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A class of novel Schiff's bases: Synthesis, therapeutic action for chronic pain, anti-inflammation and 3D QSAR analysis

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ABSTRACT

To discover analgesics for treating chronic pain 17 novel Schiff's bases, *N,N′-(Z*-allylidene-1,3-diyl)bisamino acid methyl esters were prepared from 1,1,3,3,-tetramethoxypropane and amino acid methyl esters. On tail-flick mouse model 20 μmol/kg of these Schiff's bases were orally administered, the analgesic action started 30 min after administration, reached the maximum 120 min after administration, and at 180 min this action was still observed. On a xylene-induced ear edema mouse model 20 μmol/kg of these Schiff's bases exhibited desirable anti-inflammation. Thus the present Schiff's bases are able to treat chronic pain from inflammation. The effect of the side chains of the amino acid residues of these Schiff's bases on the analgesic activity was explained with 3D QSAR.

1. Introduction

The treatments with simple analgesics or traditional agents are generally resisted by chronic pain from various etiologies including inflammation and neural destruction. Pain from neural destruction is accompanied by the hypersensitivity to mechanical and/or thermal stimuli.[1] Pain from inflammation may be accompanied by various painful responses to peripheral tissue injury.[2,3] Due to the relative lack of response to current analgesics, chronic pain represents an unmet medical need.

The biologically active Schiff's bases and derivatives are known as the leads of anti-microbial agents,[4–7] anti-virus agents,[8,9] anti-oxidants,[10] radical inhibitors,[11] anti-tumor agents,[12,13] carbonic anhydrase inhibitors,[14] xanthine oxidase inhibitors,[15] anti-bacterials,[16–19] plant-growth regulators,[20] free radical scavengers,[21] trypsin inhibitors,[22] inhibitors of cartilage matrix degeneration,[23] 5-HT6 antagonists,[24] anti-inflammatory agents[24,25] and analgesics.[26,27]

To develop the analgesics for the treatment of chronic pain and inflammation great attention has been paid to Schiff's bases and the compounds of Figure 1 are used as the leads. These leads are characterized by aromatic substitutions and the high doses. In an attempt to discover new, safer and potent Schiff's bases for treating the chronic pain and inflammatory diseases we changed the aromatic substitution to amino acid substitution, prepared a class of novel *N,N′-(Z*-allylidene-1,3-diyl)bisamino acid methyl esters via the amination of 1,1,3,3,-tetramethoxypropane (TMP) and amino acid methyl esters, evaluated the orally analgesic activities on tail-flick mouse model, measured the oral anti-inflammatory activities on xylene-induced ear edema mouse model, and analyzed the effect of the side chains of the amino acid residues on the analgesic activities with 3D QSAR.

2. Results and discussion

2.1. Preparation of *N,N′-(Z*-allylidene-1,3-diyl)bisamino acid methyl esters

In the preparation of *N,N′-(Z*-allylidene-1,3-diyl)bisamino acid methyl esters (**2a–q**) a two-step-route and the corresponding reaction conditions were used (Scheme 1).

According to a general method the solution of amino acid in methanol was treated with thionyl chloride to form 17 corresponding amino acid methyl esters (**1a–q**) in 85–99% yields. The amination of TMP and **1a–q** resulted in **2a–q**. The yields of **2a–q** are 27–85%. These data demonstrated that by using this two-step-route **2a–q** could be obtained in satisfactory yield. The structural features and yields of both **1a–q** and **2a–q** are summarized in Table 1.

2.2. Configuration assignment of 2a–q

To ensure the *Z* configuration assignment of **2a–q**, the chemical shifts and the coupling constants of the two protons on their

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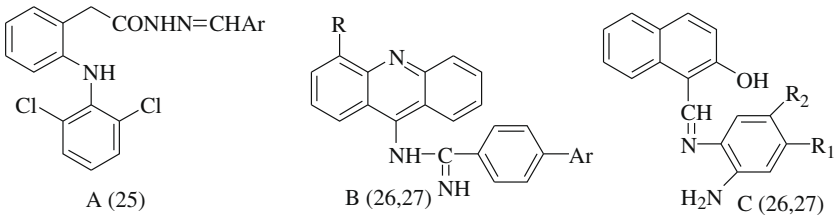
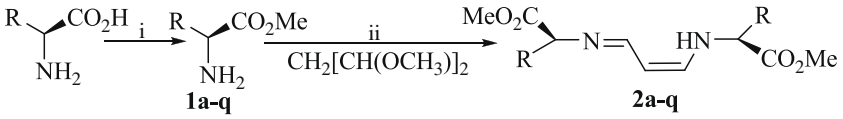
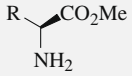
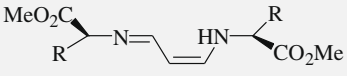
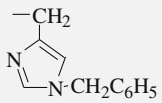
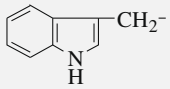


Figure 1. Schiff's bases, A–C,[25–27] for treating chronic pain and inflammation of mice.



Scheme 1. Synthetic route of *N*,*N*′-(*Z*-allylidene-1,3-diyl)bisamino acid methyl esters. Reagents: (i) thionyl chloride and methanol; (ii) glacial acetic acid.

Table 1
Structural features and yields of **1a–q** and **2a–q**

Compd	 **1,** Yield %	R	 **2,** Yield %
a	85	$-CH_3$	64
b	90	$-(CH_2)_3NHC(NH)NHNO_2$	41
c	80	$-CH_2SCH_2C_6H_4-CH_3$-p	74
d	92	$-CH_2CO_2CH_3$	53
e	96	H	46
f	98	$-CH_2CH_2CO_2CH_3$	21
g	85		85
h	88	$-(CH_2)_4NHCOOCH_2C_6H_5$	83
i	92	$-CH(CH_3)CH_2CH_3$	79
j	94	$-CH_2CH(CH_3)_2$	76
k	96	$-CH_2CH_2SCH_3$	44
l	99	$-CH_2C_6H_5$	51
m	98	$-CH_2OCH_2C_6H_5$	38
n	87	$-CH(CH_3)OCH_2C_6H_5$	85
o	89	$-CH(CH_3)_2$	60
p	90		72
q	92	$-CH_2C_6H_4-OH$-p	74

carbon–carbon double bond were examined. The ^1H NMR spectra indicate that the chemical shifts of one of these protons range from 5.86 to 6.30 ppm, while the chemical shifts of another proton of these protons range from 5.00 to 5.15 ppm. This means that these two protons are olefinic one. The coupling constants of these two protons range from 7.3 to 10.5, which are less than 12. This means that these two olefinic protons locate at the same side of the carbon–carbon double bond, that is, this carbon–carbon double bond possesses *Z* configuration. To further confirm this *Z* configuration the nuclear Overhauser effect (NOE) experiments were performed for **2a–q**. The positive NOE effects were observed between the two α-protons, as well as the two α-protons and the methoxy groups of the two amino acid methyl ester residues of each Schiff's base. This indicates that the two amino acid methyl ester residues in each Schiff's base are at the same side and the carbon–carbon double bond of these Schiff's bases is in a *Z*-configuration.

2.3. In vivo orally analgesic activities of 2a–q

On tail-flick mouse model the in vivo analgesic activities of **2a–q** were measured. The pain threshold variations stand for the activities and are listed in Table 2. As positive control the measured pain threshold variations of the mice receiving 20 μmol/kg of morphine (ip) or 165 μmol/kg of aspirin are also listed in Table 2.[28] The data indicate that 30 min after oral administration **2a–q** started to increase the pain threshold of the treated mice, 120 min after oral administration the pain threshold reached the maximum and the analgesic action was still noticed 180 min after oral administration. The maximal increases of pain threshold for **2a,i,j,l**, **2b,c,e,m,n**, **2f,g,k,q** and **2d**,**h,o,p** are 41–53%, 30–37%, 20–25% and 7–16%, respectively. The orally analgesic potencies of 20 μmol/kg of these Schiff's bases are higher than that of 165 μmol/kg of aspirin.

2.4. Dose dependence of in vivo analgesic activities of 2a

To observe the effect of the dose on the analgesic activities of **2a–q** the most potent **2a** were measured at three doses (20, 2 and 0.2 μmol/kg). In the dose-dependent responses TMP and Ala-OMe were used as the references and the data are listed in Table 3. It was noticed that with the increase of the dose the pain threshold of the mice increases, that is, the pain thresholds of 20, 2

Table 2
Pain threshold variation of mice receiving **2a–q**

Compd[a]	Pain threshold variation ($x \pm SD\%$) at					
	30 min	60 min	90 min	120 min	150 min	180 min
NS	8.30 ± 2.90	13.74 ± 2.28	11.68 ± 2.66	12.01 ± 2.19	11.71 ± 2.13	13.73 ± 2.14
Morphine	147.00 ± 47.50	177.60 ± 84.70	122.70 ± 59.80	64.90 ± 49.60	36.70 ± 41.30	32.90 ± 31.30
Aspirin	12.88 ± 1.27[b]	18.69 ± 1.49[b]	19.79 ± 0.53[b]	16.46 ± 0.90[b]	14.59 ± 0.48[b]	14.65 ± 2.65
2a	32.26 ± 4.73[b]	32.49 ± 4.16[b]	48.40 ± 5.90[b]	53.33 ± 6.52[b]	44.71 ± 5.74[b]	42.29 ± 5.65[b]
2b	27.71 ± 3.99[b]	33.98 ± 4.44[b]	36.80 ± 5.76[b]	37.20 ± 5.30[b]	32.25 ± 4.03[b]	29.48 ± 3.00[b]
2c	20.91 ± 3.47[b]	21.76 ± 2.45[b]	25.93 ± 3.68[b]	30.75 ± 4.91[b]	31.37 ± 4.15[b]	32.43 ± 4.27[b]
2d	10.31 ± 4.46	13.56 ± 4.53	11.90 ± 2.88	7.12 ± 4.71	4.20 ± 4.26	7.15 ± 4.64
2e	14.52 ± 2.51[c]	14.25 ± 2.06	27.33 ± 3.96[b]	34.77 ± 4.66[b]	33.74 ± 3.43[b]	18.16 ± 2.31[c]
2f	26.23 ± 3.51[b]	25.30 ± 3.39[b]	21.45 ± 2.78[c]	20.21 ± 3.61[c]	29.11 ± 3.85[b]	17.80 ± 2.29[c]
2g	19.61 ± 2.89[b]	21.94 ± 3.02[c]	25.64 ± 3.65[b]	25.32 ± 3.30[b]	22.31 ± 2.23[b]	20.09 ± 2.85[c]
2h	16.38 ± 2.24[b]	21.97 ± 2.29[b]	27.80 ± 3.52[b]	16.26 ± 2.73	15.84 ± 2.45	14.21 ± 2.50
2i	18.66 ± 2.36[b]	19.68 ± 2.46[c]	34.31 ± 4.21[b]	41.65 ± 4.58[b]	34.89 ± 3.85[b]	32.75 ± 3.45[b]
2j	26.52 ± 3.36[b]	29.79 ± 3.63[b]	40.37 ± 4.93[b]	45.12 ± 5.10[b]	42.38 ± 4.05[b]	41.84 ± 4.16[b]
2k	28.73 ± 3.52[b]	29.93 ± 3.72[b]	31.61 ± 3.45[b]	23.37 ± 2.17[b]	22.05 ± 2.74[b]	21.18 ± 4.53[c]
2l	28.22 ± 3.59[b]	28.92 ± 3.93[b]	35.82 ± 4.45[b]	40.67 ± 5.64[b]	35.33 ± 3.92[b]	30.66 ± 3.04[b]
2m	32.18 ± 3.99[b]	34.87 ± 4.63[b]	36.24 ± 4.43[b]	37.48 ± 4.13[b]	34.17 ± 4.12[b]	33.33 ± 4.74[b]
2n	23.15 ± 2.65[b]	24.86 ± 2.54[b]	28.03 ± 3.89[b]	30.33 ± 3.73[b]	28.39 ± 3.64[b]	26.55 ± 2.70[b]
2o	15.36 ± 2.46[c]	18.68 ± 2.54	18.37 ± 2.83[c]	11.10 ± 2.34	11.27 ± 2.86	11.57 ± 2.60
2p	21.40 ± 2.13[b]	20.87 ± 2.42[b]	14.84 ± 2.61	14.95 ± 2.95	14.54 ± 2.69	14.04 ± 2.25
2q	13.8 ± 2.19[c]	13.13 ± 2.30	23.86 ± 2.75[b]	23.47 ± 2.44[b]	24.45 ± 2.12[b]	23.06 ± 2.32[b]

[a] The statistical analyses are carried out for the data of same time point, $n = 12$, NS = vehicle, pain threshold variation is represented by mean ± SD%; Oral dose of **2a–q** = 20 μmol/kg; Intraperitoneal dose of morphine = 20 μmol/kg; Oral dose of aspirin = 165 μmol/kg.
[b] Compared to NS group, $p < 0.01$.
[c] Compared to NS group, $p < 0.05$.

Table 3
Dose-dependent effect of **2a** on the pain threshold variation of the treated mice

Compd[a]	Pain threshold variation ($\bar{X} \pm SD\%$)					
	30 min	60 min	90 min	120 min	150 min	180 min
NS	8.30 ± 2.90	13.74 ± 2.28	11.68 ± 2.66	12.01 ± 2.19	11.71 ± 2.13	13.73 ± 2.14
TMP	6.22 ± 2.35	11.84 ± 2.07	12.15 ± 2.66	11.93 ± 2.51	11.80 ± 2.60	11.89 ± 2.55
AM	7.37 ± 2.03	12.66 ± 2.51	12.02 ± 2.74	11.55 ± 2.37	11.01 ± 2.22	11.44 ± 2.38
TMP + AM	8.55 ± 2.77	13.60 ± 2.54	12.42 ± 2.81	12.34 ± 2.41	12.18 ± 2.57	12.61 ± 2.43
2a						
H	32.26 ± 4.73[b]	32.49 ± 4.16[b]	48.40 ± 5.90[b]	53.33 ± 6.52[b]	44.71 ± 5.74[b]	42.29 ± 5.65[b]
M	24.58 ± 2.89[c]	25.00 ± 3.01[c]	30.94 ± 4.05[c]	35.87 ± 4.22[c]	28.25 ± 3.13[c]	27.31 ± 3.24[c]
L	12.24 ± 2.02[e]	16.79 ± 2.11[e]	17.41 ± 2.34[d]	22.08 ± 2.45[d]	20.27 ± 2.67[d]	18.80 ± 2.00[d]

[a] The statistical analyses are carried out for the data of same time point, $n = 12$, TMP's dose = 20 μmol/kg, AM = Ala-OMe and dose = 20 μmol/kg; Dose of **2a**: H = 20 μmol/kg, M = 2 μmol/kg and L = 0.2 μmol/kg.
[b] Compare to 2 μmol/kg group, $p < 0.01$.
[c] Compare to 0.2 μmol/kg group, $p < 0.01$.
[d] Compare to NS group, $p < 0.01$.
[e] Compare to NS group, $p < 0.05$.

and 0.2 μmol/kg of **2a** treated mice show significant differences. The differences may last 180 min. TMP, Ala-OMe and the mixture of them lack analgesic action. This demonstrates that the analgesic activity of **2a** is independent of the possible metabolites.

2.5. In vivo anti-inflammatory activities of 2a–q

Schiff's bases **2a–q** were evaluated for their anti-inflammatory activity on a xylene-induced ear edema model of mouse. In this in vivo assay the solution of **2a–q** in NS were administrated orally. Each Schiff's base was initially tested at a dose of 20 μmol/kg. It was observed that all Schiff's bases showed significant inhibition against xylene-induced inflammation in mice as compared with NS (Table 4), indicating that **2a–q** possess potent anti-inflammatory action. It was especially noted that 20 μmol/kg of **2i,j** exhibited even higher anti-inflammatory activities than 165 μmol/kg of aspirin. Subsequently, the most potent **2a,i,j,l** were administrated in two lower doses to enable a detailed pharmacological activity profile (Table 5). The data indicate that they dose-dependently offer anti-inflammatory action, and even at a dose of 5 μmol/kg they still possess anti-inflammatory action.

Table 4
Anti-inflammatory activities of **2a–q** against xylene-induced ear edema in mice [a]

Compd	Edema weight	Inhibition%	Compd	Edema weight	Inhibition%
NS	94.18 ± 11.72	—	Aspirin	32.90 ± 5.75[c]	65.08
2a	44.20 ± 8.72[c]	53.09	**2j**	10.73 ± 2.90[b]	88.61
2b	61.55 ± 10.01[c]	34.65	**2k**	70.31 ± 10.71[c]	25.35
2c	55.10 ± 10.50[c]	41.50	**2l**	59.60 ± 10.29[c]	36.75
2d	82.21 ± 12.75[d]	12.71	**2m**	42.38 ± 8.01[c]	55.00
2e	49.22 ± 9.36[c]	47.74	**2n**	46.81 ± 8.90[c]	50.30
2f	65.91 ± 11.21[c]	30.02	**2o**	80.37 ± 10.94[c]	14.66
2g	69.11 ± 11.09[c]	26.62	**2p**	82.29 ± 11.00[d]	12.62
2h	79.60 ± 11.84[c]	15.48	**2q**	72.22 ± 10.41[c]	23.32
2i	21.10 ± 4.31[b]	77.61			

[a] NS = vehicle, $n = 12$, Dose of **2a–q** = 20 μmol/kg, dose of aspirin = 165 μmol/kg.
[b] Compared to NS and aspirin $p < 0.01$.
[c] Compared to NS, $p < 0.01$.
[d] Compared to NS, $p < 0.05$.

2.6. 3D QSAR analysis of 2a–q

To elucidate the effects of the side chain of amino acid on the analgesic action of **2a–q** their activities were converted into the

Table 5
Anti-inflammatory activities of **2a,i,j,l** at different doses[a]

Compd	Dose (μmol/kg)	Edema weight	Inhibition%
NS	—	94.18 ± 11.72	—
Aspirin	165	32.90 ± 5.75	65.08
2a	20	44.20 ± 8.72[b]	53.09
	10	54.73 ± 9.05[c]	41.89
	5	80.26 ± 10.01	14.78
2i	20	21.10 ± 4.31[b]	77.61
	10	30.09 ± 4.75[c]	68.05
	5	48.15 ± 8.01	48.87
2j	20	10.73 ± 2.90[b]	88.61
	10	18.01 ± 3.64[c]	80.88
	5	25.34 ± 3.89	73.09
2l	20	59.60 ± 11.29[b]	36.75
	10	70.26 ± 10.88[c]	25.40
	5	82.00 ± 10.95	12.93

(a) NS = vehicle, $n = 12$; For **2a**, (b) compared to NS and 10 μmol/kg $p < 0.05$, (c) compared to NS and 5 μmol/kg $p < 0.05$; For **2i** and **2j**, (b) compared to NS and 10 μmol/kg $p < 0.01$, (c) compared to NS and 5 μmol/kg $p < 0.01$; For **2l**, (b) compared to NS and 10 μmol/kg $p < 0.05$, (c) compared to NS and 5 μmol/kg $p < 0.05$.

corresponding pain threshold variation. For 3D QSAR the training set (**2a–i,k,l,n,o,q**)/test set (**2j,m,p**) selections were done manually such that they populate the wide range of analgesic activities in similar proportions.

2.6.1. Alignment of 2a–i,k,l,n,o,q

To establish valid 3D-QSAR model, a proper alignment procedure of the training set (**2a–i,k,l,n,o,q**) was practiced by use of target model align strategy in the align module within Cerius[2]. Based on the assumption that each structure exhibits activity at the same binding site of the receptor, the training set was aligned in a pharmacological active orientation. To obtain a consistent alignment, *N,N′-(Z*-allylidene-1,3-diyl)bisglycine methyl ester was selected as the template for superposing the training set. The method used for performing the alignment was Maximum Common Subgraph (MCS).[29] MCS looks at molecules as points and lines, and uses the techniques out of graph theory to identify the patterns. MCS then finds the largest subset of atoms in *N,N′-(Z*-allylidene-1,3-diyl)bisglycine methyl ester that shared by the training set, which was used for the alignment. A rigid fit of atom pairings was performed to superimpose each structure onto the target model *N,N′-(Z*-allylidene-1,3-diyl)bisglycine methyl ester. The stereoview of the training set is shown in Figure 2, which explores that to superimpose onto *N,N′-(Z*-allylidene-1,3-diyl)bisglycine methyl ester each structure has to take individual conformation. It is the individual conformation affects on the analgesic activity.

2.6.2. MFA based Cerius[2] QSAR module of 2a–i,k,l,n,o,q

Molecular Field Analysis (MFA) was performed for the training set using QSAR module of Cerius[2].[29] A five-step-procedure, conformer generation, energy minimization, atom match and molecule alignment, preference setting, as well as regression analysis, was automatically carried out in MFA. By use of proton, methyl and hydroxyl anion as probes, electrostatic and steric fields of molecules were created. These fields were sampled at each point of a regularly spaced grid of 1 Å. An energy cut-off of ±30.0 kcal/mol was set for both electrostatic and steric fields. Totally 672 grid points were generated. Among spatial and structural descriptors such as dipole moment, polarizability, radius of gyration, number of rotatable bonds, molecular volume, principal moment of inertia, *A*log P98, number of hydrogen bond donors and acceptors, and molar refractivity, only the proton, methyl and hydroxyl anion descriptors were used. Regression analysis was performed by use of Genetic Partial Least Squares (G/PLS) method consisted of 50,000 generations with 100-population size. The numbers of the independent variables were set to 5. Cross-validation was performed with the leave-one-out procedure. PLS analysis was scaled, with all variables normalized to a variance of 1.0.

Molecular Field Analysis (MFA) was performed for the training set using QSAR module of Cerius[2]. Based on the module, the regions where variations in electrostatic or steric features of the training set lead to the increase or decrease of pain threshold variation were specified as Figure 3.

MFA model for the training set increasing activity in terms of the most relevant descriptors including proton, methyl and hydroxyl anion is expressed with Eq. 1. In Eq. 1, the data points (n), correlation coefficient (r), square correlation coefficient (r^2) were 14, 0.983, and 0.966, respectively. The parameters of Eq. 1 indicated that in Molecular Field by using proton, methyl and hydroxyl anion as the descriptors the calculated analgesic activity highly fits the experimental one. The correlation of tested activities on tail-flick mouse model and calculated activities from Eq. 1 is shown with Figure 4.

$$\begin{aligned}\text{Activity} = {} & 29.7626 - 0.751778(H^+/753) \\ & - 0.690047(CH_3/1094) + 0.621014(CH_3/864) \\ & - 0.397531(CH_3/711) + 1.0224(HO^-/711) \end{aligned} \quad (1)$$

Eq. 1 contains 1 term from proton descriptor, 3 terms from methyl descriptor, and 1 term from hydroxyl anion descriptor. Term $0.751778(H^+/753)$ has negative coefficient, which means that at this position electron-withdrawing group decreases the activity. Term $0.621014(CH_3/864)$ has positive coefficient, which means that at this position large group increases the activity, while terms $0.690047(CH_3/1094)$ and $0.397531(CH_3/711)$ have negative coefficients, which means that at these positions small groups increase

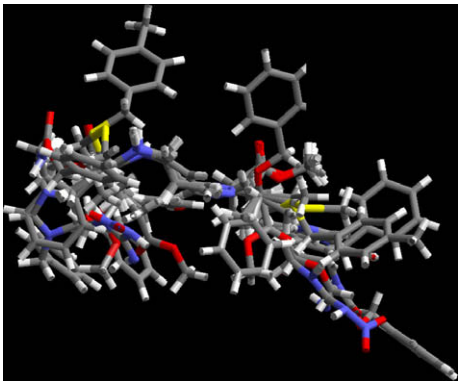


Figure 2. Alignment stereoview of **2a–i,k,l,n,o,q** generating molecular field.

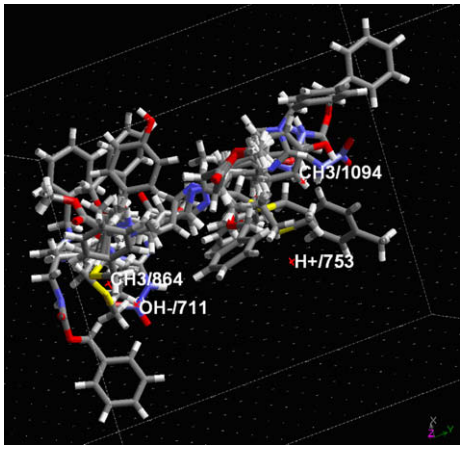


Figure 3. Steric and electrostatic features of **2a–i,k,l,n,o,q** leading to changing pain threshold variation.

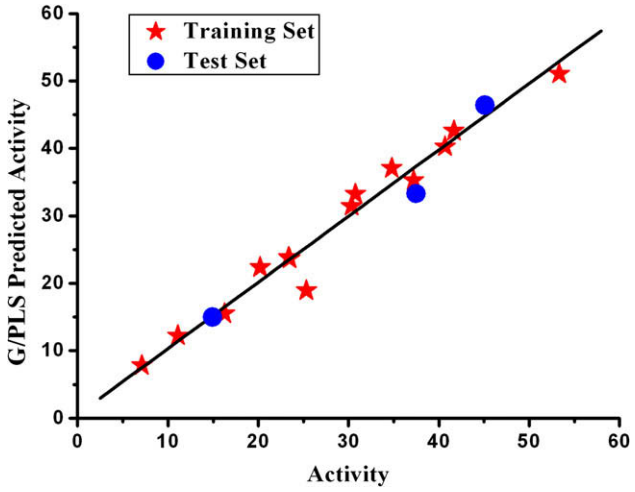


Figure 4. Graph of tested versus predicted analgesic activities of **2a–q**.

the activity. Term 1.0224(HO^-/711) has positive coefficient, which means that at this position hydrogen bond forming group increases the activity.

Figure 5 gives **2b**,**d** as diagrammatic examples of Eq. 1. Schiff's base **2b** has a large group near CH_3/864 region, a small group near CH_3/1094 region, and a hydrogen bond forming groups near HO^-/711 region and therefore possesses higher analgesic activity. Schiff's base **2d** has a large group near CH_3/864 region, a electron-withdrawing group near H^+/753 region, and no hydrogen bond forming group near HO^-/711 region and therefore possesses higher analgesic activity.

2.6.3. Predicting analgesic activities of 2j,m,p with Eq. 1

Predict power of equation 1 was demonstrated by comparing calculated and tested analgesic activities of the test set (**2j,m,p**, Table 6). The correlations of predict and test values are also shown in Figure 3. The results indicate that Eq. 1 rationally gives analgesic activities for the test set and the errors range from +0.04 to −4.14. Calculated activity is so approximate to experimental activity means that Eq. 1 is practical to accurately predict analgesic activity of bisamino acid modified Schiff's base.

3. Conclusions

Due to the relative lack of response to current analgesics the chronic pain from inflammation represents an unmet medical need. *N*,*N*′-(*Z*-Allylidene-1,3-diyl)bisamino acid methyl esters, the Schiff's bases provided here, possess both oral analgesic and anti-inflammatory actions, thus they should be capable of treating the chronic pain from inflammation. Comparing with the orally efficacious dose (53–171 μmol/kg) of the aromatic ring modified Schiff's bases reported in the literature,[25–27] the orally efficacious dose (20 μmol/kg) of the present Schiff's bases is 2.5–8.6-fold lower. Comparing with the orally efficacious dose (165 μmol/kg) of aspirin, the orally efficacious dose (20 μmol/kg) of the present Schiff's bases is 8.3-fold lower. The duration of the oral analgesic action of the present Schiff's bases is generally longer than 180 min. However the analgesic potency of the present Schiff's bases is significantly lower than that of morphine. Thus to enhance analgesic potency of the present Schiff's bases the future modification should be presented.

Table 6
Predict and test analgesic activities of **2j,m,p**

Compd	Pain threshold variation ($x \pm$ SD%)			
	Predict value	Test value	Error	Error%
2j	46.40	45.12	+1.28	+2.8
2m	33.29	37.48	−4.14	−11
2p	14.99	14.95	+0.04	+0.3

4. Experimental

4.1. General

The protected amino acids with L-configuration were purchased from Sigma Chemical Co. Chromatography was performed on Qingdao silica gel H. The purities of all Schiff's bases were measured with both TLC (Merck silica gel plates of type 60 F_{254}, 0.25 mm layer thickness) and HPLC (Waters, C_{18} column 4.6 × 150 mm), and were more than 96%. Melting points were measured on a XT5 hot stage microscope (Beijing Keyi Electro-Optic Factory). The ESI-MS was determined on Waters ZQ2000 LC–MS. Optical rotations were determined with a JASCD P-1020 Polarimeter. The IR spectra were recorded with an Avatar330 FT-IR instrument. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on AMX-500 spectrometers in $CDCl_3$ with TMS as internal standard.

4.2. General procedure of preparing amino acid methyl esters (1a–q)

At 0 °C to 20 ml of methanol 3.75 ml of thionyl chloride was added drop-wise, which took 10 min. To this mixture 42 mmol of L-amino acid was added. Reaction mixture was stirred at room temperature for 100 h and TLC ($CHCl_3$/CH_3OH, 10:1) indicated complete disappearance of amino acid. Reaction mixture was evaporated under vacuum. The residue was dissolved in 20 ml of

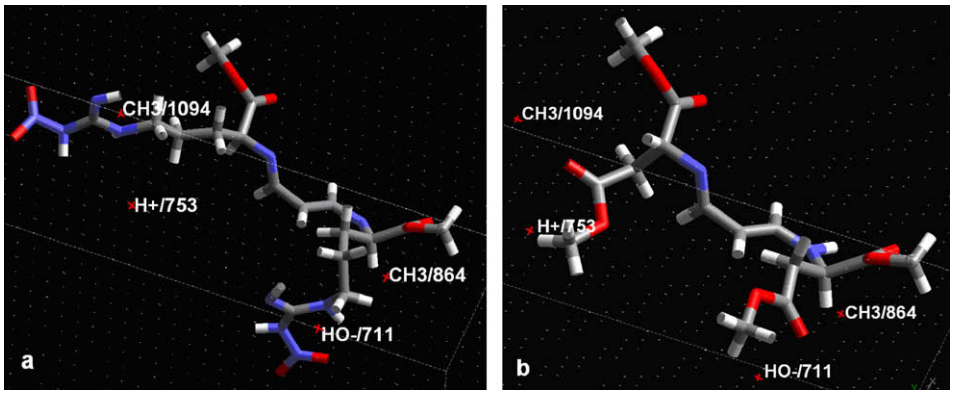


Figure 5. Electrostatic and steric environments of **2b** (a) with higher activity and **2d** (b) with lower activity within the grid with 3D points of Eq. 1.

methanol and evaporated under vacuum. This procedure was repeated for three times. Then the residue was dissolved in 20 ml of ether and evaporated under vacuum. This procedure was repeated for three times. The residue was crystallized in a mixture of methanol and ether to provide the title compounds as colorless powders in 85–99% yields.

4.3. General procedure of preparing *N,N′*-(*Z*-allylidene-1,3-diyl)bisamino acid methyl esters (2a–q)

Under argon the solution of 10 mmol of amino acid methyl ester in 15 ml of acetic acid was stirred at 90 °C for 10 min, during which a solution of 12 mmol of 1,1,3,3-tetramethoxypropane in 5 ml of acetic acid was added drop-wise and then stirred for 1 h. TLC ($CHCl_3/CH_3OH$, 10:1) indicated complete disappearance of amino acid methyl ester. Reaction mixture was cooled to room temperature, evaporated under vacuum, and the residue was separated on silica gel chromatography ($CHCl_3/CH_3OH$, 10:1) to provide the title compound.

4.3.1. *N,N′*-(*Z*-Allylidene-1,3-diyl)bisalanine methyl ester (2a)

Yellow powder. Yield: 64%. Mp 75–78 °C. $[\alpha]_D^{25} = -152.5$ (c = 0.74, MeOH); ESI/MS: 243 $[M+H]^+$; IR (KBr disk): 3148, 2946, 1751, 1605, 1528, 1450, 1342, 1211, 1134, 1049, 856, 756 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H = 7.83–8.01 (m, 2H), 5.97–6.01 (t, J = 10.9 Hz, 1H), 5.12–5.14 (d, J = 10.9 Hz, 1H), 4.25–4.38 (m, 2H), 3.76 (s, 6H), 1.54–1.61 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 171.6, 165.5, 163.8, 160.8, 95.6, 88.9, 56.4, 53.0, 52.8, 17.8, 16.9. Elemental Anal. Calcd for $C_{11}H_{18}N_2O_4$: C, 54.53; H, 7.49; N, 11.56. Found: C, 54.34; H, 7.35; N, 11.31.

4.3.2. *N,N′*-(*Z*-Allylidene-1,3-diyl)bis-N^G-NO_2-arginine methyl ester (2b)

Yellow powder. Yield: 41%. $[\alpha]_D^{25} = -165.7$ (c = 15.4, MeOH); ESI/MS: 503 $[M+H]^+$; IR (KBr disk): 3210, 2955, 1736, 1605, 1435, 1265, 787, 687 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H = 10.09–10.24 (m, 2H), 8.55 (s, 1H), 8.08–8.10 (d, J = 11.8 Hz, 3H), 7.82–7.86 (t, J = 10.7 Hz, 1H), 7.73 (s, 2H), 6.14–6.18 (t, J = 10.2 Hz, 1H), 5.01–5.03 (d, J = 10.2 Hz, 1H), 4.41–4.45 (m, 1H), 4.16–4.18 (m, 1H), 3.71 (s, 6H), 3.16 (s, 3H), 1.88–1.91 (m, 2H), 1.76–1.78 (m, 2H), 1.51–1.53 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 171.3, 170.5, 165.6, 163.5, 161.8, 92.5, 61.3, 56.4, 53.1, 49.0, 45.8, 29.1, 25.0. Elemental Anal. Calcd for $C_{17}H_{30}N_{10}O_8$: C, 40.63; H, 6.02; N, 27.88. Found: C, 40.84; H, 6.18; N, 28.12.

4.3.3. *N,N′*-(*Z*-Allylidene-1,3-diyl)bis-*S*-*p*-methylbenzylcysteine methyl ester (2c)

Yellow powder. Yield: 74%. $[\alpha]_D^{25} = -0.25$ (c = 1.5, MeOH); ESI/MS: 515 $[M+H]^+$; IR (KBr disk): 3210, 2955, 2376, 2345, 2306, 1736, 1605, 1512, 1435, 1358, 1211, 1088, 1018, 818, 725 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H = 8.24 (s, 2H), 7.53 (s, 1H), 7.19–7.21 (d, J = 6.7 Hz, 4H), 7.12–7.14 (t, J = 5.8 Hz, 2H), 6.01–6.05 (t, J = 10.0 Hz, 1H), 5.00–5.02 (d, J = 10.0 Hz, 1H), 4.22 (s, 1H), 3.56–3.88 (m, 10H), 3.44–3.45 (d, J = 3 Hz, 1H), 2.82–3.01 (m, 3H), 2.32–2.34 (d, J = 6.25 Hz, 6H), 2.03 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 176.0 (2C), 170.0 (2C), 137.0, 136.9, 134.7, 129.4 (4C), 128.8 (4C), 53.0 (2C), 36.7 (2C), 22.2 (2C), 22.1 (6C). Elemental Anal. Calcd for $C_{27}H_{34}N_2O_4S_2$: C, 63.01; H, 6.66; N, 5.44. Found: C, 62.82; H, 6.50; N, 5.21.

4.3.4. *N,N*-(*Z*-Allylidene-1,3-diyl)bisaspartic acid dimethyl ester (2d)

Yellow powder. Yield: 53%. $[\alpha]_D^{25} = -4.27$ (c = 0.75, MeOH); ESI/MS: 359 $[M+H]^+$; IR (KBr disk): 3163, 2955, 1736, 1605, 1435, 1281, 1211, 1111, 1049, 856 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H = 10.29 (s, 2H), 7.89–7.91 (m, 2H), 6.98–6.02 (t, J = 9.5 Hz, 1H), 5.15–5.17 (d, J = 9.5 Hz, 1H), 4.68–4.74 (m, 2H), 3.78 (s, 6H), 3.71 (s, 6H), 3.13–3.15 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 170.8, 170.3, 169.7, 168.7, 165.0, 161.6, 96.1, 58.3, 58.0, 53.32(2C), 52.4 (2C), 36.1, 35.4. Elemental Anal. Calcd for $C_{15}H_{22}N_2O_8$: C, 50.28; H, 6.19; N, 7.82. Found: C, 50.05; H, 6.04; N, 8.05.

4.3.5. *N,N′*-(*Z*-Allylidene-1,3-diyl)bisglycine methyl ester (2e)

Yellow powder. Yield: 46%. Mp 170–173 °C. $[\alpha]_D^{25} = -1.38$ (c = 1.0, MeOH); ESI/MS: 215 $[M+H]^+$; IR (KBr disk): 3163, 3001, 2785, 1759, 1636, 1435, 1304, 1042, 957, 826, 756.1 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H = 10.23 (s, 2H), 8.06–8.08 (d, J = 11.9 Hz, 1H), 5.92–5.97 (t, J = 11.0 Hz, 1H), 5.13–5.15 (d, J = 11.0 Hz, 1H), 4.28–4.32 (m, 3H), 3.71 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 168.7, 163.9, 92.5, 91.3, 52.8(2C), 45.2, 21.6. Elemental Anal. Calcd for $C_9H_{14}N_2O_4$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.67; H, 6.75; N, 12.84.

4.3.6. *N,N′*-(*Z*-Allylidene-1,3-diyl)bisglutamic acid dimethyl ester (2f)

Yellow powder. Yield: 21%. $[\alpha]_D^{25} = -74.58$ (c = 1.3, MeOH); ESI/MS: 387 $[M+H]^+$; IR (KBr disk): 3148, 2986, 1751, 1605, 1443, 1335, 1211, 1180, 988, 856, 779 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H = 10.51 (s, 1H), 10.08 (s, 1H), 7.77–7.87 (m, 1H), 6.09–6.13 (t, J = 9.2 Hz, 1H), 5.10–5.12 (d, J = 9.2 Hz, 1H), 4.32–4.36 (m, 1H), 3.70–3.72 (m, 6H), 3.60 (s, 6H), 2.48 (m, 4H), 2.22–2.25 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 172.8 (2C), 169.5 (2C), 163.1 (2C), 92.7, 60.6, 55.8, 51.8 (4C), 29.7 (2C), 26.2 (2C). Elemental Anal. Calcd for $C_{17}H_{26}N_2O_8$: C, 52.84; H, 6.78; N, 7.25. Found: C, 52.61; H, 6.63; N, 7.01.

4.3.7. *N,N′*-(*Z*-Allylidene-1,3-diyl)bis-N^τ-benzylhistidine methyl ester (2g)

Yellow powder. Yield: 85%. $[\alpha]_D^{25} = 0.44$ (c = 1.1, MeOH); ESI/MS: 555 $[M+H]^+$; IR (KBr disk): 3186, 2955, 1736, 1605, 1211, 1042, 733 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H = 8.96–8.98 (d, J = 8.2 Hz, 1H), 7.85–8.02 (m, 1H), 7.63–7.70 (m, 2H), 7.24–7.36 (m, 5H), 7.17–7.18 (m, 4H), 6.90–6.93 (m, 2H), 5.84–5.88 (t, J = 8.6 Hz, 1H), 5.12 (s, 3H), 4.99–5.01 (d, J = 8.6 Hz, 1H), 4.36–4.45 (m, 2H), 3.60 (s, 6H), 3.45–3.52 (m, 3H), 2.86–2.93 (m, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 171.7 (2C), 157.4, 138.2, 137.9 (2C), 137.7 (2C), 136.9 (2C), 129.1 (8C), 127.8, 127.6, 117.9, 117.7, 101.5, 56.4, 52.9, 49.9 (2C), 49.0 (2C), 30.5, 22.7. Elemental Anal. Calcd for $C_{31}H_{34}N_6O_4$: C, 67.13; H, 6.18; N, 15.15. Found: C, 67.32; H, 6.33; N, 15.38.

4.3.8. *N,N′*-(*Z*-Allylidene-1,3-diyl)bis-N^ω-*Z*-lysine methyl ester (2h)

Yellow powder. Yield: 83%. $[\alpha]_D^{25} = -90.0$ (c = 1.7, MeOH); ESI/MS: 625 $[M+H]^+$; IR (KBr disk): 3171, 2963, 1744, 1605, 1528, 1458, 1335, 1211, 1134, 1042, 741 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H = 10.08 (s, 1H), 9.91 (s, 1H), 8.02–8.04 (d, J = 11.5 Hz, 1H), 7.80–7.82 (d, J = 11.5 Hz, 1H), 7.30–7.38 (m, 11H), 6.08–6.12 (t, J = 10.5 Hz, 1H), 5.05–5.07 (d, J = 10.5 Hz, 1H), 5.01 (s, 3H), 4.57–4.58 (m, 1H), 4.34–4.36 (m, 1H), 3.71 (s, 6H), 2.98–3.00 (dt, J = 6.2 Hz, 5.95 Hz, 4H), 1.75–1.84 (m, 4H), 1.40–1.44 (m, 4H), 1.29–1.30 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 169.5, 163.4, 92.7, 65.8, 62.1, 58.2, 53.5, 52.6, 37.6, 37.1, 25.3, 24.8, 18.4, 15.5, 15.5, 11.6 (2C). Elemental Anal. Calcd for $C_{33}H_{44}N_4O_8$: C, 63.44; H, 7.10; N, 8.97. Found: C, 63.22; H, 6.96, N, 9.22.

4.3.9. *N,N′*-(*Z*-Allylidene-1,3-diyl)bisisoleucine methyl ester (2i)

Yellow powder. Yield: 79%. $[\alpha]_D^{25} = -22.1$ (c = 1.5, MeOH); ESI/MS: 327 $[M+H]^+$; IR (KBr disk): 3233, 1947, 1744, 1605, 1528, 1450, 1342, 1250, 1134, 741, 702 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H = 10.34–10.38 (t, J = 7.8 Hz, 1H), 8.07–8.12 (dd, J = 8.5 Hz, J = 11.6 Hz, 1H), 7.85–7.87 (m, 1H), 6.28–6.32 (t, J = 8.7 Hz, 1H),

5.14–5.16 (d, J = 8.7 Hz, 1H), 3.97–3.99 (m, 1H), 3.76 (s, 6H), 2.05–2.12 (m, 2H), 1.37–1.56 (m, 4H), 0.88–0.99 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 170.6, 170.1, 169.5, 165.8, 164.5, 163.3, 161.9, 92.7, 88.2, 66.7, 65.8, 62.1, 61.5, 56.4, 58.1, 53.5, 52.6, 38.2, 37.8, 25.3, 24.9, 18.4, 15.5. Elemental Anal. Calcd for $C_{17}H_{30}N_2O_4$: C, 62.55; H, 9.26; N, 8.58. Found: C, 62.76; H, 9.41; N, 8.82.

4.3.10. *N,N′*-(*Z*-Allylidene-1,3-diyl)bisleucine methyl ester (2j)

Yellow powder. Yield: 76%. $[\alpha]_D^{25} = -115.2$ (c = 1.6, MeOH); ESI/MS: 327 $[M+H]^+$; IR (KBr disk): 3388, 3186, 2955, 1736,1605, 1505, 1435, 1342, 1211, 1173, 1080, 733 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 10.44–10.48 (t, J = 7.7 Hz, 1H), 9.86–9.90 (t, J = 7.8 Hz, 1H), 8.07–8.12 (dd, J = 8.3 Hz, J = 8.3 Hz, 1H), 6.33–6.37 (t, J = 8.3 Hz, 1H), 5.04–5.06 (d, J = 8.3 Hz, 1H), 4.12–4.26 (m, 1H), 3.77 (s, 6H), 1.85–1.99 (m, 2H), 1.73–1.82 (m, 4H), 0.91–0.99 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 170.7 (2C), 163.3 (2C), 92.7, 66.6, 62.1, 52.6 (2C), 37.0, 25.3, 15.5 (4C), 11.1 (2C). Elemental Anal. Calcd for $C_{17}H_{30}N_2O_4$: C, 62.55; H, 9.26; N, 8.58; O, 19.61. Found: C, 62.78; H, 9.41; N, 8.33.

4.3.11. *N,N′*-(*Z*-Allylidene-1,3-diyl)bismethionine methyl ester (2k)

Yellow powder. Yield: 44%. $[\alpha]_D^{25} = -18.3$ (c = 0.77, MeOH); ESI/MS: 363 $[M+H]^+$; IR (KBr disk): 3171, 2963, 1744, 1605, 1435, 1335, 1211, 734 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 10.40–10.45 (m, 1H), 9.96–9.98 (m, 1H), 8.04–8.08 (t, J = 10.1 Hz, 1H), 6.16–6.20 (t, J = 9.1 Hz, 1H), 5.05–5.07 (d, J = 9.1 Hz, 1H), 4.45–4.47 (m, 1H), 3.77 (s, 6H), 2.65–3.69 (m, 2H), 2.59 (m, 2H), 2.27 (m, 4H), 2.08 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 173.4, 170.8, 166.2, 163.3, 161.7, 95.9, 92.8, 89.3, 60.2, 60.0, 55.5, 30.5, 21.1, 18.4, 15.3. Elemental Anal. Calcd for $C_{15}H_{26}N_2O_4S_2$: C, 49.70; H, 7.23; N, 7.73. Found: C, 49.50; H, 7.09; N, 7.98.

4.3.12. *N,N′*-(*Z*-Allylidene-1,3-diyl)bisphenylalanine methyl ester (2l)

Yellow powder. Yield: 51%. Mp 52–54 °C. $[\alpha]_D^{25} = -5.3$ (c = 1.0, MeOH); ESI/MS: 395 $[M+H]^+$; IR (KBr disk): 3148, 2955, 1736, 1605, 1435, 1342, 1219, 1080, 1034, 756, 703 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 10.35 (s, 1H), 10.08 (s, 1H), 7.82–7.84 (d, J = 11.9 Hz, 1H), 7.52–7.58 (m, 1H), 7.20–7.32 (m, 8H), 6.20–6.24 (t, J = 9.0 Hz, 1H), 5.09–5.11 (d, J = 9.0 Hz, 1H), 4.84–4.90 (m, 1H), 4.59 (s, 1H), 3.71 (s, 6H), 3.18–3.27 (m, 2H), 2.98–3.06 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 170.2 (2C), 163.2, 136.5, 136.2 (2C), 129.8 (4C), 128.9 (4C), 1127.4 (2C), 91.0, 57.7 (2C), 53.1 (2C), 37.7, 37.0. Elemental Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.03; H, 6.64; N, 7.10. Found: C, 69.80; H, 6.50; N, 6.87.

4.3.13. *N,N′*-(*Z*-Allylidene-1,3-diyl)bis-*O*-benzylserine methyl ester (2m)

Yellow powder. Yield: 38%. $[\alpha]_D^{25} = -24.3$ (c = 0.9, MeOH); ESI/MS: 455 $[M+H]^+$; IR (KBr disk): 3171,1736, 1620, 1435, 1327, 1281, 1204, 1080, 1011, 748, 702 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 9.38 (s, 1H), 7.78–7.82 (d, J = 11.9 Hz, 2H), 7.28–7.30 (m, 10H), 6.08–6.12 (t, J = 9.5 Hz, 1H), 5.00–5.02 (d, J = 9.5 Hz, 1H), 4.51–4.53 (m, 5H), 3.92–3.97 (m, 2H), 3.81–3.84 (m, 2H), 3.69 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 173.4 (2C), 167.4 (2C), 161.6, 137.2 (2C), 128.5 (6C), 127.9 (4C), 96.7, 73.4 (2C), 69.6, 62.4, 54.4, 53.0 (2C). Elemental Anal. Calcd for $C_{25}H_{30}N_2O_6$: C, 66.06; H, 6.65; N, 6.16. Found: C, 65.83; H, 6.50; N, 5.93.

4.3.14. *N,N′*-(*Z*-Allylidene-1,3-diyl)bis-*O*-benzylthreonine methyl ester (2n)

Yellow powder. Yield: 85%. $[\alpha]_D^{25} = 5.7$ (c = 1.3, MeOH); ESI/MS: 483 $[M+H]^+$; IR (KBr disk): 3186, 3032, 2978, 2955, 1751, 1605, 1450, 1211, 1088, 1011, 748 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 9.10–9.16 (m, 1H), 8.02–8.04 (m, 1H), 7.55–7.57 (d, J = 11.6 Hz, 1H), 7.26–7.37 (m, 10H), 6.00–6.04 (t, J = 8.2 Hz, 1H), 5.06–5.08 (d, J = 8.2 Hz, 1H), 4.56–4.61 (m, 1H), 4.39–4.45 (m, 3H), 4.19–4.21 (t, J = 5.35 Hz, 2H), 3.65–3.70 (m, 7H), 1.23–1.25 (d, J = 6.2 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 173.8, 166.1, 141.1, 137.6, 128.4 (4C), 128.1 (2C), 96.6, 93.9, 74.2, 73.9, 71.2, 66.0, 65.0, 61.2, 54.5, 52.7 (2C), 16.2 (2C). Elemental Anal. Calcd for $C_{27}H_{34}N_2O_6$: C, 67.20; H, 7.10; N, 5.81. Found: C, 67.42; H, 7.25; N, 6.06.

4.3.15. *N,N′*-(*Z*-Allylidene-1,3-diyl)bisvaline methyl ester (2o)

Yellow powder. Yield: 60%. Mp 91–93 °C. $[\alpha]_D^{25} = -86.0$ (c = 0.9, MeOH); ESI/MS: 299 $[M+H]^+$; IR (KBr disk): 3148, 2963, 2878, 1744, 1211, 1142, 856, 779, 664 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 9.24–9.26 (m, 1H), 8.02–8.06 (m, 1H), 7.78–7.82 (m, 1H), 6.12–6.16 (t, J = 9.1 Hz, 1H), 5.01–5.13 (d, J = 9.1 Hz, 1H), 4.17–4.27 (m, 1H), 3.76 (s, 6H), 2.16–2.26 (m, 2H), 0.86–1.00 (m, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 190.1, 171.8 (2C), 92.3, 67.5, 67.3, 53.0, 52.9, 31.2, 19.0 (2C), 17.8 (2C). Elemental Anal. Calcd for $C_{15}H_{26}N_2O_4$: C, 60.38; H, 8.78; N, 9.39. Found: C, 60.60; H, 8.92; N, 9.14.

4.3.16. *N,N′*-(*Z*-Allylidene-1,3-diyl)bistryptophane methyl ester (2p)

Yellow powder. Yield: 72%. Mp 126–128 °C. $[\alpha]_D^{25} = 26.4$ (c = 1.0, MeOH); ESI/MS: 473 $[M+H]^+$; IR (KBr disk): 3210, 3032, 2955, 1736, 1605, 1435, 1342, 1273, 1211, 1096, 748 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 11.08 (s, 2H), 9.90–10.42 (q, J = 8.8 Hz, 1H), 9.81–9.85 (t, J = 8.0 Hz, 1H), 7.46–7.50 (m, 2H), 7.32–7.38 (m, 2H), 7.15–7.19 (m, 2H), 7.01–7.09 (m, 3H), 6.13–6.15 (t, J = 7.3 Hz, 1H), 5.00–5.02 (d, J = 7.3 Hz, 1H), 4.54–4.57 (m, 1H), 3.70–3.72 (d, J = 5.55 Hz, 6H), 3.12–3.32 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 171.2, 169.7, 166.1, 137.6, 128.4 (4C), 128.1 (2C), 96.6, 74.2, 71.2 (4C), 66.0, 65.0, 61.2, 54.5 (2C), 52.7 (2C), 16.2 (2C). Elemental Anal. Calcd for $C_{27}H_{28}N_4O_4$: C, 68.63; H, 5.97; N, 11.86. Found: C, 68.41; H, 5.81; N, 11.60.

4.3.17. *N,N′*-(*Z*-Allylidene-1,3-diyl)bistyrosine methyl ester (2q)

Yellow powder. Yield: 74%. $[\alpha]_D^{25} = 0.8$ (c = 1.7, MeOH); ESI/MS: 427 $[M+H]^+$; IR (KBr disk): 3186, 3009, 1744, 1065, 1443, 1373, 1250, 1173, 10472, 833, 687 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ_H = 9.51 (s, 2H), 7.63–7.80 (m, 1H), 7.20–7.30 (m, 1H), 6.95–6.97 (d, J = 7.4 Hz, 4H), 6.67–6.69 (d, J = 8.3 Hz, 3H), 6.10–6.14 (t, J = 8.4 Hz, 1H), 5.76 (s, 1H), 5.04–5.08 (t, J = 8.4 Hz, 1H), 4.13 (s, 2H), 3.63 (s, 6H), 2.98–3.00 (m, 2H), 2.79–2.83 (t, J = 8.1 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C = 172.3 (2C), 172.1 (2C), 156.7 (2C), 1130.6 (4C), 115.6 (4C), 65.4, 55.4 (2C), 52.4 (2C), 22.7 (2C), 15.6 (2C). Elemental Anal. Calcd for $C_{23}H_{26}N_2O_6$: C, 64.78; H, 6.15; N, 6.57. Found: C, 64.55; H, 6.00; N, 6.32.

4.4. Pain threshold assay

Male ICR mice (weighting 20 ± 2 g) were purchased from the Experimental Animal Center of Peking University. The study described herein was performed according to a protocol reviewed and approved by the Ethics Committee of Capital Medical University. The committee assures the welfare of the animals was maintained in accordance with the requirements of the animal welfare act and according to the guide for care and use of laboratory animals.

The mice were housed in a 12/12 light/dark cycle at 21 ± 2 °C for one day before used. Each of the mice in drug receiving groups was orally given a single dose of 20 μmol/kg of one of **2a–q** in 0.2 ml of normal saline (NS). The mouse in control group was orally given 0.2 ml of NS alone or intraperitoneally given a single dose of 20 μmol/kg of morphine in NS. Analgesic effects of **2a–q** were evaluated by tail-flick test. The value of basic pain threshold of each

mouse was measured four times. After administration pain thresholds were measured for 180 min at 30 min intervals. In order to avoid the tail damage if the mouse did not respond within 10 s the test was stopped. Analgesic potency was indicated by pain threshold variation. The values were calculated according to PTV = AAPT/BPT wherein PTV stands for pain threshold variation, BPT stands for basic pain threshold and AAPT stands for the difference of pain threshold after administration minus basic pain threshold. All pain threshold variation of each mouse were averaged and constituted one sample. Statistical analysis of the data was carried out using one way ANOVA test with p <0.05 as significant cut-off.

4.5. Xylene-induced ear edema

Male ICR mice (weighting 20 ± 2 g) were randomly divided into 19 groups of 12 mice, namely the test group, vehicle control group, and positive control group. The mice in vehicle control group were administrated orally with a suspension of Aspirin in CMC at a dosage of 165 μmol/kg, while the mice in the test group were administrated orally a suspension of **2a–q** in NS at a dosage of 20 μmol/kg, 10 mg/kg, 5 mg/kg. Later (30 min), 0.03 ml of xylene was applied to both the anterior and posterior surfaces of the right ear. The left ear was considered as control. After (2 h) xylene application, mice were sacrificed and both ears were removed. Using a cork borer with a diameter of 8 mm, several circular sections were taken and weighed. The increase in weight caused by the irritant was measured through subtracting the weight of the untreated left ear section from that of the treated right ear section. The statistical analysis of the data was carried out by use of ANOVA test, p <0.05 is considered significant.

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