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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;

- 4,7-Phenantrolines;
- 2-aryl and alkylaryl substituted benzimidazoles;
- aryl/heteroarylazoenamines;
- polyhydroxylated acyclic nucleosides
- bicycloheptanoids derivatives

which were all proven to be effective, at different levels, against different *Flaviviridae*.

RESULTS: Several members of each series of compounds listed above showed an interesting activity in counteracting the viral progression in BVDV infected cells. Interestingly, a series of 2-[(Benzotriazol-1/2-yl)methyl]benzimidazoles were also found active against *Syncytial Respiratory Virus* (RSV). Finally, some compounds showed a broad range activity against BVDV, YFV, DENV and *West Nile virus*. The structure-activity relationships of the most active compounds in each series were also formulated and rationalized on the bases of 3D pharmacophore models and/or detailed modeling of target protein/drug interactions.

CONCLUSIONS: The jointed efforts of several Italian laboratories have led to the discovery of a plethora of classes of compounds with potential anti-*Flaviviridae* activity, which will eventually display anti-HCV activity. The formidable challenge posed, until recently, by the lack of an *in vitro* model for the HCV infection has been partly overcome by the efficient combination of experimental activity on a surrogate BVDV model and computer-assisted simulations. In a panorama in which very few molecules are expected to reach the clinical trials stage in the next 10 years, we believe that our results could positively contribute to the development of new active substances against these life-threatening viruses.

ABSTRACT 040

Potent Inhibitors of HCV Replication Targeting the NS5b RNA Polymerase

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BACKGROUND: Hepatitis C is a serious worldwide health problem and current therapies are insufficient, especially for the HCV subtypes 1a/b. A prime target for development of HCV specific antiviral agents is the HCV NS5b RNA polymerase, a virally encoded enzyme that is essential for viral replication. Several allosteric sites of inhibition have been described. One particular site, located at the interface between the fingerloops and the thumb domain proximal to the allosteric GTP binding [Tomei *et al., J Virol* 2003, 77, 13225], can be targeted with a class of compounds that share a benzimidazole or indole core attached to a cyclohexyl moiety. Some of the earlier representatives of this class of inhibitors exhibited a fairly flexible linker inside the inhibitor molecules.

METHODS: We have used medicinal chemistry and ligand-based design methods to find more constrained molecules with potent antiviral activity. For the evaluation of the later, candidate molecules were tested routinely (i) in an NS5b genotype 1b *in vitro* assay using a heteropolymeric RNA template and (ii) in a cell-based subgenomic replicon assay (subtype 1b). For the most potent compounds, inhibition of subtype 1a and 2a NS5b polymerases was evaluated as well.

RESULTS: We have identified a novel class of constrained compounds that target the finger-loop/thumb interface of the polymerase. In these compounds, several bicyclic heterocyles have been used to replace the earlier, more flexible linkers. The most potent compounds exhibit low nanomolar activity in an NS5b genotype 1b *in vitro* assay (IC_{50} < 100 nM) and in the subgenomic replicon assay (EC_{50} < 200 nM). The antiviral activity against NS5b subtype 2a is reduced by at least an order of magnitude, which is explained by the 1b/2a sequence differences in the allosteric inhibitor site [DiMarco *et al., JBC* 2005, 280, 29765]. Treatment of replicon bearing ET cells with some of our most potent compounds for 2 to 3 weeks led to an over 1000x reduction in viral RNA load. The initial *in vivo* pharmacokinetic data from rodent and dog studies suggests that compounds from this class have acceptable intravenous clearance rates and oral bioavailability (e.g. for compound GL59728, IV clearance is 57 ml/hr/kg and oral bioavailability is 57 %F in dogs) demonstrating their potential as anti-HCV agents.

CONCLUSION: We have discovered a novel class of allosteric inhibitors targeting the fingerloop/thumb interface of NS5b. These inhibitors are most potent against the 1a and 1b subtypes. The excellent potency in cell-based assays along with the favorable pharmacokinetic properties led us to advance two of our compounds into IND enabling studies, which are ongoing.