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Identification of the three-dimensional pharmacophore of κ-opioid receptor agonists

Noriyuki Yamaotsu *, Hideaki Fujii, Hiroshi Nagase, Shuichi Hirono

School of Pharmacy, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

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ABSTRACT

A selective κ-opioid receptor agonist might act as a powerful analgesic without the side effects of μ-opioid receptor-selective drugs such as morphine. The eight classes of known κ-opioid receptor agonists have different chemical structures, making it difficult to construct a pharmacophore model that takes them all into account. Here we propose a new three-dimensional pharmacophore model that encompasses the κ-activities of all classes, which utilizes conformational sampling of agonists by high-temperature molecular dynamics and pharmacophore extraction through a series of molecular superpositions.

1. Introduction

Opioid receptors belong to the superfamily of G-protein-coupled receptors (GPCRs), and interact with morphine and related opiate alkaloids as well as the various endogenous opioid peptides.[1,2] They are divided into at least three types: μ-opioid receptors (MORs), κ-opioid receptors (KORs), and δ-opioid receptors (DORs).[1] A selective KOR agonist could be a powerful analgesic drug without the clinically limiting side effects (e.g., dependence, respiratory depression, and constipation) of MOR-selective drugs such as morphine.[3]

The known KOR agonists belong to eight classes: peptides (e.g., dynorphin A);[4] benzomorphan derivatives (e.g., bremazocine);[5] morphinan derivatives (e.g., TRK-820);[6–8] arylacetamide derivatives (e.g., U-50,488H);[9–11] diazabicyclononanone derivatives (e.g., HZ2);[12,13] bicyclic guanidine derivatives (e.g., TPI 614-1);[14] benzodiazepine derivatives (e.g., tifluadom);[15–17] and neoclerodane diterpene derivatives (e.g., salvinorin A).[18] The endogenous peptide dynorphin A has high affinity, with a binding constant $K_i(\kappa)$ of 0.17–0.5 nM and a κ-selectivity of $K_i(\mu)/K_i(\kappa) = \mu/\kappa = 64$.[4,19,20] The benzomorphan analog bremazocine has minimal morphine-like side effects ($\mu/\kappa = 3.8–15$).[5,9,21] TRK-820 is a 4,5-epoxymorphinan derivative and a novel κ-selective agonist ($\mu/\kappa = 2.6$), which shows sedative activity with no aversive effects.[6,7] U-50,488H has high κ-selectivity ($\mu/\kappa = 630–845$),[9,21] but serious aversive effects, such as dysphoria.[22] HZ2 was synthesized based on the 3,7-diazabicyclo[3.3.1]nonan-9-one skeleton and exhibited high selectivity for the KOR ($\mu/\kappa > 66.7$).[12,13] The bicyclic guanidines were selected from the screening of a positional scanning-synthetic combinatorial library and evaluated in a KOR-binding assay.[14] Tifluadom is a 2-[(acylamino)methyl]-1,4-benzodiazepine with κ-selectivity ($\mu/\kappa = 8.8$).[15–17] Salvinorin A was isolated from *Salvia divinorum* and, although it has a notably different chemical structure from the other classes, shows high κ-selectivity ($\mu/\kappa > 526$).[18,23]

Studies of endogenous dynorphins clarified that $^{+}H_3N$-Tyr^1-Gly^2-Gly^3-Phe^4 is the common message domain for all μ-opioid, κ-opioid, and δ-opioid peptides, whereas Leu^5-Arg^6-Arg^7 is the address domain responsible for the κ-specificity.[4,19] In studies of the κ-selective antagonists norbinaltorphimine (norBNI) and 5'-guanidinonaltrindole (GNTI), a second positively charged group was considered as the address domain of morphinans (Fig. S1).[24–26] However, all of the selective κ-agonists, with the exceptions of peptides and diazabicyclononanones, lack a second positively charged group. Moreover, neoclerodane diterpenes do not have a positively charged group. Many computational studies have therefore attempted to determine the pharmacophore for κ-selective agonists. These studies have been divided into two classes: ligand-based methods[7,27–30] and docking-based methods.[15,18,30–37] With the former approach, it is difficult to superpose different skeletons onto one another; therefore, these methods have been applied to congeneric compounds or the charged NH^+ moiety corresponding to the amino (N)-terminus of peptides. The latter approach is powerful when the crystal structure of a target protein is known;[38] however, the X-ray structures of opioid receptors are not available. All docking studies of opioid ligands have therefore been based on homology models derived from other GPCRs. Homology modeling is a well-established method, and the main

* Corresponding author. Tel.: +81 3 3444 3548; fax: +81 3 3440 5246.
E-mail address: yamaotsun@pharm.kitasato-u.ac.jp (N. Yamaotsu).

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chain of a target protein is predictable.[39] However, many problems (e.g., the induced fit and the difference between agonist/antagonist binding modes) remain to be overcome before docking.[40,41] Hence, different docking protocols against KOR tend to result in different models.[18,33,34,37] Here, we propose a new ligand-based model that can account for the κ-activities of all classes, which utilizes conformational sampling of agonists by high-temperature molecular dynamics and pharmacophore extraction through a series of molecular superpositions.

2. Results and discussion

The alignment of KOR agonists in the training set at the estimated binding conformations and the three-dimensional (3D)-pharmacophore on TRK-820 are shown in Figure 1. Matched property spheres common to five and more agonists were considered as the 3D-pharmacophore of the κ-agonists. The 3D-pharmacophore of the κ-agonists required three hydrophobic groups (AR1, HP2, and HP3), one hydrogen-bond donor (HD1), and three hydrogen-bond acceptors (DA1, HA2, and HA3). On TRK-820 these were as follows: HD1 was the charged NH^+ group, AR1 was the A ring, and DA1 was the oxygen atom of the phenolic hydroxyl group; HP2 was the C ring and HA2 was the oxygen atom of the amide; and HP3 was the E ring and HA3 was the oxygen atom of the 4,5-epoxy moiety. The κ-agonists in the test set overlapped well with the 3D-pharmacophore determined by the training set (Fig. S2). The distances between matched property spheres of the 3D-pharmacophore are listed in Table S1.

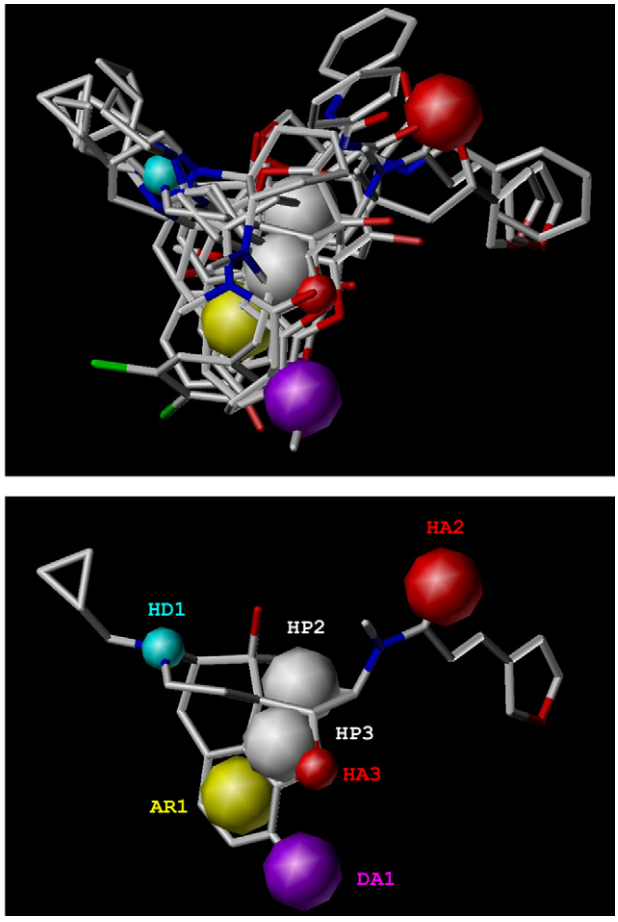

Figure 1. Alignment of estimated binding conformations of training set and 3D-pharmacophore for κ-agonists. (a) Alignment of training set. (b) 3D-pharmacophore represented by property spheres on TRK-820. The colors of the spheres indicate the properties as follows: hydrophobic (HP; white); aromatic (AR; green); hydrogen-bond donors (HD; blue); hydrogen-bond acceptors (HA; red); and hydrogen-bond donors/acceptor (DA; violet). Large and small spheres represent radii of 1 Å and 0.5 Å, respectively.

The estimated binding orientations of the κ-agonists were classified into four types (Fig. 2). Type I comprised the T-shape orientations of TRK-820, KNT-63, KNT-62, NS-22, TAN-821, MP-16 (Fig. 3a), and TPI 614-1 (Fig. 3b). Type II comprised the left-to-bottom orientations of U-69,593 (Fig. 3c), U-50,488H, and HZ2 (Fig. 3d). Type III comprised the left-to-right orientations of bremazocine (Fig. 3e) and morphine. Type IV comprised the bottom-to-right orientations of salvinorin A (Fig. 3f) and tifluadom (Fig. 3g). On the MP-16 peptide, HD1 was the charged N-terminus, AR1 and DA1 were the side chain of Tyr, and HP2 and HA2 were the side chain and the amide oxygen atom of the main chain of Phe (Fig. 3a). According to the model described by Protoghese et al.,[42,43] HD1 corresponded to the anionic (A) subsite on the receptor, AR1 and DA1 corresponded to the Tyr (T) subsite, and HP2 and HA2 corresponded to the Phe (P) subsite. Type I occupied all of the subsites. Type II and type III used the A-T and A-P subsites, respectively. Type IV had a unique binding orientation because the ligands lacked the charged NH^+ group. Therefore, the A subsite on the receptor was not used, and the ligands of type IV extended from the T subsite to the P subsite.

The matched property spheres of each κ-agonist are shown in Table 1. All of the κ-ligands included at least four out of the seven spheres. The higher κ-selective ligands had HP3 and HA3, but lacked two or more of HD1, HP1, DA1, HP2, and HA2. The affinities of the ligands to the μ-receptor might have been weakened through lacking a part of the message domain; however, the affinities to the κ-receptor might be compensated by interactions of HP3 and HA3. Therefore, HP3 and HA3 appeared to contribute to the κ-selectivity. The ligands that had an acceptor atom corresponding to HA2 showed relatively high affinities (Table S2). For AR1, the aromatic rings (AR) were seems to be better than the aliphatic rings (HP) (Table 1 and Fig. S3d and e). When a ligand has all of the property spheres, its activity might be high but its selectivity intermediate.

Moreover, when a ligand has a hydrophobic moiety beyond HA2, such as TRK-820, salvinorin A, and tifluadom, the κ-selectivity might be relatively high (Fig. 3f and g). The hydrophobic moiety might correspond to the Leu or aliphatic part of Arg in the κ-address domain. The κ-selective antagonists norBNI and GNTI had larger and positively charged moieties corresponding to Leu-Arg-Arg (Fig. S1).[24–26] Hence, if a longer but thinner positive moiety is introduced into a ligand, it might become a κ-selective agonist.

For HP3 and HA3, one of the effects of the 4,5-epoxy (E) ring introduced into the morphinan skeleton might have been maintaining the binding conformation to the κ-receptor. A similar effect was observed upon the introduction of the 10-methylene bridge (B ring) to the δ-agonist TAN-67.[44] The κ-affinity and κ-selectivity of the 10-methylene-bridged SN-28 were 72 and three times greater than those of TAN-67, respectively (Fig. S5). The oxygen atom (HA3) of the epoxy was adjacent to the hydroxyl group (DA1) of the phenol moiety. A hydroxyl group of the protein, as Tyr, Thr, or Ser, could potentially bind to both by a branched hydrogen-bond.

Although bremazocine was expected to adopt the type II binding orientation, type III was chosen by the SUPERPOSE program (Fig. 3e). This suggests that the type II orientation for bremazocine might be favorable for the μ-receptor, whereas the type III orientation might be preferable for the κ-receptor. Although compounds from all of the classes were used to construct our model, the type II orientations for arylacetamides in our model were in agreement with a model using eight arylacetamides and one benzomorphan, MPCB, reported by Lavecchia et al.[30] In their model, the phenol

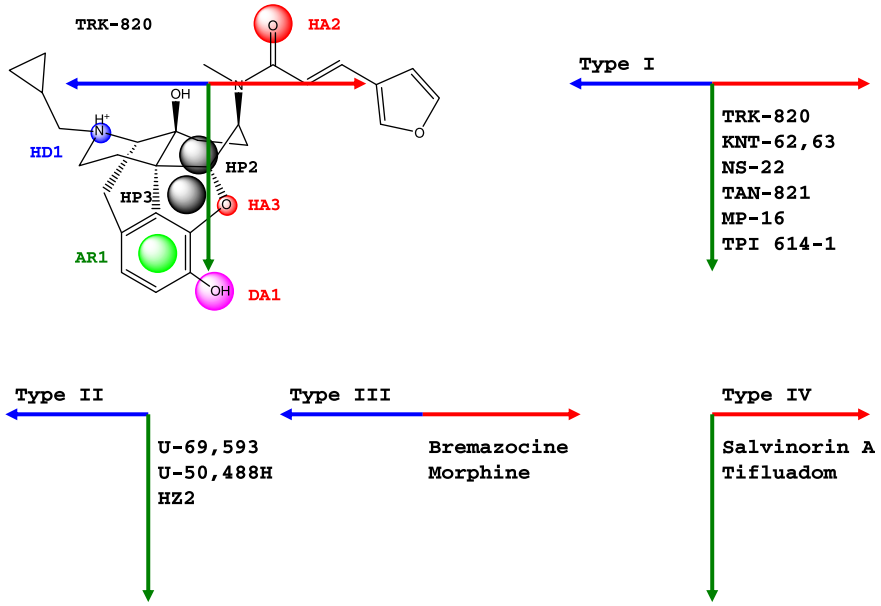

Figure 2. Classification of binding orientations for κ-agonists. The binding orientations are divided into four classes: type I with a T-shape orientation; type II with a left-to-bottom orientation; type III with a left-to-right orientation; and type IV with a bottom-to-right orientation.

moiety of MPCB was not matched with the dichlorobenzene moiety of arylacetamides. Therefore, the type III orientation for bremazocine (benzomorphan) in our model is supported by their model. This might be similar to the binding of phenylpiperidines and allylprodines to the μ-receptor.[42,43] These compounds are morphine-like but thinner. The binding mode of these ligands is thought to be the A-P orientation (type III), whereas that of morphine is the A-T orientation (type II). In our model, the binding mode of morphine was predicted to be type III (Fig. S3e). However, the binding site of the κ-receptor could not accept morphine, because it is larger than bremazocine. In addition, the conformation of the C ring of TRK-820 determined by our calculation was the boat form; by contrast, that of morphine differed because the C ring is the cyclohexene ring. The boat form is important for the κ-activity to maintain the binding conformation. In a previous study, we showed the type III orientation for arylacetamides by superimposing them on TRK-820.[7] In the current study using all κ-selective classes, type II was found to be the most likely, followed by type III. It is possible that arylacetamides adopt both in the κ-receptor.

The orientation of type IV for salvinorin A in our model was different from the docking models reported by Roth et al. and Kane et al.[18,33,34,37] In our model, salvinorin A avoided the position of the charged NH^+ group (HD1)—that is, the vicinity of the conserved Asp138 in all opioid receptors—because it has only acceptor atoms (Fig. 3f). Moreover, the furan ring of salvinorin A overlapped with that of TRK-820. It was therefore assumed that our model was reliable.

Similarly, the docking model of tifluadom reported by Cappelli et al. differed from our model.[15,31] In the latter, the position of HD1 was not occupied by tifluadom because the imine nitrogen atom were considered not to be protonated based on our acid dissociation constant (pK_a) calculation (Fig. 3g). As the thiophene ring of tifluadom in our model was matched to the furan ring of TRK-820, we considered our model to be acceptable.

Holzgrabe and Brandt proposed a docking model of HZ2, in which the two charged NH^+ groups interacted to Glu209 and Glu297 alternative to the conserved Asp138.[32] In our model, one of the two NH^+ groups occupied the HD1 position corresponding to Asp138 and the binding orientation was type II as in arylacetamides (Fig. 3c and d). This can explain the high κ-selectivity of HZ2.

Unfortunately, the κ-selectivities and κ-agonist activities of bicyclic guanidines are unknown. Only the affinities are available. In our proposed model for bicyclic guanidines, the T-shape orientation of TPI 614-1 was similar to that of TRK-820, except for the amide oxygen of HA2 (Fig. 3b). Therefore, bicyclic guanidines might be promising as κ-selective agonists.

In conclusion, we constructed a 3D-pharmacophore model for κ-agonists, which can account for all known skeletons. This might have applications for investigating why a particular κ-agonist is active and for designing new κ-selective agonists.

3. Experimental

3.1. Data sets

In order to identify the 3D-pharmacophore for κ-agonists, we prepared two data sets: the training set and the test set (Fig. 4 and Table 2). Ligands that had high affinities and high-to-intermediate selectivities were used as the training set, which consisted of four morphinans (TRK-820, KNT-63, KNT-62 and NS-22),[6–8] two arylacetamides (U-69,593 and U-50,488H),[10,11] and one neoclerodane diterpene (salvinorin A).[18] In the test set, the affinities ranged from high to intermediate, and the selectivities ranged from high to low. The test set comprised two morphinans (TAN-821[45] and morphine), one benzomorphan (bremazocine),[5] one diazabicyclononanone (HZ2),[12,13,29] one bicyclic guanidine (TPI 614-1),[14] one benzodiazepine (tifluadom),[15–17] and one peptide (MP-16).[46] The compounds which have both the charged NH^+ group and the phenolic hydroxyl group were shared between the training set (four morphinans) and the test set (two morhinan, one benzomorphan and one peptide). The skeletons which have only positive charge were divided into the training set (two arylacetamides) and the test set (one diazabicyclononanone and one bicyclic guanidine). For the skeletons without a positive charge, neoclerodane diterpene and benzodiazepine belong to the training set and the test set, respectively. Binding assays of the following compounds

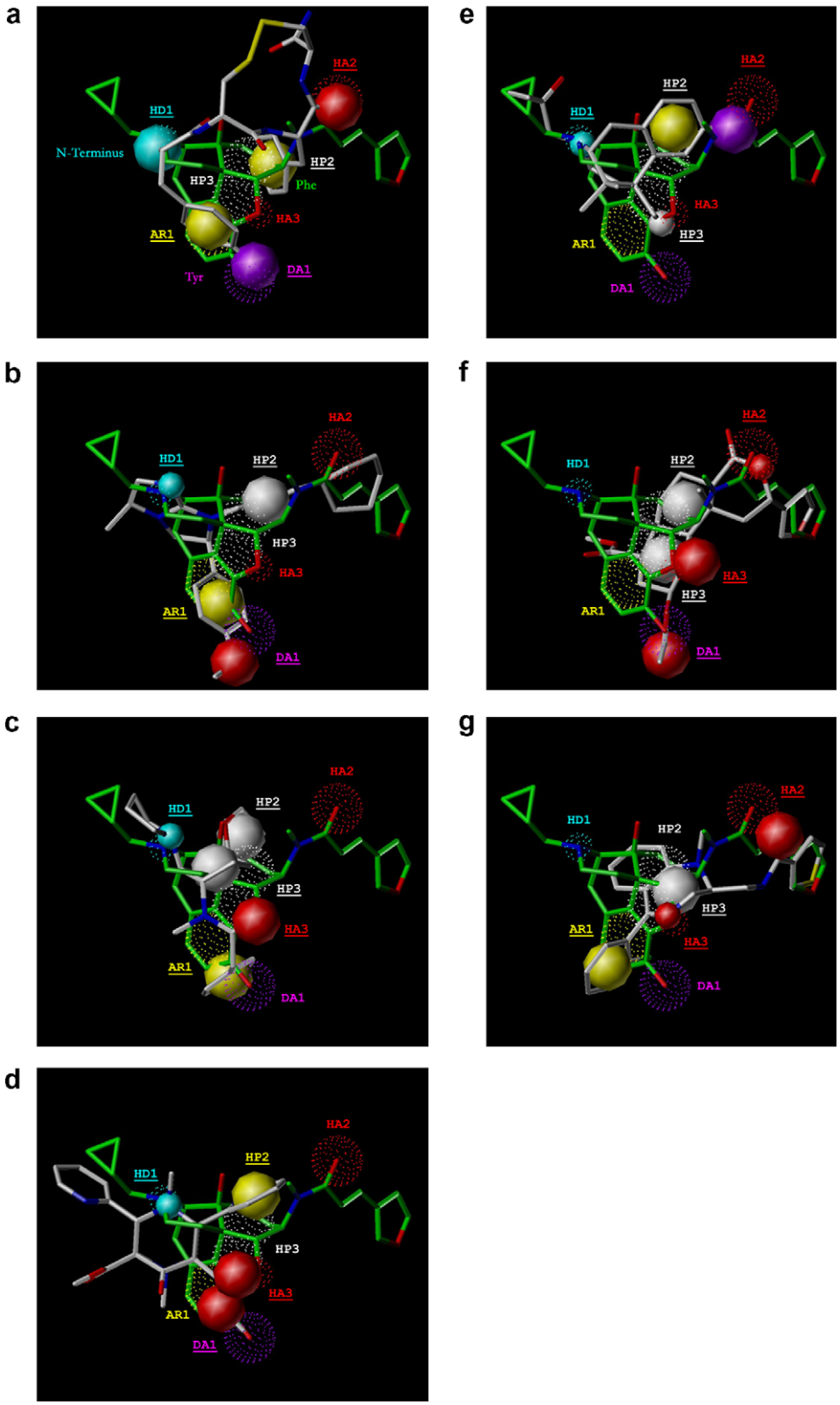

Figure 3. Superimposition of each κ-agonist skeleton on TRK-820 (morphinan) and their 3D-pharmacophores. (a) MP-16 (peptide). (b) TPI 614-1 (bicyclic guanidine). (c) U-69,593 (arylacetamide). (d) HZ2 (diazabicyclononanone). (e) Bremazocine (benzomorphan). (f) Salvinorin A (neoclerodane diterpene). (g) Tifluadom (benzodiazepine). TRK-820 is shown in green, and the other compounds are shown in white. The 3D-pharmacophores of TRK-820 and the other agonists are represented by dotted and solid spheres, respectively. Matched spheres are indicated by underlined labels.

(TRK-820, KNT-63, KNT-62, NS-22, TAN-821, morphine and U-69,593) were carried out in duplicate using guinea-pig brain homogenates (the cerebellum for κ and the forebrain for μ). The ability of each compound was evaluated by displacement of

Table 1
Matching table of property spheres sorted by κ-selectivities

Agonists	μ/κ[a]	K_i(κ) nM	HD1	AR1	DA1	HP2	HA2	HP3	HA3	Number of matches
U-69,593	292–3672	0.475–1.89	HD	AR	—	HP	—	HP	HA	5
U-50,488H	630–845	0.69–0.70	HD	AR	—	HP	—	—	HA	4
Salvinorin A	>526	1.9–16	—	—	HA	HP	HA	HP	HA	5
HZ2	>66.7	15	HD	—	HA	HP	—	—	HA	4
Bremazocine	3.8–15.0	0.04–0.12	HD	—	—	AR	DA	HP	—	4
Tifluadom	8.8	0.17	—	AR	—	—	HA	HP	HA	4
TRK-820	2.6	0.195–0.225	HD	AR	DA	HP	HA	HP	HA	7
KNT-63	1.9	0.111	HD	AR	DA	HP	HA	HP	HA	7
KNT-62	1.3	0.152	HD	AR	DA	HP	HA	HP	HA	7
TAN-821	1.2	0.279	HD	HP	—	HP	HA	HP	DA	6
NS-22	1.0	0.135	HD	AR	DA	HP	HA	—	—	5
Morphine	0.02–0.08	33.7–151	HD	HP	—	AR	DA	HP	DA	6
MP-16	0.03	38.7	HD	AR	DA	AR	HA	—	—	5
TPI 614-1	nd[b]	39	HD	AR	HA	HP	—	—	—	4

[a] $\mu/\kappa = K_i(\mu)/K_i(\kappa)$.
[b] No data.

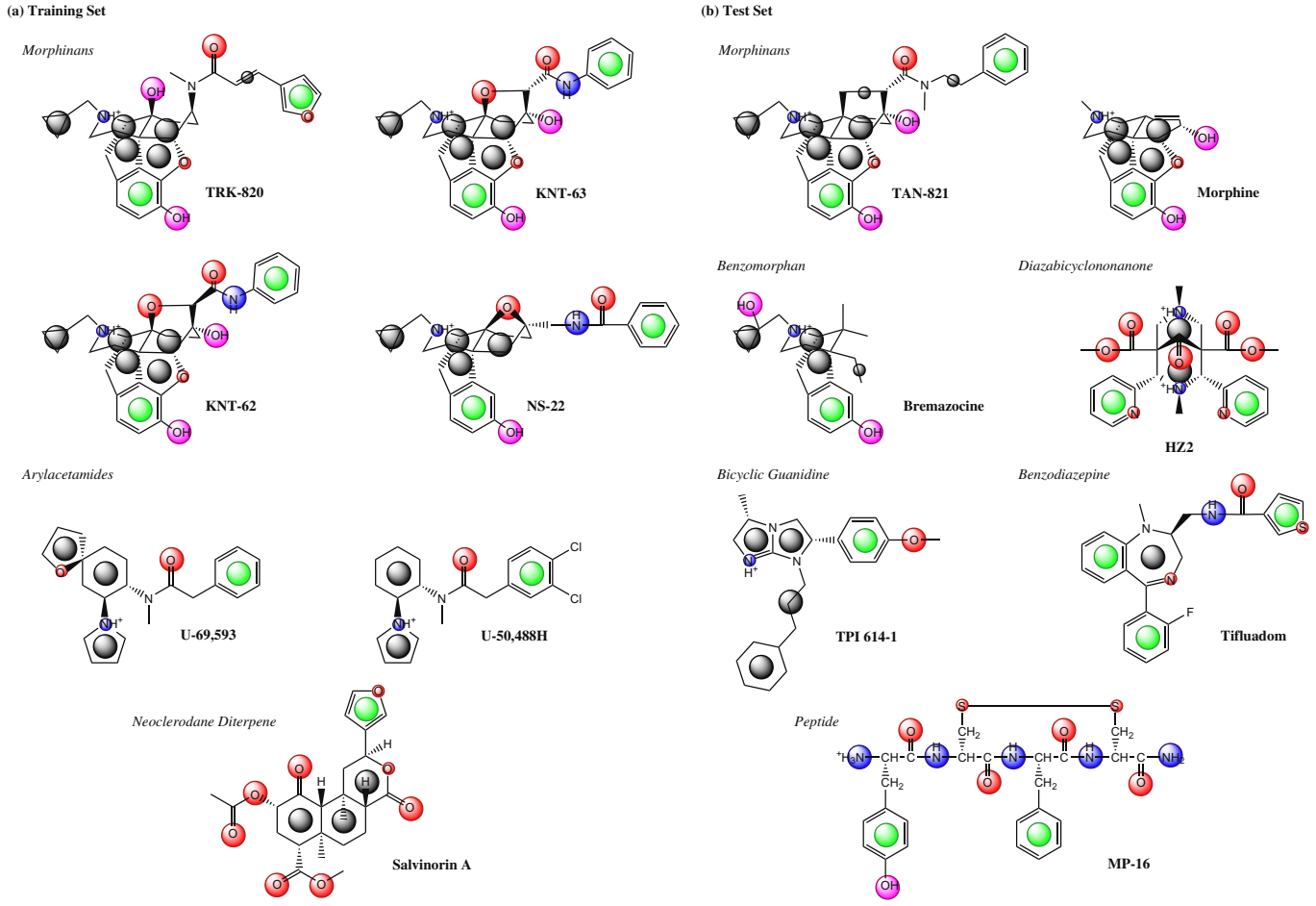

Figure 4. κ-agonists in the data set and their property spheres. (a) Training set comprising four morphinans (TRK-820, KNT-63, KNT-62, and NS-22), two arylacetamides (U-69,593 and U-50,488H), and one neoclerodane diterpene (salvinorin A). (b) Test set comprising two morphinans (TAN-821 and morphine), one benzomorphan (bremazocine), one diazabicyclononanone (HZ2), one bicyclic guanidine (TPI 614-1), one benzodiazepine ((+)-(2*S*)-tifluadom), and one peptide (MP-16; Tyr-C[D-CYS-Phe-D-Cys]-NH_2). The spheres on each molecule are property spheres for molecular superposition. The colors of the spheres indicate the properties as follows: hydrophobic (HP; black); aromatic (AR; green); hydrogen-bond donors (HD; blue); hydrogen-bond acceptors (HA; red); and hydrogen-bond donors/acceptor (DA; violet). Large and small spheres indicate radii of 1 Å and 0.5 Å, respectively.

[^{3}H]U-69,593 (κ) and [^{3}H]DAMGO (μ). Data for other compounds were taken from Refs. 7,9,12–16,18,21,23,46. All agonists were prepared by SYBYL 6.9.1 (Tripos Inc.). The ionization states of molecules were determined according to references or pK_a calculations (ADMET Predictor 4.0, Simulations Plus, Inc.).

3.2. Conformational sampling

The conformational analyses of all compounds were performed on an Apple Power Mac G5 (PowerPC G5; 2.5 GHz; two central processing units [CPUs]) using the Conformational Analyzer with

Table 2
Training set and test set

(a) Training set	μ/κ[a]	K_i(κ) nM
Morphinans		
TRK-820	2.6	0.195–0.225
KNT-63	1.9	0.111
KNT-62	1.3	0.152
NS-22	1.0	0.135
Arylacetamides		
U-69,593	292–3672	0.475–1.89
U-50,488H	630–845	0.69–0.70
Neoclerodane diterpene		
Salvinorin A	>526	1.9–16
(b) Test set		
Morphinans		
TAN-821	1.2	0.279
Morphine	0.02–0.08	33.7–151
Benzomorphan		
Bremazocine	3.8–15.0	0.04–0.12
Diazabicyclononanone		
HZ2	>66.7	15
Bicyclic guanidine		
TPI 614-1	nd[b]	39
Benzodiazepine		
Tifluadom	8.8	0.17
Peptide		
MP-16	0.03	38.7

[a] $\mu/\kappa = K_i(\mu)/K_i(\kappa)$.
[b] No data.

Molecular Dynamics And Sampling (CAMDAS) 2.1 program.[47] Ten molecular dynamics (MD) calculations were simultaneously performed using different initial structures. Each of the MD calculations was carried for 1 ns with an integral time step of 1 fs. The lengths of the covalent bonds were fixed. The temperature of the system was maintained at 1200 K in order to enhance the efficiency of the sampling. Conformers were sampled at every 100 steps. Then, each conformer was minimized until the root-mean-square of the gradients of the potential energy was below 0.001 kcal mol^{-1} $Å^{-1}$. All conformations were clustered with torsion angles of heavy atoms using a threshold of ±30°. The Merck Molecular Force Field (MMFF) 94s was used to evaluate the potential energy surface of the molecule.[48,49] A dielectric constant of 80 was used. The cut-off distance for the non-bonded interactions was not used. The conformers within 15 kcal mol^{-1} of the minimum energy of the molecule were adopted for superposition, because the probability of the existence of conformers with higher energies was low.

3.3. Molecular superposition

All conformations of the agonist molecules were superposed onto one another using the parallel version of the SUPERPOSE program.[50] This version superposes two molecules based on the physicochemical properties of the atomic groups, which is useful for elucidating the 3D-pharmacophore and estimating the binding conformation of each molecule by distinguishing it from the many conformations that are generated by CAMDAS. The program considers five types of physicochemical property: hydrophobic (HP); aromatic (AR); hydrogen-bond donors (HD); hydrogen-bond acceptors (HA); and hydrogen-bond donors/acceptors (DA). Each type is represented as a sphere with a predefined radius (1.0 or 0.5 Å) and is assigned to a functional group in a molecule (Fig. 4). HD, HA and DA were placed on nitrogen, oxygen, and sulfur atoms which are possible to hydrogen-bond. HP and AR were placed at the center of an aliphatic and aromatic ring, respectively. For a long aliphatic chain, HPs were placed on the central carbon atoms in every three sequential carbon atoms. Usually, the radii of the property spheres are 1.0 Å. However, the radius of 0.5 Å is used for a donor and/or an acceptor atom (HD, HA, DA) in a ring which have a hydrophobic or an aromatic property sphere (HP or AR) at the center. In a short aliphatic chain which consists of only two carbon atoms, an HP sphere of 0.5 Å is placed at the center. After molecular superposition, the overlaps of the spheres are scored using the scoring matrix (Table 3). When the planes of two aromatic rings match well, the score between two ARs is 4 points. Otherwise, the score of AR–AR is 3 point as that of HP–HP. One set of conformations of agonists was chosen for the binding conformations based on the sum of the scores, and the 3D-pharmacophore was represented as one set of matched spheres on the binding conformations. For the training set, we used a series of superpositions to determine the binding conformations. The binding conformations of the test set were extracted using those of the training set. The superposition calculations were performed using 28 nodes of a Dell PowerEdge 1950III (Quad Core Xeon X5460; 3.16 GHz; 56 CPUs in total).

Table 3
Scoring matrix

	HP	AR	HD	HA	DA
HP	+3	+3	−2	−2	−2
AR	+3	+4[a]	−2	−2	−2
HD	−2	−2	+2	−2	+1
HA	−2	−2	−2	+2	+1
DA	−2	−2	+1	+1	+1

[a] If the planes of two rings cannot be superposed upon each other, a score of +3 is given.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.04.069.

References and notes

1. Dhawan, B. N.; Cesselin, F.; Raghubir, R.; Reisine, T.; Bradley, P. B.; Portoghese, P. S.; Hamon, M. *Pharmacol. Rev.* **1996**, *48*, 567.
2. Kieffer, B. L. *Cell. Mol. Neurobiol.* **1995**, *15*, 615.
3. Williams, M.; Kowaluk, E. A.; Arneric, S. P. *J. Med. Chem.* **1999**, *42*, 1481.
4. Chavkin, C.; Goldstein, A. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 6543.
5. Dortch-Carnes, J.; Potter, D. E. *CNS Drug Rev.* **2005**, *11*, 195.
6. Kawai, K.; Hayakawa, J.; Miyamoto, T.; Imamura, Y.; Yamane, S.; Wakita, H.; Fujii, H.; Kawamura, K.; Matsuura, H.; Izumimoto, N.; Kobayashi, R.; Endo, T.; Nagase, H. *Bioorg. Med. Chem.* **2008**, *16*, 9188.
7. Nagase, H.; Watanabe, A.; Nemoto, T.; Yamaotsu, N.; Hayashida, K.; Nakajima, M.; Hasebe, K.; Nakao, K.; Mochizuki, H.; Hirono, S.; Fujii, H. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 121.
8. Nemoto, T.; Fujii, H.; Narita, M.; Miyoshi, K.; Nakamura, A.; Suzuki, T.; Nagase, H. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6398.
9. Hunter, J. C.; Leighton, G. E.; Meecham, K. G.; Boyle, S. J.; Horwell, D. C.; Rees, D. C.; Hughes, J. *Br. J. Pharmacol.* **1990**, *101*, 183.
10. Lahti, R. A.; Mickelson, M. M.; McCall, J. M.; Von Voigtlander, P. F. *Eur. J. Pharmacol.* **1985**, *109*, 281.
11. Szmuszkovicz, J.; Von Voigtlander, P. F. *J. Med. Chem.* **1982**, *25*, 1125.
12. Kögel, B.; Christoph, T.; Friderichs, E.; Hennies, H.-H.; Matthiesen, T.; Schneider, J.; Holzgrabe, U. *CNS Drug Rev.* **1998**, *4*, 54.
13. Siener, T.; Cambareri, A.; Kuhl, U.; Englberger, W.; Haurand, M.; Kögel, B.; Holzgrabe, U. *J. Med. Chem.* **2000**, *43*, 3746.
14. Houghten, R. A.; Pinilla, C.; Giulianotti, M. A.; Appel, J. R.; Dooley, C. T.; Nefzi, A.; Ostresh, J. M.; Yu, Y.; Maggiora, G. M.; Medina-Franco, J. L.; Brunner, D.; Schneider, J. *J. Comb. Chem.* **2008**, *10*, 3.
15. Anzini, M.; Canullo, L.; Braile, C.; Cappelli, A.; Gallelli, A.; Vomero, S.; Menziani, M. C.; De Benedetti, P. G.; Rizzo, M.; Collina, S.; Azzolina, O.; Sbacchi, M.; Ghelardini, C.; Galeotti, N. *J. Med. Chem.* **2003**, *46*, 3853.
16. Azzolina, O.; Collina, S.; Linati, L.; Anzini, M.; Cappelli, A.; Scheideler, M. A.; Sbacchi, M. *Chirality* **2001**, *13*, 606.
17. Freye, E.; Boeck, G.; Schaal, M.; Ciaramelli, F. *Pharmacology* **1986**, *33*, 241.
18. Roth, B. L.; Baner, K.; Westkaemper, R.; Siebert, D.; Rice, K. C.; Steinberg, S.; Ernsberger, P.; Rothman, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 11934.

19. Lapalu, S.; Moisand, C.; Mazarguil, H.; Cambois, G.; Mollereau, C.; Meunier, J.-C. *FEBS Lett.* **1997**, *417*, 333.
20. Raynor, K.; Kong, H.; Chen, Y.; Yasuda, K.; Yu, L.; Bell, G. I.; Reisine, T. *Mol. Pharmacol.* **1994**, *45*, 330.
21. Emmerson, P. J.; Liu, M. R.; Woods, J. H.; Medzihradsky, F. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1630.
22. Millan, M. J. *Trends Pharmacol. Sci.* **1990**, *11*, 70.
23. Harding, W. W.; Tidgewell, K.; Byrd, N.; Cobb, H.; Dersch, C. M.; Butelman, E. R.; Rothman, R. B.; Prisinzano, T. E. *J. Med. Chem.* **2005**, *48*, 4765.
24. Jones, R. M.; Hjorth, S. A.; Schwartz, T. W.; Portoghese, P. S. *J. Med. Chem.* **1998**, *41*, 4911.
25. Lin, C.-E.; Takemori, A. E.; Portoghese, P. S. *J. Med. Chem.* **1993**, *36*, 2412.
26. Metzger, T. G.; Paterlini, M. G.; Ferguson, D. M.; Portoghese, P. S. *J. Med. Chem.* **2001**, *44*, 857.
27. Filizola, M.; Villar, H. O.; Loew, G. H. *J. Comput. Aided Mol. Des.* **2001**, *15*, 297.
28. Filizola, M.; Villar, H. O.; Loew, G. H. *Bioorg. Med. Chem.* **2001**, *9*, 69.
29. Kuhl, U.; von Korff, M.; Baumann, K.; Burschka, C.; Holzgrabe, U. *J. Chem. Soc., Perkin Trans. 2* **2001**, 2037.
30. Lavecchia, A.; Greco, G.; Novellino, E.; Vittorio, F.; Ronsisvalle, G. *J. Med. Chem.* **2000**, *43*, 2124.
31. Cappelli, A.; Anzini, M.; Vomero, S.; Menziani, M. C.; De Benedetti, P. G.; Sbacchi, M.; Clarke, G. D.; Mennuni, L. *J. Med. Chem.* **1996**, *39*, 860.
32. Holzgrabe, U.; Brandt, W. *J. Med. Chem.* **2003**, *46*, 1383.
33. Kane, B. E.; McCurdy, C. R.; Ferguson, D. M. *J. Med. Chem.* **2008**, *51*, 1824.
34. Kane, B. E.; Nieto, M. J.; McCurdy, C. R.; Ferguson, D. M. *FEBS J.* **2006**, *273*, 1966.
35. Pogozheva, I. D.; Przydzial, M. J.; Mosberg, H. I. *AAPS J.* **2005**, *07*, E434.
36. Subramanian, G.; Paterlini, M. G.; Larson, D. L.; Portoghese, P. S.; Ferguson, D. M. *J. Med. Chem.* **1998**, *41*, 4777.
37. Yan, F.; Mosier, P. D.; Westkaemper, R. B.; Stewart, J.; Zjawiony, J. K.; Vortherms, T. A.; Sheffler, D. J.; Roth, B. L. *Biochemistry* **2005**, *44*, 8643.
38. Kolb, P.; Ferreira, R. S.; Irwin, J. J.; Shoichet, B. K. *Curr. Opin. Biotechnol.* **2009**, *20*, 429.
39. Michino, M.; Brooks, C. L., III *Proteins* **2009**, *77*, 448.
40. de Graaf, C.; Rognan, D. *Curr. Pharm. Des.* **2009**, *15*, 4026.
41. Katritch, V.; Rueda, M.; Lam, P. C.-H.; Yeager, M.; Abagyan, R. *Proteins* **2010**, *78*, 197.
42. Portoghese, P. S. *J. Med. Chem.* **1965**, *8*, 609.
43. Portoghese, P. S.; Alreja, B. D.; Larson, D. L. *J. Med. Chem.* **1981**, *24*, 782.
44. Nagase, H.; Osa, Y.; Nemoto, T.; Fujii, H.; Imai, M.; Nakamura, T.; Kanemasa, T.; Kato, A.; Gouda, H.; Hirono, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2792.
45. Fujii, H.; Narita, M.; Mizoguchi, H.; Murachi, M.; Tanaka, T.; Kawai, K.; Tseng, L. F.; Nagase, H. *Bioorg. Med. Chem.* **2004**, *12*, 4133.
46. Przydzial, M. J.; Pogozheva, I. D.; Bosse, K. E.; Andrews, S. M.; Tharp, T. A.; Traynor, J. R.; Mosberg, H. I. *J. Pept. Res.* **2005**, *65*, 333.
47. Tsujishita, H.; Hirono, S. *J. Comput. Aided Mol. Des.* **1997**, *11*, 305.
48. Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720.
49. Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 730.
50. Iwase, K.; Hirono, S. *J. Comput. Aided Mol. Des.* **1999**, *13*, 499.