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The anti-mycobacterial activity of *Artemisia annua* L is based on deoxyartemisinin and artemisinic acid

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Abstract: The discovery of antimalarial artemisinin from *Artemisia annua* L. is an example of how Traditional Chinese Medicine (TCM) may be exploited to meet a recognized need. In this study, we systemically investigated *A. annua* L. for its antimicrobial activity and assessed it as a source of bioactive natural products for anti-mycobacterial activity.

We used a silica gel column to perform antimicrobial activity-guided purification of the *A. annua* leaf, whose identity was confirmed by *rbcL* DNA barcoding, and used UHPLC-HRMS and NMR to elucidate the structure of purified active compounds. The antimicrobial activity of crude extracts, isolated compounds and the control artemisinin (Apollo Scientific Ltd) was assessed against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Mycobacterium smegmatis* strains by serial micro dilution method (31.25-1000 µg/mL). The isolated compounds were tested for synergistic effects against mycobacterium.

Bioactive compounds were purified and identified as deoxyartemisinin and artemisinic acid. Artemisinic acid (MIC 250 µg/mL) was more effective in comparison to deoxyartemisinin (MIC 500 µg/mL) and artemisinin (MIC 1000 µg/mL) against *M. smegmatis*. We used a molecular docking approach to investigate the interactions between selected anti-mycobacterial compounds and proteins involved in vital physiological functions in *M. tuberculosis*, namely *MtPks13*, *MtPknB*, *MtPanK*, *MtKasA*, *MtInhA* and *MtDprE1* and found artemisinic acid showed docking scores superior to the control inhibitors for *MtKasA*, suggesting it to be a potential nick for further *in vitro* biological evaluation and anti-TB drug design.

Keywords: Traditional Chinese Medicine, *Artemisia annua* L, anti-mycobacterial activity, *Mycobacterium smegmatis*, deoxyartemisinin, artemisinic acid, artemisinin

1. Introduction

Currently, tuberculosis (TB) is the leading cause of death from an infectious disease, causing 10 million incident cases globally in 2018. There were an estimated 1.2 million (range, 1.1–1.3 million) TB deaths among HIV-negative people in 2018 (27% reduction from 1.7 million in 2000), and an additional 251 000 deaths (range, 223 000–281 000) among HIV positive people (a 60% reduction from 620 000 in 2000) [1]. There remains an urgent need to discover and develop new anti-TB drugs, particularly to target drug-resistant and dormant strains of *M. tuberculosis* as well as providing a more effective and shorter duration of treatment [2].

Traditional Chinese Medicine (TCM) is one of the most comprehensive, well-documented traditional and folk medicines in human history, representing an important corpus of “folk-medicine” than could

be seen as the product of centuries of “trial-and-error” approaches used by doctors. Therefore it is attracting considerable interest throughout the world. With the increased export of Chinese medicines, it has been easier to apply the modern research ways to detect the molecules, define their mode of action and pharmacokinetic and pharmacodynamic properties, and study the natural products in Chinese herbs as one of the most important resources for developing new lead compounds.

Artemisia annua L., a Chinese herb, is known to have anti-malarial, antipyretic, analgesic and anti-inflammatory activities. The most potent anti-malarial natural product artemisinin was purified from *A. annua* L. Artemisinin and its semi-synthetic derivatives are now front line anti-malarial drugs. A common structural feature of artemisinin derivatives with antimalarial activity is the endoperoxide bridge [3]. The antimalarial mechanism of these artemisinin derivatives appears to be dependent on the breaking of an endoperoxide bridge due to haem-mediated free-radical generation [4]. Artemisinin also exhibits activity against other protozoan parasites including the blood fluke species of *Schistosoma* [5]. The antimicrobial activity of artemisinin derivatives remains poorly defined but conjugation to a siderophore improves its anti-tubercular activity [6].

Given the wide-ranging activities of artemisinins [7] and anti-mycobacterial properties of conjugated artemisinin, we sought to identify artemisinin-like molecules from *A. annua* that, by themselves, contain potent anti-microbial activity. Thus, we isolated a small library of compounds with anti-mycobacterial activity. Two compounds were identified as deoxyartemisinin and artemisinic acid, which whilst have some structural similarity to artemisinin, lack the endoperoxide bridge.

2. Results

2.1. Antimicrobial Assay:

Based on antimicrobial activity -guided purification (Supplementary Figure 1), two weakly active components were isolated and purified from crude extracts of dried *A. annua* leaves, and identified as deoxyartemisinin and artemisinic acid (Figure 1, Figure 2 and Figure 3). The antimicrobial properties of deoxyartemisinin and artemisinic acid were assessed against our panel of bacterial strains (Table 1), and showed that artemisinic acid and deoxyartemisinin had MICs of 250 and 500 µg/mL against *S. aureus*. Also, both deoxyartemisinin and artemisinic acid exhibited a MIC of 500 µg/mL against *M. smegmatis* (NCTC 333) and 500 and 250 µg/mL against *M. smegmatis* (mc²155) respectively. However, crucially, this MIC decreased 4-fold in assessments for synergy between deoxyartemisinin and artemisinic acid. The checkerboard assay, against *M. smegmatis* mc²155, proved a synergistic effect between deoxyartemisinin and artemisinic acid with FICI = 0.5 and the MIC was reduced by a factor of 4 to 125 µg/mL.

2.2. Computational studies:

To suggest a mode of action, the individual binding energies of artemisinin, deoxyartemisinin and artemisinic acid were analysed by docking against *MtDprE1*, *MtInhA*, *MtKasA*, *MtPanK*, *MtPknB* and *MtPks13*. These were compared with their respective positive controls which were the established inhibitor (control) drugs for each mycobacterial target. AutoDock Vina was used to predict binding affinities. Individual binding energy of artemisinin, artemisinic acid and deoxyartemisinin was analysed by docking against *MtDprE1*, *MtInhA*, *MtKasA*, *MtPanK*, *MtPknB* and *MtPks13*, and compared with the respective control inhibitor, retrieved from Protein Data Bank (PDB). AutoDock Vina was used to predict binding affinities (

Table 2).

Comparing the MIC data artemisinic acid shows lower binding energy against *MtKasA* in comparison to artemisinin and deoxyartemisinin and also the respective control thiolactomycin (

Table 2). To further investigate the key properties necessary for an optimal binding, the chemical space spanned by the compounds was studied for all the compounds. The physiochemical properties were studied using PaDel-Descriptor including: molecular weight (MW), partition coefficient (xLogP), rotatable bonds (nRotB), H-bond donors (nHBDon_Lipinski), H-bond acceptors (nHBAcc_Lipinski) and topological polar surface area (TopoPSA) (Supplementary Figure 2).

After analysing the binding energies and physiochemical properties, a structural study was carried out based on their binding energy by docking the compounds onto *MtKasA* and their interaction were further investigated (Figure 4) (Supplementary Figure 4).

A closer look at the interactions between artemisinic acid and *MtKasA* reveals that the C-12 carboxylic acid end of the molecule have a strong hydrogen bond with nitrogen (contact distance 3.0 Å) and oxygen (contact distance 2.77 Å) of Gly403 and oxygen (contact distance 3.25 Å) Asn 408. While the 4,7-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalen-1-yl group binds to the hydrophobic pocket of Pro280, Phe 402 and His311(Figure 5). The active site, and amino acid are in close similarity to what has been previously described as the mode of binding of the TLM control [8]

2.3. Figures, Tables and Schemes

Figure 1: The chemical structures of three bioactive natural products isolated from *Artemisa annua*.

Figure 3: Total ion chromatograms of HFE3e. A: Positive ionization, B: Negative ionization

Figure 2: Total ion chromatograms of HFE3f. A: Positive ionization, B: Negative ionization

Table 1: Antibacterial activity (MIC $\mu\text{g/mL}$) of compounds 1 (deoxyartemisinin), 2 (artemisinic acid) against Gram-positive and Gram-negative bacteria

	Deoxyartemisinin	Artemisinic acid	Artemisinin	Rifampicin	Gentamycin Sulphate
<i>Escherichia coli</i>	500	≥ 1000	≥ 1000	NT ¹	1.6
<i>Pseudomonas aeruginosa</i>	500	≥ 1000	≥ 1000	NT ¹	3.13
<i>Staphylococcus aureus</i>	500	250	≥ 1000	NT ¹	3.13
<i>Mycobacterium smegmatis</i>	500	500	≥ 1000	1.14	NT ¹
<i>Mycobacterium smegmatis</i> mc ² 155	500	250	500	1.41	NT ¹
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	500	250	≥ 1000	NT ¹	3.13

¹ NT: not tested

Table 2: Binding energies (kcal.mol⁻¹) of compounds and their respective controls

Targ ets	Artem isinic acid	Artemi sinin	Deoxy-Art emisinin	Respective controls
MtPks13	-6.7	-7.6	-8.2	I28 (ethyl 5-hydroxy-4-[(4-methylpiperidin-1-yl)methyl]-2-phenyl-1-benzofuran-3-carboxylate): -10.5
MtPknB	-6.7	-8.4	-8	Mitoxantrone: -7.7
MtPanK	-7.6	-8.7	-8.7	ZVT (2-chloro-N-[1-(5-[[2-(4-fluorophenoxy)ethyl]sulfanyl]-4-methyl-4H-1,2,4-triazol-3-yl)ethyl]benzamide): -8.3
MtKasA	-8.3	-6.4	-6.9	Thiolactomycin: -6.57
MtInhA	-8	-8.9	-8.5	Isoniazid: -10.4
MtDprE1	-7.2	-8.3	-8.3	Bedaquiline: -10.1

Figure 4: Molecular interactions of the best docking positions of Thiolactomycin (blue: top left), Artemisininic acid (Orange: top right) Artemisinin (green: down left) and Deoxyartemisinin (down right).

Figure 5: Ligplot illustration of artemisinic acid and thiolactomycin with *MtKasA*. H-bonds in green dash. Carbons are in black, nitrogens in blue and oxygens in red. Red spheres are hydrophobic interactions

3. Discussion

Artemisinin, deoxyartemisinin and artemisinic acid were originated from *A. annua* L.. Artemisinin and its several derivatives are commonly used to treat malaria, primarily as part of drug combination therapies. In antimicrobial studies against *S. aureus*, deoxyartemisinin and artemisinin have been reported with a MIC of 1000 and 2000 $\mu\text{g/mL}$ respectively [9]. These MICs were higher than those reported for these compounds against other bacterial species [9]. Such results could reflect the strains that we used and also the penetration of compounds through the microorganism membrane, as we can clearly observe lower MICs with *M. smegmatis* mc²155. However, crucially, this MIC decreased 4-fold in assessments for synergy between deoxyartemisinin and artemisinic acid. The checkerboard assay, against *M. smegmatis* mc²155, proved a synergistic effect between deoxyartemisinin and artemisinic acid with FICI = 0.5 and the MIC was reduced by a factor of 4 to 125 $\mu\text{g/mL}$. These antimicrobial activities were significantly better than shown by artemisinin, including *M. smegmatis*. No anti-mycobacterial activity has previously been demonstrated for these natural products. We observed that the anti-mycobacterial activity of both Artemisia-derived natural products was not dependent on an endoperoxide bridge. Artemisinin, artesunate, artemisone, dihydroartemisinin and artemether, all with endoperoxide bridges, have shown to be effective against *S. mansoni*, through the peroxidative activity [10,11]. However, we found neither deoxyartemisinin nor artemisinic acid exhibited any effect on *S. mansoni* viability or mobility (data not shown), which could be caused by the lack of endoperoxide bridges in both compounds.

Furthermore, we investigated the anti TB potential of the compounds using a guided molecular docking approach to find a potential target. We selected some key enzymes required for *M. tuberculosis* to grow and survive within the eukaryotic host, that were involved in essential mycobacterial pathways and were absent from mammalian cells. The purpose of molecular docking is to use scoring algorithms to estimate the likelihood of the compound to bind to the protein ligand. Based on our MIC results and binding energies, it is likely that the artemisinic acid displayed a strong predicted binding towards with the *MtKasA* enzyme in comparison to artemisinin, deoxyartemisinin and its control

thiolactomycin. Figure 4 indicates that all the compounds accept artemisinin enter the binding site of thiolactomycin and artemisinic acid forms stronger hydrogen bonding in comparison to deoxyartemisinic acid and thiolactomycin. The docking studies provided strong evidence that the molecular basis for this activity is probably due to KasA inhibitor. However, this finding needs to be further investigated and validated *in vitro* via genomics and metabolomics; followed by transformation studies.

4. Materials and Methods

4.1. Plant materials:

The dried leaf material of *A. annua* was provided by Artemisinin Research Center, Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing, China in October 2016 and was identified under light microscope by Xirong He at the Institute of Chinese Materia Medica and deposited as a voucher in that institute (Voucher Number, CMM-AberUK1). The plant sample was identified using DNA barcoding.

4.2. Flash column chromatography:

Purification of compounds by flash chromatography was performed using a Biotage Flash chromatography system with SNAP C18 silica cartridges (30 g; 40–63 μ m, 60 Å) and fraction collection controlled by photo-diode array (PDA) at 250 nm.

4.3. Semi-preparative high performance liquid chromatography (Semi-preparative HPLC):

Chromatographic separation was performed on a reverse phase (RP) ACE 10 C18 250x21.2 mm using water with 0.1% formic acid as a mobile phase solvent A and methanol with 0.1% formic acid as a mobile phase solvent B. Each sample was injected using a flow rate of 10 mL/min. Column oven temperature was set to 40 °C. Data was acquired with a photo-diode array (PDA) at 250 nm (Dionex).

4.4. Ultra high performance liquid chromatography–high resolution mass spectrometry (UHPLC-HRMS):

Fractions were analyzed on an Exactive Orbitrap (Thermo Fisher Scientific) mass spectrometer, which was coupled to an Accela Ultra High Performance Liquid Chromatography (UHPLC) system (Thermo Fisher Scientific). Chromatographic separation was performed on a reverse phase (RP) Hypersil Gold C18 1.9 μ m, 2.1 × 150 mm column (Thermo Scientific) using H₂O using 0.1% formic acid (v/v, pH 2.74) as the mobile phase solvent A and ACN: isopropanol (10:90) with 10 mM ammonium acetate as mobile phase solvent B. Each sample (20 μ L) was analysed using 0–20% gradient of B from 0.5 to 1.5 min and then to 100% in 10.5 min. After 3 min isocratic at 100% B the column was re-equilibrating with 100% A for 7 min.

4.5. Spectroscopy:

UV-visible spectra were determined using the Unicam UV-500. NMR spectra were obtained using Bruker Ultra shield-500 NMR spectrophotometer (1H-NMR 500 MHz, 13C-NMR 100 MHz) using either MeOD as the solvent reference.

4.6. DNA barcoding of plant samples:

Total genomic DNA of was extracted using a DNeasy Plant Mini kit (Qiagen, UK) in accordance with the manufacturer's instructions. The *rbcL* sequences were amplified with *rbcLa*-F: 5'-ATGTCACCACAAACAGAGACTAAAGC-3' and *rbcLa*-R: 5'-GTAAATCAAGTCCACCRGC-3';

DNA barcoding primers [12]. PCR used a total volume of 20 μ L with 10 μ L BioMix (BioLine, UK) 1 μ L forward primer (10 μ M), 1 μ L reverse primer (10 μ M), and 1 μ L of the DNA template (50 ng/ μ L) and 7 μ L distilled water. PCR involved 1 cycle (94°C for 3 min), 35 cycles (94°C for 1 min, 55°C for 1 min, and 72°C for 1 min), and 1 cycle 72°C for 7 min. The resulting 600 bp band was sequenced in both directions on an ABI3730X, using the same primers as used for PCR. The derived sequence was submitted to BLAST to confirm 100% identity to *rbcL* sequences from *A. annua* vouchers. The *rbcL* sequence of CMM-AberUK1 has been submitted to Genbank (Accession Number MH051919).

4.7. Extraction and Isolation

Dried leaf material of *A. annua* was extracted sequentially using *n*-hexane, dichloromethane (DCM), and methanol (MeOH) at room temperature with continuous stirring for 24 h respectively. Bioactivity-linked fractionation of the *n*-hexane extract was based on the ability to suppress the growth of *M. smegmatis*.

The *n*-hexane extract was fractionated on a silica gel column chromatography (CC) (4×50 cm, 150 g of SiO₂), eluted with *n*-hexane-EtOAc (1:0 to 0:1, at 5% gradient, 300 mL of each eluent), and EtOAc-MeOH (1:0 to 8:1, 300 mL of each eluent) to yield 14 fractions (designated H for “*n*-hexane” and A through to N; thus HA through to HN). The fraction MICs indicated that HF displayed the highest anti-mycobacterial activity. Fraction HF was again subjected to silica CC (1.5×50 cm, 60 g of SiO₂) eluting with *n*-hexane-EtOAc (1:0 to 0:1, 5% gradient, 300 mL of each eluent) and EtOAc-MeOH (1:0 to 8:1, 300 mL of each eluent) to yield 6 crude fractions (A through F; thus HFA-HFF). The anti-mycobacterial screen suggested that fraction HFE had the highest activity (Supplementary Figure 1). The HFE fraction was then using reversed-phase flash chromatography, eluted with a methanol-water gradient resulting in 7 fractions (HFE1 through HFE7. Fractions HFE3 and HFE7 had the highest anti-mycobacterial activity. However, fraction HFE7 was not further studied due to very low yields of isolated compounds. HFE3 was then subjected to further fractionation in a Semi-preparative HPLC, to yielding 8 fractions (HFE3a through HFE3h).

All the fractions after purification was run through UHPLC-MS to analyse their mass ions. Fraction HFE3e was found to be **1** (6 mg) and HFE3e was **2** (4.5 mg) at > 95% purity. The identification of the pure compounds was made based on their accurate mass and 1D-NMR comparison to previous studies [9,13].

1: 1H-NMR (500 MHz, CDCl₃) δ 0.93 (3H, s, H-14), 0.99 (2H, m, H-8), 1.11 (1H, m, H-9), 1.19 (3H, d, J = 7.2 Hz, H-13), 1.23 (1H, m, H-2), 1.25 (1H, m, H-10), 1.27 (3H, m, H 1), 1.51 (3H, s, H-15), 1.61 (1H, m, H-3), 1.79 (2H, m, H-3' and H-9'), 1.90 (2H, m, H-2' and H-8'), 1.99 (1H, m, H-7), 3.17 (1H, m, H-11), 5.69 (1H, s, H-5) ppm; 13C-NMR (100 MHz, CDCl₃) δ 12.74 (C-13), 18.69 (C-14), 22.20 (C-2), 23.68 (C-8), 24.09 (C-15), 32.92 (C-11), 33.66 (C-9), 34.17 (C-3), 35.53 (C-10), 42.64 (C-7), 44.85 (C-1), 82.58 (C-6), 99.80 (C-5), 109.32 (C-4), 171.88 (C-12) ppm.

2: 1H-NMR (500 MHz, MeOD) δ 0.92 (3H, d, J = 6.3 Hz, H-14), 1.07 (1H, m, H-9), 1.30-1.45 (2H, m, H-3), 1.40 (3H, m, H-1 and H-8), 1.55 (1H, m, H-10), 1.59 (3H, s, H-15), 1.70-1.83 (2H, m, H-2), 1.90 (1H, m, H-9'), 1.98 (1H, m, H-6), 2.68 (2H, m, H-7), 5.02 (1H, s, H-5), 5.45 (1H, s, H-13), 6.29 (1H, s, H-13') ppm; 13C-NMR (100 MHz, MeOD) δ 20.2 (C-14), 23.8 (C-15), 26.7 (C-2), 27.1 (C-8), 27.3 (C-3), 28.9 (C-10), 36.5 (C-9), 39.4 (C-6), 43.1 (C-1), 43.7 (C-7), 121.5 (C-5), 124.6 (C-13), 135.9 (C-4), 145.3 (C-11), 170.7 (C-12) ppm.

4.8. Antimicrobial assays

The antimicrobial activity of all the extracts and isolated compounds were assessed against *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, methicillin-resistant *S. aureus* (MRSA), *Mycobacterium smegmatis* strains NCTC 333 and mc²155. Rifampicin and gentamycin sulphate were the reference antimicrobial. The checkerboard method and the fractional inhibitory concentration index (FICI) was used to determine potential synergistic, additive or even antagonistic effects of combinations of individual compounds at different concentrations as previously described [14].

4.9. In silico studies

All chemical structures were retrieved from the PubChem compound database (NCBI) (<http://www.pubchem.ncbi.nlm.nih.gov>). The crystal structures and respective controls of MtDprE1 (PDB ID: 6HEZ)[15], MtInhA (PDB ID: 1ENY) [16], MtKasA (PDB ID: 2WGE) [17], MtPanK type 1 (PDB ID: 4BFT) [18], MtPknB (PDB ID: 2FUM) [19] and MtPks13 (PDB ID: 5V3X) [20] were retrieved from the RCSB Protein Data Bank (PDB) database (<https://www.rcsb.org>).

In silico prediction of physicochemical and structural properties of the compounds was performed using PaDEL-Descriptor [21] including the descriptors: nHBAcc_Lipinski (acceptor H-bonds), nHBDOn_Lipinski (donor H-bonds), nRotB (number of rotation bonds), TopoPSA (topological polar surface area), MW (molecular weight) and XLogP (prediction of logP based on the atom-type method).

An extended PDB format, termed as a PDBQT file, was used for coordinate files which includes atomic partial charges [22]. All file conversions were performed using the open source chemical toolbox Open Babel 2.3.2 [23]. The ligand and protein structures were optimised using AutoDock Tools software (AutoDock 1.5.6) which involved adding all hydrogen atoms to the macromolecule, which is a step necessary for correct calculation of partial atomic charges. Gasteiger charges are calculated for each atom of the macromolecule in AutoDock 1.5.6 [22].

All the compounds were docked against MtDprE1, MtInhA, MtKasA, MtPanK, MtPknB, and MtPks13 respectively along with the controls. Molecular docking calculations for all compounds with each of the proteins were performed using AutoDock Vina 1.1.2. Docking calculation was generated with the software free energy binding own scoring function. The binding affinity of the ligand was observed as a negative score with units expressed as kcal.mol⁻¹. Nine different poses were calculated for each protein with the parameters num_modes = 9 and exhaustiveness = 16. The lowest energy conformation was chosen for binding model analysis. Molecular interactions between ligand and protein were generated, analysed by LigPlot+ and depicted by PyMOL. PyMOL Molecular Graphics System, Version 2.0 Schrödinger (<http://www.pymol.org>) was used to prepare figures [24].

To provide enough space for free movements of the ligands, the grid box was constructed to cover the active sites and was defined using AutoDock 1.5.6. The grid points for MtDprE1, the grid points were set to 20 × 20 × 20, at a grid center of (x,y,z) 14.99, -20.507, 37.226 with spacing of 1 Å. For MtInhA, the grid points were set to 26 × 24 × 22, at a grid center of (x,y,z) -5.111, 33.222, 13.410 with spacing of 1 Å. For MtKasA, the grid points were set to 20 × 20 × 20, at a grid center of (x,y,z) 38.342, -7.033, 13.410 with spacing of 1 Å. For MtPanK, the grid points were set to 20 × 20 × 20, at a grid center of (x,y,z) -18.742, 13.919, 11.679 with spacing of 1 Å. For MtPknB, the grid points were set to 21 × 20 × 20, at a grid center of (x,y,z) 61.518, 2.429, -25.588 with spacing of 1 Å. For MtPks13 the grid points were set to 16 × 18 × 14, at a grid center of (x,y,z) 3.954, 27.324, 8.499 with spacing of 1 Å.

5. Conclusions

Our studies have demonstrated that the anti-mycobacterial activity of *Artemisia annua* L is based on deoxyartemisinin and artemisinic acid, but not artemisinin. It clearly indicated endoperoxide

bridge-independent activity that was exhibited at least against Mycobacterium. The interaction of KasA and the compounds is to be verified based on efficacy; toxicity and pharmacokinetic properties is needed be validated experimentally. It is possible that these natural products or their target could be exploited in the search for new anti-TB drugs.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1.

Figure S 1: Bioassay guided purification of *A. annua*

Figure S 2: Screening for anthelmintic activity against *Schistosoma mansoni* schistosomula. Using the Roboworm platform, anthelmintic activity of deoxyartemisinin (C1) and artemisinic acid (C2) were compared to those of Auranofin (AUR, 10 μ M), Praziquantel (PZQ, 10 μ M) and the negative control dimethylsulphoxide (DMSO). (A) Images of schistosomula at 72 h following treatments. Schistosomula treated with AUR and PZQ exhibited obvious phenotypic changes compared to DMSO. Treatments (two-fold dilutions; 10 μ M-0.625 μ M) with C1 and C2 showed no obvious effect. Viability for (B) C1 and (C) C2 treatments as well as assessments of motility for (D) C1 and (E) C2 were quantified. All results were tested for significance using one-way ANOVA with a Dunn's post-test. Results falling within the "hit zone" were significantly ($P < 0.05$) from negative DMSO treated controls. In no case were significant effects for C1 and C2 observed.

Figure S 3: Physiochemical properties of Artemisinin, Artemisinic acid, Deoxyartemisinin and thiolactomycin analysed by PaDel-Descriptor.

Figure S 4: Superposition of the best docking positions of Deoxyartemisinin (Red), artemisinin(green), Artemisinic acid (orange), Thiolactomycin (Blue) against KasA

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Conflicts of Interest: The authors declare that there is no conflict of interest

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SUPPLEMENTARY MATERIALS

Extracts	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Mycobacterium smegmatis</i>	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	<i>Candida albicans</i>
<i>n</i> -Hexane	≥1	0.5	0.5	≥1	≥0.5
Dichloromethane	≥1	≥1	≥1	≥1	≥0.5
Methanol	≥1	≥1	≥1	≥1	≥0.5

Extracts	<i>Mycobacterium smegmatis</i>
HFE1	0.25
HFE2	0.25
HFE3	0.125
HFE4	0.25
HFE5	0.5
HFE6	0.5
HFE7	0.125

Extracts	<i>Mycobacterium smegmatis</i>
HA-HC	Not soluble
HD-HE	0.25
HF	0.125
HG-HJ	0.5
HK	≥1
HL-HM	0.5
HN	≥1

Extracts	<i>Mycobacterium smegmatis</i>
HFA-HFD	Not soluble
HFE	0.25
HFF	0.5

Figure S 5: Bioassay guided purification of *A.annua*

Figure S 6: Screening for anthelmintic activity against *Schistosoma mansoni* schistosomula. Using the Roboworm platform, anthelmintic activity of deoxyartemisinin (C1) and arteminisc acid (C2) were compared to those of Auranofin (AUR, 10 μ M), Praziquantel (PZQ, 10 μ M) and the negative control dimethylsulphoxide (DMSO). (A) Images of schistosomula at 72 h following treatments. Schistosomula treated with AUR and PZQ exhibited obvious phenotypic changes compared to DMSO. Treatments (two-fold dilutions; 10 μ M-0.625 μ M) with C1 and C2 showed no obvious effect. Viability for (B) C1 and (C) C2 treatments as well as assessments of motility for (D) C1 and (E) C2 were quantified. All results were tested for significance using one-way ANOVA with a Dunn's

post-test. Results falling within the “hit zone” were significantly ($P < 0.05$) from negative DMSO treated controls. In no case were significant effects for C1 and C2 observed.

Name	nHBacc	nHBacc2	nHBacc3	nHBacc_Lipinski	nHBDon	nHBDon_Lipinski	nRotB	RotBFrac	nRotBt	RotBtFrac	LipinskiFailures	TopoPSA	MW	AMW	XLogP
Artemisinic acid	2	2	2	2	1	1	2	0.111111	5	0.277778	0	37.3	234.162	6.004153	4.592
Artemisinin	5	5	5	5	0	0	0	0	3	0.130435	0	53.99	282.1467	6.717779	3.039
Deoxyartemisinin	4	4	4	4	0	0	0	0	3	0.136364	0	44.76	266.1518	6.491508	2.913
Thiolactomycin	2	2	2	2	1	1	2	0.142857	6	0.428571	0	62.6	210.0715	7.502552	2.458

Figure S 7: *Physicochemical properties of Artemisinin, Artemisinic acid, Deoxyartemisinin and thiolactomycin analysed by PaDel-Descriptor*

Figure S 8: *Superposition of the best docking positions of Deoxyartemisinin (Red), artemisinin(green), Artemisinic acid (orange), Thiolactomycin (Blue) against KasA topological polar surface area (TopoPSA)*

Name	nHBAcc	nHBAcc ₂	nHBAcc ₃	nHBAcc _{Lipinski}	nHBDDon	nHBDDon_Lipinski	nRotB	RotBFrac	nRotBt	RotBtFrac	Lipinski Failures	TopoPSA	MW	AMW	XLogP
Artemisinic acid	2	2	2	2	1	1	2	0.111111	5	0.277778	0	37.3	234.162	6.004153	4.592
Artemisinin	5	5	5	5	0	0	0	0	3	0.130435	0	53.99	282.1467	6.717779	3.039
Deoxyartemisinin	4	4	4	4	0	0	0	0	3	0.136364	0	44.76	266.1518	6.491508	2.913
Thiolactomycin	2	2	2	2	1	1	2	0.142857	6	0.428571	0	62.6	210.0715	7.502552	2.458

2

3 Table 3: The physiochemical properties using PaDel-Descriptor: molecular weight (MW), partition coefficient (xLogP), rotatable bonds (nRotB), H-bond
4 donors (nHBDDon_Lipinski), H-bond acceptors (nHBAcc_Lipinski) and t