in 75 ml of boiling H_2O and the solution was filtered and neutralized at pH 8 with K_2CO_3. The crystalline base was collected on a filter, washed (H_2O), and recrystallized from 60 ml of DMF. On cooling to 0°, the crystals were filtered off, washed (cold MeOH), and dried in a vacuum desiccator, yielding 9.8 g (69.5%) of product, mp 214°.

Method F. 1-(3,4-Methylenedioxybenzyl)-4-guanidinopiperazine sulfate.—A mixture of 55 g (0.25 mole) of 1-piperonylpiperazine and 35.3 g (0.254 mole) of S-methylisothiuronium sulfate in 250 ml of H_2O was heated to boiling for 6 hr. On cooling to room temperature overnight, the crystallized product was collected on a filter and recrystallized from 60% *i*-PrOH yielding 40 g (63%) of neutral sulfate, mp 260–262°. *Anal.* ($C_{13}H_{18}N_4O_2 \cdot 0.5H_2SO_4$) C, H, N, S.

1-(3,4-Methylenedioxybenzyl)-4-(2-*o*-triazinyl)piperazine Hydrochloride (1).—A mixture of 35.6 g (0.114 mole) of the above sulfate and 150 ml of DMF was treated with 3.2 ml (0.114 mole) of concentrated H_2SO_4 and 22 g (0.154 mole) of triformamidomethane.[26] The mixture was then heated for 5 hr at 150°. There was incomplete dissolution. After cooling at 10°, the insoluble crystals were filtered off and the solution was concentrated *in vacuo*. The oily residue was taken up in 50 ml of 4 *N* NaOH and extracted several times into 150 ml of $CHCl_3$. After washing and drying (K_2CO_3), the solvent was evaporated to dryness and the residue weighing 29 g was dissolved in 75 ml of anhydrous EtOH. The solution was saturated with HCl gas and after cooling 19.5 g of crystals of salt was obtained; they were recrystallized from 325 ml of 98% MeOH to give 14.2 g (33.5%) of white product, mp 207–211°.

(26) H. Bredereck, R. Gompper, H. Rempfer, K. Kleem, and H. Heck, *Ber.*, **92**, 329 (1959).

Acetylenic Carbamates. III. The N-Cycloaliphatic Derivatives

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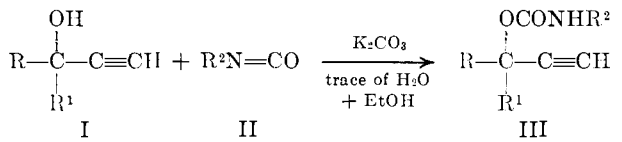
The series of acetylenic carbamates was extended with emphasis on the N-cycloaliphatic derivatives. The N-cycloaliphatics had much less cell culture cytotoxicity and neurotoxicity as determined by intrathecal studies in dogs than the NH_2 compounds, even though their acute toxicities were about the same. Most of the N-cycloaliphatic compounds exhibited antitumor activity.

It has recently been reported[1] that a series of acetylenic carbamates possessed potent antitumor activity against several experimental neoplasms in animals. From the initial structure–activity relationship study, it was apparent that the N-cycloaliphatic groups were very beneficial in promoting antitumor activity. This paper presents the investigation of the relationship of certain toxicological effects to substitution on the nitrogen and an extensive structure–activity study of the carbamates with the N-cycloaliphatic moiety, using the X5563 and C1498 tumor systems.

Chemistry.—Since the compounds in this series all involve monosubstitution on N, use was made of the reaction of the 2-propyn-1-ols with a cycloaliphatic isocyanate as previously described.[1] An improvement in this method was utilized that greatly accelerated the reaction, and yields up to 80% were obtained. This involved the use of CH_2Cl_2 or MeCN as solvent with addition of a catalytic amount of K_2CO_3 and a trace of H_2O and EtOH. All products are listed in Tables I

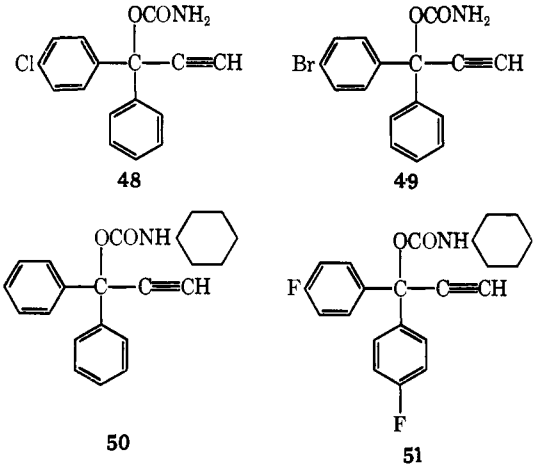


I II III

and II; their purity was determined by the usual physical methods (nmr and ir spectra and elemental analyses).

Pharmacology. Toxicity Studies.—The influence of structural changes on certain toxicological effects were investigated with particular emphasis on the NH_2 and N-cycloaliphatic carbamates. The acute toxicities[2] in mice of six of the carbamates are listed in Table III. There appear to be no significant differences in acute toxicities of the NH_2 and N-cycloaliphatic compounds (**48** and **50**, see Chart I); however, a comparison of the phenyl with *p*-fluorophenyl shows that the fluoro compound is more toxic (**50** and **51**).

CHART I[a]



48 49 50 51

Studied in diverse *in vitro* cell systems, dramatic differences in activity were observed (Table III). The NH_2 carbamates **48** and **49** showed inhibition against the nonparasitic protozoa *Tetrahymena pyriformis*, *Euglena gracilis*, and *Ochromonas malhamensis*, the algae *Chlorella vulgaris* and *Scenedesmus basiliensis*, and the human cell HeLa, previously used to detect potential antitumor agents. For the N-cycloaliphatics **50**, **51**, **29**, and **31** a complete lack of cytotoxicity was demonstrated (although these do show potent antitumor effects). This difference in cytotoxicity has been demonstrated against other tissue culture and bacterial cell systems.

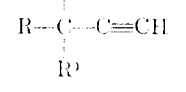
The *in vitro* cell studies were extended to all of the

(1) R. D. Dillard, G. Poore, D. R. Cassady, and N. R. Easton, *J. Med. Chem.*, **10**, 40 (1967).

(2) Long-term toxicity studies with two of the carbamates, **48** and **50**, will be published elsewhere.

TABLE I

ACETYLENIC CARBAMATES AND THEIR ANTITUMOR ACTIVITY



No.	R	R^1	R^2	Mp, °C	Formula[a]	Tumor system[b] X5563[c]	Tumor system[b] C1498[d]
1	CH_3	CH_3	Cyclohexyl	118–122	$C_{12}H_{19}NO_2$	0/150	0/150
2	CH_3	C_6H_5	Cyclohexyl	118–120	$C_{17}H_{21}NO_2$	0/150	0/150
3	Cyclohexyl	C_6H_5	Cyclohexyl	158–160	$C_{22}H_{29}NO_2$	0/300	0/300
4	ClHC=CH	C_6H_5	Cyclohexyl	100–102	$C_{18}H_{20}ClNO_2$	0/12	0/12
5	$H_2C=C(CH_3)$	C_6H_5	Cyclohexyl	122–124	$C_{19}H_{23}NO_2$	61 (5)/300	25/300
6	3-ClC_6H_4	C_6H_5	Cyclohexyl	120–122	$C_{22}H_{22}ClNO_2$	62 (5)/60	0/60
7	3-FC_6H_4	C_6H_5	Cyclohexyl	150–152	$C_{22}H_{22}FNO_2$	100 (7)/150	88/150
8	2-FC_6H_4	C_6H_5	Cyclohexyl	181–183	$C_{22}H_{22}FNO_2$	33 (6)/60	0/60
9	4-$CH_3C_6H_4$	C_6H_5	Cyclohexyl	147–149	$C_{25}H_{25}NO_2$	100 (7)/30	182/30 (3)
10	3-$CH_3C_6H_4$	C_6H_5	Cyclohexyl	128–130	$C_{23}H_{25}NO_2$	100 (7)/30	148/30 (8)
11	3,4$(CH_3)_2C_6H_3$	C_6H_5	Cyclohexyl	133–135	$C_{24}H_{27}NO_2$	100 (7)/30	146/30 (4)
12	4-[$(CH_3)_3C$]C_6H_4	C_6H_5	Cyclohexyl	124–126	$C_{26}H_{31}NO_2$	100 (7)/30	129/30
13	4-$C_6H_{11}C_6H_4$	C_6H_5	Cyclohexyl	118–120	$C_{28}H_{33}NO_2$	100 (3)/30	0/30
14	4-$CH_3OC_6H_4$	C_6H_5	Cyclohexyl	143–145	$C_{23}H_{25}NO_3$	0/75	0/75
15	4-$C_6H_5OC_6H_4$	C_6H_5	Cyclohexyl	68–70	$C_{28}H_{27}NO_3$	67 (5)/30	57/30
16	4-$C_6H_5C_6H_4$	C_6H_5	Cyclohexyl	111–112	$C_{28}H_{27}NO_2$	86 (6)/60	72/60 (1)
17	3-$O_2NC_6H_4$	C_6H_5	Cyclohexyl	120–122	$C_{22}H_{22}N_2O_4$	0/150	0/150
18	2-$C_{10}H_7$	C_6H_5	Cyclohexyl	156–158	$C_{26}H_{25}NO_2$	100 (6)/7.5	80/30 (3)
19	4-C_5H_4N	C_6H_5	Cyclohexyl	155–157	$C_{21}H_{22}N_2O_2$	0/150	0/150
20	2-C_5H_4N	C_6H_5	Cyclohexyl	144–146	$C_{21}H_{22}N_2O_2$[e]	0/300	0/300
21	2-C_4H_3S	C_6H_5	Cyclohexyl	130–132	$C_{20}H_{23}NO_2S$	78 (5)/60	25/60
22	C_6H_5	C_6H_5	2-Norbornyl	162–164	$C_{23}H_{23}NO_2$	88 (7)/30	63/30
23	4-ClC_6H_4	C_6H_5	Cycloheptyl	153–155	$C_{23}H_{24}ClNO_2$	100 (4)/150	
24	4-FC_6H_4	C_6H_5	Cyclopentyl	154–157	$C_{21}H_{20}FNO_2$	100 (7)/15	135/15 (5)
25	4-FC_6H_4	C_6H_5	Cycloheptyl	166–168	$C_{23}H_{24}FNO_2$	100 (10)/7.5	80/7.5 (1)
26	4-FC_6H_4	C_6H_5	2-Norbornyl	176–178	$C_{23}H_{22}FNO_2$	56 (2)/15	0/24 (9)
27	4-FC_6H_4	C_6H_5	Cyclooctyl	152–154	$C_{24}H_{26}FNO_2$	100 (5)/15	65/15 (3)
28	4-FC_6H_4	4-FC_6H_4	Cyclopentyl	171–173	$C_{21}H_{19}F_2NO_2$	49 (6)/6	130/12 (1)
29	4-FC_6H_4	4-FC_6H_4	Cycloheptyl	178–180	$C_{23}H_{23}F_2NO_2$	100 (8)/7.5	145/7.5
30	4-FC_6H_4	4-FC_6H_4	2-Norbornyl	185–187	$C_{23}H_{21}F_2NO_2$	100 (6)/12	40/12 (2)
31	4-FC_6H_4	4-FC_6H_4	Cyclooctyl	170–172	$C_{24}H_{25}F_2NO_2$	100 (10)/15	135/15 (3)
32	3-FC_6H_4	4-FC_6H_4	Cyclohexyl	162–164	$C_{22}H_{21}F_2NO_2$	100 (6)/150	65/150 (3)
33	2-FC_6H_4	4-FC_6H_4	Cyclohexyl	179–181	$C_{22}H_{22}F_2NO_2$	46 (6)/30	20/30
34	C_6H_5	C≡CH	Cyclohexyl	124–126	$C_{18}H_{19}NO_2$	100 (6)/30	20/30
35	4-ClC_6H_4	C≡CH	Cyclohexyl	172–174	$C_{18}H_{18}ClNO_2$	100 (7)/150	0/150
36	4-FC_6H_4	C≡CH	Cyclohexyl	143–145	$C_{18}H_{18}FNO_2$	100 (6)/37.5	0/37.5
37	4-$CH_3C_6H_4$	C≡CH	Cyclohexyl	154–156	$C_{19}H_{21}NO_2$	0/30	35/30
38	4-$CH_3OC_6H_4$	C≡CH	Cyclohexyl	128–130	$C_{19}H_{21}NO_3$	0/60	0/60
39	1-$C_{10}H_7$	C≡CH	Cyclohexyl	132–134	$C_{22}H_{21}NO_2$	100 (4)/37.5	83/37.5

[a] The analytical results obtained for C, H, and N were within ±0.4% of the theoretical values for all compounds unless noted otherwise. [b] The reported activities against the two systems are the results of a specific dose–response test for each compound and should be considered in a qualitative manner in comparing relative potencies. See ref 1. [c] The number given is the per cent inhibition of tumor growth over the dose (intraperitoneal) in milligrams per kilogram. These values were selected as described in ref 1. The number in parentheses is the number of animals surviving the test period out of ten animals. [d] The number is the per cent prolongation of life of treated animals over the dose (intraperitoneal) in milligrams per kilogram. The following number in parentheses is the number of indefinite survivors (those living for 45 days from inoculation) and these are not calculated in the per cent activity. These values are obtained as described in ref 1. [e] H: calcd, 6.63; found, 7.21.

carbamates, and with the exception of the diacetylenic derivatives (**34–39**), the N-cycloaliphatic compounds showed little, if any, cytotoxicity.

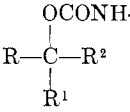
Intrathecal Toxicity.—It was reported by Adamson, *et al.*,[3] that neurotoxicity developed rapidly in dogs given single intrathecal doses of approximately 0.02–0.05 mg of vincristine/kg. Ataxia and hindlimb weakness and/or paralysis developed within 24 hr and complete paralysis within 48 hr. In the absence of other causative factors, it was concluded that vincristine was the neurotoxin. Since intrathecal administration seemed a useful and rapid technique to identify compounds with potential neurotoxicity, five acetylenic carbamates were tested by this route in dogs.

Results.—A summary of the results of the experiments appears in Table IV.

Of the five carbamates injected into the cerebrospinal fluid of dogs, two (**48** and **49**, see Chart I) were shown to be neurotoxins, causing effects qualitatively similar to those described for vincristine. The remaining three carbamates (N-cycloaliphatic) caused ony slight and transient neurotoxicity. The persistent neurotoxicity that followed the administration of 5.0 mg/kg of **50** was attributed to mechanical trauma at the injection site. This was revealed when the dog was sacrificed for autopsy.

(3) R. H. Adamson, R. L. Dixon, M. Ben, L. Crews, S. B. Dhobet, and D. P. Rall, *Arch. Intern. Pharmacodyn.*, **158**, 299 (1965).

TABLE II
RELATED CARBAMATES AND THEIR ANTITUMOR ACTIVITY



No.	R	R^1	R^2	Mp, °C	Formula[a]	Tumor system[b] X5563[c]	Tumor system[b] C1498[d]
40	C_6H_5	C_6H_5	$C{\equiv}CC_6H_5$	158–160	$C_{28}H_{27}NO_2$	51 (3)/300	38/300
41	C_6H_5	C_6H_5	$C{\equiv}CBr$	168–170	$C_{22}H_{22}BrNO_2$[e]	100 (5)/60	61/60
42	C_6H_5	C_6H_5	$C{\equiv}CCH_3$	170–172	$C_{23}H_{25}NO_2$	25 (6)/150	0/150
43	4-FC_6H_4	C_6H_5	$C{\equiv}CCH_3$	171–173	$C_{23}H_{24}FNO_2$	33 (4)/150	0/150
44	4-$CH_3C_6H_4$	C_6H_5	$C{\equiv}CCH_3$	164–166	$C_{24}H_{27}NO_2$	66 (3)/150	0/150
45	C_6H_5	C_6H_5	$CH{=}CH_2$	152–154	$C_{22}H_{25}NO_2$	100 (6)/75	0/75
46	4-FC_6H_4	4-FC_6H_4	$CH{=}CH_2$	164–166	$C_{22}H_{23}F_2NO_2$	0/15	80/15
47	C_6H_5	C_6H_5	CH_2CH_3	149–151	$C_{22}H_{27}NO_2$	0/300	0/300

[a–d] See corresponding footnotes in Table I. [e] C: calcd, 64.08; found, 64.74.

TABLE III
ACUTE TOXICITY AND CELL CULTURE TOXICITY OF THE CARBAMATES

No.	Acute toxicity,[a] mg/kg ip	Cell culture toxicity[b]
48	347 ± 137	+[c]
49	~767.4	+
50	374 ± 15	−
51	153 ± 15	−
29	307.3 ± 16.5	−
31	466 ± 36.4	−

[a] The acute toxicities were determined in ICR mice and mortality was dose responsive. Results were calculated by the method of C. I. Bliss [*Quart. J. Pharm. Pharmacol.*, **11**, 192 (1938)]. [b] Cell culture inhibitions were determined by the general method described by [I. S. Johnson, P. J. Simpson, and J. C. Cline [*Cancer Res.*, **22**, 617 (1962)]. The systems used were *Tetrahymena pyriformis, Euglena gracilis, Ochromonas malhamensis, Chlorella vulgaris, Scenedegmus basiliensis,* and human cell HeLa. The highest concentration of drug used was 40 μg/filter paper disk. [c] The + indicates at least some inhibition against all systems, and the − indicates no effect.

The antitumor effect of optimal doses are reported in Tables I and II. These data should be considered qualitative. Most of the N-cycloaliphatic compounds do show antitumor activity.

Structure–Activity Relationships.—Differences in potency and effectiveness depended not only on the particular N-cycloaliphatic group employed but also on variations in structure on the remainder of the molecule. Conclusions concerning the structural features responsible for optimum antitumor activity were based on the results reported in Tables I and II.[5]

As suggested from these tables (**1–5**, **40–47**), substitution other than 1,1-diaryl and the terminally unsubstituted triple bond do not promote antitumor activity. It is interesting to note, however, that reduction to the vinyl derivatives **45** and **46** retains activity, whereas, with full reduction to the ethyl compound, there was complete loss of activity. Substitution of one of the 1,1-diaryl groups with ethynyl (**34–39**) retains

TABLE IV
THE EFFECTS OF FIVE CARBAMATES GIVEN BY INTRATHECAL INJECTION TO DOGS

No.[a]	Concn in PEG 200, mg/ml	Dose, mg/kg	Sex	Wt, kg	Response[b]	Duration of response
29	12.5	1.25	M(A)[c]	10.5	1, 7	2 days
29	12.5	1.25	F(A)	6.2	None	...
31	12.5	1.25	F(A)	6.6	1, 2	2 days
31	12.5	1.25	M(A)	11.6	1, 9	2 days
48	50.0	5.00	M(U)	9.3	6, 10	7 min
48	25.0	2.50	M(U)	13.0	1–3, 6, 8, 10	8 days
48	12.5	1.25	M(A)	8.3	1, 2, 6, 8	7 days (S)[d]
48	12.5	1.25	F(A)	15.6	1, 6, 8, 10	2 days
49	50.0	5.00	M(U)	10.6	1–3, 6, 8, 10	36 hr
50	50.0	5.00	F(U)	10.3	1–5	103 days (S)
50	50.0	5.00	M(U)	9.1	1, 2	5 hr

[a] See Tables I and II and Chart I. [b] 1 = ataxia, 2 = tremors, 3 = head shaking, 4 = hyperirritability to sound and touch, 5 = anorexia, 6 = prostration, 7 = hypoactivity, 8 = paralysis, 9 = clonic convulsion, 10 = death. [c] (A) = anesthetized, (U) = unanesthetized. [d] (S) = sacrificed.

Larger doses of **29** and **31** were not used because of their limited solubility in PEG 200.

Antitumor Testing. Methods and Results.—Although many of the N-cycloaliphatic carbamates were tested against a variety of experimental tumor systems in these laboratories, two of these, X5563 and C1498, were selected for evaluating this series. These systems and testing methods have been previously described.[1,4]

activity. In comparing **6–21**, the effects of changes in substitution on one of the 1,1-diphenyl groups or replacing one of the phenyls with another aromatic moiety are seen. The most interesting variations include the alkylphenyl (**9–13**), the 2-naphthyl (**18**), and the fluorophenyl compounds previously described.[1] Comparing **28**, **51**,[6] **29**, **30**, and **31**, the effect of changing

(4) I. S. Johnson, H. F. Wright, G. H. Svoboda, and J. Vlantis, *Cancer Res.*, **20**, 1016 (1960).

(5) For regression analyses of the compounds reported in ref 1, see W. P. Purcell and J. M. Clayton, *J. Med. Chem.*, **11**, 199 (1968).

(6) For the antitumor results, see ref 1.

the ring size of the N-cycloaliphatic group is noted. As the size of the ring is increased, potency is decreased.

In summary, the N-cycloaliphatic carbamates in which the 1,1-diaryl substituents are phenyl, fluorophenyl, or methylphenyl and in which terminal position of the acetylene is unsubstituted represent the most desirable compounds with respect to antitumor and toxicological properties.

Experimental Section

All melting points were determined using a Mel-Temp melting point apparatus and are uncorrected. The ir and nmr spectra determined on all compounds were as expected. Analytical results obtained for C, H, and N were within ±0.4% of the theoretical values.

Pharmacological Methods for Intrathecal Studies.—Polyethylene glycol 200 (PEG 200) was used as a vehicle because of the aqueous insolubility of the carbamates. It was known that 0.2 ml/kg of PEG 200 injected into the cerebrospinal fluid caused only transient motor incoordination. Solutions were prepared by dissolving the carbamates (**29**, **31**, **48**, **49**, **50**) in warm (55°) PEG 200 in concentrations varying from 12.5 to 50.0 mg/ml.

In some experiments the dogs were unanesthetized; in others, administration of the carbamates was performed under intravenous methohexital sodium (12.5 mg/kg) anesthesia. The carbamate solutions were routinely administered in 0.1-ml/kg volumes. The dogs were loosely confined in pens and were watched for the development of any neurological deficit. The onset, duration, and intensity of effects were recorded. Dogs considered normal 2 weeks after treatment were returned to stock.

General Synthetic Procedure.—All of the compounds were made by the following general procedure and are listed in Tables I and II.

A solution of 0.1 mole of the 2-propyn-1-ol and 0.12 mole of the cycloalkyl isocyanate in 50 ml of CH_2Cl_2 to which one drop of H_2O, one drop of EtOH, and 0.01 mole of K_2CO_3 had been added was heated at reflux temperature for 2–20 hr (if MeCN was used as solvent, heating was for 0.5–1.0 hr). After cooling, the reaction mixture was diluted with more CH_2Cl_2 and washed (H_2O). After drying ($MgSO_4$), the solvent was removed at reduced pressure, and the residue was crystallized from C_5H_{12}–petroleum ether (bp 35–60°). Recrystallization using the same type of solvent was performed when necessary.

Acknowledgment.—The microanalyses were performed by Messrs. George Maciak, Raymond Cain, Buddy Cantrell, David Cline, and Robert Meister. Appreciation is given to Mrs. Virginia Bothwell and Messrs. James Mattingly, Edward Priller, and Paul Craig for assistance in testing these compounds in the animal tumor screen. Many of the intermediate compounds were prepared by Mr. Lawrence White. We are most grateful to Mr. Donald Pavey for his technical assistance in preparing these compounds.

Anticonvulsant Semicarbazides

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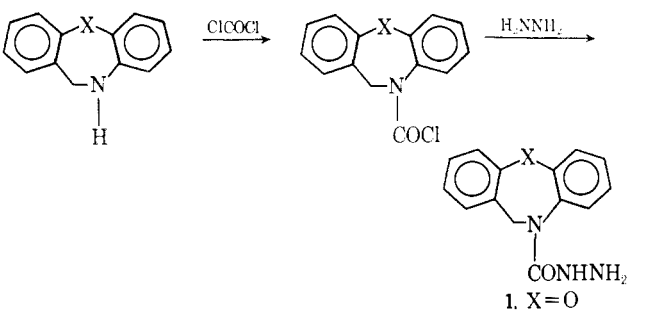
A series of semicarbazides was synthesized from various tricyclic amines and the structure–activity relationships of their anticonvulsant activity was investigated.

During the course of our investigation of carbamoyl derivatives of tricyclic amines, we prepared 10,11-dihydrodibenz[*b*,*f*][1,4]oxazepin-10-carboxylic acid hydrazide (**1**) and found it to have potent anticonvulsant and analgetic properties. The scope of this activity was examined by preparing a series of similar hydrazides in which substitution on the nitrogen functions and aryl groups was investigated as well as compounds in which the oxygen bridge was replaced by S, NCH_3, CH_2CH_2, CH=CH, and a single bond.

The desired semicarbazides were synthesized from the tricyclic amines *via* the carbamoyl chlorides as seen in Scheme I. Most of the tricyclic amines employed and the carbamoyl chlorides of these amines have been described by us previously.[1] Treatment of the carbamoyl chlorides with hydrazine or substituted hydrazines gave the expected products in good yield. Acylation of the semicarbazides obtained above gave the terminal acyl derivatives as confirmed by spectral comparison with standard compounds. The unsubstituted semicarbazides condensed readily with aldehydes but were inert to ketones such as acetone, acetophenone, and cyclohexanone.

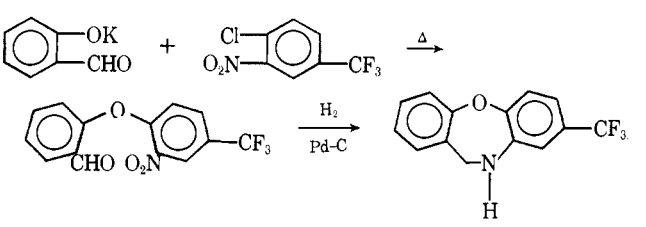
Two new tricyclic amines were prepared for structure–activity studies. 8-Trifluoromethyl-10,11-dihydrodibenz[*b*,*f*][1,4]oxazepine was obtained in a two-step synthesis shown in Scheme II. Condensation of 2-nitro-4-trifluoromethylchlorobenzene with potassium salicylaldehyde gave O-(2-nitro-4-trifluoromethylphenyl)salicylaldehyde which was subsequently hydrogenated with Pd–C to give an excellent yield of the desired amine.

SCHEME I



1. X=O

SCHEME II



(1) W. E. Coyne and J. W. Cusic, *J. Med. Chem.*, **10**, 541 (1967).