In vitro antimalarial and xanthine oxidase inhibition of 2-Aminoanthraquinone

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Abstract: In the present research study 2-Aminoanthraquinone were scrutinized for their antimalarial and Xanthine oxidase inhibitor potential. It demonstrated marked concentration dependent antimalarial activity with maximum effect of 89.06% and with IC_{50} of 34.17 µM. Regarding Xanthine oxidase inhibitor activity, it evoked significant effect with 57.45% activity with IC_{50} value of 81.57.19µM. In conclusion, 2-Aminoanthraquinone showed potent antimalarial and xanthine oxidase inhibitory activity.

Keywords: 2-Aminoanthraquinone, anti-malarial and xanthine oxidase.

INTRODUCTION

2-Aminoanthraquinone is a synthetic compound which was first made on large scale in the United States in 1921 (Monogr *et al*., 1982). It is usually employed as an intermediate in the synthesis of anthraquinone dyes. These dyes are used in high quality paints and enamels, plastics, rubber, printing inks, and in textile dyeing (Gosselin *et al*., 1984; Lewis., 2000). The human exposure to 2-aminoanthraquinone may occur in working places like industries. Studies have been shown the incidences of bladder cancer in those working in the dye manufacturing industry (Wynder *et al*., 1963; Anthony *et al*., 1970). In most of the studies, the 2-aminoanthraquinone had a melting point from 255-292^{o}C, normally decomposed at 292^{o}C. The deviation from the determined melting point ranges from those reported in the literature (303-306^{o}C) suggested that the chemicals were either of very low purity or that decomposition occurred before the melting point was reached. The UV analysis showed the presence of some impurities that might be responsible that decomposition (Doi *et al*., 2005).

Malaria is one of devastating infectious diseases of the world especially in third world nations (Khan *et al*., 2012). *Plasmodium falciparum* is a protozoan parasite, which is the most virulent form of malaria and affected a large population of the world. In endemic areas, malaria accounts for nearly one million deaths, primarily among children under the age of five. While considering the emergence of resistance to antimalarial drugs, the researchers are interested in the investigation of new effect compounds to cope with it. The antimalarial activity of aminoanthraquinone derivatives is already reported (Nor *et al*., 2013). Therefore, the purpose of present research work was to evaluate 2-Aminoanthraquinone for its antimalarial and xanthine oxidase inhibition in well-established *in-vitro* protocols.

MATERIALS AND METHODS

Synthesis

Starting from the phthalic anhydride and bromobenzene, 2-aminoanthraquinone (fig. 1) was synthesized through multi-step synthesis according to reported procedure (scheme 1) and was identified by comparing their physical (m.p 301-302^{o}C) and spectral data with the reported data (Groggins *et al*., 1931; Gounda *et al*., 2010).

Anti-malarial activity

The compounds were tested for their anti-malarial potential using well recommended methods. To sterile 96 well plate 100µl of CCM was added by multichannel pipette in each well (from 1-12 and from A to H). 100µl of sample (1mg/ml) was added in triplicate in well A1-A3, Sample 2 from A4-A6 and standard drug Chloroquine from A7 to A9 and A10-A12 total 14 dilutions (stock will be of 1µg/ml). Serial two-fold dilution from A1 to F1, A2 to F2 and so on (total seven dilutions) was then made. Infected RBCs solution was added with 2% parasitemia and 1% hematocrit. The total volume became 200µl in each well and Parasitemia will be 1% and 0.5% hematocrit. In last row, well H1 to H6 contained infected RBCs and H7-H12 have non-infected RBCs. Plate was incubated in candle jar with 5% CO_2 at 37ºC for 72h. After 72h, plate was placed in freezer for complete lysis

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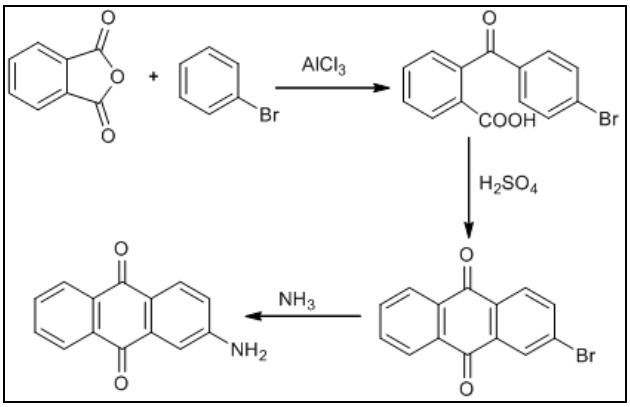
of cell for 24 hours and then after 24 hour Malstat reaction was done (Krugliak *et al*., 2000). After lysis of cell, the plate was taken out from freezer and placed in water bath at 37ºC for 1h. Malstat solution (100µl) was then added in each well of 96 well plates. ELISA plate reader was used at 650 nm. 20µl from each well of plate 1 was added to respective well of Malstat plate and then placed in shaking water bath at 37ºC for 30 min. Solution of NBT (2mg/ml) and PES (0.1mg/ml) in ratio 1:1 and 25µl of this solution were added to each well and the plate was placed in dark to complete the reaction. The plate was read at 650 nm and OD was noted. LD_{50} was calculated by EZfit computer program.



Scheme 1: Synthesis of 2-aminoanthraquinone

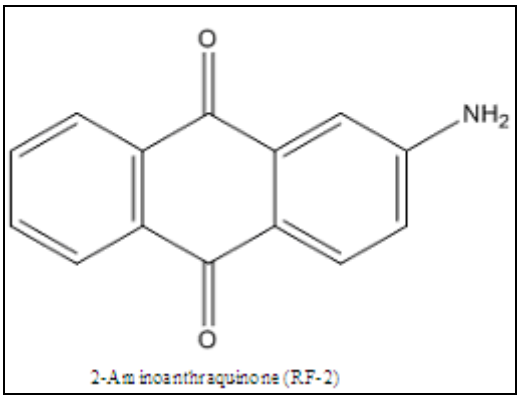


Fig. 1: Structure of 2-Aminoanthraquinone

Xanthine oxidase inhibitory assay

The xanthine oxidase activity with xanthine as the substrate was measured spectrophotometrically using the procedure of Owens and Johns (1999) with the following modifications (Owen *et al*., 1999). The positive control, allopurinol solution, was prepared by dissolving 5.0mg of allopurinol in 5.0ml of 0.15M phosphate buffer (pH 7.5). Xanthine oxidase from bovine milk was purchased from Sigma (×4500). The enzyme solution was prepared by diluting 30µl of a 5.0 U/0.2ml xanthine oxidase solution to a final volume of 3.0ml. The substrate solution was prepared by addition of 5 drops of 1.0M NaOH to 22.7 mg of xanthine to aid its dissolution with deionized water to a final volume of 250ml. The plant extracts were dissolved in 1% dimethyl sulfoxide (DMSO) to a final concentration of 1mg/ml. All solutions were prepared immediately before use.

Total volume of the assay mixture is 3.4ml and consists of the plant extract under study (apportioned concentrations of 200, 100 and 75µg/ml), 0.15 M phosphate buffer (pH 7.5) and 100µl of 0.03 U/ml xanthine oxidase enzyme solution. After preincubation of the test solution at 25°C for 10min, the reaction was initiated by addition of 1 ml of 0.6mM substrate solution of xanthine, mixed thoroughly, and monitored through absorption increments read every 30s for 10min at 295nm indicating the formation of uric acid using a Shimadzu UV-1700 series spectrophotometer. Allopurinol was used at a final concentration of 30µg/ml in the assay mixture. The percent xanthine oxidase inhibitory activity of the assayed samples was determined through the slope of the plot of absorbance against time (seconds). IC_{50} values were obtained through linear regression analysis the plot of concentration (200, 100, 25µg/mL) against percent inhibition.

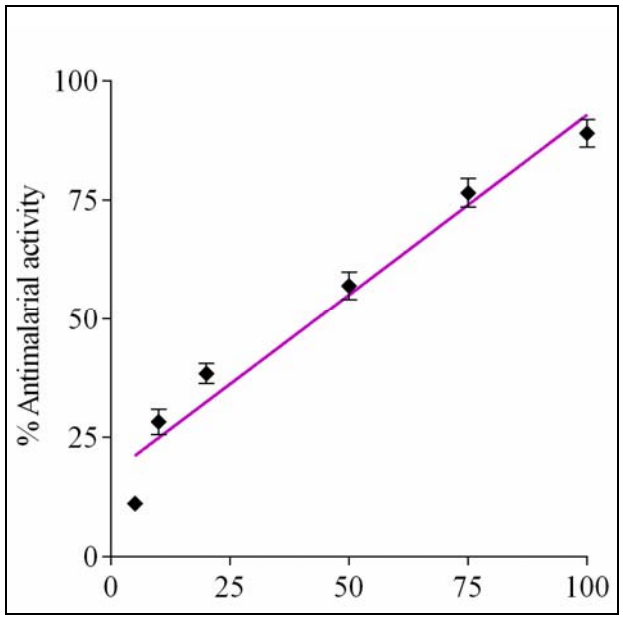


Fig. 2: Percent antimalarial activity of 2-Aminoanthraquinone. Values are expressed as mean ± SEM of three separate experiments.

RESULTS

Effect of antimalarial activity

The results of our anti-malarial test demonstrated that 2-Aminoanthraquinone has marked potential to compete against malarial parasites in a concentration dependent manner (fig. 2). It provoked the maximum inhibition of 89.06% with IC_{50} of 34.17µM as depicted in table 1.

Effect of xanthenes oxidase inhibition

The xanthenes oxidase inhibitor activity of 2-Aminoanthraquinone is presented fig. 3. It provoked

concentration dependent attenuation of the enzyme activity and caused maximum inhibition of 57.45% IC_{50} value of 81.57.19µM (table 2) in comparison to standard drug (Allupurinol, IC_{50}= 0.59±0.01µM).

DISCUSSION

Like other developing countries a large number of malarial cases are reporting annually in Pakistan (Khan *et al*., 2012), the history of malarial treatment is a strong evidence for the contribution of plants in human health. The malaria was treated first time through cinchona bark, which later proved that the antimalarial potential of cinchona bark was attributed to the presence of quinines (Meshnick *et al*., 2001). Similarly the well-known anti-malarial drug artemisinin is also plant based chemical moiety (Klayman *et al*., 1985). Keeping in view the role of aminoanthraquinone derivatives as antimalarial (Osman *et al*., 2010), we investigated 2-Aminoanthra-quinone for antimalarial activity, which showed marked activity.

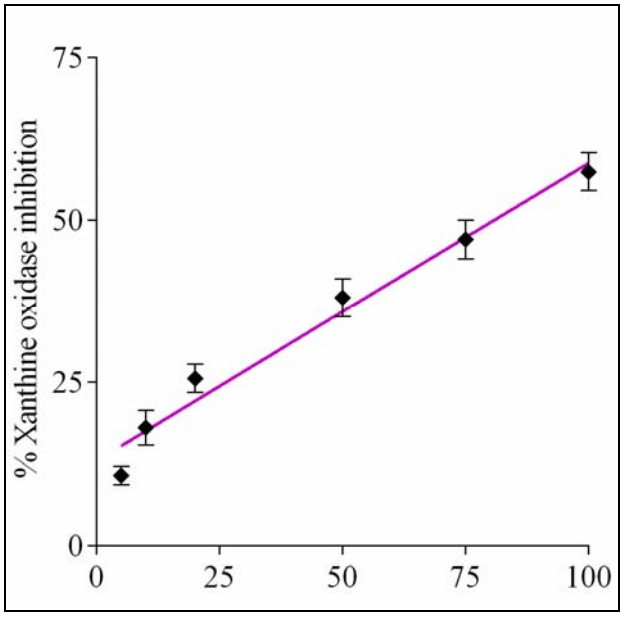


Fig. 3: Percent xanthine oxidase inhibitory profile of 2-Aminoanthraquinone. Values are expressed as mean ± SEM of three separate experiments.

Table 1: Antimalarial activity of 2-Aminoanthraquinone

Compound	IC_{50} (µM)
RF-2	34.17±0.21
Chloroquine diphosphate	0.11±0.07
Control	0.00

Table 2: Xanthine oxidase inhibitory profile of RF-2

Compound	IC_{50} (µM)
RF-2	81.57.19±0.02
Allopurinol	0.59±0.01
Control	0.00

The xanthine oxidase (XO) inhibitors such as Allupurinol have been used in the clinical management of gout and related conditions with hyperuricemia. Studies also showed XO plays an important role in various forms of ischemic and other types of tissue and vascular injuries, inflammatory diseases, and chronic heart failure (Pacher *et al*., 2006). It provoked concentration dependent attenuation of the enzyme activity.

In conclusion, 2-aminoanthraquinone exhibited marked antimalarial and xanthine oxidase inhibitory potentials. The study provided a sound foundation for medicinal chemist to synthesis such type of compounds (derivatives) in the hope of finding new and effective therapeutic agents.

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