Synthesis and *in silico* evaluation of new 3,4-dihydro-2(1H)-pyridones as SARS-CoV-2 inhibitors

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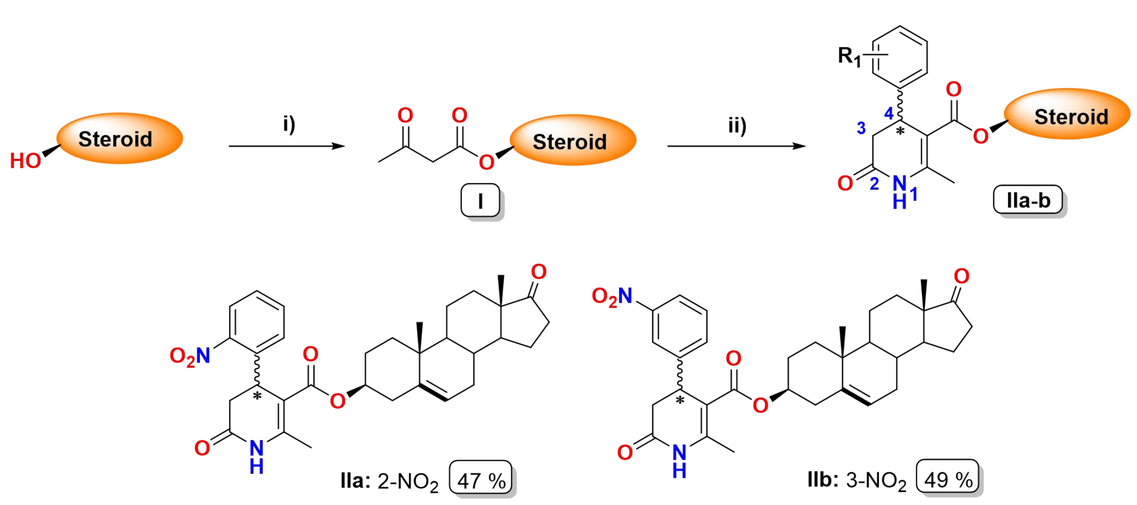
Introduction Steroids constitute an extensive and important class of biologically active polycyclic compounds that are widely used for therapeutic purposes. Due to their chemical and biological properties, they have been employed as molecular building blocks in the design of hybrid molecules that combine the characteristics of their precursors.[1]
Furthermore, 3,4-dihydro-2(1H)-pyridones have a wide range of biological activity as anti-inflammatory, antibacterial, and to treat of cardiovascular diseases.[2] Hence, the design of hybrid systems combining covalently these N-heterocyclic derivatives and steroids as molecular building blocks represents an attractive approach to synthesizing novel conjugates with outstanding bio-medical applications.

Results

Chemical synthesis

We report the synthesis of new steroid-2-(1H)-pyridone hybrids using the Hantzsch protocol in a straightforward manner by a multicomponent reaction. The synthesis of hybrid steroid-[3,4-dihydro-2(1H)-pyridones (Scheme 1) was developed using androstane derivatives endowing a hydroxyl group. First, the synthesis of steroid β-ketoesters (**I**) was performed by reaction of sterols derivatives with dioxinone. The reaction crude was purified by column chromatography.

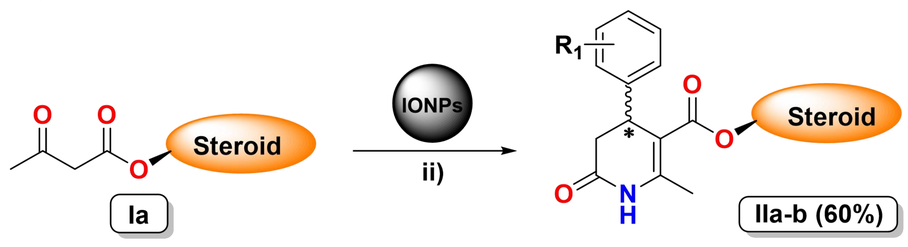


Scheme 1. Synthesis of hybrid steroid-[3,4-dihydro-2(1H)-pyridone]; i): sterol, dioxinone, toluene, reflux, 7h; ii): aromatic aldehyde, Meldrum acid, ethanol, reflux, 14h.

The synthesis of steroid-heterocycle hybrids **II(a-b)** was performed following the Hantzsch multicomponent reaction by refluxing in ethanol for 14 hours a mixture of an aromatic aldehyde, Meldrum's acid, ammonium acetate, and the β-ketoester. Isolation was achieved by flash chromatography. As the reaction to afford the pyridone ring takes place with the generation of a new stereogenic center on the C4, and the configuration of the steroid is fixed, the reaction gives rise to a diastereomeric mixture of all compounds. The new hybrid steroid-pyridones were isolated as yellow solids in moderate yields.

Magnetic nanoparticles in the Hantzsch reaction

To increase the chemical yield of multicomponent reactions, magnetic nanocatalysts based on iron oxide (IONPs) were used. Iron oxide nanoparticles were prepared by a modification of Massart's coprecipitation technique using a rapid injection method with a 1:2 molar ratio of $FeSO_4$:$FeCl_3$.[3] After a study of catalytic and reaction conditions, 50 mg of IONPs dispersed in absolute ethanol were used. Yields increased to 60% and reaction times were over 6 hours. (see Scheme 2)



Scheme 2. Synthesis of hybrid steroid-[3,4-dihydro-2(1H)-pyridone] with magnetic nanocatalyst; ii): aromatic aldehyde, Meldrum acid, ethanol, reflux, 6h.

Chemical synthesis

Theoretical calculations at B3LYP 6-311G(d,p) level were employed to predict the most stable conformation of the synthesized compounds (Figure 1). Both diastereomeric conformations (4*S* and 4*R*) for all hybrids show a pyridone ring in a screw-boat conformation with the aryl group near the orthogonal disposition to the pyridone ring pseudoplane. These results are in perfect agreement with those previously reported for related 3,4-dihydropyridones.[2]

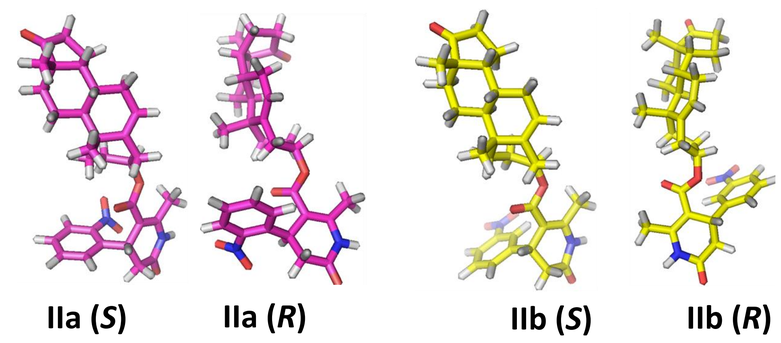


Figure 1. Minimum energy conformation of compounds **IIa-b**, obtained by the DFT method.

Molecular docking

The molecular target studied was the papain-like protease (PLpro) of SARS-CoV-2 [PDB: 4OVZ, chain A]. Rigid molecular docking simulation was performed with AutoDock-Vina. The calculated negative binding energy predicted that all hybrids have affinity for the enzyme and similar binding modes. Additionally, they display several interactions with key residues in the active site of PLpro like Tyr 208, Met 209, and Gln 233, (see Figure 2 for **IIa** (R)).

Ligand	Affinity*
IIa (R)	-9,55
IIa (S)	-7,89
IIb (R)	-8,15
IIb (S)	-8,54

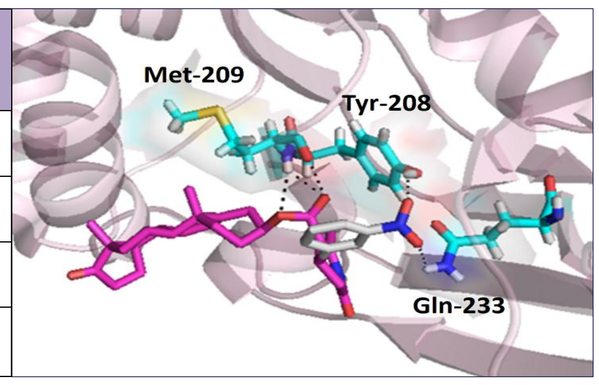


Figure 2. Molecular docking results. Binding mode of **IIa (R)**. *(kcal/mol)

Conclusions

A series of new hybrids steroid-[3,4-dihydro-2(1H)-pyridone] have been synthesized from an aromatic aldehyde, Meldrum's acid, NH_4Ac and a steroid β-ketoester in good yields by the use of magnetic nanoparticles in the Hantzsch reaction.
The affinity study with the SARS-CoV-2 PLpro and the interactions found indicate that the hybrids have potential for its implementation in further studies towards anti-viral therapies against COVID-19.

References

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2- S. Chalán-Gualán, V. Castro, R. Oropeza, M. Suárez, F. Albericio, H. Rodríguez, 3,4-Dihydro-2(1H)-Pyridones as Building Blocks of Synthetic Relevance, *Molecules* **2022**, *27(16)*, 5070.
3- A. Lagar-Sosa, M. Muñoz-Arias, A. Lugo-Calas, M. Hernández, A. Díaz-García, C. Lariot, Transmission Electron Microscopy Characterization of IONPs Modified with Polyethylene Glycol Derivatives, *Microsc. Microanal*. **2020,** *26*, 191-192.