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Novel 1-oxyl-2-substitutedphenyl-4,4,5,5-tetramethylimidazolines: Synthesis, selectively analgesic action, and QSAR analysis

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Abstract—Based on the knowledge that imidazoline can result in analgesic action due to its selective binding with the prostacyclin receptor, 20 1-oxyl-2-substitutedphenyl-4,4,5,5-tetramethylimidazolines (**3a**–**t**) were prepared in moderate yields. At 0.13 mmol/kg dose, their in vivo analgesic activities were evaluated after the mice were administered at 30, 60, 90, and 150 min. Compared with the pain threshold (12.27 ± 9.56–17.71 ± 7.00%) of normal saline (NS) receiving mice, the pain threshold (23.42 ± 8.14% to 102.58 ± 10.66%) of **3a**–**t** receiving mice increases significantly. Considering a prostacyclin receptor targeting analgesic agent usually had bleeding action and to appraise the bleeding risk, the in vivo tail bleeding time of 1.30 mmol/kg **3a**–**t** receiving mice was found to be ranged from 116.3 ± 8.2 s to 120.3 ± 9.2 s, which was substantially equal to that (117.8 ± 8.4 s to 119.0 ± 8.6 s) of NS receiving mice. Based on the possibility of imidazoline acting as vasodilator, the in vitro vasorelaxations of **3a**–**t** were tested using the rat aortic strip model. When the aortic strip contracted by noradrenaline (NE, final concentration 10^{-7} mol/l) was treated with **3a**–**t** (final concentration 5×10^{-4} mol/l), only lower percentage inhibitions (6.55 ± 5.70–37.40 ± 4.07%) were recorded, implying that the vasorelaxation of **3a**–**t** was neglectable. By selecting appropriate molecular descriptors generated from e-dragon server, the QSAR model of the analgesic activities of **3a**–**t** was constructed using the multiple linear regression method. The established QSAR model showed reasonable accuracy and thus it is promising to be used for screening new 1-oxyl-2-substitutedphenyl-4,4,5,5-tetramethylimidazoline derivatives as analgesic agents.

1. Introduction

During the past years intense efforts have been mounted to develop the structural diversity of imidazolines chosen for the discovery of antagonists of human adenosine receptors (A3 Ars),[1–3] α-adrenergic receptor subtypes,[4–7] P2X7 receptor,[8] and prostacyclin receptor.[9] Activation of these receptors could result in a series of diseases including inflammatory, glaucoma, stroke, rheumatoid arthritis, Alzheimer's disease, nasal decongestion, hypertension, hypotension, hyperglycemia, liver cell proliferation, hyperaggregability of platelets, and benign prostatic hyperplasia, nasal decongestion, hypertension, hyperglycemia, depression, liver cell proliferation, hyperaggregability of platelets, erectile dysfunction and dermal necrosis. These efforts rationally resulted in some useful relationships between a certain structure and a specific inhibition of imidazoline, for instance 2-phenyl-substituted imidazopurinone possesses high affinity for human A3 Ars,[3] idazoxan selectively blocks α_2-adrenoreceptor,[7] phentolamine mesylate improves male erectile dysfunction,[8] and 2-phenylaminoimidazoline alleviates pain,[9] which could be used as the clue for developing more selective drug of imidazoline. By understanding these relationships, the connection of the structure of 2-phenylaminoimidazoline and its selective inhibition to prostacyclin receptor was efficaciously used for discovering analgesic agent.[9,10] Following this connection, using radioligand binding technology the prostacyclin receptor affinities of 460 2-phenylaminoimidazolines were determined. As a result [(4,5-dihydro-1H-imidazol-2-yl)-[4-(4-isopropoxybenzyl)phenyl]amine] was found to have analgesic activity in the rat. The results

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told us that the 2-position modification of imidazoline may result in desirable lead compound.

With this understanding as a clue for further structural modification of analgesic imidazoline, the preparation, in vivo evaluation, and QSAR analysis of 20 analgesic 1-oxyl-2-substituted-phenyl-4,4,5,5-tetramethyl-imidazolines were performed in the present study. In order to demonstrate that the analgesic action of these 20 1-oxyl-2-substitutedphenyl-4,4,5,5-tetramethylimidazolines is selective one, their in vivo tail bleeding time of mouse and in vitro vasorelaxation activities were also reported.

2. Results and discussion

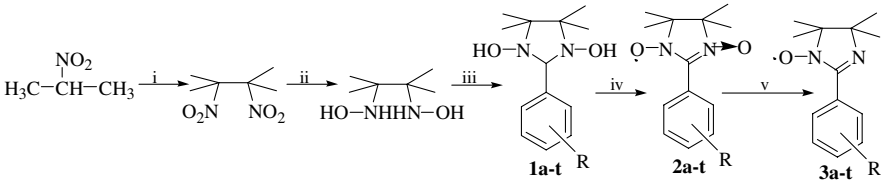
2.1. Preparations of 1-oxyl-2-substitutedphenyl-4,4,5,5-tetramethylimidazolines

1-Oxyl-2-substitutedphenyl-4,4,5,5-tetramethylimidazolines **3a**–**t** were synthesized according to the synthetic route shown in Scheme 1.[11,12] After the bromination and reduction, 2-nitropropane was smoothly converted into 2,3-bis(hydroxylamino)-2,3-dimethylbutane which was condensed with substitutedphenyl aldehydes to give 1,3-dihydroxy-2-substitutedphenyl-4,4,5,5-tetramethylimidazolidines (**1a**–**t**) in 31–85% yield. The condensation yield significantly depended on the lipophilicity and space hindrance of the substituents in the phenyl, hydrophilic and multiple substitutions usually led to lower yield (31–85%). The oxidation of **1a**–**t** with PbO_2 provided 1-oxyl-2-(substitutedphen-1′-yl)-3-oxide- 4,4,5,5-tetramethylimidazoline (**2a**–**t**) in 52–97% yield. The oxidation yield also depended on the lipophilicity and space hindrance of the substituents in the phenyl, hydrophilic and multiple substitutions again led to lower yield (52–68%). The reduction of **2a**–**t** with $NaNO_2$ provided 1-oxyl-2-(substitutedphen-1′-yl)-4,4,5,5-tetramethylimidazoline (**3a**–**t**) in 40–98% yield. The reduction yield depended on the substituent in the phenyl very much, when the phenyl was substituted by electron donating polar group, such as $N(CH_3)_2$ and OH, the yield was less than 30%, when the phenyl was substituted by electron donating nonpolar group, such as CH_3 and OCH_3, the yield was more than 50%, and when the phenyl was substituted by electrondrawing group, such as F, Cl, Br, and NO_2, the yield reached 70%.

2.2. In vivo analgesic activities of 3a–t

Considering the structural likeness between **3a**–**t** and analgesic imidazoline reported in the literature,[9] the in vivo analgesic effects of **3a**–**t** were first evaluated by the tail-flick test.13,14 After the administration of **3a**–**t** at a level of 0.13 mmol/kg, the pain thresholds of the mice were measured at 30 min intervals and total measurement was carried out for 150 min. Based on the tested basic pain threshold (BPT), the tested pain threshold after administration (PTAD), and the AAPT value, namely PTAD minus BPT, the pain threshold variation (PTV) was calculated according to the equation PTV = AAPT/BPT. All values of the PTV for each animal were averaged and constituted one sample. The data are listed in Table 1, and the statistical analysis was carried out by use of ANOVA test, $p < 0.05$ is considered significant. Under the testing condition, the pain threshold of the mice was increased by nearly all **3a**–**t** after administering them for 30 min and these analgesic effects may continue for 150 min.

As shown in Table 1, all **3a**–**t** exhibited obvious analgesic activity at the dosage of 0.13 mmol/kg body weight. Though after the administration of 30 min the enhancing pain thresholds in mice were observed for all **3a**–**t**, the maximal values appeared at different time points. The enhancing actions of **3e**,**p**–**t** reached their peaks after the administration of 60 min, of **3d**,**f**,**k** reached their peaks after the administration of 90 min, of **3b**,**c**,**g**,**h** reached their peaks after the administration of 120 min, and of **3m**,**n**,**o** reached their peaks after the administration of 150 min. The data listed in Table 2 demonstrated that the analgesic activity of **3b**–**d**,**f**,**m**,**n** was significantly higher than that of the others. From these data the effect of the substitution in the phenyl on the analgesic potency can be simply deduced. The OH, F, Cl, and multiple hydrophobic substitutions usually led to higher activities, and di-Cl and NO_2 substitutions usually led to lower activities. Comparing the effects of the substituents in the phenyl on the in vitro vasorelaxation and the in vivo analgesic actions, it was found that the two kinds of effects exhibited substantially opposite trends. Thus, the in vivo analgesic actions of **3a**–**t** are independent of their in vitro vasorelaxation. Based on the relative higher analgesic activity at relative lower dosage and the relative weaker vasorelaxation activity at relative higher concentration, it could be



Scheme 1. Synthetic route of 1-oxyl-2-(substitutedphen-1′-yl)-4,4,5,5-tetramethylimidazolines. (i) Br_2 and NaOH (6 mol/l), (ii) Zn and NH_4Cl, (iii) R-C_6H_4CHO, (iv) PbO_2. (v) $NaNO_2$ and hydrochloric acid (pH 5–6). In (**1**–**3**)**a** R = H, (**1**–**3**)**b** R = 2-OH, (**1**–**3**)**c** R = 3-OH, (**1**–**3**)**d** R = 4-OH, (**1**–**3**)**e** R = 4-CH_3, (**1**–**3**)**f** R = 4-OCH_3, (**1**–**3**)**g** R = 2,4-dimethoxyl, (**1**–**3**)**h** R = 3,4-dimethoxyl, (**1**–**3**)**i** R = 3,4-methylenedioxy, (**1**–**3**)**j** R = 3-OH-4-OCH_3, (**1**–**3**)**k** R = 3-OCH_3-4-OH, (**1**–**3**)**l** R = 4-$N(CH_3)_2$, (**1**–**3**)**m** R = 2-F, (**1**–**3**)**n** R = 4-Cl, (**1**–**3**)**o** R = 4-Br, (**1**–**3**)**p** R = 2,4-di-Cl, (**1**–**3**)**q** R = 3,4- di-Cl, (**1**–**3**)**r** R = 2-NO_2, (**1**–**3**)**s** R = 3-NO_2, (**1**–**3**)**t** R = 4-NO_2.

Table 1. The effect of **3a–t** on the pain threshold in mice

Compound	Pain threshold variation ($\bar{x} \pm$ SD%)				
	30 min	60 min	90 min	120 min	150 min
Control	12.45 ± 7.21	13.40 ± 8.47	17.71 ± 7.00	12.27 ± 9.56	17.43 ± 9.18
3a	40.60 ± 10.71[a]	36.99 ± 11.60[a]	34.00 ± 10.11[a]	32.14 ± 11.55[a]	33.48 ± 9.19[a]
3b	48.57 ± 10.59[a]	45.39 ± 11.42[b]	64.20 ± 12.46[a]	70.17 ± 12.00[a]	62.22 ± 10.50[a]
3c	49.60 ± 11.78[a]	50.30 ± 11.75[a]	53.99 ± 10.74[a]	72.72 ± 12.07[a]	70.80 ± 10.04[a]
3d	55.66 ± 10.38[a]	70.80 ± 11.37[a]	75.88 ± 12.60[a]	55.54 ± 12.40[a]	29.92 ± 9.46[b]
3e	40.16 ± 9.27[a]	53.56 ± 11.30[a]	43.72 ± 10.04[a]	35.17 ± 11.14[a]	25.47 ± 10.41
3f	35.34 ± 9.35[a]	102.58 ± 10.66[a]	99.30 ± 11.70[a]	68.70 ± 11.97[a]	53.05 ± 9.34[a]
3g	42.00 ± 10.84[a]	59.73 ± 10.45[a]	62.49 ± 11.09[a]	71.19 ± 12.22[a]	32.39 ± 9.43[a]
3h	44.92 ± 10.26[b]	60.22 ± 12.60[a]	59.50 ± 11.97[a]	78.44 ± 12.50[a]	55.57 ± 11.99[a]
3i	52.83 ± 11.27[b]	64.96 ± 11.80[a]	74.02 ± 12.43[a]	58.34 ± 11.85[a]	25.44 ± 10.60[b]
3j	33.08 ± 10.03[a]	48.18 ± 11.88[a]	48.38 ± 10.23[a]	56.42 ± 10.72[a]	59.76 ± 11.34[a]
3k	54.18 ± 11.17[a]	42.86 ± 10.38[a]	66.34 ± 11.61[a]	43.25 ± 10.64[a]	40.92 ± 10.88[a]
3l	30.37 ± 9.00[a]	35.49 ± 10.30[a]	36.19 ± 10.00[a]	34.30 ± 10.61[a]	27.87 ± 9.09[b]
3m	62.19 ± 10.40[a]	67.78 ± 11.18[a]	63.27 ± 10.21[a]	61.95 ± 10.04[a]	86.78 ± 12.10[a]
3n	59.33 ± 10.25[a]	66.29 ± 10.42[a]	61.68 ± 10.29[a]	50.78 ± 10.22[a]	84.48 ± 11.33[a]
3o	41.32 ± 9.62[a]	35.56 ± 9.23[a]	59.84 ± 10.60[a]	59.97 ± 11.22[a]	76.45 ± 11.99[a]
3p	39.64 ± 10.61[a]	63.34 ± 10.83[a]	48.37 ± 9.22[a]	30.51 ± 9.48[a]	14.75 ± 9.57
3q	50.57 ± 11.04[a]	72.76 ± 12.63[a]	55.72 ± 9.45[a]	28.78 ± 9.34[a]	26.96 ± 9.33[b]
3r	38.36 ± 9.38[a]	66.89 ± 12.23[a]	57.25 ± 11.54[a]	23.42 ± 8.14[b]	19.69 ± 9.91
3s	49.81 ± 10.75[a]	66.37 ± 11.72[a]	47.41 ± 10.19[a]	43.45 ± 10.08[a]	41.84 ± 10.97[a]
3t	56.49 ± 11.11[a]	60.55 ± 11.07[a]	57.95 ± 11.55[a]	35.90 ± 10.37[a]	52.27 ± 11.59[a]

$n = 10$.
[a] Compare to control $p < 0.01$.
[b] Compare to control $p < 0.05$.

Table 2. Effect of **3a–t** on the tail bleeding time ($\bar{x} \pm$ SD%) of mice

Compound	Before administration	After administration			
		5 min	15 min	30 min	60 min
NS	119.0 ± 8.6	117.8 ± 8.4	118.3 ± 7.7	118.2 ± 8.3	117.9 ± 8.1
3a	117.6 ± 8.1	118.2 ± 7.6	117.4 ± 7.9	119.3 ± 8.1	118.2 ± 7.6
3b	116.8 ± 8.6	116.5 ± 9.1	118.3 ± 7.6	117.1 ± 7.8	116.6 ± 8.2
3c	118.4 ± 9.0	118.8 ± 8.2	117.2 ± 8.0	119.3 ± 9.2	117.3 ± 8.0
3d	120.1 ± 9.1	118.0 ± 8.5	116.9 ± 7.6	117.3 ± 7.7	117.8 ± 8.2
3e	119.4 ± 8.9	117.6 ± 8.3	116.8 ± 8.1	118.2 ± 9.1	117.9 ± 7.6
3f	117.4 ± 8.0	117.7 ± 8.1	116.5 ± 7.9	119.3 ± 8.9	119.1 ± 9.1
3g	120.0 ± 9.3	119.3 ± 8.3	116.8 ± 8.2	117.7 ± 7.9	118.1 ± 8.4
3h	119.3 ± 8.1	118.5 ± 8.7	118.8 ± 9.1	119.5 ± 9.4	120.5 ± 9.0
3i	116.5 ± 8.3	117.4 ± 7.9	116.8 ± 8.8	117.9 ± 9.2	116.8 ± 7.5
3j	118.5 ± 8.2	117.3 ± 7.6	116.9 ± 9.1	118.0 ± 7.9	117.3 ± 7.6
3k	117.3 ± 8.0	117.8 ± 9.0	116.4 ± 9.2	117.8 ± 8.0	118.5 ± 8.3
3l	119.5 ± 9.6	119.2 ± 9.0	118.7 ± 8.9	118.5 ± 8.3	117.7 ± 9.1
3m	118.4 ± 8.5	119.3 ± 8.8	118.4 ± 8.3	117.9 ± 8.6	117.3 ± 8.5
3n	116.8 ± 9.1	119.0 ± 8.8	117.3 ± 9.2	118.0 ± 8.8	117.5 ± 8.0
3o	116.3 ± 8.2	116.7 ± 8.4	117.5 ± 8.6	116.6 ± 8.4	117.4 ± 8.3
3p	118.6 ± 9.1	118.3 ± 8.9	118.9 ± 8.9	117.3 ± 9.3	117.7 ± 9.2
3q	119.7 ± 9.3	120.2 ± 8.8	119.3 ± 9.2	119.1 ± 8.9	118.7 ± 9.0
3r	117.7 ± 8.4	117.5 ± 8.5	117.8 ± 8.1	117.2 ± 8.0	116.7 ± 8.3
3s	118.8 ± 9.0	118.4 ± 8.9	116.5 ± 8.6	117.6 ± 8.5	118.2 ± 9.0
3t	120.3 ± 9.2	119.4 ± 8.8	119.3 ± 8.9	118.6 ± 8.3	118.9 ± 8.7

$n = 10$, dose, 1.3 mmol/kg; NS (normal saline), vehicle.

postulated that **3a–t** may be selective inhibitor of the prostacyclin receptor.

2.3. In vivo tail bleeding activities of 3a–t

As the prostacyclin receptor targeting analgesic agents usually have bleeding action, to appraise the bleeding risk of **3a–t**, their tail bleeding time assays were performed on mice using a literature method.[15] The observed bleeding time in mice at all time points tested (5–60 min) fell into a range of 116–120 s which were substantially equal to that before administration (Table 2). In each case the dose was 1.30 mmol/kg body weight. This observation implied that at ten fold analgesic dose **3a–t** exhibited no bleeding risk.

2.4. In vitro vasorelaxation activities of 3a–t

Since the structure of **3a–t** is similar to that of the vasodilator imidazoline,[11,16,17] the conversions of **3a–t**

during vasoconstriction induced by noradrenaline (NE) were examined using the rat aortic strip model. Based on the model, the aortic strip contracted by NE (final concentration 10^{-7} mol/l) was treated with **3a**–**t** (final concentration 5×10^{-4} mol/l, 1×10^{-4} mol/l, and 2×10^{-5} mol/l). It was noticed that even at 5×10^{-4} mol/l the NE contracted aortic strip can be relaxed less than 40% by **3a**–**t** (Table 3). This observation demonstrates **3a**–**t** are weak vasodilators.

2.5. Dose-dependent in vivo analgesic activities of 3c,f,m

Compounds with significant activity (**3c**,**f**,**m**) were administered using three dosages to study detailed pharmacological activity profile (Table 4). Statistical analysis was carried out using one way ANOVA test with $p < 0.05$ as significant cut-off. The data indicated that the in vivo anti-thrombolysis activities of intravenous **3c**,**f**,**m** were dose-dependent.

2.6. QSAR analysis of 3a–t

Selecting the molecular descriptors generated from e-dragon webserver,[18] the multiple linear regression method was employed to construct the QSAR equation of **3a**–**t**. The resubstitution and Leave-One-Out (LOO) tests were carried out to validate the established equations. For **3a**–**t** Eq. 1 could give the highest predictive accuracy.

$$\begin{aligned} \mathrm{PTV}_{90} &= 927.3(\pm220.5) - 43.5(\pm14.7)\times \mathrm{GATS4e} \\ &\quad -610.7(\pm172.7)\times \mathrm{BELe1} - 2706.5 \\ &\quad (\pm824.2)\times \mathrm{JGI1} + 92.5(\pm36.8)\times \mathrm{R2u} \\ N &= 20,\ R = 0.79,\ S = 8.7,\ \bar{e} = 7.0,\ R_{\mathrm{LOO}} = 0.76, \\ S_{\mathrm{LOO}} &= 11.1,\ \bar{e}_{\mathrm{LOO}} = 9.3,\quad F = 6.23,\ p = 0.004. \end{aligned} \tag{1}$$

In the current QSAR analysis, the PTV_{90} (i.e., the PTV value measured at 90 min) is considered as the analgesic

Table 3. Conversion of **3a**–**t** during vasoconstriction induced by NE ($n = 6$)

Compound	Vasorelaxation ($\bar{x} \pm$ SD%) at following concentrations		
	5×10^{-4} mol/l	1×10^{-4} mol/l	2×10^{-5} mol/l
3a	17.25 ± 4.79	11.04 ± 3.02	5.44 ± 2.95
3b	20.32 ± 4.32	13.38 ± 2.99	6.01 ± 3.05
3c	35.70 ± 4.96	22.98 ± 3.11	7.06 ± 2.78
3d	8.71 ± 3.18	7.66 ± 4.03	5.36 ± 4.00
3e	31.77 ± 4.18	19.96 ± 3.21	7.00 ± 2.47
3f	35.08 ± 4.10	23.15 ± 2.79	6.88 ± 2.80
3g	37.08 ± 4.03	24.03 ± 3.13	7.33 ± 2.74
3h	33.73 ± 4.33	22.33 ± 3.05	6.66 ± 2.95
3i	37.40 ± 4.07	24.51 ± 2.99	8.10 ± 3.30
3j	16.65 ± 3.52	11.04 ± 2.86	5.36 ± 3.00
3k	6.55 ± 5.70	6.09 ± 3.33	5.98 ± 6.02
3l	39.98 ± 2.63	26.00 ± 3.36	8.02 ± 3.46
3m	23.40 ± 3.92	14.94 ± 2.89	5.00 ± 2.97
3n	36.59 ± 5.17	23.78 ± 3.03	7.19 ± 3.04
3o	34.78 ± 2.98	23.01 ± 3.22	6.89 ± 3.01
3p	34.82 ± 5.03	22.99 ± 3.30	6.86 ± 3.00
3q	34.37 ± 3.45	22.71 ± 2.98	6.74 ± 2.89
3r	35.58 ± 2.37	20.39 ± 3.07	6.98 ± 2.76
3s	34.27 ± 3.91	22.07 ± 2.99	6.72 ± 3.34
3t	36.53 ± 4.66	24.44 ± 3.32	7.36 ± 3.11

Table 4. Effect of **3c**,**f**,**m** at different dosages on the pain threshold in mice

Compound, dose (mmol/kg)	Pain threshold variation ($\bar{x} \pm$ SD%)				
	30 min	60 min	90 min	120 min	150 min
Control	12.45 ± 7.21	13.40 ± 8.47	17.71 ± 7.00	12.27 ± 9.56	17.43 ± 9.18
3c, 0.1300	49.60 ± 11.78[a]	50.30 ± 11.75[a]	53.99 ± 10.74[a]	72.72 ± 12.07[a]	70.80 ± 10.04[a]
3c, 0.0130	35.69 ± 9.30[b]	37.80 ± 10.30[b]	42.80 ± 10.60[b]	59.94 ± 10.41[b]	52.12 ± 9.06[b]
3c, 0.0013	24.16 ± 9.07[c]	25.56 ± 10.00[c]	30.72 ± 10.04[c]	35.17 ± 11.14[c]	33.47 ± 10.41[c]
3f, 0.1300	35.34 ± 9.35[a]	102.58 ± 10.66[a]	99.30 ± 11.70[a]	68.70 ± 11.97[a]	53.05 ± 9.34[a]
3f, 0.0130	25.00 ± 7.84[b]	89.73 ± 10.15[b]	82.49 ± 10.09[b]	50.19 ± 10.22[b]	34.39 ± 9.23[b]
3f, 0.0013	14.92 ± 7.26	55.22 ± 10.60[c]	50.50 ± 10.97[c]	38.44 ± 10.50[c]	22.57 ± 7.99[c]
3m, 0.1300	62.19 ± 10.40[a]	67.78 ± 11.18[a]	63.27 ± 10.21[a]	61.95 ± 10.04[a]	86.78 ± 12.10[a]
3m, 0.0130	49.33 ± 10.25[b]	51.29 ± 10.12[b]	50.68 ± 10.09[b]	49.78 ± 9.22[b]	64.48 ± 10.33[b]
3m, 0.0013	31.32 ± 9.32[c]	33.52 ± 9.33[c]	31.84 ± 9.60[c]	30.37 ± 10.02[c]	44.45 ± 9.99[c]

$n = 10$.

[a] Compare to group of 0.0130 mmol/kg dose, $p < 0.01$.

[b] Compare to group of 0.0013 mmol/kg dose, $p < 0.05$.

[c] Compare to control $p < 0.01$.

activity of a compound. The resubstitution analysis gave an absolute average error ($\bar{e}$) of 7.0, whereas the absolute average error for the LOO test ($\bar{e}_{LOO}$) is 9.3. The detailed description of the other statistical measurements in Eq. 1 is available in Section 3. Grouped into the 2D autocorrelation descriptors, the GATS2e is related to the atomic electronegativities of a molecule.[19] The BELe1 is a molecular descriptor obtained from the lowest eigenvalue of the adjacency matrix, weighting the diagonal elements with atomic Sanderson electronegativities.[20] The JGI1 belongs to the descriptors of charge indices, which evaluate the global charge transferred between pairs of atoms inside the molecule.[21] The R2u is classified into the GETAWAY (GEometry, Topology, and Atom-Weights AssemblY) molecular descriptors, which try to match 3D-molecular geometry provided by the molecular influence matrix and atom relatedness by molecular topology with chemical information by using different atomic weightings.[22]

Considering the standard deviation in measuring PTV_{90} (cf. Table 1), the current predictive accuracy of **3a**–**t** is quite reasonable (Fig. 1). Therefore, Eq. 1 can be practically used to forecast the analgesic activities of new 1-oxyl-2-substitutedphenyl-4,4,5,5-tetramethylimidazoline derivatives. In Eq. 1, three descriptors (GATS2e, BELe1, and JGI1) are significantly related to a molecule's electrostatic property, while the remaining descriptor R2u is more correlated with the steric character of a molecule. Therefore, the overall analgesic activities of **3a**–**t** can be reasonably explained by their electrostatic and steric effects.

The QSAR studies on the analgesic activities of different compounds have been widely reported in the literature.[23–25] In addition to obtain the predictable QSAR models, the molecular mechanisms of analgesic effects were also derived to some extent. Focused on obtaining the optimized accuracy, Eq. 1 is unable to provide a more detailed insight into the molecular mechanism of **3a**–**t** to exert their analgesic effects. This is mainly due to the fact that physicochemical information hidden in these selected descriptors is complicated. It can be envisioned that the analgesic effects of **3a**–**t** are caused by their interactions with the same receptor, therefore a detailed CoMFA[26] may be able to provide new insight into the SAR of these compounds. Moreover, molecular docking studies between the receptor and these compounds will be very helpful in understanding the molecular mechanisms of their analgesic effects provided that the 3D structure of the receptor is known or can be confidently predicted.

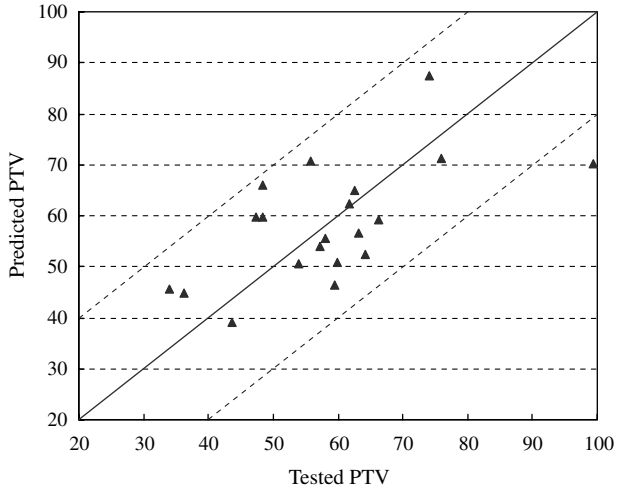


Figure 1. Comparison of the predicted analgesic activities of compounds **3a**–**t** in the LOO test with the tested activities. The data points situated at the zone between the two diagonal-dash lines correspond to the compounds, in which the absolute values of the prediction errors are less than 20.0.

3. Experimental

3.1. General

The purity of all the compounds was confirmed by TLC (Merck silica gel plates of type 60 F_{254}, 0.25 mm layer thickness) and HPLC (waters, C_{18} column 3.9×150 mm). Melting points were measured on a XT5 hot stage microscope (Beijing keyi electro-optic factory). The infrared spectra were recorded with a Perkin–Elmer 983 instrument. The EI-MS was determined by Trace MS System instrument (American Thermo Finnigan Company). The ^1H NMR was determined by Varian INOVA-300 MHz spectrometer. The ESR spectra were obtained from 10^{-5} mol/l phosphate-buffered saline, using a BRUKER 300-E spectrometer. The conditions of measurements are as follows: center field: 3440 G, sweep width: 100 G, sweep time: 60 s, modulation amplitude: 1.1 G, time constant 8.2×10^{-2} s, modulation frequency 100 kHz, microwave frequency 9.68 GHz, and microwave power: 20 MW.

3.2. Synthesis of the compounds

3.2.1. 2,3-Dimethyl-2,3-dinitrobutane. At −5 °C, 10 ml (0.19 mol) of Br_2 was added dropwise within 1 h to the solution of 34.5 g (0.39 mol) of 2-nitropropane in 65 ml (6.0 mol/l) aqueous NaOH. One hundred and twenty-eight milliliters of ethanol was added into the solution via stirring. The reaction mixture was stirred at 84 °C for 3 h. The hot reaction mixture was transferred into 400 ml of ice water. The formed colorless crystals were collected by filtration to yield 25 g (73%) of the title compound, mp 110–112 °C.

3.2.2. 2,3-Bis(hydroxylamino)-2,3-dimethylbutane. 2,3-Dimethyl-2,3-dinitrobutane (17.6 g, 0.1 mol) was dissolved in a mixture of THF (300 ml) and water (50 ml). Zn powder (27 g) was added all at once to this solution cooled to 8–10 °C in an ice bath. A solution of NH_4Cl (43 g, 0.8 mol) in H_2O (150 ml) was added dropwise to this slurry within 2 h, with continued stirring for 1 h at 10 °C, and the flask was stored in a fridge (4–6 °C) for 16 h. The slurry was filtered, and the precipitate was carefully washed with THF (4×100 ml). The precipitate was then dried by three washings with diethyl ether and carefully collected (59 g). The solution was

evaporated under vacuum until THF ceased to distill off. Then the solution was protected from air, and sodium carbonate (50 g) and sodium chloride (30 g) were added with cooling. Continuous extraction with chloroform (400 ml) was performed over 18 h. A white powder was obtained (9.4 g, 63% yield). Mp 182 °C.

3.2.3. General procedure for the preparation of 1,3-dihydroxy-2-substituted-phenyl-4,4,5,5-tetramethylimidazolidine (1a–t). A solution of 296 mg (2 mmol) of 2,3-bis(hydroxylamino)-2,3-dimethylbutane and 2 mmol of various aldehydes in 3 ml of methanol was stirred at room temperature for 16 h. The title compound was collected by filtration. The filtration was evaporated under reduced pressure to obtain the second crop of the title compound. The title compound was directly used for the next reaction without further purification.

3.2.3.1. 1,3-Dihydroxy-2-phenyl-4,4,5,5-tetramethylimidazolidine (1a). Using the general procedure mentioned above 401 mg (85%) of the title compound was obtained. Mp 168–169 °C. IR (KBr): 3340, 1606, 1500, 1450, 1381 cm^{-1}; ^1H NMR ($CDCl_3$) δ = 1.14 (s, 12H), 4.78 (s, 1H), 7.31–7.51 (m, J = 6.9 Hz, 5H), 7.71 (s, 2H); ESI-MS (m/e) = 236$[M]^+$.

3.2.3.2. 1,3-Dihydroxy-2-(2′-hydroxylphen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1b). Using the general procedure mentioned above 286 mg (57%) of the title compound was obtained. Mp 139–141°C. R_f = 0.73 ($CHCl_3$/CH_3OH, 6:1). EI-MS (m/z) 252$[M]^+$. IR (KBr) 3325, 1600, 1500, 765 cm^{-1}. ^1H NMR (DMSO-d_6) δ=1.08 (s, 12H), 4.63 (s, 1H), 6.70 (d, J = 6.3 Hz, 1H), 6.74 (t, J = 6.5 Hz, 1H), 7.14 (t, J = 6.6 Hz, 1H), 7.20 (d, J = 6.4 Hz, 1H), 8.12 (s, 1H), 8.35 (s, 2H).

3.2.3.3. 1,3-Dihydroxy-2-(3′-hydroxyphen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1c). Using the general procedure mentioned above 304 mg (60%) of the title compound was obtained. Mp 244–246°C. R_f = 0.56 ($CHCl_3$/CH_3OH, 6:1). EI-MS (m/z) 252$[M]^+$. IR (KBr) 3320, 1500, 880, 795, 695 cm^{-1}. ^1H NMR (DMSO-d_6) δ=1.03 (s, 6H), 1.05 (s, 6H), 4.51 (s, 1H), 6.89 (dd, J = 8.4 Hz, J = 8.0 Hz, 1H), 6.92 (d, J = 6.2 Hz, 1H), 7.01 (d, J = 7.3 Hz, 1H), 7.71 (s, 1H), 7.86 (s, 1H), 7.95 (s, 2H).

3.2.3.4. 1,3-Dihydroxy-2-(4′-hydroxyphen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1d). Using the general procedure mentioned above 275 mg (51%) of the title compound was obtained. Mp 240–242 °C. R_f 0.67 ($CHCl_3$/CH_3OH, 6:1). EI-MS (m/z) 252$[M]^+$. IR (KBr) 3310, 1610, 1500, 1450, 794, 693 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.03 (s, 6H), 1.05 (s, 6H), 4.39 (s, 1H), 6.70 (d, J = 6.9 Hz, 2H), 7.23 (d, J = 6.9 Hz, 2H), 7.63 (s, 1H), 7.85 (s, 2H).

3.2.3.5. 1,3-Dihydroxy-2-(4′-methylphen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1e). Using the general procedure mentioned above 350 mg (70%) of the title compound was obtained. Mp 199–201 °C. R_f = 0.61 ($CHCl_3$/CH_3OH, 10:1). EI-MS (m/z) 250$[M]^+$. IR (KBr) 3335, 2985, 1600, 815 cm^{-1}. ^1H NMR ($CDCl_3$) δ = 1.14 (s, 6H), 1.19 (s, 6H), 1.37 (s, 3H), 4.90 (s, 1H), 7.68 (d, J = 9.0 Hz, 2H), 8.22 (d, J = 8.4 Hz, 2H), 8.35 (s, 2H).

3.2.3.6. 1,3-Dihydroxy-2-(4′-methoxyphen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1f). Using the general procedure mentioned above 372 mg (70%) of the title compound was obtained. Mp 179–181 °C. R_f = 0.52 ($CHCl_3$/CH_3OH, 10:1). EI-MS (m/z) 266$[M]^+$. IR (KBr) 3340, 2835, 1500, 825 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 0.99 (s, 6H), 1.03 (s, 6H), 3.73 (s, 3H), 4.56 (s, 1H), 6.88 (d, J = 4.2 Hz, 2H), 7.38 (d, J = 5.7 Hz, 2H), 7.77 (s, 2H).

3.2.3.7. 1,3-Dihydroxyl-2-(2′,4′-dimethoxylphenyl)-4,4,5,5-tetramethylimidazolidine (1g). Using the general procedure mentioned above 349 mg (59%) of the title compound was obtained. Mp 140–141 °C. R_f = 0.49 ($CHCl_3$/CH_3OH, 10:1). EI-MS (m/z) 296$[M]^+$. IR (KBr) 3295, 2825, 1500, 865, 810 cm^{-1}. ^1H NMR (DMSO-$d6$) δ = 1.03 (s, 12H), 3.72 (s, 6H), 4.99 (s, 1H), 7.02 (s, 1H), 7.42 (d, J = 6.7 Hz, 1H), 7.45 (d, J = 6.5 Hz, 1H), 7.79 (s, 2H).

3.2.3.8. 1,3-Dihydroxy-2-(3′,4′-dimethoxyphen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1h). Using the general procedure mentioned above 390 mg (66%) of the title compound was obtained. Mp 147–149°C. R_f = 0.52 ($CHCl_3$/CH_3OH, 6:1). EI-MS (m/z) 296$[M]^+$. IR (KBr) 3310, 2825, 1610, 865, 815 cm^{-1}. ^{1}HNMR (DMSO-d_6) δ = 1.05 (s, 12H), 3.74 (s, 6H), 4.53 (s, 1H), 6.85 (s, 1H), 6.87 (d, J = 6.6 Hz, 1H), 6.92 (d, J = 6.4 Hz, 1H), 7.85 (s, 2H).

3.2.3.9. 1,3-Dihydroxyl-2-(3′,4′-methylendioxyphen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1i). Using the general procedure mentioned above 240 mg (43%) of the title compound was obtained. Mp 188–190°C, R_f = 0.47 ($CHCl_3$/CH_3OH, 10:1), EI-MS (m/z) 280$[M]^+$, IR (KBr) 3315, 1600, 915, 1235, 1105 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.05 (s, 12H), 3.74 (s, 6H), 4.53 (s, 1H), 6.82 (d, J = 7.7 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.87 (s, 1H) 7.85 (s, 2H).

3.2.3.10. 1,3-Dihydroxyl-2-(3′-hydroxyl-4′-methoxyl)-phenyl-4,4,5,5-tetramethylimidazolidine (1j). Using the general procedure mentioned above 276 mg (49%) of the title compound was obtained. Mp 245–247°C. R_f = 0.52 ($CHCl_3$/CH_3OH, 6:1). EI-MS (m/z) 282$[M]^+$. IR (KBr) 3340, 2825, 1500, 1450, 825 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.03 (s, 6H), 1.04 (s, 6H) 3.72 (s, 3H), 4.53 (s, 1H), 6.82 (s, 1H), 6.89 (d, J = 6.6 Hz, 1H), 6.94 (d, J = 6.4 Hz, 1H), 7.65 (s, 1H), 8.04 (s, 2H).

3.2.3.11. 1,3-Dihydroxyl-2-(3′-methoxyl-4′-hydroxyphen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1k). Using the general procedure mentioned above 186 mg (33%) of the title compound was obtained. Mp 235–237°C, R_f = 0.47 ($CHCl_3$/pCH_3OH, 6:1), EI-MS (m/z) 282$[M]^+$, IR (KBr) 3310, 2840, 1600, 870, 815 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.00 (s, 6H), 1.03 (s, 6H), 3.74 (s, 3H), 4.41 (s, 1H), 6.70 (d, J = 7.8 Hz, 1H),

6.85 (d, J = 8.1 Hz, 1H), 7.02 (s, 1H), 7.68 (s, 1H), 8.05 (s, 2H).

3.2.3.12. 1,3-Dihydroxyl-2-(4′-*N*,*N*-dimethylphen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1l). Using the general procedure mentioned above 173 mg (31%) of the title compound was obtained. Mp 168–169°C. R_f = 0.53 ($CHCl_3/CH_3OH$, 6:1). EI-MS (m/z) 279$[M]^+$. IR (KBr) 3345, 2840, 1595, 1450, 840 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.03 (s, 6H), 1.05 (s, 6H), 2.85 (s, 6H), 4.01 (s, 1H), 6.68 (d, J = 8.7 Hz, 2H), 7.25 (d, J = 8.7 Hz, 2H), 7.60 (s, 2H).

3.2.3.13. 1,3-Dihydroxy-2-(2′-fluorophen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1m). Using the general procedure mentioned above 366 mg (72%) of the title compound was obtained. Mp 172–173°C, R_f = 0.69 ($CHCl_3/CH_3OH$, 6:1), EI-MS (m/z) 254$[M]^+$, IR (KBr) 3310, 1600, 1130, 1235, 775 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.07 (s, 12H), 4.95 (s, 1H), 7.18 (t, J = 8.4 Hz, 1 H), 7.30 (d, J = 8.1 Hz, 1H), 7.68 (d, J = 8.7 Hz, 2H), 8.14 (s, 2H).

3.2.3.14. 1,3-Dihydroxy-2-(4′-chlorophen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1n). Using the general procedure mentioned above 330 mg (61%) of the title compound was obtained. Mp 213–215°C. R_f = 0.73 ($CHCl_3/CH_3OH$, 10:1). EI-MS (m/z) 270$[M]^+$. IR (KBr) 3325, 1600, 1500, 1085, 825 cm^{-1}. ^1H NMR ($CDCl_3$) δ = 1.03 (s, 6H), 1.07 (s, 6H), 4.50 (s, 1H), 7.38 (d, J = 8.7 Hz, 2H), 7.48 (d, J = 8.4 Hz, 2H), 7.80 (s, 2H).

3.2.3.15. 1,3-Dihydroxy-2-(4′-bromophen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1o). Using the general procedure mentioned above 320 mg (51%) of the title compound was obtained. Mp 205–207 °C. R_f = 0.69 ($CHCl_3/CH_3OH$, 10:1). EI-MS (m/z) 314$[M]^+$. IR (KBr) 3310, 1590, 1450, 1075, 830 cm^{-1}. ^1H NMR ($CDCl_3$) δ = 1.14 (s, 12H), 4.77 (s,1H), 7.37 (d, J = 8.4 Hz, 2H), 7.47 (d, J = 7.8 Hz, 2H), 7.73 (s, 2H).

3.2.3.16. 1,3-Dihydroxyl-2-(2′,4′-dichlorophenyl)-4,4,5,5-tetramethylimidazolidine (1p). Using the general procedure mentioned above 427 mg (70%) of the title compound was obtained. Mp 201–203°C. R_f = 0.69 ($CHCl_3/CH_3OH$, 10:1). EI-MS (m/z) 305$[M]^+$. IR (KBr) 3315, 1590, 1450, 995, 820, 865 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.11 (s, 12H), 5.01 (s, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.52 (d, J = 8.1 Hz, 1H), 7.58 (s, 1H), 7.79 (s, 2H).

3.2.3.17. 1,3-Dihydroxyl-2-(3′,4′-dichlorophenyl)-4,4,5,5-tetramethylimidazolidine (1q). Using the general procedure mentioned above 427 mg (70%) of the title compound was obtained. Mp 212–214°C. R_f = 0.69 ($CHCl_3/CH_3OH$, 10:1). EI-MS (m/z) 305$[M]^+$. IR (KBr) 3313, 1592, 1453, 992, 863, 822 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.06 (s, 12H), 4.99 (s, 1H), 7.66 (m, 3H), 7.92 (s, 2H).

3.2.3.18. 1,3-Dihydroxy-2-(2′-nitrophen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1r). Using the general procedure mentioned above 410 mg (73%) of the title compound was obtained. Mp 189–190°C. R_f = 0.54 ($CHCl_3/CH_3OH$, 10:1). EI-MS (m/z) 281$[M]^+$. IR (KBr) 3325, 1600, 1535, 1365, 750 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.00 (s, 6H), 1.04 (s, 6H), 5.37 (s, 1H), 7.52 (d, J = 6.6 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.92 (t, J = 7.6 Hz, 1H), 8.05 (d, J = 7.2 Hz, 1H), 8.23 (s, 2H).

3.2.3.19. 1,3-Dihydroxy-2-(3′-nitrophen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1s). Using the general procedure mentioned above 421 mg (75%) of the title compound was obtained. Mp 179–181 °C. R_f = 0.51 ($CHCl_3/CH_3OH$, 10:1). EI-MS (m/z) 281$[M]^+$. IR (KBr) 3315, 1530, 1360, 1600, 875, 790, 685 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 0.52 (s, 12H), 4.19 (s, 1 H), 7.04 (dd, J = 8.7 Hz, J = 8.1 Hz, 1H), 7.34 (d, J = 6.0 Hz, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.77 (s, 1H), 8.03 (s, 2H).

3.2.3.20. 1,3-Dihydroxy-2-(4′-nitrophen-1′-yl)-4,4,5,5-tetramethylimidazolidine (1t). Using the general procedure mentioned above 335 mg (63%) of the title compound was obtained. Mp 172–174 °C. R_f = 0.59 ($CHCl_3/CH_3OH$, 10:1). EI-MS (m/z) 281$[M]^+$. IR (KBr) 3325, 1532, 1590, 1500, 1450, 1365, 835, 790, 685 cm^{-1}. ^1H NMR (DMSO-d_6) δ = 1.00 (s, 6H), 1.05 (s, 6H), 4.71 (s, 1H), 7.70 (d, J = 9.0 Hz, 2H), 8.21 (d, J = 7.2 Hz, 2H), 8.40 (s, 2H).

3.2.4. General procedure for the preparation of 1-oxyl-2-substitutedphenyl-3-oxide-4,4,5,5-tetramethyl-imidazolidines (2a–t). The mixture of 250 mg (0.8 mmol) of 1,3-dihydroxy-2-substitutedphenyl-4,4,5,5-tetramethylimidazolidine and 0.5 g of lead dioxide in 80 ml methanol was stirred at room temperature for 20 min and TLC ($CHCl_3/CH_3OH$, 20:1) indicated the complete disappearance of 1,3-dihydroxy-2-substitutedphenyl-4,4,5,5-tetramethylimidazolidine. The reaction mixture was filtered and the filtrate was evaporated under vacuum to provide the title compound as dark blue crystals.

3.2.4.1. 1-Oxyl-2-(phen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2a). Using the general procedure mentioned above 74 mg (80%) of the title compound was obtained. Mp 89–91 °C. R_f = 0.85 ($CHCl_3/CH_3OH$, 20:1). EI-MS (m/z) 311$[M]^+$. IR (KBr) 1600, 1450, 1357, 1070, 825 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.16G, g = 2.00997.

3.2.4.2. 1-Oxyl-2-(2′-hydroxylphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2b). Using the general procedure mentioned above 111 mg (64%) of the title compound was obtained. Mp 83–85 °C. R_f = 0.75 ($CHCl_3/CH_3OH$, 20:1). EI-MS (m/z) 217$[M]^+$. IR (KBr) 3345, 1600, 1500, 1450, 760 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.16G, g = 2.00997.

3.2.4.3. 1-Oxyl-2-(3′-hydroxylphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2c). Using the general procedure mentioned above 151 mg (76%) of the title compound was obtained. Mp 137–139 °C. R_f = 0.33 ($CHCl_3/CH_3OH$, 20:1). EI-MS (m/z) 249$[M]^+$. IR (KBr) 3340, 1590, 1450, 1380, 880, 800, 690 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.18 G, g = 2.00994.

3.2.4.4. 1-Oxyl-2-(4′-hydroxylphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2d). Using the general procedure mentioned above 103 mg (52%) of the title compound was obtained. Mp 134–135 °C. R_f = 0.13 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 249[M]$^+$. IR (KBr) 3250, 1500, 1490, 1385, 882, 843, 690 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.18 G, g = 2.00994.

3.2.4.5. 1-Oxyl-2-(4′-methylphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2e). Using the general procedure mentioned above 157 mg (80%) of the title compound was obtained. Mp 86–88 °C. R_f = 0.88 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 247[M]$^+$. IR (KBr) 2980, 1610, 1500, 1365, 810 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.22 G, g = 2.00993.

3.2.4.6. 1-Oxyl-2-(4′-methoxyphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2f). Using the general procedure mentioned above 203 mg (97%) of the title compound was obtained. Mp 89–92 °C. R_f = 0.88 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 263[M]$^+$. IR (KBr) 2830, 1600, 1355, 835 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.28 G, g = 2.00991.

3.2.4.7. 1-Oxyl-2-(2′,4′-dimethoxyphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2g). Using the general procedure mentioned above 199 mg (85%) of the title compound was obtained. Mp 75–77 °C. R_f = 0.37 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 293[M]$^+$. IR (KBr) 2830, 1610, 1365, 805, 870 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.32 G, g = 2.00989.

3.2.4.8. 1-Oxyl-2-(3′,4′-dimethoxyphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2h). Using the general procedure mentioned above 194 mg (83%) of the title compound was obtained. Mp 95–97 °C. R_f = 0.34 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 293[M]$^+$. IR (KBr) 2830, 1595, 1355, 870, 820 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.28 G, g = 2.00991.

3.2.4.9. 1-Oxyl-2-(3′,4′-methylendioxyphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2i). Using the general procedure mentioned above 185 mg (84%) of the title compound was obtained. Mp 97–99 °C. R_f = 0.75 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 277[M]$^+$. IR (KBr) 1600, 1365, 910, 1240, 1100 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_n = 8.28 G, g = 2.00990.

3.2.4.10. 1-Oxyl-2-(3′-hydroxy-4′-methoxyphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2j). Using the general procedure mentioned above 151 mg (68%) of the title compound was obtained. Mp 117–119 °C. R_f = 0.34 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 279[M]$^+$. IR (KBr) 3320, 2830, 1595, 1500, 1350, 820 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_n = 8.25 G, g = 2.00991.

3.2.4.11. 1-Oxyl-2-(3′-methoxy-4′-hydroxyphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2k). Using the general procedure mentioned above 158 mg (71%) of the title compound was obtained. Mp 83–85 °C. R_f = 0.38 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 279[M]$^+$. IR (KBr) 3340, 2830, 1590, 1345, 875, 820 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_n = 8.29 G, g = 2.00990.

3.2.4.12. 1-Oxyl-2-(4′-*N*,*N*-dimethylphen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2l). Using the general procedure mentioned above 147 mg (67%) of the title compound was obtained. Mp 151–154 °C. R_f = 0.51 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 276[M]$^+$. IR (KBr) 2850, 1595, 1500, 1360, 845 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.27 G, g = 2.00992.

3.2.4.13. 1-Oxyl-2-(2′-fluorophen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2m). Using the general procedure mentioned above 147 mg (67%) of the title compound was obtained. Mp 112–114 °C. R_f = 0.52 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 251[M]$^+$. IR (KBr) 1610, 1450, 1370, 1135, 770 cm^{-1}. Anal. Calcd for $C_{13}H_{16}N_2O_4$ C 62.14, H 6.42, N 11.15. Found C 62.23, H 6.58, N 11.09. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.15 G, g = 2.00996.

3.2.4.14. 1-Oxyl-2-(4′-chlorophen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2n). Using the general procedure mentioned above 240 mg (97%) of the title compound was obtained. Mp 103–105 °C. R_f = 0.67 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 267[M]$^+$. IR (KBr) 1590, 1500, 1365, 1090, 825 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.12 G, g = 2.00997.

3.2.4.15. 1-Oxyl-2-(4′-bromophen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2o). Using the general procedure mentioned above 231 mg (93%) of the title compound was obtained. Mp 89–91 °C. R_f = 0.85 ($CHCl_3$/CH_3OH, 10:1). EI-MS (*m*/*z*) 311[M]$^+$. IR (KBr) 3310, 1600, 1450, 1070, 825 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.12 G, g = 2.00997.

3.2.4.16. 1-Oxyl-2-(2′,4′-dichlorophen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2p). Using the general procedure mentioned above 212 mg (88%) of the title compound was obtained. Mp 123–125 °C. R_f = 0.54 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 302[M]$^+$. IR (KBr) 1590, 1450, 1365, 1000, 870, 825 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.15 G, g = 2.00997.

3.2.4.17. 1-Oxyl-2-(3′,4′-dichlorophen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2q). Using the general procedure mentioned above 214 mg (89%) of the title compound was obtained. Mp 120–122 °C. R_f = 0.54 ($CHCl_3$/CH_3OH, 20:1). EI-MS (*m*/*z*) 302[M]$^+$. IR (KBr) 1593, 1452, 1363, 1004, 871, 822 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.15 G, g = 2.00997.

3.2.4.18. 1-Oxyl-2-(2′-nitrophen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2r). Using the general procedure mentioned above 161 mg (73%) of the title compound was obtained. Mp 145–147 °C. R_f = 0.49 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 278[M]$^+$. IR (KBr) 1530, 1360, 1450, 740 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.14 G, g = 2.00997.

3.2.4.19. 1-Oxyl-2-(3′-nitrophen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2s). Using the general procedure mentioned above 173 mg (78%) of the title compound was obtained. Mp 161–162 °C. R_f = 0.69 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 278[M]$^+$. IR (KBr) 1530, 1350, 1600, 1450, 880, 790, 680 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.10 G, g = 2.00998.

3.2.4.20. 1-Oxyl-2-(4′-nitrophen-1′-yl)-3-oxide-4,4,5,5-tetramethylimidazoline (2t). Using the general procedure mentioned above 162 mg (73%) of the title compound was obtained. Mp 175–176 °C. R_f = 0.65 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 278[M]$^+$. IR (KBr) 1530, 1360, 1603, 1500, 1450, 830, 790, 680 cm^{-1}. ESR: a five-line pattern with intensity ratios of 1:2:3:2:1, a_N = 8.10 G, g = 2.00998.

3.2.5. General procedure for the preparation of 1-oxyl-2-substitutedphenyl-4,4,5,5-tetramethyl-imidazolines (3a–t). The solution of 1.0 mmol of 2-substitutedphenyl-4,4,5,5-tetramethylimidazoline-3-oxide-1-oxyl and 500 mg $NaNO_2$ in 15 ml of methanol was adjusted to pH 5 with hydrochloric acid. The reaction mixture was stirred at room temperature for 1 h and TLC ($CHCl_3/CH_3OH$, 20:1) indicated the complete disappearance of 2-substitutedphenyl-4,4,5,5-tetramethylimidazoline-3-oxide-1-oxyl. The reaction mixture was evaporated under vacuum to remove most of the solvent, adjusted to pH 6–7 with aqueous sodium bicarbonate, and extracted with chloroform. The combined chloroform phase was dried by anhydrous sodium sulfate and filtered. The filtrate was evaporated under vacuum to provide the title compound.

3.2.5.1. 1-Oxyl-2-(phen-1′-yl)-4,4,5,5-tetramethylimidazoline (3a). Using the general procedure mentioned above 171 mg (79%) of the title compound was obtained as red syrup. R_f = 0.61 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 217[M]$^+$. IR (KBr) 1602, 1455, 1353, 1072, 822 cm^{-1}. Anal. Calcd for $C_{13}H_{17}N_2O_3$: C, 71.86; H, 7.89; N, 14.89. Found: C, 71.69; H, 7.75; N, 12.99.

3.2.5.2. 1-Oxyl-2-(2′-hydroxylphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3b). Using the general procedure mentioned above 177 mg (76%) of the title compound was obtained as red syrup. R_f = 0.83 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 233[M]$^+$. IR (KBr) 1610, 1503, 1450, 760 cm^{-1}. Anal. Calcd for $C_{13}H_{17}N_2O_2$: C, 66.93; H, 7.35; N, 12.01. Found: C, 66.77; H 7.11; N, 12.33.

3.2.5.3. 1-Oxyl-2-(3′-hydroxylphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3c). Using the general procedure mentioned above 182 mg (78%) of the title compound was obtained as red syrup. R_f = 0.52 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 233[M]$^+$. IR (KBr) 3338, 1588, 1451, 1380, 880, 790 cm^{-1}. Anal. Calcd for $C_{13}H_{17}N_2O_2$: C, 66.93; H, 7.35; N, 12.01. Found: C, 66.76; H 7.52; N, 12.24.

3.2.5.4. 1-Oxyl-2-(4′-hydroxylphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3d). Using the general procedure mentioned above 150 mg (65%) of the title compound was obtained as yellow powder. Mp 98–100 °C. R_f = 0.36 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 233[M]$^+$. IR (KBr) 3259, 1595, 1492, 1382, 881 845, 693 cm^{-1}. Anal. Calcd for $C_{13}H_{17}N_2O_2$: C, 66.93; H, 7.35; N, 12.01. Found: C, 66.77; H 7.54; N, 12.25.

3.2.5.5. 1-Oxyl-2-(4′-methylphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3e). Using the general procedure mentioned above 200 mg (86%) of the title compound was obtained as yellow powder. Mp 156–157 °C. R_f = 0.93 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 231[M]$^+$. IR (KBr) 2985, 1606, 1503, 1368, 804 cm^{-1}. Anal. Calcd for $C_{14}H_{19}N_2O$: C, 72.69; H, 8.28; N, 12.11. Found: C, 72.80; H 8.42; N, 12.32.

3.2.5.6. 1-Oxyl-2-(4′-methoxyphen-1′-yl)-4,4,5,5-tetra-methylimidazoline (3f). Using the general procedure mentioned above 212 mg (86%) of the title compound was obtained as red powder. Mp 42–43 °C. R_f = 0.63 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 247[M]$^+$. IR (KBr) 2830, 1610, 1352, 840 cm^{-1}. Anal. Calcd for $C_{14}H_{19}N_2O_2$: C, 67.99; H, 7.74; N, 11.33. Found: C, 67.80; H 7.55; N, 11.55.

3.2.5.7. 1-Oxyl-2-(2′,4′-dimethoxylphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3g). Using the general procedure mentioned above 177 mg (64%) of the title compound was obtained as yellow powder. Mp 77–79 °C. R_f = 0.41 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 277[M]$^+$. IR (KBr) 2850, 1610, 1590, 1500, 805, 830 cm^{-1}. Anal. Calcd for $C_{15}H_{21}N_2O_3$: C, 64.96; H, 7.63; N, 10.10. Found: C, 64.78; H 7.49; N, 10.22.

3.2.5.8. 1-Oxyl-2-(3′,4′-dimethoxylphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3h). Using the general procedure mentioned above 138 mg (50%) of the title compound was obtained as yellow powder. Mp 74–75 °C. R_f = 0.43 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 277[M]$^+$. IR (KBr) 2833, 1592, 1351, 874, 822 cm^{-1}. Anal. Calcd for $C_{15}H_{21}N_2O_3$: C, 64.96; H, 7.63; N, 10.10. Found: C, 64.80; H 7.50; N, 10.41.

3.2.5.9. 1-Oxyl-2-(3′,4′-methylendioxyphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3i). Using the general procedure mentioned above 230 mg (81%) of the title compound was obtained as red powder. Mp 46–47 °C. R_f = 0.82 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 261[M]$^+$. IR (KBr) 3420, 1600, 1450, 1365, 1260, 935 cm^{-1}. Anal. Calcd for $C_{14}H_{17}N_2O_3$: C, 64.35; H, 6.56; N, 10.72. Found: C, 64.17; H 6.44; N, 10.91.

3.2.5.10. 1-Oxyl-2-(3′-hydroxy-4′-methoxyphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3j). Using the general

procedure mentioned above 118 mg (45%) of the title compound was obtained as yellow powder. Mp 70–71 °C. R_f = 0.45 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 263[M]$^+$. IR (KBr) 3327, 2831, 1605, 1502, 1351, 818 cm^{-1}. Anal. Calcd for $C_{14}H_{19}N_2O_3$: C, 63.86; H, 7.27; N, 10.64. Found: C, 63.69; H 7.15; N, 10.81.

3.2.5.11. 1-Oxyl-2-(3′-methoxyl-4′-hydroxyphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3k). Using the general procedure mentioned above 106 mg (40%) of the title compound was obtained as yellow syrup. R_f = 0.50 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 263[M]$^+$. IR (KBr) 3337, 2835, 1596, 1342, 878, 817 cm^{-1}. Anal. Calcd for $C_{14}H_{19}N_2O_3$: C, 63.86; H, 7.27; N, 10.64. Found: C, 63.71; H 7.35; N, 10.82.

3.2.5.12. 1-Oxyl-2-(4′-*N*,*N*-dimethylphen-1′-yl)-4,4,5,5-tetramethylimidazoline (3l). Using the general procedure mentioned above 123 mg (47%) of the title compound was obtained as yellow powder. Mp 85–86 °C. R_f = 0.67 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 260[M]$^+$. IR (KBr) 3341, 2855, 1598, 1502, 1367, 841 cm^{-1}. Anal. Calcd for $C_{15}H_{22}N_3O$: C, 69.20; H, 8.52; N, 16.14. Found: C, 69.38; H 8.45; N, 16.22.

3.2.5.13. 1-Oxyl-2-(2′-fluorophen-1′-yl)-4,4,5,5-tetramethylimidazoline (3m). Using the general procedure mentioned above 147 mg (62%) of the title compound was obtained as red syrup. R_f = 0.69 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 235[M]$^+$. IR (KBr) 3345, 2850, 1611, 1455, 1376, 1130, 772 cm^{-1}. Anal. Calcd for $C_{13}H_{16}N_2OF$ C 66.36, H 6.85, N 11.91. Found C 66.25, H 6.68, N 11.74.

3.2.5.14. 1-Oxyl-2-(4′-chlorophen-1′-yl)-4,4,5,5-tetramethylimidazoline (3n). Using the general procedure mentioned above 210 mg (83%) of the title compound was obtained as red syrup. R_f = 0.73 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 251[M]$^+$. IR (KBr) 3350, 1598, 1501, 1369, 1092, 821 cm^{-1}. Anal. Calcd for $C_{13}H_{16}N_2OCl$ C 62.03, H 6.41, N 11.13. Found C 62.14, H 6.60, N 11.24.

3.2.5.15. 1-Oxyl-2-(4′-bromophen-1′-yl)-4,4,5,5-tetramethylimidazoline (3o). Using the general procedure mentioned above 290 mg (98%) of the title compound was obtained as red powder. Mp 91–92 °C. R_f = 0.95 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 296[M]$^+$. IR (KBr) 3351, 1590, 1452, 1480, 1369, 840 cm^{-1}. Anal. Calcd for $C_{15}H_{16}N_2OBr$ C 52.72, H 5.44, N 9.46. Found C 52.52, H 5.67, N 9.67.

3.2.5.16. 1-Oxyl-2-(2′,4′-dichlorophen-1′-yl)-4,4,5,5-tetramethylimidazoline (3p). Using the general procedure mentioned above 255 mg (90%) of the title compound was obtained as red powder. Mp 96–97 °C. R_f = 0.66 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 286[M]$^+$. IR (KBr) 3351, 1598, 1459, 1362, 873, 824 cm^{-1}. Anal. Calcd for $C_{13}H_{16}N_2Ocl_2$ C 54.56, H 5.28, N 9.79. Found C 54.45, H 5.22, N 9.64.

3.2.5.17. 1-Oxyl-2-(3′,4′-dichlorophen-1′-yl)-4,4,5,5-tetramethylimidazoline (3q). Using the general procedure mentioned above 215 mg (75%) of the title compound was obtained as red powder. Mp 66–67 °C. R_f = 0.71 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 286[M]$^+$. IR (KBr) 3348, 1597, 1456, 1369, 1005, 873, 820 cm^{-1}. Anal. Calcd for $C_{13}H_{16}N_2Ocl_2$ C 54.56, H 5.28, N 9.79. Found C 54.43, H 5.20, N 9.68.

3.2.5.18. 1-Oxyl-2-(2′-nitrophen-1′-yl)-4,4,5,5-tetramethylimidazoline (3r). Using the general procedure mentioned above 189 mg (72%) of the title compound was obtained as yellow powder. Mp 74–75 °C. R_f = 0.52 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 262[M]$^+$. IR (KBr) 3343, 1604, 1500, 1452, 1361, 740 cm^{-1}. Anal. Calcd for $C_{13}H_{16}N_3O_3$ C 59.53, H 6.15, N 16.02. Found C 59.69, H 6.30, N 16.18.

3.2.5.19. 1-Oxyl-2-(3′-nitrophen-1′-yl)-4,4,5,5-tetramethylimidazoline (3s). Using the general procedure mentioned above 191 mg (73%) of the title compound was obtained as yellow powder. Mp 71–72 °C. R_f = 0.78 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 262[M]$^+$. IR (KBr) 3348, 1604, 1533, 1452, 1359, 810, 791, 684 cm^{-1}. Anal. Calcd for $C_{13}H_{16}N_3O_3$ C 59.53, H 6.15, N 16.02. Found C 59.66, H 6.28, N 16.16.

3.2.5.20. 1-Oxyl-2-(4′-nitrophen-1′-yl)-4,4,5,5-tetramethylimidazoline (3t). Using the general procedure mentioned above 186 mg (71%) of the title compound was obtained as red powder. Mp 119–120 °C. R_f = 0.74 ($CHCl_3/CH_3OH$, 20:1). EI-MS (*m*/*z*) 262[M]$^+$. IR (KBr) 3340, 1604, 1520, 1500, 1452, 860, 793, 681 cm^{-1}. Anal. Calcd for $C_{13}H_{16}N_3O_3$ C 59.53, H 6.15, N 16.02. Found C 59.40, H 6.08, N 15.97.

3.3. Pain threshold in vivo assay

Male ICR mice weighing 25 ± 2 g were housed in a 12/12 light/dark cycle at a room temperature of 21 ± 2 °C for one day before being used. Each of them in drug receiving groups was given a single intraperitoneal (ip) injection of one of **3a**–**t** at a level of 0.13 mmol/kg in 0.2 ml of saline mixed with 0.01 ml DMSO and 0.02 ml Tween 80. The mouse in control group was given the same mixed solvent as the drug receiving groups received. Analgesic effects of **3a**–**t** were evaluated by the tail flick test. The basic pain threshold value of each mouse was measured for 4 times.

After **3a**–**t** were administered, the pain thresholds were measured at 30 min intervals. This measurement was carried out for 150 min. The potency of analgesia was indicated by the pain threshold variation. The values were calculated according to equation PTV = AAPT/BPT wherein PTV = pain threshold variation, BPT = basic pain threshold and AAPT = pain threshold after administration-basic pain threshold. All values of the pain threshold variation for each animal were averaged and constituted one sample.

3.4. Tail bleeding time in vivo assay

Male ICR mice weighing 25 ± 2 g were housed in a 12/12 light/dark cycle at a room temperature of 21 ± 2 °C

for one day before being used. Each of them in drug receiving groups was given a single intraperitoneal (ip) injection of one of **3a**–**t** at a level of 1.30 mmol/kg in 0.2 ml of saline mixed with 0.01 ml DMSO and 0.02 ml Tween 80. The mouse in control group was given the same mixed solvent as the drug receiving groups received. At 5, 15, 30, and 60 min, a mouse was placed in a tube holder with its tail protruding, and a 2 mm cut was made on the tail. Flowing blood until it stopped was gently wiped away with a filter paper every 30 s, yielding the bleeding time (BT). The assay reported by Dejana et al.[15] was employed.

3.5. Vasorelaxation in vitro assay of 3a–t

The conversion of **3a**–**t** during vasoconstriction induced by NE was examined using the rat aortic strip according to a published method.[13,14] In brief, immediately after decapitation, rat aortic strips were obtained and put in a perfusion bath with 5 ml of warmed (37 °C), oxygenated (95%O_2, 5%CO_2) Krebs solution (pH 7.4). The aortic strips were mounted to the tension transducers and the relaxation-contraction curves were recorded. Noradrenaline (NE, final concentration 10^{-9} mol/l) solution was added to induce contraction. When the hypertonic contraction reached to the maximum level, NE was washed and the vessel strips were stabilized for 30 min. After the renewal of the solution, NE (final concentration 10^{-9} mol/l), was added. When the hypertonic contraction value of aortic strips reached the peak, 15 μl of alcohol (vehicle), or the solution of **3a**–**t** in 15 μl of alcohol (final concentration 5×10^{-5} mol/l) were added, respectively, to observe the relaxant response of the aortic strip to them. The percentage inhibitions of NE-induced vasoconstriction by **3a**–**t** were recorded.

3.6. QSAR analysis

To perform a QSAR analysis, first the SMILES formats of compounds **3a**–**t** were generated using JME server (http://www.molinspiration.com/cgi-bin/properties). Moreover, the obtained SMILES files were submitted to e-dragon server (http://www.vcclab.org/lab/edragon/) to calculate molecular descriptors. Finally, the 1664 different molecular parameters for each compound were obtained.

The multiple linear regression method was employed to derive the QSAR equation of **3a**–**t**. Concerning the analgesic effect of a molecule, the PTV value measured at 90 min (i.e., PTV_{90}) was considered in the current QSAR analysis. As the number of compounds is only 20, the optimum number of molecular descriptors used in the QSAR analysis is around 4. To obtain the best regression equation, two-step descriptor selection procedure was applied. The first step consisted in the elimination of the descriptors that are not able to provide useful statistical information. For instance, the absolute value of the correlation coefficient between the selected descriptor (X_i) and the activity (PTV_{90}) under investigation ($|\mathrm{cor}(X_i,PTV_{90})|$) should be larger than 0.3. At the second step, the descriptor subset was optimized by means of a genetic algorithm.[27] Finally, the optimal QSAR equation describing the analgesic effects of **3a**–**t** was determined. The definitions of the selected molecular descriptors in the final QSAR equation are summarized in Table 5.

To systematically assess a QSAR model, a reliable validation is required. Usually, a QSAR model is evaluated by the predictive results for a training dataset (resubstitution test) and a testing dataset (cross-validation), respectively. By the test of resubstitution, the PTV_{90} value of each compound in a training data set is predicted using the rules derived from the same set. Although this test gives somewhat optimistic error estimate because the same compounds are used to derive the QSAR equation and to predict themselves, the resubstitution test is absolutely necessary due to its ability to reflect the self-consistency of a given method. On the other hand, a cross-validation test for an independent testing data set is also needed because it can reflect the real effectiveness of a QSAR model. Of the various cross-validation tests, the LOO test is thought to be a reliable one. By the LOO test, the PTV_{90} value of each compound in the data set is predicted by the rules derived using all other compounds except the one that is being predicted. Both tests of resubstitution and LOO were used to evaluate the QSAR models in the current work.

In the resubstitution test, three statistical parameters (R, S, and $\bar{e}$) were used to evaluate the performance. The regression coefficient R is defined as follows:

$$R = \sqrt{1 - \sum_{i=1}^{N}(y_i - \hat{y}_i)^2 / \sum_{i=1}^{N}(y_i - \bar{y})^2} \qquad (2)$$

where y_i, $\hat{y}_i$, and $\bar{y}$ are predicted, actual, and mean values of the activity (i.e., PTV_{90}), respectively. The N is the number of compounds in the resubstitution test. The root mean square of errors S was calculated with the following equation:

$$S = \sqrt{\frac{\sum_{i=1}^{N}(y_i - \hat{y}_i)^2}{N}} \qquad (3)$$

To illustrate the predictive accuracy more explicitly, the $\bar{e}$ (absolute average error) is defined as follows:

Table 5. The four molecular descriptors used in the current QSAR analysis[a]

Abbreviation	Type	Definition
GATS4e	2D autocorrelation indices	Geary autocorrelation – lag 4/weighted by atomic Sanderson electronegativities
BELe1	Burden eigenvalue descriptors	Lowest eigenvalue n. 1 of Burden matrix/weighted by atomic Sanderson electronegativities
JGI1	Topological charge indices	Mean topological charge index of order1
R2u	GETAWAY	R autocorrelation of lag 2/unweighted

[a] The definitions of these four descriptors were downloaded from the website of e-dragon (http://www.vcclab.org/lab/edragon/).

$$\bar{e} = \frac{\sum_{i=1}^{N} |y_i - \hat{y}_i|}{N} \quad (4)$$

In the LOO test, the above three statistical parameters, that is, R_{LOO},S_{LOO}, and $\bar{e}_{LOO}$, were also calculated.

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