

REVIEW

Molecular modeling of fentanyl analogs*

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Abstract: Fentanyl is a highly potent and clinically widely used narcotic analgesic. A large number of its analogs have been synthesized, some of which (sufentanil and alfentanil) are also in clinical use. Theoretical studies, in recent years, afforded a better understanding of the structure-activity relationships of this class of opiates and allowed insight into the molecular mechanism of the interactions of fentanyl analogs with their receptors. An overview of the current computational techniques for modeling fentanyl analogs, their receptors and ligand-receptor interactions is presented in this paper.

Keywords: fentanyl analogs, molecular modeling, μ -receptor, ligand-receptor interactions.

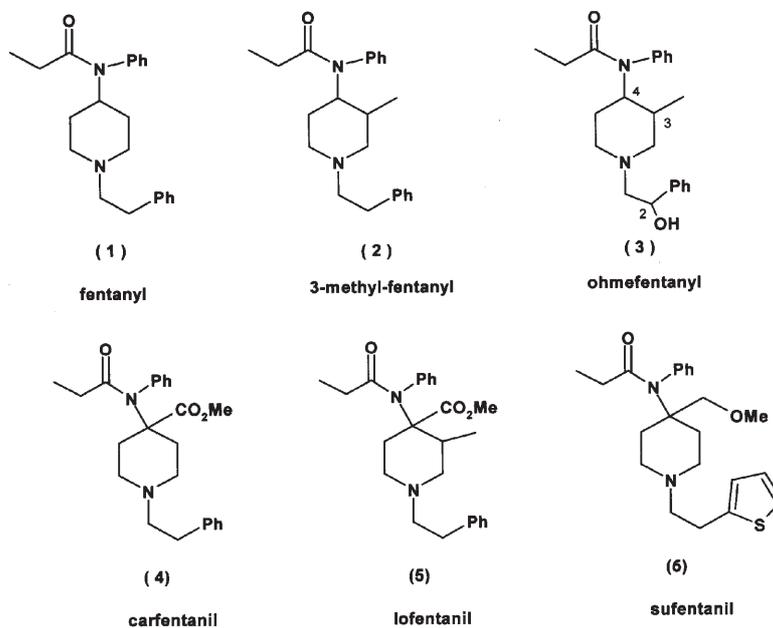
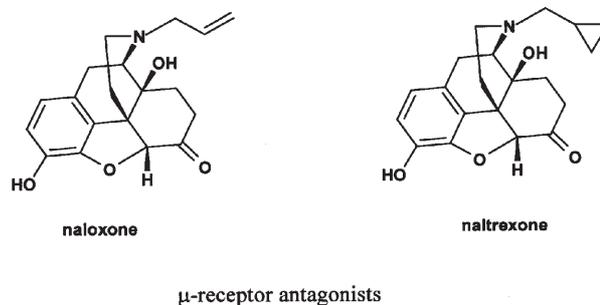
INTRODUCTION

Opioid analgesics present a very important class of drugs, widely used in surgical procedures, in the treatment of post operational pain, cancer pain and other conditions.^{1–6} According to the chemical structure, opioid analgesics are divided into several major classes¹ including: 4,5-epoxymorphinanes, morphinanes, benzomorphans, arylmorphans, pethidines, anilido-piperidines, methadone analogs, opioid peptides and a number of less important groups.

The 4-anilidopiperidines are the most potent class of opioid analgesics known to date.^{1,2} The prototype of this class, fentanyl (**1**), Fig. 1, is 50–100 times more potent than morphine in humans and some of the fentanyl analogs in Fig. 1 are even more active. Thus the (–) *cis* isomer of 3-methylfentanyl (**2**) is *ca.* 16 times more active than fentanyl,⁷ carfentanil (**4**), lofentanil (**5**) and sufentanil (**6**) are all *ca.* 10–30 times more active^{8,9} and the (2*S*,3*R*,4*S*)-isomer of ohmefentanyl (**3**) is 110 times more active¹⁰ than fentanyl. Also, the recently synthesized 3-methoxycarbonylfentanyl¹¹ (**7**), and 4-methylfentanyl¹² (**8**), Fig. 2, exhibit potencies comparable to fentanyl, while acyclic analogs, such as diampromide¹ (**9**) and 2,3-*seco*-fentanyl (**10**), are considerably less active than fentanyl.^{13,14}

* Dedicated to Professor Živorad Čeković on the occasion of his 70th birthday.

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Fentanyl (1) and the most potent fentanyl analogs, μ -receptor agonistsFig. 1. Agonists and antagonists of the μ -opioid receptor.

A very large number of fentanyl analogs have been synthesized in the past 30 years with the aim of producing novel, clinically useful drugs with a better pharmacological profile, and to establish the relation between the structure of the compounds and their pharmacological action, SAR (structure–activity relationships). To achieve this goal, one has to understand how ligands (drugs) recognize and activate the fentanyl receptor. However, until recently, there has been no knowledge of the nature of opioid receptors. Pharmacological studies suggested that there are at least three protein receptors, known as μ , δ and κ that interact with opiate ligands,¹⁵ but there is no high-resolution three-dimensional structure of any opioid receptor, to date. To overcome this difficulty, two major computational approaches

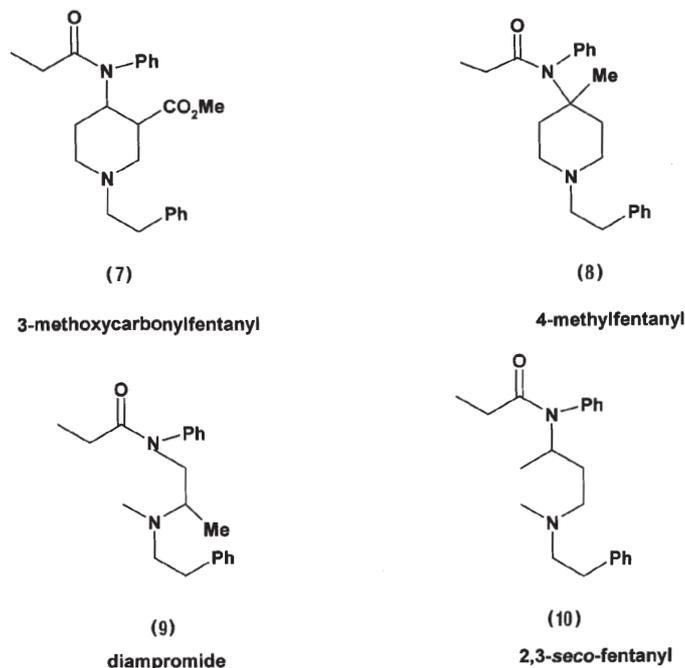


Fig. 2. Fentanyl analogs.

are now in use. They are known as: the pharmacophore modeling and receptor modeling with ligand docking methods. Both methods are based on the same idea that certain molecules can bind and activate a receptor because they comprise functional groups and structural fragments that interact favorably at a receptor binding site, the geometry of which complements the ligand. When a ligand binds to a receptor, the ligand and receptor may interact in a specific manner to produce a pharmacological effect. If, upon binding of a ligand a pharmacological effect is produced, the ligand is known as an agonist. If, on the other hand, a ligand binds to a receptor but produces no effect, that ligand is known as an antagonist. Fentanyl, for instance, binds to a μ -receptor and relieves pain; fentanyl is a μ -receptor agonist. On the other hand, naloxone, Fig. 1, binds to a μ -receptor but produces no analgesic activity; naloxone is a μ -receptor antagonist. Antagonists, like naloxone, are used to displace an opioid agonist from the receptor.

PHARMACOPHORE MODELING

The pharmacophore modeling method is an indirect approach that focuses on the properties of the ligands themselves, determining what major structural features of ligands are necessary for effective binding and receptor activation. Within this method, the three-dimensional structures of high- and low-affinity ligands are compared in order to identify the steric and electronic properties (molecular descriptors) which are required for ligand recognition by a receptor. For flexible com-

pounds, such as fentanyl and its analogs, this procedure requires conformational searching to be performed^{16–18} since receptor recognition and active forms of ligands are typically found among the low energy conformations. Each set of molecular determinants, in a spatial arrangement typical for the compounds which bind to or activate a receptor, becomes a possible three-dimensional recognition or activation pharmacophore. These pharmacophores can be used to search databases containing three-dimensional structures when looking for novel compounds that are potential ligands for the fentanyl receptor.

The great influence of the stereochemistry of the fentanyl class of compounds upon their analgesic activities is well documented.^{1,2} Experimental^{1,2,23} and theoretical studies^{17–22} led to the proposal of the fentanyl activation pharmacophore, Fig. 3, which consists of:

- a protonated amine nitrogen capable of electrostatic attraction to the negatively charged site of the receptor,
- a polar function (C=O group) capable of forming hydrogen bonds with the receptor,
- one aromatic ring involved in lipophilic interaction with the receptor,
- another aromatic ring involved, most likely, in electron transfer interactions with the receptor.

Other structural elements necessary for optimal interaction with the receptor are:

- a piperidine ring in the chair conformation,
- *N*-phenethyl and 4-*N*-phenylpropanamide substituents *trans*, and both equatorial,
- *trans* configuration of the amide group,
- extended conformation of the phenethyl substituent,
- a 4-*N*-phenylpropanamide substituent conformation with the ϕ angle in the range 0 – 30°, Fig. 3, and with the aromatic ring nearly perpendicular to the amide function.

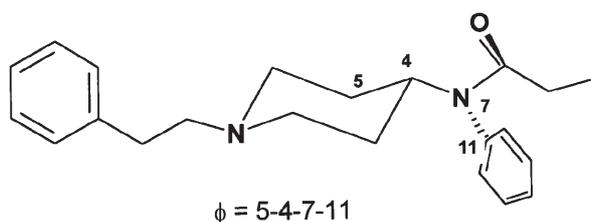


Fig. 3. Fentanyl class pharmacophore.

Favorable variations of this pharmacophore have led to the synthesis of novel potent analogs of fentanyl,^{11,12,24,25} Fig. 2

RECEPTOR MODELING

In the last two decades, experimental studies have led to the discovery of three major groups of opioid receptors, known as μ , δ and κ , previously postulated in pharmacological studies.¹⁵ In the last ten years these receptors were successfully cloned and expressed.²⁶ It was found that all opioid receptors are highly homologous and belong to the superfamily of proteins known as G-protein coupled receptors (GPCR). They consist of a single polypeptide chain containing seven transmembrane domains (TM1 – TM7), Fig. 4, looping back and forth across the lipid membrane. These transmembrane domains are hydrophobic and they have a helical secondary structure. The seven helices pack together to form a seven helix bundle having a more hydrophobic exterior facing the lipid membrane and a more hydrophilic interior suitable for ligand binding. The considerable technical difficulties, such as isolation and purification, prevented the determination of the three-dimensional structure of any of these receptors. Rhodopsin is the only GPCR whose high resolution (2.8 Å) crystal structure has recently been reported.²⁷

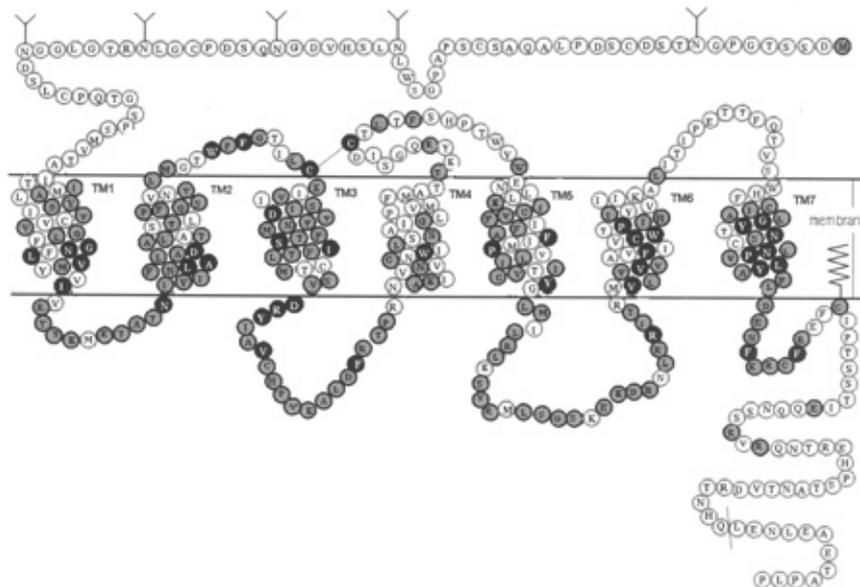


Fig. 4. Seven transmembrane helices of the μ -opioid receptor.

Due to the lack of an experimental three-dimensional structure of the opioid μ -receptor, insight into the receptor-ligand interactions must be inferred with the aid of computed receptor models. The first step in this process is the building of a receptor model. This is mainly achieved through two major strategies.²⁸ One is to build a receptor model using the structure of bacteriorhodopsin (or the structure of rhodopsin since the year 2000) as a homology template. The other is to build a receptor model *de novo*, comparing the orientation of its helix to the low resolution

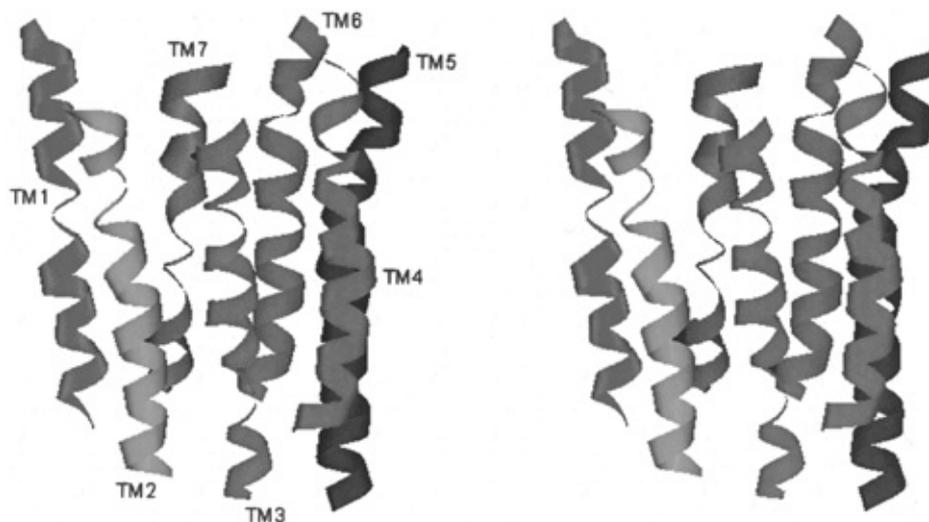


Fig. 5. Stereoview of the transmembrane helices of the μ -opioid receptor.

structure of rhodopsin and to other experimental data. Considering the extensive number of receptor models already generated, a large amount of information can be elicited from a simple sequence alignment to a receptor that has already been modeled, following the expectation that similar protein sequences will have similar three-dimensional structures. The μ -opioid receptor, Fig. 5, has recently been modeled²² based on the template available for transmembrane portion of the rhodopsin family of GPCR.²⁹ The extra- and intra-cellular loops were usually modeled by homology to proteins having a similar sequence taken from the Protein Data Bank, or they may be constructed by suitable computer software, such as the LOOP option³⁰ in the SYBYL package,³⁰ or by a profile-fed neural network³¹ system (PHDhtm) from the EMBL (European Molecular Biology Laboratory) in Heidelberg. The receptor model is usually further refined by using a series of MD (molecular dynamics) simulations, with different constraints,^{22,29-33} followed by geometrical optimization. The structure quality of the receptor model is evaluated for consistency with other opioid receptor models. Sometimes^{22,32} the PROCHECK program³³ is used to check the stereochemical quality of a protein structure.

The receptor model was also tested against the experimental data available from site-directed mutagenesis. The site-directed mutagenesis is a procedure where one amino acid is specifically replaced by another through the laboratory biosynthesis of a modified protein. Testing activity of a mutated receptor provides information on amino acids involved in ligand binding and receptor activation. For μ -opioid receptor important residues (in rat sequence) include³¹: Thr¹³⁷, Ile¹³⁸, Ile¹⁴², Ile¹⁴⁴, Asp¹⁴⁷ in TM3, and His²⁹⁷ in TM6, as well as Val¹²⁶, Asn¹²⁷ in TM2. These residues were expected to affect the receptor ligand interactions, what means that they have to be oriented towards the interior of the helical bundle. How-



ever it should be borne in mind that the results of mutagenesis studies are not necessarily related to receptor-ligand interactions. In fact, mutations can also alter the three-dimensional structure of a receptor and therefore modify the binding profile of a ligand by this mechanism.

When the receptor model is ready and the low energy conformations of a ligand have been determined: experimentally (by X-ray diffraction or from the NMR spectrum) or computationally, the ligand-receptor complex may be constructed by ligand docking to the receptor. In order to obtain meaningful docking results it is necessary that, because of the nature of empirical force field calculations, the same force field is used for the ligand, receptor and the ligand-receptor complex. This usually does not represent a problem since most of the commercially available programs have generally applicable force fields which have parameters for both: receptors and ligands. Still atomic charges for ligand molecules are often missing, they have to be determined by the same procedure as for the receptor atoms. The AMBER program,³⁸ for instance, uses the electrostatic potential-derived atomic charges, with the RESP charge-fitting formalism.^{50,51} The electrostatic potential used by AMBER is calculated by *ab initio* quantum mechanical calculations (HF/6-31G*).

Docking of a ligand into a receptor may be carried out by an automated procedure^{22,30} (using for instance the DOCK program package³⁴ or AUTODOCK set of programs³⁵) or manually.³¹ In both cases, docking is a combination of two components³⁶: a search strategy and a scoring function.

A rigorous search strategy would enable all possible binding modes between the ligand and the receptor to be found. In this procedure all six degrees of translational and rotational freedom of the ligand and the internal conformational degrees of freedom of both the, ligand and the receptor, would have to be explored. However, this is impractical due to the size of the search space. In practice, constraints and approximations were applied in order to locate the lowest energy complex as efficiently as possible. A common approximation in early docking algorithms was to treat both the ligand, and the receptor as rigid bodies. This is not very realistic since a conformational change may occur in both the ligand and receptor upon binding. For this reason, different techniques are used to incorporate conformational flexibility into docking protocol. The most frequently used search strategies are:

The systematic search method. This method is in use in the program EUDOC³⁷ for the systematic search (step by step) of ligand-receptor geometries. Both ligand and receptor are treated as rigid and the AMBER force field is used for energy calculations. Although not highly realistic, this method is useful when a large number of ligands has to be docked, *i.e.* for screening libraries of potentially active ligands.

Molecular dynamics (MD). MD involves the calculation of the solutions to the Newton equation of motion. The simulation begins by giving each atom in a molecule some kinetic energy. This makes the atoms move around. By solving the Newton

equation of motion, it is possible to predict how they move, *i.e.* it is possible to calculate next conformation from the existing one. MD mimics the way a molecule actually explores its conformational space, rather than trying to obtain a picture of the whole conformational space. It is searching local regions of the conformational space. A number of commercially available programs, such as AMBER³⁸ and CHARMM³⁹, can perform MD simulations. An MD simulation of an opioid κ -receptor complex in a phospholipid bilayer has recently been reported³².

The Monte Carlo (MC) method. The standard MC method⁴⁰ (Metropolis MC) involves randomly changing the Cartesian coordinates of the system, calculating the energy, and accepting or rejecting the new position of the system (*i.e.* the new geometry of a complex) based on Boltzman probability. Therefore, it is a stochastic optimization technique and, coupled to potential energy evaluation, it represents one of the most powerful methods for structure optimization and prediction, and for searching conformational space. Programs such as AutoDock⁴¹, Prodock⁴² and MCDOCK⁴³ use MC strategy for docking with AMBER and CHARMM force fields used for geometrical optimization.

The genetic algorithms (GA) method. This method requires the generation of initial complex structures, whereas conventional MC and MD methods require a single starting complex structure. The starting generation may be created randomly but it has to be described by a “chromosome” which is a string of numbers. The “chromosome” can be a list of dihedral angles of a ligand, for instance. The next generation is created by mixing and mutating the information in the “chromosome”. This is achieved by taking two “chromosomes” chopping them in two and recombining. The new generation is evaluated (energy may be one of the criteria) and the best are allowed to make a new generation. The program GOLD⁴⁴ uses GA for docking, as do recent version of the AutoDock program and the DARWIN program.⁴⁵

The fragment-based method. This method is less general than MD or MC but is frequently used in drug design studies. It divides the ligand into separate portions or fragments which are separately docked to the receptor. Docking is followed by linking of the fragments. Decisions have to be made regarding the importance of various functional groups in the ligand and regarding the base fragment to which the other fragments are linked. The choice of base fragment is essential in this method and can significantly affect the quality of the results. The FlexX program⁴⁶ uses the fragment-based method for docking.

All docking procedures generate a number of structures of the ligand-receptor complex while the best one is being searched for, presumably representing the real complex. Therefore complex structures have to be evaluated and this is done by a scoring function. This may be the energy of a complex calculated by a particular force field, or an empirical free energy⁴⁷ of a complex, or a knowledge based function⁴⁸ which exploits structural information of known protein–ligand complexes extracted from the Brookhaven Protein Data Bank and converts it into a distant-de-

pendent free energy of interaction of protein-ligand atom pairs. Based on the scoring function, some structures of the complex are discarded and some are retained until the best complex geometry is found. Docking may also be validated by the results of site-directed mutagenesis studies and previous structure-activity studies.

The ligand binding modes of a series of fentanyl derivatives have recently been reported.²² The low energy (largely populated) conformations in water of eight analogs of fentanyl, all with a high affinity for the μ -receptor were used for docking. The docking was performed using the automated rigid body docking procedure implemented in the DOCK 3.5 program. All predicted ligand-receptor orientations were evaluated based on the calculated energies (van der Waals and electrostatic energies were considered) and the experimental results of site-directed mutagenesis experiments. For instance, a protonated basic nitrogen in the fentanyl analogs is an important part of the pharmacophore, as was found by site-directed mutagenesis experiments. Therefore, the ligand orientations are favored if the nitrogen proton is proximal to the carboxyl group of the TM3 aspartate, enabling strong electrostatic stabilization. Refinement of the receptor-ligand complex was achieved by in vacuum energy minimization followed by MD simulation with a medium dielectric constant $\epsilon = 4$, and final geometry optimization. The final complex structure orientation of the ligand was such that the *N*-phenethyl group of fentanyl was placed deep in the crevice between TM helices 2 and 3, while the *N*-phenylpropanamide group was in the pocket formed by TM helices 2, 6 and 7. The results are consistent with ligand binding data derived from native and mutant receptor studies, as well as with structure-activity relationship data reported on a wide range of fentanyl analogs.

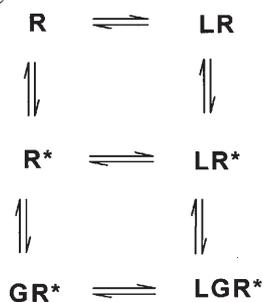


Fig. 6. The scheme of receptor activation.

However, efficient binding to a receptor does not guarantee that a ligand will produce a pharmacological action. The ligands may act as an agonist or as an antagonist, a concept slightly changed in most recent studies. A possible model of receptor activation was deduced by studying and comparing the structures of the agonist- and antagonist-receptor complexes and the structure of the ligand-free receptor. Some plausible mechanisms of receptor activation have been proposed. These range from suggestions of individual residues that act as switches, to more complex models involving conformational changes of the receptor, or receptor

dimerization.⁴⁸ Two recent developments²⁸ in this field directed research to a new model of receptor activation. The first development was the mutation-produced receptor which was active by itself (without the binding of an agonist). The second development was the discovery that a number of antagonists were capable of reversing this constitutive activity, *i.e.*, to make the receptor inactive again. Therefore, more than the simple formation of an agonist–receptor complex, receptor activation looks like series of equilibrium reactions, Fig. 6, where R is the ground state of a receptor and R* is its activated state (in a different conformation). Ligand (L) binding can favor either the ground state (LR) if the ligand is an antagonist, or the activated state (LR*) if it is an agonist. The activated, ligand bound, complex then associates with a G-protein and produces a pharmacological response.

CONCLUSION

Computational studies of the opioid ligand–receptor complex can provide new insight into the requirements for recognition and activation, especially if coupled with the results of pharmacological experiments, such as site-directed mutagenesis, and experimental structure–activity studies. They provide insight into the nature of drug–receptor interactions, help in creating models of the mechanism of action of a drug and will hopefully assist in the creation of new potent analgesics free of adverse effects.

ИЗВОД

МОЛЕКУЛСКО МОДЕЛИРАЊЕ АНАЛОГА ФЕНТАНИЛА

ЉИЉАНА ДОШЕН-МИЋОВИЋ

Хемијски факултет, Универзитет у Београду, б. бр. 158, 11000 Београд и Центар за хемију, ИХТМ, Београд

Фентанил је високо активан наркотички аналгетик у широкој клиничкој употреби. Синтетисан је велики број његових аналога, а неки од њих су (суфентанил и алфентанил) такође у клиничкој употреби. Теоријска испитивања током последњих година омогућила су боље разумевање везе између структуре и активности ове класе опијата, као и увид у молекулски механизам интеракције аналога фентанила са њиховим рецепторима. Овде је дат преглед савремених рачунских метода за моделирање аналога фентанила, њихових рецептора и лиганд–рецептор интеракција.

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