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CoMFA analyses of C-2 position Salvinorin A analogs at the kappa-opioid receptor provides insights into epimer selectivity

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ABSTRACT

The highly potent and kappa-opioid (KOP) receptor-selective hallucinogen Salvinorin A and selected analogs have been analyzed using the 3D quantitative structure–affinity relationship technique Comparative Molecular Field Analysis (CoMFA) in an effort to derive a statistically significant and predictive model of salvinorin affinity at the KOP receptor and to provide additional statistical support for the validity of previously proposed structure-based interaction models. Two CoMFA models of Salvinorin A analogs substituted at the C-2 position are presented. Separate models were developed based on the radioligand used in the kappa-opioid binding assay, [^{3}H]diprenorphine or [^{125}I]6β-iodo-3,14-dihydroxy-17-cyclopropylmethyl-4,5α-epoxymorphinan ([^{125}I]IOXY). For each dataset, three methods of alignment were employed: a receptor-docked alignment derived from the structure-based docking algorithm GOLD, another from the ligand-based alignment algorithm FlexS, and a rigid realignment of the poses from the receptor-docked alignment. The receptor-docked alignment produced statistically superior results compared to either the FlexS alignment or the realignment in both datasets. The [^{125}I]IOXY set (Model 1) and [^{3}H]diprenorphine set (Model 2) gave q^2 values of 0.592 and 0.620, respectively, using the receptor-docked alignment, and both models produced similar CoMFA contour maps that reflected the stereoelectronic features of the receptor model from which they were derived. Each model gave significantly predictive CoMFA statistics (Model 1 PSET r^2 = 0.833; Model 2 PSET r^2 = 0.813). Based on the CoMFA contour maps, a binding mode was proposed for amine-containing Salvinorin A analogs that provides a rationale for the observation that the β-epimers (*R*-configuration) of protonated amines at the C-2 position have a higher affinity than the corresponding α-epimers (*S*-configuration).

1. Introduction

Salvinorin A (Fig. 1) is a highly potent and selective kappa-opioid (KOP) receptor agonist and the most potent naturally occurring hallucinogen known [1]. The terpenoid was first isolated from the plant *Salvia divinorum* and characterized by Ortega [2] in 1982. The same compound was later isolated from *S. divinorum* by Valdes [3] in 1984 who reported its psychoactive properties in mice. *S. divinorum* has been used for centuries by the Mazatec Indians of Mexico for divination and is indigenous to a small area in the Sierra Mazateca Mountains. The plant was subsequently propagated and can now be found growing in widespread locations, sold by nurseries, and sold through the Internet for its hallucinogenic properties as both dried leaves and fortified plant extracts. The FDA has yet to schedule Salvinorin A, its extracts, or dried leaves as a controlled substance, although many countries and several states within the United States have adopted legislation banning the use of *S. divinorum* and related products.

Since the discovery that Salvinorin A is a remarkably potent and selective KOP receptor agonist [1], a large number of analogs have been synthesized, especially C-2 position analogs [4–19]. A smaller number of C-4 position analogs [4,5,7,19–21] and analogs with alterations of the furan ring [15,22,23] have also been reported in the literature. By inspection, the data suggest that very little change is tolerated at the C-4 position or the furan ring. Thus, attention was focused on C-2 modified structures for which a wide range of affinities have been reported.

Salvinorin A is unique among hallucinogens in that its chemical structure lacks a basic amine group. This is significant because such a moiety was previously thought to be required for high ligand affinity at aminergic and other closely related G Protein-Coupled Receptors (GPCRs). It is generally understood that the receptor-ligand interaction involving the amine is mediated by a conserved aspartate residue ($D^{3.32}$) on transmembrane helix 3 (TM3) through formation of a hydrogen-bonded salt bridge, anchoring the ligand in the binding site. Thus it is quite surprising that Salvinorin A's

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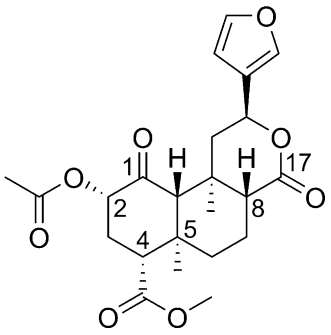

Fig. 1. Salvinorin A (**1**).

high affinity for the KOP receptor is comparable to that of amine-containing ligands [24].

The molecular mechanisms by which Salvinorin A achieves its exquisite affinity and selectivity for the KOP receptor is an active and ongoing area of research. Our working hypothesis is that by removing the amine from a ligand, its affinity for the many aminergic and related receptors decreases dramatically, resulting in high selectivity. That is, without the amine "anchor", the receptor becomes more sensitive to changes in the ligand structure, and therefore the stereoelectronic nature of the ligand and its complementarity to the target receptor become much more important factors in determining the affinity of the ligand for the receptor.

Although the affinity of hundreds of Salvinorin A analogs for the KOP receptor has been reported, there is very little published information regarding the QSAR of these compounds. In 2006, Singh et al. [25] described a quantitative and predictive structure–affinity model derived using a KOP receptor homology model and virtual screening techniques with a set of a set of 27 Salvinorin A analogs with modifications at the C-1, C-2, C-4 and C-17 positions. In the same year, Pandit et al. [26] reported a CoMFA model for C-2 position Salvinorin A analogs, though the details of this study have yet to be published.

Comparative Molecular Field Analysis (CoMFA), a three-dimensional quantitative structure–activity relationship (3D-QSAR) methodology, may be used to rationalize and predict ligand–receptor interactions when used in conjunction with homology modeling. In CoMFA, a 3D-QSAR model is constructed by correlating regions of steric and electrostatic fields with experimentally obtained affinity data for a set of aligned ligands (the training set or TSET). Information contained in the model can then be used for the design and prediction of binding affinities of new ligands (the prediction set or PSET) for the target receptor. The resulting models are critically dependent on the ligand alignment method used. If receptor structure-based ligand docking is used to generate the alignment, statistical 3D-QSAR methods like CoMFA may be used to complement and provide additional statistical support for the proposed ligand binding modes. Salvinorin A analogs are well-suited for a CoMFA study because the core of the molecule does not vary and it is conformationally constrained due to its polycyclic structure, much like the steroid system presented in the initial description of the method [27].

We report here our successful generation of statistically significant and predictive CoMFA models describing the interaction of C-2 Salvinorin A analogs with the KOP receptor and our use of these models to propose a binding mode for C-2 amine-containing Salvinorin A analogs.

2. Experimental methods

2.1. Receptor and ligand structures

CoMFA studies were performed using SYBYL software (version 7.3, Tripos Associates, St. Louis, MO) on an HP xw9400 workstation running Red Hat Enterprise Linux 4. The human KOP receptor model used here was built based on the coordinates of activated bovine rhodopsin crystal as previously described [24,28,29]. Compounds were constructed using the crystal structure [2] of Salvinorin A, (Cambridge Structural Database code = BUJJIZ) as the template and then energy-minimized using the Tripos Force Field (Gasteiger-Hückel charges; distance-dependent dielectric constant = 4.0; default parameters elsewhere).

2.2. Ligand docking and alignment

To explore the effect of ligand superimposition on the resulting statistical models, three methods of alignment were employed in each study. In the first, the automated docking routine GOLD was used to produce an alignment based on docked solutions of ligands to a previously described model [24,28,29] of the KOP receptor. Thus, the ligand ensemble is that produced by docking with no explicit ligand-ligand atom superposition performed. The second, a ligand-based method, was obtained using FlexS [30]. The third alignment method was a rigid realignment of the receptor-docked alignment.

Docking of salvinorin compounds was performed using GOLD (version 4.0, Cambridge Crystallographic Data Center, Cambridge, UK) as previously described [24,28,29]. Ten docking runs were performed for each compound in the dataset. The initial alignment was generated by selecting the docked solution in which (a) a furan oxygen-Q115$^{2.60}$ H-bond was present and (b) the stereochemical interactions appeared most reasonable for each ligand. In most cases the chosen pose was the top-ranked solution. This resulted in an alignment that resembled the previously postulated model of Salvinorin A in the KOP receptor [24,28,29] (Fig. 2). The second alignment method (using the same dataset) was performed with FlexS (version 1.20.3, BioSolveIT GmbH, Sankt Augustin, Germany). FlexS aligns the conformation and orientation of a ligand molecule relative to a reference molecule (template) that is treated as rigid. The molecule to be superimposed is partitioned into fragments. An 'anchor fragment' is placed first and the remaining fragments are added iteratively, allowing conformational flexibility at each step [30]. Compound **4** was used as the template for this alignment because it is the longest C-2 chain that still retains high affinity. The third alignment method, a realignment of the docked poses in the receptor-docked alignment, was performed by aligning all compounds to Salvinorin A using the SYBYL fit-atoms method. Carbon atoms C-2, C-4 and C-5 of Salvinorin A and the analogous atoms of each analog were selected for the fitting process.

2.3. Dataset generation

The quality and nature of the data used to construct the CoMFA model is of prime importance in obtaining an accurate, predictive model. Binding affinity data can vary from lab to lab depending on the assay methods, radioligand and cell lines employed. The choice of radiolabeled ligand can dramatically affect the values obtained [31,32], as can the level of gene expression that results in differing receptor densities in cloned cell lines [33]. Therefore pooling of data for a CoMFA study is generally discouraged. In this work, two independent CoMFA studies were undertaken, one in which [^{125}I]IOXY (6β-iodo-3,14-dihydroxy-17-cyclopropylmethyl-4,5α-epoxymorphinan) was used as the assay radioligand and a second in which [^{3}H]diprenorphine was the assay radioligand.

Compounds that are protonated at physiological pH (e.g. amines) and compounds with $K_i > 1{,}000$ nM were not included in the dataset. Protonated compounds would, perhaps, form an ion-pair interaction with D138$^{3.32}$ (Ballesteros-Weinstein numbering system [34,35]) of transmembrane helix 3 (TM3) or E209$^{xl2.49}$ of the extracellular loop 2 (EL2) that may result in a significant difference in the binding mode compared to that of