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Phytosterols as precursors for the synthesis of aromatase inhibitors: Hemisynthesis of testololactone and testolactone

Shabir H. Lone, Khursheed A. Bhat *

Bioorganic Chemistry Division, Indian Institute of Integrative Medicine (CSIR), Srinagar, 190005 Jammu and Kashmir, India

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ABSTRACT

Using β-sitosterol and stigmasterol as precursor materials, a concise and efficient hemisynthesis of aromatase inhibitors: testololactone and testolactone was accomplished in a well-established reaction scheme. It involves highly effective Oppaneur oxidation of both β-sitosterol as well as stigmasterol to generate the required enone moiety in ring 'A' of the desired steroid system. The Oppaneur oxidation products of both β-sitosterol and stigmasterol were then subjected to oxidative cleavage of the side chain to produce 4-androstene-3,17-dione. Baeyer–Villiger oxidation of 4-androstene-3,17-dione using *m*-CPBA yielded testololactone. Dehydrogenation of 4-androstene-3,17-dione using phenylselenyl chloride in ethyl acetate followed by selenoxide elimination with H_2O_2 in dichloromethane furnished androstenedienone. Baeyer–Villiger oxidation of the resulting androstenedienone yielded the desired testolactone (overall yield 33%). This expeditious reaction scheme may be exploited for the bulk production of aromatase inhibitors (especially testolactone marketed under the brand name Teslac) from the most abundant and naturally occurring phytosterols like β-sitosterol.

1. Introduction

Natural products generally represent unique scaffolds which contribute to the design, discovery and thus the synthesis of new chemical compounds. Steroids act as a repertoire of such lipophilic structures, characteristic of the living world that perform important physiological functions within the living systems such as, hormones, regulators and provitamins. These compounds exhibit a wide array of bioactivities vital for human life [1–4]. Apart from the cancers of skin, breast cancer is generally considered the most prevalent cancer in women and ranks second as a cause of tumor-related death only after lung cancer [5]. Presently, it is envisaged that one in about eight women of America will develop breast cancer some time during her life. About two-thirds of breast related tumors require estrogens to grow and hence are called hormone-dependent [6]. One approach in treating such cancers involves obstruction of hormone production. Aromatase which is also known as estrogen synthase, has always been found to be the most promising target for the treatment of breast cancer [7] because inhibition of the aromatase enzyme leads to decreased estrogen production, and thus stopping/reducing the tumor. The circulating oestrogens have been recognized as major contributors to the growth of some mammary tumors. Therefore suppressing the oestrogen action by affecting their biosynthesis at the androstenedione–oestrone aromatization step, by means of inhibitors of enzyme estrogen-synthetase, has become an effective option for the treatment of such hormone-dependent breast cancer. Among the naturally occurring steroids, which possess a moderate aromatase inhibitory activity include pollinastanol (**1**), cycloeucalenol (**2**), cycloeucalenol linolenate (**3**), 24-methylenecholesterol (**4**) and their fatty acid conjugates-24-methylenecholesterol linolenate (**5**) and 24-methylenecholesterol palmitate (**6**) (Fig. 1) extracted from *Brassica rapa* L. These natural molecules are known to inhibit the human placental aromatase with IC_{50} values ranging from 0.03 to 0.45 mM [8]. β-sitosterol (**7**) and stigmasterol (**8**) obtained from *Atractylodes macrocephala* are reported to inhibit more than 50% of aromatase activity at about 10 mM concentration [9]. 6β-hydroxystigmast-4-en-3-one (**9**) has been found to be a weak aromatase inhibitor in cell-based assays [10]. Since the naturally occurring aromatase inhibitors are weak in their action, both steroidal as well as non-steroidal aromatase inhibitors have been developed, which exhibit this enzyme inhibition at nano-molar concentrations. Among the synthesized steroidal inhibitors that cause aromatase inhibition include testolactone, testololactone and exemestane. Testolactone, marketed under the trade name

* Corresponding author at: Indian Institute of Integrative Medicine, P/O Sanat Nagar, Srinagar, 190005 Jammu and Kashmir, India. Tel.: +91 1942431253x123; fax: +91 1942441331.
E-mail address: kabhat@iiim.ac.in (K.A. Bhat).

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TESLAC is regarded as a pioneer drug to treat breast cancer [11]. So far there are a few literature reports which highlight the synthetic routes for the preparation of these aromatase inhibitors. Testololactone has been previously synthesized from a variety of substances using biotransformation approach [12–15]. A recent study highlights the multifunctional conversion of steroids using *pencillium* sps [16]. Testolactone has been synthesized in a multistep functional biotransformation from dehydroepiandrosterone (DHEA) and pregnanolone using *Fusarium oxysporum* [17]. But till now there has not been a single approach to synthesize these potent inhibitors using the naturally known abundant phytosterols like β-sitosterol and stigmasterol. Based on the aforementioned facts as well as our ongoing research program to search for natural product based medicinal leads [18–20], we turned our attention towards the hemisynthesis of steroidal based aromatase inhibitors using well known abundant sterols like β-sitosterol and stigmasterol as starting material.

2. Materials and methods

2.1. General

β-sitosterol and stigmasterol were both isolated from the underground parts of *Senecio graciliflorus* DC. However bulk of these natural products was also purchased commercially. All the chemical reagents were of analytical grade and purchased from commercial suppliers (Sigma–Aldrich) and used without further purification unless otherwise stated. All the solvents used were of LR grade. The reactions were monitored on TLC plates precoated with silica-gel G F_{254}. The spots were visualized both under 254 nm UV-light as well as by staining with ceric ammonium sulfate. Column chromatography was carried out using normal phase silica-gel (60–120). All the reaction products were characterized using ^{1}H and ^{13}C NMR spectra along with mass analysis. ^{1}H and ^{13}C NMR were recorded on 400 MHz bruker spectrometer with TMS as an internal standard. Chemical shift values were reported in ppm units and coupling constants were measured as Hz. Mass spectra were carried out on LC–MS 8030 tandem mass spectrometer manufactured by Shimadzu Corporation, Kyoto, Japan. All the compounds were analysed in full scan mode with nitrogen serving as interface gas. Detection was done in ESI mode having probe voltage of 180.0 V, with probe temperature of 400 °C. Elemental analysis was carried on vario EL III analyser.

2.2. Synthesis

2.2.1. Synthesis of 4-stigmasten-3-one (**10**)

To a solution of β-sitosterol (**7**) (200 mg, 0.483 mmol) in 9 ml of toluene and 2.5 ml (0.031 mol) of cyclohexanone, was added aluminum isopropoxide (400 mg, 1.95 mmol) in 4 ml of warm toluene. The reaction mixture was refluxed for 5 h and shaken for one minute with 5 ml of water and 2 ml of 3.6 N sulfuric acid. The organic layer was washed with 20 ml of brine, dried over sodium sulfate, filtered through celite, and concentrated under vaccum. The oily residue was purified by flash chromatography (15% ethyl acetate–hexane on silica gel) and recrystallization from aqueous acetone yielde colorless needles of 4-stigmasten-3-one (**10**) (140 mg, 0.3398 mmol, 70%). 4-Stigmasten-3-one (**10**): mp = 191–193 °C, ($C_{29}H_{48}O$; calcd C, 84.40; H, 11.72; found C, 84.43; H, 11.68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.01 (s, 1H), 2.55 (m, 4H), 2.04 (m, 4H), 1.84 (m, 3H), 1.56 (m, 8H), 1.25 (m, 9H), 1.16 (m, 9H), 0.92 (m, 5H), 0.75 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) 199.64, 171.41, 125.63, 57.24, 55.67, 54.07, 50.63, 47.16, 45.67, 42.76, 40.31, 39.63, 36.35, 34.66, 33.97, 29.32, 28.34, 26.14, 24.28, 23.68, 21.18, 19.92, 19.30, 18.93, 18.03, 12.68, 12.36. ESI-MS at m/z = 413 for $[M+1]^+$.

2.2.2. Synthesis of 4,22-stigmastdien-3-one (**11**)

To a solution of stigmasterol (**8**) (200 mg, 0.483 mmol) in 10 ml of toluene and 2.5 ml (0.03 mol) of cyclohexanone was added aluminum isopropoxide (400 mg, 1.95 mmol) in 4 ml of warm toluene. The reaction mixture was refluxed for 5 h and shaken for 1 min with 5 ml of water and 2 ml of 3.6 N sulfuric acid. The organic layer was washed with 20 ml of brine, dried over sodium sulfate, filtered through celite, and concentrated under reduced pressure. The oily residue was purified by flash chromatography (15% ethyl acetate–hexane on silica gel) and recrystallization from aqueous acetone to give colorless needles of 4,22-stigmastdien-3-one (**11**) (134 mg,

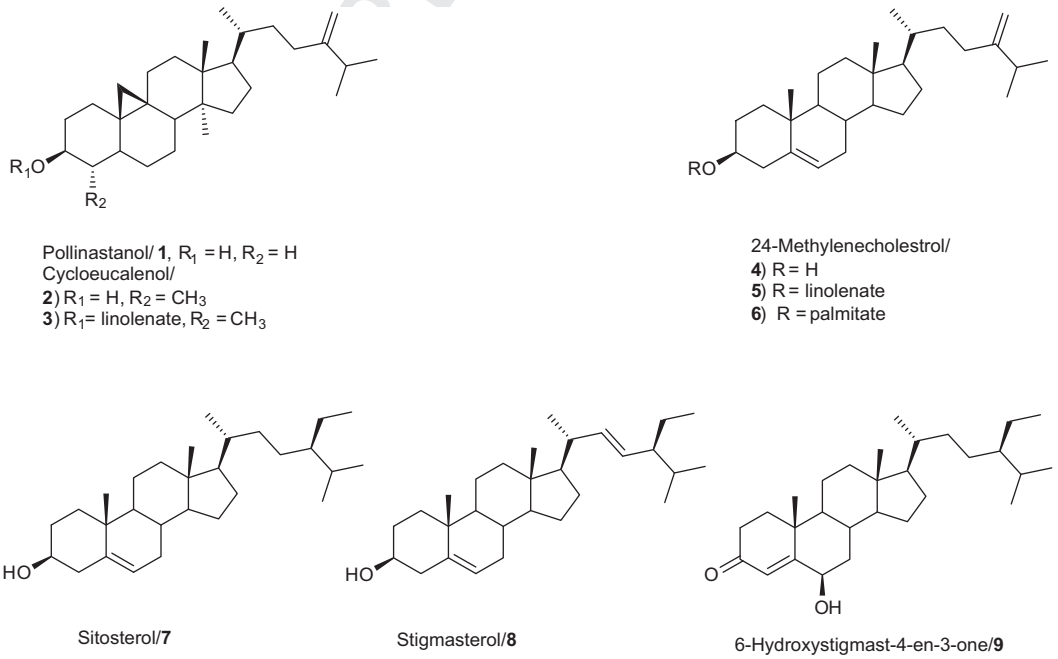

Fig. 1. Naturally occurring aromatase inhibitors.

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0.326 mmol, 67%). 4,22-Stigmastdien-3-one (**11**): mp = 184–185 °C, ($C_{29}H_{46}O$; calcd C, 84.81; H, 11.29; found C, 84.87; H, 11.34%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.15 (s, 1H), 5.05–4.97 (m, 1H), 5.18–5.10 (m, 1H), 2.67–2.64 (m, 1H), 2.48–2.44 (m, 3H), 2.05–1.85 (m, 4H), 1.6–1.5 (m, 7H), 1.25 (m, 7H), 1.16 (s, 6H), 0.92 (m, 4H), 0.75 (m, 8H). ^{13}C NMR (400 MHz, $CDCl_3$) δ 199.85, 171.91,138.00, 130.02, 125.63, 56.78, 56.10, 51.27, 47.03, 46.04, 42.76, 40.03, 39.37, 36.26, 35.77, 34.44, 34.19, 34.07, 29.39, 28.23, 26.30, 24.19, 23.30, 21.10, 19.24, 17.73, 12.19, 12.11. ESI-MS at m/z = 411 for $[M+1]^+$.

*2.2.3. Synthesis of 4-androstene-3,17-dione (**12**)*

A solution of compound **10**/**11** in DMF was distributed in various Erlenmeyer flasks and incubated with a culture containing *Mycobacterium* sps (NRRL B-3805). At the end of the inoculation the culture broth was acidified with acetic acid to a pH of 3.0 and extracted with $CHCl_3$. The combined extract layers were washed with distilled H_2O and dried over sodium sulfate and evaporated to dryness to yield a solid residue. Chromatographic separation over silica gel column and elution with hexane:ethylacetate (70:30) afforded the desired compound **12** in 80% yields. 4-Androstene-3,17-dione (**12**): mp = 171–172 °C; ($C_{19}H_{26}O_2$; calcd C, 79.68; H, 9.15; found C, 79.63; H, 9.09%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.69 (s, 1H) 2.36 (m, 4H), 2.00 (m, 4H), 1.81 (d, J = 15.8 Hz, 1H), 1.68 (m, 3H), 1.58–1.34 (m, 2H), 1.24 (m, 2H), 1.16 (s, 3H), 1.01 (m, 2H), 0.87 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 222.12, 199.35, 170.41, 124.28, 105.7, 53.97, 51.00, 47.63, 38.79, 35.88, 35.85, 35.30, 34.05, 32.70, 31.44, 30.90, 21.88, 20.46, 17.53, 13.85, 18.51. ESI-MS at m/z = 287 for $[M+1]^+$.

*2.2.4. Synthesis of testololactone (**13**)*

A solution of compound **12** (55 mg, 0.192 mmol), $NaHCO_3$ (1.5 eq) and *m*-CPBA (40 mg, 0.23 mmol) in CH_2Cl_2 (7 ml) was stirred at room temperature overnight. The reaction mixture was washed with Na_2SO_3, and saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated under pressure to give a crude residue which was crystallized from acetone to yield **13** (47 mg, 0.155 mmol) in 85% yield. Testololactone (**13**): (overall yield 46%); mp = 207–208 °C; ($C_{19}H_{26}O_3$; calcd C, 75.46; H, 8.67; found C, 75.50; H, 9.12%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (s, 1H), 2.7–2.49 (m, 4H), 2.25–2.10 (m, 2H), 2.06–1.78 (m, 7H), 1.89–1.34 (m, 5H), 1.31 (s, 3H), 1.20 (m, 1H), 1.04 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.32, 172.69, 171.41, 128.09, 82.63, 50.92, 46.56, 45.96, 41.51, 39.34, 38.42, 32.78, 31.79, 30.61, 29.32, 29.01, 23.06, 20.81, 20.20. ESI-MS at m/z = 303 $[M+1]^+$ and 342 for $[M+1+K]^+$.

*2.2.5. Synthesis of 2-phenylseleno-4-androstene-3,17-dione (**14**)*

A solution of 4-androstene-3, 17-dione (**12**) (55 mg, 0.192 mmol) and phenylselenyl chloride (110 mg, 3 equivalents) in ethyl acetate (10 ml) was stirred for 2 h at room temperature. After the completion of reaction the solvent was evaporated and the residue was subjected to flash chromatography in Hex:EtOAc (75:25) to furnish 2-phenylseleno-4-androstenedione (**14**) in 75% yields; mp = 233–235 °C; ($C_{25}H_{30}O_3Se$; calcd C, 65.64; H, 6.61; found C, 65.61; H, 6.67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30–7.24 (m, 3H), 7.11–6.87 (m, 1H), 6.20 (s, 1H), 6.13 (s, 1H), 4.88 (s, 1H), 2.48 (m, 2H), 2.29 (m, 2H), 2.09 (m, 3H), 2.00–1.74 (m, 3H), 1.69 (s, 3H), 1.40–1.19 (m, 34H), 0.96 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 219.11, 185.82, 161.32, 156.32, 132.52, 129.34, 128.07, 126.85, 50.92, 49.98, 48.72, 48.41, 47.13, 43.35, 40.58, 35.83, 31.47, 30.21, 22.65, 21.73, 21.47, 13. 39. ESI-MS at m/z = 441 for $[M+1]^+$ and 459 for $[M+1+H_2O]^+$

*2.2.6. Synthesis of 1,4-androstadi-en-3,17-dione (**15**)*

To a solution of compound **14** (45 mg, 0.149 mmol) in CH_2Cl_2 was added 30% H_2O_2 solution and stirred for 4 h until the reaction was complete as measured by TLC profiling. After completion the reaction-mixture was worked up in CH_2Cl_2 and the organic layers collected. The organic layers were then combined and concentrated under vaccum to yield crude residue which was purified using column chromatography to produce pure androstenedienone (**15**) in 90% yields (40 mg, 0.140 mmol). 1,4-androstadien-3,17-dione (**15**): mp = 139–140 °C; ($C_{19}H_{24}O_2$; calcd C, 80.24; H, 8.51; found C, 80.20; H, 8.54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (d, J = 9.8 Hz, 1H), 6.23 (s, 1H), 6.17 (d, J = 9.6 Hz, 1H), 2.54–2.39 (m, 2H), 2.37–2.27 (m, 2H), 2.15 (s, 2H), 1.97–1.81 (m, 2H), 1.48 (s, 5H), 1.30–0.88 (m, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 221.23, 186.08, 161.29, 155.98, 127.98, 127.13, 50.99, 50.31, 48.31, 47.85, 43.65, 40.04, 35.80, 31.37, 30.53, 30.33, 22.71, 22.09, 21.79, 14.09. ESI-MS at m/z = 285 for $[M+1]^+$ and 326 for $[M+1+ACN]^+$.

*2.2.7. Synthesis of testolactone (**16**)*

A solution of compound **15** (40 mg, 0.140 mmol), $NaHCO_3$ (1.2 eq) and *m*-CPBA (36 mg, 0.21 mmol) in CH_2Cl_2 (6 ml) was stirred at room temperature overnight. The reaction mixture was washed with Na_2SO_3 and saturated brine solution, dried over anhydrous Na_2SO_4 and concentrated under pressure to give a crude residue which was crystallised from acetone to yield **16** (85%). Testolactone (**16**) (overall yield 33%); mp = 216–218 °C, ($C_{19}H_{24}O_3$; calcd C, 75.97; H, 8.05; 11.72; found C, 76.00; H, 8.10%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.00 (d, J = 10.2 Hz, 1H), 6.24 (d, J = 10.9 Hz, 1H), 6.07 (s, 1H), 2.6–2.74 (m, 2H), 2.47–2.37 (m, 2H), 2.15–1.78 (m, 5H), 1.71–1.40 (m, 6H), 1.36 (s, 3H), 1.19 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 186.45, 170.80, 167.63, 154.83, 128.10, 124.72, 82.91, 51.61, 45.66, 43.39, 39.02, 37.76, 32.45, 32.10, 29.02, 23.37, 20.50, 20.21, 18.89. ESI-MS at m/z = 301 for $[M+1]^+$ and 342 for $[M+1+ACN]^+$.

3. Results and discussion

Testololactone (**13**) and testolactone (**14**) were prepared from easily available phytosterols like β-sitosterol (**7**)/stigmasterol (**8**) via a simple reaction scheme (Scheme 1). β-sitosterol (**7**) as well as stigmasterol (**8**) were subjected to Oppaneur oxidation using cyclohexanone and aluminium isopropoxide under reflux conditions in boiling toluene to yield 4-stigmasten-3-one (**10**) and 4,22-stigmastedien-3-one (**11**) respectively. The formation of both the products **10** and **11** was easily confirmed by the appearance of a downfield singlet at δ 5.85 ppm in the proton NMR spectrum corresponding to the α-proton (C-4 proton) in the enone system of both compounds **10** and **11**.

This was further confirmed by the appearance of signals at δ 199 ppm in ^{13}C NMR spectra corresponding to the 3-oxo carbon of **10** and **11** along with the disappearance of signals at δ 71 ppm corresponding to C-3 hydroxylated carbons in both sitosterol (**7**) and stigmasterol (**8**). Next step was to carry out the cleavage of side chain to furnish the C-17 keto functionality as is required in both the end products. Initially a number of chemical methods were tried but none succeeded to furnish the desired 4-androstene-3,17-dienone (**12**). Consequently biotransformation approach was used to synthesize the required compound **12**. Treatment of the either compound (**10** or **11**) with *Mycobacterium species* (NRRL B-3805) yielded 4-androstene-3,17-dienone (**12**) in excellent yields. Formation of 4-androstene-3,17-dienone (**12**) could easily be confirmed by the appearance of a very downfield carbon resonance at δ 221 ppm in ^{13}C NMR spectrum which corresponds to the C-17 keto-carbon-moiety along with the disappearance of some ten carbon signals corresponding to the loss of side chains in both **10** and **11**. Baeyer–Villiger oxidation of 4-androstene-3,17-dienone (**12**) using *m*-CPBA in presence of $NaHCO_3$ yielded the desired product testololactone (**13**) in excellent yield, the structure of which was confirmed easily by the disappearance of carbon signal at δ 221 ppm and appearance of additional peak at

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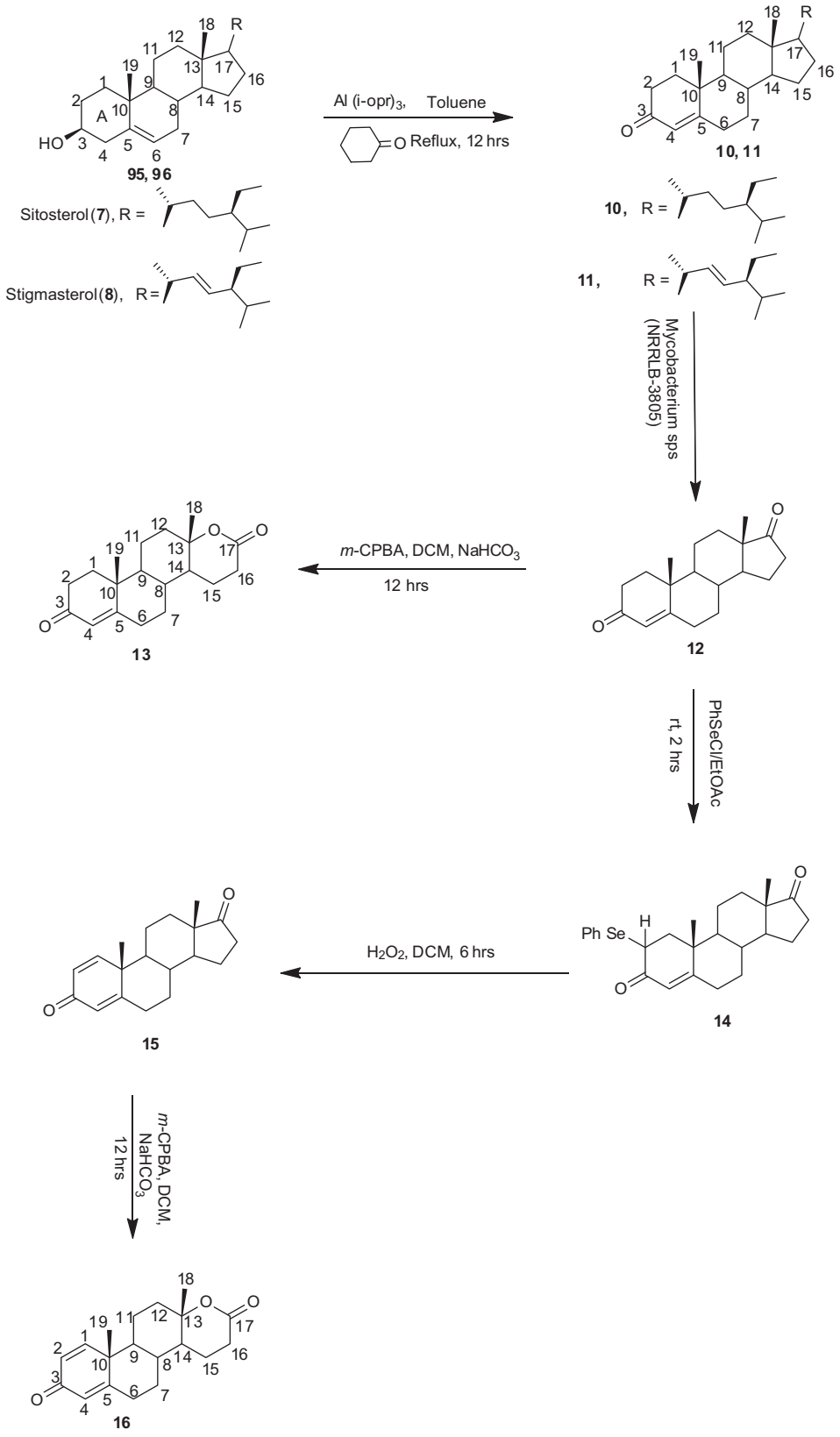

Scheme 1. Hemisynthesis of testololactone and testolactone.

δ 171 ppm corresponding to the lactonic carbonyl moiety in **13**. On the other hand a number of procedures were tested to generate the 1,2-double bond as is required in testolactone (**16**): use of both phenylselenyl chloride in presence of lithium diisopropyl amide (LDA) followed by degradation using H_2O_2 failed to generate the required 1,2 double bond. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in either dioxane or toluene also failed to furnish the required 1,2 double bond. Finally phenylselenyl chloride in

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ethyl acetate yielded the desired α-phenylselenide which was degraded with hydrogen peroxide in dichloromethane to give 1,4-androstadiene-3,17-dione (**15**). Baeyer–Villiger oxidation of 1,4-androstadiene-3,17-dione using *m*-CPBA in sodium bicarbonate successfully furnished the desired testolactone (**16**) in quantitative yield.

4. Conclusion

An efficient and highly facile route for the semi synthesis of aromatase inhibitors which include testololactone (**13**) and testolactone (**16**) was developed using the well-known and most abundant phytosterols (*β*-sitosterol, stigmasterol) as precursor materials. The synthetic route involves a highly effective Oppaneur oxidation, followed by oxidative cleavage using biotransformation approach to give (**12**) which yielded testololactone (**13**) *via* Baeyer–Villiger oxidation. 4-Androstene-3,17-dione (**12**) on further dehydrogenation using phenylselenyl chloride in EtOAc followed by selenoxide elimination with H_2O_2 in DCM furnished androstenedienone (**15**) whose Baeyer–Villiger oxidation yielded testolactone (**16**). This reaction protocol can be safely used for the bulk production of testolactone (marketed under the brand name Teslac) from the easily available and most abundant precursors like sitosterol and stigmasterol and thus offers a novel commercial route to potentially bioactive steroid lactones.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2015.02.011.

References

[1] Zeelen FJ. Medicinal chemistry of steroids, vol. 15. Elsevier; 1990.
[2] Aggarwal S, Thareja S, Bhardwaj TR, Haupenthal J, Hartmann RW, Kumar M. Synthesis and biological evaluation of novel unsaturated carboxy steroids as human 5α-reductase inhibitors: a legitimate approach. Eur J Med Chem 2012;54:728–39.
[3] Khan MOF, Lee HJ. Synthesis and pharmacology of anti-inflammatory steroidal antedrugs. Chem Rev 2008;108:5131–45.
[4] Aggarwal S, Thareja S, Verma A, Bhardwaj TR, Kumar M. An overview on 5α-reductase inhibitors. Steroids 2010;75:109–53.
[5] Cancer facts & figures 2008. American Cancer Society.
[6] Brueggemeier RW, Hackett JC, Az-Cruz ES. Aromatase inhibitors in the treatment of breast cancer. Endocr Rev 2005;26:331.
[7] Meunier B, de Visser SP, Shaik S. Mechanism of oxidation reactions catalyzed by cytochrome p450 enzymes. Chem Rev 2004;104:3947.
[8] Li YH, Yang YF, Li K, Jin LL, Yang NY, Kong DY. 5 alpha-reductase and aromatase inhibitory constituents from *Brassica rapa* L. pollen. Chem Pharm Bull 2009;57:401–4.
[9] Jiang H, Shi J, Li YY. Screening for compounds with aromatase inhibiting activities from *Atractylodes macrocephala* Koidz. Molecules 2011;16:3146–51.
[10] Balunas MJ, Su B, Riswan S, Fong HHS, Brueggemeier RW, Pezzuto JM, Kinghorn AD. Isolation and characterisation of aromatase inhibitor from *Brassaiopsis glomerulata* (Araliaceae). Phytochem Lett 2009;2:29–33.
[11] Lombardi P. Exemestane, a new steroidal aromatase inhibitor of clinical relevance. Biochim Biophys Acta 2002;1587:326–37.
[12] Prairie RL, Talalay P. Enzymatic formation of testololactone. Biochemistry 1963;2:203–8.
[13] Liu HM, Li H, Shan L, Wu J. Synthesis of steroidal lactone by penicillium citreoviride. Steroids 2006;71:931–4.
[14] Kolek T, Szpineter A, Swizdor A. Studies on Baeyer–Villiger oxidation of steroids: DHEA and pregnenolone D-lactonization pathways in *Penicillium camemberti* AM83. Steroids 2009;74:859–62.
[15] Hunter AC, Carragher NE. Flexibility of the endogenous progesterone lactonisation pathway in *Aspergillus tamarii* KITA. J Steroids Biochem Mol Biol 2003;87:301–8.
[16] Yang B, Wang Y, Chen X, Feng J, Qiaqing W, Dumming Z. Biotransformations of steroids to testololactone by a multifunctional strain *Penicillium simplicissimum* WY134-2. Tetrahedron 2014;70:41–6.
[17] Zhang H, Jie Ren, Wang Y, Sheng C, Qiaqing W, Diao A, et al. Effective multistep functional biotransformations of steroids by a newly isolated *Fusarium oxysporum* SC1301. Tetrahedron 2013;69:184–9.
[18] Lone SH, Bhat KA, Syed Naseer, Rather RA, Khuroo MA, Tasduq SA. Isolation, cytotoxicity evaluation and HPLC-quantification of the chemical constituents from *Artemisia amygdalina* Decne. J Chromatogr B 2013;940:135–41.
[19] Lone SH, Bhat KA, Shakeel-u-Rehman, Majeed R, Hamid A, Khuroo MA. Synthesis and biological evaluation of amino analogs of Ludartin: potent and selective cytotoxic agents. Bioorg Med Chem Lett 2013;23:4931–4.
[20] Lone SH, Bhat KA, Majeed R, Hamid A, Khuroo MA. Click chemistry inspired facile synthesis and bioevaluation of novel triazolyl analogs of Ludartin. Bioorg Med Chem Lett 2014;24:1047–51.

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