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Synthesis, Molecular Docking Analysis and In-Vitro Evaluation of 1, 4-Dihydroxyanthraquinone Derivatives as Anti-Trypanosomal Agents

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Abstract

Hydroxyl substituted anthraquinones are among the most important derivatives in organic synthesis. The attractive biological properties of these compounds are relevant to many therapeutic areas that are of use in clinical applications. In this present work, several amino-substituted anthraquinones were synthesized from 1,4-dihydroxyanthraquinone using a modified Marschalk reaction. Moreover, 1, 4, 5-trihydroxyanthraquinone was synthesized from anacardic acid, an agro-waste from the cashew industry. The in vitro screening of the compounds against Trypanosoma brucei parasites revealed noteworthy activity with reasonable selectivity against a human cell lines. A molecular docking study was performed to analyze the modes of interaction of the synthesized compounds to the active site of trypanothione reductase. The docked poses were examined by visual inspections, and test compounds displayed good binding affinity with the receptor protein. This in-vitro/ molecular docking evaluation suggest that substituted 1, 4-dihydroxyanthraquinone derivative can be promising starting structures in the search for active drugs against trypanosomiasis.

Keywords: 1, 4-dihydroxyanthraquinone; Anacardium occidentale; Anacardic acid; Trypanosoma brucei; Molecular docking

Introduction

Trypanosomatids are digenetic protozoan parasites that cause debilitating diseases in certain regions of tropical and subtropical countries [1]. Trypanosomatid parasites particularly African trypanosomes (Trypanosoma brucei sp.) cause serious mortality and morbidity in humans, livestock and wildlife leading to severe economic impacts in the developing world [2]. Trypanosomatid pathogens of humans are Trypanosoma cruzi, the causative agent for Chagas disease, and T. b. Gambiense and T. b. Rhodesians, causing African trypanosomiasis [3].

The world health organization (WHO) regards human African trypanosomiasis (HAT or sleeping sickness) and Chagas disease (South American trypanosomiasis) as "Neglected Tropical Diseases" (NTDs) [4].

The current clinically approved drugs such as Pentamidine, suramin, eflornithine, and melarsoprol (Figure 1) for treating HAT are far from satisfactory [5]. Pentamidine and suramin which are the proposed options for the first stage of HAT often have high toxicity effects with complex routes of administration which limit their medical applications [6, 7]. In addition this, lack of resources to cover the cost of materials needed for the intravenous administration which limits the implementation of this when the target group are the rural and poor societies [8]. Treatment for the second stage HAT has mainly been restricted on the use of melarsoprol, a toxic arsenic derivative and a combination of nifurtimox-eflornithine [9]. The use of these as the only preferred drugs for 2nd stage HAT normally suffer from problems of drug resistance; melarsoprol drug-resistant parasites were common in patients treated with this drug [8, 10].

An alarming increase in toxicity effects and in drug resistant call for the need for search for new chemotherapies effective against HAT inevitable [3]. Fexinidazole a 2-substiuted 5-nitroimidazole derivative **(Figure 1)** is a new drug, developed by the Drugs for Neglected Disease initiative (DNDi) in collaboration with Sanofi that could be safe effective oral treatment curing both acute (1st Stage) and chronic HAT (2nd Stage) [11, 12]. Despite this progress new target based and phenotypic screening based approaches to be still needed to deliver new development candidates for treating HAT [9].

Target based approaches have demonstrated to be a key strategy for drug discovery [13]. For neglected tropical diseases there has been limited success due to very few fully validated drug targets [14]. Trypanothione reductase (TR) is a genetically validated drug target enzyme of the unique Trypanothione-based thiol metabolism of Trypanosomatidae [15]. TR is a key enzyme for the parasite antioxidant defense and does not occur in mammalian hosts [16]. TR is an essential enzyme in the catalysis of the NADPH–dependent reduction of Trypanothione disulphide (T [S] 2) to Di thiol Trypanothione bis (glutathionyl) spermidine (T [SH] 2) [17]. Genetic approaches have validated TR to be essential for the proliferation of trypanosomatids [17]. The inhibition of TR is one of the strategies towards developing drugs against trypanosomatids [17].

Natural products provide attractive new chemical libraries and inspirations for development of new drugs and various industrial chemicals [18]. Anthraquinones are bioactive natural products occurring in many plants, microorganisms, insects and marine animals [19]. A large number of natural anthraquinones and their derivative

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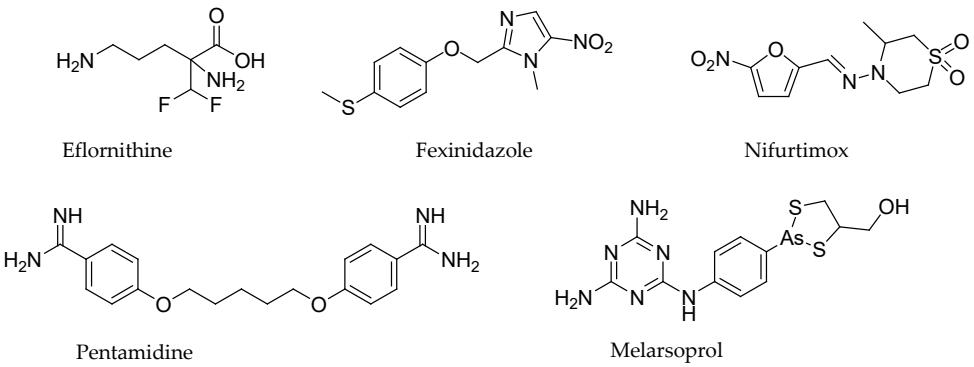


Figure 1: Some common drugs used to treat human African trypanosomiasis.

display a wide range of biological activities which include antibacterial [20], anticancer [21], antifilarial [22] and antileishmanial [23].

These interesting biological activities have in recent days raised the attention of many re-searchers from fields such as pharmaceutical and food industry and hence can be considered as target in organic synthesis [24, 25].

Many anthracycline antibiotics which are drugs used in the treatment of different types human cancer (Figure 2) have a 1, 4-dihydroxyanthraquine skeleton in their structure [26]. This core feature has caused 1, 4-dihydroxyanthraquinone to be used as a building block for the constructions of various antiparasitic drugs.

The by-product of cashew (Anacardium occidentale) agribusiness called cashew nut shell liquid is a unique source of organic compounds with potential antimicrobial and antiparasitic activities [27, 28]. CNSL contain unique unsaturated long-chain phenols that are considered as an abundant low cost starting material in organic synthesis [29].

CNSL can be natural or technical depending on the methods of extractions as sol-vent extraction or heat extraction [29]. Natural CNSL contains a mixture of anacardic acid (60–65%), cardol (15–20%), cardanol (10%) and traces of methyl cardol whilst technical CNSL obtained by roasting shells contains mainly cardanol (60–65%), cardol (15–20%), polymeric material (10%), and traces of methyl cardol [30]. In the work being reported here in, CNSL was employed as the raw material for the synthesis of compound 6 from which hydroxyanthraquinone 9 was derived.

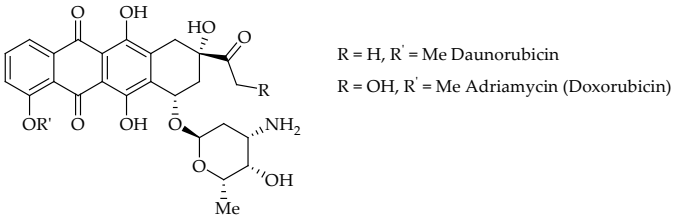
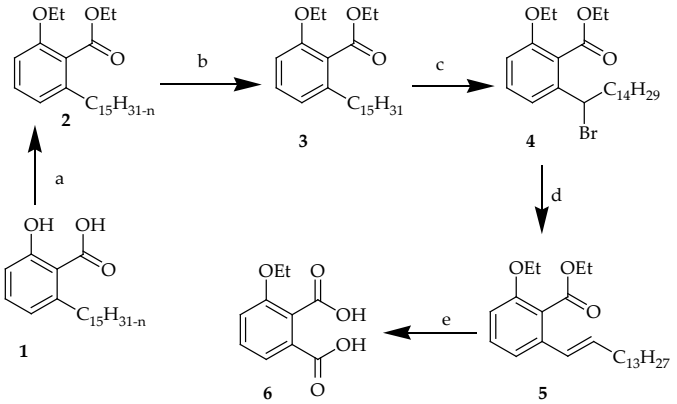


Figure 2: Anthracycline anticancer drugs daunorubicin and Adriamycin.



Scheme 1: Synthesis of compound 3-ethoxy phthalic acid 6. (a) DES, K2CO3, acetonitrile, 90 ℃, 66% (b) Pd/C, H2, methanol 96% (c) NBS, BPO, CCl4, 90 ℃, 91% (d) DBU, toluene, 110 ℃, 87% (e) FeCl3.6H2O, aq 70% TBHP, NaoH, H2O, 80℃, 64%.

Results

Chemistry

The present work reports on the synthesis of 1,4-dihydroxyanthraquinone derivatives, starting first with anacardic acid 1, the later obtained by extraction from CNS. The chemical structure of anacardic 1 (Scheme 1), contains a carboxylic acid functionality and a pentadecyl side chain Ortho to each other making it possible to structurally modify to yield 3-ethoxy phthalic acid 6.

The 3-ethoxy phthalic acid 6 on the other hand is regarded as a synthon in preparation of pharmaceutically important hydroxyanthraquinone including anticancer drugs such as Adriamycin and daunomycin **(Figure 2).** However many of the reported methods used in the preparation of this reagent suffers from the disadvantages of multiple steps, low yields and the use of expensive chemicals [31].

Thus, in this study the synthesis of 3-ethoxyphthalic acid 6, from the locally available CNSL had been accomplished. Moreover, the chemicals used in this transformation are readily available and cheap. Anacardic acid 1 **(Scheme 1)** was ethoxylated using diethyl sulphate and the double bond reduced to give the saturated ester [3].

This was further brominated at the benzylic position using N-bromosuccinamide (NBS) and a catalytic amount of benzoyl peroxide (BPO), which was prepared from benzoyl chloride [32]. The dehydrobromination of bromide 4 afforded the alkene 5, sub-sequent oxidation of this alkene using Tertbutyl Hydro Peroxide (TBHP) afforded the 3-ethoxy phthalic acid 6 in 64% yields [33], the acid was used as the precursor in the synthesis of hydroxyanthraquinone 9. The chemical structures of the synthesized com-pounds were confirmed using spectroscopic methods (NMR, IR and MS).The coupling constant (J) of alkene 5 was found to be 15.7 Hz which is characteristic to Tran's configuration [34]. The 1H NMR spectrum of carboxylic 6, showed the disappearance of the pentadecyl side chain while the 13C-NMR

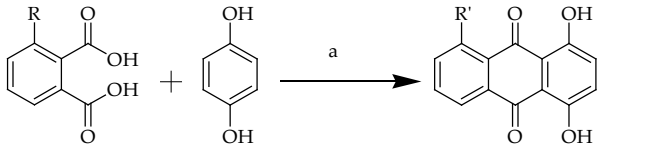
displayed two signals at chemical shift 167.1 and 168.3 ppm indicating the presence of two carboxylic acid groups [35].

The Friedel-Crafts acylation has been used by several scholars, in the synthesis of the Anthraquinone core nucleus [36, 37]. The 3-ethoxyphthalic acid 6 from anacardic acid 1 and the commercially available phthalic acid 7 were each reacted with 1,4-dihydroxybenzene 8 using a eutectic mixture of AlCl3 and NaCl to form the 1,4,5-trihydroxyanthraquinone 9 and 1,4-dihydroxyanthraquinone 10 [38] and (Scheme 2).

The 1,4,5-trihydroxyanthraquinone 9 presented two spots lying almost over one another on TLC, posing difficulty in purification. The reason for this was due to lack of regio-chemical control in the intermolecular Friedel-Crafts reaction which is the result of a Hayashi rearrangement [39].

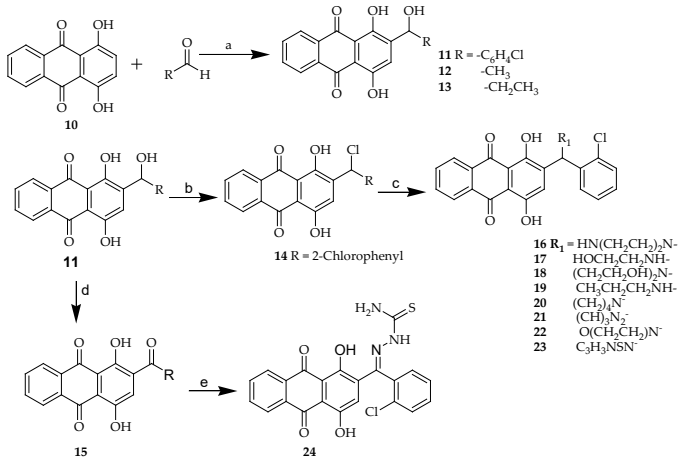
A modified Marschalk reaction was employed to introduce the hydroxyalkyl groups at position 2 of quinizarin 10 using sodium dithionite under basic conditions. To achieve the alcohol anthraquinones 11-13, aldehydes such as 2-chlorobenzaldehyde, acetaldehyde, and propionaldehyde were used (Scheme 3).

The introduction of alcohol functionality to the Anthraquinone structure offers a plausible link for structural elaborations to diverse functional groups. The course of the reaction was monitored by using TLC, and it was found that 5.2 eq of aliphatic aldehyde was needed to react with quinizarin. To avoid the decomposition of these products the reaction time was limited two hours. Additionally, a 1.5 eq of 3% aqueous H_2O_2 was required for the re-oxidation of the resultant hydroquinone to Quinone [40]. The reaction of 2-chlorobenzaldehyde with 1,4-dihydroanthraquinone afforded aryl hydroxyanthraquinone



6 R = -OCH_2CH_3, **7** R =. H **8** **9** R' = OH, **10** R' = H

Scheme 2: Friedel-Crafts acylation synthesis of hydroxyanthraquinone 9 and 10 (a) NaCl/AlCl3, 180.



Scheme 3: A modified Marschalk reaction and some elaborations (a) NaoH, Na2S2O4, aldehyde, methanol, 0°C, H2O2/HCl, 14-75 % 2-4 h (b) SOCl2, 50°C, 51% (c) amine, dichloromethane, 22-80%, 1-24 h (d) Dess-Martin periodinane/ dichloromethane, 99% (e) Thiosemicarbazide, methanol/H2SO4, 90°C.

11 at a yield of 75% which on chlorination and subsequent amination produced several Anthraquinone amines 16-23 in yields varying from 22-80%. Subjecting aryl hydroxyanthraquinone 11 to Dess-Martin oxidation furnished carbonyl 15, which was further treated with thiosemicarbizide to form Anthraquinone thiosemicarbazone 24. The 13C NMR spectra of compound 15 and 24 exhibited the chemical shift value at shift 192.2 and 178.6 ppm for the carbon of carbonyl (C=O) and thioamide –C=S groups respectively [41, 42]. The mass spectrum of each of the newly synthesized compounds was consistent with their molecular formula.

Biological evaluation

Seventeen (17) synthesized compounds were in vitro evaluated for their inhibitory activity against Trypanosoma brucei (T.b brucei) (427 Lister) parasites, using Pentamidine as a standard. The results were expressed as the % parasite viability of the treated cells against untreated cells at the initial screening concentration of 20 μM.

Test compounds that showed % viability below 20% were considered as active and were put forward to determine their IC50 (50% inhibitory concentration) values. Evaluation of activity for the hydroxyl alkyl Anthraquinone 11-13 reveals that the presence of an aliphatic group in 12 and 13, is important for their potency against T. b. brucei (IC50 4.73 and 3.61 μM) Table 1. This data suggest that presence of an aliphatic alkyl group is a key factor for the anti-infective properties of hydroxyanthraquinone [43, 44].

The activity improved when substituents such as chloride, aliphatic amines and heterocyclic were introduced to hydroxyalkyl Anthraquinone 11. Amino substituted anthraquinones derivative 17, 18 and 23 were observed to show almost the same pattern of activity against trypanosomes with IC50 values of 1.14, 1.10 and 1.20 μM, respectively. Compounds 17 and 18 have similarity in their chemical structure in terms of the groups attached to it whether is an ethanolamine or diethanolamine which is an essential structural unit for diverse pharmacologic effects [45, 46]. Test compounds 2 and 6 which were derived from anacardic acid were evaluated for their inhibitory activity against trypanosomes. Test compound 2 which is an

Table 1: Antitrypanosomal activities of the test compound against T.b brucei parasites.

Compound	*T.b brucei*		Cytotoxicity
	IC_{50}/μM	Docking score (Kcal/mol)	% Viability (125 μM)
2	3.10 ± 0.01	-5.73	72.34
6	NA	-7.59	109.06
9	NA	-7.14	59.23
11	NA	-7.21	112.00
12	4.73 ± 0.36	-7.04	15.98
13	3.61 ± 0.07	-7.28	95.14
14	2.39 ± 0.12	-6.46	95.20
15	0.98 ± 0.01	-5.40	79.90
16	3.23 ± 0.23	-7.25	15.56
17	1.14 ± 0.08	-6.65	75.43
18	1.10 ± 0.10	-7.85	15.98
19	2.59 ± 0.10	-6.71	85.99
20	NA	-6.42	99.67
21	NA	-7.41	83.37
22	NA	-5.28	85.48
23	1.2 ± 0.01	-7.35	55.11
24	0.72 ± 0.02	-5.47	14.62
Pentamidine	0.01	-4.12	-

ester of anacardic displayed inhibitory activity against trypanosomes with an IC50 of 3.10 μM, while 3-ethoxy phthalic acid 6 was found to be inactive against T. b. brucei. Test compounds 15 and 24 were found to be the most active against T. b. brucei with IC50 0.98 and 0.72 μM.

In this present work we evaluated the activity of a series 1,4-dihydroxyanthraquinone derivatives against T.b. brucei (strain 427 Lister) using a dose-response procedure. The results revealed that 1,4-dihydroxyanthraquinone derivatives bearing chloride, aliphatic amine, and nitrogen containing heterocycles such as piperazine and thiazole substituents at the alkyl group are potent against trypanosomes. The findings from this study have further demonstrated on the inhibitory activity against T.b brucei of 2-ethoxybenzoate 2 which is an ester of anacardic acid obtained from CNSL.

The cytotoxicity evaluation of the synthesized compounds was done on HeLa cell lines which the name is given for cervical cancer tumors [47]. HeLa cells are extensively used as cell lines to study the cytotoxicity of chemical compounds. Results in **Table 1** show that compounds 12, 16, 18 and 24 reduced the viability of HeLa cells below 40% and were considered active against HeLa cells at the concentration of 125 μM.

Molecular docking

The docking procedure was performed in Maestro 12.6 which comes with the Schrödinger suite software. The drug target was Trypanothione reductase; PDB Id, 6BU7. The protein is a polypeptide with two chains A and B; chain B was used in the glide grid docking and seventeen ligands were docked into the active sites of Trypanothione reductase. The obtained docking results were assessed by visual inspection to determine the mode of interaction with the receptor protein. Analysis of the docked poses was done using Biovia discovery studio, while 3D representations of the protein ligand interactions were acquired using Pymol software (Figures 3 and 4).

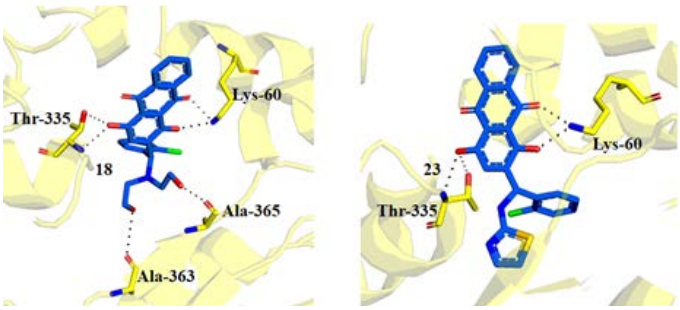


Figure 3: 3D representation for compound 18 and 23 bound to trypanothione reductase, PDB ID: 6BU7. Hydrogen bonding interaction is indicated by dotted lines.

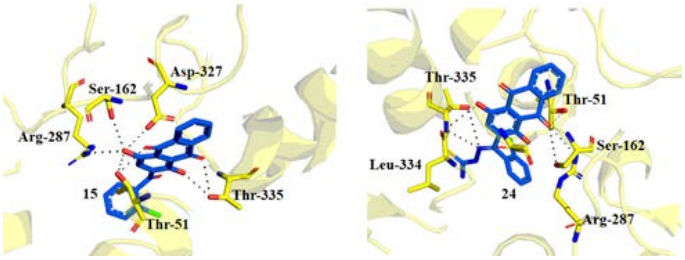


Figure 4: 3D representation for compound 15 and 24, bound to trypanothione reductase, PDB ID: 6BU7. Hydrogen bonding interaction is indicated by dotted lines.

Test compounds 18 and 23 which had displayed good activity against trypanosomes (IC50 = 1.10 and 1.20 μM) were found to produce favourable poses with good binding affinity of -7.857 and -7.351 kcal/mol. Compound 18 is found to be actively associated with hydrogen bonding interaction with amino acid LYS 60, ALA 363 and ALA 365. Whilst compound 23 is associated with two hydrogen bonding with the amino acid LYS 60 and THR 335 **(Figure 3).** Similarly test compound 12 and 13 are actively involved in hydrogen bonding with amino acid LYS 60 and ASP 327.

Compound 15 and 24 which showed a slightly higher activity against trypanosomes than the rest of the compounds IC50 = 0.98 μM and 0.78 μM respectively, displayed a moderate binding affinity of -5.4 kcal/mole with the target protein. Compounds 15 and 24 appear to be in hydrogen bonding with THR 51, ARG 287, THR 335, SER 14, and Pi-Pi Cation with PHE 198 **(Figure 4).**

Almost each one of the test compounds that had displayed activity against T. b. brucei demonstrated hydrogen bonding interaction with at least one amino acid residues such as LYS 60, ALA 363, ALA 365, ASP 327, THR 51 and THR 335. Interestingly almost each one of these 1,4-dihydroxyanthraquinone derivatives demonstrated interactions such as halogen, pi-anion, pi-alkyl with the amino acid CYS 57 a key amino acid of hydride transfer. Interaction of these ligands with Cystines disrupts the reduction of trypanothione disulphide (T [S] 2) to dihydro-trypanothione (T [SH] 2) [48].

Materials and Methods

General procedures

Raw cashew nuts were obtained from small-scale farmers in Dar es Salaam (Tanzania). All other chemicals and reagents were purchased from Sigma-Aldrich Company limited. Reactions were carried out in clean oven-dried glassware. All air or moisture sensitive reactions were carried under nitrogen or argon atmosphere. All solvents used unless specified were distilled and dried over molecular sieves. Acetaldehyde was to be distilled prior to use. Purification of methanol was done by adding a significant amount of CaH2 letting it to stand for 24 hours, distilling and drying over molecular sieves. Reactions were monitored by thin- layer chromatography (TLC) silica-gel 60 F254 using UV light as a visualizing agent. Products were purified using silica gel 60Å, 70-230 mesh, 63-200 μm. Melting points were measured by using a melting point apparatus and are reported as uncorrected.

1H-NMR and 13C-NMR spectra were recorded on Bruker Nuclear Magnetic Resonance spectrometers (300 MHz, 400 MHz and 600 MHz, 1H-NMR chemical shifts (δH) and 13C-NMR chemical shifts (δC) are recorded in parts per million (ppm) downfield from trimethylsilane (TMS) and coupling constants (J) are quoted in Hertz (Hz). Abbreviations for NMR data are s (singlet), br (broad), d (doublet), t (triplet), q (quartet), quin (quintet) and sxt (sextet)

1H-NMR and 13C-NMR spectra were assigned with the aid of HSQC, HMBC and DEPT 135 NMR experiments. Infrared (IR) spectra were recorded on a Perkin Elmer spectrum 100 FT-IR spectrometer and mass spectra were recorded on a Bruker Compact quadrupole time of flight (QToF) mass spectrometer. Raw mass spectrometry data were processed using MZmine software (version 2.38).

Trypanosoma assays and cytotoxicity assays were performed by Rhodes University Centre of Chemical and Bio-medicinal Research.

Extraction of cashew nut shell liquid (CNSL)

Dry cashew nut shells, (1000.00 g) contained in a closed bottle were

soaked in methanol for 5 days and after filtration and evaporation of solvents under reduce pressure using a rotary evaporator, 270.00 g of cashew nutshell liquid (n-CNSL) crude extract was obtained.

Isolation and purification of anacardic acid

To a solution of CNSL 100.00 g dissolved in 5% aqueous methanol (600.00 mL), calcium hydroxide (50.00 g) was added portion-wise under stirring. Afterwards the temperature was raised to 50°C and stirring was maintained for 3 h. The reaction mixture was filtered using a Buchner funnel, washed with methanol and dried to yield 110.00 g of calcium anacardate.

To 40.00 g powdered calcium anacardate dissolved in 160.00 mL distilled water, was added 21. 80 mL of 11 M HCl and the resultant mixture was stirred for 1 h. The reaction mixture was extracted with ethyl acetate; the organic layer was washed with water and dried over anhydrous Na2SO4, filtered and the solvent evaporated in vacuo. The crude product was then chromatographed over silica gel using a mixture of 10% methanol in dichloromethane to afford 23.18 g of anacardic acid 1 as a dark brown liquid which was directly used as the starting material for the synthesis of 3-ethoxyphthalic acid 6 which in turn was used to prepare 1,4,5-trihydroxyanthraquinone 9.

Synthesis of ethyl 2-ethoxy-6-((E)-pentadec-8-enyl)benzoate 2

To a solution of anacardic acid 1 (19.48 g, 0.06 mole) in acetonitrile (150 mL) was added K2CO3 (39.22 g, 0.28 mole) and diethyl sulphate (35.00 g, 0.27 mole). The content was heated to reflux at 90 °C for 24 h. The reaction mixture was cooled to room temperature, filtered and the filtrate concentrated, re-dissolved in ethyl acetate, and washed with water (2 × 200 ml). The organic layer obtained was dried over Na2SO4, filtered and the solvent evaporated in vacuo. The resultant crude product was subjected to column chromatography using silica gel (10% ethyl acetate in petroleum ether) yielding ester 2 as a reddish yellow liquid (14.90 g, 66%). FTIR υmax(ATR)/cm-1 2926-2856 (C-H) aromatic, 1728 (C=O); 1H-NMR (300 MHz, CDCl3): δ 7.21 (1H, t, J = 8.00 Hz, ArH), 6.79 (1H, d, J = 7.7 Hz, ArH), 6.73 (1H, d, J = 8.3 Hz, ArH), 5.34 (2H, m, =CH2), 4.39 (2H, q, J = 7.10 Hz, OCH2), 4.04 (2H, q, J = 6.90 Hz, OCH2), 2.84 (2H, m, CH2), 2.63 (2H, m, CH2), 1.99 (2H, m, CH2), 1.52 (2H, m, CH2), 1.37 (6H, td, J = 7.0, 3.2 Hz, 2CH3), 1.30 (10H, m, CH2) and 0.89 (3H, m, CH3). 13C- NMR (75 MHz, CDCl3): δ 167.5, 154.6, 135.8, 128.9, 128.3, 126.6, 123.1, 120.3, 108.4, 63.2, 59.9, 32.4, 30.7, 30.59, 30.2, 28.7, 28.7, 28.5, 28.4, 28.3, 28.2, 27.9, 26.2, 24.5, 13.7 and 13.3. HRMS (ESI+) m/z ESI-HRMS found 403.3207 calcd for C26H42O3 403.2042.

Synthesis of ethyl 2-ethoxy-6-pentadecylbenzoate 3

To a two neck 100 mL round bottomed flask containing ester 2 (1.27 g, 3.43 mmol) dissolved in 50 mL absolute methanol, was added powdered Pd/C (0.13 g, 10% w/w). The two necks were closed by a septum and an inlet allowed hydrogen gas from the balloon to enter. The reaction mixture was stirred for 24 h at room temperature, and it was filtered and concentrated to yield saturated compound 3 as a light-yellow liquid (1.22 g, 96 %); FTIR υmax(ATR)/cm-1 2923-2855 (C-H) aromatic, 1734 (C=O); 1H-NMR (300 MHz, CDCl3): 7.22 (1H, t, J = 8.0 Hz, ArH), 6.79 (1H, d, J = 7.7 Hz, ArH), 6.73 (1H, d, J = 8.3 Hz, ArH), 4.39 (2H, q, J = 7.1 Hz, OCH2), 4.03 (2H, q, J = 7.0 Hz, OCH2), 2.55 (2H, t, CH2), 1.57 (2H, m, CH2), 1.26 (30H, m, 12 CH2 and 2 CH3) and 0.88 (3H, t, J = 6.7 Hz, CH3). 13C- NMR (75 MHz, CDCl3): δ 168.3, 155.7, 141.0, 130.2, 124.2, 121.4, 109.8, 63.6, 60.8, 33.5, 31.9, 31.3, 29.39 - 29.7 (10C), 22.7, 14.7, 14.4 and 14.2.

Synthesis of ethyl 2-(1-bromopentadecyl)-6-ethoxybenzoate 4

To a solution of 3 (2.32 g, 5.90 mmol) in dry CCl4 was added NBS (1.27 g, 7.16 mmol) and benzoyl peroxide (0.23 g, 10% w/w). The resulting mixture was refluxed at 90°C for 3 h. The reaction mixture was filtered, and the filtrate was added with water, extracted with ethyl acetate, washed with saturated NaHCO3, brine and dried over sodium sulphate. The solvent was evaporated to afford bromide 4 as a yellowish liquid (2.57 g, 91%); FTIR υmax(ATR)/cm-1 2916-2847 (C-H); 1H-NMR (300 MHz, CDCl3): δ 7.25 (1H, t, J = 8.1 Hz, ArH), 7.12 (1H, d, J = 8.0 Hz, ArH), 6.74 (1H, d, J = 8.3 Hz, ArH), 4.89 (1H, t, J = 7.4 Hz, CH), 4.34 (2H, q, J = 7.1 Hz, CH2), 3.97 (2H, q, J = 7.0 Hz, CH2), 2.08 (2H, m, CH2), 1.28 (30H, m, 12 CH2 and 2 CH3), 0.80 (3H, t, J = 6.7 Hz, CH3). 13C-NMR (75 MHz, CDCl3): δ 167.3, 155.3, 140.4, 130.8, 123.1, 119.4, 111.4, 64.6, 61.5, 50.6, 39.9, 31.7, 28.0- 29.7 (10C), 22.2, 14.6, 14.3 and 14.1.

Synthesis of ethyl 2-ethoxy-6-((E)-pentadec-1-enyl)benzoate 5

To a solution of bromide 4 (1.25 g, 2.50 mmol) in toluene was added (1.18 g, 7.75 mmol) DBU. The reaction mixture was refluxed at 110°C for 6 h under argon atmosphere. The reaction mixture was allowed to cool down to room temperature and quenched with 50 mL of 10% hydrochloric acid and then extracted with ethyl acetate. The combined organic extracts were washed with 10% hydrochloric acid (50 mL), brine (2 mL), and dried over sodium sulphate. The organic phase was concentrated to give a yellow solid, purified on column chromatography with 5% ethyl acetate: hexane mixture to obtain alkene 5 as a lightly yellow liquid (0.91g, 87%); FTIR υmax(ATR)/cm-1 2916-2847 (C-H), 1452 (C=C) 1H-NMR (400 MHz, CDCl3): δ 7.27 (1H, t, J = 8.0 Hz, ArH), 7.14 (1H, d, J = 7.9 Hz, ArH), 6.78 (1H, d, J = 8.2 Hz, ArH), 6.44 (1H, d, J = 15.7 Hz, -CH=), 6.27 (1H, m, =C-H), 4.46 (2H, q, J = 7.1 Hz, -OCH2), 4.08 (2H, q, J = 6.9 Hz, -OCH2), 2.24 (2H, q, J = 7.1 Hz, CH2), 1.50 (2H, m, CH2), 1.33 (26H, m, 10CH2 and 2CH3), 0.95 (3H, t, J = 6.7 Hz, CH3). 13C-NMR (100 MHz, CDCl3): δ 168.1, 157.7, 136.4, 134.3, 129.9, 126.1, 122.9 , 117.5, 110.0, 64.3, 60.9, 33.2, 31.9, 29.1- 29.2 (9C), 22.2, 14.6, 14.3 and 14.1.

Synthesis of 3-ethoxyphthalic acid 6

To a mixture of olefin 5 (1.76 g, 4.37 mmol) and FeCl3.6 H_2O (0.06 g, 5 Mol %) was added 70% aqueous TBHP 3.61 mL and water 4.37 mL, after stirring for 1 h, NaOH (0.70 g, 17.40 mmol) was added, there after the temperature of the reaction was raised to 80 °C and stirring was maintained for 36 h. The reaction mixture was allowed to cool down to room temperature and extracted with ethyl acetate and the aqueous layer was treated with dilute HCl 10% and crushed ice. This mixture was then extracted with ethyl acetate and the combined organic phase was washed with saturated brine solution, dried over anhydrous Na2SO4, and concentrated under reduced pressure giving a white solid which was filtered and then washed with hexane to afford the pure carboxylic acid 6 as a white solid (0.59 g, 64%), m.p. 183-186 °C; FTIR υmax(ATR)/cm-1 3246 (O-H) carboxylic, 1734 (C=O); 1H-NMR (400 MHz, DMSO): δ 7.21 (2H, q, J = 7.5 Hz, ArHs), 7.07 (1H, dd, J = 7.3, 1.8 Hz, ArH), 3.84 (2H, q, J = 6.9 Hz, -OCH2), 1.05 (3H, t, J = 6.9 Hz, CH3). 13C-NMR (100 MHz, DMSO): δ 168.2, 167.0, 155.2, 130.1, 129.3, 127.2, 121.8, 117.1, 64.7 and 14.9. HRMS (ESI+) m/z ESI-HRMS found 211.0591 calcd for C10H10O5 211.0601.

Friedel-Crafts acylation synthesis of hydroxyanthraquinone 9 and 10

To an oven dried two neck 50 mL round bottomed flask containing mixture of aluminium chloride (3.18 g, 23.84 mmol) and sodium

chloride (0.68 g, 11.63 mmol). A con-denser was well fitted, and the other neck was fitted with a balloon containing nitrogen gas. The contents were heated at 180°C in an oil bath till molten. To this melt an intimate mixture of phthalic acid (0.43 g, 2.88 mmol), 1, 4-benzenediol (0.32 g, 2.96 mmol) and aluminium chloride (1.06 g, 7.94 mmol) were added. The temperature was raised to 220°C with stirring for 90 minutes. The reaction mixture was cooled to room temperature then poured into a mixture of ice/conc. HC1 10% and stirred for 2 h. The solids were filtered, rinsed with water, and air dried overnight. The filtrate was extracted with ethyl acetate, the organic layer dried over sodium sulphate, filtered, and concentrated to afford a solid. For the 1, 4, 5-trihydroxy Anthraquinone 9 the solids were purified over a short silica gel column and washed with 20% CHCl3: Petroleum ether to afford

1,4,5-trihydroxyanthraquinone 9 as a red-orange solid yield 0.22 g, 45%, m.p 254-256°C; FTIR υmax (ATR)/cm-1 2912-2848 (C-H), 1676 (C=O); 1H-NMR (400 MHz, CDCl3): δ 7.84 (1H, t, J = 8.0 Hz, ArH), 7.80 (1H, dd, J = 7.5, 1.0 Hz, ArH), 7.45 (2H, d, ArHs) and 7.41 (1H, dd, J = 8.0, 1.5 Hz, ArH). 13C-NMR (100 MHz, CDCl3): δ 190.0, 185.2, 161.6, 157.2, 156.7, 135.9, 132.4, 128.9, 128.5, 123.5, 118.5, 115.0, 111.6 and 111.4. HRMS (ESI+) m/z ESI-HRMS found 257.2696 calcd for C14H8O5 257.0444.

1,4-dihydroxyanthraquinone 10 as a red orange solid 0.50 g, 82% m.p 195-197°C. FTIR υmax(ATR)/cm-1 2906 (C-H), 1624 (C=O); 1H-NMR (400 MHz, DMSO): δ11.88 (2H, s, 2OH), 7.45 (2H, m, ArHs), 7.22 (2H, m, ArHs), 6.62 (2H, s, ArHs). 13C-NMR (100 MHz, DMSO): δ 187.1, 157.1, 135.5, 133.3, 129.8, 127.1 and 113.1.

General procedure for the preparation of alcohol anthraquinones 11-13

To a 0°C cold solution of Anthraquinone 10 (2.00 g, 8.3 mmol) in absolute methanol (20 mL) was added aqueous NaoH (1 M, 50 mL) and a solution of Na2S2O4 (2.89 g, 16.5 mmol) in water (16.50 mL) under nitrogen atmosphere. After 10 minutes of stirring, aldehydes (2-chlorobenzaldehyde, acetaldehyde and propionaldehyde) (4.6 g, 33.00 mmol) was added. The reaction mixture was stirred for 3 h at 0 °C. The solution was poured into cold water (50 mL) that contained 30% H_2O_2 (10 mL), and the mixture was stirred for 10 min. The mixture was acidified by the addition of HCl (1 M solution, 10 mL), forming a solid mass which was separated and was dissolved in ethyl acetate and evaporated, re-dissolved in hexane, and stirred for 2 h to form precipitates. The precipitates were filtered, washed several times with hexane to remove the starting material and some of the unreacted aldehyde, and dried to yield compound 11, which was either directly used in the next reaction or purified through column chromatography with 10% ethyl acetate/petroleum ether to afford

2-((2-chlorophenyl)(hydroxy)methyl)-1,4-dihydroxyanthracene-9,10-dione 11 as an orange solid (2.4 g, 75 %); m.p. 187-190 °C, FTIR υmax (ATR)/cm-1 3450 (OH) alcohol, 1232 (C-O-); 1H-NMR (400 MHz, DMSO): δ 8.16 (2H, m, ArHs), 7.88 (2H, m, ArHs), 7.47 (dd, 1H), 7.42 (1H, s, H3), 7.33 (3H, m, Ar-H) and 6.33 (1H, s, ArH). 13C-NMR (100 MHz, DMSO): δ 187.4, 186.5, 156.8, 154.9, 145.2, 140.2, 135.5, 135.3, 133.3, 133.2, 133.0, 129.9, 129.7, 129.3, 127.7, 127.0, 126.9, 126.3, 112.7, 112.3 and 65.8. HRMS (ESI+) m/z ESI-HRMS found 381.1482 calcd for C21H13ClO5 381.0524.

1,4-dihydroxy-2-(1-hydroxyethyl)anthracene-9,10-dione 12 as an orange solid (0.4 g, 16%), m.p. 90-92 °C; FTIR υmax(ATR)/cm-1 3246 (O-H) carboxylic, 2908 (CH_3), 1609 (Ar-C=C-); 1H-NMR (400 MHz, DMSO): δ 8.23 (2H, m, ArHs), 7.97 (2H, m, ArHs), 7.45 (1H, s, ArH), 5.05 (1H, q, J = 6.4 Hz, CH), 1.38 (3H, d, J = 6.4 Hz, CH3). 13C-NMR (100 MHz, DMSO): δ 187.3, 186.4, 157.4, 154.6, 149.7, 135.5, 135.3, 133.3 and, 127.1, 127.0, 124.7, 112.5, 111.6, 63.2 and 24.1. HRMS (ESI+) m/z ESI-HRMS found 285.1745 calcd for C16H12O5 285.0757.

1,4-dihydroxy-2-(1-hydroxypropyl)anthracene-9,10-dione 13 as an orange solid (0.07 g, 14 %) m.p. 123-125 °C; FTIR υmax (ATR)/cm-1 3183 (O-H) alcohol, 2916 (CH3), 1593 (Ar-C=C-). 1H-NMR (400 MHz, CDCl3): δ 8.32 (2H, m, ArHs), 7.81 (2H, m, ArHs), 7.41 (1H, s, ArH), 5.01 (1H, m, CH), 1.77-1.90 (2H, m, CH2) and 1.03 (3H, t, J = 6.4 Hz, CH3). 13C-NMR (100 MHz, CDCl3): δ 187.3, 186.4, 157.9, 155.5, 145.9, 134.5, 134.4, 133.5, 133.4, 127.0, 126.9, 126.0, 112.4, 111.7, 70.7, 29.5 and 10.0. HRMS (ESI+) m/z ESI-HRMS found 299.0914 calcd for C17H14O5 299.0808.

Synthesis of anthraquinone chloride 14

Thionyl chloride 2 mL was added drop wise to the parent alcohol 11 (0.50 g, 1.30 mmol) cooled in an ice bath after stirring for 10 min. The temperature of the reaction was raised to 50 °C and stirring was maintained for 2 h. Excess thionyl chloride was evaporated in vacuo. The solid obtained was dissolved in dichloromethane and evaporated to form a solid which was further re-dissolve in dichloromethane/hexane mixtures to form precipitates which were filtered washed several times with hexane and dried to obtain 2-(chloro(2-chlorophenyl) methyl)-1,4-dihydroxyanthracene-9,10-dione 14 as an orange solid (0.27 g, 51.5%) m.p 200-204 °C. FTIR υmax (ATR)/cm-1 1738 (C=O); 1H-NMR (400 MHz, CDCl3) δ 13.43 (1H, s, OH), 12.82 (1H, s, OH), 8.34 (2H, m, ArHs), 7.84 (2H, m, ArHs), 7.60 (1H, m, ArH), 7.42 (2H, m, ArHs), 7.32 (2H, m, ArHs) and 6.94 (1H, s, CH). 13C-NMR (100 MHz, CDCl3): δ 187.2, 186.7, 157.1, 155.1, 140.5, 136.3, 134.7, 134.6, 133.4, 133.3, 133.2, 130.0, 129.9, 129.5, 128.0, 127.3, 127.2, 127.1, 112.8, and 53.6. HRMS (ESI+) m/z ESI-HRMS found 399.1771 calcd for C21H12Cl2O4 399.0185.

General procedure for the preparation of Anthraquinone amines 16-23.

A solution of Anthraquinone 14 (0.10 g, 0.25 mmol) in dry dichloromethane (10 mL) and appropriate amine (6 equivalents) was added, and the resulting mixture was stirred at room temperature to completion of the reaction as indicated by TLC. The reaction mixture was poured into water and extracted with ethyl acetate. The organic layer was washed with water, dried over sodium sulphate, and concentrated. The solid obtained was purified by column chromatography using a mixture of 4% MeOH/DCM or 10% ethyl acetate/petroleum ether as a solvent system depending on the polarity of the compound.

2-((2-chlorophenyl) (piperazin-1-yl) methyl)-1,4-dihydroxyanthracene-9,10-dione 16 as a deep purple solid; (0.08 g, 71%) m.p. 100-120 °C. FTIR υmax (ATR)/cm-1 3057 (O-H) Alcohol, 1620 (Ar-C=C); 1H-NMR (400 MHz, CDCl3): δ 8.31 (2H, m, ArHs), 7.80 (2H, m, ArHs), 7.60 (1H, s, ArH), 7.51 (1H, dd, J = 7.7, 1.7 Hz, ArH), 7.36 (1H, dd, J = 7.8, 1.3 Hz, ArH), 7.19 (2H, m, ArH), 5.55 (1H, s, CH2), 2.91 (4H, m, 2CH2), 2.55 (2H, m, CH2) and 2.48 (2H, m, CH2). 13C-NMR (100 MHz, CDCl3): δ 186.0, 185.6, 156.5, 155.9, 142.1, 136.1, 134.0, 133.4, 132.5, 132.4, 129.1, 128.8, 127.6, 127.1, 126.1, 125.9, 125.8, 111.5, 111.0, 61.5, 51.4 and 45.2. HRMS (ESI+) m/z ESI-HRMS found 449.3618 calcd for C25H21ClN2O4 449.1263

2-((2-hydroxyethylamino) (2-chlorophenyl) methyl)-1, 4-dihydroxyanthracene-9,10-dione 17 as a deep purple solid. Yield = 22 %, m.p. 147-150 °C. FTIR υmax (ATR)/cm-1 3285 (O-H) alcohol, 1609 (Ar-C=C-); H-NMR (400 MHz, CDCl3): δ 8.25 (2H, m, ArHs),

7.74 (1H, m, ArHs), 7.45, (1H, d, J = 7.4 Hz, ArH), 7.33 (1H, d, J = 7.6 Hz, ArH), 7.15 - 7.22 (3H, m, ArHs), 5.67 (1H, s, H15), 3.58 (2H, m, H17) and 2.75 (2H, m, H16). 13C-NMR (100 MHz, CDCl3): δ 186.2, 186.5, 157.5, 156.3, 142.9, 137.6, 134.5, 133.4, 134.2, 133.5, 133.4, 130.0, 129.1, 128.9, 127.6, 127.2, 127.1, 127.0, 112.7, 112.0, 60.3, 55.5 and 48.7. HRMS (ESI+) m/z ESI-HRMS found 424.1253, calcd for C23H18ClNO5 424.0946.

2-((bis (2-hydroxyethyl) amino) (2-chlorophenyl) methyl)-1, 4-dihydroxyanthracene-9, 10-dione 18 as an orange solid. Yield = 72.2%, m.p. 130-140 °C. FTIR υmax (ATR)/cm-1 3332 (O-H) alcohol, 1585 (Ar-C=C 1H-NMR (400 MHz, CDCl3): δ 8.09 (2H, m, ArHs), 7.99 (2H, m, ArHs), 7.28 (1H, d, J = 7.1 Hz, ArH), 7.19 (1H, d, J = 7.4 Hz, ArH), 7.07 (2H, m, ArH), 6.84 (1H, s, ArH), 5.79 (1H, s, CH), 3.37 (2H, m, CH2), 3.28 (2H, m, CH2) and 2.83 (4H, m, 2CH2). 13C-NMR (100 MHz, CDCl3): δ 187.4, 186.6, 157.2, 156.4, 142.4, 137.1, 134.7, 134.5, 134.4, 133.5, 133.4, 130.5, 129.5, 129.1, 128.6, 127.2, 127.0, 112.7, 112.2, 60.8, 59.7 and 54.4. HRMS (ESI+) m/z ESI-HRMS found 468.1544 calcd for C25H22ClNO6 468.1208.

2-((2-chlorophenyl) (Propyl amino) methyl)-1, 4-dihydroxyanthracene-9, 10-dione 19 as an orange solid. Yield = 90 %, m.p. 98-120 °C; FTIR υmax (ATR)/cm-1 3069 (O-H) alcohol, 1581 (Ar-C=C-); 1H-NMR (400 MHz, CDCl3): δ 8.10 (2H, m, ArHs), 7.60 (2H, m, ArHs), 7.25 (1H, s, ArH), 7.22 (1H, d, J = 1.9 Hz, ArH), 7.21 (1H, dd, J = 7.5, 1.6 Hz, ArH), 7.05 (2H, m, ArHs), 5.43 (1H, s, CH), 2.47 (2H, t, J = 7.1 Hz, CH2), 1.50 (2H, dq, J = 14.9, 7.6, 7.0 Hz, CH2) and 0.78 (3H, t, J = 7.4 Hz, CH3). 13C-NMR (100 MHz, CDCl3): δ 187.0, 186.4, 158.1, 156.7, 144.1, 138.5, 134.4, 134.3, 134.0, 133.4, 129.8, 129.0, 128.7, 127.7, 127.0, 126.8, 112.4, 111.8, 57.3, 50.6, 23.4 and 11.8. HRMS (ESI+) m/z ESI-HRMS found 422.3618, calcd for C24H20ClNO4 422.1154.

2-((2-chlorophenyl) (pyrrolidin-1-yl) methyl)-1, 4-dihydroxyanthracene-9, 10-dione 20 as a dark orange solid. Yield = 44%, m.p. 148-150 °C. FTIR υmax (ATR)/cm-1 3065 (O-H) alcohol, 1577 (-Ar-C=C-); 1H-NMR (400 MHz, Acetone-d6): δ 8.34 (2H, m, ArHs), 7.84 (2H, m, ArHs), 7.55 (1H, dd, J = 7.8, 1.6 Hz, ArH), 7.50 (1H, s, H3), 7.28 (1H, dd, J = 7.9, 1.2 Hz, ArH), 7.22 (1H, (td, J = 7.6, 1.2 Hz, ArH), 7.12 (1H, td, J = 7.6, 1.7 Hz, ArH), 5.37 (1H, s, CH), 2.48 (2H, m, CH2), 2.38 (2H, m, CH2) and 1.65 (4H, m, 2CH2). 13C-NMR (100 MHz, Acetone-d6): δ 187.5, 186.8, 157.5, 156.2, 144.0, 138.7, 134.9, 134.8, 133.8, 133.5, 133.4, 130.4, 129.6, 128.7, 127.8, 127.2, 126.8, 126.7, 112.6, 111.9, 61.4, 52.2 and 23.3. HRMS (ESI+) m/z ESI-HRMS found 434.3386, calcd for C25H20ClNO4 434.1154.

2-((2-chlorophenyl) (1H-imidazol-1-yl) methyl)-1, 4-dihydroxyanthracene-9, 10-dione 21 as an orange solid. Yield = 46%, m.p. 180-200 °C. FTIR υmax (ATR)/cm-1(1617 (-C=N), 1573 (Ar-C=C-); 1H-NMR (400 MHz, CDCl3): δ 13.29 (1H, s, OH), 12.79 (1H, s, OH), 8.37 (2H, m, ArHs), 7.86 (2H, m, ArHs), 7.54 (1H, s, ArH), 7.47 (1H, d, J = 7.8 Hz, ArH), 7.35 (1H, t, J= 7.6 Hz, ArH), 7.28 (2H, m, ArHs), 7.19 (1H, s, ArH), 6.91 (2H, d, J = 7.8 Hz, ArH), and 6.76 (1H s, CH). 13C-NMR (100 MHz, CDCl3): δ 187.3, 186.8, 157.0, 155.0, 138.5, 134.9, 134.8, 134.7, 133.8, 133.4, 133.3, 130.5, 130.4, 129.2, 128.4, 127.3, 127.2, 127.1, 119.3, 113.2, 113.1 and 56.3. HRMS (ESI+) m/z ESI-HRMS found 431.0796 calcd for C24H15ClN2O4 431.0793.

2-((2-chlorophenyl) (morpholino) methyl)-1, 4-dihydroxyanthracene-9, 10-dione 22 as an orange solid. Yield = 80%, m.p. 180-182 °C. FTIR υmax (ATR)/cm-13057 (O-H) alcohol, 1577 (Ar-C=C-); 1H-NMR (400 MHz, CDCl3): δ 8.34 (2H, m, ArHs), 7.81 (2H, m, ArHs), 7.62 (1H, s, ArH), 7.53 (1H, dd, J = 7.7, 1.8 Hz, ArH), 7.37 (1H, dd, J = 7.8, 1.5 Hz, ArH), 7.20 (2H, m, ArH), 5.51 (1H, s, CH), 3.72 (4H, m, 2-CH2), 2.60 (2H, m, CH2) and 2.50 (2H, m, CH2). 13C-NMR (100 MHz, CDCl3): δ 186.1, 185.6, 156.4, 155.9, 141.8, 135.7, 134.1, 133.43, 132.4, 132.3, 129.1, 128.9, 127.7, 127.1, 126.1, 126.0, 125.9, 111.5, 111.0, 66.2, 61.3 and 50.8. HRMS (ESI+) m/z ESI-HRMS found 450.3422 calcd for C24H20ClNO5 450.1103.

2-((2-chlorophenyl) (thiazol-2-ylamino) methyl)-1, 4-dihydroxyanthracene-9,10-dione 23 as a black solid. Yield = 27%, m.p. 135-137 °C. FTIR υmax (ATR)/cm-1 3352 (-NH-), 1629 (C=N); 1H-NMR (400 MHz) CDCl3: δ 8.24 (2H, m, ArHs), 7.75 (2H, m, ArHs), 7.19 (3H, m, ArH), 6.96 (1H, d, J = 3.6 Hz, ArH) and 6.44 (1H, s, CH). 13C-NMR (100 MHz, CDCl3): δ 186.1, 185.6, 168.1, 156.3, 155.0, 139.5, 137.5, 135.2, 133.6, 133.5, 133.2, 132.4, 132.3, 129.2, 128.7, 127.5, 126.3, 126.2, 126.1, 126.0, 111.9, 111.5, 109.5 and 55.1. HRMS (ESI+) m/z ESI-HRMS found 463.0508, calcd for C24H15ClN2O4S 463.0514.

Synthesis of Anthraquinone thiosemicarbazone 24

A solution of alcohol Anthraquinone 11 (0.22 g, 0.58 mmol) in dichloromethane (20 mL) was treated at 25 °C with Dess–Martin periodinane (0.36 g, 0.84 mmol) and stirred for 20 min. Afterwards, saturated aq. NaHCO3 was added and extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulphate. The solvent was evaporated in vacuo; the crude product recrystallized from methanol/ petroleum ether to afford

2-(2-chlorobenzoyl)-1, 4-dihydroxyanthracene-9, 10-dione 15 as an orange solid (0.21 g, 99%); m.p. 140-153 °C. FTIR υmax (ATR)/cm-1 1738 (C=O); 1H-NMR (400 MHz, CDCl3): δ 8.28 (2H, m, ArHs), 7.79 (2H, m, ArHs), 7.51 (1H, s, ArH), 7.41 (1H, d, ArH) and 7.35 (2H, m, ArH). 13C-NMR (100 MHz, CDCl3): δ 192.2, 187.1, 187.0, 156.5, 156.1, 138.1, 138.0, 134.9, 134.8, 133.4, 133.2, 132.7, 132.3, 130.6, 130.4, 129.6, 127.2, 127.0, 115.3 and 113.7. HRMS (ESI+) m/z ESI-HRMS found 379.0447 calcd for C21H11ClO5 379.0368.

To the Thiosemicarbazide (0.14 g, 1.86 mmol) in absolute methanol (20 mL), carbonyl 15 (0.10 g, 0.26 mmol) was added afterward and three drops of sulphuric acid were added. The reaction mixture was refluxed at 90 °C for 24 h till the completion of the re-action as monitored by TLC. The reaction mixture was allowed to cool to room temperature. The solvent was evaporated, and the crude product was purified by column chromatography from 2:1, petroleum ether/ethyl acetate mixture to chloroform as solvent system to yield a solid which was recrystallized from CH2Cl2/petroleum ether giving (Z)-1-((2-chlorophenyl)(9,10-dihydro-1,4-dihydroxy-9,10-dioxoanthracen-3-yl)methylene)thiosemicarbazide 24 as a red-orange solid. Yield = 45%, solid m.p 137-139 °C; FTIR υmax (ATR)/cm-1 3222 (-NH2), 1624 (C=N), 1164 (C=S); 1H NMR (400 MHz, CDCl3): δ 13.68 (1H, s, OH), 12.78 (1H, s, -OH), 8.34 (2H, m, ArHs), 7.84 (2H, m, ArHs), 7.50 (3H, m, ArHs), 7.39 (1H, s, ArHs) and 7.34 (1H, dd, J = 7.3, 1.9 Hz, ArH). 13C-NMR (100 MHz, CDCl3) δC186.3, 185.7, 178.6, 156.1, 155.4, 142.5, 134.5, 133.8, 133.7, 132.3, 132.2, 131.8, 131.0, 129.9, 129.1, 128.9, 128.2, 127.1, 126.2, 126.1, 112.7 and 112.5. HRMS (ESI+) m/z ESI-HRMS found 452.0366 calcd for C22H14ClN3O4S 452.0466.

Molecular docking

The docking procedure was performed in Maestro 12.6 Schrödinger suite software. Each ligand saved in Sdf format was imported and prepared to suitable conformers using Ligprep module. The PDB file of the target protein was downloaded by going into the file menu, selecting get PDB and the protein 6BU7 was typed and downloaded. The poly-peptide which has two chains (chain A and B) was trimmed to chain

B. The solvents other chemicals such as metallic ions were removed. Before commencing the docking procedure the protease structures of the protein were subjected to preparation by protein preparation wizard. During the preparation the protein was first pre-processed, refined where the H-bonding was optimized, water was removed, and the protein was minimized based on OPLS-3e force field. The grid box was generated by going into the task menu, receptor grid generation was selected and an atom from the ligand was picked. Then each ligand prepared into conformers was docked into the receptor protein.

In vitro Antitrypanosomal assay

To assess Antitrypanocidal activity, test compounds were added to in vitro cultures of T.b brucei (strain 427 Lister) in 96-well plates at a fixed concentration of 20 μM. After an incubation period of 24 hours, resazurin was added to a final concentration of 50 μM. After further 24-hour incubation, conversion of resazurin to resorufin by viable cells was measured by fluorescence readings (Ex560/Em590) in a Spectramax M3 plate reader (Molecular Devices). Results were converted to % parasite viability – the resorufin fluorescence in compound-treated wells relative to untreated controls, after subtracting background readings from wells without parasites. For dose-response assays, the procedure was repeated, except that parasites were incubated with 3-fold serial dilutions of test compounds and IC50 values derived by non-linear regression analysis of % viability vs. log[compound] using Graph Pad Prism.

In vitro cytotoxicity assay

To assess the cytotoxicity effects of the compounds, they were incubated at a fixed concentration 125 uM in 96-well plates seeded with 2x104 HeLa cells per well for 24 hours. The numbers of cells surviving drug exposure were determined by adding resazurin to a final concentration of 50 μM and reading resorufin fluorescence (Ex560/Em590) in a multiwall plate reader.

Results were expressed as % viability-the resorufin fluorescence measured in compound-treated wells relative to untreated controls, after subtracting background readings obtained from wells without cells. Compounds were usually tested in technical duplicate.

Conclusions

In this present work we have synthesized 1, 4-dihydroxyanthraquinone derivatives and tested for their potency against trypanosomes. We have further derivatized anacardic acid from CNSL to synthesize 3-ethoxyphthalic acid which is synthon in the preparation of hydroxyanthraquinone. The in vitro biological evaluation of test compounds against Trypanosoma brucei (T.b) revealed 1, 4-dihydroxyanthraquinone derivatives to be potent against trypanosomes. In our finding we found hydroxy-alkyl-1, 4-dihydroxyanthraquinone with aliphatic substituents have activity against T. brucei when compared to its analogous hydroxyaryl-1,4-dihydroxyanthraquinone. The molecular docking analysis of compounds produced favourable poses with docking scores ranging from good to moderate. We therefore propose 1,4-dihydroxyanthraquinone derivatives as promising starting structures in the development drug candidates targeting these neglected tropical diseases.

Supplementary Materials: 1H NMR and 13C NMR-spectra of selected test compounds (Figure S1-S26) and % viability of cells against test compound (Table S1)

Author Contributions

Synthesis and molecular docking analysis of the test com-pounds, conceptualization and analysis of results LK, review and editing XSN, performed the bioassay studies TS and HCH; designed the study QM, supervision RWK.

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Conflict of Interest

The authors declare no conflict of interest.

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