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Enhancing allosteric inhibition of dihydrodipicolinate synthase through the design and synthesis of novel dimeric compounds†

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The synthesis of the first dimeric inhibitor of *E. coli* dihydrodipicolinate synthase (DHDPS) is reported herein. Inspired by 2,4-thiazolidinedione based ligands previously shown to inhibit DHDPS, a series of dimeric inhibitors were designed and synthesised, incorporating various alkyl chain bridges between two 2,4-thiazolidinedione moieties. Aiming to exploit the multimeric nature of this enzyme and enhance potency, a dimeric compound with a single methylene bridge achieved the desired outcome with low micromolar inhibition of *E. coli* DHDPS observed. This work highlights the continued importance of investigation into DHDPS as an antibacterial target. Furthermore, we demonstrate the design of dimeric ligands can provide a promising strategy to improve potency in the search for novel bioactive compounds.

Introduction

With the majority of oligomeric proteins existing as homodimers,[1] the design of dimeric inhibitors that can interact with more than one monomer of a multivalent protein is a compelling approach to the development of new therapeutics. By binding to two sites simultaneously, a dimeric ligand can increase both potency and selectivity toward the target for a single ligand.[2] This strategy has been explored in the development of treatments for influenza,[3] the synthesis of anti-cancer agents[4,5] and also for central nervous system therapeutics.[6]

Dihydrodipicolinate synthase (DHDPS) is a key enzyme of the diaminopimelate pathway. It predominately exists as a tetramer comprising of four identical monomeric units and is responsible for the production of lysine and its immediate precursor *meso*-DAP.[7,8] As an essential enzyme in both bacteria and plants, DHDPS has attracted much interest as an enzyme target for the development of new antibiotics and herbicides.[9–13] Despite many efforts in mimicking natural substrates of the pathway including pyruvate, (*S*)-aspartate semialdehyde (ASA), 2,3-dihydrodipicolinate (DHDP), 4-hydroxy-2,3,4,5-tetrahydro-(2*S*)-dipicolinic acid (HTPA), 2,3,4,5-tetrahydrodipicolinate (THDP) and (*S*)-lysine[14–25] it wasn't until the discovery of *R*,*R*-bislysine (Fig. 1) that potent sub-micromolar inhibition of DHDPS was observed.[26] Not only did *R*,*R*-bislysine demonstrate improved binding affinity in comparison to a single (*S*)-lysine ligand but also confirmed that DHDPS is susceptible to inhibition across a dimeric interface.

Recently we reported the discovery and synthesis of a series of 2,4-thiazolidinediones and analogous heterocycles as potent inhibitors of both bacterial and plant DHDPS. 2,4-Thiazolidinedione MBDTA-2 (Fig. 1) was found to have an IC_{50} value of 47.0 ± 2.3 μM against bacterial *E. coli* DHDPS and inhibited plant *A. thaliana* DHDPS1 and DHDPS2 with IC_{50} values of 66.2 ± 7.6 μM and 66.0 ± 11.0 μM respectively.[27–29] We later demonstrated MBDTA-2 is the first inhibitor of the diaminopimelate pathway with herbicidal activity and validating DHDPS as a viable target for the development of agrichemicals.[30] Herein, we expand upon our

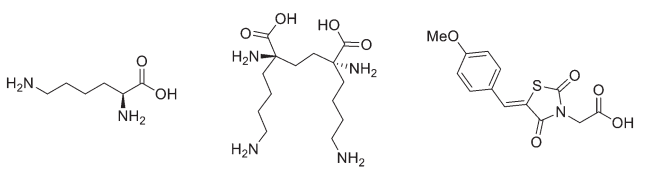

Fig. 1 Natural allosteric inhibitor (*S*)-lysine, *C. jejuni* DHDPS inhibitor *R*,*R*-bislysine and previously synthesised *E. coli* and *A. thaliana* DHDPS, inhibitor MBDTA-2.[26–30]

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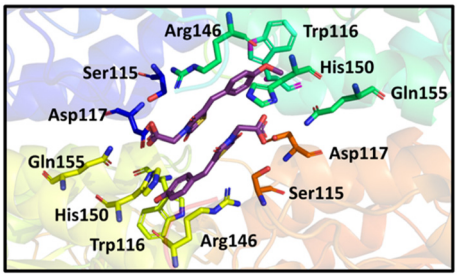


Fig. 2 Binding of two MBTA-2 ligands with DHDPS.[29]

previous work to describe the synthesis of a series of novel dimeric 2,4-thiazolidinedione compounds based on MBDTA-2, with the intent to exploit the multimeric nature of DHDPS to generate an improved inhibitor of DHDPS.

Results and discussion

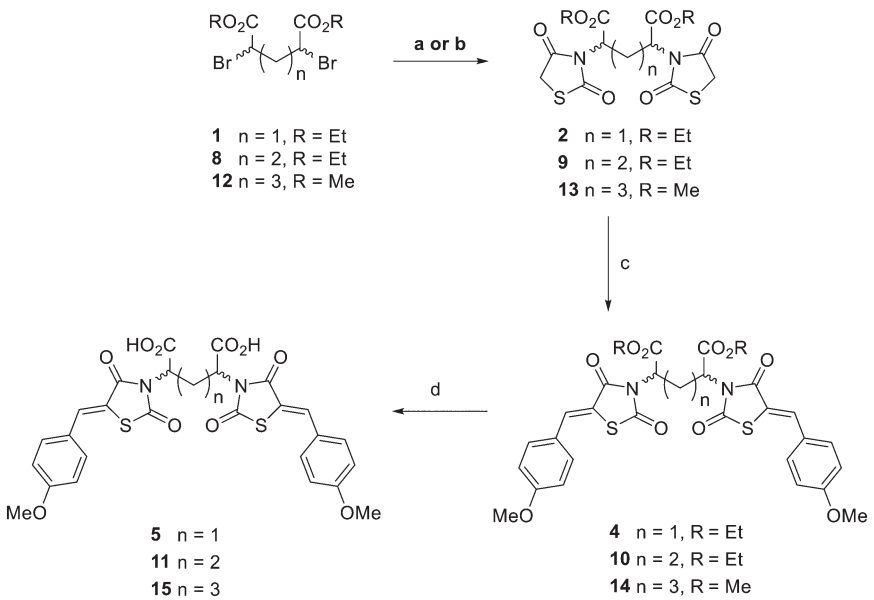
Design and synthesis of dimeric compounds

Based on X-ray data of the previously synthesised MBDTA-2 co-crystallised with *A. thaliana* DHDPS, we observed binding of two ligands in close proximity at the tight dimer interface (Fig. 2).[29] It was anticipated that the binding mode for an inhibitor to the *A. thaliana* protein would be similar to the *E. coli* protein as both have a tetrameric quaternary structure consisting of a "dimer of dimers", with the only difference being the configuration of the dimers for the plant structure ("back to back") *versus* the bacterial structure ("head to head"). We hypothesised a short linker between two inhibiting molecules of MBDTA-2 would potentially allow allosteric binding across the dimeric interface and enhance DHPDS inhibition in comparison to the binding of a single ligand. It was therefore decided to produce dimers of 2,4-thiazolidinedione MBDTA-2 with methylene, ethylene and propylene bridges between the carboxylic acid moieties.

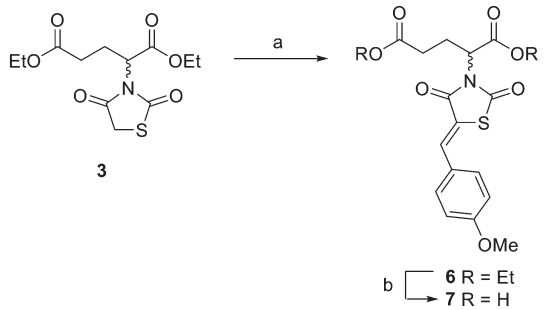
To begin the synthesis of the proposed MBDTA-2 dimer with a methylene bridge, diethyl 2,4-dibromopentanedioate **1** (Scheme 1) was produced from glutaric acid as previously reported by Guanti and Riva.[31] Subsequent alkylation of this diester with 2.1 equivalents of 2,4-thiazolidinedione in the presence of potassium carbonate under reflux conditions afforded a mixture of diastereoisomers **2**. It was observed that there was no need for separation of diastereomers at this point as Knoevenagel condensation using a single diastereoisomer with 4-methoxybenzaldehyde would result in racemic product mixture regardless. It should also be noted that the presence of a small amount of diethyl 2-bromopentanedioate in the starting material of this reaction resulted in the formation of an unwanted product which was isolated by column chromatography and confirmed as the monosubstituted compound **3** (Scheme 2).

Knoevenagel condensation of compound **2** with 4-methoxybenzaldehyde was conducted in the presence of piperidine and acetic acid in toluene. Column chromatography resulted in the elution of two compounds with the same molecular mass, presumably the *rac* and *meso* compounds. Proton NMR spectroscopy of both compounds gave, unsurprisingly, similar spectra except for the doublet of doublets (dd) signals rising from the methylene bridge. The spectra of the **4a** had this signal at 4.93 ppm while the same signal was downfield at 5.08 ppm for **4b** (ESI† data).

Acid hydrolysis of diester **4a** by reflux in concentrated hydrochloric acid and glacial acetic acid afforded the desired



Scheme 1 Synthetic route to afford one, two and three carbon bridge linked compounds. Reagents and conditions: (a) 2,4-thiazolidinedione, K_2CO_3, ACN, reflux 2–4 h, 36–39%; (b) $KHCO_3$, DMF, rt 3 days, 51%; (c) 4-methoxybenzaldehyde, piperidine, AcOH, $PhCH_3$, reflux 18 h, 24–70%; (d) conc. HCl : AcOH (1 : 2), reflux 2 h, 26–85%.



Scheme 2 Synthesis of extended analogue of MBDTA-2. Reagents and conditions: (a) 4-methoxybenzaldehyde, piperidine, AcOH, $PhCH_3$, reflux 18 h, 68%; (b) conc. HCl : AcOH (1 : 2), reflux 2 h, 78%.

final diacid product **5a** in 68% yield. Singles crystals of **5a** were grown from chloroform/diethyl ether, which were high quality and allowed for a satisfactory structural solution and refinement by X-ray crystallography. Compound **5a** was confirmed as the *rac* mixture, revealing an "open" structure with the two thiazolidine-2,4-dione groups extended in *anti* configuration relative to the pentanedioic acid moiety and as shown by the *R*,*R* enantiomer depicted in Fig. 3. Compound **4b** was also subjected to the same hydrolysis conditions to afford the diacid **5b** in 74% yield. X-ray crystallography indicated **4b** to be the expected *meso* compound with a "closed" structure with the two thiazolidine-2,4-dione groups extended in *syn* configuration relative to the pentanedioic acid moiety (ESI† Fig. S1). Notable differences in the methylene protons were also observed in the proton NMR spectra for the diastereomers. Racemic isomers of the diacid product **5a** saw splitting of the methylene protons into two separate environments meanwhile the methylene protons were isochronous for the *meso* isomer **5b**. A similar phenomenon was reported by Krasnov *et al.* in the synthesis of adipic and glutaric acid stereoisomers.[32]

In addition to the planned synthesis of the methylene bridged dimeric compound, the monosubstituted compound **3** was also reacted by Knoevenagel condensation and acid hydrolysis to afford compound **7** (Scheme 2) as an extended analogue of MBDTA-2 for biological testing.

To synthesise an ethylene bridge between dimeric ligands, the commercially available diethyl *meso*-2,5-dibromoadipate **8** was coupled with 2,4-thiazolidinedione to afford **9** as a mixture of stereoisomers. Knoevenagel condensation of the mixture with 4-methoxybenzaldehyde resulted in the synthesis of compound **10**. Trituration of this product with small amounts of methanol afforded a solid as compound **10a** which was found by proton NMR spectroscopy to have a doublet of doublets methylene signal at 4.89 ppm and was indicated to be the *meso* isomer *via* X-ray crystallography (ESI† Fig. S2). It was noted that the <5 ppm proton NMR methylene signal was indicative of the formation of an "open" crystal structure, consistent with the observations made for compound **4a**. As expected, hydrolysis of *meso* diester **10a** resulted in the corresponding diacid product **11a**.

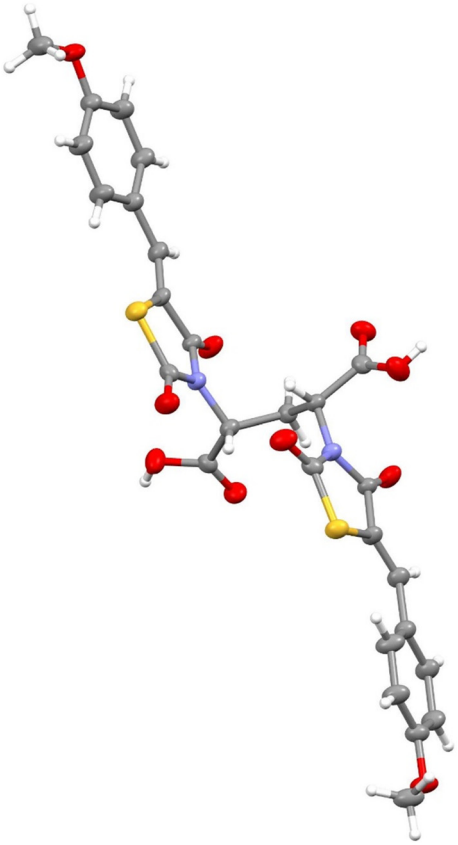


Fig. 3 Crystal structure of compound **5a** indicating an "open" configuration and *rac* stereochemistry with the *R*,*R* enantiomer shown.

The methanolic solution, from which the *meso* compound **10a** had been removed by trituration, was found to contain mostly the racemate **10b** as a mixture of *R*,*R* and *S*,*S* enantiomers but also a small amount of compound **10a**. Compound **10b** was found to have a doublet of doublets methylene signal at 5.02 ppm by proton NMR spectroscopy. This material was not purified any further but hydrolysed to give the desired diacid compound **11**, with the major product isolated by semi-preparative RP-HPLC as compound **11b** and indicated to be the *rac* mixture with a "closed" structure which was assigned as the *S*,*S* enantiomer (ESI† Fig. S3). As observed for compounds **5a** and **5b** respectively, splitting of the methylene protons into two separate environments occurred for racemic compound **11b** while the methylene protons were isochronous for the *meso* compound **11a**. In summary, both isomers of the ethylene bridged 2,4-thiazolidinedione dimers were thus synthesised and characterised alongside both of the methylene bridged, 4-thiazolidinedione dimers in preparation for biological testing (Fig. 4).

Lastly, a compound possessing a propylene bridge was synthesised *via* the *N*-alkylation of 2,4-thiazolidinedione with dimethyl 2,6-dibromoheptandioate **12** in the presence of potassium hydrogen carbonate in *N*,*N*-dimethylformamide to afford compound **13**. Knoevenagel condensation resulted in diester **14** which was followed by acid hydrolysis to afford the

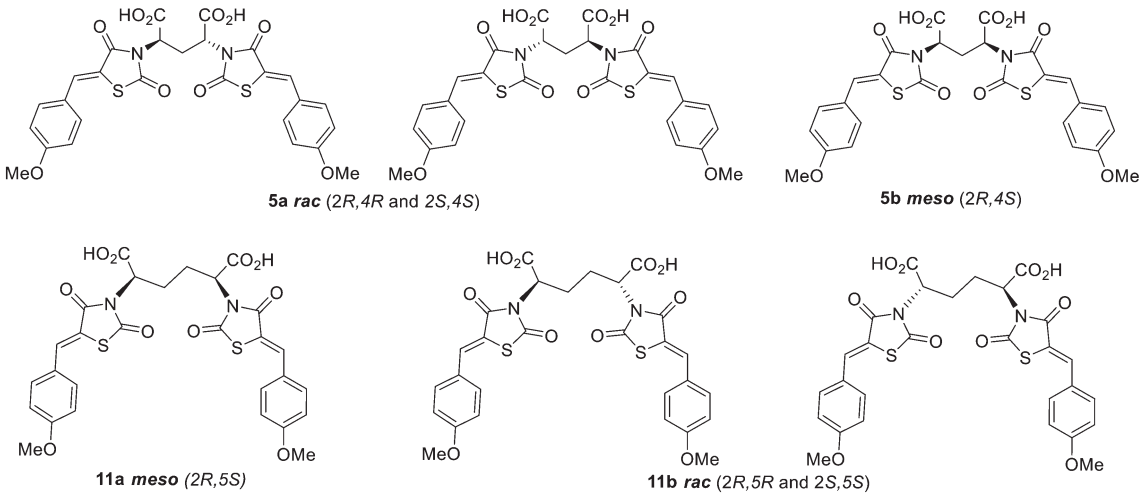


Fig. 4 Dimeric 2,4-thiazolidinediones synthesised with methylene or ethylene bridges.

desired diacid product **15** as a mixture of *rac* and *meso* diastereomers in an approximate 1 : 1 ratio according to proton NMR spectroscopy. Recrystallisation, column chromatography and semi-preparative RP-HPLC were attempted to separate the *meso* and *rac* compounds, initially as the desired acid products but also at the stage of the precursor esters. Unfortunately, these efforts went unrewarded. It was decided to test compound **15** as a mixture of diastereomers and then undertake alternative strategies for purification if it was warranted by the initial assay results.

Biological assay results

The synthesised MBDTA-2 analogues were subjected to biological testing against *E. coli* DHDPS *in vitro via* the DHDPS–DHDPR coupled enzyme assay (Table 1).[33] All dimeric compounds saw improved inhibitory *in vitro* activity towards *E. coli* DHDPS, with the exception of the extended diacid analogue **8** which was not expected to have comparable inhibition given the absence of the second 2,4-thiazolidinedione moiety. The most potent compound was found to be *meso* compound **5b** with the methylene bridge which had an improved IC_{50} value of 9.95 ± 0.6 μM against *E. coli* DHDPS followed by *rac* compound **11b** with an ethylene bridge with an IC_{50} value of 19.7 ± 1.5 μM. The mixture of stereoisomers of compound **15** with a propylene bridge did not see a significant improvement in potency over the methylene or ethylene bridged analogues with an IC_{50} of 40.6 ± 3.0 μM, thus separation of the stereoisomers of this compound was not pursued any further.

Compound **5b** was then selected for testing against the two orthologues of *Arabidopsis thaliana* to evaluate potential herbicidal activity of this series. **5b** was found to inhibit with an IC_{50} 35.6 ± 7.4 μM and 33.9 ± 8.0 μM against DHDPS1 and DHDPS2 respectively. Inhibition efficacy was found to be slightly weaker than that observed against bacterial *E. coli* DHDPS, though such variation is to be expected as the allosteric site is only semi-conserved across species.

Table 1 IC_{50} values of inhibitors against *E. coli* DHDPS

Compound	Bridge length	Stereochemistry	*E. coli* IC_{50} (μM)
MBDTA-2	—	*achiral*	47.0 ± 2.3
5a	$n = 1$	*rac* (*R*,*R* and *S*,*S*)	30.4 ± 1.8
5b	$n = 1$	*meso*	9.95 ± 0.6
8	—	*rac* (*R* and *S*)	77.4 ± 5.8
11a	$n = 2$	*meso*	42.0 ± 0.8
11b	$n = 2$	*rac* (*R*,*R* and *S*,*S*)	19.7 ± 1.5
15	$n = 3$	*meso*, *rac* (*R*,*R* and *S*,*S*)	40.6 ± 3.0

Conclusion

With DHDPS as a target of significant interest in the development of new antibiotics and herbicides, a series of dimeric chiral 2,4-thiazolidinedione compounds consisting of a methylene, ethylene and propylene bridge were designed and synthesised. Separation of the *meso* isomer for this series was achieved by trituration and/or recrystallisation while the remaining *R*,*R* and *S*,*S* enantiomers required separation by RP-HPLC in order to remove traces of the *meso* compound. By taking advantage of the symmetrical nature of the target, allosteric inhibition of DHDPS was improved by approximately five-fold over the single ligand. The most potent inhibitor was found to be compound **5b** as a *meso* dimer containing a methylene bridge which was found to possess an IC_{50} value of 9.95 ± 0.6 μM against *E. coli* DHDPS. This work describes the first low micromolar dimeric inhibitor of *E. coli* DHDPS and further supports the continued exploration of this enzyme as biological target of significant interest.

Conflicts of interest

There is no conflict of interest to declare.

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