Total Synthesis of Psoralidin, an Anticancer Natural Product

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A base-catalyzed condensation of phenyl acetate with acid chloride, followed by intramolecular cyclization and microwave-assisted cross-metathesis reaction, leads to the first total synthesis of psoralidin, a natural product with a broad range of biological activities, in a highly convergent and regioselective manner.

Introduction

Psoralidin (1) was first isolated from seeds of Psoralea corylifolia Linn in 1948 by Chakravarti et al.,1 and later its structure was corrected by Khastgir et al. in 1961.2 It was found to be an active ingredient of many Indian and Chinese traditional herbal medicines.3 Psoralidin (1) exhibits a variety of biological activities like antioxidant,4 antibacterial,5 and antidepressant activities.6,7 It also shows inhibitory activities against protein tyrosine phosphatase 1B, which plays a major role in the negative regulation of insulin signaling.8 In addition, psoralidin inhibits antigen IgE-induced degranulation in RBL-2H3 cells9 and acts as a potent antidepressant by strongly inhibiting forskolin-induced corticotrophin releasing factor (CRF) gene transcription.5,7 Psoralidin also has great potential as an anticancer agent, showing cytotoxic effects on gastric (SNU–1 and SNU–16), colon (HT–29), and breast (MCF–7) cancer cell lines.10,11 Recently, we reported that 1 inhibits protein kinase Akt phosphorylation, and thus, inhibits the growth of androgen-independent prostate cancer cells both in vitro and in vivo.12 Psoralidin 1 is a member of the coumestane family of natural products having an isopentenyl group at the C–2 position of coumestrol (4). In 1961, Nasipuri and Pyne13 reported the synthesis of dihydropsoralidin (2) and confirmed the structure of the natural product. Jain et al.14 reported the synthesis of some prenylated coumestanes as analogues of psoralidin, and most recently Fürstner et al.15 synthesized and corrected the proposed structure of phaseol (3), a structural isomer of 1.


FIGURE 1. Chemical structures of psoralidin and related natural products.
However, there are no reports on the synthesis of 1 so far. In our ongoing work on the investigation of the chemical constituents of Rasagenthi lehyam, a complementary and alternative medicine for prostate cancer used as Ayurveda and Siddha medicine in different parts of India, we isolated 1 as the active principle of this mixture of 38 different botanicals, metals, and fats.12 However, the isolated natural product was found to be insoluble in water and, therefore, less effective for in vivo studies. To overcome this problem, we plan to generate a synthetic library of psoralidin analogues that can be utilized to study structure-activity relationships (SAR), which in turn will pave the way for the synthesis of an active analogue or derivative with increased water solubility. Herein, we report an efficient and general synthesis of 1 that can be easily adopted for the synthesis of various analogues.

Many routes to prepare coumestanes can be found in literature, but most of them are complicated and require multistep syntheses.16–24 Retrosynthetically, 1 can be reduced to 5 through the opening of the lactone and furan rings; in turn, 5 can be obtained from two relatively simple starting materials, namely 2,4-dimethoxy-phenylacetic acid methylester (6) and 2,4-dimethoxy-5-prenyl benzoylchloride (7) (Scheme 1). This strategy was expected to be flexible enough for the preparation of various analogues, considering the facile preparation of the starting materials. Al-Maharik and Botting24 used a similar strategy for their synthesis of 13C-labeled 4.

Results and Discussion

We started our synthesis of 1,5-dibromo-2,4-dimethoxybenzene (9),25 which was prepared from resorcinol 8 through bromination with Br2/CHCl3 and O-methylation of the hydroxyl groups using K2CO3/MeI. Br/Mg exchange with i-PrMgCl at ∼10 °C and quenching with prenylbromide provided 10 exclusively in 65% yield. The same reaction was carried out using n-BuLi and diprenylated and monodebrominated side products were found that were not easy to separate. Acid 11 was obtained by replacing the bromine atom of 10 using n-BuLi and CO2 gas that was bubbled through the reaction mixture. The other starting molecule, compound 6, was prepared by the condensation of 2,4-dimethoxybenzaldehyde and hippuric acid following a literature procedure.26 Treatment of acid 11 with oxalyl chloride in the presence of catalytic amounts of DMF produced acid chloride 7 that, without further purification, was reacted with the anion obtained from the treatment of 6 with

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SCHEME 1. Retrosynthetic Analysis

SCHEME 2

SCHEME 3
LDA and produced the desired compound 5 in an overall 82% yield. The structure of 5 was confirmed by spectroscopic analysis. At this point, it was expected that demethylation followed by intramolecular cyclization would yield 1 in a one-pot reaction. The demethylation of the four methoxy groups was carried out with BBr3 at 0 °C, but the attempted in situ cyclization ended up with isopsoralidin (12) instead of 1. The structure of 12 was confirmed by comparison with reported data (Scheme 2). The formation of 12 can be explained by the generation of a stable tertiary carbocation produced from the prenyl group in acidic conditions, which is attacked by the ortho-hydroxy group to yield the undesired dihydropyran ring. A similar reaction of a prenyl group was reported by Molyneux and Jurd.27

To avoid this undesired reaction, we modified our strategy and planned to introduce the prenyl group at the concluding step of the reaction sequence. Thus, we started with acid 13, which was prepared from 9 using i-PrMgBr and allyl bromide. A similar reaction sequence as described by Hastings et al.29 using n-BuLi/CO2 and 6/LDA in an overall 82% yield along with very small amounts of the dibrominated side product 23 (1%, Scheme 4). Compounds 18, 19, and 21–23 were characterized by 1H and 13C NMR spectroscopy, as well as HRMS for structure verification.

In the search for a specific functional group which could survive the acidic conditions necessary for the cyclization reaction and could be easily transformed to a prenyl group, we considered the olefin cross-metathesis reaction. Recently Hastings et al.29 used an olefin cross-metathesis reaction in the presence of Grubbs second-generation catalyst to convert an allyl side chain to prenylated compound in very good yields. However, all attempts to convert 17 with i-PrMgBr/prenyl bromide under various reaction conditions ended up with complex product mixtures. A Pd(0)-catalyzed Ullmann type cross-coupling reaction also failed to give any product.


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achieved by heating a mixture of 22, Grubbs second-generation catalyst, and 2-methyl-2-butene in CH$_2$Cl$_2$ in a sealed tube microwave reactor at 100 °C for 15 min. The reaction produced 1 in 73% yield along with compound 24 as a minor side product. All spectroscopic data of the synthetic compound matched those of the natural product (Table 1 in Supporting Information) except for the melting point, which was significantly higher for the synthetic compound than reported for the natural product. Since there was no ambiguity about the structure of the synthetic compound, we assumed that the melting point of the natural product was lowered by minor impurities.

Conclusions
In summary, we achieved the first total synthesis of psoralidin 1 in a convergent and highly regioselective manner. The synthetic scheme described here is straightforward and efficient. Further structural diversification could be achieved on the level of the phenyl acetate and the allyl benzoyl chloride starter molecules to furnish numerous analogues for SAR studies. The synthetic scheme described here is straightforward and efficient. Further structural diversification could be achieved on the level of various regioisomers, are in progress.

Experimental Section

Psoralidin 1. Grubbs second-generation catalyst (3 mg, 0.0035 mmol) was added to a degassed solution of 22 (20 mg, 0.06 mmol) in CH$_2$Cl$_2$ (10 mL) and 2-methyl-2-butene (2 mL). The solution was heated in a sealed tube at 100 °C for 15 min by a microwave reactor (Biotoاجر Initiator 2.0, standard configuration, temperature control, external IR temperature sensor, fixed hold time). CH$_2$Cl$_2$ was removed in vacuum, and the mixture was purified by reverse phase semipreparative HPLC (symmetry18C, 7 to 99% CH$_3$CN:H$_2$O) to get compound 1 (16 mg, 73%) as a white solid along with a minor amount of side product 24 (1 mg, 5%). R$_f$ = 0.5 (50% ethyl acetate in hexane); mp > 400 °C (lit. 131 °C; $\nu_{max}$ (CHCl$_3$) cm$^{-1}$ 1719, 1629, 1597, 1419, 1370, 1250, 1170, 1092. $\delta$ NMR (CDCl$_3$, 125 MHz) $\nu$ 7.0 Hz, 1.64 (s, 3H). $\delta$C NMR (CDCl$_3$, 125 MHz) $\delta$ 7.0 Hz, 1.64 (s, 3H), 5.22 (t, 1H, $J$ = 7.5 Hz), 3.82 (s, 3H), 3.76 (s, 3H), 3.75 (s, 3H), 3.72 (3H, 3.70 (3H, 3.18 (2H, J = 7.5 Hz), 1.68 (s, 3H), 1.64 (s, 3H). 13C NMR (CDCl$_3$, 125 MHz) $\delta$ 193.0 (C), 171.1 (C), 162.6 (C), 160.3 (C), 159.6 (C), 157.9 (C), 132.7 (CH), 132.3 (CH), 130.3 (CH), 122.4 (CH), 118.2 (C), 115.9 (C), 104.1 (CH), 98.5 (CH), 94.2 (CH), 57.3 (CH$_3$), 55.6 (CH$_3$), 55.5 (CH$_3$), 55.3 (CH$_3$), 52.2 (CH$_2$), 27.6 (CH$_2$), 25.9 (CH$_3$), 17.8 (CH$_3$). HRMS (EI) $\nu$m/z 442.1993 ([M]+$^+$ C$_{22}$H$_{24}$O$_7$Br, requires 442.1992).

1-Bromo-2,4-dimethoxy-5-(3-methyl-but-2-enyl)-benzene (10). Compound 9 (3.10 g, 10.47 mmol) was added to a solution of i-Pr$_2$MgCl (1 mL in THF, 13 mL, 13 mmol) at −10 °C under N$_2$ atmosphere. After 45 min of stirring, prenyl bromide (1.3 mL, 11.5 mmol) was added dropwise, and the mixture was allowed to stir at the same temperature for 30 min, and then 30 min at room temperature. The reaction was quenched by the addition of 10% HCl (15 mL). THF was removed in vacuum, and the mixture was extracted with ethyl acetate (2 × 5 mL). The organic layer was washed with water (2 × 30 mL) and brine (20 mL), dried (Na$_2$SO$_4$), filtered, and concentrated. The resulting crude compound was purified by column chromatography to yield 10 (1.94 g, 65%) as a colorless liquid along with 550 mg of unresolved starting material. $\nu_{max}$ (CHCl$_3$) cm$^{-1}$ 1599, 1500, 1463, 1295, 1208, 1029. $\delta$ NMR (CDCl$_3$, 500 MHz) $\delta$ 7.21 (s, 1H), 6.44 (s, 1H), 5.21 (t, 1H, $J$ = 7.0 Hz), 3.87 (3H, 3.82 (s, 3H), 3.19 (d, 2H, $J$ = 7.0 Hz), 1.72 (s, 3H), 1.67 (s, 3H). 13C NMR (CDCl$_3$, 125 MHz) $\delta$ 157.5 (C), 154.8 (C), 133.1 (C), 133.0 (CH), 124.0 (C), 122.2 (CH), 101.6 (C), 96.8 (CH), 56.6 (CH$_3$), 55.9 (CH$_3$), 27.6 (CH$_2$), 26.0 (CH$_2$), 17.9 (CH$_3$). HRMS (EI) $\nu$m/z 284.0414 ([M]+$^+$ C$_{17}$H$_{20}$O$_4$Br, requires 284.0412).

2,4-Dimethoxy-5-(3-methyl-but-2-enyl)-benzoic acid (11). n-BuLi (2.5 M in THF, 2.1 mL, 5.34 mmol) was added dropwise to a stirred solution of 10 (1.27 g, 4.45 mmol) in THF (15 mL) at −78 °C under N$_2$ atmosphere. After 30 min, CO$_2$ gas was passed through the solution for 45 min, and then it was allowed to warm up to rt. THF was removed in vacuum, and the mixture was treated with saturated NaHCO$_3$ solution (30 mL). The water layer was washed with ethyl acetate (2 × 20 mL) and then acidified with conc. HCl. The mixture was extracted with ethyl acetate (2 × 75 mL). The organic layer was washed with water (2 × 30 mL) and brine (30 mL), dried (Na$_2$SO$_4$), filtered, and concentrated. Recrystallization from ethyl acetate produced 11 (930 mg, 84%) as a white solid. $\nu_{max}$ (film) cm$^{-1}$ 1719, 1616, 1442, 1278,1168, 1020. $\delta$ NMR (CDCl$_3$, 500 MHz) $\delta$ 7.77 (s, 1H), 6.41 (s, 1H), 5.16 (t, 1H, $J$ = 7.0 Hz), 3.97 (s, 3H), 3.84 (s, 3H), 3.14 (2H, $J$ = 7.0 Hz), 1.64 (s, 3H), 1.78 (s, 3H).
1.60 (s, 3H). 13C NMR (CDCl3, 125 MHz) δ 166.2 (C), 162.6 (C), 158.5 (C), 133.6 (C), 132.9 (CH), 123.9 (CH), 121.6 (C), 108.9 (C), 94.5 (CH), 56.7 (CH), 55.7 (CH2), 27.6 (CH), 25.8 (CH2), 17.7 (CH3). HRMS (EI+) m/z 250.1206 ([M]+ Cl3H16O4 requires 250.1205).

Methyl-3-(5-bromo-2,4-dimethoxy-phenyl)-2-(2,4-dimethoxy-phenyl)-3-oxo-propionate (15). This compound was prepared as a yellowish oil (320 mg, 74%) from compound 13 and 6, following an analogous reaction sequence as described above for the synthesis of compound 5 (for the exact procedure, see Supporting Information). Rf = 0.3 (50% ethyl acetate in hexane); mp 136–137 °C; νmax (film): cm⁻¹ 3173, 1700, 1668, 1613, 1590, 1508, 1465, 1334, 1271, 1213, 1155, 1021. 1H NMR (CDCl3, 500 MHz) δ 8.10 (s, 1H), 7.00 (d, 1H, J = 9.0 Hz), 6.43 (s, 1H), 6.42 (d, 1H, J = 9.0 Hz), 6.34 (s, 1H), 5.88 (s, 1H), 3.91 (s, 3H), 3.80 (s, 3H), 3.76 (s, 6H), 3.72 (s, 3H), 3.51 (s, 3H). 13C NMR (CDCl3, 125 MHz) δ 192.1 (C), 170.8 (C), 160.6 (C), 160.5 (C), 160.1 (C), 157.9 (C), 136.0 (CH), 130.4 (CH), 120.2 (C), 115.3 (C), 104.3 (CH), 103.2 (C), 98.7 (CH), 95.8 (CH), 57.3 (CH3), 56.6 (CH3), 55.7 (CH), 55.6 (CH), 55.4 (CH2), 52.4 (CH). HRMS (EI+) m/z 452.0477 ([M]+ C19H18O4 requires 452.0471).

2-Bromo-3,9-dihydroxy-benzo[4,5]furo[3,2-c]chromen-6-one (16). BBr3 (1 M in CH2Cl2, 1.72 mL, 1.72 mmol) was added at 0 °C to a solution of 15 (130 mg, 0.29 mmol) in CH2Cl2 (6 mL) under N₂ atmosphere. The mixture was stirred at the same temperature for 10 h, and water (10 mL) was added. CH2Cl2 was removed in vacuum, and the mixture was heated to reflux for 1 h. The water layer was extracted with ethyl acetate (2 × 30 mL), and the organic layer was washed with water (2 × 10 mL) and brine (10 mL), dried (Na2SO4), and concentrated. Crude product was purified by column chromatography to get 16 (78 mg, 78%) as a white solid. Rf = 0.3 (50% ethyl acetate in hexane); mp 362–364 °C; νmax (film) cm⁻¹ 3179, 1634, 1599, 1418, 1368, 1272, 1085. 1H NMR (DMSO-d6, 500 MHz) δ 8.08 (s, 1H), 7.68 (d, 1H, J = 8.5 Hz), 7.15 (d, 1H, J = 2.0 Hz), 7.03 (s, 1H), 6.94 (dd, 1H, J = 8.5, 2.0 Hz). 13C NMR (DMSO-d6, 125 MHz) δ 158.2 (C), 157.5 (C), 157.3 (2C, C), 156.1 (C), 153.4 (C), 125.0 (CH), 120.8 (CH), 114.5 (C), 114.2 (CH), 107.0 (C), 105.4 (C), 103.9 (CH), 102.8 (C), 98.7 (CH). HRMS (EI+) m/z 345.9475 ([M]+ C19H16O3Br requires 345.9477).

2-Bromo-3,9-bis-methoxymethoxy-benzo[4,5]furo[3,2-c]chromen-6-one (17). i-Pr2NEt (0.05 mL, 0.29 mmol) was added to a solution of 16 (40 mg, 0.11 mmol) in CH2Cl2 (6 mL) at rt. The mixture was stirred for 15 min, and then MOMCl (0.02 mL, 0.25 mmol) was added. The reaction was stirred for 10 h and quenched by the addition of water (5 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL) and the organic layer was washed with water (2 × 10 mL) and brine (10 mL), dried (Na2SO4), and concentrated. The crude product was purified by column chromatography to get 17 (35 mg, 65%) as a white solid. Rf = 0.8 (30% ethyl acetate in hexane); mp 212–214 °C; νmax (film) cm⁻¹ 1758, 1629, 1490, 1353, 1257, 1102, 957. 1H NMR (CDCl3, 500 MHz) δ 8.14 (s, 1H), 7.94 (d, 1H, J = 8.5 Hz), 7.35 (s, 1H), 7.29 (s, 1H), 7.14 (d, 1H, J = 8.5 Hz), 5.33 (s, 2H), 5.25 (s, 2H), 3.53 (s, 3H), 3.51 (s, 3H). 13C NMR (CDCl3, 125 MHz) δ 159.0 (C), 158.0 (C), 157.2 (C), 156.5 (C), 156.3 (C), 153.9 (C), 125.6 (CH), 122.0 (CH), 117.6 (C), 115.5 (CH), 109.3 (C), 108.2 (C), 104.7 (CH), 102.7 (C), 99.9 (CH), 95.5 (CH2), 95.2 (CH2), 56.9 (CH3), 56.4 (CH2). HRMS (EI+) m/z 433.9992 ([M]+ C19H16O4Br requires 434.0001).

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Supporting Information Available: General experimental methods, experimental details, and characterization data for compounds 9, 12, 13, 15, 18, 19, 21, 23, and copies of 1H and 13C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.