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Synthesis of novel vasodilatory active nicotinate esters with amino acid function

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Abstract—A variety of *N*-[(ethyl-4,6-diaryl-3-pyridinecarboxylate)-2-yl]amino acid esters **6a**–**h** were synthesized through the reaction of 2-bromonicotinates **4** with a number of primary amino acid ester hydrochlorides **5** in refluxing tetrahydrofuran in the presence of triethylamine as dehydrohalogenating agent. Similarly, reaction of **4** with *N*-glycylglycine ethyl ester hydrochloride **7** 'as a representative example of dipeptide derivative' afforded smoothly the corresponding *N*-[(ethyl-4,6-diaryl-3-pyridinecarboxylate)-2-yl]-*N*′-glycylglycine ethyl ester analogues **8**. However, reaction of **4** with **5** in refluxing pyridine yielded the unexpected 2-aminonicotinate esters **9**. Vasodilation activity screening for the synthesized nicotinate esters was investigated in vitro on the contractile response of vascular thoracic aorta smooth muscle from Wistar rats, where all the tested compounds exhibit considerable vasodilatory properties. In addition, few prepared compounds especially, **6b**, **6h** and **9b** reveal remarkable vasodilation potency (IC_{50}).

1. Introduction

Relaxation of vascular smooth muscle is the basis for the treatment of hypertension.[1] Several pharmacological agents have been synthesized, but none of them has had a specific action, free of side effects. It is important to find new vasodilators for clinical use and not associated with adverse effects. One of the most known vasodilatory active heterocycles are nicotinate esters. Where many analogues are well known as vasodilating active agents such as, micinicate '*cis*-3-pyridinecarboxylic acid, 2-oxo-1-phenyl-2-[(3,3,5-trimethylcyclohexyl)oxy]ethyl ester' (**1**), hepronicate '3-pyridinecarboxylic acid, 2-hexyl-2-[[(3-pyridinylcarbonyl)oxy]methyl]-1,3-propanediyl ester' (**2**) and inositol nicotinate '*myo*-inositol hexa-3-pyridinecarboxylate' (**3**).[2] Recently, it has been reported that, topical application of methyl nicotinate at the forearm and foot levels of diabetic neuropathic patients results in skin vasodilation, that is comparable to the maximal vasodilation that can be induced by iontophoresis of acetylcholine or sodium nitroprusside.[3] The pharmacological effect of topically applied nicotinic acid derivatives is a vasodilation of the peripheral blood capillaries which are located in the dermal papillae of upper dermis layers adjacent to the epidermis–dermis junction. The mechanism of this action involves the release of prostaglandin D_2 as an important step. However, prostaglandins have a very short half-life being rapidly metabolized and therefore act strictly locally on cells which they are released or on neighbouring cells. Considering this, it is postulated that the concentration of nicotinic acid derivatives estimated at the epidermis–dermis interface, which is in the immediate vicinity of the blood capillaries, represents the active site drug concentration.[4] In addition, many publications have been reported about the pharmacological properties of various nicotinate esters as A_3 adenosine receptor antagonists, [5] cholesteryl ester transfer protein inhibitors, [6] blood circulation promotors and anti-inflammatory.[7,8]

In continuation to our previous work directed towards construction of novel heterocycles of potential pharmacological properties,[9,10] it is intended in the present work to investigate the synthesis of novel nicotinate ester analogues with amino acid function as a natural product residue. This investigation not only deals with preparation of the mentioned heterocyclic analogues through

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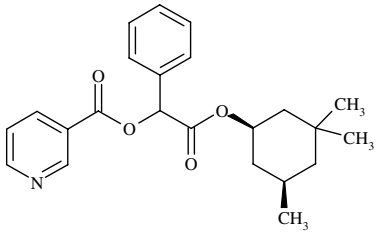
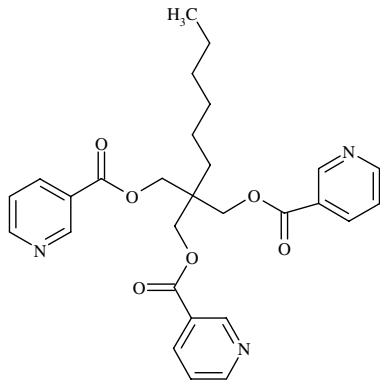
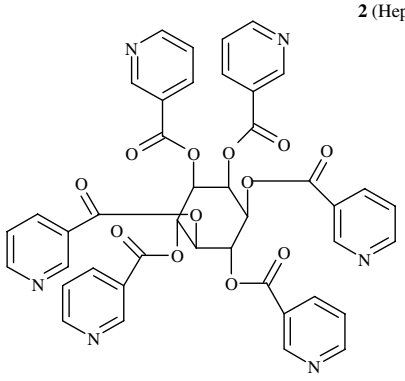


1 (Micinicate)



2 (Hepronicate)



3 (Inositol nicotinate)

adopting simple synthetic pathways and easily accessible starting materials but also, extended towards evaluation of their vasodilating properties. Also, our previously reported properties of 3-pyridinecarboxamide derivatives as anti-tumor active agents especially against breast cancer (MDA-MB-231/ATCC)[10] as well as the smooth muscle relaxation (e.g., rabbit's jejunium and rat's uterus) of 3-pyridinecarboxylates[11] prompted the present work.

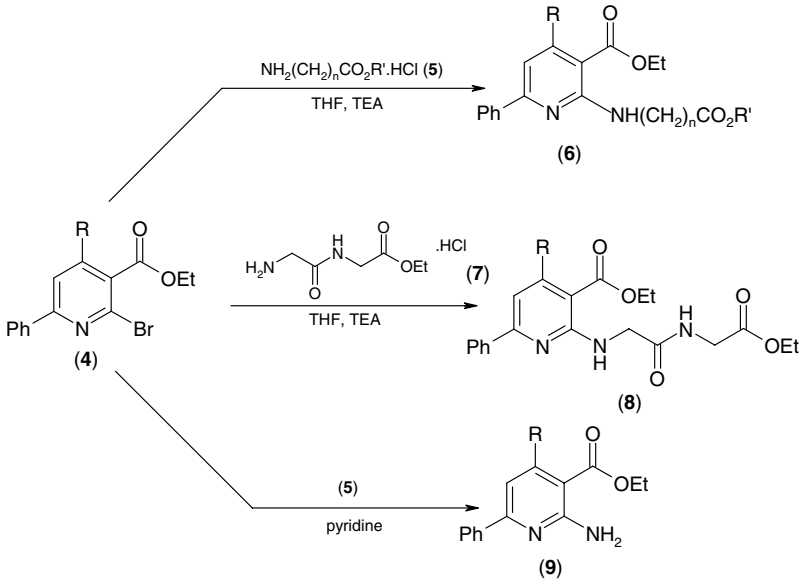
2. Results and discussion

2.1. Chemistry

We previously reported the synthesis of ethyl 2-bromo-3,5-diaryl-3-pyridinecarboxylates **4** via reaction of the easily accessible 1,3-diaryl-2-propen-1-ones 'chalcone derivatives' with ethyl cyanoacetate followed by treatment of the produced Michael adducts with bromine in glacial acetic acid.[11] In the present work, it is intended to investigate aromatic nucleophilic attack of amino acid esters **5a**–**d** at **4** under different basic catalytic conditions. So, reaction of 2-bromonicotinate esters **4a** and **b** with glycine ethyl ester hydrochloride **5a** in refluxing tetrahydrofuran in the presence of triethylamine as dehydrohalogenating agent afforded the corresponding *N*-[(ethyl-4,6-diaryl-3-pyidinecarboxylate)-2-yl]glycine ethyl esters **6a** and **b**. The structure of the latter was established through spectroscopic (IR, ^{1}H NMR) and elemental analyses data. The IR spectra of **6a** and **b** reveal the presence of two strong vibration bands (at υ = 1738–1734, 1698 cm^{-1}) assignable for the stretching vibrations of carbonyl ester functions, in addition to the imino residue (at υ = 3419–3415 cm^{-1}). ^{1}H NMR spectra of **6a** and **b** exhibit the glycinate methylene protons as a doublet signal (δ = 4.36, J = 5.1–5.4 Hz) due to the mutual coupling with the adjacent imino proton of amino acid residue. The two ethyl ester groups are also well recognized (triplets at δ = 0.83–0.87, 1.30–1.31 and quartets at δ = 3.98–4.02, 4.25–4.26 assignable for the methyl and methylene protons, respectively).

Similarly, reaction of **4a** and **b** with β-alanine- **5b**, 4-amino butyric acid **5c** and caproic acid **5d** methyl ester hydrochlorides under the same described reaction conditions yielded the *N*-[(ethyl-4,6-diaryl-3-pyridinecarboxylate)-2-yl]amino acid ester analogues **6c**–**h** whose structures were inferred through spectroscopic (IR, ^{1}H NMR) and elemental analyses data (Scheme 1). In addition, reaction of **4a** and **b** with *N*-glycylglycine ethyl ester hydrochloride **7** 'as a representative example of dipeptide derivative' in refluxing tetrahydrofuran in the presence of triethylamine as dehydrohalogenating agent gave *N*-[(ethyl-4,6-diaryl-3-pyridinecarboxylate)-2-yl]-*N*′-glycylglycine ethyl esters **8a** and **b**. ^{1}H NMR spectra of **8** show two glycinate methylene protons (at δ = 4.25–4.26, 4.55–4.56), in addition to the two ethyl ester groups (triplets at δ = 1.03–1.07, 1.41 and quartets at δ = 4.19–4.23, 4.33–4.34 corresponding to the methyl and methylene protons, respectively).

On the other hand, reaction of **4a** and **b** with amino acid ester hydrochlorides **5a**–**d** in refluxing pyridine afforded



4a, **8a**, **9a**, R = 4-ClC_6H_4

4b, **8b**, **9b**, R = 3,4-$Cl_2C_6H_3$

5a, R' = Et, n = 1

5b, R' = Me, n = 2

5c, R' = Me, n = 3

5d, R' = Me, n = 5

6a, R = 4-ClC_6H_4, R' = Et, n = 1

6b, R = 3,4-$Cl_2C_6H_3$, R' = Et, n = 1

6c, R = 4-ClC_6H_4, R' = Me, n = 2

6d, R = 3,4-$Cl_2C_6H_3$, R' = Me, n = 2

6e, R = 4-ClC_6H_4, R' = Me, n = 3

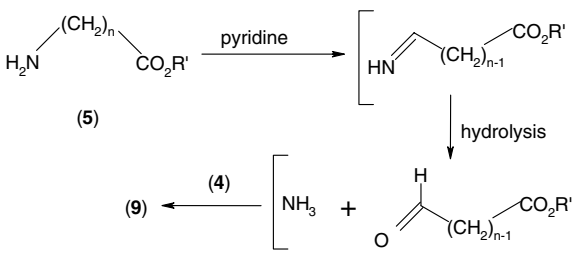
6f, R = 3,4-$Cl_2C_6H_3$, R' = Me, n = 3

6g, R = 4-ClC_6H_4, R' = Me, n = 5

6h, R = 3,4-$Cl_2C_6H_3$, R' = Me, n = 5

Scheme 1.

the unexpected ethyl-2-amino-4,6-diaryl-3-pyridinecarboxylates **9a** and **b**. Formation of **9** through this mentioned reaction could be explained analogously to the famous ninhydrin reaction with different amino acids,[12] where the amino acids isomerized to the corresponding imino-acid forms under the effect of applied basic reaction conditions. Then, upon hydrolysis due to unavoidable moisture, ammonia was liberated, which in turn interacted with 2-bromonicotinate esters **4**, giving finally **9** (Scheme 2). In fact, a similar observation was recently reported during the reaction of 2-bromonicotinamide derivatives with different primary aromatic amines in refluxing pyridine. Where the reaction afforded besides the 2-(arylamino)nicotinamides, the unexpected 2-(unsubstituted amino)-3-pyridinecarboxamide analogues.[10]



Scheme 2.

2.2. Vasodilation activity

Vasodilation activity screening for the synthesized nicotinate esters was investigated in vitro using thoracic aortic rings of male Wistar rats pre-contracted with norepinephrine hydrochloride (3×10^{-7} to 3×10^{-3} M) according to the standard known procedure.[13,14] From the observed data (Fig. 1, Table 1), it has been noticed that, all the tested compounds show considerable vasodilation properties. In addition, few prepared compounds especially **6b**, **6h** and **9b** exhibit remarkable potency (IC_{50}, concentrations necessary for 50% reduction of maximal norepinephrine hydrochloride reduced contracture) 0.16, 0.14 and 0.19 mM, respectively.

Structure–activity relationship based on the obtained results indicated that, 3,4-dichlorophenyl substitution of nicotinate ester nucleus enhances the pharmacological vasodilation properties than the case of substitution with 4-chlorophenyl function as observed in compounds **6c** and **6d** (IC_{50}, 0.51, 0.42 mM, respectively), **6g** and **6h** (IC_{50}, 0.32, 0.14 mM, respectively) and **9a** and **9b** (IC_{50}, 0.73, 0.19 mM, respectively). However, through the observed data no precise rule could be attained governing the type of used amino acid and vasodilation potency.

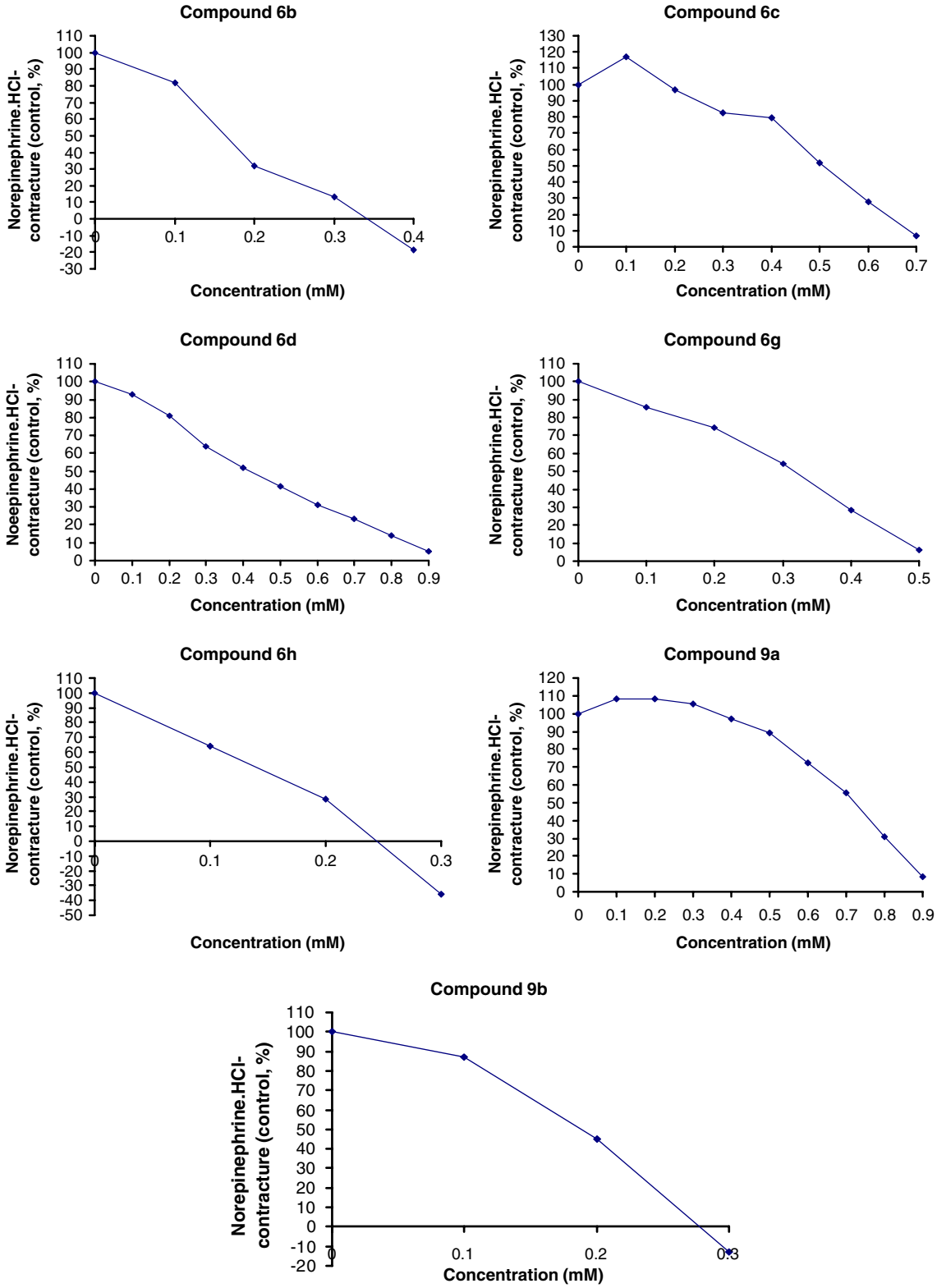


Figure 1. Effect of prepared compounds on contracture induced by Norepinephrine hydrochloride in rat aortic rings.

Generally, it could be concluded that, nicotinate ester analogues substituted with amino acid function could be a hint for constructing a promising vasodilation active agent useful for biological and/or pharmacological usage.

3. Experimental

Melting points are uncorrected and recorded on an Electrothermal 9100 digital melting point apparatus. IR spectra were recorded (KBr) on a Bruker Vector 22

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
6b	0.16	75.7
6c	0.51	223.8
6d	0.42	198.8
6g	0.32	153.9
6h	0.14	72.2
9a	0.73	257.6
9b	0.19	73.6

spectrophotometer. ^{1}H NMR spectra were recorded on a Varian MERCURY 300 (300 MHz) spectrometer. Mass spectra were recorded on a Finnigan SSQ 7000 (EI, 70 eV) spectrometer. The starting compounds **4a** and **b**[11] were prepared according to the previously reported procedures.

3.1. Synthesis of *N*-[(ethyl 4,6-diaryl-3-pyridinecarboxylate)-2-yl]amino acid esters 6a–h and their dipeptide analogues 8a and b (general procedure)

A mixture of **4a** and **b** (5 mmol) and the corresponding **5a–d** or **7** (5.5 mmol) in tetrahydrofuran (20 ml) containing triethylamine (15 mmol) was boiled under reflux for the appropriate time. The separated triethylamine salt was removed by filteration and the reaction mixture was evaporated untill dryness under reduced pressure. The remaining residue was purified on silica gel (60G F_{254}) TLC affording **6a–h**, **8a**, and **b**.

3.1.1. *N*-[[Ethyl 4-(4-chlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]glycine ethyl ester (6a). Reaction time 30 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 1:1 v/v for elution; mp: 134–136 °C, yield 64%; IR: ν_{max}/cm^{-1} 3419 (NH), 1734, 1698 (C=O), 1576, 1547 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.83 (t, 3H, CH_3, J = 7.2 Hz), 1.30 (t, 3H, CH_3, J = 7.2 Hz), 3.98 (q, 2H, OCH_2, J = 7.2 Hz), 4.25 (q, 2H, OCH_2, J = 7.2 Hz), 4.36 (d, 2H, NCH_2, J = 5.1 Hz), 6.98 (s, 1H, pyr. *H*-5), 7.21–7.46 (m, 7H, arom. H), 7.90 (br s, 1H, NH), 8.02–8.05 (m, 2H, arom. H). Anal. Calcd for $C_{24}H_{23}ClN_2O_4$ (438.893): C, 65.67; H, 5.28; N, 6.38. Found: C, 65.90; H, 5.42; N, 6.47.

3.1.2. *N*-[[Ethyl 4-(3,4-dichlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]glycine ethyl ester (6b). Reaction time 30 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 2:1 v/v for elution; mp: 107–109 °C, yield 55%. IR: ν_{max}/cm^{-1} 3415 (NH), 1738, 1698 (C=O), 1573, 1543 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H, CH_3, J = 7.2 Hz), 1.31 (t, 3H, CH_3, J = 7.2 Hz), 4.02 (q, 2H, OCH_2, J = 7.2 Hz), 4.26 (q, 2H, OCH_2, J = 7.2 Hz), 4.36 (d, 2H, NCH_2, J = 5.4 Hz), 6.96 (s, 1H, pyr. *H*-5), 7.11–8.05 (m, 9H, 8 arom. H+NH). Anal. Calcd for $C_{24}H_{22}Cl_2N_2O_4$ (473.35): C, 60.89; H, 4.69; N, 5.92. Found: C, 61.00; H, 4.77; N, 6.12.

3.1.3. *N*-[[Ethyl 4-(4-chlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]-β-alanine methyl ester (6c). Reaction time 36 h, pale yellow oils purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 2:1 v/v for elution, yield 50%. IR: ν_{max}/cm^{-1} 3383 (NH), 1719, 1684 (C=O), 1580, 1551 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.81 (t, 3H, CH_3, J = 7.2 Hz), 2.79 (t, CH_2CO, J = 6.6 Hz), 3.71 (s, 3H, OCH_3), 3.92–4.00 (m, 4H, OCH_2 + NCH_2), 6.93 (s, 1H, pyr. *H*-5), 7.20–7.47 (m, 7H, arom. H), 7.75 (br s, 1H, NH), 8.03–8.07 (m, 2H, arom. H). Anal. Calcd for $C_{24}H_{23}ClN_2O_4$ (438.893): C, 65.67; H, 5.28; N, 6.38. Found: C, 65.86; H, 5.39; N, 6.33.

3.1.4. *N*-[[Ethyl 4-(3,4-dichlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]-β-alanine methyl ester (6d). Reaction time 36 h, pale yellow oils purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 1:1 v/v for elution, yield 59%. IR: ν_{max}/cm^{-1} 3346, 3311 (NH), 1737, 1674 (C=O), 1574, 1546 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 3H, CH_3, J = 7.2 Hz), 2.78 (t, CH_2CO, J = 6.6 Hz), 3.72 (s, 3H, OCH_3), 3.95–4.03 (m, 4H, OCH_2 + NCH_2), 6.89 (s, 1H, pyr. *H*-5), 7.10–7.47 (m, 6H, arom. H), 7.90 (br s, 1H, NH), 8.04–8.07 (m, 2H, arom. H). Anal. Calcd for $C_{24}H_{22}Cl_2N_2O_4$ (473.35): C, 60.89; H, 4.69; N, 5.92. Found: C, 60.83; H, 4.64; N, 5.78.

3.1.5. *N*-[[Ethyl 4-(4-chlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]-4-aminobutyric acid methyl ester (6e). Reaction time 36 h, pale yellow oils purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 1:1 v/v for elution, yield 49%. IR: ν_{max}/cm^{-1} 3350 (NH), 1723, 1665 (C=O), 1578, 1547 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.72 (t, 3H, CH_3, J = 7.2 Hz), 1.99 (quintet, 2H, CH_2CH_2CO, J = 7.2 Hz), 2.40 (t, 2H, CH_2CO, J = 7.2 Hz), 3.58 (s, 3H, OCH_3), 3.62-3.68 (m, 2H, NCH_2), 3.86 (q, 2H, OCH_2, J = 7.2 Hz), 6.82 (s, 1H, pyr. *H*-5), 7.13–7.40 (m, 7H, arom. H), 7.49 (br s, 1H, NH), 7.96–7.99 (m, 2H, arom. H). Anal. Calcd for $C_{25}H_{25}ClN_2O_4$ (452.923): C, 66.29; H, 5.56; N, 6.19. Found: C, 66.08; H, 5.40; N, 6.29.

3.1.6. *N*-[[Ethyl 4-(3,4-dichlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]-4-aminobutyric acid methyl ester (6f). Reaction time 36 h, pale yellow oils purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 2:1 v/v for elution, yield 45%. IR: ν_{max}/cm^{-1} 3378 (NH), 1738, 1681 (C=O), 1574, 1547 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.84 (t, 3H, CH_3, J = 7.2 Hz), 2.07 (quintet, 2H, CH_2CH_2CO, J = 7.2 Hz), 2.47 (t, 2H, CH_2CO, J = 7.2 Hz), 3.66 (s, 3H, OCH_3), 3.72 (br s, 2H, NCH_2), 3.98 (q, 2H, OCH_2, J = 7.2 Hz), 6.87 (s, 1H, pyr. *H*-5), 7.10–7.48 (m, 6H, arom. H), 7.74 (br s, 1H, NH), 8.03–8.07 (m, 2H, arom. H). Anal. Calcd for $C_{25}H_{24}Cl_2N_2O_4$ (487.37): C, 61.61; H, 4.96; N, 5.75. Found: C, 61.54; H, 4.90; N, 5.56.

3.1.7. *N*-[[Ethyl 4-(4-chlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]caproic acid methyl ester (6g). Reaction time 40 h, pale yellow oils purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 2:1 v/v for elution, yield 46%. IR: ν_{max}/cm^{-1} 3384

(NH), 1738, 1680 (C=O), 1576, 1547 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.79 (t, 3H, CH_3, J = 7.2 Hz), 1.47–1.53 (m, 2H, $NCH_2CH_2CH_2$), 1.68–1.78 (m, 4H, NCH_2CH_2 + CH_2CH_2CO), 2.34 (t, 2H, CH_2CO, J = 7.5 Hz), 3.63–3.70 (m, 5H, OCH_3 + NCH_2), 3.93 (q, 2H, OCH_2, J = 7.2 Hz), 6.89 (s, 1H, pyr. *H*-5), 7.20–7.46 (m, 7H, arom. H), 7.56 (br s, 1H, NH), 8.05–8.08 (m, 2H, arom. H). Anal. Calcd for $C_{27}H_{29}ClN_2O_4$ (480.973): C, 67.42; H, 6.08; N, 5.83. Found: C, 67.56; H, 6.17; N, 5.76.

3.1.8. *N*-[[Ethyl 4-(3,4-dichlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]caproic acid methyl ester (6h). Reaction time 42 h, almost colourless oils purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 2:1 v/v for elution, yield 47%. IR: ν_{max}/cm^{-1} 3357 (NH), 1736, 1677 (C=O), 1575, 1546 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.85 (t, 3H, CH_3, J = 7.2 Hz), 1.48–1.54 (m, 2H, $NCH_2CH_2CH_2$), 1.70–1.79 (m, 4H, NCH_2CH_2 + CH_2CH_2CO), 2.37 (t, 2H, CH_2CO, J = 7.5 Hz), 3.68 (br s, 5H, OCH_3 + NCH_2), 3.99 (q, 2H, OCH_2, J = 7.2 Hz), 6.87 (s, 1H, pyr. *H*-5), 7.12–7.49 (m, 6H, arom. H), 7.74 (br s, 1H, NH), 8.06–8.09 (m, 2H, arom. H). Anal. Calcd for $C_{27}H_{28}Cl_2N_2O_4$ (515.42): C, 62.91; H, 5.48; N, 5.44. Found: C, 63.09; H, 5.60; N, 5.53.

3.1.9. *N*-[[Ethyl 4-(4-chlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]-*N′*-glycylglycine ethyl ester (8a). Reaction time 50 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 7:1 v/v for elution; mp: 131–133 °C, yield 36%. IR: ν_{max}/cm^{-1} 3424, 3343 (NH), 1760, 1690. 1655 (C=O), 1580, 1548 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.03 (t, 3H, CH_3, J = 7.2 Hz), 1.41 (t, 3H, CH_3, J = 7.2 Hz), 4.19 (q, 2H, OCH_2, J = 7.2 Hz), 4.26 (d, 2H, NCH_2, J = 5.4 Hz), 4.33 (q, 2H, OCH_2, J = 7.2 Hz), 4.55 (d, 2H, NCH_2, J = 4.5 Hz), 7.18 (br s, 1H, NH), 7.42–7.65 (m, 8H, 7 arom. H + pyr. *H*-5), 8.00 (br s, 1H, NH), 8.22–8.25 (m, 2H, arom. H). Anal. Calcd for $C_{26}H_{26}ClN_3O_5$ (495.943): C, 62.96; H, 5.28; N, 8.47. Found: C, 62.90; H, 5.23; N, 8.34.

3.1.10. *N*-[[Ethyl 4-(3,4-dichlorophenyl)-6-phenyl-3-pyridinecarboxylate]-2-yl]-*N′*-glycylglycine ethyl ester (8b). Reaction time 50 h, colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 7:1 v/v for elution; mp: 126–128 °C, yield 34%. IR: ν_{max}/cm^{-1} 3404, 3294 (NH), 1756, 1670 (C=O), 1574, 1544 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.07 (t, 3H, CH_3, J = 7.2 Hz), 1.41 (t, 3H, CH_3, J = 7.2 Hz), 4.23 (q, 2H, OCH_2, J = 6.9 Hz), 4.25 (br s, 2H, NCH_2), 4.34 (q, 2H, OCH_2, J = 7.2 Hz), 4.56 (br s, 2H, NCH_2), 7.09 (br s, 1H, NH), 7.32–7.70 (m, 7H, 6 arom. H + pyr. *H*-5), 8.22–8.24 (m, 2H, arom. H), 8.30 (br s, 1H, NH). Anal. Calcd for $C_{26}H_{25}Cl_2N_3O_5$ (530.39): C, 58.87; H, 4.75; N, 7.92. Found: C, 58.73; H, 4.69; N, 7.95.

3.2. Synthesis of ethyl 2-amino-4,6-diaryl-3-pyridinecarboxylates 9a and b (general procedure)

A mixture of equimolar amounts of **4a** and **b** and the corresponding **5a–d** (5 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 and purified on silica gel (60 G F_{254}) TLC affording **9a** and **b**.

3.2.1. Ethyl 2-amino-4-(4-chlorophenyl)-6-phenyl-3-pyridinecarboxylate (9a). Reaction time 45 h (in case of reaction of **4a** with **5a–d**), colourless crystals purified by silica gel TLC using chloroform-light petroleum (60–80 °C) mixture as 2:1 v/v for elution; mp: 170–172 °C, yield 51%, 40%, 43% and 34% (in case of reaction of **4a** with **5a–d**, respectively). IR: ν_{max}/cm^{-1} 3442, 3275, 3169 (NH_2), 1680 (C=O), 1572, 1564 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.83 (t, 3H, CH_3, J = 7.2 Hz), 3.98 (q, 2H, OCH_2, J = 7.2 Hz), 6.18 (s, 2H, NH_2), 6.97 (s, 1H, pyr. *H*-5), 7.23–8.00 (m, 9H, arom. H). MS: *m*/*z* (%) 352 (M, 100), 307 (21), 306 (18), 281 (14), 280 (39), 279 (21). Anal. Calcd for $C_{20}H_{17}ClN_2O_2$ (352.813): C, 68.08; H, 4.86; N, 7.94. Found: C, 68.12; H, 4.88; N, 8.05.

3.2.2. Ethyl 2-amino-4-(3,4-dichlorophenyl)-6-phenyl-3-pyridinecarboxylate (9b). Reaction time 50 h (in case of reaction of **4b** with **5a**), colourless crystals purified by silica gel TLC using chloroform; mp: 150–151 °C, yield 36%. IR: ν_{max}/cm^{-1} 3342 (NH_2), 1673 (C=O), 1576, 1558 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 0.77 (t, 3H, CH_3, J = 7.2 Hz), 3.90 (q, 2H, OCH_2, J = 7.2 Hz), 6.15 (br s, 2H, NH_2), 6.90 (s, 1H, pyr. *H*-5), 7.04–8.02 (m, 8H, arom. H). MS: *m*/*z* (%) 386 (M, 100), 341 (21), 340 (17), 316 (29), 315 (22), 314 (49). Anal. Calcd for $C_{20}H_{16}Cl_2N_2O_2$ (387.26): C, 62.03; H, 4.17; N, 7.24. Found: C, 62.24; H, 4.30; N, 7.50.

3.3. Vasodilation activity screening

All the experimental procedures were carried out following the guidelines of European Community Council Directive 86-609. The vasodilation activity screening procedures were carried out according to the standard reported techniques,[13,14] by testing the effects of the synthesized nicotinate ester derivatives on isolated thoracic aortic rings of male Wistar rats (250–350 g). After a 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 PoweLab 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 testing 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, demonstrated that the solvent did not affect the contractile response of isolated aorta. Prazosin 'α_1-adrenergic blocker, $IC_{50} = 1.2 \times 10^{-10}$ M', was used as a reference standard for comparison.[15,16]

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