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Synthesis of a new series of 4-aryl-1,4-dihydropyridines with calcium channel blocking and vasodilatory activity

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Synthesis of a new series of 4-aryl-1,4-dihydropyridines possessing potential calcium channel blocking activity along with good vasodilatory profile is reported. The compounds were synthesized using modified Hantzsch condensation of various aldehydes with methyl 3-aminocrotonate in the presence of a catalytic amount of trifluoroacetic acid and subsequent alkylation with various hydrochlorides of dialkylaminoalkyl chlorides. *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. Many compounds exhibited moderate to significant calcium channel blockade around 1 μM. The vasodilatory activity was assessed on isolated rat thoracic aortic rings precontracted by phenylephrine/KCl (30 mM). Most of the compounds produced a concentration-dependent inhibition of the contractile response.

1. Introduction

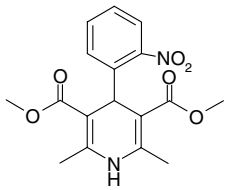
Calcium channel blockers (CCBs), particularly 4-aryl-1,4-dihydropyridines (DHP), attract the interest of medicinal chemists because of their high potency, selectivity of action and heterogeneity. Nifedipine (**1**), a prototype of this class of compounds, has been used in general medical practice worldwide for the treatment of hypertension and vasospastic angina for over two decades (Abbernethy and Schwartz 1999; Hernández-Hernández et al. 2002; Messerli 2002; Triggle 2003a, 2003b; Eisenberg et al. 2004). Despite the high potency and selectivity of action, these drugs are not free from side effects and associated with an increased mortality rate. One of the most discussed undesirable side effects of dihydropyridines is the consistent reflex activation of the adrenergic system leading to tachycardia (Bala 1982; Furberg et al. 1995; Beever and Sleight 1996; Furberg et al. 1996; Califf and Kramer 1998). A dihydropyridine type calcium channel and α-adrenoceptor blocker without revealing reflex tachycardial activity is an important target in the research of new drugs in this field.

Structure-activity relationship studies (SAR) of the dihydropyridines reveal the importance of a phenyl ring at 4-position of the basic dihydropyridine nucleus. Structural modifications at various positions of the phenyl ring affect the activity profile of this class of calcium channel blockers (Mannhold 1994; Romero et al. 2003). It has been observed that the introduction of electron-donating substituents at ortho and meta positions of the 4-phenyl ring results in a decrease in activity whereas electron-withdrawing ortho and meta substituents increases the vasorelaxant activity. Lengthy (but not bulky) substituents at meta and para positions reduce the activity (Satoh et al. 1991; Kanno et al. 1992; Loev et al. 1974). Aryl moieties bearing a basic side chain have been considered as an essential active structure in a number of known calcium channel blockers such as verapamil, diltiazem, prenylamine, fendiline, cinnarizine etc. A new generation of long acting, relatively side effect free potent dihydropyridine type calcium channel blockers (**2**, **3**), synthesized by introducing basic side chains at various positions of the 4-phenyl ring by replacing the phenolic hydroxy group, has recently been reported (Nobuhiro et al. 1999; Yeh et al. 2000; Liang et al. 2002).

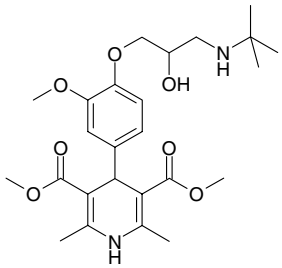
We hypothesized on the basis of these studies that the substitution of the hydroxy group at the phenyl ring at different positions can be an interesting target to introduce various basic moieties and it was thought worthwhile to discover appropriate substitutions on the 4-phenyl ring, which may affect the pharmacological activity pattern of these compounds.

We selected the dialkylaminoalkyl moiety as basic side chain because such a group is also present in many potent vasodilators (Leonardi et al. 1998).

In the present study, we report the synthesis, structural characterization, *in vitro* calcium channel blocking and vasodilatory effects of some newly synthesized 4-aryl-1,4-dihydropyridine derivatives. Furthermore, these compounds are structurally lacking a nitro group, a functionality that usually imparts chemical instability and mutagenicity to the molecules (Debnath et al. 1991).



(1)



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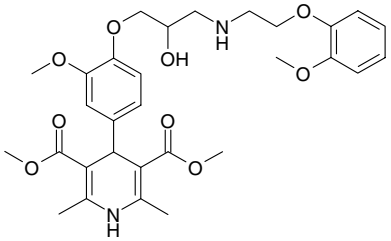


(3)

2. Investigations and results

We explored the potential of basic side chains substituted at meta and para position of the 4-phenyl ring in affecting the activity of 4-aryl-1,4-dihydropyridine type calcium channel blockers. Various new dihydropyridines have been synthesized using modified Hantzsch condensation (Gasco et al. 1996) and subsequent alkylation.

2.1. Synthesis of the compounds

Synthesis of the parent 4-aryl-1,4-dihydropyridine skeletons (**7**, **14**, **21**) has been carried out by condensing corresponding aldehydes such as vanillin, 3-hydroxybenzaldehyde and 4-hydroxybenzaldehyde with methyl 3-aminocrotonate in the presence of a catalytic amount of trifluoroacetic acid as shown in the Scheme. All of the three products exhibited sharp singlets for $-CH_3$ in the range of δ 2.31–2.33 and for $-COOCH_3$ at δ 3.64–3.66 ppm. A small singlet at δ 5.61–5.62 ppm for N–H confirmed the formation of the dihydropyridine nucleus.

These 4-aryl-dihydropyridines **7**, **14**, **21** were further alkylated with hydrochlorides of various dialkylaminoalkyl chlorides like *N,N*-dimethylaminoethyl chloride, *N,N*-diethylaminoethyl chloride, 4-(2-chloroethyl)morpholine, 1-(2-chloroethyl)pyrrolidine and 1-(2-chloroethyl)piperidine in ethyl methyl ketone using anhydrous potassium carbonate to get compounds **8**–**12**, **15**–**19**, **22**–**26** (Table 1). The cyclopentyl derivatives **13**, **20**, **27** were also synthesized using cyclopentyl bromide as alkylating agent in order to study the effect of a cyclic ring on the pharmacological profile.

The structural assignments of the newly synthesized compounds were based on IR, UV, ^{1}H NMR spectral data and elemental analyses, which were fully consistent with the proposed structures.

2.2. Pharmacological results

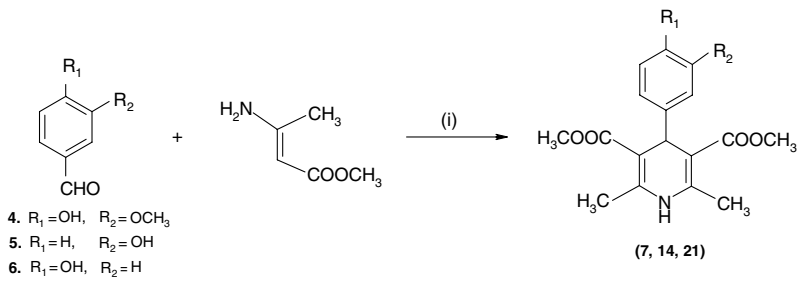
The newly synthesized compounds were screened for their inhibitory activity at L-type calcium channels activated by veratridine in neonatal rat cortical neurons maintained in cell culture. Initial concentration-response data for veratridine were collected. Veratridine (10 μM) was used as the depolarizing agent for all the experiments. IC_{50} values derived from logistical fits to the calcium flux inhibitory data from various dihydropyridines are shown in Table 2. Many of the compounds showed moderate to significant activity. The most active compound, **22**, of the series exhibited calcium channel blocking activity at 0.16 μM.

The compounds have also been screened for their vasodilatory activity (Carron et al. 1991; Perez-Vizcaino et al. 1993) using rat thoracic aortic rings, which were contracted either with phenylephrine (10^{-6} M) or with KCl (30 mM). The results are expressed as relaxation as a percentage of the maximal control phenylephrine-induced/KCl-induced responses. Many compounds produced a concentration-dependent inhibition of the contractile response of phenylephrine/KCl; their IC_{50} values are given in Table 3.

3. Discussion

Introduction of a dialkylaminoalkyl group at the 3 or 4 position of the 4-aryl moiety resulted in compounds **9**, **10**, **12**, **15**, **18**, **19**, **22**, **23**, **26** with significant calcium channel blocking activity, while compounds **11**–**13**, **17**–**19**, **26** and **27** inhibited phenylephrine-induced contractions in isolated rat aortal rings when compared with nifedipine. The compounds **11**–**13**, **18**, **19** and **27** also produced relaxation of KCl (30 mM) induced contractions at a similar concentration range as required for phenylephrine suggesting that at these concentrations, compounds may be acting at various sites other than calcium channels. Substitution

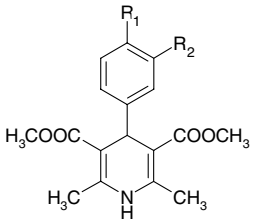
Scheme (i) trifluoroacetic acid, absolute alcohol, 0 °C



4. $R_1 = OH$, $R_2 = OCH_3$
5. $R_1 = H$, $R_2 = OH$
6. $R_1 = OH$, $R_2 = H$

(7, 14, 21)

Table 1: Chemical data of compounds 7–27



(7-27)

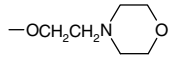
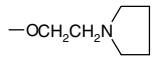
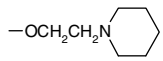
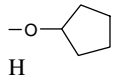
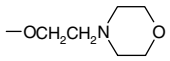
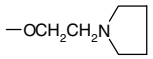
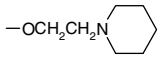
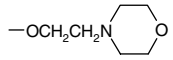
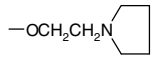
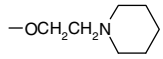
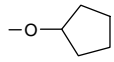
Compd.	R_1	R_2	M.P. (°C)	Crystallizing solvent
7	$-OH$	$-OCH_3$	240–245	Acetone
8	$-OCH_2CH_2N(CH_3)_2$	$-OCH_3$	98–101	Ether
9	$-OCH_2CH_2N(C_2H_5)_2$	$-OCH_3$	92–95	Ether
10		$-OCH_3$	158–160	Ether + Ethyl acetate
11		$-OCH_3$	178–180	Ether + Ethyl acetate
12		$-OCH_3$	162–165	Ether + Ethyl acetate
13		$-OCH_3$	147–149	Ether + Ethyl acetate
14	H	$-OH$	231–236	Acetone
15	H	$-OCH_2CH_2N(CH_3)_2$	120–125	Ether
16	H	$-OCH_2CH_2N(C_2H_5)_2$	110–115	Petroleum ether (60 : 80)
17	H		132–136	Petroleum ether (60 : 80)
18	H		96–98	Ether
19	H		120–124	Petroleum ether (60 : 80)
20	H		196–198	Ether + Ethyl acetate
21	$-OH$	H	231–233	Acetone
22	$-OCH_2CH_2N(CH_3)_2$	H	169–171	Ether + Ethyl acetate
23	$-OCH_2CH_2N(C_2H_5)_2$	H	122–123	Petroleum ether (60 : 80) + Ether
24		H	140–142	Ether + Ethyl acetate
25		H	155–156	Ether + Ethyl acetate
26		H	153–155	Ether + Ethyl acetate
27		H	174–176	Ethyl acetate

Table 2: IC_{50} values derived from logistical fits to the calcium inhibitory data from various dihydropyridines

Compd.	$IC_{50} \pm$ SEM (μM)	Compd.	$IC_{50} \pm$ SEM (μM)
8*	–	**19**	80 ± 20
9	90 ± 90	**20***	–
10	5.3 ± 2.6	**22**	0.161 ± 0.076
11*	–	**23**	2.5 ± 1.8
12	4.7 ± 1.3	**24***	–
13*	–	**25***	–
15	20.0 ± 4.9	**26**	70.0 ± 20.0
16*	–	**27***	–
17*	–	Nifedipine	57.7 ± 48.6 nM
18	3.2 ± 1.1	–	–

* These compounds did not show significant calcium channel blocking activity at 100 μM concentration

of different side chains at meta and para positions of the 4-aryl group led to diversified pharmacological activity with some of the compounds displaying potent calcium channel blocking activity through inhibition of L-type calcium channels and others producing good vasorelaxation, a few possibly through α_1-adrenoceptor blocking effects. The findings implied that the substitutions on the hydroxy group of the 4-phenyl ring with a dialkylaminoalkyl side chain may lead to an additional adrenoceptor blockade. It was observed that substitution of various functionalities on the 4-aryl group produced dramatic alterations in the pharmacological activity of the dihydropyridines. 4[4-(2-Dimethylaminoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**22**) exhibited maximum calcium channel blocking activity with an IC_{50} of 0.16 μM but did not produce any vasorelaxant effect in phenylephrine as well as KCl (30 mM) induced vasocon-

Table 3: IC_{50} values of various dihydropyridines to inhibit the contractions induced by phenylephrine (10^{-6} M) and KCl (30 mM) on isolated rat thoracic aortic rings

Compd.	$IC_{50} \pm$ SEM (μM) PE	$IC_{50} \pm$ SEM (μM) K-30	Compd.	$IC_{50} \pm$ SEM (μM) PE	$IC_{50} \pm$ SEM (μM) K-30
8*	–	–	**19**	4.3 ± 0.14	1.6 ± 0.09
9*	–	–	**20***	–	–
10*	–	–	**22***	–	–
11	5.8 ± 0.18	3.7 ± 0.17	**23***	–	–
12	1.8 ± .015	4.8 ± 0.06	**24***	–	–
13	8.1 ± 0.2	6.1 ± 0.08	**25***	–	–
15	–	7.9 ± 0.10	**26**	3.8 ± 0.14	–
16*	–	–	**27**	6.2 ± 0.43	4.7 ± 0.02
17	5.8 ± 0.05	–	Nifedipine	27.5 nM	2 nM
18	0.9 ± 0.14	6.7 ± 0.12	–	–	–

The asterisks (*) denotes the compounds which did not produce 50% relaxation at 10^{-5} M

striction. However, the cyclopentyl derivatives **13**, **20** and **27** completely lacked the calcium channel blocking activity but **13** and **27** produced significant vasodilatory activity. Three compounds, **12**, **18** and **19**, exhibited moderate to good calcium channel blocking activity along with additional α_1-adrenoceptor blockade. Based on these observations, it is suggested that conformational and electronic change 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. Suitable structural modifications of the 4-phenyl group in the 4-aryl-1,4-dihydropyridines can lead to a nitro group-free compound with potent calcium channel blocking activity and additional vasorelaxant properties through different mechanisms.

4. Experimental

4.1. Chemistry

Melting points were determined on a Veego melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 882 spectrophotometer model and Perkin-Elmer spectrum RX 1, FT-IR spectrophotometer models using potassium bromide pellets. Proton-NMR spectra were recorded on Bruker AC-300F, 300 MHz using deuterated-chloroform or deuterated dimethylsulfoxide-containing tetramethylsilane as internal standard (chemical shifts in δ, ppm). The purity of compounds was established by thin layer chromatography (TLC) and by elemental analyses. Elemental analyses were carried out on a Perkin-Elmer-2400 model CHN analyzer. All solvents were distilled prior to use according to standard procedures.

4.1.1. General procedure for the synthesis of parent dihydropyridines **7**, **14**, **21**

A solution of methyl 3-aminocrotonate (1 g, 7.75 mmol) in absolute alcohol (10 ml) was added drop wise to an ice-cooled solution of respective aldehyde (2.58 mmol) in absolute alcohol (10 ml) containing catalytic amount of trifluoroacetic acid (0.25 ml). The reaction mixture was further stirred at 0 °C for 3 h. The precipitate obtained was filtered off, washed with cold ethanol (25%) and crystallized using appropriate solvents (Table 1).

4.1.1.1. 4-(4-Hydroxy-3-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**7**)

From 4-hydroxy-3-methoxybenzaldehyde (0.39 g). Yield: 0.68 g, 76.40%. IR (ν_{max}): 3380, 2980, 1690, 1650, 1480, 1210, 1100, 1010 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.33 (6 H, s, 2X-C*H*$_3$), 3.65 (6 H, s, 2X-COOC*H*$_3$), 3.84 (3 H, s, −OC*H*$_3$), 4.93 (1 H, s, 4-C*H* DHP), 5.44 (1 H, s, −O*H*), 5.61 (1 H, s, −N*H*), 6.72 (2 H, m, Ar−*H*), 6.83 (1 H, d, J_m = 1.3 Hz, Ar−*H*) ppm.
$C_{18}H_{20}NO_6$

4.1.1.2. 4-(3-Hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**14**)

From 3-hydroxybenzaldehyde (0.31 g). Yield: 0.69 g, 87.89%. IR (ν_{max}): 3370, 2950, 1695, 1650, 1490, 1210, 1010 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.33 (6 H, s, 2X-C*H*$_3$), 3.65 (6 H, s, 2X-COOC*H*$_3$), 4.98 (1 H, s, 4-C*H* DHP), 5.62 (1 H, s, N*H*), 6.61 (1 H, dd, J_o = 8.2 Hz, J_m = 2.31 Hz, Ar−*H*), 6.73 (1 H, s, Ar−*H*), 6.83 (1 H, d, J_o = 7.17 Hz, Ar−*H*), 7.07 (1 H, t, J_o = 7.74 Hz, Ar−*H*) ppm.
$C_{17}H_{19}NO_5$

4.1.1.3. 4-(4-Hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**21**)

From 4-hydroxybenzaldehyde (0.31 g). Yield: 0.71 g, 87.43%. FT-IR (ν_{max}): 3330, 2940, 1701, 1652, 1484, 1227, 1017 cm^{-1}. ^{1}H-NMR ($CDCl_3$ + DMSO-d_6): δ 2.30 (6 H, s, 2X-C*H*$_3$), 3.61 (6 H, s, 2X-COOC*H*$_3$), 4.84 (1 H, s, 4-C*H* DHP), 6.64 (2 H, dd, J_o = 8.28 Hz, J_m = 1.42 Hz, Ar−*H*s), 7.03 (2 H, dd, J_o = 6.77 Hz, J_m = 1.95 Hz, Ar−*H*s), 7.89 (1 H, s, −O*H*), 8.51 (1 H, s, −N*H*) ppm.
$C_{17}H_{19}NO_5$

4.1.2. General procedure for alkylation of parent dihydropyridines **7**, **14**, **21** *to afford* **8–13**, **15–20**, **22–27**

A mixture of parent dihydropyridine (1.0 g, (**7**) 2.88 mmol, (**14**) 3.15 mmol, (**21**) 3.15 mmol) and anhydrous potassium carbonate (1.0 g, 7.24 mmol) in ethyl methyl ketone (35 ml) was refluxed for 2 h with stirring. To this, the required hydrochloride of various dialkylaminoalkyl chlorides was added and the reaction mixture was refluxed for another 6 h. The resulting slurry was filtered off, solvent was recovered under reduced pressure to obtain oily residues of the corresponding products **8–13**, **15–20** and **22–27**, which were crystallized using appropriate solvents (Table 1).

4.1.2.1. 4-[4-(2-Dimethylaminoethoxy)-3-methoxyphenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**8**)

From 2-(dimethylamino)ethyl chloride hydrochloride (0.83 g, 5.76 mmol). Yield: 0.72 g, 59.80%. IR (ν_{max}): 3350, 2950, 1700, 1650, 1500, 1380, 1210, 1020 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.31 (6 H, s, −N(C*H*$_3$)$_2$), 2.33 (6 H, s, 2X-C*H*$_3$), 2.74 (2 H, t, −C*H*$_2$N<), 3.65 (6 H, s, 2X-COOC*H*$_3$), 3.81 (3 H, s, −OC*H*$_3$), 4.04 (2 H, t, −OC*H*$_2$−), 4.94 (1 H, s, 4-C*H* DHP), 5.73 (1 H, s, −N*H*), 6.73 (2 H, s, Ar−*H*s), 6.86 (1 H, s, Ar−*H*) ppm.
$C_{22}H_{30}N_2O_6$

4.1.2.2. 4-[4-(2-Diethylaminoethoxy)-3-methoxyphenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**9**)

From 2-(diethylamino)ethyl chloride hydrochloride (0.99 g, 5.76 mmol). Yield: 0.68 g, 52.91%. IR (ν_{max}): 3350, 2950, 1700, 1660, 1470, 1210, 1020 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.04 (6 H, t, −N(CH$_2$C*H*$_3$)$_2$), 2.32 (6 H, s, 2X-C*H*$_3$), 2.61 (4 H, q, −N(C*H*$_2$CH$_3$)$_2$), 2.87 (2 H, t, −C*H*$_2$N<), 3.65 (6 H, s, 2X-COOC*H*$_3$), 3.81 (3 H, s, −OC*H*$_3$), 4.02 (2 H, t, −OC*H*$_2$−), 4.94 (1 H, s, 4-C*H* DHP), 6.16 (1 H, s, −N*H*), 6.73 (2 H, s, Ar−*H*s), 6.86 (1 H, s, Ar−*H*) ppm.
$C_{24}H_{34}N_2O_6$

4.1.2.3. 4-[3-Methoxy-4-(2-morpholin-4-yl-ethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**10**)

From 4-(2-chloroethyl)morpholine hydrochloride (1.07 g, 5.76 mmol). Yield: 0.85 g, 64.15%. IR (ν_{max}): 3250, 2950, 1690, 1650, 1500, 1210, 1100, 1010 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 2.33 (6 H, s, 2X-C*H*$_3$), 2.57 (4 H, t, 2X-C*H*$_2$ morph), 2.80 (2 H, t, −C*H*$_2$N<), 3.65 (6 H, s, 2X-COOC*H*$_3$), 3.71 (4 H, t, 2X-C*H*$_2$ morph), 3.80 (3 H, s, −OC*H*$_3$), 4.09 (2 H, t, −OC*H*$_2$−), 4.95 (1 H, s, 4-C*H* DHP), 5.73 (1 H, s, −N*H*), 6.73 (2 H, s, Ar−*H*s), 6.86 (1 H, s, Ar−*H*) ppm.
$C_{24}H_{32}N_2O_7$

4.1.2.4. 4-[3-Methoxy-4-(2-pyrrolidin-1-yl-ethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**11**)

From 1-(2-chloroethyl)pyrrolidine hydrochloride (0.98 g, 5.76 mmol). Yield: 0.54 g, 42.22%. IR (ν_{max}): 3340, 2930, 1700, 1640, 1470, 1200, 1010 cm^{-1}. ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 1.78 (4 H, p, 2X-C*H*$_2$ pyr), 2.31 (6 H, s, 2X-C*H*$_3$), 2.60 (4 H, t, 2X-C*H*$_2$ pyr), 2.88 (2 H, t, −C*H*$_2$N<),

3.62 (6 H, s, 2X-COOCH_3), 3.79 (3 H, s, −OCH_3), 4.06 (2 H, t, −OCH_2−), 4.89 (1 H, s, 4-C*H* DHP), 6.72 (2 H, s, Ar−*H*s), 6.84 (1 H, s, Ar−*H*), 8.09 (1 H, s, −N*H*) ppm.
$C_{24}H_{32}N_2O_6$

4.1.2.5. 4-[3-Methoxy-4-(2-piperidin-1-yl-ethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**12**)

From 1-(2-chloroethyl)piperidine hydrochloride (1.06 g, 5.76 mmol). Yield: 0.68 g, 51.55%. IR (ν_{max}): 3350, 2940, 1690, 1650, 1500, 1265, 1200, 1020 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.45 (d, 2 H, −CH_2 pip), 1.59 (4 H, q, 2X-CH_2 pip), 2.33 (6 H, s, 2X-CH_3), 2.51 (4 H, t, 2X-CH_2 pip), 2.80 (2 H, t, −CH_2N<), 3.65 (6 H, s, 2X-COOCH_3), 3.81 (3 H, s, −OCH_3), 4.09 (2 H, t, −OCH_2−), 4.94 (1 H, s, 4-C*H* DHP), 5.63 (1 H, s, −N*H*), 6.73 (2 H, s, Ar−*H*s), 6.86 (1 H, s, Ar−*H*) ppm.
$C_{25}H_{34}N_2O_6$

4.1.2.6. 4-(4-Cyclopentyloxy-3-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**13**)

From cyclopentyl bromide (0.617 ml, 5.76 mmol). Yield: 0.64 g, 53.55%. IR (ν_{max}): 3350, 2940, 1690, 1500, 1200, 1020 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.58 (2 H, m, −CH_2 cyclopent), 1.83 (6 H, m, 3X-CH_2 cyclopent), 2.29 (6 H, s, 2X-CH_3), 3.65 (6 H, s, 2X-COOCH_3), 3.76 (3 H, s, −OCH_3), 4.67 (1 H, s, −C*H* cyclopent), 4.85 (1 H, s, 4-C*H* DHP), 5.97 (1 H, s, −N*H*), 6.62 (2 H, s, Ar−*H*s), 6.75 (1 H, s, Ar−*H*) ppm.
$C_{23}H_{29}NO_6$

4.1.2.7. 4-[3-(2-Dimethylaminoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**15**)

From 2-(dimethylamino)ethyl chloride hydrochloride (0.9 g, 6.30 mmol). Yield: 0.68 g, 55.40%. IR (ν_{max}): 3230, 2950, 1690, 1640, 1490, 1330, 1210, 1010 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.31 (12 H, s, −N(CH_3)$_2$ and 2X-CH_3), 2.69 (2 H, t, −CH_2N<), 3.64 (6 H, s, 2X-COOCH_3), 4.01 (2 H, t, −OCH_2−), 4.98 (1 H, s, 4-C*H* DHP), 5.80 (1 H, s, −N*H*), 6.68 (1 H, dd, J_o = 8.01 Hz, J_m = 2.26 Hz, Ar−*H*), 6.86 (2 H, m, Ar−*H*s), 7.11 (1 H, t, J_o = 7.80 Hz, Ar−*H*) ppm.
$C_{21}H_{28}N_2O_5$

4.1.2.8. 4-[3-(2-Diethylaminoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**16**)

From 2-(diethylamino)ethyl chloride hydrochloride (1.08 g, 6.30 mmol). Yield: 0.65 g, 49.58%. IR (ν_{max}): 3350, 2950, 1700, 1640, 1480, 1220, 1010 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.05 (6 H, t, −N(CH$_2$CH_3)$_2$), 2.32 (6 H, s, 2X-CH_3), 2.62 (4 H, q, −N(CH_2CH$_3$)$_2$), 2.84 (2 H, t, −CH$_2$N<), 3.64 (6 H, s, 2X-COOCH_3), 4.00 (2 H, t, −OCH_2−), 4.98 (1 H, s, 4-C*H* DHP), 5.68 (1 H, s, −N*H*), 6.68 (1 H, dd, J_o = 8.05 Hz, J_m = 2.70 Hz, Ar−*H*), 6.84 (2 H, m, Ar−*H*s), 7.11 (1 H, t, J_o = 7.94 Hz, Ar−*H*) ppm.
$C_{23}H_{32}N_2O_5$

4.1.2.9. 4-[3-(2-Morpholin-4-yl-ethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**17**)

From 4-(2-chloroethyl)morpholine hydrochloride (1.17 g, 6.30 mmol). Yield: 0.69 g, 50.88%. IR (ν_{max}): 3300, 2880, 1700, 1640, 1500, 1230, 1110, 1030 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.33 (6 H, s, 2X-CH_3), 2.57 (4 H, t, 2X-CH_2 morph), 2.78 (2 H, t, −CH_2N<), 3.64 (6 H, s, 2X-COOCH_3), 3.73 (4 H, t, 2X-CH_2 morph), 4.06 (2 H, t, −OCH_2−), 4.99 (1 H, s, 4-C*H* DHP), 5.73 (1 H, s, −N*H*), 6.68 (1 H, dd, J_o = 7.98 Hz, J_m = 2.98 Hz, Ar−*H*), 6.84 (2 H, m, Ar−*H*s), 7.11 (1 H, t, J_o = 7.83 Hz, Ar−*H*) ppm.
$C_{23}H_{30}N_2O_6$

4.1.2.10. 4-[3-(2-Pyrrolidin-1-yl-ethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**18**)

From 1-(2-chloroethyl)pyrrolidine hydrochloride (1.07 g, 6.30 mmol). Yield: 0.63 g, 48.27%. IR (ν_{max}): 3380, 2950, 1700, 1660, 1460, 1210, 1010 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.83 (4 H, m, 2X-CH_2 pyr), 2.33 (6 H, s, 2X-CH_3), 2.64 (4 H, m, 2X-CH_2 pyr), 2.90 (2 H, t, −CH_2N<), 3.64 (6 H, s, 2X-COOCH_3), 4.08 (2 H, t, −OCH_2−), 4.98 (1 H, s, 4-C*H* DHP), 5.67 (1 H, s, −N*H*), 6.69 (1 H, dd, J_o = 7.91 Hz, J_m = 2.09 Hz, Ar−*H*), 6.85 (2 H, m, Ar−*H*s), 7.11 (1 H, t, J_o = 7.90 Hz, Ar−*H*) ppm.
$C_{23}H_{30}N_2O_5$

4.1.2.11. 4-[3-(2-Piperidin-1-yl-ethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**19**)

From 1-(2-chloroethyl)piperidine hydrochloride (1.16 g, 6.30 mmol). Yield: 0.59 g, 43.70%. IR (ν_{max}): 3350, 2950, 1700, 1640, 1470, 1210, 1020 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.22 (2 H, q, −CH_2 pip), 1.45 (4 H, t, 2X-CH_2 pip), 2.32 (6 H, s, 2X-CH_3), 2.49 (4 H, t, 2X-CH_2 pip), 2.74 (2 H, t, −CH_2N<), 3.64 (6 H, s, 2X-COOCH_3), 4.05 (2 H, t, −OCH_2−), 4.98 (1 H, s, 4-C*H* DHP), 5.85 (1 H, s, −N*H*), 6.67 (1 H, dd, J_o = 8.10 Hz, J_m = 2.56 Hz, Ar−*H*), 6.84 (2 H, m, Ar−*H*s), 7.10 (1 H, t, J_o = 7.80 Hz, Ar−*H*) ppm.
$C_{24}H_{32}N_2O_5$

4.1.2.12. 4-(3-Cyclopentyloxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**20**)

From cyclopentyl bromide (0.676 ml, 6.30 mmol). Yield: 0.71 g, 58.48%. IR (ν_{max}): 3351, 2948, 1705, 1651, 1486, 1220, 1012 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.60 (2 H, m, −CH_2 cyclopent), 1.81 (6 H, m, 3X-CH_2 cyclopent), 2.33 (6 H, s, 2X-CH_3), 3.65 (6 H, s, 2 X−COOCH_3), 4.69 (1 H, m, −C*H* cyclopent), 4.98 (1 H, s, 4-C*H* DHP), 5.72 (1 H, s, −N*H*), 6.64 (1 H, dd, J_o = 8.10 Hz, J_m = 2.61 Hz, Ar−*H*), 6.80 (2 H, m, Ar−*H*s), 7.09 (1 H, t, J_o = 7.83 Hz, Ar−*H*) ppm.
$C_{22}H_{27}NO_5$

4.1.2.13. 4-[4-(2-Dimethylaminoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**22**)

From 2-(dimethylamino)ethyl chloride hydrochloride (0.9 g, 6.30 mmol). Yield: 0.56 g, 45.78%. FT-IR (ν_{max}): 3266, 2947, 1696, 1644, 1504, 1307, 1211, 1025 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.31 (12 H, s, 2X-CH_3, −N(CH_3)$_2$), 2.69 (2 H, t, −OCH_2−), 3.63 (6 H, s, 2X-COOCH_3), 4.00 (2 H, t, −CH_2N<), 4.89 (1 H, s, 4-C*H* DHP), 5.87 (1 H, s, −N*H*), 6.72 (2 H, dd, J_o = 6.79 Hz, J_m = 1.95 Hz, Ar−*H*s), 7.12 (2 H, dd, J_o = 6.75 Hz, J_m = 2.81 Hz, A_γ−H_5) ppm.
$C_{21}H_{28}N_2O_5$

4.1.2.14. 4-[4-(2-Diethylaminoethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**23**)

From 2-(diethylamino)ethyl chloride hydrochloride (1.08 g, 6.30 mmol). Yield: 0.61 g, 46.49%. FT-IR (ν_{max}): 3344, 2956, 1697, 1650, 1492, 1300, 1225, 1108, 1021 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.04 (6 H, t, −N(CH$_2$CH_3)$_2$), 2.31 (6 H, s, 2 X−CH_3), 2.61 (4 H, q, −N(CH_2CH$_3$)$_2$), 2.83 (2 H, t, −CH_2N<), 3.63 (6 H, s, 2 X−COOCH_3), 3.98 (2 H, t, −OCH_2−), 4.93 (1 H, s, 4-C*H* DHP), 5.95 (1 H, s, −N*H*), 6.74 (2 H, dd, J_o = 6.77 Hz, J_m = 1.90 Hz, Ar−*H*s), 7.15 (2 H, dd, J_o = 6.71 Hz, J_m = 1.98 Hz, Ar−*H*s) ppm.
$C_{23}H_{32}N_2O_5$

4.1.2.15. 4-[4-(2-Morpholin-4-yl-ethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**24**)

From 4-(2-chloroethyl)morpholine hydrochloride (1.17 g, 6.30 mmol). Yield: 0.74 g, 54.57%. FT-IR (ν_{max}): 3338, 2958, 1696, 1628, 1498, 1379, 1222, 1104, 1052 cm^{-1}. ^1H NMR ($CDCl_3$): δ 2.31 (6 H, s, 2X-CH_3), 2.55 (4 H, t, 2X-CH_2 morph), 2.75 (2 H, t, −CH_2N<), 3.64 (6 H, s, 2X-COOCH_3), 3.69 (4 H, t, 2X-CH_2 morph), 4.04 (2 H, t, −OCH_2−), 4.89 (1 H, s, 4-C*H* DHP), 5.67 (1 H, s, −N*H*), 6.71 (2 H, dd, J_o = 6.92 Hz, J_m = 1.66 Hz, Ar−*H*s), 7.12 (2 H, dd, J_o = 6.72 Hz, J_m = 1.98 Hz, Ar−*H*s) ppm.
$C_{23}H_{30}N_2O_6$

4.1.2.16. 4-[4-(2-Pyrrolidin-1-yl-ethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**25**)

From 1-(2-chloroethyl)pyrrolidine hydrochloride (1.07 g, 6.30 mmol). Yield: 0.56 g, 41.48%. FT-IR (ν_{max}): 3299, 2938, 1697, 1639, 1497, 1379, 1234, 1050 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.79 (4 H, p, 2X-CH_2 pyr), 2.32 (6 H, s, 2X-CH_3), 2.59 (4 H, t, 2X-CH_2 pyr), 2.87 (2 H, t, −CH_2N<), 3.64 (6 H, s, 2X-COOCH_3), 4.05 (2 H, t, −OCH_2−), 4.93 (1 H, s, 4-C*H* DHP), 5.89 (1 H, s, −N*H*), 6.76 (2 H, d, J_o = 8.63 Hz, Ar−*H*s), 7.16 (2 H, d, J_o = 8.62 Hz, Ar−*H*s) ppm.
$C_{23}H_{30}N_2O_5$

4.1.2.17. 4-[4-(2-Piperidin-1-yl-ethoxy)phenyl]-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**26**)

From 1-(2-chloroethyl)piperidine hydrochloride (1.16 g, 6.30 mmol). Yield: 0.59 g, 43.70%. FT-IR (ν_{max}): 3350, 2950, 1690, 1640, 1470, 1210, 1020 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.44 (2 H, p, −CH_2 pip), 1.60 (4 H, m, 2X-CH_2 pip), 2.32 (6 H, s, 2X-CH_3), 2.49 (4 H, t, 2X-CH_2 pip), 2.74 (2 H, t, −CH_2N<), 3.64 (6 H, s, 2X-COOCH_3), 4.05 (2 H, t, −OCH_2−), 4.93 (1 H, s, 4-C*H* DHP), 5.64 (1 H, s, −N*H*), 6.75 (2 H, dd, J_o = 6.68 Hz, J_m = 2.04 Hz, Ar−*H*s), 7.15 (2 H, dd, J_o = 6.97 Hz, J_m = 1.54 Hz, Ar−*H*s) ppm.
$C_{24}H_{32}N_2O_5$

4.1.2.18. 4-(4-Cyclopentyloxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylic acid dimethyl ester (**27**)

From cyclopentyl bromide (0.676 ml, 6.30 mmol). Yield: 0.78 g, 64.46%. FT-IR (ν_{max}): 3348, 2951, 1698, 1654, 1492, 1300, 1218, 1018 cm^{-1}. ^1H NMR ($CDCl_3$): δ 1.56 (2 H, t, −CH_2 cyclopent), 1.81 (6 H, m, 3X-CH_2 cyclopent), 2.32 (6 H, s, 2X-CH_3), 3.64 (6 H, s, 2X-COOCH_3), 4.67 (1 H,

m, −C*H* cyclopent), 4.92 (1 H, s, 4-C*H* DHP), 5.65 (1 H, s, −N*H*), 6.70 (2 H, m, Ar−*H*s), 7.13 (2 H, m, Ar−*H*s) ppm.
$C_{22}H_{27}NO_5$

4.2. Biological methods

4.2.1. 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. Prior to the experiments, the cells were washed thoroughly with saline solution using a Tomtec liquid handling robot. The fluorescence screening was done using fluo-3-ester calcium dye (2 μM) which 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 was 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 are 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 then serially diluted.

4.2.2. 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 a 95% O_2-5% CO_2 gas mixture. The preparation was allowed to equilibrate for at least 60 min prior to initiation of experimental procedures and during this period the incubation media was changed every 30 min.
After equilibration aortic rings were contracted by a single concentration of 30 mM KCl. When the contractions were stable, compound was added in progressively increasing cumulative concentrations (10^{-8}–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.

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