Synthesis of Substituted Anilino-(3-methoxy-4-substituted Acetoxy)benzylidenes and Their Monoamine Oxidase Inhibitory and Anticonvulsant Properties

SHIVA P. SINGH *§, ANSHUMALI CHAUDHARI §, VIRGIL I. STENBERG *, and SURENDRA S. PARMAR ‡§x

Abstract □ Several substituted anilino-(3-methoxy-4-substituted acetoxy)benzylidenes were synthesized and characterized by their sharp melting points and elemental analyses. All substituted benzylidenes competitively inhibited the *in vitro* monoamine oxidase activity of rat brain homogenates and possessed anticonvulsant activity against pentylenetetrazol-induced convulsions in mice.

Keyphrases □ Benzylidenes, substituted—synthesized, screened for effect on monoamine oxidase activity and anticonvulsant properties □ Monoamine oxidase activity—effect of various substituted anilinobenzylidenes, rat brain homogenate □ Anticonvulsant activity—various substituted anilinobenzylidenes screened, mice □ Structure–activity relationships—various substituted anilinobenzylidenes synthesized and screened for effect on monoamine oxidase activity and anticonvulsant properties

Mixed excitatory and depressant actions on the central nervous system activity have been shown to be associated with numerous tertiary alkylamines containing one or more aromatic groups. Of the several azacycloalkylbenzaldehyde hydrazones examined, 3-[2-(4- methyl-1-piperazinyl)-5- nitrobenzylidene]amino-2-oxazolidinone produced the most marked inhibition of rat brain monoamine oxidase (1). Monoamine oxidase inhibitors also possess pronounced anticonvulsant properties (2). Earlier studies indicated that substituted benzylidenes have anticonvulsant and monoamine oxidase inhibitory activity (3). These observations prompted the synthesis of substituted anilino- (3-methoxy-4- morpholino/pyrrolidino/piperidinoacetoxy)benzylidenes. These substituted benzylidenes were evaluated for their ability to inhibit rat brain monoamine oxidase and anticonvulsant activity against pentylenetetrazol-induced convulsions in mice.

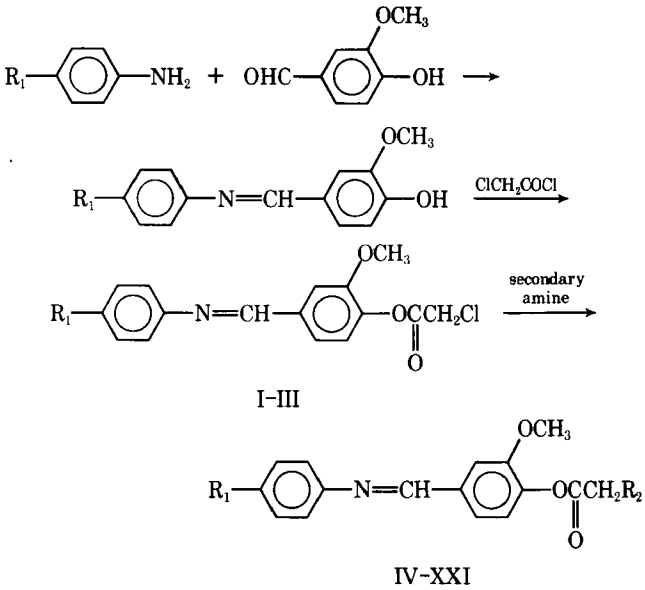
The various substituted anilino-(3-methoxy-4-substituted acetoxy)benzylidenes were synthesized by following the route outlined in Scheme I.

EXPERIMENTAL

Substituted Anilino-(3-methoxy-4-hydroxy)benzylidenes—These benzylidenes were prepared by the condensation of substituted anilines with vanillin as reported previously (3).

Substituted Anilino-(3-methoxy-4-chloroacetoxy)benzylidenes (I–III)—To a substituted anilino-(3-methoxy-4-hydroxy)-benzylidene (0.05 mole) in 25 ml of dry benzene was added chloroacetyl chloride (0.055 mole), gradually and with shaking, and the mixture was refluxed on a steam bath for 4 hr. Excess benzene was removed by distillation; the solid mass which separated was collected by filtration, washed with water to remove the traces of chloroacetyl chloride, dried, and recrystallized from ethanol. All compounds were characterized by their sharp melting points and elemental analyses (Table I).

Substituted Anilino-(3-methoxy-4-substituted Acetoxy)benzylidenes (IV–XXI)—These compounds were synthesized by refluxing a mixture of a substituted anilino-(3-methoxy-4-chloroacetoxy)benzylidene (0.005 mole) and an appropriate secondary amine (0.01 mole) in 25 ml of dry benzene on a steam bath for 6 hr. Excess benzene was removed by distillation; the solid mass which separated was collected by filtration, dried, and recrystallized from ethanol. All substituted benzylidenes were characterized by their sharp melting points and elemental analyses (Table II).



Scheme I

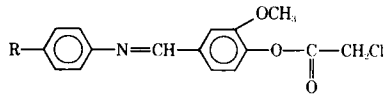
Monoamine Oxidase Activity—Monoamine oxidase activity of rat brain homogenate was determined by a spectrophotofluorometric method, using kynuramine as the substrate (4). Male albino rats, 100–150 g, were allowed food and water *ad libitum*. They were sacrificed by decapitation, and the brains were removed immediately and homogenized[1] in ice-cold 0.25 *M* sucrose (1:9 w/v). The monoamine oxidase activity of rat brain homogenate was determined by incubation at 37° in air for 30 min. The reaction mixture in a total volume of 3 ml consisted of 0.5 ml of phosphate buffer (0.5 *M*, pH 7.5), 0.1 m*M* kynuramine, and 0.5 ml of brain homogenate (equivalent to 10 mg wet weight of the tissue).

The various substituted benzylidenes were dissolved in propylene glycol (100%) and used at a final concentration of 0.166 m*M*. An equivalent amount of propylene glycol was added to the control tubes. All benzylidenes were incubated with rat brain homogenates for 10 min before the addition of kynuramine. The mixture, after the addition of kynuramine, was further incubated for 30 min. The enzymatic reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid (w/v), and the precipitated proteins were removed by centrifugation.

Suitable 1-ml aliquots of the supernatant solution were taken in 2 ml of 1 *N* NaOH solution and assayed for 4-hydroxyquinoline, formed during deamination of kynuramine. They then were measured fluorometrically[2], using activating light of 315 nm and measuring fluorescence at 380 nm. An increase in absorbance provided a direct

[1] Potter-Elvehjem.
[2] Aminco-Bowman spectrophotofluorometer.

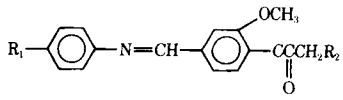
Table I—Physical Constants of Substituted Anilino-(3-methoxy-4-chloroacetoxy)benzylidenes



Compound	R	Melting Point[a]	Yield, %	Molecular Formula[b]	Analysis, % Calc.	Found
I	Cl	105°	80	$C_{16}H_{13}Cl_2NO_3$	C 56.80	56.32
					H 3.85	3.76
					N 4.14	4.12
II	CH_3	115°	82	$C_{17}H_{16}ClNO_3$	C 64.25	63.85
					H 5.04	4.92
					N 4.40	4.22
III	OCH_3	130°	85	$C_{17}H_{16}ClNO_4$	C 61.17	61.00
					H 4.79	4.57
					N 4.19	3.88

[a] Melting points were taken in open capillary tubes and are corrected. [b] All compounds were recrystallized from ethanol.

Table II—Physical Constants of Substituted Anilino-(3-methoxy-4-substituted Acetoxy)benzylidenes



Compound	R_1	R_2	Melting Point[a]	Yield, %	Molecular Formula[b]	Analysis, % Calc.	Found
IV	Cl	Morpholino	115°	78	$C_{20}H_{21}ClN_2O_4 \cdot HCl$	C 56.47	56.23
						H 5.18	4.98
						N 6.59	6.44
V	Cl	Pyrrolidino	112°	80	$C_{20}H_{21}ClN_2O_3 \cdot HCl$	C 58.68	58.28
						H 5.38	5.12
						N 6.85	6.52
VI	Cl	Piperidino	68°	74	$C_{21}H_{23}ClN_2O_3 \cdot HCl$	C 59.57	59.34
						H 5.67	5.41
						N 6.62	6.21
VII	Cl	2-Methylpiperidino	98°	58	$C_{22}H_{25}ClN_2O_3 \cdot HCl$	C 60.41	60.20
						H 5.95	5.75
						N 6.41	6.30
VIII	Cl	3-Methylpiperidino	103°	60	$C_{22}H_{25}ClN_2O_3 \cdot HCl$	C 60.41	59.98
						H 5.95	5.58
						N 6.41	6.13
IX	Cl	4-Methylpiperidino	118°	60	$C_{22}H_{25}ClN_2O_3 \cdot HCl$	C 60.41	60.38
						H 5.95	5.84
						N 6.41	6.40
X	CH_3	Morpholino	167°	76	$C_{21}H_{24}N_2O_4 \cdot HCl$	C 62.29	62.12
						H 6.18	5.83
						N 6.92	6.48
XI	CH_3	Pyrrolidino	222°	73	$C_{21}H_{24}N_2O_3 \cdot HCl$	C 64.86	64.54
						H 6.43	6.27
						N 7.21	7.15
XII	CH_3	Piperidino	226°	72	$C_{22}H_{26}N_2O_3 \cdot HCl$	C 65.59	65.37
						H 6.71	6.29
						N 6.96	6.38
XIII	CH_3	2-Methylpiperidino	215°	56	$C_{23}H_{28}N_2O_3 \cdot HCl$	C 66.27	66.00
						H 6.96	6.88
						N 6.72	6.55
XIV	CH_3	3-Methylpiperidino	235°	61	$C_{23}H_{28}N_2O_3 \cdot HCl$	C 66.27	66.22
						H 6.96	6.76
						N 6.72	6.42
XV	CH_3	4-Methylpiperidino	237°	68	$C_{23}H_{28}N_2O_3 \cdot HCl$	C 66.27	66.14
						H 6.96	6.58
						N 6.72	6.46
XVI	OCH_3	Morpholino	134°	85	$C_{21}H_{24}N_2O_5$	C 65.62	65.46
						H 6.25	6.08
						N 7.29	7.21
XVII	OCH_3	Pyrrolidino	128°	83	$C_{21}H_{24}N_2O_4$	C 68.48	68.28
						H 6.52	6.37
						N 7.61	7.48
XVIII	OCH_3	Piperidino	125°	80	$C_{22}H_{26}N_2O_5$	C 69.11	69.02
						H 6.81	6.72
						N 7.33	7.12
XIX	OCH_3	2-Methylpiperidino	122°	60	$C_{23}H_{28}N_2O_4$	C 69.69	69.35
						H 7.07	6.86
						N 7.07	6.65
XX	OCH_3	3-Methylpiperidino	124°	64	$C_{23}H_{28}N_2O_4$	C 69.69	69.29
						H 7.07	7.02
						N 7.07	6.59
XXI	OCH_3	4-Methylpiperidino	127°	72	$C_{23}H_{28}N_2O_4$	C 69.69	69.46
						H 7.07	6.88
						N 7.07	6.78

[a] Melting points were taken in open capillary tubes and are corrected. [b] All compounds were recrystallized from ethanol.

Table III—Monoamine Oxidase Inhibitory and Anticonvulsant Properties of Substituted Anilino-(3-methoxy-4-substituted Acetoxy)benzylidenes

Compound	Monoamine Oxidase Inhibition[a]		Anticonvulsant Activity[b], % Protection	Pentylenetetrazol Mortality[c], %
	% Inhibition	I_{50} Values[d] ($\times 10^{-4}$ M)		
IV	65.0 ± 0.5	1.16	40	50
V	67.5 ± 0.8	1.14	20	40
VI	51.8 ± 0.6	1.60	30	70
VII	70.1 ± 0.6	1.10	20	50
VIII	62.2 ± 1.1	1.22	20	60
IX	45.3 ± 1.0	1.76	20	80
X	57.3 ± 0.5	1.40	50	30
XI	72.7 ± 0.6	1.06	70	90
XII	65.0 ± 0.7	1.16	20	90
XIII	75.2 ± 0.8	1.00	20	80
XIV	70.1 ± 0.6	1.10	30	60
XV	66.1 ± 0.5	1.15	30	50
XVI	64.8 ± 0.9	1.15	70	20
XVII	64.8 ± 0.5	1.15	10	90
XVIII	63.5 ± 0.7	1.20	30	70
XIX	68.7 ± 0.5	1.20	50	50
XX	71.2 ± 0.6	1.11	30	80
XXI	64.5 ± 0.8	1.20	20	70
Methaqualone	—	—	60	20
Meprobamate	—	—	80	10

[a]Contents of the reaction mixture and assay procedures are as described in the text. Each experiment was done in triplicate, and the values are the mean values of three separate experiments with ±*SEM*. Kynuramine and substituted benzylidenes were used at a final concentration of 0.1 and 0.166 m*M*, respectively. [b]Screening procedures for the determination of anticonvulsant activity of substituted benzylidenes and methaqualone and meprobamate, the two reference drugs, at a dose of 100 mg/kg ip are described in the text. [c]Mortality in pentylenetetrazol-treated animals was observed during 24 hr. [d]Various substituted benzylidenes were used at final concentrations ranging between 0.05 and 0.2 m*M* for the determination of their I_{50} values.

measurement of 4-hydroxyquinoline formation, which was taken as an index of the monoamine oxidase activity. The percentage inhibition was calculated from the decrease observed in absorbance, and this value provided an index of the inhibitory property of these substituted benzylidenes.

In the preincubation studies, the rat brain homogenates in the incubation mixture were incubated with or without substituted benzylidenes at 37° for 10, 20, and 30 min prior to the addition of kynuramine. In zero-time experiments, the substituted benzylidenes and kynuramine were added simultaneously to the reaction mixture containing the brain homogenate. The I_{50} values (concentrations producing 50% inhibition) were determined graphically from the values obtained for the inhibition of rat brain monoamine oxidase by using these benzylidenes in concentrations ranging between 0.05 and 0.2 m*M*. In addition, the nature of the enzyme inhibition caused by XI, XIII, and XX was evaluated by the graphic method of Lineweaver and Burk (5) as modified by Dixon (6).

Determination of Anticonvulsant Activity—The anticonvulsant activity of substituted benzylidenes was determined against pentylenetetrazol-induced seizures. Albino mice of either sex, 25–30 g, were divided into groups of 10, keeping the group weights as near the same as possible. All substituted benzylidenes were suspended in 5% aqueous gum acacia, which was devoid of anticonvulsant activity, to give a concentration of 1% (w/v). An arbitrary dose of 100 mg/kg of benzylidene was administered intraperitoneally to one group of 10 mice.

The mice were injected with pentylenetetrazol (90 mg/kg sc) 4 hr after the administration of the test compounds (7). This dose of pentylenetetrazol has been shown to produce convulsions in almost all untreated mice and to exhibit 100% mortality during 24 hr. No mortality was observed during 24 hr for animals treated with 100 mg/kg of the benzylidenes alone. The mice were observed for 60 min for the occurrence of seizures. An episode of clonic spasm persisting for a minimum of 6 sec was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted.

Mice devoid of threshold convulsions during 60 min were considered protected. The number of mice protected in each group was recorded, and the anticonvulsant activity of these benzylidenes was represented as the percent protection. The animals were then ob-

Table IV—Preincubation Studies on the *In Vitro* Inhibition of Monoamine Oxidase by Substituted Benzylidenes

Compound	Preincubation Time, min			
	0	10	20	30
	Percent Inhibition[a]			
XI	68.2 ± 0.4	72.0 ± 0.8	71.8 ± 0.5	72.6 ± 0.7
XIII	73.5 ± 0.5	75.6 ± 0.6	74.3 ± 0.6	75.0 ± 0.9
XX	70.2 ± 0.8	71.4 ± 0.5	70.9 ± 0.8	71.7 ± 0.5

[a]Contents of the reaction mixture and the assay procedures are as indicated in Table III. The enzyme preparations were incubated with substituted benzylidenes for varying times before the addition of kynuramine. Zero-time experiments indicated that both kynuramine and benzylidenes were added to the reaction mixture containing rat brain homogenates simultaneously. Each experiment was done in triplicate, and the values are the mean values of three separate experiments with ±*SEM*. Compounds XI, XIII, and XX were used in the final concentration of 0.166 m*M*.

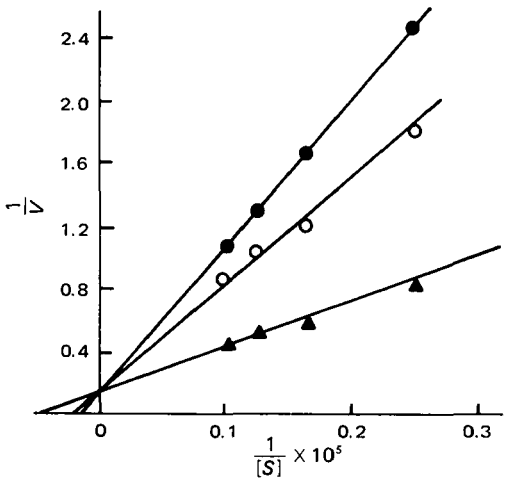


Figure 1—*Competitive inhibition of rat brain monoamine oxidase by XV. Assay procedures and the contents of the reaction mixture are as described in the text.* [S] *denotes molar concentration of kynuramine, and 1/V represents the reciprocal of the change in percent absorbance/10 mg fresh tissue wet weight/30 min. Key:* ▲, *control;* ○, *0.1 m*M *of the benzylidene (XI);* and ●, *0.15 m*M *of the benzylidene (XI). The* K_m *value for kynuramine was 0.19 m*M.

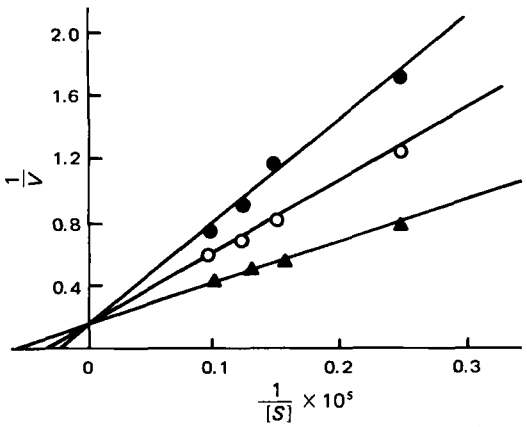


Figure 2—*Competitive inhibition of rat brain monoamine oxidase by XIII. Details of the assay procedure are as described in Fig. 1.*

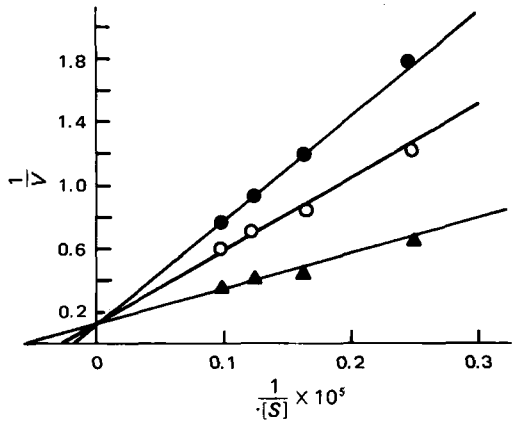


Figure 3—*Competitive inhibition of rat brain monoamine oxidase by XX. Details of the assay procedures are as described in Fig. 1.*

served for 24 hr, and the mortality of mice in each group was recorded. In the present study, methaqualone[3].(8) and meprobamate[4] (9) were used intraperitoneally at a dose of 100 mg/kg as reference anticonvulsant drugs for comparative evaluation.

RESULTS AND DISCUSSION

The monoamine oxidase inhibitory activity possessed by substituted anilino-(3-methoxy-4-morpholino/pyrrolidino/piperidinoacetoxy)benzylidenes is recorded in Table III. All benzylidenes inhibited *in vitro* monoamine oxidase activity of rat brain homogenates at a final concentration of 0.166 m*M*, and the degree of inhibition ranged from 45.3 to 75.2%. Maximum inhibition was observed with XIII, while IX was the least effective inhibitor. The effectiveness of these benzylidenes in inhibiting rat brain monoamine oxidase correlated well with their I_{50} values. The I_{50} values were 0.1 and 0.176 m*M* for XIII and IX, respectively.

Preincubation of XI, XIII, and XX at a final concentration of 0.166 m*M* for varying times prior to the addition of the substrate in *in vitro* studies in no way altered the degree of monoamine oxidase inhibition. These studies thus indicated a rapidly reversible and possibly competitive nature of the inhibition of monoamine oxidase by substituted benzylidenes (Table IV). These findings were further supported by kinetic studies with XI (Fig. 1), XIII (Fig. 2), and XX (Fig. 3), which revealed the competitive nature of inhibition of rat brain monoamine oxidase by these three substituted benzylidenes. The intercept at the $1/S$ axis was taken at $-1/K_m$; the value of 0.19 m*M* was obtained as the apparent Michaelis constant (K_m) in these experiments, using kynuramine as the substrate.

The results of anticonvulsant activity studies (Table III) demonstrated that most of these substituted benzylidenes possess significantly low anticonvulsant activity; the degree of protection against pentylenetetrazol-induced convulsions afforded by these compounds at a dose of 100 mg/kg ranged from 10 to 70%. Maximum anticonvulsant activity was observed with XI and XVI, while the rest of benzylidenes possessed low anticonvulsant activity. In the present study, intraperitoneal administration of methaqualone and meprobamate at 100 mg/kg provided protection of 60 and 80%, respectively, against pentylenetetrazol-induced convulsions in mice under similar experimental conditions. These studies also indicated that there appears to be some connection, but no uniform trend, between the percent protection afforded by substituted benzylidenes and the reference drugs against pentylenetetrazol-induced convulsions and their ability to protect against pentylenetetrazol-induced mortality during a 24-hr period.

[3] Arnar-Stone Laboratories, Mount Prospect, Ill.
[4] Wyeth Laboratories, Philadelphia, Pa.

REFERENCES

(1) T. George, C. L. Mehta, R. S. Grewal, and D. V. Mehta, *J. Med. Chem.*, **14**, 909(1971).

(2) D. J. Prokop, P. A. Shore, and B. B. Brodie, *Ann. N.Y. Acad. Sci.*, **80**, 643(1959).

(3) C. Dwivedi, R. D. Harbison, B. Ali, and S. S. Parmar, *J. Pharm. Sci.*, **63**, 1124(1974).

(4) M. Krajl, *Biochem. Pharmacol.*, **14**, 1684(1965).

(5) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658(1934).

(6) M. Dixon, *Biochem. J.*, **55**, 170(1953).

(7) S. S. Parmar, A. K. Gupta, T. K. Gupta, and V. I. Stenberg, *J. Pharm. Sci.*, **64**, 154(1975).

(8) M. L. Gujral, P. N. Saxena, and R. P. Kohli, *Indian J. Med. Res.*, **45**, 207(1957).

(9) M. A. Perlstein, *J. Am. Med. Assoc.*, **161**, 1040(1956).

ACKNOWLEDGMENTS AND ADDRESSES

Received March 17, 1975, from the **Department of Chemistry and the [1]Department of Physiology and Pharmacology, University of North Dakota, Grand Forks, ND 58202,* and the *[8]Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow 226003, India.*

Accepted for publication February 4, 1976.

Supported in part by U.S. Public Health Service Grants GM-21590, 1 T01 HL-05939-02, and 1 R01 DA-00996-01 and U.S. Public Health Service Career Development Award 1-K4-GM-09888 to V. I. Stenberg.

The authors thank Professor Roland G. Stevenson and Professor Stanley J. Brumleve for their advice and encouragement. Grateful acknowledgment is made to the Atomic Energy Commission, Bombay, India, and the Indian National Science Academy, New Delhi, India, for providing research fellowships to S. P. Singh and A. Chaudhari, respectively.

[x] To whom inquiries should be directed (at the University of North Dakota).