Supporting Information to

Screening and HPLC-Based Activity Profiling for New Antiprotozoal Leads from European Plants

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Analytical Methods
### Tab. S1. Antiplasmodial activity (growth inhibition in % ± standard deviation [SD]) of 254 plant extracts against Plasmodium falciparum. Bioassays were carried out in duplicate of three independent experiments, at test concentrations of 4.81 µg/mL and 0.81 µg/mL, respectively. The positive control was artesunate (100% inhibition in all bioassays).

| Plant Family | Plant species | Historical source | Voucher | Plant part | Growth inhib. at 4.81 µg/mL | Growth inhib. at 0.81 µg/mL |
|--------------|--------------|-------------------|---------|-----------|-----------------------------|-----------------------------|
| Adoxaceae    | Sambucus ebulus L. | Br., Lo., Ta.2 | A | leaves | 80.1 ± 2.8 | 65.3 ± 3.5 |
|              | Sambucus nigra L. | Lo., Ta.2 | A | flowers | 7.8 ± 6.2 | 6.8 ± 5.2 |
|              | Amaryllidaceae Allium ursinum L. | – | A | petals | 7.9 ± 7.6 | 6.5 ± 4.8 |
|              | Apiaceae Angelica archangelica L. | Ta.2 | A | fruits | 16.0 ± 9.6 | 12.7 ± 9.0 |
|              | Angelica sylvestris L. | Ta.2 | B | leaves | 34.0 ± 6.0 | 29.2 ± 4.5 |
|              | Coriandrum sativum L. | Br., Ta.2 | A | seed | 6.3 ± 5.0 | 5.0 ± 3.8 |
|              | Eryngium campestre L. | Ma., Ta.1, Ta.2 | A | roots | 36.9 ± 11.7 | 34.4 ± 10.0 |
|              | Apioidea Foeniculum vulgare Mill. subsp. vulgare var. dulce (Mill.) | Ta.2 | A | roots | 15.7 ± 10.0 | 9.0 ± 5.0 |
|              | Peucedanum ostruthium (L.) Koch | Bo., Br., Ma., Zw. | A | rhizome | 16.4 ± 4.2 | 2.2 ± 1.9 |
| Asparagaceae | Asparagus officinalis L. | Ta.2, Zw. | A | roots | 26.4 ± 10.3 | 15.8 ± 6.1 |
|              | Aspleniacae Asplenium scolopendrium L. | Bo., Lo., Zw. | B | roots | 7.8 ± 5.9 | 5.0 ± 3.8 |
| Asteraceae   | Achillea millefolium L. | Ta.2 | A | roots | 48.4 ± 4.1 | 32.9 ± 4.4 |
|              | Achillea moschata Wulfen | – | A | roots | 42.6 ± 17.2 | 29.2 ± 17.4 |
|              | Anacyclus pyrethrum (L.) | Bo., Ma., Ta.2, Zw. | A | roots | 15.0 ± 6.1 | 10.2 ± 4.1 |

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### Tab. S1. (Cont.)

| Plant Family | Plant species | Historical source | Voucher specimen | Plant part | extract solvent | growth inhib. \( \pm 81 \text{ pg/mL} \) | growth inhib. \( \pm 81 \) SD \% |
|--------------|---------------|-------------------|-----------------|------------|-----------------|--------------------------------------|----------------------------------|
| Asteraceae   | Arctium lappa L. | Zw. | A | P01472 leaves | PE | 17.4 ± 1.5 | 2.9 ± 2.9 |
|              |                |                  |                | P01473 leaves | EtOAc | 33.3 ± 25.7 | 0.0 ± 0.0 |
|              |                |                  |                | P01474 leaves | MeOH | 8.9 ± 8.1 | 2.3 ± 3.2 |
|              |                |                  |                | P01475 roots | PE | 50.5 ± 3.0 | 54.7 ± 2.8 |
|              |                |                  |                | P01476 roots | EtOAc | 23.3 ± 4.0 | 11.2 ± 2.0 |
|              |                |                  |                | P01477 roots | MeOH | 4.1 ± 3.0 | 0.3 ± 0.5 |
|              | Arctium nemorosum Lej. | – | B | S20010 aer. pts | MeOH | 53.6 ± 24.8 | 21.8 ± 29.1 |
|              |                |                  |                | S20011 leaves | EtOAc | 16.4 ± 3.9 | 12.8 ± 2.9 |
|              |                |                  |                | S20012 leaves | EToAc | 99.1 ± 0.4 | 14.1 ± 10.0 |
|              |                |                  |                | S20013 leaves | MeOH | 16.5 ± 3.2 | 4.3 ± 3.2 |
|              |                |                  |                | S20014 fruits | PE | 5.3 ± 3.8 | 2.3 ± 3.3 |
|              |                |                  |                | S20015 fruits | EToAc | 55.6 ± 4.8 | 3.1 ± 4.2 |
|              |                |                  |                | S20016 fruits | MeOH | 5.8 ± 3.5 | 0.0 ± 0.0 |
|              |                |                  |                | S20017 hollow stem | PE | 2.7 ± 2.0 | 1.7 ± 2.4 |
| Asteraceae   | Arnica montana L. | – | B | P01451 flowers | EtOAc | 18.2 ± 3.8 | 6.4 ± 4.7 |
|              |                |                  |                | P01452 flowers | EToAc | 30.7 ± 6.8 | 4.0 ± 5.7 |
|              |                |                  |                | P01453 flowers | MeOH | 5.7 ± 4.4 | 0.0 ± 0.0 |
|              |                |                  |                | P01454 roots | PE | 23.1 ± 0.9 | 14.1 ± 1.3 |
|              |                |                  |                | P01455 roots | EToAc | 36.1 ± 3.2 | 5.9 ± 8.3 |
|              |                |                  |                | P01456 roots | MeOH | 13.5 ± 8.0 | 5.6 ± 4.0 |
|              | Artemisia abrotanum L. | Bo., Ma., Ta.2 | A | P01433 aer. pts | PE | 50.2 ± 2.4 | 23.5 ± 8.5 |
|              |                |                  |                | P01434 aer. pts | EToAc | 69.3 ± 3.4 | 18.3 ± 7.5 |
|              |                |                  |                | P01435 aer. pts | MeOH | 17.8 ± 11.2 | 8.5 ± 6.0 |
|              | Artemisia absinthium L. | Ma., Ta.2, Zw. | B | P01672 leaves | PE | 67.9 ± 11.2 | 5.8 ± 3.8 |
|              |                |                  |                | P01673 leaves | EToAc | 55.7 ± 14.5 | 3.8 ± 4.9 |
|              |                |                  |                | P01674 leaves | MeOH | 6.3 ± 7.4 | 1.0 ± 1.5 |
|              | Artemisia dracunculus L. | – | A | P01545 aer. pts | PE | 16.5 ± 3.2 | 0.5 ± 0.7 |
|              |                |                  |                | P01546 aer. pts | EToAc | 4.3 ± 3.0 | 7.1 ± 5.0 |
|              |                |                  |                | P01544 aer. pts | MeOH | 24.5 ± 3.2 | 1.7 ± 1.8 |
| Asteraceae   | Artemisia vulgaris L. | Ta.2 | A | P01457 aer. pts | PE | 18.9 ± 7.0 | 6.3 ± 2.7 |
|              |                |                  |                | P01458 aer. pts | EToAc | 41.9 ± 11.6 | 0.0 ± 0.0 |
|              |                |                  |                | P01459 aer. pts | MeOH | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |                | P01460 roots | PE | 16.5 ± 5.9 | 5.2 ± 4.1 |
|              |                |                  |                | P01461 roots | EToAc | 44.1 ± 9.7 | 5.0 ± 4.3 |
|              |                |                  |                | P01462 roots | MeOH | 17.3 ± 3.8 | 6.6 ± 5.7 |
|              | Carthamus tinctorius L. | – | A | P01502 flowers | EToAc | 9.1 ± 9.6 | 1.2 ± 1.7 |
|              |                |                  |                | P01503 flowers | EToAc | 10.3 ± 2.1 | 0.4 ± 0.6 |
|              |                |                  |                | P01504 flowers | MeOH | 9.0 ± 6.7 | 1.0 ± 1.5 |
|              | Centaurea cyanus L. | Bo., Lo. | A | P01527 flowers | PE | 54.8 ± 9.1 | 22.3 ± 3.5 |
|              |                |                  |                | P01528 flowers | EToAc | 19.2 ± 8.0 | 9.6 ± 6.3 |
|              |                |                  |                | P01526 flowers | MeOH | 28.0 ± 6.6 | 26.4 ± 7.5 |
|              | Centaurea montana L. | – | B | P01648 flowers | PE | 40.7 ± 5.8 | 0.0 ± 0.0 |
|              |                |                  |                | P01649 flowers | EToAc | 16.6 ± 5.3 | 18.2 ± 25.7 |
|              |                |                  |                | P01650 flowers | MeOH | 16.5 ± 2.6 | 0.0 ± 0.1 |
|              |                |                  |                | P01651 leaves | PE | 25.3 ± 8.7 | 6.5 ± 3.8 |
|              |                |                  |                | P01652 leaves | EToAc | 10.0 ± 4.6 | 0.0 ± 0.0 |
|              |                |                  |                | P01653 leaves | MeOH | 8.4 ± 2.7 | 1.1 ± 1.1 |
|              | Cichorium intybus L. | Bo., Ta.2, Zw. | A | P01514 roots | PE | 6.0 ± 4.4 | 0.0 ± 0.4 |
|              |                |                  |                | P01515 roots | EToAc | 14.2 ± 1.9 | 2.0 ± 1.4 |
|              |                |                  |                | P01516 roots | MeOH | 1.8 ± 2.3 | 0.0 ± 0.0 |
|              |                |                  |                | P01517 aer. pts | PE | 9.5 ± 1.9 | 0.0 ± 0.0 |
|              |                |                  |                | P01518 aer. pts | EToAc | 18.2 ± 11.4 | 0.1 ± 0.2 |
|              |                |                  |                | P01519 aer. pts | MeOH | 6.5 ± 6.6 | 0.0 ± 0.0 |
|              |                |                  |                | P01657 aer. pts | PE | 9.7 ± 8.6 | 0.0 ± 0.0 |
|              |                |                  |                | P01658 aer. pts | EToAc | 9.7 ± 13.7 | 0.0 ± 0.0 |
|              |                |                  |                | P01659 aer. pts | MeOH | 3.5 ± 3.3 | 0.6 ± 0.8 |
|              |                |                  |                | P01660 roots | PE | 15.0 ± 12.3 | 4.5 ± 6.3 |
|              |                |                  |                | P01661 roots | EToAc | 21.0 ± 3.1 | 1.1 ± 1.0 |
|              |                |                  |                | P01662 roots | MeOH | 12.1 ± 3.6 | 3.9 ± 3.7 |
Tab. S1.  (Cont).

| Plant Family | Plant species | Historical source | Src. | Voucher specimen | Plant part | extract solvent | growth inhib. at 4.81 µg/mL ± SD | growth inhib. at 0.81 µg/mL ± SD |
|--------------|---------------|-------------------|------|------------------|------------|----------------|----------------------------------|----------------------------------|
| Asteraceae   | Echinacea angustifolia DC. | – | A | P01551 | roots | PE | 20.8 ± 6.9 | 4.2 ± 4.0 |
|             | Echinacea purpurea (L.) Moench | – | A | P01552 | roots | EtOAc | 8.9 ± 6.1 | 1.3 ± 1.9 |
|             |               | A | P01555 | roots | EtOH | 29.2 ± 12.8 | 0.0 ± 0.0 |
|             |               | A | P01553 | roots | MeOH | 19.0 ± 7.4 | 0.3 ± 0.2 |
|             | Eupatorium cannabinum L. | Ma. | A | P01569 | roots | EtOAc | 23.3 ± 6.5 | 0.0 ± 0.0 |
|             |               | A | P01570 | roots | EtOAc | 21.9 ± 2.9 | 10.1 ± 5.2 |
|             |               | A | P01571 | roots | EtOAc | 16.2 ± 6.8 | 1.4 ± 0.9 |
|             |               | B | S20023 | roots | MeOH | 38.7 ± 9.6 | 5.2 ± 4.1 |
|             |               | S20024 | roots | EtOAc | 11.2 ± 9.1 | 0.9 ± 0.6 |
|             |               | B | S20025 | roots | EtOAc | 17.4 ± 4.0 | 0.0 ± 0.0 |
|             |               | A | S20026 | roots | EtOAc | 31.6 ± 5.2 | 5.6 ± 5.3 |
|             |               | A | S20027 | leaves + flowers | EtOAc | 16.4 ± 10.2 | 0.0 ± 0.0 |
|             | Silybum marianum (L.) Gaerth. | – | A | P01490 | aers. pts. | PE | 11.4 ± 9.4 | 0.0 ± 0.0 |
|             |               | A | P01491 | aers. pts. | EtOAc | 19.8 ± 7.3 | 0.0 ± 0.0 |
|             |               | A | P01492 | aers. pts. | EtOAc | 6.8 ± 7.1 | 0.0 ± 0.0 |
|             |               | A | P01493 | fruits | EtOAc | 8.0 ± 5.1 | 11.0 ± 2.5 |
|             |               | A | P01494 | fruits | EtOAc | 24.7 ± 5.5 | 0.0 ± 0.0 |
|             |               | A | P01495 | fruits | EtOAc | 23.9 ± 9.5 | 0.5 ± 0.8 |
|             | Asteraeae Tanacetum parthenium L. | Br., Lo., Zw. | A | P01511 | aers. pts. | PE | 21.9 ± 10.3 | 0.0 ± 0.0 |
|             |               | A | P01512 | aers. pts. | EtOAc | 37.6 ± 11.4 | 0.0 ± 0.0 |
|             | Brassicaceae Armoracia rusticana | Bo., Lo., Ta.2, Zw. | A | P01513 | aers. pts. | MeOH | 3.6 ± 5.1 | 0.4 ± 0.5 |
|             | Nasturtium officinale R. Br. | Zw. | A | P01510 | aers. pts. | EtOAc | 29.8 ± 11.4 | 9.3 ± 3.8 |
|             | Cannabaceae Humulus lupulus L. | Br., Lo., Ta.2 | A | P01504 | flowers | PE | 10.7 ± 4.5 | 2.1 ± 2.9 |
|             |               | A | P01602 | aers. pts. | PE | 26.1 ± 3.8 | 5.2 ± 5.0 |
|             |               | A | P01603 | aers. pts. | EtOAc | 7.3 ± 5.2 | 4.6 ± 3.8 |
|             |               | A | P01601 | aers. pts. | MeOH | 30.7 ± 12.7 | 8.5 ± 2.0 |
|             | Caryophyllaceae Gypsophila muralis L. | – | B | P01634 | aers. pts. | PE | 4.2 ± 3.5 | 0.0 ± 0.0 |
|             |               | A | P01635 | aers. pts. | EtOAc | 10.9 ± 8.1 | 8.5 ± 12.0 |
|             |               | A | P01636 | aers. pts. | MeOH | 4.7 ± 1.6 | 0.0 ± 0.0 |
|             | Clusiaceae Hypericum perforatum L. | Bo., Fu., Lo., Ma., Ta.2 | A | P01670 | aers. pts. | EtOAc | 69.3 ± 17.2 | 32.3 ± 40.0 |
|             |               | A | P01671 | aers. pts. | EtOAc | 97.5 ± 12.7 | 20.0 ± 1.3 |
|             |               | A | P01691 | aers. pts. | MeOH | 16.8 ± 10.8 | 1.6 ± 2.3 |
|             | Cucurbitaceae Bryonia alba L. | – | A | P01487 | roots | PE | 18.0 ± 6.8 | 0.8 ± 0.6 |
|             |               | A | P01488 | roots | EtOAc | 13.7 ± 10.1 | 0.0 ± 0.0 |
|             |               | A | P01489 | roots | MeOH | 9.2 ± 5.6 | 3.1 ± 2.2 |
|             | Ericaceae Arbutus unedo L. | – | A | P01445 | roots | PE | 19.1 ± 7.8 | 0.0 ± 0.0 |
|             |               | A | P01446 | roots | EtOAc | 8.8 ± 10.4 | 0.7 ± 0.6 |
|             | Euphorbiaceae Euphorbia cyparissias L. | – | A | P01447 | roots | MeOH | 4.4 ± 6.2 | 1.5 ± 2.1 |
|             |               | A | P01572 | aers. pts. | PE | 11.0 ± 9.3 | 0.0 ± 0.0 |
|             |               | A | P01573 | aers. pts. | EtOAc | 18.4 ± 11.5 | 0.0 ± 0.0 |
|             |               | A | P01571 | aers. pts. | MeOH | 10.2 ± 7.3 | 0.0 ± 0.0 |
|             | Fabaceae Anthyllis vulneraria L. | – | A | P01439 | flowers | PE | 20.7 ± 5.0 | 13.5 ± 5.1 |
|             |               | A | P01440 | flowers | EtOAc | 20.8 ± 14.7 | 8.9 ± 5.8 |
|             |               | A | P01441 | flowers | MeOH | 15.8 ± 12.5 | 7.3 ± 3.7 |
|             | Robinia pseudoacacia L. | – | A | P01466 | flowers | PE | 26.7 ± 5.2 | 7.5 ± 1.8 |
|             |               | A | P01467 | flowers | EtOAc | 39.1 ± 5.6 | 8.0 ± 7.3 |
|             |               | A | P01468 | flowers | MeOH | 0.0 ± 0.0 | 0.0 ± 0.0 |
|             | Gentianaceae Centaurium erythraea Rafn. | Ma., Ta.1, Ta.2, Zw. | A | P01654 | aers. pts. | PE | 9.1 ± 6.5 | 5.4 ± 1.7 |
|             |               | A | P01655 | aers. pts. | EtOAc | 38.9 ± 4.2 | 0.0 ± 0.0 |
|             |               | A | P01656 | aers. pts. | MeOH | 3.6 ± 2.8 | 2.3 ± 3.2 |
|             |               | A | P01642 | roots | PE | 4.4 ± 2.1 | 7.9 ± 10.8 |
|             | Lamiaceae Galeopsis segetum Neck. | – | A | P01578 | aers. pts. | EtOAc | 18.1 ± 10.1 | 0.0 ± 0.0 |
|             |               | A | P01579 | aers. pts. | EtOAc | 7.6 ± 6.9 | 1.6 ± 2.2 |
|             |               | A | P01577 | aers. pts. | MeOH | 23.7 ± 7.6 | 2.4 ± 3.4 |
Tab. S1. (Cont).

| Plant Family | Plant species | Historical source | Src. | Voucher specimen | Plant part | extract solvent | growth inhib. at 4.81 µg/mL ± SD | growth inhib. at 0.81 µg/mL ± SD |
|--------------|---------------|-------------------|------|-----------------|------------|----------------|---------------------------------|---------------------------------|
| Lamiaceae    | Hyssopus officinalis L. | Ma.  | A | P01584 | aer. pts. | PE | 4.8 ± 3.6 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01585 | aer. pts. | EtOAc | 6.1 ± 3.1 | 3.7 ± 1.2 | 1.3 ± 0.2 |
|              | Nepeta cataria L.  | Br.  | A | P01605 | aer. pts. | PE | 3.2 ± 1.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01606 | aer. pts. | EtOAc | 6.4 ± 3.8 | 9.3 ± 2.3 | 1.6 ± 0.3 |
|              | Origanum dictamnus L. | –   | A | P01542 | aer. pts. | PE | 16.4 ± 7.2 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01543 | aer. pts. | EtOAc | 3.3 ± 1.8 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              | Origanum vulgare L.  | –   | A | P01607 | aer. pts. | PE | 57.7 ± 15.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              | Stachys officinalis (L.) Trev. | Br.  | A | P01478 | aer. pts. | PE | 30.5 ± 17.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01479 | aer. pts. | EtOAc | 30.5 ± 17.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01480 | aer. pts. | MeOH | 4.2 ± 5.8 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Piperaeae    | Piper cubeba L.F. | –   | A | P01520 | fruits | PE | 34.9 ± 2.4 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Polygonaceae | Bistorta officinalis Delarbre | –   | A | P01481 | aer. pts. | PE | 7.1 ± 8.1 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01482 | aer. pts. | EtOAc | 23.3 ± 6.8 | 4.2 ± 3.0 | 1.3 ± 0.2 |
|              |                |                  |     | P01483 | aer. pts. | MeOH | 14.1 ± 5.7 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01484 | roots | PE | 11.1 ± 8.2 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01485 | roots | EtOAc | 12.6 ± 11.2 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Ranunculaceae | Aquilegia vulgaris L. | –   | A | P01442 | aer. pts. | PE | 18.1 ± 3.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01443 | aer. pts. | EtOAc | 42.7 ± 7.4 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01444 | aer. pts. | MeOH | 18.7 ± 2.3 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Rosaceae     | Alchemilla alpina L. | –   | A | P01533 | aer. pts. | PE | 67.3 ± 2.9 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01534 | aer. pts. | EtOAc | 12.6 ± 3.6 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01535 | aer. pts. | MeOH | 27.6 ± 5.4 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              | Alchemilla vulgaris L. em. Fröhner | –   | A | P01563 | aer. pts. | PE | 43.3 ± 2.6 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01564 | aer. pts. | EtOAc | 11.4 ± 0.6 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              | Agrimonia eupatoria L. | Br., Lo., | A | P01699 | aer. pts. | PE | 47.9 ± 6.1 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |              Ma., Ta.2, Zw. |     | P01700 | aer. pts. | EtOAc | 20.4 ± 4.7 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              | Geum urbanum L. | –   | A | P01701 | roots | MeOH | 53.6 ± 17.6 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01505 | roots | PE | 8.6 ± 1.9 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01506 | roots | EtOAc | 14.7 ± 11.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              | Potentilla erecta (L.) Raeusch. | Br., Bo., Lo., | A | P01698 | roots | PE | 7.6 ± 5.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                | Ta.2 |     | P01697 | roots | EtOAc | 23.8 ± 1.8 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              | Potentilla anserina L. | –   | A | P01436 | aer. pts. | PE | 16.9 ± 7.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01437 | aer. pts. | EtOAc | 16.7 ± 15.9 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              | Potentilla aurea L. | ZW. | A | P01687 | leaves | PE | 11.1 ± 5.3 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01688 | leaves | EtOAc | 18.5 ± 16.8 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Rubiaceae    | Galium odoratum (L.) Scop. | –   | A | P01469 | aer. pts. | PE | 8.5 ± 3.7 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01470 | aer. pts. | EtOAc | 21.8 ± 4.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              | Verbena officinalis L. | Bo., Ta.2, Zw. | B | P01690 | aer. pts. | PE | 8.8 ± 8.7 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01691 | aer. pts. | EtOAc | 40.9 ± 16.8 | 0.0 ± 0.0 | 0.0 ± 0.0 |
|              |                |                  |     | P01692 | aer. pts. | MeOH | 22.5 ± 19.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |

Source A: Plants were obtained from Dixa (St. Gallen, Switzerland).
Source B: Plants were collected in and around Basel by Dr. M. Adams in the summer of 2010.

Analytical methods

TLC

Thin layer chromatography plates (TLC silica gel 60 F254) were from Merck (Darmstadt, Germany). Mobile phase: ethyl acetate/n-heptane 30:70. Detection was done in a UV

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chamber at 254 and 366 nm. Spots were also visualised with anis aldehyde-sulphuric acid reagent, which was prepared according to Wagner and Bladt [21].

**HPLC ESI-MS**

For micro fractionation and analysis of extracts an HPLC system consisting of a 1100 series low-pressure mixing pump with degasser module, column oven, and a 1100 series PDA detector (all Agilent, Waldbronn, Germany) was used. A Gilson 215 liquid handler with Gilson 819 injection module and 50 µl loop served as autosampler (Gilson; Mettmenstetten, Switzerland). The HPLC was coupled to an Esquire 3000 Plus ion trap mass spectrometer equipped with an electrospray (ESI) interface (Bruker Daltonics; Bremen, Germany). The MS parameters were as follows: Spectra were recorded under ion charge control conditions (ICCD 30 000) at a scan speed of 30 000 m/z/s with a Gauss filter with of 0.2 m/z. Nitrogen was used as a drying gas a flow rate of 10 L/min and as nebulising gas at a pressure of 30 psi. The nebulizer temperature was set 300 ° C. In the positive ion mode spectra were detected from 150–1500 m/z. Capillary voltage was set at -4500 V, endplate offset at -500 V, capillary exit at 109.8 V, skimmer voltage at 65.0 V, and trap drive at 39.8. The negative ion mode was also recorded from 150–1500 m/z. Capillary voltage was set at 4500 V, endplate offset at -500 V, capillary exit at -111.8 V, skimmer voltage at -40 V, and trap drive at 43.7. A SunFire RP-18, 3.5 µm, 3 x 150 mm (Waters GmbH, Eschborn, Germany) was used for HPLC ESI-MS. A gradient consisting of A (H₂O + 0.1% formic acid) and B (acetonitrile + 0.1% formic acid) was used, starting at 90% A–10 % B and leading to 0% A–100% B in in 30 min, followed 100% B for 5 minutes. The flow rate was 0.5mL/min. Data acquisition and processing for HPLC system was performed using HyStar 3.0. software (Bruker Daltonics).

**MPLC**

A Büchi Sepacore system consisting of a control unit C-620, a fraction collector C-660, an UV photometer C-635, and two pump modules C-605 was used, with the following method. The column consisted of a cartridge (Büchi, ø 40 x150 mm) containing pressed silica gel (Silica gel 60, 0.040-0.063 mm, Merck, Darmstadt, Germany). A gradient system was used consisting of A (heptane) and B (ethyl acetate), starting at 100 % A and 0% B, and leading to 70% A and 30% B in 33 minutes, then to 20 % A and 80 % B in 31.5 minutes. The flow rate was 30 mL/min. Fractions were collected every 30 seconds. The sample was dissolved in A:B 1:1 at a concentration of 50 mg/mL and the injection volume was 10 ml.

**Semi-preparative HPLC**

Semi-preparative HPLC was done on an Agilent 1100 series HPLC system consisting of an 1100 series quaternary low-pressure mixing pump with degasser module, column oven, and a 1100 series PDA detector with a 1000 µL loop.) using a SunFire prep RP-18 column (5 µm, 10 x 150 mm, Waters GmbH, Eschborn, Germany). A gradient starting at 85% A (H₂O + 0.1% formic acid) and 15% B (acetonitrile + 0.1% formic acid) and leading to 40% A and 60% B in 15 minutes, then to 100 % B in another 5 minutes. Finally the column was flushed with 100% B for 7 minutes. The flow rate was 5 mL/min. The sample was dissolved in MeOH at a concentration of 50 mg/mL and the injection volume was 300 µl.
Preparative HPLC

Preparative HPLC was done on a SCL-10, HPLC system from Shimadzu (Kyoto, Japan). A SunFire™ prep C18 OBD™ (5 µm, 30x 150 mm, Waters, Ireland) was used. The gradient was isocratic for 30 min and consisted of acetonitrile:H₂O 1:1 at a flow rate of 30 ml/min. UV data were recorded from 220 to 500 nm. The samples were dissolved in acetonitrile at a concentration of 100 mg/ml and the injection volume was 300 µl.

High resolution Mass Spectrometry (micrOTOF)

High-resolution mass spectra were obtained on a micrOTOF ESI-MS system (Bruker Daltonics) connected to an Agilent 1100 series HPLC. Data acquisition and processing was performed using HyStar 3.0 software (Bruker Daltonics). Conditions for LC-TOF MS were as follows: spectra were recorded in the range of m/z 150–1500 in positive mode. Nitrogen was used as a nebulising gas at a pressure of 2.0 bar and as a drying gas at a flow rate of 9.0 L/min (dry gas temperature 240 °C). Capillary voltage was at 4500 V, endplate offset at -50 V, hexapole at 250.0 Vpp, skimmer 1 at -50 V and skimmer 2 at -22.5 V. Instrument calibration was performed using a reference solution of sodium formiate 0.1% in isopropanol / water (1:1) containing 5 mM sodium hydroxide. Typical mass accuracy was ±2 ppm. The spectra were recorded in negative and positive mode in the range of m/z 150–1500.

NMR

NMR data were acquired at target temperature 18°C on a Bruker Avance III™ 500 MHz spectrometer (Bruker, Fällanden, Switzerland) operating at 500.13 MHz for ¹H, and 125.77 MHz for ¹³C. A 1mm TXI microprobe with a z-gradient was used for ¹H-detected experiments; ¹³C-NMR spectra were recorded with a 5 mm BBO probe head with z-gradient. NMR experiments were done as previously described [22]. For processing and evaluation Topspin 2.0 was used.

Bioassays

a. In vitro test against Trypanosoma brucei rhodesiense

Trypanosoma brucei rhodesiense (STIB 900) were grown in axenic medium as previously described [23]. The compounds were tested using a modified Alamar Blue assay protocol [24] to determine the 50% inhibitory concentration (IC₅₀). Serial threefold drug dilutions were prepared in 96-well micro titer plates and 50 µl of T. b. rhodesiense STIB 900 bloodstream forms were added to each well except for the negative controls. Melarsoprol (Arsobal®, Sanofi-Aventis, Meyrin, Switzerland) was used as a reference drug. After 70 h of incubation Alamar blue marker (12.5 mg resazurin dissolved in 100 mL distilled water) was added. The plates were then incubated for an additional 2 to 5 h. A Spectramax Gemini XS micro plate fluorescence reader (Molecular Devices Cooperation, Sunnyvale, CA) with an excitation wavelength of 536 nm and an emission wavelength of 588 nm was used to read the plates. The IC₅₀ values were calculated from the sigmoidal growth inhibition curves using Softmax Pro software (Molecular Devices).

b. In vitro testing against Plasmodium falciparum

A modification of the [³H]-hypoxanthine incorporation assay was used to determine the intra-erythrocytic antiplasmodial activity (Des Jardins 1979) of the extract library and
purified compounds in 96 well plates. Chloroquine (Sigma-Aldrich) and artesunate (Mepha, Switzerland) were used as standard drugs. Briefly, infected human red blood cells in RPMI 1640 medium (100 µL per well with 2.5% haematocrit and 0.3% parasitaemia) were exposed to twofold serial drug dilutions in 96-well micro titer plates. After 48 h incubation, 0.5 μCi [³H]-hypoxanthine was added to each well. The plates were incubated for further 24 h before being harvested using a Betaplate cell harvester (Wallac, Zürich, Switzerland) The radioactivity was counted with a Betaplate liquid scintillation counter (Wallac) as counts per minute per well at each drug concentration and compared to the untreated controls. IC₅₀ values were calculated from sigmoidal inhibition curves using Microsoft Excel. All assays were run in duplicate and repeated three times [25].

c. In vitro cytotoxicity testing

Cytotoxicity was assessed using a similar Alamar Blue assay protocol [23] whereby 4000 rat myoblast cells/well were seeded in RPMI 1640 medium. All following steps were according to the T. b. rhodesiense protocol. Podophyllotoxin (Sigma-Aldrich) was used as the reference drug.

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