Benzofuran–Morpholinomethyl–Pyrazoline Hybrids as a New Class of Vasorelaxant Agents: Synthesis and Quantitative Structure–Activity Relationship Study

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The benzofuran–morpholinomethyl–pyrazoline hybrids 4a–e, 5a–e and 6a–j were synthesized via reaction of a,b-unsaturated carbonyl compounds 3a–e with hydrazine hydrate, semicarbazide or thiosemicarbazide. Applying the Mannich reaction to 5-(5-aryl-4,5-dihydro-1H-pyrazol-3-yl)-4-methoxybenzofuran-6-ols 7a–e with morpholine hydrochloride and paraformaldehyde afforded positional isomeric 7-morpholinomethyl derivatives 4a–e and N-morpholinomethyl derivatives 8a–e. All the synthesized compounds showed significant vasodilatation properties in isolated thoracic aortic rings of rats precontracted using the standard norepinephrine hydrochloride technique. Compounds 3d, 3e, 5a–c, 6b, 6c, 6f, 6h and 6i exhibited activity (IC_{50} 0.3185–0.4577 mM) superior to that of prazocin (IC_{50} 0.487 mM), while 5d, 6j and 8c showed comparable activity (IC_{50} 0.4789–0.4951 mM). The quantitative structure–activity relationship study revealed a correlation between the observed vasorelaxant activities of the newly synthesized compounds and their different physicochemical parameters, especially solubility, in addition to structure connectivity and energetic quantities calculated from stored three dimensional (3D) conformations. Absorption, distribution, metabolism and elimination (ADME) evaluation showed good agreement with the biological results obtained.

Key words benzofuran; morpholinomethyl; pyrazoline; vasorelaxant; quantitative structure–activity relationship study

Vasodilators are smooth muscle relaxants which cause blood vessels to dilate. They are prominently used to treat hypertension, heart failure and angina. This group of medication is known to cause several undesirable adverse reactions. This prompted us to attempt to design and synthesis new effective vasodilators which possess minimal side effects.

Benzofurans are promising candidates for this purpose as they are known found to possess anti-arrhythmic, hypotensive and vasodilator effects. The benzofuran amidodarone (A) and its analogue KB130015 (B) were reported to relax vascular smooth muscle. A new noniodinated benzofuran derivative dronedarone, SR33589 (C) was recently approved by the Food and Drug Administration (FDA) for treatment of atrial fibrillation and atrial flutter. Khellin (D) and visnagin (E) are the principle active constituents obtained from Ammi visnaga L. which possess strong vasodilatation and spasmylctic activities. Some 6-(amoalcohol)-5-cinnamoyl-4,7-dimethoxybenzofuran (F) derivatives were previously prepared and showed vasodilating and hypotensive activities.

The morpholine scaffold is very versatile and was featured in a number of biologically and pharmacologically active products such as vasorelaxant, anticancer, antioxidant, analgesic, antimicrobial and antiparasitic agents.

Mannich bases with morpholinomethyl of synthetic flavonoids exhibited good effects during global ischemia and perfusion in rat brain. In addition, morpholinomethyl benzofurans were reported to exhibit promising hypotensive and antiarrhythmic activities. Other Mannich bases, for instance, 7-piperazinomethylbenzofuran derivative showed promising vasorelaxant activity (IC_{50} 0.21 mM) compared with prazocin is (IC_{50} 0.487 mM) in isolated thoracic aortic rings of rats pre-contracted with norepinephrine hydrochloride.

On the other hand, pyrazolines represented a common motif in pharmacologically and remarkably active compounds demonstrating a wide range of pharmacological activities including anti-inflammatory, analgesic, antibacterial, antimicrobial and antihypertensive agents. It was reported that, 4,5-dihydro-1H-pyrazole derivatives were potential inhibitors of neural nitric oxide synthase (nNOS) and endothelial cell NOS (eNOS). In addition, they played a role in neurotransmission and blood vessel dilation.

In view of the biological significance of benzofuran, morpholinomethyl and pyrazoline, hybrids whose chemical structure incorporated benzofuran directly attached with morpholinomethyl and substituted at 5-position with aryl-4,5-dihydro-1H-pyrazoles, compounds 4a–e, 5a–e and 6a–j were synthesized. Compounds 8a–e, benzofuran and morpholinomethyl systems linked through pyrazoline moiety were also synthesized. Generally, the presence of electron-donating groups on the phenyl ring intensifies the biological activities. These findings initiated the interest to explore the contribution of methoxy and hydroxyl substituents to the vasorelaxant activity. According to our hypothesis, the combination of two or more biologically active moieties or substituents may bring significant improvement in biological activity and may provide new classes of active vasorelaxant compounds.

Quantitative structure–activity relationship (QSAR) study was also considered in the present work to validate the vasorelaxant activity of the investigated compounds and also for determining the most important structure parameters controlling such activity. Furthermore, drug likeness evaluation of the synthesized compounds as well as prazocin and amidodarone.
rone was applied to reveal the relation of molecular properties, structure features and bioavailability of their vasorelaxant activity.

Results and Discussion

Chemistry $\alpha,\beta$-Unsaturated carbonyl compounds 2a,\(^{22}\) b,\(^{33}\) c,\(^{44}\) and the new 2d were synthesized by reacting 1-(6-hydroxy-4-methoxybenzofuran-5-yl)ethanone 1\(^{45}\) with the appropriate aromatic aldehydes in presence of sodium hydroxide by the conventional Claisen–Schmidt condensation.

On subjecting 2a–e to Mannich reactions using secondary amine (morpholine hydrochloride) and paraformaldehyde, the 7-morpholinomethylbenzofuran derivatives 3a, b,\(^{28}\) c–e were formed.

Upon reacting 3a–e with different nucleophiles such as hydrazine hydrate in ethanol, the corresponding pyrazolines 4a, \(^{28}\) b,\(^{33}\) c–e were obtained through 1,4-addition on $\alpha,\beta$-unsaturated carbonyl system, followed by dehydration and rearrangement.\(^{43}\) On the other hand, when 3a–e was reacted with hydrazine hydrate in glacial acetic acid, N-acetylpyrazolines 5a–e was obtained. 4,5-Dihydro-1H-pyrazole-1-carboxamides 6a–e and 1-carbothioamides 6f–j were obtained through the reaction of 3a–e with semicarbazide or thiosemicarbazide, respectively in ethanol/glacial acetic acid (few drops) (Chart 1).

Similarly, compounds 7a, b\(^{33}\) and c–e were prepared starting with compounds 2a–e. Upon reacting 5-(5-aryl)-4,5-dihydro-1H-pyrazol-3-yl)-4-methoxybenzofuran-6-ol 7a–e with...
morphine hydrochloride and paraformaldehyde (Mannich reaction), two positional isomers were obtained 4a–e and 8a–e. The later compounds were separated by application of preparative TLC technique (Chart 2).

All the new synthesized compounds were characterized by spectral and elemental analyses which were in full agreement with the proposed structures.

**Vasorelaxant Activity** All synthesized compounds 3a–e, 4a–e, 5a–e, 6a–j and 8a–e were tested for their vasorelaxant activities against nor-adrenaline-induced spasm on thoracic rat aorta rings, and were compared to the reference drug, prazocin. The results are listed in Table 1 and illustrated in Fig. 2 as IC₅₀ values (mM). These results exhibited the correct choice of compounds’ design as hybrids of benzofuran scaffold with morpholinemethyl and pyrazoline as vasorelaxant agents as all the tested compounds showed biological activity.

Regarding the morpholinemethylbenzofuran chalcones 3a–e, substitution of phenyl ring with methoxy and hydroxyl substituent, (compound 3e, IC₅₀ 0.3185 mM) followed by triacetylpyrazolines N-improvement of activity for most compounds. Results of 6a, 4a–e, 6d, 4d, 7d, 8d Ar = 3,4,5-(OCH₃)₂C₆H₄, 2d, 4e, 7e, 8e Ar = 4-(OH)-3-(OCH₃)C₆H₅

Reagents and conditions: (i) Hydrazine hydrate 98%, EtOH, reflux, 3 h, (ii) Morpholine hydrochloride, paraformaldehyde, EtOH, reflux, 24 h, preparative TLC.

**Structure Activity Relationship** Examination of the vasorelaxant activity results revealed that separation of morpholinemethyl moiety from benzofuran scaffold by pyrazoline spacer (compounds 8a–e) resulted in decrease in activity compared to their positional isomers 4a–e where morpholinemethyl moiety was directly linked to benzofuran scaffold.

Table 1. Concentration of Compounds Necessary to Reduce Maximal Norepinephrine-Induced Contracture by 50% (IC₅₀) in Rat Thoracic Aortic Rings

| Compd. No. | IC₅₀ (mM) |
|------------|-----------|
| 3a         | 0.6585    |
| 3b         | 0.5069    |
| 3c         | 0.5342    |
| 3d         | 0.4577    |
| 3e         | 0.3185    |
| 4a         | 0.7625    |
| 4b         | 0.5664    |
| 4c         | 0.5621    |
| 4d         | 0.5748    |
| 4e         | 0.5340    |
| 5a         | 0.4475    |
| 5b         | 0.4158    |
| 5c         | 0.4550    |
| 5d         | 0.3704    |
| 5e         | 0.4951    |
| 5f         | 0.5185    |
| 6a         | 0.6332    |
| 6b         | 0.4475    |
| 6c         | 0.4158    |
| 6d         | 0.5564    |
| 6e         | 0.5243    |
| 6f         | 0.4212    |
| 6g         | 0.5815    |
| 6h         | 0.4041    |
| 6i         | 0.3505    |
| 6j         | 0.4789    |
| 7a         | 0.7657    |
| 7b         | 0.6681    |
| 7c         | 0.4937    |
| 7d         | 0.6916    |
| 7e         | 0.8022    |
| 8a         | 0.4870    |
| 8b         | 0.6681    |
| 8c         | 0.4937    |
| 8d         | 0.6916    |
| 8e         | 0.8022    |
| Prazocin   | 0.4870    |

Prazocin while compounds 6a, 7a, 7b, 8a Ar = C₆H₄, 2b, 4b, 7b, 8b Ar = 4-(OCH₃)C₆H₄, 2c, 4c, 7c, 8c Ar = 3,4,5-(OCH₃)₂C₆H₄, 2d, 4d, 7d, 8d Ar = 3,4,5-(OCH₃)₂C₆H₄, 2e, 4e, 7e, 8e Ar = 4-(OH)-3-(OCH₃)C₆H₅

Vasorelaxant Activity of the chalcone system. 49–51) As for positional isomer N-morpholinemethylpyrazolines 8a–e, vasorelaxant activities were less than their isomers 4a–e. Except compound 8c (IC₅₀ 0.4937 mM) which exhibited activity comparable to prazocin and the rest of compounds 8a, b, d and e (IC₅₀ 0.6681–0.8022 mM) were less than prazocin.
mment of activity for most compounds.

N-Acetylpyrazoline derivatives 5a–e exhibited highest activity followed by pyrazoline carbothioamide 6f–j then pyrazoline carboxamide derivatives 6a–e while pyrazoline derivatives 4a–e were the least active among this class of compounds.

In addition, the influence of methoxy and hydroxyl substituted aryl pyrazolines on the vasorelaxant activity was observed. At least one methoxy group substitution was essential to increase vasorelaxant activity compared to unsubstituted ring. The effect was increased with the second methoxy substituent and to a lesser extent with additional two methoxy or one hydroxyl group substitution.

QSAR In order to correlate the vasorelaxant activity expressed as log IC$_{50}$ (mM) with the structure conformation of the synthesized benzofuran–morpholinomethyl–pyrazoline hybrids, QSAR study was undertaken. The study was performed using MOE, Molecular Operating Environment software package (MOE version 2008.10.2).

Different molecular descriptors (Table 2) were selected from an initial pole of 85 descriptors and were calculated for compounds structure aiming to cover a wide range of different electronic, hydrophobic and topological characters. In order to avoid multicolinearity between the calculated descriptors the correlation matrix was calculated. The correlation matrix indicated that some of the descriptors used were highly correlated which suggests avoiding the combinations of such intercorrelated descriptors.

Model Construction To test the best structural predictors for activity, stepwise linear regression analysis (SLRA) technique was used.

For the current dataset of 30 compounds, the QSAR model was derived by partial least square. The development of QSAR model was restricted to a maximum of four variables in accordance the general accepted rule for each compound: descriptors ratio to be around 5:1.

The simple linear regression analysis between the logIC$_{50}$ and the different descriptors yields one statistically signifi-

![Fig. 2. Vasorelaxant Activity (IC$_{50}$ Values) of Tested Compounds on Contracture Induced by Norepinephrine Hydrochloride on Thoracic Rat Aortic Rings Compared to Prazocin](image)

Table 2. The Molecular Descriptor Values of the Studied Compounds

| Compd. No. | logS  | VSA    | E$_{sol}$ | rgyr  |
|------------|-------|--------|-----------|-------|
| 3a         | 5.2602| 415.3450| −5.9096  | 4.2836|
| 3b         | 5.3106| 444.1414| −10.1487 | 5.0438|
| 3c         | 5.3610| 474.1896| −2.9714  | 5.3157|
| 3d         | 5.4113| 501.4015| 0.8602   | 5.0932|
| 3e         | 4.9486| 449.5437| −11.8534 | 5.1419|
| 4a         | 4.5772| 419.5661| −8.8021  | 4.6045|
| 4b         | 4.6276| 456.8181| −8.8021  | 4.9362|
| 4c         | 4.6780| 490.1929| −12.0295 | 5.0422|
| 4d         | 4.7542| 503.4925| −16.6703 | 4.6650|
| 5a         | 5.0658| 468.3897| −9.5429  | 4.4384|
| 5b         | 5.1161| 496.8009| −20.3672 | 4.5314|
| 5c         | 5.1665| 529.1497| −6.8252  | 4.8810|
| 5d         | 5.2169| 560.5244| −12.9473 | 4.8320|
| 5e         | 4.7542| 503.4925| −16.6703 | 4.6650|
| 6a         | 5.0056| 461.2488| −5.8186  | 4.4359|
| 6b         | 5.0560| 494.3793| −11.8881 | 4.7452|
| 6c         | 5.1064| 524.4553| −14.3439 | 4.8093|
| 6d         | 5.1568| 549.1744| −11.8380 | 4.6072|
| 6e         | 4.6940| 496.1378| −26.7391 | 4.5096|
| 6f         | 6.0407| 471.0862| 2.8766   | 4.4424|
| 6g         | 6.0911| 506.7375| −7.9529  | 4.7773|
| 6h         | 6.1414| 530.6677| −3.5103  | 4.8021|
| 6i         | 6.1918| 562.1160| −2.2115  | 4.7922|
| 6j         | 5.7291| 507.7466| −4.5321  | 4.7679|
| 7a         | 4.3847| 426.4172| −7.7219  | 4.3002|
| 7b         | 4.4351| 459.0730| −9.0937  | 4.4039|
| 7c         | 4.4855| 490.3040| 0.6237   | 4.6997|
| 7d         | 4.5359| 519.6377| 5.5265   | 4.7292|
| 7e         | 4.0732| 462.5117| −3.2103  | 4.5672|
the model. Detection of outliers was achieved through the Z score method. Z Score can be defined as absolute difference between the value of the model and the activity field, divided by the square root of the mean square error of the data set. Any compound which shows a value of Z score higher than 2.5, during generation of a particular QSAR model, is considered as an outlier.3)

\[
\log IC_{50} = 3.35834 + 0.12660 \times \log S
\]
\[
 n = 28, \text{RMSE} = 0.06457, \ r^2 = 0.5279 \quad \text{(Model 1)}
\]

\(n\): number of compounds used for construction of model. RMSE: root mean square error. \(r^2\): correlation coefficient.

Stepwise regression analyses using different combinations of \(\log S\) and other structural descriptors resulted into bi-parametric model (Model 2). The biparametric model correlated vasorelaxant activity (\(\log IC_{50}\)) with \(\log S\) and VSA. Model 2 showed better statistics than the mono-parametric model discussed above. Compounds 5a and e were omitted as outliers while deriving the model.

\[
\log IC_{50} = 3.63093 + 0.09961 \times \log S - 0.00082 \times \text{VSA}
\]
\[
 n = 26, \text{RMSE} = 0.04983, \ r^2 = 0.6934 \quad \text{(Model 2)}
\]

Stepwise regression analyses using different combinations of \(\log S\) and VSA with other structural descriptors resulted in tri-parametric model (Model 3). The triparametric model correlated \(\log IC_{50}\) with \(\log S\), VSA and \(E_{sol}\). Model 3 exhibited better statistics than the mono-parametric and biparametric models discussed above. Compounds 6d and 8c were omitted as outliers while deriving the model.

\[
\log IC_{50} = 3.79347 + 0.12247 \times \log S - 0.00084 \times \text{VSA} + 0.00409 \times E_{sol}
\]
\[
 n = 24, \text{RMSE} = 0.03662, \ r^2 = 0.8459 \quad \text{(Model 3)}
\]

Stepwise regression analyses using different combinations of \(\log IC_{50}\) with \(\log S\), VSA & \(E_{sol}\) with other structural descriptors resulted into tetraparametric model (Model 4). The tetraparametric model correlated \(\log IC_{50}\) with \(\log IC_{50}\) with \(\log S\), VSA, \(E_{sol}\) and rgyr. Model 4 revealed better statistics than the other models discussed above. Compounds 4c and 6f were omitted as outliers while deriving the model.

\[
\log IC_{50} = 4.05175 + 0.12029 \times \log S - 0.00080 \times \text{VSA} + 0.00502 \times E_{sol} - 0.05864 \times \text{rgyr}
\]
\[
 n = 22, \text{RMSE} = 0.0287, \ r^2 = 0.9074 \quad \text{(Model 4)}
\]

The \(\log IC_{50}\) was plotted against their predicted values (LOO), Table 3 and Fig. 4.

**Statistical Diagnosis** Fraction of the Variance (\(r^2\)): Represents the goodness of fit. The value of \(r^2\) may vary between 0 and 1, when multiplied by 100 gives explanation to variance in biological activity, where 0 means a perfect model explaining 100% of the variance in the data, and 0 means a model without any explanatory power. It has already been suggested that the only QSAR model having \(r^2>0.6\) will be considered for validation.55) The value of \(r^2\) for this QSAR model is 0.9074.

**Cross-Validation Test** \(q^2\): A measure of quality of the QSAR model. According to the literature, a QSAR model must have \(q^2>0.5\) for their predictive ability.55) The value of \(q^2\) for this QSAR model is 0.860459.

\[
\begin{align*}
q^2 &= 1 - \frac{\sum(\text{IC}_{50} \text{Obs.} - \text{IC}_{50} \text{Pred.})^2}{\sum(\text{IC}_{50} \text{Obs.} - \text{IC}_{50} \text{average})^2} \\
\end{align*}
\]

The \(\log IC_{50}\) (observed) was plotted against their predicted values. All tested compounds were in compliance with this rule except compounds 6h and 1 that had slight lower \(\log S\) \(-6.1414\) and \(-6.1918\), respectively but this compensated with low value of rgyr and VSA descriptors (compounds 6f and g were considered outliers). QSAR results assured that the aqueous solubility of the compounds is one of the most important determinants for the vasorelaxant activity due to increase in bioavailability.

**ADME Evaluation** As a part of our study, the compliance of the benzofturan drug amidarone, reference drug prazocin and the synthesized compounds with the Lipinski’s rule of five57 was evaluated. This was assessed using mipc—Molinspiration Property Calculator.58

Lipinski’s rule had been used in the evaluation of oral bioavailability of the compounds. Lipinski’s rule of five defines molecular properties important to the drug’s pharmacokinetics in the human body, including their (ADME) and is used to insure that drug-like physicochemical properties. The rule describes a likely oral bioavailability molecule as (i) a molecular weight (MW) less than 500 Daltons (Da), (ii) the logarithm of the octanol/water partition coefficient representing the lipophi-
licity factor (log $P$) less than 5, (iii) not more than 5 hydrogen bond donors (OH and NH groups, HBD), (iv) not more than 10 hydrogen bond acceptors (HBA) and (v) not more than 10 rotatable bonds (NRB). The number of violation to this rule must not exceed 2 and at least three parameters coincide with the rule. In addition, the topological polar surface area (TPSA) of the compounds was also calculated since it is another key property that has been linked to drug bioavailability, TPSA equal to or less than 140 Å$^2$ are considered to have good oral bioavailability.

Absorption and liver first-pass metabolism determine the bioavailability of a compound. According to Jorgensen’s rule of three, the computed parameters used to assess oral absorption should comply with the following values (log $S_{wat}$ > −5.7, BIP caco-2 > 22 nm/s and primary metabolites < 7) to be orally available. The most important parameters considered are the predicted aqueous solubility, log $S_{wat}$.

The size of a molecule, lipophilicity, capacity to make hydrogen bonds, and its ability to act as a hydrogen bond donor or acceptor are key factors. It is important to consider the presence of functional groups such as hydroxyl (OH), amino (NH), and amide (CONH) groups, as they can significantly affect the bioavailability of a drug.

Table 3. The Experimental and Predicted Activities (log IC$\text{_{50}}$), Residuals and Z-Scores for the Tested Compounds Calculated Using MLR Validation and the Corresponding Cross-Validation LOO

| Compd. No. | log Obs. IC$\text{_{50}}$ | MLR validation | | LOO validation | |
|------------|----------------|----------------|----------------|----------------|
|            | log Pred. IC$\text{_{50}}$ | Residual | Z-Score | log Pred. IC$\text{_{50}}$ | Residual | Z-Score |
| 3a | 2.8190 | 2.8063 | 0.0127 | 0.4412 | 2.7980 | 0.0210 | 0.7195 |
| 3b | 2.7050 | 2.7114 | 0.0064 | 0.2240 | 2.7140 | −0.0090 | 0.3084 |
| 3c | 2.7280 | 2.7014 | 0.266 | 0.9261 | 2.6874 | 0.0406 | 1.4240 |
| 3d | 2.6610 | 2.7059 | 0.0449 | 1.5642 | 2.7160 | −0.0550 | 2.0148 |
| 4a | 2.8820 | 2.8518 | 0.3020 | 1.0522 | 2.8454 | 0.0366 | 1.2875 |
| 4b | 2.7410 | 2.7803 | 0.0393 | 1.3705 | 2.7879 | −0.0469 | 1.6828 |
| 4c | 2.7530 | 2.7368 | 0.0162 | 0.5645 | 2.7332 | 0.0198 | 0.6800 |
| 4d | 2.7800 | 2.7355 | 0.0445 | 1.5488 | 2.7258 | 0.0542 | 1.9813 |
| 5b | 2.6580 | 2.6715 | 0.0135 | 0.4717 | 2.6752 | −0.0172 | 0.5888 |
| 5d | 2.6950 | 2.6584 | 0.0366 | 1.2760 | 2.6483 | 0.0467 | 1.6715 |
| 5e | 2.7280 | 2.7204 | 0.0076 | 0.2632 | 2.7193 | 0.0087 | 0.2972 |
| 6a | 2.8020 | 2.7783 | 0.0237 | 0.8266 | 2.7750 | 0.0270 | 0.9351 |
| 6b | 2.6510 | 2.7107 | 0.0597 | 2.0803 | 2.7144 | −0.0634 | 2.4265 |
| 6c | 2.6190 | 2.6645 | 0.0455 | 1.5867 | 2.6710 | −0.0520 | 1.8976 |
| 6e | 2.7200 | 2.6922 | 0.0278 | 0.9698 | 2.6752 | 0.0448 | 1.5796 |
| 6h | 2.6060 | 2.5898 | 0.0162 | 0.5632 | 2.5840 | 0.0220 | 0.7571 |
| 6i | 2.5450 | 2.5658 | 0.0208 | 0.7230 | 2.5769 | −0.0319 | 1.1065 |
| 6j | 2.6800 | 2.6547 | 0.0253 | 0.8826 | 2.6506 | 0.0294 | 1.0216 |
| 8a | 2.8840 | 2.8927 | 0.0087 | 0.3046 | 2.8954 | −0.0114 | 0.3893 |
| 8b | 2.8250 | 2.8476 | 0.0226 | 0.7887 | 2.8516 | −0.0266 | 0.9205 |
| 8d | 2.8400 | 2.8414 | 0.0014 | 0.0485 | 2.8425 | −0.0025 | 0.0845 |
| 8e | 2.9040 | 2.9084 | 0.0044 | 0.1520 | 2.9100 | −0.0060 | 0.2035 |

Fig. 3. Correlation of log Observed and log Predicted IC$\text{_{50}}$ Using MLR ($r^2$=0.9074)
Hydrogen bonds as well as shape and flexibility are important properties to consider when determining its oral bioavailability.59

The calculated parameters (Table 4) showed good bioavailability of studied compounds. Most compounds fulfilled Lipinski’s rule similar to the clinically used drug prazocin. Compounds 5c, 6h, j and 8d violated the rule of five in molecular weight value while compounds 5d, 6c and 1 violated...
in molecular weight value and HBA. In addition, compound 6c violated in HBA and had slightly large TPSA (143.232 Å³). Also, compound 6d violated in molecular weight value and HBA and had slightly large TPSA (141.472 Å³). Meanwhile, amiodarone violated in molecular weight value and logP.

Hence; theoretically, all of these compounds should present good passive oral absorption and differences in their bioactivity cannot be attributed to this property.

Conclusion

This study describes the design and synthesis of new benzofuran–morpholinomethyl–pyrazoline hybrids as vasorelaxant agents. All the synthesized compounds showed significant vasodilatation properties using isolated thoracic aortic rings of rats pre-contracted with norepinephrine hydrochloride standard technique and results were compared with reference drug prazocin. Results revealed that direct linked morpholinomethyl moiety to benzofuran scaffold 4a–e gave better results than their positional isomers 8a–e with morpholinomethyl moiety separated away from benzofuran scaffold by pyrazoline spacer. N-Acetylpyrazoline derivatives 5a–e exhibited highest activity followed by pyrazoline carbothioamide 6f–j then pyrazoline carboxamide derivatives 6a–e while pyrazoline derivatives 4a–e were the least active among this class of compounds. In general, methoxy and hydroxyl substitution on the phenyl ring at pyrazoline had good effect on activity.

In addition QSAR and drug likeness studies results accounted for the importance of aqueous solubility on oral bioavailability and activity of tested compounds.

Experimental

Chemistry Melting points were determined by open capillary tube method using Gallen Kamp melting point apparatus MFB-595-010M (Gallen Kamp, London, U.K.) and were uncorrected. Microanalyses were carried out at The Regional Center for Mycology and Biotechnology, Al-Azhar University.

Infrared spectra were recorded as potassium bromide discs on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan) and expressed in wave number (cm⁻¹). The NMR spectra were recorded with 300 MHz in deuterated chloroform (CDCl₃). Chemical shifts are quoted in δ as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard. Mass spectra were recorded using Hewlett Packard Varian (Varian, Polo, U.S.A.) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan) and expressed in wave number (cm⁻¹). IR (KBr) cm⁻¹; 3400 (OH), 3040 (CH Ar), 2924, 2852 (CH aliphatic), 1670 (C=O), 1548, 1522 (C=CH), 7.73 (1H, d, J = 17.1 Hz, COCH=CH), 13.10 (1H, s, OH, exch. D₂O). IR (KBr) cm⁻¹; 3414 (OH), 3100 (CH Ar), 2920, 2850 (CH aliphatic), 1670 (C=O), 1614, 1604, 1560, 1541, 1508 (C=C). MS (m/z) %: 454 (M⁺+1) 10.53%. Anal. Calcd for C₂₃H₂₀O₅: C, 66.34; H, 6.09; N, 3.18.

General Procedure for Synthesis of 1-(6-Hydroxy-4-methoxybenzofuran-5-yl)-3-(unsubstituted)phenylprop-2-en-1-one (3a–e) To a solution of the appropriate propene derivative 2a–e (10 mmol) in ethanol (20 mL), morpholine hydrochloride (1.36 g, 11 mmol) and paraformaldehyde (0.6 g, 20 mmol) were added. The mixture was refluxed for 24 h. Excess solvent was removed under vacuum then cooled and water was added. The mixture was neutralized with dilute ammonia and extracted with chloroform. Chloroform extract was dried over anhydrous sodium sulfate and evaporated under vacuum. The product was crystallized from methanol (Chart 1).

3-(4-Dimethoxyphenyl)-1-[6-Hydroxy-4-methoxy-7-(morpholin-4-ylmethyl)benzofuran-5-yl]prop-2-en-1-one (3a) Yield 70%. mp 134–136°C. ¹H-NMR (CDCl₃) δ: 2.90–3.00 (4H, m, morpholine H), 3.45–3.65 (4H, m, morpholine H), 3.94 (6H, s, 2xOCH₃), 4.11 (3H, s, OCH₃), 4.81 (2H, s, CH₂), 6.88 (1H, d, J = 15.1 Hz, H-3 furan), 6.92 (1H, d, J = 6.6 Hz, H-5' Ar), 7.70 (1H, s, H-2' Ar), 7.27 (1H, d, J = 5.4 Hz, H-6' Ar), 7.58 (1H, d, J = 17.1 Hz, COCH=CH), 7.73 (1H, d, J = 1.5 Hz, H-2 furan), 7.79 (1H, d, J = 17.1 Hz, COCH=CH), 13.10 (1H, s, OH, exch. D₂O). IR (KBr) cm⁻¹; 3414 (OH), 3100 (CH Ar), 2920, 2850 (CH aliphatic), 1670 (C=O), 1614, 1604, 1560, 1541, 1508 (C=C). MS (m/z) %: 454 (M⁺+1) 10.53%. Anal. Calcd for C₂₃H₂₀O₅: C, 66.34; H, 6.09; N, 3.18.
Yield 64%. mp 145–148°C. 1H-NMR (CDCl₃) δ: 8.88–3.00 (4H, m, morpholine H), 3.21–3.49 (4H, m, morpholine H), 3.64 (1H, dd, J=14.1, 7.85 Hz, CH₂ pyrazoline), 3.93 (6H, s, 2×OCH₃), 4.02 (3H, s, OCH₃), 4.09 (2H, s, CH₂), 4.16 (1H, dd, J=14.1, 7.85 Hz, CH₂ pyrazoline), 4.85 (1H, t, CH pyrazoline), 6.76 (1H, s, H-2' Ar), 6.84 (1H, d, J=2.4 Hz, H-3 furan), 6.95 (1H, d, J=9.4 Hz, H-5' Ar), 7.49 (1H, d, J=9.3 Hz, H-6' Ar), 7.80 (1H, d, J=2.4 Hz, H-2 furen), 11.75 (1H, s, NH, exch. D₂O), 13.00 (1H, s, NH, exch. D₂O), IR (KBr cm⁻¹): 3400 (OH), 3323 (NH), 3040 (CH Ar), 2922, 2850 (CH aliphatic), 1620, 1516 (NH, C=C), MS (m/z): 465 (M⁺–2) 2.53%. Anal. Calcd for C₂₆H₃₁N₃O₇ (497.54): C, 62.76; H, 6.11; N, 9.44. Found: C, 62.79; H, 6.31; N, 9.51.

Yield 64%. mp 124–125°C. 1H-NMR (CDCl₃) δ: 2.08 (3H, s, COCH₃), 2.34–2.43 (4H, m, morpholine H), 3.54 (1H, dd, J=18.9, 7.5 Hz, CH₁ pyrazoline), 3.71–3.85 (4H, m, morpholine H), 3.99 (3H, s, OCH₃), 4.04 (1H, d, J=17.3, 7.9 Hz, CH₂ pyrazoline), 4.11 (3H, s, OCH₃), 4.21 (2H, s, CH₂), 5.43 (1H, t, CH pyrazoline), 6.81 (1H, d, J=2.0 Hz, H-3 furan), 6.82 (2H, d, J=11.4 Hz, H-3',5' Ar), 7.18 (2H, d, J=11.7 Hz, H-2',6' Ar), 7.46 (1H, d, J=2.1 Hz, H-2 furen), 11.80 (1H, s, NH, exch. D₂O). IR (KBr cm⁻¹): 3431 (OH), 3066 (CH Ar), 2932, 2835 (CH aliphatic), 1664 (C=O), 1618, 1514 (C=C), MS (m/z): 478 (M⁺–1) 0.51%.

Yield 66%. mp 114–117°C. 1H-NMR (CDCl₃) δ: 2.06 (3H, s, COCH₃), 2.34–2.43 (4H, m, morpholine H), 3.47–3.77 (6H, m, morpholine H, CH₂ pyrazoline), 3.84 (6H, s, 2×OCH₃), 3.97 (3H, s, OCH₃), 4.21 (2H, s, CH₂), 5.41 (1H, t, CH pyrazoline), 6.78–6.97 (4H, m, H-3 furen, H-2',5',6' Ar), 7.48 (1H, d, J=2.1 Hz, H-2 furen), 11.73 (1H, s, NH, exch. D₂O). IR (KBr cm⁻¹): 3423 (OH), 3072 (CH Ar), 2924, 2850 (CH aliphatic), 1662 (C=O), 1620, 1544, 1517 (C=C), MS (m/z): 508 (M⁺–1) 1.14%. Anal. Calcd for C₁₅H₁₄N₂O₇ (509.55): C, 63.64; H, 6.13; N, 8.25. Found: C, 63.70; H, 6.18; N, 8.38.

Yield 65%. mp 88–89°C. 1H-NMR (CDCl₃) δ: 2.40–2.65 (4H, m, morpholine H), 3.20–3.80 (5H, m, morpholine H, CH₂ pyrazoline), 4.00 (1H, dd, J=14.1, 7.8 Hz, CH₂ pyrazoline), 4.06 (3H, s, OCH₃), 4.12 (3H, s, OCH₃), 4.20 (2H, s, CH₂), 4.85 (1H, t, CH pyrazoline), 6.75–6.90 (4H, m, H-3 furen, H-2',5',6' Ar), 7.45 (1H, d, J=2.1 Hz, H-2 furen), 12.00 (1H, s, NH, exch. D₂O), 13.00 (2H, s, 2×OH, exch. D₂O). IR (KBr cm⁻¹): 3392 (OH), 3296 (NH), 3066 (CH Ar), 2922, 2850 (CH aliphatic), 1618, 1560, 1540, 1508 (NH, C=C), MS (m/z): 457 (M⁺+4) 0.19%. Anal. Calcd for C₁₅H₁₄N₂O₇ (453.49): C, 63.56; H, 6.00; N, 9.27. Found: C, 63.64; H, 6.04; N, 9.39.

Yield 60%. mp 79–80°C. 1H-NMR (CDCl₃) δ: 2.09 (3H, s, COCH₃), 2.20–2.50 (4H, m, morpholine H), 3.81 (9H, s, 3×OCH₃), 3.83–3.89 (6H, m, morpholine H, CH₂ pyrazoline), 3.90 (3H, s, OCH₃), 4.10 (2H, s, CH₂), 5.40 (1H, t, CH pyrazoline), 6.45 (2H, s, H-2',6' Ar), 6.86 (1H, d, J=2.4 Hz, H-3 furen), 11.70 (1H, s, NH, exch. D₂O). IR (KBr cm⁻¹): 3421 (OH), 3066 (CH Ar), 2935, 2839 (CH aliphatic), 1660 (C=O), 1618, 1593, 1543, 1508 (C=C), MS (m/z): 539 (M⁺) 0.88%. Anal. Calcd for C₁₅H₁₄N₂O₇ (539.58): C, 62.32; H, 6.16; N, 7.79. Found: C, 62.41; H, 6.13; N, 7.88.

Yield 55%. mp 101–104°C. 1H-NMR (CDCl₃) δ: 2.12 (3H, s, COCH₃), 2.71–2.78 (4H, m, morpholine H, CH₂ pyrazoline), 3.80–4.18 (6H, m, morpholine H, CH₂ pyrazoline), 4.19
General Procedure for Synthesis of 3-[6-Hydroxy-4-methoxy-7-(morpholin-4-ylmethyl)benzofuran-5-yl]-5-(unsubstituted)phenyl-4,5-dihydro-1H- pyrazole-1-carboxamide (or Carbothioamide) (6a–j) A mixture of the appropriate Mannich base compound 3a–e (10 mmol), semicarbazide or thiosemicarbazide (10 mmol) in ethanol (20 mL) in presence of few drops glacial acetic acid was heated under reflux for 8 h. The solvent was concentrated under reduced pressure and the residue was crystallized from methanol (Chart 1).

3-[6-Hydroxy-4-methoxy-7-(morpholin-4-ylmethyl)benzofuran-5-yl]-5-phenyl-4,5-dihydro-1H-pyrazole-1-carboxamide (6a) Yield 60%. mp 167–178°C. $^1$H-NMR (CDCl$_3$) δ: 2.80–3.20 (4H, m, morpholine H), 3.60–4.00 (6H, m, morpholine H, CH$_2$ pyrazoline), 4.03 (3H, s, OCH$_3$), 4.16 (2H, s, CH$_2$), 4.50 (2H, s, NH$_2$, exch. D$_2$O), 5.10 (1H, t, CH pyrazoline), 6.95 (1H, d, J = 4.2 Hz, H-2 furan), 7.43–7.64 (4H, m, H-3′,5′ A, Ar), 7.64 (2H, d, J = 7.8 Hz, H-2′,6′ A) 7.86 (1H, d, J = 4.2 Hz, H-2 furan), 12.10 (1H, s, OH, exch. D$_2$O). IR (KBr) cm$^{-1}$: 3462 (OH), 3388, 3342 (NH$_2$), 3059 (CH Ar), 2924, 2825 (CH aliphatic), 1685 (C=O), 1618, 1570, 1543 (NH, C=C). MS (m/z) %: 449 (M$^+$–1) 6.16%. Anal. Calc. for C$_{32}$H$_{36}$N$_5$O$_7$ (540.56): C, 64.03; H, 5.89; N, 12.53.

3-[6-Hydroxy-4-methoxy-7-(morpholin-4-ylmethyl)benzofuran-5-yl]-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (6b) Yield 60%. mp 164–166°C.

$^1$H-NMR (CDCl$_3$) δ: 2.80–3.00 (4H, m, morpholine H), 3.47 (1H, dd, J = 18.9, 5.4 Hz, CH$_2$ pyrazoline), 3.71–3.82 (4H, m, morpholine H), 3.84 (3H, s, OCH$_3$), 3.97 (3H, s, OCH$_3$), 4.11 (1H, dd, J = 17.7, 4.8 Hz, CH$_2$ pyrazoline), 4.18 (2H, s, CH$_2$), 4.54 (2H, s, NH$_2$, exch. D$_2$O), 5.40 (1H, t, CH pyrazoline), 6.43 (1H, d, J = 2.2 Hz, H-3 furan), 6.80–6.94 (2H, m, H-3′,5′ A, Ar), 7.31–7.62 (2H, m, H-2′,6′ A) 7.83 (1H, d, J = 2.2 Hz, H-2 furan), 13.00 (1H, s, OH, exch. D$_2$O). IR (KBr) cm$^{-1}$: 3462 (OH), 3340, 3219 (NH$_2$), 3032 (CH Ar), 2924, 2825 (CH aliphatic), 1681 (C=O), 1620, 1604, 1583, 1543, 1512 (NH, C=C). MS (m/z) %: 483 (M$^+$+3) 1.45%. Anal. Calc. for C$_{34}$H$_{38}$N$_5$O$_7$ (580.51): C, 62.49; H, 5.87; N, 11.66. Found: C, 62.54; H, 5.94; N, 11.72.

3-[3-(4-Dimethoxyphenyl)-3-[6-hydroxy-4-methoxy-7-(morpholin-4-ylmethyl)benzofuran-5-yl]-4,5-dihydro-1H-pyrazole-1-carboxamide (6c) Yield 66%. mp 206–208°C.

$^1$H-NMR (CDCl$_3$) δ: 2.45–2.65 (4H, m, morpholine H), 3.50–3.85 (6H, m, morpholine H, CH$_2$ pyrazoline), 3.90 (6H, s, 2×OCH$_3$), 3.95 (3H, s, OCH$_3$), 4.04 (2H, s, CH$_2$), 4.40 (2H, br s, NH$_2$, exch. D$_2$O), 5.17 (1H, t, CH pyrazoline), 6.93–7.15 (4H, m, H-3 furan, H-2′,5′,6′ A) 7.60 (1H, d, J = 2.0 Hz, H-2 furan), 12.20 (1H, s, OH, exch. D$_2$O). IR (KBr) cm$^{-1}$: 3470 (OH), 3380, 3340 (NH$_2$), 3100 (CH Ar), 2924, 2825 (CH aliphatic), 1690 (C=O), 1620, 1600, 1590, 1506, 1514 (NH, C=C). MS (m/z) %: 510 (M$^+$+1) 0.01%. Anal. Calc. for C$_{36}$H$_{38}$N$_5$O$_7$ (510.54): C, 61.17; H, 5.92; N, 10.97. Found: C, 61.29; H, 5.97; N, 11.09.
pyrazole-1-carbothioamide (6h) Yield 68%. mp 135–138°C. 1H-NMR (CDCl3) δ: 2.69–2.96 (4H, m, morpholine H), 3.42–3.57 (4H, m, morpholine H), 3.65 (1H, dd, J=13.5, 7.2 Hz, CH2 pyrazoline), 3.88 (3H, s, OCH3), 3.95 (6H, s, 2×OCH3), 4.05 (1H, dd, J=15.3, 7.2 Hz, CH2 pyrazoline), 4.11 (2H, s, CH2), 4.61 (2H, s, NH2, exch. D2O), 4.82 (1H, t, CH pyrazoline), 6.80 (1H, d, J=7.8 Hz, H-5 Ar), 7.16 (1H, s, H-2 Ar), 7.68 (1H, d, J=7.8 Hz, H-6′ Ar), 7.85 (1H, d, J=2.0 Hz, H-2 furan), 13.14 (1H, s, OH, exch. D2O). IR (KBr) cm−1: 3423 (OH), 3315, 3250 (NH3), 3040 (CH aliphatic), 2926, 2852 (CH aliphatic), 1618, 1598, 1544, 1512 (NH, C=C), 1263 (C=S). MS (m/z): 526 (M+ 1) 0.15%. Anal. Calcd for C26H30N4O6S (526.60): C, 59.30; H, 5.74; N, 10.64. Found: C, 59.38; H, 5.78; N, 10.78.

4-Methoxy-5-[3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-3-yl]benzofuran-6-ol (7d) Yield 72%. mp 154–156°C. 1H-NMR (CDCl3) δ: 3.33 (1H, dd, J=17.1, 9.3 Hz, CH2 pyrazoline), 3.81 (1H, dd, J=15.3, 9.3 Hz, CH2 pyrazoline), 3.90 (9H, s, 3×OCH3), 4.04 (3H, s, OCH3), 4.80 (1H, t, CH pyrazoline), 6.65 (2H, s, H-2′′′′ Ar), 6.81 (1H, d, J=2.1 Hz, H-3 furan), 6.86 (1H, s, H-7 benzofuran), 7.43 (1H, d, J=2.1 Hz, H-2 furan), 11.60 (1H, s, NH, exch. D2O), 12.80 (1H, s, OH, exch. D2O). IR (KBr) cm−1: 3431 (OH), 3323 (NH), 3072 (CH Ar), 2939, 2831 (CH aliphatic), 1600, 1560, 1543, 1508 (NH, C=C). MS (m/z): 398 (M+ 1) 100%. Anal. Calcd for C27H32N4O7S (556.63): C, 63.31; H, 5.57; N, 7.03. Found: C, 63.37; H, 5.61; N, 7.17.

General Procedure for Synthesis of 4-Methoxy-5-[1-(morpholin-4-ylmethyl)-4,5-dihydro-1H-pyrazol-3-yl]benzofuran-6-ol (7a–e) To a solution of the appropriate compound 7a–e (10 mmol) in ethanol (20 mL), morpholine hydrochloride (1.36 g, 11 mmol) and paraformaldehyde (0.6 g, 20 mmol) were added. The mixture was refluxed for 24 h. Excess solvent was removed under vacuum then cooled and water was added. The mixture was neutralized with dilute ammonia and extracted with chloroform. Chloroform extract was dried over anhydrous sodium sulfate and the residue was crystallized from chloroform/methanol (Chart 1). TLC was prepared by mixing the Chloroform extract was dried over anhydrous sodium sulfate and the residue was crystallized from chloroform/methanol (9 : 1), the compounds were purified by preparative thin layer chromatography (TLC) (Chart 2).

Preparative TLC TLC was prepared by mixing the absorbent, silica gel, with a small amount of inert binder, calcium sulfate (gypsum) and water. This mixture is spread as thick slurry on an unreactive carrier sheet, glass (20 cm×20 cm). The resultant plate was dried and activated by heating in an oven for thirty minutes at 110°C. The compounds to be separated (about 0.5 g dissolved in chloroform) were applied to the plate as a thin even layer horizontally, 2 cm far from the bottom. When developed, the compounds separated in horizontal bands with yellow color (λmax 366, 254 nm by UV Vilber Lourmat 77202 were used to determine the compound bands). The lower band (Rf is 0.16–0.32) and the upper band (Rf is 0.82–0.95) were scraped of the baking material. The baking material was extracted with chloroform and filtered to give the isolated compound upon evaporating solvent. Lower bands yielded compounds 4a–e (with yield 60–70%) while upper bands yielded compounds 8a–e (with yield 30–40%).
4-Methoxy-5-[(1-(morpholin-4-ylmethyl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)benzofuran-6-ol (8a) Yield 32%. mp 157–159°C. 1H-NMR (CDCl3) δ: 3.20–3.44 (4H, m, morpholine H), 3.69–3.74 (4H, m, morpholine H), 3.90 (1H, dd, J=14.7, 6.3 Hz, CH2 pyrazoline), 3.98 (3H, s, OCH3), 4.09 (2H, s, CH2), 4.42 (1H, dd, J=15.0, 6.6 Hz, CH2 pyrazoline), 5.43 (1H, t, CH pyrazoline), 6.74 (1H, d, J=2.1 Hz, H-3 furan), 6.79 (1H, s, H-7 benzofuran), 7.29–7.65 (6H, m, H-2 furen, H-2',3',4',5',6' Ar), 11.60 (1H, s, OH, exch. D2O). IR (KBr) cm⁻¹: 3440 (OH), 3032 (CH Ar), 2932, 2848 (CH aliphatic), 1618, 1560, 1541, 1515 (C=C). MS (m/z) %: 499 (M+H) 94.6%. Anal. Calcd for C32H33N4O4 (567.57): C, 66.81; H, 5.90; N, 8.47. Found: C, 66.51; H, 5.88; N, 8.48.

In Vitro Vasodilatation Activity Screening The study was performed at the Pharmacology Department, National Research Centre, Dokki, Egypt, after approval from the Ethics committee of the centre and in accordance with the recommendations of the proper care and use of laboratory animals (NIH publication No. 85–23, revised 1985).

The vasodilatation activity screening procedures were carried out according to the standard reported techniques 46–48 by testing the effects of the synthesized compounds 3a–e, 4a–e, 5a–e, 6a–j and 8a–e on isolated thoracic aortic rings of male Wister rats (250–350 g). Aorta was cut in 3–5 mm long rings and placed in a vertical chamber “10 mL jacketed multi-chamber organ bath system (model no. ML780B6/C, Panlab, Spain)” filled with modified Krebse Henseleit solution composed of (in m M): NaCl, 118.0; KCl, 4.7; NaHCO3, 25.0; CaCl2, 1.8; NaHPO4, 1.2; MgSO4, 1.2; glucose, 11.0 and oxygenated with carbogen gas (95% O2/5% CO2) at 37±0.5°C.

Each aorta 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 fewer than 2 g resting tension during 2h. The lack of endothelium was confirmed by the absence of acetylcholine (1 µm) vasorelaxant action in aortic rings precontracted by noradrenalin (0.1 µm). The contractile response to norepinephrine hydrochloride (10⁻⁶m) was measured before and after exposure to increasing concentrations of the tested compounds. The tested compounds as well as prazosin (as reference standard) were dissolved in dimethyl sulfoxide (DMSO) as stock solution (10mL of 0.01M). 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 vasodilatation activity data are reported (Table 1, Fig. 2) and the potency (IC50 concentration necessary for 50% reduction of maximal norepinephrine hydrochloride induced contracture) was determined by the best fit line technique.

QSAR Computational Method All the computational works were performed on Molecular Operating Environment software (MOE version 2008.10.2). 49 The structures of 30 compounds used as training set were sketched using molecular builder of MOE and each structure was subjected to energy minimization up to 0.01 kcal/mol Å using the MMFF94x force field. Optimization methods were used followed by conformational search of each energy-minimized structure. The most stable conformer of each structure was selected and saved into the database to generate the common descriptors. QuaSAR descriptor module of MOE was used to calculate descriptors for each molecule. The probability density functions used are

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Gaussian. The RMSD tolerance was set to 0.5 Å. Regression analysis was performed using vasodilator IC₅₀ as dependent factor and the calculated descriptors as predictable variables.

In this study, the pool of descriptors was optimized utilizing principal components analysis (PCA). The optimization started with the reduction in the number of molecular descriptors by the determination of the highly inter-correlated descriptor pairs and only one from each pair was selected; then the descriptors with insignificant variance through the data set were also rejected. QSAR model was then constructed after ensuring reasonable correlation of vasodilator activity with the individual descriptors and minimum inter-correlation among the descriptors used in the derived model. The quality of the model was assessed using the statistical parameter \( r^2 \) and \( q^2 \).

**Molecular Descriptors** Log S: Log of the aqueous solubility (mol/L). This property was calculated from an atom contribution linear atom type model with \( r^2=0.90 \).

VSA: van der Waals surface area. A polyhedral representation was used for each atom in calculating the surface area. E_sol: Solvation energy. In the Potential Setup panel, the term enable parameter (Solvation menu) was ignored, but the term weight is applied.

gyr: Radius of gyration, Table 2.

**Validation and Cross-Validation of the Model** The log observed activities (log Obs. IC₅₀) together with the log predicted activities (log Pred. IC₅₀) for the tested compounds calculated using multi-linear regression (MLR) were listed in Table 3. All compounds showed very good results with Z-scores not exceeding the value of 2.5 indicating excellent predictive ability of the model. \(^3\)

The log observed IC₅₀ were plotted against their log predicted values (calculated by MLR method) with a value of \( r^2 \) found to be 0.9074, Fig. 3.

Cross-validation statistical technique was applied to estimate the quality with regard to predictive ability of the generated model. This is the most common validation technique, where a number of modified data sets were created by deleting, in each case, one or a smaller group of objects from the data in such a way that each object is taken away once and only once. For each reduced data set, the model was calculated, and responses for the deleted objects were predicted from the descriptors used in the derived model. The quality of the model was found to be 0.860459, Fig. 4.

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