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Rational design, synthesis and 2D-QSAR study of novel vasorelaxant active benzofuran-pyridine hybrids

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Keywords:

Benzofuran
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Vasodilator
Amiodarone
QSAR

Abstract

Reaction of 3-aryl-1-(benzofuran-2-yl)-2-propen-1-ones **3a**–**c** with malononitrile in the presence of sufficient amount of sodium alkoxide in the corresponding alcohol proceeds in a regioselective manner to afford 2-alkoxy-4-aryl-6-(benzofuran-2-yl)-3-pyridinecarbonitriles **4**–**37**, which also obtained by treating ylidenemalononitriles **6a**–**q** with 2-acetylbenzofuran **1** in the presence of sufficient amount of sodium alkoxide in the corresponding alcohol. The new chemical entities showed significant vasodilation properties using isolated thoracic aortic rings of rats pre-contracted with norepinephrine hydrochloride standard technique. Compounds **11**, **16**, **21**, **24** and **30** exhibited remarkable activity compared with amiodarone hydrochloride the reference standard used in the present study. CODESSA-Pro software was employing to obtain a statistically significant QSAR model describing the bioactivity of the newly synthesized analogs (N = 31, n = 5, R^2 = 0.846, R^2_{cv}OO=0.765, R^2cvMO = 0.778, F = 27.540. s^2 = 0.002).

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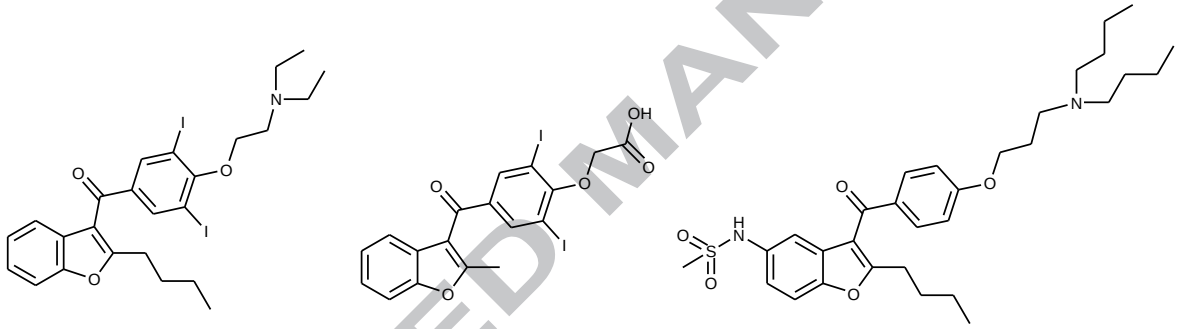
Benzofuran scaffold attracted considerable attention due to the profound chemotherapeutic properties as well as its wide spread in nature.[1] Bezofuran-containing compounds are reported to possess antihyperglycemic,[2] analgesic,[3,4] anti-inflammatory,[3,4] antiparasitic,[5] antimicrobial,[6] antitumor[7] and kinase inhibitory effects.[8] Vasodilation, anti-arrhythmic and hypotensive effects are the most interesting bioactive properties associated with benzofuran skelton.[9,10] Amiodarone and its analog KB130015 (Figure 1) are powerful antiarrhythmic agents used for various types of cardiac dysrhythmias, possesses coronary and peripheral vasodilator properties. This effect appears to be mainly due to the release of nitric oxide (NO). Amiodarone dilates preconstricted human hand veins *in vivo* and acts as a venodilator through the cyclooxygenase pathway, activation of NO synthase, and blockade of α adrenergic mechanisms.[11–14] A new noniodinated benzofuran derivative dronedarone (Fig. 1) was approved by food and drug administration (FDA) for treatment of atrial fibrillation and atrial flutter. Dronedarone differs from amiodarone by a remaining relaxant effect refractory to the inhibition of NO synthase pathway probably due to its Ca^{2+} antagonist property. Consequently, dronedarone may induce a coronary dilation involving a dual mechanism, stimulation of NO synthase pathway and putative Ca^{2+} channels inhibition.[15,16] Amiodarone and dronedarone are known to induce a variety of side effects including neurotoxicity, characterized by a set of symptoms such as headache, dizziness, fatigue, tremor, peripheral sensorimotor neuropathy, proximal muscle weakness and ataxia in addition to gastrointestinal side effects such as nausea, vomiting, and diarrhea.[17– 20]

Moreover, It is well documented that, nicotinate esters are one of the most known vasodilatory active heterocycles.[21,22] Many nicotinate analogs are well known as vasodilating active agents such as, micinicate, hepronicate and inositol nicotinate.[23] Interest in developing these scaffolds could be attributed to the fact that, 3-pyridinecarbonitrile is recognized as bioisosteric forms of nicotinate analog (pyridine-3-carboxylate) where only the cyano group replaces the acid/ester function.[23]

Based on the afore-mentioned findings and in an attempt to design new potent vasorelaxant agents, hybrids of 3-pyridinecarbonitriles and benzofuran function have been synthesized for their vasodilation properties.

Cardiovascular diseases (CVDs) remain the biggest cause of deaths worldwide. More than 17.5 million people died from CVDs in 2012 representing 31% of all global deaths. More than 3 million of these deaths occurred before the age of 60 and could have largely been prevented.[24,25] CVDs caused by disorders of the heart and blood vessels.[26]

Notably, hypertension is the most common cardiovascular disorders that represent the major risk factor for endothelial dysfunctions,[27] vasodilators are smooth muscle relaxant which causes blood vessels to dilate.[28–29] They are prominently used to treat cerebrovascular disease, peripheral arter y disease, rheumatic heart disease, congenital heart disease, heart failure and hypertension.[30,31] Uncontrolled hypertension is associated with acute end-organ damage[32] as congestive heart failure[33] or renal failure as in type-2 diabetes patients.[34] Several direct vasodilators have been synthesized but none of them has presented a specific action while being free of side effects,[35,36] reinforcing the importance of identifying new clinically safe useful vasodilator agents with higher potency and fewer side effects. Exploring the controlling factors governing the observed pharmacological properties of the synthesized hyprids and validation the observed activity will be studied by quantitative structure activity relationships (QSAR).

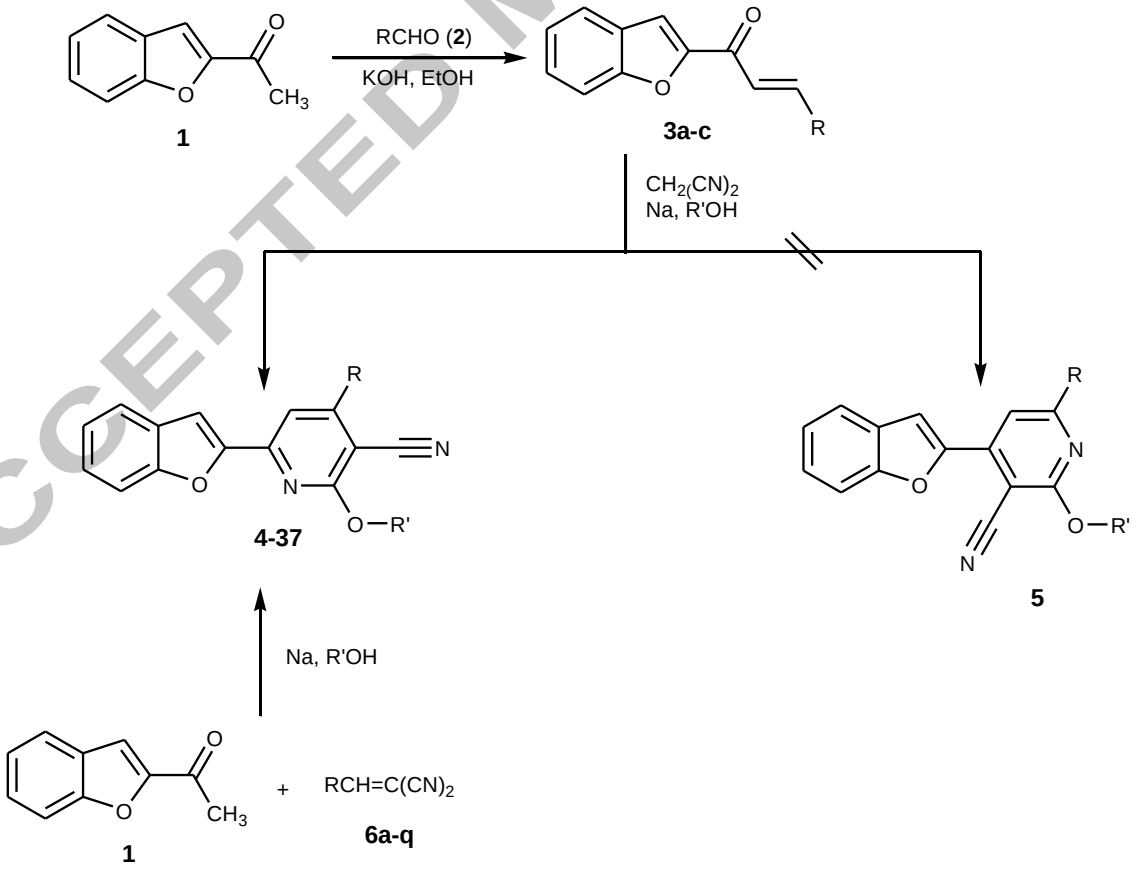


Amiodarone KB130015 Dronedarone

Figure 1 Benzofuran based Vasorelaxant agents.

In this paper, 3-aryl-1-(benzofuran-2-yl)-2-propen-1-ones **3a**–**c** were treated 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-(benzofuran-2-yl)-3-pyridinecarbonitriles **4**–**37** or their isomeric form 2-alkoxy-6-aryl-4-(benzofuran-2-yl)-3-pyridinecarbonitriles **5**, based on the observed spectroscopic (IR,^{1}H NMR,^{13}C NMR, Mass) and elemental analysis data, **4**–**37** were formed. On the other hand, the treatment of ylidenemalononitriles **6a**–**q** with 2-acetylbenzofuran **1** 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 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**–**37**) rather than Knoevenagel pathway (condensation of malononitrile's active methylene 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**).[23] IR spectrum of **4a** (as a representative example of the synthesized analogs) reveals the presence of a strong stretching nitrile vibration band at ν = 2215 cm^{-1} while, no assignable band due to carbonyl stretching vibration was detected. ^{1}H NMR spectrum of **4** exhibits the characteristic pyridinyl H-5 at δ = 7.64 in addition to the methoxide signal at δ = 4.18. ^{13}C NMR spectrum of **4** reveals the methoxide carbon at δ = 54.79, pyridinyl C-3 and C-5 at δ = 93.77 and 108.23 respectively in addition to nitrile carbon at δ = 115.59. Mass spectrum (EI) of **4** reveals the molecular ion peak 326.3 with relative intensity value 100%. The spectral data (IR, ^{1}H NMR, ^{13}C NMR, Mass) of all the other new chemical entities showed similar observations to that of compounds **4**–**37** confirming their established structures (c.f. Experimental section, Figures 3–136 of Supplementary data).



2a, **3a**; R = Ph
2b, **3b**; R = 4-ClC_6H_4

4; R = Ph, R' = Me
5; R = Ph, R' = Et

2c, **3c**, R = 4-$H_3COC_6H_4$

6a; R = Ph
6b; R = 1-Naphthyl
6c; R = 2-Naphthyl
6d; R = 4-ClC_6H_4
6e; R = 2,4-$Cl_2C_6H_3$
6f; R = 4-BrC_6H_4
6g; R = 4-FC_6H_4
6h; R = 4-$H_3CC_6H_4$
6i; R = 4-$H_3COC_6H_4$
6j; R = 2,4-$(H_3CO)_2C_6H_3$
6k; R = 2,5-$(CH_3O)_2C_6H_3$
6l; R = 3,4,5-$(CH_3O)_3C_6H_2$
6m; R= 4-$(CH_3)_2NC_6H_4$
6n; R = 2-Thienyl
6o; R = 5-Methyl-2-furanyl
6p; R = N-Metyl-3-indolyl
6q; R = N-Ethyl-3-indolyl

6; R = 1-Naphthyl, R' = Me
7; R = 1-Naphthyl, R' = Et
8; R = 2-Naphthyl, R' = Me
9; R = 2-Naphthyl, R' = Et
10; R = 4-ClC_6H_4, R' = Me
11; R = 4-ClC_6H_4, R' = Et
12; R = 2,4-$Cl_2C_6H_3$, R' = Me
13; R = 2,4-$Cl_2C_6H_3$, R' = Et
14; R = 4-BrC_6H_4, R' = Me
15; R = 4-BrC_6H_4, R' = Et
16; R= 4-FC_6H_4, R' = Me
17; R = 4-FC_6H_4, R' = Et
18; R = 4- $H_3CC_6H_4$, R' = Me
19; R = 4- $H_3CC_6H_4$, R' = Et
20; R = 4- $H_3COC_6H_4$, R' = Me
21; R = 4- $H_3COC_6H_4$, R' = Et
22; R = 2,4-$(H_3CO)_2C_6H_3$, R' = Me
23; R = 2,4-$(H_3CO)_2C_6H_3$, R' = Et
24; R = 2,5-$(H_3CO)_2C_6H_3$, R' = Me
25; R = 2,5-$(H_3CO)_2C_6H_3$, R' = Et
26; R= 3,4,5-$(H_3CO)_3C_6H_2$, R' = Me
27; R= 3,4,5-$(H_3CO)_3C_6H_2$, R' = Et
28; R = 4-$(CH_3)_2NC_6H_4$, R' = Me
29; R = 4-$(CH_3)_2NC_6H_4$, R' = Et
30; R = 2-Thienyl, R' = Me
31; R = 2-Thienyl, R' = Et
32; R = 5-Methyl-2-furanyl, R' = Me
33; R = 5-Methyl-2-furanyl, R' = Et
34; R = N-Methyl-3-indolyl, R' = Me
35; R = N-Methyl-3-indolyl, R' = Et
36; R = N-Ethyl-3-indoly, R' = Me
37; R = N-Ethyl-3-indoly, R' = Et

Scheme 1. Synthetic routes of compounds **4**–**37**.

Vasodilation properties of the synthesized 2-alkoxy-4-aryl-6-(benzofuran-2-yl)-3-pyridinecarbonitriles **4**–**37** were investigated using isolated thoracic aortic rings of rats pre-contracted with norepinephrine hydrochloride according to the standard reported procedure[37] and compared with amiodarone 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 data) reveal that, all the synthesized compounds show significant vasodilation properties. Whilst, compounds **4**, **5**, **10**, **11**, **14**, **15**, **18**–**21**, **24**, **26**, **28**, **30**, **31**, **32**, **34** and **36** exhibit remarkable activity (IC_{50}, concentration necessary for 50% reduction of maximal norepinephrine hydrochloride induced contracture = 0.163–310 mM) compared with amiodarone hydrochloride (IC_{50} = 0.312 mM). Through

the observed vasodilation properties of the synthesized hyprids a general SAR (structure activity relationship) rule could be attained, the vasodilation activity was enhanced when the 2-position of 3-pyridinecarbonitriles is occupied by methoxy group rather than the case when an ethoxy group was adopted, as shown in all of the tested analogs. In order to understand the observed pharmacological properties and determining the main controlling factors governing these activities, 2D-QSAR study was initiated**.**

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'	Potency (IC_{50}), mM
1	**4**	Ph	Me	0.232
2	**5**	Ph	Et	0.310
3	**6**	1-Naphthyl	Me	0.359
4	**7**	1-Naphthyl	Et	0.344
5	**8**	2-Naphthyl	Me	0.320
6	**9**	2-Naphthyl	Et	0.423
7	**10**	4-ClC_6H_4	Me	0.286
8	**11**	4-ClC_6H_4	Et	0.221
9	**12**	2,4-$Cl_2C_6H_3$	Me	0.322
10	**13**	2,4-$Cl_2C_6H_3$	Et	0.442
11	**14**	4-BrC_6H_4	Me	0.298
12	**15**	4-BrC_6H_4	Et	0.227
13	**16**	4-FC_6H_4	Me	0.321
14	**17**	4-FC_6H_4	Et	0.396
15	**18**	4-$H_3CC_6H_4$	Me	0.266
16	**19**	4-$H_3CC_6H_4$	Et	0.290
17	**20**	4-$H_3COC_6H_4$	Me	0.262
18	**21**	4-$H_3COC_6H_4$	Et	0.204
19	**22**	2,4-$(H_3CO)_2C_6H_3$	Me	0.425
20	**23**	2,4-$(H_3CO)_2C_6H_3$	Et	0.488
21	**24**	2,5-$(H_3CO)_2C_6H_3$	Me	0.246
22	**25**	2,5-$(H_3CO)_2C_6H_3$	Et	0.327
23	**26**	3,4,5-$(H_3CO)_3C_6H_2$	Me	0.310
24	**27**	3,4,5-$(H_3CO)_3C_6H_2$	Et	0.365
25	**28**	4-$(H_3C)_2NC_6H_4$	Me	0.278
26	**29**	4-$(H_3C)_2NC_6H_4$	Et	0.338
27	**30**	2-Thienyl	Me	0.163
28	**31**	2-Thienyl	Et	0.308
29	**32**	5-Methyl-2-furanyl	Me	0.285
30	**33**	5-Methyl-2-furanyl	Et	0.417
31	**34**	N-Methyl-3-indolyl	Me	0.262
32	**35**	N-Methyl-3-indolyl	Et	0.327
33	**36**	N-Ethyl-3-indolyl	Me	0.299
34	**37**	N-Ethyl-3-indolyl	Et	0.358
35	**Amiodarone.HCl**	–	–	0.312

Molecular descriptors of the 2D-QSAR are the physic-chemical parameters used to correlate chemical structure and property value expressed as log(IC_{50}) (Figure 2). The descriptors were obtained based on BMLR (Best Multiple Linear Regression) method. The descriptors controlling the bio-activity (property) by the established multi-linear QSAR model are presented in Table 2 and are arranged, based on their level of significance (*t*-criterion). The first descriptor controlling the BMLR-QSAR model based on its *t*-criterion value (*t* = 6.578) is minimum e-e repulsion for bond H-C which is a semi-empirical descriptor. Electron-electron repulsion between two given atoms can be determined by equation (1).[38]

$$E_{ee}(AB) = \sum_{\mu,\nu \in A} \sum_{\lambda,\sigma \in B} P_{\mu\nu} P_{\lambda\sigma} \langle \mu\nu | \lambda\sigma \rangle \quad \text{..... (1)}$$

Where, *A* stands for a given atomic species, *B* is another atomic species $P_{\mu\nu}$, $P_{\lambda\sigma}$ – isdensity matrix elements over atomic basis $\{\mu\nu\lambda\sigma\}$, $\langle \mu\nu | \lambda\sigma \rangle$ is the electron repulsion integrals on atomic basis $\{\mu\nu\lambda\sigma\}$. The second descriptor controlling the BMLR-QSAR model (t = 5.289) is charged surface area (MOPAC PC) for atom C, which is a charge-related descriptor. The partial positively/negatively charged surface areais determined by equation (2).[38]

$$PPSA1 = \sum_{A} S_A \qquad A \in \{\delta_A > 0\} \quad \text{..... (2)}$$

Where, S_A stands for positively/negatively charged solvent-accessible atomic surface area. The third descriptor controlling BMLR-QSAR model (*t* = -4.374) is WNSA1 weighted PNSA (PNSA1*TMSA/1000) (Zefirov PC). Surface weighted charged partial negative charged surface area (WNSA1) is determined by equation (3).[38]

$$WNSA1 = \frac{PNSA1 \cdot TMSA}{1000} \quad \text{..... (3)}$$

Where, *PNSA1* stands for partial negatively charged molecular surface area, *TMSA* for total molecular surface area. The fourth descriptor controlling BMLR-QSAR model (*t* = -8.078) ismaximum e-n attraction for bond C-O which is a semi-empirical descriptor. Thenuclear-electron attraction energy between two given atoms is determined by equation (4).[38]

$$E_{ne}(AB) = \sum_{B} \sum_{\mu,\nu \in A} P_{\mu\nu} \langle \mu | \frac{Z_B}{R_{iB}} | \nu \rangle \quad \text{..... (4)}$$

Where, *A* stands for given atomic species, *B* for another atomic species, $P_{\mu\nu}$ fordensity matrix elements over atomic basis $\{\mu\nu\}$, Z_B forcharge of atomic nucleus *B*, R_{iB} fordistance

between the electron and atomic nucleus *B*, $\left\langle \mu \left| \frac{Z_B}{R_{iB}} \right| \nu \right\rangle$ for electron-nuclear attraction integrals on atomic basis $\{\mu\nu\}$. The fifth descriptor controlling BMLR-QSAR model (t = -10.863) is total molecular 1-center E-N attraction which is also a semi-empirical descriptor. The total molecular one-center electron-nuclear attraction energy is determined by equation (5).[38]

$$E_{ne}(tot) = \sum_{A} E_{ne}(A) \quad \text{..... (5)}$$

Where, *A* stands for given atomic species, $E_{ne}(A)$for electron-nuclear attraction energy for atom *A*

Figure 2. BMLR-QSAR model plot of correlations representing the observed *vs.* predicted vasodilatory active agents (compound **19** is an outlier).

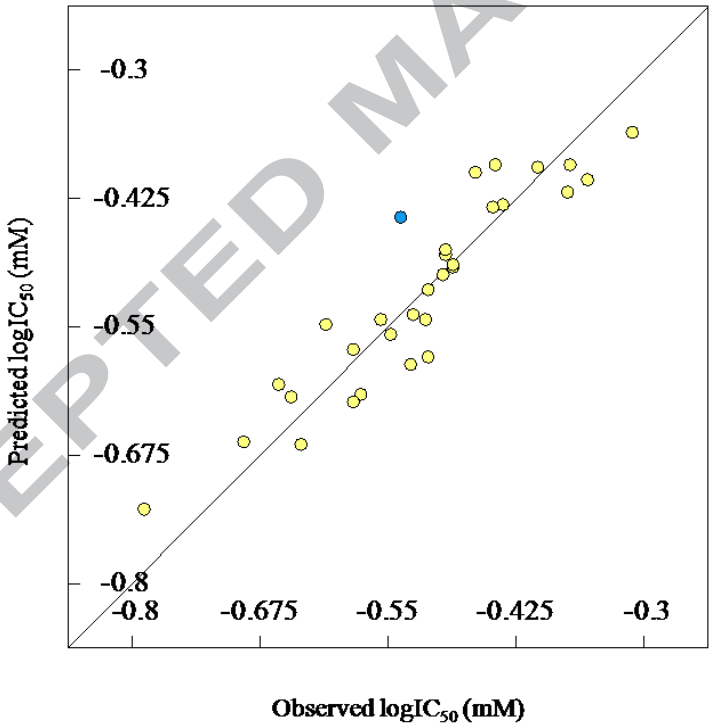


Table 2

Descriptor of the BMLR-QSAR model for the vasodilatory active agents.

Entry	N=31, n=5, R^2=0.846, R^2_{cv}OO=0.765, R^2_{cv}MO=0.778, F=27.540, s^2=0.002				
	ID	coefficient	s	t	Descriptor
1	0	16.850	2.657	6.341	Intercept
2	D_1	0.166	0.025	6.578	Min. e-e repulsion for bond H-C
3	D_2	0.115	0.022	5.289	Charged surface area (MOPAC PC)

					for atom C
4	D_3	-0.002	0.0005	-4.374	WNSA1 Weighted PNSA (PNSA1*TMSA/1000) (Zefirov PC)
5	D_4	-0.062	0.008	-8.078	Max. e-n attraction for bond C-O
6	D_5	-0.0003	2.64318E-005	-10.863	Tot. molecular 1-center E-N attraction

$\log IC_{50} = 16.850 + (0.166 \times D_1) + (0.115 \times D_2) - (0.002 \times D_3) - (0.062 \times D_4) - (0.0003 \times D_5)$

The reliability and statistical relevance of the attained BMLR-QSAR model is examined by internal and external validation procedures. Internal validation is applied by the CODESSA-Pro (Comprehensive Descriptors for Structural and Statistical Analysis) technique employing both Leave One Out (LOO), which involves developing a number of models with one example omitted at a time, and Leave Many Out (LMO), which involves developing a number of models with many data points omitted at a time (up to 20% of the total data points). The observed correlations due to the internal validation techniques are $R^2_{cv}OO = 0.765$, $R^2_{cv}MO = 0.778$. Both of them are significantly correlated with the squared correlation coefficient of the attained QSAR model ($R^2 = 0.846$). Standard deviation of the regressions ($s^2 = 0.002$) is also a measurable value for the attained model together with the Fisher test value ($F = 27.540$) that reflects the ratio of the variance explained by the model and the variance due to their errors. A high value of F-test compared with the s^2 is a validation of the model. The predicted/estimated IC_{50} values of all the training set compounds according to the attained BMLR-QSAR model are presented in table 3. From the results obtained it is obvious that, the most potent analogue **30** among all the synthesized compounds, reveals observed bio-activity comparable to its experimentally predicted one (IC_{50} = 0.163, 0.187 mM, respectively, error "difference between observed and predicted values" = -0.024). The BMLR-QSAR model has controlled the other potent training set analogues (compounds **4, 11, 15, 21** and **24**) relative to amiodarone hydrochloride (standard reference clinically used as antihypertensive, IC_{50} = 0.312 mM), exhibited estimated bio-properties matched with their observed potencies (IC_{50} = 0.204–0.246, 0.217–0.282 mM, corresponding to the observed and predicted values respectively, error = 0.013–0.036). Compounds **5** and **26** that showed bio-potency similar to the standard reference (IC_{50} = 0.310 mM), revealed predicted vasodilatory properties close to their experimentally observed ones (IC_{50} = 0.263, 0.306 mM respectively). The other synthesized compounds (**6**–**9**, **12**–**14**, **16**–**20**, **22**, **23**, **25**, **27**, **28**, **31**, **32** and **34**–**37**) with vasodilatory activities better than the standard reference used (experimentally observed

IC_{50} = 0.262–0.488 mM) showed compatible bio-data (estimated IC_{50} = 0.238–0.435 mM, error = 0.003–0.069).

Table 3

Observed and predicated values of the training set compounds **4**–**9, 11**–**28, 30**–**32** and **34**–**37** according to the multi-linear QSAR model.

Entry	Compd.	R	R'	Observed IC_{50}, mM	Predicted IC_{50}, mM	Error
1	**4**	Ph	Me	0.232	0.217	0.015
2	**5**	Ph	Et	0.31	0.263	0.047
3	**6**	1-Naphthyl	Me	0.359	0.404	-0.045
4	**7**	1-Naphthyl	Et	0.344	0.398	-0.054
5	**8**	2-Naphthyl	Me	0.32	0.316	0.004
6	**9**	2-Naphthyl	Et	0.423	0.381	0.042
7	**11**	4-ClC_6H_4	Et	0.221	0.247	-0.026
8	**12**	2,4-ClC_6H_3	Me	0.322	0.331	-0.009
9	**13**	2,4-ClC_6H_3	Et	0.442	0.390	0.052
10	**14**	4-BrC_6H_4	Me	0.298	0.259	0.039
11	**15**	4-BrC_6H_4	Et	0.227	0.240	-0.013
12	**16**	4-FC_6H_4	Me	0.321	0.335	-0.014
13	**17**	4-FC_6H_4	Et	0.396	0.403	-0.007
14	**18**	4-$H_3CC_6H_4$	Me	0.266	0.242	0.024
15	**19**	4-$H_3CC_6H_4$	Et	0.29	0.359	-0.069
16	**20**	4-$H_3COC_6H_4$	Me	0.262	0.238	0.024
17	**21**	4-$H_3COC_6H_4$	Et	0.204	0.217	-0.013
18	**22**	2,4-$(H_3CO)_2C_6H_3$	Me	0.425	0.403	0.022
19	**23**	2,4-$(H_3CO)_2C_6H_3$	Et	0.488	0.435	0.053
20	**24**	2,5-$(H_3CO)_2C_6H_3$	Me	0.246	0.282	-0.036
21	**25**	2,5-$(H_3CO)_2C_6H_3$	Et	0.327	0.322	0.005
22	**26**	3,4,5-$(H_3CO)_3C_6H_2$	Me	0.31	0.306	0.004
23	**27**	3,4,5-$(H_3CO)_3C_6H_2$	Et	0.365	0.370	-0.005
24	**28**	4-$Me_2NC_6H_4$	Me	0.278	0.286	-0.008
25	**30**	2-Thienyl	Me	0.163	0.187	-0.024
26	**31**	2-Thienyl	Et	0.308	0.287	0.021

27	**32**	5-Methyl-2-furanyl	Me	0.285	0.276	0.009
28	**34**	1-Methyl-3-indolyl	Me	0.262	0.267	-0.005
29	**35**	1-Methyl-3-indolyl	Et	0.327	0.324	0.003
30	**36**	1-Ethyl-3-indolyl	Me	0.299	0.288	0.011
31	**37**	1-Ethyl-3-indolyl	Et	0.358	0.367	-0.009

Compounds **10, 29**, and **33** were used as an external test set not only for validating the attained QSAR model but also for examining its predicative ability. The test set analogues experimentally exhibited high and low vasodilatory potencies relative to the standard reference used. The variation in potency can indicate the predication capabilities of the attained QSAR model. Table 4 summarizes the observed and predicted IC_{50} values of the test set compounds. From the observed data, it has been noticed that, compound **10** which is considered a high potent vasodilatory active agent (experimentally observed IC_{50} = 0.286 mM), relative to amiodarone hydrochloride, standard reference used, reveals a predicted IC_{50} = 0.210 mM with minimum error value = 0.076. Compounds **29**, and **33** which considered low potent analogues relative to the standard reference used (experimentally observed IC_{50} = 0.338, 0.417 mM, respectively), reveal predicted IC_{50} = 0.443, 0.305 mM, respectively (error values = -0.105, 0.112, respectively). These observations give good sign for the predictive capability of the attained BMLR-QSAR model and support the previous statement concerning its predictive power due to the statistical values.

Table 4

Observed and predicated values of the external test set compounds **10, 29** and **33** according to the multi-linear QSAR model.

Entry	Compd.	R	R'	Observed IC_{50}, mM	Predicted IC_{50}, mM	Error
1	**10**	4-ClC_6H_4	Me	0.286	0.210	0.076
2	**29**	4-$Me_2NC_6H_4$	Et	0.338	0.443	-0.105
3	**33**	5-Methyl-2-furanyl	Et	0.417	0.305	0.112

In conclusion, 2-alkoxy-4-aryl-6-(benzofuran-2-yl)-3-pyridinecarbonitriles **4**–**37** were successfully prepared *via* a regioselective reaction of 3-aryl-1-(benzofuran-2-yl)-2-propen-1-ones **3a**–**c** with malononitrile which also attained by a facile synthesis of ylidenemalononitriles **6a**–**q** with 2-acetylbezofuran **1** in the presence of sodium alkoxide in

the corresponding alcohol. All the newly synthesized compounds showed significant vasodilation properties using isolated thoracic aortic rings of rats pre-contracted with norepinephrine hydrochloride standard technique. Compounds **30**, **21**, **11**, **15** and **24** (IC_{50} = 0.163, 0.204, 0.221, 0.227 and 0.246 mM respectively) exhibited remarkable activity compared with amiodarone hydrochloride (standard reference used IC_{50} = 0.312 mM). CODESSA-Pro software was employed to obtain a statistically significant QSAR model describing the bioactivity of the newly synthesized analogues **4**–**37**, revealed a good predictive and statistically significant 5 descriptor model (R^2 = 0.846, $R^2_{observed}$ = 0.765, $R^2_{prediction}$ = 0.778). External validation supported the attained model. From all the above it could be concluded that, designing new hybrids of benzofuran scaffold incorporating 3-pyridinecarbonitrile analogs, seems highly promising approach toward developing vasodilting active hits.

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References and notes

1 Hassan, G. S.; Abdel Rahman, D. E.; Saleh, D. O.; Abdel Jaleel, G. A. *Chem. Pharm. Bull*. **2013**, *62*, 1238–1251.

2 Manna, K.; Agrawal, Y. K. *Bioorg. Med. Chem. Lett*., **2009**, *19*, 2688–2692.

3 Xie, Y.; Kumar, D.; Bodduri, V. D.; Tarani, P. S.; Zhao, B.; Miao, J.; Jang K.; Shin, D. *Tetrahedron Lett*., **2014**, *55*, 2796–2800.

4 El-Sawy, E. R.; Ebaid, M. S.; Abo-Salem, H. M.; Al-Sehemi, A. G.; Mandour, A. H. *Arab. J. Chem*., **2014**, *7*, 914–923.

5 Thevenin, M.; Thoret, S.; Grellier, P.; Dubois, J., *Bioorg. Med. Chem*., **2013**, *21*, 4885–4892.

6 Feher, D.; Barlow, R. S.; McAtee, J.; Hemscheidt, T. K. *J. Nat. Prod*., **2010**, *73*, 1963–1966.

7 Galal, S. A.; Abd El-All, A. S.; Abdallah, M. M.; El-Diwani, H. I. *Bioorg. Med. Chem. Lett*., **2009**, *19*, 2420–2628.

8 Xie, F.; Zhu, H.; Zhang, H.; Lang, Q.; Tang, L.; Huang, Q.; Yu, L. *Eur. J. Med. Chem*., **2015**, *89*, 310–319.

9 Hassan, G. S.; Abdel Rahman, D. E.; Saleh, D. O.; Abdel Jaleel, G. A. *Chem. Pharm. Bull*., **2014**, *62*, 1238–1251.

10 Campos-Toimil, M.; Orallo, F.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 783–786.

11 Grossmann, M.; Dobrev, D.; Kirch, W. *Clin. Pharmacol. Ther*., **1998**, *64*, 302–311.

12 Massie, B. M.; Fisher, S. G.; Deedwania, P. C.; Singh, B. N.; Fletcher, R. D.; Singh, S. N. *Circulation*, **1996**, *93*, 2128–2134.

13 Grossman, M.; Dobrev, D.; Kirch, W. *Clin. Pharmacol. Ther*., 1998, **64**, 302–11.

14 Guiraudou, P.; CosnierPucheu, S. C.; Gayraud, R.; Gautier, P.; Roccon, A.; Herbert, J. M.; Nisato, D. *Eur. J. Pharmacol*., **2004**, *496*, 119–127.

15 Hanafy, D. A.; Chen, Y.; Chang, S.; Lu, Y.; Lin, Y.; Kao, Y.; Chen, S.; Chen, Y. *Eur. J. Pharmacol*., **2013**, *702*, 103–108.

16 Stewart, S.; Hart, C. L.; Hole, D. J.; McMurray, J. *J. Am. J. Med*., **2002**, *113*, 359–364.

17 Pomponio, G.; Zurich, M.; Schultz, L.; Weiss, D.; Romanelli, L.; Gramowski-Voss, A.; Consiglio, D. E.; Testai, E. *Toxicology in Vitro*, **2015**, *30*, 192-202.

18 Brief, J. F.; Jimmo, S.; Brennan, F. J.; Ford, S. E.; Armstrong, P. W. *Can. J. Physiol. Pharmacol*., **1987**, *65*, 360–364.

19 Ishida, S.; Sugino, M.; Hosokawa, T.; Sato, T.; Furutama, D.; Fukuda, A.; Kimura, F.; Kuwabara, H.; Shibayama, Y.; Hanafusa, T. *Clin. Neuropathol*., **2010**, *29*, 84–88.

20 Kathofer, S.; Thomas, D.; Karle, C. A. *Cardiovasc., Drug Rev*. **2005**, *23*, 217–230.

21 Kleemann, A.; Engel, J.; Kutscher, B.; Reichert, D. *in: Pharmaceutical Substances: Syntheses Patents Applications, third ed., Thieme, Stuttgart, New York*, **1999**, pp. 938, 1007, 1245.

22 Girgis, A. S.; Kalmouch, A.; Ellithey, M. *Bioorg. Med. Chem*., **2006**, *14*, 8488–8494.

23 Nofal, Z. M.; Srour, A. M.; El-Eraky, W. I.; Saleh, D. O.; Girgis, A. S. *Eur. J. Med. Chem*., **2013**, *63*, 14–21.

24 Mendis, S.; Puska, P.; Norrving, B. Global Atlas on cardiovascular disease prevention and control, World Health Organization (**2011**).

25 http://www.who.int/mediacentre/factsheets/fs317/en/

26 Prisant, L. M. *J. Clin. Hypertens*., **2002**, *4*, 286–294

27 Garovic, V. D.; Hayman, S. R. *Nat. Clin. Pract. Nephr*., **2007**, *3*, 613–622.

28 Savoia, C.; Tabet, F.; Yao, G.; Schiffrin, E. L.; Touyz, R. M. *J. Hypertens*., **2005**, *23*, 1037–1045.

29 Deng, Y.; Ng, E. S.; Kwan, Y. W.; Lau, C. B. S.; Cheung, D. W. S.; Koon, J. C. M.; Zhang, Z.; Zuo, Z.; Leung, P. C.; Fung K. P.; Lam, F. F. Y. *Phytomedicine*, **2014**, *21*, 391–399.

30 Tang, K. M.; Wang, G. R.; Lu, P. R.; Karas, H.; Aronovitz, M.; Heximer, S. P.; Kaltenbronn, K. M.; Blumer, K. J.; Siderovski, D. P.; Zhu, Y.; Mendelsohn, M. E. *Nat. Med*., **2003**, *9*, 1506–1512.

31 Pitt, B.; Reichek, N.; Willenbrock, R.; Zannad, F.; Phillips, R. A.; Roniker, B.; Kleiman, J.; Krause, S.; Burns, D.; Williams, G. H. *Circulation*, **2003**, *108*, 1831–1838.

32 Danaei, G.; Ding, E. L.; Mozaffarian, D.; Taylor, B.; Rehm, J. R.; Murray, C. J.; Ezzat, M. *PLoS Med*., **2009**, *6*, 1–23.

33 Haider, A. W.; Larson, M. G.; Franklin, S. S.; Levy, D. *Ann. Intern. Med*., **2003**, *138*, 10–16.

34 Galan, B. E.; Perkovic, V.; Ninomiya, T.; Pillai, A.; Patel, A.; Cass, A.; Neal, B.; Poulter, N.; Harrap, S.; Mogensen, C.; Cooper, M.; Marre, M.; Williams, B.; Hamet, P.; Mancia, G.; Woodward, M.; Glasziou, P.; Grobbee, D. E.; MacMahon, S.; Chalmers, J. *J. Am. Soc. Nephrol*., **2009**, 20883–892.

35 Pettinger, W. A.; Mitchell, H. C. *Hypertension.*, **1988**, *11*, 34–36.

36 Marona, H.; Szkaradek, N.; Rapacz, A.; Filipek, B.; Dybata, M.; Siwek, A.; Cegta, M.; Szneler, E. *Bioorg. Med. Chem*., **2009**, *17*, 1345–1352.

37 Girgis, A. S.; Mishriky, N.; Farag, A. M.; El-Eraky, W. I.; Farag, H. *Eur. J. Med. Chem*., **2008**, *43*, 1818–1827.

38 CODESSA-Pro manual pp. 54, 55, 60, 73, 76.

39 Raj, P. A.; Suddendra, G.; Shakeel, A. S.; Girish, M. *IJDFR*. **2012**, *3*, 135–147.

40 Silver, R. F.; Kerr, K. A.; Frandsen, P. D.; Kelley, S. J.; Holmes, H. L. *Can. J. Chem.* **1967**, *45*, 1001–1006.

41 Mantri, M.; Graaf, O.; Veldhoven, J. V.; Goeblyoes, A.; Von Frijtag, J. T.; Kuenze, D.; Mulder-Krieger, T.; Link, R.; Vries, H.; Beukers, M. W.; Brussee, J.; Ijzerman, A. P. *J. Med. Chem*., **2008**, *51*, 4449–4455.

42 Girgis, A. S.; Panda, S. S.; Srour, A. M.; Farag, H.; Ismail, N. S. M.; Elgendy, A. K. M.; Abdel-Aziz, M.; Katritzky, A. R. *Org. Biomol. Chem.* **2015**, *13*, 6619–6633.

43 Naumov, R. N.; Panda, S. S.; Girgis, A. S.; George, R. F.; Farhat, M.; Katritzky, A. R. *Bioorg. Med. Chem. Lett*., **2015**, *25*, 2314–2320.

44 Girgis, A. S.; Panda, S. S.; Aziz, M. N.; Steel, P. J.; Hall, C. D.; Katritzky, A. R. *RSC Advances*, **2015**, *5*, 28554–28569.

45 Girgis, A. S.; Panda, S. S.; Shalaby, E. M.; Mabied, A. F.; Steel, P. J.; Hall, C. D.; Katritzky, A. R. *RSC Advances*, **2015**, *5*, 14780–14787.

46 Tiwari, A. D.; Panda, S. S.; Girgis, A. S.; Sahu, S.; George, R. F.; Srour, A. M.; La Starza, B.; Asiri, A. M.; Hall, C. D.; Katritzky, A. R. *Org. Biomol. Chem.* **2014**, *12*, 7238–7249.

47 Shalaby, E. M.; Girgis, A. S.; Moustafa, A. M.; ElShaabiny, A. M.; El-Gendy, B. E.; Mabied, A. F.; Farag, I. S. A. *Mol. J. Structure*, **2014**, *1075*, 327–334.

48 Girgis, A. S.; Stawinski, J.; Ismail, N. S. M.; Farag, H. *Eur. J. Med. Chem*., **2012**, *47*, 312–322.

49 Girgis, A. S.; Farag, H.; Ismail, N. S. M.; George, R. F. *Eur. J. Med. Chem*., **2011**, *46*, 4964–4969.

Graphical abstract

Some novel bezofuran-pyridinecarbonitrile hyprids as vasorelaxant active agents