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Synthesis and SERCA activities of structurally simplified cyclopiazonic acid analogues

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ABSTRACT

The indole alkaloid cyclopiazonic acid (CPA) is one of the few known nanomolar inhibitors of sarco(endo)plasmic reticulum Ca^{2+}-ATPase (SERCA) besides the anticancer drug thapsigargin and the antiplasmoidal terpenoid artemisinin. Due to its less complex structure CPA represents an attractive lead structure for the development of novel antimalarial drugs or for applications in the field of plant protection. We report here the first syntheses of structurally simplified CPA fragments and discuss their SERCA activities on the basis of published crystal structures of CPA–SERCA complexes.

1. Introduction

Acyltetramic acids have been known since almost a century but it was not until the 1960s that this heterocycle was identified as a key structural element in natural products, such as the SERCA inhibitor cyclopiazonic acid (CPA, **1**), the potent HIV-1 integrase inhibitor equisetin (**3**), the antiprotozoal ikarugamycin (**4**), or the antibiotic tirandamycin (**5**) to name only a few (Fig. 1).[1–4] The wide range of biological activities that have been shown in many cases to be related to the tetramic acid core renders this class of natural products prime targets for chemists and biologists.

The toxic indole alkaloid cyclopiazonic acid (CPA **1**) was first isolated from cereals and meal infected with fungi of the Aspergillus- or Penicillium genus. CPA is the cause of several food poisonings but shows on the other hand promising antiplasmoidal activities on a level comparable to chloroquin. CPA (**1**) specifically inhibits a Ca^{2+}-ATPase of the sarcoendoplasmic reticulum (SERCA) which is essential for calcium reuptake in the muscle contraction–relaxation cycle.[5,6] Calcium metabolism also represents an important target for modern insecticides as exemplified by the natural product ryanodin or flubendiamide, a synthetic benzenedicarboxamide insecticide for controlling lepidopterous insect pests.[7]

Despite of more than one hundred published natural tetramic acids, the skeleton of cyclopiazonic acid (CPA) remains unique as the acetyltetramic acid core is part of a multicyclic framework.[8] The first synthesis of CPA was reported in 1984, followed by two other racemic syntheses.[9–11] A 15-step asymmetric total synthesis of CPA based on the racemic synthesis developed by Knight and coworkers has been accomplished only very recently.[11] However, less complex CPA analogues accessible by short syntheses are a prerequisite for the development of synthetic drugs against malaria or for potential applications in crop protection.

Published CPA–SERCA structures suggest the CPA binding pocket to consist of three regions: a polar region coordinates the acyltetramic acid with Gln^{56}, Asp^{59}, Asn^{101} and Mg^{2+} as main interacting residues (Fig. 2).[13,14] The chelation of Mg^{2+} by the acyltetramic acid plays a pivotal role for SERCA inhibition.[12] The center of the pocket binds ring C of CPA and displays a preference for conjugated π-systems as documented by BHQ (2,5-di-(*tert*-butyl) hydroquinone), a small molecule inhibitor of SERCA. An extended hydrophobic region of the binding pocket houses the lipophilic indole residue and connects the CPA with the thapsigargin (TG) binding site.[15] Remarkably, the indole is fixed to its binding site only by lipophilic interactions, since the NH-group seems not to be involved in hydrogen bond formation. Thus, the indole-ring can be presumed to be more flexible to structural variations as the other CPA partial structures.

Abbreviations: ACN, acetonitrile; DMF, dimethylformamide; EGTA, ethylene glycol tetraacetic acid; IPy_2BF_4, bis(pyridine)iodine-(I)-tetrafluoroborate; MOPS, 3-(*N*-morpholino)-propanesulfonic acid; THF, tetrahydrofurane; TFA, trifluoroacetic acid; DMAP, 4-dimethylaminopyridine; DCC, *N*,*N*′-dicyclohexylcarbodiimide.

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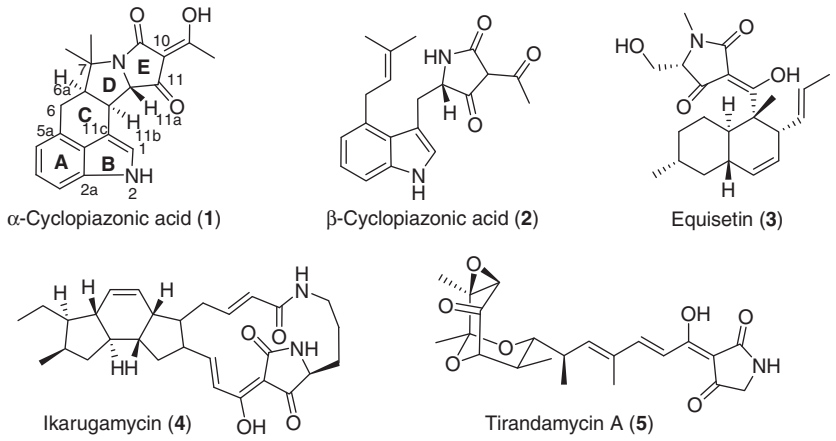


Figure 1. Natural products containing acyltetramic acids.

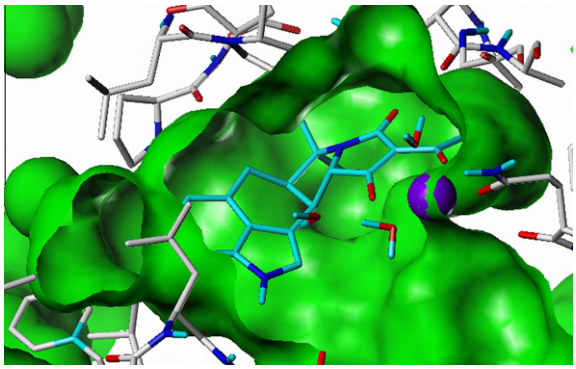


Figure 2. Homology model of *Heliothis virescens* SERCA complexed with cyclopiazonic acid.

Taking the available molecular-modelling studies and structure–activity data into account, we suggest compounds **6**–**9** (Fig. 3) as structurally simplified models for CPA (**1**). These compounds should be appropriate to elucidate the role of the indole core for SERCA inhibition.[12] Tetramic acids **6** and **7** in which the cyclohexane ring C is replaced by a phenyl ring, combine structural elements of BHQ and CPA (**1**). Both compounds address the postulated requirement for unsaturation in ring C. In addition, CPA analogue **6** has a linear arrangement of rings while the analogous compound **7** with a pyrrolizidine skeleton shows the CPA typical angulated shape. The saturated compound **9**, formally available from substance **7** by hydrogenation, is identical with the C,D,E-ring system of CPA (**1**).

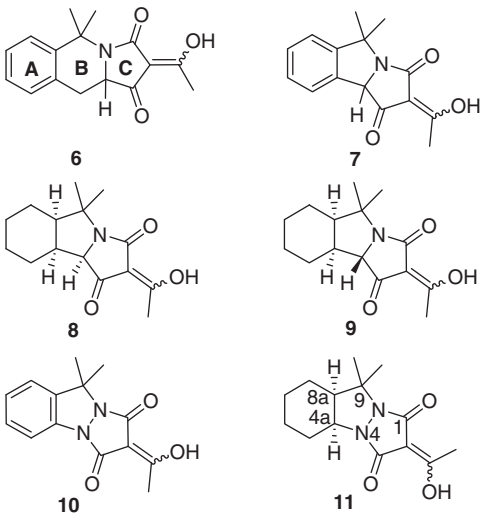


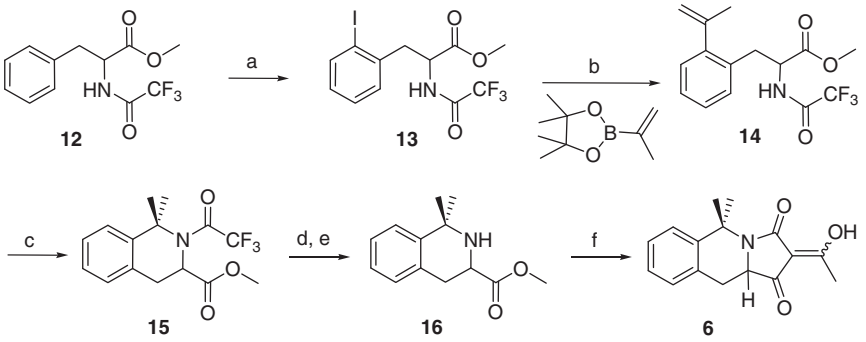
Figure 3. Structurally simplified model compounds for CPA.

An additional structural simplification we were interested in is found in compounds **10** and **11** that carry a nitrogen in position 4 instead of a carbon atom. To our surprise, no natural product with the corresponding acyl pyrazolidine-3,5-dione core is known and even reports on the synthesis of monocyclic and in particular bicyclic acylpyrazolidine-diones are rare, probably due to the very limited numbers of synthetic procedures available for this heterocyclic system.[16–18] In view of this situation we have developed a general and straightforward synthesis for the tricyclic dihydro-pyrazolo-[1,2-a]pyrazole-1,3-diones **10** and **11**.

2. Results and discussion

2.1. Chemistry

The key steps in our synthesis of acyltetramic acid **6** include an *ortho*-selective iodination of phenylalanine and a cationic cyclization (Scheme 1). IPy_2BF_4 is a mild iodination reagent which has been used previously to iodinate phenylalanine and tyrosine selectively in the *ortho* position even as residues in peptides and proteins.[19–22] With the fully protected phenylalanine **12** in hand the *ortho*-iodine derivative **13** was prepared in more than 90% yield and with an *ortho*/*para* ratio of up to 10:1. A Suzuki–Miyaura coupling with isopropenylboronic acid pinacol ester under standard conditions afforded the isopropenylated phenylalanine **14** in reasonable yields. For the ring-closure to the sterically hindered dimethyl piperidine **15** we applied a cationic cyclization procedure with triflic acid to form a benzylic cation by protonation of the double bond, which is attacked subsequently by the amino acid nitrogen. It has been shown recently that cationic cyclizations follow an overall 5-*endo*-trig course and afford excellent cyclization yields even in the case of sterically hindered rings.[11,23] The nitrogen protecting group plays a critical factor in this type of reaction since it must be strongly electron-withdrawing to prevent protonation of the basic amino acid nitrogen, but on the other hand the group should be cleavable under mild conditions. Therefore, the frequently used sulfonamide protecting groups appear to be problematic. We found that a trifluoroacetyl group, which can be removed under much milder conditions, gives comparable cyclization yields (compound **15**). Following a two-step deprotection–reprotection

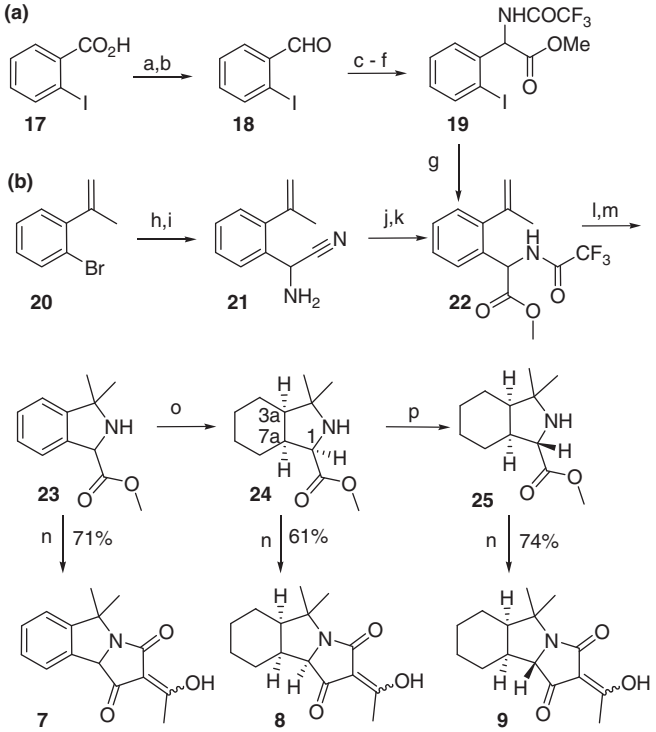


Scheme 1. Synthesis of the linear CPA analogue **6**. Reagents and conditions: (a) IPy_2BF_4, HBF_4, DCM/TFA 10:1, rt, 12 h, 92%; (b) $Pd(PPh_3)_4$ 10 mol %, Na_2CO_3 (aq), THF/toluene 1:1, 80 °C, 36 h, 55%; (c) TfOH, $CHCl_3$, rt, 6 h, 63%; (d) NaOH (aq) EtOH, rt, 12 h, 89%; (e) $SOCl_2$, MeOH, 0 °C, rt, 12 h, 69%; (f) acetyl Meldrum's acid, toluene, 110 °C, 30 min, then KO*t*Bu, 110 °C, 30 min, 67%.

sequence the tetramic acid moiety was finally built up by a one-pot reaction of the bicyclic piperidine **16** with acetyl Meldrum's acid (Scheme 1).

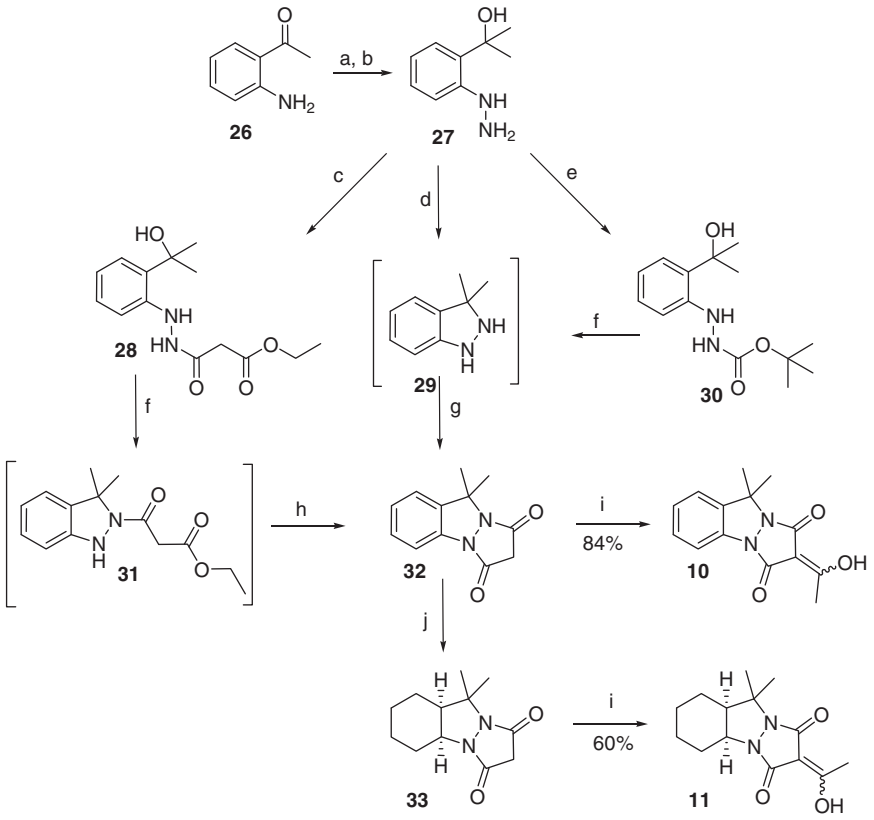
Unfortunately, all attempts to prepare the pyrrolizidine scaffold of compound **7** in the same way were unsatisfactory due to a low regioselectivity and yield in the iodination step of phenylglycine, the corresponding starting material for compounds **7**–**9**. Thus, a new synthesis for the pyrrolizidine moiety was developed, utilizing a Strecker-reaction for the introduction of the amino acid functionality.[24] The central intermediate **22** is available either from commercially available 2-iodobenzoic acid (**17**) by formation of the aminoacid ester **19** followed by a Suzuki–Miyaura coupling (Scheme 2a), similar to the synthesis of isopropenyl phenylalanine **14**, or via aminonitrile **21** which can be prepared from 1-bromo-2-isopropenyl-benzene (**20**) in two steps according to Scheme 2b. However, the Strecker reaction of iodobenzaldehyde **18** (Scheme 2a) gave only low yields of 2-iodo phenylglycine. It turned out that an inverted sequence (Scheme 2b) with formation of the isopropenyl group first followed by a Strecker reaction afforded significantly better yields of the key intermediate **22**. The cationic cyclization yields of dihydroisoindole **23** were similar to those of tetrahydroisochinoline **15**. Cycloacylation with acetyl Meldrum's acid then afforded 2-acetyl-5,9b-dihydro-pyrrolo[2,1-*a*]isoindole-1,3-dione **7**. All attempts to obtain the saturated analogues **8** or **9** directly from compound **7** by hydrogenation resulted in decomposition of the starting material. More successful was the hydrogenation of the precursor **23**. The *all-cis* stereoisomer **24** was formed almost exclusively controlled by the stereogenic center in position 1. This was subsequently equilibrated to the more stable *cis–trans* isomer **25** with NEt_3 in benzene.[10] Stronger basic conditions caused a complete decomposition of the *cis*-stereoisomer **24**. Acylation of the precursors **23**–**25** with acetyl Meldrum's acid in NaOEt/EtOH gave the CPA analogues **7**–**9** in yields of up to 74% as a mixture of *E*/*Z*-isomers around the exocyclic double bond.

As starting material for the construction of the 2,3-dihydroindazole scaffold (compounds **10** and **11**, Scheme 3), we chose aminoacetophenone **26**, which has already been used for the synthesis of a variety of heterocyclic compounds such as indoles, quinazolines and benzodiazepines.[25] Hydrazine **27** was prepared from aminoacetophenone **26** by a twofold Grignard addition followed by a diazotization and reduction of the intermediate diazonium salt with stannous chloride.[26] In principle, diketopyrazolidine **32** is accessible via two routes (Scheme 3): Firstly, by acidic cyclization of the Boc-protected hydrazine **30** to dihydroindazole **29** and acylation with malonyl chloride. Secondly, by acylation of phenylhydrazine **27** with ethylmalonyl chloride, followed by an acidic ring-closure to dihydroindazole **31** and immediate cyclization to the tricyclic pyrazolo[1,2-*a*]indazole **32**. The yields of the



Scheme 2. Syntheses of tetramic acids **7**–**9**. Reagents and conditions: (a) BH_3xSMe_2, THF, rt, 12 h, 99%; (b) PCC, CH_2Cl_2, rt, 2 h, 93%; (c) NH_4Cl, NaCN in water, MeOH, rt, 3 h, 25%; (d) 6 N HCl, reflux, 1 h, 100%; (e) $SOCl_2$, MeOH, rt, 16 h, 67%; (f) TFAA, CH_2Cl_2, Py, rt, 6 h, 85%; (g) $Pd(PPh_3)_4$, isopropenylboronic acid pinacol ester, 2 M Na_2CO_3, THF/toluene 1:1, 80 °C, 24 h, 83%; (h) Mg, THF, DMF, 20 °C, 12 h, 60%; (i) AcOH, NH_3, NaCN, 40 °C, 6 h, 57%; (j) HCl(g), MeOH, 2 h, 60%; (k) CH_2Cl_2, TFAA, pyridine, 1 h, 91%; (l) TfOH, CH_2Cl_2, rt, 1 h, 85%; (m) (i) 5 M NaOH, EtOH, reflux, 30 min, (ii) $SOCl_2$, MeOH, 14 h, 83%; (n) (i) acetyl Meldrum's acid, toluene, reflux 1 h, (ii)1 M NaOEt in EtOH, reflux 2 h; (o) PtO_2, H_2, 8 bar, 48 h, 73%; (p) NEt_3, benzene, reflux 48 h, 85%.

first route tend to be lower, because dihydroindazole **29** is instable and quickly decomposes to an aromatic indazole by air oxidation. Tetramic acid **10** was prepared from precursor **32** by a simple acylation with acetic acid using DCC as coupling reagent.[27] Not completely unexpected, the hydrogenation of the acetylpyrazolo[1,2-*a*]indazole **10** resulted in N–N bond cleavage. Instead, precursor **32** was hydrogenated and then acylated with acetic acid to afford the pyrazolidinedione **11**. Both pyrazolinediones were obtained as *E*/*Z*-mixtures around the exocyclic enolic double bond.



Scheme 3. Syntheses of acylated pyrazolidinediones **10** and **11**. Reagents and conditions: (a) CH_3MgI, Et_2O, rt, 2 h, 90%; (b) (1) $NaNO_2$, HCl, −25 °C, 30 min, (2) $SnCl_2$, −25 °C, 30 min; (c) ethyl malonyl chloride, DMAP, CH_2Cl_2, 0 °C, 1 h, 70% (for steps b and c); (d) TFA, rt, 20 min; (e) BOC_2O, DMAP, 0 °C, 1 h, 66% (for steps b and e); (f) TFA, rt, 2 h; (g) malonyl chloride, DMAP, rt, 1 h, 57% (for steps f and g); (h) NaOEt, EtOH, reflux, 6 h, 85% (for steps f and h); (i) AcOH, DCC, DMAP, Et_3N; (j) PtO_2, H_2, 7 bar, 48 h, 42%.

2.2. SERCA activities and molecular-modelling studies

All CPA derivatives were tested for *Heliothis virescens* SERCA activity 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 (Table 1). Since no experimental protein structure of *H. virescens* SERCA is available a homology model was constructed from the structure of rabbit SERCA complexed with CPA.[12,28] Since chelation of Mg^{2+} by the tetramic acid plays a pivotal role in CPA binding all CPA analogues were superimposed with their tetramic acid moieties.

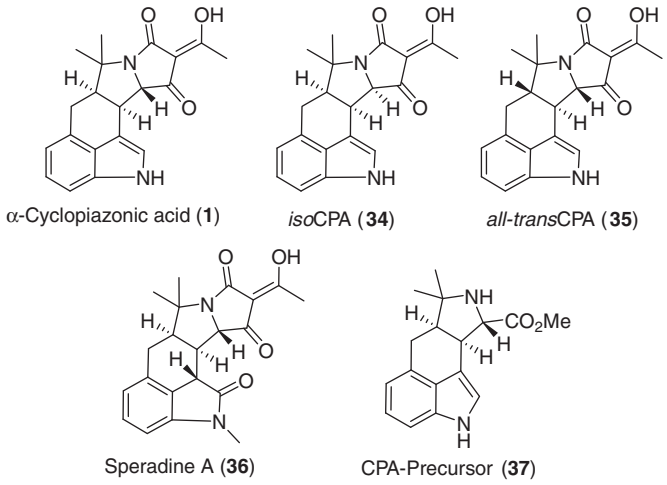
It has already been shown that epimerization of the 11a position (*iso*CPA **34**) exerts a minor effect on SERCA inhibition than epimerization at position 6a (*all-trans*CPA **35**), which results in a decline of activity by a factor of 500 compared to CPA (**1**).[12] CPA-precursor **37** is completely inactive and thus confirms that the tetramic acid is indispensable for SERCA inhibition (Table 1). Remarkably, speradine A (**36**) still shows some SERCA inhibition even though the indole system has been changed considerably by the introduction of an additional stereogenic center in position 11c, oxidation of position 1 to a carbonyl group, and methylation of the indole nitrogen. This again points to a certain structural variability of the indole system.

Most notable, a pronounced SERCA activity (IC_{50} 0.63 μM) was found for the tricyclic tetramic acid **9**, which is identical with the C,D,E-ring fragment of CPA (Fig. 4, left). Obviously, the complete indole system does not contribute to the overall SERCA activity more than the stereochemistry in position 11a of CPA. The inversion of the configuration in position 11 induces a bowl-like shape in *iso*CPA (**17**) with the consequence that the indole system now moves upwards out of the lipophilic binding channel and approximates the side-chain carboxylate-group of Asp 254 (Indol-N. . .Asp-O: 2.8 Å), probably forming a new hydrogen bond.[12] This provides an explanation for the considerable SERCA activity of *iso*-CPA **34**. Compound **8** that has the same stereochemistry as isoCPA but lacks the indole core, shows only weak SERCA inhibition (Table 1). This is because now neither the central binding pocket can be occupied nor is it possible to form an additional hydrogen bond like *iso*CPA (Fig. 4, left).

The simple linear tetramic acid **6** lacking the indole system and chirality in the corresponding CPA positions 6a and 11a shows an unexpected SERCA inhibition (IC_{50} 6.3 μM) in the range of *all-trans*-CPA **35** and Speradine A (**36**). A plausible explanation is that the six-membered piperidine ring directs the phenyl system straight into the lipophilic binding-niche fixing tetramic acid **6** in a position very similar to that of BHQ (Fig. 4, middle). A consensus orientation of BHQ obtained from docking into the SERCA structure has been published recently.[14,29] A superimposition of CPA and dihydro isoquinoline **6** onto their tetramic acid moieties together with BHQ in its published binding pocket orientation convincingly confirms the similarity between these structures. Both the *tert*-butyl group of BHQ and the phenyl ring of the linear tetramic acid **6** cover exactly the same position as the aromatic ring A of CPA (Fig. 4, middle). This model also explains the unexpected inactivity of tetramic acid **7** without any difficulties. The five-membered pyrrolidine ring causes an angulated shape of dihydro isoindole **7** with the consequence that the phenyl ring is now shifted out of the lipophilic binding niche towards the pyrrol ring B in CPA (Fig. 4, right).

The very low activity (Table 1) of the pyrazolidinediones **10** and **11**, however, is difficult to interpret with our model since steric and electronic properties have been changed by the exchange of

Table 1
SERCA (*Heliothis virescens*) activities of CPA, model compounds and related structures



Compound	SERCA (IC_{50}, μM)
BHQ	0.13 ± 0.013
CPA (**1**)	0.01 ± 0.005
*iso*CPA (**34**)[12]	0.50 ± 0.1
*all-trans*CPA (**35**)	5.0 ± 0.5
Speradine A (**36**)	8.0[3]
CPA precursor (**37**)	>50
6	6.3 ± 0.5
7	>50
8	19.9 ± 4
9	0.63 ± 0.04
10	40.0 ± 9
11	40.0 ± 10

a chiral carbon atom by a nitrogen. Most probably, also Mg binding is affected by the hydrazide group introduced in **10** and **11**.

3. Summary and conclusion

Six new CPA core structures have been prepared in order to elucidate the role of the indole moiety and the stereochemistry of the ring conjunctions. In particular for the hitherto unknown tricyclic hydrazides **10** and **11** a new synthesis was developed. CPA fragment **9**, consisting of the CPA rings C, D, and E still shows a remarkable SERCA inhibition and proves that the indole system is of minor importance for SERCA inhibition. The unexpected activity of dihydro isoquinoline **6** can be attributed to an orientation in the binding site of SERCA similar to BHQ. On the other hand, the phenyl ring of the inactive dihydro isoindole **7** is directed towards the pyrrole ring B of CPA causing unfavourable interactions with the binding site. The structure-activity data together with molecular modelling studies provide promising suggestions for future work. For instance, ring C opened CPA analogues containing an indole ring, which is linked by an acyclic spacer of two or three atoms to the pyrrolidine core, should reach the lipophilic binding niche without any problems. Starting from fragment **8** with the *iso*-stereochemistry, the introduction of polar substituents on ring A should allow the formation of additional hydrogen bonds to Asp 254 and nearby amino acids located above the plane of CPA ring C.

4. Experimental

4.1. 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 Ion-Trap 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 ppm and coupling constants are given in Hz. 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.

4.2. 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 No. 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.[30] 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) gylerol, 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

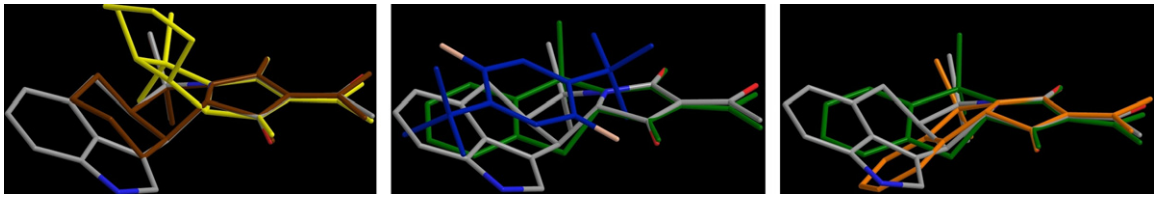


Figure 4. Superimpositions of CPA with compounds **8** and **9** (left), BHQ and **6** (middle), **6** and **7** (right). Color scheme: CPA, grey; BHQ, blue; **6**, green; **7**, orange; **8**, yellow; **9**, brown.

subsequent luciferase reaction employing 193.5 nM luciferin (Bio-Synth), 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 IC_{50} 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.

4.3. *rac*-2-(2,2,2-Trifluoroacetylamino)-3-(2-iodo-phenyl)-propionic acid methyl ester (13)

Bis(pyridine)iodonium(I) tetrafluoroborate was prepared according to the method developed by Barluenga et al.[19,20] Trifluoroacetyl protected phenylalanine **12** (2.0 g, 7.27 mmol) was dissolved in a mixture of 1450 mL CH_2Cl_2 and 145 mL TFA in a 2 L three-necked round-bottom flask. To this solution HBF_4 (3.05 mL, 21.8 mmol) was added, followed by addition of IPy_2BF_4 (4.06 g, 10.90 mmol) in several small portions. After stirring overnight at 40 °C under exclusion of light the reaction was stopped by addition of water. The organic phase was washed with a 5% $Na_2S_2O_3$ solution and two times with water. The organic phase was dried over Na_2SO_4, filtrated and evaporated. The pale yellow crude product was purified by column chromatography (cyclohexane–ethyl acetate 8.5:1.5) to afford **13** (2.691 g, 92%, *ortho*:*para* 10:1) as white crystals. Mp 108–110 °C (lit.[19] 110–111 °C); IR (KBr): 1749, 3310 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.22 (dd, J = 14.1, 8.4 Hz, 1H), 3.41 (dd, J = 14.1, 6.1 Hz, 1H), 3.78 (s, 3H), 4.95 (dt, J = 8.1 Hz, 1H), 6.85 (s, br, 1H), 6.96 (dt, J = 7.6, 1.5 Hz, 1H), 7.17 (dd, J = 7.6, 1.5 Hz, 1H), 7.30 (t, J = 7.4 Hz, 1H), 7.85 (d, J = 8.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 42.3, 52.9, 53.0, 100.9, 115.5, 128.6, 129.3, 130.3, 138.2, 140.0, 156.6, 170.4; ^{19}F NMR (376 MHz, $CDCl_3$): δ −76.3; MS (ESI): *m/z* (%) 342 (31), 402 ($[M+H]^+$, 90), 419 (100). Anal. Calcd for $C_{12}H_{11}F_3INO_3$: C, 35.93; H, 2.76. Found: C, 35.49; H, 2.59.

4.4. *rac*-2-(2,2,2-Trifluoroacetylamino)-3-(2-isopropenyl-phenyl)-propionic acid methyl ester (14)

Phenyliodine **13** (1.210 g, 3.02 mmol) and $Pd(PPh_3)_4$ (348 mg, 0.30 mmol) were dissolved in THF/toluene 1:1 (20 mL) in a Schlenk flask and carefully degassed with Helium, followed by isopropenyl boronicacid pinacol ester (1.20 mL, 6.04 mmol) and 2 M Na_2CO_3 (3.02 mL, 6.04 mmol; degassed). The reaction was stirred for 48 h at 80 °C. After cooling down to room temperature the two layers were separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4, filtrated and evaporated. The crude product was purified by column chromatography (cyclohexane–ethanol 95:5) to yield **14** (520.0 mg, 55%) as colorless crystals. Mp 68–70 °C; IR (KBr): 1709, 1752, 3311 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 2.06 (m, J = 1.0, 1.5 Hz, 3H), 3.08 (dd, J = 14.0, 8.4 Hz, 1H), 3.30 (dd, J = 14.0, 5.9 Hz, 1H), 3.73 (s, 3H), 4.84 (dt, J = 8.1, 1H), 4.92 (dt, J = 1.0 Hz, 1H), 5.28 (dt, J = 1.5, 1H), 6.70 (br s, 1H), 7.13 (m, 2H), 7.22 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.0, 35.1, 52.7, 53.6, 115.5, 116.3, 127.3, 127.5, 128.8, 129.8, 131.4, 144.4, 145.3, 156.7, 170.8; ^{19}F NMR (376 MHz, $CDCl_3$): δ −76.3; MS (ESI): *m/z* (%) 143 (18), 284 (39), 316 ($[M+H]^+$, 100), 333 ($[M+NH_4]^+$, 49). Anal. Calcd for $C_{15}H_{16}F_3NO_3$: C, 57.14; H, 5.12. Found: C, 57.00; H, 5.03.

4.5. *rac*-1,1-Dimethyl-2-(2,2,2-trifluoro-acetyl)-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid methyl ester (15)

Isopropenyl phenylalanine **14** (541.0 mg, 1.72 mmol) was dissolved in dry chloroform (50 mL). The solution was cooled down to 0 °C, triflic acid (0.153 mL, 1.72 mmol) was added at 0 °C and the mixture was stirred for 6 h without further cooling. Then the reaction was quenched by addition of saturated $NaHCO_3$ solution. The phases were separated and the aqueous layer was extracted with dichloromethane (2 × 20 mL). The combined organic phases were dried with Na_2SO_4, filtered and evaporated. The crude product was purified by column chromatography (cyclohexane–ethyl acetate 10:1) to yield **15** (340.0 mg, 63%) as an oil. IR (film): 1694, 1756 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.72 (s, 3H), 2.08 (s, 3H), 3.21 (dd, J = 15.6, 4.4 Hz, 1H), 3.37 (dd, J = 15.6, 2.8 Hz, 1H), 3.51 (s, 3H), 5.00 (m, 1H), 7.11 (d, J = 7.1 Hz, 1H), 7.18 (t, J = 7.4 Hz, 1H), 7.28 (t, J = 7.6 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.3, 30.6, 32.4, 52.5, 55.7, 62.6, 116.4, 124.8, 126.8, 128.0, 128.1, 129.8, 143.1, 156.1, 170.0; ^{19}F NMR (376 MHz, $CDCl_3$): δ −69.1; MS (ESI): *m/z* (%) 284 (31), 316 ($[M+H]^+$, 100); HR MS (ESI): calcd for $C_{15}H_{16}F_3NNaO_3$ ($[M+Na]^+$): 338.0974, found 338.0973.

4.6. *rac*-1,1-Dimethyl-1,2,3,4-tetrahydro-isoquinoline-3-carboxylic acid methyl ester (16)

Five molars of aqueous NaOH (5.8 mL) were added to a solution of tetrahydroisoquinoline **15** (276.0 mg, 0.87 mmol) in ethanol (20 mL). The reaction mixture was heated to reflux for 12 h, cooled down and concentrated in vacuo. The residue was dissolved in a small amount of ethanol and filtered through a short silica gel column. The filtrate was concentrated in vacuo. The residue (175 mg, 89%) was dissolved in methanol (5 mL) and thionyl chloride (0.32 mL, 4.36 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at room temperature for 12 h and then evaporated. The residue was dissolved in CH_2Cl_2 (30 mL) and washed successively with 1 M aqueous K_2CO_3 solution and water. The aqueous phases were combined and extracted two times with dichloromethane. The combined organic layers were dried with Na_2SO_4 and evaporated. Flash chromatography (cyclohexane–ethyl acetate 3:2) provided compound **16** (110 mg, 69%) as an oil. IR (film): 1743, 3331 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 3H), 1.53 (s, 3H), 1.72 (br s, 1H), 2.91 (dd, J = 16.2, 11.5 Hz, 1H), 3.04 (dd, J = 16.2, 4.1 Hz, 1H), 3.79 (s, 3H), 3.89 (dd, J = 11.5, 3.9 Hz, 1H), 7.07 (d, J = 7.1 Hz, 1H), 7.12 (dt, J = 7.1, 1.5 Hz, 1H), 7.18 (t, J = 7.4 Hz, 1H), 7.22 (d, J = 7.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.9, 31.8, 33.4, 51.9, 52.2, 53.9, 125.7, 126.0, 126.4, 129.2, 132.4, 143.3, 173.8; MS (ESI): *m/z* (%) 220 ($[M+H]^+$, 100); HR MS (ESI) calcd for $C_{13}H_{18}NO_2$ ($[M+H]^+$): 220.1332, found 220.1334.

4.7. *rac*-2-[1-Hydroxy-ethylidene]-5,5-dimethyl-10,10a-dihydro-5*H*-pyrrolo[1,2-b]isoquinoline-1,3-dione (6)

Acetyl Meldrum's acid was prepared as described by Crimmins[31] and recrystallized from cyclohexane. Acetyl Meldrum's acid (269 mg, 1.44 mmol) was added to a solution of isoquinoline **16** (158 mg, 0.72 mmol) in freshly dried toluene (5 mL). The reaction was heated to 110 °C and monitored by TLC every 15 min until the starting material was consumed completely. Then KO*t*Bu (122 mg, 1.08 mmol) was added and the mixture refluxed for another 2 h. After evaporation of the solvent, the residue was dissolved in methanol (2 mL) and the pH was adjusted to 1 by slow addition of 1 M HCl. The mixture was extracted with dichloromethane twice, the combined organic layers were dried with Na_2SO_4 and the solvents were removed under reduced pressure. Column chromatography of the crude product (ethyl acetate–EtOH 9:1) yielded tetramic acid **6** (131 mg, 67%) as an oil. IR (film): 3419, 1601, 1661, 879 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.79 (s, 3H), 1.84/1.87 (s, 3H), 2.43 (br s, 3H), 2.62 (t, J = 13.7 Hz, 1H), 3.17 (dd, J = 14.8, 2.5 Hz, 1H), 3.68 (m, 1H), 7.18 (m, 2H), 7.26 (t,

J = 6.1 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H); ^{13}C NMR (100 MHz, CDCl3): δ 26.7, 27.5, 31.5, 33.2, 33.5, 58.6, 58.8, 60.8, 61.3, 103.84, 126.4, 127.3, 127.4, 128.1, 128.3, 129.9, 133.9, 134.1, 145.5, 146.0, 174.5, 175.2, 195.3, 195.8; MS (ESI): m/z (%) 272.1 ([M+H]$^+$, 100); HR MS (ESI) calcd for $C_{16}H_{16}NO_3$ ([M−H]$^-$): 270.1136, found 270.1137.

4.8. *rac*-(2-Iodo-phenyl)-2,2,2-trifluoroacetylamino-acetic acid methyl ester (19)

2-Amino-2-(2′-iodophenyl) acetic acid hydrochloride, prepared from **18** according to the method described by Steiger,[32] was dissolved in MeOH (50 mL), cooled to 0 °C and thionyl chloride (10 mL, 137.68 mmol) was added dropwise. After stirring 16 h at room temperature the solvents were removed in vacuo. The residue was dissolved in dichloromethane (30 mL), washed with 1 M $NaHCO_3$ (3 × 30 mL) and brine (50 mL). The organic layer was dried with Na_2SO_4 and filtered. Chromatographic purification (cyclohexane–ethyl acetate 1:1) of the residue obtained after evaporation of the solvent gave 2-amino-2-(2′-iodophenyl) acetic acid methyl ester (4.6 g, 67%) as a yellow amorphous powder. IR (KBr): 1685, 3340 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.70 (s, 3H), 4.94 (s, 1H), 6.97 (ddd, J = 1.2, 7.4, 7.8 Hz, 1H), 7.30 (m, 2H), 7.85 (d, J = 7.8 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 52.4, 62.6, 99.8, 127.4, 128.8, 129.6, 139.9, 143.2, 173.8; MS (ESI): m/z (%) 291.1 ([M+H]$^+$, 100).

Pyridine (7.8 mL, 96.83 mmol) was added to 2-amino-2-(2′-iodophenyl) acetic acid methyl ester (4.6 g, 15.80 mmol) in dichloromethane (100 mL) followed by trifluoroacetic anhydride (11.00 mL, 79.00 mmol). The reaction was stirred 12 h at room temperature and then quenched with ice-water. The aqueous phase was extracted with dichloromethane (2 × 100 mL). The combined organic phases were washed with water, dried with Na_2SO_4 and then evaporated. The crude product was purified on a silica gel column (cyclohexane–dichloromethane 1:1) to yield compound **19** (5.2 g, 85%) as a white amorphous powder. IR (KBr): 1721, 1746, 3343 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.79 (s, 3H), 5.85 (d, J = 6.7 Hz, 1H), 7.06 (ddd, J = 1.1, 7.4, 7.6 Hz, 1H), 7.27 (ddd, J = 7.6, 7.8, 1.7 Hz, 1H), 7.38 (dd, J = 7.4, 1.7 Hz, 1H), 7.90 (dd, J = 7.8, 1.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.5, 60.6, 99.1, 115.5, 128.7, 128.8, 130.7, 137.7, 140.6, 156.3, 169.4; ^{19}F NMR (376 MHz, $CDCl_3$): δ −76.2; MS (ESI): m/z (%) 388.1 ([M+H]$^+$, 100); HR MS (ESI): calcd for $C_{11}H_9F_3NNaO_3$([M+Na]$^+$): 409.9471, found 409.9472.

4.9. *rac*-Amino-(2-isopropenyl-phenyl)-acetonitrile (21)

At 20 °C DMF (57 mL, 0.74 mol) was added to a solution of the Grignard reagent obtained from 1-bromo-2-isopropenyl-benzene **20** (73.0 g, 0.37 mol) and Mg turnings (11.0 g, 0.44 mol) in THF (450 mL).[33] The reaction mixture was stirred overnight at room temperature then poured into of 3 N aq HCl (500 mL) and extracted with dichloromethane (2 × 250 mL). The combined organic phases were washed with a saturated $NaHCO_3$ solution, water and brine. After drying over Na_2SO_4 and evaporation of the solvent, pure 2-isopropenyl-benzaldehyde (32.0 g, 60%) was obtained by Kugelrohr distillation as a colorless oil. IR (film): 1720, 2820 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.20 (s, 3H), 4.93 and 5.45 (s, each 1H), 7.36 (dd, J = 0.8, 7.6 Hz, 1H), 7.41 (ddd, J = 0.8, 7.5, 7.8, Hz, 1H), 7.57 (ddd, J = 1.1, 7.5, 7.6 Hz, 1H), 7.94 (dd, J = 1.1, 7.8 Hz, 1H), 10.2 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.0, 118.9, 127.5, 127.8, 128.6, 133.3, 140.4, 141.9, 148.0, 192.5; MS (EI) m/z (%): 146 ([M]$^+$, 38), 131 (100).

2-Isopropenyl-benzaldehyde (32.0 g, 0.22 mol) was added to an ice-cooled solution of aqueous ammonia (80 mL, 25% w/v), acetic acid (37.6 mL, 0.66 mol) and NaCN (13.1 g, 0.26 mol). The reaction was quenched with water (300 mL) after 24 h at 40 °C and extracted with dichloromethane (3 × 100 mL). The organic phase was dried over Na_2SO_4, concentrated and purified by column chromatography (cyclohexane–ethyl acetate 4:1) to yield amino-(2-isopropenyl-phenyl)-acetonitrile (**21**) (21.0 g, 57%) as a yellow oil. IR (film): 1642, 3340 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.13 (s, 3H), 5.18 (s, 1H), 5.00 and 5.34 (br s, each 1H), 7.22 (dd, J = 2.4, 7.6 Hz, 1H), 7.37 (m, 2H), 7.72 (dd, J = 1.0, 7.6 Hz, 1H), 7.66 (dd, J = 1.5, 9.0 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.4, 44.4, 116.7, 121.6, 126.9, 128.1, 128.6, 128.9, 133.8, 140.3, 143.8; MS (ESI) m/z (%):173.1 ([M+H]$^+$, 34), 156.1 (100); HR MS (ESI): calcd for $C_{11}H_{13}N_2$ ([M+H]$^+$): 173.1073, found 173.1072.

4.10. *rac*-(2-Isopropenyl-phenyl)-(2,2,2-trifluoro-acetylamino)-acetic acid methyl ester (22)

Aminoacetonitrile **21** (8.0 g, 46.45 mmol) was dissolved in MeOH (630 mL, c = 0.1 M) and cooled to 0 °C. This solution was saturated with HCl gas and stirred for 6 h at 0 °C. Then HCl and methanol were removed in vacuo. The residue was dissolved in dichloromethane (500 mL) and the solution was washed with saturated Na_2CO_3 solution (3 × 250 mL). The aqueous layer was extracted with dichloromethane (3 × 250 mL), the combined organic phases were dried with Na_2SO_4 and evaporated to yield pure amino-(2-isopropenyl-phenyl)-acetic acid methyl ester (6 g, 60%) as a brownish oil. IR (film): 1725, 3340, 3220 cm^{-1}; ^{1}H NMR (600 MHz, CD_3OD): δ 2.15 (s, 3H), 3.79 (s, 3H), 5.05 and 5.43 (br s, each 1H), 5.45 (s, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.40 (d, J = 7.8 Hz, 1H), 7.42 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD): δ 25.7, 53.9, 54.1, 118.0, 127.6, 129.3, 130.0, 130.1, 131.2, 145.1, 146.3, 170.4; MS (ESI) m/z (%): 206.1 ([M+H]$^+$, 100); HR MS (ESI): calcd for $C_{12}H_{16}NO_2$ ([M+H]$^+$): 206.1176, found 206.1175.

Trifluoroacetic anhydride (4.30 mL, 30.35 mmol) was added dropwise at 0 °C to a solution of 2-amino-2-(2′-isopropenylphenyl) acetic acid methyl ester (6.23 g, 30.35 mmol) and pyridine (4.7 mL, 58.34 mmol) in dichloromethane (100 mL). The reaction mixture was stirred for 1 h at room temperature, quenched with ice-water (200 mL) and extracted with dichloromethane (2 × 100 mL). The combined organic phases were dried with Na_2SO_4 and evaporated. Column chromatography (cyclohexane–ethyl acetate 9:1) of the residue afforded trifluoroacetate **22** (8.0 g, 91%) as an off-white amorphous powder. IR (KBr): 1754, 3333 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.14 (s, 3H), 3.74 (s, 3H), 5.03 and 5.36 (br s, each 1H), 5.94 (d, J = 6.8 Hz, 1H), 7.22–7.35 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.2, 53.2, 53.4, 114.2, 117.1, 126.3, 127.8, 128.8, 128.9, 131.6, 143.5, 144.5, 156.1, 170.5; ^{19}F NMR (376 MHz, $CDCl_3$): δ −76.3; MS (ESI): m/z (%) 242.1 (100), 302.1 ([M+H]$^+$, 35); HR MS (ESI): calcd for $C_{14}H_{14}F_3NNaO_3$ ([M+Na]$^+$): 324.0818, found 324.0814.

4.11. *rac*-3,3-Dimethyl-2,3-dihydro-1*H*-isoindol-1-carboxylic acid methyl ester (23)

Triflic acid (2.54 mL, 28.80 mmol) was added at 0 °C to a solution of ester **22** (8.7 g, 28.80 mmol) in chloroform (50 mL). The reaction was stirred without further cooling for 1 h. Then a 10% sodium carbonate solution (50 mL) was added, the phases were separated and the aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined organic phases were dried with Na_2SO_4, filtered and evaporated. Column chromatography (cyclohexane–ethyl acetate 10:1) afforded 3,3-dimethyl-2-(2,2,2-trifluoroacetyl)-2,3-dihydro-1*H*-isoindol-1-carboxylic acid methyl ester (7.4 g, 85%) as a white amorphous powder. IR (KBr): 1701, 1758, 2958, 2934 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.82 (s, 3H), 1.87 (s, 3H), 3.77 (s, 3H), 5.83 (br s, 1H), 7.16 - 7.41 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.6, 26.7, 53.1, 65.2, 72.0, 115.7, 121.3,

122.9, 128.4, 129.68, 131.5, 145.8, 155.7, 170.1; ^{19}F NMR (376 MHz, $CDCl_3$): δ -72.1; MS (ESI): *m/z* (%) 302.1 ([M+H]$^+$, 100); HR MS (ESI): calcd for $C_{14}H_{14}F_3NNaO_3$ ([M+Na]$^+$): 324.0818, found 324.0817.

3,3-Dimethyl-2-(2,2,2-trifluoroacetyl)-2,3-dihydro-1*H*-isoindol-1-carboxylic acid methyl ester (2.3 g, 7.60 mmol) was dissolved in ethanol (30 mL), 5 M aqueous NaOH (14 mL) was added and the reaction mixture was heated to reflux for 30 min. After evaporation of the solvent the residue was stirred with silica gel (20 g) in ethanol, filtered. The filtrate was concentrated in vacuo. Thionyl chloride (2.7 mL, 38.30 mmol) was added at 0 °C to a solution of the residue (1.62 g, 7.60 mmol) in methanol and the reaction mixture was stirred at room temperature for 14 h. The excess methanol and thionyl chloride were removed by evaporation. The residue was dissolved in dichloromethane (30 mL) and washed successively with 1 M aqueous Na_2CO_3 (25 mL) and then with water (25 mL). The aqueous phases were combined, extracted with dichloromethane (3 × 50 mL). And the combined organic layers were dried with Na_2SO_4, evaporated and purified by flash chromatography (dichloromethane–acetone 10:1) to provide of the isoindole **23** (1.3 g, 83%) as a colorless amorphous powder. IR (KBr): 1735, 2959, 3448 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (s, 3H), 1.54 (s, 3H), 2.62 (br s, 1H), 3.82 (s, 3H), 5.02 (s, 1H), 7.16 (d, *J* = 7.5 Hz, 1H), 7.26 (t, *J* = 7.3, 7.5 Hz, 1H), 7.33 (t, *J* = 7.3, 7.5 Hz, 1H), 7.44 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 30.1, 31.0, 52.5, 64.1, 64.3, 121.5, 123.1, 127.1, 128.3, 137.4, 149.7, 173.3; MS (ESI): *m/z* (%) 206.1 ([M+H]$^+$, 100); HR MS (ESI) calcd. for $C_{12}H_{16}NO_2$ ([M+H]$^+$): 206.1176, found 206.1177.

4.12. *rac*-(1*R*,3a*R*,7a*S*)-3,3-Dimethyl-octahydro-isoindole-1-carboxylic acid methyl ester (24)

Ester **23** (300 mg, 1.46 mmol) was dissolved in ethanol (5 mL), platinum(IV) oxide (95%) (16.5 mg, 0.07 mmol) was added and the reaction was hydrogenated (8 bar) for 48 h at room temperature. After that time, the mixture was filtrated, the catalyst was washed with ethanol (containing 5% acetic acid) and the combined solutions were evaporated. The residue was dissolved in dichloromethane (20 mL) and washed with saturated $NaHCO_3$ solution. The aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined organic phases were dried with Na_2SO_4 and evaporated to yield a raw material (280 mg) which was purified by column chromatography (cyclohexane–ethyl acetate 7:3) to yield reisolated starting material **23** (110 mg, 37%) and octahydro-isoindole **24** (130.0 mg, 45%) as a colorless oil. IR (film): 1678, 3320 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.83 (d, *J* = 9.3 Hz, 1H), 3.76 (s, 3H), 2.87 (br s, 1H), 2.74 (m, 1H), 1.73 (m, 1H), 1.59 (m, 2H), 1.59 and 1.23 (m, 1H), 1.73 and 1.52 (m, each 1H), 1.34 and 1.16 (m, each 1H), 1.24 and 1.16 (s, each 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ22.3, 23.5, 23.7, 24.0, 25.7, 29.9, 41.7, 47.2, 61.4, 61.5, 51.8, 175.2; MS (ESI): *m/z* (%): 212.1 ([M+H]$^+$, 100), HR MS (ESI): calcd. for $C_{12}H_{22}NO_2$ ([M+H]$^+$): 212.1645, found 212.1647.

4.13. *rac*-(1*S*,3a*R*,7a*S*)-3,3-Dimethyl-octahydro-isoindole-1-carboxylic acid methyl ester (25)

A solution of triethylamine (0.068 mL, 0.48 mmol) and octahydro-isoindole **24** (25 mg, 0.12 mmol) in benzene (5 mL) was refluxed for 42 h at 80 °C. Chromatographic purification (dichloromethane–MeOH 100:2) of the residue which was obtained after removal of the solvents in vacuo afforded stereoisomer **25** (20.0 mg, 85% based on conversion) as a colorless oil. IR (film): 1678, 3320; ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.79 (d, *J* = 10.4 Hz, 1H), 3.74 (s, 3H), 2.59 (m, 1H), 1.65 (m, 1H), 1.53 and 1.37 (m, each 1H), 1.75 and 1.16 (m, each 1H), 1.26 (m, 2H), 1.65 and 1.26 (m, each 1H), 1.22 and 1.16 (s, each 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 21.1, 24.8, 24.9, 25.3, 29.7, 30.4, 42.2, 48.2, 51.8, 60.6, 62.6, 175.6; MS (ESI): *m/z* (%): 212.1 ([M+H]$^+$, 100); HR MS (ESI): calcd for $C_{12}H_{22}NO_2$ ([M+H]$^+$): 212.1645, found 212.1647.

4.14. *rac*-2-(1-Hydroxyethylidene)-5,5-dimethyl-5,9b-dihydro-1*H*-pyrrolo[2,1-*a*]isoindole-1,3(2*H*)-dione (7)

Acetyl Meldrum's acid (92 mg, 0.48 mmol) was added to dihydro-isoindole **23** (50 mg, 0.24 mmol) in toluene (2 mL). The reaction mixture was heated to 80 °C and monitored every 15 min until complete consumption of the starting material. Then the solvent was evaporated and the residue was dried in high vacuum for 12 h. A 0.5 M sodium ethoxide solution (1 mL, 0.50 mmol) was added to the raw material (68 mg, 0.23 mmol) of step one in EtOH (2 mL) at 0 °C. The reaction was refluxed for 2 h, the solvent evaporated after that time and the residue dissolved in methanol (2 mL). Aq HCl (1 M) was added until pH 1. The solution was extracted with dichloromethane (3 × 25 mL), the organic phase was dried with Na_2SO_4, filtered and concentrated in vacuo. Chromatographic purification of the crude product (ethyl acetate–EtOH 100:8) afforded tetramic acid **7** (40.0 mg, 71%) as a white amorphous powder. IR (KBr): 1615, 2965, 3432 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.19/1.20 (s, 3H), 2.12/2.13 (s, 3H), 2.57/2.58 (s, 3H), 4.94 (s, 1H), 7.19–7.65 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.3/25.1, 26.7/28.9, 31.6/32.2, 66.2, 69.4, 119.3, 120.9, 122.0, 127.4, 128.0, 136.2, 148.3, 159.6, 178.2,195.3; MS (ESI): *m/z* (%) 258.1 ([M+H]$^+$, 100); HR MS (ESI): calcd for $C_{15}H_{14}NO_3$ ([M−H]$^-$): 256.0979, found 256.0978.

4.15. *rac*-(5a*R*,9a*S*,9b*R*)-2-(1-Hydroxyethylidene)-5,5-dimethyloctahydro-1*H*-pyrrolo[2,1-*a*]isoindole-1,3(2*H*)-dione (8)

A solution of octahydro-isoindole **24** (67 mg, 0.32 mmol) and acetyl Meldrum's acid (118 mg, 0.63 mmol, freshly prepared) in toluene (5 mL) was refluxed for 2 h, cooled to room temperature and evaporated. The residue was dried in high vacuum for 12 h, dissolved in 1 M NaOEt in EtOH (1 mL) and refluxed for 2 h at 80 °C. After cooling to room temperature the reaction was poured in 2 M HCl solution (20 mL) and extracted with dichloromethane (3 × 25 mL). The combined organic phases were dried with Na_2SO_4, filtered and evaporated. Pure tetramic acid **8** (52.0 mg, 61%) was obtained after reversed phase column chromatography (acetonitrile-water 6:4) as a white amorphous powder. IR (KBr): 1572, 1682, 3320 cm^{-1}; ^{1}H NMR (600 MHz, acetonitril-d_3): δ 4.24 (d, *J* = 6 Hz, 1H), 2.36 (s, 3H), 2.35 (m, 1H), 2.30 (m, 1H), 1.73 (m, 2H), 1.66 and 1.24 (m, each 1H), 1.67 (s, 3H), 1.54 and 1.04 (m, each 1H), 1.48–1.45 (m, 2H), 1.39 (s, 3H); ^{13}C NMR (150 MHz, acetonitril-d_3): δ 19.6, 22.2, 22.7, 24.3, 24.4, 24.6, 30.8, 39.5, 51.0, 63.6, 71.7, 106.8, 174.2, 184.4, 194.7; MS (ESI): *m/z* (%): 264.2 ([M+H]$^+$, 100); HR MS (ESI): calcd. for $C_{15}H_{22}NO_3$ ([M+H]$^+$): 264.1594, found 264.1596.

4.16. *rac*-(5a*R*,9a*S*,9b*S*)-2-(1-Hydroxyethylidene)-5,5-dimethyloctahydro-1*H*-pyrrolo[2,1-*a*]isoindole-1,3(2*H*)-dione (9)

A solution of octahydro-isoindole **25** (29 mg, 0.14 mmol) and acetyl Meldrum's acid (51 mg, 0.27 mmol) in toluene (5 mL) was refluxed for 2 h at 110 °C, cooled to room temperature and evaporated. Then a 0.3 M NaOEt solution (1 mL, 0.30 mmol) was added to the dried residue (12 h high vacuum) dissolved in EtOH (2 mL). The mixture was refluxed at 80 °C for 2 h, cooled to room temperature and evaporated. The resulting residue was dissolved in of 2 M HCl solution (25 mL) and extracted with dichloromethane (3 × 25 mL).

The combined organic phases were dried with Na_2SO_4 and evaporated. Chromatographic purification on a reversed phase column (acetonitrile–water 6:4) yielded tetramic acid **9** (21.0 mg, 74%) as a white amorphous powder. IR (KBr): 1572, 1682, 3320 cm^{-1}; ^1H NMR (600 MHz, acetonitril-d_3): δ 4.24 (d, J = 12 Hz, 1H), 2.34 (s, 3H), 2.33 (m, 1H), 2.14 and 1.67 (m, each 1H), 1.71 and 1.44 (m, each 1H), 1.81 and 1.21 (m, each 1H), 1.59 and 1.44 (m, each 1H), 1.49 and 1.40 (s, each 3H); ^{13}C NMR (150 MHz, acetonitril-d_3): δ 19.8, 21.5, 24.4, 24.9, 25.0, 25.9, 26.1, 38.6, 53.3, 64.5, 69.1, 106.5, 176.7, 185.3, 197.0; MS (ESI): m/z (%): 264.2 ([M+H]$^+$, 100); HR MS (ESI): calcd for $C_{15}H_{22}NO_3$ ([M+H]$^+$): 264.1594, found 264.1597.

4.17. Ethyl 3-(2-(2-(2-hydroxypropan-2-yl)phenyl)hydrazinyl)-3-oxopropanoate (28)

Ethyl malonyl chloride (0.18 mL, 1.33 mmol) was added dropwise to a solution of the crude hydrazine **27** (300.0 mg, 1.26 mmol) prepared according to a literature procedure[26], and DMAP (154 mg, 1.26 mmol) in dichloromethane (2 mL). The reaction was stirred for 30 min at room temperature and then partitioned between dichloromethane and water. The aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined organic phases were evaporated and the residue was purified by silica gel chromatography (cyclohexane–ethyl acetate 1:1) to yield oxopropanoate **28** as a yellow amorphous powder (120.0 mg, 70%). IR (KBr): 1689, 3340, 3228 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 1.30 (t, J = 7.2 Hz, 3H), 1.64 (s, 6H), 3.36 (s, 2H), 4.22 (q, J = 7.2 Hz, 2H), 6.82 (ddd, J = 1.2, 7.5, 8.0 Hz, 1H), 6.91 (dd, J = 1.2, 8.0 Hz, 1H), 7.17 (m, 1H), 7.21 (m, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 13.5, 28.9, 39.8, 40.5, 61.7, 73.3, 112.2, 119.7, 125.2, 127.7, 131.6, 145.3, 166.0, 168.0; MS (ESI): m/z (%) 263.1 ([[M−H_2O]+H]$^+$, 100), 303.1 ([M+H]$^+$, 8); HR MS (ESI): calcd for $C_{14}H_{20}N_2NaO_4$ ([M+Na]$^+$): 303.1310, found 303.1312.

4.18. *tert*-Butyl 2-(2-(2-hydroxypropan-2yl)phenyl)hydrazine carboxylate (30)

A 10% aq Na_2CO_3 solution (2 mL) was added to hydrazine **27** (150 mg, 0.90 mmol) and di-*tert*-butyl dicarbonate (200 mg, 0.90 mmol) in dichloromethane (2 mL). The two phase reaction mixture was stirred for 2 h at room temperature. Then the dichloromethane phase was separated and the water phase was extracted with dichloromethane (3 × 5 mL). The combined organic layers were dried with Na_2SO_4 filtered and evaporated. Boc protected hydrazine **30** (160.0 mg, 66%) was obtained as a yellow amorphous powder after chromatographic purification (cyclohexane–ethyl acetate 9:1) of the raw material. IR (KBr): 1520, 1689, 3228, 3340 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 1.48 (s, 9H), 1.68 (s, 6H), 2.97 (br s, 1H), 6.43 (br s, 1H), 6.82 (ddd, J = 1.1, 7.6, 7.8 Hz, 1H), 7.00 (d, J = 7.6 Hz, 1H), 7.17 (m, 2H), 8.06 (br s, 1H); ^{13}C NMR (150 MHz, $CDCl_3$): δ 28.3, 28.3, 29.4, 29.4, 29.4, 73.9, 80.7, 112.6, 119.4, 125.4, 128.1, 131.2, 147.3, 156.3; MS (ESI): m/z (%) 149.1 (100), 267.2 ([M+H]$^+$, 2), 289.2 ([M+Na]$^+$, 7); HR MS (ESI): calcd for $C_{14}H_{22}N_2NaO_3$ ([M+Na]$^+$): 289.1422, found 289.1424.

4.19. 3,3-Dimethyl-2,3-dihydro-1*H*-indazole (29)

A solution of hydrazine **30** (820 mg, 4.93 mmol) in trifluoroacetic acid (2 mL) was stirred 4 h at room temperature until the starting material has disappeared completely. Then the solvent was evaporated and the residue dried in high vacuum for 12 h. The raw product **29** was used without further purification for the next step. MS (ESI): m/z (%) 132.1 (100), 149.1 ([M+H]$^+$, 40).

4.20. Ethyl 3-(3,3-dimethyl-1*H*-indazol-2(3*H*)-yl)-3-oxopropanoate (31)

A solution of oxopropanoate **28** (90 mg, 0.32 mmol) in TFA (2 mL) was stirred at room temperature for 30 min and partitioned between dichloromethane and saturated $NaHCO_3$ after that time. The aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined organic phases were dried with Na_2SO_4, filtered and evaporated. The residue was dried in high vacuum for 12 h and used for the next step without further purification. MS (ESI) m/z (%): 263.1 ([M+H]$^+$, 100).

4.21. 9,9-Dimethylpyrazolo[1,2-*a*]indazole-1,3(2*H*,9*H*)-dione (32)

Method A: A 0.76 M NaOEt solution in ethanol (0.5 mL) was added to indazole **31** (83 mg, 0.32 mmol) dissolved in EtOH (1 mL). The solution was refluxed for 6 h. After that time the reaction mixture was partitioned between dichloromethane and saturated NH_4Cl solution. The aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined organic phases were dried with Na_2SO_4, filtered and evaporated. Silica gel chromatography (cyclohexane–ethyl acetate 2:1) yielded indazole-dione **32** as a white amorphous powder (59 mg, 85%).

Method B: A 1.0 M malonyl chloride solution (2 mL, 2.00 mmol) in dichloromethane was added dropwise to dihydroindazole **29** (701 mg, 2.00 mmol) and DMAP (490 mg, 4.00 mmol) dissolved in dichloromethane (5 mL). The reaction was stirred at room temperature for 30 min and then quenched with saturated NH_4Cl solution. The two layers were separated and the aqueous phase was extracted with dichloromethane (3 × 50 mL). The combined organic layers were dried with Na_2SO_4, filtered and evaporated. Pure indazole-dione **32** (250.0 mg, 57%) was obtained after column chromatography (cyclohexane–ethyl acetate 1:1) as a white amorphous powder. IR (KBr): 1689, 3228, 3340 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 1.84 (s, 6H), 3.52 (s, 2H), 7.19 (m, 2H), 7.35 (ddd, J = 2.2, 7.9, 8.0 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 26.9, 26.9, 42.5, 64.5, 112.4, 121.9, 125.5, 129.1, 131.5, 137.6, 159.2, 161.4; MS (ESI): m/z (%) 217.1 ([M+H]$^+$, 100), 239.1 ([M+Na]$^+$, 14); HR MS (ESI): calcd for $C_{12}H_{12}N_2NaO_2$ ([M+Na]$^+$): 239.0786, found 239.0784.

4.22. *rac*-(4a*S*,8a*R*)-9,9-Dimethylhexahydropyrazolo[1,2-*a*]indazole-1,3(2*H*,4a*H*)-dione (33)

The mixture of indazole-dione **32** (100 mg, 0.46 mmol) in EtOH (10 mL, containing 0.1% AcOH) and platinum(IV) oxide (95%) (6 mg, 0.02 mmol) was hydrogenated (7 bar) at room temperature for 72 h. After that time the reaction was filtrated, the catalyst was washed with ethanol (containing 5% acetic acid) and the combined ethanol phases were evaporated. The residue was dissolved in of dichloromethane (20 mL) and washed with saturated $NaHCO_3$ solution (2 × 20 mL). The aqueous layer was extracted with dichloromethane (3 × 25 mL). The combined organic phases were dried with Na_2SO_4, filtered and evaporated. The raw material was purified by column chromatography (cyclohexane–ethyl acetate 1:1) to yield product **33** (43.0 mg, 42%) as a white amorphous powder. IR (KBr) 1697, 1730, 2930, 3436 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$), 1.48 (s, 3H), 1.58 (s, 3H), 1.25 and 1.79 (m, each 1H), 1.39 and 1.81 (m, each 1H), 1.43 and 1.57 (m, each 1H), 1.70 and 2.73 (m, each 1H), 2.09 (m, 1H), 3.24 and 3.49 (d, J = 21 Hz, each 1H), 4.24 (d, J = 4.7, 8.3 Hz, 1H), ^{13}C NMR (150 MHz, $CDCl_3$): δ 20.4, 21.3, 23.5, 24.2, 25.5, 25.6, 43.0, 50.7, 53.7, 62.5, 163.3, 164.3; MS (ESI): m/z (%) 223.1 ([M+H]$^+$, 100); HR MS (ESI): m/z (%) calcd for $C_{12}H_{18}N_2NaO_2$ ([M+Na]$^+$): 245.1260, found 245.1262.

4.23. 2-(1-Hydroxyethylidene)-9,9-dimethylpyrazolo[1,2-*a*]indazole-1,3(2*H*,9*H*)-dione (10)

Indazole-dione **32** (50 mg, 0.23 mmol) and DMAP (5.6 mg, 0.05 mmol) were dissolved in dichloromethane (2 mL). To this solution acetic acid (0.0146 mL, 0.25 mmol), DCC (57.2 mg, 0.28 mmol) and triethyl amine (0.0387 mL, 0.28 mmol) were added at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and then 12 h at room temperature. After that time the solution was evaporated and the residue purified by column chromatography (ethyl acetate, then CH_2Cl_2–MeOH 100:5, containing 0.1% AcOH) to afford tetramic acid **10** (51 mg, 84%) as a white amorphous powder. IR (KBr): 1520, 1689, 3228, 3340 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ 1.85 (s, 6H), 2.53 (s, 3H), 7.33 (d, J = 7.6 Hz, 1H), 7.22 (t, J = 7.6, 7.6 Hz, 1H), 7.39 (t, J = 7.6, 7.9 Hz, 1H), 7.56 (d, J = 7.9 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ 19.2, 19.2, 26.8, 66.1, 100.6, 112.7, 123.3, 126.1, 130.6, 132.8, 138.5, 179.8, 187.5, 189.5; MS (ESI) m/z (%) 159.1 ($[M+H]^+$, 100), 281.1 ($[M+Na]^+$, 29); HR MS (ESI): calcd for $C_{14}H_{14}N_2NaO_3$ ($[M+Na]^+$): 281.0896, found 281.0897.

4.24. *rac*-(4a*S*,8a*R*)-2-(1-Hydroxyethylidene)-9,9-dimethylhexahydropyrazolo[1,2-a]indazole-1,3(2*H*,4a*H*)-dione (11)

Pyrazoloindazole **33** (35 mg, 0.16 mmol), DMAP (3.8 mg, 0.03 mmol), DCC (39.0 mg, 0.19 mmol) were dissolved in dichloromethane (5 mL), followed by acetic acid (0.010 mL, 0.17 mmol) and triethyl amine (0.0263 mL, 0.19 mmol) at 0 °C. The reaction mixture was filtered after 12 h at room temperature and the filtrate was washed with 1 M HCl (2 × 10 mL). The aqueous layer was extracted with dichloromethane (3 × 25 mL), the combined organic phases were dried with Na_2SO_4, filtered and the solvents were removed in vacuo. Column chromatography (ethyl acetate, then CH_2Cl_2–MeOH 100:5, containing 0.1% AcOH) of the residue afforded tetramic acid **11** (25 mg, 60%) as a white amorphous powder. IR (KBr): 1670, 3218, 3326 cm^{-1}; ^1H NMR (600 MHz, CD_3OD): δ 1.97 (s, 6H), 2.36 (s, 3H), 1.43 and 1.52 (m, each 1H), 1.29 and 1.76 (m, each 1H), 1.44 and 1.82 (m, each 1H), 1.75 and 2.74 (m, each 1H), 2.19 (m, 1H), 4.21 (m, 1H); ^{13}C NMR (150 MHz, CD_3OD): δ 21.4, 22.9, 22.9, 25.0, 25.4, 26.0, 27.8, 51.3, 55.6, 63.8, 99.9, 167.5, 179.4, 195.5; MS (ESI) m/z (%) 265.1 ($[M+H]^+$, 100); HR MS (ESI): calcd for $C_{14}H_{20}N_2NaO_3$ ($[M+Na]^+$): 287.1366, found 287.1366.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.06.001.

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