tuberculosis $H_{37}Rv$ culture. The ability of penetration and incorporation of the drug candidates and the conjugates into the lipid Langmuir monolayer, maximum amount of penetrated compounds were determined. The cellular uptake of the conjugates with fluorophore was determined by flow cytometry on MonoMac6 human monocytic cells.

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P274.

Synthesis of GSH-linked tyrosinase-activated melanoma prodrugs

P. Ruzza,[a] A. Calderan,[a] A. Nassi,[b] and L. Quintieri[b]
[a]CNR Institute of Biomolecular Chemistry, Padua Unit, Padua, Italy
[b]Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy

Melanoma is the most deadly skin cancer with an increasing incidence. Its therapy continues to be a challenge since, regardless of the treatment used, long-term survival is quite uncommon.

Melanoma cells are characterized by high levels of glutathione S-transferase P1-1 (GSTP1-1) isozyme. This enzyme is also highly expressed in solid tumours and drug-resistant cells, where a role for this protein in the regulation of cell proliferation has been described.[1]

In the past, Lyttle et al. synthesised glutathione (GSH)-linked anticancer prodrugs activated by physiological concentration of GSTs.[2] Structure and mechanistic studies showed that in the GST-GSH complex, the GST Tyr residue placed near the S atom of the Cys in GSH, is in the phenoxide form to facilitate the deprotonation of the sulfhydryl group in GSH, making it able to react with electrophilic species.[3]

By taking advantage of this active-site geometry, we synthesised two tyrosinase activated melanoma prodrugs (4-methoxyphenol and N-acetyl-4-S-cysteaminylphenol) linked to GSH. In these compounds an ethoxycarbonyl group was placed between the GSH sulphur, oxidate to sulfone, and the tyrosinase-activated prodrugs.

In this way the Tyr phenoxide (Tyr7 in GSTP and Tyr6 in GSTM isozyme) would be able to abstract one of the acidic methylene protons linked to the sulfone moiety. Such a deprotonation should trigger a beta-elimination, resulting in the release of melanoma prodrugs.

The stability of the obtained putative prodrugs has been evaluated in different pH buffers and in rat liver and kidney cytosolic fractions.

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P275.

Synthesis of new hydroxycinnamoyl oseltamivir amides and evaluation of their antioxidant and antiviral activities

M. Chochkova,[a] D. Dimitrova,[a] G. Ivanova,[b] A. Galabov,[c] T. Milkova[a]
[a]South-West University "Neofit Rilski", Blagoevgrad, Bulgaria;
[b]REQUIMTE, Departamento de Química, Faculdade de Ciências, Universidade do Porto, 4169-007 Porto, Portugal;
[c]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria

Nowadays, the neuraminidase is known to be the important target for development of novel influenza virus inhibitors. Therefore, many efforts were made in design of new anti-flu agents. Several studies confirm the positive role of administration of antioxidants in combination with approved antiviral drugs against influenza infection. The aim of this investigation is focused on preparation of new derivatives of viral sialidase inhibitor-oseltamivir by modifying its amino group with antioxidant molecule such as hydroxycinnamic acids. The evaluation of their biological activities is in progress.

P276.

Synthesis, in vitro and in vivo evaluation of new 99mTc-labeled cyclic RGDfK peptide monocationic complexes

A. Calderan,[a] B. Biondi,[a] C. Bolzati,[b] F. Refosco,[b] N. Morellato,[c] N. Salvarese,[c] P. Ruzza[a]
[a]CNR Institute of Biomolecular Chemistry, Padua Unit, Padua, Italy
[b]CNR Institute of Inorganic Chemistry and Surface, Padua, Italy
[c]Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua, Italy

Synthetic peptides represent first choice biomolecules, with respect to proteins and mAb, for the monitoring of population variability and receptor functionality associated to a tumor pathology due to their favorable pharmacokinetic profile. It has been demonstrated that the surface molecule integrin alfa-V beta-3 could be an ideal target for the interaction with melanoma directed radiolabeled peptides. This integrin is indeed overexpressed both in tumor cells and in tumor vessels endothelium, while it is not expressed in the majority of normal tissues. Cyclic peptides with the sequence Arg-Gly-Asp (RGD), are known to bind the integrin alpha-V beta-3 with high affinity and selectivity and could conveniently target a radiotherapeutic to melanoma cells.[1] We previously demonstrated that the cyclic RGDfK peptide interact with high affinity to A375 human melanoma cells.[2] The aim of this work was to conjugate the 99mTc to the peptide by using the $[^{99m}Tc(N)(PNP)]^{2+}$ system (PNP=aminodiphosphines) to obtain a radiolabeled peptide useful in melanoma imaging.[3,4] To this purpose a Cys-peptide conjugate (NS-RGDfK) was synthesized and used in preparation of monocationic $[^{99m}Tc(N)(NS\text{-}RGDfK)(PNPn)]^{+}$ complexes. In vitro stability and transchelation studies with cysteine and glutathione of radiolabeled complexes were carried out as well as their pharmacokinetic profiles in healthy rats were investigated.

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P277.

The combination of phage display and molecular grafting gives access to a novel disulfide-stabilized miniprotein as drug candidate

F. Zoller,[a,b] A. Markert,[a] P. Barthe,[c] U. Haberkorn,[a,b] W. Mier*[,b]
[a]DKFZ, Clinical Cooperation Unit Nuclear Medicine, Heidelberg, Germany
[b]University Hospital Heidelberg, Department of Nuclear Medicine, Heidelberg, Germany
[c]CNRS, Centre de Biochimie Structurale, Montpellier, France

Small disulfide-stabilized peptide formats are relevant scaffolds for drug design.[1] Due to the clinical need of anti-angiogenetic compounds, the identification of novel affinity reagents against targets associated with angiogenesis is warranted. Owing to its unique expression pattern in tumor endothelia, the delta-like ligand 4 (DLL4)[2] was selected as target for phage display[3] using a library based on the disulfide-stabilized miniprotein Min-23[4] to identify binding peptides against DLL4.

The phage-display evolved binding domain was grafted into the sunflower trypsin inhibitor (SFTI-I)[5] to facilitate synthetic access of a disulfide-stabilized molecular scaffold. Surface plasmon resonance (SPR) experiments, cell binding assays, immunohistological analysis and biodistribution studies in tumor-bearing mice were performed to characterize the peptide in vitro and in vivo.

The new SFTI derivative showed high affinity (K_D = 22 nM) against DLL4. Tumor targeting in vivo was verified by biodistribution studies in tumor-bearing mice. SPR demonstrated inhibition of the DLL4-Notch interaction, which accentuated a therapeutic potential of this new binding entity.

The grafting of the evolved affinity function into the small, stable SFTI-I provided a drug candidate readily accessible by SPPS. These results are a proof of the scaffold concept demonstrating the flexibility of miniproteins for the integration of an affinity function for hit-to-lead development.

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P278.

The influence of HIV-1 Tat protein sequences on platelet activation.

P. Stathopoulos, A. A. Dimitriou, E. Malisiova, A. D. Tselepis, V. Tsikaris*
Department of Chemistry, University of Ioannina, Ioannina, Greece

Several reports have evidenced an increased incidence of thrombotic events in HIV-infected patients, especially after an antiretroviral therapy. In these cases activation of the platelets has been observed. Wang J. et al. have investigated the role of the HIV-1 Tat protein in platelet activation and reported that it binds to β_3-platelet integrin through its $R^{78}GD^{80}$ sequence inducing their activation1. In this work we report that the Tat_{48-60} (G48RKKRRQRRR PPQ^{60}-NH_2) is also involved in platelet activation, P-selectin expression and platelet aggregation. This strongly cationic peptide sequence is a well-known cell penetrating peptide (CPP) extensively used to deliver bioactive molecules such as proteins, peptides, nanoparticles, oligonucleic acids and liposomes into cells. Therefore, the Tat_{48-60} activity on platelets activation must be taking into account if it has to be used as a CPP[2]. Moreover, in order to investigate the role of the Tat-$R^{78}GD^{80}$ adjacent peptide sequences on β3-platelet integrin binding of the Tat protein, we designed and synthesized the PRGDP, QPRGDP, QPRGDPTG, PRGDPTG, PKGDPTG, PKGDP peptide analogues in their C-terminus amide form. Evaluation of their biological behaviour is in progress.

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