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SYNTHESIS AND INVESTIGATION OF MAO INHIBITORS: HYDRAZONES OF POTENTIAL BRAIN-SPECIFIC DELIVERY

Nawal A. El-Koussi, Hoda Y. Hassan, Hassan H. Farag

Department of Pharmaceutical Medicinal Chemistry, Faculty of Pharmacy, Assiut University, Assiut, Egypt

تم تحضير سلسلة من ١-ميثيل-٣{ن-(مشتقات البنزاليمينو) كربامويل} بيريدينيم أيوديد وداى هيدروبيريدين المقابل. وقد تم أختبار فاعلية المركبات المحضرة كمثبطات لمؤكسدات الأمين الأحادية من الخارج علـى ميتاكوندريـا الكبـد بواسطة التحليـل الفلوروميترى للكينورامين. وأظهـرت النتـائج فاعلية المركبات المؤلكلة عن مثيلاتها من داى هيدروبيريدين.

A series of 1- methyl-3-[N-(substituted benzalimino)carbamoyl] pyridinium iodide and their corresponding dihydropyridines were prepared by conventional methods. The prepared compounds were tested in vitro for their MAO inhibitory activity on liver mitochondria by kynuramine fluorimetric assay. The results showed that MAOI activity of quaternaries is more than the corresponding dihydro compounds.

INTRODUCTION

Mitochondrial monoamine oxidase (MAO) is known to be the enzyme responsible within the nervous system for the metabolic inactivation of some neurotransmitters such as serotonin, norepinephrine and dopamine. MAOIs have been considered as important psychoactive drugs.

Hydrazino compounds[1,2] and hydrazones[3,4] have been extensively studied for their potentials as therapeutic agents, MAO inhibitory properties and CNS depression.[5] The clinical use of MAOIs as antidepressents has been seriously restricted because of their systemic side effects and toxicity.[6] Accordingly site-specific delivery of MAOIs to the brain would alleviate most of their side effects. Brain-specific delivery could be accomplished by using the dihydropyridine/ pyridinium salt redox system.[7-10] This redox chemical delivery system has proven to be effective in specific delivery of drugs to the brain. The drugs will be administered as their corresponding 1,4-dihydropyridine derivatives, because of their lipid solubility, are expected to cross BBB to the CNS in addition to peripheral distribution. Biological oxidation of the dihydropyridine to the quaternary locks the drug into the brain and enhances its systemic clearance through the kidney.

The prepared compounds were designed on grounds of the reported QSAR of MAOIs of substitutd benzylidines,[11,12] hydrazines[1,2] and hydrazones.[3,4]

A spectrofluorimetric method was selected for determination of the *in vitro* MAO inhibitory activity of both the quaternaries and dihydrocompounds.

EXPERIMENTAL

Melting points were determined on electrothermal melting point apparatus and are uncorrected. IR spectra were recorded on 470-Shimadzu infrared spectrophotometer as KBr discs. ^{1}H-NMR spectra were recorded on EM-360 instrument, at 60 MHz using TMS as internal standard and DMSO-d_6 as solvent. UV analysis was performed on Pye-Unicam spectrophotometer 1750 UV/VIS in methanol. Microanalysis were performed at the microanalytical center, Faculty of Science, Cairo University. TLC were performed on precoated TLC plates of silica gel 60 F-254 (MERCK).

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A- Chemistry

Ethyl nicotinate (I) was synthesized according to the reported procedure.[13]

N-Methyl-3-ethoxycarbonyl pyridinium iodide (II)[14]

To a solution of I (13.6 ml, 0.1 mole) in methanol (25 ml), solution of methyl iodide (0.2 mole) in methanol (10 ml) was dropped gradually while stirring over a period of 30 minutes. The reaction mixture was refluxed for 12 hour. The solvent was concentrated under reduced pressure, triturated with ether untill solid product was separated. It was filtered, washed with ether, dried and crystallized from methanol/ethyl acetate (1:3). Yield 75 %, m.p. 133°C.

N-Methyl-3-hydrazinocarbonyl pyridinium iodide (III)[15]

To a solution of II (29.3 g, 0.1 mole) in methanol (50 ml). Hydrazine hydrate (0.5 mole) in methanol (10 ml) was added while stirring. The mixture was refluxed under stirring for one hour. The separated product was filtered, recrystallized from methanol. Yield 78 %, m.p.170-172°C.

1-Methyl-3-[N-(substituted benzalimino) carbamoyl] pyridinium iodide (IV)

A mixture of III (5.58 g, 0.02 mole) in methanol (20 ml) was refluxed, the appropriate quantity of aldehyde or acetophenone (0.02 mole) in methanol (5 ml) was added gradually onto the hydrazide solution while stirring. A drop of glacial acetic acid was added as catalyst. The reaction mixture was refluxed while stirring for two hours. The solvent was concentrated and the formed precipitate was filtered and crystallized from methanol. IR spectra showed bands at 3485-3430 cm^{-1} (NH), 1683-1664 cm^{-1} (CO), 1552-1489 cm^{-1} (C=N) respectively. The physical constants and spectral data are listed in Table (1,2).

1-Methyl-3-[N-(substituted benzalimino) carbamoyl]-1,4-dihydropyridine (V)

To a cold solution of IV (0.01 mole) in deareated water (100 ml), sodium carbonate (6.36 g, 0.06 mole) and ethyl acetate (25 ml) were added while stirring in an ice bath. Sodium dithionite (7 g, 0.0 4 mole) was added gradually. The reaction mixture was stirred under nitrogen for 3 hours. The organic layer was separated, dried with anhydrous sodium sulphate, and evaporated under reduced pressure. The yellow to orange product was dried overnight under vacuum over P_2O_5 and stored immediatly in ice chest λ_{max} in methanol showed maximum absorption at 370-380 nm. The m.p. of dihydrocompound Va (107-110°C), Vb (113-115°C), Vc (155°C), Ve (149-150°C). ^{1}H-NMR data are listed in Table (2)

B- Biochemical assay

MAO enzyme was prepared using tissue homogenizer and centrifuged (Minifuge-2-Heraeus-type 4123). Protein concentration was estimated using Spekol II spectrophotometer. Assay of MAO inhibitory activity was performed using SFM 23/B Kontron, Switzer land spectrofluorometer.

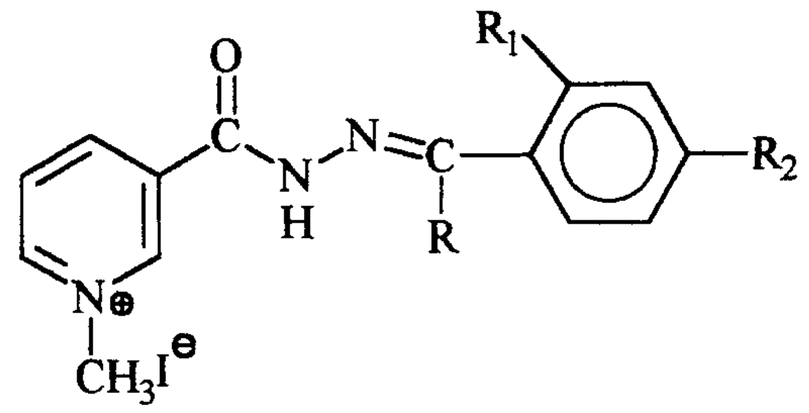
Mitochondrial preparations[16]

Crude mitochondrial fractions were obtained from the liver of male albino rats weighing 200-250 g. The animals were decapitated and the organs rapidly removed and homogenized in cold phosphate buffer (0.11 M, pH 7.4) using tissue grinder. The homogenate containing 20 % w/v of fresh liver was centrifuged at -4°C for 10 minutes at 4000 rpm. The supernatant was decanted and centrifuged at -4°C for 30 minute at 4000 rpm to sediment the mitochondrial pellets, which were then suspended in the least amount of phosphate buffer and stored at -20°C. Protein content was determined by the method of Lowry *et al*[17] using bovine serum albumin (BSA) as the standard.

Determination of MAO inhibitory activity of the prepared compounds

Into six to seven incubation tubes, different aliquots of standard solution of the tested compound and phenelzine sulphate was added to

Table 1: Physical constants of 1-Methyl-3-[N-(substituted benzalimino) carbamoyl]pyridinium iodide IVa-f.



Compd. No.	R	R_1	R_2	M.P.°C	Yield %	Molecular formula (Mol. wt.)	Microanalysis % Calcd./Found		
							C	H	N
IVa	H	OH	H	256-258	94	$C_{14}H_{14}IN_3O_2$ (383)	43.86 43.28	3.65 3.42	10.69 11.00
IVb	H	H	$N(CH_3)_2$	260	87.4	$C_{16}H_{19}IN_4O$ (410)	46.82 47.30	4.63 4.20	13.65 13.30
IVc	H	H	Cl	278-280	69.9	$C_{14}H_{13}ClIN_3O$ (401.5)	41.84 41.65	3.23 3.10	10.46 10.20
IVd	CH_3	H	H	198-200	64	$C_{15}H_{16}IN_3O$ (381)	47.24 47.50	4.19 3.90	11.02 11.31
IVe	CH_3	H	CI	246-248	77.5	$C_{15}H_{15}ClIN_3O$ (415.5)	43.32 43.23	3.61 3.03	10.10 9.96
IVf	CH_3	H	OCH_3	232-235	55.6	$C_{16}H_{18}IN_3O_2$ (411)	46.71 46.55	4.37 4.22	10.21 10.00

Table 2: ^{1}H NMR Spectral data of 1-Methyl-3-[N-(substituted benzalimino) carbamoyl] pyridinium iodide and dihydroderivatives in (DMSO-d_6).

Compd. No.	Quaternary Compounds	Compd. No.	Dihydro Compounds
IVa	12.4(s,1H,NH); 10.8(s,1H,OH); 9.53(s,1H,C_2); 9.36-8.8 (m, 2H,C_6, C_4); 8.73 (s,1H, N=CH); 8.6-8.1(m,1H,C_5); 7.8-6.76 (m,4ArH); 4.5 (s,3H , $^{+}NCH_3$)		
IVb	12(s,1H,NH); 9.43(bs,1H,C_2); 9.2-8.7(m,2H,C_6, C_4); 8.46-8 (m, 2 H , C_5,N=CH); 7.6(d,2ArH,C_2,C_6); 6.8 (d, 2ArH, C_3,C_5) ; 4.46(s,3H,$^{+}NCH_3$); 3 (s,6H,N(CH_3)$_2$)	Vb	10.1(s,1H,NH); 8.2(s,1H,N=CH); 7.63(d,2ArH,C_2,C_6); 7.19 (s,1H, C_2); 6.63 (d,2ArH,C_3,C_5); 5.7(d,1H,C_2); 4.76(m, 1H, C_5); 3.06(bs,8H, N(CH_3)$_2$$C_4$); 2.86(s,3H,$NCH_3$)
IVc	12.4 (s,1H,NH); 9.5(s,1H,C_2); 9.33-8.8(m,2H,C_6,C_4); 8.56-8 (m,2H,C_5, N=CH);7.76-7.43(d,4ArH);4.5(s,3H,^{+}N-CH_3)	Vc	10.76(s,1H,NH); 8.3(s,1H,N=CH); 7.86-7.3(d,4ArH); 7.1(s, 1H,C_2); 5.86(d,1H,C_6); 4.76(m,1H,C_5); 3.06(m,2H,C_4); 2.9(s, 3H,N-CH_3).
IVd	11.46(s,1H,NH); 9.5(s,1H,C_2); 9.33-8.7(m,2H,C_6,C_4); 8.43-8(m,1H,C_5); 8-7.26(m,5ArH);4.5(s,3H,$^{+}NCH_3$) ;2.45(s,3H , N=CH3)		
IVe	11.33(s,1H,NH); 9.5(s,1H,C_2); 9.3-8.8(m,2H,C_6,C_4); 8.4-8(m, 1H, C_5); 7.8 (d,2ArH,C_3,C_5); 7.5(d,2ArH,C_2,C_6); 4.5(s,3H , ^{+}N CH_3); 2.48(s,3H , CH_3)	Ve	9.36(s,1H,NH); 8.03-7.6(d,2ArH,C_3,C_5); 7.6-7.2(d, 2ArH ,C_2, C_6); 7.1 (s,1H,C_2); 5.83(d,1H,C_6); 4.8(m,1H,C_5); 3.03(m,2H, C_4); 2.9(s,3H, NCH_3);2.5(s, 3H,CH_3).
IVf	11.2(s,1H,NH); 9.5(s,1H,C_2);9.2-8.7(m,2H,C_6,C_4); 8.36-8 (m, 1H, C_5) ; 7.86(d,2ArH,C_2,C_6);6.96(d,2ArH,C_3,C_5); 4.5(s,3H, $^{+}NCH_3$); 3.86(s,3H , OCH_3); 2.45(s,3H,CH_3)		

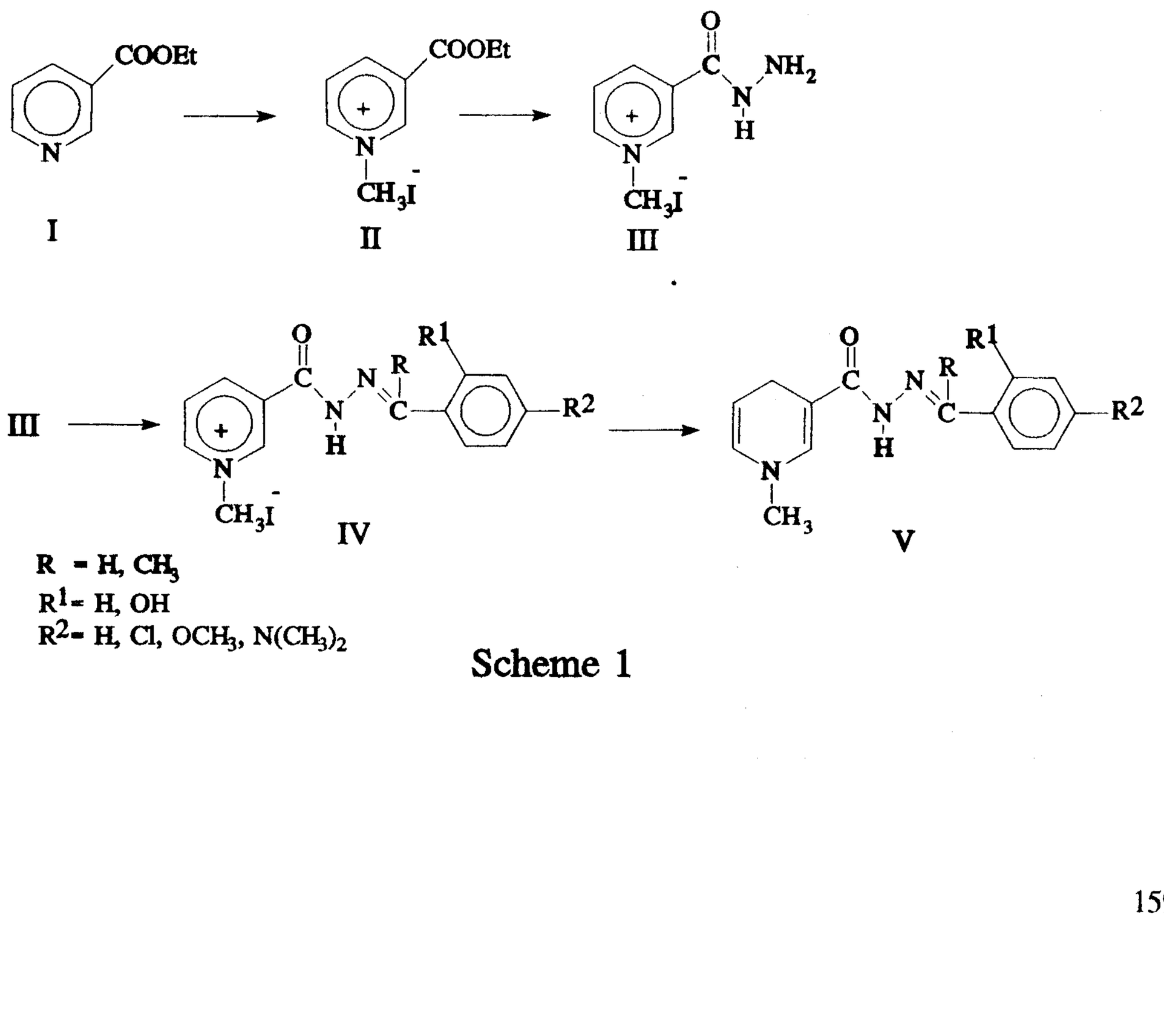
0.5 ml of enzyme preparation (300 μg/ml) then incubated at 37°C for 10 minutes. To each tube 0.4 ml of kynuramine solution (100 μg/ml) and 0.5 ml of phosphate buffer were added and the volume was then completed to 3 ml with water. The mixture was vortexed and incubated at 37°C for 30 minutes. The reaction was then stopped by addition of 2 ml of trichloroacetic acid (TCA, 10%) and the precipitated protein was spun down by centrifugation at 3000 rpm for 10 minutes, 1 ml of the supernatant was pipetted into 2 ml of 1N Na OH and mixed. A control experiment was prepared for each compound at the same time and conditions except replacing the compound with water. The solution was activated at 315 nm and measuring the fluorescence intensity at 380 nm against a blank prepared in the same manner but replacing the enzyme preparation with the phosphate buffer. pI_{50} for each compound was estimated by plotting logarithm of the molar concentration of MAOIs against percentage inhibition where

$$\% \text{ of inhibition} = \frac{\text{Control - Experiment}}{\text{Control}} \times 100$$

RESULT AND DISCUSSION

A- Chemistry

The designed compounds were prepared according to scheme (1). The structure of the prepared compounds were confirmed by microanalysis IR, UV, ^{1}H-NMR which revealed the presence of geometric isomers (E,Z) in unequal portions. Reduction of the quaternary hydrazones using sodium dithionite, sodium carbonate and ethyl acetate gave the corresponding dihydroderivatives (V). The 1,4-dihydro pyridine compounds showed absorption maxima at 370-380 nm. characteristic for the 1,4-dihydropyridine derivatives,[18] they were found to be readily oxidized with alcoholic silver nitrate to their corresponding quaternaries. The compounds were identified by their ^{1}H-NMR spectra.



Scheme 1

B- *In vitro* MAO inhibition

The fluorimetric method of Krajel[19] for *in vitro* determination of MAO inhibitory activity were selected to test both quaternaries and dihydrocompounds. Phenelzine sulphate was used as reference compound. The test depends on oxidative deamination of kynuramine substrate by the MAO enzyme to 4-hydroxy quinoline.

The amount of the product formed can be measured spectrofluorimetrically at 315 nm for excitation and 380 nm for emission. Under controlled standard conditions the fluorescence intensity is directly proportional to enzyme activity. Mitochondrial MAO was obtained by homogenising liver rat. Protein concentration of the enzyme preparation was estimated by the method of Lowery *et al*,[17] using crystalline BSA to construct a standard calibration curve. Standard conditions were selected after investigating the effect of protein concentration, substrate concentration and incubation time on the enzyme activity. Optimal conditions were found to be 300 μg protein concentration, 100 μg kynuramine concentration and 30 mintues incubation time. The pI_{50} (-log molar concentration of inhibitor required to produce 50% inhibition of MAO activity) were determined for both quaternaries and the dihydrocompounds by plotting the percentage inhibition against log molar concentration as showen in Table (3). All the examined compounds display a MAOI activity at molar concentration of 10^{-5}. Both the dihydro pyridine derivatives (V) and their corresponding quaternaries (IV) showed pronounced MAOI activity. The quaternaries showed slightly higher activity than their corresponding dihydro-derivatives, except for compound (IVf) where the quaternary is more than three times the activity of the corresponding dihydro-compound. The increase in the activity of the quaternaries may be attributed to the increase of the electrophilicity of the aromatic ring and/or the nitrogen atom. Although the dihydro compounds are active as MAOIs they will not be a source for systemic side effects since they are very labile towards biological oxidation to the corresponding quaternaries. The sequestering and locking-in the hydrophilic counterpart in the CNS using the redox CDS will increase the concentration of the more active quaternary compound inside the brain. The quaternary, beside being at lower concentrations in the blood and other tissues, is expected to be easily excreted through the kidney because of their hydrophilicity and accordingly lower systemic side effects.

Table 3: *In vitro* MAOI activity on rat liver of 1-Methyl-3-[N-(substituted benzalimino) carbamoyl] pyridinium iodide and dihydrocompounds by kynuramine fluorimetric assay.

Activity: [molar conc. (pI_{50})]			
Compd. No.	Quaternary derivatives	Compd. No.	Dihydro derivatives
IVa	$1.440x10^{-5}$ (-4.84)	Va	$1.600x10^{-5}$ (-4.79)
IVb	$1.080x10^{-5}$ (-4.96)	Vb	$1.300x10^{-5}$ (-4.88)
IVc	$7.480x10^{-6}$ (-5.12)	Vc	$8.000x10^{-6}$ (-5.09)
IVd	$2.275x10^{-5}$ (-4.64)	Vd	$6.920x10^{-5}$ (-4.15)
IVe	$1.420x10^{-5}$ (-4.84)	Ve	$2.000x10^{-5}$ (-4.69)
IVf	$3.055x10^{-5}$ (-4.51)	Vf	$9.300x10^{-5}$ (-4.03)
Ref. St*	$7.100x10^{-5}$ (-5.14)		

* Phenelzine sulfate.

REFERENCES

1- R. Pignatello, S. Mazzone, F. Castelli, Patrizia Mazzone Giuseppina Raciti and G. Mazzone: J. Pharmazie, 49, 272 (1994).

2- G. Mazzone, R. Pignatello, Annamaria Panico, S. Mazzon G.Puglisi, Giuseppao Pennisi, Giuseppina Raciti, Patrizia Mazzone and M. Matera: J. Pharmazie 47, 902 (1992).

3- T. George, C. L. Kaul, R. S. Grewal and D.V. Mehta; J. Med. Chem. 14, 909 (1971).

4- T. George, C. L. Kaul, R. S. Grewal and R. Tahilramani; J. Med. Chem. 14, 913 (1971).

5- C. Kaiser, C. L. Zirkle in A. Burger, "Medicinal Chemistry" 3rd Ed., Wiley interscience, New York. 1970, p. 1470.

6- P. Dostert in "Mono Amine Oxidase and Disease", K. F. Tipton, P. Dostert and M.S. Benedetti, eds., Academic Press, New York, 1984, p. 9.

7- N. Bodor, H. Farag and M. Brewster; J. Science, 214, 1370 (1981).

8- N. Bodor and H. Farag; J. Med. Chem., 26, 313 (1983).

9- N. Bodor and M. Brewster; Eur. J. Med. Chem., 18, 235 (1983).

10- N. Bodor and M. Brewster, "Chemical Approaches to Brain Trgeting of Biologically Active Compounds", Chapter 16 Drug Design for Neuroscience, A. Kozikowski, Ed., Raven Press, Ltd., New York, pp.435-467, 1993.

11- S. P. Singh, A. Chaudhari, V. I. Stenberg, and S. S. Parmar; J. Pharm. Sci, 65, 1678 (1976).

12- C. Dwivedi, R. D. Harbison, B. Ali and S. S. Parmar; J. Pharm. Sci, 63, 1124 (1974).

I3- A.I.Vogel. "Practical Organic Chemistry", 5 th Ed., London 1991, P. 1076.

14- R. Adams and J. E. Mahan; J. Am. Chem. Soc., 64, 2588 (1942).

15- A. C. Lovesey; J. Med. Chem. 13, 693 (1970).

16- A. N. Davison; J. Biochem., 67, 316 (1957).

17- O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall; J. Biochem., 193, 265 (1951).

18- U. Eismer and J. Kuthan: Chem. Rev., 72, 25 (1972).

19- M. Krajel; J. Biochem., Pharmacol., 14, 1683 (1965).