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# Synthesis and structure–activity relationships of 6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide derivatives as a novel class of NCX inhibitors: a QSAR study

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**Abstract**—The sodium–calcium exchanger (NCX) transports $Na^+$ and $Ca^{2+}$ ions, and controls the $Ca^{2+}$ concentration in myocytes. Calcium overload is induced via activation of reverse NCX, and is responsible for reperfusion injury in heart failure. Hence, NCX is an attractive target for prevention and treatment of reperfusion arrhythmias, myocardial contracture, and necrosis. We have synthesized a series of 6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide derivatives, and evaluated their inhibitory activity against the reverse and forward modes of NCX. *N*-(3-Aminobenzyl)-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide (**8**) was shown to be a potent inhibitor of reverse NCX activity, with an $IC_{50}$ value of 0.24 μM. A QSAR study showed that inhibition of reverse NCX activity by 6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide derivatives is multiply dependent on the hydrophobicity ($\pi$) and the shape ($B_{1v}$) of the substituent at the 3-position of the phenyl ring.


## 1. Introduction

Ischaemia is a state of oxygen deficit in tissues that occurs due to shortage of blood flow. In the development of ischaemia, a decrease in ATP and initiation of acidosis induce cell necrosis. In the heart, myocardial infarction is caused by coronary occlusion, and reperfusion techniques, such as percutaneous transluminal coronary angioplasty (PTCA) and percutaneous transluminal coronary recanalization (PTCR), are performed to aid recovery from ischaemia in myocardial tissue. However, it has become apparent that reperfusion can cause other injuries.[1–3] Such injuries not only occur during reperfusion treatment itself, but also in cases of temporary ischaemia requiring perioperative reperfusion for blood vessel occlusion, and in organ transplantation. Reperfusion injuries are caused by calcium overload, which is in turn induced by dysbolism of ions. Hence, it is known that acidosis induced by anaerobic metabolism leads to enhanced exchange of intracellular $H^+$ for $Na^+$ via the sodium-hydrogen exchanger (NHE) during ischaemia.[4,5] $Na^+$ ions are then transported to the extracellular fluid and $Ca^{2+}$ is taken up intracellularly via the sodium–calcium exchanger (NCX). This leads to calcium overload in cardiac myocytes, and such overload is responsible for contractile dysfunction and arrhythmia.[6–14] NCX typically functions in a forward mode, and inhibition of this activity of NCX may have potential antiarrhythmic effects in pathophysiological states such as heart failure or myocardial ischaemia and reperfusion. However, NCX can also function in a reverse mode, which has a more important role in induction of calcium overload. Consequently, selective inhibition of reverse NCX activity may provide a novel therapeutic approach to the prevention and treatment of reperfusion arrhythmias, myocardial contracture, and necrosis. Indeed, reverse mode NCX inhibitors are currently considered to be beneficial in treating these disease states.[15,16]

Recently, a quinazoline derivative (**1**)[17] and several benzyloxyphenyl derivatives (**2**–**4**)[18–20] have been reported as reverse mode NCX inhibitors (Fig. 1). However, the

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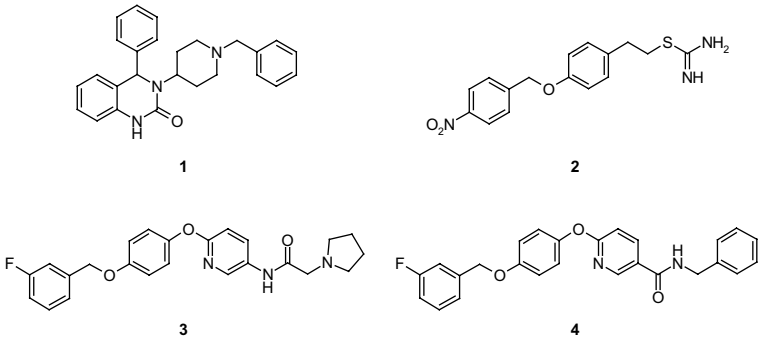


**Figure 1.** Several inhibitors of sodium–calcium exchanger. (**1**) SM-15811; (**2**) KB-R7943; (**3**) patented compound of JP11092454; (**4**) nicotinamide derivative.

SAR of the benzyloxyphenyl derivatives has not been studied in detail. In a previous paper, we reported a partial SAR for a series of 6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide derivatives with reverse NCX inhibitory activity.[20] *N*-Benzyl-6-{4-[(3-fluorobenzyl)-oxy]phenoxy}nicotinamide (**4**) was found to be a highly potent and selective reverse NCX inhibitor, compared to KB-R7943 (**2**) and compound **3**. In this paper, we describe the synthesis, biological activities and SAR of the nicotinamide derivatives, and use a traditional quantitative structure–activity relationship (QSAR) study for discovery of more potent reverse NCX inhibitors.

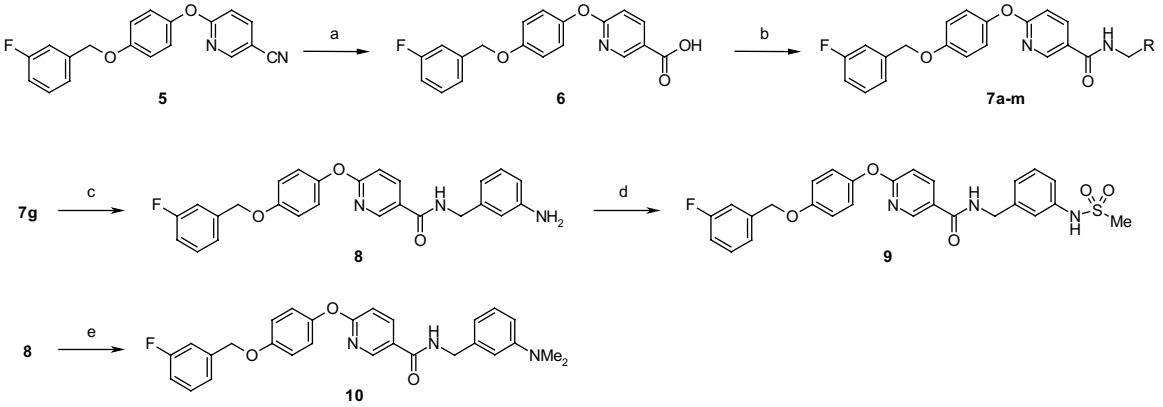
## 2. Chemistry

The synthesis of the novel 6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide derivatives **7a**–**m** and **8**–**10** is summarized in Scheme 1. Starting material **5**[20] was converted into compound **6** by hydrolysis of the cyano group in high yield (99%). Compounds **7a**–**m** were prepared from compound **6** using amidation reactions. Compound **7g** was reduced by hydrogenation to give compound **8** in 52% isolated yield. This compound was transformed to the sulfonyl amide derivative **9** using methanesulfonyl chloride in 78% isolated yield. Compound **10** was afforded from **8** by reductive alkylation with formaldehyde and sodium-borohydride in 17% isolated yield.

## 3. Results and discussion

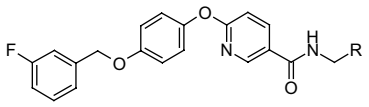
To measure the inhibitory effect of the synthesized compounds on reverse mode NCX activity, a $Na^+$-dependent $Ca^{2+}$ influx assay was performed according to reported protocols, using $^{45}Ca$ and CCL39 cells that stably express NCX1.1.[18,20] The inhibitory effect on forward mode NCX activity was assayed in a cell necrosis assay, in which NCX1.1-expressing CCL39 cells were also used.[20,21] The inhibitory potencies of our novel compounds were thus evaluated in both reverse mode and forward mode NCX assays. These compounds were then compared to KB-R7943 (**2**) and compounds **3** and **4**. In this paper, the substituent on the benzyl moiety connected to the nicotinamide moiety of compound **4** was optimized to create novel reverse mode NCX inhibitors. We also performed a traditional QSAR study based on the results.

The structure–activity relationships of our novel series of NCX inhibitors are summarized in Table 1. The phenyl moiety of compound **4** was optimized by introduction of various substituents into the phenyl ring. Introduction of methyl groups at the 2-, 3-, and 4-positions was performed to investigate which substituent position is most appropriate (**7a**–**c**). Among these molecules, **7b** (substituted at the 3-position) displayed the most potent inhibitory activity against reverse NCX. This result prompted us to introduce other substituents



**Scheme 1.** Reagents and conditions: (a) 5 M NaOH, EtOH, reflux; (b) WSC·HCl, HOBt, THF, $RCH_2NH_2$; (c) Pd–C, $H_2$, EtOH; (d) $MeSO_2Cl$, pyridine; (e) $NaBH(OAc)_3$, formaldehyde, AcOH, THF.

**Table 1.** Inhibitory activities of nicotinamide derivatives against the sodium–calcium exchanger



| Compd | R | $^{45}$Ca influx[a] $IC_{50}$ (μM)[c] | Cell necrosis[b] $EC_{50}$ (μM)[c] | Selectivity[d] |
|---|---|---|---|---|
| **7a** | 2-Me–$C_6H_4$ | 5.2 | >100 | >19 |
| **7b** | 3-Me–$C_6H_4$ | 1.2 | >100 | >83 |
| **7c** | 4-Me–$C_6H_4$ | 11 | >100 | >9.0 |
| **7d** | 3-Br–$C_6H_4$ | 8.3 | NT[e] | — |
| **7e** | 3-Cl–$C_6H_4$ | 3.5 | >100 | >28 |
| **7f** | 3-F–$C_6H_4$ | 0.79 | 72 | 91 |
| **7g** | 3-$NO_2$–$C_6H_4$ | 1.3 | 18 | 14 |
| **7h** | 3-CN–$C_6H_4$ | 0.79 | 56 | 71 |
| **7i** | 3-$CF_3$–$C_6H_4$ | 15 | NT[e] | — |
| **7j** | 3-$CO_2$Et–$C_6H_4$ | 160 | NT[e] | — |
| **7k** | 3-OH–$C_6H_4$ | 0.63 | 21 | 33 |
| **7l** | 3-MeO–$C_6H_4$ | 2.5 | >40 | >16 |
| **9** | 3-$MeSO_2NH$–$C_6H_4$ | 3.9 | NT[e] | — |
| **8** | 3-$NH_2$–$C_6H_4$ | 0.24 | 92 | 380 |
| **7m** | 3-MeNH–$C_6H_4$ | 2.4 | >100 | >41 |
| **10** | 3-$Me_2N$–$C_6H_4$ | 3.0 | >100 | >33 |
| **4** | $C_6H_5$ | 0.79 | >100 | >120 |
| KB-R7943 (**2**) | | 5.1 | 24 | 4.7 |
| **3** | | 0.94 | 34 | 36 |

[a] Activity at the NCX1.1 expressed in CCL39 cells. $^{45}$Ca influx mean NCX inhibitory activity for reverse mode.
[b] Activity at the NCX1.1 expressed in CCL39 cells. Cell necrosis mean NCX inhibitory activity for forward mode.
[c] $IC_{50}$ values and $EC_{50}$ values were determined in a single experimental run in triplicate.
[d] Ratio of $EC_{50}$ value of Cell necrosis and $IC_{50}$ value of $^{45}$Ca influx.
[e] Not tested.

at the 3-positon of the phenyl ring. Introduction of halogen substituents (bromo (**7d**), chloro (**7e**), and fluoro (**7f**)) resulted in one compound, **7f**, that had similar inhibition of reverse NCX activity to that of compound **4**. Nitro (**7g**) and cyano (**7h**) substituents were introduced as representative strong electron-withdrawing groups, and compounds **7g** and **7h** had $IC_{50}$ values of 1.3 and 0.79 μM, respectively. However, compounds with $CF_3$ (**7i**) and $CO_2$Et (**7j**) groups were detrimental to reverse NCX activity. Compounds with hydroxyl (**7k**) and methoxy (**7l**) substituents, which were chosen as typical electron-donating groups, had $IC_{50}$ values of 0.63 and 2.5 μM, respectively. A $MeSO_2NH$ substituent (**9**) was unfavorable, but compound (**8**) with amino group, chosen as representatives of basic substituent, had an $IC_{50}$ value of 0.24 μM.

From the above results, we found that compound **8** had the most potent inhibitory activity against reverse NCX function, and this compound also showed high selectivity for the reverse versus the forward mode. On the other hand, the results show no relationship between potency and the electronic properties of substituents at the 3-position. A QSAR study is basically concerned with correlation of structure with property/activity. Several physicochemical descriptors, such as hydrophobicity, topology, electronic parameters, and steric effects, are usually used in QSAR studies in many disciplines, with many pertaining to drug design. Consequently, we performed correlation analysis using Hansch substituent parameters: $\pi$ (hydrophobicity), MR (steric bulk), $\sigma_m$ (electronic property), and Verloop parameters: $L$ (length), $B_i$–$B_{iv}$ (shape of each substituent)[22,23] as shown in Table 2. A correlation of substituent constants with $pIC_{50}$ ($-\log IC_{50}$) is shown in Table 3. From the correlation study it was found that $\pi$, MR, $L$, $B_i$, and $B_{iv}$ have individual correlation to reverse NCX inhibitory activity ($pIC_{50}$). Multicollinearities may cause difficulties in certain aspects of forming a QSAR model, and if a model contains multicollinearities, it is generally discarded. As MR, $L$, $B_i$, $B_{iii}$, and $B_{iv}$ were highly multicollinear with each other we tried to use separately MR, $L$, $B_i$, $B_{iii}$, and $B_{iv}$ with other independent parameters to get statistically good QSAR models. We performed multiple linear regression analysis using more standard parameters ($\pi$ and MR). When $\pi$ and MR were used, Eq. 1 was obtained given below:[24]

$$\begin{aligned} pIC_{50} &= -0.624(\pm 0.300)\pi - 0.085(\pm 0.038)MR \\ &\quad + 6.268(\pm 0.360) \\ n &= 15, \quad r = 0.879, \quad r^2 = 0.773, \\ s &= 0.352, \quad F = 20.4 \end{aligned} \tag{1}$$

Eq. 1 shows a good correlation coefficient ($r = 0.879$), and explains 77.3% of the variance in reverse NCX inhibitory activity data. Although this suggests that reverse NCX inhibitory activity seems to be related to hydrophobic and steric characters of the substituents, the explained variance ($r^2 = 0.773$) of Eq. 1 do not give an enough explanation for the relationship between

**Table 2.** Substituent parameters and $pIC_{50}$

| Compd | Substituent | $\pi$ | MR | $\delta_m$ | $L$ | $B_i$ | $B_{ii}$ | $B_{iii}$ | $B_{iv}$ | $pIC_{50}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| **7b** | Me | 0.56 | 5.65 | −0.07 | 3.00 | 1.52 | 2.04 | 1.90 | 1.90 | 5.92 |
| **7d** | Br | 0.86 | 8.88 | 0.39 | 3.83 | 1.95 | 1.95 | 1.95 | 1.95 | 5.08 |
| **7e** | Cl | 0.71 | 6.03 | 0.37 | 3.52 | 1.80 | 1.80 | 1.80 | 1.80 | 5.46 |
| **7f** | F | 0.14 | 0.92 | 0.34 | 2.65 | 1.35 | 1.35 | 1.35 | 1.35 | 6.10 |
| **7g** | $NO_2$ | −0.28 | 7.36 | 0.71 | 3.44 | 1.70 | 1.70 | 2.44 | 2.44 | 5.89 |
| **7h** | CN | −0.57 | 6.33 | 0.56 | 4.23 | 1.60 | 1.60 | 1.60 | 1.60 | 6.10 |
| **7i** | $CF_3$ | 0.88 | 5.02 | 0.43 | 3.30 | 1.98 | 2.61 | 2.44 | 2.44 | 4.82 |
| **7j** | $CO_2Et$ | 0.51 | 17.47 | 0.37 | 5.96 | 1.90 | 1.90 | 2.36 | 4.29 | 3.80 |
| **7k** | OH | −0.67 | 2.85 | 0.12 | 2.74 | 1.35 | 1.93 | 1.35 | 1.35 | 6.20 |
| **7l** | MeO | −0.02 | 7.87 | 0.12 | 3.98 | 1.35 | 2.87 | 1.90 | 1.90 | 5.60 |
| **9** | $MeSO_2NH$ | −1.18 | 18.17 | 0.20 | 4.06 | 1.50 | 1.90 | 3.59 | 3.88 | 5.41 |
| **8** | $NH_2$ | −1.23 | 5.42 | −0.16 | 2.93 | 1.50 | 1.50 | 1.84 | 1.84 | 6.62 |
| **7m** | MeNH | −0.47 | 10.33 | −0.30 | 3.53 | 1.50 | 3.08 | 1.90 | 1.90 | 5.62 |
| **10** | $Me_2N$ | 0.18 | 15.55 | −0.15 | 3.53 | 1.50 | 2.56 | 2.80 | 2.80 | 5.52 |
| **4** | H | 0.00 | 1.03 | 0.00 | 2.06 | 1.00 | 1.00 | 1.00 | 1.00 | 6.10 |

**Table 3.** Correlation matrix for the correlation of substituent parameters and their correlation with $pIC_{50}$

| | $pIC_{50}$ | $\pi$ | MR | $\delta_m$ | $L$ | $B_i$ | $B_{ii}$ | $B_{iii}$ | $B_{iv}$ |
|---|---|---|---|---|---|---|---|---|---|
| $pIC_{50}$ | 1 | | | | | | | | |
| $\pi$ | −0.566 | 1 | | | | | | | |
| MR | −0.618 | −0.092 | 1 | | | | | | |
| $\delta_m$ | −0.286 | 0.272 | −0.072 | 1 | | | | | |
| $L$ | −0.767 | 0.130 | 0.760 | 0.323 | 1 | | | | |
| $B_i$ | −0.667 | 0.489 | 0.360 | 0.514 | 0.591 | 1 | | | |
| $B_{ii}$ | −0.345 | 0.162 | 0.396 | −0.317 | 0.287 | 0.264 | 1 | | |
| $B_{iii}$ | −0.455 | −0.113 | 0.830 | 0.086 | 0.490 | 0.425 | 0.377 | 1 | |
| $B_{iv}$ | −0.735 | 0.003 | 0.900 | 0.152 | 0.776 | 0.487 | 0.241 | 0.847 | 1 |

the activity data and parameters. $L$ and $B_i$–$B_{iv}$ are similar to MR with respect to showing substituent structural character. In the further stepwise multiple linear regression analysis, $L$ and $B_i$–$B_{iv}$ were used instead of MR. Eqs. 2 and 3[24] were obtained when $\pi$, $L$, and $B_{iv}$ were taken as variables to get QSAR models. Eq. 3 is the best model giving higher correlation coefficient ($r = 0.926$).

$$pIC_{50} = -0.471(\pm 0.276)\pi - 0.538(\pm 0.211)L + 7.490(\pm 0.766)$$
$$n = 15, \quad r = 0.900, \quad r^2 = 0.809,$$
$$s = 0.322, \quad F = 25.5 \qquad (2)$$

$$pIC_{50} = -0.560(\pm 0.236)\pi - 0.552(\pm 0.179)B_{iv} + 6.788(\pm 0.418)$$
$$n = 15, \quad r = 0.926, \quad r^2 = 0.857,$$
$$s = 0.279, \quad F = 36.0 \qquad (3)$$

Eq. 3 explains 85.7% of the variance in reverse NCX inhibitory activity data. A graph depicting the observed versus calculated activities of the molecules is shown in Figure 2 ($r = 0.926$). The result indicates that the activity is related to the hydrophobicity and shape of phenyl substituent at position 3.

## 4. Conclusion

A series of nicotinamide derivatives were prepared and evaluated for their inhibition of reverse mode NCX activity and for their selectivity versus forward mode

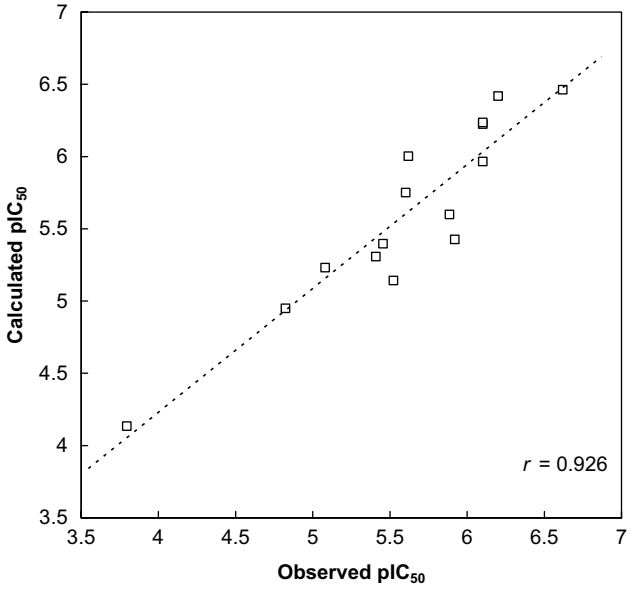



**Figure 2.** Observed versus calculated inhibitory activities against reverse NCX ($pIC_{50}$ values), for **4**, **7b**, **7d**–**m**, **8**–**10** using Eq. 3.

NCX activity. Compound **8** was found to be a potent inhibitor of reverse NCX activity, with an $IC_{50}$ value of 0.24 μM. In a QSAR study, inhibition of reverse NCX activity was found to be dependent on the nature of the substituent at the 3-position of the phenyl ring adjacent to the nicotinamide, with parameters such as $\pi$ and $B_{iv}$ being of importance. Such SAR-based methodology may be useful for the rapid optimization of lead candidates, and we believe that this study provides a novel approach to discovery of potent inhibitors for reverse NCX.

## 5. Experimental

### 5.1. Chemistry

Melting points were determined with a Yanaco MP-500D melting point apparatus or a Büchi B-545 melting point apparatus and are uncorrected. $^1$H NMR spectra were recorded on a JEOL JNM-LA300 or a JNM-EX400 spectrometer and the chemical shifts are expressed in $\delta$ (ppm) values with tetramethylsilane as an internal standard (in NMR description, s = singlet, d = doublet, t = triplet, m = multiplet, and br = broad peak). Mass spectra were recorded on a Hitachi M-80 or a JEOL JMS-LX2000 spectrometer. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and were within ±0.4% of theoretical values. Drying of organic solutions during workup was done over anhydrous $Na_2SO_4$.

**5.1.1. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}nicotinic acid (6).** The mixture of **5** (12.0 g, 37.5 mmol), EtOH (80 mL), and 5 M NaOH (75 mL, 375 mmol) was stirred at 100 °C for 1.5 h. After cooling at room temperature, the mixture was concentrated in vacuo. To the mixture was added 1 M HCl at 0 °C. The precipitate was filtered and dried in vacuo to afford **6** as a beige powder (12.6 g, 99%): $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 5.15 (2H, s), 7.04–7.20 (6H, m), 7.27–7.33 (2H, m), 7.42–7.49 (1H, m), 8.26 (1H, dd, $J$ = 8.6, 2.4 Hz), 8.65 (1H, d, $J$ = 2.4 Hz), 13.16 (1H, br s); MS (FAB) $m/z$ 340 $(M+H)^+$.

**5.1.2. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(2-methylbenzyl)nicotinamide (7a).** To the mixture of HOBt (57 mg, 0.42 mmol), WSC·HCl (177 mg, 0.92 mmol), and THF (4 mL), (3-methylbenzyl)amine (0.11 mL, 0.88 mmol) was added **6** (285 mg, 0.84 mmol) at room temperature. The mixture was stirred at room temperature for 2.5 h. The mixture was partitioned between $CHCl_3$ and aqueous HCl. The organic layer was washed with aqueous NaOH, dried, and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 1:0–99:1) to give pale yellow oil. The material was recrystallized from hexane–AcOEt to afford **7a** as a white powder (258 mg, 69%): mp 116–118 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.31 (3H, s), 4.46 (2H, d, $J$ = 5.8 Hz), 5.15 (2H, s), 7.03–7.21 (9H, m), 7.21–7.26 (1H, m), 7.28–7.34 (2H, m), 7.42–7.49 (1H, m), 8.28 (1H, dd, $J$ = 8.3, 2.4 Hz), 8.63 (1H, d, $J$ = 2.4 Hz), 8.92–8.97 (1H, m); MS (FAB) $m/z$ 443 $(M+H)^+$. Anal. Calcd for $C_{27}H_{23}N_2O_3F$: C, 73.29; H, 5.24; N, 6.32; F, 4.19. Found: C, 73.39; H, 5.19; N, 6.33; F, 4.07.

**5.1.3. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(3-methylbenzyl)nicotinamide (7b).** Compound **7b** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7b** was obtained as a white powder (75%): mp 108–110 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.28 (3H, s), 4.44 (2H, d, $J$ = 5.8 Hz), 5.15 (2H, s), 7.03–7.13 (8H, m), 7.14–7.23 (2H, m), 7.28–7.33 (2H, m), 7.42–7.49 (1H, m), 8.27 (1H, dd, $J$ = 8.8, 2.5 Hz), 8.63 (1H, d, $J$ = 2.4 Hz), 9.05 (1H, t, $J$ = 5.8 Hz); MS (FAB) $m/z$ 443 $(M+H)^+$. Anal. Calcd for $C_{27}H_{23}N_2O_3F$: C, 73.29; H, 5.24; N, 6.32; F, 4.19. Found: C, 73.42; H, 5.30; N, 6.34; F, 4.16.

**5.1.4. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(4-methylbenzyl)nicotinamide (7c).** Compound **7c** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7c** was obtained as a white powder (71%): mp 117–119 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.27 (3H, s), 4.43 (2H, d, $J$ = 5.8 Hz), 5.15 (2H, s), 7.03–7.16 (8H, m), 7.17–7.22 (2H, m), 7.28–7.34 (2H, m), 7.42–7.48 (1H, m), 8.26 (1H, dd, $J$ = 8.8, 2.4 Hz), 8.62 (1H, d, $J$ = 2.5 Hz), 9.04 (1H, t, $J$ = 5.8 Hz); MS (FAB) $m/z$ 443 $(M+H)^+$. Anal. Calcd for $C_{27}H_{23}N_2O_3F$: C, 73.29; H, 5.24; N, 6.32; F, 4.19. Found: C, 73.31; H, 5.25; N, 6.28; F, 4.16.

**5.1.5. *N*-(3-Bromobenzyl)-6-{4-[(3-fluorobenzyl)oxy]-phenoxy}nicotinamide (7d).** Compound **7d** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7d** was obtained as a white powder (39%): mp 137–139 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.47 (2H, d, $J$ = 5.9 Hz), 5.15 (2H, s), 7.03–7.20 (6H, m), 7.27–7.35 (4H, m), 7.42–7.52 (3H, m), 8.26 (1H, dd, $J$ = 8.8, 2.5 Hz), 8.63 (1H, t, $J$ = 2.0 Hz), 9.13 (1H, t, $J$ = 5.9 Hz); MS (GC) $m/z$ 507, 509 $(M+H)^+$. Anal. Calcd for $C_{26}H_{20}N_2O_3FBr$·HBr: C, 53.08; H, 3.60; N, 4.76; F, 3.23; Br, 27.17. Found: C, 52.73; H, 3.47; N, 4.73; F, 3.32; Br, 27.36.

**5.1.6. *N*-(3-Chlorobenzyl)-6-{4-[(3-fluorobenzyl)oxy]-phenoxy}nicotinamide (7e).** Compound **7e** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7e** was obtained as a white powder (70%): mp 88–90 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.48 (2H, d, $J$ = 5.9 Hz), 5.15 (2H, s), 7.03–7.14 (5H, m), 7.14–7.20 (1H, m), 7.26–7.39 (6H, m), 7.42–7.49 (1H, m), 8.27 (1H, dd, $J$ = 8.8, 2.5 Hz), 8.63 (1H, d, $J$ = 2.5 Hz), 9.12 (1H, t, $J$ = 5.9 Hz); MS (FAB) $m/z$ 463 $(M+H)^+$. Anal. Calcd for $C_{26}H_{20}N_2O_3ClF$: C, 67.46; H, 4.35; N, 6.05; F, 4.10; Cl, 7.66. Found: C, 67.40; H, 4.37; N, 6.09; F, 3.93; Cl, 7.61.

**5.1.7. *N*-(3-Fluorobenzyl)-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide (7f).** Compound **7f** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7f** was obtained as a white powder (79%): mp 107–109 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.50 (2H, d, $J$ = 5.8 Hz), 5.15 (2H, s), 7.04–7.20 (9H, m), 7.28–7.34 (2H, m), 7.34–7.41 (1H, m), 7.42–7.49 (1H,

m), 8.27 (1H, dd, $J = 8.8$, 2.4 Hz), 8.64 (1H, d, $J = 2.4$ Hz), 9.12 (1H, t, $J = 5.8$ Hz); MS (FAB) *m/z* 447 $(M+H)^+$. Anal. Calcd for $C_{26}H_{20}N_2O_3F$: C, 69.95; H, 4.52; N, 6.27; F, 8.51. Found: C, 69.84; H, 4.54; N, 6.25; F, 8.66.

**5.1.8. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(3-nitrobenzyl)nicotinamide (7g).** Compound **7g** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7g** was obtained as a white powder (93%): mp 79–81 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.61 (2H, d, $J = 5.9$ Hz), 5.15 (2H, s), 7.05–7.14 (5H, m), 7.14–7.20 (1H, m), 7.28–7.34 (2H, m), 7.42–7.49 (1H, m), 7.64 (1H, t, $J = 7.8$ Hz), 7.80 (1H, d, $J = 7.8$ Hz), 8.10–8.14 (1H, m), 8.17–8.20 (1H, m), 8.28 (1H, dd, $J = 8.3$, 2.5 Hz), 8.65 (1H, d, $J = 2.4$ Hz), 9.24 (1H, t, $J = 5.9$ Hz); MS (FAB) *m/z* 474 $(M+H)^+$. Anal. Calcd for $C_{26}H_{20}N_3O_5F$: C, 65.69; H, 4.26; N, 8.88; F, 4.01. Found: C, 65.97; H, 4.21; N, 8.68; F, 4.10.

**5.1.9. *N*-(3-Cyanobenzyl)-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide hydrochloride (7h).** Compound **7h** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7h** was obtained as white powder (16%): mp 101–109 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.53 (2H, d, $J = 5.9$ Hz), 5.15 (2H, s), 7.04–7.13 (5H, m), 7.14–7.21 (1H, m), 7.28–7.33 (2H, m), 7.43–7.49 (1H, m), 7.53–7.58 (1H, m), 7.65–7.69 (1H, m), 7.72–7.75 (1H, m), 7.71 (1H, s), 8.28 (1H, dd, $J = 8.8$, 2.5 Hz), 8.65 (1H, d, $J = 2.5$ Hz), 9.19 (1H, t, $J = 5.9$ Hz); MS (FAB) *m/z* 454 $(M+H)^+$. Anal. Calcd for $C_{27}H_{20}N_3O_3F{\cdot}0.5HCl{\cdot}0.1H_2O$: C, 68.49; H, 4.41; N, 8.87; F, 4.01; Cl, 3.74. Found: C, 68.34; H, 4.38; N, 8.88; F, 3.86; Cl, 3.55.

**5.1.10. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-[3-(trifluoromethyl)benzyl]nicotinamide hydrochloride (7i).** Compound **7i** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7i** was obtained as a beige amorphous (26%): $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.57 (2H, d, $J = 5.9$ Hz), 5.15 (2H, s), 7.04–7.13 (5H, m), 7.13–7.20 (1H, m), 7.28–7.33 (2H, m), 7.43–7.49 (1H, m), 7.55–7.68 (4H, m), 8.27 (1H, dd, $J = 8.8$, 2.4 Hz), 8.64 (1H, d, $J = 2.4$ Hz), 9.19 (1H, t, $J = 5.8$ Hz); MS (FAB) *m/z* 497 $(M+H)^+$; HRMS: $(M+H)^+$Calcd for $C_{27}H_{21}O_3N_2F_4$, 497.1488. Found: 497.1470.

**5.1.11. Ethyl 3-({[(6-{4-[(3-fluorobenzyl)oxy]phenoxy}-pyridin-3-yl)carbonyl]amino}methyl)benzoate hydrobromide (7j).** Compound **7j** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7j** was obtained as a beige powder (83%): mp 150–156 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 1.31 (3H, t, $J = 7.2$ Hz), 4.31 (2H, q, $J = 7.3$ Hz), 4.54 (2H, d, $J = 5.8$ Hz), 5.15 (2H, s), 7.04–7.14 (5H, m), 7.14–7.21 (1H, m), 7.28–7.34 (2H, m), 7.43–7.51 (2H, m), 7.58–7.62 (1H, m), 7.84 (1H, d, $J = 8.8$ Hz), 7.92 (1H, s), 8.27 (1H, dd, $J = 8.8$, 2.5 Hz), 8.64 (1H, d, $J = 2.4$ Hz), 9.18 (1H, t, $J = 6.9$ Hz); MS (FAB) *m/z* 501 $(M+H)^+$. Anal. Calcd for $C_{29}H_{25}N_2O_5F{\cdot}HBr$: C, 59.91; H, 4.51; N, 4.82; F, 3.27; Br, 13.74. Found: C, 59.69; H, 4.56; N, 4.77; F, 3.32; Br, 13.70.

**5.1.12. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(3-hydroxybenzyl)nicotinamide (7k).** Compound **7k** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7k** was obtained as a white powder (36%): mp 173–175 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.46 (2H, d, $J = 5.8$ Hz), 5.15 (2H, s), 6.60–6.64 (1H, m), 6.70–6.74 (2H, m), 7.03–7.13 (6H, m), 7.14–7.20 (1H, m), 7.28–7.33 (2H, m), 7.42–7.49 (1H, m), 8.27 (1H, dd, $J = 8.8$, 2.4 Hz), 8.64 (1H, d, $J = 2.4$ Hz), 9.04 (1H, t, $J = 5.9$ Hz), 9.31 (1H, s); MS (FAB) *m/z* 445 $(M+H)^+$. Anal. Calcd for $C_{26}H_{21}N_2O_4F$: C, 70.26; H, 4.76; N, 6.30; F, 4.27. Found: C, 70.17; H, 4.85; N, 6.22; F, 4.44.

**5.1.13. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-(3-methoxybenzyl)nicotinamide (7l).** Compound **7l** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7l** was obtained as a white powder (81%): mp 125–127 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.73 (3H, s), 4.45 (2H, d, $J = 5.8$ Hz), 5.15 (2H, s), 6.79–6.84 (1H, m), 6.86–6.90 (2H, m), 7.03–7.13 (5H, m), 7.14–7.21 (1H, m), 7.21–7.27 (1H, m), 7.28–7.34 (2H, m), 7.42–7.49 (1H, m), 8.27 (1H, dd, $J = 8.8$, 2.5 Hz), 8.63 (1H, d, $J = 2.4$ Hz), 9.06 (1H, t, $J = 5.8$ Hz); MS (FAB) *m/z* 459 $(M+H)^+$. Anal. Calcd for $C_{27}H_{23}N_2O_4F$: C, 70.73; H, 5.06; N, 6.11; F, 4.14. Found: C, 69.82; H, 4.99; N, 6.06; F, 4.03.

**5.1.14. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-[3-(methylamino)benzyl]nicotinamide (7m).** Compound **7m** was prepared from **6** by a procedure similar to that described for **7a**. Compound **7m** was obtained as a white powder (80%): mp 162–166 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.92 (3H, s), 4.57 (2H, d, $J = 5.3$ Hz), 5.15 (2H, s), 7.05–7.13 (5H, m), 7.14–7.21 (1H, m), 7.28–7.40 (5H, m), 7.43–7.51 (2H, m), 8.29 (1H, dd, $J = 8.8$, 2.5 Hz), 8.66 (1H, d, $J = 2.5$ Hz), 9.21 (1H, t, $J = 5.9$ Hz); MS (FAB) *m/z* 458 $(M+H)^+$. Anal. Calcd for $C_{27}H_{24}N_3O_3F{\cdot}2HBr{\cdot}0.2H_2O$: C, 52.06; H, 4.27; N, 6.75; F, 3.05; Br, 25.65. Found: C, 51.91; H, 4.16; N, 6.74; F, 2.96; Br, 25.62.

**5.1.15. *N*-(3-Aminobenzyl)-6-{4-[(3-fluorobenzyl)oxy]-phenoxy}nicotinamide hydrochloride (8).** The mixture of **7g** (450 mg, 0.95 mmol), EtOH (6 mL), Fe powder (265 mg, 4.75 mmol), and $NH_4Cl$ (153 mg, 2.85 mmol), $H_2O$ (1 mL) was stirred at 100 °C for 90 min. The mixture was filtered through a Celite pad. The filtrate was concentrated in vacuo. The residue was partitioned between $CH_3Cl$ and aqueous NaOH. The organic layer was dried and concentrated. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 1:0–92:8) to give the free base of **8** as a pale yellow solid. This material was converted to its hydrochloride salt by treating it with HCl/AcOEt in AcOEt–MeOH. The mixture was concentrated in vacuo. The residue was recrystallized from AcOEt–EtOH–hexane to give **8** as a gray powder (237 mg, 52%): mp 156–158 °C; $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 4.50 (2H, d, $J$ =5.8 Hz), 5.15 (2H, s), 7.03–7.13 (5H, m), 7.14–7.23 (2H, m), 7.24–7.34 (4H, m), 7.38–7.49 (2H, m), 8.30 (1H, dd, $J = 8.8$, 2.5 Hz), 8.67 (1H, d, $J = 2.5$ Hz), 9.28 (1H, t, $J = 5.8$ Hz), 10.02 (2H, br s); MS (FAB) *m/z*

444 $(M+H)^+$. Anal. Calcd for $C_{26}H_{22}N_3O_3F{\cdot}HCl{\cdot}0.2H_2O$: C, 64.58; H, 4.88; N, 8.68; F, 3.93; Cl, 7.33. Found: C, 64.53; H, 5.05; N, 8.58; F, 3.95; Cl, 7.17.

**5.1.16. 6-{4-[(3-Fluorobenzyl)oxy]phenoxy}-*N*-{3-[(methylsulfonyl)amino]benzyl}nicotinamide hydrobromide (9).** To the mixture of pyridine (5 mL), *N*-(3-aminobenzyl)-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide (443 mg, 1.0 mmol) was added methanesulfonyl chloride (0.077 mL, 1.0 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min. The mixture was concentrated in vacuo. The residue was partitioned between AcOEt and $H_2O$. The organic layer was washed with aqueous NaOH and aqueous HCl, and then dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 98:2–96:4) to give the free base of **9** as a light yellow foam. This material was converted to its hydrobromide salt by treating it with aqueous HBr in acetone. The mixture was concentrated in vacuo. The residue was recrystallized from AcOEt–$CH_3CN$ to give **9** as a beige powder (467 mg, 78%): mp 135–139 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 2.97 (3H, s), 4.45 (2H, d, $J = 5.8$ Hz), 5.15 (2H, s), 7.03–7.20 (9H, m), 7.26–7.34 (3H, m), 7.42–7.48 (1H, m), 8.26 (1H, dd, $J = 8.3$, 2.4 Hz), 8.63 (1H, d, $J = 2.5$ Hz), 9.11 (1H, t, $J = 5.9$ Hz), 9.72 (1H, s); MS (FAB) *m/z* 522 $(M+H)^+$. Anal. Calcd for $C_{27}H_{24}N_3O_5SF{\cdot}HBr$: C, 53.83; H, 4.18; N, 6.97; S, 5.32; Br, 13.26; F, 3.15. Found: C, 53.86; H, 4.04; N, 6.99; S, 5.27; Br, 13.29; F, 3.06.

**5.1.17. *N*-[3-(Dimethylamino)benzyl]-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide hydrobromide (10).** To the mixture of 35% HCHO in $H_2O$ (429 mg, 5.0 mmol), THF (5 mL), and *N*-(3-aminobenzyl)-6-{4-[(3-fluorobenzyl)oxy]phenoxy}nicotinamide (443 mg, 1.0 mmol) were added AcOH (0.286 mL, 5.0 mmol) and $NaBH(OAc)_3$ at 0 °C. The mixture was stirred at room temperature for 6 h. To the mixture was added 1 M NaOH at 0 °C. The mixture was partitioned between $CHCl_3$ and $H_2O$. The organic layer was dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 99:1–98:2) to give the free base of **10** as a light yellow syrup. This material was converted to its hydrobromide salt by treating it with aqueous HBr in acetone. The mixture was concentrated in vacuo. The residue was recrystallized from AcOEt–$CH_3CN$–MeOH to give **10** as a beige powder (108 mg, 17%): mp 141–146 °C; $^1H$ NMR (400 MHz, DMSO-$d_6$) $\delta$ 3.11 (6H, s), 4.51 (2H, d, $J = 5.4$ Hz), 5.15 (2H, s), 7.04–7.20 (7H, m), 7.28–7.34 (3H, m), 7.35–7.49 (3H, m), 8.28 (1H, dd, $J = 8.8$, 2.4 Hz), 8.65 (1H, d, $J = 2.5$ Hz), 9.11–9.19 (1H, br s); MS (FAB) *m/z* 472 $(M+H)^+$. Anal. Calcd for $C_{28}H_{26}N_3O_3F{\cdot}2HBr$: C, 53.10; H, 4.46; N, 6.63. Found: C, 52.86; H, 4.25; N, 6.57.

## 5.2. QSAR

In the present study liner mathematical methods are developed to study quantitative structure–activity relationships (QSAR). Multiple regression analysis calculations were performed with an Excel (Microsoft Co., version 2003). Multicollinear parameters were not used to get statistically certain QSAR models. All possible combinations of parameters were considered for multiple regression analysis. Statistical evaluations of all equations and individual parameters and intercepts were examined by *F*-values and *t*-values. If a model did not give a statistical confidence, it was discarded. Where the number in parentheses is the 95% confidence interval, *n* is the number of compounds, *r* is correlation coefficient, $r^2$ is explained variance, *s*, *F*, *p* are standard deviation, ratio between the variances of observed and calculated activities, probability factor related to *F*-ratio.

## 5.3. Pharmacology: $^{45}Ca$ influx assay and Cell necrosis assay

The methods were described in previous report.[20]

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## References and notes

1. Forman, M. B.; Perry, J. M.; Wilson, B. H.; Verani, M. S.; Kaplan, P. R.; Shawl, F. A.; Friesinger, G. C. *J. Am. Coll. Cardiol.* **1991**, *18*, 911.
2. Forman, M. B.; Puett, D. W.; Virmani, R. *J. Am. Coll. Cardiol.* **1989**, *13*, 450.
3. Forman, M. B.; Velasco, C. E.; Jackson, E. K. *Cardiovas. Res.* **1993**, *27*, 9.
4. Karmazyn, M.; Gan, X. T.; Humphreys, R. A.; Yoshida, H.; Kusumoto, K. *Circ. Res.* **1999**, *85*, 777.
5. Eigel, B. N.; Hadley, R. W. *Am. J. Physiol.* **1999**, *277*, H1817.
6. Ver Donck, L.; Marcal, B.; Verdonck, F. *Cardiovasc. Res.* **1993**, *27*, 349.
7. Allen, D. G.; Cairns, S. P.; Turvey, S. E.; Lee, J. A. *Adv. Exp. Med. Biol.* **1993**, *346*, 19.
8. Scholz, W.; Albus, U.; Lang, H. J.; Linz, W.; Martorana, P. A.; Englert, H. C.; Schölkens, B. A. *Br. J. Pharmacol.* **1993**, *109*, 562.
9. Kawada, T.; Yoshida, Y.; Sakurai, H.; Imai, S. *Jpn. J. Pharmacol.* **1992**, *59*, 191.
10. Dennis, S. C.; Coetzee, W. A.; Cragoe, E. J., Jr.; Opie, L. H. *Circ. Res.* **1990**, *66*, 1156.
11. Haigney, M. C. P.; Miyata, H.; Lakatta, E. G.; Stern, M.G.; Silverman, H. S. *Circ. Res.* **1992**, *71*, 547.
12. Tani, M.; Neely, J. R. *Circ. Res.* **1989**, *65*, 1045.
13. Pogwizd, S. M.; Schlotthauer, K.; Li, L.; Yuan, W.; Bers, D. M. *Circ. Res.* **2001**, *88*, 1159.
14. Köster, O. F.; Szigeti, G. P.; Beuckelmann, D. J. *Cardiovasc. Res.* **1999**, *41*, 175.
15. Kaczorowski, G. J.; Garcia, M. L.; King, R.; Slaughter, R. S. In *Sodium–Calcium Exchange*; Allen, F. J., Noble, D., Reuter, H., Eds.; Oxford University Press: Oxford, 1989; pp 66–101.

16. Pogwizd, S. M. *Drugs* **2003**, *63*, 439.
17. Hasegawa, H.; Muraoka, M.; Matsui, K.; Kojima, A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3471.
18. Iwamoto, T.; Watano, T.; Shigekawa, M. *J. Biol. Chem.* **1996**, *271*, 22391.
19. Ohta, T.; Nakanishi, M.; Aibe, I.; Taguchi, M.; Tomisawa, K. JP Patent 11092454, 1999.
20. Kuramochi, T.; Kakefuda, A.; Yamada, H.; Sato, I.; Taguchi, T.; Sakamoto, S. *Bioorg. Med. Chem.* **2004**, *12*, 5039.
21. Iwanoto, T.; Wakabayashi, S.; Imagawa, T.; Shigekawa, M. *Eur. J. Cell. Biol.* **1998**, *76*, 228.
22. (a) Skagerberg, B.; Bonelli, D.; Clementi, S.; Cruciani, G.; Ebert, C. *Quant. Struct.-Act. Relat.* **1989**, *8*, 32; (b) Verloop, A.; Hoogenstraaten, W.; Tipker, J. In *Drug Design*; Ariëns, E. J., Ed.; Academic: New York, 1976; Vol. 7, pp 165–207.
23. Hansch, C.; Fujita, T. *J. Am. Chem. Soc.* **1964**, *86*, 1616.
24. All the equations have regression coefficients, intercepts, and variance ratio ($F$) significant to more than 99% level. All the coefficients of parameters and intercepts in all equations are of over 99% confidence intervals as supported by their $t$- and $p$-values. Eq. 1 $\pi$ ($t$-value = −4.54, $p < 0.00$), MR ($t$-value = −4.89, $p < 0.00$), intercept ($t$-value = 38.0, $p < 0.00$); Eq. 2 $\pi$ ($t$-value = −3.73, $p < 0.00$), $L$ ($t$-value = −5.55, $p < 0.00$), intercept ($t$-value = 21.3, $p < 0.00$); Eq. 3 $\pi$ ($t$-value = −5.16, $p < 0.00$), $B_{iv}$ ($t$-value = −6.72, $p < 0.00$), intercept ($t$-value = 35.4, $p < 0.00$).