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Synthesis of Sildenafil Analogues from Anacardic Acid and Their Phosphodiesterase-5 Inhibition

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Anacardic acid (6-pentadecylsalicylic acid), a major component of cashew nut shell liquid, consists of a heterogeneous mixture of monoenes, dienes, and trienes. The enes mixture of anacardic acid was hydrogenated to a saturated compound. Using saturated anacardic acid as a starting material, analogues of sildenafil [a potent phosphodiesterase-5 (PDE_5) inhibitor and an orally active drug for the treatment of erectile dysfunction] were synthesized, to observe the effect of the pentadecyl side chain on PDE_5 inhibition. The synthesized compounds were characterized by spectral studies and tested for PDE_5 inhibition, and the results were compared with those obtained with sildenafil.

KEYWORDS: Anacardic acid; cashew nut shell liquid; sildenafil; PDE_5 inhibitor; erectile dysfunction

INTRODUCTION

Phosphodiesterases (PDEs) are the key enzymes in the regulation of smooth muscle tone and play a very important physiological role by regulating the intracellular level of cyclic nucleotides. They are classified into five isoenzymes (*1*, *2*), PDE_1, PDE_2, PDE_3, PDE_4, and PDE_5, according to their specificity toward the hydrolysis of cyclic 3′,5′-adenosine monophosphate (cAMP) and cyclic 3′,5′-guanosine monophosphate (cGMP). PDEs catalyze the hydrolysis of cyclic nucleotides [3′,5′-guanosine monophosphate (cGMP) or adenosine monophosphate (cAMP)] to the corresponding 5′-nucleotides. Among phosphodiesterase isoenzymes, PDE_5 plays a very important role in male "erectile dysfunction" by inhibiting the hydrolysis of cGMP (*3*).

Erectile dysfunction is a fairly common medical problem (*4*), affecting about 40% of men over the age of 40 and 70% of men over the age of 70. It is also estimated that the number of men who have this condition will more than double in the next 25 years, ultimately affecting more than 330 million men worldwide (*5*). Sildenafil (**Figure 1**) is a potent PDE_5 inhibitor and an orally active drug in the treatment of erectile dysfunction (*6*, *7*), but it is associated with several side effects (*8*).

Anacardic acid (6-pentadecylsalicylic acid), a major component of cashew nut shell liquid (CNSL), is obtained by solvent extraction of cashew nut shells. It exists as a heterogeneous mixture of monoenes, dienes, and trienes along with toxic phenols (*9*). The presence of a long alkyl chain in anacardic acid is attributed to a variety of biological activities, such as antibacterial activity (*10*, *11*), antimicrobial activity (*12*), prostaglandin synthase inhibition (*13*), and tyrosinase (*14*) and lipoxygenase inhibition (*15*). To explore the potential of anacardic acid, it was extensively derivatized to drug analogues by several researchers (*16*, *17*). By considering biological and industrial application of CNSL constituents, we have developed a novel method for comprehensive isolation of all major phenolic constituents of CNSL (*18*). To make use of abundantly available anacardic acid for pharmaceutical application, our group has been working on the synthesis of drug analogues, and recently we reported the synthesis of 1,4-dihydropyridine derivatives as T-type calcium channel blockers (*19*).

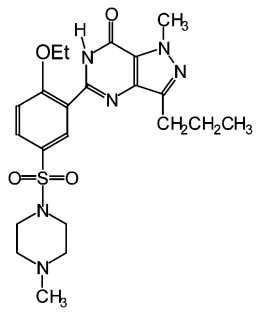

Figure 1. Structure of sildenafil (**18**).

In continuation of our ongoing effort to make a druglike candidate, we have designed and synthesized analogues of sildenafil from anacardic acid instead of salicylic acid. The presence of a long alkyl chain in these compounds may enhance the fat solubility, which in turn may increase the bioavailability of the resultant compound. Hence, to determine the effect of the pentadecyl side chain on PDE_5 inhibition, synthesis of sildenafil analogues from anacardic acid was attempted, and our preliminary results are described in this communication.

MATERIALS AND METHODS

Chemicals and Solvents. 2-Pentanone, diethyl oxalate, *N*-methylpiperazine, and dicyclohexylcarbodiimide were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI). Hydrazine hydrate, triethylamine, ferric chloride, potassium *tert*-butoxide, ammonium

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hydroxide, and chlorosulfonic acid were purchased from Merck (India). Radioactive cGMP (specific activity, 40.0 Ci/mol) and cold cGMP were purchased from Amersham Pharmacia Biotech Ltd. (UK). $MgCl_2$, EDTA, and DEAE cellulose anion-exchange resin were purchased from Sigma Chemicals (St. Louis, MO). Alkaline phosphatase (specific activity, 3143 u/mg) was purchased from Genei (Bangalore, India).

Instrumentation. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 200 MHz on a Bruker A G spectrometer, and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (Me_4Si) as internal standard. IR spectra were recorded with a Galaxy series FT-IR, and mass spectra were taken using GC−MS (Hewlett-Packard HP 5890/Shimadzu QP 5050A) and electrospray mass spectrometry (quadrupole mass spectrometer, Quattro II). Phosphodiesterase assay was performed using a liquid scintillation counter (LKB, Wallac Beta, Bruker). Melting points are uncorrected and were determined with a melting point apparatus (Arco Steels Pvt Ltd.). Elemental analyses were performed on a Carlo Erba Strumentazones elemental analyzer (MOD-1106).

Preparation of Saturated Anacardic Acid (1). To a solution of an ene mixture of anacardic acid (500 g, 1300 mmol) in methanol (2.0 L) was added 5% palladium−carbon (25 g), and then hydrogen gas was passed through the solution at 2.5 kg/cm^2 until consumption of hydrogen gas ceased (4−5 h). The reaction mass was filtered over a Celite bed and washed with methanol (100 mL). The filtrate was concentrated under reduced pressure to give an off-white solid (490 g), which on recrystallization from hexane yielded 475 g (95%) of the title compound. Mp: 89−90 °C [lit. (*18*) mp 90 °C].

Preparation of Ethyl 2-Ethoxy-6-pentadecylbenzoate (2). To a stirred solution of anacardic acid **1** (50 g, 143 mmol) in acetone (300 mL) was added anhydrous powdered potassium carbonate (80 g, 580 mmol). Diethyl sulfate (44.25 g, 290 mmol) was added in portions for about 10 min at room temperature. After the addition was complete, the solution was heated to reflux temperature on a water bath and maintained for 3 h. The solution was cooled to room temperature and then concentrated under reduced pressure. Distilled water (200 mL) was added to the reaction mixture, which was then extracted with ethyl acetate (200 mL). The organic layer was washed with distilled water (2 × 200 mL), dried over anhydrous sodium sulfate, and concentrated to yield the title compound (48 g, 82%). Mp: 34 °C. IR (KBr): 3010, 2920, 2863, 1730, 1588, 1463, 1260, 1185, 1109, 1070, 742 cm^{-1}. GC-MS: *m*/*z* (relative intensity) 404 (M^+, 23), 362 (55), 330 (20), 287 (16), 199 (12), 161 (100), 134 (30), 43 (60).

Preparation of 2-Ethoxy-6-pentadecylbenzoic Acid (4). To a stirred solution of compound **2** (10 g, 240 mmol) in dimethyl sulfoxide (40 mL) was added potassium *tert*-butoxide (10 g, 890 mmol) in portions. The solution was heated to 70 °C on a water bath for 2 h, and the progress of the reaction was monitored by TLC using a hexane−ethyl acetate (8:2) solvent system. The reaction mass was cooled to 10 °C, poured into ice water, and then acidified with 5% dilute hydrochloric acid. The precipitated solid was filtered and washed thoroughly with distilled water, and the crude mass was recrystallized in hexane (50 mL) to yield an off-white solid (7.5 g, 80%). Mp: 58−60 °C. IR (KBr): 2920, 2851, 1708, 1649, 1591, 1419, 1388, 1305, 1246, 1121, 1076, 800 cm^{-1}. GC-MS: *m*/*z* (relative intensity) 390 (M^+, 14), 359 (18), 245 (5), 189 (15), 175 (100), 134 (10), 43 (12) (for methyl ester).

Preparation of 2-Ethoxy-6-pentadecylbenzoyl Chloride (6). To a stirred solution of compound **4** (6.5 g, 16 mmol) in hexane (60 mL) were added thionyl chloride (2.5 g, 21 mmol) and *N*,*N*-dimethylformamide (0.5 mL). The reaction mixture was heated to reflux for 1 h. After the reaction was complete, the solvent was evaporated under reduced pressure to yield the desired acid chloride, which was redissolved in dichloromethane (50 mL) and used for the next step without further isolation. Mp: 46−48 °C. IR: 2920, 2850, 1792, 1586, 1470, 1270, 1248, 846, 648 cm^{-1}.

Preparation of 4-(2-Ethoxy-6-pentadecylbenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (9). To the cold solution of carboxamide **8** (3 g, 16 mmol) containing triethylamine (4.35 g, 43 mmol) in dichloromethane (50 mL) was added acid chloride **6** (6.5 g, 16.4 mmol) while maintaining the temperature at 0 °C. The reaction mixture was kept at 0−5 °C for 1 h and then brought to room temperature (28−30 °C). The progress of the reaction was monitored by TLC using a chloroform−methanol (9:1) solvent system. After the reaction was complete, distilled water (50 mL) was added. The organic layer was washed with 5% hydrochloric acid (100 mL), followed by distilled water (100 mL). The dichloromethane layer was dried over anhydrous sodium sulfate and then concentrated under reduced pressure to yield a crude mass. The solid was recrystallized from hexane to give an off-white solid (6.5 g, 72%). Mp: 145−148 °C. IR (KBr): 3527, 3484, 3383, 2962, 2922, 1688, 1606,1512, 1462, 1387, 1300, 1250, 1192, 1142, 1092, 1056, 870 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.84−0.90 (3 H, t, CH_3, J = 6.8 Hz), 0.93−1.011 (3 H, t, CH_3, J = 7.4 Hz), 1.24 (24 H, bs, $(CH_2)_{12}$), 1.35−1.42 (3 H, t, OCH_2CH_3), 1.58 (2 H, m, CH_2), 1.64−1.75 (2 H, m, CH_2), 2.53−2.61 (2 H, t, $ArCH_2$, J = 8.0 Hz), 2.66−2.70 (2 H, t, J = 8.1 Hz, $PyCH_2$), 4.06−4.17 (2 H, q, OCH_2, J = 7.0 Hz), 4.08 (3 H, s, N−CH_3), 5.6 (2 H, bs, NH_2), 6.78−6.82 (1 H, d, Ar H, J = 8.2 Hz), 6.88−6.92 (1 H, d, Ar H, J = 7.2 Hz), 7.27−7.35 (1 H, t, Ar H, J = 8.1 Hz), 7.6 (1 H, bs, NH). ES-MS: *m*/*z* 541.5 (M + 1).

Preparation of 4-[2-Ethoxy-5-(chlorosulfonyl)-6-pentadecylbenzamido]-1-methyl-3-*n*-propylpyrazole-5-carboxamide (11). To the chlorosulfonic acid (42 g, 2790 mmol) was added compound **9** (6 g, 11.4 mmol) while maintaining the temperature at 0−5 °C. The reaction was allowed to proceed at 5 °C until TLC analysis indicated the absence of starting material. After the reaction was complete, it was quenched on ice. The precipitated milky solid was filtered, washed thoroughly with distilled water (200 mL), and dried under suction to yield an off-white solid. The resultant solid was redissolved in dichloromethane and washed with distilled water (50 mL), and the organic layer was dried over anhydrous sodium sulfate and concentrated to yield the title compound as a milky white solid (6 g, 84%). Mp: 116−118 °C. IR (KBr): 3525, 3483, 3382, 2960, 2924, 1686, 1606,1622, 1480, 1380, 1320, 1280, 1190, 1140, 1092, 1050, 875 cm^{-1}.

Preparation of 4-[2-Ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)-6-pentadecylbenzamido]-1-methyl-3-*n*-propylpyrazole-5-carboxamide (13). To a stirred solution of compound **11** (6 g, 9.3 mmol) in distilled water (20 mL) was added *N*-methylpiperazine (1.4 g, 14 mmol) while maintaining the temperature at 5 °C. The reaction was allowed to proceed for 2 h, and the precipitated solid was filtered, washed thoroughly with distilled water, and dried under vacuum. The crude mass was purified on a silica gel column using chloroform−methanol to yield a yellowish-brown solid (2.4 g, 36%). Mp: 65−70 °C. IR (KBr): 3449, 3306, 2960, 1688, 1579, 1464, 1288, 1152 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.84−0.90 (3 H, t, CH_3, J = 6.7 Hz), 0.95−1.031 (3 H, t, CH_3, J = 7.4 Hz), 1.23 (24 H, bs, $(CH_2)_{12}$), 1.40−1.47 (3 H, t, OCH_2CH_3, J = 7.0 Hz), 1.67−1.78 (2 × 2 H, m, CH_2, CH_2), 2.26 (3 H, s, N−CH_3), 2.44 (4 H, ds t, $(CH_2)_2$), 2.53−2.61 (2 H, t, $ArCH_2$, J = 8.1 Hz), 2.97 (2 H, ds t, $PyCH_2$), 3.1 (4 H, ds t, $(CH_2)_2$), 4.05 (3 H, s, N−CH_3), 4.13−4.23 (2 H, q, OCH_2, J = 6.9 Hz), 5.82 (2 H, bs, NH_2), 6.84−6.88 (1 H, d, Ar H, J = 9.1 Hz), 7.27 (1 H, s, NH), 7.88−7.93 (1 H, d, Ar H, J = 9.0 Hz). ES-MS: *m*/*z* 703.4 (M + 1).

Preparation of 5-[2-Ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)-6-pentadecylphenyl]-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (15). To a stirred solution of compound **13** (1.2 g, 1.7 mmol) in dry xylene (20 mL) was added dicyclohexylcarbodiimide (DCC) (2 g, 9.6 mmol). The solution was heated to reflux until the reaction was complete (38 h). The progress of the reaction was monitored by TLC analysis using chloroform−methanol (9:1) as the mobile phase. The reaction mixture was concentrated to remove the solvent under reduced pressure. Distilled water (25 mL) was added to the residue and extracted with ethyl acetate (25 mL). The organic layer was dried over anhydrous sodium sulfate, concentrated to dryness, and then purified by column chromatography on silica gel by eluting with chloroform−methanol (1−5%) of increasing polarity. The resultant compound was recrystallized in ethyl alcohol to give the title compound (0.6 g, 51.7%). Mp: 154−156 °C. IR (KBr): 3370, 3308, 2942, 2859, 1663, 1602, 1456, 1336, 1289, 1155 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.84−0.90 (3 H, t, CH_3, J = 6.7 Hz), 0.97−1.04 (3 H, t, CH_3, J = 7.4 Hz), 1.24 (24 H, bs, $(CH_2)_{12}$), 1.32−1.39 (3 H, t, OCH_2CH_3, J = 7.0 Hz), 1.61 (2 H, s, CH_2), 1.73−1.80 (2 H, m, CH_2), 2.27 (3 H, s, N−CH_3), 2.43 (4 H, ds t, $(CH_2)_2$), 2.84−2.88 (2 H, t, $ArCH_2$, J = 8.0 Hz), 2.91−2.95 (2 H, ds t, $PyCH_2$), 3.17 (4 H, ds t, $(CH_2)_2$), 4.03−4.10 (2 H, q, J = 7.0 Hz, OCH_2), 4.11 (3 H, s, N−CH_3), 6.72−6.82 (1 H, d, Ar H, J = 9.0 Hz),

7.93−7.98 (1 H, s, J = 8.9 Hz), 9.52 (1 H, s, NH, D_2O exchangeable). ^{13}C NMR (300 MHz, $CDCl_3$): δ 14.05, 14.43, 14.45, 21.7, 22.6, 24.6, 24.2, 25.2, 29.2, 29.6, 29.7, 30.7, 31.88, 32.0, 32.8, 39.59, 44.9, 45.5, 50.08, 54.1, 64.1, 108.2, 120.1, 127.6, 130.3, 130.7, 133.0, 143.2, 144.7, 157.9, 159.2, 164.2, 170.27. ES-MS: m/z 685.45 (M + 1). Anal. Found: C, 64.97; H, 8.86; N, 12.30. Calcd for $C_{37}H_{60}N_6O_4S$: C, 64.87; H, 8.82; N, 12.26.

Preparation of 5-(2-Ethoxy-6-pentadecylphenyl)-1-methyl-3-*n*-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (17). To a solution of compound **9** (0.5 g, 0.92 mmol) in dry xylene (10 mL) was added dicyclohexylcarbodiimide (1 g, 4.8 mmol). The reaction mixture was heated to reflux until the reaction was complete (38 h). After the reaction was complete, the reaction mixture was concentrated under reduced pressure. Distilled water (10 mL) was added to the residue and extracted with ethyl acetate (20 mL). The organic layer was dried over anhydrous sodium sulfate, concentrated under reduced pressure to yield a brown residue, and then purified by column chromatography on silica gel by eluting with chloroform−methanol (1−5%). The resultant solid was recrystallized in ethyl alcohol (10 mL) to give the colorless title compound (0.3 g, 62%). Mp: 126−127 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 0.84−0.9 (3 H, t, CH_3, J = 6.6 Hz), 0.96−1.03 (3 H, t, CH_3, J = 7.4 Hz), 1.24 (24 H, bs, $(CH_2)_{12}$), 1.28−1.35 (3 H, t, OCH_2CH_3, J = 7.0 Hz), 1.60 (2 H, bs, CH_2), 1.72−1.84 (2 H, m, CH_2), 2.61−2.69 (2 H, t, $ArCH_2$, J = 8.1 Hz), 2.85−2.93 (2 H, t, $PyCH_2$, J = 8.1 Hz), 3.92−4.03 (2 H, q, J = 7.0 Hz, OCH_2), 4.10 (3 H, s, N−CH_3) 6.68−6.72 (1 H, d, Ar H, J = 7.9 Hz), 6.79−6.82 (1 H, d, Ar H, J = 7.9 Hz), 7.16−7.26 (1 H, t, Ar H, J = 8.0 Hz), 9.37 (1 H, s, NH, D_2O exchangeable).

Preparation of Methyl 2-Methoxy-6-pentadecylbenzoate (3). In a procedure similar to that for the synthesis of **2**, anacardic acid **1** (50 g, 143 mmol) was treated with dimethyl sulfate (36.2 g, 280 mmol) and anhydrous potassium carbonate (80 g, 580 mmol) in acetone to yield the title compound (51 g, 94.4%). Mp: 37−38 °C. IR (KBr): 3006, 2922, 2853, 1732, 1588, 1465, 1268, 1185, 1109, 1071, 752 cm^{-1}. GC-MS: *m*/*z* (relative intensity) 376 (M^+, 35), 345 (46), 317 (3), 273 (2), 204 (9), 161 (100), 121 (15), 91 (10).

Preparation of 2-Methoxy-6-pentadecylbenzoic Acid (5). In a procedure similar to that for the synthesis of **4**, compound **3** (20 g, 53 mmol) was hydrolyzed in dimethyl sulfoxide (80 mL) with potassium *tert*-butoxide (20 g, 178 mmol) to give an off-white solid (14 g, 73%). Mp: 78−79 °C. IR (KBr): 2920, 2851, 1708, 1649, 1591, 1419, 1388, 1305, 246, 1121, 1076, 800 cm^{-1}. GC-MS: *m*/*z* (relative intensity) 376 (M^+, 35), 345 (46), 317 (3), 273 (2), 204 (9), 161 (100), 121 (15), 91 (10) for methyl ester.

Preparation of 2-Methoxy-6-pentadecylbenzoyl Chloride (7). In a procedure similar to that for the synthesis of **6**, compound **5** (5.0 g, 13.8 mmol) in hexane (50 mL) was treated with thionyl chloride (2 g, 16.8 mmol) to give an acid chloride, which was taken up for the next step. Mp: 52−54 °C. IR (KBr): 2919, 2850, 1794, 1585, 1472, 1271, 1247, 1078, 847, 645 cm^{-1}.

Preparation of 4-(2-Methoxy-6-pentadecylbenzamido)-1-methyl-3-*n*-propylpyrazole-5-carboxamide (10). In a procedure similar to that for the synthesis of **9**, compound **8** (2.6 g, 14 mmol) was condensed with **7** to yield an off-white solid (5.2 g, 75%). Mp: 150−152 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 0.84−0.90 (3 H, t, CH_3, J = 6.8 Hz), 0.94−1.01 (3 H, t, CH_3, J = 7.4 Hz), 1.24 (24 H, bs, $(CH_2)_{12}$), 1.64 (2 H, bs, CH_2), 1.67−1.75 (2 H, m, CH_2), 2.54−2.61 (2 H, t, $ArCH_2$, J = 8.0 Hz), 2.66−2.73 (2 H, t, $PyCH_2$, J = 8.1 Hz), 3.85 (3 H, s, OCH_3), 4.08 (3 H, s, NCH_3), 5.73 (2 H, bs, NH_2), 6.79−6.83 (1 H, d, Ar H, J = 8.2 Hz), 6.89−6.93 (1 H, d, Ar H, J = 7.8 Hz), 7.29−7.39 (1 H, t, Ar H, J = 8.0 Hz), 7.63 (1 H, bs, NH, D_2O exchangeable).

Preparation of 4-[2-Methoxy-5-(chlorosulfonyl)-6-pentadecylbenzamido]-1-methyl-3-*n*-propylpyrazole-5-carboxamide (12). In a procedure similar to that for the synthesis of **11**, compound **10** (4.5 g, 8.5 mmol) was treated with chlorosulfonic acid to yield an off-white solid (4 g, 75%). Mp: 158−160 °C. IR (KBr): 3524, 3482, 3380, 2966, 2921, 1685, 1602, 1627, 1485, 1382, 1322, 1280, 1195, 1144, 1090, 1050, 874 cm^{-1}.

Preparation of 4-[2-Methoxy-5-(4-methylpiperazin-1-ylsulfonyl)-6-pentadecylbenzamido]-1-methyl-3-*n*-propylpyrazole-5-carboxamide (14). In a procedure similar to that for the synthesis of **13**, compound **12** (4 g, 6.4 mmol) was condensed with *N*-methylpiperazine (0.9 g, 8.9 mmol) to yield an off-white solid (2.6 g, 59%). Mp: 80−85 °C. IR (KBr): 3450, 3310, 2960, 1688, 1579, 1464, 1288, 1152 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.84−0.90 (3 H, t, CH_3, J = 6.8 Hz), 0.94−1.01 (3 H, t, CH_3, J = 7.2 Hz), 1.23 (24 H, bs, $(CH_2)_{12}$), 1.63 (2 H, m, CH_2), 1.72−1.80 (2 H, m, CH_2), 2.25 (3 H, s, N−CH_3), 2.42 (4 H, ds t, $(CH_2)_2$), 2.54−2.62 (2 H, t, $ArCH_2$, J = 8.0 Hz), 2.92−2.97 (2 H, ds t, $PyCH_2$), 3.14 (4 H, ds t, $(CH_2)_2$), 3.86, (3H, s, OCH_3), 4.12 (3 H, s, N−CH_3), 5.84 (2 H, bs, NH_2), 6.82−6.84 (1 H, d, Ar H, J = 8.9 Hz), 7.25 (1 H, bs, NH), 7.86−7.90 (1 H, d, Ar H, J = 9.0 Hz).

Preparation of 5-[2-Methoxy-5-(4-methylpiperazin-1-ylsulfonyl)-6-pentadecylphenyl]-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (16). In a procedure similar to that for the synthesis of **15**, compound **14** (2 g, 2.9 mmol) was cyclized with dicyclohexylcarbodiimide (3 g, 14.5 mmol) to give the title compound (0.5 g, 25%).

Mp: 150−152 °C. IR (KBr): 3370, 3308, 2942, 2859, 1663, 1602, 1456, 1336, 1155 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 0.84−0.90 (3 H, t, CH_3, J = 6.8 Hz), 0.97−1.04 (3 H, t, CH_3, J = 7.4 Hz), 1.25 (24 H, bs, $(CH_2)_{12}$), 1.63 (2 H, m, CH_2), 1.72−1.83 (2 H, m, CH_2), 2.27 (3 H, s, N−CH_3), 2.43 (4 H, ds t, $(CH_2)_2$), 2.83−2.87 (2 H, t, $ArCH_2$, J = 8.0 Hz), 2.91−2.95 (2 H, ds t, $PyCH_2$), 3.18 (4 H, ds t, $(CH_2)_2$), 3.83 (3 H, s, OCH_3), 4.11 (3 H, s, N−CH_3), 6.80−6.85 (1 H, d, Ar H, J = 9.0 Hz), 7.96−8.01 (1 H, d, Ar H, J = 9.0 Hz), 9.48 (1 H, s, NH, D_2O exchangeable). GC-MS: *m*/*z* (relative intensity) 670 (M^+, 6), 462 (10), 373 (13), 319 (10), 232 (8), 146 (100), 135 (29), 104 (10), 70 (23). Anal. Found: C, 64.68; H, 8.81; N, 12.45. Calcd for $C_{36}H_{58}N_6O_4S$: C, 64.44; H, 8.71; N, 12.52.

Isolation of PDE_5 Isoenzyme. Freshly dissected goat penile tissue was chopped into small pieces using a sterilized knife and homogenized in a buffer containing 0.2 mM Tris-hydrochloride, 0.2 mM EDTA, and 0.25 mM mercaptoethanol. The homogenate was filtered through cheesecloth and centrifuged at 8 k/20′/4 °C in a Beckman centrifuge. The supernatant was filtered through cheesecloth, partially purified by DEAE-Sepharose column chromatography (*20*), and frozen at −20 °C. This sample was used in each phosphodiesterase inhibition assay.

Phosphodiesterase$_5$ Inhibition Assay. Synthesized compounds were tested for PDE_5 inhibition by the method of Kincaid and Manganiello (*21*); sildenafil was used as an active control in the PDE_5 inhibition assay.

RESULTS AND DISCUSSION

The ene mixture of anacardic acid isolated from cashew nut shell liquid was hydrogenated to saturated anacardic acid **1** (*18*) (saturated anacardic acid is generally termed as tetrahydroanacardic acid or anacardic acid). During the synthesis of sildenafil analogues from anacardic acid, different methods were attempted for selective O-alkylation of anacardic acid with a variety of alkylating agents, such as methyl iodide, ethyl iodide, dimethyl sulfate, and diethyl sulfate. In all of these experiments, alkoxy ester formation was observed. Further hydrolysis of alkoxy ester **2**/**3** to acid **4**/**5** was not achieved with either sodium hydroxide or hydrochloric acid/sulfuric acid, probably due to steric hindrance. Finally, it was achieved with potassium *tert*-butoxide in anhydrous DMSO, a method described for the hydrolysis of sterically hindered ester (*22*). Acid chloride **6** was prepared by heating a mixture of alkoxyanacardic acid **4** and thionyl chloride in the presence of a catalytic amount of *N*,*N*-dimethylformamide (**Figure 2**). 4-Amino-1-methyl-3-*n*-propylpyrazole-5-carboxamide was prepared according to the reported procedure (*23*).

Compound **9** was prepared by condensation of amine **8** with acid chloride **6** in dichloromethane in the presence of triethylamine as acid scavenger. This was further reacted with chlorosulfonic acid at 5 °C to give the corresponding chlorosulfonated compound **11**, which was condensed with *N*-methylpiperazine to yield compound **13**. Cyclization of **13** was not effected by either the method reported for the preparation of sildenafil (*22*, *23*) or the reaction with potassium *tert*-butoxide in *t*-BuOH/

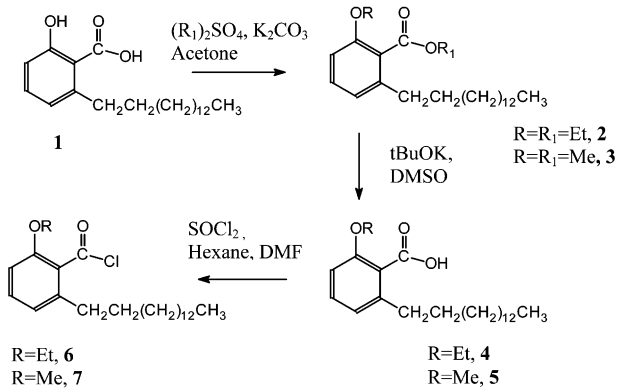

Figure 2. Synthesis of 2-alkoxy-6-pentadecylbenzoyl chloride (**6/7**).

DMSO/DMF at reflux temperature. The reaction with phosphoric acid or with phosphorous pentoxide yielded an undesired product. Finally, cyclization was achieved with dicyclohexylcarbodiimide in xylene to yield sildenafil analogue **15** (**Figure 3**). Sildenafil **18** was synthesized by the reported method (*23*, *24*) and characterized by spectral studies. This sample was used as a reference sample for comparison and for PDE_5 inhibition assay.

The ^1H and ^{13}C NMR spectra of analogue **15** were similar to those of sildenafil, except for a few additional peaks due to the pentadecyl side chain. In the ^1H NMR spectra of **15**, the triplet at δ 0.84−0.90 is assigned to the terminal methyl of the pentadecyl side chain and that at δ 0.97−1.04 to methyl protons of propyl chain, while another triplet at δ 1.32−1.39 is assigned to the methyl group of the ethoxy group. The broad singlet at δ 1.24 is attributed to 12-methylene groups of the C_{15} alkyl side chain. The methylene group adjacent to the benzylic group in the C_{15} side chain appeared as a distorted multiplet at δ 1.61, while that on the propyl chain on pyrazolopyrimidone appeared as a multiplet at δ 1.73−1.80. Benzylic protons are seen as a triplet at δ 2.84−2.88, and methylene protons on the pyrazole ring also appeared as a triplet at δ 2.91−2.95. The four methylene groups of the *N*-methylpiperazine ring appeared as two broad singlets (distorted triplets) at δ 2.43 and 3.17. The methylene protons of the ethoxy group are seen as a quartet at δ 4.03−4.1. The *N*-methyl protons on pyrazolopyrimidone and the piperazine ring appeared as singlets at δ 4.11 and 2.27, respectively. Two aromatic protons on the phenyl ring appeared as doublets at δ 6.72−6.82 and 7.93−7.98, and the NH proton on the pyrazolopyrimidone ring appeared as a singlet at δ 9.52, as compared to δ 10.5 for sildenafil, which was readily exchangeable with D_2O. The IR spectrum bands at 3370 (N−H), 3036 (Ar C−H), and 1663 cm^{-1} (C═O) were similar to bands observed for sildenafil. Also, electrospray mass spectrometry showed an M + 1 peak, similar to that observed for sildenafil.

Sildenafil analogues (**Figure 4**) synthesized from anacardic acid were evaluated on partially purified PDE_5 enzyme. The enzyme was isolated by a method similar to that reported by Burns et al. (*20*) from the penile tissue of a goat. PDE_5 inhibition was studied by the method of Kincaid and Manganiello (*21*), and the results are summarized in **Table 1**.

Compounds **15** and **16**, which have a pentadecyl side chain on the phenyl ring, showed an IC_{50} of 145 and 125 μM, respectively, whereas the reference molecule sildenafil **18** has shown 38 μM under similar experimental conditions. Compound **17**, an analogue of **15** without the *N*-methylpiperazinesulfonamide moiety, inhibited the PDE_5 enzyme with an IC_{50} of 160 μM.

The sildenafil analogues **15** and **16**, synthesized from anacardic acid, have shown less potency compared to sildenafil **18**. Hence, introduction of a pentadecyl side chain may alter

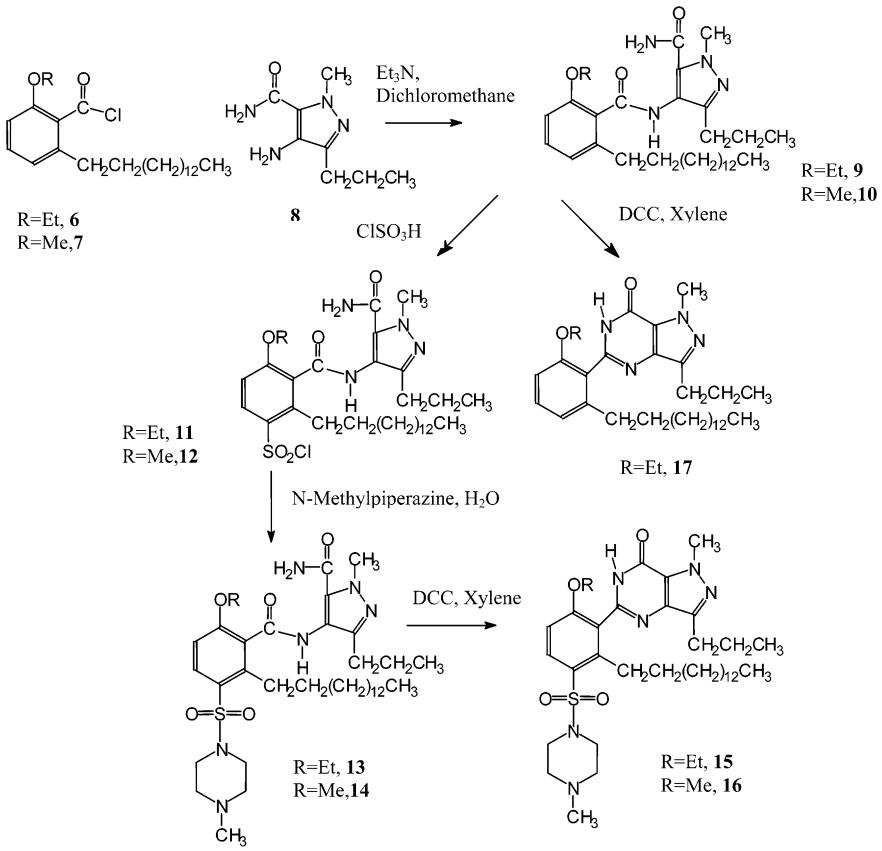

Figure 3. Synthesis of sildenafil analogues (**15**, **16**, and **17**).

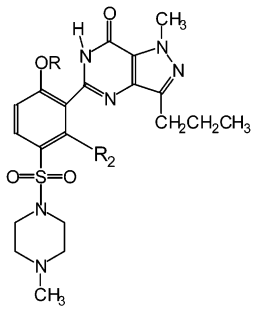

Figure 4. Structure of sildenafil analogues.

Table 1. Results of PDE_5 Inhibition of Sildenafil Analogues

compound	R	R_2	IC_{50} (μM)
15	CH_2CH_3	$C_{15}H_{31}$	145
16	CH_3	$C_{15}H_{31}$	125
17[a]	CH_2CH_3	$C_{15}H_{31}$	160
18	CH_2CH_3	H	38

[a] *N*-Methylpiperazinesulfonamide moiety is absent.

the orientation of the sildenafil molecule at the site of action, resulting in a decrease in the PDE_5 inhibition. Compound **17** is less potent compared to **15**, indicating the essentiality of the *N*-methylpiperazinesulfonamide group in the molecule, and this observation is consistent with the SAR reported on sildenafil (*6*).

SAFETY PRECAUTIONS

Use protective apron, hand gloves, goggles, and air respirator during the preparation of compounds **6**, **7**, **11**, and **12**. Special care must be taken to avoid the inhalation of vapors of thionyl chloride and chlorosulfonic acid.

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Supporting Information Available: ^{1}H NMR spectra of **9**, **13**, **16**, and **17** and comparative ^{1}H NMR spectra of **15** and sildenafil **18**; ^{13}C NMR spectrum of **15**; and mass spectra of **9**, **13**, and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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