www.chemmedchem.org

CHEMMEDCHEM

CHEMISTRY ENABLING DRUG DISCOVERY

EFMC | ISMC 2012

XXIInd International Symposium on Medicinal Chemistry

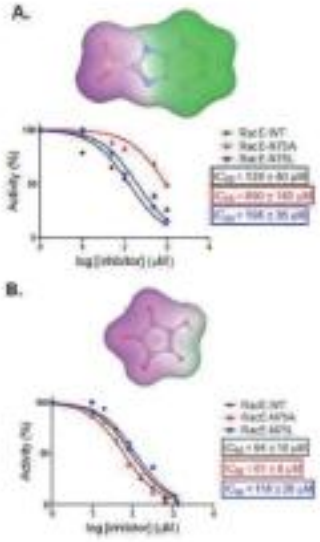
Berlin, Germany September 2-6, 2012

ISMC 2012 Book of Abstracts

A Journal of

ChemPubSoc Europe

WILEY-VCH



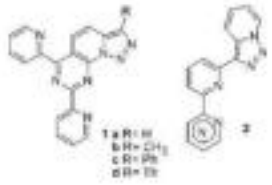
P263

[1,2,3]Triazolo[1,5-*a*]pyridines and Their Copper(II) Complexes. Studies of DNA Interactions

Rosa Adam,[a] Gloria Alzuet,[b] Rafael Ballesteros,[a] Belén Abarca,[a] Pablo Bilbao,[c] Mª Auxiliadora Dea-Ayuela,[c,d] Mª Eugenia González-Rosende[d]

[a] Department of Organic Chemistry, [b] Department of Inorganic Chemistry, Faculty of Pharmacy, University of Valencia, Avda. Vicente Andrés Estellés s/n, Burjassot, Valencia, Spain [c] Department of Parasitology, University Complutense of Madrid, Plaza Ramón y Cajal, s/n 28040, Madrid, Spain [d] Department of Pharmacy, University CEU Cardenal Herrera, Avda. Seminario s/n, 46113 Moncada, Valencia, Spain

DNA, as carrier of genetic information, is a major target for drug interaction due to the ability to interfere with important biological processes governed by this biomolecule.[1] The main DNA interacting compounds are either polycyclic, aromatic and planar ligands,[2] or Ru, Pt or Cu complexes of this kind of ligands.[3] In our synthetic work focused on generating new derivatives from [1,2,3]triazolo[1,5-*a*] pyridine nucleus, we have synthesized two series of compounds with general structures **1**,[4] and **2**. These molecules and their Cu^{2+} complexes have been evaluated as possible DNA binders.



DNA binding tests have been done studying variations in fluorescence emission and UV absorption of the compounds, when DNA is added. Spectrophotometric titrations have also been done using specific DNA (poly(dA-dT)$_2$ and poly(dG-dC)$_2$), to determine interaction specifity. DNA viscosimetry titrations were also performed, revealing that compounds **1** interact with DNA in a non-intercalative way. Finally, compounds **1** have been tested as antileishmanial agents, obtaining good results against four species of *Leishmania* [*L. infantum*, *L. braziliensis*, *L. guyanensis* and *L. amazonensis* promastigotes] for **1b** and **1c**.

References

[1] D. S. Johnson, D. L. Boger in *DNA Binding Agents in Comprehensive Supramolecular Chemistry*, (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle), Pergamon, Oxford, **1996**.
[2] T. Phillips, I. Haq, A. J. H. M. Meijer, H. Adams, I. Soutar, L. Swanson, M. J. Sykes, J. A. Thomas, *Biochemistry* **2004**, *43*, 13657.
[3] a) M. L. Clark, R. L. Green, O. E. Johnson, P. E. Fanwick, D. R. McMillin, *Inorg. Chem.* **2008**, *47*, 9410; b) J. L. García-Giménez, G. Alzuet, M. González-Álvarez, M. Liu-González, A. Castiñeiras, J. Borrás, *J. Inorg. Biochem.* **2009**, *103*, 243; c) A. Ghosh, P. Das, M. R. Gill, P. Kar, M. G. Walker, J. A. Thomas, A. Das, *Chem. Eur. J.* **2011**, *17*, 2089.
[4] B. Abarca, R. Ballesteros, M. Chadlaoui, E. García-España, C. Ramírez de Arellano, *ARKIVOC* **2005**, *xiv*, 71.

P264

Discovery of a New Small-Molecule Inhibitor of p53–MDM2 Interaction

Emília Sousa,[a] Mariana Leão,[a] Ana Paiva,[b] Joana Soares,[a] Alessandra Bisio,[c] Yari Ciribilli,[c] Frederico Silva,[d] Luis Gales,[d,e] Madalena Pinto,[b] Alberto Inga,[c] Lucília Saraiva[d]

[a] REQUIMTE, Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Porto, Portugal [b] CEQUIMED-UP, Department of Chemistry, Laboratory of Organic and Pharmaceutical Chemistry, Faculty of Pharmacy, University of Porto, Porto, Portugal [c] CIBIO, Centre for Integrative Biology, Laboratory of Transcriptional Networks, University of Trento, Trento, Italy [d] IBMC, Instituto de Biologia Molecular e Celular, Porto, Portugal [e] ICBAS, Instituto de Ciências Biomédicas de Abel Salazar, Universidade do Porto, Porto, Portugal

The p53 tumour suppressor is a major regulator of cell proliferation and death. In tumours that retain a wild-type (wt) p53, the activity of this protein can be inhibited by the endogenous negative regulator MDM2. In this case, inhibitors of p53–MDM2 interaction have been

considered promising drugs for cancer therapy.[1,2] Developing small molecules that modulate protein–protein interactions is difficult, owing to issues that include the typical flatness of the interface, the difficulty of distinguishing real from artefactual binding, and the size and character of typical small-molecule libraries.

Yeast assays consisting of *Saccharomyces cerevisiae* cells co-expressing human wt p53 and MDM2 have been used for the screening of small-molecule inhibitors of p53–MDM2 interaction. In these assays, inhibitors of p53–MDM2 interaction, such as Nutlin-3A, revert the inhibitory effect of MDM2 on p53-induced growth inhibition/cell cycle arrest as well as on p53-dependent transcriptional activity of a reporter gene (described in [3]). Using this approach, a chemical library of small molecules synthesised by the CEQUIMED-UP group was tested and the small molecule LEM1 was identified as inhibitor of p53–MDM2 interaction.

The identified compound (LEM1), with favourable apparent permeability coefficient and no cytotoxicity on normal human cell lines, exhibited promising activities as inhibitor of p53–MDM2 interaction in two human tumour cell lines derived from breast cancer (MCF7) and colon carcinoma (HCT116 p53+/+). The results obtained confirmed that 10 μM LEM1 treatment stimulated p53-dependent transcriptional activity, led to p53 protein stabilization, increased p21 and Bax protein levels, and induced caspase-7 activation in human tumour cell lines. Notably, these effects were not observed in the HCT116 p53-/-derivative cell line.

Though the molecular mechanism of action of this compound was validated in human tumour cell lines, the molecular interaction site of LEM1 is still unknown. In order to evaluate the molecular basis of disruption of p53–MDM2 interaction by LEM1, X-ray crystallographic studies will be carried out by checking possible molecular interactions between LEM1 and MDM2. For that, several attempts to obtain a recombinant human MDM2 (amino acid residues 17–125) expressed in *Escherichia coli* BL21 (DE3) RIL, using the pEX-N-His expressing vector, were performed. This MDM2 fragment was purified by affinity chromatography, using Ni-NTA agarose column.

The higher simplicity of the synthetic process of LEM1 when compared with that of other inhibitors of p53–MDM2 interaction (e.g., Nutlin 3A) will certainly guarantee economic advantages for the commercialization of this compound as an additional research tool in the p53 field. Additionally, LEM1 represents a promising small molecule to be further explored as anticancer drug and/or as a lead compound toward the synthesis of more potent and selective inhibitors of p53–MDM2 interaction.

Acknowledgements: This work was supported by the FCT (Fundação para a Ciência e a Tecnologia) through REQUIMTE (PEst-C/EQB/LA0006/2011) and CEQUIMED-UP (Pest-OE/SAU/UI4040/2011), FEDER funds through the COMPETE program under the projects FCOMP-01-0124-FEDER-015752 and FCOMP-01-0124-FEDER-011057, and by U. Porto/Santander Totta, and in part by the Italian Association for Cancer Research, AIRC (IG #9086). A.M. Paiva thanks for a grant (PTDC/SAU-FCT/100930/2008) in the scope of the project. M. Lello (SFRH/BD/64184/2009) and J. Soares (SFRH/BD/78971/2011) are recipients of FCT fellowships.

References

[1] W. Wang, Y. Hu, *Med. Res. Rev.* **2011**, *in press*, DOI: 10.1002/med.20236.
[2] Q. Yu, *Drug Resist. Updates* **2006**, *9*, 19–25.
[3] V. Andreotti, et al., *Plos One* **2011**, *6*, e20643.

P265

Exploring the Orthosteric nACh Receptor Binding Site by Conformational Restriction of the nACh Agonist DMABC

Mario de la Fuente Revenga, Anders A. Jensen, Thomas Balle, Bente Frølund

[a] Instituto de Química Médica (CSIC), Juan de la Cierva 3, 28006, Madrid, Spain; e-mail: fuente.revenga@gmail.com
[b] Department of Drug Design and Pharmacology, Faculty of Health and Medicinal Sciences, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark

If we are to talk about classic approaches in Medicinal Chemistry conformational restriction and controlled geometry of ligands is a must. Old but never outdated, this approach is being used for a better understanding of the topography and interactions that occurs at the orthosteric binding site of nicotinic acetylcholine receptors (nAChR). The orthosteric binding site of these ligand-gated ion channels is located in the interface of α–β or α–α subunits. The amino acid sequences that form this binding site are highly conserved among the different receptor subtypes, therefore, achieving a high degree of selectivity turns out to be a challenge that requires a fine tuning in the design of potential selective ligands in order to exploit the small differences found in the receptor cavity.

DMABC is a small synthetic agonist related to acetycholine (ACh) and exhibits a significant selectivity towards the $\alpha_4\beta_2$ subtype. The predicted linear binding conformation of this molecule, similar to that of ACh or epibatidine, was shown to be in disagreement with a recent X-ray crystallography study, which revealed a folded conformation of DMABC to ACh-binding protein. Based on these new findings, three series of DMABC analogues, cyclopropane, piperazine/piperidine and azepine/azepane containing derivatives, were designed, synthesized and pharmacologically characterized in a [^{3}H]-epibatidine binding assay at the $\alpha_4\beta_2$, $\alpha_3\beta_2$ and $\alpha_4\beta_4$ subtypes and a FLIPR membrane potential blue assay at the $\alpha_4\beta_2$ and $\alpha_3\beta_4$ subtypes.

The synthesized compounds represent different degrees of conformational restriction of DMABC, and in general the results reveal strict structural requirements regarding stereochemistry and conformation for activating the nAChR.