Purpurogallin-A heme binding component of oak galls

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Abstract

Recently, it has been shown that Purpurogallin (PPG), (Fig.1) an orange benztropolone constituent of oak galls and its derivative, CU-CPT22, can compete with the binding of the specific lipoprotein ligand to toll-like receptors (TLRs), which are type I transmembrane proteins. These recognize pathogen-derived macromolecules that play a key role in the innate immune system. This system provides an attractive target for the treatment of various immune disorders. Notably, PPG also interacts with various metals and its mode of action against HIV in vitro may involve inhibition of metal containing integrases. In the current study, an optimised synthesis of PPG is presented together with its gas phase behaviour (probed by mass spectrometry) as well as its redox behaviour with porphyrins such as heme. This interaction may also explain its effects at metal containing integrases within HIV in vitro as well as its action during processing of iron complexes within Plasmodia. This compound could serve as a novel prototype for the synthesis of novel redox active antimalarials.

Introduction

Certain Purpurogallin (PPG) analogues, which are benztropolones derivatives with two or more hydroxyl groups can act as antimalarial agents [1]. They are known to bind metal ions including iron but the structure of such complexes has been little investigated [2]. Since *Plasmodium* parasites, the most dangerous variant of malarial infections, rely on catabolism of host haemoglobin as a food source, PPG and related derivatives could disrupt these highly conserved metabolic pathways by hydrogen bonding and then translocating heme and/or free iron to sites susceptible to oxidative stress [1]. Considering the ready accessibility of PPG by total synthesis [3], we sought to quantify its potential as an affordable, potential heme binding lead substance. Since the molecular mechanism of action of PPG derivatives against parasites remains unproven, the current study investigates the ability of heme to interact with heme using vibrational, nuclear magnetic and collision induced electrospray mass spectrometry (+ve and –ve ion mode) *in vitro*. Using ion mobility mass spectrometry, it is also possible to determine if predicted geometries coincide with either structures from crystallography databases and/or DFT calculations. PPG has been used in iron-gall inks in many historical documents such as DaVinci's Vitruvian Man and occurs naturally in various galls (Fig. 2).

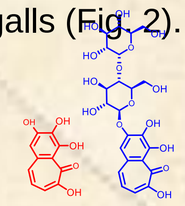

Fig.1 PPG (red):dryophantin (Blue)

Fig. 2 Oak leaf

Materials & Methods

Compounds were purchased from Sigma-Aldrich Co. or synthesised using literature methods. All masses were detected in -ve ion mode at high resolution using Waters LCT Classic (LC/MS) and Quattro Premier (LC/MS-MS) instruments. PPG:Hm complexes in the solid state were produced by co-grinding in an agate mortar under wet acetone (5 min), evaporated and re-ground repeatedly for 30 minutes. A fragment was then dissolved in wet methanol and subjected to –ve Ion Electrospray Mass Spectra. Complexes between Hm and purpurogallin [1] in solution (e.g. chloroquine and cryptolepeine (Cryp) [2-5]) were simultaneously assessed by FT-IR/MS as internal controls (data not shown).

Results & Discussion

Drugs were shown to interact in a 1:1 complex with monomeric Hm in the liquid, gas and solid phase (FT-IR, UV & MS data). Therefore, the putative geometry and thermodynamics of the interaction was proposed from previous computational investigations using PPG using both nominal ES mass spectrometry (+ve and -ve ion mode). Complex formation with Hm may then add either oxygen or methanol. Accurate ion cyclotron studies are planned to determine if the adduct is oxygen or methanol. Various investigators have demonstrated quenching of the Soret band (UV) upon addition of drugs as evidence of π…π interactions (i.e. the Dewar-Chatt-Duncanson model) as opposed to anticipated bathochromic shift [1]. ES-MS suggests that hydrogen bonding stabilised both the drug receptor complex and monomer, and dimers of PPG.

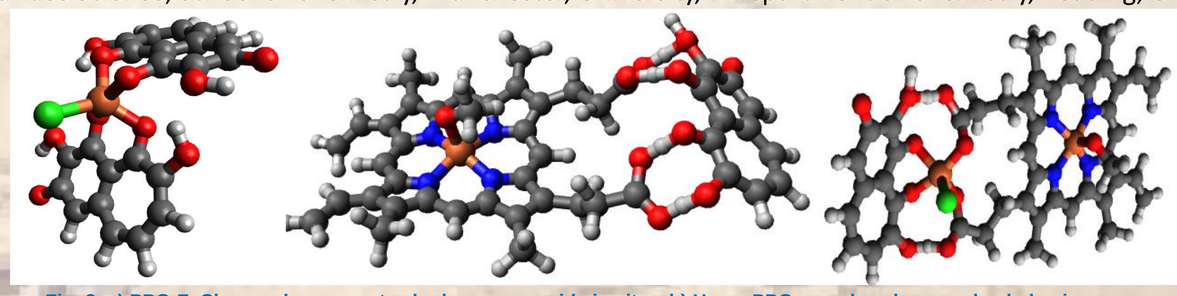

Fig. 3. a) PPG-$FeCl_3$ complex generates hydrogen peroxide in vitro; b) Heme-PPG complex shows redox behaviour; c) Proposed new complex that may generate hydrogen peroxide and then from hydroxyl radicals to kill cells.

Our earlier investigations have shown DFT [1,3] quantifies such non-covalent interactions (Fig.3) and we found that lowest energy structures closely match spectroscopic observations which are more likely to be stabilised by hydrogen bonds rather than π…π interactions (Fig 4). The –ve ion electrospray spectrum for PPG-Hm complexes revealed an isotope cluster ($C_{45}H_{39}FeN_4O_9^-$, 835 Da) matching theoretical calculations. The collision induced dissociation (CID) spectrum of the complex at 835 Da fragmented into anions at 219 and 615 Da. Currently, it is believed that quinoline based drugs modulate biomineralization of haem to haemozoin. However, phenolic substances such as PPG have not, to our knowledge, been investigated for haem binding activity. The negative ion mass spectra of the putative PPG-HmCl aggregate indicated that PPG binds strongly with Hm (835 Da), probably through H-bonding, an observation previously noted in buffered DMSO (Fig 4). Unusually, CID spectra produced fragment ions at 217 Da and 615 Da, which corresponds with 8-hydroxy-2,3,9-trioxo-3,9-dihydro-*2H* benzo[7]annulen-1-olate or 4,6-dihydroxy-*2H*-benzo[7]annulene-2,3,5-trione anion and Hm anions respectively. MS of adducts shows that the axial oxygen if any is displaced by the methoxide ion.

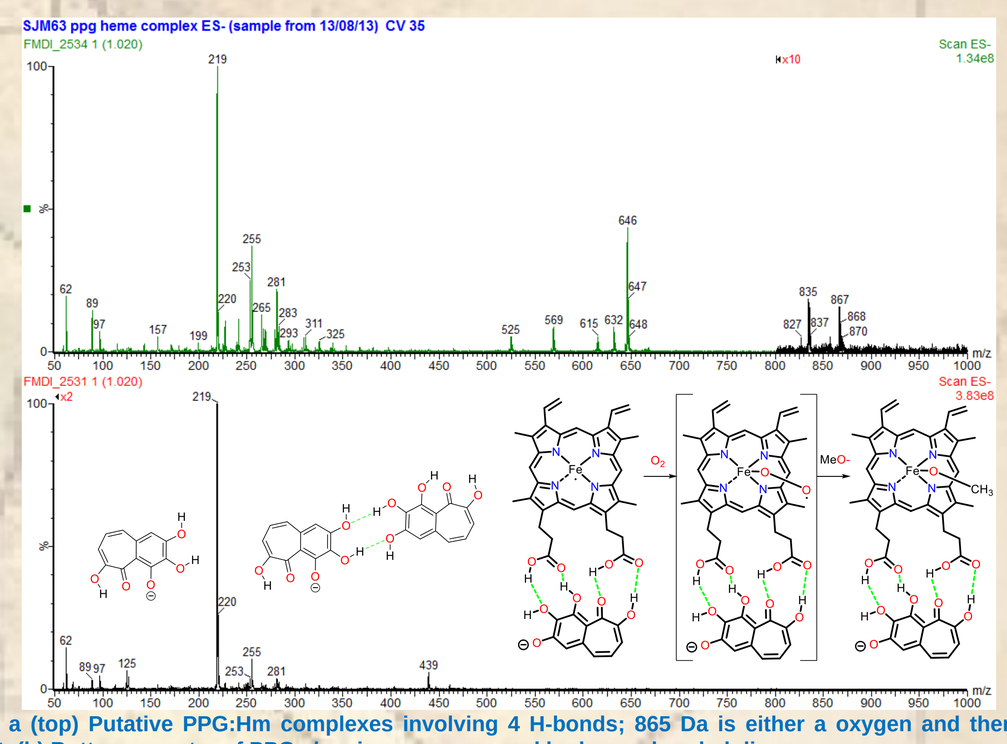

Fig. 4. a (top) Putative PPG:Hm complexes involving 4 H-bonds; 865 Da is either a oxygen and then methanol adduct. (b) Bottom, spectra of PPG showing monomer and hydrogen bonded dimer

Conclusion

Quinoline based antimalarials have previously been suggested to interact with monomeric Hm via π…π interactions (MM2, UV) but our study such interaction were energetically unfavourable. This observation indicates such interactions are unlikely to be involved in the antimalarial action of phenolics acting on Hm with the propionic acid side chains of Hm (edge on) [3] by hydrogen bonding [1,3,6] and may have important consequences for understanding the antimalarial mechanism of action as well as the design of new drugs [4,5,6]. F Ongoing work suggest that PPG also targets other receptors such as melanin involved in certain tumorogenic processes

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