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Research Article
doi.org/10.1002/slct.202204816

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Synthesis of Selagibenzophenone A and Its Derivatives for Evaluation of Their Antiproliferative, RORγ Inverse Agonistic, and Antimicrobial Effect**

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We report a modular synthetic approach towards novel derivatives of the naturally occurring arylated benzophenone selagibenzophenone A. The initial strategy for the construction of the carbon framework of the derivatives relied on the Suzuki reaction of 2,4,6-tribromobenzonitrile, and the addition of the aryl lithium species to nitrile to generate imine. However, the formed imines showed remarkable stability toward hydrolysis. Therefore, Suzuki cross-coupling was carried out with 2,4,6-tribromobenzaldehyde and the subsequent addition of organometallic species to the aldehyde. Oxidation of the resulting alcohol ensured the access to desired ketones. The importance of the developed modular strategy is underlined by the discovery of several derivatives with selective cytotoxic effects and potential anti-inflammatory activity superior to the effect of the natural product.

Introduction

About 10% of the FDA-approved drugs are natural products and the number increases to about one-third if compounds derived from natural products are taken into consideration.[1] Bearing in mind the clinical potential of such compounds, it is necessary to develop robust synthetic strategies that not only allow access to natural products but also derivatives thereof. For the latter, synthesis is often the only means to obtain them.[2] Plants from the genus *Selaginella (Selaginellaceae)* are living fossils, with an estimated age of 400 million years.[3] They consist of more than 700 species and many of them were used in various traditional folk medicines to treat different diseases, such as jaundice, gonorrhea, acute hepatitis, asthma, dysmenorrhea, or traumatic injuries.[4,5] Extracts from *Selaginella* have demonstrated anticancer, anti-inflammatory, antimicrobial, antioxidant, antiviral, and other *in vitro* and *in vivo* effects.[6–8]

More than 100 structurally diverse polyphenolic structures have been identified in plants from this genus, including selaginellins (**1**, Figure 1)[9] and selaginpulvilins (**2**, Figure 1),[10] compounds unique for *Selaginella*.[11] The isolated constituents displayed a wide variety of biological activities, among which the most extensively investigated is the inhibitory effect on phosphodiesterase 4 (PDE4), an enzyme involved in the degradation of cytosolic cyclic adenosine monophosphate (cAMP).[12,13] The second messenger cAMP is involved in the regulation of many pro- and anti-inflammatory factors.[14] Moreover, the expression levels of PDE4D isoforms were shown to be increased in prostate cancer patients, therefore it is

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[**] *RORγ = retinoic acid receptor-related orphan receptor-gamma.*

Supporting information for this article is available on the WWW under https://doi.org/10.1002/slct.202204816

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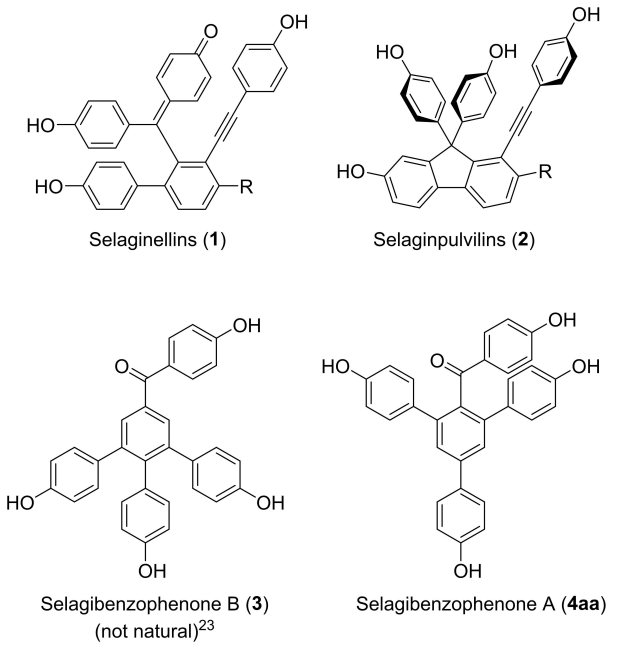

Figure 1. Natural products from Selaginella plans.

proposed to be used as a biomarker for prostate cancer progression.[15] A remarkably high potency for inhibition of the catalytic domain of PDE4D2 was found in particular for selaginpulvilin derivatives **2**, with IC_{50} values as low as 0.11 μM[10] or 0.22 μM.[16]

Besides that, some selaginellins were shown to be cytotoxic towards various cancer cell lines,[11] as well as against different bacterial and fungal strains with IC_{50} values in the low micromolar range.[17]

Recently, the arylated benzophenone selagibenzophenone A (**4aa**, Figure 1) was isolated by Liu et al. and described to inhibit PDE4D2 with an IC_{50} value of 1.04 μM in a cell-free enzymatic *in vitro* assay.[18] At the same time, Liu et al.[19] and Wang et al.[20] reported the isolation of the regioisomeric selagibenzophenone B (**3**, Figure 1, in the latter report referred to as selaphenine A). Compound **3** showed potential antimetastatic and cytotoxic properties with moderate activity. In 2020, Chen et al. reported the isolation of selagibenzophenone C.[21]

The reported therapeutic potential of *Selaginella*-derived plant products as well as their structural peculiarity sparked interest in the synthesis of these natural products and derivatives thereof. We have recently become interested in the chemistry of *Selaginella* constituents and reported the formal total synthesis of selaginpulvilins C and D.[22] Furthermore, we prepared selagibenzophenones A (**4aa**), B (**3**),[23] and C[24] and demonstrated that the structure of selagibenzophenone B was incorrectly elucidated. The compound reported as selagibenzophenone B (**3**)[19] (or selaphenin A[20]) was, in fact, selagibenzophenone A (**4aa**).[23]

In this study, we report a modular synthesis of selagibenzophenone A (**4aa**) and derivatives, which were further evaluated for various biological effects described for the natural product or the plant of origin. These include inhibition of intracellular PDE4, cytotoxic activities towards various cancer cell lines, an inverse agonistic activity on the nuclear receptor RORγ, and an antimicrobial activity. We found that the biological properties of some of the synthetic derivatives are superior to the ones of the natural product, underlining the importance of the development of modular synthetic strategies allowing the synthesis of natural products and in particular derivatives thereof.

Results and Discussion

Retrosynthetic analysis revealed that the construction of the carbon framework can rely on Suzuki cross-coupling and the addition of aryl lithium species to carbonyl **5** or nitrile **6** (Figure 2). Employing different boronic acids **7a–c** and different aryl lithium species **8a–c** allows us to access the derivatives with different substituents at rings C, D, and E (R^1), compared to ring A (R^2, Figure 2). We decided to synthesize a series of derivatives, which would bear either original hydroxy groups (hydrogen bond donor), methoxy ether (hydrogen bond acceptor), or simple hydrogen as a neutral substituent.

Our initial approach was based on a conversion of aniline **9** to nitrile **6** by a treatment of **9** with copper cyanide in the presence of t-BuONO (Scheme 1). Unfortunately, the reaction provided only 25% of the desired cyanide **6**. However, from an economical point of view, even such a low yield is advantageous over the use of aldehyde **5** (Figure 2). Therefore, we proceeded with the cross-coupling reaction for the introduction of the aryl moieties. In the presence of tetrakis(triphenylphosphine)palladium(0) and potassium carbonate, the reaction with 4-methoxyphenylboronic acid (**7b**) provided 73% of the desired trisarylated benzonitrile **10b** and the reaction with phenylboronic acid (**7c**) furnished benzonitrile **10c** in 77% yield. Subsequently, the benzonitrile **10b** was subjected to the addition of organolithiated species, formed *in situ* in the reaction of aryl bromide **11a**, containing *tert*-butyldimethylsilyl (TBS) protected phenol in the *para* position. The crude reaction mixture, which contained the imine **12** was subjected to sulfuric acid hydrolysis to obtain the corresponding ketone **4ba** (Scheme 1).

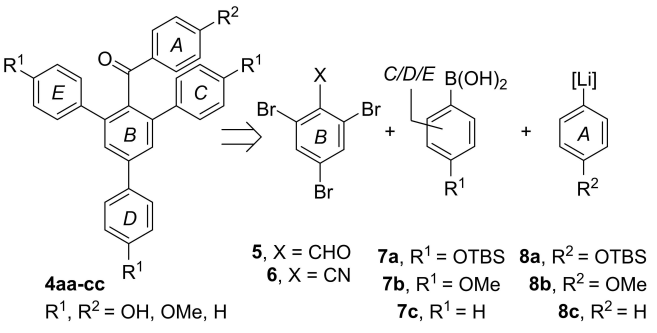

Figure 2. General structures of synthesized derivatives. The retrosynthetic plan allows access to derivatives with a different substitution of the ring A and rings C, D, and E.

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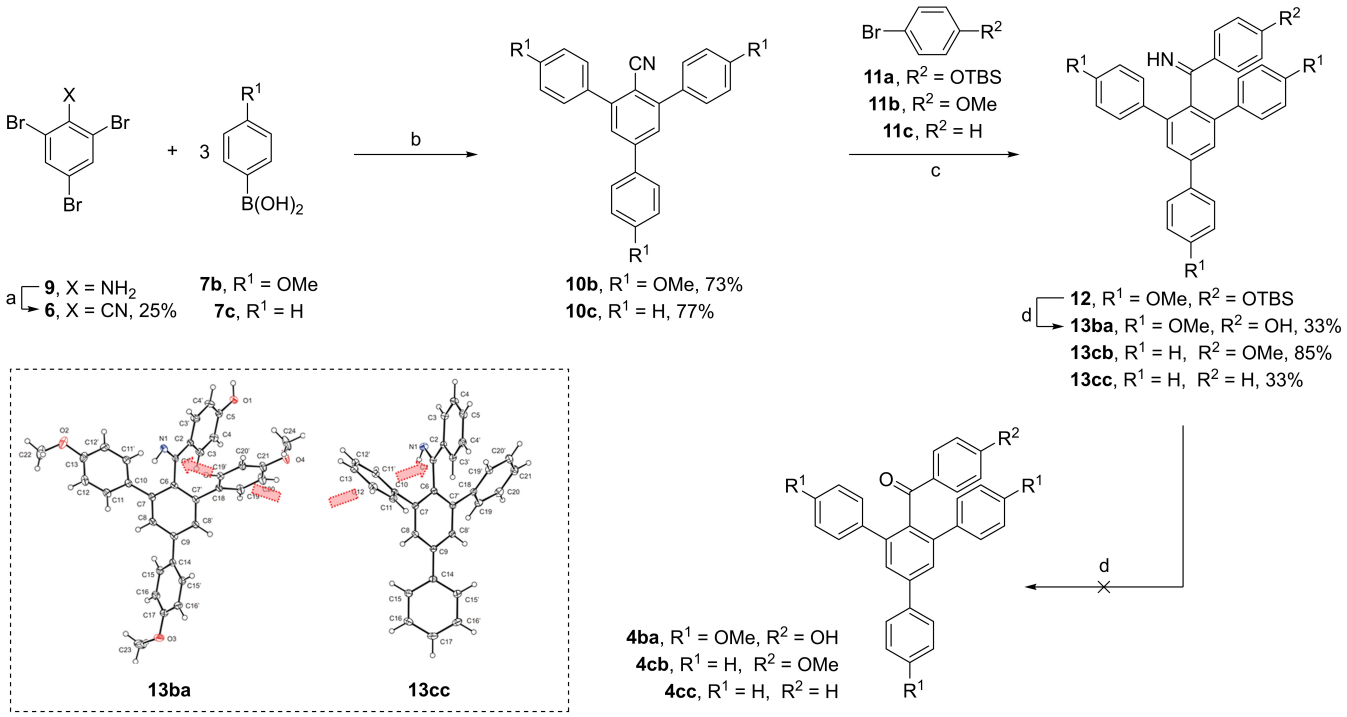

Scheme 1. Synthesis of the imine derivatives and failed attempts of their hydrolysis, and view on the molecules of **13ba** and **13cc** with the atom numbering scheme. The displacement ellipsoids at 30% probability level. The red arrows depict the approximate trajectory of the nucleophile, which is blocked by the aromatic rings in both *ortho* positions.

To our surprise, after the isolation of the reaction product, the analysis of the ^{13}C NMR spectrum revealed a signal with a chemical shift of 177.4 ppm, typical for imines. On the other hand, we did not observe any signal corresponding to the expected ketone group, suggesting that the hydrolysis of the imine did not take place and only TBS group was cleaved, providing the imine **13ba** instead of the desired ketone **4ba**. A similar resistance towards hydrolysis was observed for the imine **13cb**, which was prepared from nitrile **10c** by the addition of organolithiated species derived from 4-bromoanisole (**11b**), and as well for imine **13cc**, obtained from a reaction of nitrile **10b** and organolithiated species derived from bromobenzene (**11c**, Scheme 1). We confirmed the structure of the imines **13ba** and **13cc** by X-Ray crystallography. The analysis of the X-Ray structures confirmed the presence of imines, and it became obvious, that the electrophilic imine center is very effectively shielded by the two aromatic rings in the two *ortho* positions of ring B. The unusual stability of the imines towards the hydrolysis may therefore be of a kinetic origin, resulting from this steric shielding, which hinders the approach of the nucleophile to the electrophilic imine carbon or to the corresponding carbocation, derived from the protonation of the imine.[25] Alternatively, the tetrahedral hemiaminal, formed upon the addition of water to the imine, might be sterically crowded, causing thermodynamic instability of the system. Notably, the imines proved to be unreactive in lithium aluminum hydride-mediated reduction, suggesting that these imines are resistant to the attack of different nucleophiles as well.

We, therefore, focused on the synthetic strategy employing 2,4,6-tribromobenzaldehyde (**5**) as starting material (Scheme 2). Aldehyde **5** was first subjected to Suzuki cross-coupling with three different boronic acids **7a–c**, yielding three tris-arylated aldehydes **14a–c**, bearing a TBS-protected phenol in *para* positions, in the case of **14a**, methoxy groups in case of **14b**, and finally a hydrogen atom in the case of **14c**, in the respective yields of 79%, 93%, and 77%. We first synthesized a set of derivatives, which contained hydroxy groups in the *ortho* positions of aromatic rings C, D, and E (R^1=OH) and differed in the substitution at aromatic ring A (R^2). Aldehyde **14a** was subjected to the addition of organolithiated species, generated from aryl bromide **11a**. The formed secondary alcohol was used without additional purification in the next two steps, namely oxidation with PCC and removal of TBS protecting groups by Olah's reagent (HF/pyridine), to furnish selagibenzophenone A (**4aa**) in 82% (after three steps). Similarly, derivatives **4ab** and **4ac** were obtained by the same reaction sequence, using different aryl bromides for the generation of the organometallic species. In the case of **4ab**, aldehyde **14a** was subjected to the addition of an organolithiated compound, generated from 4-bromoanisol (**11b**). The oxidation of the formed alcohol **15ab** and deprotection led to the formation of monomethoxylated (R^2=OMe) derivative **4ab** in 53% (after three steps). The reaction of aldehyde **14a** with organometallic

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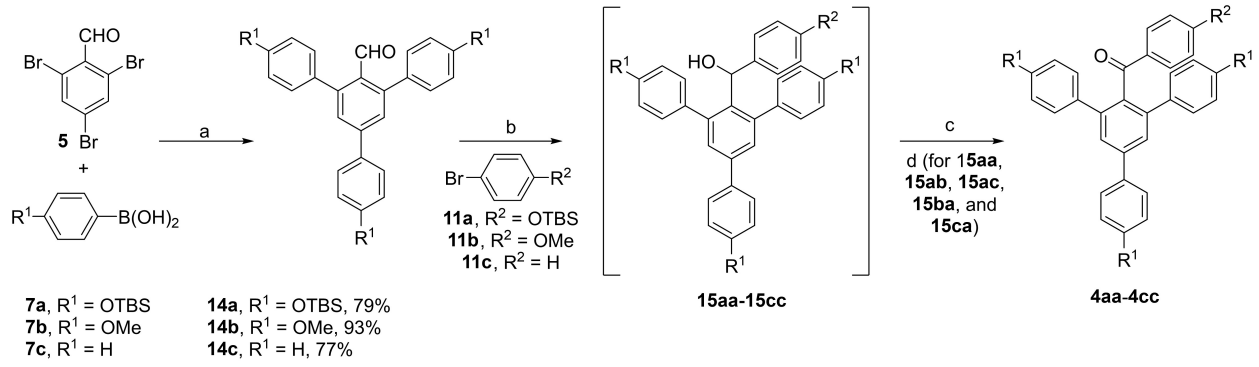
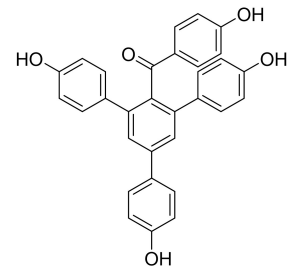
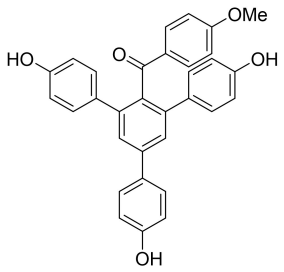


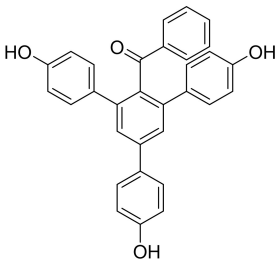
a) $Pd(PPh_3)_4$, K_2CO_3, PhH:H_2O (5:1), 90 °C; b) *t*-BuLi, THF, -78 °C; c) PCC, celite, CH_2Cl_2, r.t.; d) HF/pyridine, THF, r.t.



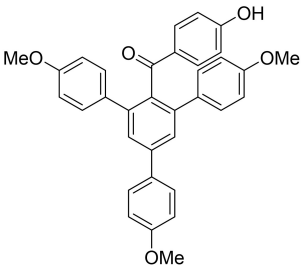
4aa, 82% (three steps)



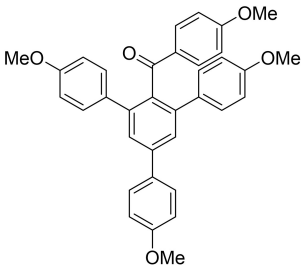
4ab, 53% (three steps)



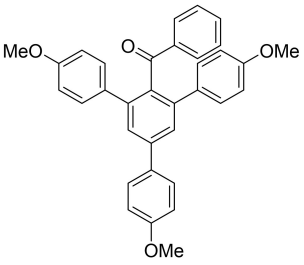
4ac, 40% (three steps)



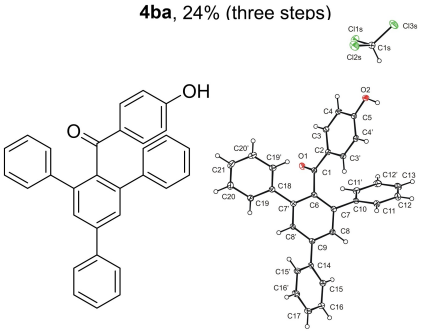
4ba, 24% (three steps)



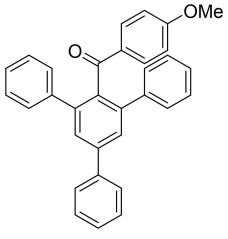
4bb, 79% (two steps)



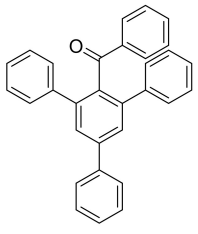
4bc, 74% (two steps)



4ca, 80% (three steps)



4cb, 87% (two steps)



4cc, 81% (two steps)

Scheme 2. Synthesis of the desired derivatives and view on the molecule of **4ca** with atom numbering scheme. The displacement ellipsoids at 30% probability level.

species generated from bromobenzene (**11c**) resulted in the formation of secondary alcohol **15ac**, which was subsequently oxidized and deprotected to yield deoxyselagibenzophenone **4ac** (R^2=H) in 40% after three steps.

Next, the derivatives **4ba**, **4bb**, and **4bc**, bearing the methoxy group in positions *para* of the aromatic rings C, D, and E (R^1=OMe), were prepared from aldehyde **14b**. When aldehyde **14b** was subjected to the reaction with lithiated species, generated from aryl bromide **11a**, secondary alcohol **15ba** was formed, which upon oxidation with PCC and removal of the TBS group with HF in pyridine, provided 24% (after three steps) of compound **4ba**, bearing free hydroxy group in the *para* position of aromatic ring A (R^2=OH). Treatment of 4-bromoanisol (**11b**) with *t*-BuLi and subsequent addition of

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aldehyde **14b** resulted in the formation of alcohol **15bb**, which upon oxidation furnished desired ketone **4bb** with *para* methoxy substitution of the ring A (R^2=OMe) in 79% yield (after two steps). The reaction of **14b**, with phenyllithium, generated from bromobenzene (**11c**), subsequent oxidation with PCC led to the formation of ketone **4bc**, without any substitution at the ring A (R^2=H) in 74% yield (after two steps).

Last but not least, derivatives **4ca**, **4cb**, and **4cc** without any substitution at rings C, D, and E (R^1=H) were prepared from aldehyde **14c**. First, **14c** reacted with an organolithiated compound generated from aryl bromide **11a**. Subsequent oxidation and TBS group cleavage furnished derivative **4ca** with a hydroxy group in the *para* position of ring A (R^2=OH) in 80% (after three steps). Compound **4cb** with a methoxy group at ring A (R^2=OMe) was obtained from aldehyde **14c** in the same sequence of two steps, namely addition to ketone and oxidation, in 87% (after two steps) using 4-methoxybromide (**11b**) for generation of the organolithium reagent. The last prepared derivative **4cc** with no substitution at the ring A (R^2=H) was prepared from the reaction of aldehyde **14c** and phenyllithium generated from bromobenzene **11c** and oxidation of the formed secondary alcohol **15cc** by PCC. Ketone **4cc** was obtained in 81% yield (after two steps). Notably, the desired derivatives were obtained in a sequence of three or four steps, but only two chromatographic purifications (of the Suzuki coupling products **14a–c** and the final products **4aa–cc**) were necessary in all the cases.

The structure of compound **4ca** was confirmed by X-Ray spectroscopy. The suitable crystal was obtained by slow evaporation of $CDCl_3$ solution of **4ca**. The compound crystallized as a solvate with one molecule of $CDCl_3$ (Scheme 2).

All of the synthesized ketones **4aa–cc** and three imines **13ba**, **15cb**, and **15cc** were evaluated for their biological activities. First, the effect on cellular levels of cAMP as an indirect measure of PDE4 activity was measured. Notably, none of the synthetic compounds (including selagibenzophenone A **4aa**) altered cellular levels of cAMP in HEK293 cells at the tested concentrations (10–30 μM, SI, Figure S1), despite the fact, that selagibenzophenone A (**4aa**) was recently described to inhibit PDE4D2 in a cell-free enzymatic *in vitro* assay with an IC_{50} value of 1.04 μM.[18] This lack of activity in the cellular model, in contrast to the described activity of selagibenzophenone A on recombinant PDE4 enzyme, could be (at least partly) due to a low cellular uptake of compounds and thus a resulting lower effective concentration at the intracellular target site.

Using a RORγ–Gal4 mammalian one-hybrid luciferase assay, compounds were further investigated for a potential inverse agonistic activity on this nuclear receptor. Thereby, eight compounds: **4aa** (10 μM), **4ab** (10 μM), **4ac** (3 μM), **4ba** (10 μM). **4bb** (3 μM), **4ca** (10 μM), **13cb** (10 μM), and **13cc** (10 μM) showed a statistically significant inhibition of the RORγ–Gal4 transcriptional activity, suggesting a direct binding of the compounds to the ligand-binding domain of this receptor (SI, Figure S2).[26]

The most interesting results were obtained from cytotoxicity studies. Compounds were evaluated for their cytotoxic effect on a colon cancer cell line (HT-29), a prostate cancer cell line (PC3), and a breast cancer cell line (MCF-7) using the SRB method, as described previously.[27] In addition, the cytotoxicity against healthy human umbilical vein endothelial cells (HUVEC) was assessed. Cells were initially treated with 100 μM of each of the derivatives (SI, Figure S3). Among these **4aa**, **4ab**, **4ac**, **4ba**, **4ca**, **13cb**, and **13cc** were selected as the most promising compounds and concentration-response curves were determined to obtain their IC_{50} values (SI, Figures S4–6). The selectivity index (SI) of a compound was calculated by division of the IC_{50} value obtained for a non-cancerous cell line by that obtained for a specific cancer cell line. The obtained results are summarized in Table 1. The natural compound **4aa** showed only weak cytotoxicity towards the tested cancer cell lines with no or very low selectivity (Table 1, Entry 1). Derivatives **4ab** and **4ac** showed a moderate cytotoxicity towards the investigated cancer cell lines (EC_{50} = 22.0 to 54.7 μM) and for **4ac** the selectivity index increased to 4.1 for the PC3 cell line (Table 1, Entry 2, 3). The best results were obtained with derivatives **4ba** and **4ca**. The former showed a promising potency (EC_{50} = 17.7 μM) against HT-29 with a good selectivity index of 8.2 (Table 1, Entry 4) and the latter showed good potency (7.8 μM) against PC3 cell line with a selectivity index of 3.5 (Table 1, Entry 5). The investigated imines showed only limited or no activity at all with no significant selectivity.

Table 1. Cytotoxicity and selectivity index[a] of selected derivatives.

ENTRY	COMPOUND	IC_{50} ± SE (μM) HT-29[b]	PC3[c]	MCF-7[d]	HUVEC[e]	Selectivity Index (SI)[a] HT-29[b]	PC3[c]	MCF-7[d]
1	**4aa**	84.3 ± 1.2	55.3 ± 1.9	51.1 ± 1.4	72.8 ± 1.4	0.9	1.3	1.4
2	**4ab**	27.8 ± 1.3	32.1 ± 1.3	44.4 ± 1.7	26.8 ± 1.3	1.0	0.8	0.6
3	**4ac**	24.3 ± 1.3	22.0 ± 1.2	54.7 ± 1.3	89.7 ± 1.3	3.7	4.1	1.6
4	**4ba**	17.7 ± 1.2	43.4 ± 1.2	56.7 ± 1.3	144.5 ± 1.1	8.2	2.6	2.6
5	**4ca**	11.9 ± 1.2	7.8 ± 1.1	25.7 ± 1.3	27.5 ± 1.4	2.3	3.5	1.1
6	**13cc**	168.8 ± 1.1	58.0 ± 1.4	80.7 ± 1.4	113.9 ± 1.2	0.8	2.3	1.7
7	**13cb**	29.4 ± 1.5	48.0 ± 1.1	213.8 ± 1.1	79.3 ± 1.1	2.7	1.7	0.4

[a] Selectivity index calculated as EC_{50} against HUVEC cells divided by EC_{50} against the cancer cell line.
[b] Colon cancer cell line.
[c] Prostate cancer cell line.
[d] Breast cancer cell line.
[e] Healthy human umbilical vein cells.

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Synthesized compounds were also evaluated for their antimicrobial potential. The minimal inhibitory concentrations were determined according to previous studies.[28,29] Obtained data showed that none of the synthesized compounds exhibited an antimicrobial effect against *Staphylococcus aureus* ATCC 25930, *Escherichia coli* ATCC 25922, or clinically relevant *Candida albicans* strains (SI, Figure S7).

Conclusion

In conclusion, we developed a modular synthetic approach towards several derivatives of the natural product selagibenzophenone A (**4aa**). The initial synthetic plan, based on a synthesis of imine analogs and their hydrolysis to desired ketones, failed as a consequence of the unusual stability of imines. The synthetic strategy based on the use of 2,4,6-tribromobenzaldehyde allowed us to prepare derivatives with a differently substituted aromatic ring A and rings C, D, and E. We synthesized derivatives bearing hydrogen bond donors, hydrogen bond acceptors, and neutral hydrogen substituents in these positions and evaluated them for various biological effects. To our surprise, we did not observe an inhibition of PDE4 in a cAMP accumulation assay performed in HEK293A cells, despite the previously described activity of the natural product in a cell-free enzymatic assay. However, we were able to identify a few derivatives with selective cytotoxicity towards prostate or colon cancer cell lines with somewhat lower cytotoxicity towards the non-cancerous cell lines. Moreover, some of the derivatives were shown to be inverse agonists of the nuclear receptor RORγ. On the other hand, none of the derivatives exhibited any antimicrobial activity. The fact that the derivatives of selagibenzophenone A exhibited superior biological activity compared to the natural product itself underlines the importance of the development of the modular synthetic strategies allowing a quick access towards unnatural derivatives. The compounds with a selective cytotoxic profile will serve as lead compounds for further development of potential anticancer agents as well as for the investigation related to the molecular mechanism responsible for the observed selectivity.

Experimental Section

General: All the chemicals for the synthesis were purchased from the common sources: Sigma Aldrich, Acros Organics, Alfa Aesar, Strem Chemicals, PENTA Chemicals, Fluorochem, Cambridge Isotope Laboratories, Inc. Unless otherwise noted, all the reagents were used without further purification. Solvents used in the reactions were distilled and dried prior the use. The reactions were monitored by TLC using Merck TLC silica gel 60 F_{254} plates, using a UV lamp (254 nm) detection and Hanessian's stain (CAM). NMR spectra were recorded on a Bruker Avance III spectrometer (400 MHz and 600 MHz for ^{1}H NMR and 100 MHz and 150 MHz for ^{13}C NMR, respectively) and Varian NMR Solutions 300 (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR). All chemical shifts δ are reported in ppm with a reference to a residual solvent. Mass spectrometry was performed on a VG-Analytical ZAB SEQ. Infrared spectrum were measured in KBr with a Hermo Nicolet AVATAR 370 FT-IR spectrometer. Melting points were determined using Kofler apparatus KB T300. For reaction that require heating, it was carried out with the oil bath as the heat source. X-ray diffraction experiments for **13ba**, **13cc** and **4ca** were performed on Bruker D8 VENTURE Kappa Duo PHOTONIII by IμS micro-focus sealed tube MoKα (λ = 0.71073) at temperature 120 K of measured crystals. The structures were solved by direct methods (XT)[30] and refined by full matrix least squares based on F^2 (SHELXL2018).[31] The hydrogen atoms on carbon were fixed into idealized positions (riding model) and assigned temperature factors either $H_{iso}(H) = 1.2\ U_{eq}$(pivot atom) or $H_{iso}(H) = 1.5\ U_{eq}$ (pivot atom) for methyl moiety. The hydrogen atoms on N and O were found on difference Fourier map and refined with assumptions of riding model. Crystallographic data are summarized in Table S1X-ray crystallographic data have been deposited with the Cambridge Crystallographic Data Centre. Deposition Number(s) 2190278 (for **13ba**), 2190280 (for **13cc**), 2190279 (for **4ca**), contain(s) the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service. The corresponding ccdc numbers were included into Table S1PC-3 (human Caucasian prostate adenocarcinoma cells), HT-29 (human Caucasian colon adenocarcinoma cells), MCF-7 (human breast cancer cells) and HUVEC (human umbilical vein endothelial cells) were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Dulbecco's modified Eagle medium (DMEM; 4.5 g/L glucose), fetal bovine serum (FBS), penicillin-streptomycin solution, trypsin-EDTA were purchased from Gibco/Thermo Fisher Scientific (Waltham, MA, USA). Sulforhodamine B (SRB) was obtained from Sigma-Aldrich (St. Louis, MO, USA). HEK293 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). For the measurement of intracellular cAMP levels, a HEK293A cell line stably expressing an EPAC-based FRET biosensor was employed as described before.[32] Dulbecco's modified Eagle medium (DMEM; 4.5 g/L glucose) was purchased from Lonza (Basel, Switzerland). Fetal bovine serum (FBS) was acquired from biowest (Nuaillé, FR). Glutamine solution, penicillin, and streptomycin were obtained from Lonza (Basel, Switzerland). Trypsin and Na_2-EDTA were purchased from Gibco/Thermo Fisher Scientific (Waltham, MA, USA). RORγ-Gal4 and tk(MH1000) 4×LUC plasmids were kind gifts from Dr. Fabio R. Santori (Center for Molecular Medicine, University of Georgia, Athens, GA, USA) and Dr. Ronald Evans (Salk Institute for Biological Studies, La Jolla, CA, USA), respectively. pEGFP–N1 was acquired from Takara Bio USA (Mountain View, CA, USA).

Representative example of the synthesis of imine

2,4,6-tribromobenzonitrile 6. CuCN (1.3 eq, 19.7 mmol, 1.77 g) and amine **9** (1 eq, 15.2 mmol, 5 g) were dissolved in anhydrous DMSO (40 mL) at 50 °C, followed by addition of *t*-BuONO (3 eq, 45.6 mmol, 5.4 mL). After two hours stirring at 50 °C, the reaction mixture was diluted with water (300 mL) and the product was extracted with EA (4×50 mL). The organic phase was dried over Na_2SO_4 and concentrated. Product **6** was then purified with column chromatography (EA:Hex 1:200). Reaction yielded 1.29 g (25 %) of the product as a brown solid. $R_f = 0.2$ [EA:Hex (1:100)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.8 (2C), 128.3, 127.2 (2C), 117.9; 115.6. Recorded data agree with the literature.[33]

4,4′′-dimethoxy-5′-(4-methoxyphenyl)-[1,1′:3′,1′′-terphenyl]-2′-carbo-nitrile (10b). Nitrile **6** (1 eq, 0.72 mmol, 246 mg), $Pd(PPh_3)_4$ (5 mol%, 0.036 mmol, 42 mg), K_2CO_3 (3.5 eq, 2.52 mmol, 348 mg) and (4-methoxyphenyl)boronic acid (3.15 eq, 2.27 mmol, 345 mg) were dissolved in a degassed mixture of benzene and water (5:1,

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6 mL). The reaction was heated in a closed vial at 90 °C for 16 h. Then, the reaction mixture was concentrated and product was purified with column chromatography (EA:Hex 1:10 to 1:8). Reaction yielded 223 mg (73 %) of nitrile **10 b** as a yellow solid. R_f= 0.4 [EA:Hex (1:5)]; mp = 87–94 °C (DCM); IR (KBr) 3035, 3001, 2958, 3993, 2837, 2544, 2216, 2044, 1894 1512, 1252, 829 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.64–7.55 (m, 8H), 7.06–7.97 (m, 6H), 3.88 (s, 6H), 3.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.4, 160.2, 147.2, 144.7, 131.7, 131.43, 130.4, 128.6, 126.6, 118.9, 114.7, 114.3, 108.2, 55.6, 55.5. HRMS (ESI) calculated for $C_{28}H_{24}NO_3$ ($M+H^+$): 422.1751; found 422.1750.

4-((4,4′′-dimethoxy-5′-(4-methoxyphenyl)-[1,1′:3′,1′′-terphenyl]-4′-yl)(imino)methyl)phenol (13 ba). A solution of *t*-BuLi (1.2 eq, 0.4 mmol, 0.25 mL, 1.6–1.7 M in hexanes) was added dropwise to a solution of (4-bromophenoxy)(*tert*-butyl)dimethylsilane (**11 a**) (1.2 eq, 0.4 mmol, 115 mg,) in THF (2 mL) at −78 °C. After 10 min of stirring, a solution of nitrile **10 b** (1 eq, 0.33 mmol, 140 mg) in THF (2 mL) was added to the reaction mixture. The reaction mixture was then allowed to warm up to the 22 °C. After 16 hours of stirring, a solution of H_2SO_4 (20 %, 14 mL) was added to the reaction mixture. After refluxing the reaction mixture for 16 hours, it was cooled down and phases were separated. The organic layer was washed with brine (30 mL), then concentrated, and the product was purified with column chromatography (DCM:MeOH 50:1 to 10:1). Reaction yielded 56 mg (33 %) of imine **13 ba** as a yellow solid. R_f = 0.2 [DCM:MeOH (30:1)]; mp = 390 °C (decomp. DCM); IR (KBr) 3269, 3001, 2952, 2925, 2852, 2841, 2676, 2364, 1608, 1252, 833 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.65–7.58 (m, 2H), 7.54 (s, 2H), 7.20–7.14 (m, 4H), 7.02–6.96 (m, 2H), 6.76–6.70 (m, 4H), 6.39–6.30 (m, 2H), 3.86 (s, 3H), 3.71 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 159.6, 158.8, 140.8, 133.1, 130.1, 130.1, 128.2, 127.5, 115.0, 114.4, 113.5, 55.4, 55.2. HRMS (ESI) calculated for $C_{34}H_{30}NO_4$ ($M+H^+$): 516.2169; found 516.2167.

Representative example of the synthesis of ketone

4,4′′-dimethoxy-5′-(4-methoxyphenyl)-[1,1′:3′,1′′-terphenyl]-2′-carbaldehyde (14 b). Aldehyde **5** (1 eq, 0.5 mmol, 170 mg), Pd-$(PPh_3)_4$ (5 mol%, 0.025 mmol, 29 mg), K_2CO_3 (3.5 eq, 1.75 mmol, 242 mg) and (4-methoxyphenyl)boronic acid (**7 b**, 3.2 eq, 1.6 mmol, 243 mg) were dissolved in a degassed mixture of benzene and water (5:1, 6 mL). Reaction was heated in a closed vial at 90 °C for 16 h. Then reaction mixture was concentrated and product was purified by column chromatography (EA:Hex 1:30). Reaction yielded 198 mg (93 %) of aldehyde **14 b** as a colorless glassy oil. R_f = 0.3 [EA:Hex (1:4)]; IR (KBr) 3033, 2999, 2954, 2933, 2906, 2835, 2754, 1693, 1608 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 9.97 (s, 1H), 7.70–7.59 (m, 2H), 7.54 (s, 2H), 7.41–7.32 (m, 4H), 7.10–6.91 (m, 6H), 3.88 (s, 6H), 3.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.4, 160.2, 159.4 (2C), 145.1 (2C), 143.9, 132.3 (2C), 132.0, 131.3, 131.0 (4C), 128.6 (2C), 128.4 (2C), 114.6 (2C), 113.8 (4C), 55.5 (3C); HRMS (ESI) calcd for $C_{28}H_{25}O_4$ ($M+H^+$): 425.1747; found 425.1746.

(4,4′′-dimethoxy-5′-(4-methoxyphenyl)-[1,1′:3′,1′′-terphenyl]-4′-yl)(4-hydroxyphenyl)methanone (4 ba). A solution of *t*-BuLi (1.5 eq, 1.7 M in hexanes, 0.21 mmol, 0.12 mL) was added to a solution of bromide **11 a** (1.5 eq, 0.21 mmol, 60 mg) in THF (2 mL) at −78 °C. After 10 min of stirring, the aldehyde **14 b** (1 eq, 0.14 mmol, 60 mg) in THF (1 mL) was added to the reaction mixture. It was then allowed to warm up to room temperature and stirred for 10 h. The reaction was quenched with a saturated solution of NH_4Cl (10 mL). Crude alcohol was extracted with EA (3× 10 mL) and combined organic phases were dried over Na_2SO_4, filtered, and concentrated. The resulting mixture was then dissolved in DCM (2 mL) and it was followed by the addition of PCC (1.5 eq, 0.21 mmol, 45 mg) and Cellite (43 mg). After 16 hours of stirring, the reaction mixture was filtrated and concentrated. The remaining mixture was then dissolved in THF (2 mL) and HF:py (70:30, 1.6 mmol, 0.1 mL) was added. After 3 hours, a saturated solution of $NaHCO_3$ (10 mL) was added to the reaction mixture. The aqueous phase was extracted with EA (3×10 mL) and combined organic phases were washed with 2M HCl (10 mL). The product was purified with column chromatography (EA:Hex 1:5). Reaction yielded 18 mg (25 %) of ketone **4 ba** as a white solid. R_f = 0.2 [EA:Hex (1:5)]; mp = 117–121 °C (DCM); IR (KBr) 3423, 3251, 3072, 3033, 2924, 2933, 2835, 1510, 1026 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.66–7.61 (m, 2H), 7.55 (s, 2H), 7.52–7.47 (m, 4H), 7.25–7.20 (m, 4H), 7.00–6.96 (m, 2H), 6.77–6.73 (m, 4H), 6.60–6.55 (m, 2H), 3.87 (s, 3H), 3.74 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 198.0, 159.7, 159.7, 158.9, 141.4, 140.9, 136.7, 133.0, 132.8, 132.2, 131.8, 130.4, 128.4, 127.2, 115.1, 114.5, 113.7, 55.5, 55.3. HRMS (ESI) calculated for $C_{34}H_{29}O_5$ ($M+H^+$): 517.2009; found 217.2011.

Supporting Information Summary

In the supplementary information the following information can be found: extended describtion of the materials and methods, crystallographic details for compounds **13 ba**, **13 cc** and **4 ca**, information on cellular cAMP and RORγ-Gal4 luciferase assays, and detailed description, and results from cytotoxicity and antimicrobial studies. In addition, detailed experimental protocols for synthesis of all compounds with the full analytical characterization, including copies of ^1H and ^{13}C NMR spectra are provided.

Acknowledgements

R. L., Š. M., and L. R. thank the Charles University Primus program, grant number PRIMUS/20/SCI/017, and the Charles University Research Centre program grant number UNCE/SCI/014 for funding. Studies related to cytotoxicity of the compounds were financially supported by a research grant from Çanakkale Onsekiz Mart University (Scientific Research Projects, ID: FIA-2021-3666).

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: antiproliferation · biological activity · drug discovery · medicinal chemistry · natural products

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Submitted: December 12, 2022
Accepted: January 31, 2023

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