

Inhibition of SARS Coronavirus Helicase by Baicalein

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The severe acute respiratory syndrome (SARS) occurred in the southern part of China in the late 2002 and rapidly spread to Canada and Southeast Asian countries, including Singapore, Vietnam, Hong Kong, and Taiwan. During the outbreak, clinicians were unaware of which treatments were appropriate to treat SARS patients because they had no prior experience in dealing with this disease. Therefore, they prescribed the SARS patients with general antiviral medications, such as ribavirin, type-I interferon and corticosteroids. The SARS pandemic was finally controlled within a year of outbreak through progressive global efforts, including patient quarantine. A retrospective case-controlled analysis showed that these antiviral agents had minimal beneficial effects or even worsened the symptoms of SARS patients,¹ suggesting that it is necessary to develop effective anti-SARS agents for another SARS outbreak in the future.

A novel coronavirus (CoV) was identified as a causal factor for the SARS. Coronaviruses are members of a family of enveloped viruses that replicate in the cytoplasm of host cells. Analysis of the full sequence of the SARS coronavirus (SCV) revealed that it is a large, single-stranded, and positive-sense RNA virus, which bears a moderate resemblance with other human coronaviruses, HCoV-OC43 and HCoV-229E. Notably, the first 2/3 of the SCV genome consists of the viral replicase genes that encode 16 non-structural proteins (nsPs), including the NTPase/helicase (nsP13).² The SCV helicase, nsP13 is a critical component for viral replication and currently regarded as a feasible drug target for potential SCV chemical inhibitors. Helicase is a molecular motor protein that separates double-stranded nucleic acid (NA), using the free energy generated from nucleoside triphosphate (NTP) hydrolysis during translocation on single-stranded NA.³ The nsP13 is considered as a RNA helicase because SCV is a positive-strand ssRNA virus and comprise three major domains: Zn²⁺ ion binding domain, hinge domain and NTPase/helicase domain.⁴

Previous studies have identified numerous natural or synthetic chemicals that suppress the nsP13 activity with different modes of actions. For example, Tanner *et al.* have demonstrated that the bananin derivatives are non-competitive inhibitors of the ATPase activity of nsP13.⁵ Yang *et al.* have demonstrated that bismuth complexes compete for the Zn²⁺

ion binding sites, thereby exhibiting significant inhibitory effects on both the ATPase and DNA duplex-unwinding activities of nsP13.⁶ Lee *et al.* have found that aryl diketone (ADK) analogs suppress the nsP13 activity.⁷ Because ADKs are well-known metal chelators, authors initially speculated that ADKs would inhibit the activity of nsP13 in an analogous manner to the bismuth complexes: ADKs bind to Zn²⁺ ion binding site, thereby inhibiting both the ATPase and DNA duplex-unwinding activities. Contrary to their expectations, however, they observed that ADKs selectively inhibited the duplex DNA-unwinding activity, but not the ATPase activity of nsP13 and the anti-SCV helicase activities of ADKs were dependent on regiochemistry as well as the substituent of ADKs, leading to the conclusion that alternative inhibitory mechanism(s) of the nsP13 activity by ADKs might exist. In accordance with the above observations, we have recently conducted *in vitro* biochemical experiments to find out dietary or medicinal compounds that possess significant inhibitory effects on the nsP13 helicase activity.⁸ As a result, we found that both myricetin and scutellarein (Fig. 1) strongly inhibited the ATPase activity, but not the DNA unwinding activity of nsP13. IC₅₀ values of myricetin and scutellarein against the ATPase activity of nsP13 were observed to be 2.71 ± 0.19 μM and 0.86 ± 0.48 μM, respectively.

It is notable that myricetin and scutellarein are natural flavonoids. Flavonoids are secondary plant metabolites that

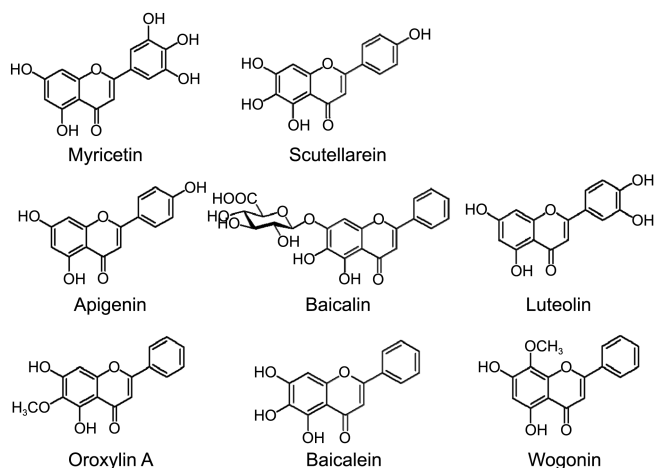


Figure 1. Chemical Structure of myricetin and scutellarein together with six additional natural flavonoids, which were used in our study.

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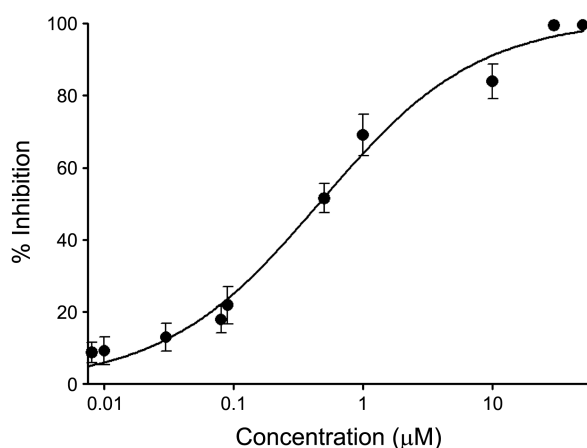


Figure 2. Baicalein is a new natural flavonoid that has significant inhibitory effects on the ATPase activity of nsP13. IC_{50} value of nsP13 ATPase inhibitory activity of baicalein was determined by a serial dilution of baicalein in the experiment setup.

have been reported to exhibit numerous beneficial effects.⁹ In the present study, we have attempted to further examine whether there exist any other natural flavonoid(s) that might possess the significant inhibitory effects on the nsP13 activity. In line with this idea, we have prepared six additional flavonoids (Fig. 1) whose chemical structures are similar to myricetin and scutellarein and tested their potential inhibitory effects on the nsP13 activity. Utilizing the Fluorescence Resonance Energy Transfer (FRET)-based DNA unwinding assay and ATP hydrolysis assay,¹⁰ we have examined the possible inhibitory effects of individual natural compounds on the dsDNA-unwinding and ATP hydrolysis by measuring the emitting fluorescent intensity at a wavelength of 535 nm (dsDNA-unwinding assay) and the release of inorganic phosphate (P_i) by colorimetric analysis (ATP hydrolysis assay). We have conducted both experiments at an initial concentration of 10 μ M. As a result, we found that none of flavonoids tested in our study exhibited significant inhibitory effects against the dsDNA-unwinding activity of nsP13 (data not shown). However, we observed that baicalein inhibited the ATPase activity of nsP13 by more than 60%, while luteolin and wogonin exhibited a little more than 20% of inhibition at this concentration (data not shown). In order to determine the IC_{50} value of baicalein in suppressing the ATPase activity of nsP13, we serially diluted the concentration of baicalein and measured their inhibitory effects on the ATPase activity of nsP13 *in vitro*. As a result of this

analysis, we have determined the IC_{50} value of baicalein to be $0.47 \pm 0.09 \mu$ M (Fig. 2).

In conclusion, we have demonstrated that baicalein is a novel chemical inhibitor of the ATPase activity of nsP13 protein, whose pharmacological activity against ATPase activity of nsP13 is superior to that of myricetin and scutellarein. While it is currently unclear why baicalein selectively suppressed the ATPase activity, but not the DNA unwinding activity of nsP13, we have found that baicalein shares a common structural feature with myricetin and scutellarein: these molecules have three consecutive hydroxyl moieties in the A or C aromatic ring of flavonoid structure, while other inactive compounds tested in both the previous and present studies do not. Our previous modeling analysis suggested that hydroxyl groups of myricetin, existing in B ring of flavonoid structure could directly interact with several residues located in the ATPase domain of nsP13 and contribute to displacing a correct ATP/ADP positioning in the ATP-binding pocket.⁸ While this assumption requires experimental verifications, it is possible to assume that three hydroxyl groups in the A or C ring of flavonoid would affect the ATPase function of nsP13 in a manner other than positioning the ADP/ATP in the ATP-binding pocket, if this structural hypothesis is true.

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