New Azacannabinoids Highly Active in the Central Nervous System

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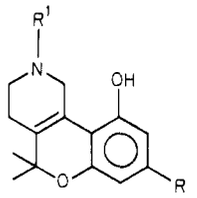
Pursuit of the hypothesis that optimum central nervous system (CNS) activity in 2-azacannabinoids (I and II) requires a moderately basic nitrogen atom (pK = 5–7) has led to several very active α-amino amide derivatives, namely, 10-hydroxy-*N*,5,5-trimethyl-8-(1,2-dimethylheptyl)-1,2,3,4-tetrahydro-5*H*-[1]benzopyrano[4,3-*c*]pyridine-2-acetamide (**3a**), 8-[5-(4-fluorophenyl)-2-pentyl]-10-hydroxy-*N*,5,5-trimethyl-1,2,3,4-tetrahydro-5*H*-[1]benzopyrano[4,3-*c*]-pyridine-2-acetamide (**3b**), and the corresponding acetylurea, **7b**. In a battery of seven CNS tests, **7b** is one of the most potent cannabinoids so far reported.

Prior studies of the CNS pharmacology of a series of nitrogen analogues of the tetrahydrocannabinols (THC's) revealed that optimum potency was achieved in the 7-propynyl derivatives, Ia[1] and Ib.[2]



Ia, R^1 = $CH_2C{\equiv}CH$; R = $CH(CH_3)CH(CH_3)$-*n*-C_5H_{11} = C_9H_{19}
b, R^1 = $CH_2C{\equiv}CH$; R = $CH(CH_3)(CH_2)_3C_6H_4F$-*p* = $C_{11}H_{14}F$
IIa, R^1 = H; R = C_9H_{19}
b, R^1 = H; R = $C_{11}H_{14}F$

Because CNS activity was greatly reduced both in simple alkyl and in acyl[3] analogues of this series, it was concluded that a nitrogen atom of moderate basicity was required for high potency (i.e., 2-propynylamines are 2–3 pK units less basic than the corresponding alkylamines[4]). This notion gained further credence from the observation[5] that incorporation of the nitrogen atom in a fully aromatic pyridine ring also served to maintain high CNS potency. (It should be noted, however, that although moderate basicity may be a necessary condition for high potency, it is clearly not a sufficient one.)

Because α-amino esters and amides are of the same order of basicity[4,6] as 2-propynylamines, a series of such derivatives was made by alkylation of IIa and IIb with the requisite chloro ester or amide derivative. These are listed in Table I. As is previous work,[1,2,5] these compounds were put to a battery of standard pharmacological CNS tests in mice and rats. Results are summarized in Table II.

The rat tail-flick and mouse writhing tests are measures of the ability of a substance to counteract the effects of painful stimuli. The mouse fighting, rat motor activity, methamphetamine antagonism, and serotonin antagonism tests all represent different measures of the tranquilizing activity of a substance. The rat hyperexcitability test measures a CNS response that appears to be peculiar to cannabinoid compounds. It is characterized by lowered activity in undisturbed animals but exaggerated motor and vocal responses in animals that are mechanically or aurally stimulated.

The ester 1 clearly shows cannabinoid-like CNS activity, but its potency is much lower than that of Ia,b in the five tests tried. The slightly higher potency of the primary amide **2** still falls short of Ia,b, but the *N*-methyl amides, **3a,b**, on the other hand, are roughly equal to the activities of Ia,b in all but the serotonin antagonism test. Further methylation on the amide nitrogen atom, however, is counterproductive. Compounds **4a,b** are markedly less potent than **3a,b** and are not any better than the primary amide **2**. N-Phenylation of the amide group virtually destroys activity (e.g., **6**), and the more basic[4] β-amino amide **5** is inactive in all but the methamphetamine antagonism test.

The most striking rise in potency, however, is evident with the acetylurea, **7b**. In all seven CNS tests it tends to exceed the potency of Ia,b, especially with regard to antiwrithing and antifighting activities. This compound thus represents one of the most potent derivatives so far reported in this azacannabinoid series.[7] Methylation at either the terminal nitrogen atom (i.e., **8**) or at the α-carbon atom (i.e., **9**) lowers potency roughly to that of the *N*-methyl amides (**3a,b**), and N-phenylation (i.e., **10**) destroys activity. Inserting a methylene group in the urea system, as in the diglycyl compound **11**, also results in an inactive compound.

Experimental Section

General experimental conditions and instrumentation have been reported.[3] NMR spectra of all target compounds were consistent with their structural assignments. Ethyl chloroacetate, chloroacetamide, and 2-chloropropionamide were commercially available. Compounds IIa[1] and IIb,[2] *N*-methyl-[8] and *N*,*N*-dimethylchloroacetamide,[8] chloroacetylurea,[9] 1-(chloroacetyl)-3-methylurea,[9] 1-(chloroacetyl)-3-phenylurea,[9] α-chloropropionylurea,[9] and chloroacetylglycine amide[10] were prepared by reported methods. The following procedure for the preparation of **7b** serves as a general method for all compounds listed in Table I.

[[8-[5-(4-Fluorophenyl)-2-pentyl]-10-hydroxy-5,5-dimethyl-1,2,3,4-tetrahydro-5*H*-[1]benzopyrano[4,3-*c*]-pyridin-2-yl]acetyl]urea (7b). A solution of 1.50 g (0.011 mol) of chloroacetylurea in 20 mL of dimethylformamide (DMF) was added dropwise to a stirred solution of 3.95 g (0.01 mol) of IIb

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(7) Compound **7a** was not prepared in time to permit subjecting it to the battery of tests. Nevertheless, a neuropharmacological screen in mice produced overt CNS symptomatology (i.e., depression, decreased activity, hypothermia) down to 0.3 mg/kg oral doses, the same as **7b**. Thus, **7a** is probably comparable to **7b** in potency.
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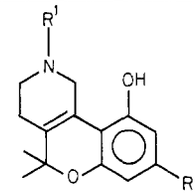
Table I. 2-Substituted 8-Alkyl-5,5-dimethyl-10-hydroxy-1,2,3,4-tetrahydro-5*H*-[1]benzopyrano[4,3-*c*]pyridines



compd	R[a]	R¹	mp,[b] °C	yield, %	formula[c]
1	C_9H_{19}	$CH_2CO_2C_2H_5$	amorph	35	$C_{27}H_{41}NO_4$
2	C_9H_{19}	CH_2CONH_2	165-168	78	$C_{25}H_{38}N_2O_3$
3a	C_9H_{19}	$CH_2CONHCH_3$	131-134	63	$C_{26}H_{40}N_2O_3$
3b	$C_{11}H_{14}F$	$CH_2CONHCH_3$	amorph	98	$C_{28}H_{35}FN_2O_3$
4a	C_9H_{19}	$CH_2CON(CH_3)_2$	122-124	48	$C_{27}H_{42}N_2O_3$
4b	$C_{11}H_{14}F$	$CH_2CON(CH_3)_2$	amorph	79	$C_{29}H_{37}FN_2O_3$
5	C_9H_{19}	$CH_2CH_2CONH_2$	178-180	37	$C_{26}H_{40}N_2O_3$
6	C_9H_{19}	$CH_2CONHC_6H_5$	125-127	18	$C_{31}H_{42}N_2O_3$
7a	C_9H_{19}	$CH_2CONHCONH_2$	131-133	70	$C_{26}H_{39}N_3O_4$
7b	$C_{11}H_{14}F$	$CH_2CONHCONH_2$	133-134	67	$C_{28}H_{34}FN_3O_4$
8	C_9H_{19}	$CH_2CONHCONHCH_3$	145-147	79	$C_{27}H_{41}N_3O_4$
9	C_9H_{19}	$CH(CH_3)CONHCONH_2$	95-100	37	$C_{27}H_{41}N_3O_4$
10	C_9H_{19}	$CH_2CONHCONHC_6H_5$	159-161	33	$C_{32}H_{43}N_3O_4$
11	$C_{11}H_{14}F$	$CH_2CONHCH_2CONH_2$	119-123	53	$C_{29}H_{36}FN_3O_4$

[a] C_9H_{19} = $CH(CH_3)CH(CH_3)$-*n*-C_5H_{11}; $C_{11}H_{14}F$ = $CH(CH_3)(CH_2)_3$-C_6H_4-F-*p*. [b] Compounds **6**, **7a,b**, **8**, and **10** were purified by recrystallization of crude product from acetonitrile, all others were purified by chromatography on a column of Florisil (60-100 mesh) with graded chloroform-methanol (1-5%) mixtures for elution, followed, where possible, by recrystallization from acetonitrile. [c] C, H, and N analyses for all compounds were within ±0.4% of calculated values.

Table II. Biological Activity[a]

compd	analgesia[b] RTF	analgesia[b] W	mouse fighting[c]	rat motor act.[d]	methamphetamine antagonism in rat[d]	serotonin antagonism in rat[d]	rat hyperexcitability[e]
1		35 (10), 51 (40)		49, 19, 60	18, 54, 81	60, 56, 89	0, 12, 81
2	132 (40)	6 (10), 82 (40)	10, 21	28, 78, 86	42, 58, 71	71, 83, 78	65, 79, 98
3a	92 (20)	(5.1 ± 2.1)[f]	11, 34	33, 66, 93	72, 72, 72	−54, 21, 97	92, 100, 100
3b		71 (10)	56, 93	73, 66, 94	51, 63, 73	6, 63, 92	92, 100, 100
4a	84 (40)	19 (10), 86 (40)	10, 13	53, 65, 78	−43, −36, 26		0, 13, 69
4b		24 (10)	3, 12	0, 27, 68	−35, 34, 54	11, 12, 55	0, 10, 69
5		0 (10)		49, 65, 83	−4, 17, 13	−73, −13, −71	0, 0, 67
6		12 (10)		4, 18, 6	0, 15, −9	3, −28, −13	0, 0, 0
7b	(1.6 ± 1.5)[f]	(0.7 ± 0.3)[f]	(2 ± 1)[f]	55 (5), 88 (2)	64 (1), 75 (2)	50 (1), 72 (2)	46, 90, 100
8				63, 68, 88	38, 66, 54	34, 50, 89	35, 71, 81
9				67, 67, 83	47, 50, 81	11, 43, 98	50, 63, 77
10				37, 42, 51	−37, −24, −28	−56, −15, −17	0, 0, 0
11				−11, 12, 9	1, 2, 25	−17, −10, 26	0, 0, 0
Ia	(14 ± 9)[f]	(4.3 ± 1.2)[f]	48 (5), 62 (10)	51 (10)	59, 80, 73	81, 84, 33	29 (5), 71 (10)
Ib	(3.7 ± 4)[f]	(5.3 ± 1.3)[f]	−29 (5), 47 (10)	60 (1), 77 (4)	8, 34, 84		60 (5), 100 (10)
Δ⁹-THC	(27 ± 20)[f]	(43 ± 13)[f]	36 (5)	15 (5), 72 (20)	15 (5), 41 (10)		29 (5), 100 (20)

[a] Numbers in parentheses are oral doses in milligrams per kilogram. Solids were administered as suspensions in 0.5% methylcellulose; Δ⁹-THC was given as a suspension in olive oil and 0.5% methylcellulose. When doses are not specified, two successive activities are at 5 and 20 mg/kg, respectively, and three successive responses are at 5, 20, and 80 mg/kg, respectively. Negative numbers represent percent increases in motor activity vs. controls. [b] Percent increase in nociceptive response threshold vs. controls. RTF = rat tail-flick test; W = mouse writhing test. [c] Percent decrease in fighting vs. controls. [d] Percent decrease in motor activity vs. controls. [e] Percent of maximum number (48) of possible responses actually observed. [f] ED_{50} (oral) in milligrams per kilogram (with 95% confidence limits where indicated) determined by an unpaired *t* test.

in 25 mL of DMF, followed by 1.11 g (0.011 mol) of triethylamine. After being stirred at room temperature for 22 h, the mixture was diluted with water (45 mL) and extracted with ether. Combined ether extracts were washed with water, dried over anhydrous Na_2SO_4, and filtered, and the filtrate was evaporated to dryness. The residue was recrystallized from acetonitrile.

Pharmacology. Except for the serotonin antagonism test, all tests have been described previously.[5,11] In the serotonin antagonism test, three Long–Evans hooded adult rats (100–150 g) per dose level were pretreated with pargyline (50 mg/kg, orally), followed in 2 h by the test drug and 5-hydroxytryptophan at a dose of 100 mg/kg ip. Controls were given vehicle without drug. Activity of the rats was then measured for 60 min with Stoelting electromagnetic sensors. A 50% inhibition of the serotonin-induced motor activity is required for significance.

The statistical test used for determination of the significance of the biological data was by one-way analysis of variance, followed by Duncan's Multiple Range Test to make comparisons among the treatment groups.

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F. Will, Ms. P. Morse, and Mr. W. Jochimsen.

Registry No. 1, 69061-10-5; 2, 69061-14-9; 3a, 69061-15-0; 3b, 69061-04-7; 4a, 69061-05-8; 4b, 69061-06-9; 5, 69061-08-1; 6, 69061-12-7; 7a, 69124-34-1; 7b, 69124-33-0; 8, 69124-37-4; 9, 69124-39-6; 10, 69124-38-5; 11, 83845-02-7; IIa, 26685-53-0; IIb, 60926-09-2; $ClCH_2CO_2C_2H_5$, 105-39-5; $ClCH_2CONH_2$, 79-07-2; $ClCH_2CONHCH_3$, 96-30-0; $ClCH_2CON(CH_3)_2$, 2675-89-0; $ClCH_2CH_2CONH_2$, 5875-24-1; $ClCH_2CONHC_6H_5$, 587-65-5; $ClCH_2CONHCONH_2$, 4791-21-3; $ClCH_2CONHCONHCH_3$, 4791-22-4; $ClCH(CH_3)CONHCONH_2$, 24224-16-6; $ClCH_2CONHCONHC_6H_5$, 4791-23-5; $ClCH_2CONHCH_2CONH_2$, 41312-83-8.

2′-*O*-Nitro-1-β-D-arabinofuranosylcytosine. A New Derivative of 1-β-D-Arabinofuranosylcytosine That Resists Enzymatic Deamination and Has Antileukemic Activity

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To overcome the susceptibility of the anticancer drug 1-β-D-arabinofuranosylcytosine (*ara*-C) to enzymatic deamination, and hence deactivation, we prepared the 2′-*O*-nitro-1-β-D-arabinofuranosylcytosine (termed *nitrara*-C) and evaluated it for biological activity. *Nitrara*-C was resistant to enzymatic deamination and inhibited the proliferation of several strains of human leukemic T and B lymphoblasts grown in culture. Moreover, it substantially extended the life spans of mice with L1210 leukemia. Studies with *ara*-C-resistant human leukemic lymphoblasts deficient in deoxycytidine kinase activity disclosed that the inhibitory activity of the new compound depends on its phosphorylation.

1-β-D-Arabinofuranosylcytosine (*ara*-C), a synthetic nucleoside, is widely used in the treatment of acute myeloblastic and lymphoblastic leukemias.[1-4] The drug is deaminated very rapidly by cytidine deaminase, present in body tissues and some neoplastic cells as well, to 1-β-D-arabinofuranosyluracil (*ara*-U), a metabolite with little or no antitumor activity in most tumor cells.[5-9] Attempts to circumvent the problem of inactivation have included structural modifications of *ara*-C,[10-17] as well as the development of cytidine deaminase inhibitors to be used in combination with the S-phase agent.[18,19] One deaminase-resistant derivative of *ara*-C is 2,2′-anhydro-1-β-D-arabinofuranosylcytosine (cyclo-C),[20-22] which, although not effective against *ara*-C-resistant tumor lines,[22,23] is more

Table I. Substrate Specificity of Cytidine Deaminase[a]

compd[b]	rel initial rate[c]
cytidine	100[d]
2′-deoxycytidine	39
ara-C	15
nitrara-C	<1

[a] Cytidine deaminase was isolated and partially purified from mouse kidney acetone powder (Sigma Chemical Co., St. Louis, MO) essentially by the procedure reported in ref 33 and 34. The amount of 1.3 mg of enzyme protein in a final volume of 1.0 mL of phosphate buffer (pH 7.0, 0.05 M) was used per assay. [b] The concentration of each substrate was 2.5×10^{-4} M. [c] Direct spectrophotometric assay was performed at 37 °C by the procedure reported in ref 35 and 36. [d] Deaminase activity is expressed as a proportion of the most active substrate, cytidine, which was arbitrarily assigned a value of 100.

Table II. Cytotoxicity of *Nitrara*-C and *Ara*-C against Various Human Leukemic Lymphoblastoid Cell Lines

cell line	IC_{50}[a] *nitrara*-C	IC_{50}[a] *ara*-C	ref[b]
CCRF-CEM	6×10^{-7}	2×10^{-8}	37
MOLT-4	4×10^{-7}	1×10^{-8}	38
RPMI-6410	6×10^{-6}	6×10^{-8}	39
CCRF-CEM/*ara*-C[c]	1×10^{-3}	1×10^{-4}	40

[a] Molar concentration for 50% inhibition of cell growth. [b] Culture conditions used were essentially similar to those reported in the references. [c] Mutant deficient in deoxycytidine kinase.

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