Indole-Based Compounds as Promising Inhibitors of SARS-CoV-2

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Abstract: This review focused on the recent development of indole-based compounds as anti- SARS-CoV-2 agents with focus on the following objectives: 1) present the design strategy of indole-based compounds as promising inhibitors; 2) focus on recent developments of indole-based compounds and their anti-severe acute respiratory syndrome coronavirus 2 and anti-severe acute respiratory syndrome coronavirus activities; 3) summarize molecular docking and the structure–activity relationship, in hopes to inspire the development of new and more creative approaches; and 4) offer perspectives on how indole scaffolds might be exploited in the future.

Keywords: Indole; Protease; Inhibitor; SARS-CoV-2

1. Introduction

Indole is an important structural motif in drug discovery and is described as a "privileged scaffold," a term first introduced by Evans *et al*. to define scaffolds capable of serving as a ligand for a diverse array of receptors. Furthermore, indole derivatives can mimic the structures of peptides and bind reversibly to protease, providing tremendous opportunities to discover novel drugs with different modes of action. Indole scaffold is widely used in the design and synthesis of antiviral drugs and shows high biological activities; it can be used as a framework to design SARS-CoV-2 inhibitors. Therefore, indole-based scaffolds have been extensively used in drug discovery and have resulted in the development of related drugs [1].

2. Design strategy of indole-based compounds as inhibitors of SARS-CoV-2

The drug design strategy included screening for indole-based bioactive compounds with reported antiviral activities. First, their chemical structures and activities were investigated. Subsequently, the structure-based approach for repurposing these compounds against SARS-CoV-2 co-crystallized proteins was addressed. The departure point started with docking indole-based molecules with Mpro, RdRp, and spike proteins. Then, full binding modes and poses were discussed for the highest-scoring compounds. Finally, the structure–activity relationship was derived to find the link between the chemical structure of the indole-based molecule and its cytotoxic activity. These bioactive compounds were made publicly accessible to facilitate further studies and optimization by the scientific community [2].

3. Indole-based compounds as promising inhibitors of SARS-CoV-2

3.1 Indole-based compounds as Mpro inhibitors

The active sites of Mpro are highly conserved among all coronavirus Mpros and are usually composed of the following

four sites: S1′, S1, S2, and S4. Dai group [3] have designed and synthesized inhibitors targeting SARS-CoV-2 Mpro by analyzing the substrate-binding pocket of SARS-CoV Mpro (Fig. 1). An indole group was introduced into P3 to form new hydrogen bonds with S4 and improve drug-like properties. Compounds **1** and **2** exhibited high SARS-CoV-2 Mpro inhibition activity, reaching 100% for **1** and 96% for **2** at 1 μM, respectively. The X-ray crystal structures of SARS-CoV-2 Mpro in complex with **1** and **2** showed that the aldehyde groups of **1** and **2** are covalently bound to cysteine 145 of Mpro. Both compounds showed good pharmacokinetic properties *in vivo*, suggesting that these compounds are promising drug candidates.

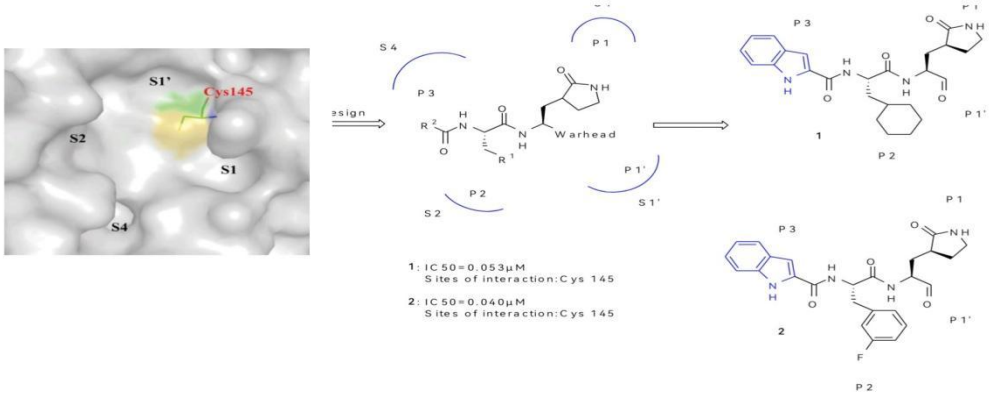


Fig. 1. The design strategy of SARS-CoV-2 main protease (Mpro) inhibitors a6nd the chemical structures of **1** and **2**.

A set of indole-based compounds (**3a–3o**) was synthesized and tested by Singhal *et al.* (Fig. 2) [4]. **3o** had higher antibacterial action at 50 μg/mL, verifying that the inhibitory effect increased with an increasing number of carbon atoms on the linker chain. The protease from SARS-CoV has a protein data bank code 1UK4, whereas SARS-CoV-2 has protein data bank code 6LU7. The key residues in this protease substrate-binding pocket are Thr45, Met49, Phe140, Asn142, Cys145…His41 dyad, Met165, His172, Glu166, Asp187, Arg188, and Gln189. **3o** was most effective with 6LU7. Three conventional H-bonds occurred between NH of the benzimidazole rings and indole carbonyl of **3o;** PHE140, ASN142, CYS145 of the enzyme proved its efficacy as potential DNA binders and anti-SARS-CoV-2 agents.

R n	2	3	4	5	6
H	a	d	g	j	m
Cl	b	e	h	k	n
Br	c	f	i	l	o

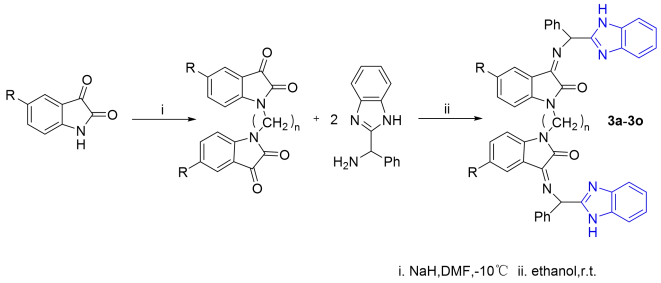
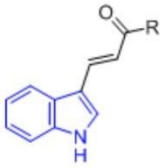


Fig. 2. The synthetic protocol for **3a–3o**.

3.2 Indole-based compounds as RdRp inhibitors

The SARS-CoV-2 replication mechanism is chiefly led by RdRp, which is a complex of nsp12, nsp8, and nsp7. RdRp is largely carried over by SARS-CoV and provides certain targets of opportunity for the selected indole-based compounds. The key catalytic residue sequence of Ser759, Asp760, and Asp761 is the binding site of RNA on the palm region, partly assisted by Asp618, which is a divalent cation-binding residue; these are essential to replication. In addition, residues Lys545 and Arg555 stabilize the incoming orientation of RNA, whereas Lys500 and Ser501 mobilize to accommodate its approach. In addition to these residues, 29–50 on β-hairpin of nsp12 are responsible for RdRp structural stabilization by interacting with other nsp12 domains.

Vijayakumar group studied a set of indole-based compounds 4a–4x (Fig. 3) [5]. They all obeyed Veber's rules and showed excellent DLM properties. Postdocking analysis shows that library members **4d**, **4h**, **4l**, **4p**, **4q**, **4t**, **4v,** and **4w** interact with β-hairpin residues. The results suggested that the indole-based compounds fight SARS-CoV-2.



4a–4x

4a R=4-F-C_6H_5 4b.R=4-CF_3-C_6H_5 4c.R=3-CF_3-C_6H_5 4d.R=4-Cl-C_6H_5
4e.R=2,4-Cl-C_6H_5 4f.R=4-Br-C_6H_5 4g.R=4-NO_2-C_6H_5 4h.R=4-OH-C_6H_5
4i.R=3-NH_2-C_6H_5 4j.R=4-CH_3-C_6H_5 4k.R=3-OCH_3-C_6H_5 4l.R=3,4-OCH_3-C_6H_5
4m.R=4-pyridyl 4n.R=3-pyridyl 4o.R=2-pyridyl
4p.R=4-OH-4-C_6H_5-piperazine
4q.R=1-C_6H_5-imidazole 4r. R=3-furyl 4s. R=2-pyroyl 4t. R=2-thiophenyl

Fig. 3. Synthetic indole-based compounds.

4. Conclusion and prospects

In this study, first, a panel of indole compounds based on the previously known structures was synthesized, and their inhibitory activity against SARS-CoV-2 was demonstrated. Then, using molecular docking, the binding of the indole-based compounds was assessed against their proteins, namely, RdRp, Mpro, and spike protein. If the compounds showed better binding affinity and overall dynamic stability, further *in vitro* and *in vivo* studies were conducted and the structure–activity relationships of such compounds were derived to discover a link between chemical structures and their corresponding activities.

So far, the research and development of new drugs usually require many human, material, and financial resources and time. Considering the urgent need for drugs in the current COVID-19 epidemic, the strategy of "new use of old drugs" can save resources and time. Then, through structure–activity relationship research, structural optimization, and drug-ready evaluation, we can discover lead compounds, candidate drugs, and clinically effective drugs that can enrich the "repository" of anti-SARS-CoV-2 drugs.

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