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One-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one and evaluation of acetal derivatives as antibacterial and antifungal agents

Abstract: A one-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one (lactol) **3** by oxidation of indene using tungstic acid-hydrogen peroxide is reported. The synthesized lactol was converted into acetal derivatives **5a–h**, which were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes* (Gram-positive), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative) strains, and antifungal activity against *Candida albicans* and *Aspergillus niger* by broth dilution method. The compounds show potent activity against *S. aureus* in comparison with ampicillin and exhibit modest antifungal activity.

Keywords: acetals; antibacterial; antifungal; 3,4-dihydro-3-hydroxyisochroman-1-one; lactol.

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Introduction

The lactol scaffold is a core structural unit in various biologically active compounds such as callipeltoside A [1], ginkgolides [2, 3], dysidiolide [4], cladocorans A and B [5], acuminolide [6], spongianolide A [7], manoalide [8], cacospongionolide B₂ [9], and peniolactol [10]. Lactols are also employed as important synthetic precursors to several compounds such as illudalic acid [11], benzopyran-1-ones [12], caronaldehyde [13], mevalonate, and mevaldate [14].

The lactol unit has been assembled *via* different routes such as photosensitized oxygenation of furan, oxidative cleavage of olefins or diols, followed by cyclization using

various reagents like sodium metaperiodate [11], aqueous potassium hydroxide [13], trifluoroacetic acid [14], and ozonolysis [15] in several steps. Schöpf and Kühne [16] have been the first to report the synthesis of lactol **3** in four steps. Abe and colleagues [17–19] have also synthesized lactol **3** by photooxygenation in three steps from indene, and its crystal structure has been subsequently reported by Valente et al. [20].

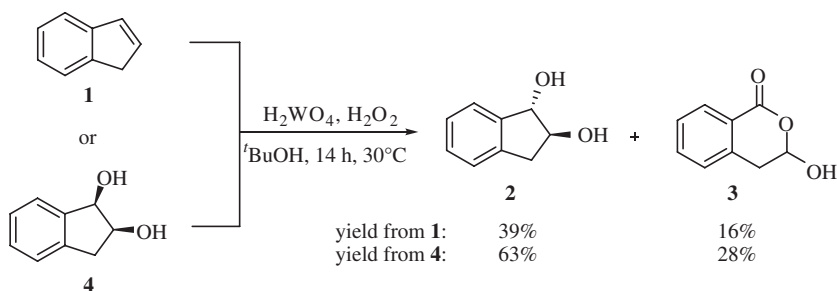
Earlier, our group has demonstrated the synthetic utility of tungstic acid-hydrogen peroxide in *t*-butanol to furnish the corresponding *trans*-diol of (*Z,Z*)-cycloocta-1,5-diene in 65% yield [21]. The treatment of *endo*-dicyclopentadiene with this reagent combination in *t*-butanol unexpectedly resulted in the formation of polycyclic oxetanes [22]. Our continued interest in the dihydroxylation of olefins prompted us to explore the reaction of indene (**1**) with a tungstic acid-hydrogen peroxide mixture in order to prepare its *trans*-diol, which is an important intermediate for indinavir (Crixivan), an HIV protease inhibitor [23]. However, this reaction resulted in the formation of not only *racemic* indan-*trans*-1,2-diol **2** but also *racemic* lactol **3** (Scheme 1). This serendipitous formation of lactol encouraged us to further investigate this reaction.

Results and discussion

Because certain 4- and 5-oxoacids exist as cyclic lactols or pseudoacids [20], it is clear that **3** is formed as a result of cleavage of indene followed by *in situ* cyclization of the intermediate 2-carboxyphenylethanal. To ascertain the source of the lactol **3**, the experiments were designed as follows. Initially, it was thought that indan-*trans*-1,2-diol **2** could be oxidized to **3** *via* intermediacy of 2-carboxyphenylethanal, but the treatment of **2** with tungstic acid-hydrogen peroxide at 30°C in *t*-butanol left it unchanged even after 14 h. It was then contemplated that the *cis*-diol of indene could be the source of **3**. Thus, indan-*cis*-1,2-diol **4** was prepared by a reported method [24] and treated with tungstic acid-hydrogen peroxide. This reaction gave

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Scheme 1

a mixture of lactol **3** and *trans*-diol **2** in 28% and 63% yields, respectively (Scheme 1). A similar conversion of **4** to **2** in the presence of nickel at 60°C has been reported [25]. It should be noted that the present method (Scheme 1) furnishes **2** from **4** in better yield (63%) under milder conditions.

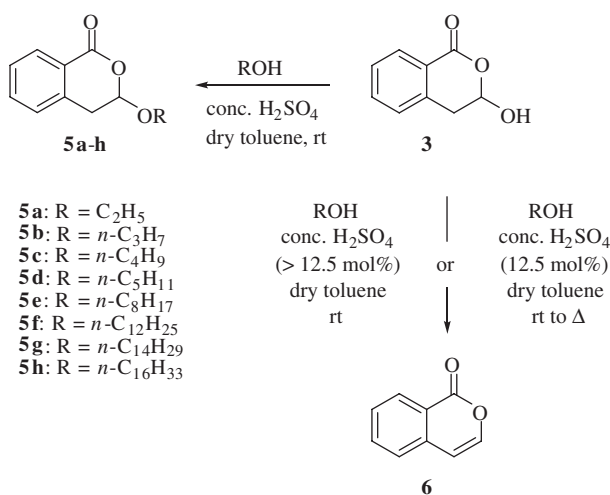
These observations also implied that **4** could be transformed into lactol **3** via oxidative cleavage followed by cyclization under the reaction conditions. A similar cleavage of **2** does not take place during the reaction, perhaps due to the *trans* geometry of the hydroxyl groups. To the best of our knowledge, this one-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one **3** from indene **1** under such mild conditions is described here for the first time.

Lactol derivatives show a wide variety of biological activities [4–11]. These interesting biological properties prompted us to prepare the acetal derivatives **5a–h** (Scheme 2) and examine their antimicrobial activity. The cyclic lactols are known to coexist with open-chain structures having free aldehyde functionality that can react with alcohols to form acetals [20]. Various acetal

derivatives of lactol **5a–h** were synthesized to examine the effect of spacer groups on their biological potency. The catalyst quantity and the reaction temperature play an important role in the formation of acetal derivatives. It was observed that lactol **3** underwent dehydration to **6** by increasing either the temperature or the catalyst quantity (>12.5 mol%) (Scheme 2).

All synthesized compounds (**2**, **3**, and **5a–h**) were tested for their *in vitro* antimicrobial activities. The minimum inhibitory concentration (MIC) of the compounds were recorded against two Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*), two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and fungi (*Candida albicans* and *Aspergillus niger*) by broth dilution method [26, 27]. Standard antibacterial drug ampicillin and antifungal drug griseofulvin were also screened under identical conditions for comparison. Individual MIC values of all the compounds and for the standards are listed in Table 1. The results reveal that all compounds show significant antibacterial activity. All compounds show good activity against *S. aureus* (Gram-positive bacteria). Derivatives **5d** and **5e** are potent against *E. coli* (MIC=62.5 $\mu\text{g/mL}$) and *P. aeruginosa* (MIC=62.5 $\mu\text{g/mL}$), respectively.

The antifungal screening data show that a few compounds are potent against fungal strains. Compounds **2**, **5d**, and **5e** are equipotent against *C. albicans* in comparison with griseofulvin.



Scheme 2

Conclusion

A one-pot synthesis of 3,4-dihydro-3-hydroxyisochroman-1-one (lactol) **3** from indene **1** is reported along with its probable route of formation. Various acetal derivatives **5a–h** were prepared and tested for biological activity. All products exhibit moderate to good antibacterial and antifungal activity.

Table 1 Results of antibacterial and antifungal screening of compounds **2**, **3**, and **5a–h**.

Compound	MIC (μg/mL)					
	<i>E. coli</i> MTCC 443	<i>P. aeruginosa</i> MTCC 1688	<i>S. aureus</i> MTCC 96	<i>S. pyogenes</i> MTCC 442	<i>C. albicans</i> MTCC 227	<i>A. niger</i> MTCC 282
2	200	500	200	250	500	>1000
3	125	250	100	100	>1000	500
5a	250	200	200	200	1000	>1000
5b	200	250	100	100	1000	1000
5c	200	250	200	200	1000	>1000
5d	62.5	100	200	200	500	>1000
5e	200	62.5	250	250	500	500
5f	200	200	250	250	>1000	200
5g	250	250	100	100	>1000	250
5h	250	200	250	250	>1000	1000
Ampicillin	100	100	250	100	–	–
Griseofulvin	–	–	–	–	500	100

MICs of ampicillin and griseofulvin are used as standards for comparison and hence are in bold.

Experimental

IR spectra were obtained using KBr pellets on a Shimadzu 8400S FTIR spectrometer. ^1H NMR spectra (400 MHz) and ^{13}C NMR spectra (100 MHz) were determined on a Bruker-400 FT NMR spectrometer in CDCl_3 . Mass spectra were obtained on a Shimadzu QP-5050 mass spectrometer. Column chromatography was carried out using Acme's silica gel (60–120 mesh size) and eluting with light petroleum and ethyl acetate mixtures. Thin-layer chromatography (TLC) was performed using Acme's silica gel for TLC and spots were visualized with the iodine vapor.

Synthesis of indan-*trans*-1,2-diol (**2**) and 3,4-dihydro-3-hydroxyisochroman-1-one (**3**)

To a stirred solution of indene (5 mL, 43 mmol) in *t*-butanol (25 mL) was added a suspension of tungstic acid (0.5 g) and hydrogen peroxide (30%, 140 mmol). The mixture was stirred for 14 h at room temperature ($\sim 30^\circ\text{C}$). After completion of the reaction, as monitored by TLC, the mixture was filtered through a celite pad to remove the suspended catalyst. The filtrate was diluted with water (20 mL) and extracted with ethyl acetate (4×25 mL). The combined organic extracts were washed with water (15 mL), brine (10 mL), and dried over anhydrous sodium sulfate. Removal of solvent and column chromatography of the residue furnished the indan-*trans*-1,2-diol (**2**, 2.10 g, 39%) and 3,4-dihydro-3-hydroxyisochroman-1-one (**3**, 0.90 g, 16%). Prolonged reaction periods did not appreciably alter the product ratio.

2,3-Dihydro-1H-indan-*trans*-1,2-diol (2**)** White crystalline solid; mp $156\text{--}160^\circ\text{C}$; IR (ν_{max} , cm^{-1}): 3226, 2911, 2849, 1559, 1477, 1458, 1354, 1057, 746, 645; ^1H NMR: δ_{H} 2.74 (dd, 1H, CH_2 geminal, $J_1 = 15.5$ Hz, $J_2 = 7.5$ Hz), 3.18 (dd, 1H, CH_2 geminal, $J_1 = 15.5$ Hz, $J_2 = 7.5$ Hz), 4.25 (m, 1H, CH-OH), 5.04 (d, 1H, Ar-CH-OH , $J = 5.6$ Hz), 4.88 and 4.86 (s, 2H, OH, both exchange with D_2O), 7.32 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 37.7, 80.6, 81.1, 123.9, 124.3, 126.3, 127.4, 138.9, 143.1. MS (EI): m/z 150 (M^+). Anal. Calcd for $\text{C}_9\text{H}_{10}\text{O}_2$: C, 71.98; H, 6.71. Found: C, 71.80; H, 6.74.

3,4-Dihydro-3-hydroxyisochroman-1-one (3**)** White crystalline solid; mp 95°C ; IR (ν_{max} , cm^{-1}): 3276, 2923, 1702, 1603, 1439, 1393, 1133, 1067, 735. ^1H NMR: δ_{H} 1.70 (broad s, 1H, OH, exchanges with D_2O), 3.15 (dd, 1H, CH_2 , $J_1 = 16.6$ Hz, $J_2 = 4.6$ Hz), 3.35 (dd, 1H, CH_2 , $J_1 = 16.6$ Hz, $J_2 = 4.6$ Hz), 5.95 (t, 1H, CH-OH , $J = 4.8$ Hz), 7.54 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 34.2, 96.2, 124.7, 127.9, 128.6, 130.2, 134.5, 136.6, 165.5; MS (EI): m/z 164 (M^+). Anal. Calcd for $\text{C}_9\text{H}_8\text{O}_3$: C, 65.85; H, 4.91. Found: C, 65.78; H, 4.84.

Synthesis of indan-*trans*-1,2-diol (**2**) and 3,4-dihydro-3-hydroxyisochroman-1-one (**3**) from *cis*-diol (**4**)

The reaction was conducted and the products isolated as described above. Column chromatography furnished the indan-*trans*-1,2-diol (**2**, 0.63 g, 63%) and 3,4-dihydro-3-hydroxyisochroman-1-one (**3**, 0.31 g, 28%).

Synthesis of acetal derivatives **5a–h**

To a mixture of the lactol **3** (3.05 mmol) and an alcohol (3.65 mmol) in dry toluene (20 mL) was added concentrated H_2SO_4 (0.4 mL) at room temperature under stirring for 5 h. After completion of the reaction (TLC), the solvent was removed and the mixture was neutralized with saturated solution of sodium bicarbonate and extracted with ethyl acetate. The combined organic extracts were washed with water, brine, and dried over anhydrous sodium sulfate. Removal of solvent followed by chromatography of the residue furnished **5a–h**.

3-Ethoxy-3,4-dihydroisochroman-1-one (5a**)** Light yellow liquid; yield 72%; IR (ν_{max} , cm^{-1}): 2946, 2912, 2834, 1729, 1628, 1464, 1310, 1274, 1104, 1092, 738; ^1H NMR: δ_{H} 1.20 (t, 3H, OCH_2CH_3 , $J = 6.9$ Hz), 3.15 (dd, 1H, CH_2 , $J_1 = 16.2$ Hz, $J_2 = 4.8$ Hz), 3.35 (dd, 1H, CH_2 , $J_1 = 16.2$ Hz, $J_2 = 4.8$ Hz), 3.70 (m, 1H, OCH_2CH_3), 3.98 (m, 1H, OCH_2CH_3), 5.55 (t, 1H, CH-OR , $J = 4$ Hz), 7.52 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 15.3, 33.7, 65.3, 101.1, 125.0, 127.7, 128.3, 129.9, 134.1, 136.6, 164.2; MS (EI): m/z 192 (M^+). Anal. Calcd for $\text{C}_{11}\text{H}_{12}\text{O}_3$: C, 68.74; H, 6.29. Found: C, 68.76; H, 6.23.

3,4-Dihydro-3-propoxyisochroman-1-one (5b) Light yellow liquid; yield 78%; IR (ν_{\max} , cm^{-1}): 2964, 2933, 2879, 1728, 1610, 1462, 1379, 1271, 1016, 910, 731; ^1H NMR: δ_{H} 0.88 (t, 3H, $\text{OCH}_2\text{CH}_2\text{CH}_3$, $J = 7.2$ Hz), 1.60 (m, 2H, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 3.12 (dd, 1H, CH_2 , $J_1 = 16.3$ Hz, $J_2 = 4.4$ Hz), 3.31 (dd, 1H, CH_2 , $J_1 = 16.3$ Hz, $J_2 = 4.4$ Hz), 3.59 (m, 1H, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 3.91 (m, 1H, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 5.57 (t, 1H, $\text{CH}-\text{OCH}_2\text{CH}_2\text{CH}_3$, $J = 4.4$ Hz), 7.54 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 15.3, 22.6, 33.4, 71.1, 101.0, 124.9, 127.5, 128.1, 129.9, 133.9, 136.5, 164.2; MS (EI): m/z 206 (M^+). Anal. Calcd for $\text{C}_{12}\text{H}_{14}\text{O}_3$: C, 69.88; H, 6.84. Found: C, 69.79; H, 6.88.

3-Butoxy-3,4-dihydroisochroman-1-one (5c) Light yellow liquid; yield 74%; IR (ν_{\max} , cm^{-1}): 2958, 2935, 2874, 1728, 1610, 1460, 1379, 1271, 1128, 1053, 732, 692; ^1H NMR: δ_{H} 0.89 (t, 3H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$, $J = 7.2$ Hz), 1.30 (m, 2H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 1.60 (m, 2H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3.11 (dd, 1H, CH_2 , $J_1 = 18.2$ Hz, $J_2 = 4.4$ Hz), 3.30 (dd, 1H, CH_2 , $J_1 = 18.2$ Hz, $J_2 = 4.4$ Hz), 3.62 (m, 1H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 3.97 (m, 1H, $\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$), 5.56 (t, 1H, $\text{CH}-\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3$, $J = 4.1$ Hz), 7.52 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 13.7, 19.0, 31.3, 33.4, 69.3, 101.0, 124.9, 127.5, 128.1, 129.9, 133.9, 136.4, 164.2; MS (EI): m/z 220 (M^+). Anal. Calcd for $\text{C}_{13}\text{H}_{16}\text{O}_3$: C, 70.89; H, 7.32. Found: C, 70.81; H, 7.26.

3,4-Dihydro-3-(pentyloxy)isochroman-1-one (5d) Light yellow liquid; yield 71%; IR (ν_{\max} , cm^{-1}): 2956, 2933, 2872, 1732, 1620, 1460, 1383, 1271, 1136, 1072, 732; ^1H NMR: δ_{H} 0.86 (t, 3H, $\text{OCH}_2(\text{CH}_2)_3\text{CH}_3$, $J = 6.4$ Hz), 1.28 (m, 4H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_2\text{CH}_3$), 1.56 (m, 2H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_2\text{CH}_3$), 3.10 (dd, 1H, CH_2 , $J_1 = 17.4$ Hz, $J_2 = 4.4$ Hz), 3.29 (dd, 1H, CH_2 , $J_1 = 17.4$ Hz, $J_2 = 4.4$ Hz), 3.60 (m, 1H, $\text{OCH}_2(\text{CH}_2)_3\text{CH}_3$), 3.94 (m, 1H, $\text{OCH}_2(\text{CH}_2)_3\text{CH}_3$), 5.55 (t, 1H, $\text{CH}-\text{OCH}_2(\text{CH}_2)_3\text{CH}_3$, $J = 4$ Hz), 7.48 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 12.3, 22.5, 27.9, 29.0, 34.2, 69.6, 101.0, 123.4, 127.5, 128.1, 129.9, 133.9, 136.5, 164.2; MS (EI): m/z 234 (M^+). Anal. Calcd for $\text{C}_{14}\text{H}_{18}\text{O}_3$: C, 71.77; H, 7.74. Found: C, 71.68; H, 7.71.

3,4-Dihydro-3-(octyloxy)isochroman-1-one (5e) Light yellow liquid; yield 76%; IR (ν_{\max} , cm^{-1}): 3018, 2928, 1723, 1612, 1464, 1320, 1244, 1045, 794, 689; ^1H NMR: δ_{H} 0.86 (t, 3H, $\text{OCH}_2(\text{CH}_2)_6\text{CH}_3$, $J = 6.4$ Hz), 1.25 (m, 10H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_5\text{CH}_3$), 1.55 (m, 2H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_5\text{CH}_3$), 3.09 (dd, 1H, CH_2 , $J_1 = 17.1$ Hz, $J_2 = 4.4$ Hz), 3.28 (dd, 1H, CH_2 , $J_1 = 17.1$ Hz, $J_2 = 4.4$ Hz), 3.59 (m, 1H, $\text{OCH}_2(\text{CH}_2)_6\text{CH}_3$), 3.93 (m, 1H, $\text{OCH}_2(\text{CH}_2)_6\text{CH}_3$), 5.54 (t, 1H, $\text{CH}-\text{OCH}_2(\text{CH}_2)_6\text{CH}_3$, $J = 3.6$ Hz), 7.52 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 14.0, 22.6, 25.8, 29.1, 29.2, 29.2, 31.7, 33.4, 69.6, 101.0, 124.9, 127.5, 128.1, 129.8, 133.8, 136.5, 164.0; MS (EI): m/z 276 (M^+). Anal. Calcd for $\text{C}_{17}\text{H}_{24}\text{O}_3$: C, 73.88; H, 8.75. Found: C, 73.82; H, 8.78.

3-(Dodecyloxy)-3,4-dihydroisochroman-1-one (5f) Light yellow liquid; yield 78%; IR (ν_{\max} , cm^{-1}): 2968, 2931, 2868, 1728, 1637, 1487, 1251, 1045, 792, 688; ^1H NMR: δ_{H} 0.89 (t, 3H, $\text{OCH}_2(\text{CH}_2)_{10}\text{CH}_3$, $J = 6.8$ Hz), 1.26 (m, 18H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_9\text{CH}_3$), 1.60 (m, 2H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_9\text{CH}_3$), 3.12 (dd, 1H, CH_2 , $J_1 = 16.6$ Hz, $J_2 = 4.8$ Hz), 3.31 (dd, 1H, CH_2 , $J_1 = 16.6$ Hz, $J_2 = 4.8$ Hz), 3.62 (m, 1H, $\text{OCH}_2(\text{CH}_2)_{10}\text{CH}_3$), 3.96 (m, 1H, $\text{OCH}_2(\text{CH}_2)_{10}\text{CH}_3$), 5.56 (t, 1H, $\text{CH}-\text{OCH}_2(\text{CH}_2)_{10}\text{CH}_3$, $J = 4$ Hz), 7.54 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 14.1, 22.6, 25.7, 29.3, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 31.8, 32.7, 62.9, 107.0, 125.5, 128.5, 129.5, 134.8, 136.4, 144.6, 162.2; MS (EI): m/z 332 (M^+). Anal. Calcd for $\text{C}_{21}\text{H}_{32}\text{O}_3$: C, 75.86; H, 9.70. Found: C, 75.78; H, 9.66.

3,4-Dihydro-3-(tetradecyloxy)isochroman-1-one (5g) White crystalline solid; yield 80%; IR (ν_{\max} , cm^{-1}): 3020, 2938, 1720, 1637, 1487, 1330, 1251, 1045, 794, 688; ^1H NMR: δ_{H} 0.89 (t, 3H, $\text{OCH}_2(\text{CH}_2)_{12}\text{CH}_3$, $J = 6.8$ Hz), 1.28 (m, 22H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_{11}\text{CH}_3$), 1.58 (m, 2H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_{11}\text{CH}_3$), 3.12 (dd, 1H, CH_2 , $J_1 = 16.6$ Hz, $J_2 = 4.4$ Hz), 3.31

(dd, 1H, CH_2 , $J_1 = 16.6$ Hz, $J_2 = 4.4$ Hz), 3.62 (m, 1H, $\text{OCH}_2(\text{CH}_2)_{12}\text{CH}_3$), 3.95 (m, 1H, $\text{OCH}_2(\text{CH}_2)_{12}\text{CH}_3$), 5.56 (t, 1H, $\text{CH}-\text{OCH}_2(\text{CH}_2)_{12}\text{CH}_3$, $J = 4.4$ Hz), 7.52 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 14.1, 22.7, 25.8, 29.3, 29.3, 29.3, 29.5, 29.5, 29.6, 29.6, 29.7, 31.9, 33.4, 69.7, 101.0, 124.9, 127.5, 128.1, 129.9, 133.9, 136.4, 164.1; MS (EI): m/z 360 (M^+). Anal. Calcd for $\text{C}_{23}\text{H}_{36}\text{O}_3$: C, 76.62; H, 10.06. Found: C, 76.58; H, 10.10.

3-(Hexadecyloxy)-3,4-dihydroisochroman-1-one (5h) White crystalline solid; yield 78%; IR (ν_{\max} , cm^{-1}): 3090, 2941, 2837, 1720, 1637, 1487, 1251, 1045, 794, 688; ^1H NMR: δ_{H} 0.89 (t, 3H, $\text{OCH}_2(\text{CH}_2)_{14}\text{CH}_3$, $J = 6.9$ Hz), 1.26 (m, 26H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_{13}\text{CH}_3$), 1.57 (m, 2H, $\text{OCH}_2\text{CH}_2(\text{CH}_2)_{13}\text{CH}_3$), 3.11 (dd, 1H, CH_2 , $J_1 = 16.6$ Hz, $J_2 = 4.4$ Hz), 3.30 (dd, 1H, CH_2 , $J_1 = 16.6$ Hz, $J_2 = 4.4$ Hz), 3.61 (m, 1H, $\text{OCH}_2(\text{CH}_2)_{14}\text{CH}_3$), 3.96 (m, 1H, $\text{OCH}_2(\text{CH}_2)_{14}\text{CH}_3$), 5.56 (t, 1H, $\text{CH}-\text{OCH}_2(\text{CH}_2)_{14}\text{CH}_3$, $J = 4$ Hz), 7.54 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 14.1, 22.7, 25.7, 25.8, 29.3, 29.3, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 29.7, 31.9, 32.8, 33.4, 69.7, 101.0, 124.9, 127.5, 128.1, 129.9, 133.9, 136.5, 164.1; MS (EI): m/z 388 (M^+). Anal. Calcd for $\text{C}_{25}\text{H}_{40}\text{O}_3$: C, 77.27; H, 10.38. Found: C, 77.22; H, 10.30.

1H-Isochroman-1-one (6) [28] White crystalline solid; mp 46°C ; IR (ν_{\max} , cm^{-1}): 2962, 1732, 1610, 1462, 1246, 1008, 731; ^1H NMR: δ_{H} 6.52 (d, 1H, $\text{CH}=\text{CH}-\text{O}$, $J = 5.6$ Hz), 7.28 (d, 1H, $\text{CH}=\text{CH}-\text{O}$, $J = 5.2$ Hz), 7.62 (m, 4H, aromatic H); ^{13}C NMR: δ_{C} 107.0, 121.8, 125.5, 128.6, 129.7, 134.8, 136.4, 144.7, 162.2.

Antibacterial assay

Compounds **2**, **3**, and **5a–h** were screened for their antibacterial activity against Gram-positive bacteria [*S. aureus* (MTCC-96) and *S. pyogenes* (MTCC-442)] and Gram-negative bacteria [*Escherichia coli* (MTCC-443) and *P. aeruginosa* (MTCC-1688)]. All MTCC cultures were collected from the Institute of Microbial Technology, Chandigarh, India. The activity of compounds was determined as per National Committee for Clinical Laboratory Standards (NCCLS) protocol using Mueller Hinton broth (Becton Dickinson, Franklin Lakes, NJ, USA). Compounds were screened for their antibacterial activity as primary screening in five sets against *S. aureus*, *S. pyogenes*, *E. coli*, and *P. aeruginosa* at different concentrations of 1000, 500, and 250 $\mu\text{g}/\text{mL}$. The compounds found to be active in primary screening were similarly diluted to obtain 200, 125, 100, 62.5, 50, 25, and 12.5 $\mu\text{g}/\text{mL}$ concentrations for secondary screening to test in a second set of dilution against all microorganisms. Inoculum size for test strain was adjusted to 10^6 colony forming unit (CFU)/mL by comparing the turbidity (turbidimetric method). Mueller Hinton broth was used as nutrient medium to grow and dilute the compound suspension for test bacteria. DMSO (2%) was used as a diluent/vehicle to obtain the desired concentration of synthesized compounds and standard drugs to test against standard microbial strains. The compounds were diluted to 2000- $\mu\text{g}/\text{mL}$ concentration as a stock solution. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of test organisms. The tubes were then put for incubation at 37°C for 24 h for bacteria. Suspensions (10 $\mu\text{g}/\text{mL}$) were further inoculated on an appropriate media, and growth was noted after 24 and 48 h. The highest dilution (lowest concentration) preventing appearance of turbidity was considered as MIC ($\mu\text{g}/\text{mL}$), i.e., the amount of growth from the control tube before incubation (which represents the original inoculum) was compared. A set of tubes containing only seeded broth and solvent controls were

maintained under identical conditions to ensure that the solvent had no influence on strain growth. The result was greatly affected by the size of the inoculum. The test mixture should contain 10^6 CFU/mL organisms. The standard drug used in the present study was ampicillin for evaluating antibacterial activity.

Antifungal assay

Compounds **2**, **3**, and **5a–h** were also tested for antifungal activity in primary screening in five sets against *C. albicans* and *A. niger* at various concentrations of 1000, 500, and 250 µg/mL. Compounds found to be active in the primary screening were similarly diluted to obtain 200-, 125-, 100-, 62.5-, 50-, 25-, and 12.5-µg/mL concentrations for a secondary screening to test in a second set of dilution against all microorganisms. For fungal growth, in the present protocol, Sabourauds dextrose broth was used at 28°C in aerobic condition for 48 h. Griseofulvin was used as a standard drug for antifungal activity.

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References

- [1] Evans, D. A.; Hu, E.; Tedrow, J. S. An aldol-based approach to the asymmetric synthesis of L-callipeltose, the deoxyamino sugar of L-callipeltoside A. *Org. Lett.* **2001**, *3*, 3133–3136 [and references cited therein].
- [2] Stromgaard, K.; Nakanishi, K. Chemistry and biology of terpene trilactones from *Ginkgo Biloba*. *Angew. Chem. Int. Ed.* **2004**, *43*, 1640–1658.
- [3] Nakanishi, K. Terpene trilactones from *Ginkgo biloba*: from ancient times to the 21st century. *Bioorg. Med. Chem.* **2005**, *13*, 4987–5000.
- [4] Boukouvalas, J.; Cheng, Y. X.; Robichaud, J. Total synthesis of (+)-disidiolide. *J. Org. Chem.* **1998**, *63*, 228–229.
- [5] Yamada, Y.; Miyaoka, H.; Yamanishi, M.; Kajiura, Y. Total synthesis of cladocorans A and B: a structural revision. *J. Org. Chem.* **2003**, *68*, 3476–3479.
- [6] Lee, I.-S.; Ma, X.; Chai, H.; Madulid, D. A.; Lamont, R. B.; Cordell, G. A.; Pezzuto, J. M.; Kinghorn, A. D. Novel cytotoxic labdane diterpenoids from *Neouvaria acuminatissima*. *Tetrahedron* **1995**, *51*, 21–28.
- [7] He, H.; Kulanthaivel, P.; Baker, B. J. New cytotoxic sesterterpenes from the marine sponge *Spongia* sp. *Tetrahedron Lett.* **1994**, *35*, 7189–7192.
- [8] De Silva, E. D.; Scheuer, P. J. Manoalide, an antibiotic sesterterpenoid from the marine sponge *Luffariella variabilis* (Pole-jaeff). *Tetrahedron Lett.* **1980**, *21*, 1611–1614.
- [9] De Rosa, S.; Crispino, A.; De Giulio, A.; Iodice, C.; Pronzato, R.; Zavodnik, N. Facospongionolide B, a new sesterterpene from the sponge *Faciospongia cavernosa*. *J. Nat. Prod.* **1995**, *58*, 1776–1780.
- [10] Saeed, A. A short total synthesis of rac-peniolactol. *Monatsh Chem.* **2003**, *134*, 457–463.
- [11] Shen, J.; Ling, Q.; Huang, Y.; Zhou, Y.; Cai, Z.; Xiong, B.; Zhang, Y.; Maa, L.; Wang, X.; Li, X.; Li, J. Illudalic acid as a potential LAR inhibitor: synthesis, SAR, and preliminary studies on the mechanism of action. *Bioorg. Med. Chem.* **2008**, *16*, 7399–7409.
- [12] Hauser, F. M.; Baghdanov, V. M. A new procedure for regio-specific syntheses of benzopyran-1-ones. *J. Org. Chem.* **1988**, *53*, 4676–4681.
- [13] Tkachev, A. V.; Kolesnik, V. D.; Rukavishnikov, A. V. Synthesis of (-)-caronaldehyde from (+)-3-carene. *Mendeleev Commun.* **1995**, *5*, 179–180.
- [14] Kondratov, I. S.; Gerus, I. I.; Kukhar, V. P.; Manoilenko, O. V. New synthetic approach to mevalonate and mevaldate fluoro-analogues. *Tetrahedron: Asymmetry* **2007**, *18*, 1918–1925.
- [15] Rousha, W. R.; Roth, J.; Madoux, F.; Hodderb, P. Synthesis of small molecule inhibitors of the orphan nuclear receptor steroidogenic factor-1 (NR5A1) based on isoquinolinone scaffolds. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2628–2632.
- [16] Schöpf, C.; Kühne, R. Notiz über 2-oxy-hydrindon-(1). *Chem. Ber.* **1950**, *83*, 390–394.
- [17] Einaga, H.; Nojima, M.; Abe, M. Photooxygenation (electron transfer) of silyl enol ethers derived from 1-indanone: silyl group and medium effects on the competitive formation of 3-siloxy-1,2-dioxetane and α -peroxy ketone. *Main Group Metal Chem.* **1999**, *22*, 539–544.
- [18] Einaga, H.; Nojima, M.; Abe, M. Photooxygenation (1O_2) of silyl enol ethers derived from indan-1-ones: competitive formation of tricyclic 3-siloxy-1,2-dioxetane and silylperoxy ketone. *J. Chem. Soc. Perkin Trans. 1* **1999**, *17*, 2507–2512.
- [19] Abe, M.; Inakazu, T.; Munakata, J.; Nojima, M. ^{18}O -Tracer studies of Fe(II)-induced decomposition of 1,2,4-trioxolanes (ozonides) derived from cyclopentenones and indenones. Inner-sphere electron transfer reduction of the peroxide linkage. *J. Am. Chem. Soc.* **1999**, *121*, 6556–6562.
- [20] Valente, E. J.; Fuller, J. E.; Ball, J. D. Pseudoacids I. 4- and 5-Oxoacids. *Acta Cryst.* **1998**, *B54*, 162–173.
- [21] Deota, P. T.; Singh, V. K. Tungstic acid-hydrogen peroxide. A simple reagent for oxidation of cycloocta-1,5-diene preparation of (Z)cyclooct-5-ene-trans-1,2-diol and 1,6-dicarbomethoxy(Z) hex-3-ene. *Synth. Commun.* **1988**, *18*, 617–624.
- [22] Deota, P. T.; Desai, R.; Valodkar, V. B. Reaction of tungstic acid-hydrogen peroxide with endo-dicyclopentadiene: an unusual observation. *J. Chem. Res. (S)* **1998**, *9*, 562–563.
- [23] Senanayake, C. H. Applications of cis-1-amino-2-indanol in asymmetric synthesis. *Aldrichim. Acta* **1998**, *31*, 1–32.
- [24] Brewster, D.; Myers, M.; Ormerod, J.; Otter, P.; Smith, A. C. B.; Spinner, M. E.; Turner, S. Prostaglandin synthesis: design and execution. *J. Chem. Soc. Perkin Trans* **1973**, *1*, 2796–2804.
- [25] Taylor, J. E. Catalytic cis to trans conversions; dihydroxybicyclo[2.2.1]heptanes and 1,2-dihydroxyindane. *Synthesis* **1985**, 1142–1144.
- [26] National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard, third ed. NCCLS Publication M7-A3; NCCLS: Villanova, PA, 1993.
- [27] National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal testing of yeasts, proposed standard. NCCLS Document M27-P; NCCLS: Villanova, PA, 1992.
- [28] Narasimhan, N. S.; Mali, R. S. Synthetic application of lithiation reaction; ix. A simplified synthesis of isocoumarin. *Synthesis* **1975**, 796–797.