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PART 1: DESIGN, SYNTHESIS AND EVALUATION OF NEW SITE-SPECIFIC MONOAMINE OXIDASE INHIBITORS

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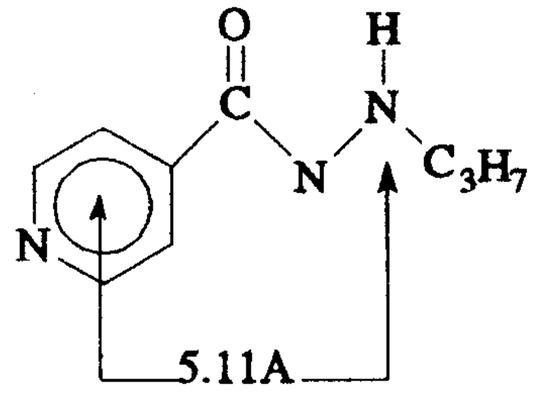
في هذا البحث تم تصميم بعض مثبطات أنزيم مؤكسد الأمينات الأحادية والتى تحتوى على البيريدينيم كجزء من تركيبها الأساسى. وقد تم تشييد مركبات البيريدينيم (Q) ومشتقات الدايهيدروبيريدين المقابلة لها (CDS) وكذلك مركبات البيريدين المصدرية لها (P). وقد طبقت طريقة فلورومترية لأختبار مدى تثبيط أنزيم مؤكسد الأمينات الأحادية. هذا وقد أثبتت الأختبارات التى أجريت أن للمركبات المشيدة فاعلية معقولة كمضادات للإكتئاب.

Certain new monoamine oxidase inhibitors (MAOIs) containing a pyridinium moiety as an integral part of their structures (Q) were designed. The quaternaries, their corresponding 1,4-dihydropyridine derivatives (CDS) and their parent pyridine compounds (P) were synthesized. A spectrofluorimetric method was used to test their in vitro monoamine oxidase inhibition. The compounds showed reasonable activities by the test performed.

INTRODUCTION

Depression is one of the most common mental illnesses which reflect the human condition.[1] The MAOIs[2] are one of the most useful drugs developed for the treatment of such ailment. Quantitative structure-activity relationships (QSAR) studies on many classes of MAOIs have been carried out.[3-6] According to these studies, the minimal requirements essential for activity are; an aromatic ring, which acts as an electron acceptor[3], connected to an electron rich functional group e.g., amino, imino or acetylenic carbon. The electron rich group should be in the same plane of the ring and at approximately 5.25 A° from its center[4] e.g., Iproniazid (1). A relation between the activity and electronegativity of the aromatic ring was found, i.e., decrease of the electron density on the ring increases activity. Substituted benzylidene moiety proved also to be a good moiety in the pharmacophore.[7]



(1)

The clinical use of MAOIs as anti-depressants has been seriously restricted because of their systemic side effects and toxicity.[8] 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.[9-12] This redox chemical delivery system has proven to be effective in specific delivery of drugs to the brain. Two major approaches are known for the redox CDS. The first involves the use of

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dihydropyridine derivative as a carrier to which the drug is chemically connected.[10] The second approach deals with drugs which contain pyridinium moiety as an integral part of their structure (Scheme 1).[11] Many MAOIs have a pyridine nucleus in their structure, e.g., Iproniazid and Nialamide. Hence we used the second approach to design the new brain specific MAOIs (Q_{1-3}) (Chart I). The compounds were designed to keep the essential structural requirements for MAO inhibition, i.e., aromatic ring, basic nitrogen and the distance in between. Quaternization of the pyridine ring is expected to increase activity by increasing the electronegativity of the ring.[3] The drugs will be administered as their corresponding 1,4-dihydropyridine derivatives (CDS_{1-3}). The latter because of their lipid solubility, are expected to cross the BBB to the CNS in addition to peripheral distribution. Biological oxidation of the dihydropyridine to the corresponding hydrophilic quaternary locks the drug into the brain and in the same time enhances its systemic clearance through the kidney. Accordingly the onset and duration of action of the compounds will depend mostly on the rates of delivery and oxidation of the CDS and rate of clearance of the quaternary form the CNS. The parent nonquaternized derivatives (P_{1-3}) were also prepared to test the effect of quaternization on delivery and enzyme inhibition.

A spectrofluorimetric method[13] was utilized for determination of the *in vitro* MAO-inhibitory activity of both the quaternaries (Qs) and parent (Ps) compounds.

EXPERIMENTAL

Chemistry

Melting points were determined in open capillaries on an Electrothermal melting point apparatus and are uncorrected. UV spectra were recorded using Cary 210 spectrophotometer. NMR spectra were recorded on a Varian EM360/390 instrument using DMSO-d_6 and D_2O as solvent and TMS as internal standard. Elemental microanalysis was performed by Atlantic Microlab, Atlanta, GA. USA. All reagents and solvents used were of analytical grades.

N-Methyl-3-hydrazinocarbonylpyridinium idodide (4)

To a solution of N-methyl-3-ethoxycarbonylpyridinium iodide[14] (15.0 g, 0.054 mole) in methanol (30 ml), a solution of hydrazine hydrate (2.7 g, 0.054 mole) in methanol (5 ml) was added gradually while stirring. The mixture was stirred further for 1 h at room temperature and the yellow solid separated was filtered off, recrystallized from methanol, dried under vacuum over P_2O_5/ KOH pellets to give 10.8 g (75.6 % yield) of hydrazide, mp 170-2°C.

3-{N-[(Benzyl- and substituted benzyl) imino]carbamoyl} 1-methyl pyridinium iodides (Q_{1-3})

Compound 4 (18.1 mmole) was dissolved in 200 ml of methanol by heating on a water bath under reflux. A solution of the appropriate aldehyde (18.1 mmole of benzaldehyde, anisaldehyde and vanillin for CDS_1, CDS_2 and CDS_3 respectively) in 50 mL methanol was added gradually to the hydrazide solution while stirring. A drop of glacial acetic acid was added and the mixture was refluxed while stirring for 2 h. The yellow crystalline solid which separated either immediately (Q_3) or after concentration of the reaction mixture ($Q_{1,2}$) was filtered off and recrystallized from the appropriate solvent (Table 1). The product is slightly soluble in water, ethanol and methanol; insoluble in ether, chloroform and ethyl acetate.

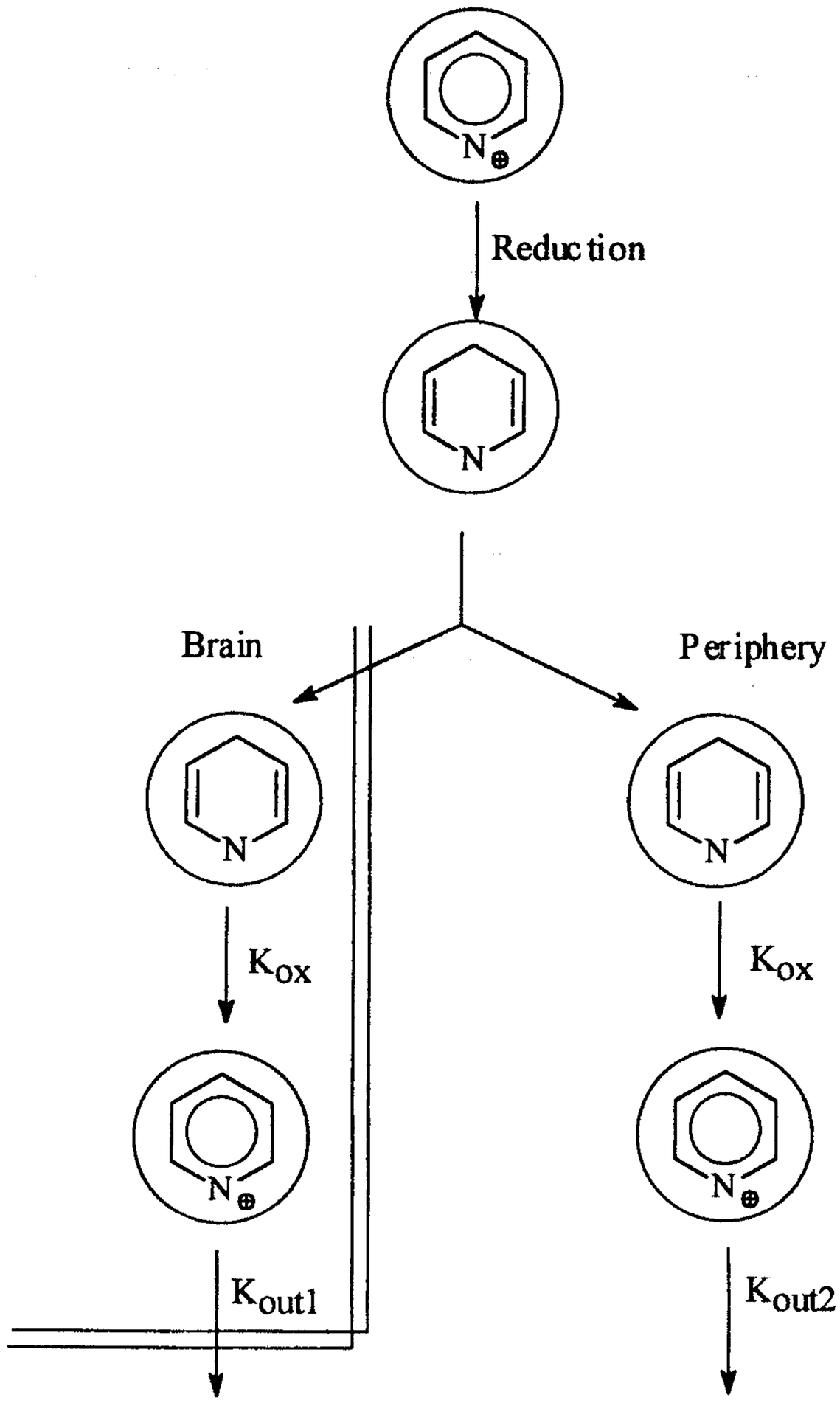
3-{N-[(benzyl- and substituted benzyl) imino]carbamoyl} 1-methyl-1,4-dihydro-pyridines (CDS_{1-3})

To a cold solution of the appropriate quaternary compound (Q_{1-3}) (1 mmole) in 100 ml of deareated water, sodium bicarbonate (6 mmole) and ethyl acetate (25 ml) were added while stirring in an ice bath. Sodium dithionite (4 mmole) was then added gradually and the reaction mixture was stirred under nitrogen for 3 hrs (for CDS_1), 2 hrs (for CDS^2) and 1.5 hrs

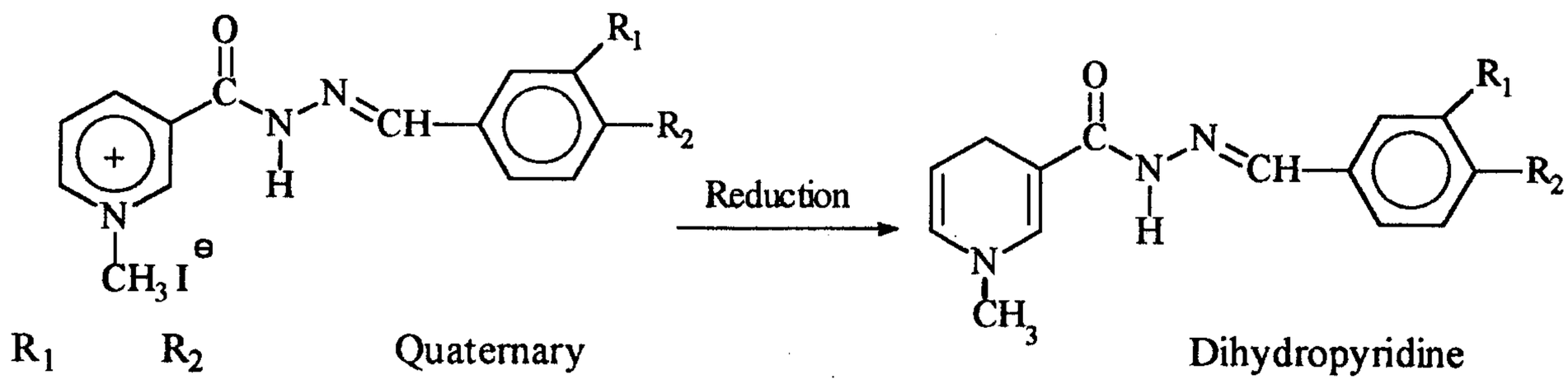
Table 1: Physical properties and microanalysis data of the studied quaternaries and their corresponding dihydropyridine compounds.

Compound	Yield %	m.p °C	Solvent of cyst.	Mol. Formula/ Mot. wt.	Microanalysis (Calc./Found)		
					%C	%H	%N
Q_1	89.7	198	methanol	$C_{14}H_{14}IN_3O$ 367	44.02 44.72	4.02 4.09	11.1 11.01
Q_2	67.8	245	distilled water	$C_{15}H_{16}IN_3O_2$ 397	45.35 45.23	4.03 4.12	10.58 10.51
Q_3	91.2	252	ethanol	$C_{15}H_{16}IN_3O_3$ 413	43.58 43.66	3.87 3.91	10.17 10.12
CDS_1	49.8	-	-	$C_{14}H_{15}N_3O$ 241	69.71 68.26	6.22 6.32	17.43 16.89
CDS_2*	40.6	-	-	$C_{15}H_{17}N_3O_2$ 271	-	-	-
CDS_3	52.3	-	-	$C_{15}H_{17}N_3O_3/½H_2O$ 296	60.81 60.77	6.08 5.81	14.18 14.15

* Could not be crystallized for elemental microanylsis and its purity was proved by HPLC.



Scheme 1: The proposed Drug Delivery System.[11]



R_1	R_2	Quaternary	Dihydropyridine
H	H	Q_1	CDS_1
H	OCH_3	Q_2	CDS_2
OCH_3	OH	Q_2	CDS_3

Chart I

(for CDS_3). Ethyl acetate was then separated, dried with anhydrous sodium sulfate and distilled under reduced pressure. The yellow to orange product was dried overnight under vacuum over P_2O_5 and then kept in closed container in the refrigerator. Elemental microanalysis and PMR spectra were listed in Tables 1 and 2 respectively.

3-{N-[(Benzyl- and substituted benzyl) imino]carbamoyl} 1- methyl-pyridines (P_{1-3})

To a solution of nicotinic acid hydrazide (2.0 g, 14.6 mmole) in methanol (100 ml), a solution of the appropriate aldehyde (14.6 mmole) in methanol (25 ml) was gradually added while stirring. A drop of glacial acetic acid was added and the reaction mixture was refluxed while stirring for 2 h. The solvent was then distilled off under reduced pressure and the product was dried in vacuum desiccator over P_2O_5/ KOH pellets to give the corresponding hydrazone[15] (Table 3).

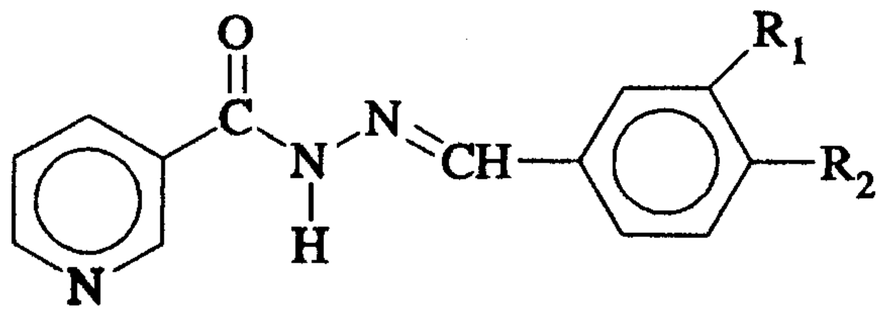
Biology

MAO enzyme was prepared using Tekmar Tissumizer and Beckman J2-21 centrifuge. Protein concentration was estimated using Beckman DU-7 spectrophotometer. Assay of MAO inhibitory activity was performed using Perkin-Elmer LS-3B fluorescence spectrophotometer. All reagents and solvents were of analytical grade.

Table 2: Proton magnetic resonance spectra of some of the studied compounds.

Compound	PMR (DMSO-d_6)
Q_1	δ; 12.30 (bs, 1H, CONH, disappeared with D_2O), 9.60 (s, 1H, C_2 pyridine proton), 9.30 (d, 1H, J= 6 Hz, C_6 pyridine proton), 9.10 (d, 1H, J= 8 Hz, C_4 pyridine proton), 8.60 (s, 1H, HC=N), 8.40 (dd, 1H, J= 8 Hz, C_5 pyridine proton), 7.90 (m, 2H, o-phenyl protons), 7.55 (m, 3H, m- and p-phenylprotons), 4.50 (s, 3H, N^+-CH_3). Small peaks appear at 9.50 and 8.98 which may be attributed to the presence of minute amount of a different geometric isomer.
Q_2	δ; 12.30 (bs, 1H, CONH, disappeared with D_2O), 9.50 (s, 1H, C_2 pyridine proton), 9.20 (d, 1H, J= 6 Hz, C_6 pyridine proton), 9.00 (d, 1H, J= 8 Hz, C_4 pyridine proton), 8.48 (s, 1H, HC=N), 8.30 (m, 1H, C_5 pyridine proton), two doublets at 7.80 and 7.15 typical for p-substituted phenyl and integrated for four protons, 4.50 (s, 3H, N^+-CH_3), 3.83 (s, 3H, OCH_3). The spectrum shows small peaks and doublets at 9.45, 7.60 and 7.00 which may be attributed to minute amount of a different geometric isomer.
Q_3	δ; 9.50 (s, 1H, C_2 pyridine proton), 9.20 (d, 1H, J= 6 Hz, C_6 pyridine proton), 9.00 (d, 1H, J= 8 Hz, C_4 pyridine proton), 8.40 (s, 1H, HC=N), 8.28-8.10 (m, 1H, C_5 pyridine proton), 7.40-6.80 (m, 3H, phenyl protons), 4.46 (s, 3H, N^+-CH_3), 3.43 (s, 3H, OCH_3)
CDS_1	δ; 8.80 (bs, 1H, CONH), 8.20 (s, 1H, HC=N), 7.80-7.30 (m, 5H, phenyl protons), 7.35 (s, 1H, C_2 dihydropyridine proton), 5.00 (d, 1H, J= 6 Hz, C_6 dihydropyridine proton), 4.85 (m, 1H, C_5 dihydropyridine proton), 3.35 (m, 2H, C_4 dihydropyridine protons), 3.05 (s, 3H, N-CH_3)
CDS_2	δ; 9.25 (bs, 1H, CONH), 8.10 (s, 1H, HC=N), two doublets at 7.65 and 6.85 (typical for p-substituted phenyl), 7.25 (s, 1H, C_2 dihydropyridine proton), 5.70 (d, 1H, J= 6 Hz, C_6 dihydropyridine proton) 4.80-4.68 (m, 1H, C_5 dihydropyridine proton), 3.80 (s, 3H, OCH_3), 3.30 (bs, 2H, C_4 dihydropyridine protons), 2.93 (s, 3H, N-CH_3)

Table 3: Physical properties and yields of the prepared non quaternized compounds.



R_1	R_2	Compound	Yield %	Solvent of cryst.	Melting point		λ_{max} **
					noticed	Reported*	
H	H	P_1	68	ethanol	150-152	154	320
H	OCH_3	P_2	50	ethanol	112-115	115	316
OCH_3	OH	P_3	60	ethanol	214-216	218	323

* Reference 15.

** Solvent is 5% dimethylsulfoxide / acetonitrile.

Preparation of MAO enzyme[16]

Male adult Sprague Dawley rats weighing 150-200 g were sacrificed by decapitation. Livers were quickly removed, washed with ice cold 5% sucrose solution, minced with scissor and homogenized in ice cold phosphate buffer (0.01 M, pH 7.6) using tissue grinder. The homogenate containing about 20% W/W of fresh liver was centrifuged at -4°C for 2 minutes at 4000 x G to remove the cell debris. The supernatant was decanted and centrifuged at -4°C for 10 minutes at 8500 x G to sediment the mitochondrial pellets, which were then suspended in the least amount of phosphate buffer and stored at -20°C.

Standard protein solution

A stock solution of bovine serum albumin containing 1 mg/ml in distilled water was prepared. Serial dilutions were obtained by diluting different aliquots of the stock solutions with distilled water. These dilutions were used to construct a calibration curve for protein concentrations using a reported spectrophotometric method.[16,17]

Preparation of the MAO enzyme samples

Twenty microliter of enzyme preparation was diluted with 480 μl of 0.1 N NaOH, then 100 μl of this preparation was diluted with 400 μl of distilled water. The protein content of the sample was determined using the spectrophotometric method[16,17] and extrapolation of the calibration curve of standard protein solution (bovine serum albumin).

Effect of protein concentration on MAO activity

Into six incubation tubes, different aliquots; 0.05, 0.1, 0.2, 0.3, 0.5, 0.75 and 1 ml of enzyme preparaion containing 437.6 μg protein /ml were pipetted and incubated for 10 minutes at 37°C. To each of the tubes, 0.5 ml of kynuramine solution (200 μg/ml) and 0.5 ml of phosphate buffer (0.5 M, pH 7.6) were added and the volume was completed with water to 3 ml. The mixture was vortexed and incubated at 37°C for 30 minutes with air as the gas phase. 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 6000 rpm for 10 minutes. One

milliliter of the supernatant was pipetted into 2 ml of 1 N NaOH in a quartz cuvette and mixed. The solution was then 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. The relation between protein concentration and MAO enzyme activity was obtained by plotting milliliters of enzyme or protein concentration against the corresponding fluorescence intensity.

Effect of substrate concentration on MAO activity

Into each of six incubation tubes, 0.5 ml of the enzyme preparation was measured and the tubes were incubated at 37°C for 10 minutes and then different aliquots; 0.05, 0.1, 0.2, 0.5, 0.75 and 1 ml, of kynuramine solution (200 μg/mL) were added. To each tube 0.5 ml of the phosphate buffer was added and the volume was completed to 3 ml with water. The tubes were then treated exactly as under; effect of protein concentration experiment. The relation between substrate concentration and MAO activity was obtained by plotting concentration of kynuramine against the corresponding fluorescence intensity.

Determination of MAO inhibitory activity of the prepared compounds

Into six to seven incubation tubes, different aliquots of standard solution of the tested compound were added to 0.5 ml of the enzyme preparation then incubated at 37°C for 10 minutes. To each tube 0.1 ml of kynuramine solution (100 μg) and 0.5 ml of phosphate buffer were added and the volume was then completed to 3 ml with water. The experiment was then completed exactly as under effect of protein concentration experiment. A control experiment was prepared for each compound at the same time and conditions except replacing the compound standard solution with, water in case of $Q_{1\text{-}3}$ or $MeOH/H_2O$ (1:1) in case of $P_{1,2}$. A blank experiment was also prepared in the same time and under the same conditions except replacing the enzyme preparation with phosphate buffer and replacing the compound standard solutions with, water for the quaternaries or $MeOH/H_2O$ for the non quaternaries. pI_{50} for each compound was estimated by plotting the logarithm of the molar concentration of the MAOI against percentage inhibition where:

$$\% \ Inhiibtion = \frac{control - experiment}{control} X\ 100$$

RESULTS AND DISCUSSION

Chemistry

The parent non quaternized compounds ($P_{1\text{-}3}$) were prepared according to reported procedures.[15] Trials to prepare the quaternaries ($Q_{1\text{-}3}$) by quaternization of the pyridine moiety in the parent compounds were unsuccessful. Alkylation of the hydrazone nitrogen atom and/or addition to the double bond occurred. Scheme 2 summarizes the route followed for the preparation of these quaternary compounds. The yield of the crystalline final quaternaries was about 70% calculated on nicotinic acid starting material (Table 1). They show high UV molar absorbtivity with the following λ_{max} nm and (ϵ) Q_1; 300 (18014), Q_2; 316 (19920), Q_3; 332 (22314) (Figs. 1,2&3). Their PMR spectra (DMSO-d_6, Table 2) revealed the presence of geometric isomers (E and Z) in unequal proportions. no efforts were made to isolate and identify the isomers. The designed 1,4-dihydro-pyridines chemical delivery systems (CDS_1, CDS_2 and CDS_3) were prepared by reduction of their corresponding quaternaries (Q_1, Q_2 and Q_3) using sodium dithionite in alkaline medium (sodium bicarbonate) in the presence of ethyl acetate (Chart I). The role of ethyl acetate is to extract the CDS as soon as it is formed so protecting it from degradation in the aqueous medium. To determine the appropriate time needed to complete reduction, the reaction of Q_2 (as a representative example) was monitored with UV (Fig. 4). The spectra show that after fifteen minutes a band at 378 nm started to appear and increased until reached maximum within two hours, after which very small change in the spectrum was observed.

The UV spectra of the isolated dihydropyridines (Figs. 1-3) show an absorption band around 375 nm which is expected to be the characteristic absorption of 1,4-dihydropyridine

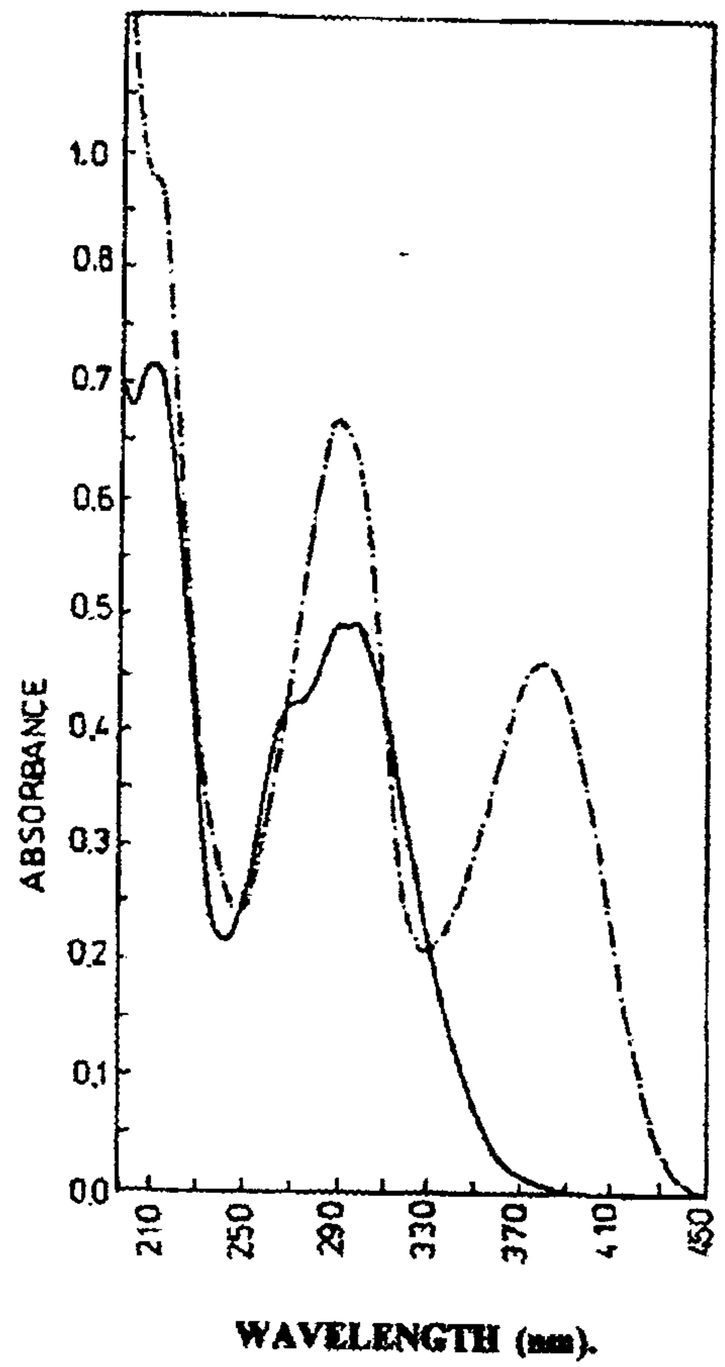


Fig. 1: UV spectra of: (————) Q_1 and (-.-.) CDS_1 methanol.

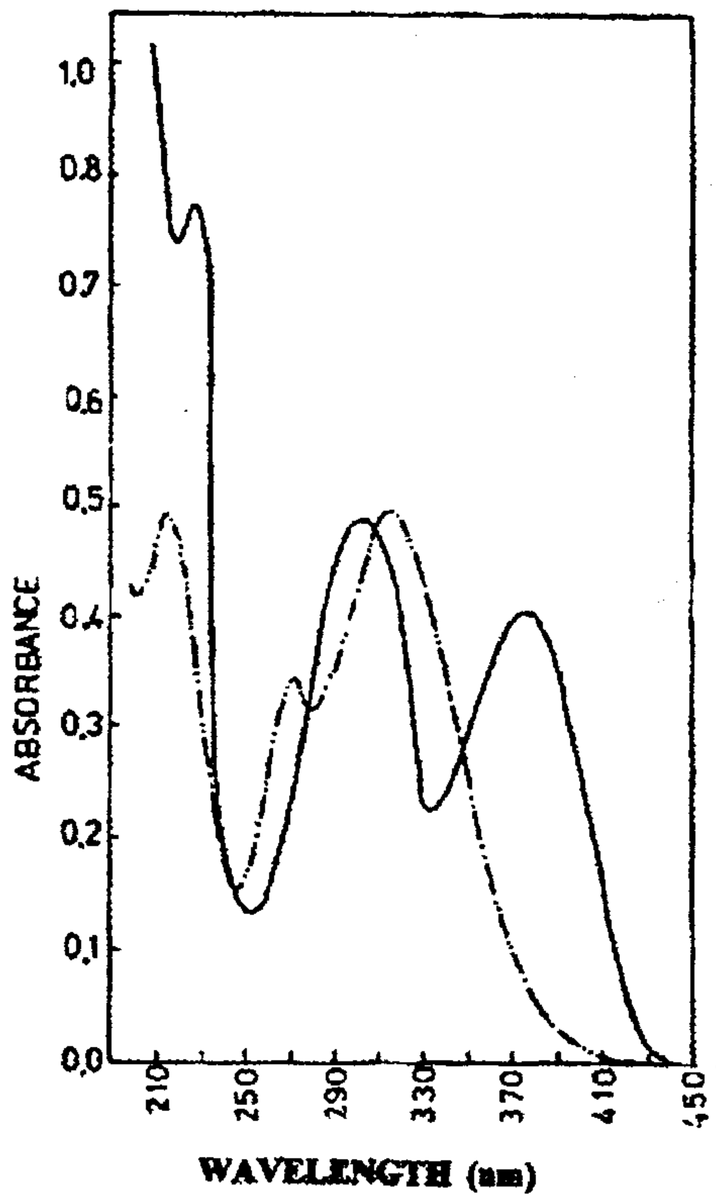


Fig. 2: UV spectra of: (————) Q_2 and (-.-.) CDS_2 methanol.

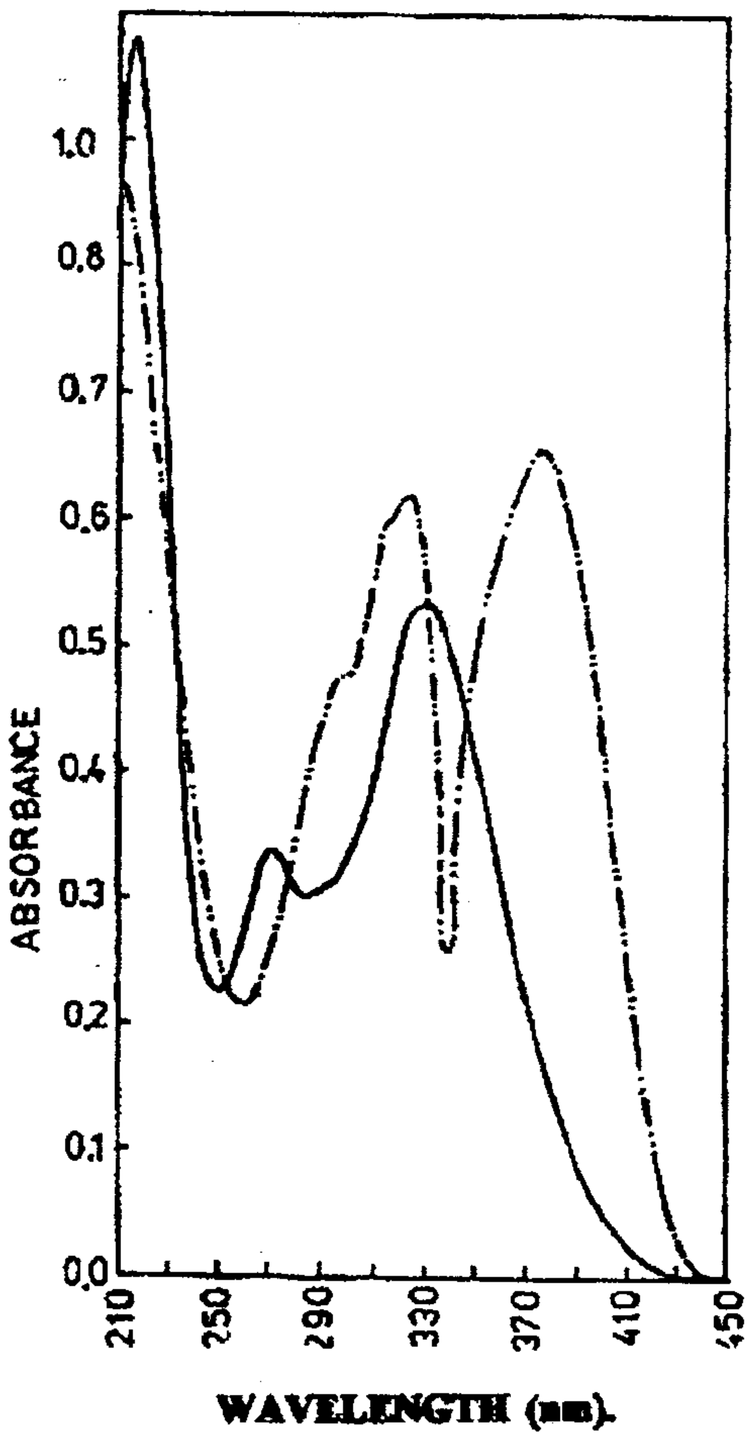


Fig. 3: UV spectra of: (————) Q_3 and (-.-.) CDS_3 methanol.

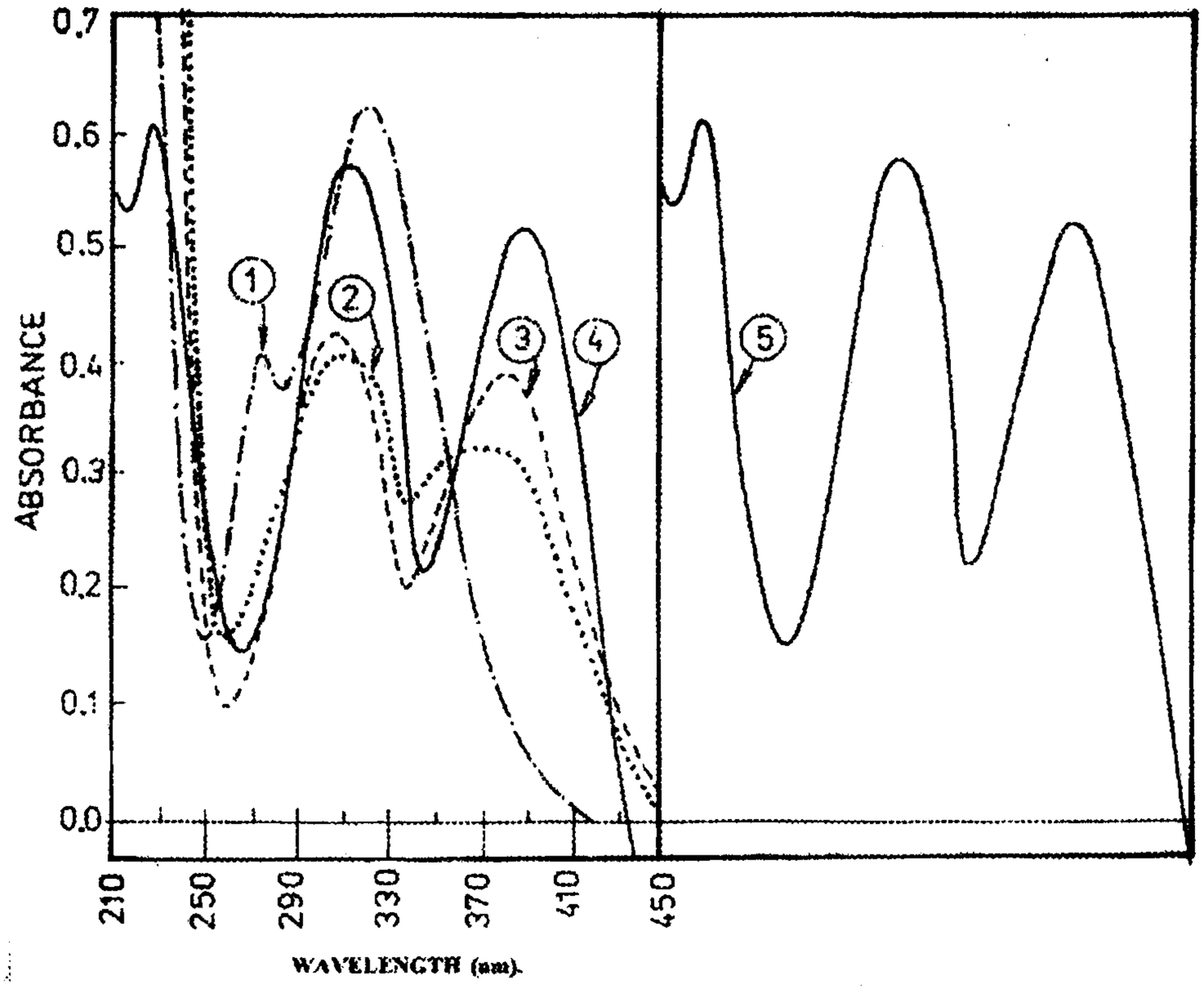
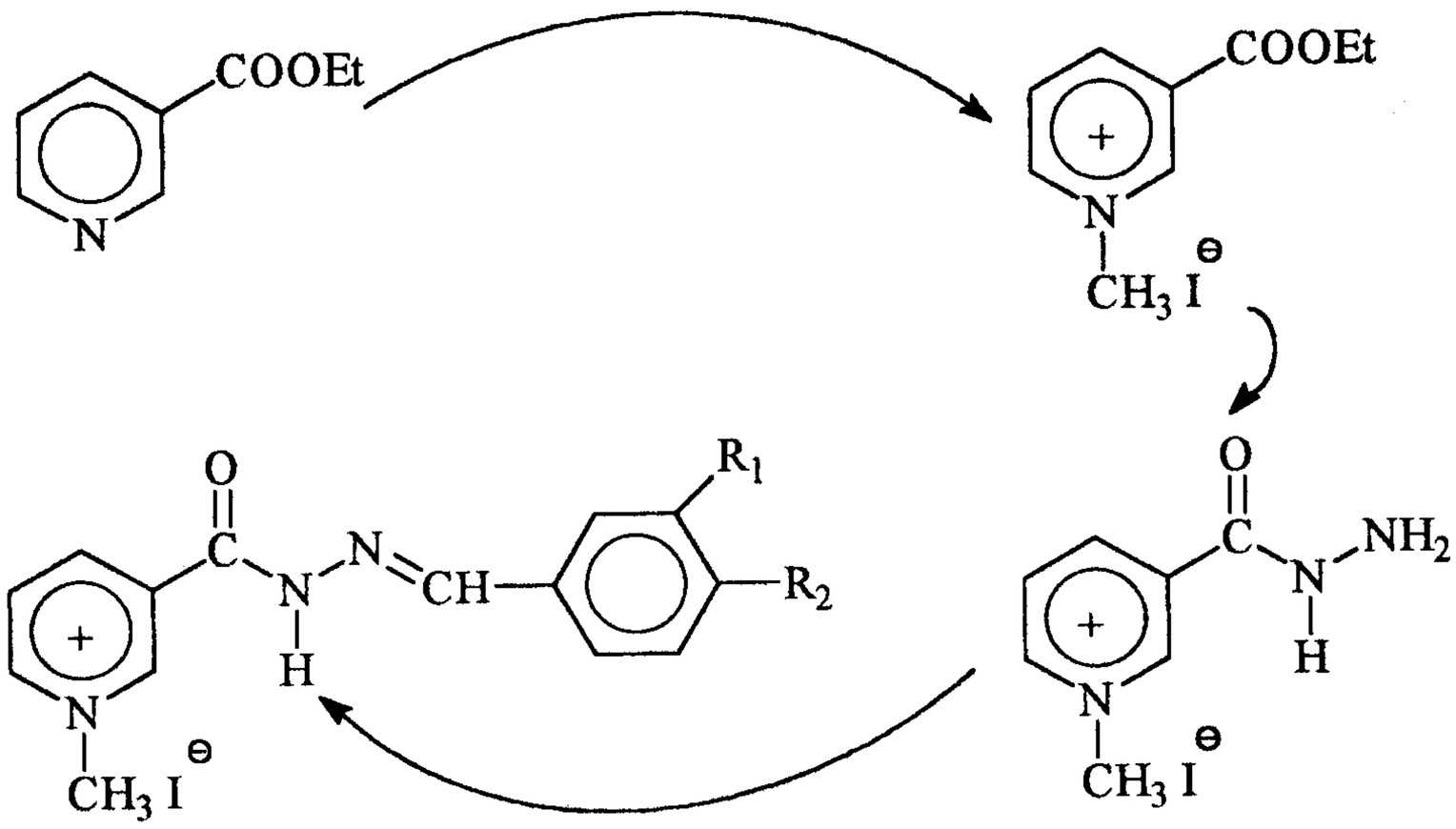


Fig. 4: UV spectra of the reaction mixture of the reduction of Q_2 at different time intervals 1- (-.-.) 0 min.; 2- (.....) 15 min.; 3- (----) 1 hr.; 4- (————) 2 hrs. and 5- (————) 5 hrs.



Q_1 : R_1, R_2 = H, H
Q_2 : R_1, R_2 = 3-H, 4-OCH_3
Q_3 : R_1, R_2 = 3-OCH_3, 4-OH

Scheme 2

systems.[18] This band is about 50 nm red shifted from the nearst absorption of the spectrum of the corresponding quaternary. This made it possible to monitor the dihydropyridine derivatives even in the presence of its oxidative product, the quaternary compound. On monitoring stabilities of these CDS under nitrogen and in dry atmosphere for one week, no change was observed in their UV spectra which prooved reasonable stabilities to carry out any *in vitro* and *in vivo* studies.

The parent non quaternized compounds (P_{1-3}) were prepared as reported in order to compare their MAO inhibition activity, if any, with that of the designed CDS. Parent compounds, as benzylidenes derivatives, have highly conjugated structures which allow their detection by UV spectroscopy (Table 3).

In vitro MAO inhibition

The fluorimetric method of Krajel[13] for determination of MAO inhibitory activity was selected to test both the quaternary (Q_{1-3}) and the parent (P_{1-3}) compounds. The test depends on oxidative deamination of kynuramine substrate by the MAO enzyme to 4-hydroxyquinoline (4-HOQ) which is highly fluorescent compound (Scheme 3).

Accordingly, the amount of 4-HOQ formed can be measured spectrofluorimetrically at 315 nm for excitation and 380 nm for emission. Under controlled standard conditions, the increase in fluorescence intensity provided a direct measurement of the 4-HOQ which was taken as an index of enzyme activity. The percent inhibition was calculated from the observed decrease in the fluorescence intensity in the presence of inhibitor for a specific time. This provides an index of the MAO inhibitory property of the compound tested. Mitochondrial MAO was obtained by differential centrifugation of homogenized rat liver[16]. Protein concentration of the enzyme preparation was estimated by the method of Lowry *et al.*[16], using crystalline Bovine serum albumin (BSA) to construct a standard calibration curve. Standard conditions were selected after investigating the effects of protein concentration, substrate concentration and incubation time on the enzyme activity. The optimal conditions were found to be; 200-300 μg protein concentration (Fig. 5), 100 μg kynuramine concentration (Fig. 6) and 30 minutes incubation time (Fig. 7).

The pI_{50} (-log molar concentration of inhibitor required to produce 50% inhibition of MAO activity) were determined for the quaternaries and the parent compounds by plotting the percentage inhibition against log molar concentration of the tested compounds (Fig. 8) The results (Table 4) show that both the quaternaries and the parent compounds inhibit MAO (a control experiment was performed using phenelzine sulfate) but the quaternaries have higher activities (14 to 22 times more active) which was expected due to higher electronegativity of the pyridinum ring.

The highest activity was shown by Q_3. The inhibitory activity of P_3 could not be determined due to solubility problems.

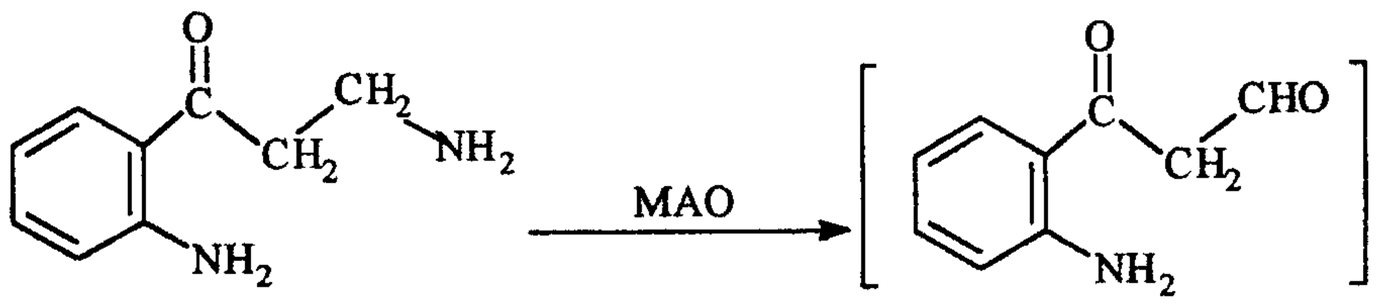
The correlations between enzyme inhibitory activity of the tested quaternaries, calculated as log 1/c, and certain physicochemcial parameters (π, σ, S, P and MR) of substituents on the phenyl ring (Table 5) were performed using microstate program. π is a measure of the relative hydrophobic effect[19] of a substituent, S represents the inductive or field effect, P the resonance effect,[20] Hammet π values are a linear combination of S and P values, and MR: molar polarizability, is a rough measure of the size of the substituent.[21]

Table 6 shows the results of these correlations and reveals that the best correlation could be obtained with the parameter P where:

$$\text{Activity (log 1/c)} = 3.858 - 0.126\ P\ (\pm 0.0099)$$

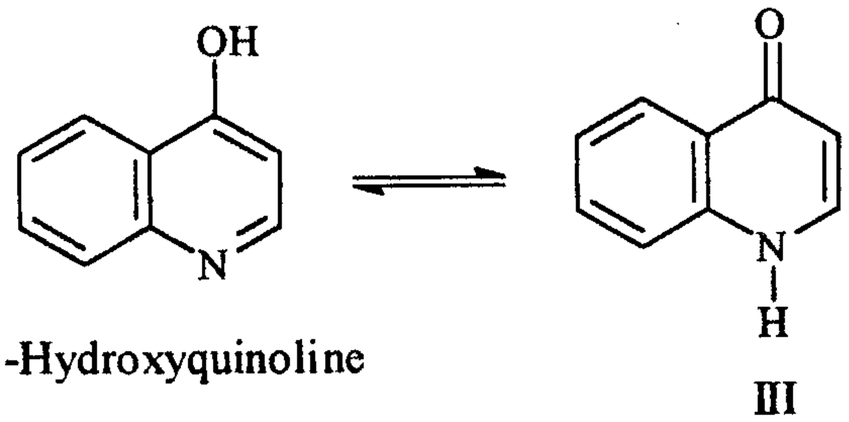
Table 7 shows the observed and calculated activity together with the corresponding residual. Low correlations were obtained with other parameters and this may be due to either the absence of any relation between activity and those parameters or due to the small number of compounds tested (n = 5).

The dihydropyridine/pyridinium redox sysem is similar to the naturally occurring reduced nicotinamide adenine dinucleotide



Kynuramine
I

II



4-Hydroxyquinoline

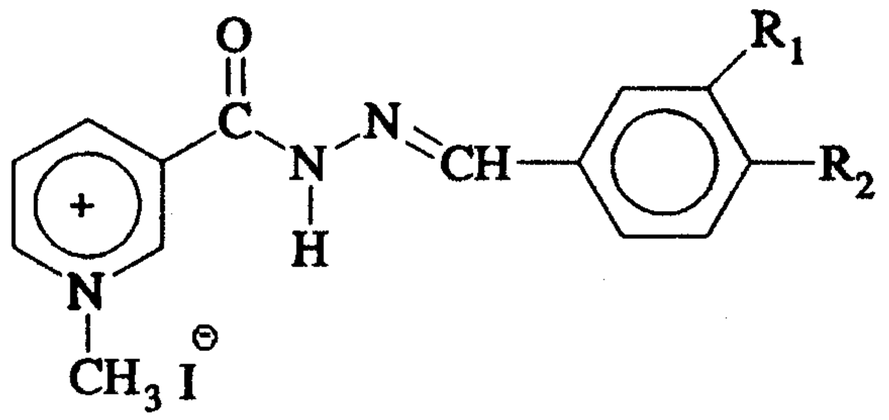
III

Scheme 3

Table 4: The pI_{50} of the tested compound.

n	Activity [pI_{50} (molar conc.)]	
	Pn	Qn
1	3.5 (3.1×10^{-4})	3.8 (14.0×10^{-5})
2	3.9 (1.4×10^{-4})	4.0 (9.8×10^{-5})
3	- -	4.2 (6.6×10^{-5})

Table 5: Values of substitution constants of aromatic substituents of the tested quaternary compounds.



No	R_1	R_2	Substitution constant[a,b]				
			π	σ	S	P	MR
1	H	H	0.000	0.000	0.000	0.000	1.030
2	H	OCH_3	-0.040	-0.270	0.634	-1.125	7.870
3	OCH_3	OH	-0.490	-0.250	1.116	-2.573	10.720

a: The algebric sum of R_1 and R_2 constants.
b: References 19, 20, 21.

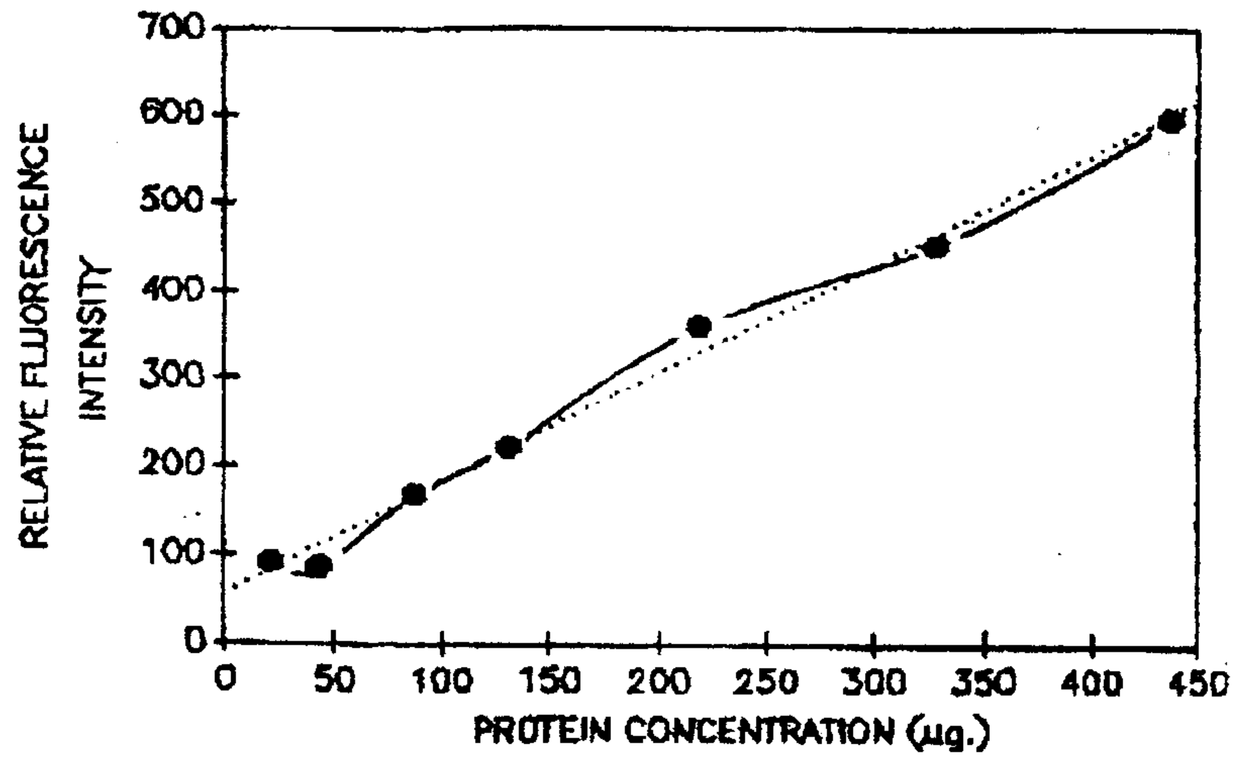


Fig. 5: Relationship between protein concentration and the measured fluorescence intensity.

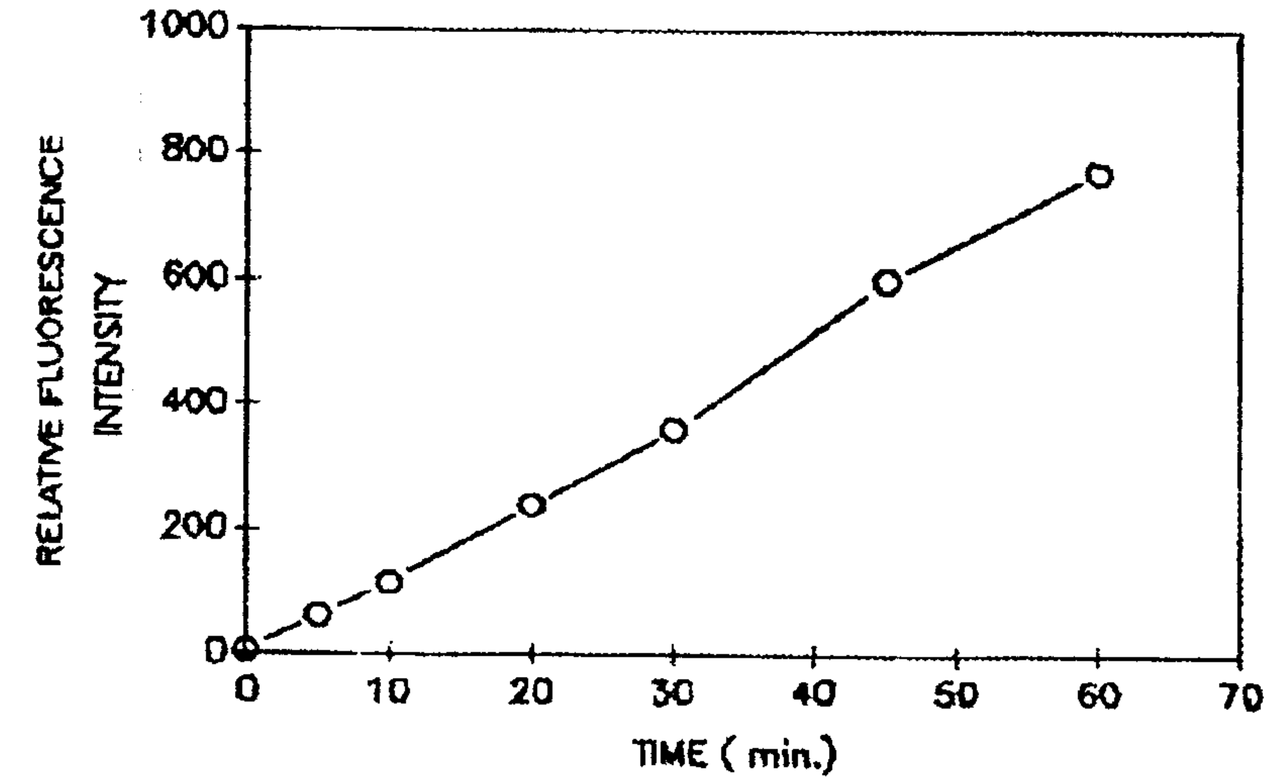


Fig. 7: Effect of incubation time on the measured fluorescence intensity.

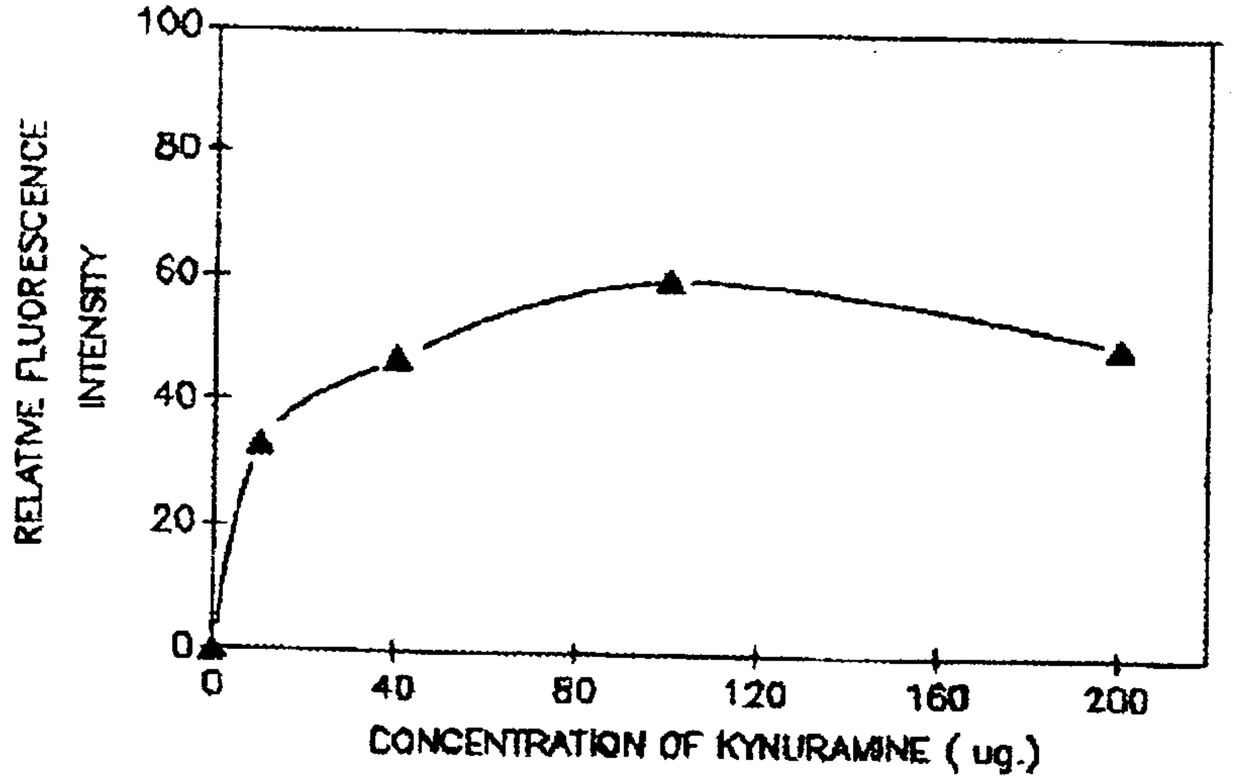


Fig. 6: Effect of substrate concentration on the measured fluorescence intensity.

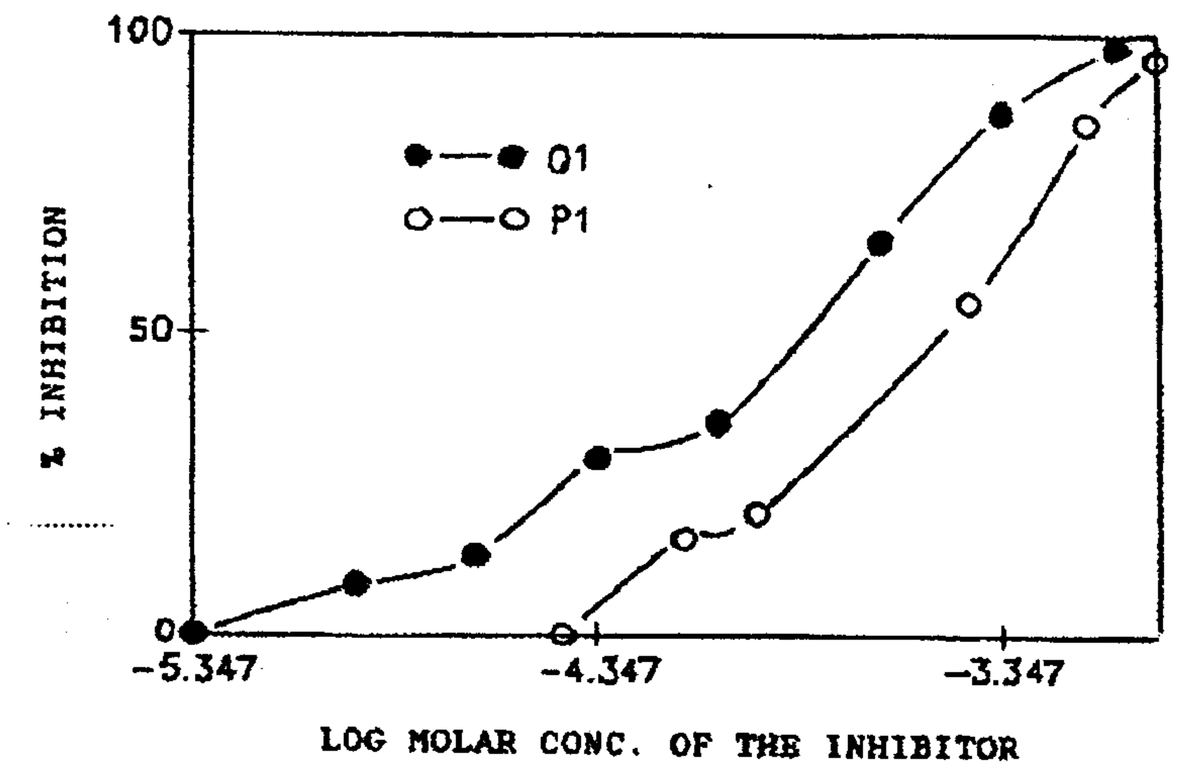


Fig. 8: Inhibition curve of: (●-●) Q_1 and (○-○) P_1.

Table 6: Results of linear regression of activity (log 1/C) of quaternary compounds against certain selected substitution constants.

Variable substitution constant	r	F	Prob.
π	0.913	4.999	0.268
σ	0.814	1.966	0.394
S	0.994	85.174	0.069
P	0.999	542.901	0.027
MR	0.966	13.811	0.167

Table 7: The regression line, the observed, calculated activity and the residual.
Activity (log 1/C) = 3.858 - 0.126 P ($\pm$ 0.0099)

No.	Observed	Calculated	Residual
1	3.854	3.858	-0.0045
2	4.009	4.001	0.0081
3	4.180	4.184	-0.0035

(NADH) ⇌ oxidized dinucleotide (NAD^+) system. Their suitability for the site specific CDS is related to the chemistry of dihydro-pyridine and enamine. This system was, therefore applied successfully for the site specific delivery of the antineoplastic alkaloid, berberine,[11] to the brain. Therefore, *in vitro* and *in vivo* investigations of the prepared MAOIs to explore their brain preferential delivery and retention will be continued in part 2.

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