"УТВЕРЖДАЮ"

Директор ИНЭОС РАН

чл.-корр. А. А. Трифонов

21 мая 2019

N12111-6252/129

ЭКСПЕРТНОЕ ЗАКЛЮЧЕНИЕ О ВОЗМОЖНОСТИ ОПУБЛИКОВАНИЯ

Руководитель-эксперт Федерального государственного бюджетного учреждения науки Института элементоорганических соединений им. А.Н.Несмеянова Российской академии наук, рассмотрев *статью* авторов A. A. Korlyukov, M. Malinska, A. V. Vologzhanina, M. S. Goizman, D.Trzybinski, K. Wozniak «Charge density view on bicalutamide molecular interactions in the monoclinic polymorph and androgen receptor binding pocket», подготовленную для печати в журнале **IUCrJ**, подтверждает, что в материале *не содержатся* сведения, предусмотренные Постановлением Правительства РФ №1233 от 30.11.1994г. и на публикацию материала *не следует* получать разрешение *Минобрнауки и/или Президиума РАН*

Заключение: *статья может* ***быть опубликована в открытой печати, т.к. она не содержит сведений, не подлежащих открытой публикации***

Руководитель-эксперт _____

(подпись)

Нелюбина Ю.В., зав. лаб.

(ф. и. о., должность)

IUCrJ
ISSN 2052-2525
BIOLOGY | MEDICINE

Charge density view on bicalutamide molecular interactions in the monoclinic polymorph and androgen receptor binding pocket

Alexander A. Korlyukov,[a]‡ Maura Malinska,[b]‡ Anna V. Vologzhanina,[a] Mikhail S. Goizman,[c] Damian Trzybinski[b] and Krzysztof Wozniak[b]*

[a]A. N. Nesmeyanov Institute of Organoelement Compounds of Russian Academy of Sciences, Vavilov St. 28, Moscow 119991, Russian Federation, [b]Biological and Chemical Research Centre, Department of Chemistry, University of Warsaw, Żwirki i Wigury 101, Warszawa 02089, Poland, and [c]Drug Technology Co, 2a Rabochaya Street, Chimki, Moscow Oblast 141400, Russian Federation. *Correspondence e-mail: kwozniak@chem.uw.edu.pl

Received 23 May 2019
Accepted 22 October 2019

Edited by Z.-J. Liu, Chinese Academy of Sciences, China

‡ These authors contributed equally to this study.

Keywords: structure determination; drug discovery; protein structures; X-ray crystallography; intermolecular interactions.

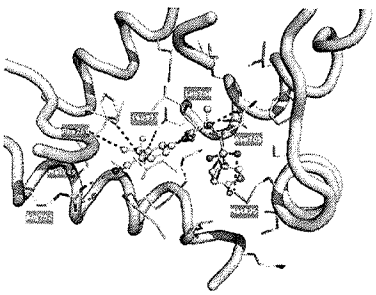
CCDC reference: 1954089

Supporting information: this article has supporting information at www.iucrj.org

High-resolution single-crystal X-ray measurements of the monoclinic polymorph of bicalutamide and the aspherical atom databank approach have served as a basis for a reconstruction of the charge density distribution of the drug and its androgen receptor (AR) and albumin complexes. The contributions of various types of intermolecular interactions to the total crystal energy or ligand:AR energy were estimated. The cyan and amide groups secured the ligand placement in the albumin (Lys-137) and the AR binding pocket (Leu-704, Asn-705, Arg-752), and also determined the packing of the small-molecule crystals. The total electrostatic interaction energy on average was -230 kJ mol^{-1}, comparable with the electrostatic lattice energy of the monoclinic bicalutamide polymorph. This is the result of similar distributions of electropositive and electronegative regions on the experimental and theoretical molecular electrostatic potential maps despite differences in molecular conformations. In general, bicalutamide interacted with the studied proteins with similar electrostatic interaction energies and adjusted its conformation and electrostatic potential to fit the binding pocket in such a way as to enhance the interactions, *e.g.* hydrogen bonds and $\pi\cdots\pi$ stacking.

1. Introduction

One of the most challenging problems in molecular biology is the search for the mechanism of the agonistic or antagonistic activities of genes related to the growth of tumour tissue. For example, in prostate cancer, many tumours are hormone-dependent, meaning that drugs that can block or inhibit androgen receptors might have potential as chemotherapies. These potential drugs can be divided into two groups: steroidal (those that contain steroid fragments) and non-steroidal. Herein, we present the results of charge density studies of the compound most commonly used to treat prostate cancer: racemic monoclinic bicalutamide (**Bic**), commercially available as *Casodex* (Scheme 1). **Bic** is a non-steroidal drug that possesses low solubility in water (5 mg l^{-1}, according to https://www.drugbank.ca/drugs/DB01128) and demonstrates antiandrogen activity and is a selective antagonist of the androgen receptor (AR). Antiandrogens are AR ligands that antagonize the actions of androgens by competing for AR binding sites. Antiandrogens can be both steroidal and non-steroidal drugs. Toluidide derivatives such as **Bic** are anti-androgens without themselves having androgenic properties; this lack of androgenic properties makes them suitable for use in the treatment of prostate cancer (Tan *et al.*, 2012).



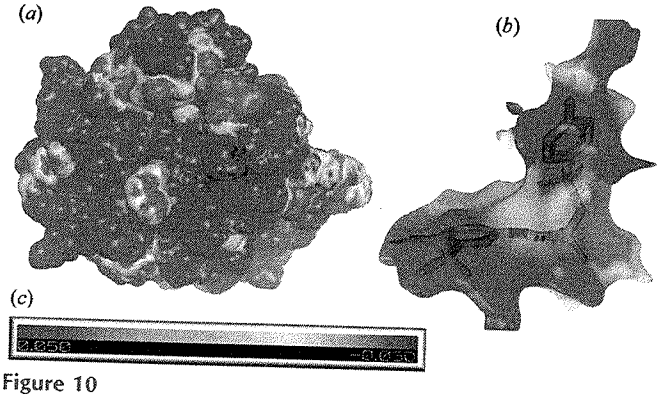


Figure 10
Electrostatic potential (e Å^{-1}) mapped onto the density isosurface (0.067 e Å^{-3}) for (*a*) AR and (*b*) the binding site of AR. (*c*) ESP scale for all figures. The ESP of AR was calculated without the ligand in the binding pocket. Ligands are shown in stick representation to orient the binding pocket.

This similarity can also be observed in the total E_{el}. The electrostatic lattice energy calculated by the EP/MM method based on the experimental charge density was calculated to be −210.5 kJ mol^{-1}, which is very close to the averaged electrostatic interaction energy in the AR binding pocket, −198.9 kJ mol^{-1}. However, the total lattice energy of the monoclinic polymorph based on periodic DFT calculations is −498.5 kJ mol^{-1}, highlighting the importance of dispersive interactions in the crystal structure that play an important role in the binding interactions. The lattice energy for the triclinic polymorphs is −497.8 kJ mol^{-1}. Nevertheless, the role of electrostatic interactions in the stabilization of crystal packing and the ligand–receptor complex can be suitably investigated using EP/MM, QTAIM and NCI methods.

4. Conclusions

The non-steroidal drug bicalutamide is on the World Health Organization's List of Essential Medicines. Crystal structures of two polymorphs, several co-crystals and the **Bic** protein complexes showed vast molecular flexibility, confirmed by quantum chemical calculations and MD simulations. Although the formally single bonds connect two phenyl rings in the molecule, their conformation is rather rigid. The lowest energy conformation of the drug with two intramolecular hydrogen bonds was found in the complex with albumin. In different environments the orientation of the phenyl-CF_3 ring changes and behaves like a canopy, whereas the other phenyl ring has two possible orientations. Here, we present an experimental study of the electron-density distribution of **Bic** in its monoclinic polymorph and protein-bound conformation which reveal the conserved nature of the molecular electrostatic potential and intermolecular bonding based on their propensities and energies. For instance, while bicalutamide bound to the AR exhibits different spatial arrangements, the MEP distribution is unchanged compared with the lowest energy state. The orientation of the hydrogen bond donors and acceptors differ, which allows the formation of the most favourable interactions. This conformation complements the MEP of the binding pocket in a constructive way. In terms of hydrogen bonding propensity, the most likely interaction, the hydroxyl–amide pair, was also found to be the strongest of all the intermolecular interactions found in the monoclinic structure and in the AR complexes. The conformational angle of the nitrile group of the drug molecule causes the formation of numerous C—H···N≡C interactions in the crystal structure, and its interaction with Arg-752 is part of the strongest set of interactions in AR complexes. Although numerous, the role of water molecules in the direct stabilization of the drug molecule in the binding pocket was found to be negligible. While these interactions can be classically described as hydrophobic, interactions with Met-749 and Met-895 have significant electrostatic energy values that are probably additionally stabilized by dispersive forces.

Hydrogen bonds and stacking interactions were found to play a crucial role in the formation of the polymorphs and protein complexes. However, we showed that the description of charge density in terms of QTAIM cannot provide all the information that is necessary to describe intermolecular bonding due to their non-directional character. Therefore, this study based on the NCI approach plays a crucial role in understanding the different binding modes of **Bic**.

Funding information

Single-crystal X-ray diffraction analysis was carried out at the Biological and Chemical Research Centre, University of Warsaw, established within the project co-financed by theEuropean Union from the European Regional Development Fund under the Operational Programme Innovative Economy, 2007–2013. KW, MM and DT are thankful for the support within the Polish National Science Centre (NCN) MAESTRO (grant No. DEC-2012/04/A/ST5/00609). The data were collected at the Core Facility for crystallographic and biophysical research to support the development of medicinal products sponsored by the Foundation for Polish Science (FNP). AAK and AVV acknowledge support by the Ministry of Science and Higher Education of the Russian Federation.

References

Agilent (2014). *CrysAlisPRO*. Agilent Technologies Ltd, Yarnton, Oxfordshire, England.
Allen, F. H. & Bruno, I. J. (2010). *Acta Cryst.* B**66**, 380–386.
Andrews, P. R., Craik, D. J. & Martin, J. L. (1984). *J. Med. Chem.* **27**, 1648–1657.
Bader, R. W. F. (1990). *Atoms in Molecules: A Quantum Theory*, New York: Oxford University Press.
Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. & Bourne, P. E. (2000). *Nucleic Acids Res.* **28**, 235–242.
Bis, J. A., Vishweshwar, P., Weyna, D. & Zaworotko, M. J. (2007). *Mol. Pharm.* **4**, 401–416.
Bohl, C. E., Gao, W., Miller, D. D., Bell, C. E. & Dalton, J. T. (2005). *Proc. Natl Acad. Sci.* **102**, 6201–6206.
Bonomo, S., Hansen, C. H., Petrunak, E. M., Scott, E. E., Styrishave, B., Jørgensen, F. S. & Olsen, L. (2016). *Sci. Rep.* **6**, 29468.
Carver, F. J., Hunter, C. A., Carver, F. J. & Seward, E. M. (1998). *Chem. Commun.* 775–776.