosomal fractions and the determinations of [^{3}H]NE and [^{3}H]-5-HT uptake activities were measured essentially by the method of Kuhar *et al.* [11], with the modifications described by Gal *et al.* [12].

Adenylate cyclase (AC) activity was assayed in striatal tissues by the method described by Kebabian *et al.* [13], in the presence or absence of dopamine (DA) and/or test compounds. The amount of cyclic adenosine-3′,5′-monophosphate (cAMP) formed was determined by the protein binding assay of Gilman [14]. The preparation of membrane fractions and the determinations of [^{3}H]spiroperidol binding were described in an earlier report [15]. Protein content in the tissue samples was determined by the method of Lowry *et al.* [16].

As can be seen in Table 1, amoxapine, 7-OH-Amox and 8-OH-Amox substantially inhibited the synaptosomal uptake of [^{3}H]NE, while having little effect on [^{3}H]-5-HT uptake. Imipramine, on the other hand, influenced the uptake of both monoamines to approximately the same degree. Amoxapine and 7-OH-Amox were equipotent with imipramine in inhibiting [^{3}H]NE uptake, whereas 8-OH-Amox was about half as potent as imipramine. No significant inhibitory effects were detected for amoxapine or 8-OH-Amox on the DA-induced stimulation of AC in striatal homogenates (Table 2). However, 7-OH-Amox and loxapine were very active in inhibiting the rise in cyclic adenosine-3′,5′-monophosphate formation induced by 30 μM DA. Moreover, 7-OH-Amox and loxapine

Table 2. Effects of amoxapine and its analogs on dopamine-stimulated adenylate cyclase of rat striatal homogenates*

Drug	cAMP formed (pmoles/mg protein/2.5 min ± S.E.M.) Basal activity	Stimulation by 30 μM dopamine	Per cent inhibition of stimulation by 30 μM dopamine ±S.E.M.
None	66.89 ± 7.008	143.91 ± 12.490	
Amoxapine	75.17 ± 8.734	128.31 ± 9.180	NS†
7-OH-Amox	78.10 ± 8.770	106.37 ± 3.930‡	65.2 ± 6.70
8-OH-Amox	77.60 ± 6.114	142.93 ± 7.361	NS
Loxapine	59.57 ± 7.658	91.26 ± 2.870§	60.0 ± 3.73

*All drugs were tested at 0.2 μM. Values represent the means ±S.E.M. of four replicate experiments. The mean increment in cAMP values induced by 30 μM dopamine in control samples was normalized to 100 percent enzyme stimulation.

†NS, not significantly different from stimulated control values.

‡Statistically different from stimulation control values at $P < 0.05$ by two-tailed Student's *t*-test.

§$P < 0.01$.

displayed high affinities for [^{3}H]spiroperidol binding sites (Table 3). The potency of 7-OH-Amox was twice that of loxapine for displacing [^{3}H]spiroperidol. Neither imipramine nor 8-OH-Amox was active in the spiroperidol binding assay. Amoxapine, however, showed moderate activity in this test.

The results of these *in vitro* studies tend to confirm the findings of Greenblatt *et al.* [9], and also those of Chermat *et al.* [8], on the dual spectrum of activity of amoxapine in laboratory animals. In common with imipramine, amoxapine and its two hydroxylated metabolites influenced rather strongly the synaptosomal uptake of [^{3}H]NE. However, unlike imipramine, these dibenzoxazepine derivatives had little effect on the uptake of [^{3}H]-5-HT.

Our results also indicate that amoxapine possesses neurochemical properties in common with loxapine, a major neuroleptic. It appears, however, that amoxapine may not be a neuroleptic *per se*. For example, amoxapine is inactive in preventing the stimulation of adenylate cyclase by DA in striatal homogenates, but it is moderately active in displacing [^{3}H]spiroperidol from its sites. In contrast, 7-OH Amox is strongly active in both assay procedures. In fact, 7-OH-Amox displays greater affinities than loxapine for the DA postsynaptic receptors. It is possible that the moderate activity shown by amoxapine in the spiroperidol assay is due to partial enzymic conversion to 7-OH-Amox in the incubation mixture. Further experiments are in progress to verify this assumption. Nevertheless, the results of these *in vitro* experiments on the differential effects of the two hydroxylated metabolites of amoxapine on synaptosomal uptake of [^{3}H]NE and [^{3}H]-5-HT and on DSAC and [^{3}H]spiroperidol binding suggest a major role for 7-OH-Amox in the mediation of the anti-dopamine effects of the parent compound [8, 9].

Table 3. Calculated IC_{50} values for the displacement of specific [^{3}H]spiroperidol binding from rat striatal membranes*

Drug	IC_{50} (nM ± S.E.M.)
Amoxapine	196 ± 2
7-OH-Amox	50 ± 11
8-OH-Amox	1800 ± 190
Loxapine	101 ± 11
Imipramine	5870 ± 505

*The IC_{50} values shown are the concentration of each drug required to inhibit the specific binding of 0.75 nM [^{3}H]spiroperidol by 50 per cent. Blanks to determine nonspecific binding constitute binding measured in the presence of 1.0 μM unlabeled spiroperidol. The IC_{50} values were determined by log-probit analysis of data, using five or six concentrations of drug in triplicate.

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REFERENCES

1. S. Gershon, L. J. Hekimian, E. I. Burdock and S. S. Kim, *Curr. ther. Res.* **12**, 280 (1970).
2. M. P. Bishop and D. M. Gallant, *Curr. ther. Res.* **12**, 594 (1970).
3. J. Coupet, V. A. Szucs and E. N. Greenblatt, *Brain Res.* **116**, 177 (1976).
4. D. M. Gallant, M. P. Bishop and R. Guerrero-Figueroa, *Curr. ther. Res.* **13**, 364 (1971).
5. K. D. Charlampous, *Curr. ther. Res.* **14**, 657 (1972).
6. G. L. Sathananthan, R. Matz, H. Thompson and S. Gershon, *Curr. ther. Res.* **15**, 919 (1973).
7. R. C. Smith, *Curr. ther. Res.* **18**, 346 (1975).
8. R. Chermat, P. Simon and J. R. Boissier, *Collegium Internationale Neuropharmacologicum*, Tenth Congress, Quebec, Canada, Abstr., p. 162, (1976).
9. E. N. Greenblatt, A. S. Lippa and A. C. Osterberg, *Archs int. Pharmacodyn. Thér.* **233**, 107 (1978).
10. T. B. Cooper and R. G. Kelly, *J. pharm. Sci.*, in press.
11. M. J. Kuhar, R. J. Roth and G. K. Aghajanian, *J. Pharmac. exp. Ther.* **181**, 36 (1972).
12. E. M. Gal, P. A. Christiansen and L. M. Yunger, *Neuropharmacology* **14**, 31 (1975).
13. J. W. Kebabian, G. L. Petzold and P. Greengard, *Proc. natn. Acad. Sci. U.S.A.* **60**, 2145 (1972).
14. A. G. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
15. J. Coupet and C. E. Rauh, *Eur. J. Pharmac.*, **55**, 215 (1979).
16. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).