THEORETICAL STUDY OF AROMATIC COMPOUNDS WITH INHIBITORY ACTIVITY OF β-HEMATIN FORMATION

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Summary

Malaria is the most important parasitic diseases, affecting almost half of the world. This disease increases in the population of Colombia each year. 5 aromatic compounds were sinthetized, they were evaluated as inhibitor of β-hematin formation and we reported the theoretical study of the compounds-hematin aggregated in order to determine how the interaction drug-receptor could be established. Theoretical study using Gaussian 03 shows the interaction between carbonyl oxygen and Fe in the porphyrin ring of hemin. The modification of funtional groups in the compounds changes the inhibition of compounds-hemin aggregated. The geometrical parameters of the complexes are relationed with the inhibitory activity.

Keyword: *P. falciparum,* theoretical study, β-hematin, electronic profile

More than 5 million people live in endemic areas in Colombia, for example, the Pacífic and Atlantic Coast, San Andrés island, East LLanos, Amazon, Orinoquia, Urabá, Bajo Cauca, Middle Magdalena, North Santander, Casanare, Bolívar, Cundinamarca, Caquetá and Tolima.(1,2). Actually, the mechanism of inhibit hemozoin formation remains as an attractive target for the design of new antimalarial drugs(3).

Methods

Computational Details: The geometric parameters and energy of the compounds were optimized using Gaussian 03 software with B3LYP functional and the LANL2DZ basis set.

General procedure. 5 compounds were synthetized following the Brian et al procedure (4): 1-(4-chlorophenyl)-3-phenylprop-2-en-1-one (**1**), 1-(4-chlorophenyl)-3-[4-(dimethylamino)phenyl]prop-2-en-1-one (**2**), 4-(4-chlorophenyl)-4-oxo-2-phenylbutanoic acid (**3**), 1-(4-methylphenyl)-3-pyridin-4-yl-prop-2-en-1-one (**4**), 1-(4-chloro-phenyl)-3-pyridin-4-yl-prop-2-en-1-one (**5**) and chloroquine which was obtained commercially.

1-(4-chloro-phenyl)-3-phenyl-2-propenone (1, 2, 4 and 5). 4-Chloro (or 4-methyl)-acetophenone (5.0 g. 32.34 mmol) and Benzaldehyde (or p-$(CH_3)_2N$-C_6H_4) (3.43 g. 32.34 mmol) were added to NaOH (1.43 g. 35.6 mmol) disolved in water (15 ml) and ethanol (5 ml). The mixture was stirred for 2 hr. at 25°C, kept in the refrigerador overnight, filtered, washed with water:metanol (1:1) and dried. (**1**): (7.8 g, 32.34 mmol, 99%, Tf= 96 °C). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.42-7.48 ppm (m, 5H), δ 7.99 ppm (d, 1H), δ 7.66 ppm (d, 1H), δ 7.5-7.65 ppm (dd, 4H). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 122.02, δ 128.9, 129.39, 129.44, 130.34, 131.16, δ 145.79.

4-(4-chloro-phenyl)-4-oxo-2-phenyl-butyric acid (3). (**1**) (500 mg. 2.1 mmol) and NaCN (2.7 g. 54.4 mmol) were disolved in acetone:water (2:3), the mixture was acidified with H_2SO_4 0.1 M at pH 4-5, stirred for 5 days at room temperature, filtered, washed with brine and dried. The compound formed in the previous step (2.7 g. 10.6 mmol) and KOH (1.8 g. 31.7 mmol) were disolved in water (300 ml), heated under reflux for 16 hr, diluted, acidified at pH 2, and purified with Hex:AcOEt (1:1) (2.5 g, 8.7 mmol, 82%, Tf= 106 °C). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.25 ppm (dd, 1H), δ 3.9 ppm (dd, 1H), δ 4.3 ppm (m, 1H), δ 7.2-7.4 ppm (m, H), δ 7.9 ppm (d, H), δ 9.8 ppm (s, OH). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 42.7 ppm, 46.7 ppm, 127.9-129.9 ppm, 135.0 ppm., 138.0 ppm, 140.3 ppm, 178.9 ppm, 196.8 ppm.

β-hematin Inhibitory activity: the compunds were evaluated using the method described by Deharo et al (5-8). Hemin (100 μl. 6.5 mM) was disolved in NaOH (0.2 N), acetic acid glacial (50 μl, 17.4 M), and compound or chloroquine to different concentrations (50 μl, 0.5-2.0

mg/ml) in acetate buffer pH 4.0 (200 µl. 3.0 M). The mixture was incubated for 1 hr at 60 °C, centrifuged at 9250 rpm for 25 min, and washed with DMSO (400 µl) and the pellet dissolved in NaOH (600µl. 0.1N). This sample was read at 386 nm in a spectrophotometer.

Antimalarial testing *in vitro*: The compounds **1-3** were evaluated as antimalarial, and based on the Carmona et al method (8,9). The *P. falciparum* (strain NF54) parasites were cultured continuously. The 50% effective concentration (EC_{50}) of the compounds was calculated according to the method of Deharo et al (5-8). EC_{50} value was the result of at least three separate experiments in triplicate.

Results

Values of β-hematin inhibitory and biological activity *in vitro* of the aromatic compounds are given in table 1.

Table 1-β-haematin inhibition, EC_{50} against *P. falciparum* parasite and Compound-hemin complex energy.

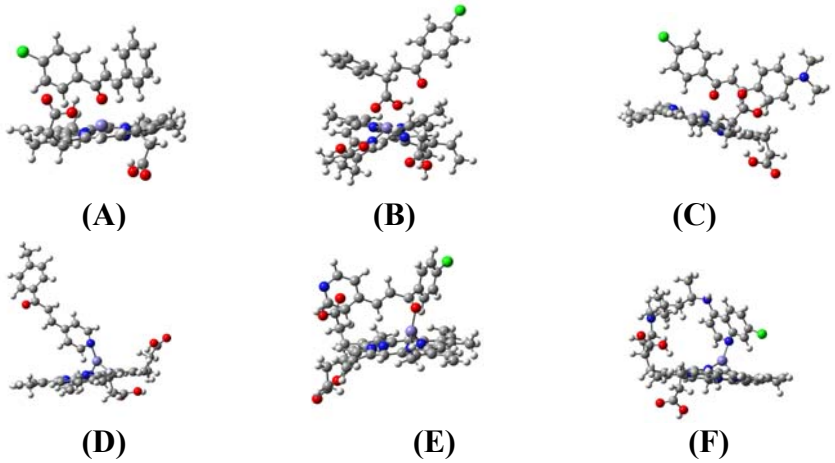
Compounds	Inhibitory Concentration IC_{50} (mg/ml)	β-hematin Inhibition (%)	EC_{50} against *P. falciparum* (µg/ml)	Energy (hartrees)
(**1**)	0.5	72.0	1.51	-2618.27
(**2**)	0.5	7.0	3.10	-2750.98
(**3**)	1.0	17.0	5970.00	-2815.42
(**4**)	2.0	52.0	---	-2657.08
(**5**)	2.0	76.0	---	-2631.79
chloroquine	1.8	50.0	1.30	-2869.86

The complexes more active with the highest bond energy were the compunds (**1**), (**4**) and (**5**), therefore the reaction between these compounds and hemin is more favourable, this results are correlated to the inhibitory activity of the compounds.

The molecular modelling of the compounds suggests interaction between Fe center in hemin and carbonyl oxygen of the compounds (**1**), (**2**), (**3**) and (**5**), C1=O---Fe, the information of the figure 1 supports the structures of the aggregated. The compound (**3**) is bounded with Fe center through of the carboxylic oxygen and the distance between hydroxyl group of carboxylic group and Fe center, O=C1-OH---Fe, is higher than the distance

between carbonyl oxygen and Fe center, C4=O---Fe. This result shows the preference of Fe of the porphyrin ring to C=O group. The change in binding group of the compound (**3**) with the hemin influences inhibitory activity.

Figure 1: Optimized compounds-hemin complexes. **(A)** Compound(**1**)-hemin, **(B)** Compound(**2**)-hemin, **(C)** Compound(**3**)-hemin, **(D)** Compound(**4**)-hemin**, (E)** Compound(**5**)-hemin, **(F)** Chloroquine-hemin. N= dark blue, Fe= violet, O= red, C= gray, H= white.



The compound (**5**) establishes interaction between carbonyl oxygen group and Fe center of porphyrin ring of hemin. When the Cl (electron-withdrawing group) was substituted for the CH_3 (electron-donating group), the interaction was stablished between the pyridinyl N and Fe. This change is because of the center of the most negative potential lies in the pyridinyl N when the electron-donating group is at 1-(p-methylphenyl) position of the molecule. In accordance with Mulliken charges the pyridinyl N is charged more negatively than the carbonyl oxygen in the compound (**4**), because of inductive effect of CH_3 group. On the other hand the electron-withdrawing group reinforces a negative potential smaller on the phenyl ring than on the pyridinyl ring, doing the oxygen more succeptible to electophilic attack. This shows that the compounds with an electron-withdrawing group in the phenyl ring locates a strong negative potential on the pyridinyl ring which by inductive effect does the oxygen atom more succeptible to electrophilic attack. This change of electrostatic potential between the atoms of this compounds exhibits a strong influence in inhibitory activity of the compounds over the hematin aggregation process.

Discussion

According to the theoretical study there are interactions between Fe center porphyrin ring and the oxygen atom of C1 of the compounds, C1=O---Fe, in order to form the complexes, this interaction is important to β-hematin inhibition activity of the compounds. The aromatic ring of the compounds (**1**), (**2**), (**3**) and (**5**) are interacting perpendicularly through C=O with the π-π system of porphyrin Fe center of hemin.

In accordance with the electronic features of the compounds (**4**) and (**5**) have resulted the following aspect: (a) the electron-withdrawing groups in p-phenyl position could contribute to increase inhibitory activity of the compounds. (b) the electron-donating groups in p-phenyl position change the place of electrophilic attack in the compounds and this change modifies the inhibitory activity of the compounds (**4**) and (**5**). (c) the electron-withdrawing groups form an area with a strong negative potential on the pyridinyl ring doing the carbonyl oxygen of the compound (**5**) in a succeptible place to electrophilic attack. (d) the compounds with inhibitory activity of β-hematin formation should have a carbonyl oxygen together with the points refered above which do the carbonyl group more succeptible of electrophilic attack.

We conclude that the electronic features could control the inhibitory activity of β-hematin formation, and it is a point of view to determine the antimalarial activity. This part of the theoretical study corroborates that our molecular modelling and the biological evaluation results were carried out succesfully and therefore are valid, also the theoretical study is an important tool to design new compounds with antimalarial activity.

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References

1. Poveda G, Rojas W, Quiñones ML, et al. Coupling between Annual and ENSO Timescales in the Malaria–Climate Association in Colombia. Environmental Health Perspectives 2001; 109 (5):489-493.
2. Olano VA, Brochero HL, Sáenz R, et al. Mapas preliminares de la distribución de especies de *Anopheles* vectores de malaria en Colombia. Biomédica 2001; 21: 402-408.
3. Egan TJ. Structure-Function Relationships in Chloroquine and Related 4-Aminoquinoline Antimalarials. Drug Desing Review 2004; 1: 93-110.
4. Brian SF, Hannaford AJ, Smith PW, Tatchell Austin R. . Vogel's Textbook of Practical Organic Chemistry. 5th edition. 1989, 1034.
5. Egan TJ, Hunter R, Kaschula CH, et al. Structure-Function Relationships in Aminoquinolines: Effect of Amino and Chloro Groups on Quinoline-Hematin Complex Formation, Inhibition of β-Hematin Formation, and Antiplasmodial Activity. J. Med. Chem 2000; (43):283-291.
6. Deharo E, Gautret Ph., Muñoz V, Sauvain M. Técnicas de laboratorio para la selección de sustancias antimaláricas. 1 ed. Ed. CYTED-IRD. La Paz, Bolivia 2000: 19-20, 72, 84-88.
7. Muñoz V, Sauvain M, Bourdy G, et al. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach: Part I. Evaluation of the antimalarial activity of plants used by the Chacobo Indians. Journal of Ethnopharmacology 2000; 69: 127-137.
8. Baelmns R., Deharo E, Bourdy G, et al. A search for natural bioactive compounds in Bolivia through a multidisciplinary approach: Part IV. Is a new haem polymerisation inhibition test pertinent for the detection of antimalarial natural products?. Journal of Ethnopharmacology 2000; 73: 271-275.
9. Carmona D, Sáez J, Granados H, et al. Antiprotozoal 6-Substituted-5,6-Dihydro-a-Pyrones from Raimondia CF. Monoica. Natural Product Research 2003:17(4):275-280.