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RESULTS: Synthesis of DABO's analogues bearing diverse substituents at position 6 of the 4-oxopyrimidine ring lead to compounds having nanomolar (nM) activity against the HIV-1 WT virus with a selectivity index for the single and double mutations below 10. Replacement of methyl at position 5 by various groups did not improve activity or selectivity. Exploration of position 2 was carried out with the best substituent obtained from modification at position 6. No synergistic effect was observed. Substitution at that position had no detrimental effect on the activity that remains in the nM range for HIV-1 WT with a good selectivity index.

CONCLUSION: Structure based design of new DABOs analogues lead to very potent inhibitors of the RT with high selectivity against single mutant K103N and Y181C but also double mutant K103N/Y181C. Substitution at position 2 of the 4-oxopyrimidine ring enabled the exploration of a different pocket of the protein that does not improve the activity of the inhibitor but can be further exploited for modulation of the physicochemical properties of the drug.

ABSTRACT 56

A New Twist for an Old Song. The DABO Story Revisited

S Pricl[1], M Ferrone[1], P Posocco[1], GM Pavan[1], M Fermeglia[1], P La Colla[2] and R Loddo[2]

1 Molecular Simulation Engineering (MOSE) Laboratory, DICAMP, University of Trieste, Trieste, Italy; 2 Department of Biomedical Sciences and Technologies, University of Cagliari, Cagliari, Italy

BACKGROUND: Dihydro-alkoxy-benzyl-oxopyrimidines (DABOs), structurally related to 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT), belong to an NNRTI class disclosed firstly in 1992. Since then, many structural modifications of DABO compounds have been performed, aimed at obtaining more potent derivatives. In particular, the *in vitro* anti-HIV-1 potency, selectivity and knocking out activity of some new DABOs make them particularly attractive for further development as microbicides to prevent sexual HIV transmission/acquisition.

Recently, a series of 6-(1-naphtylmethyl) substituted S-DABOs featuring a β-carbonyl on the C-2 side chain were designed and synthesized, which exhibited a significant HIV-1 RT inhibitory activity *in vitro*. Some of these compounds showed activity against clinically relevant resistant variants carrying the K103N and Y181C mutations, suggesting that these DABOs may inhibit RT in a different manner with respect to other NNRTIs.

METHODS: Using an *ad hoc* developed procedure based on advanced molecular simulation techniques, we screened among different, possible binding modes of the DABOs derivatives, and calculated the relevant free energy of binding to HIV-1 RT. The putative docking modes of the DABO compounds were checked against blank tests performed on known NNRTIs, whose crystal structures in complex with RT were available. The free energy of binding, its components, and the corresponding value of IC50, were evaluated using the Molecular Mechanics/Poisson Boltzmann Surface Area (MM/PBSA) theory.

RESULTS: The overall simulation results show that the simulation technique adopted can reproduce the experimental RT/NNRTI complex structure used as blank tests unambiguously well. Further, our methodology reveals that three, alternative binding modes are possible for this DABO series of compounds, two of which are more favorable from the energetic standpoint. The access to multiple binding modes is a consequence of the NNIRT binding pocket (BP) flexibility, which can adapt to the binding mode of the inhibitor. Moreover, the protein conformational flexibility permits a wide range of inhibitor modifications that, at least virtually, can retain activity and provide opportunity for evading drug-resistance mutations.

CONCLUSIONS: Our study allows to conclude that: i) three binding modes to HIV-1 RT, two of which are equivalent from the energetical standpoint, are

possible for a series of S-DABO compounds; ii) the NNIRT BP is flexible, and adapts to accommodate different ligand conformations via readjustment of the neighboring residues, which in turn modulate the conformation of the bound drug because of induce-fit effect; iii) although these virtual evidences still await experimental confirmation, they could be used as good starting point for *in silico* design of new, more potent RT inhibitors.

ABSTRACT 57

Chasing the Evaders. Design, Synthesis, Activity and Molecular Modeling of a New Small Molecule with Activity Against Drug-Resistant HIV-1 mutants

S Pricl[1], M Ferrone[1], P Posocco[1], M Fermeglia[1], G Sanna[2], G Collu[2], P La Colla[2], R Loddo[2], A Sias[3], S Piras[3], and A Carta[3]

1 Molecular Simulation Engineering (MOSE) Laboratory, DICAMP, University of Trieste, Trieste, Italy; 2 Department of Biomedical Sciences and Technologies, University of Cagliari, Cagliari, Italy; 3 Department of Medicinal and Toxicological Chemistry, University of Sassari, Sassari, Italy

BACKGROUND: A major challenge facing medicinal chemistry over the last few years has been (and still remains) the development of antiretroviral drugs with new modes of action and/or significantly improved cross-resistance profiles and barrier to resistance for chronic use in combination therapy. An important component of such regimens are non-nucleoside inhibitors of the HIV-1 reverse transcriptase (NNRTIs). The high selectivity of NNRTIs for HIV-1 RT over HIV-2 RT and cellular polymerases contributes to lower cellular toxicity levels of these antiretroviral agents. However, their selectivity, together with the relatively unconserved aminoacid sequence in the NNRTI binding site, renders the different classes of NNRTIs susceptible to the rapid selection of drug-resistant variants. All reported NNRTI-resistant mutations occur in residues surrounding the inhibitor-binding pocket on the enzyme. A commonly observed mutation is the single aminoacid mutation Y181C; additionally, a K103N mutation appears relatively frequently *in vivo*, conferring resistance to many NNRTIs.

In this work, we present the results obtained from the design, synthesis, antiviral activity evaluation, and molecular modeling of a new, small molecule, which exhibits interesting activity against wild type (wt) HIV-1, as well as against single, double and triple mutant variants, including those containing clinically relevant NNRTI mutations.

METHODS: Compound PS994 was synthesized using a two-step, original strategy starting from 7,8-dichloro-6-nitroquinoline. The activity of this compound was tested against wt HIV-1, as well as against variants resistant to NRTI and NNRTI drugs. Using an *ad hoc* developed procedure based on advanced molecular simulation techniques, we docked PS994 in the NNRTI binding pocket and calculated the relevant free energy of binding to HIV-1 RT.

RESULTS: Compound PS994 can be obtained with high yield following the new synthetic route proposed. The results of its broad spectrum activity against HIV-1 wt and resistant mutants are quite interesting. An *in silico* model of this compound docked into the NNIRT binding site of HIV-1 reveals that its binding mode does not rely on fundamental contacts with the muted residues at positions 181 and 103, thus yielding a possible rationale for its activity against viruses carrying the above mutations.

CONCLUSIONS: Our joint experimental and modeling efforts have led to the discovery of a new, small molecule endowed with potential anti-HIV-1 activity against the wild type virus and variants containing clinically relevant NNRTI mutations. Overall, the information obtained could be used for the development of a second generation of more potent NNRTIs.