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activity of these inhibitors with the variation of their respective NS5B binding sites through an array of studies that combine biochemistry, biophysics and computational simulation. The present studies provide insights into the rational drug design of inhibitors that can be accommodated into highly variable binding sites.

ABSTRACT 037

Identification and Optimization of a Novel Class of Non-nucleoside Inihibitors of HCV NS5B RNA Polymerase

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BACKGROUND: The HCV NS5B polymerase is essential for HCV infectivity and is a major focus of antiviral drug discovery efforts. We have previously identified a novel, dihydropyrone-containing inhibitor using high throughput screening against NS5B genotype 1b polymerase (IC_{50} = 1.0 µM) [*J Virol* 2003 77(13) 7575-81]. A co-crystal structure revealed the inhibitor bound to the 'thumb' site of the polymerase, approximately 30 Ä removed from the active site.

METHODS: To improve the potency of this inhibitor, SAR studies were performed exploring 3 key binding pockets (designated A, B and C) of NS5B BK1b, utilizing structure-based design, parallel synthesis and traditional medicinal chemistry. Other sets of analogs were prepared to define the dihydropyrone pharmacophore.

RESULTS: It was found that the cyclopentyl ring was critical for activity, tightly filling the hydrophobic "A" pocket. The para phenol in the "B" pocket could be removed and a combination of meta-halogens and para phenol ethers improved enzyme and antiviral potency. Although sulfur linked aromatic fragments in the "C" pocket improved enzyme potency, the analog with the most potent antiviral activity incorporated a carbon linker; this modification resulted in a ~ 10-fold increase in antiviral potency.

CONCLUSION: A synergistic approach utilizing structure-based design, parallel synthesis and traditional medicinal chemistry afforded > 400-fold improvement in potency against the enzyme and > 400-fold improvement in the antiviral 1b replicon assay.

ABSTRACT 038

Novel Aryl Diketo Acids (ADKs) as Inhibitors of Hepatitis C Virus NS5b RNA-dependent RNA-polymerase: Better Understanding through Molecular Modeling

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BACKGROUND: Structure-based drug design refers to the intricate process of using the information contained in the 3D structure of a macromolecular target and of related ligand-target complexes to design novel drugs for important diseases. Computational methods are required to extract all of the relevant information from the available structures and to use it an efficient and intelligent manner to design improved ligands for the target. Currently, there are intense efforts toward the development of new drugs targeting essential HCV enzymes, and in particular the RNA-dependent RNA-polymerase (NS5b), responsible for the replication of the viral genome. Under these perspectives, in this work we used molecular modeling methods to derive QSARs for diketo acid analogues to carry out design of new, potential anti HCV-agents using computer models of the receptors, and *in silico* screening and scoring of the drug candidates.

METHODS: A molecular modeling strategy using aryl diketo acid (ADK) derivatives recently reported in literature as HCV polymerase inhibitors was designed.

A 3D chemical feature-based pharmacophore model was developed, consisting of two hydrogen bond acceptors, one negative ionizable moiety, and two hydrophobic aromatics. This model was used to predict the anti-RNA-dependent RNA polymerase (RdRp) activity of a series of ADK derivatives previously synthesized in our laboratories as HIV-1 integrase inhibitors. Furthermore, experimental IC_{50} of 9 compounds, tested *in vitro* against recombinant HCV polymerase, were compared with the corresponding values predicted using the 3D pharmacophore model. Finally, we determined the three-dimensional structure of a small subset of the most promising proposed compounds bound to the target, and calculated the free energy of interaction using a molecular dynamics-based, continuum solvent method (MM/PBSA).

RESULTS: In this work we built 3D pharmacophore hypotheses from a training set of 40 diketo acids active as inhibitors of the HCV RNA-dependent RNA-polymerase. We verified that the more active compounds map well onto all features of the best hypothesis Hypo1. Taken together, two hydrogen bond acceptors, a negative ionizable group, and two hydrophobic aromatics located on the molecules seem to be essential for high activity in inhibition of HCV RNA-dependent RNA-polymerase. The same hypothesis has been applied to the prediction of the activity of recently synthesized diketo acid derivatives. For those compounds for which the activity was obtained concomitantly, the results obtained were both qualitatively and quantitatively in good agreement. The binding modes and affinities of the most promising set of compounds have also been predicted using an ansatz that combines MM/PBSA and normal mode calculations. Given the quality of the results achieved, the combination of 3D pharmacophore modeling and detailed atomistic MD simulations can be considered the most promising tool in designing new leads for hopefully more active compounds, and work is in progress in this respect.

ABSTRACT 039

Non-nucleoside Anti-*Flaviviridae* Agents from Different Molecular Classes: A Joint Experimental/ Modeling Effort

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BACKGROUND: *Flaviviridae* are enveloped, positive single-stranded RNA viruses. This virus family contains three genera: *Hepacivirus, Flavivirus, and Pestivirus*. Currently, more that 70 *Flaviviruses* have been discovered, and many of them cause important human diseases. HCV has infected an estimated 1-3% of the world population, which means that more than 170 million chronic carriers are at risk of developing cirrhosis and/or liver cancer. Although an efficient vaccine exists against YFV, this virus is still a leading cause of hemorrhagic fever and related mortality worldwide. Another *Flavivirus*, DENV, is currently threatening up to 2.5 billion people, and is still emerging throughout the world. Thus, there is an urgent need for more (cost-)effective treatments and/or immunoprophylaxis for these viral diseases. Accordingly, the Italian FIRB group of researchers has spent intense, jointed efforts in the discovery of compounds that inhibit the replication of *Flaviviridae* in general, with the hope that they will result in the discovery of selective HCV inhibitors.

METHODS: In this work we present the combined results obtained from the synthesis, biological studies, and *in silico* screening and modeling of the following classes of compounds:

- Mannich bases of 7-hydroxycoumarins;
- hindered nucleoside analogs;