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Synthesis of Substituted Cinnamides: Relationship between Anticonvulsant and Monoamine Oxidase Inhibitory Properties

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Abstract ☐ Several esters and hydrazides of substituted cinnamides were synthesized and characterized. Evaluation of their anticonvulsant activity indicated appreciable activity of hydrazides where no correlation could be observed between anticonvulsant activity possessed by these hydrazides and their ability to inhibit monoamine oxidase.

Keyphrases ☐ Cinnamides of substituted amino acid esters and their hydrazides—synthesis, relationship between anticonvulsant and monoamine oxidase inhibitory properties ☐ Monoamine oxidase inhibitors, cinnamides of amino acid esters and their hydrazides—synthesis, anticonvulsant activity ☐ Anticonvulsant activity—cinnamides of substituted amino acid esters and their hydrazides, relationship to monoamine oxidase inhibitory properties ☐ Structure-activity relationships—cinnamides, relationship between anticonvulsant and monoamine oxidase inhibitory properties

Presence of a styryl group, responsible for high π-electron density, has been postulated to account for monoamine oxidase [EC 1.4.3.4 monoamine–O_2 oxido reductase (deaminating)] inhibitory property of styrylquinoliniums (1). The ability of monoamine oxidase inhibitors to possess anticonvulsant activity (2, 3) led to the synthesis of α-benzoylamino-*N*-[*p*-(4-aryl semicarbazide/thiosemicarbazide)-benzoyl]-*p*-substituted cinnamides possessing both an ethylenic moiety and the hydrazide group in an attempt to correlate their anticonvulsant activity with *in vitro* monoamine oxidase inhibitory effectiveness (4). In the present study, substituted cinnamides of amino acid esters and their corresponding hydrazides were synthesized as possible anticonvulsants to investigate correlation between anticonvulsant activity possessed by these hydrazides with their ability to inhibit monoamine oxidase.

CHEMISTRY

The various cinnamides of substituted amino acid esters and their corresponding hydrazides (Tables I and II) were synthesized by following the methods outlined in Scheme I.

Substituted oxazolones (I*a*) were obtained by treatment by hippuric acid with appropriate aromatic aldehydes and acetic anhydride in the presence of anhydrous sodium acetate. Compounds I*a* were treated with ethyl esters of amino acids (I*b*) in the presence of 2–3 drops of triethylamine to give esters (I–XII) which were converted into the corresponding hydrazides (XIII–XXIV) by refluxing with 99–100% hydrazine hydrates (1:2 molar ratio) in absolute ethanol for 6–8 hr.

EXPERIMENTAL

Ethyl Esters of Amino Acids (I*b*)—These were prepared by following the method of Kupryszewski and Sokolowska (5). Thionyl chloride, 0.1 mole, was added at 5° to 50 ml. of an absolute ethanolic solution of the appropriate amino acid (0.1 mole) and the mixture was refluxed for 3 hr. The excess thionyl chloride was removed under reduced pressure. The free ester, which precipitated by passing a slow stream of dry ammonia into a suspension of the ester hydrochloride in chloroform, was filtered, and excess chloroform was removed by distillation under reduced pressure.

2-Phenyl-4-(substituted benzylidene)-oxazole-5-ones (I*a*)—The various oxazolones were prepared by heating a mixture of an appropriate aromatic aldehyde (0.96 mole), powdered dry hippuric acid (1.07 moles), freshly powdered sodium acetate (0.98 mole), and acetic anhydride (2.9 moles) in a flask on an electric hot plate (6, 7). As soon as the material had liquefied completely, the flask was transferred to a steam bath and heated for 2 hr. The reaction mixture was allowed to stand overnight, and the solid mass which precipitated was filtered with suction and washed first with two 100-ml. portions of ice-cold ethanol and finally with two 100-ml. portions of boiling water. After drying, the various oxazolones were used without further purification.

α-Benzoylamino-*N*-(substituted esters)-substituted Cinnamides (I–XII)—Equimolar quantities (1 mole) of the appropriate 2-phenyl-4-(substituted benzylidene)-oxazole-5-ones and ethyl esters of amino acids were refluxed in absolute ethanol in the presence of 2–3 drops of triethylamine on a steam bath for 6–8 hr. Excess ethanol was distilled, and the cinnamides which separated on cooling were filtered and recrystallized with either ethanol or the mixture of ethanol and water. The cinnamides were characterized by their sharp melting points and elemental analyses (Table I).

α-Benzoylamino-*N*-(substituted hydrazides)-substituted Cinnamides (XIII–XXIV)—A mixture of the appropriate α-benzoylamino-*N*-(substituted esters)-substituted cinnamides (0.1 mole) and 99–100% hydrazine hydrate (0.12 mole) in absolute ethanol was refluxed on a water bath for 6–8 hr. The solid mass which separated on cooling was filtered and recrystallized from suitable solvents (Table II).

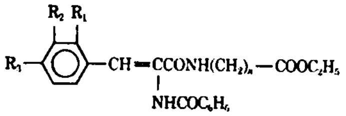

Table I—Physical Constants of α-Benzoylamino-*N*-(substituted esters)-substituted Cinnamides

Compound Number	R_1	R_2	R_3	*n*	Melting Point[a]	Yield, %	Recrystallization Solvent	Formula	Analysis, % Calc.	Analysis, % Found
I	H	H	H	1	114–115°	70	Ethanol–water	$C_{20}H_{20}N_2O_4$	C 68.01 H 5.68 N 7.95	67.96 5.38 7.56
II	Cl	H	H	1	105°	75	Ethanol–water	$C_{20}H_{19}ClN_2O_4$	C 62.09 H 4.91 N 7.24	62.13 5.03 7.03
III	H	H	Cl	1	200–202°	80	Ethanol	$C_{20}H_{19}ClN_2O_4$	C 62.09 H 4.91 N 7.24	61.09 4.63 6.95
IV	H	Cl	Cl	1	199–200°	80	Ethanol	$C_{20}H_{18}Cl_2N_2O_4$	C 57.00 H 4.27 N 6.65	56.56 4.13 6.77
V	H	H	H	2	135°	79	Ethanol–water	$C_{21}H_{22}N_2O_4$	C 68.85 H 6.01 N 7.65	68.72 6.11 7.25
VI	Cl	H	H	2	125°	75	Ethanol–water	$C_{21}H_{21}ClN_2O_4$	C 62.89 H 5.24 N 6.98	62.65 5.24 6.80
VII	H	H	Cl	2	191–192°	80	Ethanol	$C_{21}H_{21}ClN_2O_4$	C 62.89 H 5.24 N 6.98	62.77 5.34 6.78
VIII	H	Cl	Cl	2	190°	82	Ethanol	$C_{21}H_{20}Cl_2N_2O_4$	C 57.93 H 4.59 N 6.43	57.22 4.09 6.07
IX	H	H	H	3	144–145°	70	Ethanol–water	$C_{22}H_{24}N_2O_4$	C 69.47 H 6.31 N 7.36	69.04 6.08 7.22
X	Cl	H	H	3	122–123°	75	Ethanol–water	$C_{22}H_{23}ClN_2O_4$	C 63.69 H 5.54 N 6.75	62.97 5.33 6.66
XI	H	H	Cl	3	116–117°	75	Ethanol	$C_{22}H_{23}ClN_2O_4$	C 63.69 H 5.54 N 6.75	63.57 5.34 6.63
XII	H	Cl	Cl	3	140–141°	80	Ethanol	$C_{22}H_{22}Cl_2N_2O_4$	C 58.79 H 4.89 N 6.23	58.05 4.72 6.00

[a] All melting points were taken in open capillary tubes and are corrected.

PHARMACOLOGY

Anticonvulsant activity was determined in mice of either sex weighing 25–30 g. The mice were divided in groups of 10, keeping the group weights as near the same as possible. Each hydrazide or its precursor ester was suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compound was injected in a group of 10 animals at a dose of 100 mg./kg. i.p. Four hours after administration of the hydrazide or ester, the mice were injected with pentylenetetrazol (90 mg./kg. s.c.). This dose of pentylenetetrazol has been shown not only to produce convulsions in almost all untreated mice but also to exhibit 100% mortality during 24 hr. On the other hand, no mortality was observed during 24 hr. in animals treated with 100 mg./kg. of the test compounds alone. The mice were then observed 60 min. for seizures. An episode of clonic spasm that persisted at least 5 sec. was considered a threshold convulsion. Transient intermittent jerks or tremulousness was not counted. Animals devoid of threshold convulsions during the 60 min. were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of these α-benzoylamino-*N*-(substituted hydrazides)-substituted cinnamides and their corresponding esters was represented as percent protection. The animals were then observed for 24 hr. and their mortality was recorded.

BIOCHEMISTRY

Spectrophotofluorometric Method—A spectrophotofluorometric method was used for the determination of monoamine oxidase activity of rat brain homogenate, using kynuramine as the substrate (8). The 4-hydroxyquinoline formed during oxidative deamination of kynuramine was measured fluorometrically with a spectrophotofluorometer[1], using activating light of 315 nm. and measuring fluorescence at the maximum of 380 nm.

Male adult rats, weighing approximately 150–200 g., were killed by decapitation. Brains were quickly removed and homogenized in ice-cold 0.25 *M* sucrose with the Potter–Elvehjem homogenizer. The reaction mixture, in a final concentration, consisted of 0.5 ml. phosphate buffer (0.2 *M*, pH 7.5), 1 × 10^{-4} *M* kynuramine, and 0.5 ml. brain homogenate (equivalent to 10 mg. of wet weight of the tissue). The monoamine oxidase activity of the brain homogenate was determined by incubation at 37° in air for 30 min. The various α-benzoylamino-*N*-(substituted hydrazides)-substituted cinnamides were added to the brain homogenate to produce a final concentration of 5 × 10^{-4} *M* and incubated for 10 min. before adding kynuramine. The mixture was then incubated for an additional 30 min. The 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 supernate were taken in 2 ml. of 1 *N* NaOH solution and were assayed for 4-hydroxyquinoline. An increase in the absorbance provided a direct measurement of the 4-hydroxyquinoline formation, which was taken as an index of the enzyme activity. The percent inhibition was calculated from the decrease observed in the absorbance and this provided an index of the inhibitory property of these α-benzoylamino-*N*-(substituted hydrazides)-substituted cinnamides.

Warburg Manometric Method—The monoamine oxidase activity of rat brain homogenate was determined by the conventional Warburg manometric technique, using tyramine as the substrate (9). The decrease in the oxygen uptake for 1 hr. during oxidative deamination of tyramine in the presence of substituted cinnamides was used as an index of enzyme inhibition. The reaction mixture,

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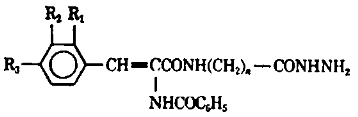
[1] Aminco-Bowman.

Table II—Physical Constants of α-Benzoylamino-*N*-(substituted hydrazides)-substituted Cinnamides



$R_3-C_6H_2(R_2)(R_1)-CH{=}C(NHCOC_6H_5)CONH(CH_2)_n-CONHNH_2$

Compound Number	R_1	R_2	R_3	*n*	Melting Point[a]	Yield, %	Recrystallization Solvent	Formula	Analysis, %	
									Calc.	Found
XIII	H	H	H	1	217°	60	Ethanol	$C_{18}H_{18}N_4O_3$	C 63.90 H 5.32 N 16.56	63.73 5.03 16.45
XIV	Cl	H	H	1	250–251°	60	Ethanol	$C_{18}H_{17}ClN_4O_3$	C 58.01 H 4.56 N 15.03	58.31 4.41 15.00
XV	H	H	Cl	1	236°	70	Butanol	$C_{18}H_{17}ClN_4O_3$	C 58.01 H 4.56 N 15.03	57.93 4.50 14.91
XVI	H	Cl	Cl	1	210°	80	Ethanol	$C_{18}H_{16}Cl_2N_4O_3$	C 53.07 H 3.93 N 13.75	53.12 3.72 13.62
XVII	H	H	H	2	171°	73	Ethanol	$C_{19}H_{20}N_4O_3$	C 64.77 H 5.68 N 15.90	64.63 5.24 15.72
XVIII	Cl	H	H	2	191°	69	Ethanol	$C_{19}H_{19}ClN_4O_3$	C 58.99 H 4.91 N 14.48	58.77 4.70 14.03
XIX	H	H	Cl	2	205–206°	65	Ethanol	$C_{19}H_{19}ClN_4O_3$	C 58.99 H 4.91 N 14.48	58.63 4.81 14.32
XX	H	Cl	Cl	2	209–210°	70	Ethanol	$C_{19}H_{18}Cl_2N_4O_3$	C 54.15 H 4.27 N 13.30	54.03 4.07 13.36
XXI	H	H	H	3	185°	80	Ethanol	$C_{20}H_{22}N_4O_3$	C 68.30 H 6.01 N 15.30	68.14 6.15 15.41
XXII	Cl	H	H	3	260°	76	Ethanol	$C_{20}H_{21}ClN_4O_3$	C 59.97 H 5.24 N 13.58	59.27 5.02 13.09
XXIII	H	H	Cl	3	249–250°	75	Ethanol	$C_{20}H_{21}ClN_4O_3$	C 59.97 H 5.24 N 13.58	59.76 5.32 13.42
XXIV	H	Cl	Cl	3	229–230°	80	Ethanol	$C_{20}H_{20}Cl_2N_4O_3$	C 55.17 H 4.59 N 12.87	55.21 4.09 12.67

[a] All melting points were taken in open capillary tubes and are corrected.

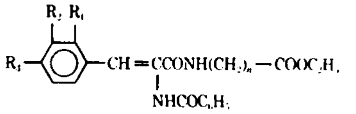
in a final concentration, consisted of 50 m*M* phosphate buffer (pH 7.4), 5 m*M* tyramine, and the homogenate (equivalent to 100 mg. of fresh tissue) in a total volume of 1.2 ml. The inhibitors, used at a final concentration of 1 × 10^{-3} *M*, were incubated with the enzyme preparation for 20 min. before adding the substrate. The enzyme system was then incubated an additional hour at 37° using oxygen as the gaseous phase. Readings of the oxygen uptake were taken every 10 min.

RESULTS AND DISCUSSION

In the present study, α-benzoylamino-*N*-(substituted esters)-substituted cinnamides and their corresponding hydrazides were synthesized and evaluated for anticonvulsant activity. The degree of protection afforded by these compounds against pentylenetetrazol-induced seizures and the 24-hr. mortalities are recorded in Tables III and IV. The data seem to indicate a trend of more protection from convulsions with less mortality; however, it is not sufficiently uniform to conclude that definitely (*e.g.*, Table III: 0% protection = 70% mortality, 10% protection = 90% mortality, and 30% protection = 70% mortality). Each hydrazide or its precursor ester was found to be devoid of not only the sedative or central nervous system (CNS) depressant effect but also of 24-hr. mortality in the 100-mg./kg. dose used in the present study. Thus, 24-hr. mortality in these experimental animals accounts for the toxic effects of pentylenetetrazol.

These results indicate a greater anticonvulsant activity of α-benzoylamino-*N*-(substituted hydrazides)-substituted cinnamides as compared to their corresponding esters. These esters were either devoid of activity or showed less than 30% protection. The protection afforded by these hydrazides ranged from 30 to 70%, where the greatest protection was observed when the aliphatic chain of these α-benzoylamino-*N*-(substituted hydrazides)-substituted cinnamides possessed the glycine hydrazide moiety (Compound XIII). Elongation of the aliphatic chain resulted in a decrease of anticonvulsant activity. Evaluation of monoamine oxidase inhibitory activity of these hydrazides with a view to elucidating the biochemical basis for anticonvulsant activity indicated that all α-benzoylamino-

Table III—Anticonvulsant Activity of α-Benzoylamino-*N*-(substituted esters)-substituted Cinnamides[a]

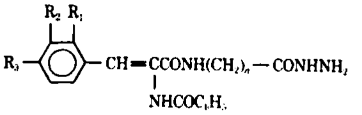


$R_3-C_6H_2(R_2)(R_1)-CH{=}C(NHCOC_6H_5)CONH(CH_2)_n-COOC_2H_5$

Compound Number	Anticonvulsant Activity, % Protection	Pentylenetetrazol Mortality, %
I	10	80
II	20	70
III	20	80
IV	30	70
V	10	90
VI	Nil	70
VII	10	70
VIII	10	70
IX	20	60
X	30	60
XI	20	70
XII	10	70

[a] The compound numbers are those recorded in Table I. The experimental procedures are as indicated in the text.

Table IV—Anticonvulsant and Monoamine Oxidase Inhibitory Properties of α-Benzoylamino-*N*-(substituted hydrazides)-substituted Cinnamides[a]

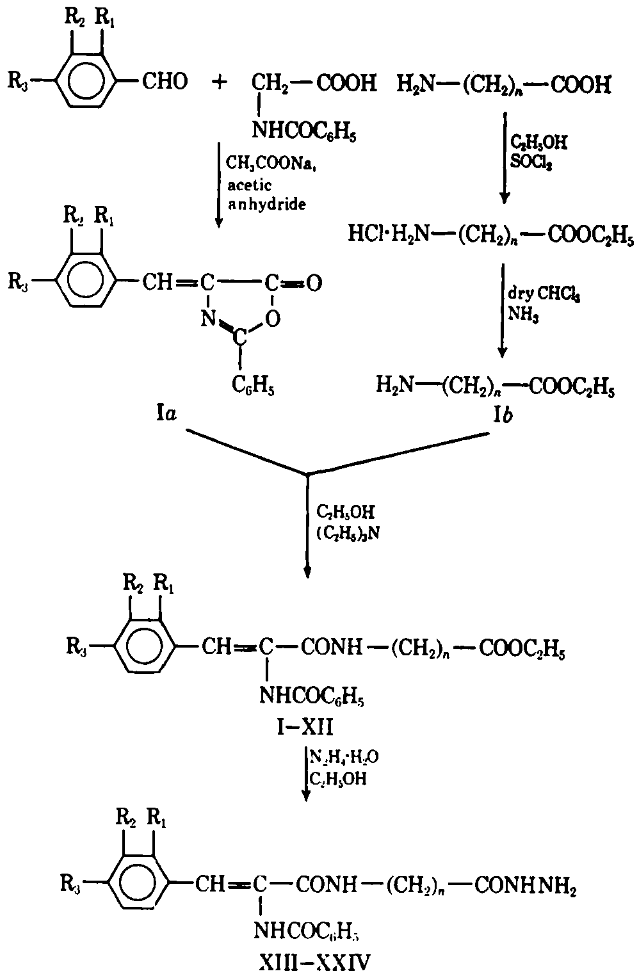


Compound Number	Anticonvulsant Activity, % Protection	Pentylenetetrazol Mortality, %	Monoamine Oxidase Inhibition, % Kynuramine[a]	Monoamine Oxidase Inhibition, % Tyramine[a]
XIII	70	30	44.5 ± 0.85	18.5 ± 0.68
XIV	60	50	56.0 ± 0.76	24.0 ± 0.32
XV	50	70	51.2 ± 1.21	23.0 ± 0.43
XVI	40	40	22.2 ± 0.44	19.5 ± 0.65
XVII	20	80	25.8 ± 0.21	26.6 ± 0.43
XVIII	40	60	19.8 ± 0.66	17.3 ± 0.77
XIX	40	50	17.4 ± 0.58	26.0 ± 0.85
XX	50	60	15.9 ± 0.44	23.7 ± 0.43
XXI	50	60	40.2 ± 0.98	24.0 ± 0.24
XXII	40	70	55.0 ± 1.0	45.6 ± 1.23
XXIII	50	60	51.5 ± 0.78	26.4 ± 0.21
XXIV	30	60	80.8 ± 0.65	42.3 ± 1.11

[a] Compound numbers are those recorded in Table II. The final concentrations of the inhibitors used were 5×10^{-4} and 1×10^{-3} *M* during oxidative deamination of kynuramine and tyramine, respectively. Vessel contents and assay procedures are as indicated in the text. Each experiment was done in duplicate, and figures indicate mean values of three separate experiments with ± standard error of the mean.

N-(substituted hydrazides)-substituted cinnamides inhibited *in vitro* oxidative deamination of kynuramine and tyramine by rat brain homogenate. The results, indicating greater sensitivity of these hydrazides for monoamine oxidase when kynuramine was used as the substrate, are in agreement with other studies (1, 10) which also found greater degree of inhibition with kynuramine as compared to tyramine from the α-benzoylamino-*N*-(1-benzhydrazide)-*p*-substituted cinnamides (4).

Elongation of the aliphatic chain was found to have no significant effect on the monoamine oxidase inhibitory effectiveness of α-benzoylamino-*N*-(substituted hydrazides)-substituted cinnamides. Maximum inhibition was observed with α-benzoyl-*N*-(substituted hydrazides)-substituted cinnamides possessing the *ortho*-chloro substituent (Compound XXII) using tyramine, while substituted cinnamide possessing chloro substituents at both the 2- and 3-positions of the benzene ring (Compound XXIV) exhibited maximum inhibition of monoamine oxidase during oxidative deamination of kynuramine. CNS stimulation, a property associated with many monoamine oxidase inhibitors, was not observed with these compounds in the doses employed, presumably due to a relatively weak monoamine oxidase inhibitory activity of these compounds. These data failed to provide a relationship between anticonvulsant activity of α-benzoyl-*N*-(substituted hydrazides)-substituted cinnamides and their ability to inhibit monoamine oxidase. Further studies dealing with the detailed pharmacological and toxicological properties and the effects upon the activity of other enzymes may possibly reflect a biochemical basis for anticonvulsant activity of these substituted cinnamides.



Scheme I

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