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Design, synthesis and biological evaluation of novel riccardiphenol analogs

Srinivas K. Kumar,[a] Maria Amador,[a] Manuel Hidalgo,[a] Sujata V. Bhat[b] and Saeed R. Khan[a,*]

[a]*Division of Chemical Therapeutics, The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21231, USA*
[b]*Department of Chemistry, Indian Institute of Technology Bombay, Mumbai, 400 076 India*

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Abstract—A novel, facile, high yield, and less cumbersome synthesis of riccardiphenol analogs is described. The synthesized compounds were characterized and assessed for its in vitro activity in a panel of human cancer cell lines of differing origin: HuCCT-1, BxPC3, Panc-1, Mia-Paca, A431, Hep2, and HN006. HuCCT-1 was derived from an intrahepatic cholangiocarcinoma; BxPC3, Mia-Paca, and Panc-1 were derived from pancreatic cancers; A431 was derived from a vulvar epithelial carcinoma; and Hep2 and HN006 were derived from squamous cell carcinomas of the head and neck. The cytotoxicity of a newly developed riccardiphenol analog against human cancer cell lines was assessed. The cancer cells exhibited varying sensitivities to the compound, with IC_{50} values from 30 to 50 μM. This susceptibility was particularly interesting in the case of lines such as Hep2 and BxPC3 that are resistant to classic cytotoxic drugs as well as some targeted agents. These results demonstrate that the novel riccardiphenol analog has effective action against human-derived cancer cell in vitro.

1. Introduction

In the course of their phytochemical study of bryophytes,[1–3] Toyota and Asakawa[4] found that liverworts, including the Jungermanniales, are rich sources of terpenoids with a variety of carbon skeletons. These Riccardiaceae, belonging to the order Metzgeriales, occasionally contain aromatic compounds. The compounds isolated from liverworts show very interesting biological activities, including anti-fungal, anti-microbial, and anti-tumor activity, as well as the ability to inhibit 5-lipoxygenase, calmodulin, and plant growth.

From a Japanese collection of *Riccardia crassa*, Toyota and Asakawa[4] isolated riccardiphenols A (**1**) and B (**2**), which contain a sesquiterpene portion attached to a quinol ring, and later Perry et al.[5] isolated riccardiphenol C (**3**) from a New Zealand collection of *R. crassa* (Chart 1).

Riccardiphenols, which have a *seco*-eudesmane skeleton attached to phenol, have been shown to have both cytotoxic and anti-bacterial activity. Though such sesquiterpene–quinone and sesquiterpene–quinol skeletons are rare in terrestrial plants, they are common in marine sources[6] and include zonarol,[7] avarol,[8] and ilimaquinones.[6] Tori et al.[9] have reported total synthesis of riccardiphenols A (**1**) and B (**2**) and have elucidated their absolute configuration.[10,11] In this approach, the *seco*-eudesmane skeleton was synthesized first, and then the benzylic component was attached.

Here we report a novel approach to synthesize riccardiphenol B analogs and have tested the cytotoxic activity of one of these analogs against a variety of cancer cell lines.

Conjugated dienes are versatile building blocks in the synthesis of organic natural products, especially as a component of the Diels–Alder reaction in the synthesis of six-membered cyclic compounds. 3-Methyl-3-sulfolene,[12,13] the isoprene–sulfur dioxide adduct, and its derivatives are attractive as masked diene synthons, since they generate dienes readily by thermal desulfonylation under relatively mild conditions.[14,15] The terminal CH bonds of the original dienes are activated by the adjacent

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* Corresponding author. Tel.: +1 410 614 0200; fax: +1 410 614 8397; e-mail: khansa@jhmi.edu

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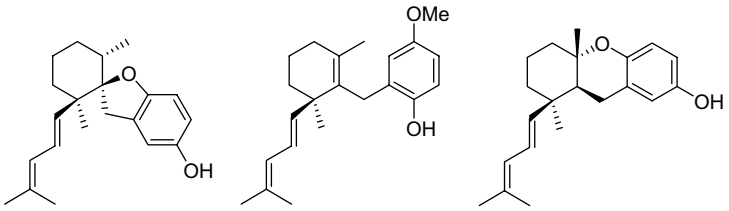


Chart 1. Structures of riccardiphenols.

sulfonyl group,[16] suggesting the possibility of modifying the terminal position of the dienes.

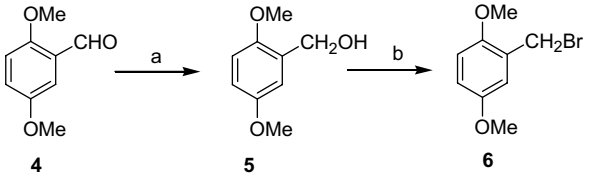
Thus, by introducing the appropriate electrophile in 3-methyl-3-sulfolene, the required terminally substituted conjugated dienes can be generated. We decided to use this strategy for the construction of the substituted six-membered ring in the synthesis of riccardiphenol B. Since sulfolenes react only with alkyl iodides or alkyl bromides and not with alkyl chlorides,[13] we had to start with benzyl bromide or iodide.

2. Results and discussion

2.1. Chemistry

In our approach (Scheme 3) 3-methyl-3-sufolene was first alkylated with the benzylic bromides **6** and **10** to give 2-benzylsulfone **12**. Diels–Alder reaction of the diene **13** generated from the thermolysis of sulfolene **12** with acrolein gave benzylic-substituted six-membered ring **14**. This, upon methylation and a Wittig reaction with isoprenyl bromide gave the skeleton of riccardiphenol **2**.

2,5-Dimethoxybenzyl bromide **6** was easily prepared from 2,5-dimethoxybenzaldehyde **4** as the starting material (Scheme 1). Aldehyde **4**, on reduction with $NaBH_4$ in methanol, gave the alcohol **5**. The alcohol **5**, upon treatment with aqueous hydrobromic acid, gave 2,5-dimethoxybenzyl bromide **6**.

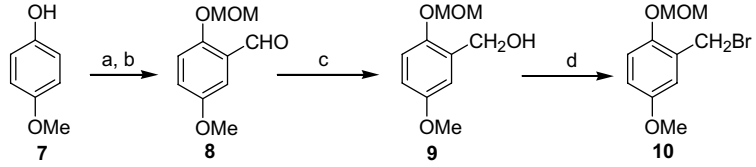


Scheme 1. Reagents: (a) $NaBH_4$, MeOH; (b) aq HBr.

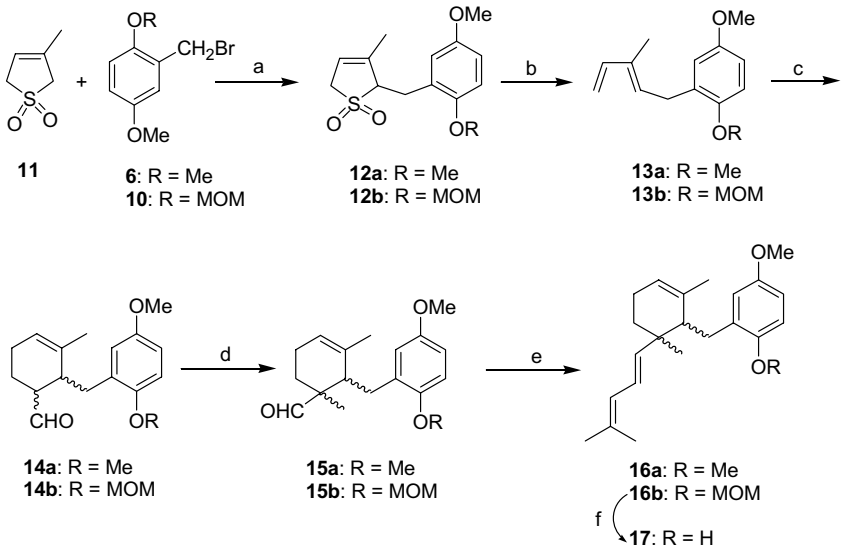
Benzyl bromide **10** is highly unstable, and its preparation by treating the corresponding alcohol **9** with PPh_3 in $CCBr_4$ resulted in a very poor yield (9%). Therefore, we followed Corey's procedure[17] for preparing benzyl bromides from benzyl alcohol, followed by immediate reaction with 3-methyl-3-sulfolene. Benzyl bromide **10** was synthesized from *p*-hydroxy anisole as shown in Scheme 2. The phenolic hydroxyl group was protected as a MOM derivative, followed by ortholithiation and formylation with BuLi and DMF, to give compound **8**.[18,19] Compound **8**, on further reduction with $NaBH_4$, gave the benzyl alcohol **9**.

Benzyl alcohol **9**, when treated with N-bromosuccinamide in the presence of dimethyl sulfide in dry dichloromethane at 10 °C, gave the benzyl bromide **10**.[17] Almost all reports dealing with deprotonation/substitution reactions of 3-sulfolenes describe the use of HMPA as cosolvent when using *n*-BuLi or LiHMDS as the base. Under these conditions, the sulfolene carbanion is thought to be stabilized by the cation-trapping action of the HMPA. Chou and co-workers[12] have reported the alkylation of 3-methyl-3-sulfolene (2.5 equiv) with geranyl bromide (1 equiv) in the presence of HMPA (4 equiv) at −78 °C while using LiHMDS (1 equiv) as the base.

Following the above procedure, the 3-methyl-3-sulfolene was alkylated with substituted benzyl bromide. 3-Methyl-3-sulfolene was prepared by heating a methanolic solution of isoprene and liquid SO_2 at 80 °C for 4 h in a sealed tube. A THF solution of 3-methyl-3-sulfolene (1 equiv), benzyl bromide (2 equiv) **10**, and HMPA (2 equiv) was cooled to −98 °C (MeOH/liq. N_2), and a THF solution of LiHMDS was added at the same temperature. The reaction mixture was allowed to warm to 0 °C over 30 min and was then quenched with saturated aqueous NH_4Cl solution. A standard work-up, followed by silica gel chromatographic (SGC) purification, gave a colorless, viscous liquid, which was characterized as 2-(2-methoxymethoxy-5-methoxybenzyl)-3-methyl-3-sulfolene **12b** from spectral data. The ^1H NMR spectrum of this compound displayed a broad singlet at δ 5.7 due to the olefinic proton



Scheme 2. Reagents: (a) NaH, MOMCl, diethylether; (b) BuLi, DMF, diethylether; (c) $NaBH_4$, MeOH; (d) Me_2S, NBS, DCM.



Scheme 3. Reagents: (a) LiHMDS, HMPA, THF; (b) pyridine, reflux; (c) sealed tube, toluene; (d) NaH, MeI, THF; (e) prenyl phosphonate; (f) HCl, THF.

and four distinct singlets at δ 5.16 (2H), 3.76 (3H), 3.48 (3H), and 1.71 (3H), corresponding to the methylene of the MOM group, the methoxy group attached to the aromatic ring, the methoxy group of MOM, and the methyl group of the sulfolene nucleus, respectively. A triplet and multiplet appeared at δ 3.92 (1H) and 3.74 (2H), respectively, due to the methine and methylene of the sulfolene moiety, and three aromatic protons in the region of δ 6.9 supported the formation of adduct **12b**.

Takayama and co-workers[13] have usually opened sulfolenes by heating them in 95% ethanol in the presence of $NaHCO_3$ in a sealed tube at 125 °C to obtain quantitative yields of the corresponding dienes. A much cleaner and milder condition for the extrusion of SO_2 was reported by Bhat and co-workers[15] by refluxing sulfolene in pyridine at 80 °C. This method provided an optimal reaction temperature, and the SO_2 liberated was expelled and could be monitored very easily, unlike that in a sealed tube.

The adduct **12b** was subjected to thermolysis by refluxing in pyridine gave compound **13b**. The ^{1}H NMR spectrum showed typical double doublets at δ 6.39 (1H) (J = 17.39 and J = 10.8 Hz), due to the H-2 olefinic proton, as well as a triplet at δ 5.64 (1H) (J = 6.96 Hz), which was assigned to the H-4 of the olefin. Two one-proton doublets at δ 5.1 (J = 17.39 Hz) and 4.96 (J = 10.8 Hz) were ascribed to *trans*- and *cis*-protons at C-1, and the appearance of three aromatic protons in the region δ 7.01–6.68 indicated the formation of conjugated diene **13b**.

The use of a Diels–Alder reaction to construct an appropriately functionalized cyclohexyl system in a concise manner is especially attractive, in light of the fact that the ease of cycloaddition of the diene and dienophile, the rapid accumulation of polyfunctionality in a relatively small molecular framework, the extraordinary stereochemical control of the cycloaddition, and the predictability of the regiochemistry.

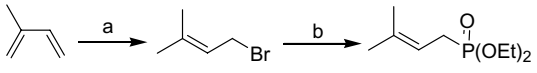
In the Diels–Alder reaction, the addition of 1-substituted isoprenes to the dienophilic carbon–carbon bond is asymmetrical, leading to the possible formation at least two regioisomers. However, the *ortho* rule predicts that the isomer in which the carbonyl group of the dienophile and the C-1 substituent on the diene are *ortho* to each other would be preferentially formed over the *meta* adduct. The Diels–Alder reaction was carried out between the diene **13b** and acrolein as the dienophile, with a mixture of the diene **13b** and acrolein in toluene being heated in the presence of hydroquinone in a sealed tube at 90 °C for 2 h. TLC analysis of the reaction mixture showed the complete disappearance of the diene and the formation of a more polar product. Removal of the toluene, followed by chromatographic purification of the residue, yielded the adduct **14b**. ^{1}H NMR spectrum showed an aldehydic proton at δ 9.36; multiplets at δ 2.91 (2H), 2.46 (2H), and 1.8–2.2 (2H); and a broad singlet at δ 5.41 (1H), due to seven protons of the cyclohexene ring, as well as four distinct singlets at δ 5.14 (2H), 3.74 (3H), 3.48 (3H), and 1.71 (3H) corresponding to the methylene of the MOM group, the methoxy group attached to the aromatic ring, the methoxy group of MOM, and the methyl group of the cyclohexene nucleus, respectively; double doublets at δ 2.79 (1H) (J = 13.54 and 5.4 Hz) and 2.6 (1H) (J = 13.54 and 8.42 Hz), corresponding to benzylic protons and three aromatic protons in the region δ 6.82–7.01 (3H), indicated structure **14b**.

The compound **14b** was methylated using methyl iodide and NaH in dry THF. To the slurry of NaH in dry THF was added compound **14b** and methyl iodide at 0 °C; the reaction mixture was stirred for 4 h and quenched with aqueous NH_4Cl solution. The usual work-up and SGC purification yielded **15b** as a colorless, viscous liquid. The structure of **15b** was indicated from the ^{1}H NMR

spectrum. It displayed an absence of a multiplet at δ 2.91 (1H), corresponding to the proton α to the aldehydic group, and the appearance of a methyl group singlet at δ 1.01 (3H). The structure of compound **15b** was further confirmed from its MS analysis, which displayed a molecular ion peak at *m/z* 318.

As is well known, in the Wittig reaction an aldehyde or ketone is treated with phosphorane ylide (also called a phosphorane) to give an olefin and phosphine oxide. The Horner–Emmons modification involves the use of other ylides, the most important being prepared from phosphonates; this modification has several advantages over the use of phosphoranes: these ylides are more reactive than the corresponding phosphoranes, and the compounds often react with ketones that are inert to phosphoranes. In addition, the product is a water-soluble phosphate ester, unlike Ph_3PO, which makes it easy to separate it from the olefin product. Moreover, phosphonates are less expensive than the phosphonium salt and can be easily prepared by the Arbuzov reaction.

Initially, the reaction of aldehyde **15b** with the Wittig salt of isoprenyl bromide using NaH/BuLi as the base gave back the starting material: the reaction did not proceed, probably because of the bulkiness of the tri-phenyl phosphine groups of the Wittig salt. Hence, the Horner–Emmons method was used. First, the prenyl phosphonate **20** was prepared by refluxing prenyl bromide **19** with triethyl phosphite in toluene for 12 h[20] (Scheme 4). The required prenyl bromide was obtained from isoprene using the reported procedure.[21] The reaction of phosphonate **20** with the LDA in THF at −23 °C gave the corresponding ylide, which was treated with aldehyde **15b** to give the required product **16b** in 17% yield. The ^{1}H NMR spectrum also displayed the absence of an aldehydic proton and showed the presence of six distinct singlets at δ 5.12 (2H), 3.75 (3H), 3.48 (3H), 1.8 (3H), 1.75 (3H), 1.74 (3H), and 0.98 (3H), corresponding to the methylene of MOM, the methoxy group attached to the aromatic ring, the methoxy group of MOM, the methyl group attached to the olefin of the cyclohexene ring, the *gem*-dimethyls and the methyl group attached to the cyclohexene nucleus, respectively. Double doublets at δ 6.15 (J = 15.7 and 10.2 Hz) due to the proton at C-2, and two doublets at δ 5.75 (J = 10.25 Hz) and 5.53 (J = 15.7 Hz) due to C-1 and C-3 of the 1,3-pentadienyl unit indicated the formation of the olefin in the *trans* confirmation. Two double doublets of the benzylic proton at δ 2.77 (J = 13.5 and 5.5 Hz) and 2.38 (J = 13.5 and 5.5 Hz) and multiplets at δ 6.85–7.02 due to aromatic protons indicated the structure of compound **16b**. The structure of compound **16b** was further supported by the MS analysis, which showed a molecular ion peak at *m/z* 370. Further, the deprotection of MOM with HCl in THF gave compound **17**.



Scheme 4. Reagents: (a) 48% HBr, CuBr; (b) $P(OEt)_3$, toluene.

Compound **16a** was similarly synthesized, and its structure was deduced.

2.2. Biology

A panel of seven different human cancer cell lines from different origins was used for this study. The cell lines included: HuCCT-1 cell line obtained from the Health Science Research Resources Bank (Japan); BxPC3, Panc-1, Mia-Paca, A431, and Hep2 cell lines were purchased to the American Tissue Culture Collection (Manassas, VA), and HN006 was obtained from Dr. David Sidransky's laboratory at Johns Hopkins. Of these, HuCCT-1 was derived from an intrahepatic cholangiocarcinoma; BxPC3, Mia-Paca, and Panc-1 were derived from pancreatic cancers; A431 was derived from a vulvar epithelial carcinoma; and Hep2 and HN006 were derived from squamous cell carcinomas of the head and neck. Cells were grown in monolayer culture in different media (Eagle's minimum essential medium for Mia Paca, Dulbelccos's modified Eagle's medium for A431 and Panc 1; and RPMI 1640 for SNU 308, HuCCT-1, HN006, and BxPC3 cell lines). Media were supplemented with 10% FBS and 50UI Penicillin/Streptomycin. Cell cultures were maintained at 37 °C in an humified atmosphere of 5% CO_2 in air. In all assays, cells were plated for 24 h before drug administration. Stock solutions were prepared for each compound in dimethyl sulfoxide (DMSO) and stored at −20 °C.

2.2.1. Growth inhibition studies. In vitro drug sensitivity was assessed using the tetrazolium (MTT) dye conversion method. The MTT assay is a colorimetric method[22] that quantify viable cells through their ability to reduce a salt, a process that requires active mitochondrial function. The intensity of the dye is proportional to the number of cells. Briefly, cells were trypsinized, seeded at 5×10^3 cells/well in 96-well plate and allowed to grow for 24 h before the treatment with exponential increasing concentrations of the drugs in the presence of 10% FBS. After a 96-h period of treatment, 20 μL of MTT solution (5 mg/mL in PBS) (Sigma, St. Louis, MO) were added to each well, and the plates were then incubated for 3 h at 37 °C. Medium was then replaced with 100 μL of DMSO per well. Plates were shaken and the optical density was measured at 570 nm using a multiwell plate reader (Bio-Rad, Model 550, bio-Rad Inc., Hercules, CA). Each experiment were performed in triplicate for each drug concentration and was carried out independently at least three times. The IC_{50} value was defined as the concentration needed for a 50% reduction in the absorbance calculated based on the survival curves. Response to drug treatment was assessed by standardizing treatment groups to untreated controls.

MTT assay in a panel of seven human-derived cancer cell lines from different origins. Relative growth after exposure to increasing concentrations of compounds. The results indicate a susceptibility of some of the cell lines in our panel to the compound. This finding is especially interesting in the case of some of these cell lines, such as Hep2 and BxPC3, which are known to be resistant to classic cytotoxic drugs and to some targeted

agents. These results suggest that at least one of the compounds discussed in this paper has activity against certain human-derived cancer cell lines. Compounds **16a** and **16b** showed mild anti-tumor activity against these cancer cell lines.

3. Conclusions

A novel, facile, high yield, and less cumbersome synthesis of riccardiphenol analogs, **16a**, **16b**, and **17** is described. The synthesized compounds were characterized and assessed for its in vitro activity in a panel of human cancer cell lines of differing origin. In this study, we demonstrate that one of the leading compound, **17**, significantly inhibits the growth of different human cancer cells (Fig. 1). The fact that some of these cell lines, including Hep2 and BxPC3 are known to be resistant to classic cytotoxic drugs, as well as some targeted agents, makes this sensitivity to the riccardiphenol analog particularly intriguing. These results demonstrate that the novel riccardiphenol analog has effective action against human-derived cancer cell in vitro. Further investigations on structural activity relationship of the diastereomers are underway and will be reported in due course.

4. Experimental section

4.1. Synthesis of (2,5-dimethoxyphenyl)methan-1-ol (5)

2,5-Dimethoxybenzaldehyde (**4**) (7 g, 0.042 mol) in methanol (100 mL) was placed in a round bottom flask and cooled to 0 to −10 °C. To this, sodium borohydride (1.9 g, 0.05 mol) was added in portions, and the reaction mixture was allowed to stir for 3 h at room temperature. The reaction mixture was quenched with water, and the methanol was removed under vacuum. The residual mixture was then extracted with diethyl ether (3 × 50 mL), and the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure and column chromatography of the residue over silica gel (100–200 mesh) using a petroleum ether–EtOAc gradient gave 2,5-dimethoxybenzyl alcohol (**5**) in 95% yield. IR (neat): ν_{max} 3400, 2940, 2900, 1590, 1450 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.85 (3H, m), 4.2 (2H, s), 3.8 (6H, s). Anal. ($C_9H_{12}O_3$) C, H, N. Mass spectrum: *m*/*z* 168 (M^+).

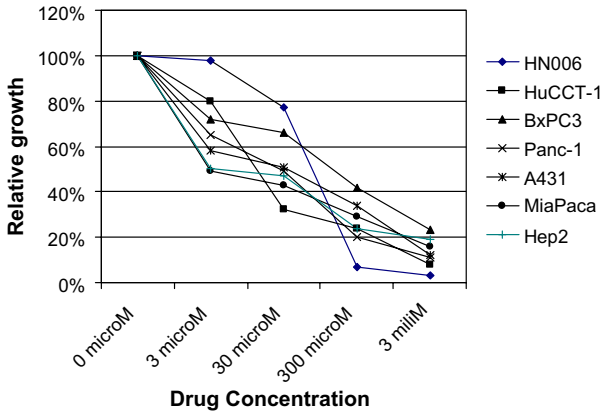


Figure 1. MTT assay in a panel of seven human-derived cancer cell lines from different origins. Relative growth after exposure to increasing concentrations of compound **17**.

4.2. Synthesis of 2-(bromomethyl)-1,4-dimethoxybenzene (6)

Hydrobromic acid (40%, 20 mL) was added to the 2,5-dimethoxybenzylalcohol (**5**) (7 g, 0.042 mol) at 0–5 °C, and the reaction mixture was stirred for 30 min. The reaction mixture was extracted with dichloromethane (3 × 50 mL), and the organic layer was washed with $NaHCO_3$, water, and brine and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure and column chromatography of the residue over silica gel (100–200 mesh) using a petroleum ether–EtOAc gradient gave compound (**6**) in 96% yield. IR (neat): ν_{max} 2940, 2900, 1590, 1450, 1420 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.83 (3H, m), 4.1 (2H, s), 3.8 (6H, s). Anal. ($C_9H_{11}BrO_2$) C, H, N. Mass spectrum: *m*/*z* 230 and 232 (M^+).

4.3. Synthesis of 5-methoxy-2-(methoxymethoxy)benzaldehyde (8)

N-butyl lithium (15%, 4.13 g, 0.06 mol) was added to the 4-methoxymethoxy anisole (5.56 g, 0.034 mol) in dry diethylether (30 mL) under argon at room temperature and stirred for 3 h. Dimethylformide (DMF) (7.30 g, 0.09 mol) was added to this reaction mixture and stirred for a further 30 min. The reaction mixture was quenched with saturated NH_4Cl solution, and the residual mixture was extracted with diethyl ether (3 × 30 mL). The organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure yielded compound (**8**) in 80% yield (crude). IR (neat): ν_{max} 2940, 2900, 2730, 1686, 1607, 1512, 1450 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 10.4 (1H, s), 7.0 (2H, s), 7.2 (1H, s), 5.2 (2H, s), 3.8 (3H, s), 3.4 (3H, s). Anal. ($C_{10}H_{12}O_4$) C, H, N. Mass spectrum: *m*/*z* 196 (M^+).

4.4. Synthesis of (5-methoxy-2-(methoxymethoxy)phenyl)-methan-1-ol (9)

2-Methoxymethoxy-5-methoxybenzaldehyde (**8**) (3 g, 0.015 mol) in methanol (100 mL) was placed in a round bottom flask and cooled to 0 to −10 °C. Sodium borohydride (1 g, 0.03 mol) was added in portions, and the reaction mixture was allowed to stir for 3 h at room temperature. The reaction mixture was quenched with water, and methanol was removed under vacuum. The residual mixture was extracted with diethyl ether (3 × 50 mL), and the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure and column chromatography of the residue over silica gel (100–200 mesh) using a petroleum ether–EtOAc gradient gave compound (**9**) in 95% yield. IR (neat): ν_{max} 3400, 2940, 2900, 1590, 1450 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ

6.83 (3H, m), 5.2 (2H, s), 4.2 (2H, s) 3.8 (3H, s), 3.4 (3H, s). Anal. ($C_{10}H_{14}O_4$) C, H, N. Mass spectrum: *m/z* 198 (M^+).

4.5. Synthesis of 2-(bromomethyl)-4-methoxy-1-(methoxymethoxy)benzene (10)

Dimethyl sulfide (1.9 g, 0.03 mol) was added to a suspension of NBS (4.56 g, 0.025 mol) in dry dichloromethane (10 mL) at 0 °C under argon and stirred for 10 min. The reaction mixture was cooled to −20 °C; alcohol (**9**) (0.017 mol) in dichloromethane (10 mL) was added, and the mixture was further stirred at 0 °C for 4 h. The reaction mixture was poured into ice-cold water (10 mL) and extracted with dichloromethane (3 × 50 mL). The organic layer was washed with saturated $NaHCO_3$, water, and brine and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure gave compound (**10**) in 90% yield. IR (neat): ν_{max} 2938, 2900, 1588, 1450, 1420 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.8 (3H, m), 5.2 (2H, s), 4.1 (2H, s) 3.8 (3H, s), 3.3 (3H, s). Anal. ($C_{10}H_{13}BrO_3$) C, H, N. Mass spectrum: *m/z* 260 and 262 (M^+).

4.6. General procedure for the preparation of sulfones 12a–b

3-Methyl-3-sulfolene (4.5 g, 0.057 mol), HMPA (2 equiv), and benzyl bromide **6/10** (0.038 mol) in dry THF (75 mL) were placed in a three-necked round bottom flask and cooled to −92 °C. To this mixture LiHMDS (0.038 mol) was added and stirred for 25 min at the same temperature. The reaction mixture was quenched with saturated NH_4Cl solution, and THF was removed under vacuum. The residual mixture was extracted with diethyl ether (3 × 30 mL), and the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure and silica gel (100–200 mesh) column chromatography of the residue using a petroleum ether–EtOAc gradient gave compound **12a**–**b**.

4.6.1. 2-((2,5-Dimethoxyphenyl)methyl)-3-methylthiolene-1,1-dione (12a). 73%; IR (neat): ν_{max} 2942, 2900, 1592, 1450, 1420, 1310, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.85 (3H, m), 5.7 (1H, s), 3.94 (3H, s), 3.83 (3H, s) 3.75 (2H, m), 3.1 (3H, m), 1.7 (3H, s). Anal. ($C_{14}H_{18}O_4S$) C, H, S.

4.6.2. 2-((5-Methoxy-2-methoxymethoxyphenyl)methyl)-3-methylthiolene-1,1-dione (12b). 70%; IR (neat): ν_{max} 2942, 2900, 1592, 1450, 1420, 1310, 1130 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.02 (1H, d, J = 8.97 Hz), 6.86 (1H, d, J = 3.11 Hz), 6.74 (1H, q, J = 8.97 and 3.11 Hz), 5.67 (1H, s), 5.16 (2H, s), 3.92 (1H, t), 3.76 (3H, s), 3.74 (2H, m), 3.48 (3H, s), 3.28 (1H, dd, J = 7.14 and 14.28 Hz), 2.92 (1H, dd, J = 6.92 and 14.28 Hz), 1.71 (3H, s). Anal. ($C_{15}H_{20}O_5S$) C, H, S.

4.7. General procedure for the preparation of diene 13a–b

Compound **12a**–**b** (27.3 mmol) in dry pyridine (15 mL) was placed in a round bottom flask and refluxed for 3 h. Pyridine was removed under reduced pressure, and flash chromatography of the residue over silica gel (100–200 mesh) using a petroleum ether–EtOAc gradient gave compound **13a**–**b**.

4.7.1. 1,4-Dimethoxy-2-(3-methylpenta-2,4-dienyl)benzene (13a). 90%; IR (neat): ν_{max} 2950, 2880, 1650, 1610, 1590, 1420 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.76 (3H, m), 6.4 (1H, dd, J = 10.62 and 17.57 Hz), 5.63 (1H, t, J = 7.34 Hz), 5.11 (1H, d, J = 17.57 Hz), 4.95 (1H, d, J = 10.62 Hz), 3.78 (3H, s), 3.75 (3H, s), 3.45 (2H, d, J = 7.35 Hz), 1.83 (3H, s). Anal. ($C_{14}H_{18}O_2$) C, H, N. Mass spectrum: *m/z* 218 (M^+).

4.7.2. 4-Methoxy-1-(methoxymethoxy)-2-(3-methylpenta-2,4-dienyl)benzene (13b). 86%; IR (neat): ν_{max} 2950, 2880, 1650, 1610, 1590, 1420 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.01 (1H, d), 6.68 (2H, m), 6.39 (1H, dd, J = 10.8 and 17.39 Hz), 5.64 (1H, t, J = 6.96 Hz), 5.12 (2H, s), 5.1 (1H, d, J = 17.38 Hz), 4.96 (1H, d, J = 10.8 Hz), 3.75 (3H, s), 3.47 (5H, br s), 1.84 (3H, s). Anal. ($C_{15}H_{20}O_3$) C, H, N. Mass spectrum: *m/z* 248 (M^+).

4.8. General procedure for the preparation of the Diels–Alder adduct 14a–b

The diene **13a**–**b** (9.1 mmol), acrolein (1 g, 17 mmol), and hydroquinone (1 mmol) were taken in toluene (8 mL) was placed in a sealed tube and heated at 90 °C for 2 h. After the reaction, the toluene was removed under vacuum, and silica gel (100–200 mesh) column chromatography of the residual mixture, with petroleum ether and ethyl acetate as eluent, gave compounds **14a**–**b**.

4.8.1. 2-((2,5-Dimethoxyphenyl)methyl)-3-methylcyclohex-3-enecarbaldehyde (14a). 80%; IR (neat): ν_{max} 2940, 2897, 1722, 1592, 1500, 1461 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.46 (1H, s), 6.75 (3H, m), 5.41 (1H, s), 3.76 (3H, s) 3.74 (3H, s), 2.9 (1H, m), 2.78 (1H, dd, J = 13.54 and 5.12 Hz), 2.65 (1H, dd, J = 13.54 and 8.42 Hz), 2.43 (1H, m), 2.0 (5H, m), 1.73 (3H, s). Anal. ($C_{17}H_{22}O_3$) C, H, N.

4.8.2. 2-((5-Methoxy-2-(methoxymethoxy)phenyl)methyl)-3-methylcyclohex-3-enecarbaldehyde (14b). 78%; IR (neat): ν_{max} 2940, 2897, 1722, 1592, 1500, 1461 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.48 (1H, s), 7.01 (1H, d, J = 8.77 Hz), 6.82 (2H, m), 5.41 (1H, s), 5.14 (2H, s), 3.74 (3H, s), 3.48 (3H, s), 2.91 (1H, m), 2.79 (1H, dd, J = 5.4 and 13.54 Hz), 2.66 (1H, dd, J = 8.4 and 13.54 Hz), 2.0 (5H, m), 1.71 (3H, s); Anal. ($C_{18}H_{24}O_4$): C, H, N.

4.9. General procedure for the preparation of compounds 15a–b

NaH (50%, 0.5 g, 10.5 mmol) was placed in a three-neck round bottom flask and washed with dry petroleum ether. Dry THF (30 mL) was added and the mixture cooled to 0 °C. To this was added compound **14a**–**b** (5 mmol) and methyl iodide (1.5 g, 10.5 mmol) in THF

(20 mL), and the reaction mixture was allowed to stir for 4 h at room temperature. The reaction mixture was quenched with saturated NH_4Cl solution, and THF was removed under vacuum, then the residual mixture was extracted with diethyl ether (3×30 mL), and the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure and column chromatography of the residue over silica gel (100–200 mesh) with petroleum ether and ethyl acetate as eluent gave compound **15a–b**.

4.9.1. 2-((2,5-Dimethoxyphenyl)methyl)-1,3-dimethylcyclohex-3-enecarbaldehyde (15a). 50%; IR (neat): ν_{max} 2940, 2895, 2707, 1722, 1592, 1500, 1461 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.43 (1H, s), 6.73 (3H, m), 5.35 (1H, br s), 3.78 (3H, s), 3.74 (3H, s), 2.63 (1H, dd, $J = 14$ and 6.59 Hz), 2.56 (1H, dd, $J = 14$ and 6.77 Hz), 2.39 (1H, t), 2.0 (4H, m), 1.6 (3H, s), 1.0 (3H, s). Anal. ($C_{18}H_{24}O_3$) C, H, N. MS: *m/z* 288 (M^+).

4.9.2. 2-((5-Methoxy-2-(methoxymethoxy)phenyl)methyl)-1,3-dimethylcyclohex-3-enecarbaldehyde (15b). 55%; IR (neat): ν_{max} 2940, 2895, 2707, 1722, 1592, 1500, 1461 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 9.36 (1H, s), 6.98 (1H, d, $J = 8.42$ Hz), 6.65 (2H, m), 5.36 (1H, s), 5.12 (2H, s), 3.75 (3H, s), 3.48 (3H, s), 2.64 (1H, dd, $J = 13.8$ and 6.408 Hz), 2.6 (1H, dd, $J = 13.8$ and 6.591 Hz), 2.41 (1H, m), 2.0 (4H, m), 1.57 (3H, s), 1.02 (3H, s). Anal. ($C_{19}H_{26}O_4$) C, H, N. MS: *m/z* 318 (M^+).

4.10. General procedure for the preparation of compounds 16a–b

LDA (0.20 mmol) was generated in THF at −23 °C, and prenyl phosphite (0.20 mmol), obtained by refluxing prenyl bromide and triethlyphosphite in toluene (5 mL) for 12 h, was added and stirred for 30 min. To this mixture aldehyde **15a–b** (50 mg, 0.17 mmol) was added and stirred for 4 h. The reaction mixture was quenched with saturated NH_4Cl, and THF was removed under vacuum. The residual mixture was extracted with diethyl ether (3×20 mL), and the organic layer was washed with water and brine and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure and column chromatography of the residue over silica gel (100–200 mesh) with petroleum ether and ethyl acetate as eluent gave compound **16a–b**.

4.10.1. 2-((2,6-Dimethyl-6-(4-methylpenta-1,3-dienyl)cyclohex-2-enyl)methyl)-1,4-dimethoxybenzene (16a). 40%; IR (neat): ν_{max} 2940, 2895, 1670, 1620, 1510 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.68 (3H, m), 6.15 (1H, dd, $J = 15.7$ and 10.2 Hz), 5.7 (1H, d, $J = 10.25$ Hz), 5.55 (1H, d, $J = 15.7$ Hz), 5.28 (1H, br s), 3.75 (3H, s), 3.73 (3H, s), 2.38 (1H, dd, $J = 13.5$ and 5.5 Hz), 2.77 (1H, dd, $J = 13.5$ and 7.14 Hz), 2.07 (1H, m), 1.8 (3H, s), 1.74 (3H, s), 1.75 (3H, s), 0.97 (3H, s); ^{13}C NMR ($CDCl_3$): δ 19.4, 21.3, 23, 23.5, 24.4, 25.4, 37.5, 41.0, 51.8, 56.0, 56.3, 112.3, 114.9, 114.5, 122.1, 124.3, 126.0, 135.1, 141.1, 142.3, 154.1, 154.4. Anal. ($C_{23}H_{32}O_2$) C, H, N. MS: *m/z* 340 (M^+).

4.10.2. 2-((2,6-Dimethyl-6-(4-methylpenta-1,3-dienyl)cyclohex-2-enyl)methyl)-4-methoxymethoxybenzene (16b). 19%; IR (neat): ν_{max} 2940, 2895, 1670, 1620, 1510 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 6.75 (3H, m), 6.15 (1H, dd, $J = 15.7$ and 10.2 Hz), 5.75 (1H, d, $J = 10.25$ Hz), 5.53 (1H, d, $J = 15.7$ Hz), 5.4 (1H, s), 5.1 (2H, s), 3.7 (3H, s), 3.4 (3H, s), 2.38 (1H, dd, $J = 13.5$ and 5.5 Hz), 2.77 (1H, dd, $J = 13.5$ and 7.23 Hz), 2.07 (1H, m), 1.8 (3H, s), 1.74 (3H, s), 1.75 (3H, s), 0.98 (3H, s); ^{13}C NMR ($CDCl_3$): δ 19.5, 21.2, 23.1, 23.5, 23.9, 25.8, 37.2, 41.6, 51.8, 56.0, 58.3, 112.3, 114.9, 114.5, 122.1, 124.3, 126.2, 135.3, 141.1, 141.7, 154.1, 154.4, 171.2. Anal. ($C_{24}H_{34}O_3$) C, H, N. MS: *m/z* 370 (M^+).

4.11. 2-[2,6-Dimethyl-6-(4-methyl-penta-1,3-dienyl)-cyclohex-2-enylmethyl]-4-methoxy-phenol (17)

Compound **16b** (25 mg; 67 μM) was treated with THF:3 M HCl = 1:1 (3 mL) at 50 °C for 3 h. The solvent was evaporated, and the residue was extracted with ether. The organic phase was washed with water, dried ($NaSO_4$), and evaporated. The residue was purified by HPLC to give **17**(8 mg) (yield; 30%). ^1H NMR (400 MHz, $CDCl_3$): δ 6.57 (1H, d, $J = 8$ Hz), 6.46 (1H, s), 6.42 (1H, d, $J = 8$ Hz), 6.05 (1H, dd, $J = 15.7$ and 10.2 Hz), 5.72 (1H, d, $J = 10.25$ Hz), 5.51 (1H, d, $J = 15.7$ Hz), 5.38 (1H, s), 3.73 (3H, s), 2.36 (1H, dd, $J = 13.5$ and 5.5 Hz), 2.75 (1H, dd, $J = 13.5$ and 7.23 Hz), 2.06 (1H, m), 1.82 (3H, s), 1.73 (3H, s), 1.75 (3H, s), 0.98 (3H, s). Anal. ($C_{22}H_{30}O_2$) C, H, N. MS: *m/z* 326 (M^+).

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