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Synthesis and Pharmacological Properties of New Tetracyclic Forskolin Analogues

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New tetracyclic analogues of forskolin have been prepared by derivatization of the natural product. Treatment of a forskolin-derived cyclic thionocarbonate with 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine resulted in the formation of a seven-membered cyclic carbonate derivative by an unprecedented rearrangement of an intermediate dialkoxycarbene or 1,3-dipole, whereas radical deoxygenation was followed by intramolecular cyclization with the double bond to form a third analogue. Two of the new analogues were investigated for their ability to activate adenylyl cyclases 1, 2 and 5. The introduction of another ring into the forskolin skeleton did not lead to a loss of binding affinity to the enzyme. Although the new compounds are much more spacious than forskolin, they still seem to fit into the binding pocket and were found to be partial agonists.

Introduction

The roots of *Coleus forskohlii*, a member of the mint family that can be found in the subtropical areas of India, Pakistan, Sri Lanka and Brazil, have long been used for the treatment of asthma, glaucoma and cardiovascular diseases.[1] In 1977 Bhat et al. isolated the labdane diterpenoid forskolin as the major component,[2] which displayed antihypertensive and positive inotropic effects.[3] Forskolin was shown to directly activate the enzyme adenylyl cyclase (AC), thereby increasing the intracellular level of cAMP and leading to various physiological effects such as hypotension, cardiac inotropy, bronchodilation and the reduction of intraocular pressure.[4] Nine membranous AC isoforms with distinct tissue distribution and regulatory properties are known to date.[5] Although ACs 1–8 represent interesting pharmaceutical targets that are directly activated by forskolin, the lack of AC isoform selectivity resulting in numerous side-effects has prevented the clinical use of forskolin so far. Recently we have shown that AC isoforms are not uniformly modulated by forskolin analogues, which demonstrates the feasibility of developing highly desired isoform-selective AC activators and inhibitors.[6] Herein we report the synthesis of new tetracyclic forskolin analogues during which we discovered an unprecedented rearrangement of a dialkoxycarbene intermediate. The ability of one of the resulting new forskolin derivatives and its precursor to activate ACs 1, 2 and 5 have been investigated.

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Results and Discussion

Synthesis

During our investigations on forskolin (**1**) analogues as isoform-selective adenylyl cyclase modulators we intended to prepare 1-deoxyforskolin (**2**) and 1,9-dideoxyforskolin (**3**) (Figure 1) directly from forskolin, which has not been described so far. Both compounds were isolated as minor constituents from *Coleus forskohlii*.[2,7] 1,9-Dideoxyforskolin (**3**) has been synthesized starting from (*E*,*E*)-farnesol[8] as well as the natural products larixol[9] and ptychantin A,[10] and has itself been used as the starting material for the preparation of 1-deoxyforskolin (**2**).[7]

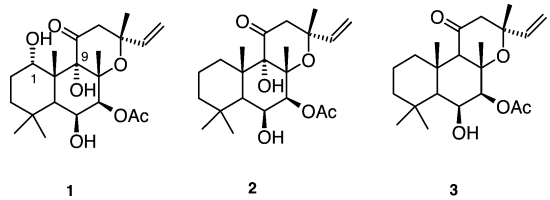
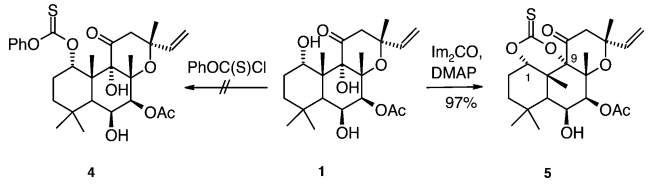


Figure 1. Structures of forskolin (**1**), 1-deoxyforskolin (**2**) and 1,9-dideoxyforskolin (**3**).

Of the OH group functionalities on forskolin it is known that apart from a few exceptions 1-OH is generally more reactive than the sterically hindered 6-OH and especially

the 9-OH group.[4b,11] We therefore planned to selectively convert the 1-OH group into a suitable thiocarbonyl precursor, which could then be used for radical deoxygenation to provide 1-deoxyforskolin (**2**). Although the reaction of **1** with *O*-phenyl chlorothionoformate to yield thionocarbonate **4** was unsuccessful, quantitative conversion into the 1,9-disubstituted cyclic product **5** was observed after exchanging *O*-phenyl chlorothionoformate for thiocarbonyldiimidazole (Scheme 1).



Scheme 1. Attempted formation of **4** and the preparation of the cyclic thionocarbonate **5**.

Compound **5**, in which the two axial hydroxy groups are connected via a thionocarbonate bridge (see Figure 2 for the X-ray crystal structure), was subsequently treated with $HSnBu_3$/AIBN to remove the 9-OH group. This reaction resulted not only in the cleavage of the C9–O bond, but at the same time led to the formation of a new bicyclic ring system (compound **6**, Scheme 2).

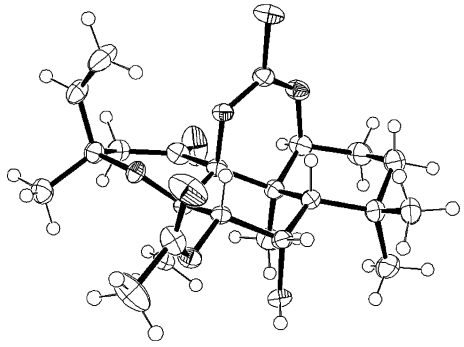
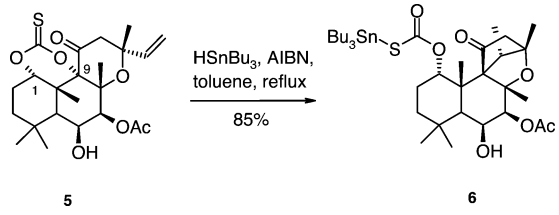
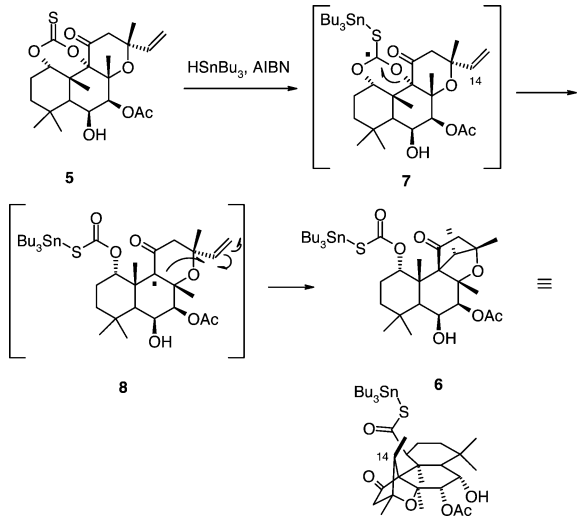


Figure 2. X-ray crystal structure of thionocarbonate **5**.



Scheme 2. Formation of the new forskolin analogue **6** via the radical precursor **5**.

The proposed mechanism for the formation of **6** is shown in Scheme 3. As expected, the initial radical **7** fragments in such a way that the more stable tertiary radical at C9 (compound **8**) is formed as opposed to the potential secondary C1 radical. This new radical intermediate then intramolecularly attacks the double bond at C14 in a 5-*exo-trig* cyclization to give after hydrogen abstraction the new tetracyclic forskolin analogue **6**.



Scheme 3. Proposed mechanism for the formation of **6**.

The X-ray crystal structure of compound **6** revealed that the newly formed stereocentre at C14 is *S*-configured (Figure 3). Attempts to hydrolyse the tributyltin-containing thionocarbonate and thus obtain the free alcohol at C1 were not successful and resulted in either only C7 ester cleavage or in decomposition products.

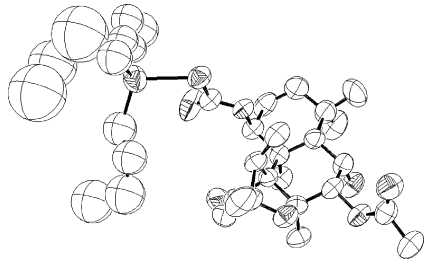
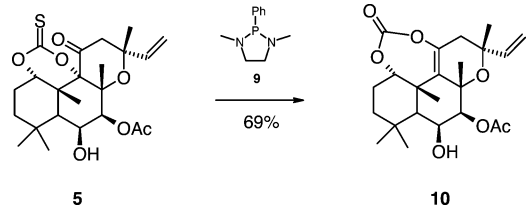


Figure 3. X-ray crystal structure of compound **6**. Hydrogen atoms have been omitted for clarity.

In addition to this reaction, **5** was also treated with 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine (**9**), which is generally used in a mild variant of the Corey–Winter olefination of thionocarbonates derived from 1,2-diols,[12] to find out whether the thionocarbonate group in **5** could be removed. After stirring **5** with **9** at 50 °C, a new product was isolated. X-ray structure analysis revealed that the C9–O bond was again cleaved and a new seven-membered cyclic carbonate was formed (compound **10**, Scheme 4, see Figure 4 for the crystal structure).



Scheme 4. Formation of the new forskolin analogue **10**.

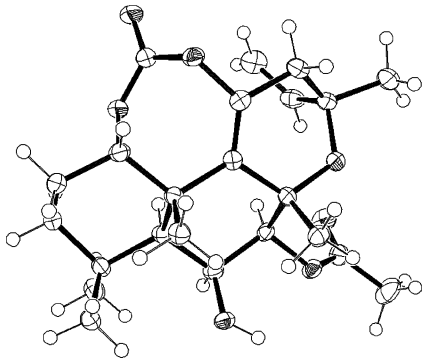
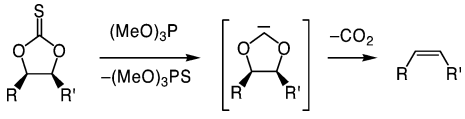


Figure 4. X-ray crystal structure of compound **10**.

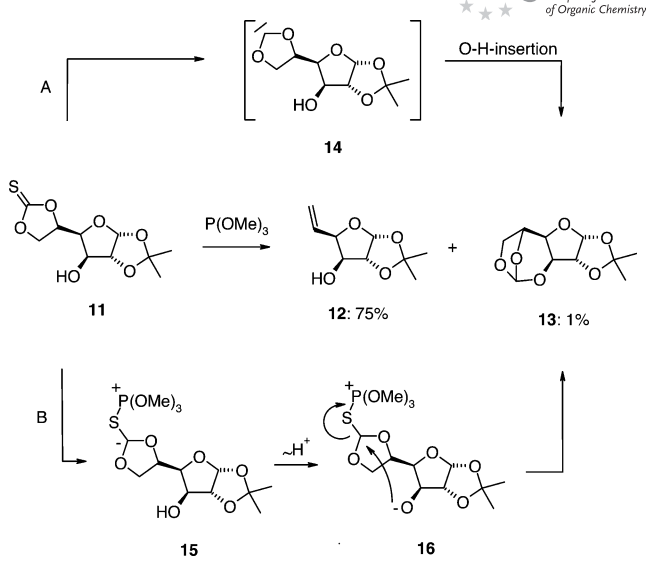
The Corey–Winter olefination is postulated to proceed via carbene intermediates (Scheme 5),[13] but the exact mechanism seems to be more complex and has not been fully clarified.[14]



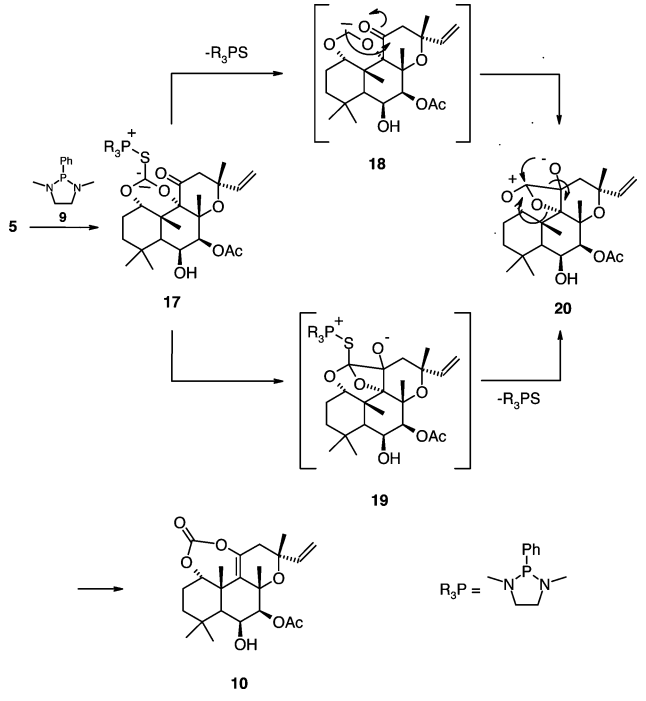
Scheme 5. Proposed mechanism of the Corey–Winter olefination.

In the case of trithiocarbonates, Corey and Märkl suggested the formation of phosphite ylide intermediates.[15] The results of Scherowsky and Weiland on the other hand point to the existence of 1,3-dipoles as the reactive intermediates and argue against carbenes.[16] However, the existence of carbenes in the case of thionocarbonates is supported by the detection of a small amount of the orthoester **13** in the reaction of **11** with $P(OMe)_3$ to form **12**[17] (Scheme 6), which is generally cited as direct evidence for the intermediacy of carbenes.[14] However, this result may not only be explained by the carbene intermediate **14** (path A), which is claimed by the authors, but also by the formation of the intermediate 1,3-dipole **15**, which first deprotonates the free alcohol and then undergoes nucleophilic attack by the alkoxide with elimination of thionophosphate (path B, Scheme 6).

The mechanism proposed for the formation of **10** on the basis of these results is shown in Scheme 7. Thiophilic attack on 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine (**9**) leads to the 1,3-dipole **17**, which then either loses diazaphospholidine sulfide to give the dialkoxycarbene intermediate **18** or directly attacks the C11 carbonyl group to form **19**. Elimination of diazaphospholidine sulfide from **19** would then lead to the same zwitterion **20** that is formed after nucleophilic attack of the carbene **18** on the carbonyl group. Such inter- and intramolecular reactions of dialkoxycarbenes with carbonyl compounds are well known in the literature.[18] Finally, in a concerted mechanism, the alkoxide attacks the carbenium ion, the C11–C(O)O bond is broken and a new seven-membered ring is formed. An orthoester intermediate resulting from the attack of the alkoxide on the carbenium ion or in analogy to the formation of **13** from **16** (Scheme 6) can probably be ruled out due to the very high ring strain that would be generated in such a compound. To the best of our knowledge, such a rearrangement of a dialkoxycarbene or 1,3-dipole generated from thionocarbonates has not been reported before.



Scheme 6. Possible reaction pathways that could explain the formation of the side-product **13**.



Scheme 7. Proposed mechanism for the formation of **10**.

Adenylyl Cyclase Activity Assay

The effects of compounds **5** and **10** on ACs 1, 2 and 5 were investigated to study the AC isoform specificity profiles of these new tetracyclic and more spacious forskolin analogues and to find out whether such compounds still fit into the binding pocket of the enzyme. Owing to the

toxicological properties that are associated with organotin compounds,[19] analogue **6** was not used in the pharmacological investigations. In our previous studies forskolin was found to have a higher potency towards AC 1 than towards AC 2 and AC 5.[6] AC 1 is predominantly expressed in hippocampal areas of the brain and a decreased AC 1 activity is measured in Alzheimer's disease.[20] The identification of AC 1 specific activators could therefore be useful for the treatment of Alzheimer's disease. For a better comparison of the molecular interactions of the compounds in recombinant membrane and tissue preparations we also studied the effect of the new analogues in mouse cardiac membranes. Because it has been shown in several studies that cardiac membranes are enriched in ACs 5 and 6 but have smaller quantities of ACs 1, 3, 4 and 7,[5b,21] we wished to find out whether isoform selectivity might be observed for AC 5. The results of the AC activity assays are shown in Table 1 and Figure 5.

Table 1. Calculated potencies and efficacies of the new forskolin analogues **5** and **10**.

	5	**10**	Forskolin
AC	EC_{50} [μM] Efficacy [%]	EC_{50} [μM] Efficacy [%]	EC_{50} [μM] Efficacy [%]
1	20.8 ± 0.7 17.1 ± 4.6	8.03 ± 1.08 45.3 ± 1.9	0.74 ± 0.07 100
2	228.6 ± 35 –18.7 ± 1.7	46.6 ± 11.1 19.7 ± 4.5	14.3 ± 3.0 100
5	65.2 ± 16.1 21.5 ± 1.7	7.3 ± 0.9 19.0 ± 2.5	4.65 ± 0.6 100
Cardiac	100.8 ± 30 40.1 ± 4.2	41.7 ± 3.7 62 ± 3.9	4.75 ± 0.8 100

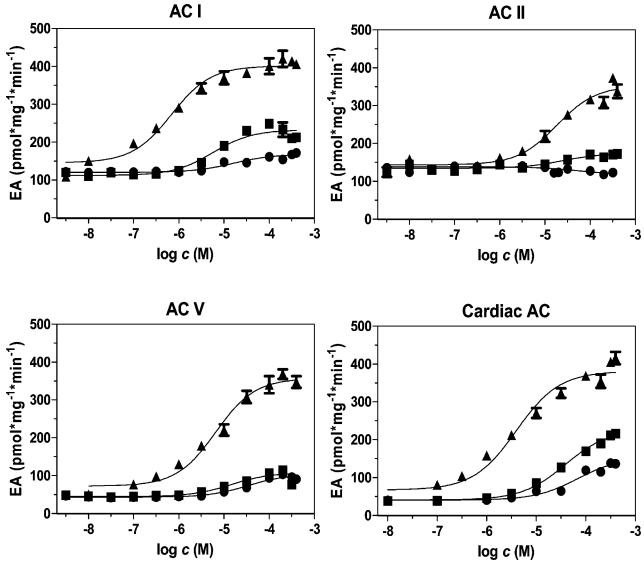


Figure 5. AC stimulation of recombinant AC isoforms 1, 2 and 5 as well as cardiac membranes by compounds **5** (filled circle) and **10** (filled squares) with 10 mM Mn^{2+} in comparison with forskolin (filled triangles).

Compared with forskolin, compounds **5** and **10** showed lower potencies and efficacies for all the isoforms examined. The cyclic thionocarbonate **5** is a weak partial agonist on AC 1 and AC 5 and even inhibits AC 2. Interestingly, **5** shows a stronger efficacy but lower potency towards the cardiac membrane preparation. This effect could result from an activation of other existing AC isoforms. The cyclic carbonate **10** has a higher potency towards ACs 1, 2 and 5 compared with compound **5**, however, the EC_{50} values obtained with ACs 1 and 5 are similar and do not significantly differ from AC 2. Compound **5** has a three-fold higher potency towards AC 1 than AC 5. Neither compound shows substantial selectivity towards any of the investigated specific AC isoforms.

Conclusions

New tetracyclic forskolin analogues have been prepared by direct modification of the natural product. Whereas radical deoxygenation of a thionocarbonate precursor was followed by intramolecular cyclization with the terminal olefin to form analogue **6**, the reaction with a diazaphospholidine resulted in an unprecedented rearrangement and the formation of a seven-membered cyclic carbonate **10**. Interestingly, the introduction of an additional ring into the forskolin skeleton did not lead to a loss of binding affinity towards AC. Although the new compounds are much more spacious they still seem to fit into the binding pocket, which is situated close to the catalytic domains C1 and C2 and lead to enzyme activation. This clearly demonstrates that the binding site offers enough space for larger modifications of the forskolin skeleton. Despite their lack of isoform specificity the new analogues are interesting because they are one of the few known partial AC agonists.

Experimental Section

General: Commercial reagents and starting materials were purchased from Aldrich, Fluka or Acros and used without further purification. Flash chromatography was performed on silica gel (Merck silica gel Si 60 40–63 μm). Products were detected by TLC on alumina plates coated with silica gel (Merck silica gel 60 F_{254}, thickness 0.2 mm) and visualized after staining with phosphomolybdic acid. Melting points were determined with a Büchi SMP 20 apparatus and are uncorrected. NMR spectra were recorded with a Bruker Avance 300 instrument (^{1}H: 300.1 MHz, ^{13}C: 75.5 MHz, T = 300 K). Chemical shifts are reported in δ (ppm) relative to external standards and coupling constants J are given in Hz. The relative number of protons was determined by integration. Error in the reported values: chemical shift 0.01 ppm (^{1}H NMR), 0.1 ppm (^{13}C NMR) and coupling constant 0.1 Hz. The solvent used for each spectrum is reported. Mass spectra (ESI) were recorded with a Finnigan MAT TSQ 7000 spectrometer and IR spectra with a Bio-Rad FT-IR-FTS 155 spectrometer.

Compound 5: An oven-dried Schlenk tube was charged with forskolin (100 mg, 0.24 mmol) and dry DCM (2 mL). DMAP (60 mg, 0.49 mmol) and thiocarbonyldiimidazole (46 mg, 0.26 mmol) were added under nitrogen and the mixture was stirred under nitrogen at room temperature for 4 h. After consumption of all the starting material, as judged by TLC, the solvent was removed and the crude product was purified by flash chromatography on silica gel (petroleum ether/acetone, 19:6, R_f = 0.27) to yield the product as a white

crystalline solid (107 mg, 97%); m.p. >225 °C. ^{1}H NMR (300 MHz): δ = 1.05 (s, 3 H, CH_3), 1.26 (s, 3 H, CH_3), 1.29–1.31 (m, 1 H, CH), 1.41 (s, 3 H, CH_3), 1.48–1.53 (m, 2 H, CH/OH), 1.69 (s, 3 H, CH_3), 1.73 (s, 3 H, CH_3), 1.85–2.00 (m, 2 H, CH), 2.08–2.16 (m, 1 H, CH), 2.18 [s, 3 H, C(O)CH_3], 2.52 (d, ^2J = 16.0 Hz, 1 H, CH), 3.11 (d, ^2J = 16.0 Hz, 1 H, CH), 4.49–4.52 (m, 1 H), 4.85 (t, J = 2.7 Hz, 1 H), 5.07 (dd, J_{cis} = 10.7, ^1J = 0.8 Hz, 1 H), 5.33 (dd, J_{trans} = 17.0, ^1J = 0.8 Hz, 1 H), 5.34 (d, ^3J = 4.1 Hz, 1 H), 6.01 (dd, J_{trans} = 17.0, J_{cis} = 10.6 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, DEPT 135, $CDCl_3$): δ = 17.8 (+), 21.1 (+), 23.0 (+), 23.2 (+), 29.9 (+), 32.5 (+), 33.8 (C_{quat}), 36.2 (–), 36.6 (C_{quat}), 44.1 (+), 50.6 (–), 68.8 (+), 76.0 (+), 76.9 (C_{quat}), 80.3 (C_{quat}), 84.1 (+), 89.0 (C_{quat}), 111.9 (–), 144.7 (+), 169.3 (C_{quat}), 187.0 (C_{quat}), 200.1 (C_{quat}) ppm. IR: $\tilde{\nu}$ = 3407, 2931, 1721, 1375, 1251 cm^{-1}. MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): *m*/*z* (%) = 453 (100) $[MH]^+$, 470 (67) $[MNH_4]^+$. $C_{23}H_{32}O_7S$ (452.57): C 61.04, H 7.13, S 7.08; found C 61.12, H 7.00 S 7.29.

Compound 6: A solution of thionocarbonate **5** (25 mg, 0.06 mmol), tributyltin hydride (0.47 mL, 0.17 mmol) and azoisobutyronitrile (1 mg, 6 μmol) in toluene (1.5 mL) under nitrogen was heated at 100 °C for 4 h. After consumption of all the starting material, as judged by TLC, the reaction was quenched by adding petroleum ether. Column chromatography (petroleum ether followed by petroleum ether/diethyl ether, 1:4) afforded the crystalline compound **3** (35 mg, 85%); m.p. >225 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.89 (t, ^3J = 7.7 Hz, 9 H, 3 CH_3), 1.04 (s, 3 H, CH_3), 1.13–1.19 (m, 2 H), 1.21–1.39 (m, 17 H), 1.51–1.63 (m, 13 H), 1.67 (s, 3 H, CH_3), 1.70–1.75 (m, 2 H), 1.97–2.07 (m, 3 H), 2.18 [s, 3 H, C(O)CH_3], 2.29 (dd, J = 1.4, J = 17.8 Hz, 1 H), 4.38–4.43 (m, 1 H, CH), 5.08–5.12 (m, 1 H, CH), 5.44 (d, ^3J = 3.8 Hz, 1 H, CH) ppm. ^{13}C NMR (75 MHz, DEPT 135, $CDCl_3$): δ = 13.7 (+), 15.0 (–), 15.4 (+), 17.5 (+), 19.6 (+), 21.3 (+), 22.9 (–), 25.4 (+), 25.8 (+), 27.0 (–), 27.9 (C_{quat}), 28.6 (–), 34.2 (+), 35.9 (–), 42.3 (C_{quat}), 45.7 (+), 51.5 (–), 53.3 (+), 69.4 (+), 71.5 (C_{quat}), 78.3 (+), 79.2 (+), 80.9 (C_{quat}), 81.4 (C_{quat}), 170.0 (C_{quat}), 171.3 (C_{quat}), 210.2 (C_{quat}) ppm. IR: $\tilde{\nu}$ = 2863, 1741, 1369, 1238 cm^{-1}. MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): *m*/*z* (%) = 745 (31) $[MH]^+$. Despite several attempts satisfactory combustion analysis or HRMS data could not be obtained for this compound.

Compound 10: Thionocarbonate **5** (50 mg, 0.1 mmol) and 1,3-dimethyl-2-phenyl-1,3,2-diazaphospholidine (**9**; 107 mg, 0.5 mmol) were mixed in a small screw-capped glass vial and stirred at 50 °C for 12 h. The crude product was purified by flash chromatography on silica gel (petroleum ether/acetone, 4:1, R_f = 0.24) and isolated as a white solid (29 mg, 69%). Crystallization for X-ray analysis was achieved from *n*-hexane; m.p. 198 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.00 (s, 3 H, CH_3), 1.17–1.23 (m, 1 H, CH_2), 1.22 (s, 3 H, CH_3), 1.22 (s, 3 H, CH_3), 1.48 (d, ^3J = 2.5 Hz, 1 H, CH), 1.63 (s, 3 H, CH_3), 1.63 (s, 3 H, CH_3), 1.68–1.78 (m, 1 H, CH_2), 1.82–1.90 (m, 1 H, CH_2), 2.12–2.24 (m, 1 H, CH_2), 2.19 (s, 3 H, CH_3), 2.40–2.58 (m, 2 H, CH_2), 4.37–4.39 (m, 1 H, CH), 4.68–4.70 (m, 1 H, CH), 4.98–5.05 (m, 3 H, CH), 5.88–5.97 (m, 1 H, CH) ppm. ^{13}C NMR (75 MHz, DEPT 135, $CDCl_3$): δ = 21.3 (+), 23.7 (+), 23.9 (–), 24.7 (+), 27.5 (+), 29.6 (+), 32.4 (+), 34.1 (C_{quat}), 36.3 (–), 38.3 (–), 43.5 (C_{quat}), 46.7 (+), 69.5 (+), 74.3 (C_{quat}), 77.2 (C_{quat}), 77.6 (+), 83.3 (+), 112.9 (–), 127.8 (C_{quat}), 140.7 (C_{quat}), 142.6 (+), 152.6 (C_{quat}), 169.8 (C_{quat}) ppm. IR: $\tilde{\nu}$ = 2950, 1762, 1739, 1224, 1036 cm^{-1}. MS (ESI, DCM/MeOH + 10 mmol/L NH_4OAc): *m*/*z* (%) = 438 (100) $[MNH_4]^+$, 421 (44) $[MH]^+$. $C_{23}H_{32}O_7$ (420.51): calcd. C 65.70, H 7.67; found C 65.48, H 7.80.

Materials: Baculoviruses for the expression of ACs 1, 2 and 5 were kindly provided by Drs. G. Gilman and R. K. Sunahara (University of Texas Southwestern Medical Center, Dallas, TX). Insect cells of *Spodoptera frugiperda* (Sf9) were from the American Type Cell Culture Collection (Rockville, MD). Guanosine-5′-[γ-thio]-triphosphate (GTPγS), creatine kinase and adenosine triphosphate (ATP) were purchased from Roche Diagnostics (Mannheim, Germany). Isobutylmethylxanthine (IBMX) and cyclic adenosine monophosphate (cAMP) were purchased from Sigma Aldrich (Seelze, Germany) and forskolin was purchased from LC Laboratories (Woburn, MA). Forskolin and the forskolin analogues **5** and **10** were prepared in DMSO stock solutions (20 mM each) and stored at –20 °C. The final DMSO concentration in the assay was 2% (v/v) DMSO. [α-^{32}P]-ATP (800 Ci/mmol) was from Perkin–Elmer (Wellesley, MA) and neutral alumina (N Super I) was purchased from MP Biomedicals (Eschwege, Germany). The highest quality $MnCl_2$ was purchased from Merck (Darmstadt, Germany). Protein concentration was determined by using the DC protein assay kit (Bio-Rad, Hercules, CA).

AC Expression and Membrane Preparation of Sf9 Cells: The cell culture of Sf9 cells, the expression of AC 1, 2 and 5 in Sf9 membranes and membrane preparation were performed as described previously.[22] The membrane preparations were stored in 1 mL aliquots at –80 °C. After thawing, the membranes were centrifuged for 15 min at 4 °C and 13000*g* and the sediments were resuspended in 75 mM TRIS buffer (pH 7.4) to obtain a concentration of 30 μg membrane protein/tube.

Preparation of Cardiac AC: Female CD1 mice were housed in a controlled environment according to the German animal protection law. At the age of 10 weeks the mice were sacrificed by cervical dislocation and hearts were removed, shock-frozen in liquid nitrogen and stored at –80 °C. The hearts were thawed and rinsed in ice-cold homogenization buffer containing 5 mM Tris·HCl (pH 7.4) and 5 mM EDTA. The ratio of buffer volume to heart tissue was 20-fold. The hearts were fragmented and homogenized in a glass-glass homogenizer (Braun, Melsungen, Germany) at 1500 rpm. After removal of organ debris (centrifugation at 500*g*, 8 min) the supernatant was centrifuged at 40000 *g* for 30 min. The suspended membranes were washed three times to remove residual endogenous ligands and nucleotides and then resuspended in assay buffer consisting of 50 mM triethanolamine, 10 mM $MnCl_2$ and 1 mM ethyleneglycol tetraacetic acid (EGTA; pH 7.4). The membrane preparation was shock-frozen in liquid nitrogen and stored at –80 °C. The frozen cardiac membranes were thawed and centrifuged to reach a concentration of 20 μg cardiac membrane protein/tube.

AC Activity Assay: AC activity was determined as described previously.[6] For the AC 1, 2, and 5 membrane preparation, the assay tubes contained forskolin analogue (10 μL), membrane preparation (20 μL, 30 μg protein/tube) and a reaction mixture (20 μL) consisting of 2.7 mM phosphoenolpyruvate, 0.125 IU pyruvate kinase and 1 IU myokinase. The assay with cardiac ACs had the same composition, but the reaction mixture contained 0.4 mg/mL creatine kinase, 9 mM phosphocreatine and 100 μM IBMX. All tubes additionally contained 10 mM Mn^{2+}, 10 μM GTPγS, 40 μM ATP, 100 μM cAMP and 0.2–1.0 μCi [α-^{32}P]ATP. The tubes were preincubated for 2 min at 30 °C. The reaction with the AC isoform preparation was initiated by the reaction mixture and the cardiac membrane preparation was initiated by the addition of 20 μL membrane preparation (20 μg protein/tube). The AC activities of AC 1, 2 and 5 were measured at time intervals of 20 min at 37 °C. To ensure a linear reaction progress for the cardiac AC, the assays were incubated for only 10 min at 30 °C. The reactions were terminated by the addition of 20 μL 2.2 N HCl to denature the protein and by centrifugation at 12000*g* for 2 min. [^{32}P]cAMP was separated from

[α-^{32}P]ATP by transfer of the sample to a 1.4 g neutral alumina column and elution with 4 mL of 0.1 M ammonium acetate (pH 7.0). After addition of double-distilled water (10 mL), Čerenkov radiation was determined with a liquid scintillation counter Tri-Carb 2800 TR (Perkin–Elmer, Wellesley, MA). The concentration–response curves and potency/efficacy values shown in Table 1 and Figure 5 were obtained by non-linear regression analysis with the Prism 5.01 software (Graphpad, San Diego, CA).

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[1] V. Shah, S. V. Bhat, B. S. Bajwa, H. Dornauer, N. J. de Souza, *Planta Med.* **1980**, *39*, 183–185.
[2] S. V. Bhat, B. S. Bajqwa, H. Dornauer, N. J. do Scusa, H.-W. Fehlhaber, *Tetrahedron Lett.* **1977**, *18*, 1669–1672.
[3] S. V. Bhat, A. N. Dohadwalla, N. K. D. Balbir, S. Bajwa, H. Dornauer, N. J. de Souza, *J. Med. Chem.* **1983**, *26*, 486–492.
[4] a) T. Onda, Y. Hashimoto, M. Nagai, H. Kuramochi, S. Saito, H. Yamazaki, Y. Toya, I. Sakai, C. J. Homcy, K. Nishikawa, Y. Ishikawa, *J. Biol. Chem.* **2001**, *276*, 47785–47793; b) J. D. Robbins, D. L. Boring, W.-J. Tang, R. Shank, K. B. Seamon, *J. Med. Chem.* **1996**, *39*, 2745–2752.
[5] a) N. Defer, M. Best-Belpomme, J. Hanoune, *Am. J. Physiol. Renal Physiol.* **2000**, *279*, 400–416; b) J. Hanoune, N. Defer, *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 145–174.
[6] C. Pinto, D. Papa, M. Hübner, T.-C. Mou, G. H. Lushington, R. Seifert, *JPET* **2008**, *325*, 27–36.
[7] Y. Khandelwal, E. R. Jotwani, P. K. Inamdar, N. J. de Souza, R. H. Rupp, *Tetrahedron* **1989**, *45*, 763–766.
[8] S. Zimmermann, S. Bick, P. Welzel, H. Meuer, W. S. Sheldrick, *Tetrahedron* **1995**, *51*, 2947–2952.
[9] B. M. F. Lagnel, A. de Groot, C. Morin, *Tetrahedron Lett.* **2001**, *42*, 7225–7227.
[10] a) H. Hagiwara, F. Takeuchi, T. Hoshi, T. Suzuki, T. Hashimotoc, Y. Asakawac, *Tetrahedron Lett.* **2003**, *44*, 2305–2306; b) H. Hagiwara, F. Takeuchi, M. Kudou, T. Hoshi, T. Suzuki, T. Hashimoto, Y. Asakawa, *J. Org. Chem.* **2006**, *71*, 4619–4624.
[11] a) R. W. Kosley, R. J. Cherill, *J. Org. Chem.* **1989**, *54*, 2972–2975; b) T. Tatee, A. Narita, K. Narita, G. Izumi, T. Takahira, M. Sakurai, A. Fujita, M. Hosono, K. Yamashita, K. Enomoto, A. Shiozawa, *Chem. Pharm. Bull.* **1996**, *44*, 2274–2279; c) B. Lal, A. K. Gangopadhyay, R. Rajagopalan, A. V. Ghateb, *Bioorg. Med. Chem.* **1998**, *6*, 2061–2073.
[12] E. J. Corey, B. Hopkins, *Tetrahedron Lett.* **1982**, *23*, 1979–1982.
[13] E. J. Corey, R. A. E. Winter, *J. Am. Chem. Soc.* **1963**, *85*, 2677–2678.
[14] E. Block, in: *Reactions of Organosulfur Compounds*, Academic Press, New York, **1978**, pp. 231–235.
[15] E. J. Corey, G. Märkl, *Tetrahedron Lett.* **1967**, *8*, 3201–3204.
[16] G. Scherowsky, J. Weiland, *Chem. Ber.* **1974**, *107*, 3155–3163.
[17] D. Horton, C. G. Tindall Jr., *J. Org. Chem.* **1970**, *35*, 3558–3559.
[18] a) R. A. Braun, *J. Am. Chem. Soc.* **1965**, *87*, 5516–5517; b) D. L. Pole, J. Warkentin, *J. Org. Chem.* **1997**, *62*, 4065–4067; c) M. Dawid, P. C. Venneri, J. Warkentin, *Can. J. Chem.* **2001**, *79*, 110–113; d) P. C. Venneri, J. Warkentin, *Can. J. Chem.* **2000**, *78*, 1194–1203; e) M. Dawid, J. Warkentin, *Can. J. Chem.* **2003**, *81*, 598–606.
[19] K. G. Jensen, A. Önfeldt, M. Wallin, V. Lidums, O. Andersen, *Mutagenesis* **1991**, *6*, 409–416.
[20] a) M. Yamamoto, M. E. Götz, H. Ozawa, C. Luckhaus, T. Saito, M. Rösler, P. Riederer, *Biochim. Biophys. Acta* **2000**, *1535*, 60–68; b) M. Yamamoto, H. Ozawa, T. Saito, S. Hatta, P. Riederer, N. Takahata, *J. Neur. Transmiss.* **1997**, *104*, 721–732.
[21] a) D. Rottländer, J. Matthes, S. F. Vatner, R. Seifert, S. Herzig, *J. Pharmacol. Exp. Ther.* **2007**, *321*, 608–615; b) X. Liu, M. Thangavel, S. Q. Sun, J. Kaminsky, P. Mahautmr, J. Stitham, J. Hwa, R. S. Ostrom, *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2007**, *377*, 359–369; c) S. Okumura, G. Takagi, J. Kawabe, G. Yang, M. C. Lee, C. Hong, J. Liu, D. E. Vatner, J. Sadoshima, S. F. Vatner, Y. Ishikawa, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 9986–9990.
[22] A. Gille, G. H. Lushington, T. C. Mou, M. B. Doughty, R. A. Johnson, R. Seifert, *J. Biol. Chem.* **2004**, *279*, 19955–19969.

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