amino group in **3c** reduces the in vitro potency 4–16-fold for all strains tested when compared to **3a**. The dimethylamino and acetylamino analogues are inactive—an ~8000-fold reduction in potency! The lost activity of **3e** and **3d** is visible at the enzyme level where methylation or acetylation of the 5-amino group has a devastating effect on enzyme inhibition. The same trends are witnessed in the pyrrolidine series as well (**4e** and **4f**).

While the 5-amino group in the 6,8-difluoro quinolones confers excellent overall spectrum and potency with major improvements over a 5-hydrogen substituent, this phenomena cannot be generalized to other quinolone nuclei. The 8-amino analogue of ofloxacin (**2b**) is substantially less active than ofloxacin (**2a**) in vitro, in vivo, and in the 50% inhibition of the gyrase enzyme.

The 5-amino group in the quinolones **3** and **4** tends to be nonbasic (pK_a for **4a** of 2.7) and nonnucleophilic. Water solubility did not vary much from the corresponding 5-hydrogen analogues.

In summary, we have shown that a 5-amino group in the 6,8-difluoroquinolone series enhances in vitro potency, especially for the piperazinyl side chains and most significantly against the Gram-positive bacterial strains. Several other extremely potent agents were prepared, such as 5-amino-7-(3-amino-1-pyrrolidinyl)-1-cyclopropyl-6,8-difluoro-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid (**4a**) and the 7-[3-(aminomethyl)-1-pyrrolidinyl] analog **4b**. Studies are currently under way to further define the structure–activity relationships of R_5 to delineate the interaction between R_5 and the quinolone nucleus itself.

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John M. Domagala,* Susan E. Hagen, Carl L. Heifetz
Marland P. Hutt, Thomas F. Mich
Joseph P. Sanchez, Ashok K. Trehan
Parke-Davis Pharmaceutical Research Division
Warner-Lambert Company
Ann Arbor, Michigan 48105
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Articles

Synthesis and Biological Characterization of Pyridohomotropanes. Structure–Activity Relationships of Conformationally Restricted Nicotinoids†

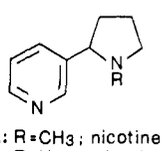
David B. Kanne*§ and Leo G. Abood⊥

Stauffer Chemical Company,‡ Western Research Center—Richmond, 1200 So. 47th Street, Box Number 4023, Richmond, California 94804-0023, and Center for Brain Research, The University of Rochester Medical Center, Rochester, New York 14642. *Received April 3, 1987*

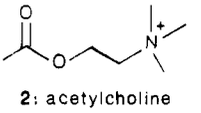
The recently discovered nicotinic agonist pyrido[3,4-*b*]homotropane (PHT) as well as its *N*-methyl and 2′-methyl derivatives (syntheses reported herein) were compared with nicotine, nornicotine, and anatoxin *a* in a series of in vitro and in vivo assays. The results reveal that PHT possesses activity comparable to that of the highly potent agonist, anatoxin *a*. The inactivity observed relative to PHT of *N*-methyl- and 2′-methyl-PHT has helped to further define the structure–activity requirements of conformationally restricted nicotinoids.

There is considerable evidence that nicotine (**1a**) can mimic the actions of acetylcholine (**2**) at the autonomic ganglia, the neuromuscular junction, and some areas of the central nervous system.[1-4] The receptor sites for nicotine in mammalian brain, moreover, appear to exist in multiple forms and are pharmacologically different from those at the neuromuscular junction and electroplax.[4,5] The binding of such nicotinic agonists is believed to be controlled by two factors: (1) an electrostatic interaction involving the alkylammonium group and (2) a hydrogen bond mediated by an unshared pair of electrons on the agonist.[6,7] Efforts to determine the precise structural requirements of the receptor have resulted in the synthesis of conformationally restricted derivatives.[8,9]

We have recently reported the synthesis and biological activity of pyrido[3,4-*b*]homotropane (PHT, **3a**)—the first



1a: R=CH_3; nicotine
b: R=H; nornicotine



2: acetylcholine

derivative of either nicotine or nornicotine to possess both high activity and conformational rigidity.[10] In the present

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‡ Now part of the ICI group of companies.
§ Stauffer Chemical Company.
⊥ The University of Rochester Medical Center.

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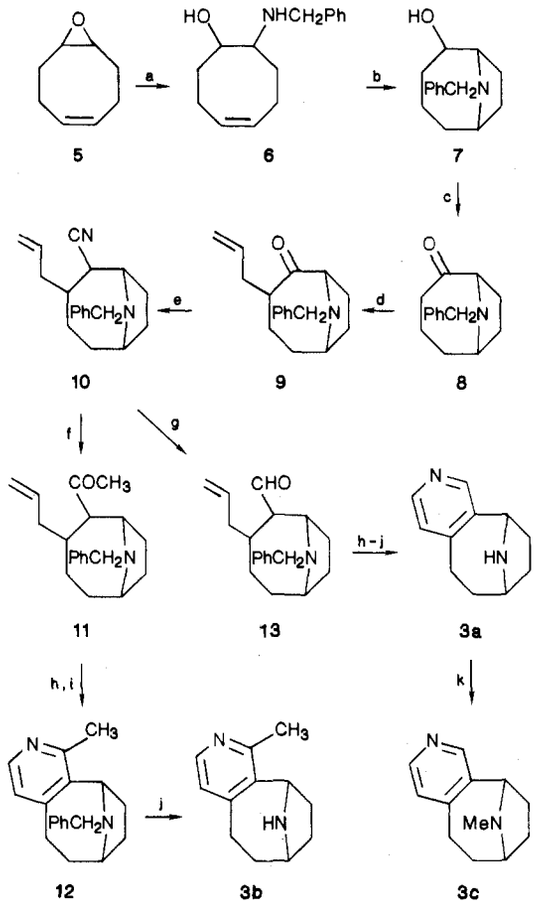
Scheme I[a]



[a] (a) 4 equiv of benzylamine, MeOH, 72 °C, 100 h, 82%; (b) 1 equiv of $Hg(OAc)_2$, THF, 4 °C, 1.5 h, NaOH, $NaBH_4$, 70%; (c) Jone's oxidation, 45%; (d) KH, Et_3B, allyl bromide, THF, 0–25 °C, 75%; (e) 2 equiv of Tos Mic, 5 equiv of potassium *tert*-butoxide, DME, 45 °C, 74%; (f) MeLi, ether, Δx, 64%; (g) 2 equiv of DIBAL, benzene, 25 °C, 5 h, 75% (h) O_3, CH_2Cl_2, −78 °C, dimethyl sulfide; (i) 3 equiv of NH_2OH·HCl, glacial HOAC, 100 °C, 20 min, **12** was produced in 39% yield over two steps (h and i); (j) H_2–$Pd(OH)_2$/C, 1 equiv of HCl, EtOH, **3b** was produced in 71% yield; (k) 37% HCHO, glacial HOAc, $NaCNBH_3$, 58%.

paper, we have examined PHT in an expanded array of in vitro and in vivo assays encompassing both peripheral and central affects. Each agonist was tested on both *Torpedo* electroplax and rat brain membranes, utilizing (−)-[^{3}H]nicotine as well as [^{3}H]-*N*-(methylcarbamyl)choline ([^{3}H]MCC), a nicotinic ligand closely resembling acetylcholine.[5] Intraventricular injection of rat brain and subsequent observation of the characteristic prostration syndrome served as the in vivo assay.[4] Anatoxin *a* (**4**),[11-13]

Table I. Inhibition of (−)-[^{3}H]Nicotine Binding

	rat brain membranes		*Torpedo* membranes	
	IC_{50},[a] M	rel potency	IC_{50},[a] M	rel potency
(±)-nornicotine	8×10^{-8}	0.062	1×10^{-7}	0.01
(±)PHT	5×10^{-9}	1.0	1×10^{-9}	1
(±)-2′-Me-PHT	9×10^{-7}	0.0056	3×10^{-7}	0.0033
(±)-N-Me-PHT	1×10^{-6}	0.0050	8×10^{-7}	0.00125
(±)-anatoxin *a*	8×10^{-9}	0.62	1×10^{-9}	1
(±)-nicotine	7×10^{-9}	0.71	4×10^{-9}	0.25

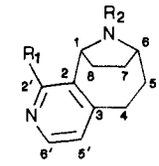
[a] IC_{50} = concentration of agent required to produce 50% inhibition of (−)-[^{3}H]nicotine binding to rat brain or *Torpedo* membrane (concentration of (−)-[^{3}H]nicotine in assay was 1×10^{-9} M).

Table II. Inhibition of [^{3}H]MCC Binding[a]

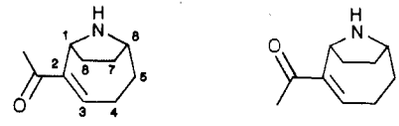
	rat brain membranes		*Torpedo* membranes	
	IC_{50}, M	rel potency	IC_{50}, M	rel potency
(±)-nornicotine	1×10^{-7}	0.1	3×10^{-7}	0.0003
(±)-PHT	1×10^{-8}	1.0	1×10^{-10}	1.0
(±)-2′-Me-PHT	7×10^{-7}	0.014	3×10^{-7}	0.0003
(±)-*N*-Me-PHT	3×10^{-6}	0.0033	2×10^{-6}	0.0005
(±)-anatoxin *a*	1×10^{-8}	1.0	1×10^{-10}	1.0
(±)-nicotine	3×10^{-9}	3.3	1×10^{-9}	0.1

[a] Concentration of [^{3}H]MCC in assay was 1×10^{-9} M.

nicotine, and nornicotine were used as comparison standards throughout.



3a: R_1=R_2=H; pyrido[3,4-*b*]homotropane (PHT)
b: R_1=CH_3; R_2=H; 2′-methyl-PHT
c: R_1=H; R_2=CH_3; *N*-methyl-PHT



4a: *s-cis*-anatoxin-*a* **4b**: *s-trans*-anatoxin-*a*

Pyrido[3,4-*b*]homotropane represents the conceptual combination of nornicotine (**1b**) and anatoxin *a*.[10] It was, therefore, also of interest to determine the effect on PHT of substituents possessed by its antecendents. First, since anatoxin *a* possesses a methyl group α to the carbonyl, it was important to establish the effect of such an "acetylcholine" methyl in the pyridohomotropane series. Second, because it is known that N-methylation of nornicotine leads to a significantly more active compound (nicotine),[4] it was necessary to determine if this same trend would be followed by its conformationally rigid counterpart. The synthesis and biological characterization of these key derivatives, 2′-methyl-PHT (**3b**) and *N*-methyl-PHT (**3c**), is described below.

Synthesis

The synthesis followed the route shown in Scheme I. The branch point to *N*-Me-PHT on the one hand and

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Table III. Psychotropic Evaluation (Prostration Assay)

	ED_{50}[a]	rel potency
(±)-nornicotine	75	0.133
(±)-PHT	10	1
(±)-2′-Me-PHT	500	0.02
(±)-*N*-Me-PHT	500	0.02
(±)-anatoxin *a*	20	0.5
(±)-nicotine	10	1

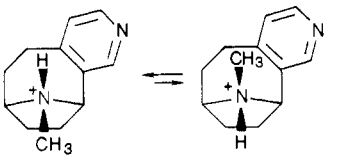
[a] ED_{50} = dose (nmol) of agent required to produce prostration after injection into the rat's fourth ventricle.

2′-Me-PHT on the other comes at the stage of the cyano compound **10**.[10] Treatment of **10** with methyllithium[14] gives the ketone **11** whereas DIBAL reduction of the nitrile gives the aldehyde **13**.[10] Elaboration to the fused pyridine ring and debenzylation yields **3a**[10] and **3b**. Final N-methylation of **3a** was accomplished by sodium cyanoborohydride reduction of the intermediate imine generated by treatment with 37% aqueous formaldehyde.[8] Nornicotine[15] and anatoxin-*a*[16] were synthesized by literature methods.

Results and Discussion

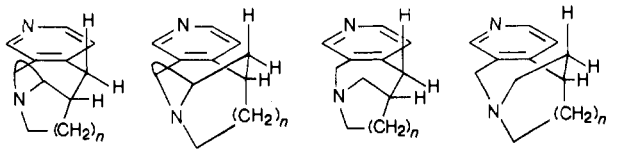
The binding of the various pyridohomotropane derivatives and related nicotinoids to both rat brain and *Torpedo* membranes was determined by using both (−)-[^{3}H]nicotine (Table I) and [^{3}H]MCC (Table II) as radioligands. With (−)-[^{3}H]nicotine as the ligand, PHT had a 16-fold greater affinity for rat brain and 100-fold greater affinity for *Torpedo* membranes than did nornicotine (Table I). Methylation of PHT at either the 2′-carbon or the pyrrolidine nitrogen resulted in a 200-fold decrease in binding to rat brain and a 300–800-fold decrease to *Torpedo* membranes (Table I). Anatoxin *a* was similar to PHT in its effect on the binding of [^{3}H]nicotine. The psychotropic potency of the various agents, as determined by prostration in rats, correlated well with the relative affinity of [^{3}H]-nicotine (Table III). With [^{3}H]MCC as the ligand, the binding affinity as well as their rank order was similar to that observed with [^{3}H]nicotine with a few exceptions: the affinity of both PHT and anatoxin *a* was 100-fold greater for *Torpedo* than for rat brain membranes (Table II), the affinity of both agents for *Torpedo* membranes with [^{3}H]MCC as the ligand was 10-fold greater than that for nicotine, and the IC_{50} values were 10-fold less than those obtained with [^{3}H]nicotine as the ligand. This difference in the affinities of the nicotinoids for *Torpedo* and rat brain membranes may be a reflection of the differential nature of the nicotinic receptors, while their differences in relative affinities with the two radioligands is indicative of the more complex nature of the recognition sites for nicotine as compared to acetylcholine or MCC.[5]

The causes of the lowered activity of 2′-Me-PHT are not yet clear but are most likely related to steric effects at the receptor. The lowered activity of *N*-Me-PHT[17] may arise from conformational effects produced by the bridge. Since the bridge protons of **3c** will favor a conformation where the *N*-methyl is anti to the pyridine ring (structure **14**), its low activity may suggest that the receptor interaction of pyridohomotropanes is more efficient when there is a relatively unencumbered approach from a position above



14 **15**

and anti to the pyridine ring. This proposal could be tested by synthesizing structures where an *N*-alkyl group is frozen in the syn conformation (structures **16–19**). These compounds would also allow a comparison of the relative receptor affinities of the twist-chair (**16** and **17**) and twist boat (**18** and **19**) conformations of the pyridohomotropane ring system.



16, *n* = 1, 2 **18**, *n* = 0,1 **17**, *n* = 1,2 **19**, *n* = 0,1

Conclusion

The significant loss in activity resulting from the incorporation of a methyl group at either the 2′-C or the pyrrolidine nitrogen of PHT has served to further delineate the structure–activity relationships of the pyridohomotropane series. Our results also demonstrate that PHT is comparable to the highly potent agonist anatoxin *a* in its pharmacologic potency and its binding affinity to both the electroplax nicotinic cholinergic receptor and brain nicotinic sites. The receptor "map" generated by PHT is, however, more precise than that of anatoxin *a* since the position and orientation of its H-bond acceptor and pyrrolidine nitrogen are more strictly defined.

Experimental Section

Chemistry. ^{1}H NMR spectra were obtained on a Varian XL-400 (400 MHz) spectrometer. ^{13}C NMR spectra were obtained on the same instrument at 100 MHz. High-resolution mass spectroscopy was carried out on a vacuum generator-70EHF mass spectrometer. Elemental analyses are reported by symbols of element; results are within ±0.4% of the calculated values.

***N*-Benzyl-2-acetyl-3-allyl-9-azabicyclo[4.2.1]nonane (11).** A solution of 560 mg (2.0 mmol) of nitrile **10** and 8.0 mmol of methyllithium in 6.0 mL of ether was refluxed with stirring for 15 h. After cooling, the mixture was poured into cold 10% sulfuric acid and stirred for 24 h. The mixture was then poured onto ice, and 50 mL of methylene chloride was added followed by 10% NaOH to pH >11. The aqueous phase was extracted a total of three times with ether, dried, and stripped to yield 380 mg, 64% of the desired ketone (**11**). The GC trace as well as the ^{1}H and ^{13}C NMR spectra revealed that the product was a mixture of diastereomers. ^{1}H NMR (400 MHz; $CDCl_3$, 7.24 ppm): δ 2.0 (3 H, s), 1–4 (16 H, m), 4.9–5.9 (3 H, m), 7.2–7.4 (5 H, m). HRMS: *m/e* calcd for $C_{20}H_{27}NO$ (M^+) 297.2093, found 297.2105.

***N*-Benzyl-2′-methyl-9-azabicyclo[4.2.1]nona[2,3-*c*]pyridine (12).** To a solution of 300 mg (1.01 mmol) of **11** in 2.5 mL of methylene chloride was added with ice cooling 128 mg (1.06 mmol) of trifluoroacetic acid. The solution was cooled to −78 °C and treated with ozone until GC of a miniworkup indicated that starting material was consumed. After purging of the mixture with nitrogen, excess dimethyl sulfide was added, and the mixture was allowed to come to room temperature. After 2 h, the mixture was concenrtated, and the residue (in 0.5 mL of glacial HOAc) was added dropwise to 347 mg (5.0 mmol) of hydroxylamine

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(17) Consistent with this result is the observation by B. Witkop and co-workers (personal communication) that *N*-methylanatoxin *a* is practically inactive.

hydrochlorine in 3 mL of refluxing glacial acetic acid. After about 30 min, the solution was cooled and poured into ether. The glacial HOAc was neutralized (ice cooling) with potassium carbonate and a little 10% NaOH. (Alternatively, the glacial HOAc could be evaporated and the residue basified as described.) The aqueous layer was saturated with salt and then extracted a total of three times with ether. The organic layers were combined, dried ($MgSO_4$), and evaporated to yield a light brown oil which solidified on standing. This material was chromatographed on silica gel 5 (CH_2Cl_2/MeOH, 95/5) to yield 110 mg (39% over two steps) of a clear colorless oil, which solidified on standing. ^1H NMR (400 MHz; $CDCl_3$, 7.24 ppm): δ 8.15, (d, J = 4.8, 1 H). 6.92 (d, J = 4.8, 1 H), 4.52 (dd, 1 H), 3.62 (d, J = 14, 1 H), 3.38 (d, J = 14, 1 H), 3.05 (m, 1 H), 2.68 (m, 1 H), 2.5 (m, 1 H), 2.2 (m, 1 H), 2.0 (s, 3 H), 1.4–2 (m, 4 H). HRMS: m/e calcd for $C_{19}H_{22}N_2$ (M^+) 278.1783, found 278.1775.

2′-Methyl-9-azabicyclo[4.2.1]nona[2,3-*c*]pyridine (3b). To 25.0 mg (0.0899 mmol) of **12** in 250 μL of EtOH was added 1 equiv of concentrated HCl. To this solution was added 6.5 mg of $Pd(OH)_2$ on carbon (Pearlman's catalyst). The solution was placed in a Paar apparatus and shaken under H_2 (45 psi) until a mini-workup indicated the completion of the reaction. The reaction was filtered through dicalite to remove the catalyst and washed thoroughly. After concentration to a small volume, the residue was partitioned between methylene chloride and 10% K_2CO_3 (with a small amount of 10% NaOH). The aqueous layer was extracted a total of three times with methylene chloride, and the organic phases were combined, dried ($MgSO_4$), and evaporated to yield a light oil. Chromatography of **3b** on silica gel (CH_2Cl_2/MeOH, 95/5) afforded 12 mg (71%) of a colorless, clear oil. ^{13}C NMR (100 MHz; $CDCl_3$, 77.0 ppm): δ 23.5, 31.2, 32.2, 32.3, 34.0, 57.2, 58.1, 124.3, 141.2, 146.5, 149.2, 154.0. ^1H NMR (400 MHz; $CDCl_3$, 7.24 ppm) δ 8.13 (d, J = 4.8, 1 H), 6.88 (d, J = 4.8, 1 H), 4.78 (dd, 1 H), 3.77 (m, 1 H), 2.98 (m, 1 H), 2.73 (m, 1 H), 2.60 (s, 3 H), 1.5–2.5 (m, 7 H). HRMS: m/e calcd for $C_{12}H_{16}N_2$ (M^+) 188.1314, found 188.1313. Anal. ($C_{12}H_{16}N_2$) C, H, N.

Pyrido[3,4-*b*]homotropane (PHT, 3a). PHT was synthesized as shown in Scheme I. ^{13}C NMR, ^1H NMR, and HRMS analyses have been reported by us previously.[10] Anal. ($C_{11}H_{14}N_2$) C, H, N.

***N*-Methyl-9-azabicyclo[4.2.1]nona[2,3-*c*]pyridine (3c).** To a solution of 0.120 g (0.689 mmol) of **3a**, 0.26 mL (3.24 mmol) of 37% aqueous formaldehyde, and 2 mL of acetonitrile was added 65 mg (1.04 mmol) of sodium cyanoborohydride in portions over 3 min. The mixture was stirred at 0 °C for 10 min followed by addition of 32 μL of acetic acid. After an additional 15 min at 0 °C, the reaction was stirred for 18 h at room temperature. To this mixture was added 1 mL of 10% aqueous HCl, followed by concentration to a small volume. The residue was then partitioned between methylene chloride and 10% aqueous NaOH. The aqueous layer was extracted a total of three times with methylene chloride, and the organic phases were combined, dried ($MgSO_4$), and evaporated to a light yellow oil. Chromatography on silica gel (CH_2Cl_2/MeOH, 95/5) afforded 75 mg (58%) of **3c** as a clear, colorless oil. ^{13}C NMR (100 MHz; $CDCl_3$, 77.0 ppm): δ 24.8, 28.9, 31.0, 32.5, 35.7, 62.4, 65.6, 127.1, 143.8, 145.0, 145.1, 156.3. ^1H NMR (400 MHz; $CDCl_3$, 7.24 ppm) δ 8.27 (d, J = 5.6, 1 H), 8.23 (s, 1 H), 7.38 (J = 5.6, 1 H), 4.13 (dd, J = 1.6 and 9.2, 1 H), 3.51 (m, 1 H), 3.13 (m, 1 H), 2.85 (m, 1 H), 2.6 (m, 1 H), 2.28 (m, 1 H), 2.20 (s, 3 H), 2.03 (m, 1 H), 1.83 (m, 2 H), 1.57 (m, 1 H). HRMS: m/e calcd for $C_{12}H_{16}N_2$ (M^+) 188.1314, found 188.1306. Anal. ($C_{12}H_{16}N_2$) C, H, N.

Measurement of [^{3}H]Nicotine and [^{3}H]MCC Binding. The procedures for preparation of membranes from rat brain and *Torpedo* electric organ for the measurement of [^{3}H]nicotine and [^{3}H]MCC binding are described elsewhere.[4,5] Briefly, membranes were obtained from whole rat brain or electric organ after homogenization in 30 volumes of 0.05 M NaH_2PO_4, pH 7.0, and centrifuged at 50000*g* for 30 min. To a polypropylene tube were added either 2 mg of brain membrane protein or 1 mg of electric organ membrane protein and a final concentration of 1×10^{-9} M [^{3}H]nicotine (New England Nuclear, specific activity = 75 Ci/mmol) or 1×10^{-9} M [^{3}H]MCC (specific activity = 80 Ci/mmol) with or without various concentration of the nicotinic agents, in a volume of 1.2 mL and final concentration of 0.05 M $NaPO_4$, pH 7.0. All assays were performed in triplicate. After incubation in an ice bath for 30 min, the tubes were centrifuged in an Eppendorf centrifuge for 2 min, and the pellet was washed twice by filling the tubes with buffer and aspirating. The bottom of the tubes were then cut off (animal nail clipper) and counted by liquid scintillation.

Psychotropic Evaluation of Various Agents. The psychotropic action of the various agents was determined by administering various doses into the fourth ventricle of the rat brain through chronically implanted cannulae, as described elsewhere.[4] A dose of 6 nmol of (−)-nicotine in 1 μL resulted in prostration of all four limbs, while 4 nmol (IC_{50}) produced prostration of hind limbs with weakness in the forelimbs.

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