Original Articles

SYNTHESIS AND INVESTIGATION OF NOVEL SHELF-STABLE, BRAIN-SPECIFIC CHEMICAL DELIVERY SYSTEM

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نصف هنا نظام ريدوكس (اختزال- أكسدة) من نوع 4,1-دايهيدروبيريدين ⇌ ملح بيريدينيوم كطريقة عامة ومرنة للإيصال المستمر والنوعي للأدوية إلى مواقع تأثيرها في المخ. وقد تم استخدام مثبطات إنزيم أوكسيداز أمين الأحادي كمثال نموذجي لايصاله إلى المخ. وتم دراسة الأكسدة الكيميائية والحيوية لهذه المركبات، حيث تم تعريض مركبات 4,1-دايهيدروبيريدين المحضرة إلى مختلف أنواع الأكسدة الكيميائية والحيوية وذلك لتقدير مدى قدرتها على اختراق حاجز الدم إلى المخ، ولكي يتم أكسدتها حيوياً إلى المركبات الرباعية المقابلة. وقد أثبت المركب 31 من بين تلك المركبات على قدرته على عبور حاجز الدم إلى المخ بمعدل كاف حيث تحول بواسطة الإنزيمات المؤكسدة إلى الملح الرباعي المقابل وهو المركب 20. وقد أظهرت دراسات الثباتية على أنظمة التوصيل الكيميائي المشيدة عند درجات حموضة (أس هيدروجيني) ودرجات حرارة مختلفة أن عمر تخزين المحلول المحتوي على المركب 31 هو 20.53 يوماً وذلك عند درجة حرارة 5°م، وهذا يؤكد وجوب استخدام درجة حرارة تخزين منخفضة لمثل هذه المحاليل. كما أثبتت أنظمة التوصيل هذه ثباتيتها المعقولة عند تخزينها على هيئة مسحوق. ويمكن أن نعزو ثباتية المركبات المحضرة إلى اقتران مجموعتي الكاربوكسيل عند ذرات الكربون 3 و 5 في حلقة البيريدين مع الروابط الثنائية المجاورة لها. وتتوافق هذه النتائج مع التصميم المنطقي الأصلي للدراسة.

A 1,4-dihydropyridine ⇌ pyridinium salt type redox system is described as a general and flexible method for site-specific and sustained delivery of drugs into the brain. Monoamine oxidase inhibitors (MAOIs) were used as a model example to be delivered into the brain. Chemical and biological oxidations of these compounds were investigated. The prepared 1,4-dihydropyridines were subjected to various chemical and biological oxidation to evaluate their ability to cross blood brain barrier (BBB), and to be oxidized biologically into their corresponding quaternary compounds. 1-(Ethoxy-carbonylmethyl)-3,5-bis[N-(2-fluoro-benzylideneamino)carbamoyl]-1,4-dihydropyridine (**31**) proved to cross BBB in adequate rate and converted by the oxidizing enzymes into the corresponding quaternary salt N-(ethoxycarbonylmethyl)-3,5-bis[N-(2-fluorobenzylideneamino)carbamoyl]pyridinium bromide (**20**). Stability studies of the synthesized chemical delivery systems (CDSs) at various pH values and temperatures showed that the shelf life time of a solution containing compound **31** is 20.53 days at 5°C, which recommend a lower storage temperature for such solutions. The prepared CDSs proved to be fairly stable for powder form storage. The stability of the prepared compounds is attributed to the conjugation of the two carboxylic functions at C3 and C5 of the pyridine ring with their adjacent double bonds. These results are in consistency with the original rationale design.

Key words: Synthesis, chemical delivery systems, MAO inhibitors

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Introduction

Chemical delivery systems (CDSs) are developing approach aiming toward the delivery of drugs into their action sites in an adequate concentration and to protect the drugs against their metabolic inactivation. So many drug delivery systems are now known in the literature such as: methacrylic carriers to deliver the nonsteroidal anti-inflammatory drugs (1), the formation of phenylephrine in the iris-ciliary body from phenylephrine chemical delivery systems as a new mydriatic agent (2), and the lipid vesicle-mediated drug delivery system of administration of topical drugs through the skin (3). In addition, many drug delivery systems are specialized to deliver drugs into the brain (4,5), including redox CDSs (6).

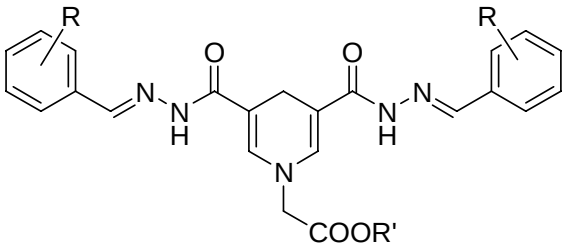
A dihydropyridinium-type CDS or a redox analog of the drug is sufficiently lipophilic to enter the brain by passive transport, then undergoes an enzymatic oxidation to an ionic pyridinium compound, which promotes retention in the central nervous system (CNS). At the same time, peripheral elimination of the entity is accelerated due to facile conversion of the CDS in the body (7,8). *In vivo* and *in vitro* studies and preliminary clinical data of several novel derivatives have been promising, particularly in the areas of neurotransmitters, steroids, anticonvulsants, antibiotics, antiviral, anticancer and antidementia agents (9-13).

Monoamine oxidase (MAO) deaminates a variety of biogenic amines and plays an important role in the regulation of their intracellular concentration (14-16). Drugs such as MAOIs are used for the treatment of depression, but they suffer from adverse side effects and food incompatibilities. One of the solutions to overcome these side effects is to deliver those inhibitors, specifically into the brain, by the use of a dihydropyridine CDSs.

In the present investigation a novel carrier, bearing MAOIs as a model example, was studied, keeping in mind all the prospects of the requirements for a safe and useful brain specific CDS, which are: a reasonable shelf-life time, a good rate of oxidation, and most importantly a reasonable rate of excretion from the brain without causing any sign of neurotoxicity. This carrier is a derivative of 1,4-dihydropyridine-3,5-dicarboxylic acid, which resemble the nifedipine analogs known to be stable against both air oxidation and hydration. The only expected obstacle for the use of this carrier system, was its slow rate of oxidation and accordingly, low efficiency for brain specific delivery (13).

The suggested approach to solve this problem and to increase the rate of oxidation was the inclusion of an alkoxycarbonylmethyl group on the nitrogen atom of the 1,4-dihydropyridne carrier (**A**, chart 1), without any substitution at C2 and C6. These compounds are expected to be stable against hydration and air oxidation during formulation and storage, since the conjugation of the two carboxylic functions at C3 and C5 with their adjacent double bonds stabilizes the compounds against these reactions.

The synthesis of these new compounds was conducted in addition to their kinetics of chemical oxidation and their oxidation in biological fluids, organ homogenates as well as their stability studies.



A

R = H, Cl, F, NO_2, CH_3, OCH_3
R' = H, Et

Chart 1.

Experimental

Synthesis:

Melting points were determined on a Mettler FP80 melting point apparatus and are uncorrected. Unless otherwise specified all chemicals were of commercial grade, used without further purification, and were obtained from Aldrich Chemical Co. (Milwaukee, WI). Solvents used for work ups were dried over $MgSO_4$, filtered and removed on a rotary evaporator. Elemental analyses were performed at College of Pharmacy, King Saud University, Central Laboratory. ^{1}H and ^{13}C NMR spectra were obtained at 400 and 100 MHz. JEOL instrument, respectively, using TMS as an internal standard. Thin layer and

flash chromatography were performed using E. Merck silica gel (230-400 mesh). Preparative thin layer chromatography was performed on a Harrison model 7924 A chromatotron using Analtech silica gel GF rotors. KUBOTA 6800 compact high speed refrigerated centrifuge (20000 rpm) was used for the centrifugation of the samples for 10 min at 4°C. High performance liquid chromatograph (WATERS), consist of a 600E system controller, Rheodyne 7161 injector, and tunable absorbance detector 486 and 746 data module, and fitted with μBondapak™ C-18, 10 μM (4.6 mm id × 250 mm) column, run at ambient temperature, with 20 μl injection volume, and at 1.5 ml/min. flow rate, was used for the detection of the 1, 4-dihydropyridines and the corresponding quaternary compounds in both chemical and biological investigations. The peak area integrations were performed using a chromatographic data module.

3, 5-Bis[N-(substituted benzylideneamino) carbamoyl]pyridines (**4–14).**

A solution of pyridine-3,5-dicarboxylic acid hydrazide (**3**, 1.0 g, 0.006 mol) and the appropriate benzaldehyde derivatives (0.006 mol) in glacial acetic acid (30 ml) was heated under reflux for 10 h. The reaction mixture was concentrated in vacuo, cooled and the obtained solid was filtered dried and recrystallized from the appropriate solvents, to give **4-14** (Table 1). ^{1}H NMR (DMSO-d_6), **4:** δ 7.45 (s, 6H, ArH), 7.71 (s, 4H, ArH), 8.47 (s, 2H, pyridine-H), 8.73 (s, 1H, pyridine-H), 9.21 (s, 2H, CH=N), 11.72 (brs, 2H, NH). **5:** δ 7.22-7.63 (m, 8H, ArH), 8.52 (s, 2H, pyridine-H), 8.89 (s, 1H, pyridine-H), 9.42 (s, 2H, CH=N), 10.62 (brs, 2H, NH). **6:** δ 7.31-7.66 (m, 8H, ArH), 8.61 (s, 2H, pyridine-H), 8.91 (s, 1H, pyridine-H), 9.40 (s, 2H, CH=N), 11.23 (brs, 2H, NH). **7:** δ 7.62-7.93 (m, 6H, ArH), 8.25 (s, 2H, ArH), 8.64 (s, 2H, pyridine-H), 8.96 (s, 1H, pyridine-H), 9.47 (s, 2H, CH=N), 11.52 (brs, 2H, NH). **8:** δ 7.65-7.92 (m, 6H, ArH), 8.32 (s, 2H, ArH), 8.74 (s, 2H, pyridine-H), 9.01 (s, 1H, pyridine-H), 9.51 (s, 2H, CH=N), 10.51 (brs, 2H, NH). **9**: δ 7.24-7.68 (m, 8H, ArH), 8.55 (s, 2H, pyridine-H), 8.91 (s, 1H, pyridine-H), 9.46 (s, 2H, CH=N), 10.32 (brs, 2H, NH). **10**: δ 7.21-7.71 (m, 8H, ArH), 8.53 (s, 2H, pyridine-H), 8.89 (s, 1H, pyridine-H), 9.52 (s, 2H, CH=N), 10.46 (brs, 2H, NH). **11**: δ 2.12 (s, 6H, $ArCH_3$), 7.46-7.81 (m, 8H, ArH), 8.45 (s, 2H, pyridine-H), 8.64 (s, 1H, pyridine-H), 9.43 (s, 2H, CH=N), 11.85 (brs, 2H, NH). **12**: δ 2.15 (s, 6H, $ArCH_3$), 7.32-7.45 (m, 4H, ArH), 7.62-7.76 (m, 4H, ArH), 8.40 (s, 2H, pyridine-H), 8.58 (s, 1H, pyridine-H), 9.45 (s, 2H, CH=N), 12.01 (brs, 2H, NH). **13**: δ 3.85 (s, 6H, OCH_3), 7.34-7.76 (m, 8H, ArH), 8.39 (s, 2H, pyridine-H), 8.59 (s, 1H, pyridine-H), 9.56 (s, 2H, CH=N), 12.02 (brs, 2H, NH). **14**: δ 3.87 (s, 6H, OCH_3), 7.35-7.46 (m, 4H, ArH), 7.64-7.72 (m, 4H, ArH), 8.43 (s, 2H, pyridine-H), 8.61 (s, 1H, pyridine-H), 9.50 (s, 2H, CH=N), 10.31 (brs, 2H, NH).

N-(Ethoxycarbonylmethyl)-3,5-Bis[N-(substituted benzylideneamino) carbamoyl]pyridinium bromides (**15–25).**

A mixture of the prepared substituted benzylidene aminocarbamoylpyridine analogs (4-14, 0.001 mol) and ethyl bromoacetate (0.23 g, 0.001 mol) in dimethylformamide (25 ml) were heated under reflux for 36 h. The solid separated upon cooling was recrystallized from the appropriate solvent to give **15–25** (Table 2). ^{1}H NMR (DMSO-d_6), **15:** δ 1.31 (t, 3H, $C\underline{H}_3CH_2$), 3.85 (s, 2H, NCH_2CO), 4.12 (q, 2H, $CH_3C\underline{H}_2$), 7.41 (m, 6H, ArH), 7.71 (s, 4H, ArH), 8.45 (s, 2H, pyridine-H), 8.70 (s, 1H, pyridine-H), 9.10 (s, 2H, CH=N), 10.52 (brs, 2H, NH). **16:** δ 1.32 (t, 3H, $C\underline{H}_3CH_2$), 3.87 (s, 2H, NCH_2CO), 4.12 (q, 2H, $CH_3C\underline{H}_2$), 7.44-7.78 (m, 8H, ArH), 8.50 (s, 2H, pyridine-H), 8.83 (s, 1H, pyridine-H), 9.00 (s, 2H, CH=N), 10.52 (brs, 2H, NH). **17:** δ 1.34 (t, 3H, $C\underline{H}_3CH_2$), 3.90 (s, 2H, NCH_2CO), 4.19 (q, 2H, $CH_3C\underline{H}_2$), 7.46-7.82 (m, 8H, ArH), 8.52 (s, 2H, pyridine-H), 8.85 (s, 1H, pyridine-H), 9.12 (s, 2H, CH=N), 11.20 (brs, 2H, NH). **18:** δ 1.35 (t, 3H, $C\underline{H}_3CH_2$), 3.95 (s, 2H, NCH_2CO), 4.23 (q, 2H, $CH_3C\underline{H}_2$), 7.62-7.91 (m, 6H, ArH), 8.32 (s, 2H, ArH), 8.61 (s, 2H, pyridine-H), 9.10 (s, 1H, pyridine-H), 9.13 (s, 2H, CH=N), 10.82 (brs, 2H, NH). **19:** δ 1.36 (t, 3H, $C\underline{H}_3CH_2$), 3.92 (s, 2H, NCH_2CO), 4.25 (q, 2H, $CH_3C\underline{H}_2$), 7.60-7.93 (m, 6H, ArH), 8.35 (s, 2H, ArH), 8.61 (s, 2H, pyridine-H), 9.00 (s, 1H, pyridine-H), 9.11 (s, 2H, CH=N), 9.98 (brs, 2H, NH). **20**: δ 1.32 (t, 3H, $C\underline{H}_3CH_2$), 3.87 (s, 2H, NCH_2CO), 4.12 (q, 2H, $CH_3C\underline{H}_2$)7.40-7.75 (m, 8H, ArH), 8.52 (s, 2H, pyridine-H), 8.80 (s, 1H, pyridine-H), 9.38 (s, 2H, CH=N), 11.23 (brs, 2H, NH). **21**: δ 1.33 (t, 3H, $C\underline{H}_3CH_2$), 3.85 (s, 2H, NCH_2CO), 4.15 (q, 2H, $CH_3C\underline{H}_2$), 7.36-7.45 (m, 4H, ArH), 7.52-7.71 (m, 4H, ArH), 8.49 (s, 2H, pyridine-H), 8.76 (s, 1H,

pyridine-H), 9.51 (s, 2H, CH=N), 11.41 (brs, 2H, NH). **22**: δ 1.31 (t, 3H, $C\underline{H}_3CH_2$), 2.14 (s, 6H, $ArCH_3$), 3.89 (s, 2H, NCH_2CO), 4.13 (q, 2H, $CH_3C\underline{H}_2$), 7.45-7.82 (m, 8H, ArH), 8.46 (s, 2H, pyridine-H), 8.68 (s, 1H, pyridine-H), 9.52 (s, 2H, CH=N), 11.02 (brs, 2H, NH). **23**: δ 1.32 (t, 3H, $C\underline{H}_3CH_2$), 2.12 (s, 6H, $ArCH_3$), 3.87 (s, 2H, NCH_2CO), 4.12 (q, 2H, $CH_3C\underline{H}_2$), 7.25-7.32 (m, 4H, ArH), 7.42-7.72 (m, 4H, ArH), 8.40 (s, 2H, pyridine-H), 8.55 (s, 1H, pyridine-H), 9.45 (s, 2H, CH=N), 11.08 (brs, 2H, NH). **24**: δ 1.32 (t, 3H, $C\underline{H}_3CH_2$), 3.86 (s, 2H, NCH_2CO), 3.88 (s, 6H, OCH_3), 4.14 (q, 2H, $CH_3C\underline{H}_2$), 7.32-7.74 (m, 8H, ArH), 8.40 (s, 2H, pyridine-H), 8.60 (s, 1H, pyridine-H), 9.48 (s, 2H, CH=N), 11.05 (brs, 2H, NH). **25**: δ 1.30 (t, 3H, $C\underline{H}_3CH_2$), 3.85 (s, 2H, NCH_2CO), 3.90 (s, 6H, OCH_3), 4.12 (q, 2H, $CH_3C\underline{H}_2$), 7.34-7.40 (m, 4H, ArH), 7.42-7.70 (m, 4H, ArH), 8.45 (s, 2H, pyridine-H), 8.62 (s, 1H, pyridine-H), 9.54 (s, 2H, CH=N), 10.91 (brs, 2H, NH).

1-(Ethoxycarbonylmethyl)-3,5-Bis[N-(substituted benzylideneamino)carbamoyl]-1.4-dihydro-pyridines (**26–36**)**.**

A suspension of 1-(ethoxycarbonyl methyl)-3,5-Bis[N-(substituted benzylideneamino)carbamoyl]-pyridinium bromides (**15–25**, 0.01 mol), in deaeriated water (200 ml) and methylene chloride (100 ml) was cooled to 0°C and stirred under nitrogen stream. Na_2CO_3 (6.4 g, 0.06 mol) was added portionwise over a period of 10 min $Na_2S_2O_4$ (7.0 g, 0.04 mol) was then added portionwise over a period of 15 min. Stirring was continued, under nitrogen at 0°C, for another 1 h. The organic layer was separated, washed with cold deaeriated water, dried and evaporated in vacuo. The obtained solid was recrystallized from the appropriate solvent to give **26-36** (Table 3). ^{1}H NMR (DMSO-d_6), **26**: δ 1.29 (t, 3H, $C\underline{H}_3CH_2$), 2.92-3.02 (m, 2H, C_4-H), 3.75 (s, 2H, NCH_2CO), 4.12 (q, 2H, $CH_3C\underline{H}_2$), 5.05 (s, 2H, C_2-H&C_6-H), 7.41-7.71 (m, 10H, ArH), 9.10 (s, 2H, CH=N), 10.52 (brs, 2H, NH). **27**: δ 1.32 (t, 3H, $C\underline{H}_3CH_2$), 2.93-3.12 (m, 2H, C_4-H), 3.87 (s, 2H, NCH_2CO), 4.12 (q, 2H, $CH_3C\underline{H}_2$), 5.25 (s, 2H, C_2-H&C_6-H), 7.44-7.78 (m, 8H, ArH), 9.40 (s, 2H, CH=N), 11.20 (brs, 2H, NH). **28**: δ 1.34 (t, 3H, $C\underline{H}_3CH_2$), 3.0-3.22 (m, 2H, C_4-H), 3.90 (s, 2H, NCH_2CO), 4.19 (q, 2H, $CH_3C\underline{H}_2$), 5.26 (s, 2H, C_2-H&C_6-H), 7.46-7.82 (m, 8H, ArH), 9.12 (s, 2H, CH=N), 10.50 (brs, 2H, NH). **29**: δ 1.31 (t, 3H, $C\underline{H}_3CH_2$), 2.94-3.02 (m, 2H, C_4-H), 3.85 (s, 2H, NCH_2CO), 4.12 (q, 2H, $CH_3C\underline{H}_2$), 4.82 (s, 2H, C_2-H&C_6-H), 5.12 (s, 4H, NH_2), 7.24-7.69 (m, 8H, ArH), 9.10 (s, 2H, CH=N), 10.15 (brs, 2H, NH). **30**: δ 1.30 (t, 3H, $C\underline{H}_3CH_2$), 2.95-3.08 (m, 2H, C_4-H), 3.87 (s, 2H, NCH_2CO), 4.14 (q, 2H, $CH_3C\underline{H}_2$), 4.91 (s, 2H, C_2-H & C_6-H), 5.15 (s, 4H, NH_2), 7.22-7.71 (m, 8H, ArH), 9.42 (s, 2H, CH=N), 11.21 (brs, 2H, NH). **31**: δ 1.33 (t, 3H, $C\underline{H}_3CH_2$), 3.01-3.24 (m, 2H, C_4-H), 3.87 (s, 2H, NCH_2CO), 4.12 (q, 2H, $CH_3C\underline{H}_2$), 5.24 (s, 2H, C_2-H&C_6-H), 7.41-7.76 (m, 8H, ArH), 9.34 (s, 2H, CH=N), 11.34 (brs, 2H, NH). **32**: δ 1.31 (t, 3H, $C\underline{H}_3CH_2$), 3.02-3.26 (m, 2H, C_4-H), 3.89 (s, 2H, NCH_2CO), 4.13 (q, 2H, $CH_3C\underline{H}_2$), 5.26 (s, 2H, C_2-H&C_6-H), 7.36-7.42 (m, 4H, ArH), 7.50-7.70 (m, 4H, ArH), 9.42 (s, 2H, CH=N), 10.25 (brs, 2H, NH). **33**: δ 1.30 (t, 3H, $C\underline{H}_3CH_2$), 2.13 (s, 6H, $ArCH_3$), 3.10-3.19 (m, 2H, C_4-H), 3.87 (s, 2H, NCH_2CO), 4.11 (q, 2H, $CH_3C\underline{H}_2$), 5.24 (s, 2H, C_2-H&C_6-H), 7.32-7.61 (m, 8H, ArH), 9.52 (s, 2H, CH=N), 10.06 (brs, 2H, NH). **34**: δ 1.32 (t, 3H, $C\underline{H}_3CH_2$), 2.12 (s, 6H, $ArCH_3$), 3.13-3.23 (m, 2H, C_4-H), 3.89 (s, 2H, NCH_2CO), 4.13 (q, 2H, $CH_3C\underline{H}_2$), 5.24 (s, 2H, C_2-H&C_6-H), 7.36-7.42 (m, 4H, ArH), 7.50-7.73 (m, 4H, ArH), 9.32 (s, 2H, CH=N), 10.15 (brs, 2H, NH). **35**: δ 1.32 (t, 3H, $C\underline{H}_3CH_2$), 3.10-3.25 (m, 2H, C_4-H), 3.87 (s, 2H, NCH_2CO), 3.92 (s, 6H, OCH_3), 4.12 (q, 2H, $CH_3C\underline{H}_2$), 5.26 (s, 2H, C_2-H&C_6-H), 7.31-7.72 (m, 8H, ArH), 9.50 (s, 2H, CH=N), 10.23 (brs, 2H, NH). **36**: δ 1.31 (t, 3H, $C\underline{H}_3CH_2$), 3.13-3.32 (m, 2H, C_4-H), 3.82 (s, 2H, NCH_2CO), 3.95 (s, 6H, OCH_3), 4.13 (q, 2H, $CH_3C\underline{H}_2$), 5.30 (s, 2H, C_2-H&C_6-H), 7.33-7.41 (m, 4H, ArH), 7.43-7.72 (m, 4H, ArH), 9.42 (s, 2H, CH=N), 10.51 (brs, 2H, NH).

Kinetics of chemical oxidation of the 1,4-dihydropyridines CDSs:

Oxidation by silver nitrate

To 5 ml of saturated $AgNO_3$/acetonitrile solution was added 1 ml of 5% acetonitrile solution of the tested 1, 4-dihydropyridine analogs. The mixture was shaken, left for 5 min to complete precipitation of silver and then centrifuged, and an aliquot was taken to check the UV spectrum.

Oxidation by hydrogen peroxide

To 3 ml of 30% H_2O_2, an aliquot volume (0.2 ml) of a stock solution of the 1,4-dihydropyridine derivative (5 mg/10 ml) was added to make the final concentration 3.33 mg%. The mixture was stirred and samples were taken at appropriate time intervals

to be checked for the disappearance of the 1, 4-dihydropyridine and the formation of the corresponding quaternary salt.

Oxidation by potassium ferricyanide:

To 10 ml of buffered 40% aqueous acetonitrile solution of 0.1 mM $K_4Fe(CN)_6$, 60 mM KCl and 1.0 mM K_2CO_3, containing 5 mM $K_3Fe(CN)_6$, 150 µl of 10^{-4} M solution of the 1,4-dihydropyridine derivative in acetonitrile was added. The resulting solutions were maintained at 37°C all the time of the experiment. At the appropriate time intervals samples were taken and analyzed by UV for the disappearance of the 1, 4-dihydropyridine. The second order rate constant and the corresponding t½ were determined.

Oxidation by diphenyl picrylhydrazyl free radical:

To 3 ml of 9×10^{-5} M of 2,2-diphenyl-1-picrylhydrazyl in acetonitrile, equilibrated at 26°C, was added 50 µl of a 1.5×10^{-2} M solution of the 1,4-dihydropyridine derivative in acetonitrile to make a final concentration of 1.48×10^{-4} M. The mixture was monitored at 525 nm against a reference sample, containing exactly the same concentrations previously prepared and left for at least 30 min (reference for A_∞).

Kinetics of oxidation of the 1,4-dihydropyridines CDSs in biological fluids

In plasma:

A freshly prepared solution of the 1, 4-dihydropyridine derivatives (0.2 ml, 6.25×10^{-4} mol) in methyl alcohol was diluted to 10 ml with 20% plasma (diluted with phosphate buffer, pH 7.4). The solution was kept at 37°C and the UV spectrum was scanned from 400 to 250 nm, at the appropriate time intervals against a reference sample made by dilution of 0.2 ml of methyl alcohol with 20% plasma to 10 ml (7, 8).

In whole blood

In each of five tubes containing 0.1 ml of a 10×10^{-4} mol methanolic solution of the freshly prepared 1,4-dihydropyridine derivative was added 2 ml of fresh heparinized whole human blood, and the tubes were kept at 37°C in a water bath. At the end of the time period to be investigated, 8 ml of acetonitrile was added, and the tubes were then shaken vigorously and centrifuged (7, 8). The UV absorption of the supernatant solution was scanned at 400-250 nm, at the appropriate time intervals. A reference sample was made by addition of 0.1 ml of methyl alcohol instead of the sample solution following the same procedure.

In brain homogenate:

Rat brain tissue (2.0 g) was homogenized in 10 ml of phosphate buffer, pH 7.4. The homogenate was centrifuged for 15 min at 3000 rpm, decanted, heated in a water bath at 50°C for 5 min, and centrifuged again. The supernatant solution was diluted to 100 ml with phosphate buffer, pH 7.4. To 10 ml of the freshly prepared homogenate was added 0.2 ml of a 6.25×10^{-4} mol methanolic solution of the freshly prepared 1, 4-dihydropyridine derivative (7, 8). The mixture was UV scanned at 37°C from 400-250 nm, at the appropriate time intervals. A reference sample was made by addition of 0.2 ml of methyl alcohol, instead of the sample solution, and diluted to 10 ml with the brain homogenate solution.

In liver homogenate:

Rat liver tissue (5.0 g) was homogenized in 50 ml of phosphate buffer, pH 7.4. The homogenate was centrifuged, decanted, heated in water bath at 50°C for 5 min, and then centrifuged again. The supernatant homogenate was diluted to 250 ml with phosphate buffer, pH 7.4. A solution of the freshly prepared 1,4-dihydropyridine derivative (0.2 ml, 6.25×10^{-4} mol) in methyl alcohol was diluted to 10 ml with liver homogenate solution (7,8).The mixture was UV scanned at 37°C from 400-250 nm at appropriate time intervals. A reference sample was made by addition of 0.2 ml of methyl alcohol, instead of sample solution, and diluted to 10 ml with the liver homogenate solution.

*In vivo brain-delivery studies of the 1,4-dihydropyridine analog **31**:*

Twenty five rats (obtained from the animal house college of pharmacy, KSU and were fed Purina chow pellets and water ad libitum) of both sexes weighing 250-300 g were divided into five groups each of five rats. Rats were anesthetized with ethyl carbamate (Urethane, 10 mg/kg of 25% solution). The neck area was opened, jugular vein exposed and cannulated. A freshly prepared solution of compound **31**, dissolved in ethanol and further diluted with phosphate buffer solution, was injected through the cannulated jugular vein at a dose of 200 mg/kg

body weight. At appropriate time intervals (0, 5, 10, 15 and 20 min), rats were decapitated and the brains were rapidly removed, weighed and immediately placed on dry ice. Each brain was then homogenized in 2 ml distilled water and then 8 ml of acetonitrile was added, and the mixture was homogenized again and centrifuged. The supernatant was analyzed in duplicate using HPLC as mentioned under stability studies (7, 8). The above mentioned procedure was repeated in all five groups to have five points at each time interval.

*In vitro determination of MAO inhibitory activity of compound **31:***

Male rats weighing 250-300 g were sacrificed by a blow on the head and exsanguinated. The abdomens were opened and few grams of the liver were quickly removed, weighed and placed in a boiling tube with 5 times its mass cold distilled water and surrounded with crushed ice. The liver was then homogenized for 1 min and the homogenate was kept in ice throughout the experiment. Two silica cells, one designated reference and the other sample, were used for the same purpose throughout the experiment. The reference cell was always prepared first, stoppered, mixed by inverting twice, wiped with tissue and placed in the rear cell hold of the UV spectrophotometer. Kynuramine (the substrate) was added to the sample cell last, stoppered, mixed, wiped and placed in the front cell holder of the spectrophotometer. Scanning of the samples was performed at room temperature using a fixed wave length of 360 nm which is the peak of the substrate (17). All drug solutions and the homogenate were kept on ice throughout the experiment. Tranylcypromine MAO inhibitor, as well as, compound **31** were dissolved in least amount of ethanol and further dilutions were made by distilled water. Kynuramine was dissolved in distilled water. Compound **31** was freshly prepared on the day of the experiment. Cells were prepared according to the following schedule (Tables A & B). Different concentrations of the test drugs or tranylcypromine were dissolved in a volume of 0.3 ml and added to the cells.

Stability studies of the 1,4-dihydropyridine analogs
Stability in buffers:

Phosphate buffers (0.05 M) at various pH (5.8, 7.4, and 10) was used as media for the kinetics. A stock solution (5 mg/10 ml) of 1,4-dihydropyridines in acetonitrile were prepared and aliquots of 1 ml were added to 10 ml of the respective buffer solution at 37°C. Samples (50 µl) were taken at time intervals, diluted with 3 ml of appropriate buffer and analyzed by UV. The t½ for each compound in each buffer were determined.

Stability studies of the 1,4-dihydropyridine analogs
Stability in buffers:

Phosphate buffers (0.05 M) at various pH (5.8, 7.4, and 10) was used as media for the kinetics. A stock solution (5 mg/10 ml) of 1,4-dihydropyridines in acetonitrile were prepared and aliquots of 1 ml were added to 10 ml of the respective buffer solution at 37°C. Samples (50 µl) were taken at time intervals, diluted with 3 ml of appropriate buffer and analyzed by UV. The t½ for each compound in each buffer were determined.

Table A: Determination of the rate of reaction on substrate concentration, cells were prepared according to the following schedule.

Additions	Reference cell (ml)	Sample cells (ml)			
0.5 M Phosphate buffer (pH 7.4)	0.3	0.3	0.3	0.3	0.3
Homogenate	0.1	0.1	0.1	0.1	0.1
Distilled water	2.6	2.5	2.4	2.3	2.2
Kynuramine (2 mM)	0.0	0.1	0.2	0.3	0.4

Table B: Determination of the effect of test drug or tranylcypromine on the dependence of rate of reaction on substrate concentration.

Additions	Reference cell (ml)	Sample cells (ml)			
0.5 M Phosphate buffer (pH 7.4)	0.3	0.3	0.3	0.3	0.3
Homogenate	0.1	0.1	0.1	0.1	0.1
Distilled water	2.3	2.2	2.1	2.0	1.9
Test drugs (10 μg/ml) or tranylcypromine (1 μg/ml)	0.3	0.3	0.3	0.3	0.3
Kynuramine (2 mM)	0.0	0.1	0.2	0.3	0.4

Accelerated stability:

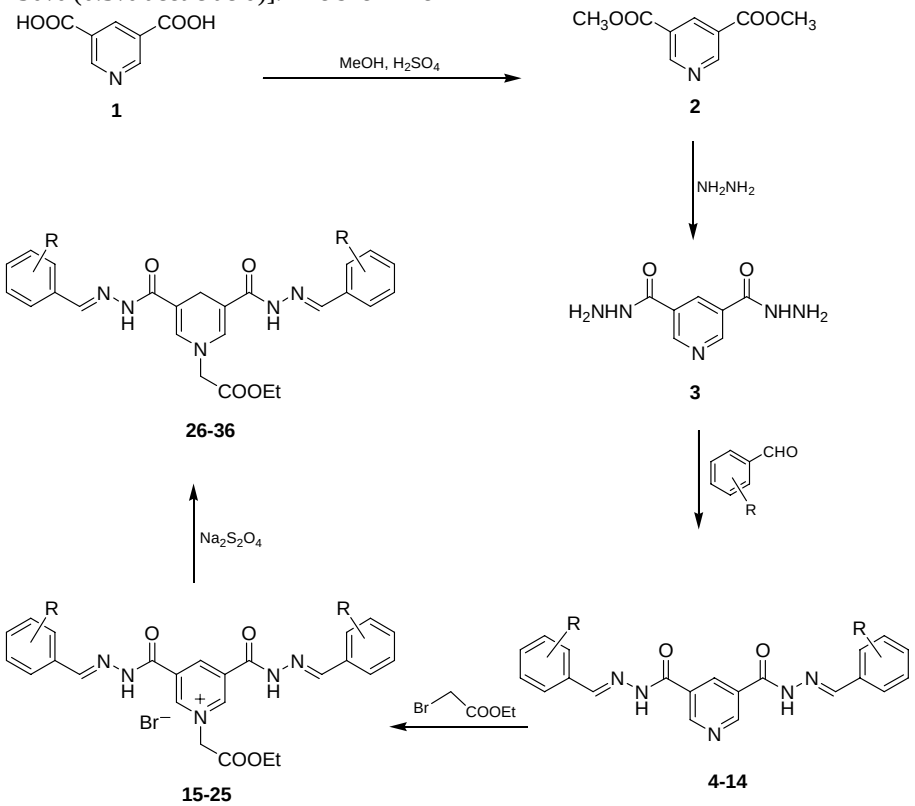
The previous experiment was performed for buffer of pH 7.4 and at different temperatures. Mixtures were kept at 55°C, 65°C and 75°C in closed containers. Samples were taken at appropriate time intervals for analysis by HPLC [50% acetonitrile + 50% (0.5% acetic acid)]. The shelf-life times ($t_{10\%}$) were determined from the Arrhenius plot.

Stability on storage:

The crystalline sample was tested for stability at different storage conditions. Samples from the prepared 1,4-dihydropyridine derivatives were dried and stored in dark brown bottles and stored under nitrogen and dry conditions at room temperatures (25°C) and in the refrigerator. At one month interval, each sample was analyzed for its content of the 1,4-dihydropyridine derivative using UV spectral analyses.

*Acute toxicity of the 1,4-dihydroyridine analog **31:***

Five groups of mice (obtained from the animal house college of pharmacy, KSU and were fed Purina chow pellets and water ad libitum), each consisted of six animals, were used. The test compounds were given up in a dose of 200, 400, 800, 1600 and 3200 mg/kg, respectively. Twenty four hours later, the percentage mortality in each group was recorded and the median lethal dose (LD_{50}) was calculated using the method described by Litchfield and Wilcoxon (18).



Scheme 1

Results and Discussion

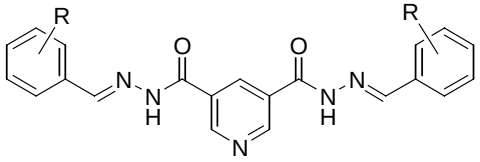
Chemistry:

The synthetic strategy to synthesize the target compounds is depicted in Scheme 1. The diacid **1** was used to prepare the dimethyl ester analog **2** (19, 20) which was subsequently reacted with hydrazine hydrate to give the diacid hydrazide derivative **3** (20). Reacting **3** with benzaldehyde analogs afforded the bis-hydrazones **4-14** (Scheme 1, Table 1) which were then quaternarized using ethyl bromoacetate and gave the quaternary salts **15-25** (Scheme 1, Table 2). The designed 1,4-dihydropyridine CDSs were obtained by the reduction of the quaternary salts **15-25**, using sodium dithionite in alkaline medium to give **26-36** (Scheme 1, Table 3). The nitro groups of compounds **18** and **19** were reduced into amino functions (**29**, **30**) upon the reaction with sodium dithionite. It was decided to exclude them from the biological investigation, as they become too polar to cross into the brain.

Chemical and biological investigations:

The prepared 1,4-dihydropyridines **26-36** were subjected to various chemical and biological investigations to evaluate the ability of these compounds to cross BBB, and to be oxidized biologically into their corresponding quaternary compounds (**15**–**25**). This oxidation process is very crucial to predict the ability of the 1,4-dihydropyridine CDSs to release the model example MAOIs at the site of action i.e. the brain. The rate of oxidation of the 1, 4-dihydropyridines CDSs needed to be neither too fast nor too slow. Fast oxidation process will convert the carrier CDS into the corresponding quaternary salt in the blood before reaching the brain, while slow oxidation process will allow the crossing of the carrier CDS into the brain, but it will delay the release of the carried drug. Accordingly, the moderate rate of oxidation will ensure the survival of the 1, 4-dihydropyridine species in the blood till reaching the brain.

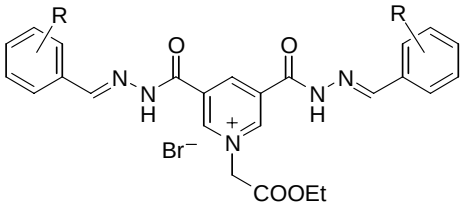
Table 1: The new synthesized 3,5-bis[N-(substituted benzylidene amino) carbamoyl]pyridines (**4-14**).



Compound	R	MP (°C)	Yield (%)	Crystallization Solvents	Molecular Formulae[a]
4	H	250-2	23	DMF	$C_{21}H_{17}N_5O_2$
5	2-Cl	235-7	32	AcOH	$C_{21}H_{15}Cl_2N_5O_2$
6	4-Cl	251-3	57	AcOH	$C_{21}H_{15}Cl_2N_5O_2$
7	2-NO_2	276-8	48	DMF	$C_{21}H_{15}N_7O_6$
8	4-NO_2	>300	59	DMF	$C_{21}H_{15}N_7O_6$
9	2-F	242-3	35	AcOH	$C_{21}H_{15}F_2N_5O_2$
10	4-F	261-2	41	AcOH	$C_{21}H_{15}F_2N_5O_2$
11	2-CH_3	188-9	65	EtOAc	$C_{23}H_{21}N_5O_2$
12	4-CH_3	173-5	70	EtOAc	$C_{23}H_{21}N_5O_2$
13	2-OCH_3	210-1	52	EtOH/EtOAc	$C_{23}H_{21}N_5O_4$
14	4-OCH_3	226-8	48	EtOH/EtOAc	$C_{23}H_{21}N_5O_4$

[a]Analyzed for C, H, N; results are within ± 0.4% of the theoretical values for the formulae given.

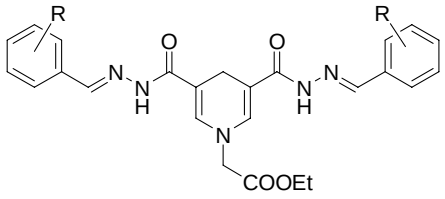
Table 2: The newly synthesized N-(ethoxycarbamoylmethyl)-3,5-bis[N-(substituted benzylideneamino) carbamoyl] pyridinium bromides (**15-25**).



Compound	R	MP (°C)	Yield (%)	Crystallization Solvents	Molecular Formulae[a]
15	H	262-4	28	DMF/dioxane	$C_{25}H_{24}BrN_5O_4$
16	2-Cl	286-8	36	DMF/dioxane	$C_{25}H_{22}BrCl_2N_5O_4$
17	4-Cl	275-7	40	DMF/dioxane	$C_{25}H_{22}BrCl_2N_5O_4$
18	2-NO_2	280-2	45	DMF	$C_{25}H_{22}BrN_7O_8$
19	4-NO_2	293-5	42	DMF	$C_{22}H_{22}BrN_7O_8$
20	2-F	280-2	36	DMF/EtOH	$C_{25}H_{22}BrF_2N_5O_4$
21	4-F	291-3	42	DMF/EtOH	$C_{25}H_{22}BrF_2N_5O_4$
22	2-CH_3	234-5	48	EtOH	$C_{27}H_{28}BrN_5O_4$
23	4-CH_3	249-50	38	EtOH	$C_{27}H_{28}BrN_5O_4$
24	2-OCH_3	262-3	51	MeOH	$C_{27}H_{28}BrN_5O_6$
25	4-OCH_3	273-5	62	MeOH	$C_{27}H_{28}BrN_5O_6$

[a]Analyzed for C, H, N; results are within ± 0.4% of the theoretical values for the formulae given.

Table 3: The new synthesized 1-(ethoxycarbonylmethyl)-3,5-bis[N-(substituted benzylideneamino) carbamoyl]-1,4-dihydropyridines (**26-36**).



Compound	R	MP (°C)	Yield (%)	Crystallization Solvents	Molecular Formulae[a]
26	H	112-3	26	EtOH/H_2O	$C_{25}H_{25}N_5O_4$
27	2-Cl	115-6	32	EtOH/H_2O	$C_{25}H_{23}Cl_2N_5O_4$
28	4-Cl	128-9	28	EtOH	$C_{25}H_{23}Cl_2N_5O_4$
29	2-NH_2	97-100	25	CH_2Cl_2/hexane	$C_{25}H_{27}N_7O_4$
30	4-NH_2	87-89	28	CH_2Cl_2/hexane	$C_{25}H_{27}N_7O_4$
31	2-F	130-2	32	MeOH	$C_{25}H_{23}F_2N_5O_4$
32	4-F	152-3	35	MeOH	$C_{25}H_{23}F_2N_5O_4$
33	2-CH_3	126-8	40	EtOAc	$C_{27}H_{29}N_5O_4$
34	4-CH_3	134-6	36	EtOAc	$C_{27}H_{29}N_5O_4$
35	2-OCH_3	161-2	23	MeOH	$C_{27}H_{29}N_5O_6$
36	4-OCH_3	185-6	25	MeOH	$C_{27}H_{29}N_5O_6$

[a]Analyzed for C, H, N; results are within ± 0.4% of the theoretical values for the formulae given.

Table 4: UV λ_{max} (nm) for the investigated 1,4-dihydropyridines **26-28, 31, 34** and their corresponding quaternary compounds **15–17, 20, 23** in acetonitrile.

Compound	1,4-Dihydropyridines		Compound	Quaternary salts
	Band I	Band II		
26	293.8	359.8	**15**	290.2
27	296.4	361.2	**16**	292.0
28	297.0	363.0	**17**	295.0
31	297.0	364.4	**20**	296.4
34	296.6	359.0	**23**	296.8

Table 5: Rates of oxidative conversion of the 1,4-dihydropyridines **26**–**28**, **31** and **34** into their corresponding quaternary salts **15**–**17**, **20** and **23**.

a. Hydrogen peroxide

Compound	R	Regression data		K_{app} min^{-1} × 10^{-3} ± S.E.	$t_{½}$ (min.) ± S.E.
		n	r		
26	H	9	0.999	33.41	2.09
27	2-Cl	9	0.999	27.27	2.54
28	4-Cl	9	0.999	32.46	2.14
31	2-F	9	0.994	31.74	2.18
34	4-CH_3	9	0.999	30.54	2.27

b. Potassium ferricyanide (5 mM concentration)

Compound	R	Regression data		$^{*}K_{app}$ min^{-1} × 10^{-2} ± S.E.	$t_{½}$ (min.) ± S.E.
		n	r		
26	H	8	0.996	10.57	16.60
27	2-Cl	5	0.993	5.34	32.86
28	4-Cl	5	0.933	6.96	25.80
31	2-F	7	0.998	6.79	24.55
34	4-CH_3	8	0.996	11.65	13.20

c. Diphenyl picrylhydrazyl free radical

Compound	R	Regression data		$^{*}K_{app}$ min^{-1} × 10^{-1} ± S.E.	$t_{½}$ (min.) ± S.E.
		n	r		
26	H	10	0.986	2.81	14.40
27	2-Cl	10	0.986	13.84	6.02
28	4-Cl	10	0.997	23.06	2.16
31	2-F	10	0.984	10.55	5.85
34	4-CH_3	10	0.985	4.45	11.20

n = no. of determinations, r = correlation coefficient, S.E. is ± 10% of the presented values.
$^{*}K_{app}$ calculated from 2nd order plots

UV spectrophotometry and HPLC were used to detect and monitor the oxidation of the dihydropyridines into their corresponding quaternary salts either chemically or in biological fluids. The mobile phase was chosen after several trials using various proportions of acetonitrile, water and acetic acid. The HPLC chromatogram showed that the 1,4-dihydropyridines were separated at retention time 5.1-5.25 min, while the quaternary salts were separated at retention time of 2.6-2.65 min. The used chromatographic system, allowed a complete base line separation with good resolution of the peaks. A calibration curve was plotted, regression equation was derived, and the amount of the quaternary salts was calculated.

Table 6: Bathochromic shifts of the investigated 1,4-dihydropyridines**26**–**28**, **31** and **34** in the used buffered biological fluids (λ_{max}, nm).

Compound	1,4-Dihydropyridines		Biological fluids*
	Acetonitrile	Phosphate buffer pH 7.4	
26	359.8	368.4	368.8
27	361.2	374.8	375.0
28	363.0	375.0	376.6
31	364.4	371.8	373.8
34	359.0	364.6	364.0

*Plasma, blood, brain homogenate, and liver homogenate.

Table 7: Percentage disappearance of the prepared 1,4-dihydropyridines **26**–**28**, **31** and **34** in various biological fluids.

Compound	% Disappearance of 1,4-dihydropyridines			
	Plasma	Blood	Brain homogenate	Liver homogenate
26	67.9	61.86	32.46	93.54
27	42.1	63.54	21.36	81.98
28	65.5	63.64	59.38	92.83
31	69.3	20.94	0.23	40.54
34	30.4	39.66	12.65	57.58

Table 8: Percentage MAO inhibition activity of the 1,4-dihydropyridine **31**.

Concentration (μM)	% MAO Inhibition ± S.E.	
	Compound **31**	Tranylcypromine
0.67	5.8 ± 1.1	40.2 ± 9.3
1.34	24.7 ± 3.1	46.6 ± 8.4
2.01	36.2 ± 1.2	59.0 ± 6.1
2.68	60.0 ± 4.3	82.0 ± 8.7

Table 9: Stability studies of the 1,4-dihydropyridines **26**, **31** and **34** in phosphate buffers (0.05 M) at various pH.

Compound	pH 5.8		pH 7.4		pH 10	
	K_{app} (h^{-1}) $\times 10^{-2}$	$t_{½}$ (h)	K_{app} (h^{-1}) $\times 10^{-2}$	$t_{½}$ (hr)	K_{app} (h^{-1}) $\times 10^{-2}$	$t_{½}$ (h)
26	4.04	17.15	2.72	25.5	0.92	75.5
31	7.43	9.33	3.56	19.5	1.71	40.5
34	7.38	9.32	2.52	27.8	1.69	40.8

S.E. for K_{app} and $t_{½}$ are ± 10% of the presented values.

Table 10: Accelerated stability study for the 1,4-dihydropyridines **31** and **34** at different temperatures (pH 7.4).

Compound	55°C			65°C			75°C		
	r	K_{app} (h^{-1}) $\times 10^{-1}$ ± S.E.	$t_{½}$ (h) ± S.E.	r	K_{app} (h^{-1}) $\times 10^{-1}$ ± S.E.	$t_{½}$ (h) ± S.E.	r	K_{app} (h^{-1}) $\times 10^{-1}$ ± S.E..	$t_{½}$ (h) ± S.E.
31	0.998	1.10	6.29	0.999	3.15	2.20	0.998	8.118	0.85
34	0.999	1.34	5.16	0.999	3.49	1.98	0.999	7.77	0.89

n (no. of determinations) = 8, r = correlation coefficient, S.E. is ± 10% of the presented values.

Table 11: Stability of compounds **26-28**, **31** and **34** upon storage.

Compound	Room Temperature			Refrigerator (4°C)		
	Zero days	60 days	% Decomposed	Zero days	60 days	% Decomposed
26	1.080	0.959	11.2	1.08	1.053	2.5
27	0.648	0.576	11.1	0.648	0.628	3.1
28	1.023	0.770	24.7	1.023	0.943	7.8
31	0.949	0.789	16.9	0.949	0.925	2.5
34	0.922	0.661	28.3	0.922	0.867	6.0

Chemical oxidation of 1, 4-dihydropyridines CDSs:

The studies of the process of oxidation were monitored using UV spectrophotometer based on the difference of the zero absorbance curve of the 1,4-dihydropyridine derivatives versus their quaternary compounds. Table 4 shows λ_{max} for reference samples for each of the 1,4-dihydropyridine derivatives and their corresponding quaternary compounds. All of the studied 1,4-dihydropyridines showed two maxima, one of them (band I) is overlapped with the corresponding quaternary absorption band. Consequently, band II was selected to monitor the disappearance of the 1,4-dihydropyridine compounds.

Silver nitrate oxidation showed that all of the 1,4-dihydropyridines were oxidized with the loss of their characteristic UV peak and the promotion of a new UV peak belongs to their corresponding quaternary salts. Hydrogen peroxide oxidation of the 1,4-dihydropyridines could give an idea about the different behavior of various derivatives toward *in vivo* oxidation which facilitate the release of the carried drug (7,8). A freshly prepared solution of the tested 1, 4-dihydropyridine derivatives **26-28**, **31** and **34** with specific concentrations, were mixed with 30% hydrogen peroxide solution. The decrease in the concentration of the 1, 4-dihydropyridines was monitored (Table 5a). Repeating the oxidation process using potassium ferricyanide at 5 mM concentration (Table 5b) and diphenyl picryl-hydrazyl free radical (Table 5c) showed a slight variation in the oxidation rates. The obtained data indicated the facile oxidative conversion of all the synthesized 1,4-dihydropyridine analogs.

Kinetics of oxidation of the 1,4-dihydropyridines chemical delivery systems (CDSs) in biological fluids:

The *in vitro* experiments were conducted to evaluate the extent of stability of the synthesized 1,4-dihydropyridine compounds towards oxidation at the selected wavelengths after incubation with different biological fluids and tissue homogenates (plasma, whole blood, liver homogenate and brain homogenate) under controlled buffer and temperature conditions.

The 1,4-dihydropyridine compounds showed a slight bathochromic shift when incubated with

different biological fluids and in buffer solution used (phosphate buffer pH 7.4) compared to the zero-time incubated sample. The UV scanning was done using a background subtraction under the same conditions (Table 6). This bathochromic shift may be related to enolization of the diamide moiety (Chart 2).

Each of the 1, 4-dihydropyridines **26-28, 31** and **34** were spiked in biological fluids and certain aliquots were monitored at appropriate time intervals by the aid of UV spectrophotometer to follow the rate of disappearance of each compound, i.e. the rate of its oxidation (Table 7). All compounds were completely disappeared after 120 min in liver homogenate; this may be attributed to the high liver enzyme activity. The lowest rate of oxidation of the 1,4-dihydropyridines was observed in brain homogenate. In overall, compounds **31, 34** showed moderate stability toward oxidation followed by **27**.

*In vivo brain-delivery studies of the 1,4-dihydropyridine analog **31:***

Compound **31**, at a dose of 200 mg/kg, was injected (i.v.) to rats. At selected time intervals, blood samples and the brains were collected. The concentrations of compound **31** and its corresponding quaternary salt **(20)** which produced in blood after administration, were measured in both blood and brain homogenate using HPLC assay. Figure 1a,b shows the mean concentration-time profiles of compounds **31** and **20** in blood and brain homogenate samples.

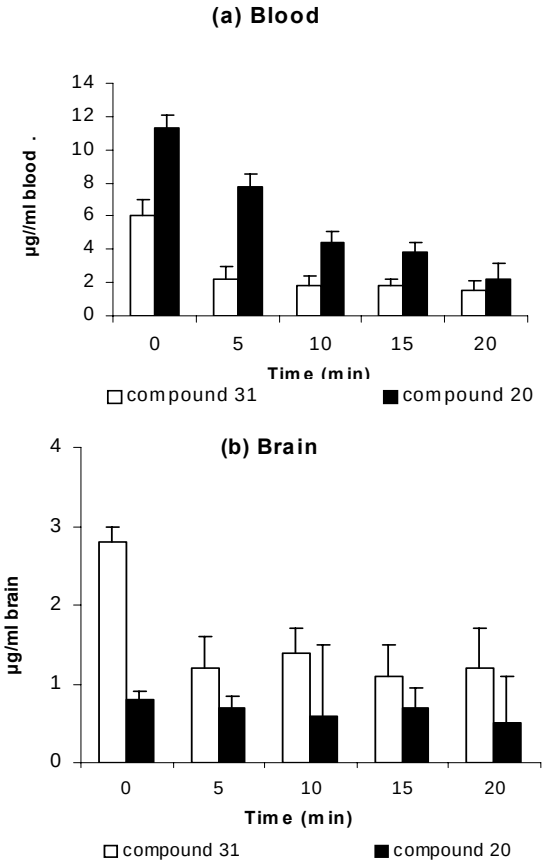


Figure 1: The concentration of the quaternary compound **20** in (a) blood and (b) brain of rats after administration of the 1,4-dihydropyridine compound **31**.

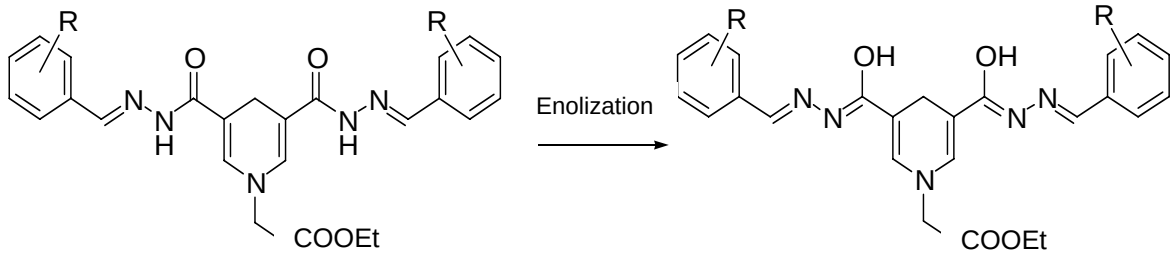


Chart 2.

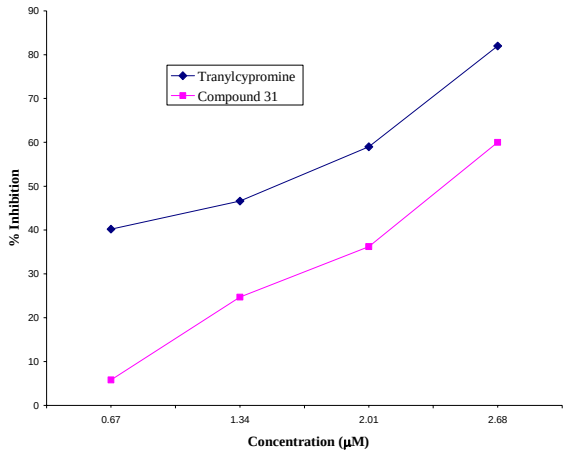


Figure 2: Inhibitory effect of compound **31** and tranylcypromine on hepatic MAO activity of male rates.

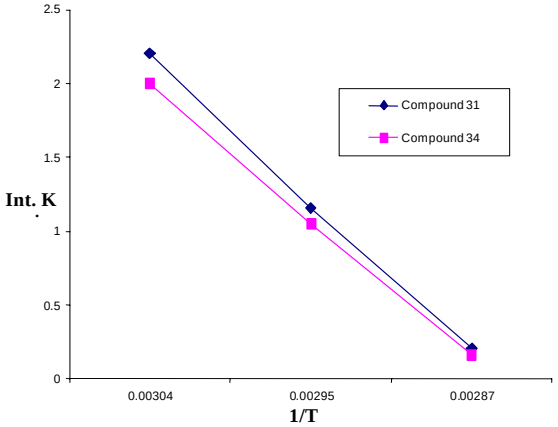


Figure 3: Stability study Arrhenius plot for compounds **31** and **34**. (K = rate of reaction, and T = absolute temperature).

Compound **31** was oxidized into the quaternary salt **20**, immediately after administration, and then both concentrations were declined steadily in blood and not detected after 20 min. This rapid disappearance may be attributed to fast metabolic reaction with the production of polar metabolites and consequently rapid elimination. Meanwhile, both compounds were also detected in brain immediately after injection, which suggest the rapid crossing of compound **31** to the BBB, followed by rapid decline of its concentration. Both compounds were not detected in brain after 20 min which suggest the rapid conversion of the 1,4-dihydropyridine **31** into its quaternary salt **20** (Figure 1). The latter compound was presumably hydrolyzed releasing the corresponding benzylidine MAO inhibitor.

*In vitro determination of MAO inhibitory activity of the 1,4-dihydropyridine analog **31**:*

The MAO inhibitory activity was determined according to the method described by Weissbach *et al* [17]. The method based on the measurement of the rate of disappearance of the MAO substrate "kynuramine" by the action of the enzyme. Percentage reduction of MAO activity by the aid of the test compound **31** was determined using tranylcypromine as positive control. The method is simple, reliable, accurate and reproducible. Table 8 and Figure 2 show the percentage inhibition of MAO activity caused by four different molar concentrations ranged from 0.67-2.68 µM, calculation based on the total volume of the experiment using a stock solution of 20 µM. The *in vitro* determination of MAOI activity revealed that compound **31** possess a MAOI potency with lower magnitude than the positive control tranylcypromine with LD_{50} (95% C.L.) value of 700.34 mg/kg.

Stability studies of the investigated CDSs:

Compounds **26**, **31** and **34** were subjected to stability studies in phosphate buffer at various pH values (5.8, 7.4 and 10). Table 9 shows that compound **31** is relatively stable at pH 7.4 ($t_{½}$ = 19.5 h). Compounds **31** and **34** were further subjected to accelerated stability studies (Table 10), at pH 7.4 and various temperatures (55°C, 65°C and 75°C). Arrhenius plot was made (Figure 3), from which it could be concluded that the shelf-life time for compound **31** is 20.53 days at 5°C and 1.3 days at 25°C, while compound **34** showed 8 days at 5°C and 0.7 day at 25°C.

A parallel study of the storage stability of compounds **26**–**28**, **31** and **34** at room temperature and refrigerator (4°C) over a period of 60 days was conducted. The acetonitrile solutions of these compounds were UV checked over the specified periods of time. All compounds showed good stability at both room temperature and refrigerator storage except compound **28** and **34** which 25% and 28% of their contents were decomposed at room temperature, respectively (Table 11).

Structure activity correlation of the investigated compounds revealed that the stability of these compounds could be manipulated by the type of substituents on the benzylidine moiety. Compounds **31** and **34** proved to be more stable than the unsubstituted **26**, also compound **31** with the electron withdrawing fluorine atom is more stable than **34** with the electron donating methyl group. On the other hand, storage stability studies emphasized the idea that electron withdrawing substituent (2-Cl of **27** and 2-F of **31**) favour the stability of the compounds rather than electron donating group (4-Me of **34**), also the position of the electron withdrawing group could control the stability of the compound as shown in the 2-Cl compound **27** (11.1% decomposition) if compared with the 4-Cl compound **28** (24.7% decomposition). Electron withdrawing group at position 2- favor the stability of such system.

The obtained results (Figure 3, Table 11) implies the relative stability of the prepared 1,4-dihydro-pyridine CDSs against hydration and air oxidation. The stability of the prepared compounds is attributed to the conjugation of the two carboxylic functions at C3 and C5 with their adjacent double bonds.

In conclusion, and based on the obtained results, the designed compounds proved to be stable CDSs against hydration and air oxidation, fairly stable for powder form storage, and are able to carry MAOIs across BBB into the brain. Accordingly, future study on this CDSs should be focused on more derivatizations at positions 3- and 5- of the 1,4-dihydropyridine system with various electronically different aryl groups aiming to produce more stable compounds, with optimized oxidation rates, to deliver other important drugs across BBB into the brain.

Acknowledgements

The authors would like to express their gratitude and thanks to King Abdulaziz City for Science and Technology, Riyadh, KSA, for their generous Grant No. AR–19–044.

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