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Asymmetric total synthesis of the indole alkaloid cyclopiazonic acid and first structure–activity data

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ABSTRACT

The indole alkaloid α-cyclopiazonic acid (CPA) is one of the few known inhibitors of sarco(*endo*)plasmic reticulum Ca^{2+}-ATPase (SERCA) besides the terpenoids thapsigargin and artemisinin. We report here the first asymmetric total synthesis of cyclopiazonic acid by a modification of the Knight synthesis, currently the most efficient route to CPA. First structure–activity data of CPA derivatives and stereoisomers are presented and will be discussed in connection with the published crystal structures of CPA–SERCA complexes.

1. Introduction

The indole alkaloid α-cyclopiazonic acid (CPA, **1**) has been isolated from stored grain and cereal products infected with the fungus *Penicillium cyclopium* Westling.[1,2] Toxicity and mode of action of CPA have been studied intensively as a consequence of several food poisonings caused by ingestion of contaminated feed.[3,4] Cyclopiazonic acid blocks the calcium access channels of Ca^{2+}-ATPases of the sarcoendoplasmic reticulum (SERCA), which are essential for calcium reuptake in the muscle.[5,6] Other known inhibitors of SERCA enzymes are the terpenoids thapsigargin (**3**, anticancer activity), artemisinin (**4**, antimalaria drug) and the CPA-related indole alkaloid speradine A (**2**), which shows significant antibacterial and histone deacetylase activity (Fig. 1).[7–11] In addition, calcium metabolism is an important target for modern insecticides, such as the natural product ryanodin or the benzenedicarboxamide flubendiamide, a novel insecticide for controlling lepidopterous insect pests.[12] Apparently, sarcoendoplasmic reticulum Ca^{2+}-ATPases are promising targets for the development of new drugs against various diseases and insect pests.

Although the structure of CPA is known since 1968 only three racemic syntheses have been published to date and almost nothing is known on structure–activity relationships. The Kozikowski synthesis (1984) comprises almost twenty steps and is based on a stepwise formation of ring C by a stereoselective Michael addition

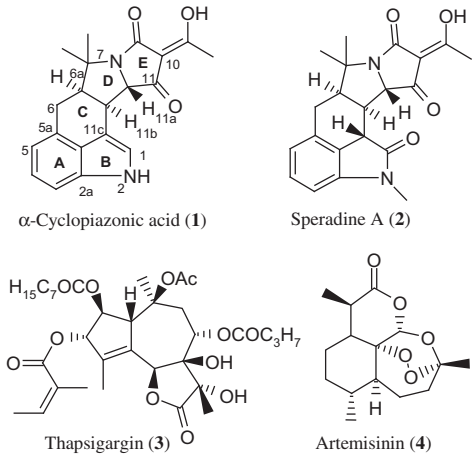

Fig. 1. Known inhibitors of SERCA.

Abbreviations: ACN, acetonitrile; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DCM, dichloromethane; DMF, dimethylformamide; EGTA, ethylene glycol tetraacetic acid; KHMDS, potassium hexamethyldisilazide; MOPS, 3-(N-morpholino)-propanesulfonic acid; Ns, *p*-nosyl; TBDPS, *tert*-butyldiphenylsilyl; TfOH, trifluoromethanesulfonic acid; THF, tetrahydrofurane; Ts, tosyl.

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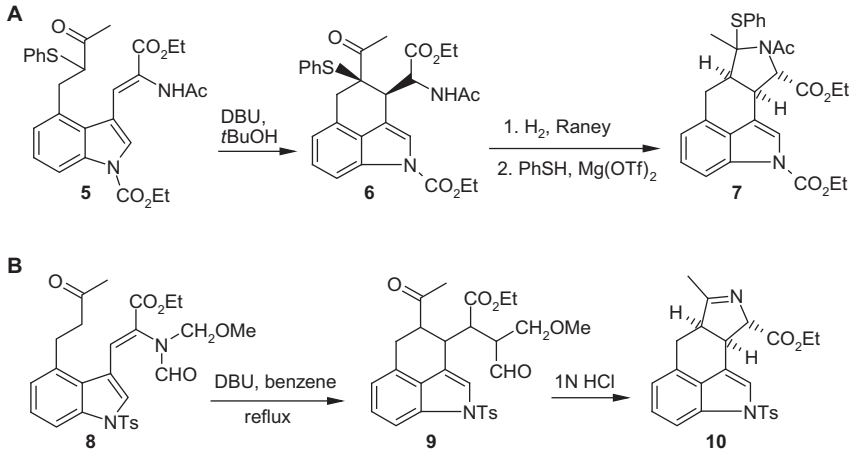


Scheme 1. Key steps of the Kozikowski (**A**) and Natsume syntheses (**B**) of CPA.

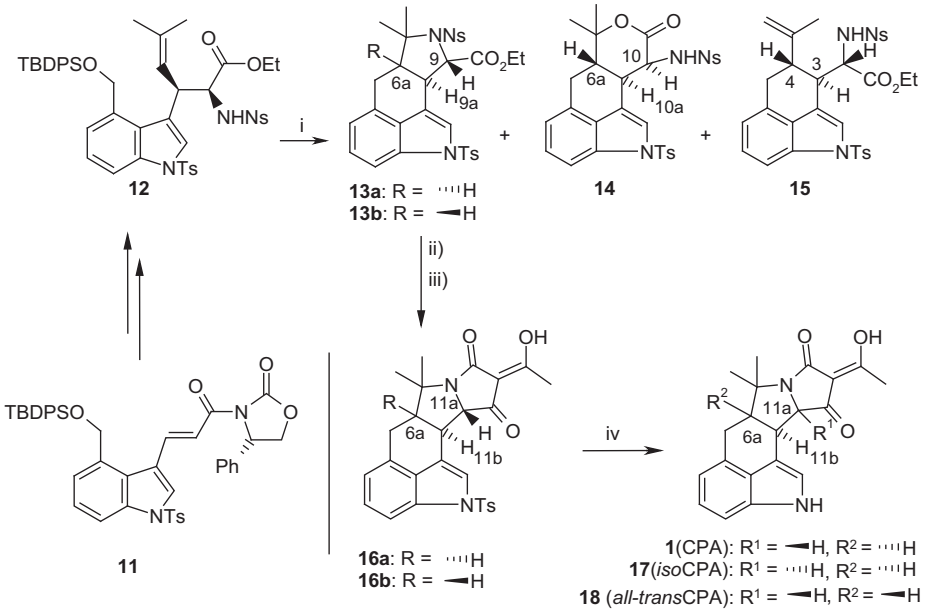
(Scheme 1A) followed by a three step sequence to establish the pyrrolidine ring D and to introduce the second methyl group.[13] Similar to the Kozikowski synthesis, also the Natsume synthesis (1985) utilizes a Michael addition (Scheme 1B) for the construction of ring C, but due to the higher temperature of the cyclization reaction a mixture of tricyclic stereoisomers **9** was obtained.[14] The Knight synthesis, published in 2005, represents a significant shorter route to CPA.[15] The key step here is a simultaneous formation of the ring C- and D-framework by a cationic cyclization of the bicyclic intermediate **12** (Scheme 2), which is available in enantiomeric pure form on a multigram scale by a 1,4-addition (dr 94:6) of isopropenylcuprate to the chiral indolyl acrylic acid **11** (Scheme 2), followed by a 1,2-addition of trisylazide (dr 98:2) as detailed recently by our group.[16] Remarkably, both stereogenic centres are established in the correct configuration by one and the same Evans auxiliary with high diastereofacial control.

We wish to report now the first asymmetric total synthesis of CPA following the Knight route with some modifications as well as first structure–activity data of several CPA derivatives and stereoisomers. The inhibitory activities against *Heliothis virescens* SERCA are discussed on the basis of published X-ray structures of CPA–SERCA complexes.

2. Results and discussion

2.1. Chemistry

The diastereomeric pyrrolidines **13a** and **13b** (62%) are formed as a 1:1 mixture accompanied by lactone **14** (27%) and, depending on the reaction conditions, minor amounts of the monocyclization product **15** on addition of triflic acid to the key intermediate **12** (Scheme 2). The latter compound clearly demonstrates a stepwise cyclization process, initiated from a benzylic carbenium ion formed by acidic cleavage of the silanyloxy-group.[17] The C/D trans-ring junction in **13b** ($J_{6a,9a}$ 12.5 Hz), **14** ($J_{6a,10a}$ 12.7 Hz) and **15** ($J_{3,4}$ 9.1 Hz) originates from a *s*-trans conformation in the transition-state of the



i) TfOH (1.0 eq), DCM, rt, 1h, 62% **13a** and **13b**, 27% **14**; ii) DBU (3 eq.), $HSCH_2CH_2OH$ (3 eq.), DMF, rt, 24h, 68%, iii) acetyl Meldrums acid (2.5 eq.), dioxane, 80°C, 90min then KOH in MeOH (0.1 M, 2.5 eq.), 80°C, 60min, 80%; iv) Cs_2CO_3 (6 eq.), THF/MeOH 1/1, reflux, 3d, 61%.

Scheme 2. Asymmetric synthesis of cyclopiazonic acid (**1**).

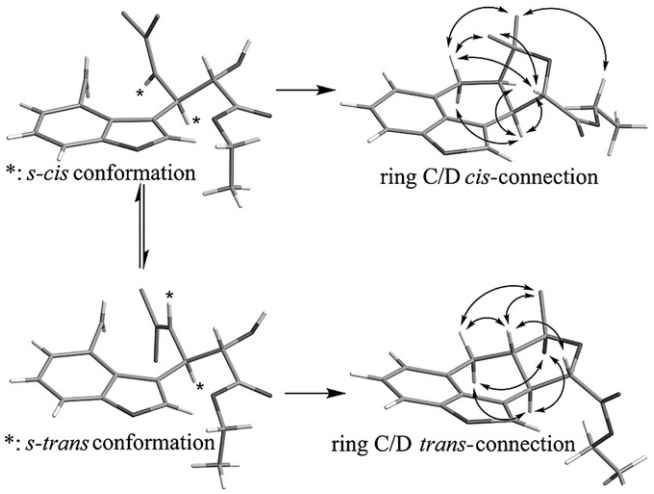


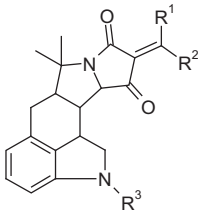
Fig. 2. Formation of pyrrolidines **13a** and **13b** (NOE contacts indicated with arrows).

cyclization reaction (Fig. 2). Due to lower steric strain of trans-fused six-membered rings a significant amount of cyclic lactone **14** is formed via acidic ester cleavage and subsequent attack of a carboxylic oxygen to the *tert*-butyl carbenium ion in position 7.[18] Furthermore, the carbenium ion turns away from N-8 in the *s*-trans conformation which additionally disfavours the formation of the smaller pyrrolidine ring (**13b**). The coupling constants indicate a trans-arrangement of H-9 and H-9a in **13b** ($J_{9,9a}$ 9.8 Hz) and a cis-configuration in lactone **14** ($J_{10,10a}$ 5.6 Hz) as a direct consequence of the suggested cyclization mechanism (Fig. 2).

Consistent with this model, the C/D-ring cis-fusion is established by a ring-closure via a *s*-cis conformation between the olefinic and the β-proton (Fig. 3). In this case, the carbenium ion is oriented towards the nitrogen and thus the formation of the strainless, cis-connected pyrrolidine ring is facilitated. The cis-ring connection in **13a** is supported by a $J_{6a,9a}$ coupling constant of 5.6 Hz. NOESY spectra reveal a β-position for H-9 and thus a trans-configuration of H-6a and H-9a. Most indicative for this stereochemistry are strong NOEs between H-9 and 7β-Me, H-6a with H-9a, as well as CH_2-ester and 7α-Me (Fig. 2).

The subsequent steps towards CPA (**1**) comprise a denosylation reaction with mercaptoethanol (68%), construction of the tetramic acid moiety in a one-pot reaction with acetyl-Meldrum's acid (80%) and finally the cleavage of the tosyl group (Scheme 2).[19,20] Three stereoisomers could be isolated from the detosylation reaction: CPA **1** ($J_{11a,11b}$ 11.0 Hz, $J_{6a,11b}$ 5.8 Hz), *alltrans*CPA **18** ($J_{11a,11b}$ 10.8 Hz, $J_{6a,11b}$ 11.5 Hz) both resulting from the 6a epimer-mixture used throughout the final steps, and *iso*CPA **17** ($J_{11a,11b}$ 5.1 Hz, $J_{11a,11b}$ 5.1 Hz), which is formed obviously by epimerization of H-11a under the cleavage conditions. Strong NOE's between H-11a, H-11b and H-6a as well as between H-11b and H_α-6 are in perfect agreement with the structure of *iso*CPA (**17**). Additionally, spectroscopic data of CPA (**1**) and *iso*CPA (**17**) were identical with those already published.[21,22]

Table 1
CPA analogues and SERCA inhibition



Compound	R^1	R^2	R^3	SERCA (IC_{50}, μM)
1 (Reference)	OH	Me	H	0.01±0.005
1 (Synthetic)	OH	Me	H	0.01±0.006
17 (*iso*CPA)	OH	Me	H	0.50±0.1
18 (*alltrans*CPA)	OH	Me	H	5.0±0.5
2 (Speradine A)				8.0[7]
16 (*tosyl*CPA)	OH	Me	Tosyl	0.40±0.05
19	OH	Cyclopropyl	H	0.14±0.02[a]
20	OH	Cyclohexyl	H	>50[a]
21	OH	Benzyl	H	1.9±0.4[a]
22	OH	Benzyl	Me	1.7±0.2[a]
23	OH	Phenyl	H	>50
24	NH_2	Me	H	0.50±0.08[a]
25	NH–NH_2	Me	H	0.78±0.08[a]
26	NH–NH–4Cl–Ph	Me	H	7.0±1.3[a]
27	NH–OH	Me	H	16.0±2[a]
28	NH–OMe	Me	H	8.8±1.4[a]

[a] Contains minor amounts of *iso*- and *alltrans*-CPA.

In order to evaluate the significance of the acyl tetramic acid moiety for SERCA inhibition two series of CPA derivatives were prepared. In the first series the acetyl group was replaced for larger acyl residues, such as cyclopropyl, benzyl and phenyl by acylation of the cyclization product **13** with the corresponding acyl Meldrum's acids, which were prepared according to literature procedures.[23–25] In the second series of derivatives the acetyl group of CPA (**1**) was reacted with various nitrogen nucleophiles to yield the corresponding oximes, hydrazones and imines. These derivatives were expected to show different complexation behaviour of Mg^{2+} ions and thus should have a major influence on SERCA inhibition (Table 1).

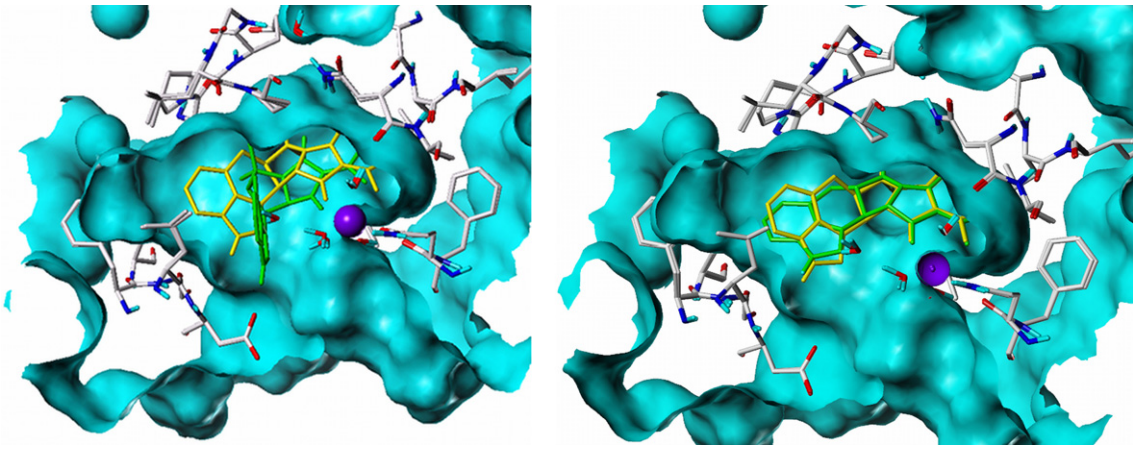


Fig. 3. Superimposition of CPA **1**/*iso*CPA **17** (left side) and CPA **1**/*alltrans*CPA **18** (right side).

For all derivatization reactions the mixture of stereoisomers as obtained from the synthesis was used since the pure stereoisomers were available only in small amounts. Unfortunately, the final preparative HPLC separation of the CPA derivatives turned out to be difficult due to slowly interconverting tautomers and *E*/*Z* isomers in the acyl tetramic acid causing a considerable peak broadening. Therefore the derivatives except **23** used for biological testing still contained some *iso*- and *alltrans*-CPA.

2.2. Biological data and structure–activity relationships

All CPA derivatives were tested in a functional *H. virescens* biochemical SERCA assay using a luciferin/luciferase-coupled assay that measures the ATP-level within the reaction. ATP-consumption of the ATPase enzyme results in reduced ATP levels for the subsequent luciferase assay and consequently a loss of luminescence signal is observed. Dose-response assays allowed the determination of IC_{50} values for each inhibitor.

Synthetic CPA was found to be equally active (IC_{50} 0.01 μM) as a natural reference sample (Table 1). *iso*CPA **17** showed a reduced SERCA inhibition by a factor of around 50 albeit still on a high level (IC_{50} 0.5 μM). In contrast to *iso*CPA **17** the *alltrans*CPA isomer **18** was found to have a drastically reduced activity (5.0 μM), much lower than the tosylate **16** (0.40 μM), which is still in the range of *iso*CPA **17**. The low activity of *alltrans*CPA **18** is not unexpected, since the trans-conjunction of the six-membered ring C and pyrrolidine ring D induces a significant change in the shape of the CPA structure resulting in drastically reduced SERCA binding.

Neither the pyrrolidines **13** nor lactone **14** or the monocyclization product **15** showed any SERCA inhibition, proofing the tetramic acid (ring E) essential for full SERCA activity. Thus, modifications, which negatively interfere with hydrogen bonding to SERCA or Mg^{2+} coordination of the acetyl tetramic acid (ring E) are expected to have a major impact on SERCA inhibition. In fact, CPA analogues with bulky acyl residues, such as phenyl (**23**) or cyclohexyl (**20**) were shown to be inactive. On the contrary, CPA analogue **19** with a small cyclopropyl-acyl group expressed only a slightly diminished activity, indicating at least a minor variability of the acyl binding pocket. This hypothesis is underlined by the benzyl-derivative **21**, which had a considerably reduced but still significant activity in the low micromolar range.

The critical role of the acyl-group is also demonstrated by the second series of CPA derivatives. Reaction of CPA with ammonia and hydrazine afforded the enamines **24** and **25**, respectively. Both CPA analogues were found to have a somewhat reduced SERCA activity (IC_{50} 0.5 μM and 0.8 μM) but still on the level of *iso*CPA **17**. CPA derivatives **26**–**28** obtained by reacting CPA with hydroxylamine, *O*-methyl hydroxylamine, and 4-chlorophenyl hydrazine showed only weak SERCA inhibition (IC_{50} 7–16 μM), but were still more active than the cyclohexyl- and phenyl-analogues **20** and **23**. Apparently, also an intact enol- or an unsubstituted enamine-function is essential for high SERCA inhibition. Taking these results together, the acetyl tetramic acid can be regarded as the pharmacophoric unit of cyclopiazonic acid. It should be emphasized that the minor amounts of *iso*- and *alltrans*-CPA, still left in several CPA derivatives influence the SERCA inhibition data, which range over almost three orders of magnitude only marginally and the structure–activity relationships provided not at all.

The role of the indole system still remains somewhat speculative. On the one hand the oxoindole speradine A (**2**), which differs from CPA (**1**) only in the indole system, shows a remarkably reduced SERCA inhibition (IC_{50} 8.0 μM) by a factor of around 800. On the other hand it remains unclear whether this loss of activity is caused by the 1-oxo function and/or the additional stereogenic centre in position 11c. From both *tosyl*CPA **16** and *N*-methylindolyl derivative **22**, which was isolated as a byproduct from the detosylation reaction in methanol, it can be concluded that substituents at the indole nitrogen are well tolerated.[26] This clearly points to a certain structural variability of the indole system in CPA.

2.3. Molecular-modelling studies

The sarcoplasmic reticulum Ca^{2+}-ATPase is an integral membrane protein consisting of 995 amino acids, arranged in three cytoplasmic domains containing the ATP binding site and 10 transmembrane helices with two calcium binding sites. Despite the fact that X-ray structures of SERCA, capturing different states of the protein, have been published, no experimental protein structure of *H. virescens* SERCA is available.[27] Hence, a homology model was constructed, using the protein modelling suite (FUGUE, ORCHESTRAR) provided by Tripos (SYBYLX1.1).[28] The structure of rabbit SERCA complexed with CPA was chosen because of the identification of a metal ion in the CPA binding niche.[29,30] However, by choosing this template, which marks the dynamical endpoint of ligand binding, the model is limited to a static picture of the binding niche. No attempt was made to model the helical movements upon binding of new ligands. The spatial neighborhood of the CPA binding niche is well conserved between rabbit and *H. virescens*. Within 5 Å of CPA 24 amino acids (L253 D254 F256 G257 L260 I307 P308 E309 G310 L311 P312 I315 I761 Q56 F57 D59 L61 V62 L65 I97 L98 I99 N101 A102), 5 water molecules and one Mg^{2+} ion can be found. Only on position 58, slightly outside the 5 Å region, the acid Glu (rabbit) is replaced by the slightly smaller acid Asp (Heliothis). Thus, it is not expected that additional uncertainties will be introduced by analyzing the orientation of different ligands based on this homology model.

Previous published crystallographic structures demonstrated that CPA fills the calcium access channel in the E2 calcium free-form of the enzyme and stabilizes the M1- and M2-helices in positions that are incompatible with calcium binding.[29,30] The CPA binding pocket, a cavity between transmembrane segments M1, M2, M3 and M4 formed by Leu^{61} and Val^{62} on M1, Asn^{101}, Ala^{102} on M2, Gly^{257} on M3 and Leu^{311} and Pro^{312} on M4, consists of three regions, each accommodating distinct substructures of CPA. A polar region including the residues Gln^{56}, Asp^{59}, Asn^{101} on M1 and M2 as well as Mg^{2+} coordinates the acyl tetramic acid by a network of hydrogen bonds. The centre of the pocket, which houses ring C of CPA and BHQ (2,5-di-(*tert*-butyl) hydroquinone), a small-molecule inhibitor of SERCA, expresses a preference for conjugated cyclic π-systems. The lipophilic indole group is located in an extended hydrophobic groove between M3 and M4, primarily formed by Leu^{61} and Val^{62}. The indole nucleus locks the M1 and M4 helices and connects the CPA with the thapsigargin (TG) binding site.[29,30] Since the chelation of Mg^{2+} plays a pivotal role in CPA binding all CPA analogues were superimposed with their tetramic acid moieties.

Due to cis-connection between the six-membered ring C and pyrrolidine ring D natural CPA (11a*S*-configuration) shows a distinct bend, which allows the indole system to orient upwardly to the M4-helix in the binding pocket. The nonpolar interactions between Leu^{311}, Pro^{308} on M4 and Leu^{61}, Val^{62} on M1 with the indole system lock the M1-against the M4-helix and can be regarded as vital for the SERCA inhibitory action of CPA. The 11a*R*-configuration imposes a bowl-like form on *iso*CPA (**17**) with the pyrrolidine ring at the bottom and the indole ring and tetramic acid forming the walls. Due to its drastically changed shape, *iso*CPA (Fig. 3, green colour) is expected to orient differently in the binding pocket compared to CPA (Fig. 3, yellow colour). In fact, the indole system moves completely out of the lipophilic binding channel connecting the TG and CPA binding site. On the other side, the indole-nitrogen comes now much closer to the side-chain carboxylate-group of Asp 254 (Indol-N···Asp-O: 2.8 Å) and should be able to form an additional

hydrogen bond (Fig. 3, left side). This, together with the unhindered Mg^{2+} coordination provides an explanation for the only slightly reduced SERCA activity of *iso*CPA (**17**). To find a plausible explanation for the low SERCA inhibitory action of *alltrans*CPA (**18**) appears to be more difficult since at a first glance the overall shape of *alltrans*CPA resembles that of CPA (**1**). However, *alltrans*CPA (**18**) adopts an almost planar and more extended conformation compared to the corrugated CPA (**1**) with the consequence that the indole system shifts away from M4 and now points directly to Leu^{61} and Gly^{257} on M1 and M3 (Fig. 3, right side). As a result, the formation of nonpolar interactions with the respective residues on M4 is reduced and at the same time the indole ring probably causes a sterical clash with Leu^{61} (M1) and possibly Gly^{257} (M3). The critical role of Gly^{257} for CPA binding has already been demonstrated by previous mutagenesis studies.[31]

Upon introducing bulky acyl residues (compounds **19**–**23**), two effects can be observed in our model, both of which might be responsible for reduced activity. Firstly, contrary to cyclopropyl, the phenyl, benzyl or cyclohexyl moieties cause a pronounced shift of the ligand towards the hydrophobic pocket, leading to a modified hydrogen bond pattern as compared to CPA (Fig. 4). Secondly, the hydrophobic and bulky substituents interact with the phenyl side-chain of F57, which in turn could modify the orientation of the alpha-helix M1 close to its kink. In our model the cyclopropyl-analogue **19** fits perfectly to CPA, both sterically and electronically.

In compound **23** the phenyl substituent matches the cyclopropyl ring rather smoothly (Fig. 4, left). However, while cyclopropyl-CPA **19** exhibits exactly the same H-bond pattern as CPA, due to the larger phenyl group **23** is shifted towards the lipophilic part of the binding niche and looses the ability to chelate Mg^{2+} with its two carbonyl functionalities (Fig. 4, middle). In addition, Mg^{2+} itself has moved and disturbs the original H-bonding pattern. Differences in Mg^{2+} complexation are not so pronounced for the bulkier benzyl- and cyclohexyl-compounds, but here the interactions with F57 seem to become more important.

Regarding the indole N–H Laursen and Bublitz found a distance of 5.6 Å between the indole nitrogen and the carboxylate-group of aspartate 254, which is too long for a hydrogen bond and thus was attributed to a polar interaction.[30] In accordance with the biological data of *tosyl*CPA **16** and the *N*-methylindole **22** also molecular modelling considerations suggest that substitutions of the indole hydrogen by other polar groups should be possible without immediate loss of activity. Additionally, the lipophilic binding channel connecting the CPA and TG binding site appears to be an attractive starting point for the design of structurally different and less complex CPA analogues.

3. Experimental

3.1. General

Solvents and reagents were purchased from commercial sources. Triethylamine and diisopropylethylamine were dried over CaH_2 and distilled prior to use. Methanol was distilled from magnesium. Chloroform and dichloromethane were dried by passing the solvents through a basic aluminium oxide filled column. Precoated plates (silica gel 60 F_{254}, 250 μm, Merck, Darmstadt, Germany) were used for thin layer chromatography (TLC). Silica gel 0.04–0.063 mm from Macherey–Nagel was used for chromatographic purification. HPLC–MS was performed on a Varian 500 IonTrap LC–ESI-MS system or on a Bruker MicroTOF (column: 5 μm RP18) with ACN/H_2O gradient 10/90 to 90/10. High-resolution MS were measured on a Bruker MicroTOF. Semipreparative HPLC was either performed on a Kromasil 100 (C18 5 μm, 250×20 mm) or on a Zorbax ODS (250×21.2 mm) column with a Gilson Abimed System. Analytical ^1H and ^{13}C NMR spectra were recorded at 25 °C on a BRUKER AVANCE 400 (400.13 MHz for ^1H NMR, 100.62 MHz for ^{13}C NMR) or BRUKER AVANCE 600 (600.13 MHz for ^1H NMR, 150.90 MHz for ^{13}C NMR) spectrometer using tetramethylsilane as an internal standard. Spectra are reported in units of parts per million and coupling constants are given in hertz. Due to tautomeric- and *E*/*Z*-equilibria caused by the tetramic acid (ring E) in particular the proton NMR signals were considerably broadened. This made a complete assignment impossible for some CPA analogues. Melting points (mp) were determined with a Buechi 535 melting point apparatus and are uncorrected. IR-spectra were measured on a Nicolet Protégé 460 Spectrometer E.S.P.

3.2. Inhibition assay of SERCA activity

Sf9 cells (Invitrogen) were maintained under serum-free conditions at 27 °C with Sf-900 II SFM according to the manufacturers manual. For expression, Sf9 cells were coinfected with baculovirus of the *H. virescens* full-length SERCA (aa 2–1000, accession number AF115572) with a MOI of 0.3. After three days cells were harvested and microsomal membranes prepared according to standard procedures and stored at −80 °C.[32] A typical inhibition assay contained varying concentrations of the inhibitor (from 3.2 nM to 50 μM final concentration) and 0.4 μg/mL recombinant enzyme together with 0.05 mM ATP in the reaction buffer (45 mM MOPS pH 7.5, 5 mM $MgSO_4$, 1 mM KCl, 0.41 mM $CaCl_2$, 0.4 mM EGTA, 5% (w/v) gylerole, 0.01% (w/v) Tween 20, 0.05 mg/mL BSA and 0.002 mM A23187) in a microtiter plate. After 60 min incubation at room temperature, the ATP depletion was detected by a subsequent luciferase reaction

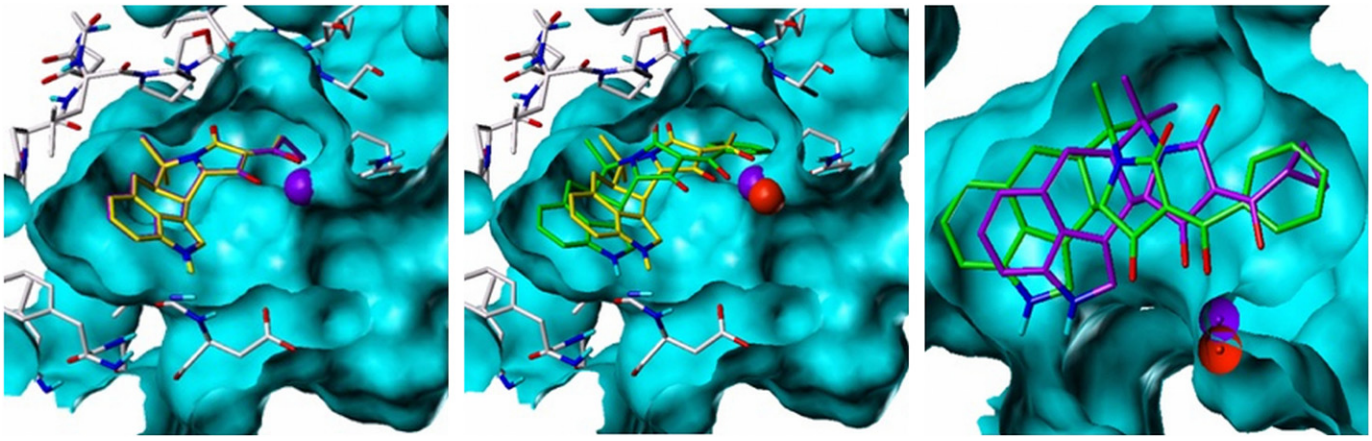


Fig. 4. Superimpositions of CPA with cyclopropyl-CPA **19** (left), CPA with phenyl-CPA **23** (middle), **19** and **23** (right).

employing 193.5 nM luciferin (BioSynth), 38.29 ng/mL luciferase (Promega) and 111 nM coenzyme A. Luminescence signal was detected on a Tecan infinity M1000 with an integration time of 100 ms. Assays were performed in quadruplicates and after normalization data were fitted to a sigmoid equation to determine IC50 values using Graph Pad Prism software (Graph Pad Prim Inc., San Diego, USA). In order to exclude direct luciferase inhibition, a luciferase-assay lacking the SERCA enzyme was performed with all observed inhibitors.

3.3. General procedure for the synthesis of acyl Meldrum's acid[23]

Pyridine (2 equiv) was added to a solution of Meldrum's acid (1 equiv) in DCM (0.7 M). The acyl chloride (1 equiv) was dissolved in DCM (1.4 M) and was added dropwise to the Meldrum's acid solution at 0 °C. The reaction was stirred for 1.5 h at 0 °C and 1.5 h at room temperature. An additional portion acyl chloride (0.5 equiv) was added and stirring was continued for 12 h at room temperature. Then the reaction was stopped by adding HCl (2 M) and diluted with DCM. The organic phase was separated, dried over Na_2SO_4 and evaporated. The crude product was either purified by column chromatography or crystallized to yield the pure acyl Meldrum's acid.

*3.3.1. (6aR,9S,9aR)-Pyrrolidine (**13a**), (6aS,9S,9aR)-pyrrolidine (**13b**) and (6aS,10S,10aR)-lactone (**14**).* Compound **12** (600 mg, 671 μmol) was dissolved in DCM (500 mL) under inert gas atmosphere, TfOH (62.2 μL, 671 μmol) was added and the reaction stirred for 1 h at room temperature. Sodium bicarbonate solution was added and the mixture was extracted twice with DCM. The combined organic layers were dried over Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude product (690 mg) was purified by column chromatography (DCM, then DCM/MeOH 20/1) and yielded a 1:1 mixture of the diastereomeric pyrrolidines **13** (265 mg, 62%) as yellowish foam together with lactone **14** (109 mg, 27%). R_f (cyclohexane/ethyl acetate 1/1): 0.48. A sample of this mixture was separated by semipreparative HPLC (ACN/H_2O 40/60). *6aR,9S,9aR-pyrrolidine* **13a** (cis-C/D ring connection): ^{1}H NMR (600 MHz; $CDCl_3$): δ=8.33 (d, J=8.8 Hz, 2H), 8.06 (d, J=8.8 Hz, 2H), 7.78 (d, J=8.4 Hz, 2H), 7.71 (d, J=8.3 Hz, 1H), 7.27–7.26 (m, 3H), 7.23 (s, 1H), 6.99 (d, J=7.3 Hz, 1H), 4.42 (d, J=5.7 Hz, 1H), 4.34 (q, J=7.1 Hz, 1H), 3.72 (Ψt, J=5.6 Hz, 1H), 3.01 (dd, J=16.2, 5.0 Hz, 1H), 2.64–2.56 (m, 2H), 2.38 (s, 3H), 1.65 (s, 3H), 1.42 (t, J=7.1 Hz, 3H), 1.00 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ=171.8, 150.0, 147.0, 145.1, 135.3, 133.1, 129.9 (CH), 129.1, 128.7 (CH), 128.2, 126.8 (CH), 126.1 (CH), 124.1 (CH), 121.1 (CH), 120.8 (CH), 116.8, 111.6 (CH), 69.3, 66.5 (CH), 62.0 (CH_2), 48.2 (CH), 37.8 (CH), 30.0 (CH_3), 25.5 (CH_2), 23.3 (CH_3), 21.6 (CH_3), 14.2 (CH_3); HR-ESI-MS: m/z=660.1445 (calcd 660.1445 for $C_{31}H_{31}N_3NaO_8S_2$); IR: ν=1747 cm^{-1}. *6aS,9S,9aR-Pyrrolidine* **13b** (trans-C/D ring connection): ^{1}H NMR (600 MHz; $CDCl_3$): δ=8.34 (d, J=8.9 Hz, 2H), 8.16 (d, J=8.9 Hz, 2H), 7.78 (d, J=8.3 Hz, 2H), 7.71 (d, J=8.3 Hz, 1H), 7.37 (d, J=1.9 Hz, 1H), 7.29–7.26 (m, 3H), 7.01 (d, J=7.3 Hz, 1H), 4.45 (d, J=9.8 Hz, 1H), 4.53–4.42 (m, 2H), 3.41 (ddd, J=12.8, 9.8, 1.9 Hz, 1H), 2.84 (dd, J=15.5, 4.0 Hz, 1H), 2.76 (dd, J=15.4, 12.1 Hz, 1H), 2.40 (s, 3H), 1.98 (ddd, J=12.4, 12.3, 4.0 Hz, 1H), 1.49 (t, J=7.1 Hz, 3H), 1.43 (s, 3H), 1.40 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ=172.1, 149.9, 147.9, 145.0, 135.5, 133.5, 130.4, 129.9 (CH), 129.2, 128.8 (CH), 126.8 (CH), 125.9 (CH), 124.2 (CH), 121.4 (CH), 119.0 (CH), 118.6, 111.9 (CH), 67.4, 65.6 (CH), 62.2 (CH_2), 54.7 (CH), 41.2 (CH), 27.6 (CH_2), 25.7 (CH_3), 23.7 (CH_3), 21.6 (CH_3), 14.2 (CH_3); HR-ESI-MS: m/z=660.1457 (calcd 660.1445 for $C_{31}H_{31}N_3NaO_8S_2$); IR: ν=1751 cm^{-1}. (*6aS,10S,10aR*)-*Lactone* **14**: R_f (cyclohexane/ethylacetate 1/1): 0.39; ^{1}H NMR (600 MHz; $CDCl_3$): δ=8.24 (d, J=8.8 Hz, 2H), 8.01 (d, J=8.9 Hz, 2H), 7.89 (d, J=8.4 Hz, 2H), 7.73 (d, J=8.3 Hz, 1H), 7.65 (d, J=1.8 Hz, 1H), 7.25–7.23 (m, 3H), 6.97 (d, J=7.3 Hz, 1H), 5.08 (br d, J=8.7 Hz, 1H), 4.78 (dd, J=8.7, 5.6 Hz, 1H), 3.62 (ddd, J=12.7, 5.6, 1.8 Hz, 1H), 2.98 (dd, J=15.8, 4.0 Hz, 1H), 2.75 (dd, J=15.6, 12.0 Hz, 1H), 2.30 (s, 3H), 2.15 (Ψdt, J=12.2, 12.2, 4.0 Hz, 1H), 1.51 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ=166.8, 150.1, 145.7, 144.9, 135.0, 132.9, 130.0 (CH), 128.8, 128.7, 128.6 (CH), 127.3 (CH), 125.6 (CH), 124.1 (CH), 122.1 (CH), 120.2 (CH), 114.4, 111.7 (CH), 87.1, 53.3 (CH), 41.0 (CH), 34.4 (CH), 29.0 (CH_2), 29.0 (CH_3), 23.0 (CH_3), 21.5 (CH_3); HR-ESI-MS: m/z=632.1132 (calcd 632.1132 for $C_{29}H_{27}N_3NaO_8S_2$); IR: ν=1735 cm^{-1}.

3.3.2. (6aR,9S,9aR)-7,7-Dimethyl-2-(toluene-4-sulfonyl)-2,6a,7,8,9,9a-hexahydro-6H-isoindolo[4,5,6-cd]indole-9-carboxylic acid ethyl ester and (6aS,9S,9aR)-7,7-dimethyl-2-(toluene-4-sulfonyl)-2,6a,7,8,9,9a-hexahydro-6H-isoindolo[4,5,6-cd]indole-9-carboxylic acid ethyl ester. Pyrrolidine **10** (190 mg, 297.9 μmol) was dissolved in DMF (3 mL), DBU (133 μL, 894 μmol) was added followed by mercaptoethanol (62 μL, 894 μmol). The resulting mixture was stirred for 24 h at room temperature. Then diethyl ether was added and the solution was washed with saturated sodium bicarbonate solution, water and brine. The combined aqueous layers were extracted twice with diethyl ether and the combined organic layers were dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure gave a crude product (240 mg), which afforded the denosylated product (92 mg, 68%) as yellowish foam after chromatographic purification (cyclohexane/ethyl acetate 1/1). R_f (cyclohexane/ethyl acetate 1/1): 0.19. *6aR,9S,9aR-isomer* (cis-C/D ring connection): ^{1}H NMR (600 MHz; $CDCl_3$): δ=7.77 (d, J=8.4 Hz, 2H), 7.74 (d, J=8.2 Hz, 1H), 7.35 (d, J=0.9 Hz, 1H), 7.29–7.26 (m, 1H), 7.22 (d, J=8.1 Hz, 2H), 7.03 (d, J=7.4 Hz, 1H), 4.39–4.30 (m, 2H), 3.79 (d, J=8.0 Hz, 1H), 3.74 (Ψt, J=7.5 Hz, 1H), 3.04 (dd, J=16.8, 6.3 Hz, 1H), 2.84 (dd, J=16.8, 7.8 Hz, 1H), 2.35 (s, 3H), 2.29 (Ψq, J=7.3 Hz, 1H), 1.41 (t, J=7.2 Hz, 3H), 1.34 (s, 3H), 0.95 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ=174.6, 144.7, 135.5, 134.6, 133.1, 130.4, 129.7 (CH), 128.6, 126.7 (CH), 125.5 (CH), 121.1 (CH), 120.6 (CH), 120.6, 111.1 (CH), 65.0 (CH), 61.4 (CH_2), 48.3 (CH), 40.0 (CH), 30.0 (CH_3), 26.0 (CH_2), 24.3 (CH_3), 21.5 (CH_3), 14.3 (CH_3); HR-ESI-MS: m/z=453.1838 (calcd 453.1843 for $C_{25}H_{29}N_2O_4S$); IR: ν=1731 cm^{-1}. *6aS,9S,9aR-Isomer* (trans-C/D ring connection): ^{1}H NMR (600 MHz; $CDCl_3$): δ=7.79–7.73 (m, 3H), 7.41 (d, J=1.7 Hz, 1H), 7.27–7.21 (m, 3H), 7.05 (d, J=7.6 Hz, 1H), 4.45–4.29 (m, 2H), 3.90 (d, J=10.1 Hz, 1H), 3.15 (ddd, J=12.4, 9.9, 1.7 Hz, 1H), 2.92 (dd, J=15.6, 3.9 Hz, 1H), 2.75 (ddd, J=15.4, 12.6 Hz, 1H), 2.36 (s, 3H), 2.03 (Ψdt, J=12.4, 12.3, 3.8 Hz, 1H), 1.44 (t, J=7.1 Hz, 3H), 1.30 (s, 3H), 1.20 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ=174.1, 144.6, 135.3, 133.5, 132.1, 130.1, 129.6 (CH), 128.5, 126.5 (CH), 125.4 (CH), 121.1, 121.0 (CH), 119.4 (CH), 111.4 (CH), 61.4 (CH), 61.2 (CH_2), 58.7, 54.6 (CH), 43.5 (CH), 29.3 (CH_3), s28.0 (CH_2), 25.0 (CH_3), 21.3 (CH_3), 14.1 (CH_3); HR-ESI-MS: m/z=453.1843 (calcd 453.1843 for $C_{25}H_{29}N_2O_4S$); IR: ν=1731 cm^{-1};

*3.3.3. N-Tosyl-CPA (**16**).* Acetyl-Meldrum's acid (309 mg, 1.66 mmol) was added to a solution of the denosylated precursor from the preceding reaction (300 mg, 663 μmol) in dioxane (15 mL). The reaction was stirred for 90 min at 80 °C. Then a solution of KOH in MeOH (0.1 M, 16.6 mL, 1.66 mmol) was added and stirring was continued for another 60 min at 80 °C. The solvent was removed under reduced pressure and the residue separated between DCM and aqueous ammonium chloride. The aqueous phase was extracted twice with DCM and the combined organic layers were dried over Na_2SO_4. Filtration and evaporation of the solvent under reduced pressure gave a crude product (347 mg), which yielded **16** (260, 80%) as a 1:1 mixture of diastereomers after chromatographic purification (DCM/MeOH 25/1). The diastereomers of a small sample were separated by semipreparative HPLC (ACN/H_2O 70/30). R_f (DCM/MeOH 95/5): 0.45. (*6aR,11aS,11bR*)-*N-tosyl-CPA* **16a** (cis-C/D ring connection): ^{1}H NMR (600 MHz; $CDCl_3$): δ=7.85 (d, J=8.4 Hz,

2H), 7.78 (d, *J*=8.3 Hz, 1H), 7.55 (s, 1H), 7.30–7.27 (m, 1H), 7.25 (d, *J*=8.2 Hz, 2H), 7.05 (d, *J*=7.3 Hz, 1H), 4.00 (d, *J*=10.9 Hz, 1H), 3.58 (dd, *J*=10.9, 6.0 Hz, 1H), 3.02 (dd, *J*=16.3, 5.8 Hz, 1H), 2.94 (dd, *J*=16.1, 11.7 Hz, 1H), 2.59 (Ψdt, *J*=11.6, 5.9, 5.9 Hz, 1H), 2.49 (s, 3H), 2.36 (s, 3H), 1.67 (s, 3H), 1.58 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): *δ*=194.3, 185.0, 175.4, 144.7, 135.6, 133.1, 129.8 (CH), 129.3, 128.4, 126.9 (CH), 125.5 (CH), 122.9 (CH), 120.6 (CH), 116.2, 111.6 (CH), 105.4, 70.7 (CH), 63.6, 52.4 (CH), 35.4 (CH), 26.1 (CH_3), 26.1 (CH_2), 24.4 (CH_3), 21.5 (CH_3), 19.8 (CH_3); HR-ES-MS: *m*/*z*=513.1449 (calcd 513.1455 for $C_{27}H_{26}N_2NaO_5S$); IR: *ν*=3415, 1709, 1614 cm^{-1}. (6*aS*,11*aS*,11*bR*)-*N*-*Tosyl-CPA* **16b** (trans-C/D ring connection): ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=7.85 (d, *J*=8.4 Hz, 2H), 7.82 (d, *J*=8.2 Hz, 1H), 7.55 (d, *J*=1.7 Hz, 1H), 7.29 (Ψt, *J*=7.8 Hz, 1H), 7.25 (d, *J*=8.1 Hz, 2H), 7.06 (d, *J*=7.3 Hz, 1H), 4.09 (d, *J*=10.7 Hz, 1H), 2.95 (Ψdt, *J*=11.4, 1.7 Hz, 1H), 2.92 (dd, *J*=15.6, 4.6 Hz, 1H), 2.88 (dd, *J*=15.5, 11.8 Hz, 1H), 2.52 (s, 3H), 2.37 (s, 3H), 2.32, (Ψdt, *J*=11.8, 11.8, 4.6 Hz, 1H), 1.60 (s, 3H), 1.53 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): *δ*=193.6, 185.6, 174.6, 144.7, 135.7, 133.7, 130.9 (CH), 129.9, 129.4, 126.9 (CH), 125.6 (CH), 121.3 (CH), 121.2 (CH), 118.0, 111.9 (CH), 105.3, 71.2 (CH), 62.1, 58.0 (CH), 38.4 (CH), 28.0 (CH_3), 27.2 (CH_2), 21.5 (CH_3), 19.8 (CH_3), 19.7 (CH_3); HR-ES-MS: *m*/*z*=489.1497 (calcd 489.1490 for $C_{27}H_{25}N_2O_5S$); IR: *ν*=3362, 1611 cm^{-1}.

3.3.4. N-Tosyl-cyclopropyl-CPA. *N*-Tosyl-cyclopropyl-CPA was prepared as described for *N*-tosyl-CPA (**16**). The reaction was monitored by TLC and continued until the open chain intermediate was consumed completely. ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=7.96–7.43 (m br, 4H), 7.35–6.82 (m br, 4H), 4.14–3.76 (m br, 1H), 3.79–3.08 (m br, 2H), 3.00–2.00 (m br, 7H), 1.75–0.72 (m br, 10H); HR-ES-MS: *m*/*z*=515.1645 (calcd for 515.1646 $C_{29}H_{27}N_2O_5S$); IR: *ν*=3399, 1608 cm^{-1}.

3.3.5. N-Tosyl-cyclohexyl-CPA. *N*-Tosyl-cyclohexyl-CPA was prepared as described for *N*-tosyl-CPA (**16**). The reaction was monitored by TLC and continued until the open chain intermediate was consumed completely. ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=7.91–7.51 (m br, 4H), 7.31–7.20 (m br, 3H), 7.09–7.04 (m br, 1H), 4.06–3.48 (m br, 2H), 3.25–2.74 (m br, 3H), 2.38–2.28 (m br, 5H), 1.93–0.88 (m br, 16H); HR-ESI-MS: *m*/*z*=559.2261 (calcd 559.2261 for $C_{32}H_{35}N_2O_5S$); IR: *ν*=3423, 1685, 1611 cm^{-1}.

3.3.6. N-Tosyl-benzyl-CPA. Additional amounts of KOH/MeOH and prolonged reaction times were needed for the complete conversion of the 3-oxo-3-phenyl-propionamide intermediate. ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=7.86–7.61 (m br, 3H), 7.47–6.88 (m br, 10H), 5.29–3.31 (m br, 3H), 2.89–2.05 (m br, 5H), 1.72–0.74 (m br, 9H); HR-ESI-MS: *m*/*z*=567.1949 (calcd 567.1948 for $C_{33}H_{31}N_2O_5S$); IR: *ν*=3349, 1713, 1673, 1614 cm^{-1}.

*3.3.7. N-Tosyl-phenyl-CPA and phenyl-CPA (**23**).* Additional amounts of KOH/MeOH and prolonged reaction times were needed for the complete conversion of the 3-oxo-3-phenyl-propionamide intermediate. Under this reaction conditions a significant cleavage of the *N*-tosyl group was observed, yielding directly two phenyl-CPA diastereomers. The reaction mixture was separated by semi-preparative HPLC (ACN/H_2O 75/25) after work-up as described above. 6*aR*,11*S*,11*aR*-*Phenyl-CPA* (cis-C/D ring connection): ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=8.21 (d, *J*=7.7 Hz, 2H), 8.13 (s br, 1H), 7.61 (t, *J*=7.2 Hz, 1H), 7.51 (t, *J*=7.5 Hz, 2H), 7.23 (d, *J*=7.9 Hz, 1H), 7.19–7.17 (m, 2H), 6.95 (d, *J*=6.7 Hz, 1H), 4.18 (d, *J*=11.0 Hz, 1H), 3.73 (dd, *J*=10.9, 5.7 Hz, 1H), 3.12–3.11 (m, 2H), 2.71–2.67 (m, 1H), 1.76 (s, 3H), 1.71 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): *δ*=193.4, 181.1, 177.3, 133.5 (CH), 133.4, 132.2, 129.6 (CH), 128.6, 128.1 (CH), 125.9, 123.0 (CH), 121.0 (CH), 116.5 (CH), 110.1, 108.7 (CH), 103.6 (CH), 71.1 (CH), 64.0 (C), 53.0 (CH), 36.2 (CH), 26.6 (CH_2), 26.4 (CH_3), 24.3 (CH_3); HR-ESI-MS: *m*/*z*=399.1703 (calcd 399.1703 for $C_{25}H_{23}N_2O_3$); IR *ν*=3402, 1694, 1594, 1557 cm^{-1}. 6*aS*,11*S*,11*aR*-*Phenyl-CPA* (trans-C/D ring connection): ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=8.24 (d, *J*=7.6 Hz, 2H), 8.03 (s br, 1H), 7.63 (t, *J*=7.3 Hz, 1H), 7.54 (t, *J*=7.6 Hz, 2H), 7.34 (s, 1H), 7.23 (d, *J*=7.8 Hz, 1H), 7.18 (Ψt, *J*=7.4 Hz, 1H), 6.95 (d, *J*=6.9 Hz, 1H), 4.19 (d, *J*=10.5 Hz, 1H), 3.16 (Ψt, *J*=11.2 Hz, 1H), 3.03–3.01 (m, 2H), 2.48 (ddd, *J*=11.6, 9.7, 6.8 Hz, 1H), 1.68 (s, 3H), 1.68 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): *δ*=192.2, 181.6, 176.6, 134.0, 133.5 (CH), 132.3, 130.2, 129.5 (CH), 128.1 (CH), 127.1, 123.1 (CH), 119.6 (CH), 117.1 (CH), 112.1, 108.9 (CH), 103.5, 71.6 (CH), 62.8, 58.9 (CH), 39.2 (CH), 27.9 (CH_3), 27.5 (CH_2), 19.9 (CH_3); HR-ESI-MS: *m*/*z*=399.1702 (calcd 399.1703 for $C_{25}H_{23}N_2O_3$); IR *ν*=3391, 1695, 1593, 1558 cm^{-1}. 6*aS*,11*S*,11*aR*-*N*-*Tosyl-phenyl-CPA*: ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=8.23 (d, *J*=7.6 Hz, 2H), 7.83 (d, *J*=8.3 Hz, 2H), 7.82 (d, *J*=8.4 Hz, 1H), 7.68 (s, 1H), 7.66 (t, *J*=7.3 Hz, 1H), 7.58 (t, *J*=7.5 Hz, 2H), 7.29 (t, *J*=7.6 Hz, 1H), 7.23 (d, *J*=8.0 Hz, 2H), 7.07 (d, *J*=7.2 Hz, 1H), 4.17 (d, *J*=10.4 Hz, 1H), 2.99 (Ψt, *J*=11.3, 11.3 Hz, 1H), 2.95 (dd, *J*=15.5, 4.5 Hz, 1H), 2.90 (dd, *J*=15.5, 11.9 Hz, 1H), 2.37 (Ψdt, *J*=11.9, 11.9, 4.4 Hz, 1H), 2.36 (s, 3H), 1.64 (s, 3H), 1.58 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): *δ*=191.5, 184.6, 176.6, 144.7, 135.7, 133.7 (CH), 133.7, 132.1, 130.9, 129.8 (CH), 129.5 (CH), 129.4, 128.2 (CH), 126.9 (CH), 125.5 (CH), 121.4 (CH), 121.3 (CH), 118.0, 111.9 (CH), 103.3, 70.6 (CH), 62.6, 57.9 (CH), 38.6 (CH), 27.9 (CH_3), 27.2 (CH_2), 21.5 (CH_3), 19.7 (CH_3); HR-ESI-MS: *m*/*z*=553.1791 (calcd 553.1792 for $C_{32}H_{29}N_2O_5S$); IR *ν*=1695, 1595, 1563 cm^{-1}.

*3.3.8. Cyclopiazonic acid (**1**), iso-cyclopiazonic acid (**17**) and alltrans-cyclopiazonic acid (**18**).* Compound **16** (50 mg, 102 μmol) was dissolved in MeOH/THF (1:1, 6 mL) and Cs_2CO_3 (118 mg, 362 μmol) was added. The resulting solution was stirred for 94 h under reflux. Then the solvent was removed and the residue separated between DCM and aqueous ammonium chloride. The aqueous phase was extracted five times with DCM. The combined organic layers were dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. Chromatographic purification (DCM/MeOH 9/1) of the crude material (38 mg) yielded CPA (20.4 mg, 60%) as a mixture of three stereoisomers, which was separated by semipreparative HPLC (ACN/$H_2$0 65/35+0.1% HCOOH). *Cyclopiazonic acid*:[1] R_f (DCM/MeOH 1/1): 0.22; ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=8.09 (s, br, 1H), 7.21 (d, *J*=8.1 Hz, 1H), 7.17 (s, 1H), 7.16 (dd, *J*=8.1, 7.0 Hz, 1H), 6.91 (d, *J*=7.0 Hz, 1H), 4.07 (d, *J*=11.1 Hz, 1H), 3.66 (dd, *J*=11.0, 5.8 Hz, 1H), 3.09–3.02 (m, 2H), 2.66–2.62 (m, 1H), 2.45 (s, 3H), 1.68 (s, 3H), 1.64 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): *δ*=195.1, 184.8, 175.3, 133.4, 128.7, 125.9, 123.1 (CH), 120.8 (CH), 116.5 (CH), 110.1, 108.7 (CH), 105.6, 71.8 (CH), 63.4, 53.1 (CH), 36.1 (CH), 26.6 (CH_2), 26.4 (CH_3), 24.4 (CH_3), 19.7 (CH_3); HR-ESI-MS: *m*/*z*=335.1404 (calcd 335.1401 for $C_{20}H_{19}N_2O_3$); IR: *ν*=3394, 1704, 1608 cm^{-1}. *iso-Cyclopiazonic acid*:[17] ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=7.91 (s, br, 1H), 7.13–7.12 (m, 2H), 6.90 (s, 1H), 6.87–6.86 (m, 1H), 4.51 (d, *J*=5.6 Hz, 1H), 3.84 (Ψt, *J*=5.1 Hz, 1H), 3.32 (dd, *J*=17.4, 6.2 Hz, 1H), 2.98 (d, *J*=17.6 Hz, 1H), 2.96 (Ψt, *J*=5.6 Hz, 1H), 2.47 (s, 3H), 1.51 (s, 3H), 0.90 (s, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): *δ*=194.4, 184.4, 173.0, 133.5, 128.8, 127.3, 123.3 (CH), 120.0 (CH), 116.3 (CH), 108.5, 108.2 (CH), 106.2, 71.7, 62.6 (CH), 52.9 (CH), 35.6 (CH), 30.0 (CH_3), 26.4 (CH_2), 21.5 (CH_3), 19.9 (CH_3); HR-ESI-MS: *m*/*z*=337.1531 (calcd 337.1547 for $C_{20}H_{21}N_2O_3$); IR: *ν*=3444, 1682, 1599 cm^{-1}. *alltrans-Cyclopiazonic acid*:[18] ^{1}H NMR (600 MHz; $CDCl_3$): *δ*=8.02 (s, br, 1H), 7.31 (s, 1H), 7.25 (d, *J*=8.1 Hz, 1H), 7.19–7.17 (m, 1H), 6.95 (d, *J*=7.0 Hz, 1H), 4.12 (d, *J*=10.8 Hz, 1H), 3.11 (Ψt, *J*=11.3 Hz, 1H), 3.01–2.98 (m, 2H), 2.51 (s, 3H), 2.44 (ddd, *J*=11.7, 10.1, 6.6 Hz, 1H), 1.64 (s, 3H), 1.58 (s, 3H); HR-ESI-MS: *m*/*z*=335.1402 (calcd 335.1401 for $C_{20}H_{19}N_2O_3$); IR: *ν*=3407, 1659, 1590 cm^{-1}.

The acyl tetramic acids **19**, **20** and **21** were prepared as described for CPA (**1**). The reactions were monitored by TLC and continued until complete conversion of the starting material. Cyclopropyl-CPA

19: R_f (DCM/MeOH 9/1): 0.38; ESI-MS: m/z=745.3 ([(M−H)$_2$+Na]$^-$, 22), 361.2 ([M−H]$^-$, 100); HR-ESI-MS: m/z=361.1557 (calcd 361.1558 for $C_{22}H_{21}N_2O_3$). Cyclohexyl-CPA **20**: ESI-MS: m/z=829.4 ([(M−H)$_2$+Na]$^-$, 6), 403.2 ([M−H]$^-$, 100); HR-ESI-MS: m/z=405.2179 (calcd 405.2173 for $C_{25}H_{29}N_2O_3$). Benzyl-CPA **21**: ESI-MS: m/z=845.3 ([(M−H)$_2$+Na]$^-$, 3), 411.2 ([M−H]$^-$, 100); ESI-MS: m/z=413.2 ([M+H]$^+$, 35), 280.2 ([$C_{17}H_{16}N_2O_2$]$^+$, 100). During the tosylate cleavage reaction small amounts of *N*-methyl-benzyl-CPA **22** were isolated: ESI-MS: m/z=873.4 ([(M−H)$_2$+Na]$^-$, 2), 425.2 ([M−H]$^-$, 100).

*3.3.9. CPA-amine (**24**).* CPA (19.3 mg, 57.4 μmol) was dissolved in dioxane (2 mL) and aqueous ammonia solution (25%, 2.2 mL) was added. The reaction was stirred for 18 h at room temperature, diluted with water (10 mL) and HCl (37%, 0.5 mL). The solution was extracted four times with DCM (5 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure to yield **24** (11.8 mg, 61%). R_f (DCM/MeOH 9/1): 0.48; ^{1}H NMR (600 MHz; $CDCl_3$): δ=10.07–9.67 (m, 1H), 8.19–7.99 (m, 1H), 7.23–7.11 (m, 3H), 6.95–6.92 (m, 1H), 5.94–5.68 (m, 1H), 4.10–4.02 (m, 1H), 3.68–2.37 (m, 4H), 2.56–2.50 (3×s, 3H), 1.68–0.90 (6×s, 6H); HR-ESI-MS: m/z=336.1707 (calcd 336.1707 for $C_{20}H_{22}N_3O_2$); IR: ν=3307, 1669, 1616 cm^{-1}.

*3.3.10. CPA-hydrazine (**25**).* CPA (19.0 mg, 56.5 μmol) was dissolved in $CHCl_3$/MeOH (1:1, 3 mL) and hydrazine hydrate (55 μL, 1.13 mmol) was added. The solution was stirred for 18 h at room temperature, diluted with water (10 mL) and HCl (37%, 0.5 mL) and then extracted four times with DCM (5 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure to give **25** (16.7 mg, 84%). R_f (DCM/MeOH 9/1): 0.46; ^{1}H NMR (600 MHz; $CDCl_3$): δ=8.47–8.06 (m, 1H), 7.22–6.85 (m, 4H), 4.45–3.61 (m, 2H), 3.08–2.88 (m, 3H), 2.69–2.65 (m, 3H), 1.64–0.86 (6×s, 6H); ESI-MS: m/z=699.3 ([2M−H]$^-$, 9), 349.2 ([M−H]$^-$, 100); HR-ESI-MS: m/z=351.1817 (calcd 351.1816 for $C_{20}H_{23}N_4O_2$); IR: ν=1677, 1586 cm^{-1}.

*3.3.11. CPA-p-chlorphenylhydrazine (**26**).* CPA (10.0 mg, 29.7 μmol) was dissolved in $CHCl_3$/MeOH (1:1, 3 mL), *p*-chlorphenylhydrazine (106 mg, 595 μmol) was added and the suspension was stirred for 90 h at room temperature. After filtration the solution was diluted with water (10 mL) and HCl (1 M, 10 mL) and extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford a crude product (12.6 mg). Chromatographic purification (DCM, then DCM/MeOH gradient) yielded **26** (7.0 mg, 51%). R_f (DCM/MeOH 20/1): 0.32; ESI-MS: m/z=921.4 ([2M−H]$^-$, 5), 919.4 ([2M−H]$^-$, 7), 461.2 ([^{37}ClM−H]$^-$, 38), 459.3 ([^{35}ClM−H]$^-$, 100), HR-ESI-MS: m/z=459.1584 (calcd 459.1593 for $C_{26}H_{24}{}^{35}ClN_4O_2$); IR: ν=3294, 1669, 1607, 1579 cm^{-1}.

*3.3.12. CPA-hydroxylamine (**27**).* CPA (18.4 mg, 54.7 μmol) was dissolved in dioxane (2 mL) and hydroxylamine solution (1.7 mL) was added. The reaction mixture was stirred for 18 h at room temperature, diluted with water (10 mL) and HCl (37%, 0.5 mL) and extracted four times with DCM (5 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure to yield **27** (18.0 mg, 94%). R_f (DCM/MeOH 9/1): 0.58; ^{1}H NMR (600 MHz; $CDCl_3$): δ=8.26–8.03 (3×s br, 1H), 7.18–6.89 (m, 4H), 4.56–1.91 (m, 8H), 1.57–0.79 (m, 6H); ESI-MS: m/z=701.3 ([2M−H]$^-$, 14), 350.2 ([M−H]$^-$, 100); HR-ESI-MS: m/z=352.1658 (calcd 352.1656 for $C_{20}H_{22}N_3O_3$); IR: ν=3391, 1682, 1622 cm^{-1}.

*3.3.13. CPA-O-methylhydroxylamine (**28**).* CPA (15.0 mg, 44.6 μmol) was dissolved in $CHCl_3$/MeOH (1:1, 3 mL) and *O*-methyl-hydroxylamine (74.5 mg, 892 μmol) added. The reaction mixture was stirred for 90 h at room temperature. After filtration the solution was diluted with water (10 mL) and HCl (1 M, 10 mL) and extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure to afford a crude product (11.9 mg), which yielded **28** (6.6 mg, 41%) after chromatographic purification (DCM, then DCM/MeOH gradient). R_f (DCM/MeOH 6/1): 0.72; ^{1}H NMR (600 MHz; $CDCl_3$): δ=13.60–11.49 (m br, 1H), 8.16–7.96 (3×s, 1H), 7.25–6.86 (m, 4H), 4.66–4.03 (m, 1H), 3.94–3.77 (6×s, 3H), 3.68–2.93 (m, 3H), 3.17–2.93 (m, 2H), 2.64–2.41 (6×s, 3H), 1.66–0.87 (11×s, 6H); HR-ESI-MS: m/z=366.1813 (calcd 366.1812 for $C_{21}H_{24}N_3O_3$); IR: ν=3307, 1668, 1626, 1574 cm^{-1}.

4. Conclusion

The first enantioselective synthesis of cyclopiazonic acid (CPA, **1**), an inhibitor of sarcoplasmic reticulum Ca^{2+}-ATPase, has been accomplished by a modification of the Knight synthesis. Several CPA analogues were prepared by varying the acyl residues in the tetramic acid unit and by direct modification of the acetyl group with nitrogen nucleophiles. The structure–activity data of these analogues proof the tetramic acid moiety the pharmacophoric unit of CPA. Any negative interference with the Mg^{2+} binding and hydrogen bond-forming properties of the tetramic acid results in at least considerable losses of SERCA activity. Nevertheless, structure–activity data as well as the binding model of CPA suggest structural modifications at the indole system or ring C to be possible without immediate loss of activity. Thus, future work should aim at designing structurally less complex CPA analogues as novel antimalarial or insecticidal drugs.

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