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Rational design of pyridyl derivatives of vanillin for the treatment of sickle cell disease

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ABSTRACT

Hypoxia-induced polymerization of sickle hemoglobin (Hb S) is the principal phenomenon that underlays the pathophysiology and morbidity associated with sickle cell disease (SCD). Opportunely, as an allosteric protein, hemoglobin (Hb) serves as a convenient and potentially critical druggable target. Consequently, molecules that prevent Hb S polymerization (Hb modifiers), and the associated erythrocyte sickling have been investigated–and retain significant interest–as a viable therapeutic strategy for SCD. This group of molecules, including aromatic aldehydes, form high oxygen affinity Schiff-base adducts with Hb S, which are resistant to polymerization. Here, we report the design and synthesis of novel potent antisickling agents (SAJ-009, SAJ-310 and SAJ-270) based on the pharmacophore of vanillin and INN-312, a previously reported pyridyl derivative of vanillin. These novel derivatives exhibited superior *in vitro* binding and pharmacokinetic properties compared to vanillin, which translated into significantly enhanced allosteric and antisickling properties. Crystal structure studies of liganded Hb in the R2 quaternary state in complex with SAJ-310 provided important insights into the allosteric and antisickling properties of this group of compounds. While these derivatives generally show similar *in vitro* biological potency, significant structure-dependent differences in their biochemical profiles would help predict the most promising candidates for successful *in vivo* pre-clinical translational studies and inform further structural modifications to improve on their pharmacologic properties.

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1. Introduction

Hemoglobin (Hb) functions in equilibrium between the R-state (relaxed) possessing high oxygen (O_2) affinity, and T-state (tense) possessing low-O_2 affinity.[1–3] The R-state is an ensemble of high-affinity relaxed conformational states, including the R2, R3, RR2, RR3, and the classical R.[4–7] The equilibrium between the T and R states can be regulated by synthetic allosteric effectors that stabilize one state relative to the others.[6,7] Stabilization of the R-state, usually via the R2 or R3 or classical R conformation by allosteric effectors shifts the Hb oxygen equilibrium curve (OEC) to the left, producing a high-O_2 affinity Hb.[6–9] These types of allosteric effectors are potentially useful for treating sickle cell disease (SCD) since high-O_2-affinity sickle Hb (Hb S) does not undergo polymerization and subsequent RBC sickling.[6–19] The surface located αF-helix in Hb has been shown to enhance inter-strand polymer contact stabilization through a hydrogen-bond interaction between αAsn78 of the helix and a Hb molecule from an adjacent polymer strand. Consequently, the variant αAsn78Gly (Hb Stanleyville) is known to increase solubility of sickle Hb by abrogating the αAsn78 mediated hydrogen-bond interaction.[20,21] Hence, compounds that bind to Hb and stereospecifically destabilize polymer contacts are potential antisickling agents.[8,22]

Aromatic aldehydes, such as vanillin, Tucaresol, 5-HMF (and furan derivatives), and the most recent GBT-440 (Fig. 1), are some of the most well-studied molecules that increase Hb oxygen affinity to prevent the hypoxia-induced sickle Hb polymerization and/or directly destabilize polymer contacts.[6–19] 5-HMF was investigated in phase I and II clinical trials for the treatment of SCD

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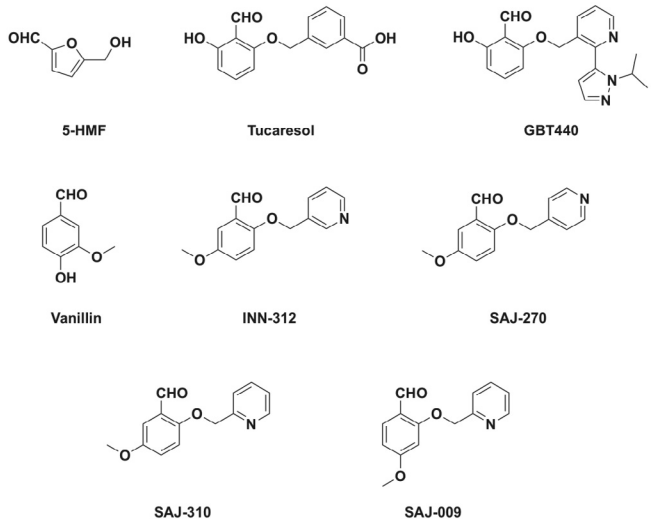


Fig. 1. Structures of 5-HMF, Vanillin, Tucaresol, GBT-440, INN-312, SAJ-270, SAJ-310 and SAJ-009.

but was hampered by extensive oxidative metabolism.[10,23] GBT-440 is currently undergoing phase III clinical studies for the treatment of SCD (clinicaltrials.gov, NCT03036813). Early studies by Zaugg et al. reported the antisickling activities of vanillin and several of its analogs.[24] A subsequent study by Abraham and co-workers further studied vanillin for the treatment of SCD.[25] Vanillin undergoes extensive metabolism resulting in low bioavailability, low potency, and overall reduces its pharmacologic effect. To understand the molecular basis of aromatic aldehyde antisickling activity, our group studied the co-crystal structures of aromatic aldehydes with Hb and showed that the compounds preferentially bind to the α-cleft of liganded R2-state Hb by forming Schiff-base interactions with the *N*-terminal αVal1 nitrogen atoms.[9] This mode of binding provides additional inter-subunit interactions across the subunit interfaces of liganded Hb that lead to stabilization of the R-state and increase the O_2 affinity of Hb. Based on these crystallographic findings that indicated a potential for appropriate modifications to vanillin (or its analogs), we designed and synthesized several pyridyl derivatives of vanillin, termed INN compounds, which demonstrated superior pharmacologic properties compared to vanillin[8,26] The modification resulted in additional hydrophobic contacts with the binding site residues, explaining the much improved pharmacologic effect.[8] Some of the compounds, such as INN-312 appeared to exhibit dual antisickling effects by increasing the oxygen affinity of Hb, and by stereospecifically destabilizing polymer contacts via interactions with a surface located F-helix.[8] INN-312, the most potent from the early group of compounds, evolved as a unique and promising pharmacophore, suggesting not only the importance of a pyridinylmethoxy substitution but also the ortho placement of this moiety (relative to the aldehyde group); some properties also shared by GBT-440.[17] In the present study we investigated INN-312 analogs (SAJ-310, SAJ-009 and SAJ-270) to further improve on the pharmacophore as a novel lead for development, and perhaps, provide newer knowledge on structure-activity relationships with mechanistic implications. We investigated these novel compounds for the general nature of their binding interactions with Hb, their effect on Hb O_2 affinity properties, inhibition of RBC sickling, as well as their *in vitro* pharmacokinetic/pharmacodynamic properties. We also conducted detailed crystallographic studies on SAJ-310 complexed with Hb to gain insights into the compound's functional and biological activities.

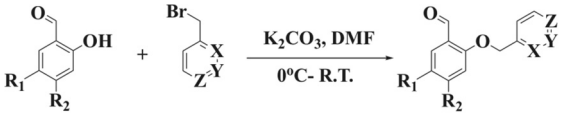
2. Results and discussion

2.1. Design and synthesis of SAJ compounds

We have previously studied several pyridyl derivatives of benzaldehyde-designated as INN compounds by incorporating a pyridinylmethoxy moiety on the vanillin pharmacophore (Fig. 1).[8,26] The derivatives potently increased Hb affinity for oxygen and resulted in several-fold increase in antisickling effect when compared to the parent compound vanillin. Structural analysis demonstrated that like vanillin, two molecules of INN compounds form Schiff-base adducts with the *N*-terminal Val1 nitrogen atom of the α-subunits of the R2 conformer of Hb and stabilize the R-state.[8] As anticipated from our design, this class of compounds made additional interactions with the protein, in part contributing to their enhanced functional and biological activities. Interestingly, the derivatives with the pyridinylmethoxy moiety at the *ortho*-position of the aldehyde group were generally more potent than the analogous *para*- or *meta*-positioned pyridinylmethoxy derivatives, with INN-312 being the most potent.[8] INN-312 binds and directs its *ortho*-positioned pyridinylmethoxy (pyridin-2-ylmethoxy) substituent (relative to the aldehyde moiety) towards the surface of Hb to make weak hydrophobic interaction with the surface located polymer stabilizing F-helix.[8] The enhanced antisickling potency of INN-312 is therefore likely due, in part, to the interactions with the F-helix.[8] Based on this promising lead, we further studied three *ortho*-substituted pyridinylmethoxy INN-312 analogs: SAJ-009, SAJ-270 and SAJ-310, for their functional and pharmacological properties (Fig. 1). Our intent was to explore the position of the nitrogen atom on the pyridine ring, which influences the electronic property of the ring system and possibly interaction of the compound with the protein, and study its role in the pharmacological function of these types of compounds (SAJ-270 and SAJ-310). Also, to confirm that the change in the activity is due to the change of the position of the nitrogen atom on the pyridine ring alone, SAJ-009, carrying a para position methoxy group on the aromatic aldehyde was synthesized. Finally, it was anticipated that like INN-312, the pyridin-2-ylmethoxy of SAJ-270, SAJ-310 and SAJ-009 would direct towards the surface of the protein, resulting in not only improved binding interaction with the protein to increase Hb affinity for oxygen but also make interactions with the F-helix to directly destabilize the polymerization process. The scheme for synthesizing the compounds is shown in Scheme 1.

2.2. SAJ-310 binds to the α-subunit of liganded Hb in the R2 state conformation

We have previously shown that aromatic aldehydes that bind to Hb and increase the protein's affinity for oxygen with concomitant antisickling effect act by forming Schiff-base interactions with αVal1 of liganded R2 Hb.[8,9,11] In the present study, co-crystallization experiments were conducted with human carbonmonoxy Hb (COHb) complexed with SAJ-310, SAJ-009 and SAJ-270 using low salt precipitant to give needle crystals.[12] Unlike SAJ-310, which gave relatively larger needle crystals, crystals from SAJ-009 and



INN-312: R_1= OCH_3, R_2= H, X= CH, Y= N, Z= CH
SAJ-270: R_1= OCH_3, R_2= H, X=CH, Y= CH, Z= N
SAJ-310: R_1= OCH_3, R_2= H, X= N, Y= CH, Z= CH
SAJ-009: R_1= H, R_2= OCH_3, X= N, Y= CH, Z= CH

Scheme 1.