Supplementary Material

Synthesis and Trypanocidal Activity of Substituted 2,4-Diarylquinoline Derivatives

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II. Bioassay protocols and data

Cytotoxicity determination
To assess the overt cytotoxicity of the compounds, they were incubated at a fixed concentration of 20 µM in 96-well plates containing HeLa (human cervix adenocarcinoma) cells for 48 hours. After the incubation, residual cell viability was determined using a resazurin assay, as previously described (Veale and Hoppe, 2018). Results were expressed as % cell viability – the resorufin fluorescence in compound-treated wells relative to untreated controls, after subtracting background readings obtained from wells without cells. Compounds were tested in duplicate wells. Emetine was used as a control drug standard.

Anti-trypanosomal Assay
To assess trypanocidal activity, compounds were incubated with in vitro cultures of T. b. brucei (strain 427) in 96-well plates at a fixed concentration of 20 µM or as 3-fold serial dilutions, and parasite viability assessed by a resazurin assay as described previously (Veale and Hoppe). Pentamidine was used a control drug standard.

Veale C.G.L., Hoppe H.C. Med. Chem. Commun. 2018, 9, 2037.

The following key correlates compound numbers in the paper with the sample codes in the following Bioassay reports

4a - AKO-Q-18
4b - AKO-Q-19
4c - sinalo 3
4d - AKO-Q-1
4e - AKO-Q-2
4f - AKO-Q-10
4g - AKO-Q-5
5h - AKO-Q-13a1
5i - AKO-Q-13a2
Cytotoxicity assay – single concentration screen

Background

To assess the overt cytotoxicity of the compounds, they are incubated at a fixed concentration of 20uM for Pure compounds and 50ug/ml for extracts (unless otherwise stated) in 96-well plates containing HeLa (human cervix adenocarcinoma) cells/cells for 48 hours. The numbers of cells surviving drug exposure are also determined by using the resazurin based reagent and reading resorufin fluorescence in a multiwell plate reader.

Results are expressed as % viability – the resorufin fluorescence in compound-treated wells relative to untreated controls. Compounds are usually tested in duplicate wells, and a standard deviation (SD) is also included. Emetine (which induces cell apoptosis) is used as a positive control drug standard.

(Note: for publication purposes, a detailed description of the method is available on request)

Assay details

Date: 16 June 2017
Concentration used: 20uM

Results

The bar graph and table below show the % HeLa cell viability ±SD obtained for the individual compounds.
Compound at 20uM unless otherwise specified | Viability % | SD
---|---|---
AKO-Q-1 | 101.7 | 0.8
AKO-Q-2 | 113.4 | 5.2
AKO-Q-4 | 116.1 | 6.6
AKO-Q-5 | 107.2 | 9.4
AKO-7 | 99.5 | 10.8
AKO-Q-8 | 101.8 | 3.4
AKO-Q-9 | 109.2 | 8.3
AKO-Q-10 | 109.3 | 1.4
AKO-Q-11 | 99.0 | 0.4
AKO-Q-12 | 109.2 | 10.5
AKO-Q-13a1 | 116.7 | 4.3
AKO-Q-13a2 | 117.1 | 6.0
AKO-Q-13a4 | 105.4 | 0.5
AKO-Q-16 | 120.6 | 3.9
AKO-Q-17 | 119.4 | 0.03
AKO-Q-18 | 110.4 | 1.1
AKO-Q-19 | 99.9 | 0.7
AKO-Q-20 | 109.4 | 1.4
Sinalo3 | 113.2 | 5.9
## Emetine

| Conc (µM) | Log(Conc) | Percentage Viability |
|-----------|-----------|----------------------|
| 1         | 0         | 28.2                 |
| 0.333333  | -0.47712  | 38.5                 |
| 0.111111  | -0.95424  | 50.1                 |
| 0.037037  | -1.43136  | 77.4                 |
| 0.012346  | -1.90849  | 93.1                 |
| 0.004115  | -2.38561  | 115.8                |
| 0.001372  | -2.86273  | 118.1                |
| 0.000457  | -3.33985  | 104.3                |

**IC50** 0.047

## Conclusion

None of the samples caused significant cytotoxic effects at a concentration of 20 µM (none reduced the viability of HeLa cells to below 50%).
Trypanosoma brucei assay – single concentration screen

Background
Trypanosoma brucei (T.b.) parasites are the causative agents of African sleeping sickness (human African trypanosomiasis) in humans and Nagana (animal African trypanosomiasis) in cattle. The subspecies responsible for Nagana (T.b. brucei) is not infective to humans and is commonly used for drug screening. To assess anti-trypanocidal activity, compounds are added to in vitro cultures of T.b. brucei in 96-well plates at a fixed concentration of 20µM for pure compounds or 25µg/mL for natural extracts (unless otherwise stated). After an incubation period of 48 hours, the numbers of parasites surviving drug exposure are determined by adding a resazurin based reagent. The reagent contains resazurin which is reduced to resorufin by living cells. Resorufin is a fluorophore (Exc560/Em590) and can thus be quantified in a multiwell fluorescence plate reader.

Results are expressed as % parasite viability – the resorufin fluorescence in compound-treated wells relative to untreated controls. Compounds are usually tested in duplicate wells, and a standard deviation (SD) is also included. Generally, compounds/extracts that reduce parasite viability to < 10-20% may be considered for further testing (e.g. dose-response and cytotoxicity assays). Pentamidine (an existing drug treatment for trypanosomiasis) is used as a positive control drug standard.

Assay details
Date: 15 June 2017
Concentration used: 20 uM

Results
The bar graph and table below show the residual % parasite viability ±SD obtained for the individual compounds.
| Compound at 20uM | Viability % | SD |
|------------------|-------------|----|
| AKO-Q-1          | 66.4        | 4.4|
| AKO-Q-2          | 42.9        | 4.5|
| AKO-Q-4          | 72.6        | 8.5|
| AKO-Q-5          | 103.5       | 0.06|
| AKO-7            | 64.8        | 3.2|
| AKO-Q-8          | 73.4        | 6.3|
| AKO-Q-9          | 89.4        | 3.0|
| AKO-Q-10         | 73.2        | 0.8|
| AKO-Q-11         | 97.6        | 1.2|
| AKO-Q-12         | 111.4       | 7.9|
| AKO-Q-13a1       | 0.49        | 0.7|
| AKO-Q-13a2       | 0.53        | 0.5|
| AKO-Q-13a4       | 7.07        | 1.0|
| AKO-Q-16         | 76.1        | 8.5|
| AKO-Q-17         | 72.8        | 4.1|
| AKO-Q-18         | 51.9        | 2.8|
| AKO-Q-19         | 5.8         | 1.7|
| AKO-Q-20         | 90.5        | 7.9|
| Sinalo 3         | 106.8       | 3.6|
Pentamidine standard:

| Concentration (uM) | Log      | Percentage activity | Std dev |
|--------------------|----------|---------------------|---------|
| 1                  | 0        | -0.3                | 0.08    |
| 0.333333           | -0.47712 | 2.0                 | 0.16    |
| 0.111111           | -0.95424 | 2.1                 | 0.16    |
| 0.037037           | -1.43136 | 2.7                 | 0.39    |
| 0.012346           | -1.90849 | 4.0                 | 1.2     |
| 0.004115           | -2.38561 | 14.8                | 0.93    |
| 0.001372           | -2.86273 | 88.9                | 2.2     |
| 0.000457           | -3.33985 | 84.4                | 2.6     |
| **IC50**           | 0        | **0.0037**          |         |

**Conclusion**

The samples highlighted in red significantly affected the viability of the Trypanosomes at 20 μM. These samples will be subjected to IC50 evaluations if they are not cytotoxic (the results need to be looked at in conjunction with the Cell toxicity assay results).
Trypanosoma brucei assay – dose response

Background

Trypanosoma brucei (T.b.) parasites are the causative agents of African sleeping sickness (human African trypanosomiasis) in humans and Nagana (animal African trypanosomiasis) in cattle. The subspecies responsible for Nagana (T.b. brucei) is not infective to humans and is commonly used for drug screening. To determine the anti-trypanocidal potency of test compounds, serial dilutions of the compounds are added to in vitro cultures of T.b. brucei in 96-well plates and incubated for 48 hours. The numbers of parasites surviving exposure to the individual compound concentrations are determined by adding a resazurin based reagent. The reagent contains resazurin which is reduced to resorufin by living cells. Resorufin is a fluorophore (Exc<sub>560</sub>/Em<sub>590</sub>) and can thus be quantified in a multiwell fluorescence plate reader.

For each compound concentration, % parasite viability – the resorufin fluorescence in compound-treated wells relative to untreated controls – is calculated. Compounds are usually tested in duplicate wells, and a standard deviation (SD) is derived. For each compound, percentage viability is then plotted against Log(compound concentration) and the IC<sub>50</sub> (50% inhibitory concentration) obtained from the resulting dose-response curve by non-linear regression. For comparative purposes, pentamidine (an existing drug treatment for trypanosomiasis) is used as a drug standard and yields IC<sub>50</sub> values in the range 0.001-0.005 μM.

Assay details

Date: 22 June 2017

Concentration used: 3-fold serial dilutions with a starting concentration of 100 μM
Results
The table below shows the IC\textsubscript{50} values obtained for individual compounds, followed by the dose-response plots and % viability ±SD data used to prepare the graphs.

| Compound       | IC\textsubscript{50} (uM) |
|----------------|--------------------------|
| AKO-Q-13a1 (5i)| 4.5                      |
| AKO-Q-13a2 (5j)| 2.8                      |
| AKO-Q-13a4 (5k)| 3.4                      |
| AKO-Q-19 (4b)  | 6.2                      |
| Pentamidine    | 0.005                    |

* Approximate
| uM   | log | %Viab  | SD    | %Viab  | SD    | %Viab  | SD    |
|------|-----|--------|-------|--------|-------|--------|-------|
| 100  | 2   | -0.6594| 0.0247| -3.1205| 0.4771| 0.2123| 0.2441|
| 33.3333| 1.522879| 6.479433| 0.493322| 4.828215| 0.757764| 9.155692| 0.44266|
| 11.1111| 1.045757| 14.71262| 0.814962| 9.761726| 0.488219| 17.31352| 2.330072|
| 3.703704| 0.568636| 57.74025| 0.523188| 29.56382| 1.264962| 43.34812| 6.399081|
| 1.234568| 0.091515| 91.97546| 1.528426| 90.82199| 3.35642| 87.65957| 1.139119|
| 0.411523| -0.38561| 95.96166| 3.627241| 95.76089| 0.808916| 95.95919| 3.523715|
| 0.137174| -0.86273| 95.98511| 3.989633| 95.32986| 4.47013| 94.10931| 3.579952|
| 0.045725| -1.33985| 93.40788| 0.56873| 93.5491| 0.863685| 93.4525| 1.997981|

### AKO-Q-19

| uM   | log | %Viab  | SD    |
|------|-----|--------|-------|
| 100  | 2   | -7.4997| 2.972549|
| 33.3333| 1.522879| 0.222726| 3.86748|
| 11.1111| 1.045757| 17.4995| 3.650694|
| 3.703704| 0.568636| 68.00697| 5.707235|
| 1.234568| 0.091515| 94.13371| 3.88022|
| 0.411523| -0.38561| 93.31949| 0.911201|
| 0.137174| -0.86273| 94.13203| 0.116965|
| 0.045725| -1.33985| 93.18314| 1.021578|

### Pentamidine

| Conc (µM) | Log(Conc) | %Viab |
|-----------|-----------|-------|
| 1         | 0         | -0.89291|
| 0.333333  | -0.47712  | 5.04438|
| 0.111111  | -0.95424  | 4.610519|
| 0.037037  | -1.43136  | 5.654776|
| 0.012346  | -1.90849  | 7.847016|
| 0.004115  | -2.38561  | 64.11454|
| 0.001372  | -2.86273  | 102.5856|
| 0.000457  | -3.33985  | 102.797|

**Conclusion**

The IC\textsubscript{50} values of the samples are listed in the first table.