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SYNTHESIS OF SUBSTITUTED 5-AMINO-8-PHENYL-3H,6H-1,4,5a,8a-TETRAAZAACENAPHTHALEN-3-0NES, A NEW CLASS OF AGENTS FOR THE IMPROVEMENT OF COGNITION.

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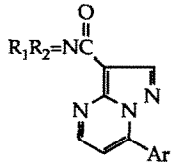
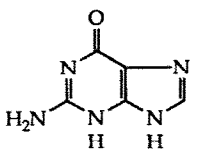
ABSTRACT: The synthesis of the novel 5-amino-8-phenyl-3H,6H-1,4,5a,8a-tetraazaacenaphthalen-3-one ring system has yielded several compounds which display activity in the reversal of cognition deficits in rats and mice as compared to existing reference agents. Activity was determined by improvements in Hypoxic Survival and Anoxic-Induced Amnesia tests.

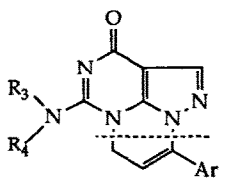
Pyrazolo[1,5-a]pyrimidines have been reported in the literature to show activity as anxiolytics, antischistosomal agents, and phosphodiesterase inhibitors[3]. A series of substituted pyrazolo[1,5-a]pyrimidines **1** studied in our laboratories had displayed good activity in our cognition screens; however, they were unstable and readily hydrolyzed back to the parent amides **2**. In an effort to improve the stability of this template while retaining activity, we endeavored to synthesize the corresponding tricyclic ring system **3**. This approach was particularly attractive since **3** resembles a ring-annelated pyrazolopyrimidine analog of guanine, and guanine nucleotides have recently been implicated in cellular receptor coupling mechanisms[4] and receptor regulation in muscarinic neurons[5], systems known to be affected in Alzheimer's Disease. As a result of this study we now describe the synthesis of a series of compounds with the previously unknown tricyclic 3H,6H-1,4,5a,8a-tetraazaacenaphthalen-3-one ring system, some of which possess significant biological activity in tests for the reversal of cognition deficits, and therefore may be useful in the treatment of conditions of impaired cognitive ability such as Alzheimer's disease.



1, R_1R_2 = $CHNMe_2$,
2, $R_1 = R_2 = H$
(Ar = 3-pyridyl)



Guanine



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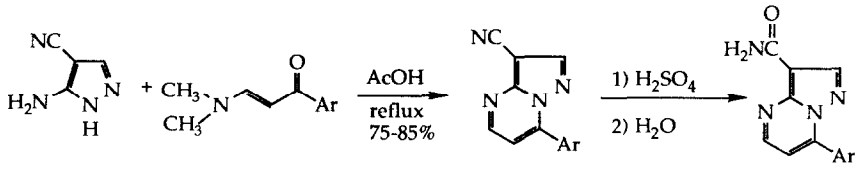
Previous work in these laboratories had demonstrated that 5-unsubstituted-7-aryl-pyrazolo[1,5-a]pyrimidines could be easily constructed in a regiospecific manner and in high yield through the reaction of substituted 3-aminopyrazoles **4** with aryl enaminones **5**, available from the corresponding aryl methyl ketones and DMF dimethyl acetal[6], in refluxing acetic acid. The regiochemistry of this reaction was confirmed by X-ray analysis of the cyano compound **6a**[7]. The cyano compounds were then hydrolyzed to amides **7** with concentrated sulfuric acid.

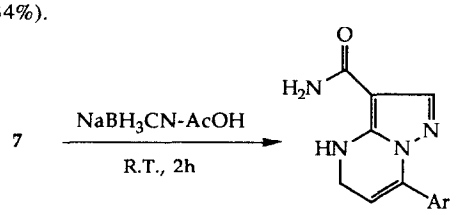


4

	Ar	
5 a	3-pyridyl	**6 a**
5 b	3-CF_3-phenyl	**6 b**
5 c	phenyl	**6 c**

7

A method for the selective reduction of the pyrimidine imine double bond of compounds **7** was now required. It was found that sodium borohydride in methanol or catalytic hydrogenation of compounds **7** resulted in mixtures of several products. However, sodium cyanoborohydride (1.5 equiv.) in acetic acid at room temperature for two hours gave the desired 4,5-dihydro-pyrazolo[1,5-a]pyrimidines **8** in 85% yield. The use of $NaBH_3CN$-MeOH gave the same product in greatly reduced yield (34%).

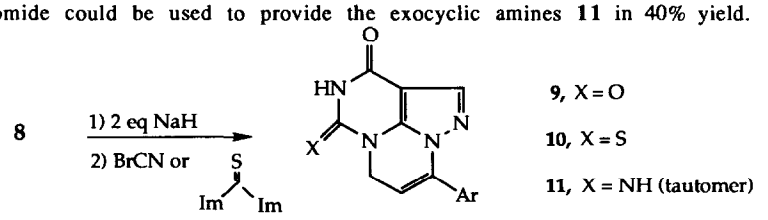


8

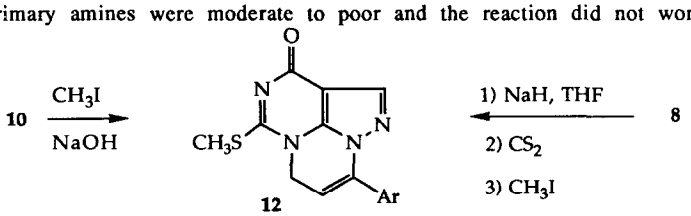
It should be noted that the dihydro compounds were somewhat unstable, being air oxidized at room temperature back to the pyrimidines **7**.

With the dihydropyrimidines in hand, several approaches to the tricyclic ring system were available. In fact, it was found that **8** could be reacted with carbonyl diimidazole in refluxing dioxane to give diones **9** in 72% yield. Unfortunately, the analogous reaction with thiocarbonyldiimidazole gave pyrimidines **7** as the only product. However, when amines **8** were reacted with two equivalents of sodium hydride in THF at -78°C, followed by the addition of

thiocarbonyl diimidazole and slowly warming the reaction to room temperature, the cyclic thiones **10** could be obtained in 85% yield with only minor amounts of pyrimidines **7** formed[8]. Similarly, cyanogen bromide could be used to provide the exocyclic amines **11** in 40% yield.

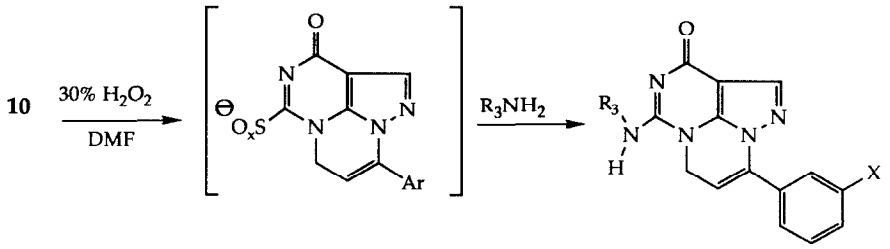


Although amines **11** could be acylated with aroyl chlorides in pyridine, or alkyl acid chlorides and Proton Sponge®, they proved exceptionally resistant to alkylation. All attempts to alkylate amines **11**, or the more soluble trifluoroacetamide derivatives, failed to produce any of the desired mono- or dialkylated amines, resulting only in the recovery of starting material. It was therefore necessary to use thiones **10** as synthons for alkylamine derivatives. Methylation of **10** with MeI/DMF/NaOH or MeI/NaH/THF provided the desired S-methyl compounds **12**, but yields were moderate due to competing N-methylation. Alternatively, the S-methyl compounds could be prepared in 76% yield by reacting carboxamides **8** with sodium hydride (3.1 equiv.) and carbon disulfide (1.5 equiv., -10°C, 0.5h) in THF followed by the addition of methyl iodide (3.1 equiv., R.T., 18h). However, this compound did not prove to be a generally useful intermediate[9]. Displacement of the S-methyl moiety by amines required using the amine as the reaction solvent at 70°C for 12 h. Yields for primary amines were moderate to poor and the reaction did not work at all for



aromatic or secondary amines. Oxidation of the S-methyl group of **12** to the corresponding sulfoxide or sulfone[10], to provide a better leaving group for displacement, could not be accomplished without subsequent hydrolysis to afford the unwanted diones **9**.

Fortunately, by analogy with work done by Pfleiderer and Maryanoff, it was found that thiones **10** could be reacted with 30% hydrogen peroxide (3 equiv., 0°C) followed by addition of the desired amine (7.0 equiv., O°C --> R.T., 12 h), to give the corresponding exocyclic alkylamines **3**[11]. This reaction presumably proceeds via the sulfinic or sulfonic acids **13**, but all attempts to isolate these intermediates gave only diones **9**. Although the yields for this reaction were moderate to poor, this process was so operationally simple as to make it practical (see **Table 1**)[12].



13 **3a-3i**

The activity of these compounds as cognition activators was determined by two *in vivo* screens. **Hypoxic Survival Test.** The rationale for this test is that drugs which enhance survival under hypoxic conditions, without concomitant depression or sedative side-effects, may be functioning by the enhancement of energy metabolism or by preserving normal brain function under conditions of reduced energy metabolism. Given the dependence of the brain on a constant supply of energy, drugs which have this property may have many far-reaching therapeutic indications including the treatment of stroke and closed head injury, as well as reducing the deleterious effects of aging on the central nervous system. For example, in aged and senile demented patients energy metabolism is known to be deficient and is thought to contribute significantly to the neurochemical and neurophysiological dysfunctions of aging. Activity in this screen is demonstrated by the enhanced survival rates of test animals subjected to a hypoxic environment after treatment with drug, as compared to saline-treated control animals.

Extensive testing has demonstrated that under hypoxic conditions (10% O_2/90% CO_2) only 5-20% of control mice (treated with saline) survive after 5 minutes whereas 60-80% of mice treated with physostigmine survive. Thus, groups of 20 Royal Hart mice (6-8 weeks of age) are given intraperitoneal injections with test compound, normally at 10 and 100 mg/kg, 30 minutes prior to placing them in a hypoxic atmosphere and measuring survival after 5 minutes. A separate group of 20 mice is given intraperitoneal injections with saline solution (0.01 cc/g of body weight) and processed as described above. Still another group of 20 mice is given intraperitoneal injections with a known active dose of a reference compound, e.g., 0.125 mg/kg of physostigmine and processed as described above. A compound is judged to be active if greater than 30% of test animals survive.

Passive-Avoidance Anoxic-Induced-Amnesia Test. This test is used to determine the attenuation of anoxic-induced amnesia in mice treated with drug, as compared to saline treated control animals. A shock-motivated, single trial, step-through passive avoidance procedure is used. Groups of 21 Royal Hart and Taconic Farms middle-aged mice (9 mo. of age) are placed singly in the front chamber of a 2-chamber box and are allowed to voluntarily cross into the rear chamber. As soon as the mouse enters the rear chamber, a door automatically traps the animal and

a mild electric shock (0.4 mA for 4 sec.) is delivered to its feet. Following the foot shock, the mice are initially placed in an anoxic environment (100% CO_2) for 12 seconds, which quickly induces unconsciousness. They are then placed in a hypoxic environment (15% O_2) for four minutes which allows the mice to resuscitate slowly. All testing is performed 24 hours later, and in all cases the mice appear fully recovered from the previous anoxic/hypoxic treatment. All test compounds are administered intraperitoneally (i.p.) 30 minutes prior to training and testing. Control animals are injected i.p. with saline at 0.01 mL/g of body weight. The elapsed time before entering the rear chamber is recorded for both training and testing. Presumably, the more the animal remembers being shocked the greater will be its delay in re-entering. An improvement of 30% over saline control scores is considered active.

Table 1. Biological Data[c]

Compd	R_3	X	yield	Hypoxic Survival, i.p., mice			Anoxic Amnesia, i.p., mice[a]			
			%	% survival	mg/kg	no. of tests	latency, sec	mg/kg	% improv.	no. of tests
3a	Et	CF_3	7	65	100	2	----			nt
3b	i-Pr	CF_3	28	15	10	1	----			nt
3c	n-Bu	CF_3	18	58	100	2	----			nt
3d	i-Bu	CF_3	59	50	50	2	51, 218	100	153-209	2
3e	Me	H	21	95	100	1	----			nt
3f	Et	H	57	73	100	2	----			nt
3g	i-Pr	H	14	20	100	1	----			nt
3h	n-Bu	H	44	70	100	2	127, 132	25	62-67	2
3i	i-Bu	H	44	30	100	2	----			nt
3j	H	CF_3	38	68	100	2	102, 180	10	17[b]-207	2
physostigmine				60-80	0.125	>2		0.1	36	>2
saline				0-15	---	>10	33-181			>10

[a]nt = not tested, [b]saline control showed longer delay than usual, [c]data for the dose with the best response is shown

As can be seen in Table 1, many of the compounds that were tested displayed activity in the two indicated screens. However, the biological data do not indicate a strict structure-activity relationship. One of these analogs, **3d**, demonstrated significant oral activity in a variety of behavioral tests used to evaluate improvements in cognition in mice, young (cognition impaired) and aged rats, and is currently undergoing clinical evaluation as a drug for the improvement of cognition. The compounds described in this paper act via some unknown mechanism. It is known that they are not inhibitors of cholinesterase[13], nor do they stimulate the release of acetylcholine[14]. They also do not bind to muscarinic receptors as determined in an *in vitro* assay using ^{3}H-quinuclidinyl benzylate as the ligand.

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12. a) All new compounds exhibited satisfactory IR, MS, ^{1}H-NMR and elemental analyses. **SPECTRAL DATA** for **3d**: ^{1}H-NMR (DMSO-d_6, 200 MHz, δ-ppm rel. to TMS) 0.9(d, 6H, J=6.6Hz, C<u>H</u>$_3$), 1.95(m, 1H, i-Bu C<u>H</u>), 3.15(t, 2H, J= 6.6Hz, i-Bu C<u>H</u>$_2$), 4.70(d,2H, J= 3.4Hz, C6 C<u>H</u>$_2$), 5.89(t,1H, J=3.6Hz, C7 <u>H</u>), 7.29(t, 1H, J=5.5Hz, N<u>H</u>), 7.65(m, 3H, aromatic), 8.05(m,2H, aromatic); IR (KBr) 1625, 1565, 1320 cm^{-1}; mass spectrum (EI) m/Z 389, 346, 290.
13. Private communication from Dr. Donald E. Moss, Alzheimer's Disease Research Project, The University of Texas at El Paso.
14. Private communication from Dr. Jack R. Cooper, Department of Pharmacology, Yale University School of Medicine.