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Original article

Synthesis of new 3-pyridinecarboxylates of potential vasodilation properties

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Abstract

2-(Alicyclic-amino)-4,6-diaryl-3-pyridinecarboxylates **5a–d** were prepared via aromatic nucleophilic substitution reaction of secondary amines (piperidine or morpholine) with 2-bromo-3-pyridinecarboxylate derivatives **3a**,**b**. The latters were obtained through bromination of 3-aryl-4-benzoyl-2-cyanobutyrates **2a** and **2b**, which were obtained from the base promoted addition of ethyl cyanoacetate to 2-propen-1-ones **1a** and **1b**, with bromine in glacial acetic acid. Reaction of **3** with piperazine hexahydrate in 2:1 molar ratio afforded 1,4-bis[(ethyl 4,6-diaryl-3-pyridinecarboxylate)-2-yl]piperazines **6a**,**b**. Reaction of **3** with anilines in refluxing pyridine unexpectedly gave 2-(aryl-amino)-3-pyridinecarboxylates **8a–g** and 2-amino-3-pyridinecarboxylates **9a** and **9b**. Vasodilation activity screening for the synthesized pyridinecarboxylates using isolated thoracic aortic rings' standard method of rats shows considerable properties. Compounds **5b**, **5c**, **6b** and **8g** reveal remarkable vasodilation potency (IC_{50}, concentrations necessary for 50% reduction of maximal norepinephrine hydrochloride induced contracture) 0.175, 0.146, 0.229 and 0.233 mM, respectively.

Keywords: 3-Pyridinecarboxylates; 2-Propen-1-ones; Aromatic nucleophilic substitution; Vasodilation

1. Introduction

3-Pyridinecarboxylate derivatives have occupied a unique place in the field of medicinal chemistry. They have a wide range of pharmacological applications. For example, ciclonicate ''3-pyridinecarboxylic acid 3,3,5-trimethylcyclohexyl ester'', nicametate ''3-pyridinecarboxylic acid 2-(dimethylamino)ethyl ester'', hepronicate ''3-pyridinecarboxylic acid 2-hexyl-2-[[(3-pyridinylcarbonyl)oxy]methyl]-1,3-propanediyl ester'' and inositol niacinate ''*myo*-inositol hexa-3-pyridinecarboxylate'' are well known vasodilating drugs [1] (Scheme 1). In addition, many research articles reported about the pharmacological properties of nicotinate esters as antihyperlipidemic [2] and cholesteryl ester transfer protein inhibitors [3]. Pharmacological compositions containing nicotinate esters have also been reported as stimulating hair growth due to their blood circulation promoter properties [4].

In the present work, it is intended to investigate synthesis of novel ethyl nicotinate derivatives, especially those substituted with fluorophenyl residue adopting easily accessible starting materials and facile synthetic approaches. The interest for construction of fluorine-containing compounds is originated due to their unique properties such as high thermal stability and lipophilicity [5]. This investigation is considered a continuation of our previous work directed towards preparation of pharmacologically active agents [6–10]. So, the vasodilation properties of the prepared compounds will be screened in an attempt to determine a new heterocyclic agent which could be useful as a hint in a drug discovery program.

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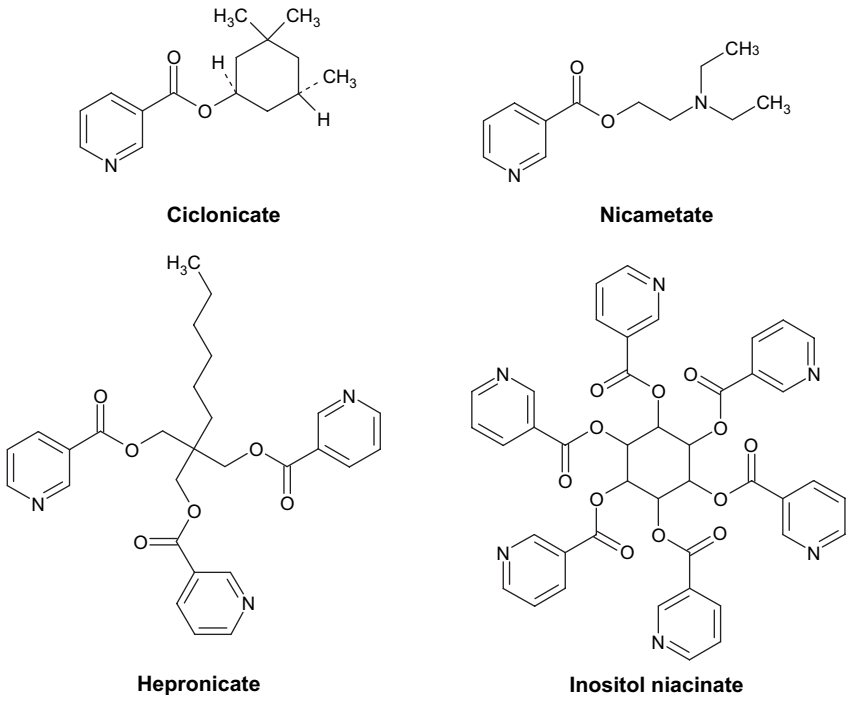


Scheme 1.

2. Results and discussion

2.1. Chemistry

Reaction of 1,3-diaryl-2-propen-1-ones **1a**,**b** with ethyl cyanoacetate in refluxing ethanol in the presence of piperidine as a basic catalyst afforded ethyl 3-aryl-4-benzoyl-2-cyanobutyrates **2a**,**b** in good yields. The structure of **2** was established through spectroscopic (IR, ^{1}H NMR) and elemental analyses data. The IR spectra of **2a**,**b** reveal the presence of two carbonyl functions at $\nu = 1729$, 1691–1690 cm^{-1} assignable to the stretching vibrations of ester and ketonic residues, respectively. A nitrile stretching vibration band is also recognized at $\nu = 2250$–2249 cm^{-1} confirming isolation of the open-chain Michael adduct product. ^{1}H NMR spectra of **2a**,**b** exhibit the ethyl ester beside the methylene protons of H_2C-4 as two sets of double doublet signals "at $\delta = 3.48$–3.51, 3.69–3.70" due to the mutual coupling with each other ($J = 18.2$–18.3 Hz) and in turn with the vicinal methine proton *H*C-3 ($J = 5.1$–5.5, 8.6–9.3 Hz). The methine proton of *H*C-2 appears as a doublet signal ($\delta = 4.33$, $J = 5.4$ Hz). However, the methine proton of *H*C-3 appears as a multiplet signal hidden under the quartet of methylene protons of ethyl ester group. Single crystal X-ray diffraction of **2a** adds a good support for the established structure (Fig. 1).

Bromination of **2a**,**b** with bromine in glacial acetic acid at 60–70 °C afforded directly ethyl 2-bromo-4,6-diaryl-3-pyridinecarboxylates **3a**,**b** in good yields. The structure of **3** was inferred from IR, ^{1}H NMR, MS and elemental analyses data. The IR spectra of **3** lack any band assignable for nitrile function confirming its involvement in the cyclization process. However, only one carbonyl stretching vibration band is observed at $\nu = 1719$ cm^{-1} corresponding to the stretching vibration of ester residue. ^{1}H NMR spectra of **3a**,**b** exhibit the ethyl ester group in addition, to the characteristic pyridinyl *H*-5 as a singlet signal at $\delta = 7.67$–7.69. Mass spectra (EI) of **3a**,**b** show (M + 1) fragmentation peaks with recognizable relative intensity values, beside the naturally isotopic abundance peaks due to the bromine atom, which add a conclusive evidence to the achieved structures (Scheme 2).

Reaction of 2-bromo-3-pyridinecarboxylates **3a**,**b** with secondary amines (piperidine or morpholine) **4a**,**b** in refluxing tetrahydrofuran led to aromatic nucleophilic substitution giving 2-(alicyclic-amino)-3-pyridinecarboxylates **5a**–**d**, whose structures were deduced through spectroscopic (IR, ^{1}H NMR, MS) and elemental analyses data. ^{1}H NMR spectra of **5a**–**d** reveal the alicyclic-amino function (piperidinyl as multiplet signals at $\delta = 1.66$–1.73, 3.45–3.49 and morpholinyl as triplet signals at $\delta = 3.50$–3.51, 3.84–3.86) beside the ethyl ester signals (triplet at $\delta = 1.04$–1.07 and quartet at $\delta = 4.07$–4.09 corresponding to the methyl and methylene protons, respectively).

Meanwhile, reaction of **3a**,**b** with piperazine hexahydrate in refluxing pyridine in 2:1 molar ratio afforded the corresponding 1,4-bis[(ethyl 4,6-diaryl-3-pyridinecarboxylate)-2-yl]piperazines **6a**,**b**. ^{1}H NMR spectra of **6** exhibit the four piperazinyl methylene protons as a singlet signal at $\delta = 3.69$–3.72.

However, reaction of **3a**,**b** with primary aromatic amines **7a**–**d** in refluxing pyridine gave 2-(aryl-amino)-3-pyridinecarboxylates **8a**–**g** beside the unexpected 2-(unsubstituted amino)-3-pyridinecarboxylates **9a**,**b** (Scheme 3). Formation of **9** during the mentioned reaction seems similar to what

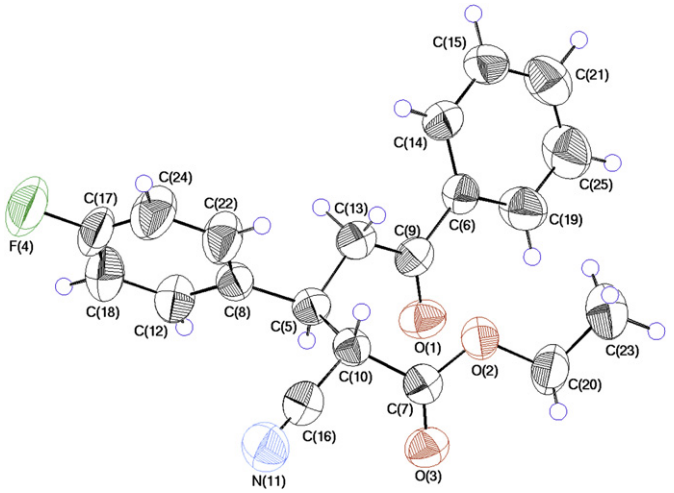
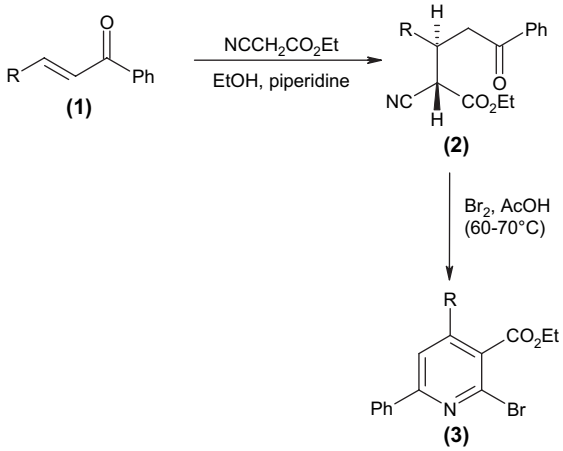


Fig. 1. ORTEP projection of single crystal X-ray diffraction of **2a**. Selected intramolecular bond lengths (Å) and bond angles (°) of **2a**. O(1)–C(9) = 1.220(2), O(2)–C(7) = 1.328(2), O(2)–C(20) = 1.470(2), O(3)–C(7) = 1.207(2), F(4)–C(17) = 1.365(2), C(5)–C(8) = 1.512(2), C(5)–C(10) = 1.5559, C(5)–C(13) = 1.5326, C(6)–C(9) = 1.489(2), C(6)–C(14) = 1.391(3), C(6)–C(19) = 1.374(3), C(7)–C(10) = 1.514(3), C(8)–C(12) = 1.381(3), C(8)–C(22) = 1.373(3), C(9)–C(13) = 1.509(3), C(10)–C(16) = 1.473(3), N(11)–C(16) = 1.145(2), C(12)–C(18) = 1.380(3), C(14)–C(15) = 1.378(3), C(15)–C(21) = 1.356(3), C(17)–C(18) = 1.351(3), C(17)–C(24) = 1.352(3), C(19)–C(25) = 1.385(3), C(20)–C(23) = 1.469(3), C(21)–C(25) = 1.358(3), C(22)–C(24) = 1.366(3), C(5)–H(5) = 0.9600(15), C(10)–H(10) = 0.91(2), C(7)–O(2)–C(20) = 114.6(2), C(9)–C(6)–C(14) = 123.2(2), C(9)–C(6)–C(19) = 119.4(2), C(14)–C(6)–C(19) = 117.4(2), O(2)–C(7)–O(3) = 124.1(2), O(2)–C(7)–C(10) = 111.8(2), O(3)–C(7)–C(10) = 124.1(2), C(5)–C(8)–C(12) = 120.2(2), C(5)–C(8)–C(22) = 122.1(2), C(12)–C(8)–C(22) = 117.6(2), O(1)–C(9)–C(6) = 121.1(2), O(1)–C(9)–C(13) = 121.1(2), C(6)–C(9)–C(13) = 117.8(2), C(7)–C(10)–C(16) = 108.3(2), C(8)–C(12)–C(18) = 121.0(2), C(6)–C(14)–C(15) = 121.3(2), C(14)–C(15)–C(21) = 119.8(2), C(10)–C(16)–N(11) = 179.7(2), F(4)–C(17)–C(18) = 118.9(2), F(4)–C(17)–C(24) = 119.3(2), C(18)–C(17)–C(24) = 121.9(2), C(12)–C(18)–C(17) = 118.9(2), C(6)–C(19)–C(25) = 120.9(2), O(2)–C(20)–C(23) = 108.0(2), C(15)–C(21)–C(25) = 120.3(2), C(8)–C(22)–C(24) = 121.8(2), C(17)–C(24)–C(22) = 118.9(2), C(19)–C(25)–C(21) = 120.2(2), C(8)–C(5)–H(5) = 108.23(14), C(7)–C(10)–H(10) = 109.9(11), C(16)–C(10)–H(10) = 109.0(11).

was previously reported about the formation of 2-amino-3-pyridine derivatives through either reaction of 2-bromonicotinonitriles or 2-bromonicotinate ester analogues with α-amino acid esters in refluxing pyridine [8,11]. It was assumed in the latter reaction that, the mechanistic pathway proceeded analogously to the famous ninhydrin reaction with α-amino acids [12]. Where, the amino acids isomerize to the corresponding imino acid forms under the effect of applied reaction conditions. Then, upon hydrolysis, due to unavoidable moisture, ammonia liberates which in turn interacts with 2-bromopyridines yielding the 2-amino derivatives. Similar observation was recently reported about the formation of 2-aminonicotinamides during the reaction of 2-bromonicotinamides with primary aromatic amines in refluxing pyridine [7].

2.2. Vasodilation activity

Vasodilation activity screening for the synthesized pyridinecarboxylates was investigated in vitro using isolated



1a,2a,3a, R = 4-FC_6H_4
1b,2b,3b, R = 4-$H_3CC_6H_4$

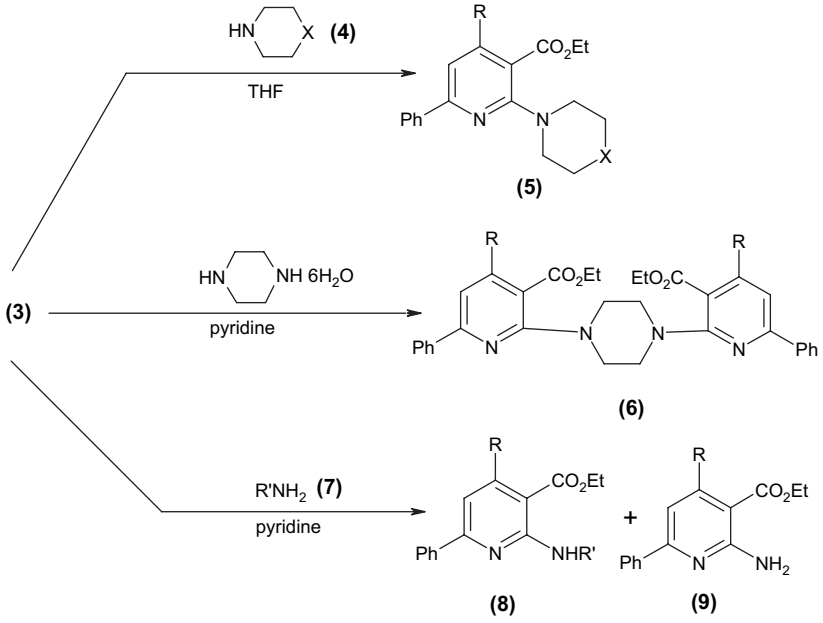
Scheme 2.

thoracic aortic rings of male Wister rats pre-contracted with norepinephrine hydrochloride according to the standard known procedure [8,13,14]. From the observed data (Table 1, Figs. 2 and 3), it has been noticed that all the tested compounds show considerable vasodilation properties. Compound **5c** reveals the best vasodilation potency effect (IC_{50}, concentration necessary for 50% reduction of maximal norepinephrine hydrochloride induced contracture) among the whole tested compounds ($IC_{50} = 0.146$ mM). In addition, few synthesized compounds especially **5b**, **6b** and **8g** show remarkable vasodilation potency ($IC_{50} = 0.175$, 0.229 and 0.233 mM, respectively).

Structure–activity relationship based on the observed results indicated that, substitution of pyridinecarboxylate nucleus with alicyclic-amino function (piperidinyl, morpholinyl or piperazinyl) at the 2-position enhances the vasodilation properties (IC_{50} of **5a**, **5b**, **5c**, **5d** and **6b** is 0.346, 0.175, 0.146, 0.340 and 0.229 mM, respectively) than the case of using either phenyl- or 4-chlorophenylamino residue (IC_{50} of **8a**, **8b** and **8c** is 0.367, 0.715 and 0.266 mM, respectively). In addition, substitution of the phenylamino group with an electron-donating function (e.g. methoxy residue) (IC_{50} of **8f** and **8g** is 0.254 and 0.233 mM, respectively) enhances the observed vasodilation effects than the case of using electron-withdrawing function (e.g. chloro residue) (IC_{50} of **8b** and **8c** is 0.715 and 0.266 mM, respectively). However, there is no precise rule could be attained, through the observed data, governing the type of substitution of the phenyl group (either fluoro or methyl function) attached to the 4-position of 3-pyridinecarboxylates in affecting the total observed vasodilation properties.

3. Experimental

Melting points are uncorrected and recorded on an Electrothermal 9100 digital melting point apparatus. IR spectra (KBr) were recorded on a Nexus 670 FT-IR spectrophotometer.



4a, X = CH_2
4b, X = O

5a, R = 4-FC_6H_4, X = CH_2
5b, R = 4-$H_3CC_6H_4$, X = CH_2
5c, R = 4-FC_6H_4, X = O
5d, R = 4-$H_3CC_6H_4$, X = O

6a, 9a, R = 4-FC_6H_4
6b, 9b, R = 4-$H_3CC_6H_4$

7a, R' = Ph
7b, R' = 4-ClC_6H_4
7c, R' = 4-$H_3CC_6H_4$
7d, R' = 4-$H_3COC_6H_4$

8a, R = 4-FC_6H_4, R' = Ph
8b, R = 4-FC_6H_4, R' = 4-ClC_6H_4
8c, R = 4-$H_3CC_6H_4$, R' = 4-ClC_6H_4
8d, R = 4-FC_6H_4, R' = 4-$H_3CC_6H_4$
8e, R = R' = 4-$H_3CC_6H_4$
8f, R = 4-FC_6H_4, R' = 4-$H_3COC_6H_4$
8g, R = 4-$H_3CC_6H_4$, R' = 4-$H_3COC_6H_4$

Scheme 3.

H NMR spectra were recorded on Varian GEMINI 200 MHz and MERCURY 300 MHz spectrometers in $CDCl_3$. Mass spectra were recorded on Shimadzu GCMS-QP 1000 EX and Finnigan SSQ 7000 spectrometers (EI, 70 eV). The starting compounds **1a**,**b** [15,16] were prepared according to the previously reported procedures.

*3.1. Synthesis of ethyl 3-aryl-4-benzoyl-2-cyanobutyrates **2a**,**b***

A mixture of **1a**,**b** (10 mmol) and ethyl cyanoacetate (11 mmol) in absolute ethanol (20 ml) containing piperidine (3–5 drops) was boiled under reflux for the appropriate time. The separated solid upon cooling the reaction mixture (4 °C) was collected and crystallized from a suitable solvent affording **2a**,**b**.

*3.1.1. Ethyl 4-benzoyl-2-cyano-3-(4-fluorophenyl) butyrate (**2a**)*

Colourless crystals from benzene–light petroleum (60–80 °C) mixture as 1:2 v/v, mp 86–88 °C, yield 77%. IR: ν_{max}/cm^{-1} 2249 (C≡N), 1729 (C=O ester), 1690 (C=O ketone), 1597, 1580 (C=C). ^{1}H NMR: δ 1.12 (t, 3H, CH_3CH_2, $J = 7.1$ Hz), 3.48 (dd, 1H, upfield H of CH_2CO, $J = 5.5$, 18.2 Hz), 3.70 (dd, 1H, downfield H of CH_2CO, $J = 8.6$, 18.2 Hz), 4.00–4.20 (m, 3H, $OCH_2CH_3 + CHCH_2CO$), 4.33 (d, 1H, $CHCHCH_2$, $J = 5.4$ Hz), 6.95–8.00 (m, 9H, arom. H). Anal. Calcd for $C_{20}H_{18}FNO_3$ (339.35): C, 70.78; H, 5.35; N, 4.13. Found: C, 70.91; H, 5.45; N, 4.12.

*3.1.2. Ethyl 4-benzoyl-2-cyano-3-(4-methylphenyl) butyrate (**2b**)*

Colourless crystals from methanol, mp 84–86 °C, yield 84%. IR: ν_{max}/cm^{-1} 2250 (C≡N), 1729 (C=O ester), 1691 (C=O ketone), 1596, 1579 (C=C). ^{1}H NMR: δ 1.14 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 2.33 (s, 3H, $ArCH_3$), 3.51 (dd, 1H, upfield H of CH_2CO, $J = 5.1$, 18.3 Hz), 3.69 (dd, 1H, downfield H of CH_2CO, $J = 9.3$, 18.3 Hz), 4.07–4.15 (m, 3H, $OCH_2CH_3 + CHCH_2CO$), 4.33 (d, 1H, $CHCHCH_2$, $J = 5.4$ Hz), 7.13–7.98 (m, 9H, arom. H). Anal. Calcd for $C_{21}H_{21}NO_3$ (335.39): C, 75.20; H, 6.31; N, 4.18. Found: C, 75.02; H, 6.18; N, 4.02.

Table 1
Concentration of compounds necessary to reduce maximal norepinephrine-induced contracture by 50% (IC_{50}) in thoracic rat aorta

Compound	Potency (IC_{50})	
	mM	mg/l
5a	0.346	139.9
5b	0.175	70.1
5c	0.146	59.3
5d	0.340	136.8
6b	0.229	164.2
8a	0.367	151.4
8b	0.715	319.5
8c	0.266	117.8
8f	0.254	112.4
8g	0.233	102.2
9a	0.335	112.7
9b	0.327	108.7

3.2. Synthesis of ethyl 2-bromo-4,6-diaryl-3-pyridinecarboxylates *3a,b*

To a solution of **2a**,**b** (5 mmol) in glacial acetic acid (10 ml) warmed at 60–70 °C, a solution of bromine (5.5 mmol) in glacial acetic acid (5 ml) was added dropwise while stirring at such a rate maintaining the same adjusted temperature within a period of 10 min. After complete addition, the reaction was kept at the same temperature for 3 h. Then, stored at room temperature overnight and poured into water (200 ml). The separated solid was collected, washed with water and crystallized from a suitable solvent giving **3a**,**b**.

3.2.1. Ethyl 2-bromo-4-(4-fluorophenyl)-6-phenyl-3-pyridinecarboxylate (*3a*)

Colourless crystals from ethanol, mp 151–153 °C, yield 75%. IR: ν_{max}/cm^{-1} 1719 (C=O), 1605, 1525 (C=N, C=C). ^1H NMR: δ 1.2 (t, 3H, CH_3CH_2, $J = 7.6$ Hz), 4.25 (q, 2H, OCH_2CH_3, $J = 7.6$ Hz), 7.10–7.50 (m, 7H, arom. H), 7.67 (s, 1H, pyridinyl *H*-5), 8.00–8.10 (m, 2H, arom. H). MS: *m/z* (%) 402 [(M + 3), 59], 401 [(M + 2), 60], 400 [(M + 1), 66], 399 (M, 59), 370 (13), 354 (78), 326 (5), 320 (4). Anal. Calcd for $C_{20}H_{15}BrFNO_2$ (400.24): C, 60.01; H, 3.78; N, 3.50. Found: C, 60.22; H, 3.70; N, 3.47.

3.2.2. Ethyl 2-bromo-4-(4-methylphenyl)-6-phenyl-3-pyridinecarboxylate (*3b*)

Colourless crystals from light petroleum (40–60 °C), mp 126–128 °C, yield 81%. IR: ν_{max}/cm^{-1} 1719 (C=O), 1559, 1523 (C=N, C=C). ^1H NMR: δ 1.19 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 2.43 (s, 3H, $ArCH_3$), 4.26 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.26–7.50 (m, 7H, arom. H), 7.69 (s, 1H, pyridinyl *H*-5), 8.02–8.05 (m, 2H, arom. H). MS: *m/z* (%) 398 [(M + 3), 19], 397 [(M + 2), 87], 396 [(M + 1), 21], 395 (M, 84), 366 (12), 350 (99), 322 (0.9), 316 (3). Anal. Calcd for $C_{21}H_{18}BrNO_2$ (396.27): C, 63.65; H, 4.58; N, 3.53. Found: C, 63.79; H, 4.68; N, 3.46.

3.3. Reaction of *3* with *4*

A mixture of **3a**,**b** (2.5 mmol) and the corresponding **4a**,**b** (5 mmol) in tetrahydrofuran (20 ml) was boiled under reflux for the appropriate time. The clear reaction mixture was evaporated till dryness under reduced pressure. The separated solid, upon triturating the remaining residual material with methanol (5 ml), was collected and crystallized from light petroleum (60–80 °C) affording **5a–d** as colourless crystals.

3.3.1. Ethyl 4-(4-fluorophenyl)-6-phenyl-2-(1-piperidinyl)-3-pyridinecarboxylate (*5a*)

Reaction time 20 h, mp 104–106 °C, yield 89%. IR: ν_{max}/cm^{-1} 1716 (C=O), 1606, 1545 (C=N, C=C). ^1H NMR: δ 1.05 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 1.68–1.72 (m, 6H, piperidinyl $3CH_2$), 3.46–3.49 (m, 4H, piperidinyl $2NCH_2$), 4.07 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.07–8.05 (m, 10H, 9 arom. H + pyridinyl *H*-5). MS: *m/z* (%) 404 (M, 42), 375 (100), 359 (9), 331 (27), 248 (44). Anal. Calcd for $C_{25}H_{25}FN_2O_2$ (404.47): C, 74.23; H, 6.23; N, 6.93. Found: C, 74.37; H, 6.29; N, 7.12.

3.3.2. Ethyl 4-(4-methylphenyl)-6-phenyl-2-(1-piperidinyl)-3-pyridinecarboxylate (*5b*)

Reaction time 24 h, mp 93–95 °C, yield 80%. IR: ν_{max}/cm^{-1} 1717 (C=O), 1585, 1543 (C=N, C=C). ^1H NMR: δ 1.07 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 1.66–1.73 (m, 6H, piperidinyl $3CH_2$), 2.42 (s, 3H, $ArCH_3$), 3.45–3.49 (m, 4H, piperidinyl $2NCH_2$), 4.09 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.21–8.07 (m, 10H, 9 arom. H + pyridinyl *H*-5). MS: *m/z* (%) 400 (M, 52), 371 (100), 355 (11), 327 (21), 244 (13). Anal. Calcd for $C_{26}H_{28}N_2O_2$ (400.50): C, 77.97; H, 7.05; N, 7.00. Found: C, 78.06; H, 7.13; N, 7.11.

3.3.3. Ethyl 4-(4-fluorophenyl)-2-(4-morpholinyl)-6-phenyl-3-pyridinecarboxylate (*5c*)

Reaction time 20 h, mp 134–136 °C, yield 79%. IR: ν_{max}/cm^{-1} 1716 (C=O), 1606, 1544 (C=N, C=C). ^1H NMR: δ 1.04 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 3.50 (t, 4H, morpholinyl $2NCH_2$, $J = 4.5$ Hz), 3.84 (t, 4H, morpholinyl $2OCH_2$, $J = 4.5$ Hz), 4.07 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.09–8.05 (m, 10H, 9 arom. H + pyridinyl *H*-5). MS: *m/z* (%) 406 (M, 93), 377 (83), 361 (51), 333 (88), 248 (100). Anal. Calcd for $C_{24}H_{23}FN_2O_3$ (406.44): C, 70.92; H, 5.70; N, 6.89. Found: C, 70.72; H, 5.59; N, 7.03.

3.3.4. Ethyl 4-(4-methylphenyl)-2-(4-morpholinyl)-6-phenyl-3-pyridinecarboxylate (*5d*)

Reaction time 24 h, mp 103–105 °C, yield 80%. IR: ν_{max}/cm^{-1} 1720 (C=O), 1585, 1541 (C=N, C=C). ^1H NMR: δ 1.05 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 2.42 (s, 3H, $ArCH_3$), 3.51 (t, 4H, morpholinyl $2NCH_2$, $J = 4.5$ Hz), 3.86 (t, 4H, morpholinyl $2OCH_2$, $J = 4.5$ Hz), 4.09 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.25–8.07 (m, 10H, 9 arom. H + pyridinyl *H*-5). MS: *m/z* (%) 402 (M, 100), 373 (76),

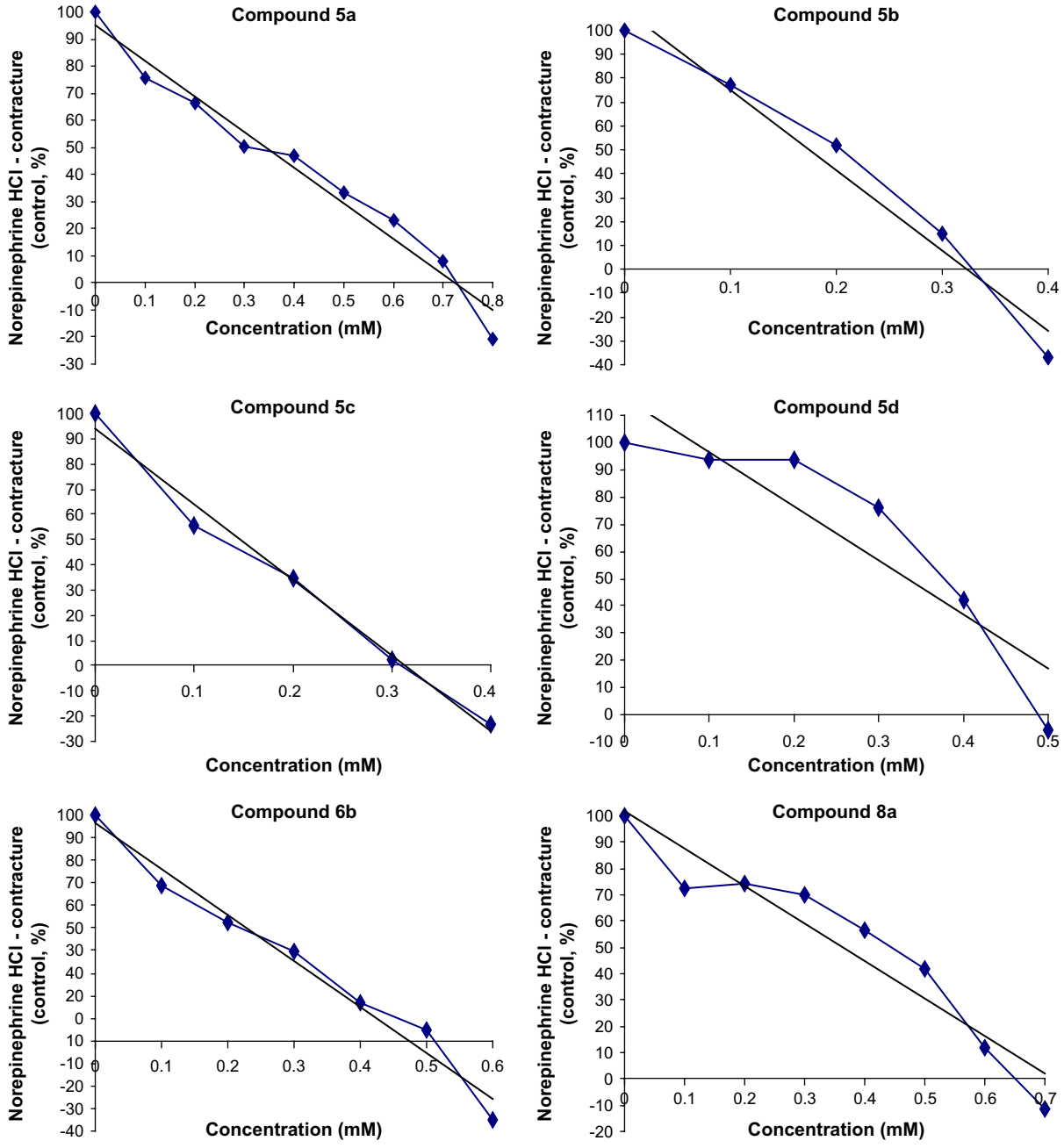


Fig. 2. Effect of tested compounds on contracture induced by norepinephrine hydrochloride in thoracic rat aortic rings.

357 (53), 329 (74), 244 (31). Anal. Calcd for $C_{25}H_{26}N_2O_3$ (402.48): C, 74.60; H, 6.51; N, 6.96. Found: C, 74.52; H, 6.44; N, 6.80.

3.4. Reaction of **3** with piperazine hexahydrate

A mixture of **3a**,**b** (5 mmol) and piperazine hexahydrate (2.5 mmol) in pyridine (20 ml) was boiled under reflux for the appropriate time. The separated solid upon pouring the reaction mixture in ice-cold water (200 ml) acidified with dil. HCl (5%), was collected, washed with water and purified on silica gel TLC (F_{254}) using chloroform–light petroleum (60–80 °C) as 3:1 v/v for elution, giving **6a**,**b** as colourless crystals.

3.4.1. 1,4-Bis[[ethyl 4-(4-fluorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]piperazine (**6a**)

Reaction time 25 h, mp 246–248 °C, yield 41%. IR: ν_{max}/cm^{-1} 1716 (C=O), 1575, 1544 (C=N, C=C). ^1H NMR: δ 1.08 (t, 6H, $2CH_3CH_2$, $J = 7.2$ Hz), 3.69 (s, 8H, piperazinyl $4NCH_2$), 4.11 (q, 4H, $2OCH_2CH_3$, $J = 7.2$ Hz), 7.10–8.08 (m, 20H, 18 arom. H + 2 pyridinyl *H*-5). MS: *m/z* (%) 723 [(M-1), 0.8], 679 (2), 651 (2), 387 (100), 360 (29), 332 (4), 302 (71). Anal. Calcd for $C_{44}H_{38}F_2N_4O_4$ (724.77): C, 72.91; H, 5.28; N, 7.73. Found: C, 73.02; H, 5.38; N, 7.53.

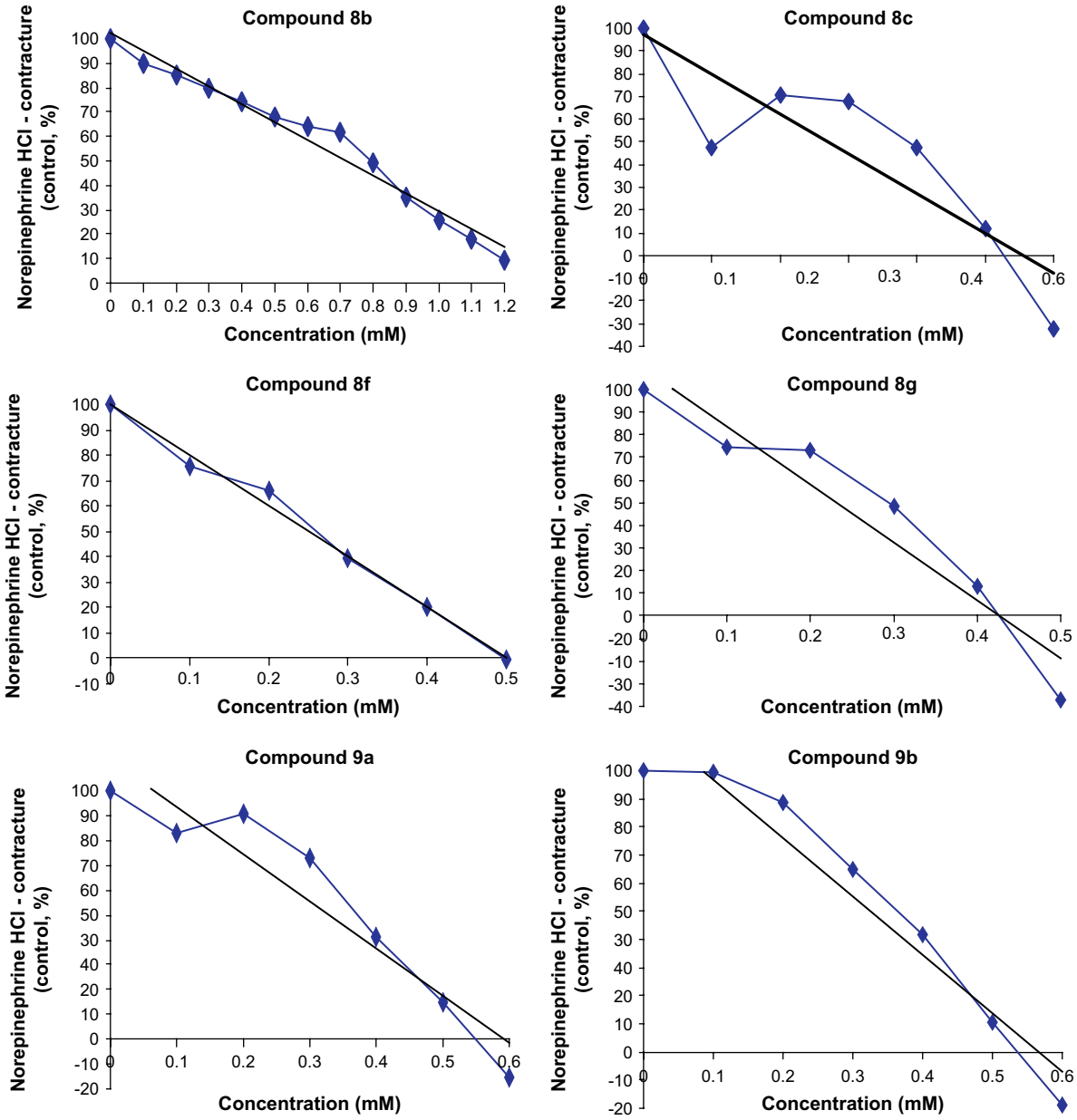


Fig. 2. (*continued*).

*3.4.2. 1,4-Bis[[ethyl 4-(4-methylphenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]piperazine (**6b**)*

Reaction time 30 h, mp 224–225 °C, yield 45%. IR: ν_{max}/cm^{-1} 1724 (C=O), 1577, 1542 (C=N, C=C). ^{1}H NMR: δ 1.08 (t, 6H, 2CH_3CH$_2$, $J = 7.5$ Hz), 2.43 (s, 6H, 2 ArCH$_3$), 3.72 (s, 8H, piperazinyl 4NCH$_2$), 4.13 (q, 4H, 2OCH_2CH$_3$, $J = 7.2$ Hz), 7.24–8.08 (m, 20H, 18 arom. H + 2 pyridinyl *H*-5). MS: *m/z* (%) 715 [(M-1), 0.7], 671 (3), 643 (3), 383 (100), 356 (30), 328 (4), 298 (67). Anal. Calcd for $C_{46}H_{44}N_4O_4$ (716.84): C, 77.07; H, 6.19; N, 7.82. Found: C, 77.25; H, 6.32; N, 7.89.

*3.5. Reaction of **3** with **7***

A mixture of **3a**,**b** (5 mmol) and the corresponding **7a–d** (10 mmol) in pyridine (20 ml) was boiled under reflux for the appropriate time. The separated solid, upon pouring the reaction mixture into ice-cold water (200 ml) acidified with dil. HCl (5%), was collected, washed with water and purified on silica gel TLC (F_{254}) affording **8a–g** and **9a**,**b**.

*3.5.1. Ethyl 4-(4-fluorophenyl)-6-phenyl-2-(phenylamino)-3-pyridinecarboxylate (**8a**)*

Reaction time 45 h, pale yellow crystals purified by silica gel TLC (F_{254}) using chloroform–light petroleum (60–80 °C) mixture as 2:3 v/v for elution, mp 92–94 °C, yield 39%. IR: ν_{max}/cm^{-1} 3303 (NH), 1674 (C=O), 1601, 1578 (C=N, C=C). ^{1}H NMR: δ 0.92 (t, 3H, CH_3CH$_2$, $J = 7.2$ Hz), 4.08 (q, 2H, OCH_2CH$_3$, $J = 7.2$ Hz), 7.13–8.18 (m, 15H, 14 arom. H + pyridinyl *H*-5), 9.68 (s, 1H, NH). MS: *m/z* (%) 412 (M, 100), 367 (7), 366 (16), 339 (15), 338

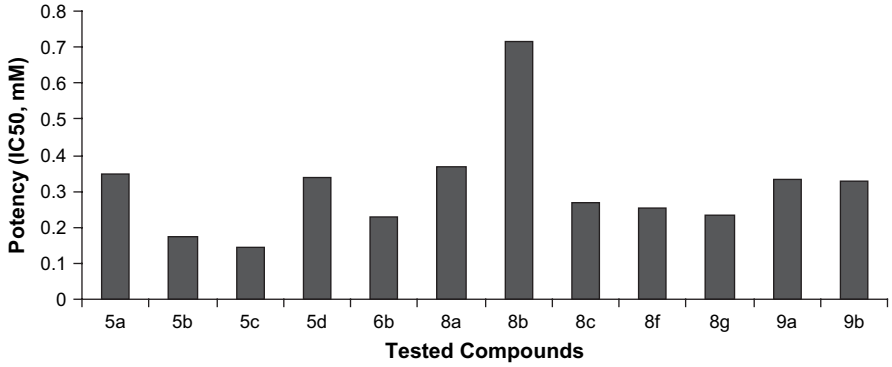


Fig. 3. Potency (IC_{50}, mM) of the tested compounds on contracture induced by norepinephrine hydrochloride in thoracic rat aortic rings.

(18), 335 (2). Anal. Calcd for $C_{26}H_{21}FN_2O_2$ (412.45): C, 75.71; H, 5.13; N, 6.79. Found: C, 75.52; H, 5.02; N, 6.85.

3.5.2. Ethyl 2-[(4-chlorophenyl)amino]-4-(4-fluorophenyl)-6-phenyl-3-pyridinecarboxylate (**8b**)

Reaction time 60 h, yellow crystals purified by silica gel TLC (F_{254}) using chloroform–light petroleum (60–80 °C) mixture as 2:3 v/v for elution, mp 144–146 °C, yield 20%. IR: ν_{max}/cm^{-1} 3261 (NH), 1682 (C=O), 1615, 1573 (C=N, C=C). ^1H NMR: δ 0.83 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 3.99 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.10–8.07 (m, 14H, 13 arom. H + pyridinyl *H*-5), 9.68 (s, 1H, NH). MS: *m/z* (%) 448 [(M + 2), 29], 446 (M, 100), 401 (20), 400 (47), 373 (18), 372 (11), 335 (5). Anal. Calcd for $C_{26}H_{20}ClFN_2O_2$ (446.89): C, 69.87; H, 4.51; N, 6.27. Found: C, 69.81; H, 4.44; N, 6.11.

3.5.3. Ethyl 2-[(4-chlorophenyl)amino]-4-(4-methylphenyl)-6-phenyl-3-pyridinecarboxylate (**8c**)

Reaction time 60 h, pale yellow crystals purified by silica gel TLC (F_{254}) using chloroform–light petroleum (60–80 °C) mixture as 1:2 v/v for elution, mp 122–124 °C, yield 23%. IR: ν_{max}/cm^{-1} 3246 (NH), 1681 (C=O), 1617, 1574 (C=N, C=C). ^1H NMR: δ 0.80 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 2.44 (s, 3H, $ArCH_3$), 3.98 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.21–8.08 (m, 14H, 13 arom. H + pyridinyl *H*-5), 9.60 (s, 1H, NH). MS: *m/z* (%) 444 [(M + 2), 44], 442 (M, 100), 397 (22), 396 (67), 369 (17), 368 (10), 331 (5). Anal. Calcd for $C_{27}H_{23}ClN_2O_2$ (442.92): C, 73.21; H, 5.23; N, 6.33. Found: C, 73.05; H, 5.14; N, 6.19.

3.5.4. Ethyl 4-(4-fluorophenyl)-2-[(4-methylphenyl)amino]-6-phenyl-3-pyridinecarboxylate (**8d**)

Reaction time 45 h, pale yellow crystals purified by silica gel TLC (F_{254}) using chloroform–light petroleum (60–80 °C) mixture as 2:3 v/v for elution, mp 103–105 °C, yield 40%. IR: ν_{max}/cm^{-1} 3313 (NH), 1681 (C=O), 1614, 1576 (C=N, C=C). ^1H NMR: δ 0.83 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 2.36 (s, 3H, $ArCH_3$), 3.98 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.09–8.09 (m, 14H, 13 arom. H + pyridinyl *H*-5), 9.57 (s, 1H, NH). MS: *m/z* (%) 426 (M, 100), 381 (12), 380 (36), 353 (16), 352 (19), 335 (2). Anal. Calcd for $C_{27}H_{23}FN_2O_2$ (426.47): C, 76.04; H, 5.44; N, 6.57. Found: C, 75.92; H, 5.36; N, 6.61.

3.5.5. Ethyl 4-(4-methylphenyl)-2-[(4-methylphenyl)amino]-6-phenyl-3-pyridinecarboxylate (**8e**)

Reaction time 45 h, pale yellow crystals purified by silica gel TLC (F_{254}) using chloroform–light petroleum (60–80 °C) mixture as 1:1 v/v for elution, mp 97–99 °C, yield 40%. IR: ν_{max}/cm^{-1} 3345 (NH), 1677 (C=O), 1597, 1576 (C=N, C=C). ^1H NMR: δ 0.70 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 2.27 (s, 3H, $ArCH_3$), 2.34 (s, 3H, $ArCH_3$), 3.87 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 7.07–8.01 (m, 14H, 13 arom. H + pyridinyl *H*-5), 9.38 (br s, 1H, NH). MS: *m/z* (%) 422 (M, 45), 421 (94), 377 (64), 376 (78), 349 (60), 348 (83), 331 (18). Anal. Calcd for $C_{28}H_{26}N_2O_2$ (422.51): C, 79.59; H, 6.20; N, 6.63. Found: C, 79.68; H, 6.29; N, 6.74.

3.5.6. Ethyl 4-(4-fluorophenyl)-2-[(4-methoxyphenyl)amino]-6-phenyl-3-pyridinecarboxylate (**8f**)

Reaction time 40 h, yellow crystals purified by silica gel TLC (F_{254}) using ethyl acetate–*n*-hexane mixture as 1:9 v/v for elution, mp 129–131 °C, yield 50%. IR: ν_{max}/cm^{-1} 3275 (NH), 1683 (C=O), 1618, 1581 (C=N, C=C). ^1H NMR: δ 0.83 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 3.84 (s, 3H, OCH_3), 3.97 (q, 2H, OCH_2CH_3, $J = 6.9$ Hz), 6.92–8.07 (m, 14H, 13 arom. H + pyridinyl *H*-5), 9.55 (br s, 1H, NH). MS: *m/z* (%) 442 (M, 100), 397 (14), 396 (41), 369 (5), 368 (6), 335 (2). Anal. Calcd for $C_{27}H_{23}FN_2O_3$ (442.47): C, 73.29; H, 5.24; N, 6.33. Found: C, 73.45; H, 5.31; N, 6.23.

3.5.7. Ethyl 2-[(4-methoxyphenyl)amino]-4-(4-methylphenyl)-6-phenyl-3-pyridinecarboxylate (**8g**)

Reaction time 40 h, pale yellow crystals purified by silica gel TLC (F_{254}) using chloroform–light petroleum (60–80 °C) mixture as 1:2 v/v for elution, mp 88–90 °C, yield 55%. IR: ν_{max}/cm^{-1} 3335 (NH), 1681 (C=O), 1596, 1578 (C=N, C=C). ^1H NMR: δ 0.81 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 2.44 (s, 3H, $ArCH_3$), 3.86 (s, 3H, OCH_3), 3.98 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 6.94–8.09 (m, 14H, 13 arom. H + pyridinyl *H*-5), 9.40 (br s, 1H, NH). MS: *m/z* (%) 438 (M, 100), 393 (78), 392 (10), 365 (26), 364 (34), 331

(6). Anal. Calcd for $C_{28}H_{26}N_2O_3$ (438.51): C, 76.69; H, 5.98; N, 6.39. Found: C, 76.82; H, 6.06; N, 6.48.

3.5.8. Ethyl 2-amino-4-(4-fluorophenyl)-6-phenyl-3-pyridinecarboxylate (**9a**)

Colourless crystals, mp 169–171 °C, yield 36, 48, 30 and 18% "during preparation of **8a**, **8b**, **8d** and **8f**, respectively". IR: ν_{max}/cm^{-1} 3448, 3280, 3169 (NH_2), 1681 (C=O), 1615, 1570 (C=N, C=C). ^1H NMR: δ 0.84 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 3.98 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 6.26 (s, 2H, NH_2), 6.99 (s, 1H, pyridinyl *H*-5), 7.07–8.01 (m, 9H, arom. H). MS: *m/z* (%) 336 (M, 100), 291 (29), 263 (25). Anal. Calcd for $C_{20}H_{17}FN_2O_2$ (336.36): C, 71.41; H, 5.10; N, 8.33. Found: C, 71.56; H, 5.21; N, 8.27.

3.5.9. Ethyl 2-amino-4-(4-methylphenyl)-6-phenyl-3-pyridinecarboxylate (**9b**)

Colourless crystals, mp 119–121 °C, yield 54, 24 and 15% "during preparation of **8c**, **8e** and **8g**, respectively". IR: ν_{max}/cm^{-1} 3437, 3277, 3172 (NH_2), 1679 (C=O), 1619, 1567 (C=N, C=C). ^1H NMR: δ 0.82 (t, 3H, CH_3CH_2, $J = 7.2$ Hz), 2.43 (s, 3H, $ArCH_3$), 3.99 (q, 2H, OCH_2CH_3, $J = 7.2$ Hz), 6.23 (s, 2H, NH_2), 7.05 (s, 1H, pyridinyl *H*-5), 7.23–8.03 (m, 9H, arom. H). MS: *m/z* (%) 332 (M, 100), 287 (30), 259 (73). Anal. Calcd for $C_{21}H_{20}N_2O_2$ (332.39): C, 75.88; H, 6.07; N, 8.43. Found: C, 75.83; H, 6.03; N, 8.26.

3.6. Single crystal X-ray crystallographic data of **2a**

For X-ray crystallographic studies, compound **2a** was recrystallized as prismatic colourless crystals from benzene–light petroleum (60–80 °C) mixture as 1:2 v/v. The crystallographic data were collected at $T = 298$ K on a Kappa CCD Enraf Nonius FR 590 diffractometer using a graphite monochromator with Mo Kα radiation ($\lambda = 0.71073$ Å). The crystal structure was determined by SIR92 [17] and refined by maXus [18] (Bruker Nonius, Delft and MacScience, Japan). Chemical formula $C_{20}H_{18}FNO_3$, $M_r = 339.366$, monoclinic, crystallizes in space group $P2_1/c$, cell lengths "$a = 9.6989(4)$, $b = 19.8809(8)$, $c = 9.3483(3)$ Å", cell angles "$\alpha = 90.00$, $\beta = 98.070(2)$, $\gamma = 90.00°$", $V = 1784.72(12)$ Å^3, $Z = 4$, $D_c = 1.263$ mg/m^3, θ values 2.910–26.022°, absorption coefficient μ (Mo Kα) = 0.09 mm^{-1}, $F(000) = 712$. The unique reflections measured 4234 of which 1346 reflections with threshold expression $I > 3\sigma(I)$ were used in the structural analysis. Convergence for 265 variable parameters by least-squares refinement on F^2 with $w = 1/[\sigma^2(F_o^2) + 0.10000F_o^2]$. The final agreement factors were $R = 0.039$ and $wR = 0.070$ with a goodness-of-fit of 1.435. Full crystallographic details, excluding structure factors, have been deposited at Cambridge Crystallographic Data Centre (CCDC) as supplementary publication number CCDC 222213.

3.7. Vasodilation activity screening

The vasodilation activity screening procedures were carried out according to the standard reported techniques [8,13,14] by testing the effects of the synthesized pyridinecarboxylate derivatives on isolated thoracic aortic rings of male Wister rats (250–350 g). After light ether anaesthesia, the rats were sacrificed by cervical dislocation and bleeding. The aortae were immediately excised, freed of extraneous tissues and prepared for isometric tension recording. Aorta was cut in 2–3 mm ring and placed in a vertical chamber filled with modified Krebs–Henseleit solution composed of (in mM): NaCl, 118.0; KCl, 4.7; $NaHCO_3$, 25.0; $CaCl_2$, 1.8; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; glucose, 11.0 and oxygenated with carbogen gas (95% O_2/5% CO_2) at 37 ± 0.5 °C. Each aorta ring was mounted between two hooks in which one was attached to a force transducer (AD Instruments, model MLT0201/D) connected with an organ bath (LETICA, Organ Bath LE01.086). The transducer signals were displayed and stored on a computer for further analysis using AD Instruments PowerLab software.

Preparations were stabilized under 2 g resting tension during 2 h and then the contractile response to norepinephrine hydrochloride (3×10^{-7} to 3×10^{-3} M) was measured before and after exposure to increasing concentrations of the tested synthesized compounds. The tested compounds were dissolved in dimethylsulfoxide (DMSO) as stock solution (50 mg/ml). Removal of functional endothelium was achieved by acetylcholine-induced relaxation test of pre-contracted aorta (relaxation < 10%). Control experiments were performed in the presence of DMSO alone, at the same concentrations at those used with the derivatives tested, which demonstrated that the solvent did not affect the contractile response of isolated aorta.

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References

[1] M.J. O'Neil, A. Smith, P.E. Heckelman, J.R. Obenchain Jr., J.A.R. Gallipeau, M.A. D'Arecca, S. Budavari, The Merck Index, An Encyclopedia of Chemicals, Drugs and Biologicals, 13th ed. Merck Research Laboratories Division of Merck & Co., Whitehouse Station, NJ, USA, 2001, p. 393, 831, 894 and 1164.

[2] Z. Zhao (Lunan Pharmaceutical Co., Ltd., Peop. Rep. China). Faming Zhuanli Shenqing Gongkai Shuomingshu CN 1,457,786 (Cl. A61K31/505), 26 Nov. 2003; Chem. Abstr. 142 (2005) 379358.

[3] L.F. Lee, K.C. Glenn, D.T. Connolly, D.G. Corley, D.L. Flynn, A. Hamme, S.G. Hegde, M.A. Melton, R.J. Schilling, J.A. Sikorski, N.N. Wall, J.A. Zablocki (G.D. Searle & Co., USA). PCT Int. Appl. WO 99 41.237 (Cl. CO7D213/80), 19 Aug. 1999; Chem. Abstr. 131 (1999) 157711.

[4] T. Omori, S. Takeda, Y. Tsuji, H. Kanokogi (Shiseido Co., Ltd., Japan). Jpn. Kokai Tokkyo Koho JP 2005 206,475 (Cl. A61K7/06), 4 Aug. 2005; Chem. Abstr. 143 (2005) 179114.

[5] T. Haga, K. Fujikawa, T. Koyanagi, T. Nakajima, K. Hayashi, Heterocycles 22 (1984) 117.

[6] F.F. Barsoum, H.M. Hosni, A.S. Girgis, Bioorg. Med. Chem. 14 (2006) 3929–3937.

[7] A.S. Girgis, H.M. Hosni, F.F. Barsoum, Bioorg. Med. Chem. 14 (2006) 4466–4476.

[8] A.S. Girgis, A. Kalmouch, M. Ellithey, Bioorg. Med. Chem. 14 (2006) 8488–8494.
[9] A.S. Girgis, M. Ellithey, Bioorg. Med. Chem. 14 (2006) 8527–8532.
[10] A.S. Girgis, N. Mishriky, M. Ellithey, H.M. Hosni, H. Farag, Bioorg. Med. Chem. 15 (2007) 2403–2413.
[11] A.S. Girgis, A. Kalmouch, H.M. Hosni, Amino Acids 26 (2004) 139–146.
[12] J.P. Greenstein, M. Winitz, in: , Chemistry of the Amino Acids, vol. 2, John Wiley and Sons Inc., New York, 1961 p. 1301.
[13] J.M. Raimundo, R.T. Sudo, L.B. Pontes, F. Antunes, M.M. Trachez, G. Zapata-Sudo, Eur. J. Pharmacol. 530 (2006) 117–123.
[14] A.G. Silva, G. Zapata-Sudo, A.E. Kummerle, C.A.M. Fraga, E.J. Barreiro, R.T. Sudo, Bioorg. Med. Chem. 13 (2005) 3431–3437.
[15] S. Toma, Collect. Czech. Chem. Commun. 34 (1969) 2771–2781.
[16] H.H. Szmant, A.J. Basso, J. Am. Chem. Soc. 74 (1952) 4397–4400.
[17] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M.C. Burla, G. Polidori, M. Camalli, J. Appl. Cryst. 27 (1994) 435.
[18] S. Mackay, C.J. Gilmore, C. Edwards, N. Stewart, K. Shankland, maXus Computer Program for the Solution and Refinement of Crystal Structures, Bruker Nonius/MacScience/The University of Glasgow, The Netherlands/Japan, 1999.