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Substituted Thiadiazolines as Inhibitors of Central Nervous System Carbonic Anhydrase

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A series (**24–30**) of substituted thiadiazolines was synthesized and tested for *in vitro* carbonic anhydrase inhibition and for protective ability against pentylenetetrazole-induced convulsions. ED_{50} (pentylenetetrazole protection), TD_{50}, and LD_{50} values are reported for each compound. With the exception of 30, all compounds approximated the model compound methazolamide as *in vitro* carbonic anhydrase inhibitors. Several of the compounds produced extended protection against pentylenetetrazole-induced convulsions. Ring methoxy substitution in the ortho position appeared to produce maximum activity.

Mann and Kellin[1] demonstrated the inhibitory effects of unsubstituted sulfonamides on carbonic anhydrase in 1940. Subsequent work led to the development of clinically important diuretics, a few of which revealed potential clinical usefulness as anticonvulsants. In a series of thiadiazole derivatives methazolamide (**1**) (2-acetylimino-3-methyl-Δ^4-1,3,4-thiadiazoline-5-sulfonamide, Neptazane, Lederle Laboratories) showed the highest concentration in the brain.[2] This compound served as a model for the design of the compounds in this paper. Previous work[3] had revealed increased carbonic anhydrase inhibition in those compounds bearing an aromatic ring in the 2-substituents. Aromatic ring methoxy substitutions were modeled after known hallucinogenic compounds. Halogen substitutions were prepared to provide opposite electronic effects for SAR comparisons and the unsubstituted compound was prepared as a standard.

Experimental Section

Methazolamide was obtained through the courtesy of Lederle Laboratories. Melting points were observed on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were taken on a Beckman Microspec Model 1485 in Nujol mull. Nmr spectra were determined on a Hitachi Perkin-Elmer spectrometer Model R-24. Elemental analyses were performed by Baron Consulting Company, Orange, Conn. Synthesized compounds are summarized in Tables I and II.

2-Amino-5-benzylmercapto-1,3,4-thiadiazole (2).[4] KOH pellets (24 g, 0.43 mol) were added to a slurry of 2-amino-1,3,4-thiadiazole-5-thiol (40 g, 0.3 mol) in 100 ml of water. After cooling, 50 ml EtOH was added and benzyl chloride (48 ml, 0.38 mol) was added dropwise. The mixture became viscous and a "curdled" white product separated. After stirring for an additional 30 min at 10°, the mixture was diluted with 200 ml of water. Filtration yielded 64 g (95%) of white crystals (EtOH) melting at 157–158°. *Anal.* ($C_9H_9N_3S_2$) C, H, N.

Substituted 2-Benzamido-5-benzylthio-1,3,4-thiadiazoles (3–9)[5] **(General Procedure).** To 25 ml of pyridine was added the appropriate acid (0.02 mol) and **2** (4.5 g, 0.02 mol). With constant stirring $SiCl_4$ (2 g, 0.023 mol) was added dropwise resulting in temperature elevation to *ca.* 85°. Stirring was continued at room temperature for 10 hr and the mixture was poured into ice water. Silica was removed by filtration and the filtrate was concentrated. The residual solid was recrystallized from EtOH–H_2O. Yields ranged from 46 to 55%.

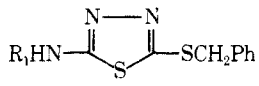
The same amides were synthesized by reacting **2** with acid anhydrides in aqueous acid (5–16% yield), with acid chlorides in aqueous hydroxide (14–32% yield), and with acid chlorides in pyridine (16–22% yield).

Substituted 2-Benzoylimino-3-methyl-5-benzylthio-Δ^4-1,3,4-thiadiazolines (10–16) (General Procedure). The appropriate thiadiazole **3–9** (0.01 mol) was dissolved in 60 ml of water containing KOH (0.7 g, 0.0125 mol) and the solution was diluted with 35 ml of EtOH. Dimethyl sulfate (1.3 g, 0.01 mol) was added, the mixture was refluxed for 20 min, and it was then cooled to 10°. Cold NaOH solution (100 ml, 1.5 *M*) was added until a slurry formed. Water (150 ml) was added and the mixture refrigerated for 1 hr. The precipitate was collected by filtration and recrystallized from MeOH–H_2O. Yields ranged between 46 and 62%. Similar methylations with CH_3I and K_2CO_3 in acetone resulted in yields between 29 and 43%.

Substituted 2-Benzoylimino-3-methyl-Δ^4-1,3,4-thiadiazoline-5-sulfonyl Chloride (17–23) (General Procedure). The appropriate thiadiazoline **10–16** (0.05 mol) was added to 40 ml of HOAc in a three-necked flask fitted with a thermometer, an inlet tube, and an outlet tube leading to a water trap. The reaction was maintained at 5° as chlorine was introduced by the inlet tube terminating about 1 in. above the reaction mixture. Chlorine was added in excess over a period of 15 min until the compound went into solution and the solution attained a yellow color. The solution was poured in water and the precipitate filtered and blotted dry. Yields ranged from 47 to 58%. The compounds were not purified for subsequent reactions.

Substituted 2-Benzoylimino-3-methyl-Δ^4-1,3,4-thiadiazoline-5-sulfonamide (24–30) (General Procedure). The appro-

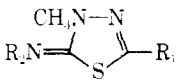
Table I. Derivatives of 2-Amino-5-benzylthio-1,3,4-thiadiazole



Compd no.	R_1	Mp, °C	Formula[a]
3	3,4,5-$(CH_3O)_3C_6H_2CO$-	209–211	$C_{19}H_{19}O_4N_3S_2$
4	2,4,6-$(CH_3O)_3C_6H_2CO$-	195–196	$C_{19}H_{19}O_4N_3S_2$
5	2,4,5-$(CH_3O)_3C_6H_2CO$-	200–201	$C_{19}H_{19}O_4N_3S_2$
6	4-ClC_6H_4CO-	230–231	$C_{16}H_{12}ON_3S_2Cl$
7	4-BrC_6H_4CO-	240–242	$C_{16}H_{12}ON_3S_2Br$
8	4-IC_6H_4CO-	224–226	$C_{16}H_{12}ON_3S_2I$
9	C_6H_5CO-	192–193	$C_{16}H_{12}ON_3S_2$

[a] All compounds analyzed within ±0.4% of theory (C, H, N).

Table II. Derivatives of 2-Imino-3-methyl-Δ^4-1,3,4-thiadiazoline



Compd no.	R_t	R_2	Mp, °C[a]	Formula[b]
10	$C_6H_5CH_2S$-	3,4,5-$(CH_3O)_3C_6H_2CO$-	155–157	$C_{20}H_{21}O_4N_3S_2$
11	$C_6H_5CH_2S$-	2,4,6-$(CH_3O)_3C_6H_2CO$-	140–141	$C_{20}H_{21}O_4N_3S_2$
12	$C_6H_5CH_2S$-	2,4,5-$(CH_3O)_3C_6H_2CO$-	147–149	$C_{20}H_{21}O_4N_3S_2$
13	$C_6H_5CH_2S$-	4-ClC_6H_4CO-	178–180	$C_{17}H_{14}ON_3S_2Cl$
14	$C_6H_5CH_2S$-	4-BrC_6H_4CO-	185–187	$C_{17}H_{14}ON_3S_2Br$
15	$C_6H_5CH_2S$-	4-IC_6H_4CO-	170–172	$C_{17}H_{14}ON_3S_2I$
16	$C_6H_5CH_2S$-	C_6H_5CO-	123–124	$C_{17}H_{15}ON_3S_2$
24	H_2NSO_2-	3,4,5-$(CH_3O)_3C_6H_2CO$-	248–250	$C_{13}H_{16}O_6N_4S_2$
25	H_2NSO_2-	2,4,6-$(CH_3O)_3C_6H_2CO$-	231–233	$C_{13}H_{16}O_6N_4S_2$
26	H_2NSO_2-	2,4,5-$(CH_3O)_3C_6H_2CO$-	235–237	$C_{13}H_{16}O_6N_4S_2$
27	H_2NSO_2-	4-ClC_6H_4CO-	274–276	$C_{10}H_9O_3N_4S_2Cl$
28	H_2NSO_2-	4-BrC_6H_4CO-	280–282	$C_{10}H_9O_3N_4S_2Br$
29	H_2NSO_2-	4-IC_6H_4CO-	268–270	$C_{10}H_9O_3N_4S_2I$
30	H_2NSO_2-	C_6H_5CO-	253–255	$C_{10}H_{10}O_3N_4S_2$

[a] Compounds **10–16** were recrystallized from MeOH–H_2O. Compounds **24–30** were purified by dissolving in dilute base, treating with activated charcoal, and acidification. [b] All compounds analyzed within ±0.4% of theory (C, H, N).

priate sulfonyl chloride **17–23** (0.03 mol) in crude damp form was added slowly to liquid NH_3 (50 ml). Vigorous reaction ensued and solution formed. Excess NH_3 was removed by warming and the residue dissolved in weak base, treated with activated charcoal, filtered, and acidified. The products isolated by filtration were obtained in yields from 67 to 76% based on damp starting material.

pK_a Determinations. These determinations were conducted according to published[6] procedures and are listed in Table III for compounds **24–30**.

***In Vitro* Determinations of Brain Carbonic Anhydrase Content.** The brains of mice (white, male, Royal Hart, North Hampton, N.Y., used for entire study) were removed immediately upon bleeding, rinsed with isotonic NaCl solution, and quick-frozen in liquid nitrogen. The weight of each brain averaged 450–500 mg. Homogenates (10% w/v) of the brains were made with ice-cold isotonic NaCl solution in a hand-held glass homogenizer fitted with a Teflon pestle (10 strokes/min for 8 min). Then 10-ml portions were centrifuged at 8700g for 20 min to remove any intact red blood cells trapped within the brain.

Table III. pK_a and I_{50} of Compounds

Compd no.	pK_a (S.E.)[a]	Mean amt[c] of compd, μg (±S.E.)	I_{50},[d] mol
24	7.86 (±0.02)	0.0126 (±0.0037)	$4.0\ (\pm1.3) \times 10^{-8}$
25	7.69 (±0.03)	0.0080 (±0.0012)	$2.6\ (\pm0.4) \times 10^{-8}$
26	8.01 (±0.02)	0.0073 (±0.0007)	$2.3\ (\pm0.2) \times 10^{-8}$
27	7.62 (±0.02)	0.0140 (±0.0023)	$5.2\ (\pm0.9) \times 10^{-8}$
28	7.46 (±0.04)	0.0150 (±0.0029)	$5.0\ (\pm1.0) \times 10^{-8}$
29	7.71 (±0.04)	0.0160 (±0.0023)	$4.7\ (\pm0.7) \times 10^{-8}$
30	7.45 (±0.05)	0.0170 (±0.0024)	$7.1\ (\pm1.0) \times 10^{-8}$
Methazolamide	7.08 (±0.05)[b]	0.0087 (±0.0007)	$4.5\ (\pm0.4) \times 10^{-8}$

[a] Each value represents three determinations ± standard error. [b] Lit.[10] pK_a = 7.3. [c] The listed amount with standard error is the average of three concentrations of compound which increased the color change reaction time from approximately 31 sec indicating two enzyme units of activity to approximately 55 sec indicating one enzyme unit of activity in the 0.8-ml micromethod determination of inhibitory capacity. It is equal to the amount of the test compound which provides 50% inhibition of the enzyme present in the *in vitro* system. [d] Concentration causing 50% inhibition in above system.

Ice-cold CO_2 solution was prepared by passing CO_2 into deionized water cooled in an ice bath for 1 hr. A mixture of 0.2 ml of CO_2 solution and 0.3 ml of Phenol Red indicator solution was diluted to 0.7 ml with deionized water. Ice-cold carbonate buffer (0.1 ml) (prepared from 1.73 g of $NaHCO_3$, 3.18 g of Na_2CO_3, and deionized water to make 100 ml) was added and the time recorded for a color change of red to yellow was recorded. This uncatalyzed reaction was conducted six times and was determined to require 110 ± 3.38 sec.

The amount of carbonic anhydrase present in the brain homogenate recorded as enzyme units (1 eu is defined as the quantity of carbonic anhydrase which will double the rate of uncatalyzed reaction under specified conditions) was determined by conducting similar color change determinations on 0.1-ml samples of various brain homogenate dilutions.[7]

The brain homogenates prepared as described were diluted (1 ml of homogenate diluted to 2, 4, 8, 16, 32, 62.5, 125, 250, 500, 1000 ml) to provide the test solutions. Ice-cold carbon dioxide solution (0.2 ml) and Phenol Red indicator (0.3 ml) were mixed and the solution was immersed in ice and kept below 5°. The solution was brought to a volume of 0.6 ml with deionized water. An addition of 0.1 ml of brain homogenate dilution was followed immediately by 0.1 ml of buffer. That dilution which brought about a decrease in time required for color change from 110 sec to 56 was defined as containing one enzyme unit. One enzyme unit was found to be contained in 0.1 ml of a solution prepared by diluting 1 ml of brain homogenate to 4 ml. A color change in 31 sec was observed when 2 eu were present.

***In Vitro* Determination of I_{50} of Synthesized Compounds.** Solutions of the synthesized compounds in deionized water (0.001 μg/0.1 ml to 1 μg/0.1 ml) were prepared. Phenol Red indicator (0.3 ml) was mixed with 0.2 ml of CO_2 solution and 0.1 ml of deionized water. To this were added 0.1 ml of brain homogenate containing 2 eu and 0.1 ml of test compound solution. The concentration of test compound required to delay the color change time from 31 (2 eu) to 55 sec (1 eu) was recorded and used to calculate the I_{50} of the synthesized compounds. These values appear in Table III.

TD_{50} and LD_{50} Determination. Toxicity of the test compounds was defined as changes in respiratory patterns or any signs of unsteady gait. TD_{50} values were determined according to published procedures.[8] LD_{50} values were determined in the same fashion and

Table IV. ED_{50}, TD_{50}, and LD_{50} of Compounds

Compd no	ED_{50} ($p = 0.05$),[a] mg/kg	TD_{50} ($p = 0.05$),[b] mg/kg	LD_{50} ($p = 0.05$), g/kg
24	62.5 (42–94)	332 (276–398)	1.93 (1.64–2.26)
25	53.9 (43–68)	319 (253–402)	2.04 (1.72–2.42)
26	50.0 (21–122)	332 (283–389)	2.15 (1.90–2.45)
27	56.0 (39–79)	251 (211–298)	1.43 (1.21–1.70)
28	62.5 (51–77)	237 (202–277)	1.21 (1.02–1.45)
29	52.0 (46–73)	265 (226–310)	1.35 (1.14–1.61)
30	62.5 (48–82)	296 (253–346)	1.72 (1.47–2.02)
Methazolamide	53.9 (43–68)	296 (261–336)	2.42 (2.06–2.83)

[a] Protection from pentylenetetrazole CD_{95} challenge at dose + 1 hr. [b] Appearance of changes in respiratory patterns or unsteady gait.

are reported with the TD_{50} in Table IV. The confidence limits ($p = 0.05$) are broad but adequate for this study.

***In Vivo* Determination of Pentylenetetrazole Protection.** The test mice were stabilized for 1 week with food and water as desired. A CD_{95} (convulsive dose for 95% of the animals) dose of pentylenetetrazole was determined[9] to be 85 mg/kg given subcutaneously.

Using the method of Weil,[8] solutions of test compounds were prepared in 0.25 *N* NaOH to provide 0.1-ml doses containing 40, 50, 62.5, and 78 mg/kg. The vehicle itself was shown not to offer any protection against pentylenetetrazole-induced convulsions. Sixty minutes after intraperitoneal dosing with test compound the animals were challenged with the CD_{95} of pentylenetetrazole. The animals were observed for a 30-min period to determine the presence of any seizure episodes. Any episode of clonic spasm lasting for at least 5 sec was considered a threshold convulsion. The test animals which did not exhibit any threshold convulsions during 60 min of observation were considered protected. Any transient body jerk or intermittent tremor by the test animal was not taken as a seizure. None of the animals exhibited any toxic symptoms at doses used to determine pentylenetetrazole protection. ED_{50} protective doses at 1 hr after administration are reported in Table IV.

Determination of Duration of Protection. Groups of ten mice were injected ip with predetermined ED_{50}'s of the test compounds and one group was injected with vehicle alone. At even hour intervals after injection, five animals were challenged with a CD_{95} of pentylenetetrazole. At the same time five animals receiving only vehicle were also challenged. The other five test animals were challenged at odd hour intervals along with the remaining five control animals. All control animals convulsed with each challenge. The results of this duration study are reported in Table V.

Determination of Test Compound in Brain Tissue. Four test and four control animals were used for each determination. Test compounds were injected ip at their respective ED_{50} doses. At the time described by the duration study as "peak effect" time the animals were sacrificed and brain homogenates prepared as previously described. After centrifugation the supernatant fraction was heated at 100° for 5 min to destroy carbonic anhydrase activity present in the brain. A previous determination had demonstrated boiling of the test compounds in water for 5 min not to cause degradation.

The colorimetric pH changing micromethod as described in the *in vitro* determination of brain carbonic anhydrase was used in this determination. Saturated carbon dioxide solution (0.2 ml) maintained at 5° was mixed with 0.3 ml of Phenol Red indicator and 0.1 ml of deionized water. The appropriate dilution of mouse brain tissue (0.1 ml) containing two enzyme units of carbonic anhydrase activity was added. This addition was immediately followed with the addition of 0.1 ml of the supernatant material as described above and 0.1 ml of buffer solution. The time required to obtain a color change of the indicator from red to yellow was noted for the test animals and control. Three such color change readings were determined for each animal. The results are reported in Table VI.

Results and Discussion

pK_a and *in Vitro* Carbonic Anhydrase Inhibition

Table V. Duration of Protection Study of Compounds

Compd no.	Dose, mg/kg	Hours of protection[a] 2	3	4	5	6	7	8	9	10	11
24	62.5	2	2	4	4	5	5	3	1	1	1
25	53.9	2	2	3	4	4	5	3	1	1	1
26	50.0	3	3	4	4	4	4	5	5	2	1
27	56.0	2	3	4	3	1	1	0	0	0	0
28	62.5	1	2	4	2	1	1	0	0	0	0
29	52.0	1	1	3	4	3	1	1	0	0	0
30	62.5	1	1	2	2	0	0	0	0	0	0
Methazolamide	53.9	2	2	3	5	3	2	1	0	0	0

[a] The figures in the hours of protection columns refer to the number of the group of five test animals that were protected against pentylenetetrazole-induced seizures at that hour after administration of the respective ED_{50} of each compound at 0 hr.

Table VI. Color Change Reaction Times in the Determination of the Test Compounds' Presence in Brain Tissue

Compd no.	Control animals[a] color change rxn time, sec	Dosed animals[a] color change rxn time, sec
24	36.75 ± 1.03	42.25 ± 0.85[b]
25	36.25 ± 1.55	41.00 ± 1.87
26	35.75 ± 0.95	48.00 ± 1.08[b]
27	33.25 ± 0.85	41.25 ± 0.63[b]
28	33.50 ± 0.87	37.25 ± 1.03[b]
29	34.00 ± 1.08	37.75 ± 0.63[b]
30	33.25 ± 1.18	33.50 ± 0.65
Methazolamide	34.25 ± 0.85	45.00 ± 0.82[b]

[a] Four control and four dosed mice were used for each compound. [b] Significant at $p = 0.05$.

(Table III). There appeared to be no significant difference in the pK_a values for the test compounds in spite of the different substituent effects of methoxy and halogen groups. This is not surprising, due to the distance between the substituent R group and the acidic group as well as the absence of any conjugative transmission of the electronic effects. Thus, the pK_a values vary only slightly.

In a similar fashion, no apparent relationship existed between the pK_a values and enzyme-inhibiting capacity. Compound **30** had the lowest pK_a (7.45) and the weakest enzyme inhibition ($I_{50} = 7.1 \times 10^{-8}$) and compound **26** had the highest pK_a (8.01) and the strongest enzyme inhibition ($I_{50} = 2.3 \times 10^{-8}$); yet methazolamide with even a lower pK_a (7.08) demonstrated intermediate enzyme inhibition ($I_{50} = 4.5 \times 10^{-8}$). There is a significant difference between the inhibitory capacity of compounds **25** ($I_{50} = 2.6 \times 10^{-8}$), **26** ($I_{50} = 2.3 \times 10^{-8}$), and the halogen-substituted compounds, the unsubstituted compounds, and methazolamide suggesting increased activity brought about by a noncoplanar side amide unit. These preliminary results also indicated an increase in activity as the size of the para substituent increased: Cl ($I_{50} = 5.2 \times 10^{-8}$), Br ($I_{50} = 5.0 \times 10^{-8}$), I ($I_{50} = 4.7 \times 10^{-8}$); however, the standard error of the determination did not permit a definitive conclusion.

ED_{50}, TD_{50}, and LD_{50} Values (Table IV). Since the *in vivo* evaluation of the test compounds was to involve protection against pentylenetetrazole-induced convulsions, it was necessary to demonstrate that at the challenge doses

the compounds were not convulsants. After determining neurological fitness of the animals,[9] they were challenged with increasing doses of the compounds until subjective evidence (changing or difficulty in breathing patterns or unsteady gait) of toxicity was noted. Subsequent analysis[5] of these data demonstrated no significant difference between the compounds but did reveal significant difference between the eventual ED_{50} range (21–122 mg/kg) and the TD_{50} range (202–402 mg/kg).

Further increases in doses finally resulted in death of the test animals. Death, which usually occurred within 5 min of injection, was preceded by tonic convulsive seizures associated with respiratory problems and ultimate collapse. Significant differences were noted between the lethal range (1.02–2.83 g/kg) and the toxicity range, although no significant difference between the compounds at either the ED_{50}, TD_{50}, or LD_{50} was identified.

At the ED_{50} dose, the test animals did not demonstrate salivation, lacrimation, or excessive urination nor were any tremors, twitches, or convulsions noted prior to pentylenetetrazole. Subtle nonquantifiable subjective behavioral changes were noted after dosage with the test compounds which were not observed in the control animals. The methoxy derivatives appeared to enhance alertness, whereas the halogen derivatives rendered the animals more passive.

The seizures induced in control animals were characterized by initial loss of righting followed by rigid tonic extension of the hind legs lasting for 2–3 min. More than one seizure occurred during the 30-min observation period. Three of the control animals died of respiratory failure within 5 min of pentylenetetrazole challenge. The vehicle alone provided no seizure protection.

Duration of Anticonvulsant Action (Table V). The unsubstituted compound did not provide satisfactory protection against pentylenetetrazole-induced convulsions. Maximum activity resulted in only two of five animals being protected. That compound showed the weakest *in vitro* carbonic anhydrase activity ($I_{50} = 7.1 \times 10^{-8}$) and is the only one subject to metabolic para hydroxylation.

Although there was no difference between the time of onset of activity with halogen or methoxy substitution, the methoxy derivatives, particularly the 2,4,5 isomer, persisted for a longer duration. The greater contribution to lipid solubility by a single halogen atom *vs.* three methoxy groups would afford the halogen derivative's more rapid passive transport and subsequent removal from the area of activity. In addition, the electron-rich trimethoxy aromatic rings would more readily form charge-transfer complexes retarding removal.

At the time of peak activity, the animals were sacrificed and their brains assayed for test compound. Presence of unsubstituted compound could not be determined after two separate attempts. The low order of carbonic anhydrase inhibition which is the basis of the analytical procedure demonstrated by this compound would indicate that the failure of identification was due to a lack of test sensitivity.

The test animals were maintained for 90 days of observation without the appearance of toxic effects.

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Potential Inhibitors of L-Asparagine Biosynthesis. 2. Chemistry and Biological Activity of β-Hydroxyaspartic Acid and Its Derivatives[1]

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Several derivatives of *erythro*-β-hydroxy-DL-aspartic acid (1) were prepared as potential inhibitors of L-asparagine synthetase (ASase) from rat Novikoff hepatoma. Benzylation of 1 gave the dibenzyl ester **2** which upon coupling with carbobenzoxyglycine afforded the blocked dipeptide **3**. Deblocking of 3 gave glycyl-*erythro*-β-hydroxyl-DL-aspartic acid (4) which could not be diazotized. The dimethyl ester of 1 was coupled with carbobenzoxyglycine to give the blocked dipeptide **7a** which was deblocked to give dimethyl glycyl-*erythro*-β-hydroxy-DL-aspartate hydrochloride (8). Diazotization of 8 gave impure diazo compound **9** which on reaction with HCl gave the chloro compound **10**. The methods of isolation, assay, and inhibition of ASase are described. At 10 m*M* concentrations **10**, 1, and its D and L enantiomers inhibit ASase by 45, 47, 36 and 66%, respectively.

The enzyme L-asparaginase has been shown to be an effective antitumor agent in both murine and human neoplasms,[2] the mechanism being the depletion of exogenous stores of L-asparagine.[2] It has also been shown that resistance to L-asparaginase therapy in certain murine tumors[3] and human neoplastic tissues[4] is related to the synthesis of endogenous L-asparagine *via* the enzyme L-asparagine synthetase (ASase). Mammalian ASase synthesizes L-asparagine from L-aspartic acid, utilizing L-glutamine as the primary source of nitrogen plus certain other cofactors.[5] We have thus embarked on a program aimed at the development of amino acid analogs related to aspartic acid and

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