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Selective induction of secondary metabolism in *Phaseolus lunatus* by 6-substituted indanoyl isoleucine conjugates☆

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Abstract

A novel and highly efficient route to new indanoyl isoleucine conjugates is described, which allows a wide range of substituents to be attached to the 6-position of the indanoyl moiety. We report the synthesis of conjugates with methyl, methoxy, propoxy, allyloxy, pentoxy, and 2-(2-methoxy-ethoxy)-ethoxy 6-position substituents. Preliminary biological activities of the novel compounds with significantly enhanced water solubility were determined using the Lima bean (*Phaseolus lunatus*) volatile bioassay. The compounds induce variable volatile patterns, and structure-activity relationships show an ability to differentially induce separate pathways leading to secondary metabolites.

Keywords: Elicitors; Coronatine; *Phaseolus lunatus* (Fabaceae); Induced defense; Indanoyl isoleucine conjugates

1. Introduction

The phytotoxin coronatine (**1**) and its analogs are strong inducers of defensive responses in many plants, among them commercially valuable species including tomato, corn, and potato. The modification of coronatine analogs is effective in inducing unique response patterns, setting the stage for potentially agriculturally useful control of plant physiology. Coronatine (**1**) is a conjugate of the polyketide, coronafacic acid (Jiralerspong et al., 2001), and the rare amino acid, coronamic acid (Mitchell et al., 1994; Parry et al., 1991). Coronafacic acid has also been found in conjugation with other amino acids, these conjugates also being bioactive compounds (Mitchell, 1991; Mitchell and Ford, 1998). Coronatine (**1**) is produced by several pathovars of the plant bacteria, *Pseudomonas syringae*, in order to increase virulence (Bender et al., 1987). The most obvious symptom is chlorosis of the leaves. When administered in low concentrations, effects include tuber induction in the potato plant (Toshima et al., 1998), tendril coiling in *Bryonia dioica* (Weiler et al., 1994), suppression of a tobacco peroxidase (Hiraga et al., 2000), root growth inhibition (Feys et al., 1994), increased paclitaxel production in *Taxus media* cells (Takahito and Hara, 1996), and volatile emission (Boland et al., 1995). Volatile emission is an indirect defense that doesn't affect herbivores directly, but rather attracts the predators of herbivores (Dicke et al., 1990; Kessler and Baldwin, 2001; Thaler, 1999) and may up regulate defense genes in neighbored still uninfested plants (Arimura et al., 2000). In previous work we introduced indanoyl isoleucine conjugates **2a**–**2c** as effective analogs of coronatine.

A major advantage of these analogs is the ease of synthesis as compared to that of coronatine itself. Coronatine has been synthesized by several methods (Ichihara and Toshima, 1999), but the routes are difficult and not likely to be practical on a large scale. The aromatic ring strategy of the indanoyl isoleucine based molecules eliminates the difficulties associated with creating the correct stereoisomer of coronafacic acid. The likewise difficult to access coronamic acid is replaced by the methyl ester of isoleucine without significant loss of activity.

Abbreviations: DMNT, 4,8-dimethyl-1,3,7-nontriene; JA, jasmonic acid; SA, salicylate; SAR, systemic acquired resistance; TMTT, 4,8,12-trimethyltrideca-1,3,7,11-tetraene.

☆ This is part V of a series. See Schüler et al. (2001) for part IV.

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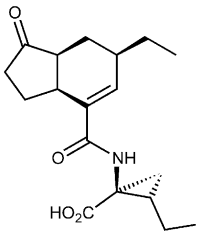
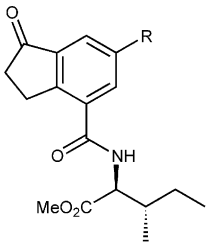


1 coronatine



2a R = H
2b R = N_3
2c R = CH_2CH_3

Previously, our routes to bioactive analogs of coronatine yielded the synthesis of the unsubstituted (Krumm and Boland, 1996; Krumm et al., 1995), the 6-azido (Schüler et al., 1999), and the 6-ethyl (Schüler et al., 2001) indanoyl isoleucine conjugates. Interestingly, the profiles of volatiles induced by each compound were significantly different, showing that there was potential for selectively inducing responses in plants. A major goal became the design of many 6-substituted analogs to examine the range of responses that could be obtained. At the same time the new compounds should possess increased water solubility since the most active 6-ethyl conjugate **2c** sometimes crystallized from emulsions, resulting in inactive preparations. As a further goal, we wanted to test the possibility of using a linker in the 6-position for attaching indanoyl isoleucine conjugates to affinity chromatography gels or fluorescent markers. Such molecules would represent valuable tools for the analysis of the involved signaling pathways and would allow the isolation of coronatine binding proteins via affinity approaches. To enhance the synthetic versatility and flexibility to generate different 6-substituted indanoyl-conjugates of type **2**, we developed a novel route starting from 6-methoxy-1,2-dihydronaphthalene (**3**). Here we report the details of the novel route and introduce the aryl triflate **13** as a new and versatile central intermediate that will give direct access to a large variety of 6-substituted indanoyl-conjugates.

2. Results and discussion

2.1. Synthesis of 6-alkoxy 1-oxo-indanoylcarboxylic acids

As in previous approaches we based our new route to 6-substituted 1-oxo-indan-4-carboxylic acids on the very reliable intramolecular Friedel–Crafts acylation of 3-phenyl-propanoic acids (Heaney, 1991). As the starting material was chosen 6-methoxy-1,2-dihydro-naphthalene (**3**), which is readily available from commercial 7-methoxy-1-tetralone by reduction with $NaBH_4$ followed by pTSA catalyzed dehydration in benzene with azeotropic removal of water (Hauser and Prasanna, 1980; Okumura et al., 1998). The double bond of **3** was cleaved with catalytic $RuCl_3$ using sodium periodate as the stoichiometric oxidant (Carlsen et al., 1981), yielding a mixture of aldehydes and carboxylic acids that was subjected to further oxidation using $AgNO_3$ and KOH (Colombo et al., 1981). The desired dicarboxylic acid **4** was thus obtained in 55% overall yield. An excess of sodium periodate did not increase the yield of the dicarboxylic acid, but resulted in a significant attack onto the aromatic nucleus. Intramolecular Friedel–Crafts acylation was achieved with simultaneous ether cleavage using a salt melt of $AlCl_3$ and NaCl at 140 °C. The resulting crude product mixture could not be separated, but was used immediately for one of two possible reactions, depending upon the desired 6-position substituent. For methoxy, propoxy, allyloxy, and pentoxy substituted indanoyl isoleucine conjugates, the strategy outlined in Fig. 1 was employed. The crude phenol was sonicated in DMF with K_2CO_3 or Cs_2CO_3 along with

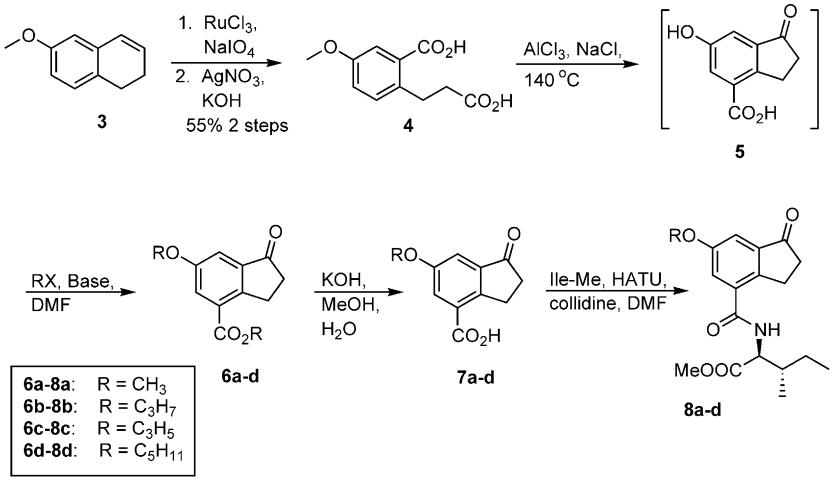


Fig. 1. Synthesis of 6-alkoxy indanoyl isoleucine conjugates.

the appropriate alkyl bromide and KI or alkyl sulfonate, causing substitution by both the phenol and carboxylic acid groups of the starting indanone.

Propyl, allyl, and pentyl bromides were used with success, but dimethyl sulfate had to be used for the synthesis of the 6-methoxy compound **6a** since methyl iodide led to substitutions next to the carbonyl group of the indanone. The products created in this step were easily purified by chromatography. Hydrolysis with KOH gave the free carboxylic acids. The final step was the attachment of isoleucine methyl ester. Stirring the carboxylic acid with isoleucine methyl ester, *sym*-collidine and HATU [*O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluroniumhexafluorophosphate] in DMF overnight at room temperature gave the desired conjugates in good yield (Schüler et al., 2001). As anticipated, the novel 6-alkoxy derivatives **8a**–**d** (except of the virtually insoluble pentyloxy derivative **8d**) displayed enhanced water solubility and gave stable solutions for induction experiments.

As a model of a typical polyether linker, 2-(2-methoxy-ethoxy)-ethyl bromide was used for alkylation of the crude phenol **5** (Fig. 1) using the same conditions that were used for the other 6-alkoxy compounds **6a**–**d**. However, the product of this reaction proved to be impossible to purify. The solution was provided by masking the carboxylic acid of the crude phenol by refluxing in MeOH with sulfuric acid to give the methyl ester **9**, which could then be purified by chromatography (Fig. 2).

The pure phenol was then subjected to the same conditions as before, this time yielding the 6-[2-(2-methoxy-ethoxy)-ethoxy]indanone **10** in 47% yield. Cleavage of the methyl ester and conjugation to isoleucine methyl ester were accomplished as described for **8a**–**d**.

2.2. 6-Alkyl 1-oxo-indanoyl carboxylic acids

The phenol **9** could be further elaborated into a versatile intermediate for subsequent modifications, namely the triflate **13** which allows a multitude of transition metal catalyzed transformations into 6-alkyl, 6-aryl, or 6-heteroatom substituted indanoyl conjugates (Ritter, 1993). Aryl triflates can undergo substitution by boronic acids in the Suzuki reaction, by sulfur compounds, by amines, and by tin compounds in the Stille reaction (Fig. 3).

Any of these reactions could be applied to the aryl triflate **13**, allowing the production of a large library of 6-substituted indanoyl isoleucine conjugates if desired. Here we report on the Stille reaction with **13** using tetramethyl tin (Saa et al., 1992). The resulting 6-methyl indanoyl ester **14** was hydrolyzed and subsequently conjugated to isoleucine methyl ester as described for **8a**–**d**. The reaction is well suited to introduce a large number of different substituents including (hetero)aromatic systems to create strongly fluorescent compounds with high biological activities as elicitors of plant secondary metabolism. First results will be reported in due course.

2.3. Biological activities

Induction of volatile emissions of the Lima bean provided the bioassay for the current and previously published coronatine analogs (Krumm et al., 1995). Since the complexity of the volatile pattern appears to be regulated by different pathways (Koch et al., 1999), the systematic structural modification of bioactive compounds such as **8a**–**d** is expected to give insight into the mode of activation and the usefulness of each pathway. Shown in Fig. 5 is a GC–MS trace of the volatiles collected after the application of 6-allyloxy indanoyl isoleucine methyl ester. Application is done by placing a

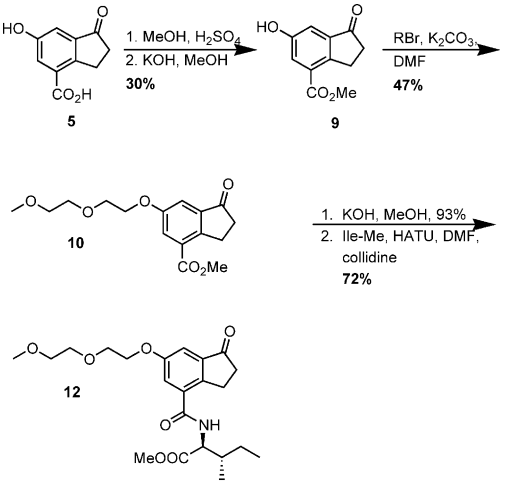


Fig. 2. Synthesis of a polyether linker of indanoyl isoleucine conjugates.

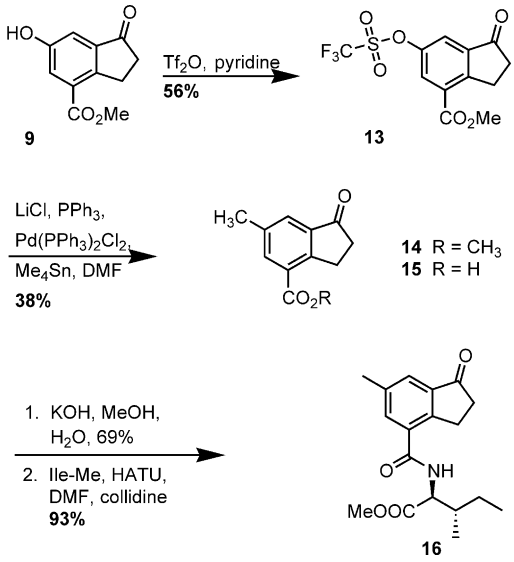


Fig. 3. Transition metal catalyzed alkylation of the 6-triflate of indanoyl isoleucine.

freshly cut plantlet of a 2-week old Lima bean into an aq. solution of **8c** (100 μM) (Figs. 4 and 5).

The 6-allyloxy conjugate **8c** induces a blend of compounds matching that resulting from application of the 6-ethyl derivative **2c** as described (Schüler et al., 2001). The terpenoids DMNT and TMTT are normally

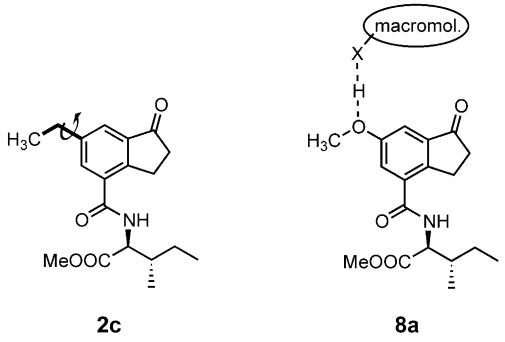


Fig. 4. Stabilization of conformations by hydrogen bonding. An additional hydrogen bond may be responsible for the reduced biological activity of the 6-methoxy indanoyl isoleucine **8a**.

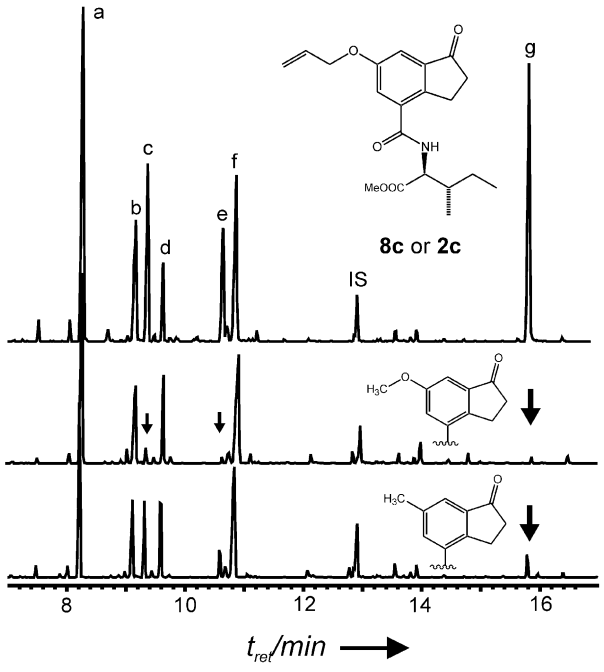


Fig. 5. Volatile blends emitted from leaves of the Lima bean *P. lunatus* after treatment with methyl esters of selected 6-substituted indanoyl conjugates. (A): 6-Allyloxy- (**8c**) or 6-ethylindanoyl isoleucine (**2c**); (B): 6-methoxyindanoyl isoleucine (**8a**); (C): 6-methylindanoyl isoleucine (**16**). Identification of compounds: (a) β-ocimene, (b) linalool, (c) 4,8-dimethyl-nona-1,3,7-triene (DMNT), (d) 2,6-dimethyl-octa-1,3,5,7-tetraene, (e) methyl salicylate, (f) 2,6-dimethyl-octa-3,5,7-trien-2-ol, IS: internal standard (1-bromodecane), (g) 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT). Volatiles were collected by absorption onto carbon traps (Donath and Boland, 1995; Kunert et al., 2002). Separation and identification of the compounds were achieved by GLC–MS using authentic references. Arrows indicate major changes in the blends.

induced by early intermediates (or their metabolites) of the octadecanoid pathway (Koch et al., 1999) upstream of the ultimate product, jasmonic acid (JA). JA itself can induce ocimene, linalool, 3,7-dimethyl-1,3,5,7-octatetraene, and 2,6-dimethyl-3,5,7-octatrien-2-ol (Boland et al., 1999). However, coronatine and most of the 6-substituted indanoyl isoleucine conjugates can induce the full spectrum of compounds to various degrees, notably at much lower concentrations than are needed for jasmonic acid. Activity of, for example, **8c** is 30–50 times that of JA. Moreover, unlike JA-related induction, methyl salicylate is also produced and emitted. Salicylate is an important regulatory compound in plant defense, and it controls systemically acquired resistance (SAR) to plant pathogens (Maleck and Dietrich, 1999) and may also act on neighboring plants (Shulaev et al., 1997).

Of the six coronatine analogs used in this study, the 6-allyloxy **8c** analogue was the most active, with activity almost identical to that of the previously investigated 6-ethyl analogue (Fig. 5A). The 6-pentoxy analogue **8d** was insoluble and, hence, did not induce biosynthesis of volatiles. The 6-[2-(2-methoxy-ethoxy)-ethoxy] analogue **12** was soluble, indeed, but failed to induce production of volatiles. Since **12** was synthesized as a model for linker attachment at the 6-position, the prospects of using this position for attachment to an affinity chromatography gel or fluorophore are not promising.

The 6-propoxy analogue **8b** induced a volatile pattern similar to that of the 6-allyloxy compound, but the levels of DMNT and TMTT were significantly reduced. Electronic factors control this difference, as both substituents are about equal in size, ruling out steric hindrance. The increased electron density of the double bond of the allyloxy group must provide extra affinity for the corresponding macromolecular targets. The 6-methoxy conjugate **8a** gave the most surprising result. The methoxy substituent is about the same size as the ethyl group, but in contrast to the 6-ethyl analogue **2c**, the 6-methoxy analogue **8a** does not induce the diterpenoid derived TMTT and only small amounts of DMNT and MeSA (Fig. 5B). It induces only ocimene, linalool, the tetraene, and the trienol at significant levels. We attribute this lack of activity to hydrogen bonding of residues of the macromolecular target to the electron lone pairs of the methoxy oxygen, holding the group in a rigid position that prevents the terminal carbon atom from achieving the conformation necessary for activity (Figs. 4 and 5C). Support for this conclusion is given by the 6-methyl analogue **16**, which induces a volatile pattern with severely reduced TMTT production and moderately reduced level of DMNT (Fig. 5C). Larger substituents such as the propyloxy analogue **8b** interact with the binding site in the usual manner and induce the full spectrum of volatiles albeit with reduced intensity (vide supra).

The individual and independent effects of the various indanoyl conjugates on the reduction of DMNT and TMTT emissions may be indicative of the existence of a network of (interdependent) signaling pathways leading to the induction of the biosynthesis of these compounds. The exploration of volatile patterns induced by the coronatine analogs presented in this work illustrate the effectiveness with which metabolic activities in the Lima bean can be controlled. Further exploration of the plant signaling network with different elicitor-active compounds is greatly facilitated by the flexibility of our synthetic strategy. Research into the effects of a variety of 6-substituted indanoyl isoleucine conjugates on other coronatine related responses and in other plants could help to evaluate the extent of selective manipulations of plant secondary metabolism. Detailed information on the currently established biological activities of the 6-substituted indanoyl conjugates along with information on their molecular targets will be reported in due course.

3. Experimental

3.1. General

Petroleum ether (40–60 °C), Et_2O, and EtOAc were distilled prior to use. DMF was dried over 0.3 or 0.4 nm molecular sieves. Analytical instruments and procedures were described previously (Schüler et al., 2001).

3.2. 2-(2-Carboxy-ethyl)-5-methoxy-benzoic acid (*4*)

A mixture of 6-methoxy-1,2-dihydronaphthalene (0.97 g, 6.1 mmol), sodium periodate (5.5 g, 25.7 mmol), ruthenium trichloride hydrate (30 mg, 2.2 mol%), CH_3CN (12 ml), CCl_4 (12 ml), and water (18 ml) was stirred for 2 h. The temperature, which was initially room temperature, rose during the reaction. The reaction mix was extracted several times with dichloromethane and filtered. The solvent was removed under low pressure to give an oil, which was dissolved in 10 ml dioxane/water (1:1). To this mixture was added aq. $AgNO_3$ (2 g in 10 ml) and aq. KOH (3 g in 10 ml). Stirring at room temp for 1 day, filtering through celite 545, acidification with conc. HCl, and extraction with EtOAc gave the product as a tan solid. Yield: 0.75 g (55%). Mp (decomp.) 174 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 2.48 (2H, *t*, $J=7.7$ Hz), 3.07 (2H, *t*, $J=7.7$), 3.77 (*s*, 3H), 7.05 (1H, *dd*, $J=8.5$ Hz, $J=3.0$ Hz), 7.25 (1H, *d*, $J=8.5$ Hz), 7.31 (1H, *d*, $J=2.9$ Hz). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 28.34, 35.60, 55.22, 115.05, 117.63, 131.33, 132.02, 133.72, 157.32, 168.42, 173.83. IR (KBr) ν_{max} cm^{-1}: 3700–2400 br., 2940, 2625, 1690, 1614, 1571, 1500, 1419, 1310, 1234, 1076, 1044, 892, 821, 765, 685, 538. EIMS 70 eV, *m*/*z* (rel. int.): 224 (24), 206 (11), 178 (100), 165 (57), 161 (14), 149 (18), 135 (12), 121 (7), 109 (9). HREIMS: *m*/*z* 224.06837 (calc. for $C_{11}H_{12}O_5$ 224.06847).

3.3. Intramolecular Friedel–Crafts acylation and esterification; general procedure for esters *6a–d*

3.3.1. Intramolecular acylation

The dicarboxylic acid **4**, $AlCl_3$ (28.0 g) and NaCl (4.0 g) were thoroughly mixed and placed in a 250 ml round bottomed flask connected to a balloon filled with argon. The mixture was heated to 140 °C for 3 h with occasional manual stirring and releasing of gas pressure. After cooling, the flask was chilled and ice (ca. 100 g), followed by conc. HCl (25 ml) were slowly added. Stirring was continued for 6 h and the product was extracted with EtOAc. After drying ($MgSO_4$) the solvent was removed i.v. to give **5** as a brown solid.

3.3.2. Alkylation

The crude product was suspended in DMF (40 ml), and an appropriate base (K_2CO_3, $CsCO_3$) along with an alkyl bromide with KI, or a sulfonate were added. The mixture was sonicated or stirred until reaction was complete (TLC). The reaction mix was poured into Et_2O and washed several times with water and satd. aq. NaCl. After drying over $MgSO_4$, the solvent was removed i.v. and the product purified by flash chromatography.

3.4. Methyl 6-methoxy-1-oxo-indan-4-carboxylate (*6a*)

Prepared from **4** (1.00 g, 4.5 mmol) as described above. Alkylation was achieved in the presence of K_2CO_3 (1.68 g) and dimethyl sulfate (4 ml, 42 mmol). After stirring for 1 day, the product was worked up and purified by flash chromatography using petroleum ether–EtOAc (2:1) for elution. Crystallization from petroleum ether–EtOAc provided slightly yellow needles of **6a**. Yield: 0.204 g (21%). Mp 130.0–131.0 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 2.65 (2H, *m*), 3.26 (2H, *m*), 3.85 (3H, *s*), 3.87 (3H, *s*), 7.33 (1H, *d*, $J=2.3$ Hz), 7.69 (1H, *d*, $J=2.8$ Hz). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 26.02, 36.14, 52.23, 55.91, 110.21, 123.16, 128.61, 139.43, 148.08, 158.83, 165.31, 205.47. IR (KBr) ν_{max} cm^{-1}: 3071, 3018, 2973, 2952, 2844, 1717, 1435, 1326, 1233, 1123, 1066, 1035, 779. EIMS 70 eV, *m*/*z* (rel. int.): 220 (100), 205 (78), 188 (31), 177 (14), 161 (39), 133 (15). HREIMS: *m*/*z* 220.073559 (calc. for $C_{12}H_{12}O_4$ 220.07356).

3.5. Propyl 6-propoxy-1-oxo-indan-4-carboxylate (*6b*)

Prepared from **4** (1.04 g, 4.5 mmol) as described above. Alkylation was achieved in the presence of Cs_2CO_3 (1.68 g) and 1-bromopropane (5 ml, 55 mmol). After sonication until completion (TLC), the product

was worked up and purified by flash chromatography using petroleum ether–EtOAc (2:1) for elution. Yield: 0.42 g (33%). Orange crystals. Mp 44.6–47.1 °C. ^{1}H NMR spectral data (500 MHz, $CDCl_3$): δ 1.04 (6H, *m*), 1.82 (4H, *sept*, $J=7.2$ Hz), 2.70 (2H, *m*), 3.38 (2H, *m*), 3.97 (2H, *t*, $J=6.4$ Hz), 4.29 (2H, *t*, $J=6.6$ Hz), 7.36 (1H, *d*, $J=2.8$ Hz), 7.84 (1H, *d*, $J=2.8$ Hz). ^{13}C NMR spectral data (125 MHz, $CDCl_3$): δ 10.54, 10.75, 22.21, 22.51, 26.60, 36.73, 66.97, 70.37, 110.83, 125.37, 129.31, 139.66, 148.64, 158.92, 165.79, 206.60. IR (KBr) ν_{max} cm^{-1}: 3077, 2962, 2929, 2874, 1721, 1700, 1607, 1470, 1312, 1230, 1208, 1120, 1060, 902, 776. EIMS 70 eV, *m*/*z* (rel. int.): 276 (84), 248 (32), 233 (40), 217 (20), 192 (99), 191 (100), 174 (16), 164 (20), 147 (28), 119 (16). HREIMS: *m*/*z* 276.13624 (calc. for $C_{16}H_{20}O_4$ 276.13616).

3.6. Allyl 6-allyloxy-1-oxo-indan-4-carboxylate (**6c**)

Prepared from **4** (1.00 g, 4.5 mmol) as described above. Alkylation was achieved in the presence of Cs_2CO_3 (3.0 g) and allyl bromide (5 ml, 61 mmol). After sonication until completion (TLC), the product was worked up and purified by flash chromatography using petroleum ether–Et_2O (2:1) giving the product as a faint yellow oil. Yield: 0.214 g (18%). ^{1}H NMR spectral data (500 MHz, $CDCl_3$): δ 2.72 (2H, *m*), 3.41 (2H, *m*), 4.60 (1H, *t*, $J=1.5$ Hz), 4.62 (1H, *t*, $J=1.5$ Hz), 4.85 (1H, *t*, $J=1.4$ Hz), 4.86 (1H, *t*, $J=1.4$ Hz), 5.31 (1H, *qnt*, $J=1.4$ Hz), 5.33 (1H, *qnt*, $J=1.4$ Hz), 5.42 (1H, *sxt*, $J=1.5$ Hz), 5.45 (1H, *sxt*, $J=1.5$ Hz), 6.05 (2H, *m*), 7.40 (1H, *d*, $J=2.4$ Hz), 7.91 (1H, *d*, $J=2.4$ Hz). ^{13}C NMR spectral data (125 MHz, $CDCl_3$): δ 26.59, 37.00, 65.93, 69.44, 111.49, 118.38, 118.90, 125.42, 129.02, 132.01, 132.43, 139.72, 149.07, 158.32, 165.21, 206.40. IR (NaCl) ν_{max} cm^{-1}: 3086, 3021, 2987, 2935, 2875, 1717, 1610, 1580, 1476, 1420, 1313, 1227, 1128, 1059, 1024, 930. EIMS 70 eV, *m*/*z* (rel. int.): 272 (35), 231 (100), 215 (11), 191 (7), 146 (6), 89 (7). HREIMS: *m*/*z* 272.10457 (calc. for $C_{16}H_{16}O_4$ 272.10486).

3.7. Pentyl 6-pentoxy-1-oxo-indan-4-carboxylate (**6d**)

Prepared from **4** (1.00 g, 4.5 mmol) as described above. Alkylation was achieved in the presence of Cs_2CO_3 (5.3 g) and *n*-pentyl bromide (4.5 ml, 36 mmol). After sonication to completion (TLC), the product was worked up and purified by flash chromatography using petroleum ether–Et_2O (2:1) for elution. Yield: 0.48 g (32%). Orange solid. Mp: 45.3–48.0 °C. ^{1}H NMR spectral data (500 MHz, $CDCl_3$): δ 0.93 (6H, 2*t*, $J=6.7$ Hz), 1.41 (8H, *m*), 1.80 (4H, *m*), 2.71 (2H, *m*), 3.38 (2H, *m*), 4.01 (2H, *t*, $J=6.6$ Hz), 4.33 (2H, *t*, $J=6.7$ Hz), 7.36 (1H, *d*, $J=2.4$ Hz), 7.84 (1H, *d*, $J=2.4$ Hz). 1H NMR spectral data (125 MHz, $CDCl_3$): δ 14.08, 14.10, 22.45, 22.53, 26.61, 28.23, 28.35, 28.54, 28.86, 36.76, 65.53, 68.92, 110.75, 125.39, 129.34, 139.68, 148.66, 158.95, 165.81, 206.64. IR (KBr) ν_{max} cm^{-1}: 3074, 2957, 2935, 2868, 1715, 1609, 1482, 1326, 1226, 1130, 1066, 1027, 899, 722, 583. EIMS 70 eV, *m*/*z* (rel. int.): 332 (63), 262 (24), 261 (21), 245 (11), 192 (100), 174 (10), 164 (5), 147 (15), 119 (7). HREIMS: *m*/*z* 332.198021 (calc. for $C_{20}H_{28}O_4$ 332.19876).

3.8. 6-Methoxy-1-oxo-indan-4-carboxylic acid (**7a**)

A chilled solution of **6a** (0.165 g, 0.75 mmol) in MeOH (25 ml) was gradually treated with aq. KOH (20% soln. in water, 25 ml). Stirring was continued at room temperature for 50 min. The solvent was then reduced i.v. by ca. 30% with gentle warming (35 °C). Unreacted ester was extracted with EtOAc. Following acidification with conc. HCl, the acid was extracted with EtOAc. The organic extract was washed with water and sat. NaCl solution. After removal of solvent, the product was adsorbed onto celite 545 and purified by flash chromatography, using petroleum ether–Et_2O (2:1, with 5% HOAc) for elution. The pure acid was obtained as an off-white solid. Yield: 0.101 g (65%). Mp (decomp.) 220 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 2.68 (2H, *m*), 3.31 (2H, *m*), 3.88 (3H, *s*), 7.33 (1H, *d*, $J=2.4$ Hz), 7.73 (1H, *d*, $J=2.8$ Hz). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 26.16, 36.20, 55.80, 109.60, 123.40, 130.16, 139.27, 148.30, 158.78, 166.58, 205.70. IR (KBr) ν_{max} cm−1: 3600–2750 br., 3123, 3072, 2973, 2848, 1711, 1685, 1481, 1436, 1333, 1038, 694. EIMS 70 eV, *m*/*z* (rel. int.): 206 (100), 188 (7), 178 (15), 161 (40), 149 (12), 135 (19), 121 (10). HREIMS: *m*/*z* 206.05781 (calc. for $C_{11}H_{14}O_4$ 206.05791).

3.9. 6-Propoxy-1-oxo-indan-4-carboxylic acid (**7b**)

A chilled solution of **6b** (0.152 g, 0.61 mmol) in MeOH (25 ml) was gradually treated with aq. KOH (20% soln. in water, 25 ml). Stirring was continued at room temperature for 3 h. The solvent was then reduced i.v. by ca. 30% with gentle warming (35 °C). Unreacted ester was extracted with Et_2O. Then, the aq. phase was acidified with conc. HCl and the free acid was extracted with Et_2O. After drying ($MgSO_4$), the product was adsorbed onto celite 545 and purified by flash chromatography using petroleum ether–Et_2O (2:1 with 5% HOAc) for elution. The pure acid was obtained as an off-white solid. Yield: 0.090 g (70%). Mp 211.5–213.3 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 0.98 (3H, *t*, $J=7.5$ Hz), 1.73 (2H, *sxt*, $J=7.0$ Hz), 2.63 (2H, *m*), 3.26 (2H, *m*), 4.00 (2H, *t*, $J=6.4$ Hz), 7.26 (1H, *d*, $J=2.4$ Hz), 7.67 (1H, *d*, $J=2.4$ Hz). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 10.27, 21.88, 26.15, 36.19, 69.71, 110.25, 123.86, 129.79, 139.29, 148.21, 158.15, 166.50, 205.64. IR (KBr) ν_{max} cm^{-1}: 3700–2400 br., 3171, 3088, 2971, 2943, 2882, 1724, 1691, 1580, 1307, 1201, 1135, 823, 695. EIMS 70 eV, *m*/*z* (rel. int.):

234 (64), 192 (100), 174 (21), 164 (20), 147 (25), 121 (11). HREIMS: *m/z* 234.08919 (calc. for $C_{13}H_{14}O_4$ 234.08921).

3.10. 6-Allyloxy-1-oxo-indan-4-carboxylic acid (*7c*)

A chilled solution of **6c** (0.084 g, 0.34 mmol) in MeOH (25 ml) was gradually treated with aq. KOH (20% soln. in water, 25 ml). Stirring was continued at room temperature for 3 h. The solvent was then reduced i.v. by ca. 30% with gentle warming (35 °C). Unreacted ester was extracted with Et_2O. Then, the aq. phase was acidified with conc. HCl and the free acid extracted with Et_2O. After drying ($MgSO_4$), the product was adsorbed onto Celite 545 and purified by flash chromatography using petroleum ether–Et_2O (2:1 with 5% HOAc) for elution. The free acid was obtained as an off-white solid. Yield: 0.050 g (70%). Mp 193.2–194.8 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 2.65 (2H, *m*), 3.28 (2H, *m*), 4.69 (2H, *m*), 5.30 (1H, *m*), 5.42 (1H, *m*), 6.05 (1H, *m*), 7.31 (1H, *d*, $J=2.4$ Hz), 7.73 (1H, *d*, $J=2.4$ Hz). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 26.18, 36.20, 68.76, 110.80, 117.65, 124.06, 129.84, 133.18, 139.29, 148.47, 157.64, 166.49, 205.64. IR (KBr) ν_{max} cm^{-1}: 3700–2300 br., 3148, 3082, 2924, 2585, 1721, 1683, 1610, 1579, 1487, 1426, 1306, 1200, 1137, 1025, 940, 705. EIMS 70 eV, *m/z* (rel. int.): 232 (100), 217 (14), 204 (21), 187 (11), 177 (8), 159 (25), 145 (11). HREIMS: *m/z* 232.073643 (calc. for $C_{13}H_{12}O_4$ 232.07256).

3.11. 6-Pentoxy-1-oxo-indan-4-carboxylic acid (*7d*)

A chilled solution of **6d** (0.103 g, 0.37 mmol) in ethanol (25 ml) was gradually treated with aqueous KOH (20% soln. in water, 5 ml). Stirring was continued at room temperature for 4 h. The solvent was then reduced to ca. 30% with gentle stream of Ar. Unreacted ester was extracted with ether. Then, the aqueous phase was acidified with conc. HCl and the free acid extracted with ether. After drying ($MgSO_4$), the product was adsorbed onto celite 545 and purified by flash chromatography using petroleum ether–Et_2O (3:1 with 5% acetic acid) for elution. The free acid was obtained as an off-white solid. Yield: 0.054 g (67%). Mp 165.4–166.2 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 0.89 (3H, *t*, $J=7.1$ Hz), 1.36 (4H, *m*), 1.72 (2H, *qnt*, $J=7.0$ Hz), 2.64 (2H, *m*), 3.28 (2H, *m*), 4.05 (2H, *t*, $J=6.4$ Hz), 7.28 (1H, s), 7.69 (1H, *s*). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 13.88, 21.83, 26.14, 27.58, 28.15, 36.19, 68.25, 110.27, 123.87, 129.88, 139.30, 148.20, 158.16, 166.52, 205.66. IR (KBr) ν_{max} cm^{-1}: 3700–2400 br., 3169, 3082, 2962, 2935, 2874, 2590, 1721, 1689, 1612, 1579, 1481, 1432, 1306, 1230, 1197, 1137, 825, 694. EIMS 70 eV, *m/z* (rel. int.): 262 (43), 192 (100), 174 (16), 164 (15), 147 (15), 121 (7). HREIMS: *m/z* 262.120444 (calc. for $C_{15}H_{18}O_4$ 262.12051).

3.12. Indanoyl isoleucine conjugates; general procedure

A mixture of the carboxylic acid **8a**–**d** and 1.1 equiv of isoleucine methyl ester hydrochloride in DMF was stirred at 0 °C under Ar and *sym*-collidine (2.2 equiv.) was added. HATU (*O*-(7-aza-benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluroniumhexafluorophosphate) (1.1 equiv.) was added in one portion, and the reaction was stirred overnight at room temp. The reaction was then cooled with an ice bath and a volume of sat. aq. $NaHCO_3$ corresponding to half that of the DMF was added. After 10 min, the mixture was poured into Et_2O, washed with water, twice with 2N HCl, again with water, and finally with satd. aq. NaCl. After drying ($MgSO_4$) the solvent was removed i.v. and the conjugate purified by flash chromatography.

3.13. 6-Methoxy-1-oxo-indanoyl-L-isoleucine methyl ester (*8a*)

Prepared from **7a** (0.077 g, 0.37 mmol) in DMF (5 ml) as described. The product was purified by chromatography on silica gel using petroleum ether–EtOAc (2:1) for elution. Yield: 0.097 g (78%). ^{1}H NMR spectral data (500 MHz, CDCl3): δ 0.95 (6H, *m*), 1.25 (1H, *m*), 1.51 (1H, *m*), 2.01 (1H, *m*), 2.70 (2H, *t*, $J=5.6$ Hz), 3.26 (2H, *m*), 3.77 (3H, *s*), 3.85 (3H, *s*), 4.79 (1H, *dd*, $J=8.4$ Hz, $J=4.7$ Hz), 6.61 (1H, *d*, $J=8.2$ Hz), 7.28 (1H, *d*, $J=2.1$ Hz), 7.44 (1H, *d*, $J=2.2$ Hz). ^{13}C NMR spectral data (125 MHz, $CDCl_3$): δ 11.61, 15.57, 25.34, 25.37, 36.63, 38.14, 52.18, 55.89, 56.78, 108.00, 121.90, 133.69, 139.58, 146.08, 159.41, 160.61, 166.25, 172.37, 206.24. IR (NaCl) ν_{max} cm^{-1}: 3338, 3061, 2964, 2936, 2877, 2842, 1744, 1716, 1651, 1480, 1338, 1207, 1149, 772. EIMS 70 eV, *m/z* (rel. int.): 333 (40), 274 (14), 216 (5), 205 (23), 189 (100), 188 (75), 161 (31), 133 (13), 116 (25). HREIMS: *m/z* 333.15762 (calc. for $C_{18}H_{23}NO_5$ 333.15762).

3.14. 6-Propoxy-1-oxo-indanoyl-L-isoleucine methyl ester (*8b*)

Prepared from **7b** (0.155 g, 0.66 mmol) in DMF (10 ml) as described. The product was purified by chromatography on silica gel using petroleum ether–EtOAc (2:1) for elution. Yield: 0.239 g (84%). Colorless solid. Mp 97.8–98.9 °C. ^{1}H NMR spectral data (500 MHz, $CDCl_3$): δ 0.97 (6H, *m*), 1.04 (3H, *t*, $J=7.3$ Hz), 1.26 (1H, *m*), 1.52 (1H, *m*), 1.82 (2H, *sxt*, $J=7.0$ Hz), 2.02 (1H, *m*), 2.71 (2H, *t*, $J=5.8$ Hz), 3.31 (2H, *m*), 3.78 (3H, *s*), 3.97 (2H, *t*, $J=6.6$ Hz, 4.81 (1H, *dd*, $J=8.6$ Hz, $J=4.6$ Hz), 6.58 (1H, *d*, $J=8.3$ Hz), 7.28 (1H, *d*, $J=2.4$ Hz), 7.45 (1H, *d*, $J=2.4$ Hz). ^{13}C NMR spectral data (125 MHz, $CDCl_3$): δ 10.56, 11.78, 15.72, 22.52, 25.50, 25.52, 36.80, 38.32, 52.44, 56.91, 70.41, 108.64, 122.45, 133.76, 139.70, 146.17, 159.06, 166.47, 172.56, 206.51. IR (KBr) ν_{max} cm^{-1}: 3227, 3070, 2966, 2937, 2883, 1745,

1706, 1632, 1558, 1258, 1154. EIMS 70 eV, *m/z* (rel. int.): 361 (45), 302 (12), 233 (25), 217 (90), 216 (100), 188 (39), 147 (26), 119 (22). HREIMS: *m/z* 361.18905 (calc. for $C_{20}H_{27}NO_5$ 361.18892).

3.15. 6-Allyloxy-1-oxo-indanoyl-L-isoleucine methyl ester (**8c**)

Prepared from **7c** (0.025 g, 0.11 mmol) in DMF (2 ml) as described. The product was purified by chromatography on silica gel using petroleum ether–EtOAc (2:1) for elution. Yield: 0.027 g (70%). Colorless solid. Mp 95.6–96.3 °C. ^{1}H NMR spectral data (500 MHz, $CDCl_3$): δ 0.99 (6H, *m*), 1.26 (1H, *m*), 1.52 (1H, *m*), 2.02 (1H, *m*), 2.73 (2H, *m*), 3.33 (2H, *m*), 3.79 (3H, *s*), 4.60 (2H, *d*, $J=5.2$ Hz), 4.81 (1H, *dd*, $J=8.4$ Hz, $J=4.8$ Hz), 5.32 (1H, *d*, $J=10.4$ Hz), 5.43 (1H, *d*, $J=16$ Hz), 6.05 (1H, *m*), 6.55 (1H, *d*, $J=7.5$ Hz), 7.31 (1H, *d*, $J=2.5$ Hz), 7.50 (1H, *d*, $J=2.5$ Hz). ^{13}C NMR spectral data (125 MHz, (CDCl3): δ 10.79, 14.74, 24.51, 24.56, 36.81, 37.35, 51.47, 55.93, 68.52, 108.17, 117.61, 121.56, 131.41, 132.87, 138.76, 145.49, 157.49, 165.38, 171.54, 205.36. IR (KBr) ν_{max} cm^{-1}: 3227, 3075, 2971, 2927, 1745, 1706, 1637, 1607, 1558, 1341, 1292, 1255, 1200, 1150, 1026. EIMS 70 eV, *m/z* (rel. int.): 359 (54), 300 (13), 258 (7), 231 (15), 215 (100), 214 (95), 186 (24), 146 (14). HREIMS: *m/z* 359.173295 (calc. for $C_{20}H_{25}NO_5$ 359.17327).

3.16. 6-Pentoxy-1-oxo-indanoyl-L-isoleucine methyl ester (**8d**)

Prepared from **7d** (0.034 g, 0.13 mmol) in DMF (2 ml) as described. The product was purified by chromatography on silica gel using petroleum ether–EtOAc (2:1) for elution. Yield: 0.040 g (79%). Colorless solid. Mp: 100.6–101.7 °C. ^{1}H NMR spectral data (500 MHz, $CDCl_3$): δ 0.97 (9H, *m*), 1.26 (1H, *m*), 1.40 (4H, *m*), 1.51 (1H, *m*), 1.79 (1H, *m*), 2.02 (1H, *m*), 2.70 (2H, *t*, $J=5.5$ Hz), 3.29 (2H, *m*), 3.77 (3H, *s*), 4.00 (2H, *t*, $J=6.4$ Hz), 4.80 (2H, *dd*, $J=8.2$ Hz, $J=4.6$ Hz), 6.59 (1H, *d*, $J=8.2$ Hz), 7.27 (1H, *s*), 7.44 (1H, *s*). ^{13}C NMR spectral data (125 MHz, $CDCl_3$): δ 11.96, 14.29, 15.91, 22.71, 25.69, 25.70, 28.41, 29.05, 36.98, 38.50, 39.73, 52.62, 57.11, 69.10, 108.81, 122.67, 133.94, 139.99, 146.35, 159.24, 166.67, 172.74, 196.70, 206.70. IR (KBr) ν_{max} cm^{-1}: 3242, 3060, 2966, 2937, 2878, 1750, 1701, 1640, 1607, 1550, 1341, 1297, 1258, 1198, 1149, 888. EIMS 70 eV, *m/z* (rel. int.): 389 (54), 330 (11), 261 (20), 245 (78), 244 (100), 216 (30), 191 (12), 175 (12), 146 (18), 145 (19), 119 (13). HREIMS: *m/z* 389.21956 (calc. for $C_{22}H_{31}NO_5$ 389.22022).

3.17. Methyl 6-hydroxy-1-oxo-indan-4-carboxylate (**9**)

The dicarboxylic acid **4** (1.00 g, 4.5 mmol) was converted into the indanone as described and the crude product dissolved in MeOH (34 ml). 12 drops of conc. H_2SO_4 were added and the mixture was refluxed overnight under Ar. After cooling EtOAc (100 ml) was added. The organic layer was washed with water twice, then satd. aq. $NaHCO_3$, and satd. aq. NaCl. After drying ($MgSO_4$), the product was adsorbed onto celite 545, and purified by flash chromatography using petroleum ether–EtOAc (2:1) for elution. Yield: 0.280 g (30%). Faintly yellow solid. Mp 186.3–187.3 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 2.55 (2H, *m*), 3.14 (2H, *t*), 3.81 (3H, *s*), 7.09 (1H, *d*, $J=2.0$ Hz), 7.56 (1H, *d*, $J=2.0$ Hz), 10.10 (1H, *s*). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 25.97, 36.08, 52.07, 112.39, 123.47, 128.38, 139.32, 146.52, 156.96, 165.46, 205.61. IR (KBr) ν_{max} cm^{-1}: 3700–2700 br., 3177, 3149, 3065, 2954, 2843, 1713, 1685, 1585, 1468, 1429, 1318, 1207, 1129, 1068, 1001, 890, 829, 573. EIMS 70 eV, *m/z* (rel. int.): 206 (100), 191 (67), 174 (41), 163 (15), 147 (41), 146 (34), 135 (7), 119 (17), 107 (6). HREIMS: *m/z* 206.057999 (calc. for $C_{11}H_{10}O_4$ 206.05791).

3.18. Methyl 6-[2-(2-methoxy-ethoxy)-ethoxy]-1-oxo-indan-4-carboxylate (**10**)

A suspension of **9** (102 mg, 0.49 mmol), K_2CO_3 (80 mg), and 1-bromo-2-(2-methoxy-ethoxy)-ethane (1 ml, about 7 mmol) in DMF (5 ml) was stirred at room temperature under Ar for 2 days. Then, the mixture was poured into ethyl acetate and water, and the organic layer was washed with water and satd. aq. NaCl. After drying ($MgSO_4$) and removal of solvent i.v., the residue was purified by flash chromatography using petroleum ether–Et_2O (2:1). Colorless oil. Yield: 0.071 g (47%). ^{1}H NMR spectral data (500 MHz, $CDCl_3$): δ 2.72 (2H, *m*), 3.40 (5H, *m*), 3.59 (2H, *m*), 3.73 (2H, *m*), 3.89 (2H, *t*, $J=4.7$ Hz), 3.94 (3H, *s*), 4.21 (2H, *t*, $J=4.7$ Hz), 7.41 (1H, *d*, $J=2.4$ Hz), 7.90 (1H, *d*, $J=2.7$ Hz). ^{13}C NMR spectral data (125 MHz, $CDCl_3$): δ 25.40, 35.59, 51.13, 58.07, 67.20, 68.54, 69.83, 70.94, 110.09, 124.20, 127.85, 138.56, 147.97, 157.49, 164.93, 205.36. IR (NaCl) ν_{max} cm^{-1}: 3076, 2929, 2880, 1718, 1613, 1582, 1479, 1438, 1401, 1317, 1232, 1132, 1067, 1001, 966, 875, 826, 778, 758. EIMS 70 eV, *m/z* (rel. int.): 308 (43), 276 (38), 250 (75), 232 (17), 206 (6), 191 (15), 173 (6), 146 (6), 129 (7), 103 (27), 59 (100). HREIMS: *m/z* 308.12581 (calc. for $C_{16}H_{20}O_6$ 308.125989).

3.19. 6-[2-(2-Methoxy-ethoxy)-ethoxy]-1-oxo-indan-4-carboxylic acid (**11**)

A chilled and well stirred solution of the ester **10** (0.055 g, 0.18 mmol) in methanol (10 ml) was treated with KOH (5 ml, 20% aqueous solution). The mixture was allowed to stir for 2.5 h at room temperature and, then, poured into ethyl acetate and water. The aqueous layer was acidified with conc. HCl and extracted with ethyl acetate. The organic layer was washed with satd.

aq. NaCl and dried ($MgSO_4$). After adsorption onto celite 545, the acid was purified by flash chromatography using petroleum ether–EtOAc (1:1). Yield: 0.049 g (93%). Colorless solid. Mp: 101–107 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): 2.65 (2H, *m*), 3.24 (3H, *s*), 3.30 (2H, *m*), 3.46 (2H, *m*), 3.59 (2H, *m*), 3.76 (2H, *m*), 4.20 (2H, *m*), 7.32 (1H, *s*), 7.72 (1H, *s*). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 26.15, 36.21, 58.04, 67.99, 68.76, 69.72, 71.25, 110.17, 123.95, 130.62, 139.22, 148.40, 157.99, 166.64, 205.73. IR (KBr) ν_{max} cm^{-1}: 3700–2750 br., 3199, 3080, 2973, 2940, 2897, 2811, 1709, 1687, 1477, 1434, 1311, 1235, 1203, 1133, 1069, 977, 886, 827, 697. EIMS 70 eV, *m*/*z* (rel. int.): 294 (36), 276 (14), 236 (9), 218 (31), 192 (10), 177 (17), 133 (6), 103 (26), 89 (16), 59 (100). HREIMS: *m*/*z* 294.11028 (calc. for $C_{15}H_{18}O_6$ 294.11034).

3.20. 6-[2-(2-Methoxy-ethoxy)-ethoxy]-1-oxo-indanoyl-L-isoleucine methyl ester (**12**)

A solution of the acid **11** (0.030 g, 0.10 mmol) in DMF (2 ml) was reacted with 1.1 equiv of isoleucine methyl ester hydrochloride as described above. The residue was purified by flash chromatography using ethyl acetate. Yield: 0.031 g (72%). ^{1}H NMR spectral data (500 MHz, $CDCl_3$): δ 0.99 (6H, *m*), 1.27 (1H, *m*), 1.53 (1H, *m*), 2.03 (1H, *m*), 2.72 (2H, *t*, J = 5.7 Hz), 3.33 (2H, *m*), 3.39 (3H, *s*), 3.59 (2H, *m*), 3.73 (2H, *m*), 3.79 (3H, *s*), 3.89 (2H, *t*, J = 4.6 Hz), 4.21 (2H, *t*, J = 4.5 Hz), 4.81 (1H, *dd*, J = 8.3 Hz, J = 4.6 Hz), 6.61 (1H, *d*, J = 8.3 Hz), 7.31 (1H, *d*, J = 1.8 Hz), 7.52 (1H, *d*, J = 2.2 Hz). ^{13}C NMR spectral data (125 MHz, $CDCl_3$): δ 11.61, 15.54, 25.27, 25.35, 36.61, 38.11, 52.29, 56.72, 59.03, 67.98, 69.48, 70.70, 71.84, 108.50, 122.45, 133.52, 139.50, 146.59, 158.49, 166.22, 172.32, 206.35. IR (KBr) ν_{max} cm^{-1}: 3307, 2963, 2932, 2877, 1744, 1651, 1531, 1475, 1338, 1305, 1258, 1208, 1150, 1109, 1066, 983, 881, 825. EIMS 70 eV, *m*/*z* (rel. int.): 421 (58), 362 (8), 277 (46), 276 (100), 201 (7), 174 (10), 146 (22), 103 (11). HREIMS: *m*/*z* 421.21005 (calc. for $C_{22}H_{31}NO_7$ 421.21046).

3.21. Methyl 1-oxo-6-trifluoromethanesulfonyloxy-indan-4-carboxylate (**13**)

A well stirred solution of **9** (85 mg, 0.41 mmol) in pyridine (3 ml) was gradually treated with triflic anhydride (0.08 ml, 0.48 mmol) and heated to 40 °C for 1.5 h. A second portion of triflic anhydride (0.07 ml, 0.42 mmol) was added and heating continued for 1 h. After cooling, the mixture was poured into Et_2O and water. The organic layer was washed with water and satd. aq. NaCl. After drying ($MgSO_4$) and adsorption onto celite 545, the crude product was purified by flash chromatography using petroleum ether–EtOAc (2:1). Yield: 0.078 g (56%). Brown solid. Mp 75.5–77.2 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6: δ 2.76 (2H, *m*), 3.43 (2H, *m*), 3.93 (3H, *s*), 8.00 (1H, *d*, J = 2.2 Hz), 8.22 (1H, *d*, J = 2.2 Hz). ^{13}C NMR spectral data (500 MHz, DMSO-d_6): δ 28.64, 37.99, 54.56, 118.81, 121.36, 121.96, 129.84, 131.82, 142.13, 150.18, 157.93, 166.02, 206.19. IR (KBr) ν_{max} cm^{-1}: 3102, 3069, 3008, 2960, 2851, 1734, 1722, 1593, 1472, 1435, 1291, 1218, 1140, 1001, 942, 737, 638, 598, 514. EIMS 70 eV, *m*/*z* (rel. int.): 338 (100), 307 (32), 206 (22), 177 (12), 173 (35), 145 (48), 89 (20). HREIMS: *m*/*z* 306.988393 (calc. for $C_{11}H_5F_3O_5S$ [M−MeOH+] 306.98881).

3.22. Methyl 6-methyl-1-oxo-indan-4-carboxylate (**14**)

A sealed glass tube containing **13** (67 mg, 0.20 mmol), LiCl (45 mg, 0.11 mmol), PPh3 (34 mg, 0.13 mmol), $Pd(PPh3)Cl_2$ (about 20 mg), tetramethyl tin (0.15 ml, 1.1 mmol), DMF (3 ml), and a few crystals of 2,6-di-*tert*-butyl-*p*-cresol under Ar was heated to 90 °C for 1 day. After cooling, the reaction mixture was poured into Et_2O and washed several times with water and satd. aq. NaCl. The organic layer was dried ($MgSO_4$) and the product was adsorbed onto celite 545. Purification by flash chromatography using petroleum ether–EtOAc (4:1) for elution afforded the free acid as a colorless solid. Yield: 0.016 g (38%). Mp 111.4–116.0 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 2.41 (3H, *s*), 2.63 (2H, *m*), 3.29 (2H, *m*), 3.88 (3H, *s*), 7.67 (1H, *s*), 8.02 (1H, *s*). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 20.25, 26.43, 35.91, 52.04, 127.46, 127.53, 136.54, 137.54, 138.22, 153.48, 165.64, 205.69. IR (KBr) ν_{max} cm^{-1}: 3021, 2953, 2928, 1712, 1609, 1581, 1478, 1435, 1310, 1201, 1124, 1066, 826, 776, 517. EIMS 70 eV, *m*/*z* (rel. int.): 204 (100), 189 (35), 173 (41), 172 (62), 161 (16), 145 (38), 115 (31), 91 (11). HREIMS: *m*/*z* 204.07879 (calc. for $C_{12}H_{12}O_3$ 204.07864).

3.23. 6-Methyl-1-oxo-indan-4-carboxylic acid (**15**)

To a stirred, ice cooled solution of **14** (15.5 mg, 0.076 mmol) of in MeOH (5 ml) was added aq. KOH (5 ml, 20% aq. soln.). Then, the mixture was allowed to stir at room temperature for 2.5 h and poured into Et_2O and water. The water layer was acidified with conc. HCl and extracted with Et_2O. The organic layer was washed with satd. aq. NaCl and dried ($MgSO_4$). Removal of solvent afforded the free acid as a colorless solid. Yield: 0.010 g (69%). Mp (decomp.) 217–219 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 2.41 (3H, *s*), 2.62 (2H, *m*), 3.32 (2H, *m*), 7.63 (1H, *s*), 8.01 (1H, *s*). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 20.30, 26.55, 35.96, 127.05, 128.66, 136.90, 137.39, 138.14, 153.65, 166.86, 205.89. IR (KBr) ν_{max} cm^{-1}: 3700–2400 br., 3403, 2969, 2926, 2717, 2610, 1707, 1695, 1582, 1483, 1437, 1312, 1257, 1128, 931, 826, 725, 571, 513, 474, 442. EIMS 70 eV, *m*/*z* (rel. int.): 190 (100), 172 (24), 162 (39), 145 (22), 133 (22), 115 (30), 105 (14), 91 (16). HREIMS: *m*/*z* 190.06299 (calc. for $C_{11}H_{10}O_3$ 190.06299).

3.24. 6-Methyl-1-oxo-indanoyl-L-isoleucine methyl ester (**16**)

A solution of the acid **15** (0.010 g, 0.053 mmol) in DMF (1 ml) was reacted with 1.1 equiv of isoleucine methyl ester hydrochloride as described above. The residue was chromatographed on silica gel using petroleum ether–EtOAc (2:1) for elution. Yield: 0.016 g (93%). Colorless solid. Mp 135.8–137.3 °C. ^{1}H NMR spectral data (500 MHz, DMSO-d_6): δ 0.88 (3H, *t*, $J=7.5$ Hz), 0.92 (3H, *d*, $J=6.8$ Hz), 1.29 (1H, *m*), 1.49 (1H, *m*), 1.94 (1H, *m*), 2.42 (3H, *s*), 2.63 (2H, *t*, $J=5.6$ Hz), 3.20 (2H, *m*), 3.67 (3H, *s*), 4.40 (1H, *t*, $J=7.3$ Hz), 7.57 (1H, *s*), 7.74 (1H, *s*), 8.64 (1H, *d*, $J=7.5$ Hz). ^{13}C NMR spectral data (125 MHz, DMSO-d_6): δ 10.96, 15.52, 20.39, 25.06, 25.10, 35.82, 36.01, 51.64, 57.02, 124.85, 133.28, 134.31, 137.03, 137.52, 151.20, 167.08, 172.01, 205.95. IR (KBr): $\nu=3306$, 3050, 2968, 2932, 2878, 1745, 1734, 1642, 1536, 1264, 1201, 1145, 827, 569. EIMS 70 eV, *m*/*z* (rel. int.): 317 (33), 258 (24), 200 (6), 189 (17), 173 (100), 172 (56), 145 (25), 115 (20), 91 (7). HREIMS: *m*/*z* 317.16292 (calc. for $C_{18}H_{23}NO_4$ 317.16271).

3.25. Plant material

Induction experiments were performed with plantlets of the Lima bean *Phaseolus lunatus* (Ferry Morse cv. Jackson Wonder Bush). Individual plants were grown from seed in a plastic pot (Ø = 5.5 cm) at 23 °C and 80% humidity using daylight fluorescent tubes at ca. 270 μE m^2 s^{-1} with a photophase of 14 h. Experiments were conducted with 12–16 day-old seedlings showing two fully developed leaves (Hopke et al., 1994).

3.26. Induction experiments

Stock solutions of the test compounds were made in DMSO at 50 mmol. Test solutions used for plant induction were made by adding 20 μl of stock solution to 10 ml of vigorously stirred tap water, giving a final concentration of 100 μM. Plantlets of *P. lunatus* were cut with razor blades and immediately transferred into 5 ml vials containing the test solution. These were enclosed in small desiccators (750 ml) and maintained at room temperature for 24 h. Experiments were started in the afternoon and the light dark cycle completed as above. Control experiments were conducted under identical conditions by placing freshly cut plantlets into tap water with 20 μl DMSO. All experiments were carried out on 4–6 plants on at least 2 different days.

3.27. Collection and analysis of headspace volatiles

The volatiles emitted from the plants were collected continuously on small charcoal traps (1 mg charcoal, CLSA-Filter, Le Ruisseau de Montbrun, F-09350 Daumazan sur Arize, France) over a period of 23–24 h using air circulation as described (Donath and Boland, 1995; Kunert et al., 2002). After desorption of the volatiles from the carbon trap with 2×20 μl of a solution of 1-bromodecane (internal standard, 200 μg/ml) in dichloromethane. The extracts were directly analyzed by GC–MS. GC-conditions: Fused-silica capillary (30 m×0.25 mm) coated with DB 5 (0.25 μm). Helium at 40 cm min^{-1} served as carrier gas. Separation of the compounds was under programmed conditions (40 °C for 2 min, then at 10 °C min^{-1} to 200 °C, finally at 30 °C min^{-1} to 280 °C and held for 2 min). MS: Finnigan GCQ; GC-interface at 265 °C; scan range 35–450 Da. Individual compounds (peak area) were quantified with respect to the peak area of the internal standard.

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