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Research Article

In vitro Cytotoxicity Evaluation and Docking Studies of Benzofurooxepines' Derivatives against Human Cancer Cell lines

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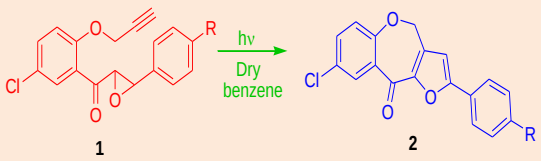
Abstract

The cytotoxic activity of some diversely substituted benzofurooxepines' derivatives has been evaluated *in vitro* using the MTT colorimetric method against four human cancer cell lines (human breast adenocarcinoma MCF-7, human colorectal cancer HT-29, human breast cancer MDA-MB-231 and human lung cancer NCI-H446 cell lines) in 72 h drug exposure assays. A molecular docking study was also performed to assess their potential growth inhibitory effect against human cancer cells. Among the studied compounds, benzofurooxepine derivative 2b exhibited very potent cytotoxicity *in vitro* against three different tumor cell lines that was clearly evinced by the results of molecular docking.

Keywords: Benzofurooxepines, cytotoxicity, molecular docking, photolysis, MTT assay.



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Introduction

Cancer, the second cause of worldwide mortality, can crudely be defined as a manifold of diseases where abnormal cells ensue, spread and proliferate uncontrollably. It is characterized by a deregulation of the cell cycle, which results in a progressive loss of the cellular differentiation and a non-controlled cellular growth [1]. According to World Health Organization, more than 10 million new cases of cancer are diagnosed every year, and the statistical trends indicate that this number would double by 2020 [2]. This growing trend indicates scarcity in the present cancer therapies which include surgical operation, radiotherapy and chemotherapy. This infers that design and development of new anticancer drugs, drug combinations and treatment modalities especially those that are based on current knowledge of cancer biology as well as that taking advantage of the cancer cells phenotype, described by Hanahan and Weinberg [3], is still the need for effective treatment of this leading life threatening pathology. Despite of such aggressive treatments, there is a critical need for anticancer agents with higher efficacy and less side effects that can be acquired at an affordable cost [4]. Indeed, the struggle to combat cancer is one of the utmost challenges of mankind [5].

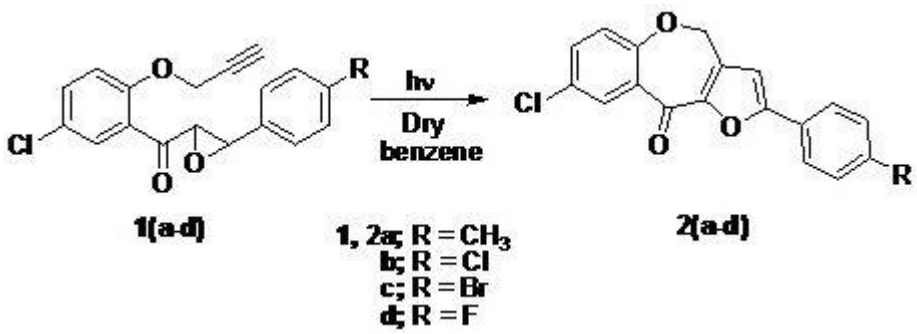
Chemoprevention is recognized as an important approach to control malignancy and recent studies have focused on the search for desirable chemopreventive agents. Oxepines, seven-membered heterocyclic motifs are frequently present in the wide array of biologically active molecule [6]. The oxepines and their derivatives (fused and substituted) constitute an important class of heterocyclic compounds and are known to possess many biological activities like antileishmanial [7], antiallergic [8], anti-depressant [9], antipsychotic [10], anti-inflammatory [11] and antifungal [12] activities. To the best of our knowledge, very few studies have explored the potential effects of oxepines on cancer therapy [13]. Keeping in view the deadly effect of cancer and pharmaceutical activities elicited by oxepines' derivatives, herein, an attempt has been made to derive a good therapeutic strategy to use as an antagonist for treatment of this dreaded disease. In the framework of our research program on the determination of the therapeutic potential of this heterocyclic scaffold, herein, some benzofurooxepinones derivatives were evaluated as the chemopreventive pool of efficacious agents to control the malignancy. These compounds were synthesized [14] through a novel, environmentally benign and single-step process by the photochemical irradiation of 2-{5-chloro-2-(prop-2-ynyloxy)benzoyl}-3-aryloxiranes developed in our laboratory.

Experimental

The benzofuroxepinones 2(a-d) were synthesized in appreciably high yields through a green eco-friendly approach [14] by the photolysis (**Scheme 1**) of the benzoyl oxiranes 1(a-f) in dry benzene with pyrex filtered UV-light from 125W Hg lamp under nitrogen atmosphere and their structures were confirmed by the support of their spectral data (IR, ^{1}H NMR, ^{13}C NMR and Mass etc.).

Scheme 1 Synthesis of Benzofurooxepinones 2(a-f).

Results and Discussion



Cytotoxic Activity

The cytotoxic activity of these synthesized benzofurooxepinones 2(a-d) was evaluated against a panel of human cell lines, including human breast adenocarcinoma MCF-7, human colorectal cancer HT-29, human breast cancer MDA-MB-231 and human lung cancer NCI-H446 cell lines. The apparent growth inhibition against MCF-7, HT-29, NC1-H446 and MDA-MB-231 cell lines was observed for these compounds.

The cancer cell lines were cultured in minimum essential medium (MEM) supplement with 10% fetal bovine serum (FBS). Approximately 4 x 10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37°C for 24 h. The subject compounds at indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT), was added to each well at a terminal concentration of 5 mg/mL and incubated with cells at 37 °C for 4 h. The formazan crystals were dissolved in100 mL DMSO each well, and the absorbance at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured with the ELISA reader. All of the compounds were tested three times in each of the cell lines. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software. The results were illustrated in the **Table 1** and **Figure 1** with Sorafenib as the positive control.

Table 1 Results of MTT cytotoxicity assay against different cancer cell lines

	IC_{50} (µMol/L)			
Compound	**MCF-7**	**HT-29**	**NCI-H446**	**MDA-MB-231**
2a	-	-	-	2.79±0.30
2b	0.009±0.30	0.69±0.29	-	0.019±0.25
2c	3.30±0.35	4.04±0.40	-	0.98±0.06
2d	24.99±0.34	4.28±0.2.5	-	4.77±0.20
Sorafenib	36.14±2.40	4.30±0.34	-	0.94±0.13

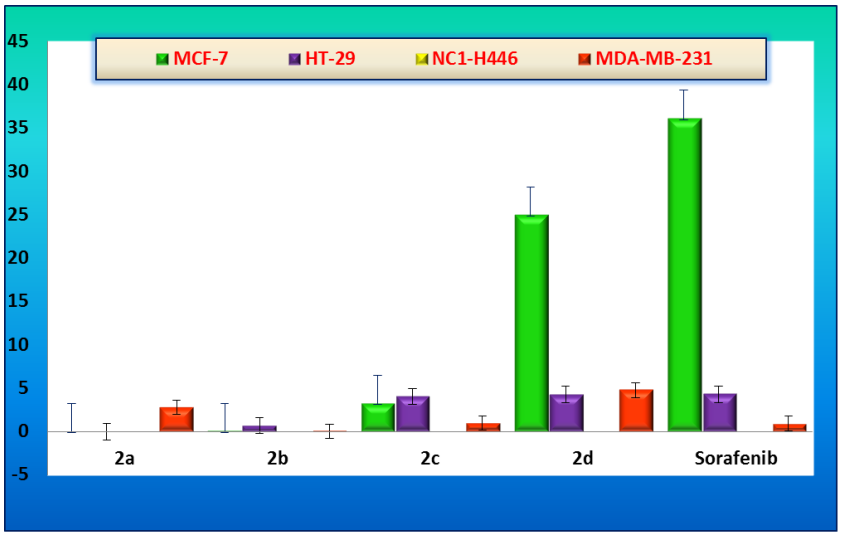


Figure 1 Comparative cytotoxic activity against different cell lines

The furobenzoxepinones 2b and 2c exhibited the promising antitumor activities against MCF-7 and HT-29 and MB-231 cell lines. Especially, the compound 2b displayed excellent antitumor activities against MCF-7, HT-29 and MDA-MB-231 cell lines with IC_{50} values of 0.009, 0.69 and 0.019µMol/L respectively. In contrast to Sorafenib, the tested compounds displayed a broader spectrum of antitumor activity. The cytotoxic activity data shows that all these compounds exhibited negligible inhibitory effect against NC1-H446. No significant influence on these cell lines was observed when a fluorine atom is substituted at *p*-position (compound 2d). It can be inferred from the above study that the introduction of a methyl group in furobenzoxepinones (compound 2a) leads to the diminished antitumour activity as it is effective only against MDA-MB-231 cell lines that too very marginally. The order of effectiveness for the halogen substituents is -Br > -Cl > -F which points toward the steric influence of the substituent.

Docking studies

Material & Methods

Receptor: The three-dimensional crystal structure of different receptors taken from Protein Databank (PDB) [15] are as follows: Estrogen Receptor (PDB ID: 1ERE) and Human Tyrosine phosphatase mutated in colorectal cancer (PDB: 1WCH). All the PDB's were loaded in the Molegro Virtual Docker (MVD) with the removal of all water molecules. Standard Molegro algorithm was employed for attaining the missing charges and protonation states of the receptor.

Ligands: Structures of ligands were sketched in marvin sketch using the 2D-structure draw application and converted to 3D-structures (Marvin sketch 2012). All the structures were minimized and optimized with the Merck Molecular Force Field (MMFF) method taking the root mean square gradient (RMS) of 0.01 kcal/mol Å and the iteration limit to 10,000. All the structures were ionized at neutral pH 7. Conformers for each structure were generated using Monte Carlo by applying MMMF force field method and least energy conformer was selected for further study [16]. One major advantage of MVD is that it helps in assigning the missing bond orders, charges, bonds, and hybridization states of the imported ligands.

Molinspiration: Molinspiration, an online tool, used to perform QSAR studies in order to identify potential activators of biological targets. It offers free on-line services for calculation of important molecular properties (log*P*, polar surface area, number of hydrogen bond donors and acceptors), as well as prediction of bioactivity score for the most important drug targets. Molinspiration tool was used to calculate properties of ligands such as log*P*, molecular weight, H-bond donors and H-bond acceptors [17]. These filters help in early preclinical development and could help in avoid costly late step preclinical and clinical failure. Lipinski's rule of five was applied to select probable ligands [18]. The constituent that had more than one violation was eliminated from the present study.

Molecular Docking of Ligands: We used MVD, which has been recently introduced and gained attention among medicinal chemists [19]. Benchmark results of MVD software provides very accurate predictions of ligand binding modes (87.0%) compared with other docking software such as Glide (81.8%), GOLD (78.2%), Surflex (75.3%) and FlexX2 (57.9%) [20]. MVD is based on a differential evolution algorithm called MolDock. MolDock

Score energy, E_{score}, is defined by Equation 1, where E_{inter} is the ligand-receptor interaction energy and E_{intra} is the internal energy of the ligand. E_{inter} is calculated according to Equation 2.

$$E_{score} = E_{inter} + E_{intra} \quad (1)$$

$$E_{inter} = \sum_{i=ligand} \sum_{j=protein} [E_{PLP}(rj) + 333.2 \frac{qiqj}{4r_{1j}^2}] \quad (2)$$

The E_{PLP} term is a ''piecewise linear potential'' [21] that uses two different parameters, one for the approximation of the steric term (Van der Waals) between atoms and another for the potential for hydrogen bonds; it describes the electrostatic interactions between charged atoms [20]. E_{intra} is calculated according to Equation 3.

$$E_{intra} = \sum_{i=ligand} \sum_{j=protein} [E_{PLP}(rj)] + \sum_{flexible\ bond} A[1 - \cos(m\theta - \theta_0)] + E_{clash} \quad (3)$$

The first term in Equation 3 calculates all the energies involving pairs of atoms of the ligand, except those connected by two bonds. The second term represents the torsional energy, where h is the torsional angle of the bond. The average of the torsional energy bond contributions is used if several torsions can be determined. The last term, E_{clash}, assigns a penalty of 1,000 kcal/mol if the distance between two heavy atoms (more than two bonds apart) is smaller than 2.0 Å, ignoring infeasible ligand conformations [20].

The molecular docking was performed for all the constituents with the predicted cavities of the receptor. The MolDock Score (GRID) function was used with a grid resolution (Å) of 0.30 and a binding site radius of 12 Å with respect to the origin of the respective cavities. The "MolDock SE" searching algorithm 10 runs using a maximum of 1500 iterations with a total population size of 50 was used. The energy threshold used for the minimized final orientation is 100. The Simplex evaluation with 300 maximum steps of neighbor distance factor 1 was completed.

Data Analysis

All compounds passed the Lipinski's rule of five, which should allow for the development of additional anticancer analogues (**Table 2**). Their advantage includes: (i) physical properties known to be compatible with desirable pharmacokinetics (low molecular weight, favorable log*P*, and favorable hydrogen bond-donating and accepting capabilities), (ii) simple synthetic access and thus low production costs, and (iii) polar groups improving the likehood of reasonable solubility.

Table 2 Lipinski's rule of five for benzofuroxepinones' derivatives

Sr. No	log*P*	*P*sa	HBD	HBA	Molecular Weight	Violations
Rule	<5	<140	<5	<10	<500	<1
2a	4.77	39.44	0	3	324.758	0
2b	4.86	39.44	0	3	345.176	0
2c	5.03	39.44	0	3	389.627	1
2d	4.40	39.44	0	3	328.722	0

Docking results obtained for each ligand with the receptors were analyzed with the help of docking energy, binding modes and interaction of each ligand with the functional residues of Estrogen Receptor (PDB ID: 1ERE), Human Tyrosine phosphatase mutated in colorectal cancer (PDB ID: 1WCH) were analyzed in detail by visually inspecting the docked complexes using MVD. The hydrogen bonds involved with bond length were also considered in docking results. All ligands were embedded in the hydrophobic pocket formed by the amino acids. Docking studies reveals following information with respect to inhibitors given in **Table 3** and **4**.

Mol dock score value of internal ligand, 17-*β*-Estradiol was -96.4808 and having H-bond interaction with Arg394, Glu353, His524 and Gly521 which are the main active sites of receptor. Oxygen atom of the furan ring of all compounds forms a H-bond with amino acid Arg394 having a distance range of 3.10-3.17 Å, except compound 2a which also forms one extra H-bond with oxygen atom of carbonyl group with distance 3.10 Å.

Table 3 Docking results of benzofuroxepinones' derivatives on Estrogen receptor by Molegro Virtual Docker

	Mole Dock Score	No. of H-bond	Amino acid involve	Atom of Ligand	Distance
Estradiol-600 (internal ligand)	-96.4808	4	Arg394 (N)	O of OH	2.95
			Glu353 (O)	O of OH	2.60
			His524 (N)	O of OH	2.82
			Gly 521 (N)	O of OH	3.10
2a	-80.9831	2	Arg394 (N)	O of furan ring	3.17
			Arg394 (N)	O of C=O	3.10
2b	-83.126	1	Arg394 (N)	O of furan ring	3.10
2c	-80.6842	1	Arg394 (N)	O of furan ring	3.10
2d	-80.8807	1	Arg394 (N)	O of furan ring	3.10

Table 4 Docking results of benzofuroxepinones' derivatives on Human Tyrosine phosphatase mutated in colorectal cancer receptor by Molegro Virtual Docker

1WCH	Mole Dock Score	No. of H-bond	Amino acid involve	Atom of Ligand	Distance
2a	-63.6131	2	Arg2396	O of furan ring	3.35
			His2399	O of oxepinone ring	3.10
2b	-63.8848	2	Arg2396	O of furan ring	3.36
			His2399	O of oxepinone ring	3.09
2c	-63.8815	2	Arg2396	O of furan ring	3.42
			His2399	O of oxepinone ring	3.06
2d	-63.6553	2	Arg2396	O of furan ring	3.43
			His2399	O of oxepinone ring	3.07

Docking with Human Tyrosine phosphatase mutated in colorectal cancer (PDB: 1WCH) was performed. Oxygen atom of the furan and oxepinone ring forms two H-bonds with amino acids Arg2396 and His2399 with a distance range of 3.35-3.43 Å and 3.06-3.10 Å respectively.

The docking of ligands with estrogen and Human Tyrosine phosphatase mutated in colorectal cancer indicated the strong interactions among them, hence suggested good level of inhibiting ability of ligands. From the results of docking score values on different receptors for cytotoxic activity it is observed that compound 2b showed best docking results on almost all the receptors, and having good cytotoxic activity. The interactions of the Compound 2b with both receptors are given in the **Figures 2** and **3**.

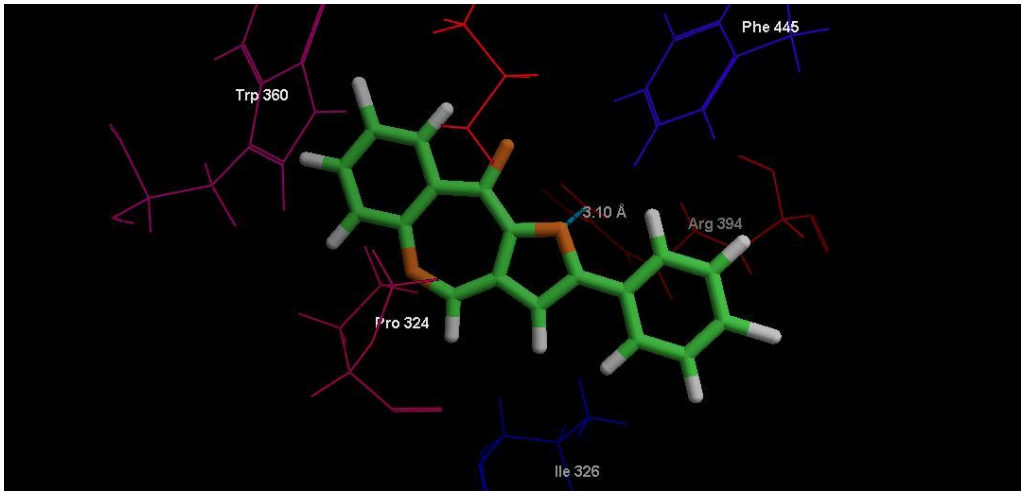


Figure 2 Binding mode of 2b (green) into the binding site of Estrogen receptor (PDB ID: 1ERE).

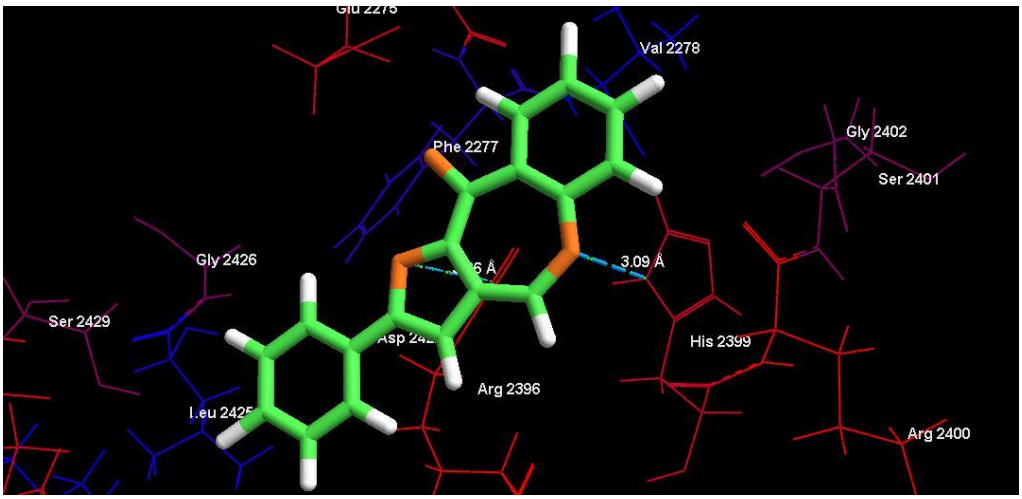


Figure 3 Binding mode of 2b (green) into the binding site of Human Tyrosine phosphatase mutated in colorectal cancer (PDB ID: 1WCH).

Conclusions

In conclusion, several diversely substituted benzofurooxepines' derivatives have been assessed for their cytotoxicity against different human cancer cell lines. The chloro-substitution of phenyl ring of these structural constructs exhibits appreciable cytotoxic potency in almost all the evaluated cell lines. Docking studies supported the *in vitro* MTT assay results and revealed the possible mechanism of their anticancer activity. Some structural modifications in the ligands may lead to development of better cytotoxic agents in future. The exploration of this intriguing cytotoxicity in these heterocyclic scaffolds encouraged us to continue travelling on this productive road. For further study of these constrained structures, we are currently investigating diverse structural modifications in the synthesis of benzofurooxepinones, and the results and structure-activity relationships will be reported in due course.

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References

[1] Valderrama JA, Ibacache JA, Arancibia V, Rodriguez J, Theoduloz C, Bioorg Med Chem 2009, 17, 2894-2901.
[2] Mignogna MD, Fedele S, Lo Russo L, Eur J Cancer Prev 2004, 13, 139-142.
[3] Hanahan D, Weinberg RA, Cell 2000, 100, 57-70.
[4] Fadeyi SA, Fadeyi OO, Adejumo AA, Okoro C, Myles EL, BMC Complementary and Alternative Medicine 2013, 13:79.
[5] Divisi D, Di TS, Salvemini S, Acta Biomed 2006, 77, 118-123.
[6] Levai A, Heterocycles 2008, 75, 2155-2185.
[7] Akendengue B, Roblot F, Loiseau PM, Bories C, Ngou-Milama E, Laurens A, Hocquemiller R, Phytochemistry 2002, 59, 885-888.
[8] Ohmori K, Hyashi KI, Kaise T, Ohshima E, Kobayashi S, Yamazaki T, Mukouyama A, Jpn J Pharmacol 2002, 88, 379-397.
[9] (a) Bloom BM, Tretter JR, Belg Pat 641498, Jun. 18, 1964, cf. Chem Abstr 1966, 64, 719c; (b) Tretter JR, USA Pat 3509175, Apr 28, 1970, cf. Chem Abstr 1970, 73, 35239u.
[10] Boehringer CF, Soehne G, Olnd Pat 6407758, Jan 11, 1965, cf Chem Abstr 1965, 62, P16216a.
[11] (a) Ueno K, Ueno S, Kojima H, Tsukada W, Jap Pat 74124086, Nov 27, 1974; cf. Chem Abstr 1975, 82, 170735d; (b) Sadakatsu S, Sotaro K, Chem Abstr 1976, 85, 192592u.
[12] Hoehn H, USA Pat 4169205, Sept 25, 1979, cf Chem Abstr 1980, 92, 146762w.

[13] (a) Lee S-H, Van HTM, Yang SH, Lee K-T, Kwon Y, Cho W-J, Bioorg Med Chem Lett 2009, 19, 2444-2447; (b) Yamazaki H, Rotinsulu H, Kaneko T, Murakami K, Fujiwara H, Ukai K, Namikoshi M, Mar Drugs 2012, 10, 2691-2697.
[14] Kamboj RC, Jindal P, Kumar D, Khullar S, Mandal SK, J Photochem Photobiol A: Chem 2014, 278, 31-38.
[15] http: //www.rcsb.org/home.
[16] Metropolis N, Rosenbluth AW, Rosenbluth MN, Teller AH, Teller E, J Chem Phys, 1953, 21, 1087.
[17] Molinspiration cheminformatics [homepage on the internet], Nova ulica, SK-900 26 Slovensky Grob, Slovak Republic; [cited 2013 Aug 28], Available from http://www.molinspiration.com
[18] Lipinski CA, Lombardo F, Dominy BW, Feeney PJ, Adv Drug Deliv Rev 2001, 46, 3-26.
[19] ApS, Molegro ApS, Molegro Virtual Docker. Aarhus C; Denmark: Vol 2.4, 2008.
[20] Thomsen R, Christensen MH, J Med Chem 2006, 49, 3315-3321.
[21] (a) Gehlhaar DK, Verkhivker G, Rejto PA, *Lect Notes Comput Sci* 1995, 1447, 449-461; (b) Yang J, Chen C, *Proteins* 2004, 55, 288-304.

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