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Synthesis and anti-H_5N_1 activity of chiral gossypol derivatives and its analogs implicated by a viral entry blocking mechanism

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ABSTRACT

A series of chiral gossypol derivatives and its analogs were synthesized and tested in vitro for their anti-H_5N_1 activity. Interestingly, (+)-gossypol derivatives and its analogs were more active against H_5N_1 than the corresponding (−)-gossypol derivatives and its analogs. Through a simple chemical modification with amino acids, less active chiral gossypol could be converted into more active derivatives, and most of chiral gossypol derivatives were more potent against H_5N_1 than 1-adamantylamine. With regard to the mechanism of action, chiral gossypol derivatives and its analogs might impair the virus entry step of cell infection, likely targeting to HA2 protein.

The H_5N_1 subtype of avian influenza virus, which is a potentially serious threat to human health in the near future because of the high mortality and potential human-to-human transmission, is spreading in Southern China, Africa and European Union mainly imputing to migratory birds. To date, only four approved anti-influenza drugs, which belong to two categories, M2 ion channel blockers and neuraminidase (NA) inhibitors, are clinically available.[1] It has been reported that most of the isolated H_5N_1 influenza A virus strains are resistant to M2 inhibitors and may rapidly develop resistance to NA inhibitors.[1,2] Therefore, it is urgent to identify and develop effective anti-H_5N_1 compounds with novel mechanisms of action.

The earliest stage of influenza virus infection is viral entry, which is mediated by interaction of viral envelope protein hemagglutinin (HA) and its receptor on host cell surface, sialic acid sugars.[3] Hemagglutinin is a trimeric glycoprotein in nature and can be cleaved into HA1 and HA2 subunits by host proteases.[4] Although HA1 is the target for developing influenza A virus entry inhibitors, the high variability and mutation rate limit its application. Nevertheless, HA2 is for virus–endosome membrane fusion in the acid environment after the virus has been endocytosed into the cell and more conserved so that it may be a better target for developing influenza virus entry inhibitors.[3,4]

Since gossypol displayed good biological activities including antiferility, anticancer and antiviral activities, gossypol was no longer viewed as a detrimental compound contained in cottonseed, but as a potentially valuable natural product with useful physiological and chemical properties to be exploited.[5] So far, many gossypol derivatives and its analogs have been obtained and tested for their antipsoriatic, antimalarial, anticancer, interferon-inducing as well as anti-HIV activity.[6,7]

Of relevance to our present study are the reports of the antiviral activities against enveloped viruses, including herpes simplex virus type 2, para-influenza virus type 3, influenza virus and HIV-1, but not against non-enveloped poliovirus.[7–9] However, there are almost few reports on the anti-H_5N_1 activity of chiral gossypol derivatives against H_5N_1. Recently, we found that some amino acids substituting the aldehyde groups of gossypol not only reduced the cytotoxicity of gossypol but also enhanced the antiviral activities of gossypol against HIV-1 and H_5N_1.[10] Our study further indicated that amino acid derivatives of (−)-gossypol could bind to the gp41 hydrophobic pocket and blocked the formation of the cell fusion-activated gp41 core to inhibit HIV-1 mediated membrane fusion and subsequent viral entry,[11] but their mechanism of action against H_5N_1 needed further investigation.

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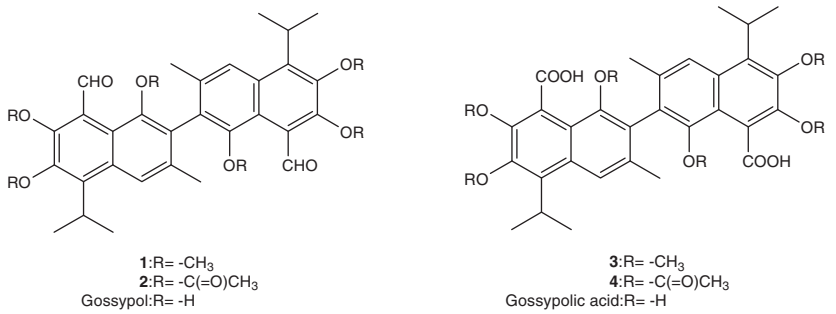


Figure 1. The structures of gossypol derivatives and its analogs.

For understanding the function of aldehyde groups and phenolic groups in gossypol, gossypolic acid and compounds **1**–**4** were synthesized and tested in vitro for their anti-H_5N_1 activity(Fig. 1).[5,8] Gossypol (IC_{50} = 2.14 μM, SI = 1.65) was more active against H_5N_1 than 1-adamantylamine (IC_{50} = 3.84 μM, SI >57.39), but gave higher cytotoxicity on MDCK cells. Meanwhile, gossypolic acid (IC_{50} = 2.37 μM, SI = 10.51) also showed the same anti-H_5N_1 activity as gossypol but lower cytotoxicity on MDCK cells. The results indicated that oxidation of the aldehyde groups in gossypol did not significantly influence the anti-H_5N_1 activity but greatly reduced the cytotoxicity on MDCK cells. It was reported that substituting the hydroxyls of gossypol decreased the antiviral activity compared with the unsubstituted gossypol (flu virus A, PR-8 strain).[12] In contrast, our results showed that methylation of gossypol and its analogs, such as compound **1** (IC_{50} = 103.25 μM, SI >0.54) and compound **3** (IC_{50} = 157.55 μM, SI >0.33), almost abolished their anti-H_5N_1 activity, while acetylation of gossypol and its analogs, such as compound **2** (IC_{50} = 2.72 μM, SI >13.35) and compound **4** (IC_{50} = 2.98 μM, SI >8.70), still retained the anti-H_5N_1 activity. Therefore, introduction of alkyl groups in the hydroxyls of gossypol and its analogs should be avoided, while acylation of hydroxyls in gossypol and its analogs merited for further study.

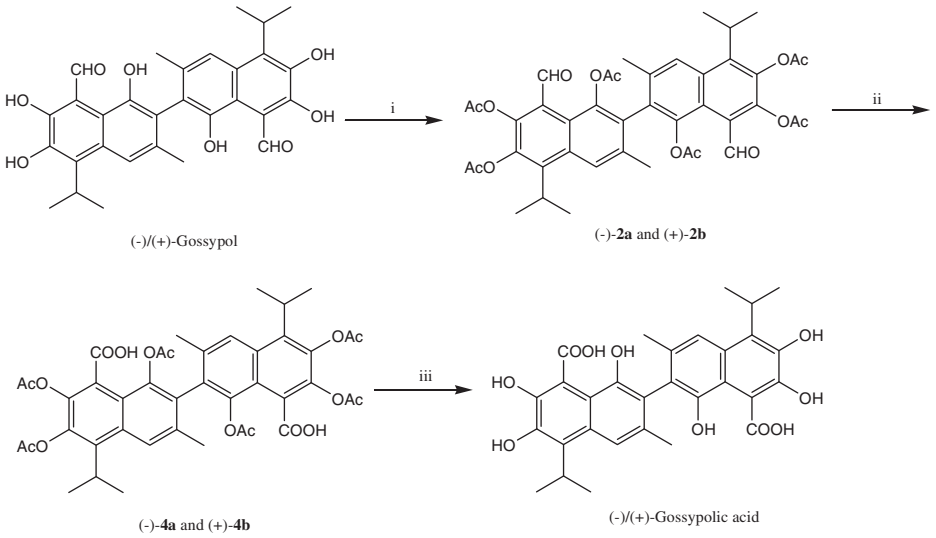
Because of restricted rotation around the C_2–C_{20} internaphthyl bond, gossypol has two optically active forms: the (−) and the (+)-enantiomers, respectively.[13] Previous studies suggested that (−)-gossypol was usually more potent in most biological evaluations in comparison with (+)-gossypol or racemic gossypol.[7,9]

In this study, a very interesting phenomenon attracted us, (+)-gossypol showed comparable activity with (−)-gossypol. The result prompted us to carry out comprehensive comparisons of the anti-H_5N_1 activity of chiral gossypol derivatives and its analogs. Therefore, a series of chiral gossypol derivatives and its analogs were synthesized and tested to evaluate their anti-H_5N_1 activity.

The preparation of chiral gossypolic acid was shown in Scheme 1. A series of chiral gossypol derivatives were prepared by treating chiral gossypol with the corresponding amino acids and D-glucosamine in a suitable solvent to form adduct in high yields (>85%) (Scheme 2).[10,14] All compounds were evaluated for their capability to inhibit the H_5N_1 Influenza A virus strain A/Vietnam/1194/2004 replication in MDCK cells (Table 1).[10]

As shown in Table 1, it was observed that (+)-gossypol to be very inhibitory against H_5N_1 (IC_{50} = 1.87 μM), whereas (−)-gossypol was less active (IC_{50} = 3.82 μM). The cytotoxicity (CC_{50}) for the (+)-gossypol on MDCK was 3.53 and 35.35 μM for the (−)-gossypol. When compared with chiral gossypol, chiral gossypolic acid displayed the similar activity against H_5N_1 but less cytotoxicity. Hence, oxidation of the aldehyde groups in chiral gossypol could not improve the anti-H_5N_1 activity.

The preliminary SAR study of gossypol against H_5N_1 indicated that methylation of gossypol led to the disappearance of inhibitory activity but acetylation of gossypol did not influence its anti-H_5N_1 activity. Therefore, compounds **2a**, **2b**, **4a** and **4b** were also designed and synthesized to test their inhibitory effect. As summarized in Table 1, acetylation of (−)-gossypol (compound **2a**) did not change the anti-H_5N_1 activity of (−)-gossypol but slightly



Scheme 1. Reagents and conditions: (i) $(Ac)_2O$, DMAP, CH_2Cl_2, room temp, 12 h, 70.6% of (−)-**2a**, 71.2% of (+)-**2b**; (ii) $NaClO_2$, H_2O_2, NaH_2PO_4, CH_3CN, room temp, overnight, then 4 M aq HCl, 60.5% of (−)-**4a**, 61.5% of (+)-**4b**; (iii) 20% Na_2CO_3, THF, 80 °C, 10 h, then 4 M aq HCl, 63.6% of (−)-gossypolic acid, 64.5% of (+)-gossypolic acid.