Synthesis and Binding Properties of 2*H*-1-Benzopyran-2 one Fluorophore-Linked Calcium Channel Antagonist Nifedipine (1,4-Dihydropyridine) Analogs

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Dedicated to Professor S. Seshadri, on the occasion of his 60th birthday.

Nifedipine analogs **I**, **II** and **III** with the 2*H*-1-benzopyran-2-one (coumarin) fluorophore linked at the 2, 2 and 6 and 4 positions of the dihydropyridine ring were synthesized by the modified Hantzch condensation procedures. Attempts to synthesize dihydropyridine with the fluorophore at position 3 (**IV**) were unsuccessful.

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Introduction.

The 1,4-dihydropyridine structure represents both antagonists and activators potently active at the L-class of voltage dependent Ca^{2+} channel [1-3]. Structure-activity relationship studies established that the NH group in the dihydropyridine unit is necessary for activity and that substituents on the ring nitrogen decrease activity [4]. To investigate further the role of the NH group of 1,4-dihydropyridines in their action on calcium channels an attempt has been made to freeze the hydrogen of NH function through hydrogen bonding rather than by alkylation.

2*H*-1-Benzopyran-2-one (coumarin) derivatives are well known for their intense fluorescence [5,6] and, furthermore, the lactone carbonyl can be involved in hydrogen bond formation with the NH of the 1,4-dihydropyridine unit. Coumarin fluorophore substitutions are made at positions 2 and 6 of the 1,4-dihydropyridine could result in intramolecular hydrogen bonding. Design and synthesis of coumarin fluorophore-conjugated nifedipine analogs could give further insight to 1,4-dihydropyridine structure-activity relationships and the derived coumarinyl 1,4-dihydropyridines may also be useful as tools to assay the Ca^{2+} channels by fluorescence spectroscopy [7].

We report our results on the synthesis of 2, 3, 4 and 2,6 coumarin fluorophore-linked 1,4-dihydropyridines and radioligand binding studies carried out using these compounds to verify our hypothesis.

Results and Discussion.

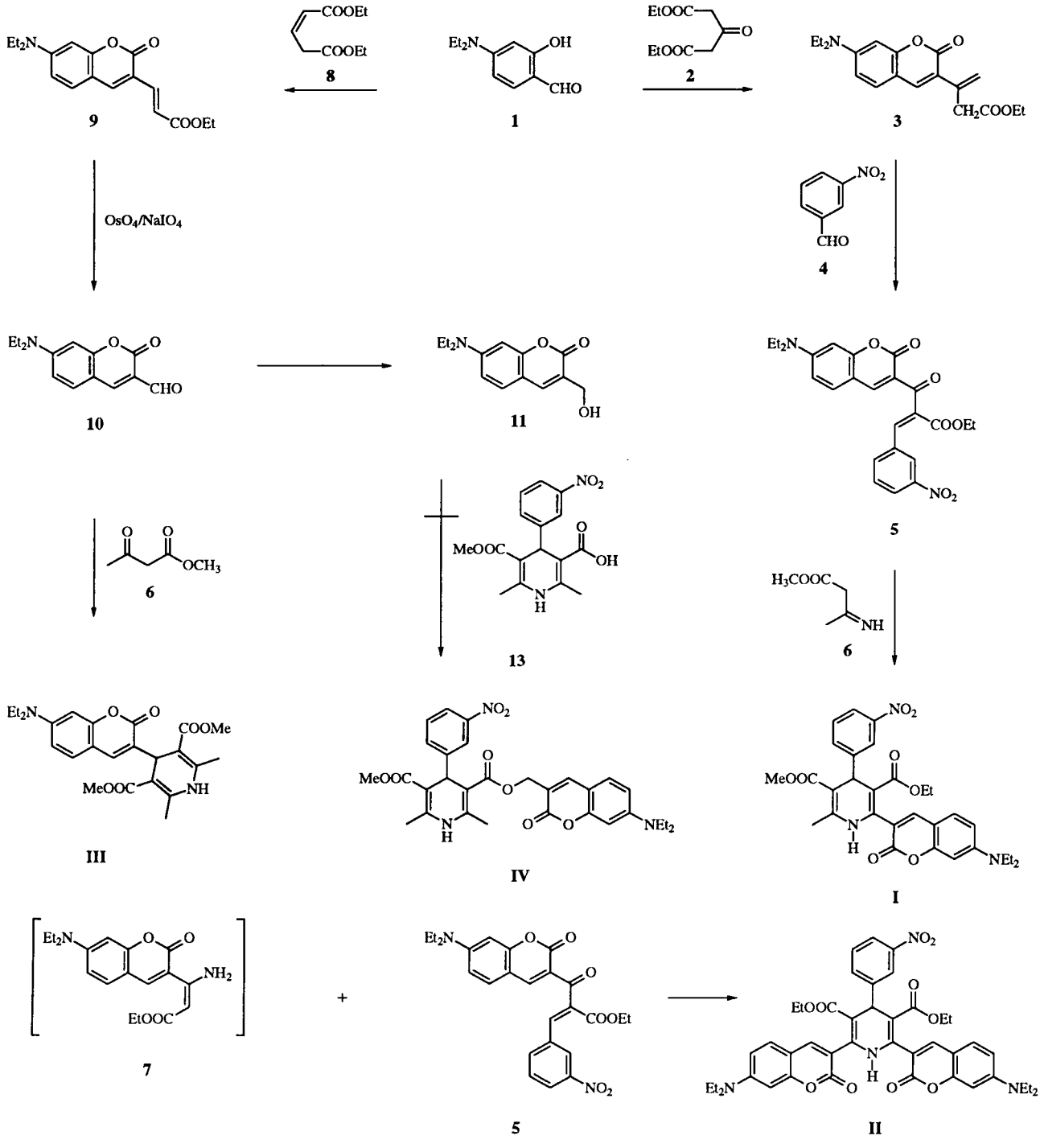
Condensation of 4-diethylamino-2-hydroxybenzaldehyde (**1**) with diethyl 3-oxopentanedicarboxylate (**2**) in refluxing ethanol in the presence of a catalytic amount of piperidine gave the corresponding β-ketoester **3** [8,9]. Condensation of the ketoester **3** with 3-nitrobenzaldehyde (**4**) in the presence of piperidine and acetic acid in 2-propanol gave the condensation product, **5** which was characterized by ^1H nmr and taken directly for the next condensation step. Reaction of **5** with methyl 3-amino-2-butenoate (**6**) in 2-propanol under refluxing conditions resulted in the isolation of the dihydropyridine derivative **I**. Synthesis of **I** was also achieved through a one pot reaction of 3-nitrobenzaldehyde, β-ketoester **3** and aminobutenoate **6** (Scheme).

Coumarinyldihydropyridine **II** was prepared by the condensation of the enamine derivative **7** (obtained from the reaction of β-ketoester, **3** and ammonium acetate in ethanol) with **5** in a "one-pot" reaction (Scheme).

Synthesis of nifedipine analogs **III** and **IV** require the key intermediate, 7-diethylamino-3-formylcoumarin (**10**). Knoevanegal condensation of 2-hydroxybenzaldehydes with diethyl 2-pentenedicarboxylate leading to the corresponding 2*H*-1-benzopyran-2-one (coumarin) and 2*H*-1-benzopyran (chromene) derivatives have been investigated [10,11]. Based on that recently we reported [10] a facile route to 3-formylcoumarin **10** through the osmium tetroxide catalyzed periodate cleavage of the unsaturated ester **9** in aqueous tetrahydrofuran at room temperature. Condensation of **10** with methyl 3-oxobutanoate (**12**) and methyl 3-amino-2-butenoate (**6**) in refluxing 2-propanol gave the coumarin analog **III** (Scheme).

Preparation of the 1,4-dihydropyridine (**IV**) with the coumarin fluorophore linked at the 3 position posed several problems. Sodium borohydride reduction of the aldehyde **10** in ethanol tetrahydrofuran gave the expected alcohol **11**. It was anticipated that esterification of the 1,4-dihydropyridine carboxylic acid **13** [12] with the alcohol **11** should lead to the 1,4-dihydropyridine **IV**. Attempted esterification of the 1,4-dihydropyridine acid **13** to yield **IV**, through the acid chloride (under different conditions), dicyclohexylcarbodiimide derivative, 1,1-carbonyldiimidazole and in the presence of *p*-toluenesulfonic acid in toluene under refluxing conditions were unsuccessful. An alternate route using diketene was therefore explored. Reaction of the alcohol **11** with diketene in *N,N*-dimethylformamide

Scheme



(DMF) in the presence of triethylamine gave the desired β-ketoester **14** in 45% yield, identified by the ^{1}H nmr and taken for the next step without further characterization. Attempted one-pot synthesis of the 1,4-dihydropyridine **IV** through the Hantzsch condensation of **14** with 3-nitrobenzaldehyde and 3-amino-2-butenoate in 2-propanol was unsuccessful. This is in contrast to the usual facile esterification of the 1,4-dihydropyridine acid **13** through the acid chloride route with many other alcohols [12,13] (Scheme).

Molecular modeling reveals one dynamic intramolecular hydrogen bond between the lactone carbonyl and the NH of the 1,4-dihydropyridine in the case of **I** and two dynamic intramolecular hydrogen bonds with the coumarinyl 1,4-dihydropyridine **11**. If the hydrogen on the nitrogen of

1,4-dihydropyridine is absolutely essential for activity, one should not observe any activity when the coumarin is substituted at either position 2 or disubstituted at positions 2 and 6 of 1,4-dihydropyridine. However, having a coumarin substitution at position 3 of 1,4-dihydropyridine does not result in any hydrogen bonding and hence no effect on the activity. Thus we can sequentially eliminate the activity by using the same substituent at different positions of 1,4-dihydropyridine. This would further suggest that free NH is essential for activity and suggest that free NH is involved in hydrogen bond formation with the channel protein in 1,4-dihydropyridine calcium antagonists.

Coumarinyl 1,4-dihydropyridines **I**, **II** and **III** were used in competition binding assays against ^{3}[H] PN200-110 in rat cerebral cortex membranes. Compounds **II** and **III** did not inhibit (+)-[5-Methyl-^{3}H] 2,6-dimethyl-3-isopropoxycarbonyl-1,4-dihydropyridinecarboxylate (PN 200-110) binding to the membranes even at concentrations up to 1μ*M*. Compound **I**, however did inhibit PN binding with Ki = 1.44 ± 0.34 x 10^{-7} *M* and a Hill slope of 0.42 ± 0.03. This accords with our hypothesis. These compounds did not exhibit any fluorescence in day light.

EXPERIMENTAL

Melting points are all uncorrected and were determined in capillary tubes using a MelTemp apparatus. The ^{1}H nmr spectra were recorded on a Varian Gemini 300 spectrometer in deuteriochloroform containing tetramethylsilane as an internal standard. Elemental analyses were carried out at Atlantic Microlabs. Inc., Norcross, GA and Quanatitiative Technologies Inc., Whitehouse, NJ. Yields are of pure products and are not optimized.

4-Diethylamino-2-hydroxybenzaldehyde (**1**), diethyl 3-oxopentanedicarboxylate (**2**), diethyl 2-pentenedicarboxylate (**8**) were obtained from Aldrich Chemical Company. The β-ketoester **3** [8,9] and the formylcoumarin **10** [10] were prepared following reported procedures. The 1,4-dihydropyridine acid **13** was synthesized in two steps from 3-nitrobenzaldehyde, diketene, 3-hydroxypropionitrile and methyl 3-amino-2-butenoate following the procedure reported in the literature [12].

Condensation of 3-nitrobenzaldehyde with the β-Ketoester **3**. Preparation of **5**.

A solution of the ketoester **3** (1 g, 3.02 mmoles) and 3-nitrobenzaldehyde (456 mg, 3.02 mmoles) in the presence of piperidine (0.2 ml) and acetic acid (0.2 ml) in 2-propanol (8 ml) was refluxed overnight under nitrogen. The reaction mixture was concentrated and purified by column chromatography using hexanes:ethyl acetate as eluent to give the condensed product **5** 550 mg (39%), mp 65-68°; ^{1}H nmr: δ 1.35 (m, 9H), 3.48 (m, 4H), 4.35 (q, 2H), 6.47 (s, 1H), 6.65 (dd, 1H), 7.45 (m, 2H), 7.65 (dd, 1H), 7.72 (s, 1H), 8.1 (d, 1H), 8.25 (s, 1H), 8.62 (s, 1H).

Preparation of Ethyl 2-(7-Diethylamino-2*H*-1-benzopyran-2-one-3-yl)-6-methyl-3-carbomethoxy-4-(3-nitrophenyl)-1,4-dihydropyridine-3-carboxylate (**I**).

A mixture of the benzylidene derivative **5** (100 mg, 0.215 mmole) and methyl 3-aminocrotonate (25 mg, 0.217 mmole) in 2-propanol (3 ml) was refluxed under nitrogen overnight and cooled overnight in refrigerator. Product **I** separated was filtered, washed with cold ethanol and was practically pure by nmr, yield 80 mg (66%), mp 236-240° dec; ^{1}H nmr: δ 1.03 (t, 3H), 1.17 (t, 6H), 2.42 (s, 3H), 3.43 (q, 4H), 3.69 (s, 3H), 3.98 (q, 2H), 5.25 (s, 1H), 6.5 (s, 1H), 6.60 (d, 1H), 7.27 (s, 1H), 7.44 (d, 1H), 7.57 (d, 1H), 7.81 (d, 1H), 8.04 (dd, 1H), 8.27 (s, 1H).

Anal. Calcd. for $C_{30}H_{31}N_3O_8$: C, 64.14; H, 5.52; N, 7.48. Found C, 64.16; H, 5.56; N, 7.51.

One-Pot Synthesis of the Coumarinyl 1,4-Dihydropyridine (**I**).

A mixture of the, β-ketoester **3** (331 mg, 1 mmole) and 3-nitrobenzaldehyde (151 mg, 1 mmole) in 2-propanol (5 ml) was refluxed in the presence of 2 drops of piperidine overnight followed by the addition of methyl 3-amino-2-butenoate (115 mg, 1 mmole). Refluxing was continued for 6 hours and the cooled reaction mixture was concentrated to give the crude product. Purification by column chromatography using hexanes initially, gradually shifting to hexanes:ethyl acetate (1:1) gave the product after concentration. The oily product was crystallized by dissolution in ethyl acetate followed by the addition of anhydrous ether with subsequent cooling to yield **I** as a yellow powder 116 mg (21%). This product was found to be identical by tlc, mp and ^{1}H nmr with the sample, obtained through the condensation of the benzylidene derivative **5** with 3-amino-2-butenoate.

Preparation of Diethyl 2,6-Bis-(7-diethylamino-2*H*-1-benzopyran-2-one-3-yl)-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**II**).

A solution of the β-ketoester **3** (331 mg, 1 mmole) and 3-nitrobenzaldehyde (151 mg, 1 mmole) in 2-propanol (5 ml) was refluxed overnight in the presence of 2 drops of piperidine The enamine, generated by refluxing a mixture of the ketoester **3** (331 mg, 1 mmole) and ammonium acetate (154 mg, 2 mmoles) for 3 hours, was added and refluxing of the reaction mixture was continued for 6 hours. The cooled reaction mixture was concentrated and purification of the crude product by column chromatography using hexanes initially and shifting to hexanes:ethyl acetate (3:7) gave the product after concentration. The product was crystallized from aqueous methanol as reddish yellow powder 100 mg (13%), mp 117-122°; A satisfactory elemental analysis for N could not be obtained even after repeated attempts. ^{1}H nmr: δ 1.20 (t, 18H), 3.4 (q, 8H), 4.03 (m, 4H), 5.38 (s, 1H), 6.41 (s, 2H), 6.56 (dd, 2H), 7.32 (m, 2H), 7.90 (m, 2H), 8.19 (s, 1H), 8.34 (s, 1H), 8.45 (dd, 2H).

Anal. Calcd. for $C_{43}H_{44}N_4O_9 \cdot H_2O$: C, 64.98: H, 5.83; N, 7.05. Found C, 64.64; H, 5.89; N, 4.95.

Preparation of 3,5-dicarbomethoxy-2,6-dimethyl-4-(7-diethylamino-2*H*-1-benzopyran-2-on-3-yl)-1,4-dihydropyridine (**III**).

A solution of the coumarincarboxaldehyde **10** (100 mg, 0.41 mmole), methyl acetoacetate (47.4 mg, 0.41 mmole) in 2-propanol (3 ml) was refluxed for 6 hours in presence of piperidine (4 drops) and acetic acid (4 drops). To this reaction mixture methyl 3-amino-2-butenoate (47 mg, 0.41 mmole) was added and refluxed overnight under nitrogen. On cooling the product separated was filtered, washed with cold ethanol. The product **III**, obtained as reddish yellow powder, 60 mg (33%) was pure

by nmr, mp 257-260°; ^{1}H nmr: δ 1.23 (t, 6H), 2.26 (s, 6H), 3.38 (q, 4H), 3.63 (s, 6H), 5.03 (s, 1H), 5.92 (s, 1H), 6.40 (s, 1H), 6.55 (dd, 1H), 7.2 (dd, 1H), 7.4 (s, 1H).

Anal. Calcd. for $C_{24}H_{28}N_2O_6$: C, 65.42; H, 6.36; N, 6.36. Found: C, 65.43; H, 6.46; N, 6.27.

Preparation of 7-Diethylamino-2*H*-1-benzopyran-2-one-3-methanol (**11**).

To a solution of the formyl derivative **10** (300 mg, 1.22 mmoles) in ethanol (30 ml) and tetrahydrofuran (25 ml) with stirring at room temperature, sodium borohydride (35 mg, 0.93 mmole) was added in one lot and after the effervescence had ceased, the colorless reaction mixture was stirred an additional 3 hours and concentrated. The crude reaction mixture was partitioned between water (10 ml) and ethyl acetate (25 ml) and the aqueous layer was repeatedly extracted with ethyl acetate (3 x 10 ml) and organic layer was washed with brine, dried and concentrated. The crude product was purified by column chromatography using dichloromethane containing ethyl acetate (10%) to give the alcohol **11** as a dull yellow solid 220 mg (73%), mp 65-67°; ^{1}H nmr: δ 1.16 (t, 6H), 3.19 (br t, 1H), 3.36 (q, 4H), 4.52 (s, 2H), 6.44 (s, 1H) 6.52 (d, 1H), 7.21 (d, 1H), 7.55 (s, 1H).

Preparation of [7-Diethylamino-2*H*-1-benzopyran-2-one-3-methyl] 3-Oxobutanoate (**14**).

A solution of the alcohol **11** (200 mg, 0.81 mmole) in anhydrous DMF (1 ml) containing anhydrous triethyl amine (0.05 ml) was heated in an oil-bath to 85-90° followed by the addition of diketene (0.08 ml, 0.97 mmole) slowly and maintained overnight at this temperature under nitrogen. The reaction mixture was cooled, worked up by adding water (10 ml) and repeated extraction with dichloromethane (2 x 10 ml) and later with ethyl acetate (10 ml). The combined organic extracts were washed with brine (10 ml), dried and purified by column chromatography using a mixture of hexanes and ethyl acetate (3:2) to elute the product, **14**, yield 45%; ^{1}H nmr: δ 1.15 (m, 6H), 2.22 (s, 3H), 3.36 (q, 4H), 3.45 (s, 2H), 5.02 (s, 2H), 6.43 (s, 1H), 6.53 (d, 1H), 7.22 (d, 1H), 7.62 (s, 1H).

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