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The Effect of *Cis*-2-(1H-imidazole-2-yl)-1H-imidazole Dichloro Platinum (II) on the *in-vitro* Formation of β-Hematin

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Abstract: This study aimed at assessing the effect of two *Cis*-Dichloro Platinum complexes on the *in-vitro* formation of the Malaria pigment. According to World Health Organization, death rates of Malaria decreased by 20%, even with this decrease Malaria is still considered to be the leading killer in Africa. Reports show nearly 90% of children die before they have the chance to reach their fifth year. *Plasmodium*; the Malaria parasite, forms during its intra-erythrocytic stage a pigment called Hemozoin. This pigment acts as a protection shield against oxygen radical-mediated stress that leads to the death of the parasite. Many drugs targeting the formation of this pigment are still effective such as Chloroquine and Amodaquine, but recently strains of *Plasmodium* have gained resistance to such drugs. As an attempt to find new anti-malarial drugs , the potential inhibitory effect of *Cis*-Dichloro Platinum complexes such as *Cis*- 2-(1H-imidazol-2-yl)-1H-imidazole Dichloro Platinum (II) and *Cis*-1-methyl-2-(1-methyl-1H-imidazole-2-yl)-1H-imidazole Dichloro Platinum (II) on Ferriprotoporphyrin IX (FP) biomineralisation was examined. A previously self-developed quantitative *in-vitro* method was used to detect the efficiency of these two complexes. Results showed that the efficiency of *Cis*- 2-(1H-imidazol-2-yl)-1H-imidazole Dichloro Platinum (II) and *Cis*-1-methyl-2-(1-methyl-1H-imidazole-2-yl)-1H-imidazole Dichloro Platinum (II) in preventing the formation of β-Hematin were 69.4 and 45%, respectively. The efficiency of Chloroquine as a standard drug using the same method was reported to give 95.9%

Key words: Antimalarial drugs, chloroquine, *cis*-Dichloro platinum complexes, ferriprotoporphyrin (IX), hemozoin

INTRODUCTION

Malaria has been one of the most devastating and highly reported vector-borne diseases occurring to mankind. According to the World Health Organization and the World Malaria Report of 2011 malaria caused the death of over 600,000 people and affected more than 200 million putting 3.3 billion people at risk (WHO, 2011) WMR report.

This disease is caused by a one-cell parasite of the genus *Plasmodium*. Among the many species of this genus four species commonly cause disease among humans; *P. vivax*, *P. ovale* and *P. malariae* and *P. falciparum* which causes 90% of the total deaths (Rathore, 2006).

The life cycle of *Plasmodium* species is a bit complicated. First the parasite invades the liver of the host where they undergo maturation before released into the bloodstream. During the intra-erythrocytic stage the malaria parasite resides inside the erythrocytes of the infected host where they change form and multiply forming “ring stage" (Pagola *et al*., 2000). During this ring stage, Plasmodium species degrade hemoglobin for their biosynthetic requirements, large amounts of free heme or known as Ferriprotoporphyrin (IX) (FePPIX) is released (Pagola *et al.*, 2000; Rathore, 2006).

Free heme is considered to be highly reactive and toxic to the malaria parasite. If allowed to accumulate it will cause the generation of reactive oxygen species which may induce oxidative stress leading to parasitic death (Kumar *et al*., 2007). To overcome this toxicity, The Plasmodium parasite has evolved a mechanism for the detoxification of free heme through its biomineralisation into a non-toxic, un-reactive, insoluble crystalline form called Hemozoin or “Malaria pigment” (Pagola *et al.*, 2000; Rathore, 2006; Kumar *et al*., 2007).

Hemozoin is a polymer made of dimers of hematin molecules that are joined together by hydrogen bonds to form larger structures (Pagola *et al.*, 2000). These dimers are formed through an iron-oxygen coordinate bond that

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links the central ferric iron of one hematin to the carboxylate side group oxygen of another. These reciprocal ferric-oxygen bonds are highly unusual and have not been observed in any other porphyrin dimer. This process is held within the digestive vacuole of the parasite, an acidic compartment with an estimated pH in the range of 4.5 to 5.0 (Slater *et al.*, 1991; Pagola *et al.*, 2000; Sullivan, 2000). This pigment has emerged as an important target in the search and finding of new anti-malarial drugs.

A synthetic polymer structure known as "β- Hematin" that is made from Ferriprotoporphyrin- IX is believed to be structurally, chemically and spectroscopically identical to purified Hemozoin. Since it is a process that is essential to the survival of the malaria parasite it acts as an excellent target for *in-vitro* study. Drugs are thought to act by inhibiting the formation of Hemozoin in the food vacuole. This prevents the detoxification of the free heme released in this compartment, killing the parasite (Slater *et al.*, 1991; Pagola *et al.*, 2000).

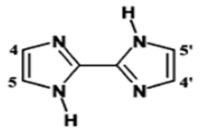
Through the years Quinoline-ring drugs such as Chloroquine have proved their effectiveness as anti-malarial drugs by accumulating inside the food vacuoles of the parasite preventing the formation of Hemozoin, killing the parasite (Slater *et al.*, 1991; Kumar *et al*., 2007).

Recently strains of Plasmodium falciparum formed resistance to Chloroquine, drawing attention to find new anti-malarial drugs. We had previous attempts to finding new anti-malarial drugs in our research laboratory, such attempts spotted light on the effect of Pyrimidine derivatives in the *in-vitro* inhibition of β-hematin (Aljazzar *et al.*, 2010).

Recently, studies have shown that transitional metal complexes such as Cisplatin are biologically effective as drugs against cancer and also may act as lead compounds for developing future treatment of malaria (Rafique *et al*., 2010).

Choosing 2-(1H-imidazol-2-yl) -1H-imidazole or what is known as 2, 2'-Biimidazole as a ligand in our studies is based on the fact that this ligand is derived from Imidazole. Different researchers have studied the derivatives of the compound finding different interesting properties, along with their ability to exert anti-cancer, antibacterial, antimyoctic and antiparasitic activity (Pfaller and Krogstad, 1983; Ashish and Pandeya, 2011). Studies have shown that Imidazoles may exert their anti-malarial effect through increasing the oxidant stress on the malaria parasites (Pfaller and Krogstad, 1983).

Therefore, this study was carried out to investigate the effect of Cisplatin complexes such as *Cis*- 2-(1H-imidazol-2-yl)-1H-imidazole Dichloro Platinum (II) and Cis-1-methyl-2-(1-methyl-1H-imidazole-2-yl)-1H-imidazole Dichloro platinum (II) on the *in-vitro* formation of β-hematin.



H_2bim

Fig. 1: Structural formula of 2-(1H-imidazol-2-yl)-1H-imidazole (H_2bim)

MATERIALS AND METHODS

Location and duration of study: This study was carried out at the Biochemical Research laboratory of the Life Sciences Department at Al-Quds University (Jerusalem, Palestine). Preliminary studies, preparation of H_2bim, Me_2bim, the *Cis*-Dichloro Platinum complexes and the *in-vitro* testing of potential drugs, lasted for a period of 10 months.

Preparation of 2-(1H-imidazol-2-yl)-1H-imidazole (2, 2'-Biimidazole) (H_2bim): According to literature (Debus and Liebigs, 1858; Walker *et al.*, 2009). 2, 2'-Biimidazole was the first reported biheterocycle, prepared by Debus, who named it glycosine, by the condensation action of ammonia on glyoxal (Debus and Liebigs, 1858; Katritzky, 1997). A light tan powder of 2, 2'-Biimidazole was produced (Fig. 1). All products were analytical reagent grade obtained from Sigma.

Preparation of *Cis*- 2-(1H-imidazol-2-yl)-1H-imidazole Dichloro Platinum (ll): The Cis-2- (1H-imidazol-2-yl) -1H-imidazole Dichloro Platinum (ll) complex was prepared according to a method described in literature with some modifications, the Cisplatin product was prepared by mixing two solutions of 1N HCl, 10 mL each, the first one containing 1.2 mmol (498.23 mg) of Potassium tetrachloroplatinate (K_2PtCl_4) and the second containing 1.3 mmol (174.38 mg) of 2, 2'-Biimidazole. These were left overnight with stirring at room temperature; the solid product was filtered out, washed with cold ultra pure water, ethanol and ether and then dried in vacuo. IR spectrum of the product υ (O-H) at 3450 cm^{-1}, (secondary amine 3320 cm^{-1}) (3100 3150 C-H of thearomaticring)/cm^{-1}, (υ (C = C) at 1650 and 1500 cm^{-1}) (1380 C-N) (Salas *et al.*, 2001; Casas *et al.*, 2003; Magan *et al.*, 2005).

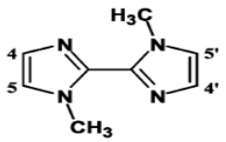


Fig. 2: Structural formula of 1, 1'-Dimethyl- 2, 2'-Biimidazole (Me_2bim)

Preparation of 1,1'-Dimethyl- 2,2'-Biimidazole (Me_2bim): According to literature (Mohanty *et al.*, 1994). The end result: colorless flakes of Me_2bim appeared (Fig. 2). It was filtered and then washed with the minimum amount of hexane and finally air-dried. All products were analytical reagent grade obtained from Sigma.

Preparation of *Cis*-1-methyl-2- (1-methyl-1H-imidazole-2-yl) -1H-imidazole Dichloro Platinum (II): This complex was prepared in a similar way as Cis- 2-(1H-imidazol-2-yl) -1H-imidazole Dichloro Platinum (ll), the Cisplatin product was prepared by mixing two solutions of 1N HCl, 5 mL each, the first containing (18.5 mg) of Potassium tetrachloroplatinate (K_2PtCl_4) and the second containing (7.21 mg) of 1,1'-Dimethyl- 2,2'-Biimidazole. These were stirred together at room temperature until fully dissolved; the liquid solution was used.

Quantitative *in-vitro* method used in testing of potential drugs: According to (Blauer and Akkawi, 1997) , freshly prepared stock solution of hemin chloride was prepared by dissolving the salt in 0.4 N aqueous NaOH and incubated for 30 min at 37ºC stock solution of the Cisplatin complex used was prepared using ultra-pure water. The final concentration of hemin and the Cis-dichloro platinum complexes were 2 and 4 mM respectively, aqueous HCl was also included in order to obtain the required pH (ionic strength was 0.1235 M).

The reaction was equilibrated at 37ºC for 10 min, finally 4 µL of glacial acetic acid (purchased from Fluka) were added under gentle mixing (Blauer and Akkawi, 2000). The whole mixture was left for 2 h at 37ºC without stirring. The total volume of the reaction mixture was 4 mL and the final pH was 4.9 to 5.2.

Samples were centrifuged for 10 min using a serological (Jouan B4) centrifuge. The supernatant was discarded and the precipitate was washed with ultra-pure water and quantitatively transferred to a Millipore Swinnex 13 filter containing Whatman filter paper No. 50, already lyophilized to a constant weight in freeze-drying machine (Labconco Freezone). DMSO was passed slowly through the filter until the filtrate remained feebly colored and washed again with ultra-pure water. The remaining was then lyophilized to a constant weight (Blauer and Akkawi, 1997).

RESULTS AND DISCUSSION

According to method of use, the result of the tested Cis-dichloro platinum complex compared to water (as negative control) and Chloroquine (CQ as positive control) in terms of β-Hematin formation is shown in Fig. 3. Comparison in terms of drug efficiency is seen in Fig. 4.

The transitional metal platinum complex is a typical square-planar compound (Fig. 5), coordinated by two

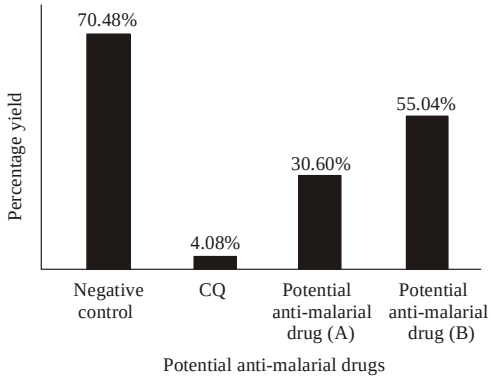


Fig. 3: Column diagram representing the percentage yields of potential anti-malarial drug (A): *Cis*- 2-(1H-imidazol-2-yl)-1H-imidazole Dichloro Platinum (ll) and potential anti-malarial drug (B):*Cis*-1-methyl-2-(1-methyl-1H-imidazole-2-yl)-1H-imidazole Dichloro Platinum (ll) compared to Chloroquine and water at 4mM. Yields are inversely proportional to drugs efficiency, the lower the yield is, the drug is considered to be more efficient

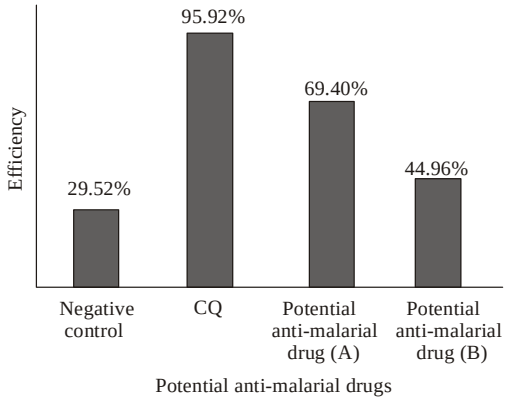


Fig. 4: Column diagram representing the efficiencies of potential anti-malarial drug (A): *Cis*- 2-(1H-imidazol-2-yl)-1H-imidazole Dichloro Platinum (ll) and potential anti-malarial drug (B): *Cis*-1-methyl-2-(1-methyl-1H-imidazole-2-yl)-1H-imidazole dichloro Platinum (ll) compared to Chloroquine, all at a concentration of 4 mM

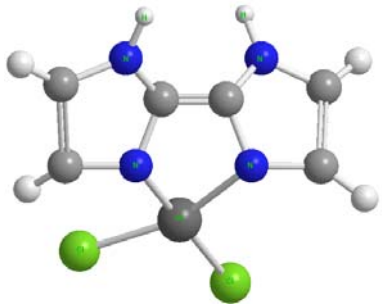


Fig. 5: The proposed structure of *Cis*- 2-(1H-imidazol-2-yl)-1H-imidazole Dichloro Platinum (ll) complex

chloride anions and two N3-bonded H_2bim ligands (Fig. 1) in a *cis* position. The relative orientation of the ligands is head-head, most probably stabilized by the presence of a water molecule, which acts as acceptor of hydrogen bonds of the N4-H groups of both ligands (Salas *et al.,* 2001; Magan *et al.,* 2005).

The anti-malarial property of platinum-containing drugs is attributable to the kinetics of their ligand displacement reactions forming coordination compounds. Studies have shown heterocyclic nitrogen plays an important role in coordination chemistry. Ring-fused heterocycles which contain more than one nitrogen atom are key structures in a large variety of biochemical processes. These ligands exhibit various coordination modes in metal complexes and it is even possible that they can function by forming stable complexes more readily that water and chlorine in catalytic reactions (Ashish and Pandeya, 2011).

Cisplatin is a relatively un-reactive molecule and is believed to remain in its neutral state, as it does not react directly with any of the molecules present in biological systems that will bind to platinum through nitrogen or oxygen donor groups. However in aqueous solutions the chloro-ligand of Cisplatin (as in 2, 2'-Biimidazole) are replaced in a stepwise manner by water to form Pt-OH_2. Pt-OH_2 bond is much more reactive than Pt-Cl, the aqua complexes therefore react readily with N-donor present in the ring of heme forming coordination bonds leading to retardation, which will affect the formation of β-hematin by preventing its polymerization (Berners-Price and Appleton, 2000).

The presence of the two methyl groups of 1,1'-Dimethyl- 2,2'-Biimidazole (Fig. 2) on the N, N' of *Cis*-1-methyl-2-(1-methyl-1H-imidazole-2-yl)-1H-imidazole Dichloro platinum (II) would probably retard the complex from coordinating to nitrogen donor atoms that are present on the heme and this can be clearly noted from the experimental results that were obtained for the dimethyl derivative of the 2, 2'-biimidazole. Also this can be supported by the fact of the in vitro studies that showed the anti-leishmanial activity of the Cisplatin complexes, which revealed that the activity may be due to the nature of the ligand carried in their structure (Salas *et al.*, 2001). This can, at least in part, affect the formation of β-hematin by interrupting and preventing its polymerization.

CONCLUSION

In this attempt of searching for new anti-malarial drugs to eliminate this dreadful disease we found that Cisplatin complexes not only do they have anti-tumor activities, as proposed by others, but also they have the ability to inhibit the formation of β-hematin in *in-vitro* systems, which should be given more attention. This study revealed that *Cis*- 2- (1H-imidazol-2-yl) -1H-imidazole Dichloro Platinum (ll) was more effective against β-hematin formation than *Cis*-1-methyl-2- (1-methyl-1H-imidazole-2-yl)-1H-imidazole dichloro Platinum (ll). It should be illustrated that ongoing research is being carried out in our laboratory to study the toxicity and the *in-vivo* effects of these two compounds to gain a further understanding of their biological action. Results will be published in the near future.

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