Hexafluoroisopropanol-Mediated Domino Reaction for the Synthesis of Thiazolo-androstenones: Potent Anticancer Agents

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ABSTRACT: A cascade reaction of thioamides with 6β-bromoandrostenedione in hexafluoroisopropanol formed substituted thiazolo-androstenones. This is a simple and mild protocol to synthesize novel molecules by using readily available reagents and substrates. Feasibility of the reaction has been rationalized by density functional theory calculations. Moreover, these compounds are potent growth inhibitors of colon, central nervous system, melanoma, ovarian, and renal cancer cell lines with 50% growth inhibition values as low as 1.04 μM.

INTRODUCTION

Several hormones of the steroidal skeleton are found in biological signaling in mammals.1,2 A large number of bioactive steroidal natural products have been isolated from various plants and microorganisms.3,4 Numerous synthetic derivatives have also been reported in literature in a quest of drugs, drug candidates, and other valuable materials including herbicides.5−11 Natural and synthetic steroidal derivatives are known to show a number of useful pharmacological properties such as agonists of cell-surface G-protein-coupled bile acid receptor,12 neuroprotective,13 anticancer,14 and anti-Alzheimer15 properties.7,16 Unnatural steroidal derivatives are one of the broadest spectra of therapeutic classes of compounds, which are used to treat different diseases including cancer.17 Thiazone derivatives are another class of important compounds with several approved drugs such as dasatinib, fanetizole, and nizatidine.18,19 Several steroidal drugs contain heterocyclic moieties: oxazole in Emflaza (deflazacort) and pyridine in Zytiga (abiraterone acetate).20−22 Thiazone-attached progesterone derivatives have been reported as potent SKOV-3 (ovarian cancer) growth inhibitor.23 Pyrazole-fused sterone, stanazolol, derivative is known for potent anabolic activities (Figure 1).24 Not surprisingly, syntheses of heterocycle-incorporated steroidal derivatives have been reported in a large number of literature.25−31 Novel molecules based on the steroidal core structure are synthesized in a multistep synthesis7,12,32−34 and using catalyst.29

RESULTS AND DISCUSSION

In our quest to synthesize bioactive molecules35−37 and to develop new domino reactions to synthesize heterocycles,38,39

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Figure 1. Representative examples of heterocycle containing steroidal drugs and pharmacologically active molecules.
we planned the synthesis of thi azolino-androstanedione derivatives by using our recently reported methodology, the synthesis of thiazoline derivatives (2) by reacting thioamides with γ-bromoenones (1). Reaction of 6β-bromoandrostanedione (3) with thiourea derivatives formed aminothiazolando- drostenone derivative (4) by an unexpected mechanism. Surprisingly, reaction of thioamide derivative (5) with the electrophile (3) did not form the product in re fluxing ethanol, as we expected from our previous report (Scheme 1). To our delight, the reaction happened in hexafluoroisopropanol (HFIP) in 61% yield, and the reaction did not require an anhydrous solvent and inert atmosphere. The products formed cleanly, and the pure material was isolated simply by distilling out HFIP followed by recrystallizing with methanol (Scheme 2). Column chromatography was not required to obtain the pure product (6). After identifying the product as thiazolo-androstenone derivative in HFIP, we carried out the reaction in different solvents including different alcohols and polar aprotic solvents: tetrahydrofuran, dimethyl sulfoxide (DMSO), and N,N-dimethylformamide (DMF); however, the reaction was not successful in any solvent except trifluoroethanol in moderate yield. Re fluxing the reaction mixture in DMF gave the unidentifiable decomposed products. On the basis of these observations, we can conclude that a very polar protic solvent is required for the product formation of this domino methodology, and HFIP has optimum properties for the success of this protocol.

Furthermore, dihydroxy thiobenzamide also reacted smoothly to give the expected product (19) in 58% yield. Thus, the number and position of the electron-donating groups did not alter the outcome of the corresponding products. Products containing electron-withdrawing substituents were obtained under the established reaction conditions. 3-Fluoro-substituted thiazolo-androstenone derivative (20) formed in 60% yield, and 4-fluorophenyl-substituted compound (21) was obtained in 61% yield. A complex thioamide, 4-[5-(trifluoromethyl)pyrid-2-yloxy]thiobenzamide, did not hamper the reaction, and the product (14) was obtained in good yield. 4-Methoxy-substituted product (15) was obtained in 60% yield. Hydroxy-substituted products (16, 17, and 18) were obtained expectantly.

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electron-withdrawing group do effect the product formation of this methodology. Finally, the limitation we found in this methodology is that pyridine-4-carbothioamide and pyrimidine-2-carbotioamide failed to give the corresponding products (29 and 30). Thus, this methodology has the potential to generate a new class of novel molecules based on the fused thiazolo-androstane scaffold.

These molecules can be further transformed into new entities by simple reactions (Scheme 4). 17-Hydroxy and 17-aceloxo derivatives of androstane skeleton are integral parts of drugs, hormones, natural products, and synthetic bioactive molecules.\textsuperscript{44−46} Ketone derivative (20) was reduced with NaBH\textsubscript{4} and led stereoselectively to the corresponding hydroxy product (31) in an excellent yield. Further acetylation with Ac\textsubscript{2}O/pyridine afforded the acetylated product (32) in quantitative yield.

The structures of compounds (20, 21, 25, and 32) were confirmed by single-crystal X-ray diffraction analysis, which has helped to establish the regiochemistry and stereochemistry of the reactions. Crystal structures (20, 21, and 25) have helped to confirm the regiospecificity of this methodology. The final product (32) confirmed the formation of substrate-controlled β-hydroxy product exclusively in NaBH\textsubscript{4} reduction (Figure 2).

**Computational Analysis.** All density functional theory calculations were carried out using Gaussian 09 suite of programs.\textsuperscript{46} The hybrid density functional method (M06-2X)/6-311++G(d,p) + PCM (solvent = HFIP) has been used to compute the feasibility of all four pathways. We have used polarizable continuum model (PCM) using the integral equation formalism variant as the self consistent reaction field method. This method creates solute cavity via a set of overlapping spheres. The free energy of all of the species was...
Figure 2. Oak ridge thermal ellipsoid plot diagrams of 20 (CCDC 1859241), 21 (CCDC 1859222), 25 (CCDC 1861085), and 32 (CCDC 1859223).

Scheme 5. Plausible Mechanism for the Formation of Product (7) Using M06-2X/6-311++G(d,p) + PCM (Solvent = HFIP) Level of Theory
calculated using the PCM solvent model i.e., HFIP. The calculations were done at 1 atm pressure and 298 K.

HFIP is a very strong hydrogen bond donor, which makes hydrogen bonding with the carbonyl oxygen of the enone of β-bromoandrostenedione (3). This hydrogen bonding makes the enone carbonyl group a better electrophile for the nucleophilic addition of thioacetamide, which is the key for the success of this methodology. Sulfur or nitrogen atom of thioacetamide can undergo nucleophilic addition to the carbonyl group of β-bromoandrostenedione (3) to form two possible intermediates, hemithioacetal (A1) or hemiaminal (B1), respectively. Both of these two reactions are endergonic, and the Gibb’s free energy for the formation of hemithioacetal (A1) and hemiaminal (B1) is achieved by refluxing the reaction mixture in HFIP. These reactions for the formation of hemithioacetal (A1) and hemiaminal (B1) are reversible under the reaction condition. Hemithioacetal (A1) and hemiaminal (B1) undergo intramolecular SN2′ reaction to form the thiazoline regioisomers (A2 and B2). This SN2′ reaction of hemithioacetal (A1) is more favorable than that of hemiaminal (B1) (−25.57 vs −22.07 kcal/mol). Dehydration, the final step, is more favorable for the hemithioacetal than the hemiaminal derivative (−28.01 vs −22.52 kcal/mol) to form the final products A3 and 7, respectively. Among the three steps for the formation of possible products, the last two steps are irreversible and exergonic (Scheme 5). Hence, the less-energy barrier for the formation of hemiaminal (B1) in the first step is the deciding factor for the formation of the actual product (7). The other possible pathway (C1 → B2) for the formation of expected product (7) is the least favorable. The expected product (D3) based on our previous report is also thermodynamically not favorable (Figure 2). Probable potential energy surface for all of the possible pathways is shown in Figure 3.

**In Vitro Anticancer Studies.** After the successful synthesis of these novel molecules, some of them have been tested against NCI-60 cancer cell lines. Many of these compounds have shown promising activity against several cancer cell lines at 10 μM concentration. Selected growth inhibition data for compound 20 are shown in Table 1. This compound has

### Table 1. Cytotoxic Data of Compound 20 against NCI-60 Cell Lines

| cancer panel | cell line | GI50 (μM) | TGI (μM) | LC50 (μM) |
|--------------|-----------|-----------|----------|-----------|
| leukemia     | HL-60(TB) | 2.00      | 5.35     | >10       |
|              | K-562     | 1.82      | 9.08     | >10       |
|              | MOLT-4    | 1.69      | 7.89     | >10       |
|              | RPMI-8226 | 1.91      | 5.65     | >10       |
|              | HOP-92    | 2.44      | 5.63     | >10       |
|              | NCI-H226  | 1.94      | 4.39     | >10       |
|              | NCI-H460  | 2.79      | 8.27     | >10       |
| colon cancer | COLO 205  | 2.42      | 5.46     | >10       |
|              | HCT-116   | 1.73      | 3.56     | 7.33      |
|              | HCT-15    | 1.56      | 4.17     | >10       |
|              | SW-620    | 2.14      | 4.50     | >10       |
| CNS cancer   | SF-295    | 1.59      | 4.91     | >10       |
| melanoma     | SF-539    | 1.04      | 2.48     | 5.90      |
| ovary cancer | LOX IMVI  | 1.74      | 3.36     | 6.47      |
|              | MALME-3M  | 1.80      | 4.13     | 9.48      |
|              | SK-MEL-28 | 1.53      | 4.07     | >10       |
|              | UACC-62   | 1.90      | 5.70     | >10       |
| ovarian cancer| IGROV1   | 2.09      | 4.88     | >10       |
|              | OVCAR-3   | 1.92      | 3.79     | 7.47      |
| renal cancer | SKOV-3    | 2.91      | 9.13     | >10       |
| prostate cancer | 786-0    | 1.30      | 2.80     | >10       |
|              | ACHN      | 1.64      | 3.01     | 5.53      |
|              | CAKI-1    | 1.87      | 3.76     | 7.57      |
|              | RXF 393   | 1.41      | 2.94     | 6.14      |
|              | TK-10     | 2.77      | 8.29     | >10       |
| prostate cancer | DU-145   | 2.01      | 4.13     | >10       |
| breast cancer | MCF7     | 1.78      | 4.26     | >10       |
|              | MDA-MB-231/ATCC | 2.60 | 7.34 | >10 |
|              | BT-549    | 2.20      | 8.06     | >10       |
|              | T-47D     | 2.00      | 5.24     | >10       |
|              | MDA-MB-468| 1.95      | 4.27     | >10       |
inhibited the growth of 31 of 60 cell lines with 50% growth inhibition (GI_{50}) value <2.80 μM concentration. The total growth inhibition (TGI) value is also in the low micromolar range. The compound (20) has also shown good lethal concentration (LC_{50}) values against nine cancer cell lines. HCT-116 and SF-539 were inhibited with LC_{50} values of 7.30 and 5.90 μM concentrations, respectively.

Colorectal cancer is the third most common cancer in men and women in the US, nevertheless, this cancer is second leading cause of cancer-related deaths in this country.47 Central nervous system (CNS) cancer is one of the most lethal forms of cancer with very limited treatment options.48 Our tested molecule (20) has shown significant activity against four colon cancer cell lines with GI_{50} values as low as 1.56 μM concentration. This molecule inhibited the growth of HCT-116 cell line with TGI and LC_{50} values 3.56 and 7.33 μM, respectively. Growth of two of the six CNS cancer cell lines were also inhibited significantly with GI_{50} values ~1 μM concentration. In vitro growth inhibition of SF-539 cancer cell line of the CNS panel is very significant with TGI and LC_{50} values of 2.48 and 5.90 μM, respectively.

Melanoma is the most serious type of skin cancer. It is the 5th–7th most common cancer in the United States, and the incidence of this cancer is increasing rapidly. Other than the skin, this cancer can also develop in the eyes and in internal organs such as the intestines.49 Development of new therapeutic options is urgently needed to treat this rapidly rising malignancy.50 Our lead compound (20) has shown promising growth inhibition activity against four melanoma cell lines with GI_{50} less than 2 μM concentration. TGI values are also in low micromolar concentration for these four melanoma cell lines. This compound also inhibited the two melanoma cell lines: LOX IMVI and MALME-3M cell lines with LC_{50} values of 6.47 and 9.48 μM, respectively. We have found several lead molecules such as 20 to generate a library of molecules to develop potent antitumor agents.

In addition, compound 20 has shown promising activity against four ovarian cancer cell lines with GI_{50} values at low micromolar concentration. The LC_{50} value for ovarian cancer cell line, OVCAR-3, is less than 10 μM. Renal cancer is the most common type of kidney cancer.51 The fluophenyl derivative (20) has shown potent activity against six cancer cell lines with GI_{50} and TGI values as low as 1.30 and 2.80 μM, respectively. Four renal cancer cell lines were inhibited with LC_{50} values less than 10 μM. Significant growth inhibition of prostate and breast cancer cell lines was also observed by this lead molecule (20). Complete data are shown in the Supporting Information. Detailed findings and mode of action of potent molecules will be reported soon.

**CONCLUSIONS**

In summary, we have discovered a one-pot protocol to synthesize novel thiazolo-androstenediones by using readily available starting materials and benign reaction conditions. On the basis of the availability of a number of thioamide starting materials and ease of reaction conditions, a large number of novel molecules as potent anticancer agents can be synthesized. Furthermore, these new scaffolds can be easily transformed into a variety of potential bioactive molecules. Further derivatization, structure–activity relationship, anticancer, and toxicity studies of these novel compounds are in progress and will be reported soon.

**EXPERIMENTAL SECTION**

**General Consideration.** All of the reactions were carried out under air atmosphere in round-bottom flasks. Solvents, reagents, and the substrate were bought from Fisher Scientific and Oakwood chemical.

**Characterization.** ^1^H NMR and ^13^C NMR spectra were recorded with a Varian Mercury-300 MHz and Varian Mercury-75 MHz, respectively, with tetramethylsilane (TMS) as internal standard. CDCl₃ (>99.9%), DMSO-d₆ (>99.8%), or mixture of both were used to record NMR spectra. In some spectra, trifluoroacetic acid-d₄ was also used to increase the solubility of the samples. The electrospray ionization-Fourier transform mass spectra (ESI-FTMS) were recorded using Bruker ApexII-FTMS system.

Crystals were grown in chloroform–methanol mixture for single-crystal diffraction.

**General Procedure for the Synthesis of Thiazolo-androstenedones (6–28).** A mixture of β-bromoandrostenedione (1 mmol), thioamide derivative (1.1 mmol), and sodium acetate (82 mg, 1.0 mmol) in 10 mL of hexafluoroisopropanol was refluxed for 12 h to complete the reaction. Progress of the reaction was monitored by thin-layer chromatography. After the completion of the reaction, HFIP was distilled out and methanol (10 mL) was added. The solid precipitate was filtered following by washing with ~10 mL of methanol and ~20 mL of water under vacuum to afford the pure product.

**Characterization Data.** (15,2R,13R,14S,18S)-2,18-Dimethyl-7-phenyl-8-thia-6-azapentacyclo[11.7.0.0²,10.05,9.014,18]icosa-5(9),7,10-trien-17-one (6). Yellowish solid, ^1^H NMR (300 MHz, CDCl₃) δ ppm: 7.92–7.90 (m, 2H), 7.46–7.40 (m, 3H), 7.36–7.24 (m, 1H), 3.05–2.86 (m, 2H), 2.56–2.34 (m, 2H), 2.20–1.80 (m, 6H), 1.68–1.16 (m, 7H), 1.09 (s, 3H), 0.95 (s, 3H); ^13^C NMR (75 MHz, CDCl₃) δ ppm: 220.8, 164.2, 150.4, 136.6, 133.8, 131.6, 129.7, 128.8, 126.3, 121.4, 51.7, 48.1, 47.6, 36.7, 35.8, 34.3, 34.1, 31.9, 30.8, 24.1, 21.8, 20.7, 18.7, 13.6. HRMS (ESI-FTMS, m/z): calcld for C₂₆H₃₀NOS [M + H]^+ 404.2042, found 404.2046. Yield (245 mg, 61%).

(15,2R,13R,14S,18S)-2,7,18-Trimethyl-8-thia-6-azapentacyclo[11.7.0.0²,10.05,9.014,18]icosa-5(9),7,10-trien-17-one (7). Yellowish solid, ^1^H NMR (300 MHz, CDCl₃) δ ppm: 5.70–5.69 (m, 1H), 2.91–2.71 (m, 2H), 2.62 (s, 3H), 2.54–2.21 (m, 2H), 2.18–1.66 (m, 7H), 1.63–1.14 (m, 6H), 1.03 (s, 3H), 0.94 (s, 3H); ^13^C NMR (75 MHz, CDCl₃) δ ppm: 220.8, 162.7, 148.7, 138.6, 130.9, 120.5, 51.7, 48.1, 47.6, 36.7, 35.8, 34.3, 31.3, 31.0, 26.3, 23.9, 21.8, 20.7, 19.4, 18.6, 13.8. HRMS (ESI-FTMS, m/z): calcld for C₁₂H₁₃NOS [M + H]^+ 342.1886, found 342.1890. Yield (177 mg, 52%).

(15,2R,13R,14S,18S)-2,18-Dimethyl-7-phenyl-8-thia-6-azapentacyclo[11.7.0.0²,10.05,9.014,18]icosa-5(9),7,10-trien-17-one (8). Yellowish solid, ^1^H NMR (300 MHz, CDCl₃) δ ppm: 7.40–7.21 (m, 5H), 5.67–5.65 (m, 1H), 4.25 (s, 2H), 2.95–2.74 (m, 2H), 2.50 (dd, J = 8.7, 19.1 Hz, 1H), 2.34–2.25 (m, 1H), 2.18–1.29 (m, 12H), 1.22–1.10 (m, 1H), 1.03 (s, 3H), 0.93 (s, 3H); ^13^C NMR (75 MHz, CDCl₃) δ ppm: 220.8, 166.9, 148.9, 137.9, 136.5, 131.6, 129.0, 128.7, 127.0, 120.7, 51.7, 48.1, 47.6, 40.7, 36.7, 35.8, 34.3, 31.3, 31.1, 30.6, 24.0, 21.8, 20.7, 18.6, 13.6. HRMS (ESI-FTMS, m/z): calcld for C₁₃H₁₁NOS [M + H]^+ 418.2199, found 418.2192. Yield (233 mg, 56%).
(15,2R,13R,14S,18S)-2,18-Dimethyl-7-[(5-trifluoromethyl)-2-pyridyl]oxy]phenyl]-8-thia-6-azapentacyclo[11.7.0.2,10.5,9,0.14,18]icos-5(9),7,10-trien-17-one (14). Yellowish solid, 1H NMR (300 MHz, CDCl3) δ ppm: 7.84 (s, 1H), 7.99–7.92 (m, 3H), 7.21 (d, J = 8.4 Hz, 2H), 7.07 (d, J = 8.5 Hz, 1H), 5.86–5.85 (m, 1H), 3.04–3.02 (m, 2H), 2.56–2.35 (m, 2H), 2.20–1.19 (m, 13H), 1.09 (s, 3H), 0.95 (s, 3H); 13C NMR (75 MHz, CDCl3) δ ppm: 219.7, 163.4, 158.0, 150.5, 145.5 (J = 43 Hz), 136.8, 136.5, 131.7, 127.9, 125.4, 122.1, 121.8, 121.7, 121.6, 111.6, 51.7, 48.1, 47.6, 36.7, 35.8, 34.3, 31.3, 31.0, 30.8, 24.1, 21.8, 17.0, 13.6. HRMS (ESI-FTMS, m/z): calculated for C25H23NO3S [M + H]+ 563.2135, found 563.2125. Yield (366 mg, 65%).

(15,2R,13R,14S,18S)-7-(4-Methoxyphenyl)-2,18-dimethyl-8-thia-6-azapentacyclo[11.7.0.2,10.5,9,0.14,18]icos-5(9),7,10-trien-17-one (15). Yellowish solid, 1H NMR (300 MHz, CDCl3) δ ppm: 7.86 (d, J = 8.6 Hz, 2H), 6.94 (d, J = 8.7 Hz, 2H), 5.82–5.81 (m, 1H), 3.86 (s, 3H), 3.02–2.86 (m, 2H), 2.55–2.33 (m, 2H), 2.19–1.81 (m, 1H), 1.68–1.16 (m, 13H), 1.09 (s, 3H), 0.95 (s, 3H); 13C NMR (75 MHz, CDCl3) δ ppm: 220.7, 164.2, 160.9, 150.2, 136.5, 136.3, 131.4, 130.5, 128.7, 126.8, 123.6, 121.3, 117.3, 113.2, 112.7, 105.8, 82.7, 47.6, 36.7, 35.8, 34.3, 31.4, 31.1, 30.8, 24.1, 21.8, 20.7, 18.7, 13.6. HRMS (ESI-FTMS, m/z): calculated for C26H29NO3S [M + H]+ 434.2148, found 434.2153. Yield (259 mg, 60%).

(15,2R,13R,14S,18S)-7-(3-Hydroxy)-2,18-dimethyl-8-thia-6-azapentacyclo[11.7.0.2,10.5,9,0.14,18]icos-5(9),7,10-trien-17-one (16). Yellowish solid, 1H NMR (300 MHz, CDCl3 + DMSO-d6) δ ppm: 12.13 (br s, 1H), 7.49 (d, J = 7.7 Hz, 1H), 7.19 (t, J = 8.2 Hz, 1H), 6.87 (d, J = 8.2 Hz, 1H), 6.81 (t, J = 7.4 Hz, 1H), 5.78 (br s, 1H), 2.87–2.74 (m, 2H), 2.51–2.31 (m, 2H), 2.07–1.71 (m, 6H), 1.60–1.12 (m, 7H), 0.98 (s, 3H), 0.84 (s, 3H); 13C NMR (75 MHz, CDCl3 + DMSO-d6) δ ppm: 220.3, 164.9, 156.6, 147.6, 135.6, 130.9, 129.2, 126.7, 121.7, 118.7, 117.2, 116.7, 51.2, 47.6, 47.1, 56.3, 35.3, 33.6, 30.9, 30.7, 30.3, 23.2, 21.4, 20.3, 18.3, 13.2. HRMS (ESI-FTMS, m/z): calculated for C25H23NO3 [M + H]+ 420.1992, found 420.1997. Yield (230 mg, 55%).
(15,2R,13R,14S,18S)-7-(3,4-Dihydroxyphenyl)-2,18-dimethyl-8-thia-6-azapentacyclo[11.7.0.02,10.0.5.0.14,18]icoso-5(9),6,10-trien-17-one (19). Yellowish solid, \(^1\)H NMR (300 MHz, CDCl\(_3\) + DMSO-d\(_6\)) \(\delta\) ppm: 8.95 (s, 1H), 8.78 (s, 1H), 7.30 (d, \(J = 2.0\) Hz, 1H), 7.13 (d, \(J = 8.1\) Hz, 1H), 6.74 (d, \(J = 8.2\) Hz, 1H), 5.68 – 2.71 (m, 2H), 2.50 – 2.29 (m, 3H), 2.07 – 1.73 (m, 6H), 1.56 – 1.12 (m, 6H), 0.98 (s, 3H), 0.84 (s, 3H); \(^1\)C NMR (75 MHz, CDCl\(_3\) + DMSO-d\(_6\)) \(\delta\) ppm: 220.1, 164.2, 149.8, 147.6, 145.5, 136.4, 130.0, 125.7; 120.9, 118.2, 115.9, 113.6, 51.4, 48.0, 47.4, 46.6, 36.5, 34.2, 31.4, 31.0, 30.6, 24.1, 21.7; 20.6, 18.7, 13.6. HRMS (ESI-FTMS, m/z): calcd for C\(_{26}\)H\(_{29}\)NO\(_3\)S \([\text{M + H}]^+\) 436.1941, found 436.1927. Yield (252 mg, 58%).

(15,2R,13R,14S,18S)-7-(3,5-Dichlorophenyl)-2,18-dimethyl-8-thia-6-azapentacyclo[11.7.0.02,10.0.5.0.14,18]icoso-5(9),6,10-trien-17-one (20). Yellowish solid, \(^1\)H NMR (300 MHz, CDCl\(_3\) + DMSO-d\(_6\)) \(\delta\) ppm: 7.69 (m, 2H), 7.42 – 3.75 (m, 1H), 7.12 – 7.06 (m, 1H), 5.88 – 5.85 (m, 1H), 3.04 – 2.88 (m, 2H), 2.53 – 2.11 (m, 2H), 2.08 – 1.81 (m, 7H), 1.69 – 1.20 (m, 6H), 1.09 (s, 3H), 0.95 (s, 3H); \(^1\)C NMR (75 MHz, CDCl\(_3\) + DMSO-d\(_6\)) \(\delta\) ppm: 226.0, 163.0 \((^1\text{JC} = 449.86\) Hz), 162.5, 150.7, 137.6, 135.9; \(^1\)JC \(= 8.0\) Hz, 132.3, 130.4 \((^1\text{JC} = 8.3\) Hz), 122.0, 121.9, 116.5 \((^1\text{JC} = 21.3\) Hz), 113.0 \((^1\text{JC} = 23.3\) Hz), 51.7, 48.1, 47.6, 36.7, 35.8, 34.2, 31.4, 31.1, 30.8, 24.1, 21.8, 20.7, 18.7, 13.6. HRMS (ESI-FTMS, m/z): calcd for C\(_{19}\)H\(_{18}\)Cl\(_2\)NO\(_3\)S \([\text{M + H}]^+\) 482.1148, 484.1128, found 482.1149, 484.1129 respectively. Yield (216 mg, 45%).

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A solution of compound 20 (210.5 mg 0.5 mmol) in methanol was cooled in ice and NaBH₄ (189 mg 5 mmol) was added portionwise, and the reaction mixture was stirred for 8 h. After completion of the reaction, aqueous 10% HCl was added, and the reaction was stirred for 2 h to precipitate the product. Filtration and washing with water gave the pure product (203 mg, 96%). Yellowish solid, ¹H NMR (300 MHz, CDCl₃) δ ppm: 7.69–7.62 (m, 2H), 7.42–7.34 (m, 1H), 7.12–7.05 (m, 1H), 5.85–5.83 (m, 1H), 3.69 (t, J = 8.3 Hz, 1H), 3.04–2.81 (m, 2H), 2.31–2.22 (m, 1H), 2.18–2.05 (m, 2H), 1.93–1.28 (m, 1H), 1.22–1.11 (m, 3H), 1.07 (s, 3H), 0.83 (s, 3H) ¹³C NMR (75 MHz, CDCl₃) δ ppm: 163.5 (J = 244.9 Hz), 162.4 (J = 3.1 Hz), 150.5, 136.3, 135.9 (J = 8.0 Hz), 132.5, 130.4 (J = 8.2 Hz), 122.6, 121.1 (J = 7.8 Hz), 116.2 (J = 21.1 Hz), 113.1 (J = 23.3 Hz), 81.7, 51.3, 48.1, 42.8, 36.7, 36.5, 34.3, 34.1, 31.5, 30.5, 24.1, 23.4, 21.0, 18.7, 11.0. HRMS (ESI-FTMS, m/z): calcd for C₂₈H₃₃FNO₂S [M+H]^+ 466.2211, found 466.2214.

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**REFERENCES**

(1) Terán-Pérez, G.; Arana-Lechuga, Y.; Esqueda-León, E.; Santana-Miranda, R.; Rojas-Zamorano, J. A.; Mocetxuma, J. V. Steroid hormones and sleep regulation. *Mini-Rev. Med. Chem.* 2012, 12, 1040–1048.

(2) Wilkenfeld, S. R.; Lin, C.; Frigo, D. E. Communication between genomic and non-genomic signaling events coordinate steroid hormone actions. *Steroids* 2018, 133, 2–7.

(3) Dai, J.; Yoshida, W. Y.; Kelly, M.; Williams, P. Pregnane-10,20-carbolactones from a Hawaiian Marine Sponge in the Genus Myrmekioderma. *J. Nat. Prod.* 2016, 79, 1464–1467.

(4) Simoben, C. V.; Ibezim, A.; Ntie-Kang, F.; Nwodo, J. N.; Lifongo, L. L. Exploring Cancer Therapeutics with Natural Products from African Medicinal Plants, Part I: Xanthones, Quinones, Steroids, Coumarins, Phenolics and other Classes of Compounds. *Anti-Cancer Agents Med. Chem.* 2015, 15, 1092–1111.

(5) Liu, J.; Zhang, D.; Sun, X.; Ding, T.; Lei, B.; Zhang, C. Structure-activity relationship of brassinosteroids and their agricultural practical usages. *Steroids* 2017, 124, 1–17.

(6) Calle, J. M.; Pérez, A. J.; Simont, A. M.; Guerra, J. O.; Macías, F. A. Steroidal Saponins from Furcraea hexapetala Leaves and Their Phytotoxic Activity. *J. Nat. Prod.* 2016, 79, 2903–2911.

(7) Qian, M.; Krishnan, K.; Kudova, E.; Li, P.; Manion, B. D.; Taylor, A.; Elias, G.; Akk, G.; Evers, A. S.; Zorumski, C. F.; Mennerick, S.; Covey, D. F. Neurosteroid analogues. 18. Structure-activity studies of ent-steroid potentiators of gamma-aminobutyric acid type A receptors and comparison of their activities with those of alphalone and allopropalone. *J. Med. Chem.* 2014, 57, 171–190.

(8) Bansal, R.; Acharya, P. C. Man-made cytotoxic steroids: exemplary agents for cancer therapy. *Chem. Rev.* 2014, 114, 6986–7005.

(9) Le Bideau, F.; Dагor, S. Synthesis of transition-metal steroid derivatives. *Chem. Rev.* 2013, 113, 7793–805.

(10) El-Desoky, E. S. I.; Reyad, M.; Afsah, E. M.; Dawidaw, A. A. Synthesis and chemical reactions of the steroid hormone 17α-alpha-methyltestosterone. *Steroids* 2016, 105, 68–95.

(11) Hamilton, N. M.; Dawson, M.; Fairweather, E. E.; Hamilton, N. S.; Hitchin, J. R.; James, D. I.; Jones, S. D.; Jordan, A. M.; Lyons, A. J.; Small, H. F.; Thomson, G. J.; Waddell, I. D.; Ogilvie, A. J.; Novellino, E.; Limongelli, G.; Arana-Lechuga, Y.; Esqueda-León, E.; Santana-Miranda, R.; Rojas-Zamorano, J. A.; Mocetxuma, J. V.; Cipriani, S.; Di Leva, F. S.; Monti, M. C.; Novellino, E.; Limongelli, G.; Zampella, A.; Fiorucci, S. Modification on ursodeoxycholic acid α-androstane-3,17β-diol. Inhibits Neurototoxicity in SH-SY5Y Human Neuroblastoma Cells and Mouse Primary Cortical Neurons. *Endocrinology* 2016, 157, 4570–4578.

(12) Sepe, V.; Renga, B.; Festa, C.; D’Amore, C.; Masullo, D.; Cipriani, S.; Di Leva, F. S.; Monti, M. C.; Novellino, E.; Limongelli, G.; Zampella, A.; Fiorucci, S. Modification on ursodeoxycholic acid α-androstane-3,17β-diol. Inhibits Neurototoxicity in SH-SY5Y Human Neuroblastoma Cells and Mouse Primary Cortical Neurons. *Endocrinology* 2016, 157, 4570–4578.

(13) Mendell, A. L.; Creighton, C. E.; Kalisch, B. E.; Malaukis, N. J. Ster-Androstane-3β,17β-Diol Inhibits Neurototoxicity in SH-SY5Y Human Neuroblastoma Cells and Mouse Primary Cortical Neurons. *Endocrinology* 2016, 157, 4570–4578.

(14) Ning, X.; Yang, Y.; Deng, H.; Zhang, Q.; Huang, Y.; Su, Z.; Fu, Y.; Xiang, Q.; Zhang, S. Development of 17β-hydroxy steroid.
dehydrogenase type 3 as a target in hormone-dependent prostate cancer therapy. Steroids 2017, 121, 10–16.

(15) Ji, Z. H.; Xu, Z. Q.; Zhao, H.; Yu, X. Y. Neuroprotective effect and mechanism of daucosterol palmitate in ameliorating learning and memory impairment in a rat model of Alzheimer’s disease. Steroids 2017, 119, 31–35.

(16) Larik, F. A.; Saeed, A.; Shahzad, D.; Faisal, M.; El-Seedi, H.; Mehdooz, H.; Channar, P. A. Synthetic approaches towards the multi target drug spironolactone and its potent analogues/derivatives. Steroids 2017, 118, 76–92.

(17) Moreno, Y. B. L.; Urban, E.; Gelbcke, M.; Dufrasne, F.; Kopp, B.; Kiss, R.; Zehl, M. Structure-activity relationship analysis of bufadienolide-induced in vitro growth inhibitory effects on mouse and human cancer cells. J. Nat. Prod. 2013, 76, 1078–1084.

(18) Rouf, A.; Tanyeli, C. Bioactive thiazole and benzothiazole analogues. Eur. J. Med. Chem. 2015, 95, 911–927.

(19) Ayati, A.; Emami, S.; Asadipour, A.; Shafiee, A.; Foroumadi, A. Recent applications of 1,3-thiazole core structure in the identification of new lead compounds and drug discovery. Eur. J. Med. Chem. 2015, 97, 699–718.

(20) FDA Approves Drug To Treat Duchenne Muscular Dystrophy. https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm540945.htm (Feb 9, 2017).

(21) Madhira, M. K.; Siriram, H. M.; Inamdar, M.; Sharma, M. K.; Prasad, M.; Joseph, S. Improved Procedure for Preparation of Abiraterone Acetate. Org. Proc. Res. Dev. 2014, 18, 555–558.

(22) Szychowski, J.; Truchon, J.-F.; Bennani, Y. L. Natural Products in Medicine: Transformational Outcome of Synthetic Chemistry. J. Med. Chem. 2014, 57, 9292–9308.

(23) Fan, N.-J.; He, Q.-R.; Duan, M.; Bai, Y.-B.; Tang, J.-J. Synthesis and antiproliferative activity of D-ring substituted steroidal anabolic steroid that does not influence parathyroid hormone response to hypercalcemia in postmenopausal women. Calcif. Tissue Int. 1994, 54, 521–522.

(24) Vitellozzi, L.; McAllister, G. D.; Genski, T.; Taylor, R. J. K. Organometallic Routes to Novel Steroids Containing Heterocyclic C-17 Side-Chains. Synthesis 2015, 48, 48–56.

(25) Zhang, B. L.; Song, L. X.; Li, Y. F.; Li, Y. L.; Guo, Y. Z.; Zhang, E.; Liu, H. M. Synthesis and biological evaluation of dehydroepiandrosterone-fused thiazole, imidazo[2,1-b]thiazole, pyridine steroidal analogues. Steroids 2014, 80, 92–101.

(26) Martinez Botella, G.; Salturo, P. G.; Harrison, B. L.; Beres, R. T.; Bai, Z.; Shen, K.; Bellort, G. M.; Loya, C. M.; Ackley, M. A.; Grossman, S. J.; Hofmann, E.; Jia, S.; Wang, J.; Doherty, J. J.; Rocha, D. D.; Steinbach, J. H.; Reichert, D. E.; Evers, A. S.; Zorumski, C. F.; Mennerick, S.; Covey, D. F. Neurosteroid Analogues. 17. Inverted Steroids and antiproliferative activity of D-ring substituted steroidal anabolic steroid that does not influence parathyroid hormone response to hypercalcemia in postmenopausal women. Calcif. Tissue Int. 1994, 54, 521–522.

(27) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.

(28) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.

(29) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.

(30) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.

(31) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.

(32) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.

(33) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.

(34) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.

(35) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.

(36) Alsharif, Z.; Ali, M. A.; Alkhattabi, H.; Jones, D.; Delaney, E.; Gottspooner, A.; Yang, T. Hexafluorosopropyl alcohol mediated synthesis of 2,3-dihydro-4H-pyridol[1,2-a]pyrimidin-4-ones. Sci. Rep. 2016, 6, No. 36316.
Potentiation Site on \( \gamma \)-Aminobutyric Acid Type A Receptors. J. Med. Chem. 2012, 55, 1334–1345.

(47) CDC Colorectal (colon) Cancer. https://www.cdc.gov/cancer/colorectal/statistics/index.htm (April 10, 2018).

(48) Heffron, T. P. Small Molecule Kinase Inhibitors for the Treatment of Brain Cancer. J. Med. Chem. 2016, 59, 10030–10066.

(49) Mayo Clinic Melanoma. http://www.mayoclinic.org/diseases-conditions/melanoma/basics/definition/con-20026009 (July 11, 2016).

(50) Reedy, J. L.; Hedlund, D. K.; Gabr, M. T.; Henning, G. M.; Pigge, F. C.; Schultz, M. K. Synthesis and Evaluation of Tetraaryl-ethylene-based Mono-, Bis-, and Tris(pyridinium) Derivatives for Image-Guided Mitochondria-Specific Targeting and Cytotoxicity of Metastatic Melanoma Cells. Bioconj. Chem. 2016, 2424–2430.

(51) CDC Kidney Cancer. https://www.cdc.gov/cancer/kidney/index.htm (April 10, 2018).