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The Structural Analysis and Optimization of Gefitinib

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ABSTRACT

In order to decrease the clinical side effects of Gefinitib, firstly, the structure of Gefinitib was optimized based on the analyzing result of hydrophobic rings distribution in the active site. Secondly, based on a small molecule library, the Gefinitib structure and the pharmacophore were optimized and obtained 34 optimized structures. Finally, 3 drug molecules that have better indicators and less drug toxicity than Gefinitib were screened based on the calculations of molecular docking, pharmacokinetics, toxicity prediction and molecular dynamics simulation.

Key words：Gefinitib, hydrophobic ring, small molecule library, optimization, screening

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INTRODUCTION

Gefitinib is a target drug molecule for non-small-cell lung cancer (NSCLC) [1], and the target of it is the epidermal growth factor receptor tyrosine kinase (EGFR-TK) [2]. When Gefitinib combined with its target receptors, the phosphorylation and transphosphorylation of EGFR dimers were blocked [3], and then the intracellular signal transduction were inhibited, in this way the Gefitinib can prevent the proliferation of malignant cells [4,5]. The specific structure which caused cancer is the therapeutic target of drugs which only kill tumor cells and do no harm to normal cells [6].

During the process of clinical medicine, the common adverse reactions of Gefitinib are rash, diarrhea, fatigue, loss of appetite, skin rough or itching and paronychia [7, 8]. The side effects may due to two reasons. For one thing, the specificity of Gefitinib is not high enough, when Gefitinib acts on EGFR; it may also act on other targets. For the other, it is because of the limitation of drug molecule structure, when the drug combines with its target, it may not obtain the best combination. Indeed, the combination of drug molecular and target is to achieve the geometry identification and energy matching between functional groups and its target [9]. Only all reasonable functional groups of the drug molecule are located at the appropriate target binding sites, could the drug's pharmacophore achieve the best results.

In this paper, Gefitinib molecular structure was optimized based on the distribution of hydrophobic ring in its target and a small molecule library of Discovery Studio (version 2.5), and 3 drug molecules better than Gefitinib were screened.

1. The assessment of hydrophobic ring in Gefitinib

Gefitinib (Figure 1) is an oral epidermal growth factor receptor tyrosine kinase (EGFR-TK) inhibitor produced by Astra Zeneca Corporation. It is a 4-arylamine quinazoline derivative. As a target drug for non-small cell lung cancer (NLCLC) [10], Gefitinib has a good therapeutic effect in phase I clinical treatment [11]. The experimental results of Fry [12, 13] show that the quinazoline ring is the essential pharmacophore of Gefitinib.

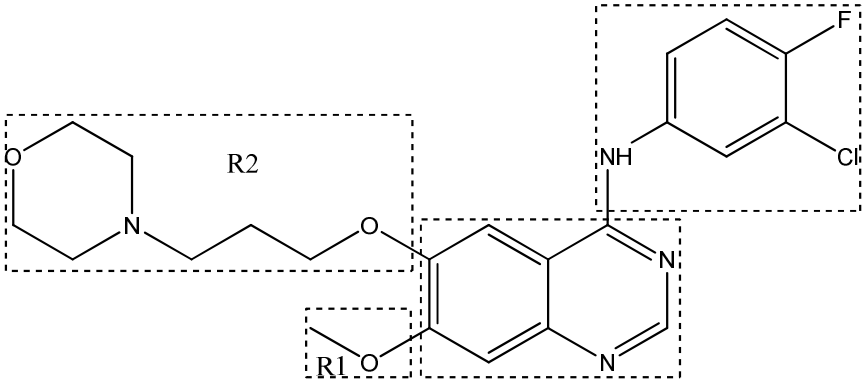


Fig.1 The plane structure of Gefitinib molecular

In order to examine the distributions of every hydrophobic ring in the activity pocket, the structure of Gefitinib was firstly divided into 4 fragments, i.e., R1, R2, 4-aromatic amines and quinazoline ring (as shown in the dashed box in Figure 1), Then, these fragments were put into the activity pocket of EGFR-TK step by step, and their distributions and dock scores were calculated by Discovery Studio. The structure of Gefitinib was optimized according to the scores.

(1) The distribution analysis of Quinazoline ring

Quinazoline ring is the pharmacophore of Gefitinib [12], and it is also the important hydrophobic ring in molecular structure. By using multiple copy simulation searches (MCSS) method the best position for fragments was searched in the active pocket [14] as follows:

a) A number of quinazoline rings were put into the active pocket randomly (Figure 2).

b) The best position of Quinazoline rings was found with Monte Carlo simulation and molecular mechanics optimization [15] in Chemistry at Harvard Macromolecular Mechanics (CHARMm) force field [16].

c) After cluster analysis and scoring of these fragments (Figure 3), the location that Quinazoline rings should exist theoretically were found in the active site.

According to the MCSS score and the distribution of quinazoline ring in the activity pocket, it is believed that there should be a hydrophobic ring at the end of the R2 substituent (Figure 4).

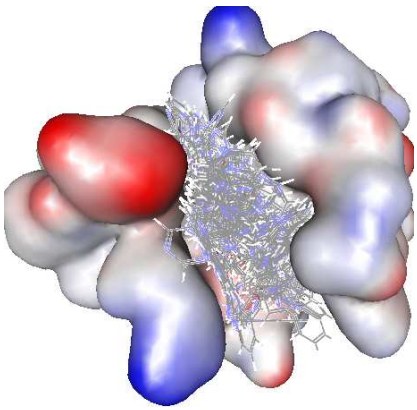


Fig.2 Quinazoline ring in the active pocket

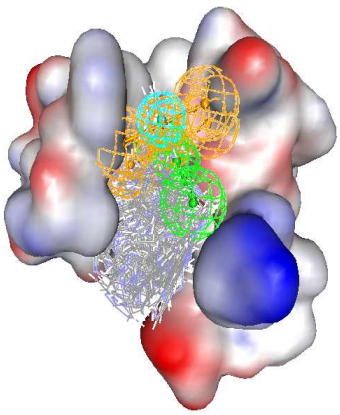


Fig.3 Cluster analysis results

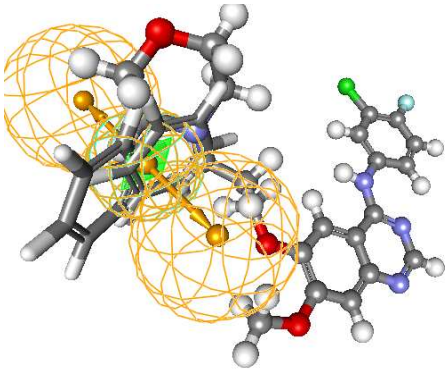


Fig.4 The location of the hydrophobic ring

(2) The distribution of benzene ring

Quinazoline ring is the pharmacophore of Gefitinib, in addition, if it connected to the R2 substituent directly, it would make the molecular weight of the drug become so large that it would become much more difficult to be synthesized. Meanwhile, the benzene ring, as a hydrophobic fragment, is often found in molecular structure of drugs. So, in this research, the benzene rings are put into the active pocket (Figure 5) as hydrophobic rings, and the distribution of them are calculated. The calculation results showed that there is a highest score position for the rings and the red benzene ring in Figure 6 signified the optimized location. (Figure 6)

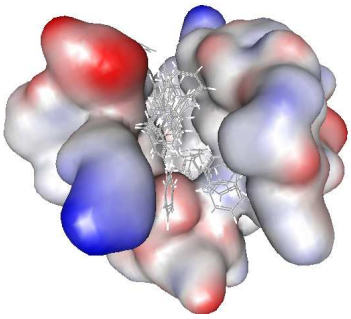


Fig.5 Benzene ring in the active pocket

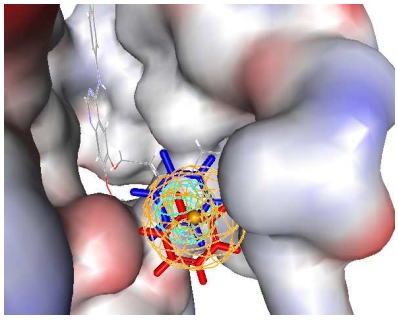


Fig.6 The best distribution of benzene in the activitypocket

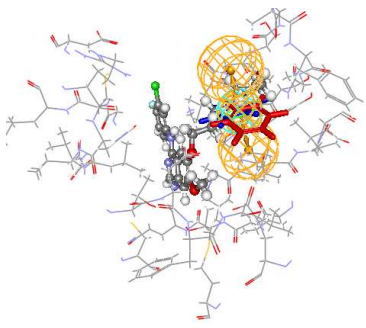


Fig.7 The position of benzene

The feature analysis indicates that a benzene ring should be put at the end of the R2 substituent to enhance the hydrophobic function (Figure 7). By using fragments connection tool, the benzene rings showed in figure 8 were connected to the Gefinitib molecule (Figure 8) and a new molecule was obtained. But the molecular weight of the new one is so large that make it difficult to be

synthesized. In order to increase the hydrophobicity of R2 as well as keep a relatively smaller molecular weight of the new molecule, the idea to replace the morpholine ring by benzene ring (Figure 9) was being considered.

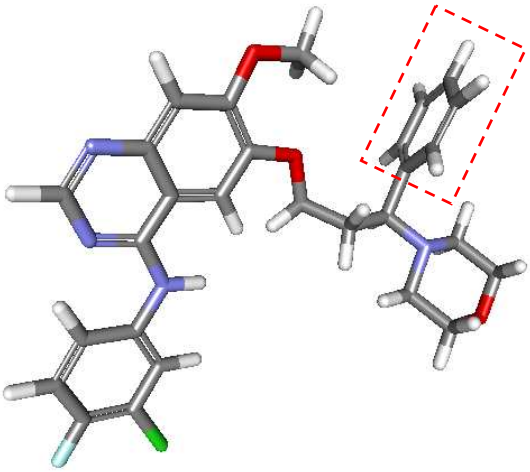


Fig.8 101st molecular structure

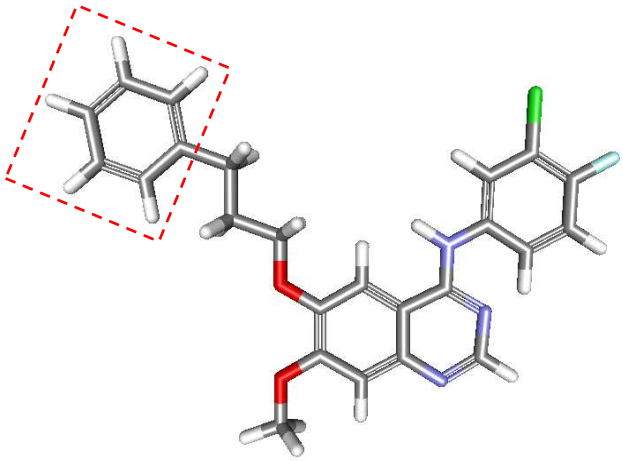


Fig.9 102nd molecular structure

(3) Structural optimization of Gefitinib based on four functional groups

Except for the 2 structures in Fig.8 and Fig. 9, the paper also carried out other structural optimizations of Gefitinib based on four functional groups. Keeping the main structure of quinazoline ring stable, the four sub-structures of Gefitinib were docked into the EGFR-TK active pocket. Then the cluster analysis and optimize the structure of Gefitinib molecule were carried out. At last, 3 optimal structures were got (Figure 10).

According to the above 5 structures, the parameters of them were calculate, including the molecular weight, LogP, molecular minimum energy, hydrogen bond acceptor and donor. The results are listed in Table 1.

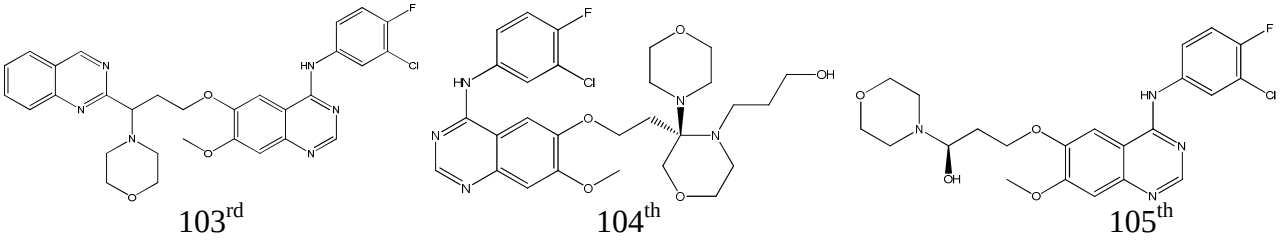


Fig.10 Molecular structure of three drugs

Tab. 1 the parameters of the five molecules

Drugs	Molecular Weight	LogP	H-bond Donor	Molecular Minimum Energy(kJ/mol)	H-bond Acceptor
101st	523.00	6.28	1	16.62	5
102nd	437.13	7.025	1	10.62	4
103rd	575.03	6.309	1	22.01	6
104th	576.06	3.588	1	29.68	6
105th	462.90	4.014	2	12.03	5

2. The optimization of lead compounds based on Gefitinib

On the basis of Gefitinib molecular structure and the small molecule library of Discovery Studio (version 2.5), the structure of Gefitinib is optimized. Firstly, the small molecules are docked into the

EGFR-TK active pocket. Secondly, through cluster analysis of small molecule locations, the necessary pharmacophore features are obtained (Figure 11, 12). In both figures, the green ball is the hydrogen bond acceptor and the light blue ball is the hydrophobic center, the dark blue ball indicates the negative center and the orange ball shows the position of benzene ring. Finally, Gefitinib and the pharmacophore structures are optimized.

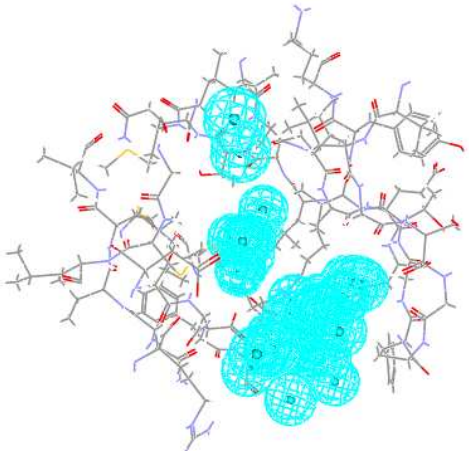


Fig.11 The position of hydrophobic groups in the active pocket

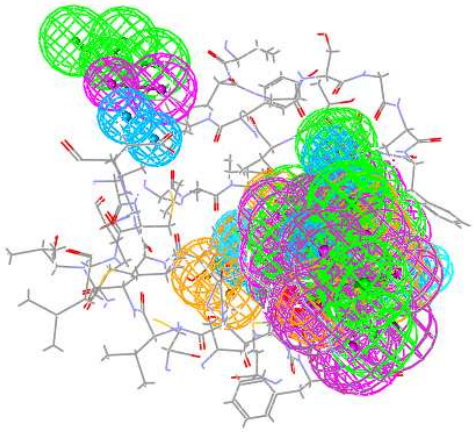


Fig.12 The structure of pharmacophore

Tab. 2 the parameters of the 21 molecules

Drugs	Molecular Weight	LogP	H-bond Donor	Molecular Minimum Energy(kJ/mol)	H-bond Acceptor
2101st	488.98	5.772	1	15.6	5
2102nd	484.95	5.445	1	22.9	5
2103rd	503.95	3.382	2	16.81	6
2104th	489.90	4.244	2	13.84	6
2105th	546.04	2.985	4	19.17	5
2106th (101st)	523.00	6.28	1	17.88	5
2107th	515.02	6.025	1	50.71	5
2108th	529.05	6.353	1	14.29	5
2109th	490.95	4.657	1	12.81	6
2110th	503.95	5.226	2	12.03	6
2111th	526.99	5.387	2	39.79	5
2112th	488.98	5.656	1	11.31	5
2113th	526.00	3.732	2	36.34	5
2114th	489.97	4.365	1	21.79	5
2115th (105th)	462.90	4.014	2	12.03	5
2116th	521.05	6.042	1	14.66	5
2117th	530.03	5.031	1	33.35	5
2118th	474.96	5.306	1	10.93	5
2119th	490.95	3.816	2	16.79	5
2120th	560.06	4.664	2	33.57	5
2121st	470.92	5.01	1	18.89	5

2.1 The optimization of Gefitinib molecular

The Gefitinib was put into the activity pocket and the structural optimization was carried out. 21 optimal structures are obtained at last (see appendix figure 1), and then the molecular weight, LogP, molecular energy and the receptor of hydrogen bonding (Table 2) of 21 drugs were calculated.

2.2 The optimization of 4-phenylamino quinazoline molecular

The 4-phenylamino quinazoline ring was put into the active pocket and then connected to the nearest small segment, six optimal structures (see appendix figure 2) were obtained. The relevant parameters of these structures are also calculated and listed in Table 3.

Tab. 3 the parameters of 6 molecules

Drugs	Molecular Weight	Log P	H-bond Donor	Molecular Minimum Energy(kJ/mol)	H-bond Acceptor
2201st	273.33	5.853	1	12	2
2202nd	303.40	7.321	1	15.77	2
2203rd	306.36	3.452	2	6.38	3
2204th	249.31	5.523	1	4.11	2
2205th	350.42	7.374	2	65.58	2
2206th	320.39	4.245	2	7.27	3

2.3 The optimization of quinazoline molecular

Same as the above process, the quinazoline ring was put into the active pocket and then connected to the nearest small segment, and obtain four optimal structures (appendix figure 3). The relevant parameters of these structures were also calculated (Table 4).

Tab. 4 the parameters of the four molecules

Drugs	Molecular Weight	Log P	H-bond Donor	H-bond Acceptor
2301st	212.29	4.057	0	2
2302nd	215.25	0.231	1	3
2303rd	158.20	2.258	0	2
2304th	259.31	4.112	1	2

3. The evaluation and prediction of the optimized structures

According to the "Rule of Five" of Lipinski [17], that is, if drug molecules are good at absorption and penetration, they must meet several conditions as follows:

a) Hydrogen-bond donor (the number of hydrogen atoms connected with the N and O) should less than 5;

b) Relative molecular mass should less than 500;

c) Log P <5;

d) Hydrogen bond acceptor (number of N and O atoms) is less than 5.

According to the rule, a total of 13 drugs (105th, 2103rd, 2104th, 2109th, 2114th, 2119th, 2121st, 2203rd, 2206th, 2301st, 2302nd, 2303rd and 2304th) were selected for further analysis. In addition, taking the distribution of hydrophobic ring into account, 101st and 102nd were also selected for further analysis.

3.1 The docking

The molecular docking of the drug with its target is a process to achieve complementary in energy, geometry structure and the surrounding chemical environment. The docking results were evaluated with a score function [18]. 15 selected structures were docked to EGFR-TK active pocket and the results of the highest dock score for these selected drugs were obtained (Table 5), among which the dock score of Gefitinib was 125.0.

Tab. 5 the docking score of the selected molecular and the intermolecular hydrogen bond number

Drugs	101st	102nd	105th	2103rd	2104th	2109th	2114th
Dock Score	134.5	125.7	132.9	136.6	136.4	130.2	131.3
Intermolecular H-bond	2	1	2	4	2	4	1

Drugs	2119th	2121st	2203rd	2206th	2301st	2302nd	2303rd	2304th
Dock Score	132.0	133.7	103.3	106.1	76.0	79.0	61.2	85.7
Intermolecular H-bond	3	3	0	1	0	0	2	1

According to the results of molecular docking and the formation of intermolecular hydrogen bonds, 8 drugs with higher dock score were selected (101st, 105th, 2103rd, 2104th, 2109th, 2114th, 2119th and 2121st) for the further study.

3.2 Pharmacokinetic characteristics and toxicity prediction

Pharmacokinetic parameters quantitatively describe the absorption, distribution, metabolism, excretion and other properties of drugs in vivo. The toxicity of drugs includes mutagenicity, skin sensitization, skin and eye irritation, carcinogenicity, etc [19]. The calculation results of the 8 screened drugs listed in table 6.

The calculation results show that the blood-brain barrier (BBB) level of drug 101st is 4, which signified a very high barrier level; and the LD50 of rat is 145.0 mg/kg, which signified the lowest concentration of the Lethal Dose 50 comparing with other drugs. The 101st structure has a higher dock score than others, but meanwhile it has a higher level of liver toxicity and the lipid-water partition coefficient is higher than 5. The mutagenicity and skin sensitization of 101st are also higher than those of Gefitinib, and its molecular weight is relatively bigger.

The BBB level of drug 105th and 2103rd are 2 and 3 respectively, which signifies that the two drugs were better than Gefitinib in this indicator. The liver toxicity level are 0.45 and 0.47 for

105th and 2103rd respectively, slightly higher than Gefitinib (0.39), but the two drugs will not cause significant liver toxicity if the dose increased. The LogP indicator of 105th is 4.0 and 3.4 for 2103, both are much better than that of Gefitinib. Aerobic biodegradability level of 105th is 1, significantly higher than Gefitinib, which implies that 105th has obvious bio-degradable advantages. Both 2103rd and 105th have the possibility of alleviating or eliminating the skin irritation and itching, because their corresponding indicators are lower than Gefitinib.

Comparing with Gefitinib, 2119th has a level of 2 of the BBB and 3.8 of the Log P, which are lower than those of Gefitinib. The skin irritation and skin sensitization indicators of 2119th equal 0, which signifies that 2119th might alleviate or eliminate symptoms of skin allergies and pruritus. The developmental toxicity potential (DTP) indicator of 2119th is very low, which indicates that the potential toxic effect for growth and development was lower.

Analysis shows that the 105th, 2103rd and 2119th drugs are better than Gefitinib in parameters of pharmacokinetics and toxicity. Therefore, the three drugs are screened for further analysis.

4 Molecular dynamics simulation.

In order to get a better understanding of the dynamics and thermodynamic process of the three screened drugs and the change information of all kinds of small molecules over time of the system, the molecular dynamics simulation were carried out. First of all, three drug structures were treated in the same way, that is, all of them were studied in an environment of water, chloride and sodium ions (Figure 13), and the force fields of them are calculated with CHARMm method [15]. Secondly, the energy optimization was calculated by using the method of the Steepest Descent [20] and Conjugate Gradient algorithms [21] respectively, the dynamic equilibrium was calculated for the entire system, and the simulation were done in a condition of isothermal-isobaric. Finally, the changes along with time of the system temperature, total energy, Van der Waals energy and potential energy were analyzed (Fig.14, Fig.15 and Fig.16), the simulation results are shown in table 7.

The simulation results show that the intermolecular hydrogen bond number between 105th (or 2119th) and the drug target is less than 3, and the initial potential energy of 105th drug molecule is the lowest among the 3 drugs, that the total energy of 105th drug is the lowest when the whole system reached a steady state. Comparing with Gefitinib, the system temperature of 105th drug is lower by 3.57K and 2103rd drug is lower by 1.68K. The potential energy and kinetic energy of 105th drug are slightly lower than the other two drugs. From the change over time of the system temperature, total energy, Van der Waals energy and potential energy, we find that the dynamic process of the 3 drugs became stable after initial fluctuations.

Tab. 6 the pharmacokinetics characteristics and the toxicity prediction of the selected drug

Drugs and parameters		Gefitinib	101st	105th	2103rd	2104th	2109th	2114th	2119th	2121st
Pharmacokinetic	BBB* level	1	4	2	3	3	2	1	2	1
Characteristics	HIA* level	0	1	0	0	0	0	0	0	0
	AS*-level	2	1	2	2	2	2	2	2	1
	Hepatotoxicity	0.39	0.65	0.45	0.47	0.46	0.44	0.45	0.55	0.45
	PPB*-Level	1	2	1	0	0	1	1	0	2
	CYP2D6*	0.59	0.45	0.59	0.58	0.59	0.64	0.74	0.59	0.62
Toxicity	LogP	4.5	6.3	4.0	3.4	4.2	4.7	4.4	3.8	5.0
Prediction	AB* level	0	0	1	0	0	0	0	0	0
	Ames Mutagenicity	0	0.98	0	0	0	0	0	0	1
	Skin Sensitization	0.271	0.991	0.002	0.001	1	0.860	0.001	0	0.977
	Skin irritation	0.002	0	0.001	0	0.011	0	0	0	0
	Rat inhalational LC50(mg/kg)	347.2	145.0	552.3	406.1	1800.0	449.7	388.0	542.7	987.2
	NTP* Carcinogenicity	0	0	0	0	0		0	0	0
	DTP*	0.80	0.96	0.99	1	0.92	0.94	1	0.57	0.15

Note: 1. BBB (Blood Brain Barrier) *2. HIA (Human Intestinal Absorption)*
3. AS (Aqueous Solubility) *4. PPB (Plasma Protein Binding)*
5. CYP2D6 (Cytochrome P450 2D6) *6. AB (Aerobic Biodegradability)*
7. NTP (the National Toxicology Program) *8. DTP (Developmental Toxicity Potential)*

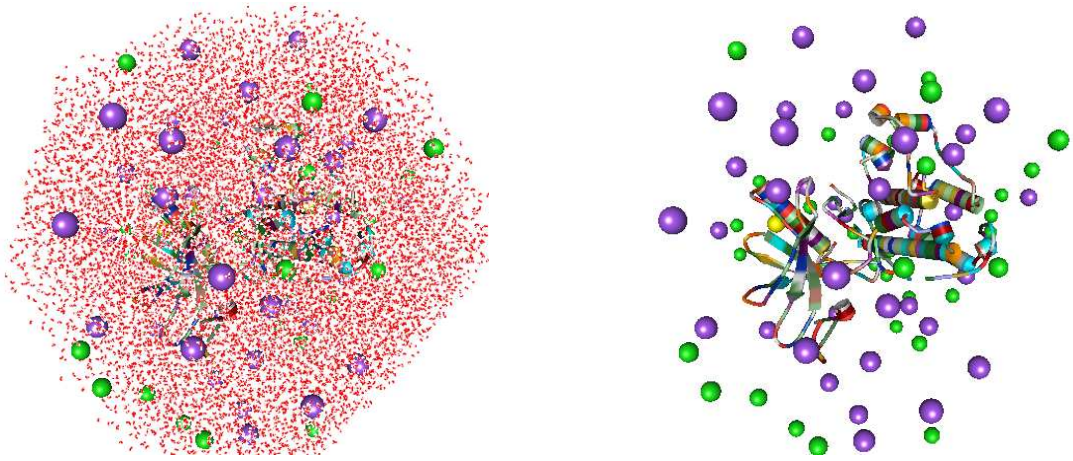


Fig. 13 The situation after imposed water and ion environment

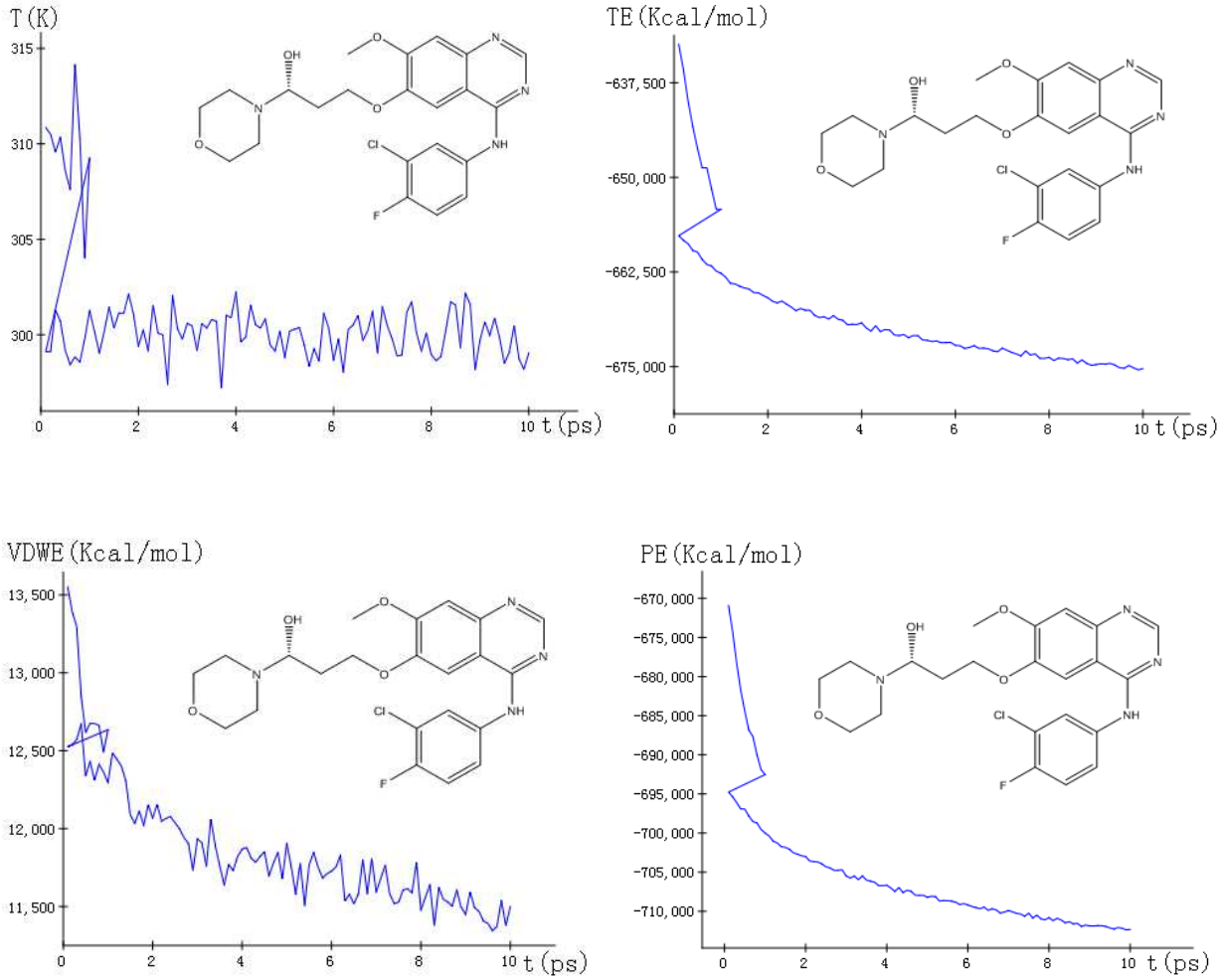
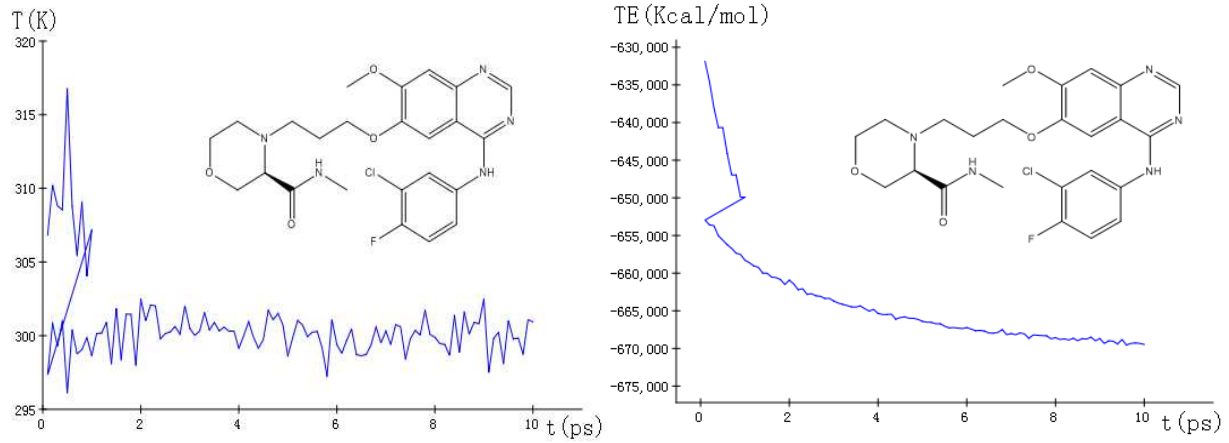


Fig 14 Molecular dynamics simulation of 105th drugs



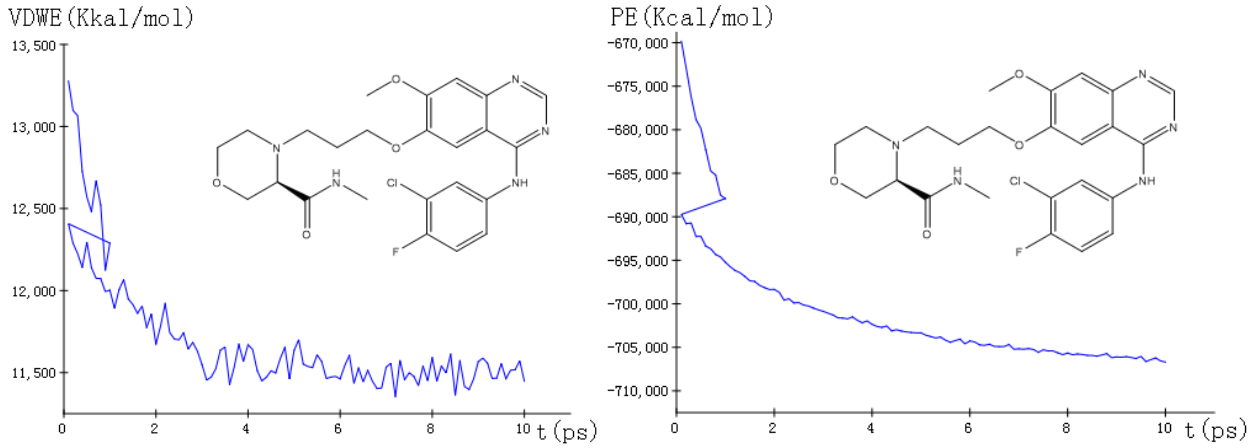


Fig 15Molecular dynamics simulation of 2103rd drugs

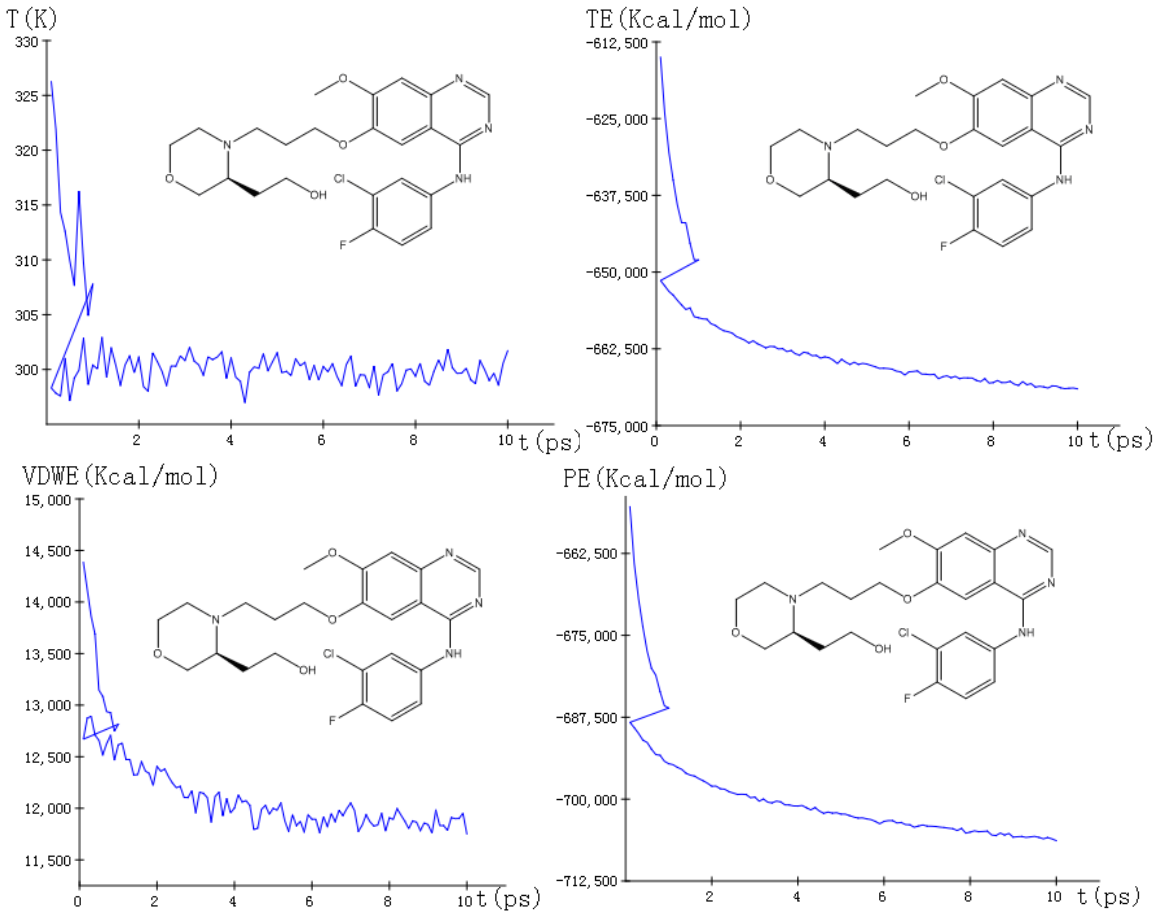


Fig 16 Molecular dynamics simulation of 2119th drugs

Tab. 7 the comparison of molecular dynamics simulation results

Drugs	Gefitinib	105th	2103rd	2119th
Force field	CHARMm	CHARMm	CHARMm	CHARMm
Initial Potential Energy (kcal/mol)	-689819.76	-692534.93	-689271.48	-687476.22
Total Energy(kcal/mol)	-670173.94	-675271.93	-669472.11	-669011.10
Potential Energy (kcal/mol)	-707656.02	-712320.22	-706695.52	-706335.26
Kinetic Energy (kcal/mol)	37482.08	37048.28	37223.41	37324.16
Temperature (K)	302.61	299.04	300.93	301.70
Van der Waals Energy (kcal/mol)	11666.59	11499.37	11447.82	11751.34
Electrostatic Energy (kcal/mol)	-637179.59	-638354.89	-634797.55	-633145.14

RESULTS

Firstly, the location of hydrophobic ring in the active pocket was analyzed, and the R2 substituent of the Gefitinib was optimized. The results show that the dock score of 101st is significantly higher than Gefitinib, and the connection of 101st molecule to its target is better than Gefitinib, that the BBB indicator of 101st increases significantly, and that the LD50 decreases from 347.2 mg/kg to 145.0mg/kg. Research results also indicate that the change of certain indicators is negative, for example, the liver toxicity level increases, and Log P becomes higher than 5, and the skin sensitization indicator increases to a degree. So the 101st molecule was given up without further study.

Except for the excellent blood-brain barrier, the optimal lipid-water partition coefficient and the good intestinal absorption, the 105th drug has better indicators than Gefitinib. The degree of skin sensitization and skin stimulus of 105th drug decrease significantly, and its indicator of aerobic biodegradability keeps the highest. Optimization result shows that the 2103rd and 2119th drug have better blood-brain barrier indicator than that of 105th, and their skin sensitization and skin irritation are rather low or 0 solely. These drugs have the potential to eliminate skin irritation, and have the good application prospect in the treatment of non-small-cell lung cancer (NSCLC).

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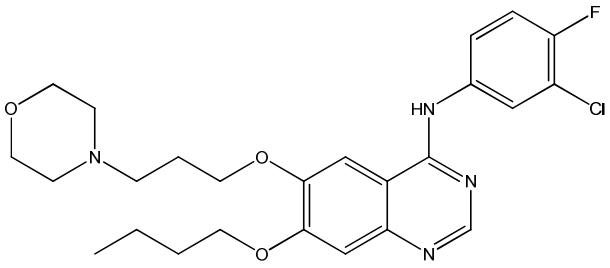
Additional figure 1 The optimization structure of 21 drug molecules based on Gefitinib



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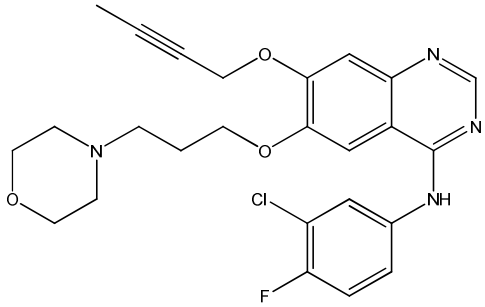

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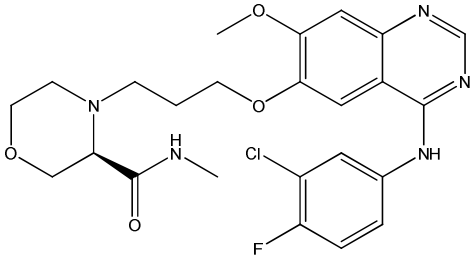

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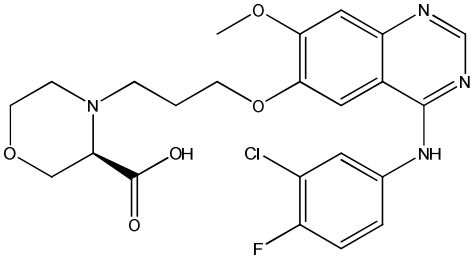

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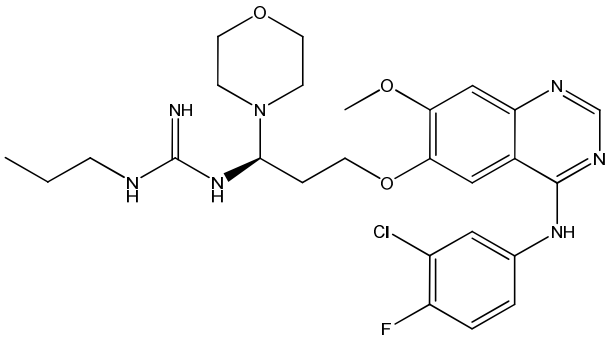

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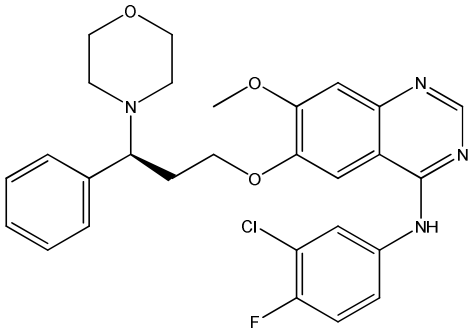

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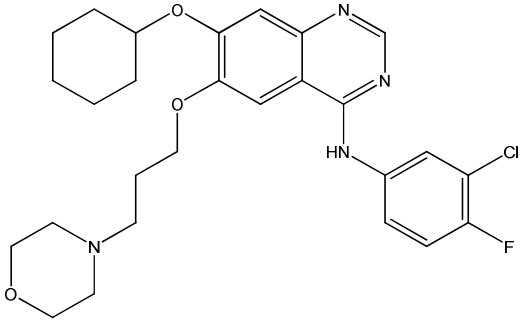

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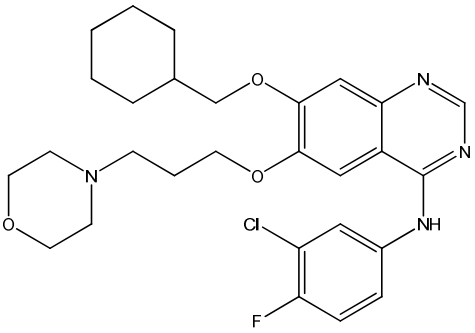

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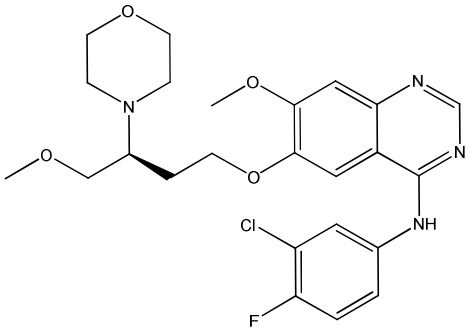

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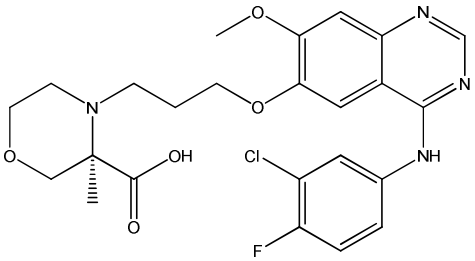

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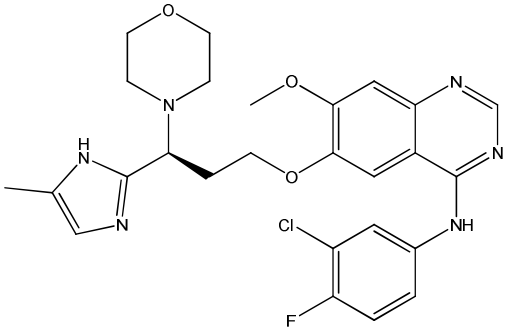

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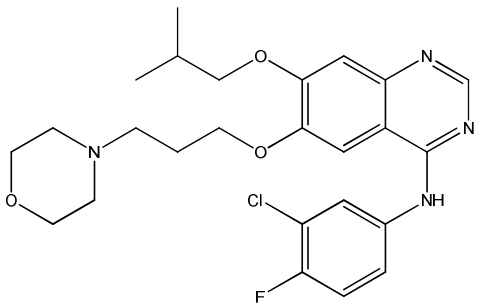

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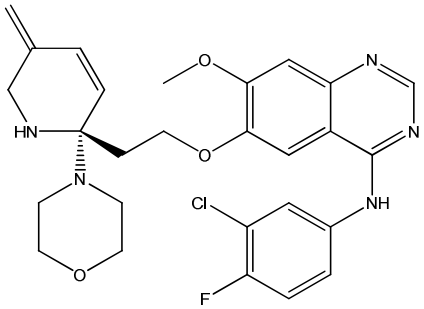

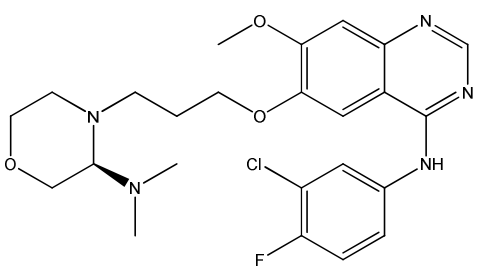

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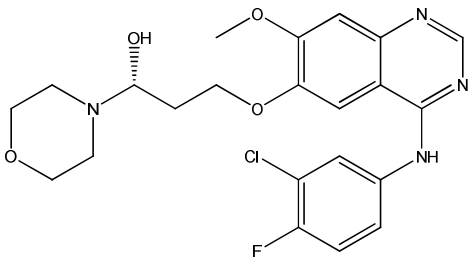

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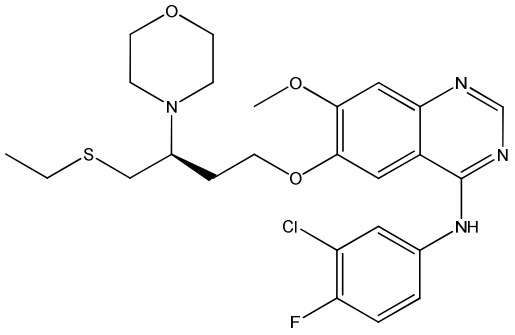

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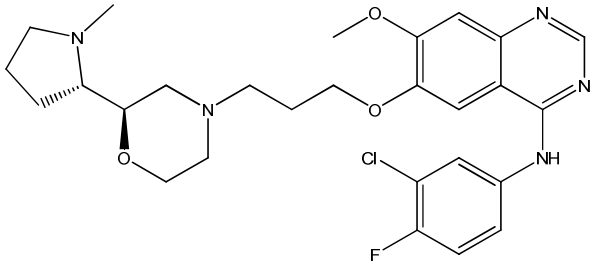

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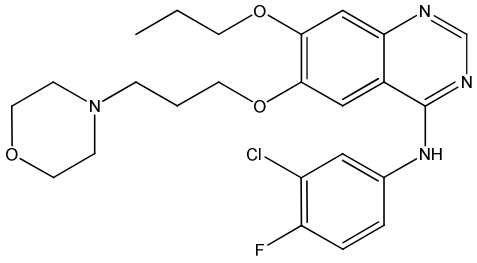

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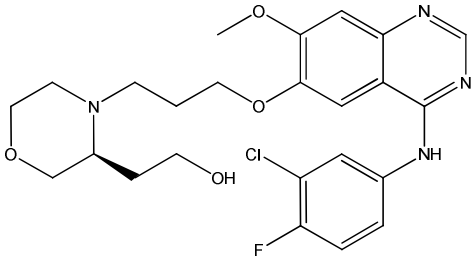

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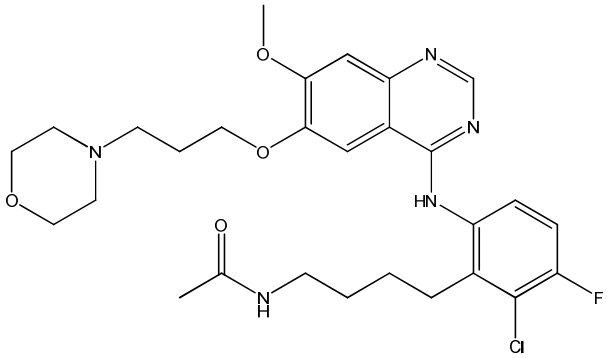

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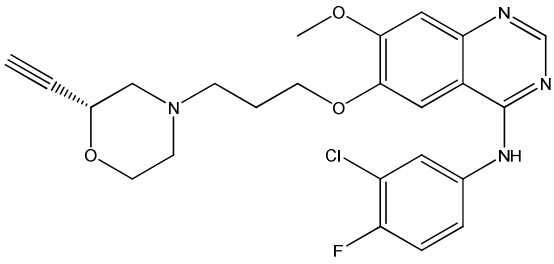

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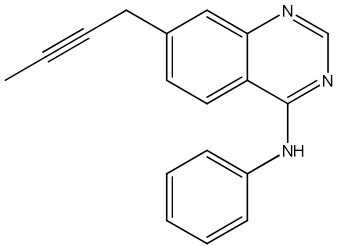
Additional figure 2 The optimization results based on 4 - Anilinoquinazoline



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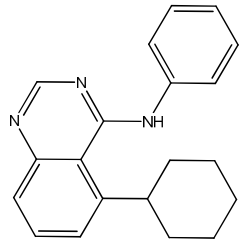

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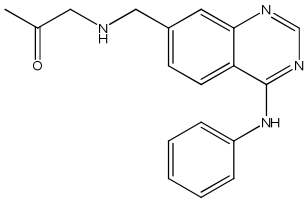

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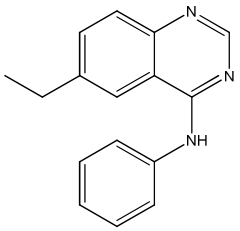

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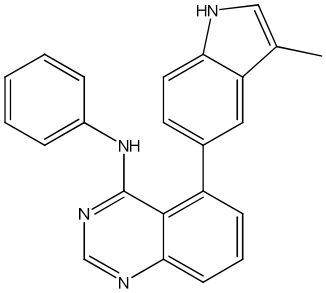

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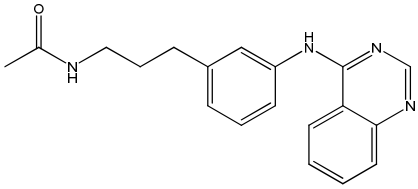

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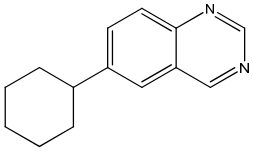
Additional figure 3 The optimization results based on quinazoline



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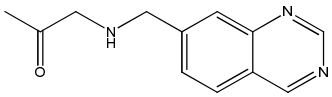

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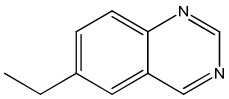

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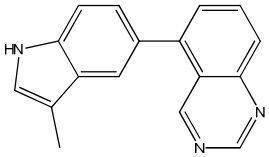

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