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Original article

Rational design, synthesis and QSAR study of vasorelaxant active 3-pyridinecarbonitriles incorporating 1*H*-benzimidazol-2-yl function

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ABSTRACT

A variety of 2-alkoxy-4-aryl-6-(1*H*-benzimidazol-2-yl)-3-pyridinecarbonitriles **4a**–**r** were prepared via either regioselective reaction of 3-aryl-1-(1*H*-benzimidazol-2-yl)-2-propen-1-ones **3** with malononitrile or ylidenemalononitriles **6** with 2-acetyl-1*H*-benzimidazoles **1** in the presence of sodium alkoxide in the corresponding alcohol. All the synthesized compounds showed significant vasodilation properties using isolated thoracic aortic rings of rats pre-contracted with norepinephrine hydrochloride standard technique. Compounds **4d**, **4p**, **4l**, and **4f** exhibited remarkable activity compared with prazosin hydrochloride, which was used as a reference standard in the present study. QSAR studies revealed a good predictive and statistically significant 3 descriptor model (r^2 = 0.913, $r^2_{adjusted}$ = 0.8808, $r^2_{prediction}$ = 0.7911).

1. Introduction

Cardiovascular and cerebrovascular disorders are the main reason for morbidity and death in recent years. These include coronary heart diseases, hypertension, and peripheral artery diseases in addition to others [1]. Hypertension is the most common cardiovascular disease that represents the major risk factor for endothelial dysfunction, metabolic syndrome, renal dysfunction, congestive heart failure, coronary artery disease, and stroke [2]. Hypertension affects approximately billions of people all around the world. Hypertension is defined as repeatedly elevated systolic and/or diastolic blood pressure above 140/90 mm Hg [3]. Consistent control of blood pressure is of crucial importance and should be achieved throughout the 24 h dosing interval where, uncontrolled hypertension is associated with acute end-organ damage [4] as congestive heart failure [5] or renal failure as in type-2 diabetes patients [6]. Drugs currently used for hypertension include diuretics [7]; drugs that prevent the action of peripheral sympathetic activity as β-adrenergic [8,9] and α-adrenergic [10] blocking agents; centrally sympathetic α_2-adrenoceptors [11]; angiotensin-converting enzyme inhibitors [12,13]; angiotensin II receptor blockers [13,14] and calcium channel blockers [15]; in addition to drugs directly dilating the blood vessels (arterial dilators) [16]. Relaxation of vascular smooth muscles is considered one of the main strategies for treatment of hypertension [17]. Several agents have been developed; however they are all associated with side effects such as fatigue, mood change, sleep disturbances, dry mouth, blurry vision, impotence etc. [1]. Therefore, there is a continuous need to explore, search and develop new vasorelaxant agents with minimal side effects.

One of the most known vasodilatory active heterocycles are nicotinate esters. Where many nicotinate analogs 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', hepronicate '3-pyridinecarboxylic acid, 2-hexyl-2-[[(3-pyridinylcarbonyl)oxy]methyl]-1,3-propanediyl ester' and inositol nicotinate '*myo*-inositol hexa-3-pyridinecarboxylate' [18]. In continuation of our reports directing toward developing vasorelaxant active agents [19–23], it is intended in the present work to describe synthesis as well as vasodilation properties of novel 3-pyridinecarbonitriles incorporating 1*H*-benzimidazol-2-yl function. Interest in developing these agents could be attributed to the fact that, 3-pyridinecarbonitriles are recognized as bioisosteric forms of nicotinate analogs (3-pyridinecarboxylates),

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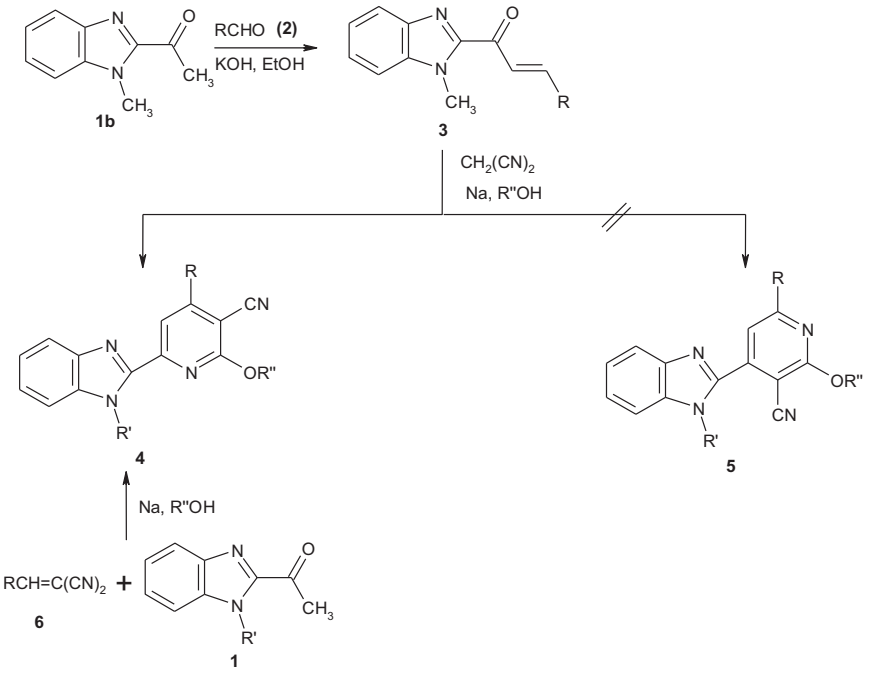
where only the cyano group replaces the acid/ester function. This may develop enhanced pharmacological active agents with higher potency and fewer side effects. Recent publications describing vasorelaxant properties of 4,4′-[1,2-ethanediylbis(oxy-2,1-phenylene)]bis(2-alkoxy-6-aryl-3-pyridinecarbonitriles) also support the present investigation [24]. Bioactivity and applications as pharmaceutical active agents and agricultural materials of pyridinecarbonitriles also prompted the present study [25,26]. Additionally, 4-amino-3-pyridinecarbonitriles were reported as PKCθ inhibitor [27–33]. PKCθ, a novel isoform, is crucial for the activation and survival of T cells [34–36]. Proof-of-concept studies with mice where the PKCθ gene was deleted or 'knocked out' validated that the inhibition of this kinase could have therapeutic utility in diseases such as multiple sclerosis [37,38], arthritis [39], asthma [40,41], inflammatory bowel disease [42], and transplant rejection [43,44]. Pyridinecarbonitriles analogs were also reported as fluorescent materials useful as security markers for treatment of paper [45–47]. Moreover, benzimidazole containing-compounds were reported to exhibit promising pharmacological properties such as antihypertensive [48] and vasodilation [49] in addition to antitumor [50–53], anti-inflammatory [54], antibacterial [55,56], and antiviral [57–59] activity. Additionally, quantitative structure–activity relationships (QSAR) will be also considered in the present study not only to explore the controlling factors governing the observed pharmacological properties of the synthesized analogs, but also to validate the observed activity.

2. Results and discussion

2.1. Chemistry

Reaction of 3-aryl-1-[(1-methyl-1*H*-benzimidazol)-2-yl]-2-propen-1-ones **3a**,**b** with malononitrile in the presence of sufficient amount of sodium alkoxide in the corresponding alcohol afforded only one product which structure was assigned to be either 2-alkoxy-4-aryl-6-[(1-methyl-1*H*-benzimidazol)-2-yl]-3-pyridinecarbonitriles **4** or their isomeric form 2-alkoxy-6-aryl-4-[(1-methyl-1*H*-benzimidazol)-2-yl]-3-pyridinecarbonitriles **5**, based on the observed spectroscopic (IR, ^{1}H NMR, ^{13}C NMR, MS) and elemental analysis data (Scheme 1). Formation of **4a**–**r** via reaction of ylidenemalononitriles **6a**–**h** with 2-acetyl-1*H*-benzimidazoles **1a**,**b** in the presence of sufficient amount of sodium alkoxide in the corresponding alcohol not only adds good support for the assumed structures but also confirms that the reaction of 2-propen-1-ones **3** with malononitrile proceeds in a regioselective



1a; R' = H
1b; R' = Me

2a, 3a; R = 4-ClC$_6$H$_4$
2b, 3b; R = 4-H$_3$COC$_6$H$_4$

6a; R = Ph
6b; R = 4-ClC$_6$H$_4$
6c; R = 2,4-Cl$_2$C$_6$H$_3$
6d; R = 4-FC$_6$H$_4$
6e; R = 4-H$_3$CC$_6$H$_4$
6f; R = 4-H$_3$COC$_6$H$_4$
6g; R = 2-thienyl
6h; R = 5-methyl-2-furanyl

4a; R = Ph, R', R" = Me
4b; R = Ph, R' = Me, R" = Et
4c; R = 4-ClC$_6$H$_4$, R' = H, R" = Me
4d; R = 4-ClC$_6$H$_4$, R', R" = Me
4e; R = 4-ClC$_6$H$_4$, R' = Me, R" = Et
4f; R = 2,4-Cl$_2$C$_6$H$_3$, R', R" = Me
4g; R = 2,4-Cl$_2$C$_6$H$_3$, R' = Me, R" = Et
4h; R = 4-FC$_6$H$_4$, R', R" = Me
4i; R = 4-FC$_6$H$_4$, R' = Me, R" = Et
4j; R = 4-H$_3$CC$_6$H$_4$, R', R" = Me
4k; R = 4-H$_3$CC$_6$H$_4$, R' = Me, R" = Et
4l; R = 4-H$_3$COC$_6$H$_4$, R', R" = Me
4m; R = 4-H$_3$COC$_6$H$_4$, R' = Me, R" = Et
4n; R = 2-thienyl, R', R" = Me
4o; R = 2-thienyl, R' = Me, R" = Et
4p; R = 5-methyl-2-furanyl, R' = H, R" = Me
4q; R = 5-methyl-2-furanyl, R', R" = Me
4r; R = 5-methyl-2-furanyl, R' = Me, R" = Et

Scheme 1. Synthetic routes of compounds **4a**–**r**.

manner via Michael addition (due to active methylene malononitrile attack at the β-carbon of **3** with subsequent cyclization via nucleophilic attack of the alkoxide at one of the nitrile groups followed by dehydration and dehydrogenation to give eventually **4**) rather than Knoevenagel pathway (condensation of the active methylene malononitrile with the ketonic residue of **3** with subsequent cyclization due to alkoxide attack at one of the nitrile groups followed by dehydrogenation to give **5**) (Scheme 2).

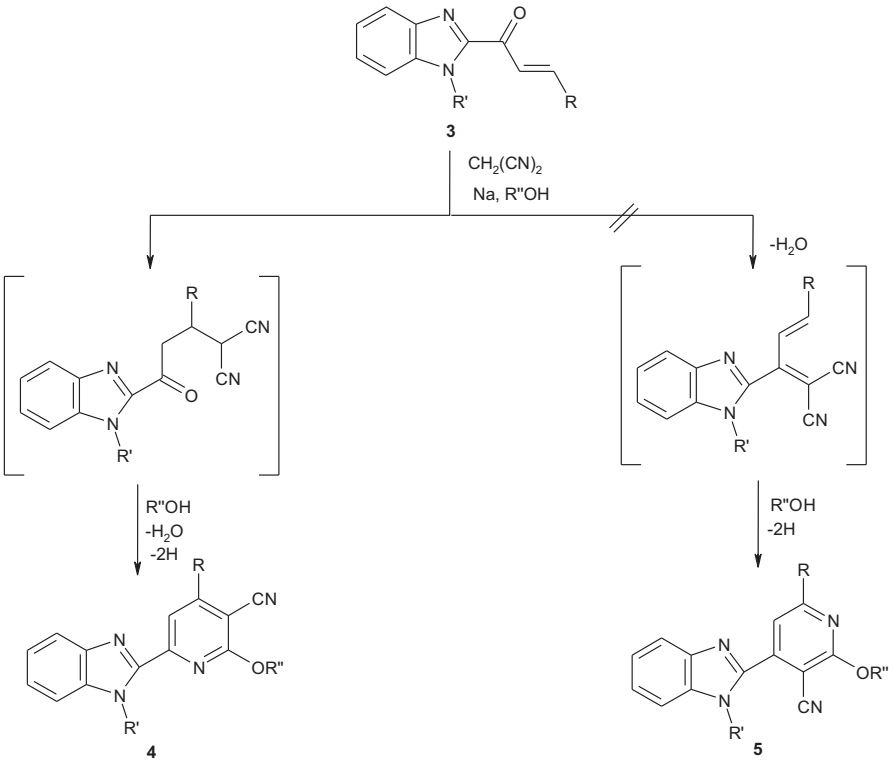
IR spectrum of **4a** (as a representative example of the synthesized analogs) reveals the presence of a strong stretching nitrile vibration band at $\nu = 2220\ cm^{-1}$ while, no assignable band due to carbonyl stretching vibration was detected. ^1H NMR spectrum of **4a** exhibits the characteristic pyridinyl *H*-5 at $\delta = 8.24$ in addition to the methoxide signal at $\delta = 4.28$. ^{13}C NMR spectrum of **4a** reveals the pyridinyl *C*-3 and *C*-5 at $\delta = 94.4$ and 110.2 in addition to the methoxide and nitrile carbons at $\delta = 55.2$ and 115.3, respectively. Mass spectrum (EI) of **4a** reveals the parent ion peak with a considerable relative intensity value. The spectral data (IR, ^1H NMR, ^{13}C NMR, MS) of all the other synthesized analogs exhibit similar observations to that of compound **4a** confirming their established structures (*c.f.* Experimental section).

2.2. Vasodilation properties

Vasodilation properties of the synthesized 2-alkoxy-4-aryl-6-(1*H*-benzimidazol-2-yl)-3-pyridinecarbonitriles **4a**–**r** were investigated using isolated thoracic aortic rings of rats pre-contracted with norepinephrine hydrochloride standard reported procedure [20–23] and compared with prazosin hydrochloride (highly selective α_1-adrenoceptor antagonist), which was used as a reference standard. The observed data (Table 1, Figures 1 and 2 of Supplementary material) reveal that, all the synthesized compounds show significant vasodilation properties. Meanwhile, compounds **4d**, **4p**, **4l**, and **4f** exhibit remarkable activity (IC_{50}, concentration necessary for 50% reduction of maximal norepinephrine hydrochloride induced contracture = 0.145, 0.202, 0.210, 0.214 mM, respectively), that seem more potent than prazosin hydrochloride (IC_{50} = 0.487 mM [60]), the used reference standard in the present study. In order to understand the observed pharmacological properties and determining the main controlling factors governing these activities, QSAR study was initiated. Meanwhile, through the observed vasodilation properties of the synthesized compounds few SAR rules could be attained. Insertion of a methoxy function at 2-position of the synthesized 3-pyridinecarbonitriles afforded more vasorelaxant active agents than the case when ethoxy group was adopted, as exhibited in all the tested analogs, except compound **4o**. Additionally, 4-chlorophenyl substitution at the 4-position of 3-pyridinecarbonitriles seems the best choice for optimization of a vasorelaxant active hit as exhibited in compounds **4a**, **4d**, **4f**, **4h**, **4j**, **4l**, **4n** and **4q** (IC_{50} = 0.300, 0.145, 0.214, 0.343, 0.295, 0.210, 0.312 and 0.283, respectively) and compounds **4b**, **4e**, **4g**, **4i**, **4k**, **4m**, **4o** and **4r** (IC_{50} = 0.364, 0.293, 0.305, 0.362, 0.400, 0.436, 0.249 and 0.336, respectively).

2.3. QSAR study

This study was performed using Discovery Studio 2.5 software (Accelrys Inc., San Diego, CA, USA), which permits search for diverse structural descriptors (connectivity, topological, etc.) capable of explaining the controlling factors governing bio-activity observations. A set of 16 compounds (**4a**,**c**,**e**–**r**) was used as a training set for a QSAR modeling. The remaining 2 synthesized analogs (**4b** and **4d** "mild and high vasodilation active agents, respectively") were adopted as an external test subset for validating the QSAR models. Many molecular descriptors were calculated for each compound employing calculate molecular properties module. The calculated descriptors including various simple and valence connectivity



Scheme 2. Schematic diagram explaining the proposed mechanistic pathways toward the synthesized pyridinecarbonitriles.

Table 1
Concentration of compounds necessary to reduce maximal norepinephrine hydrochloride induced contracture by 50% (IC_{50}) in rat thoracic aortic rings.

Entry	Compound	R	R′	R″	Potency (IC_{50}), mM
1	**4a**	Ph	Me	Me	0.300
2	**4b**	Ph	Me	Et	0.364
3	**4c**	4-ClC_6H_4	H	Me	0.286
4	**4d**	4-ClC_6H_4	Me	Me	0.145
5	**4e**	4-ClC_6H_4	Me	Et	0.293
6	**4f**	2,4-$Cl_2C_6H_3$	Me	Me	0.214
7	**4g**	2,4-$Cl_2C_6H_3$	Me	Et	0.305
8	**4h**	4-FC_6H_4	Me	Me	0.343
9	**4i**	4-FC_6H_4	Me	Et	0.362
10	**4j**	4-$H_3CC_6H_4$	Me	Me	0.295
11	**4k**	4-$H_3CC_6H_4$	Me	Et	0.400
12	**4l**	4-$H_3COC_6H_4$	Me	Me	0.210
13	**4m**	4-$H_3COC_6H_4$	Me	Et	0.436
14	**4n**	2-Thienyl	Me	Me	0.312
15	**4o**	2-Thienyl	Me	Et	0.249
16	**4p**	5-Methyl-2-furanyl	H	Me	0.202
17	**4q**	5-Methyl-2-furanyl	Me	Me	0.283
18	**4r**	5-Methyl-2-furanyl	Me	Et	0.336
19	Prazosin hydrochloride	–	–	–	0.487

indices, electro-topological state indices, single point quantum-mechanical descriptors (via the AM1 model) and other molecular descriptors were considered. Genetic function approximation (GFA) was employed to search for the best possible QSAR regression equation capable of correlating the variations in biological activities of the training set compounds with variations in the generated descriptors, i.e. multiple linear regression modeling [61]. The present equation shows our best-performing QSAR model (Fig. 1 exhibits the corresponding scatter plots of experimental versus estimated bioactivity values for the training set compounds).

$$IC_{50} = -0.203 + 0.798\ \text{Shadow YZfrac} + 0.491\ \text{Jurs FPSA 2} - 0.025\ \text{Jurs PPSA 3}$$

Where; Shadow YZfrac, is the area of the molecular shadow in the YZ plane, Jurs FPSA 2, is the Fractional charged partial surface areas "set of six descriptors obtained by dividing descriptors 1–6 by the total molecular solvent-accessible surface area", and Jurs PPSA 3, is the atomic charge weighted positive surface area "sum of the product of solvent-accessible surface area × partial charge for all positively charged atoms" (Table 2 exhibits the calculated descriptor values and the estimated bio-activity value due to the mentioned model).

The goodness of the model was validated by squared correlation coefficient (r^2) = 0.913, $r^2_{adjusted}$ = 0.8808, $r^2_{prediction}$ = 0.7911, RMS residual error (root mean square) = 0.02253, for *N* "number of molecules in the training set" = 16.

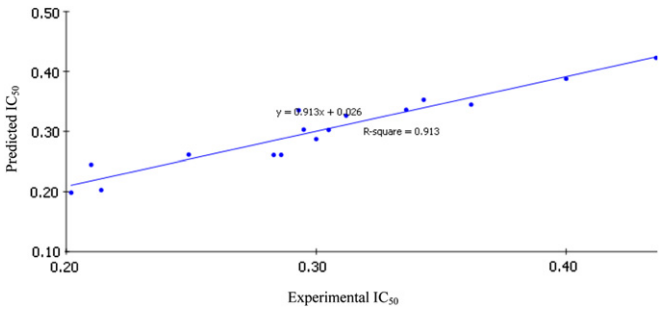


Fig. 1. Predicted versus experimental IC_{50} values of the training set compounds.

2.4. Validation of QSAR

External validation of the determined QSAR model was performed utilizing two of our synthesized analogs exhibiting mild (**4b**) and potent (**4d**) vasodilation activity. The observed activities and those provided by QSAR study, was presented in Table 3.

3. Conclusion

2-Alkoxy-4-aryl-6-(1*H*-benzimidazol-2-yl)-3-pyridinecarbonitriles **4a**–**r** were successfully prepared via either regioselective reaction of 3-aryl-1-(1*H*-benzimidazol-2-yl)-2-propen-1-ones **3** with malononitrile or ylidenemalononitriles **6** with 2-acetyl-1*H*-benzimidazoles **1** in the presence of sodium alkoxide in the corresponding alcohol. All the synthesized compounds showed significant vasodilation properties using isolated thoracic aortic rings of rats pre-contracted with norepinephrine hydrochloride standard technique. Compounds **4d**, **4p**, **4l**, and **4f** exhibited remarkable activity compared with prazosin hydrochloride, (highly selective α_1-adrenoceptor antagonist), which was used as a reference standard in the present study. QSAR studies revealed a good predictive and statistically significant 3 descriptor model (r^2 = 0.913, $r^2_{adjusted}$ = 0.8808, $r^2_{prediction}$ = 0.7911). External validation of the determined QSAR model utilizing two of our synthesized analogs exhibiting mild (**4b**) and potent (**4d**) vasodilation activity supported the established model. From all the above it could be concluded that, designing 3-pyridinecarbonitrile analogs possessing 1*H*-benzimidazol-2-yl function, seems highly promising approach toward developing vasodilation active hits.

4. Experimental

Melting points were recorded on a Stuart SMP3 melting point apparatus. IR spectra (KBr) were recorded on a JASCO 6100 spectrophotometer. NMR spectra were recorded on a JEOL AS 500 (^{1}H: 500, ^{13}C: 125 MHz) spectrometer. Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX (EI, 70 eV) spectrometer. Compounds **1a**,**b** [62], **3a**,**b** [63], and **6a**–**h** [64] were prepared according to the previously reported procedures (Figures 3–73 of Supplementary material exhibit the spectral features of the synthesized compounds).

4.1. Synthesis of 2-alkoxy-4-aryl-6-(1H-benzimidazol-2-yl)-3-pyridinecarbonitriles *4a*–*r*

4.1.1. Method A

A mixture of equimolar amounts of **3a**,**b** (10 mmol) and malononitrile in the appropriate alcohol (25 ml) containing sodium (0.46 g; 20 mmol) was stirred at room temperature (25–30 °C) for the proper time (TLC control). The separated solid was collected, washed with water and crystallized from *n*-butanol affording the corresponding **4d**,**e**,**l**,**m**.

4.1.2. Method B

A mixture of equimolar amounts of **1a**,**b** (10 mmol) and the corresponding ylidenemalononitrile **6a**–**h** in the appropriate alcohol (25 ml) containing sodium (0.46 g; 20 mmol) was stirred at room temperature (25–30 °C) for the proper time (TLC control). The separated solid was collected, washed with water and crystallized from *n*-butanol affording the corresponding **4a**–**r**.

*4.1.2.1. 2-Methoxy-6-(1-methyl-1H-benzimidazol-2-yl)-4-phenyl-3-pyridinecarbonitrile (**4a**).* Reaction time 24 h, colorless crystals, mp 219–221 °C, yield (2.10 g) 62%. IR: ν_{max}/cm^{-1} 2220 (C≡N), 1586,

Table 2
Estimated activity values of the training set and calculated descriptors governing activity according to the QSAR model.

Entry	Compd.	R	R′	R″	Observed activity (IC_{50}), mM	Estimated activity	Descriptors		
							Shadow YZfrac	Jurs FPSA 2	Jurs PPSA 3
1	**4a**	Ph	Me	Me	0.300	0.282	0.6879	1.1328	24.7913
2	**4c**	4-ClC_6H_4	H	Me	0.286	0.261	0.6810	0.9906	24.6475
3	**4e**	4-ClC_6H_4	Me	Et	0.293	0.335	0.7080	1.0187	21.6573
4	**4f**	2,4-$Cl_2C_6H_3$	Me	Me	0.214	0.203	0.5902	0.9157	21.8512
5	**4g**	2,4-$Cl_2C_6H_3$	Me	Et	0.305	0.302	0.6254	0.9380	20.4677
6	**4h**	4-FC_6H_4	Me	Me	0.343	0.353	0.6984	1.0920	24.0665
7	**4i**	4-FC_6H_4	Me	Et	0.362	0.345	0.6365	1.1406	22.9951
8	**4j**	4-$H_3CC_6H_4$	Me	Me	0.295	0.303	0.6691	1.1859	24.3749
9	**4k**	4-$H_3CC_6H_4$	Me	Et	0.400	0.408	0.7462	1.2119	23.1048
10	**4l**	4-$H_3COC_6H_4$	Me	Me	0.210	0.245	0.6051	1.3475	27.6895
11	**4m**	4-$H_3COC_6H_4$	Me	Et	0.436	0.423	0.7695	1.3329	25.3009
12	**4n**	2-Thienyl	Me	Me	0.312	0.326	0.6849	1.0194	22.8361
13	**4o**	2-Thienyl	Me	Et	0.249	0.261	0.6780	1.0197	21.3766
14	**4p**	5-Methyl-2-furanyl	H	Me	0.202	0.198	0.5769	1.2233	25.7432
15	**4q**	5-Methyl-2-furanyl	Me	Me	0.283	0.261	0.6287	1.2296	23.8522
16	**4r**	5-Methyl-2-furanyl	Me	Et	0.336	0.336	0.6765	1.2763	23.2495

1547 (C═N, C═C). ^{1}H NMR ($CDCl_3$): δ 4.16 (s, 3H, NCH_3), 4.28 (s, 3H, OCH_3), 7.32–7.82 (m, 9H, arom. H), 8.24 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 33.2 (NCH_3), 55.2 (OCH_3), 94.4 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 115.3 (*C*≡N), 117.7, 120.4, 123.3, 124.4, 128.6, 129.0, 130.4, 135.5, 137.4, 142.6, 148.2, 150.5, 156.7, 164.7 (arom. *C*). MS: *m*/*z* (%) 340 (M, 87), 339 (100). Anal. Calcd. For $C_{21}H_{16}N_4O$ (340.39): C, 74.10; H, 4.74; N, 16.46. Found: C, 74.32; H, 4.88; N, 16.52.

*4.1.2.2. 2-Ethoxy-6-(1-methyl-1H-benzimidazol-2-yl)-4-phenyl-3-pyridinecarbonitrile (**4b**).* Reaction time 24 h, pale yellow crystals, mp 228–230 °C, yield (1.95 g) 55%. IR: ν_{max}/cm^{-1} 2220 (C≡N), 1584, 1549 (C═N, C═C). ^{1}H NMR ($CDCl_3$): δ 1.55 (t, 3H, OCH_2CH_3, J = 6.5 Hz), 4.35 (s, 3H, NCH_3), 4.61 (q, 2H, OCH_2, J = 6.5 Hz), 7.38–7.88 (m, 9H, arom. H), 8.32 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 14.6 (OCH_2CH_3), 33.1 (NCH_3), 64.0 (OCH_2), 94.7 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 115.3 (*C*≡N), 117.7, 120.5, 123.4, 124.4, 128.6, 129.0, 130.3, 135.7, 137.4, 142.5, 148.4, 150.5, 156.8, 164.5 (arom. *C*). MS: *m*/*z* (%) 354 (M, 35). Anal. Calcd. For $C_{22}H_{18}N_4O$ (354.41): C, 74.56; H, 5.12; N, 15.81. Found: C, 74.75; H, 5.23; N, 15.97.

*4.1.2.3. 6-(1H-Benzimidazol-2-yl)-4-(4-chlorophenyl)-2-methoxy-3-pyridinecarbonitrile (**4c**).* Reaction time 48 h, pale yellow crystals, mp 273–275 °C, yield (2.10 g) 58%. IR: ν_{max}/cm^{-1} 3343 (NH), 2221 (C≡N), 1593, 1545 (C═N, C═C). ^{1}H NMR (DMSO-d_6): δ 4.24 (s, 3H, OCH_3), 7.23–7.78 (m, 8H, arom. H), 8.00 (s, 1H, pyridinyl *H*-5), 13.12 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 55.9 (OCH_3), 94.6 (pyridinyl *C*-3), 112.9 (pyridinyl *C*-5), 115.5 (*C*≡N), 114.8, 120.3, 123.1, 124.8, 129.6, 130.9, 134.7, 135.5, 135.9, 144.5, 149.3, 149.5, 155.5, 165.2 (arom. *C*). MS: *m*/*z* (%) 360 (M, 18), 362 [(M + 2), 11]. Anal. Calcd. For $C_{20}H_{13}ClN_4O$ (360.81): C, 66.58; H, 3.63; N, 15.53. Found: C, 66.64; H, 3.72; N, 15.73.

*4.1.2.4. 4-(4-Chlorophenyl)-2-methoxy-6-(1-methyl-1H-benzimidazol-2-yl)-3-pyridinecarbonitrile (**4d**).* Reaction time 24 h (Methods A & B), almost colorless crystals, mp 259–261 °C, yield (2.16, 2.30 g) 58, 61% (Methods A & B, respectively). IR: ν_{max}/cm^{-1} 2220 (C≡N), 1586, 1545 (C═N, C═C). ^{1}H NMR ($CDCl_3$): δ 4.20 (s, 3H, NCH_3), 4.35 (s, 3H, OCH_3), 7.33–7.84 (m, 8H, arom. H), 8.23 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 33.3 (NCH_3), 55.3 (OCH_3), 94.4 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 115.1 (*C*≡N), 117.5, 120.6, 123.4, 124.5, 129.4, 130.0, 134.0, 136.8, 137.5, 142.7, 148.1, 150.9, 155.5, 164.9 (arom. *C*). MS: *m*/*z* (%) 374 (M, 100), 376 [(M + 2), 49]. Anal. Calcd. For $C_{21}H_{15}ClN_4O$ (374.83): C, 67.29; H, 4.03; N, 14.95. Found: C, 67.15; H, 3.93; N, 15.12.

*4.1.2.5. 4-(4-Chlorophenyl)-2-ethoxy-6-(1-methyl-1H-benzimidazol-2-yl)-3-pyridinecarbonitrile (**4e**).* Reaction time 24 h (Methods A & B), colorless crystals, mp 243–245 °C, yield (1.94, 2.10 g) 50, 54% (Methods A & B, respectively). IR: ν_{max}/cm^{-1} 2223 (C≡N), 1580, 1546 (C═N, C═C). ^{1}H NMR ($CDCl_3$): δ 1.54 (t, 3H, OCH_2CH_3, J = 6.88 Hz), 4.30 (s, 3H, NCH_3), 4.60 (q, 2H, OCH_2, J = 6.9 Hz), 7.32–7.83 (m, 8H, arom. H), 8.20 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 14.6 (OCH_2CH_3), 33.2 (NCH_3), 64.2 (OCH_2), 94.7 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 115.1 (*C*≡N), 117.4, 120.5, 123.4, 124.5, 129.4, 130.0, 134.1, 136.7, 137.4, 142.6, 150.8, 155.5, 164.5 (arom. *C*). MS: *m*/*z* (%) 388 (M, 38), 390 [(M + 2), 13]. Anal. Calcd. For $C_{22}H_{17}ClN_4O$ (388.86): C, 67.95; H, 4.41; N, 14.41. Found: C, 67.86; H, 4.28; N, 14.50.

*4.1.2.6. 4-(2,4-Dichlorophenyl)-2-methoxy-6-(1-methyl-1H-benzimidazol-2-yl)-3-pyridinecarbonitrile (**4f**).* Reaction time 24 h, colorless crystals, mp 240–242 °C, yield (2.30 g) 56%. IR: ν_{max}/cm^{-1} 2226 (C≡N), 1582, 1532 (C═N, C═C). ^{1}H NMR ($CDCl_3$): δ 4.19 (s, 3H, NCH_3), 4.34 (s, 3H, OCH_3), 7.26–7.82 (m, 7H, arom. H), 8.17 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 33.2 (NCH_3), 55.3 (OCH_3), 96.6 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 114.2 (*C*≡N), 118.6, 120.6, 123.5, 124.6, 127.7, 130.2, 131.3, 133.3, 136.6, 137.5, 142.7, 148.0, 150.9, 153.8, 164.2 (arom. *C*). MS: *m*/*z* (%) 408 (M, 100), 410 [(M + 2), 57], 412 [(M + 4), 13]. Anal. Calcd. For $C_{21}H_{14}Cl_2N_4O$ (409.28): C, 61.63; H, 3.45; N, 13.69. Found: C, 61.43; H, 3.35; N, 13.87.

*4.1.2.7. 4-(2,4-Dichlorophenyl)-2-ethoxy-6-(1-methyl-1H-benzimidazol-2-yl)-3-pyridinecarbonitrile (**4g**).* Reaction time 24 h, colorless crystals, mp 250–251 °C, yield (2.25 g) 53%. IR: ν_{max}/cm^{-1} 2225

Table 3
External validation for the established QSAR model utilizing mild (**4b**) and potent (**4d**) vasodilatory active agents.

Entry	Compd.	R	R′	R″	Observed activity (IC_{50}), mM	Estimated activity	Descriptors		
							Shadow YZfrac	Jurs FPSA 2	Jurs PPSA 3
1	**4b**	Ph	Me	Et	0.364	0.440	0.79613	1.24693	24.0706
2	**4d**	4-ClC_6H_4	Me	Me	0.145	0.118	0.650737	0.87069	24.9979

(C≡N), 1589, 1562 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.56 (t, 3H, OCH_2CH_3, J = 6.88 Hz), 4.35 (s, 3H, NCH_3), 4.63 (q, 2H, OCH_2, J = 6.88 Hz), 7.34–7.84 (m, 7H, arom. H), 8.17 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 14.5 (OCH_2CH_3), 33.2 (NCH_3), 64.2 (OCH_2), 96.7 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 114.2 (*C*≡N), 118.4, 120.6, 123.4, 124.6, 127.7, 130.2, 131.3, 133.3, 133.4, 136.6, 137.5, 142.7, 148.1, 150.9, 153.8, 163.9 (arom. *C*). MS: *m*/*z* (%) 422 (M, 34), 424 [(M + 2), 22], 426 [(M + 4), 5]. Anal. Calcd. For $C_{22}H_{16}Cl_2N_4O$ (423.31): C, 62.42; H, 3.81; N, 13.24. Found: C, 62.53; H, 3.98; N, 13.32.

*4.1.2.8. 4-(4-Fluorophenyl)-2-methoxy-6-(1-methyl-1H-benzimidazol-2-yl)-3-pyridinecarbonitrile (**4h**).* Reaction time 24 h, colorless crystals, mp 254–256 °C, yield (2.25 g) 63%. IR: ν_{max}/cm^{-1} 2222 (C≡N), 1586, 1549 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 4.21 (s, 3H, NCH_3), 4.43 (s, 3H, OCH_3), 7.25–8.03 (m, 9H, 8 arom. H + pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 33.2 (NCH_3), 55.3 (OCH_3), 94.5 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 115.1 (*C*≡N), 116.2, 116.4, 117.8, 120.5, 123.5, 124.6, 130.7, 130.8, 131.6, 137.4, 142.4, 148.1, 150.6, 155.7, 164.9 (arom. *C*). MS: *m*/*z* (%) 358 (M, 93), 357 (100). Anal. Calcd. For $C_{21}H_{15}FN_4O$ (358.38): C, 70.38; H, 4.22; N, 15.63. Found: C, 70.47; H, 4.33; N, 15.79.

*4.1.2.9. 2-Ethoxy-4-(4-fluorophenyl)-6-(1-methyl-1H-benzimidazol-2-yl)-3-pyridinecarbonitrile (**4i**).* Reaction time 24 h, almost colorless crystals, mp 243–245 °C, yield (2.10 g) 56%. IR: ν_{max}/cm^{-1} 2220 (C≡N), 1585, 1549 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.54 (t, 3H, OCH_2CH_3, J = 6.9 Hz), 4.30 (s, 3H, NCH_3), 4.61 (q, 2H, OCH_2, J = 6.88 Hz), 7.19–7.87 (m, 8H, arom. H), 8.21 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 14.6 (OCH_2CH_3), 33.2 (NCH_3), 64.1 (OCH_2), 94.6 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 115.2 (*C*≡N), 116.1, 116.3, 117.5, 120.5, 123.4, 124.5, 130.7, 131.8, 137.4, 142.5, 148.2, 150.7, 155.7, 163.0, 164.5, 165.0 (arom. *C*). MS: *m*/*z* (%) 372 (M, 38). Anal. Calcd. For $C_{22}H_{17}FN_4O$ (372.41): C, 70.96; H, 4.60; N, 15.04. Found: C, 70.80; H, 4.47; N, 14.87.

*4.1.2.10. 2-Methoxy-6-(1-methyl-1H-benzimidazol-2-yl)-4-(4-methylphenyl)-3-pyridinecarbonitrile (**4j**).* Reaction time 24 h, colorless crystals, mp 211–213 °C, yield (1.95 g) 55%. IR: ν_{max}/cm^{-1} 2217 (C≡N), 1581, 1545 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 2.40 (s, 3H, $ArCH_3$), 4.11 (s, 3H, NCH_3), 4.22 (s, 3H, OCH_3), 7.26–7.80 (m, 8H, arom. H), 8.19 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 21.5 ($ArCH_3$), 33.2 (NCH_3), 55.2 (OCH_3), 94.2 (pyridinyl *C*-3), 110.1 (pyridinyl *C*-5), 115.5 (*C*≡N), 117.5, 120.4, 123.3, 124.3, 128.5, 129.7, 132.6, 137.4, 140.7, 142.6, 148.3, 150.4, 156.6, 164.8 (arom. *C*). MS: *m*/*z* (%) 354 (M, 100). Anal. Calcd. For $C_{22}H_{18}N_4O$ (354.41): C, 74.56; H, 5.12; N, 15.81. Found: C, 74.49; H, 5.08; N, 15.68.

*4.1.2.11. 2-Ethoxy-6-(1-methyl-1H-benzimidazol-2-yl)-4-(4-methylphenyl)-3-pyridinecarbonitrile (**4k**).* Reaction time 24 h, colorless crystals, mp 226–228 °C, yield (1.95 g) 53%. IR: ν_{max}/cm^{-1} 2219 (C≡N), 1580, 1546 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.54 (t, 3H, OCH_2CH_3, J = 6.88 Hz), 2.42 (s, 3H, $ArCH_3$), 4.29 (s, 3H, NCH_3), 4.59 (q, 2H, OCH_2, J = 6.9 Hz), 7.30–7.84 (m, 8H, arom. H), 8.22 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 14.6 (OCH_2CH_3), 21.5 ($ArCH_3$), 33.1 (NCH_3), 64.0 (OCH_2), 94.4 (pyridinyl *C*-3), 110.1 (pyridinyl *C*-5), 115.5 (*C*≡N), 117.4, 120.4, 123.3, 124.3, 128.5, 129.7, 132.8, 137.4, 140.6, 142.6, 148.4, 150.4, 156.7, 164.5 (arom. *C*). MS: *m*/*z* (%) 368 (M, 45). Anal. Calcd. For $C_{23}H_{20}N_4O$ (368.44): C, 74.98; H, 5.47; N, 15.21. Found: C, 75.14; H, 5.54; N, 15.02.

*4.1.2.12. 2-Methoxy-4-(4-methoxyphenyl)-6-(1-methyl-1H-benzimidazol-2-yl)-3-pyridinecarbonitrile (**4l**).* Reaction time 24 h (Methods A & B), almost colorless crystals, mp 238–239 °C, yield (1.81, 2.00 g) 49, 54% (Methods A & B, respectively). IR: ν_{max}/cm^{-1} 2217 (C≡N), 1585, 1545 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 3.86 (s, 3H, NCH_3), 4.17 (s, 3H, OCH_3), 4.32 (s, 3H, OCH_3), 7.01–7.84 (m, 8H, arom. H), 8.23 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 33.2 (NCH_3), 55.2 (OCH_3), 55.5 (OCH_3), 94.1 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 115.6 (*C*≡N), 114.5, 117.6, 120.4, 123.4, 124.4, 127.8, 130.2, 137.4, 142.4, 148.4, 150.2, 156.4, 161.4, 164.9 (arom. *C*). MS: *m*/*z* (%) 370 (M, 100). Anal. Calcd. For $C_{22}H_{18}N_4O_2$ (370.41): C, 71.34; H, 4.90; N, 15.13. Found: C, 71.45; H, 5.03; N, 14.97.

*4.1.2.13. 2-Ethoxy-4-(4-methoxyphenyl)-6-(1-methyl-1H-benzimidazol-2-yl)-3-pyridinecarbonitrile (**4m**).* Reaction time 24 h (Methods A & B), colorless crystals, mp 224–226 °C, yield (1.96, 2.10 g) 51, 55% (Methods A & B, respectively). IR: ν_{max}/cm^{-1} 2220 (C≡N), 1585, 1548 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.54 (t, 3H, OCH_2CH_3, J = 6.88 Hz), 3.86 (s, 3H, NCH_3), 4.30 (s, 3H, OCH_3), 4.59 (q, 2H, OCH_2, J = 6.88 Hz), 7.01–7.85 (m, 8H, arom. H), 8.22 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 14.6 (OCH_2CH_3), 33.2 (NCH_3), 55.5 (OCH_3), 64.0 (OCH_2), 94.3 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 115.6 (*C*≡N), 114.5, 117.5, 120.3, 123.4, 124.4, 127.9, 130.2, 137.3, 148.5, 150.1, 156.4, 161.4, 164.6 (arom. *C*). MS: *m*/*z* (%) 384 (M, 44). Anal. Calcd. For $C_{23}H_{20}N_4O_2$ (384.44): C, 71.86; H, 5.24; N, 14.57. Found: C, 71.92; H, 5.35; N, 14.40.

*4.1.2.14. 2-Methoxy-6-(1-methyl-1H-benzimidazol-2-yl)-4-(2-thienyl)-3-pyridinecarbonitrile (**4n**).* Reaction time 24 h, colorless crystals, mp 215–217 °C, yield (2.00 g) 58%. IR: ν_{max}/cm^{-1} 2223 (C≡N), 1582, 1550 (C=N, C=C). ^{1}H NMR (DMSO-d_6): δ 4.09 (s, 3H, NCH_3), 4.33 (s, 3H, OCH_3), 7.23–7.99 (m, 7H, arom. H), 8.17 (s, 1H, pyridinyl *H*-5). ^{13}C NMR (DMSO-d_6 + TFA): δ 34.1 (NCH_3), 55.8 (OCH_3), 94.3 (pyridinyl *C*-3), 111.9 (pyridinyl *C*-5), 113.4 (*C*≡N), 114.2, 116.5, 118.8, 127.1, 127.5, 129.1, 131.5, 132.4, 134.5, 136.0, 143.7, 145.2, 148.8, 165.3 (arom. *C*). MS: *m*/*z* (%) 346 (M, 10). Anal. Calcd. For $C_{19}H_{14}N_4OS$ (346.41): C, 65.88; H, 4.07; N, 16.17. Found: C, 66.02; H, 4.13; N, 16.37.

*4.1.2.15. 2-Ethoxy-6-(1-methyl-1H-benzimidazol-2-yl)-4-(2-thienyl)-3-pyridinecarbonitrile (**4o**).* Reaction time 24 h, almost colorless crystals, mp 234–236 °C, yield (2.20 g) 61%. IR: ν_{max}/cm^{-1} 2219 (C≡N), 1581, 1548 (C=N, C=C). ^{1}H NMR ($CDCl_3$): δ 1.54 (t, 3H, OCH_2CH_3, J = 6.9 Hz), 4.30 (s, 3H, NCH_3), 4.59 (q, 2H, OCH_2, J = 6.9 Hz), 7.21–8.02 (m, 7H, arom. H), 8.34 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 14.6 (OCH_2CH_3), 33.1 (NCH_3), 64.1 (OCH_2), 92.2 (pyridinyl *C*-3), 110.2 (pyridinyl *C*-5), 115.8 (*C*≡N), 116.3, 120.4, 123.4, 124.4, 128.7, 129.9, 130.1, 137.2, 137.5, 142.4, 148.2, 150.5, 165.0 (arom. *C*). MS: *m*/*z* (%) 360 (M, 44), 362 [(M + 2), 3]. Anal. Calcd. For $C_{20}H_{16}N_4OS$ (360.44): C, 66.65; H, 4.47; N, 15.54. Found: C, 66.53; H, 4.38; N, 15.35.

*4.1.2.16. 6-(1H-Benzimidazol-2-yl)-2-methoxy-4-(5-methyl-2-furanyl)-3-pyridinecarbonitrile (**4p**).* Reaction time 48 h, pale yellow crystals, mp 284–286 °C, yield (1.95 g) 59%. IR: ν_{max}/cm^{-1} 3303 (NH), 2222 (C≡N), 1603, 1555 (C=N, C=C). ^{1}H NMR (DMSO-d_6): δ 2.34 (s, 3H, $ArCH_3$), 4.12 (s, 3H, OCH_3), 6.35–7.69 (m, 6H, arom. H), 8.09 (s, 1H, pyridinyl *H*-5), 12.90 (s, 1H, NH). ^{13}C NMR (DMSO-d_6): δ 14.0 ($ArCH_3$), 55.6 (OCH_3), 87.7 (pyridinyl *C*-3), 109.2 (pyridinyl *C*-5), 116.0 (*C*≡N), 110.4, 112.9, 116.6, 120.2, 123.0, 124.5, 135.4, 142.7, 144.5, 146.3, 149.4, 156.7, 165.5 (arom. *C*). MS: *m*/*z* (%) 330 (M, 100). Anal. Calcd. For $C_{19}H_{14}N_4O_2$ (330.35): C, 69.08; H, 4.27; N, 16.96. Found: C, 69.22; H, 4.29; N, 16.89.

*4.1.2.17. 2-Methoxy-6-(1-methyl-1H-benzimidazol-2-yl)-4-(5-methyl-2-furanyl)-3-pyridinecarbonitrile (**4q**).* Reaction time 24 h, colorless crystals, mp 286–288 °C, yield (2.10 g) 61%. IR: ν_{max}/cm^{-1} 2218 (C≡N), 1577, 1550 (C=N, C=C). ^{1}H NMR (DMSO-d_6): δ 2.25 (s, 3H, $ArCH_3$), 3.98 (s, 3H, NCH_3), 4.26 (s, 3H, OCH_3), 6.19–7.73 (m, 6H, arom. H), 8.14 (s, 1H, pyridinyl *H*-5). MS: *m*/*z* (%) 344 (M, 82). Anal.

Calcd. For $C_{20}H_{16}N_4O_2$ (344.38): C, 69.76; H, 4.68; N, 16.27. Found: C, 69.84; H, 4.81; N, 16.46.

*4.1.2.18. 2-Ethoxy-6-(1-methyl-1H-benzimidazol-2-yl)-4-(5-methyl-2-furanyl)-3-pyridinecarbonitrile (**4r**).* Reaction time 24 h, colorless crystals, mp 225–227 °C, yield (2.20 g) 61%. IR: ν_{max}/cm^{-1} 2218 (C≡N), 1577, 1551 (C=N, C=C). ^1H NMR ($CDCl_3$): δ 1.51 (t, 3H, OCH_2CH_3, $J = 7.25$ Hz), 2.43 (s, 3H, $ArCH_3$), 4.27 (s, 3H, NCH_3), 4.55 (q, 2H, OCH_2, $J = 6.85$ Hz), 6.21–7.87 (m, 6H, arom. H), 8.46 (s, 1H, pyridinyl *H*-5). ^{13}C NMR ($CDCl_3$): δ 14.1, 14.6 ($ArCH_3$, OCH_2CH_3), 33.2 (NCH_3), 63.9 (OCH_2), 88.3 (pyridinyl *C*-3), 109.6 (pyridinyl *C*-5), 115.9 (C≡N), 110.1, 112.5, 116.5, 120.3, 123.4, 124.3, 137.3, 143.2, 146.5, 148.6, 150.2, 156.3, 164.7 (arom. *C*). MS: *m/z* (%) 358 (M, 43). Anal. Calcd. For $C_{21}H_{18}N_4O_2$ (358.40): C, 70.38; H, 5.06; N, 15.63. Found: C, 70.57; H, 5.14; N, 15.69.

4.2. Vasodilation activity screening

The vasodilation activity screening procedures were carried out according to the standard reported techniques [20–23] by testing the effects of the synthesized 2-alkoxy-4-aryl-6-(1*H*-benzimidazol-2-yl)-3-pyridinecarbonitriles **4a**–**r** on isolated thoracic aortic rings of male Wister rats (250–350 g). After light ether anesthesia, the rats were sacrificed by cervical dislocation. The aortae were immediately excised, freed of extraneous tissues and prepared for isometric tension recording. Aorta was cut into (3–5 mm width) rings and each ring was placed in a vertical chamber "10 ml jacketed automatic multi-chamber organ bath system (Model no. ML870B6/C, Panlab, Spain)" filled with Krebs 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 aortic ring was mounted between two stainless steel hooks passed through its lumen. The lower hook was fixed between two plates, while the upper one was attached to a force displacement transducer (Model no. MLT0201, Panlab, Spain) connected to an amplifier (PowerLab, AD Instruments Pty. Ltd.) which is connected to a computer. The Chart for windows (v 3.4) software was used to record and elaborate data.

Preparations were stabilized under 2 g resting tension during 2 h and then the contracture response to norepinephrine hydrochloride (10^{-6} 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 (10 ml of 0.005 M). Control experiments were performed in the presence of DMSO alone, at the same concentrations as those used with the derivatives tested, which demonstrated that the solvent did not affect the contractile response of isolated aorta. The observed vasodilation activity screening data are reported (Table 1, Figures 1 and 2 of Supplementary material) and the potency (IC_{50}, concentration necessary for 50% reduction of maximal norepinephrine hydrochloride induced contracture) was determined.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.01.042.

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