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Wei Li · Yun Tang · Qiong Xie · Wei Sheng · Zhui-Bai Qiu

3D–QSAR studies of orvinol analogs as κ-opioid agonists

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Abstract Orvinols are potent analgesics that target opioid receptors. However, their analgesic mechanism remains unclear and no significant preference for subtype opioid receptor has been achieved. In order to find new orvinols that target the κ-receptor, comparative 3D–QSAR studies were performed on 26 orvinol analogs using comparative molecular field analysis (CoMFA) and comparative molecular similarity indices analysis (CoMSIA). The best predictions for the κ-receptor were obtained with the CoMFA standard model (q^2=0.686, r^2=0.947) and CoMSIA model combined steric, electrostatic, hydrophobic, and hydrogen bond donor/acceptor fields (q^2=0.678, r^2=0.914). The models built were further validated by a test set made up of seven compounds, leading to predictive r^2 values of 0.672 for CoMFA and 0.593 for CoMSIA. The study could be helpful for designing and prepare new category κ-agonists from orvinols.

Keywords κ-opioid agonists · Opiate analgesics · CoMFA · CoMSIA

Introduction

Orvinols, derived from the structure of thebaine, are potent analgesics. Their structure and activity relationships were thoroughly investigated by Bentley and Colman in the 1960–1970s, and some of them were several thousand times more potent than morphine in analgesic assays of the rat tail pressure test [1, 2]. Similar to morphine, their biological targets were suggested to be opioid receptors. However, because opioid receptors had not been identified at that time, the detailed analgesic mechanism was difficult to elucidate. Furthermore, only rodent antinociceptive models were available to evaluate the analgesic effects of newly prepared compounds.

Until the early 1990s as opioid receptors were cloned gradually, at least three subtypes μ, δ and κ were identified, which greatly facilitated studies of opioid receptors and their ligands. Typical analgesics, such as morphine and fentanyl, are found to be potent μ-opioid agonists. However, it has long been recognized that μ-opioids have some notorious side effects such as respiratory depression, drug addiction and dependence. Recent studies on the κ-opioid receptor revealed that κ-agonists show potent analgesic effects but lack the above-mentioned side effects, which indicate that κ-selective agonists might be developed as a new generation of analgesics without addiction [3, 4].

As semi-synthetic opiates, orvinols also target multiple opioid receptors with diverse efficacy profiles [5]. However, BU46, one of the orvinols, displayed some κ-like induced analgesic mechanism in vivo [6]. In order to investigate κ-selective agonists, since the late 1990s, Lewis, successor of Bentley, has focused on orvinols and synthesized a large number of new derivatives and structurally related analogs of BU46. They then proposed a triple binding-site model [7] to explain the pharmacological phenomena of orvinols on κ-receptor, in combination with other moderately κ-selective compounds derived from orivinols (Fig. 1, except for KT-95 and TRK-820) [8–10] and all structure-activity relationship (SAR) analyses were carried out qualitatively on these orvinols.

Therefore in this paper, in order to guide the synthesis of new orvinol derivatives as κ-specific agonists, we investigated orvinol analogs with comparative 3D–QSAR methods in order to inspect the 3D–QSAR for the same series of compounds with affinities against the κ-opioid receptor, using comparative molecular field analysis

W. Li · Y. Tang · Q. Xie · W. Sheng · Z.-B. Qiu
Department of Medicinal Chemistry, School of Pharmacy,
Fudan University,
138 Yixueyuan Road,
Shanghai 200032, People's Republic of China
e-mail: zbqiu@shmu.edu.cn

Y. Tang (✉)
School of Pharmacy,
East China University of Science & Technology,
130 Meilong Road,
Shanghai 200237, People's Republic of China
e-mail: ytang234@yahoo.com.cn
Tel.: +86-21-54237419

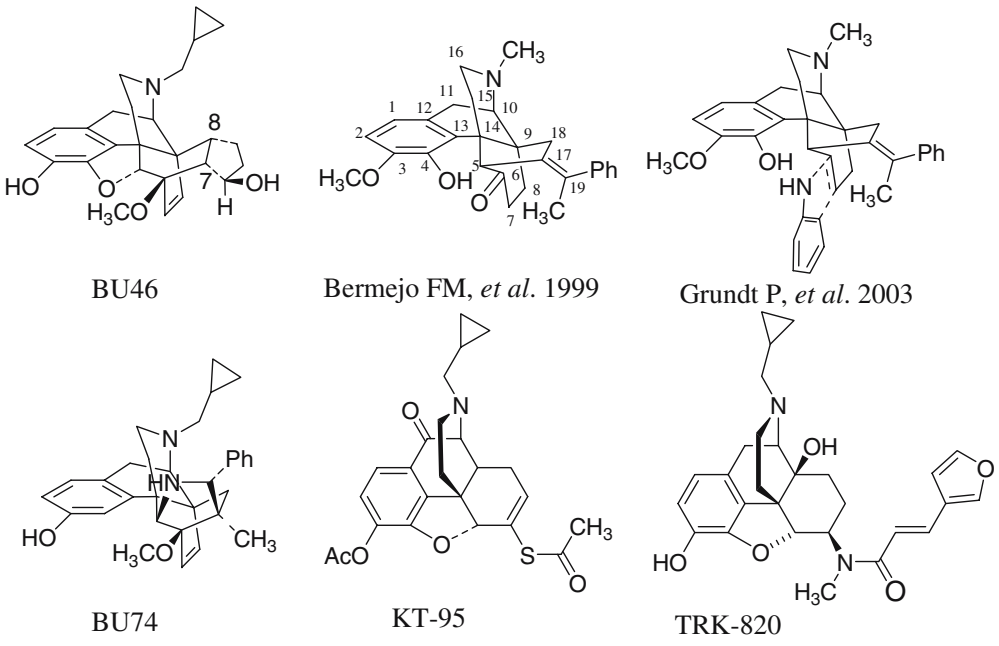


Fig. 1 Some opioids with potent κ-agonist potency

(CoMFA) and comparative molecular similarity indices analysis (CoMSIA).

Materials and methods

Data set

Thirty-three compounds were collected from several reports by the Lewis and Husbands group (see Table 1) [7–9, 11–13]. The binding affinities to the κ-opioid receptor were determined by displacement binding assays of cloned human opioid receptors transferred onto CHO Cells with [^{3}H]U69593 as the label.

Seven of 33 compounds from the dataset were randomly selected to form the test set, with the remaining 26 compounds used to make the training set.

Molecular modeling and structural alignment

All calculations were carried out on a R14000 SGI Fuel workstation running the molecular modeling software package SYBYL v6.9 [14].

The initial structures of the 33 molecules were built based on crystal structures of their analogs etorphine, diprenorphine and oxymorphone [15]. All molecules were set in their unprotonated states and assigned Gasteiger–Hückel charges available in SYBYL. Except for the rigid morphine-like skeleton, random searches were performed on additional ring systems to ensure that their conformations were energetically favorable. For molecules with more flexibility, systematic searches were carried out with an interval of 15° on rotatable bonds to obtain their lowest energy conformations. Finally, all molecules were minimized using the Tripos force field [16]. The energy-minimized conformation of molecule 17, which showed the highest binding affinity to the κ-receptor of all compounds studied in this paper, served as the template. A well-known opiate analgesic pharmacophore—a tyramine fragment [17] was common among all molecules. This fragment was used as the common substructure for structural alignments. The alignment resulting from the alignment facility in SYBYL is shown in Fig. 2.

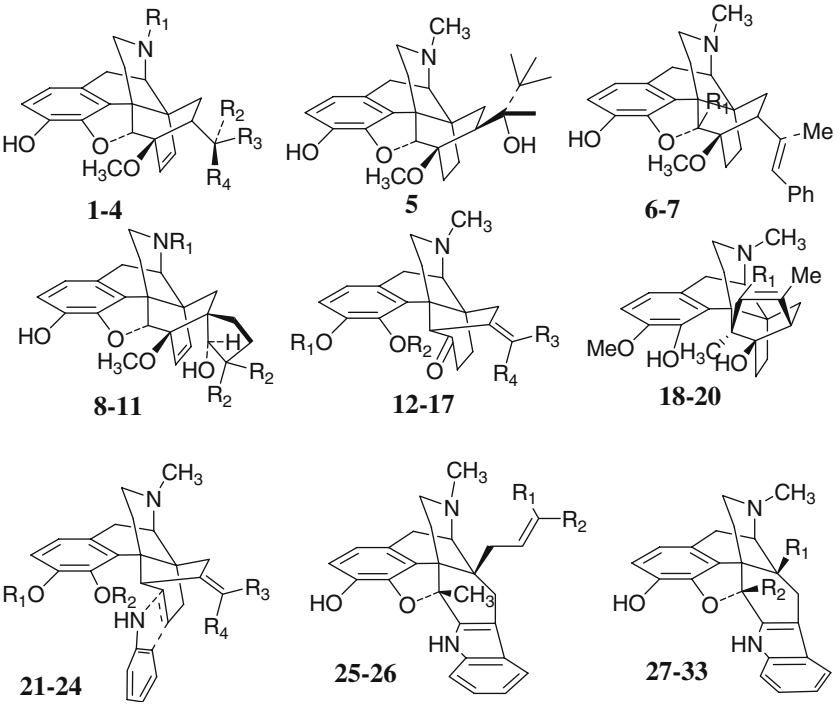
PLS analysis

The K_i value to the κ-receptor of each molecule was converted to pK_i($-\log K_i$) and set as dependent values, while CoMFA and CoMSIA descriptors were set as independent variables to perform PLS regression analyses. The CoMFA cutoff values were set to 30 kcal mol^{-1} for both steric and electrostatic fields and all fields were scaled by the default options in SYBYL.

The initial predictive coefficient q^2 values and the optimal number of components were obtained by the LOO (leave-one-out) cross-validation method. The cross-validated coefficient q^2 was calculated using the following formula:

$$q^2 = 1.0 - \frac{\sum_{\gamma} (\gamma_{pred} - \gamma_{actual})^2}{\sum_{\gamma} (\gamma_{actual} - \gamma_{mean})^2}$$

Table 1 Structures and binding affinities of molecules used in training and test sets



Compound	r_1	r_2	r_3	r_4	K_i (nM) (κ)
Training set					
1	Me	Me	OMe	H	3.9±1.1
2	Me	Me	OBn	H	0.84±0.04
4	Me	H	OBn	Me	1.1±0.08
5					2.4±1.3
6	Me				899±150
7	H				24.1±3.1
8	Me	H			0.87±0.13
10	CPM	H			1.04±0.01
11	CPM	Me			3.12±0.20
12	Me	H	Me	Ph	5.3±2.7
13	H	H	Me	Ph	2.0±1.0
15	Me	Me	Ph	Me	0.3±0.2
16	Me	H	Ph	H	0.2±0.02
17	Me	H	Ph	Me	0.14±0.10
18	Me				4841±303
19	Et				555±35
20	Ph				2608±28
22	Me	H	Ph	CH_3	0.61±0.22
23	Me	H	Me	H	239±88
24	Me	H	Me	Me	28.4±2.6
25	H	Ph			94.2±0.49
27	$NHCH_2$-nBu	H			36.1±5.6
28	$NHCH_2Bn$	H			13.8±0.78
30	NHCOBn	H			148±58
31	CH_2CH_2Bn	H			63.3±16.4
33	CH_2-iBu	Me			457±110
Test Set					

Table1 (continued)

Compound	r_1	r_2	r_3	r_4	K_i (nM) (κ)
3	Me	H	OMe	Me	43.3±2.5
9	Me	Me			1.28±0.01
14	H	Me	Ph	Me	0.2±0.1
21	Me	H	Ph	H	34.6±4.1
26	Me	Me			440±119
29	$NHCH_2CH_2Bn$	H			7.79±0.34
32	CH_2CH_2Bn	Me			36.4±0.16

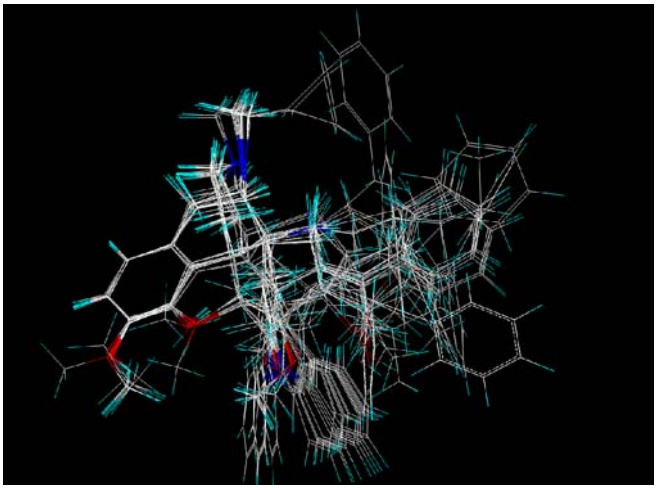


Fig. 2 Structual alignment of 33 orvinols and structurally related κ-agonists (containing compounds in the test set)

where γ_{pred}, γ_{actual} and γ_{mean} are predicted, actual, and mean values of the target property (pK_i), respectively. And $PRESS = \sum_{\gamma} (\gamma_{pred} - \gamma_{actual})^2$ is the sum of predictive sum of squares. The Column Filtering box was checked at a typical value of 2.0, to reduce analysis time with small effect on the q^2 values. Overall fits of this alignment were improved to ensure the highest q^2 values corresponded to a suitable optimum number of components. According to the optimum number of components with lowest PRESS values obtained, PLS regression models were derived. Finally, the confidence intervals for the parameters (mean and standard derivation) were estimated by bootstrap in ten runs.

QSAR model validation

In addition to LOO method to validate QSAR models, the established test set was used for further evaluation. The overall predictive performance of models on the test set is often reported as a predictive r^2 value, defined analogously to the cross-validated q^2 by comparing the accuracy of predictions with the variation in the actual data in the test set. The value of predictive r^2 is computed by the following equation:

$$predictive\ r^2 = \frac{SSD - PRESS}{SSD}$$

where SSD is the sum of squared deviation between the pK_i values of test set molecules and PRESS is the sum of squared deviations between the observed and the predicted pK_i values.

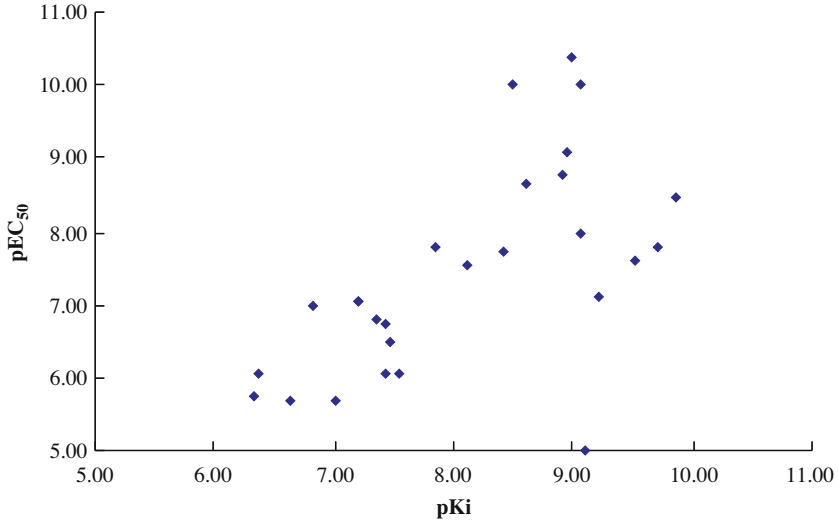


Fig. 3 Analysis on pK_i versus pEC_{50} values from 27 orvinols derived kappa agonists

Table 2 CoMFA and CoMSIA results

Model	q^{2a}	N^b	r^{2c}	SEE[d]	F^e	SEE bs[f]	q^2 bs[g]	SD[h]
CoMFA(standard)	0.686	4	0.947	0.330	94.645	0.265	0.962	0.019
CoMSIA(steric+electro)	0.705	2	0.861	0.512	71.220	0.418	0.903	0.040
CoMSIA(steric+electro+ hydrophobic)	0.713	2	0.874	0.488	79.747	0.427	0.895	0.015
CoMSIA(steric+electro+ hydrophobic+donor)	0.682	3	0.895	0.465	62.345	0.335	0.938	0.032
CoMSIA(steric+electro+ Hydrophobic+acceptor)	0.692	3	0.906	0.430	70.911	0.384	0.921	0.030
CoMSIA(all descriptors)	0.678	4	0.914	0.421	55.975	0.337	0.946	0.019

[a] q^2: leave-one-out (LOO) cross-validated correlation coefficient,
[b] N: optimum number of components,
[c] r^2: noncross-validated correlation coefficient,
[d] SEE: standard error of estimate,
[e] F: F-test value,
[f] SEEbs: standard error of estimate for boot strapping analysis,
[g] q^2bs: mean r squared of boot strapping analysis (ten runs),
[h] SD: standard deviation

QSAR coefficient contour maps

The results of all the CoMFA and CoMSIA models were visualized using the 'stDev*Coeff' mapping option. And molecule 17 was set as the template to validate these contour maps.

Results and discussion

CoMFA and CoMSIA models for κ-opioid receptor

In order to establish reliable 3D–QSAR models, analgesic activities (in vitro EC_{50} values) were also collected from the literature. However, there was no obviously linear relationship between pEC_{50} and pK_i values (shown in Fig. 3), and QSAR models established from pEC_{50} seemed to be poor (q^2=0.269, N=2 and r^2=0.530 for 27 molecules in CoMFA, data not shown), so only pK_i was used as the dependent value. The reason was possibly that both CoMFA and CoMSIA methods are based on the assumption that changes in binding affinities of ligands are related to changes in molecular properties represented by multiple fields, steric, electrostatic, etc., and other factors besides binding affinity might be involved in determining the analgesic potency of those compounds on the κ-opioid receptor.

3D–QSAR models of orvinol analogs were investigated by their binding affinities to the κ-opioid receptor with K_i values ranging from 0.14 to 4841 nM. The best predictions were obtained by the CoMFA standard model (q^2=0.686, r^2=0.947) and CoMSIA combined steric, electrostatic, hydrophobic, and donor/acceptor fields (q^2=0.678, r^2=0.914). All CoMFA and CoMSIA analysis parameters and results are shown in Table 2. In addition, Table 3 and Fig. 4 show the table and graph of the observed values versus conventional fit values in the training set and the observed values versus predicted values in the test set. All pK_i values in the test set were among the scope of the training set and both QSAR models here were well validated from the test set methods.

Characteristics of orvinol analogs binding to the κ-opioid receptor

From κ-agonists that retain the full morphine structure, as well as the compounds in this study, some potent and selective κ-agonists were found through structural modifications on two specific regions. Since compound 17 ([8] in Fig. 1) was selected as the template for QSAR contour demonstration. The two special regions mapped to this compound were 6-substituted groups in the parent morphine structure and additional 19-substituted groups (see Fig. 1). The influence of these substitutent groups on their binding to the κ-receptor are discussed below from QSAR contour plots:

From the Electrostatic Fields contours shown in Fig. 5a (CoMFA contours), it is clear that any introduction of six substitution or 6, 7-ring constrained structure could facilitate binding only if they contain electronegative groups. This is true since some potent κ-agonists such as KT-95 [18] and TRK-820 [19] (see Fig. 1) contain highly electronegative oxygen in this region. Interestingly, CoMSIA contours showed replacing C-6 with more electron-donating groups may favor binding to the κ-receptor. However, no predominant electrostatic contours around the C-19 region were observed in Fig. 5a.

Both yellow contours found in CoMFA and CoMSIA plots (Fig. 5b) showed that large steric groups may reduce compound binding affinities in six substitutions. The binding affinities of all compounds with 6, 7-ring constrained indole structures decreased considerably when compared to the compounds without such structures in this

Table 3 Actual versus conventional fit values (predicted values) activities of CoMFA standard and CoMSIA combined model

Compound number	Actual pK_i	CoMFA[a]		CoMSIA[b]	
		Conventional fit[c]	Res.[d]	Conventional fit[c]	Res.[d]
Training set					
1	8.409	8.639	−0.230	8.698	−0.289
2	9.076	9.167	−0.091	9.039	0.037
4	8.959	9.095	−0.136	8.973	−0.014
5	8.620	8.498	0.122	8.791	−0.171
6	6.046	6.732	−0.686	6.625	−0.579
7	7.618	6.895	0.723	6.762	0.856
8	9.060	8.735	0.325	8.618	0.442
10	8.983	9.018	−0.035	9.267	−0.284
11	8.506	8.805	−0.299	8.665	−0.159
12	8.276	8.320	−0.044	8.293	−0.017
13	8.699	8.527	0.172	8.612	0.087
15	9.523	9.596	−0.073	9.258	0.265
16	9.699	9.802	−0.103	9.794	−0.095
17	9.854	9.787	0.067	9.808	0.046
18	5.315	5.482	−0.167	5.651	−0.336
19	6.256	5.905	0.351	5.824	0.432
20	5.584	5.539	0.045	5.58	0.004
22	9.215	8.748	0.467	8.432	0.783
23	6.622	7.058	−0.436	7.46	−0.838
24	7.547	7.333	0.214	7.431	0.116
25	7.026	6.731	0.295	6.548	0.478
27	7.442	7.607	−0.165	7.842	−0.400
28	7.860	7.569	0.291	7.688	0.172
30	6.830	6.949	−0.119	6.982	−0.152
31	7.199	7.372	−0.173	7.332	−0.133
33	6.340	6.652	−0.312	6.588	−0.248
Test set					
Compound number	Actual pK_i	CoMFA[a]		CoMSIA[b]	
		Pred.[e]	Res.[d]	Pred.[e]	Res.[d]
3	7.364	7.871	−0.507	7.917	−0.553
9	8.893	8.294	0.599	8.481	0.412
14	9.699	9.955	−0.256	9.463	0.236
21	7.461	8.394	−0.933	8.427	−0.966
26	6.357	6.569	−0.212	6.522	−0.165
29	8.108	7.735	0.373	7.366	0.742
32	7.439	6.621	0.818	6.468	0.971

[a]CoMFA standard model,
[b]CoMSIA combined (steric, electrostatic, hydrophobic, donor and acceptor) model,
[c]Conventional fitted value,
[d]Difference between actual and fitted (predicted) pK_i values,
[e]Predicted pK_i value

study. However, the introduction of large steric groups obviously could help to improve the binding affinities in the region of C-19 substitutions. Both geometric isomers of bulky C-19 substitution contribute to binding, but the *trans*-isomer with large steric substitution was more active than the *cis*- isomer. The bulky *trans*-geometric isomer in C-19 is important for κ-receptor binding.

In CoMSIA hydrophobic fields contours (Fig. 6a), we found that the attachment of hydrophobic groups to C-6 may favor compound binding, but more hydrophilic group may be preferred in C-19 substitution. The contours of hydrogen bond fields (Fig. 6b) showed that there should be a hydrogen-acceptor site on the κ-opioid receptor near C-6 that is essential for binding. The whole compound 17 structure was surrounded by unfavorable hydrogen-acceptor field contours (Plot not shown).

In addition, we also performed QSAR studies on selectivities between κ and μ with (p$K_i\kappa$−p$K_i\mu$) as the dependent values, also considering their available binding affinities to the μ-opioid receptor. However, the poor cross-

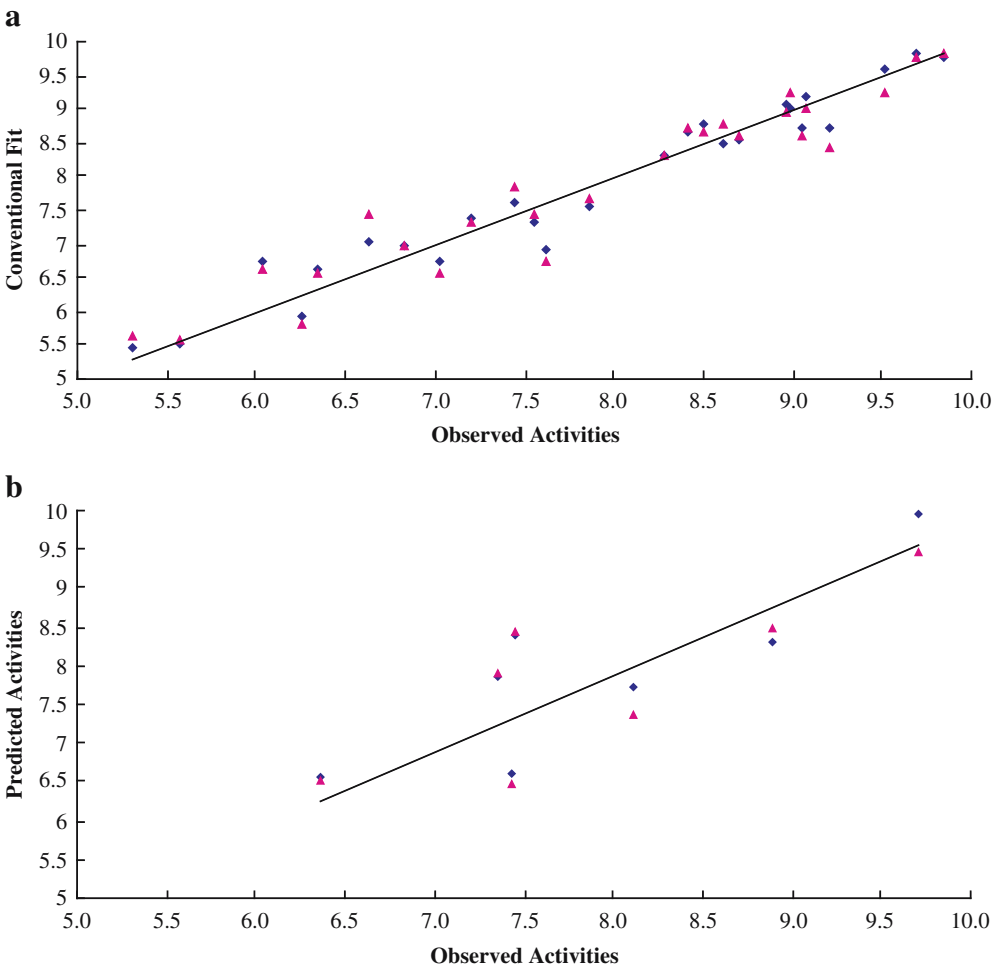


Fig. 4 Plot of observed versus conventional fitting predictions (predicted activities) of training set (**a**) and test set (**b**). *Blue rhombs* show predictions (conventional fit) of CoMFA standard model and *pink triangles* show those of CoMSIA combined model

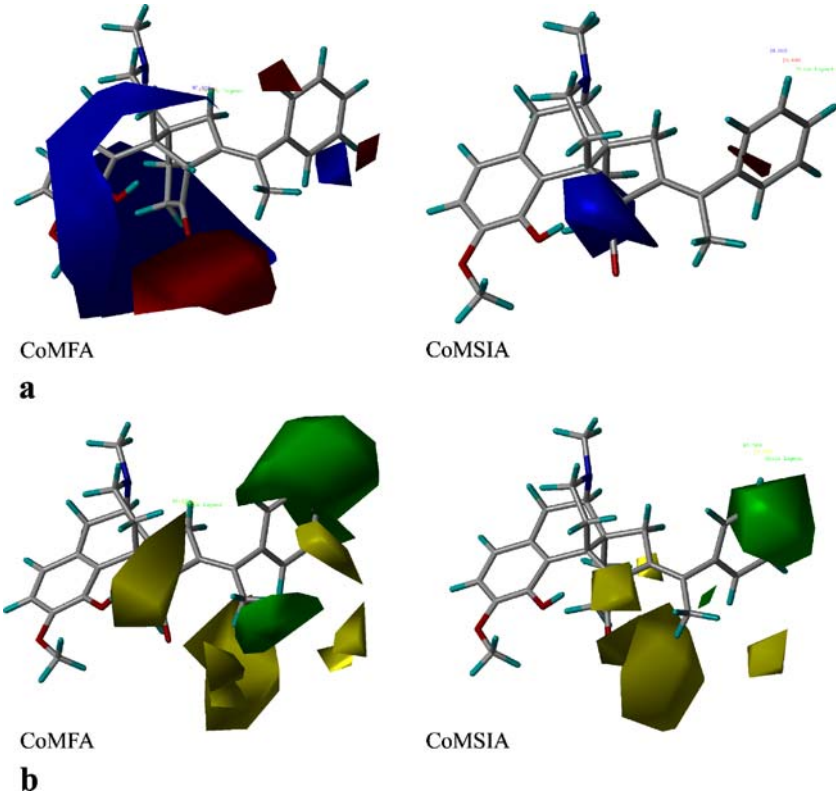


Fig. 5 CoMFA std and CoMSIA combined stdev* coeff contour plots: **a** *red contours* indicate negative charge increase binding affinity, whereas *blue contours* indicate positive charge favor binding affinity; **b** *green contours* indicate bulk favored for binding affinity, whereas *yellow contour* indicates less bulk favored for binding

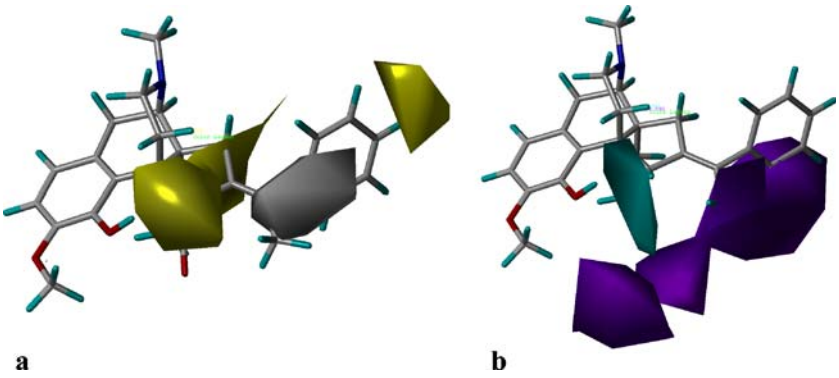


Fig. 6 CoMSIA combined stdev*coeff hydrophobic (**a**) and H-bond donor (**b**): *yellow contours* indicate hydrophobic groups increase binding affinity whereas *white contours* indicate hydrophilic groups favored; *cyan contours* indicate a H-bond acceptor groups in the receptor increase binding

validation values (q^2=0.313, N=2 for 33 compounds of this paper) and narrow selectivity range (from −1.53 to 1.51), only lead to negative results. These may result from the low selectivity in these compounds for κ and μ.

To date no highly selective potent κ-agonist from the morphine structure has been revealed. These QSAR studies also proved to be too poor to establish selective QSAR models. This leads us to conclude that orvinols' parent structure (morphine) is a non-selective pharmacophore targeting opioid receptors.

Conclusions

In this paper CoMFA and CoMSIA 3D-QSAR models were established successfully for orvinol analogs as κ-agonists. The influence of six and 19 substitutions on binding affinities were also highlighted by these QSAR studies. Negative, small steric and hydrophobic groups in the position six and hydrophilic 19-substitutions facilitate binding. Bulky *trans*-geometric isomer is also important for κ-receptor binding. This study could be helpful for designing and prepare new category κ-agonists from orvinols.

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