Med Chem Res (2012) 21:908–921
DOI 10.1007/s00044-011-9600-x

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of a New Series of 4-Aryl-1,4-Dihydropyridines with Calcium Channel Blocking and Vasodilatory Activity

Ranju Bansal · Priyanka Jain · Carmen Calle · Rosalia Carron · Karen Pemberton · Alan L. Harvey

Received: 2 September 2010 / Accepted: 7 February 2011 / Published online: 20 February 2011

Abstract Several new amide derivatives of 4-aryl-1, 4-dihydropyridine carboxylic congeners have been synthesized in this study to obtain therapeutically useful compounds. The changes in pharmacological properties of dihydropyridines by the presence of polar groups at different positions of 4-phenyl substituent and also by introduction of unsymmetrical ester groups in the synthesized symmetrical analogs have been studied. In vitro calcium channel blocking activity has been evaluated in cultures of neonatal rat cortical neurons by measuring the inhibitory response at L-type calcium channels activated by veratridine. The newly synthesized dihydropyridines displayed moderate calcium channel blockade with IC_{50} values ranging from 2 to 10 μM in comparison to nifedipine (IC_{50} = 57.7 nM). The vasodilatory activity was evaluated on isolated rat thoracic aortic rings precontracted by phenylephrine/KCl (30 mM). The symmetrically substituted dihydropyridine **8a** exhibited maximum activity with IC_{50} = 0.64 μM but was found to be approximately 24 times less active in comparison to standard drug nifedipine with IC_{50} = 27.5 nM.

R. Bansal (✉) · P. Jain
University Institute of Pharmaceutical Sciences,
Panjab University, Chandigarh 160014, India
e-mail: ranju29in@yahoo.co.in

C. Calle · R. Carron
Departamento de Fisiología y Farmacología,
Facultad de Farmacia, Campus "Miguel de Unamuno",
37007 Salamanca, Spain

K. Pemberton
Thistle Technologies, 11 Sycamore Way, Branford 06405, USA

A. L. Harvey
Strathclyde Institute for Drug Research and Department
of Physiology and Pharmacology, University of Strathclyde,
27 Taylor Street, Glasgow G4 0NR, UK

Keywords Dihydropyridines · Calcium channel blockers · Vasodilators · Unsymmetrical esters

Introduction

4-Aryl-1,4-dihydropyridines attract the interest of medicinal chemists due to their high potency and selectivity of action as calcium channel blockers (Mannhold, 1994; Triggle, 2003). Many modifications of the prototypical structure of nifedipine (Fig. 1) have been carried out leading to the formation of several novel dihydropyridines (Steffen, 1999; Meredith, 1999). Structural variations of this lead molecule have been focused mainly on ester functions and variations in phenyl substitutions. The envisaged goal of these new developments was an optimization of vascular selectivity. A new series of 4-aryl-1,4-dihydropyridines possessing vanilloid-based moieties has attracted much attention lately (Yeh *et al.*, 2000; Liang *et al.*, 2000; Liang *et al.*, 2002). These vanilloid-based hybrid molecules, e.g., vanidipinedilol and labedipinedilol (Fig. 1) have produced potent vasodilatory effects with reduced sympathetic activation-associated reflex tachycardia in the heart.

Aryl moieties bearing a basic side chain have been considered as an essential active structural component in a number of known calcium channel blockers such as verapamil, diltiazem, prenylamine etc. (Janis and Triggle, 1983). Even in dihydropyridines, the presence of basic moieties has led to the development of long acting, relatively side effect free and potent calcium channel blockers (Klotz, 2002). A series of 4-aryl-1,4-dihydropyridines substituted with basic moieties possessing potent calcium channel blocking and vasodilatory activity have also been reported by us (Jain *et al.*, 2006). This is attributed to improve bioavailability of the molecules. Considering the

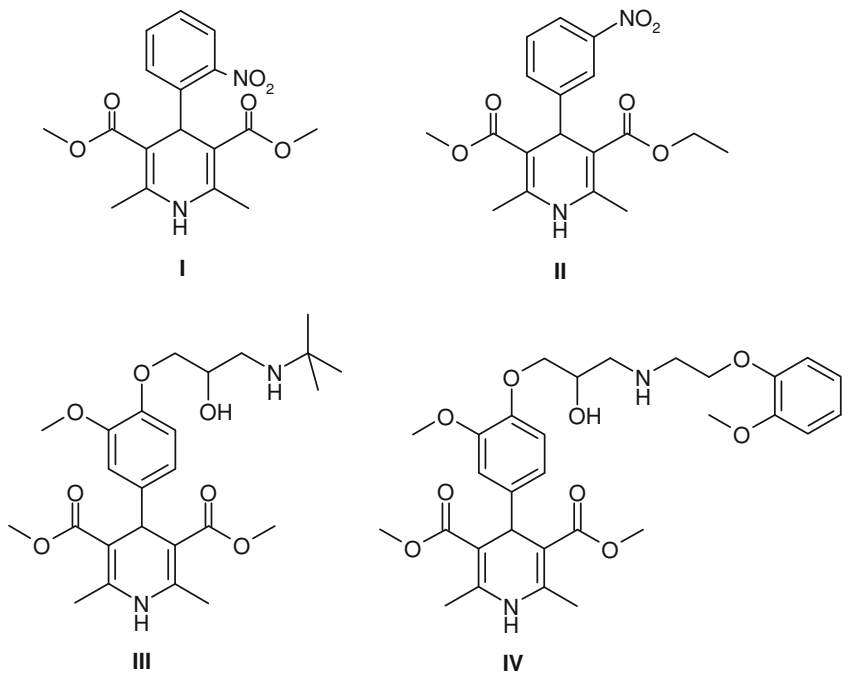


Fig. 1 Structures of Nifedipine (**I**), Nitrendipine (**II**), Vanidipinedilol (**III**), and Labedipinedilol (**IV**)

substitution of hydroxy group present at different positions of the 4-phenyl ring an interesting route to incorporate such moieties, several new amide derivatives of 4-aryl-1,4-dihydropyridine carboxylic congeners have been synthesized in this study to obtain calcium channel blockers of better therapeutic profile. The main objective of this study is to study the effect of these weakly basic substituents on pharmacological activity of dihydropyridines.

Further, the outstanding importance of the ester functions with respect to the pharmacological properties of dihydropyridines initiated the synthesis of innumerable compounds with varied ester substitutions in 3- and 5-position of the dihydropyridine skeleton (Ramesh *et al.*, 1998). Dihydropyridines such as nitrendipine (Fig. 1) with an unsymmetrical ester substitution are, in general, more potent than symmetrically substituted derivatives due to a gain in bulk at the port side ester, which presumably interacts with a hydrophobic receptor domain (Mannhold, 1994). Therefore, it was also endeavored to introduce asymmetrical ester groups in the synthesized symmetrical analogs.

Materials and Methods

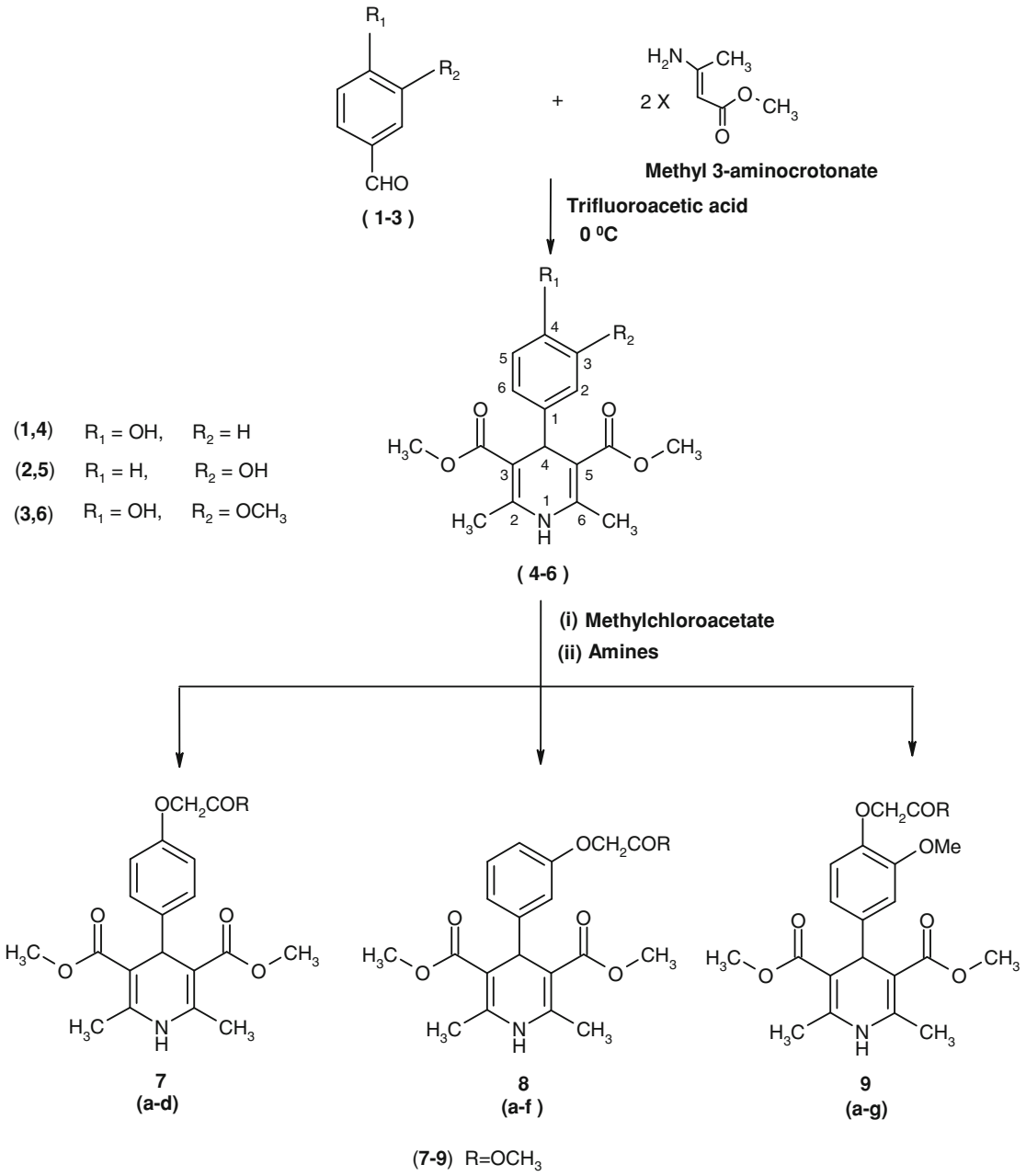
Chemistry

Melting points were determined on a Veego melting point apparatus (MP I, Veego, Mumbai, India) and are uncorrected. IR spectra were recorded on Perkin-Elmer spectrum RX 1, FT-IR spectrophotometer model (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, UK) using potassium bromide pellets. Proton-NMR spectra were recorded on Bruker AC-300F, 300 MHz (Bruker AG, Fallanden, Switzerland) using deuterated-chloroform or deuterated dimethylsulfoxide-containing (Sigma-Aldrich, St. Louis, USA) tetramethylsilane (Me_4Si) as internal standard (chemical shifts in δ, ppm). The purity of compounds was established by thin layer chromatography (TLC) (E. Merck, India) and elemental analyses. Elemental analyses were carried out on a Perkin-Elmer-2400 model CHN analyzer (Perkin-Elmer, Norwalk, CT, USA). Heterocyclic amines morpholine from E. Merck, India; pyrrolidine, piperidine, and 1-methylpiperazine from Himedia, India, and 3-pipecoline from Sigma-Aldrich, Chemical Co. inc., Milwankee, wis. were purchased. *Tert*-butylamine, methylchloroacetate, isopropanol, aldehydes, and methyl 3-aminocrotonate were from E. Merck, India.

All solvents were distilled before use according to standard procedures. Symmetric dihydropyridines **4–6** were prepared according to the reported procedure (Jain *et al.*, 2006).

Amide derivatives **7a–d**, **8a–d** and **9a–d** and **9g** were synthesized by fusing the respective dihydropyridine carboxylate **7–9** with various heterocyclic amines like morpholine, pyrrolidine, piperidine, 1-methylpiperazine, and 3-pipecoline at 80–90°C for 4 h. The 4-[(carbmethoxymethyl)oxy]phenyl-dihydropyridines **7–9** in turn were prepared by refluxing symmetrical dihydropyridines **4–6** (Jain *et al.*, 2006) with methylchloroacetate in ethyl methyl

ketone using anhydrous potassium carbonate as shown in Scheme 1. In case of amide derivatives, the prominent carbonyl absorption band of tertiary amides appeared at a lower wavenumber $\sim 1640\ cm^{-1}$ than "normal" carbonyl absorption due to the resonance effect. Methylenes of the heterocyclic ring attached to nitrogen appeared downfield



Compd No.	a	b	c	d	e	f	g
R	-N O	-N	-N	-N N—	-N N	-NHC(CH3)3	-N

Scheme 1 Synthetic route to symmetric dihydropyridines **7–9**

at $\sim\delta$ 3.50 ppm for these derivatives due to the presence of adjacent carbonyl group.

It was further endeavored to introduce a heteroaromatic ring, e.g., Imidazole and also a primary amine such as *tert*-butylamine, to observe the effect of such functionalities on the pharmacological profile since such type of moieties form an integral part of many of the known cardiovascular agents (Huber *et al.*, 1991). The synthesis of imidazolyl substituted dihydropyridines (**8e**, **9e**) required a longer time of 75 h and higher reaction temperature of 90–110°C. *tert*-Butylamine substituted dihydopyridines **8f**, **9f** were prepared by thermal fusion of **8** and **9** with excess of *tert*-butylamine in dimethylformamide (DMF) for 70 h.

The synthesis of asymmetric dihydropyridines **10–12** was carried out by modified hantzsch condensation (Iwanami *et al.*, 1979) by continuously refluxing the equimolar quantities of appropriate aldehyde, methyl 3-aminocrotonate and ethylacetoacetate in 2-propanol for 8–9 h to generate the parent dihydropyridines (Scheme 2). Target amide derivatives **13a–e**, **14a–d,** and **15a–e** of asymmetric 4-phenyldihydropyridines were prepared by treating compounds **10–12** with methyl chloroacetate to afford carboxylic congeners **13–15** and then further fusion with various cyclic amines in a similar way as is followed for symmetrical analogs.

General Procedure for the Synthesis of Parent Asymmetric Dihydropyridines **10–12**

A solution of appropriate aldehydes **1–3** (8.19 mmol), methyl 3-aminocrotonate (8.19 mmol) and ethyl acetoacetate (8.19 mmol) in isopropanol (10 mL) were refluxed for 9 h with continuous stirring. The completion of the reaction was monitored with TLC. The solvent was removed under vacuum and the resultant solid was repeatedly washed with water, filtered, dried, and crystallized from ethyl acetate to obtain **10–12**.

Ethyl methyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**10**). Yield: 81.54%; m.p. 205–206°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3347, 3101, 2987, 2946, 1699, 1664, 1592, 1510, 1489, 1438, 1371, 1335, 1227, 1128, 1020, 843, 788, 760; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.21 (t, 3H, –COOCH$_2$CH_3, J = 6.36 Hz), 2.29 (s, 6H, 2 × CH_3), 3.61 (s, 3H, –COOCH_3), 4.04 (m, 2H, –COOCH_2CH$_3$), 4.84 (s, 1H, 4-CH, dihydropyridine), 6.63 (d, 2H, J_o = 8.00 Hz, 3-CH and 5-CH, aromatic), 7.04 (m, 2H, 2-CH and 6-CH, aromatic), 7.82 (t, 1H, –NH, J = 17.62 Hz); Anal. calcd. for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23; found: C, 65.12; H, 6.11; N, 3.89.

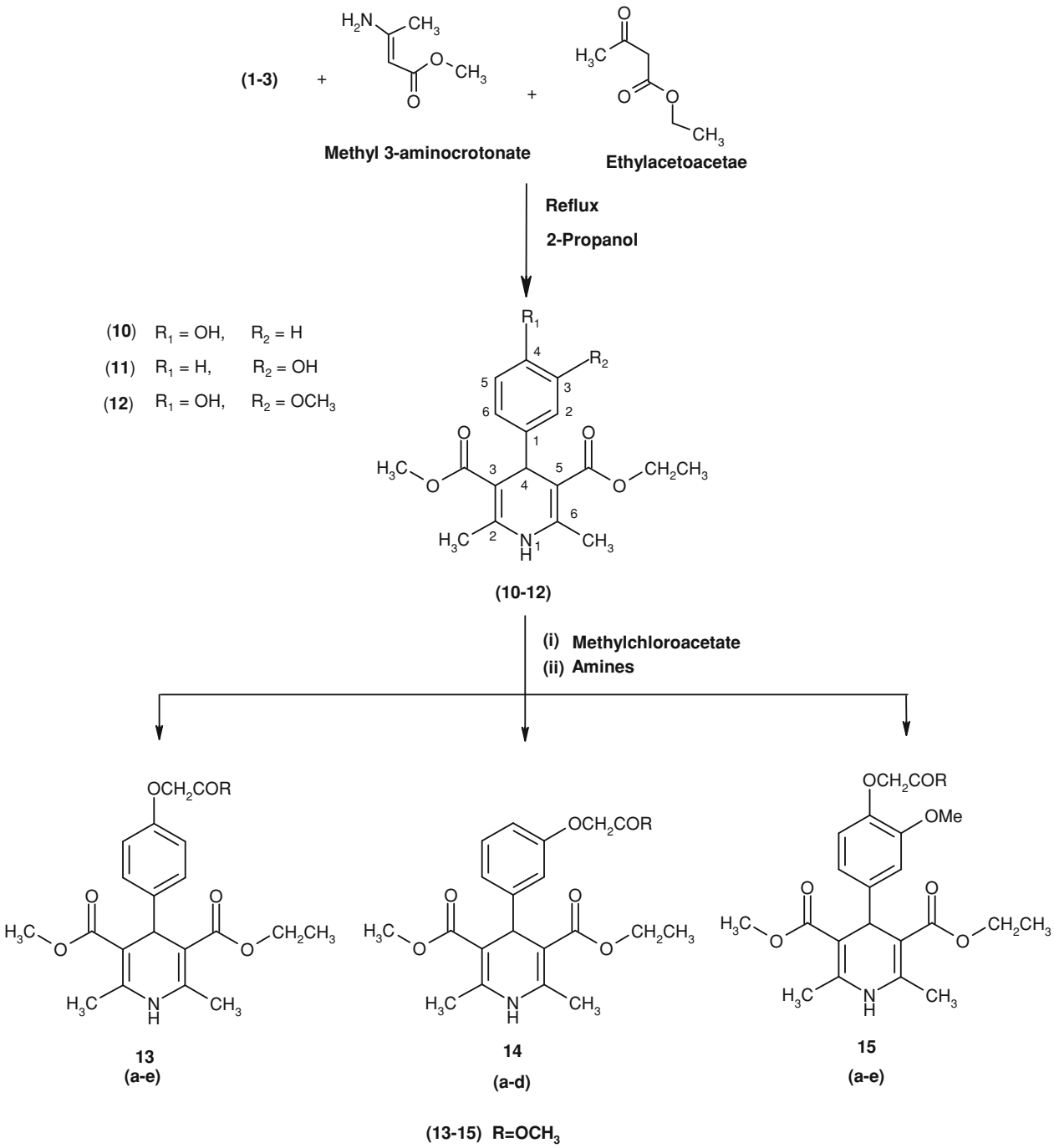
Ethyl methyl 4-(3-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***11***)**.** Yield: 81.18%; m.p. 215–216°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3380, 3272, 2941, 1689, 1659, 1497, 1450, 1221, 1096, 1025, 952, 876, 816, 706; ^{1}H NMR ($CDCl_3$, 300 MHz**,** δ, ppm): 1.22 (t, 3H, –COOCH$_2$CH_3, J = 5.70 Hz), 2.29 (s, 6H, 2 × CH_3), 3.64 (s, 3H, –COOCH_3), 4.09 (m, 2H, –COOCH_2CH$_3$), 4.98 (s, 1H, 4-CH, dihydropyridine), 5.46 (brs, 1H, –OH), 5.80 (t, 1H, NH, J = 12.52 Hz), 6.60 (d, 1H, J_o = 8.20 Hz, 6-CH, aromatic), 6.76 (s, 1H, 2-CH, aromatic), 6.84 (d, 1H, J_o = 7.43 Hz, 4-CH, aromatic), 7.05 (t, 1H, J_o = 7.82 Hz, 5-CH, aromatic); Anal. calcd. for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23; found: C, 65.05; H, 5.94; N, 4.12.

Ethyl methyl 4-(4-hydroxy-3-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***12***)**.** Yield: 65.21%; m.p. 100–101°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3377, 2982, 1730, 1695, 1650, 1624, 1490, 1432, 1380, 1302, 1209, 1091, 1051, 1024, 861, 768, 607; ^{1}H NMR ($CDCl_3$, 300 MHz**,** δ, ppm): 1.25 (m, 3H, –COOCH$_2$CH_3), 2.34 (s, 6H, 2 × CH_3), 3.67 (s, 3H, –COOCH_3), 3.86 (s, 3H, –OCH_3), 4.12 (m, 2H, –COOCH_2CH$_3$), 4.94 (s, 1H, 4-CH, dihydropyridine), 5.48 (s, 1H, –OH), 5.61 (t, 1H, –NH, J = 8.55 Hz), 6.74 (m, 2H, 5-CH and 6-CH, aromatic), 6.86 (s, 1H, 2-CH, aromatic); Anal. calcd. for $C_{19}H_{23}NO_6$: C, 63.15; H, 6.41; N, 3.88; found: C, 62.98; H, 6.40; N, 3.53.

General Procedure for Alkylation of Parent Dihydropyridines **4–6** and **10–12** (Formation of **7–9** and **13–15**):

Methylchloroacetate (6.30 mmol) was added to a stirred and refluxing suspension of respective dihydropyridine **4–6** and **10–12** (3.15 mmol) and anhydrous potassium carbonate (2 g) in ethyl methyl ketone (35 ml). The reaction mixture was further refluxed for 6 h. The resulting slurry was filtered off, and the solvent was removed under reduced pressure to obtain a solid product, which was crystallized from a mixture of diethyl ether and ethyl acetate to afford the corresponding dihydropyridine **7–9** and **13–15**.

Dimethyl 4-[4-(2-methoxy-2-oxoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-di-carboxylate (**7**). Yield: 40.98%; m.p. 139–141°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3339, 2946, 1741, 1699, 1643, 1499, 1438, 1384, 1306, 1219, 1095, 849, 674, 603; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.28 (s, 6H, 2 × CH_3), 3.63 (s, 6H, 2× COOCH_3), 3.79 (s, 3H, –OCH$_2$COOCH_3), 4.57 (s, 2H, –OCH_2COOCH$_3$), 4.94 (s, 1H, 4-CH, dihydropyridine), 6.33 (s, 1H, –NH), 6.73 (dd, 2H, J_o = 6.74 Hz, J_m = 1.97 Hz, 3-CH and 5-CH, aromatic), 7.18 (dd, 2H, J_o = 6.70 Hz, J_m = 2.01 Hz, 2-CH and 6-CH, aromatic); Anal. calcd. for $C_{20}H_{23}NO_7$: C, 61.69; H, 5.95; N, 3.60; found: C, 61.59; H, 5.64; N, 3.55.



Compound No.	a	b	c	D	e
R	-N O	-N	-N	-N N—	-N

Scheme 2 Synthetic route to asymmetric dihydropyridines **13–15**

Dimethyl 4-[3-(2-methoxy-2-oxoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-di-carboxylate (**8**). Yield: 81.96%; m.p. 170–172°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3370, 2950, 1770, 1700, 1650, 1480, 1430, 1300, 1210, 1100, 1010, 790, 710, 570; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.32 (s, 6H, 2 × CH_3), 3.64 (s, 6H, 2× $COOCH_3$), 3.79 (s, 3H, –OCH_2COOCH_3), 4.58 (s, 2H, –OCH_2COOCH_3), 4.99 (s, 1H, 4-CH, dihydropyridine), 5.81 (s, 1H, –NH), 6.65 (dd, 1H, $J_o = 8.13$ Hz, $J_m = 2.50$ Hz, 6-CH, aromatic), 6.84 (m, 1H, 2-CH, aromatic), 6.92 (d, 1H, $J_o = 7.93$ Hz, 4-CH, aromatic), 7.13 (t, 1H, $J_o = 7.82$ Hz, 5-CH, aromatic); Anal. calcd. for $C_{20}H_{23}NO_7$: C, 61.69; H, 5.95; N, 3.60; found: C, 61.42; H, 5.74; N, 3.49.

Dimethyl 4-[3-methoxy-4-(2-methoxy-2-oxoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**9**). Yield: 66.66%; m.p. 178–180°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3370, 2920, 1770, 1690, 1660, 1510, 1470, 1210, 1120, 1020, 810, 750, 690; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.32 (s, 6H, 2 × CH_3), 3.65 (s, 6H, 2× $COOCH_3$), 3.77 (s, 3H, –OCH_2COOCH_3), 3.83 (s, 3H, –OCH_3), 4.63 (s, 2H, –OCH_2COOCH_3), 4.96 (s, 1H, 4-CH, dihydropyridine), 5.78 (s, 1H, –NH, D_2O exchangeable), 6.66 (d, 1H, $J_o =$ 8.45 Hz, 5-CH, aromatic), 6.72 (dd, 1H, $J_o = 8.24$ Hz, $J_m = 1.97$ Hz, 6-CH, aromatic), 6.88 (d, 1H, $J_m = 1.62$ Hz, 2-CH, aromatic); Anal. calcd. for $C_{21}H_{25}NO_8$: C, 60.13; H, 6.01; N, 3.34; found: C, 59.85; H, 5.96; N, 3.16.

Ethyl methyl 4-[4-(2-methoxy-2-oxoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**13**). Yield: 74.38%; m.p. 165–166°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3340, 3112, 2948, 1744, 1695, 1640, 1504, 1283, 1214, 1094, 850, 742; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.21 (t, 3H, –$COOCH_2CH_3$, $J = 7.07$ Hz), 2.31 (s, 6H, 2 × CH_3), 3.63 (s, 3H, –$COOCH_3$), 3.79 (s, 3H, –OCH_2COOCH_3), 4.07 (m, 2H, –$COOCH_2CH_3$), 4.58 (s, 2H, –OCH_2COOCH_3), 4.93 (s, 1H, 4-CH, dihydropyridine), 5.85 (s, 1H, –NH), 6.75 (d, 2H, $J_o = 7.59$ Hz, 3-CH and 5-CH, aromatic), 7.18 (m, 2H, 2-CH and 6-CH, aromatic); Anal. calcd. for $C_{21}H_{25}NO_7$: C, 62.52; H, 6.25; N, 3.47; found: C, 62.35; H, 6.11; N, 3.37.

Ethyl methyl 4-[3-(2-methoxy-2-oxoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**14**). Yield: 78.51%; m.p. 134–135°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3340, 3094, 2949, 1742, 1698, 1645, 1598, 1441, 1382, 1283, 1207, 1093, 861, 797; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.22 (t, 3H, –$COOCH_2CH_3$, $J =$ 6.01 Hz), 2.31 (s, 6H, 2 × CH_3), 3.64 (s, 3H, –$COOCH_3$), 3.79 (s, 3H, –OCH_2COOCH_3), 4.09 (m, 2H, –$COOCH_2CH_3$), 4.58 (s, 2H, –OCH_2COOCH_3), 4.98 (s, 1H, 4-CH, dihydropyridine), 6.06 (t, 1H, –NH, $J = 12.87$ Hz), 6.64 (dd, 1H, $J_o = 8.06$ Hz, $J_m = 1.55$ Hz, 6-CH, aromatic), 6.85 (d, 1H, $J_m = 1.60$ Hz, 2-CH, aromatic), 6.94 (d, 1H, $J_o = 7.77$ Hz, 4-CH, aromatic), 7.14 (t, 1H, $J_o = 7.69$ Hz, 5-CH, aromatic); Anal. calcd. for $C_{21}H_{25}NO_7$: C, 62.52; H, 6.25; N, 3.47; found: C, 61.88; H, 5.95; N, 3.44.

Ethyl methyl 4-[3-methoxy-4-(2-methoxy-2-oxoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**15**). Yield: 92.43%; m.p. 105–107°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3373, 2981, 1730, 1694, 1497, 1376, 1296, 1208, 1095, 1024, 860, 768, 670, 610; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.23 (t, 3H, –$COOCH_2CH_3$, $J = 7.27$ Hz), 2.31 (s, 6H, 2 × CH_3), 3.65 (s, 3H, –$COOCH_3$), 3.77 (s, 3H, –OCH_2COOCH_3), 3.82 (s, 3H, –OCH_3), 4.09 (m, 2H, –$COOCH_2CH_3$), 4.63 (s, 2H, –OCH_2COOCH_3), 4.95 (s, 1H, 4-CH, dihydropyridine), 5.88 (t, 1H, –NH, $J = 12.60$ Hz), 6.66 (d, 1H, $J_o =$ 8.24 Hz, 6-CH, aromatic), 6.73 (m, 1H, 5-CH, aromatic), 6.89 (d, 1H, $J_m = 1.84$ Hz, 2-CH, aromatic); Anal. calcd. for $C_{22}H_{27}NO_8$: C, 60.96; H, 6.28; N, 3.23; found: C, 60.90; H, 6.18; N, 3.15.

General Procedure for the Synthesis of Amide Derivatives **7a–d, 8a–f,** and **9a–g/13a–e**, **14a–d,** and **15a–e**

A mixture of **7–9** (2.57 mmol) and requisite amine **a–d** and **g** (5.14 mmol) was thermally fused at 80–90°C for 7 h. After the completion of the reaction, which was monitored by TLC, ice-cold water was added to remove excess of amines. The solid product thus obtained was filtered, dried, and crystallized from a mixture of diethyl ether and ethyl acetate to obtain **7a–d, 8a–d,** and **9a–d** and **9g**. The synthesis of imidazolyl substituted dihydropyridines **8e**, **9e** required a longer time of 75 h and higher reaction temperature 90–110°C. *Tert*-butylamine substituted dihydopyridines **8f**, **9f** were prepared by thermal fusion of **8** and **9** with excess of *tert*-butylamine in DMF for 70 h.

Thermal fusion of **13–15** (12.48 mmol) and amines **a–e** (4.96 mmol) at 80–90°C for 4 h afforded corresponding amide derivatives **13a–e**, **14a–d,** and **15a–e**.

Dimethyl 2,6-dimethyl-4-[4-(2-morpholin-4-yl-2-oxoethoxy)phenyl]-1,4-dihydropyridine -3,5-dicarboxylate (***7a***)**.** Yield: 74.56%; m.p.: 183–185°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3274, 3217, 3093, 2943, 2868, 1695, 1651, 1505, 1437, 1377, 1306, 1277, 1215, 1097, 1029, 849, 746.4; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.32 (s, 6H, 2 × CH_3), 3.61 (m, 8H, –$N(CH_2)_2$– and $O(CH_2)_2$– morpholine and s (merged), 6H, 2 × $COOCH_3$), 4.62 (s, 2H, –OCH_2CO–), 4.94 (s, 1H, 4-CH, dihydropyridine), 5.72 (s, 1H, –NH), 6.77 (dd, 2H, $J_o = 6.74$ Hz, $J_m = 1.94$ Hz, 3-CH and 5-CH, aromatic), 7.17 (dd, 2H, $J_o = 6.70$ Hz, $J_m = 2.00$ Hz, 2-CH and 6-CH aromatic); Anal. calcd. for $C_{23}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.30; found: C, 62.01; H, 5.96; N, 5.83.

Dimethyl 2,6-dimethyl-4-[4-(2-oxo-2-pyrrolidin-1-ylethoxy)phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (***7b***). Yield: 45.45%; m.p.: 223–225°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3276, 3214, 3092, 2950, 1697, 1647, 1506, 1444, 1383, 1306, 1277, 1212, 1189, 1136, 1098, 1024, 851, 756, 686; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm: 1.87 (p, 2H, $–CH_2–$ pyr), 1.95 (p, 2H, $–CH_2–$, pyr), 2.31 (s, 6H, 2 × CH_3), 3.51 (t, 4H, $–N(CH_2)_2–$ pyr, $J = 3.25$ Hz), 3.63 (s, 6H, 2 × $COOCH_3$), 4.55 (s, 2H, $–OCH_2CO–$), 4.94 (s, 1H, 4-CH, dihydropyridine), 6.26 (s, 1H, –NH), 6.77 (dd, 2H, $J_o = 6.68$ Hz, $J_m = 2.01$ Hz, 3-CH and 5-CH, aromatic) and 7.16 (dd, 2H, $J_o = 6.73$ Hz, $J_m = 2.01$ Hz, 2-CH and 6-CH aromatic); Anal. calcd. for $C_{23}H_{28}N_2O_6$: C, 64.47; H, 6.59; N, 6.54; found: C, 63.96; H, 6.55; N, 5.98.

Dimethyl 2,6-dimethyl-4-[4-(2-oxo-2-piperidin-1-ylethoxy)phenyl]- 1,4-dihydropyridine-3,5-dicarboxylate (***7c***). Yield: 41.59%; m.p.: 201–203°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3275, 3214, 3094, 2937, 2857, 1696, 1644, 1505, 1444, 1381, 1305, 1228, 1098, 1017, 850, 781, 756; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.63 (m, 6H, 3-$CH_2–$ pip), 2.30 (s, 6H, 2 × CH_3), 3.46 (brt, 2H, $–NCH_2–$, pip, $J = 5.17$ Hz), 3.55 (brt, 2H, $–NCH_2–$, pip, $J = 5.15$ Hz), 3.63 (s, 6H, 2 × $COOCH_3$), 4.61 (s, 2H, $–OCH_2CO–$), 4.93 (s, 1H, 4-CH, dihydropyridine), 6.17 (s, 1H, –NH), 6.77 (dd, 2H, $J_o = 6.80$ Hz, $J_m = 1.90$ Hz, 3-CH and 5-CH, aromatic) and 7.16 (dd, 2H, $J_o = 6.79$ Hz, $J_m = 1.91$ Hz, 2-CH and 6-CH, aromatic); Anal. calcd. for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33; found: C, 64.79; H, 6.46; N, 6.55.

Dimethyl 2,6-dimethyl-4-{4-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]- phenyl}-1,4-dihydropyridine-3,5-dicarboxylate (***7d***). Yield: 48.80%; m.p. 164–165°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3278, 3217, 3095, 2948, 2847, 2801, 1700, 1649, 1506, 1444, 1380, 1230, 1095, 1036, 866, 752, 685; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.30 (s, 8H, 2 × CH_3 and > N–CH_3), 2.38 (brs, 4H, $CH_3–N(CH_2)_2–$, piperazine), 3.56 (brt, 2H, $–NCH_2–$, piperazine, $J = 4.84$ Hz), 3.63 (s, 8H, 2 × $COOCH_3$ and $–NCH_2–$, piperazine), 4.62 (s, 2H, $–OCH_2CO–$), 4.94 (s, 1H, 4-CH, dihydropyridine), 6.17 (brs, 1H, –NH), 6.77 (dd, 2H, $J_o = 8.63$ Hz, $J_m = 2.81$ Hz, 3-CH and 5-CH, aromatic), 7.18 (dd, 2H, $J_o = 8.57$ Hz, $J_m = 2.85$ Hz, 2-CH and 6-CH, aromatic); Anal. calcd. for $C_{24}H_{31}N_3O_6$: C, 63.00; H, 6.83; N, 9.18; found: C, 63.00; H, 6.59; N, 9.45.

Dimethyl 2,6-dimethyl-4-[3-(2-morpholin-4-yl-2-oxoethoxy)phenyl]- 1,4-dihydropyridine-3,5-dicarboxylate (***8a***). Yield: 47.37%; m.p. 165–168°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3346, 3092, 2954, 2865, 1700, 1653, 1488, 1437, 1348, 1298, 1220, 1121, 1019, 794, 686; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.33 (s, 6H, 2 × CH_3), 3.60 (s, 8H, $–N(CH_2)_2–$ and $O(CH_2)_2–$, morpholine), 3.64 (s, 6H, 2 × $COOCH_3$), 4.63 (s, 2H, $–OCH_2CO–$), 4.98 (s, 1H, 4-CH, dihydropyridine), 5.91 (s, 1H, –NH), 6.69 (dd, 1H, $J_o = 7.99$ Hz, $J_m = 3.02$ Hz, 6-CH, aromatic), 6.87 (m, 1H, 2-CH, aromatic), 6.93 (d, 1H, $J_o = 7.54$ Hz, 4-CH, aromatic), 7.13 (t, 1H, $J_o = 7.84$ Hz, 5-CH, aromatic); Anal. calcd. for $C_{23}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.30; found: C, 61.77; H, 6.51; N, 5.88.

Dimethyl 2,6-dimethyl-4-[3-(2-oxo-2-pyrrolidin-1-ylethoxy)phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (***8b***). Yield: 76.45%; m.p.: 157–160°C; Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3330, 3083, 2960, 1695, 1651, 1479, 1379, 1346, 1305, 1206, 1095, 1019, 858, 783, 714, 588; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.84 (p, 2H, $–CH_2–$, pyr), 1.95 (p, 2H, $–CH_2–$, pyr), 2.32 (s, 6H, 2 × CH_3), 3.50 (t, 4H, $–N(CH_2)_2–$, pyr, $J = 6.81$ Hz), 3.64 (s, 6H, 2 × $COOCH_3$), 4.55 (s, 2H, $–OCH_2CO–$), 4.98 (s, 1H, 4-CH, dihydropyridine), 6.44 (s, 1H, –NH), 6.68 (dd, 1H, $J_o = 7.92$ Hz, $J_m = 2.88$ Hz, 6-CH, aromatic), 6.88 (m, 1H, 2-CH aromatic), 6.92 (d, 1H, $J_o = 7.79$ Hz, 4-CH, aromatic), 7.11 (t, 1H, $J_o = 7.95$ Hz, 5-CH, aromatic); Anal. calcd. for $C_{23}H_{28}N_2O_6$: C, 64.47; H, 6.59; N, 6.54; found: C, 64.09; H, 6.25; N, 6.16.

Dimethyl 2,6-dimethyl-4-[3-(2-oxo-2-piperidin-1-ylethoxy)phenyl]- 1,4-dihydropyridine-3,5-dicarboxylate (***8c***). Yield: 58.40%; m.p. 158–161°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3288, 3225, 3094, 2939, 1696, 1645, 1492, 1437, 1379, 1308, 1214, 1096, 1019, 858, 784, 715; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.53 (brt, 4H, $–CH_2–$, pip, $J = 5.06$ Hz), 1.60 (brt, 2H, $–CH_2–$, pip, $J = 4.96$ Hz), 2.33 (s, 6H, 2 × CH_3), 3.48 (t, 2H, $–NCH_2–$, pip, $J = 5.18$ Hz), 3.53 (t, 2H, $–NCH_2–$ pip, $J = 5.39$ Hz), 3.64 (s, 6H, 2 × $COOCH_3$), 4.61 (s, 2H, $–OCH_2CO–$), 4.97 (s, 1H, 4-CH, dihydropyridine), 5.97 (s, 1H, –NH), 6.69 (dd, 1H, $J_o = 8.04$ Hz, $J_m = 1.97$ Hz, 6-CH, aromatic), 6.89 (d, 1H, $J_m = 2.74$ Hz, 2-CH, aromatic), 6.92 (s, 1H, 4-CH, aromatic), 7.11 (t, 1H, $J_o = 7.80$ Hz, 5-CH, aromatic); Anal. calcd. for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33; found: C, 64.88; H, 6.81; N, 5.95.

Dimethyl 2,6-dimethyl-4-{3-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]-phenyl}-1,4-dihydropyridine-3,5-dicarboxylate (***8d***). Yield: 57.69; m.p. 173–175°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3299, 3233, 3097, 2944, 1703, 1650, 1595, 1494, 1433, 1307, 1212, 1118, 1093, 1023, 857, 812, 795, 713; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.27 (s, 3H, > N–CH_3), 2.35 (s, 6H, 2 × CH_3 and t (merged), 4H, $CH_3–N(CH_2)_2–$ piperazine, $J = 2.33$ Hz), 3.63 (t, 4H, $–N(CH_2)_2–$ piperazine, $J = 3.64$ Hz and s (merged), 6H, 2 × $COOCH_3$), 4.62 (s, 2H, $–OCH_2CO–$), 4.98 (s, 1H, 4-CH, dihydropyridine), 5.90 (s, 1H, –NH), 6.69 (dd, 1H, $J_oJ_o = 8.00$ Hz, $J_m = 1.90$ Hz, 6-CH, aromatic), 6.87 (m, 1H, 2-CH, aromatic), 6.91 (d, 1H, $J_o = 7.52$ Hz, 4-CH, aromatic), 7.12 (t, 1H, $J_o = 7.77$ Hz, 5-CH, aromatic); Anal. calcd. for $C_{24}H_{31}N_3O_6$: C, 63.00; H, 6.83; N, 9.18; found: C, 63.01; H, 6.11; N, 8.97.

Dimethyl 4-{3-[2-(1H-imidazol-1-yl)-2-oxoethoxy]phenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***8e***). Yield: 22.93; m.p. 175–177°C. Spectroscopic analysis: IR (ν_{max}): 3345, 3147, 2957, 1694, 1650, 1567, 1486, 1439, 1307, 1226, 1107, 1057, 870, 830, 779, 715, 630; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz, δ, ppm): 2.30 (s, 6H, 2 × CH_3), 3.64 (s, 6H, 2 × $COOCH_3$), 4.59 (s, 2H, –OCH_2CO–), 4.99 (s, 1H, 4-CH, dihydropyridine), 6.10 (s, 1H, –NH), 6.67 (dd, 1H, $J_o = 7.97$ Hz, $J_m = 2.02$ Hz, 6-CH, aromatic), 6.81 (s, 1H, 2-CH, aromatic), 6.92 (d, 1H, $J_o = 8.00$ Hz, 4-CH, aromatic), 7.12 (t, 1H, $J_o = 7.85$ Hz, 5-CH, aromatic), 7.17 (s, 2H, 4-CH and 5-CH, imidazole), 8.41 (s, 1H, 2-CH, imidazole); Anal. calcd. for $C_{22}H_{23}N_3O_6$: C, 62.11; H, 5.45; N, 9.88; found: C, 62.01; H, 4.98; N, 9.31.

Dimethyl 4-{3-[2-(tert-butylamino)-2-oxoethoxy]phenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***8f***). Yield: 12.96%; m.p. 233–235°C. Spectroscopic analysis: FT-IR (KBr) ν_{max}/cm^{-1}: 3380, 3275, 2984, 1689, 1659, 1596, 1496, 1450, 1310, 1255, 1221, 1122, 1096, 1026, 875, 782, 610; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz, δ, ppm): 1.29 (s, 9H, –NHC(CH_3)$_3$), 2.33 (s, 6H, 2 × CH_3), 3.63 (s, 6H, 2 × $COOCH_3$), 4.17 (s, 2H, –OCH_2CO–), 4.91 (s, 1H, 4-CH, dihydropyridine), 6.59 (d, 1H, $J_o = 8.10$ Hz, 6-CH, aromatic), 6.66 (s, 1H, 2-CH, aromatic), 6.71 (d, 1H, $J_o = 7.14$ Hz, 4-CH, aromatic), 7.11 (t, 1H, $J_o = 7.80$ Hz, 5-CH, aromatic), 8.40 (brs, 1H, –NH), 9.01 (s, 1H, –NH); Anal. calcd. for $C_{23}H_{30}N_2O_6$: C, 64.17; H, 7.02; N, 6.51; found: C, 63.95; H, 6.75; N, 6.26.

Dimethyl 4-[3-methoxy-4-(2-morpholin-4-yl-2-oxoethoxy) phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***9a***). Yield: 56.64%; m.p.: 190–193°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3350, 2950, 1700, 1650, 1490, 1450, 1220, 1110, 1010, 850, 800, 650, 600; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.33 (s, 6H, 2 × CH_3), 3.65 (s, 6H, 2 × $COOCH_3$ and t (merged), 8H, –$N(CH_2)_2$– and $O(CH_2)_2$– morph, $J = 3.65$ Hz), 3.81 (s, 3H, –OCH_3), 4.67 (s, 2H, –OCH_2CO–), 4.95 (s, 1H, 4-CH, dihydropyridine), 5.75 (s, 1H, -NH, D_2O exchangeable), 6.73 (dd, 1H, $J_o = 8.33$ Hz, $J_m = 1.70$ Hz, 6-CH, aromatic), 6.79 (d, 1H, $J_o = 8.22$ Hz, 5-CH, aromatic), 6.88 (d, 1H, $J_m = 1.87$ Hz, 2-CH, aromatic); Anal. calcd. for $C_{24}H_{30}N_2O_8$: C, 60.75; H, 6.37; N, 5.90; found: C, 60.32; H, 6.49; N, 6.19.

Dimethyl 4-[3-methoxy-4-(2-oxo-2-pyrrolidin-1-ylethoxy) phenyl]-2,6-dimethyl-1,4,-dihydropyridine-3,5-dicarboxylate (***9b***). Yield: 55.96%; m.p. 192–194°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3350, 2960, 1700, 1650, 1500, 1430, 1320, 1210, 1110, 1010, 750, 650; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.85 (m, 2H, –CH_2–, pyr), 1.95 (m, 2H, –CH_2–, pyr), 2.26 (s, 6H, 2 × CH_3), 3.48 (t, 2H, –NCH_2–, pyr, $J = 6.83$ Hz), 3.55 (t, 2H, –NCH_2–, pyr, $J = 6.64$ Hz), 3.64 (s, 6H, 2 × $COOCH_3$), 3.75 (s, 3H, –OCH_3), 4.56 (s, 2H, –OCH_2CO–), 4.88 (s, 1H, 4-CH, dihydropyridine), 6.62 (dd, 1H, $J_o = 8.28$ Hz, $J_m = 1.66$ Hz, 6-CH, aromatic), 6.70 (d, 1H, $J_o = 8.22$ Hz, 5-CH, aromatic), 6.84 (d, 1H, $J_m = 1.89$ Hz, 2-CH, aromatic), 7.25 (s, 1H, –NH, D_2O exchangeable); Anal. calcd. for $C_{24}H_{30}N_2O_7$: C, 62.87; H, 6.59; N, 6.11; found: C, 62.41; H, 6.61; N, 6.23.

Dimethyl 4-[3-methoxy-4-(2-oxo-2-piperidin-1-ylethoxy) phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***9c***). Yield: 56.25%; m.p. 210–214°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3350, 2920, 1700, 1640, 1500, 1270, 1210, 1110, 1020, 790, 700, 630; ^{1}H NMR (CCl_4 + $CDCl_3$, 300 MHz, δ, ppm): 1.61 (m, 6H, 3-CH_2–pip), 2.27 (s, 6H, 2 × CH_3), 3.53 (t, 4H, –$N(CH_2)_2$–, pip, $J = 5.20$ Hz), 3.64 (s, 6H, 2 × $COOCH_3$), 3.77 (s, 3H, –OCH_3), 4.62 (s, 2H, –OCH_2CO–), 4.89 (s, 1H, 4-CH, dihydropyridine), 6.64 (dd, 1H, $J_o = 8.35$ Hz, $J_m = 1.82$ Hz, 6-CH, aromatic), 6.74 (d, 1H, $J_o = 8.27$ Hz, 5-CH, aromatic), 6.84 (d, 1H, $J_m = 2.00$ Hz, 2-CH, aromatic), 6.98 (s, 1H, –N*H*, D_2O exchangeable); Anal. calcd. for $C_{25}H_{32}N_2O_7$: C, 63.54; H, 6.83; N, 5.93; found: C, 63.11; H, 6.59; N, 5.51.

Dimethyl 4-{3-methoxy-4-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]-phenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***9d***). Yield: 43.10%; m.p. 202–204°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3314, 3088, 2948, 2800, 1694, 1634, 1489, 1380, 1256, 1215, 1095, 1031, 866, 764, 706, 629; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.30 (s, 3H, > N–CH_3), 2.31 (s, 6H, 2 × CH_3), 2.37 (t, 4H, –CH_3–N(CH_2)$_2$–, piperazine, $J = 4.79$ Hz), 3.65 (s, 10H, 2 × $COOCH_3$ and –$N(CH_2)_2$–, piperazine), 3.80 (s, 3H, –OCH_3), 4.66 (s, 2H, –OCH_2CO–), 4.95 (s, 1H, 4-CH, dihydropyridine), 6.05 (s, 1H, –NH, D_2O exchangeable), 6.71 (dd, 2H, $J_o = 8.33$ Hz, $J_m = 1.79$ Hz, 6-CH, aromatic), 6.78 (d, 1H, $J_o = 8.25$ Hz, 5-CH, aromatic), 6.88 (d, 1H, $J_m = 1.61$ Hz, 2-CH, aromatic); Anal. calcd. for $C_{25}H_{33}N_3O_7$: C, 61.59; H, 6.82; N, 8.62; found: C, 61.49; H, 6.59; N, 8.31.

Dimethyl 4-[4-[2-(1 h-imidazol-1-yl)-2-oxoethoxy]-3-methoxyphenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***9e***). Yield: 37.96%; m.p. 216–220°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3357, 3146, 2947, 1680, 1655, 1491, 1212, 1117, 1023, 865, 611; ^{1}H NMR ($CDCl_3$ + DMSO-d_6, 300 MHz, δ, ppm): 2.29 (s, 6H, 2 × CH_3), 3.61 (s, 6H, 2 × $COOCH_3$), 3.79 (s, 3H, –OCH_3), 4.52 (s, 2H, –OCH_2CO–), 4.87 (s, 1H, 4-CH, dihydropyridine), 6.66 (s, 2H, 5-CH and 6-CH, aromatic), 6.83 (s, 1H, 2-CH, aromatic), 7.04 (s, 2H, 4-CH and 5-CH, imidazole), 7.74 (s, 1H, 2-CH, imidazole), 8.50 (s, 1H, –NH, D_2O exchangeable); Anal. calcd. for $C_{23}H_{25}N_3O_7$: C, 60.65; H, 5.53; N, 9.23; found: C, 59.99; H, 4.95; N, 9.30.

Dimethyl 4-{4-[2-(tert-butylamino)-2-oxoethoxy]-3-methoxyphenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***9f***). Yield: 71.55%; m.p. > 270°C. Spectroscopic

analysis: IR (KBr) ν_{max}/cm^{-1}: 3150, 2897, 1660, 1492, 1301, 1259, 1079, 787, 680, 623; ^{1}H-NMR ($CDCl_3$, 300 MHz, δ, ppm): 2.17 (s, 9H, –$NH(CH_3)_3$), 2.34 (s, 6H, 2 × CH_3), 3.66 (s, 6H, 2 × $COOCH_3$), 3.87 (s, 3H, –OCH_3), 4.59 (s, 2H, –OCH_2CO–), 4.98 (s, 1H, 4-CH, dihydropyridine), 5.64 (s, 1H, –NH, D_2O exchangeable), 6.80 (m, 2H, 5-CH and 6-CH aromatic), 6.92 (d, 1H, $J_m = 1.16$ Hz, 2-CH, aromatic), 7.26 (s, 1H, –$NH(CH_3)_3$); Anal. calcd. for $C_{24}H_{32}N_2O_7$: C, 62.59; H, 7.00; N, 6.08; found: C, 62.11; H, 7.02; N, 5.86.

Dimethyl 4-{3-methoxy-4-[2-(3-methylpiperidin-1-yl)-2-oxoethoxy]- phenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***9g***). Yield: 72.17%; m.p. 148–150°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3306, 2948, 1700, 1631, 1479, 1214, 1029, 767; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 0.90 (t, 3H, –C(C*H*$_3$)H–, pipec, $J = 4.54$ Hz), 1.16 (m, 1H, –C(CH_3)*H*–, pipec), 1.54 (m, 1H, –CH(H)–, pipec), 1.68 (m, 1H, –CH(H)–, pipec), 1.79 (m, 1H, –CH(H)–, pipec), 1.98 (m, 1H, –CH(H)–, pipec), 2.32 (s, 6H, 2 × CH_3), 2.64 (m, 1H, –NCH(H)–, pipec), 3.00 (q, 1H, –NCH(H)– pipec), 3.65 (s, 6H, 2 × $COOCH_3$), 3.81 (s, 3H, –OCH_3), 3.92 (t, 1H, –NCH(H)– pipec, $J = 9.45$ Hz), 4.34 (t, 1H, –NCH(H)– pipec, $J = 10.72$ Hz), 4.66 (s, 2H, –OCH_2CO–), 4.95 (s, 1H, 4-CH, dihydropyridine), 6.08 (s, 1H, –NH, D_2O exchangeable), 6.71 (dd, 1H, $J_o = 8.30$ Hz, $J_m = 1.92$ Hz, 6-CH aromatic), 6.78 (d, 1H, $J_o = 8.29$ Hz, 5-CH aromatic), 6.88 (d, 1H, $J_m = 1.63$ Hz, 2-CH aromatic); Anal. calcd. for $C_{26}H_{34}N_2O_7$: C, 64.18; H, 7.04; N, 5.76; found: C, 63.95; H, 6.51; N, 5.91.

Ethyl methyl 2,6-dimethyl-4-[4-(2-morpholin-4-yl-2-oxoethoxy)-phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (***13a***). Yield: 59.29%; m.p.: 165–167°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3281, 3223, 3097, 2952, 1694, 1646, 1504, 1445, 1375, 1275, 1217, 1106, 1028, 853, 747; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.22 (t, 3H, –$COOCH_2CH_3$, $J = 5.83$ Hz), 2.27 (t, 4H, –$N(CH_2)_2$–, morph, $J = 14.39$ Hz and s (merged), 6H, 2 × CH_3), 3.63 (m, 4H, $O(CH_2)_2$–, morph and s (merged), 3H, –$COOCH_3$), 4.08 (m, 2H, –$COOCH_2CH_3$), 4.62 (s, 2H, –OCH_2CO–), 4.92 (s, 1H, 4-CH, dihydropyridine), 5.81 (s, 1H, –NH), 6.77 (d, 2H, $J_o = 8.43$ Hz, 3-CH and 5-CH, aromatic), 7.18 (m, 2H, 2-CH and 6-CH, aromatic); Anal. calcd. for $C_{24}H_{30}N_2O_7$: C, 62.87; H, 6.59; N, 6.11; found: C, 62.55; H, 6.16; N, 6.10.

Ethyl methyl 2,6-dimethyl-4-[4-(2-oxo-2-pyrrolidin-1-ylethoxy)-phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (***13b***). Yield: 64.22%; m.p. 189–190°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3280, 3219, 3095, 2980, 1694, 1646, 1504, 1382, 1304, 1275, 1210, 1098, 1026, 856, 756; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.21 (t, 3H, –$COOCH_2CH_3$, $J = 5.39$ Hz), 1.88 (m, 2H, –CH_2–, pyr), 1.97 (m, 2H, –CH_2–, pyr), 2.30 (s, 6H, 2 × CH_3), 3.50 (m, 4H, –$N(CH_2)_2$–, pyr), 3.63 (s, 3H, –$COOCH_3$), 4.08 (m, 2H, –$COOCH_2CH_3$), 4.56 (s, 2H, –OCH_2CO-), 4.93 (s, 1H, 4-CH, dihydropyridine), 6.53 (s, 1H, –NH), 6.76 (d, 2H, $J_o = 8.52$ Hz, 3-CH and 5-CH, aromatic), 7.19 (m, 2H, 2-CH and 6-CH aromatic); Anal. calcd. for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33; found: C, 65.02; H, 6.52; N, 6.03.

Ethyl methyl 2,6-dimethyl-4-[4-(2-oxo-2-piperidin-1-ylethoxy)- phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (***13c***). Yield: 56.63%; m.p. 187–188°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3278, 3221, 3097, 2939, 2859, 1695, 1643, 1505, 1445, 1382, 1304, 1274, 1227, 1096, 1018, 855, 781, 688; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.21 (t, 3H, –$COOCH_2CH_3$, $J = 5.89$ Hz), 1.62 (m, 6H, 3-CH_2–, pip), 2.31 (s, 6H, 2 × CH_3), 3.45 (t, 2H, –NCH_2–, pip, $J = 4.92$ Hz), 3.55 (t, 2H, –NCH_2–, pip, $J = 4.75$ Hz), 3.63 (s, 3H, –$COOCH_3$), 4.08 (m, 2H, –$COOCH_2CH_3$), 4.62 (s, 2H, –OCH_2CO–), 4.93 (s, 1H, 4-CH, dihydropyridine), 6.00 (brs, 1H, –NH), 6.77 (d, 2H, $J_o = 8.52$ Hz, 3-CH and 5-CH, aromatic), 7.17 (m, 2H, 2-CH and 6-CH, aromatic); Anal. calcd. for $C_{25}H_{32}N_2O_6$: C, 65.77; H, 7.07; N, 6.14; found: C, 65.70; H, 6.56; N, 6.01.

Ethyl methyl 2,6-dimethyl-4-{4-[2-(4-methylpiperazin-1-yl)-2-oxo- ethoxy]phenyl}-1,4-dihydropyridine-3,5-dicarboxylate (***13d***). Yield: 44.82%; m.p. 176–178°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3280, 3222, 3096, 2942, 2801, 1694, 1649, 1503, 1444, 1377, 1273, 1225, 1095, 1039, 862, 826, 754; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.21 (t, 3H, –$COOCH_2CH_3$, $J = 6.12$ Hz), 2.30 (s, 9H, > N–CH_3 and 2 × CH_3), 2.39 (brs, 4H, –$N(CH_2)_2$– piperazine), 3.61 (m, 7H, –$COOCH_3$ and –$N(CH_2)_2$– piperazine), 4.08 (m, 2H, –$COOCH_2CH_3$), 4.61 (s, 2H, –OCH_2CO–), 4.93 (s, 1H, 4-CH, dihydropyridine), 5.90 (t, 1H, –NH, $J = 15.83$ Hz), 6.76 (d, 2H, $J_o = 8.59$ Hz, 3-CH and 5-CH, aromatic), 7.18 (m, 2H, 2-CH and 6-CH, aromatic). Anal. calcd. for $C_{25}H_{33}N_3O_6$: C, 63.68; H, 7.05; N, 8.91; found: C, 62.86; H, 6.75; N, 8.46.

Ethyl methyl 4-(4-{2-[(3-methylpiperidin-1-yl)oxy]-2-oxoethoxy}- phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***13e***). Yield: 65.51%; m.p. 166–167°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3281, 3218, 3095, 2939, 1692, 1645, 1505, 1444, 1382, 1304, 1207, 1095, 1051, 1022, 854, 825, 781, 752, 687; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 0.89 (t, 3H, –C(C*H*$_3$)H–, pipec, $J = 5.71$ Hz), 1.15 (m, 1H, –C(CH_3)*H*–, pipec), 1.21 (t, 3H, –$COOCH_2CH_3$, $J = 6.22$ Hz), 1.52 (m, 2H, –CH_2–, pipec), 1.69 (m, 1H, –CH(H)–, pipec), 1.81 (m, 1H, –CH(H)–, pipec), 2.31 (s, 6H, 2 × CH_3), 2.66 (m, 1H, –NCH(H)–, pipec), 2.99 (m, 1H, –NCH(H)–, pipec), 3.63 (s, 3H, –$COOCH_3$), 3.80 (m, 1H, –NCH(H)–, pipec), 4.08 (m, 2H, –$COOCH_2CH_3$), 4.34 (m, 1H, –NCH(H)–, pipec), 4.59 (s, 2H, –OCH_2CO–), 4.93 (s, 1H, 4-CH, dihydropyridine), 5.95 (t, 1H, –NH, $J = 18.73$ Hz), 6.76 (d, 2H, $J_o = 8.50$ Hz, 3-CH and 5-CH aromatic), 7.18 (m, 2H,

2-CH and 6-CH, aromatic); Anal. calcd. for $C_{26}H_{34}N_2O_6$: C, 66.36; H, 7.28; N, 5.95; found: C, 66.33; H, 6.65; N, 5.36.

Ethyl methyl 2,6-dimethyl-4-[3-(2-morpholin-4-yl-2-oxoethoxy)- phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (***14a***). Yield: 54.36%; m.p. 137–139°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3301, 3231, 3098, 2944, 1701, 1650, 1493, 1434, 1307, 1211, 1167, 1094, 1002, 778, 756, 711; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.22 (t, 3H, –$COOCH_2CH_3$, $J = 6.04$ Hz), 2.33 (brs, 10H, 2 × CH_3 and –$N(CH_2)_2$–, morph), 3.58 (t, 4H, $O(CH_2)_2$–, morph, $J = 4.51$ Hz), 3.64 (s, 3H, –$COOCH_3$), 4.09 (m, 2H, –$COOCH_2CH_3$), 4.61 (s, 2H, –OCH_2CO–), 4.97 (s, 1H, 4-CH, dihydropyridine), 5.89 (t, 1H, –NH, $J =$ 11.17 Hz), 6.68 (dd, 1H, $J_o = 7.99$ Hz, $J_m = 1.63$ Hz, 6-CH, aromatic), 6.88 (d, 1H, $J_m = 2.05$ Hz, 2-CH, aromatic), 6.92 (m, 1H, 4-CH aromatic), 7.10 (t, 1H, $J_o =$ 7.93 Hz, 5-CH, aromatic); Anal. calcd. for $C_{24}H_{30}N_2O_7$: C, 62.87; H, 6.59; N, 6.11; found: C, 62.58; H, 6.36; N, 5.83.

Ethyl methyl 2,6-dimethyl-4-[3-(2-oxo-2-pyrrolidin-1-ylethoxy)-phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (***14 b***). Yield: 67.27%; m.p. 87–89°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3273, 3211, 3086, 2981, 2891, 1692, 1648, 1596, 1493, 1441, 1304, 1271, 1205, 1094, 1021, 863, 760, 719; ^{1}H NMR ($CDCl_3$): δ 1.22 (t, 3H, –$COOCH_2CH_3$, $J = 7.20$ Hz), 1.84 (p, 2H, –CH_2–, pyr), 1.95 (p, 2H, –CH_2–, pyr), 2.32 (s, 6H, 2 × CH_3), 3.50 (t, 4H, –$N(CH_2)_2$–, pyr, $J = 6.74$ Hz), 3.64 (s, 3H, –$COOCH_3$), 4.08 (m, 2H, –$COOCH_2CH_3$), 4.55 (s, 2H, –OCH_2CO–), 4.97 (s, 1H, 4-CH, dihydropyridine), 6.44 (t, 1H, –NH, $J = 17.62$ Hz), 6.67 (dd, 1H, $J_o = 7.94$ Hz, $J_m = 1.46$ Hz, 6-CH, aromatic), 6.92 (m, 2H, 2-CH and 4-CH, aromatic), 7.11 (t, 1H, $J_o = 7.81$ Hz, 5-CH, aromatic). Anal. calcd. for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33. Found: C, 64.84; H, 6.54; N, 6.12.

Ethyl methyl 2,6-dimethyl-4-[3-(2-oxo-2-piperidin-1-ylethoxy)-phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (14c). Yield: 78.31%; m.p. 157–158°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3289, 3220, 3095, 2938, 1701, 1645, 1495, 1435, 1307, 1210, 1115, 1041, 811, 781 and 712. ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.22 (t, 3H, –$COOCH_2CH_3$, $J = 5.95$ Hz), 1.52 (brs, 4H, 2-CH_2– pip), 1.61 (m, 2H, –CH_2– pip), 2.33 (s, 6H, 2 × CH_3), 3.51 (m, 4H, –$N(CH_2)_2$– pip), 3.64 (s, 3H, –$COOCH_3$), 4.08 (m, 2H, –$COOCH_2CH_3$), 4.63 (s, 2H, –OCH_2CO–), 4.97 (s, 1H, 4-CH, dihydropyridine), 6.04 (t, 1H, –NH, $J = 13.09$ Hz), 6.69 (dd, 2H, $J_o = 8.00$ Hz, $J_m = 1.61$ Hz, 6-CH, aromatic), 6.91 (m, 2H, 2-CH and 4-CH, aromatic), 7.11 (t, 1H, $J_o = 7.84$ Hz, 5-CH, aromatic); Anal. calcd. for $C_{25}H_{32}N_2O_6$: C, 65.77; H, 7.07; N, 6.14; found: C, 65.65; H, 7.00; N, 6.13.

Ethyl methyl 2,6-dimethyl-4-{3-[2-(4-methylpiperazin-1-yl)-2-oxoethoxy]phenyl}-1,4-dihydropyridine-3,5-dicarboxylate (***14d***). Yield: 45.51%; m.p. 135–136°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3301, 3234, 3099, 2943, 1700, 1650, 1493, 1306, 1212, 1168, 1095, 1027, 780, 711; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.22 (t, 3H, –$COOCH_2CH_3$, $J = 6.61$ Hz), 2.33 (s, 9H, 2 × CH_3 and > N–CH_3), 2.46 (brt, 4H, –$N(CH_2)_2$–, piperazine, $J = 11.59$ Hz), 3.64 (s, 3H, –$COOCH_3$), 3.66 (brs, 4H, –$N(CH_2)_2$–, piperazine), 4.08 (m, 2H, –$COOCH_2CH_3$), 4.63 (s, 2H, –OCH_2CO–), 4.97 (s, 1H, 4-CH, dihydropyridine), 5.87 (t, 1H, –NH, $J = 11.18$ Hz), 6.70 (dd, 2H, $J_o = 6.62$ Hz, $J_m = 1.39$ Hz, 6-CH, aromatic), 6.87 (d, 1H, $J_m =$ 1.59 Hz, 2-CH, aromatic), 6.93 (m, 1H, 4-CH, aromatic), 7.11 (t, 1H, $J_o = 7.66$ Hz, 5-CH aromatic); Anal. calcd. for $C_{25}H_{33}N_3O_6$: C, 63.68; H, 7.05; N, 8.91; found: C, 63.23; H, 6.68; N, 8.52.

Ethyl methyl 4-{3-methoxy-4-[2-(morpholin-4-yloxy)-2-oxoethoxy]- phenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***15a***). Yield: 85.71%; m.p.: 157–158°C; Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3328, 2966, 1692, 1642, 1509, 1330, 1219, 1118, 1027, 794, 757, 696; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.24 (t, 3H, –$COOCH_2CH_3$, $J = 7.65$ Hz), 2.32 (s, 6H, 2 × CH_3), 3.65 (s, 11H, –$COOCH_3$ and –N–$(CH_2)_2$–, O–$(CH_2)_2$–, morph), 3.81 (s, 3H, –OCH_3), 4.10 (m, 2H, –$COOCH_2CH_3$), 4.67 (s, 2H, –OCH_2CO–), 4.95 (s, 1H, 4-CH, dihydropyridine), 6.05 (brs,1H, –NH), 6.74 (m, 2H, 5-CH and 6-CH, aromatic), 6.89 (d, 2H, $J_m = 1.27$ Hz, 2-CH, aromatic); Anal. calcd. for $C_{25}H_{32}N_2O_8$: C, 61.46; H, 6.60; N, 5.73; found: C, 61.00; H, 6.49; N, 5.49.

Ethyl methyl 4-{3-methoxy-4-[2-oxo-2-(pyrrolidin-1-yloxy)ethoxy]- phenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***15b***). Yield: 94.49%; m.p. 176–177°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3283, 3087, 2944, 2881, 1692, 1649, 1508, 1307, 1259, 1211, 1092, 1036, 863, 765.6; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.24 (t, 3H, –$COOCH_2CH_3$, $J = 7.61$ Hz), 1.88 (m, 4H, 2-CH_2– pyr), 2.31 (s, 6H, 2 × CH_3), 3.52 (p, 4H, –$N(CH_2)_2$– pyr), 3.65 (s, 3H, –$COOCH_3$), 3.81 (s, 3H, –OCH_3), 4.10 (m, 2H, –$COOCH_2CH_3$), 4.62 (s, 2H, –OCH_2CO–), 4.94 (s, 1H, 4-CH, dihydropyridine), 6.25 (t, 1H, –NH, $J = 20.89$ Hz), 6.74 (m, 2H, 5-CH and 6-CH aromatic), 6.89 (s, 1H, 2-CH, aromatic); Anal. calcd. for $C_{25}H_{32}N_2O_7$: C, 63.54; H, 6.83; N, 5.93; found: C, 62.96; H, 7.01; N, 5.43.

Ethyl methyl 4-{3-methoxy-4-[2-oxo-2-(piperidin-1-yloxy)ethoxy]- phenyl}-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***15c***). Yield: 60.71%; m.p. 159–160°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3295, 3223, 3095, 2942, 1695, 1641, 1507, 1442, 1264, 1212, 1090, 1031, 857 and 789; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.23 (t, 3H, –$COOCH_2CH_3$, $J = 7.11$ Hz), 1.59 (m, 6H, 3-CH_2–, pip), 2.31 (s, 6H, 2 × CH_3), 3.53 (m, 4H, –$N(CH_2)_2$–, pip), 3.65 (s, 3H, –$COOCH_3$), 3.80 (s, 3H,

–OCH_3), 4.09 (m, 2H, –$COOCH_2CH_3$), 4.67 (s, 2H, –OCH_2CO–), 4.94 (s, 1H, 4-CH, dihydropyridine), 6.42 (t, 1H, –NH, $J = 21.30$ Hz), 6.73 (m, 2H, 5-CH and 6-CH, aromatic), 6.89 (d, 1H, $J_m = 1.26$ Hz, 2-CH aromatic); Anal. calcd. for $C_{26}H_{34}N_2O_7$: C, 64.18; H, 7.04; N, 5.76; found: C, 64.08; H, 7.00; N, 5.45.

Ethyl methyl 4-(3-methoxy-4-{2-[(4-methylpiperazin-1-yl)oxy]-2-oxoethoxy}phenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***15d***). Yield: 73.04%; m.p. 153–155°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3321, 2941, 1695, 1631, 1510, 1260, 1214, 1094, 1033, 798, 760; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 1.24 (t, 3H, –$COOCH_2CH_3$, $J = 6.96$ Hz), 2.32 (m, 4H, –$N(CH_2)_2$– piperazine and s (merged), 9H, $2 \times CH_3$ and > N–CH_3), 3.65 (brs, 7H, –$COOCH_3$ and –$N(CH_2)_2$–, piperazine), 3.81 (s, 3H, –OCH_3), 4.10 (m, 2H, –$COOCH_2CH_3$), 4.67 (s, 2H, –OCH_2CO–), 4.94 (s, 1H, 4-CH, dihydropyridine), 6.00 (t, 1H, –NH, $J = 15.27$ Hz), 6.76 (m, 2H, 5-CH and 6-CH, aromatic), 6.90 (s, 1H, 2-CH, aromatic); Anal. calcd. for $C_{26}H_{35}N_3O_7$: C, 62.26; H, 7.03; N, 8.38; found: C, 61.96; H, 6.59; N, 7.95.

Ethyl methyl 4-(3-methoxy-4-{2-[(3-methylpiperidin-1-yl)oxy]-2-oxoethoxy}phenyl) 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (***15e***). Yield: 85.21%; m.p. 155–157°C. Spectroscopic analysis: IR (KBr) ν_{max}/cm^{-1}: 3380, 3272, 2942, 1687, 1659, 1596, 1497, 1310, 1221, 1123, 1026, 875, 703; ^{1}H NMR ($CDCl_3$, 300 MHz, δ, ppm): 0.88 (t, 3H, –$C(CH_3)$H–, pipec, $J = 5.56$ Hz), 1.13 (m, 1H, –$C(CH_3)$*H*–, pipec), 1.23 (t, 3H, –$COOCH_2CH_3$, $J = 7.11$ Hz), 1.46 (m, 2H, –CH_2–, pipec), 1.69 (m, 2H, –CH_2–, pipec), 2.32 (s, 6H, $2 \times CH_3$), 2.64 (m, 1H, –NCH(H)–, pipec), 2.99 (m, 1H, –NCH(H)–, pipec), 3.65 (s, 3H, –$COOCH_3$), 3.81 (s, 3H, –OCH_3), 3.89 (m, 1H, –NCH(H)–, pipec), 4.10 (m, 2H, –$COOCH_2CH_3$), 4.35 (m, 1H, –NCH(H)–, pipec), 4.67 (s, 2H, –OCH_2CO–), 4.93 (s, 1H, 4-CH, dihydropyridine), 5.92 (t, 1H, –NH, $J =$ 18.50 Hz), 6.71 (m, 1H, 6-CH, aromatic), 6.79 (d, 1H, $J_o =$ 8.28 Hz, 5-CH, aromatic), 6.88 (d, 1H, $J_m = 1.12$ Hz, 2-CH, aromatic); Anal. calcd. for $C_{27}H_{36}N_2O_7$: C, 64.78; H, 7.25; N, 5.60; found: C, 64.66; H, 6.91; N, 5.12.

Biological Methods

Calcium Channel Blocking Activity

The calcium channel blocking activity of newly synthesized compounds was assessed using 96-well plate fluorimetery. Primary cultures of cortex were prepared from neonatal rat pups. The isolated cells were placed onto poly-D-lysine coated 96-well plates (Becton-Dickenson) using a fluid-handling robot (Quadra 96, Tomtec). Each well was loaded with approximately 25,000 cells. The plates were placed into a humidified 5% CO_2 incubator at 37°C and kept for at least 4 days before fluorescence screening. Before the beginning of the experiments, the cells were washed thoroughly with saline solution using Tomtec liquid handling robot. The fluorescence screening was done using fluo-3-ester calcium dye (2 μM). It was loaded into cells with pluronic acid (20%). Once in the cytoplasm, esterases cleave the ester from the fluo-3 dye effectively trapping the dye within the cell. The cells were incubated for 45 min at 37°C. The plates were washed thoroughly before carrying out the experiment. Initial readings were taken with a Cytofluor 4000 HT fluorimeter (Perceptives) set at 36°C to assess the background fluorescence in each of the plates. The excitation/emission settings were 483/530 nm.

Cells were exposed to veratridine for 2 min before readings were taken. For those experiments that require pretreatment, cells were pre-treated for 30 s with the compound before application of veratridine. In each plate, a saline and a veratridine lane were included. The data were analyzed using Microsoft Excel. The initial background readings were subtracted from the treatment readings. The saline control was then subtracted, and the results were expressed as % of the control veratridine response. Increase in intracellular calcium measured with the fluo-3 dye is reflected as rises in fluorescence and decreases in fluorescence represent a drop in intracellular calcium. All compounds were brought up to 10 mM stocks in 100% DMSO and then serially diluted. All the results are expressed as means ± the standard error of the mean (SEM), and statistical analysis was performed with Student's *t*-test. Dose–response curves were analyzed by a sigmoidal curve-fitting analysis to give the IC_{50} values.

Vasodilatory Activity Using KCl (30 mM) (a) and using Phenylephrine (b)

(a) Wistar rats of either sex weighing 300–400 g were killed by a blow on the head. The descending thoracic aorta was rapidly dissected and placed in a physiological saline solution (PSS) of the composition (mM): NaCl 118, $NaHCO_3$ 25, $MgSO_4$ 1.2, $CaCl_2$ 2.5, KH_2PO_4 1.2, KCl 4.75, and glucose 11. After excess of fat and connective tissue was removed, the aorta were cut into rings (4–5 mm in length), mounted under basal tension of 2 g in 5 ml organ baths containing PSS and attached to force–displacement transducers to measure isometric contractile force. The tissue bath was maintained at 37°C and bubbled with 95% O_2–5% CO_2 gas mixture. The preparation was allowed to equilibrate for at least 60 min before initiation of experimental procedures, and during this period the incubation media was changed every 30 min.

After equilibration, aortic rings were contracted by single concentration of 30 mM KCl. When the contractions were stable, compound was added in progressively increasing cumulative concentrations (10^{-8} to 10^{-5} M) at 30-min intervals. The results were expressed as a percentage of maximal control KCl-induced responses, and statistical analysis was performed with Student's *t*-test. Differences between control and experimental values were considered significant when $P < 0.05$. Dose–response slopes were analyzed to give the concentration of compounds producing a 50% inhibition of the maximal contractile response (IC_{50}) by linear regression analysis (method of least squares). The compounds were dissolved in dimethyl sulfoxide (DMSO). DMSO had no effect on KCl-contractile response.

(**b**) Vasodilatory activity was carried out using descending thoracic aortic rings of Wistar rats. The aortic rings were contracted by phenylephrine (10^{-6} M). After stabilization, compounds were added in progressively increasing cumulative concentrations (10^{-8} to 10^{-5} M) at 30-min intervals. All the results were expressed as a percentage of the maximal control phenylephrine-induced responses, and statistical analysis was performed with Student's *t*-test.

Results

Calcium Channel Blocking Activity

The calcium channel blocking activity of newly synthesized compounds **7–9, 7a–d, 8a–f, 9a–g, 10–12, 13–15, 13a–e, 14 a–f,** and **15a–e** was assessed using 96-well plate fluorimetery at L-type calcium channels activated by veratridine in neonatal rat cortical neurons maintained in cell culture. Veratridine (10 μM) was used as the depolarizing agent for all the experiments. Initially, the compounds were screened for calcium channel blocking activity at a single concentration of 10 μM on NG108-15 cells (Neuroblastoma X Glioma). Veratridine-induced increase (5 min read) in intracellular calcium was inhibited with 10 μM nifedipine ($IC_{50} = 57.7 \pm 48.6$ nM) indicating a large component of the veratridine response occurring through L-type channels. Percentage veratridine response produced by various compounds was observed. The compounds that showed more than 50% inhibition of the veratridine response at 10 μM have been selected for further detailed testing to calculate their IC_{50} values. IC_{50} values derived from logistical fits to the calcium flux inhibitory data from various dihydropyridines are shown in Table 1.

Table 1 IC_{50} values derived from logistical fits to the calcium inhibitory data of various dihydropyridines

Compound No. (Code)	$IC_{50} \pm$ SEM (μM)
7 (DPJ-RG-1212)	2.1 ± 4.2
8 (DPJ-RG-1126)	10.0 ± 3.0
8a (DPJ-RG-1161)	9.9 ± 10.0
8b (DPJ-RG-1162)	3.6 ± 3.0
8c (DPJ-RG-1163)	10.0 ± 5.7
8d (DPJ-RG-1164)	10.0 ± 2.9
9b (DPJ-RG-1144)	5.6 ± 1.2
9c (DPJ-RG-1145)	10.0 ± 5.7
9d (DPJ-RG-1160)	9.1 ± 2.1
9e (DPJ-RG-1201)	10.1 ± 7.1
9f (DPJ-RG-1203)	2.5 ± 1.3
9g (DPJ-RG-1159)	10.0 ± 5.8
Nifedipine	57.7 ± 48.6 nM

$P < 0.05$ versus DMSO

Vasodilatory Activity

The compounds **8, 8a, 8b, 8d, 9a, 9b, 9d, 9f**, and **9g** have been screened for vasodilatory activity (Carron *et al.*, 1991; Perez-Vizcaino *et al.*, 1993) using rat thoracic aortic rings, precontracted with phenylephrine (10^{-6} M). The results are expressed as relaxation as a percentage of the maximal control phenylephrine-induced responses. The compounds produced a concentration-dependent inhibition of the contractile response of phenylephrine (Table 2). Relaxation (% of control) and pD_2 ($-\log IC_{50}$) of various compounds to inhibit the contractions induced by phenylephrine (10^{-6} M) are given in Table 3. Compounds **14c**, **15**, and **15c** were also tested for their vasorelaxant action using KCl (30 mM) as the contractile agent. The relaxation (% of control) and pD_2 ($-\log IC_{50}$) values of various compounds to inhibit the contractions produced by KCl (30 mM) are depicted in Table 3. IC_{50} values of the various compounds showing potential vasodilatory activity using phenylephrine (10^{-6} M) and KCl (30 mM) have been calculated and are given in Tables 2 and 3, respectively.

Discussion

Although the carboxylates **7** and **8** substituted at *para* and *meta* positions of the 4-aryl group of symmetrical dihydropyridines displayed medium calcium channel blocking activity, the vanilloid-based derivative **9** with an *ortho*-methoxy group did not exhibit significant activity at 10 μM. The compound **7** displayed the most potent calcium channel inhibiting activity with $IC_{50} = 2.1$ μM.

Table 2 Relaxation (% of control), pD_2 ($-\log IC_{50}$), and IC_{50} (μM) values to inhibit the contractions induced by phenylephrine (10^{-6} M)

Compound No. (Code)	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	pD_2	IC_{50} ± SEM (μM)
8 (DPJ-RG-1126)	0	0	3.1 ± 4.9	47.6 ± 4.4	81.6 ± 1.3	5.84 ± 0.01	1.44 ± 0.01
8a (DPJ-RG-1161)	0	5.6 ± 3.5	26.6 ± 3.2	55.7 ± 2.7	81.5 ± 5.1	6.19 ± 0.11	0.64 ± 0.11
8b (DPJ-RG-1162)	0	0	0.0 ± 0.0	36.2 ± 4.6	60.3 ± 3.0	5.34 ± 0.10	4.57 ± 0.10
8d (DPJ-RG-1164)	0	0	4.1 ± 4.1	26.9 ± 4.2	52.5 ± 6.4	5.02 ± 0.19	9.54 ± 0.19
9a * (DPJ-RG-1143)	0	0.4 ± 1.7	36.1 ± 6.8	56.5 ± 6.3	5.25 ± 0.25	–	–
9b (DPJ-RG-1144)	0	8.1 ± 3.3	30.6 ± 3.1	54.8 ± 5.2	76.1 ± 8.6	6.08 ± 0.20	0.83 ± 0.20
9d (DPJ-RG-1160)	0	0	0.2 ± 0.2	37.5 ± 3.2	51.3 ± 5.6	5.16 ± 0.18	6.91 ± 0.18
9f * (DPJ-RG-1203)	0	0	0.9 ± 0.6	4.3 ± 1.4	8.8 ± 2.4	–	–
9g (DPJ-RG-1159)	0	0	0.4 ± 0.4	36.7 ± 4.3	83.1 ± 2.6	5.76 ± 0.04	1.73 ± 0.04
Nifedipine	0	39.5 ± 8.7	55.7 ± 7.7	68.0 ± 8.5	78.3 ± 4.7	7.56 ± 0.63	27.5 nM

Values are expressed as mean ± SEM, $n = 5$

$P < 0.05$ versus DMSO

*These compounds did not produce 50% relaxation up to 10^{-5} M

Table 3 Relaxation (% of control), pD_2 ($-\text{Log } IC_{50}$), and IC_{50} (μM) values to inhibit the contractions induced by KCl 30 mM on rat aortic rings

Compound No. (Code)	10^{-8} M	10^{-7} M	10^{-6} M	10^{-5} M	3×10^{-5} M	10^{-4} M	pD_2	IC_{50} ± SEM (μM) (K-30)
14c (DPJ-RG-1335)	0	11.42 ± 7.79	44.92 ± 8.97	92.12 ± 2.43	99.50 ± 0.27	100 ± 0.0	5.90 ± 0.10	1.25 ± 0.10
15 (DPJ-RG-1263)	0	5.53 ± 2.57	12.28 ± 4.28	39.96 ± 7.52	64.31 ± 7.77	80.38 ± 6.59	4.89 ± 0.15	12.8 ± 0.15
15c (DPJ-RG-1267)	0	4.27 ± 1.47	23.99 ± 6.43	56.30 ± 10.84	76.18 ± 8.76	85.92 ± 7.82	5.41 ± 0.17	3.89 ± 0.17

	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M	pD_2	IC_{50} ± SEM (μM) (K-30)
Nifedipine	0	8.71 ± 3.17	28.88 ± 3.53	83.63 ± 3.70	99.47 ± 0.29	8.59 ± 0.07	2 nM

Values are expressed as means ± SEM, $n = 5$–8

$P < 0.05$ versus DMSO

Formation of amides of carboxylates resulted in compounds with moderate calcium channel blocking activity with IC_{50} values ranging from 2 to 10 μM; however, there was loss of potency in comparison to nifedipine ($IC_{50} = 57.7$ nM). Even though introduction of unsymmetrical ester groups in the dihydropyridines has been reported to impart more potency (Mannhold, 1994), the introduction of asymmetry in compounds **13a–e**, **14a–d,** and **15a–e** did not even produce 50% inhibition of veratridine response at 10 μM in this study. In general, the newly synthesized dihydropyridines displayed only marginal activity in comparison to nifedipine; however, these compounds are nitro group free and represent a suitable lead to be structurally modified to get therapeutically useful dihydropyridines.

As vasodilators, the compounds are producing significant relaxation of KCl as well as phenylephrine induced contractions suggesting that compounds may be acting at various sites other than calcium channels. Substitution of esters, heterocyclic ring, heteroaromatic, or acyclic amine did not improve upon the vasodilatory potential of the dihydropyridines, rather decreased it in comparison to nifedipine. The symmetrical pyrrolidinyl substituted derivative **8a** displayed maximum potency of 0.64 μM but was found to be approximately 24 times less active in comparison to standard drug nifedipine with $IC_{50} = 27.5$ nM. It seems that conformational and electronic changes caused by the substitution at different sites of the phenyl ring might affect the interaction of the concerned molecule with drug receptors and thereby influence their potency.

Overall, it was observed that incorporation of an ester/amide group on 4-phenyl ring in all the three series of compounds resulted in decreased calcium channel blocking and vasodilatory effects of dihydropyridines in comparison to nifedipine.

Conclusions

Amide derivatives of symmetrical and unsymmetrical 4-aryl-1,4-dihydropyridine carboxylic congeners have been synthesized in this study. The newly synthesized nitro

group free dihydropyridines exhibited moderate calcium channel blocking and vasodilatory activity. Further structural modifications might lead to a new series of therapeutically useful dihydropyridines.

Acknowledgments The authors are thankful to University Grants Commission, India for providing financial support.

References

Carron R, Perez-Vizcaino F, Delphon E, Tamargo J (1991) Effects of propafenone on 45 Ca movements and contractile responses in vascular smooth muscle. Br J Pharmacol 103:1453–1457

Huber D, Ehrhardt JD, Decker N, Himber J, Andermann G, Leclerc G (1991) Synthesis and ocular antihypertensive activity of new imidazolidine derivatives containing a beta-blocking side chain. J Med Chem 34:3197–3204

Iwanami M, Shibanuma T, Fujimoto M, Kawai R, Tamazawa K, Takenaka T, Takahashi K, Murakami M (1979) Synthesis of new water-soluble dihydropyridine vasodilators. Chem Pharm Bull 27:1426–1440

Jain P, Naran G, Jindal DP, Bansal R, Calle C, Carron R, Pemberton K, Harvey AL (2006) Synthesis of a new series of 4-aryl-1,4-dihydropyridines with calcium channel blocking and vasodilatory activity. Pharmazie 61:400–405

Janis RA, Triggle DJ (1983) New developments in Ca^{2+} channel antagonists. J Med Chem 26:775–785

Klotz V (2002) Interaction potential of lercanidipine, a new vasoselective dihydropyridine calcium antagonist. Arzneim Forsch/Drug Res 52:155–161

Liang JC, Yeh JL, Chiang LC, Yang YC, Sheu SH, Lai WT, Chen IJ (2000) Labedipinedilol-A: a vanilloid-based α/β-adrenoceptor blocker with calcium entry blocking and long-lasting antihypertensive properties. Drug Dev Res 49:94–108

Liang JC, Yeh JL, Wang CS, Liou SF, Tsai CH, Chen IJ (2002) The new generation dihydropyridine type calcium blockers, bearing 4-phenyl oxypropanolamine, display alpha-/beta-adrenoceptor antagonist and long-acting antihypertensive activities. Bioorg Med Chem 10:719–730

Mannhold R (1994) Medicinal chemistry of DHP-like calcium antagonist. Drugs Today 30:103–122

Meredith PA (1999) Lercanidipine: a novel lipophilic dihydropyridine calcium antagonist with long duration of action and high vascular selectivity. Expert Opin Investig Drugs 8:1043–1062

Perez-Vizcaino F, Carron R, Delphon E, Tamargo J (1993) Effects of (S)-nafenodone on 45 Ca^{2+} fluxes and contractions in rat isolated vascular smooth muscle. Eur J Pharmacol 232:105–111

Ramesh M, Matowe WC, Akula MR, Vo D, Dagnino L, Li-Kwong-Ken MC, Wolowyk MW, Knaus EE (1998) Synthesis and calcium channel-modulating effects of alkyl (or cycloalkyl)1,4-dihydro-2,6-dimethyl-3-nitro-4-pyridyl-5-pyridine-carboxylate racem-ates and enantiomer. J Med Chem 41:509–514

Steffen HM (1999) Amlodipine: a third generation dihydropyridine calcium antagonist. J Clin Basic Cardiol 2:45–52

Triggle DJ (2003) 1,4-dihydropyridines as calcium channel ligands and privileged structures. Cell Mol Neurobiol 23:293–303

Yeh JL, Liou SF, Liang JC, Huang YC, Chiang LC, Wu JR, Lin YT, Chen IJ (2000) Vanidipinedilol: a vanilloid-based beta-adrenoceptor blocker displaying calcium entry blocking and vasorelaxant activities. J Cardiovasc Pharmacol 35:51–63