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Expeditious synthesis of isolated steroids-fluorine prodrugs, their single crystal X-ray crystallography, DFT studies and mathematical modeling

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In the present research article, we have synthesized novel steroidal-fluorine prodrugs using cholesterol (**1**), stigmasterol (**4**) and hydrocortisone acetate (**7**) isolated from the flowers of *Allamanda violacea*. These isolated compounds were converted into their fluorinated prodrugs Cholest-5-en-3β-yl-2-fluoro-benzoate **(2)**, Cholest-5-en-3β-yl-4-fluoro-benzoate **(3)**, 24α-ethylcholest-5, 22*E*-dien-3β-yl-2-fluoro benzoate **(5)**, 24α-ethylcholest-5, 22*E*-dien-3β-yl-4-fluoro benzoate **(6)**, 11, 17-dihydroxy-21-(2-fluoro benzoyloxy)-pregn-4-en-3, 20-dione **(8)** and 11, 17-dihydroxy-21-(4-fluoro benzoyloxy)-pregn-4-en-3, 20-dione **(9)**. We also synthesized fluorinated prodrugs 25R-spirost-5-en-3β-yl-2-fluoro-benzoate **(11)** and 25R-spirost-5-en-3β-yl-4-fluoro-benzoate **(12)** from diosgenin (**10**) a steroidal sapogenin. The structure and stereochemistry of 25R-spirost-5-en-3β-yl-4-fluoro-benzoate (**12**) was confirmed with help of single crystal X-ray crystallography. The characterizations of compounds have been carried out with the help of ^{1}H, ^{13}C NMR, FT-IR spectroscopy and mass spectrometry. Density functional theory (DFT) with 6-31G (d, p) basis set and B3LYP functional has been used for the quantum chemical calculations. Global reactivity descriptors have also been calculated to find the electrophilicity and nucleophilicity of a molecule. Dipole moment, polarizability and first static hyperpolarizability have been calculated to get NLO properties of synthesized prodrugs. Lastly a nonlinear mathematical model has been proposed and analyzed to study the effect of catalysts on these reactions. Local and global stability analysis of the mathematical model along with the persistence of the system is checked using theory of nonlinear ordinary differential equations.

Keywords: Fluorine-prodrugs, Hydrocortisone, X-ray crystallography, Reactivity descriptors, NLO

Phytosterols the steroidal derivatives have shown potential biological activity as anti-inflammatory, antioxidant and anticancer[1-3]. A cholesterol derivative, pentalinonsterol (I) and polyoxygenated pregnane sterol glycoside (II), isolated from roots of *Pentalinon andieuxii* (Fig. 1) were found to have antileishmanial activity against amastigotes of *L. Mexicana* [4]. The oxime-ether derivatives of cholesterol (III, Fig. 1) were evaluated for antibacterial activity[5]. Polyamine conjugates (IV) of stigmasterol were evaluated for antimicrobial and cytotoxic activity[6] whereas corticosteroids (CS) are most widely used for various inflammatory and autoimmune disorders. Transformation of 21-hydroxy group of corticosteroids into ester with carboxylic acids (V) has been found to increase its clinical utility[7]. The dexamethasone-21-isonicotinate (VI) aerosol was effective in treatment of bronchial asthma[8].

In recent years, bioactivity of potential drugs were modified by introduction of fluorine atom/group e.g., 9a-fluorohydrocortisone acetate, desoximetasone, dexamethasone, betamethasone etc. Incorporation of fluorine into drug candidates generally improves their bioavailability, potency and metabolic stability. These fluorinated drugs have been reported to possess anti-cancer, antidepressants, antiinflammatory and anaesthetics activity[9-13] . Furthermore the flowers of *Allamanda violacea* have previously been reported to possess antidyslipidemic, antioxidant and antidiabetic activities[1,2]. Due to vast varieties of biological activity of fluorinated derivative, we planned to synthesize novel fluorinated steroidal prodrugs, starting from two phytosterols (cholesterol, stigmasterol) and one corticosteroid (hydrocortisone acetate) which were

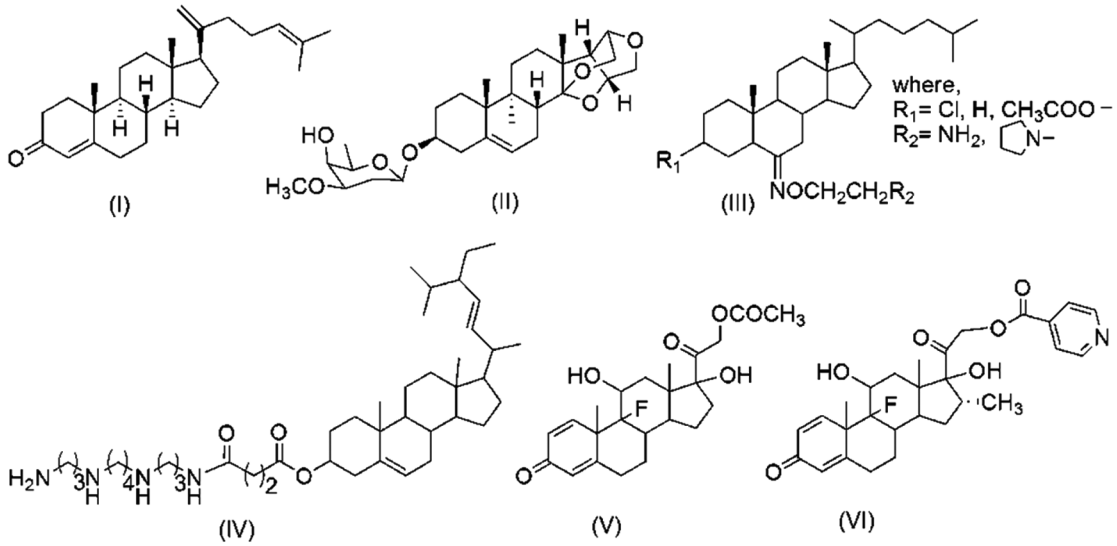


Fig. 1 — Biologically active molecules having steroidal skeleton and ester linkages

isolated from ether, pet. ether and chloroform extract of the flowers of *Allamanda,* respectively[1,2]. Besides the synthesis of title compounds, theoretical studies such as NLO and global reactivity descriptors have also been carried out on these molecules. Quantum chemical calculations were carried out by density functional theory (DFT) using B3LYP functional and 6-31G (d, p) basis set. Nonlinear optical (NLO) property of synthesized compounds was also calculated. Energy gap between HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) characterized the chemical stability and charge transfer interaction in these molecules. On the basis of HOMO-LUMO energy gap, global reactivity descriptors have also been calculated.

Experimental Details

Materials and methods

The whole plant *A. violacea* was collected in the month of October 2010 from Lucknow, India and its identity was confirmed by Dr. Tariq Hussain, Scientist and Head, Department of Taxonomy and Herbarium, National Botanical Research Institute, Lucknow, India where Voucher specimen, no-97108 was deposited[1,2]. All the solvent and reagents were taken as milligram (mg), millimole (mmol), millilitre (mL). Thin layer chromatography (TLC) was performed on silica gel G coated plates to detect progress of reaction and purity of compounds. The compounds were purified by column chromatography using silica gel (60-120 mesh) (Merck, India) as stationary phase.^{1}H NMR (proton nuclear magnetic resonance) spectra were recorded in $CDCl_3$ (deuterated chloroform) as solvent on a Bruker DRX-300 MHz (mega hertz) spectrometer and ^{13}C NMR (carbon nuclear magnetic resonance) spectra were recorded on JOEL AL 300 FT-NMR (75 MHz) Varian Inova spectrometer using $CDCl_3$ as the solvent where the chemical shifts were reported in parts per million (ppm) units with respect to TMS (tetra methyl silane) as internal standard and the multiplicity has been reported as m = multiplet, s = singlet, d = doublet, dd = double doublet, t = triplet, q= quartet. FT-IR (Fourier transform-Infrared) in ν_{max} (frequency of maximum absorption), spectra were recorded on Perkin Elmer FT-IR spectrometer within the range of 4000-450 cm^{-1}. The spectra were analyzed using Spectrum^{TM} Software suite and measured with 4 cm^{-1} resolution and 1 scan co-addition. ESI-MS (electron spray ionization-mass spectrometry) were recorded on Agilent 6520 Q-TOF mass spectrometer. Ultraviolet (UV) absorption spectra were obtained (in the range of 200-400 nm, nanometer) using ELICO BL-200 UV-Vis spectrophotometer equipped with a 10 mm quartz cell in chloroform ($CHCl_3$). Melting point (mp) was determined using open capillary tube method and uncorrected.

Crystal structure determination and refinement

The crystal data were collected on *X'calibur*CCD area-detector diffractometer equipped with graphite monochromated Mo K/α radiation (λ = 0.71073 Å). The structure was solved by direct method using

SHELXS97[28]. All the hydrogen atoms were geometrically fixed, and allowed to ride on the corresponding non-H atoms with C-H= 0.93-0.98 Å and U_{iso}= 1.2U_{eq}(C). Full-matrix least-square refinement was done using SHELXL97[28]. The crystallographic data are summarized in Table 1. The geometry of the molecule was calculated using the WINGX[29], PARST[30] and PLATON[31] softwares.

Computational studies

Theoretical studies of the compounds have been carried out using GAUSSIAN 09 program package[32] and gauss view[33]. Geometries of compounds have been optimized by density functional theory (DFT) using 6-31G (d, p) basis set and B3LYP functional.

Synthesis

Cholest-5-en-3β-yl-2-fluoro-benzoate (2)

A solution of 2-fluoro benzoic acid (2.17 mg, 0.0155 mmol), DCC (N, N′-Dicyclohexylca rbodiimide) (3.19 mg, 0.0154 mmol), DMAP (4-dimethylaminopyridine) (1.89 mg, 0.0154 mmol) and cholesterol (**1**) (6 mg, 0.0155 mmol) in $CHCl_3$ (chloroform, 10 ml) were stirred mechanically at room temperature for 3 h (progress of reaction was monitored by TLC). The side product *N,N′*-dicyclohexylurea (DCU) formed during the reaction was filtered off and the filtrate washed first with 5% HCl and then with water and then dried over anhydrous Na_2SO_4. The organic layer was evaporated under reduced pressure and the crude product obtained was purified by column chromatography (CC) using ethyl acetate : hexane (1:99) as eluent to give Cholest-5-en-3β-yl-2-fluoro-benzoate **(2)**[34] as white crystalline solid. yield 7.13 mg, 90.39%, m.p. 212-214°C; IR (ν_{max}, cm^{-1}): 3086, 3032, 2942, 2864, 2848, 1711, 1672, 1609, 1575, 1483, 1455. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.9 (H-34, dt, J=7.5, 7.5, 1.8 Hz), 7.5-7.4 (H-32, m), 7.2-7.0 (H-31, H-33, m), 5.4(H-6, br d, J= 4.8Hz), 4.9-4.8 (H-3, m), 2.4 (H-4, br d, J= 7.8Hz), 1.0 (CH_3-19, s), 0.9 (CH_3-21, d, J= 6.6Hz), 0.8 (CH_3-27, d, J= 1.2 Hz), 0.8 (CH_3-26, d, J= 1.5 Hz), 0.6 (CH_3-18, s). ^{13}C NMR (75 MHz , $CDCl_3$): δ_C C-28 (164.0), C-30 (160.4), C-5 (139.7), C-32 (134.4), C-34 (132.1), C-33 (124.0), C-6 (123.0), C-29 (119.7), C-31 (117.2), C-3 (75.2), C-17 (56.9), C-14 (56.3), C-9 (42.5), C-13 (39.9), C-24 (39.7), C-4 (38.3), C-10 (37.2), C-12 (36.8), C-22 (36.3), C-20 (36.0), C-8 (32.1), C-1 (32.0), C-7 (29.9), C-2 (28.4), C-25 (28.2), C-15 (28.0), C-16 (24.5), C-23 (24.0), C-19 (23.0), C-11 (22.7), C-27 (21.2), C-26 (19.5), C-18 (18.9), C-21 (12.0). ESI-MS: m/z 508 [M^+], 472, 375, 244, 242.

Cholest-5-en-3β-yl-4-fluoro-benzoate (3)

Compound **3**[35] was obtained by the reaction of 4-fluoro benzoic acid (2.17 mg, 0.0155 mmol), DCC (3.19 mg, 0.0154 mmol), DMAP (1.89 mg, 0.0154 mmol) and cholesterol (**1**) (6 mg, 0.0155 mmol) in $CHCl_3$ (10 mL) as white crystalline solid, yield 7.02 mg, 89.09%, m.p. 151-152°C; IR (ν_{max}, cm^{-1}): 2948, 2911, 2911, 2867, 2848, 1927, 1718, 1601,

Table 1 — Crystallographic data and structural refinement for compound **12**

CCDC no.	1044050
Crystal size	0.3 x 0.2 x 0.2 mm
Empirical formula	$C_{34}H_{45}FO_4$
Formula weight	536.70
Radiation, Wavelength	Mo *K*α, 0.71073 Å
Unit cell dimensions	a= 7.8117(7), b= 8.4207(7), c= 22.8108(19) Å, α=90°, β=98.266°(8), γ=90°
Crystal system	monoclinic
Space group	$P2_1$
Unit cell volume	1484.9(2)
No. of molecules per unit cell, Z	2
Temperature	293(2) K
Absorption coefficient	0.081mm^{-1}
F(000)	580
Scan mode	ω scan
θ range for entire data collection	3.821 <θ< 27.80
Range of indices	h= -9 to 9, k=-9 to 10, l= -17 to 28
Reflections collected / unique	5903 / 4532
Reflections observed (I > 2σ(I))	2094
R_{int}	0.0594

1505, 1463, 1444. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.08-8.02 (H-30 &H-34, m), 7.13-7.03 (H-31&H-33, m), 5.4 (H-6, br d, J=4.5Hz), 4.8-4.7 (H-3, m), 4.4-4.3 (H-16, q, J=7.8Hz), 3.5-3.4 (H-26e, m), 3.3 (H-26a, t, J=10.8Hz), 2.4 (H-4, br d, J=7.8Hz), 1.0 (CH_3-19, s), 0.9 (CH_3-21, d, J=6.9Hz), 0.8 (CH_3-27, d, J=6.0Hz), 0.7 (CH_3-18, s). ^{13}C NMR (75 MHz , $CDCl_3$): δ_C C-32& C-28 (165.5), C-5 (139.7), C-34 (132.3), C-30 (132.2), C-29 (127.3), C-6 (123.0), C-33 (115.7), C-31 (115.4), C-3 (74.9), C-17 (56.9), C-14 (56.3), C-9 (50.2), C-13 (42.5), C-24 (39.9), C-4 (39.7), C-10 (38.4), C-12 (37.2), C-22 (36.8), C-20 (36.4), C-8 (36.0), C-1 (32.1), C-7 (32.0), C-2 (28.4), C-25 (28.2), C-15 (28.1), C-16 (24.5), C-23 (24.0), C-19 (23.0), C-11 (22.7), C-27 (21.2), C-26 (19.5), C-18 (18.9), C-21 (12.0). ESI-MS: m/z 508 [M^+], 475, 431, 405, 337, 295, 262.

24α-ethylcholest-5,22E-dien-3β-yl-2-fluoro benzoate (5)

It was obtained by the reaction of 2-fluoro benzoic acid (2.37 mg, 0.0169 mmol), DCC (3.49 mg, 0.0169 mmol), DMAP (2.06 mg, 0.0168 mmol) and stigmasterol (**4**) (7 mg, 0.0169 mmol) in $CHCl_3$ (10 mL) as white crystalline solid, yield 8.32 mg, 91.84%, m.p. 256-258°C; IR (ν_{max}, cm^{-1}): 3052, 2951, 2920, 2850, 1715, 1668, 1612, 1579, 1458. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 7.9 (H-36, dt, J=7.5, 7.5, 1.8 Hz), 7.5-7.4 (H-34, m), 7.2-7.0 (H-33, H-35, m), 5.4 (H-6, br d, J=4.8 Hz), 5.2-5.1 (H-22, dd, J=8.4, 15.0 Hz), 5.0-4.9 (H-23, dd, J=8.7, 15.3 Hz), 4.9-4.8 (H-3, m), 2.4 (H-4, br d, J=7.8 Hz), 1.0 (CH_3-19, s), 1.01 (CH_3-21, d, J=6.6 Hz), 0.8 (CH_3-29, t, J=6.6 Hz), 0.8 (CH_3-27 & 26, d, J=6.3 Hz), 0.7 (CH_3-18, s). ^{13}C NMR (75 MHz , $CDCl_3$): δ_C C-32& C-28 (165.5), C-5 (139.7), C-34 (132.3), C-30 (132.2), C-29 (127.3), C-6 (123.0), C-33 (115.7), C-31 (115.4), C-3 (74.9), C-17 (56.9), C-14 (56.3), C-9 (50.2), C-13 (42.5), C-24 (39.9), C-4 (39.7), C-10 (38.4), C-12 (37.2), C-22 (36.8), C-20 (36.4), C-8 (36.0), C-1 (32.1), C-7 (32.0), C-2 (28.4), C-25 (28.2), C-15 (28.1), C-16 (24.5), C-23 (24.0), C-19 (23.0), C-11 (22.7), C-27 (21.2), C-26 (19.5), C-18 (18.9), C-21 (12.0). ESI-MS: m/z 534 [M^+], 502, 488, 430, 370.

24α-ethylcholest-5, 22E-dien-3β-yl-4-fluoro benzoate (6)

It was obtained by the reaction of 4-fluoro benzoic acid (2.37 mg, 0.0169 mmol), DCC (3.49 mg, 0.0169 mmol), DMAP (2.06 mg, 0.0168 mmol) and stigmasterol (**4**) (7 mg, 0.0169 mmol) in $CHCl_3$ (10 mL) as white crystalline solid, yield 7.99 mg, 88.11%, m.p. 186-188°C; IR (ν_{max}, cm^{-1}): 3056, 2939, 2871, 1712, 1651, 1610, 1508, 1485, 1454. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.09-8.02 (H-32 & H-36, m), 7.1-7.0 (H-33 & H-35, m), 5.4 (H-6, br d, J= 4.2Hz), 5.2-5.1 (H-22, dd, J= 8.4, 15.3 Hz), 5.0-4.9 (H-23, dd, J=8.4, 15.0 Hz), 4.9-4.7 (H-3, m), 2.4 (H-4 (br d, J=7.8 Hz), 1.0 (CH_3-19, s), 1.0 (CH_3-21, d, J=6.6 Hz), 0.8 (CH_3-29, t, J= 8.4Hz), 0.8 (CH_3-27 & 26, d, J=6.3 Hz), 0.7 (CH_3-18, s). ^{13}C NMR (75 MHz , $CDCl_3$): δ_C C-32& C-28 (165.5), C-5 (139.7), C-34 (132.3), C-30 (132.2), C-29 (127.3), C-6 (123.0), C-33 (115.7), C-31 (115.4), C-3 (74.9), C-17 (56.9), C-14 (56.3), C-9 (50.2), C-13 (42.5), C-24 (39.9), C-4 (39.7), C-10 (38.4), C-12 (37.2), C-22 (36.8), C-20 (36.4), C-8 (36.0), C-1 (32.1), C-7 (32.0), C-2 (28.4), C-25 (28.2), C-15 (28.1), C-16 (24.5), C-23 (24.0), C-19 (23.0), C-11 (22.7), C-27 (21.2), C-26 (19.5), C-18 (18.9), C-21 (12.0). ESI-MS: m/z 534[M^+], 487, 362, 246, 218.

11,17,21-tri-hydroxy-3,20-diketo-pregn-4-ene (Deacetylated hydroxycartisone) (7)

11,17,21-tri-hydroxy-3,20-diketo-pregn-4-ene (7) was synthesized by dissolving 29 mg (0.071 mmol) of hydrocortisone acetate in 1.5 mL of CH_3OH followed by addition of 0.3 mL of $CH_3O^-Na^+$ as a catalyst. The reaction mixture was kept at room temperature for 30 minutes, neutralized with 'IR 120 H^+ resin' and filtered. Filtrate was concentrated and compound 7 was purified with column chromatography using Chloroform/methanol as eluent yielding 27.5 mg (94.82%) of compound **7** in pure state[10].

11,17-dihydroxy-21-(2-fluoro benzoyloxy)-pregn-4-en-3,20-dione (8)

11,17,21-tri-hydroxy-3,20-diketo-pregn-4-ene (4.5 mg, 0.0124 mmol) (**7**) was dissolved in 2 mL of chloroform and then 2-fluoro benzoic acid (1.73 mg, 0.0123 mmol), DCC (2.55 mg, 0.0124 mmol) and DMAP (1.51 mg, 0.0123 mmol) were added. The crude product was purified by column chromatography (CC) using methanol–chloroform (2:98) as eluent to afford the pure product **8** as white crystalline solid, yield 5.53 mg, 92.10%, mp 183-185 °C; IR (ν_{max}, cm^{-1}): 3436, 2920, 2848, 1719, 1653, 1606, 1489, 1453. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.0-7.9 (1H, td, H-28, J=7.5, 1.8 Hz), 7.5-7.5 (1H, m, H-26), 7.2-7.1 (2H, m, H-27 & H-25), 5.6 (1H, br

d, H-4, J=0.9 Hz), 5.3 (1H, d, H-21A, J= 17.4 Hz), 5.1 (1H, d, H-21B, J=17.4Hz), 4.5-4.4 (1H, q, H-11, J= 6.0 Hz, 3.0 Hz), 1.4 (3H, s, CH_3-19), 1.0 (3H, s, CH_3-18). ^{13}C NMR (75 MHz , $CDCl_3$): δ_C 204.5 (C-20), 199.8 (C-3), 172.3 (C-5 & C-22), 164.1 (C-24), 135.2 (C-26), 132.5 (C-28), 124.3 (C-27), 122.6 (C-4), 118.0 (C-23), 117.4 (C-25), 90.0 (C-17), 68.6 (C-21), 56.5 (C-17), 52.2 (C-14), 47.9 (C-9), 40.0 (C-13), 39.4 (C-10), 35.2 (C-12), 35.0 (C-8), 34.0 (C-1), 32.9 (C-2), 32.2 (C-6), 31.6 (C-7), 29.9 (C-15), 23.8 (C-16), 21.2 (C-19), 17.4 (C-18). ESI-MS: m/z 485 $[M+H]^+$.

11,17-dihydroxy-21-(4-fluoro benzoyloxy)-pregn-4-en-3, 20-dione (9)

It was obtained by the reaction of 11,17,21-tri-hydroxy-3,20-diketo-pregn-4-ene (4.5 mg, 0.0124 mmol) (**7**) with 4-fluoro benzoic acid (1.73 mg, 0.0123 mmol), DCC (2.55 mg, 0.0124 mmol) and DMAP (1.51 mg, 0.0123 mmol) in 2 mL chloroform as white crystalline solid, yield 5.24 mg, 87.26%, m.p. 216-218°C; IR (ν_{max}, cm^{-1}): 3442, 3076, 2930, 2846, 1717, 1679, 1603, 1507, 1435. ^{1}H NMR (300 MHz, $CDCl_3$): δ_H 8.1-8.0 (2H, m, H-24 & H-28), 7.1-7.1 (2H, t, H-25 & H-27, J = 8.7 Hz), 5.6 (1H, s, H-4), 5.3 (1H, d, H-21A, J= 17.4 Hz), 5.1 (1H, d, H-21B, J=17.4 Hz), 4.5 (1H, br s, H-11), 1.4 (3H, s, CH_3-19), 1.0 (3H, s, CH_3-18). ^{13}C NMR (75 MHz , $CDCl_3$): δ_C 204.7 (C-20), 199.8 (C-3), 172.2 (C-5), 167.0 (C-26), 165.5 (C-22), 132.7 (C-24), 132.6 (C-28), 125.8 (C-23), 122.6 (C-4), 116.0 (C-25), 115.7 (C-27), 90.0 (C-17), 68.5 (C-11, C-21), 56.2 (C-9), 52.2 (C-14), 47.9 (C-13), 40.0 (C-12), 39.4 (C-1), 35.2 (C-2), 35.0 (C-6), 34.0 (C-7), 32.9 (C-10), 32.2 (C-16), 31.6 (C-8), 23.8 (C-19), 21.2 (C-15), 17.4 (C-18). ESI-MS: m/z 485 $[M+H]^+$.

25R-spirost-5-en-3β-yl-2-fluoro-benzoate (11)

It was obtained by the reaction of 2-fluoro benzoic acid (6.74 mg, 0.0169 mmol), DCC (6.98 mg, 0.0169 mmol), DMAP (4.12 mg, 0.0168 mmol) and diosgenin (**10**) (14 mg, 0.0169 mmol) in $CHCl_3$ (10 mL) as white crystalline solid, yield 18.5 mg, 89%, m.p. 192°C, FT-IR ν_{max} (in cm^{-1}): 3090, 3038, 2942, 2904, 2872, 2846, 1711, 1670, 1615, 1583, 1487, 1461, 1379, 1295, 1250, 1161, 1129, 1051, 981, 959, 913, 901, 837, 815, 793, 753, 689, 657, 635, 623, 577, 543, 539, 523, 479. ^{1}H NMR ($CDCl_3$, 300MHz) δ (ppm): 7.91 (1H, t, H-34, J= 7.2 Hz), 7.51-7.48 (1H, m, H-32), 7.20 (1H, t, H-33, J= 8.0 Hz), 7.12 (1H, t, H-31, J= 9.6 Hz), 5.42 (1H, br d, H-6, J= 4.8 Hz), 4.89- 4.86 (1H, m, H-3), 4.42 (1H, q, H-16, J= 15.2 Hz), 3.48- 3.50 (1H, m, H-26e), 3.39 (1H, t, H-26a, J= 11.2 Hz), 2.488-2.481 (2H, m, H-4), 1.08 (3H, s, CH_3-19), 0.98 (3H, d, CH_3-21, J= 7.2 Hz), 0.79 (3H, d, CH_3-27, J= 7.2 Hz), 0.78 (3H, s, CH_3-18). ^{13}C NMR (75MHz,$CDCl_3$) δ (ppm): 164.05 (C-28), 160.40 (C-30), 139.86 (C-5), 134.42 (C-32), 132.19 (C-34), 124.07 (C-33), 122.77 (C-6), 119.72 (C-29), 117.28 (C-31), 109.48 (C-22), 81.02 (C-16), 75.19 (C-3), 67.06 (C-26), 62.33 (C-17), 56.67 (C-9), 50.18 (C-14), 41.84 (C-20), 40.49 (C-4), 39.96 (C-13), 38.33 (C-10), 37.21 (C-12), 37.01 (C-15), 32.29 (C-8), 32.07 (C-23), 31.64 (C-25), 30.52 (C-1), 29.91 (C-7), 29.03 (C-2), 28.01 (C-24), 21.06 (C-19), 19.59 (C-11), 17.35 (C-18), 16.49 (C-27), 14.74 (C-21). ESI-MS: m/z= 537 $[M + H]^+$, 486, 485, 397, 379.

25R-spirost-5-en-3β-yl-4-fluoro-benzoate (12)

It was obtained by the reaction of 4-fluoro benzoic acid (6.74 mg, 0.0169 mmol), DCC (6.98 mg, 0.0169 mmol), DMAP (4.12 mg, 0.0168 mmol) and diosgenin (**10**) (14 mg, 0.0169 mmol) in $CHCl_3$ (10 mL) as white crystalline solid, yield 19 mg, 91%, m.p. 184°C, FT-IR ν_{max}(in cm^{-1}): 3065, 2931, 2904, 2876, 2854, 1713, 1667, 1609, 1509, 1455, 1383, 1347, 1323, 1291, 1125, 1185, 1173, 1163, 1125, 1093, 1071, 1049, 1023, 1005, 989, 911, 899, 845, 801 ,763, 729, 691, 643, 599, 515, 495, 467. ^{1}H NMR ($CDCl_3$, 300MHz) δ (ppm): 8.08-8.01 (2H, m, H-30 & H-34), 7.13-7.05 (2H, m, H-31 & H-33), 5.42 (1H, br d, H-6, J=4.5 Hz), 4.89-4.78 (1H, m, H-3), 4.43 (1H, q, H-16, J=7.8 Hz), 3.50-3.45 (1H, m, H-26e), 3.37 (1H, t, H-26a, J=10.8 Hz), 2.47 (2H, br d, H-4, J=7.8 Hz), 1.08 (3H, s, CH_3-19), 0.99 (3H, d, CH_3-21, J=6.9 Hz), 0.80 (3H, d, CH_3-27, J=6.0 Hz), 0.78 (3H, s, CH_3-18).^{13}C NMR (75MHz,$CDCl_3$) δ (ppm): 167.52 (C-32), 165.17 (C-28), 139.79 (C-5), 132.29 (C-30), 132.17 (C-34), 127.25 (C-29), 122.77 (C-6), 115.70 (C-31), 115.41 (C-33), 109.44 (C-22), 80.99 (C-16), 74.86 (C-3), 67.03 (C-26), 62.52 (C-17), 56.64 (C-9), 50.16 (C-14), 41.82 (C-20), 40.82 (C-4), 39.93 (C-13), 38.38 (C10), 37.18 (C-12), 36.98 (C-15), 32.27 (C-8), 32.05 (C-23), 31.62 (C-25), 30.49 (C-1), 29.89 (C-7), 29.01 (C-2), 28.05 (C-24), 21.04 (C19), 19.57 (C-11), 17.33 (C-18), 16.47 (C-27), 14.72 (C-21). ESI-MS: m/z= 474, 471, 449, 226, 248, 247.

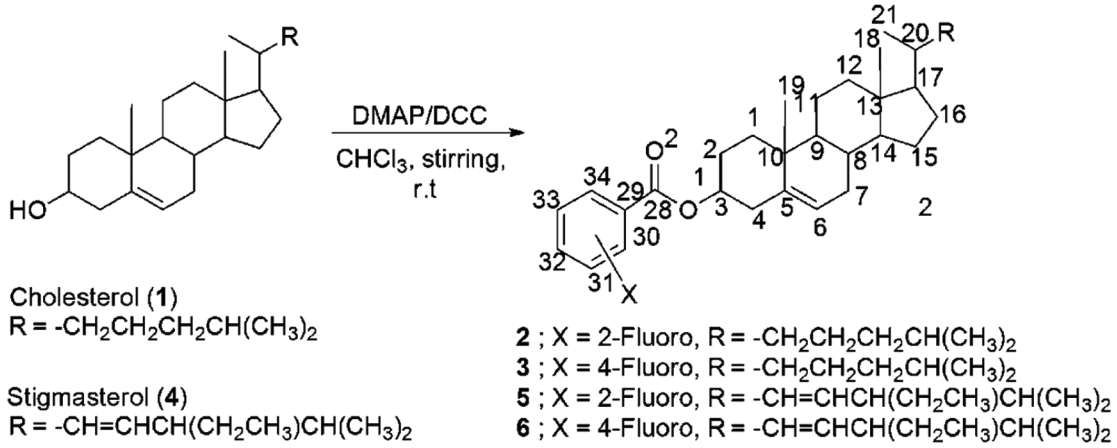
Result & Discussion

Chemistry

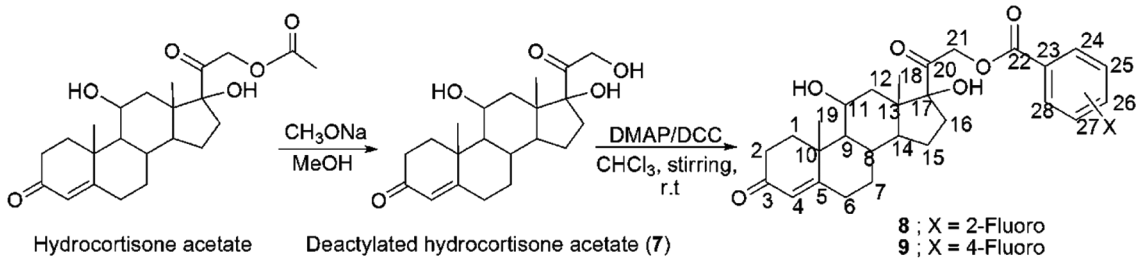
Extracts of *A. violacea* were prepared as earlier reported by Sethi *et al*[14]. Cholesterol (**1**) (12 mg) and Stigmasterol (**4**) (15 mg) and hydrocortisone acetate

(**7**) (32 mg) were obtained in pure form by repeated column chromatography[15]. Synthesis of desired fluorinated steroidal prodrugs were carried out with the help of Steglich esterification method using *N, N*′-Dicylcohexylcarbodiimide (DCC) and 4 Dimethylam inopyridine (DMAP) (Scheme 1, 2 and 3).

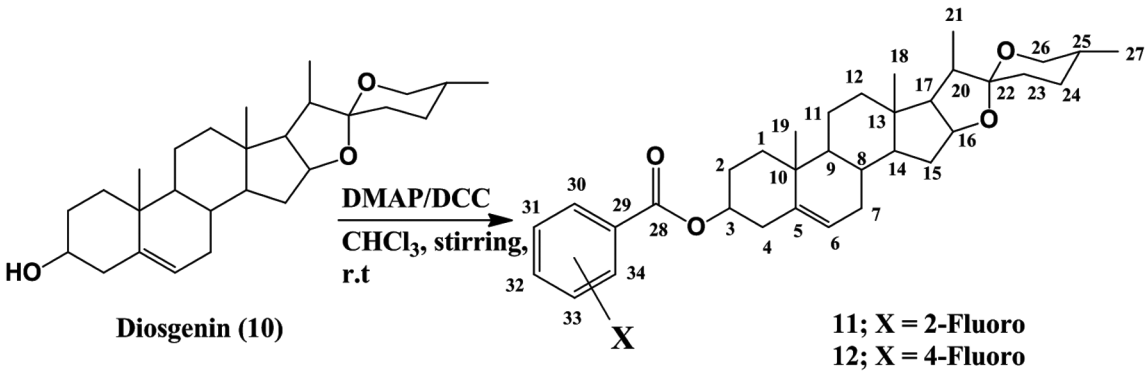
The assignment of peaks in the NMR and IR spectra with the help of earlier synthesized steroidal compounds[16,17]. The structure of all synthesized derivatives **2**, **3**, **5**, **6**, **8**, **9**, **11** and **12** were established using IR, ^{1}H-NMR,^{13}C-NMR, 2D NMR, MS spectra, which are described in the Experimental section. For example, in the ^{1}H NMR spectrum of 25R-spirost-5-en-3β-yl-4-fluoro-benzoate **(12)**, the downfield shifting of H-3 methine proton as a multiplet at δ 4.89- 4.78 (as compared to C-3 methine proton at δ 4.44-4.37 in compound **10**) along with the signals for aromatic ring protons as multiplet at δ 8.08-8.01 (2H, H-30 & H-34) and δ 7.13-7.05 (2H, H-31 & H-33) confirmed the esterification of the C-3 hydroxyl group of compound **10** with 4-fluoro benzoic acid. In the ^{13}C NMR of **12**, the introduction of the ester group at C-3 of compound **10** was confirmed by the signals for C-28 (C=O) of ester at δ 165.17 along with the signals



Scheme 1 — Synthesis of fluorinated prodrugs from cholesterol and stigmasterol



Scheme 2 — Synthesis of fluorinated prodrugs from hydrocortisone acetate



Scheme 3 — Synthesis of fluorinated prodrugs from Diosgenin

of aromatic carbons C-32 (δ 167.52), C-29 (δ 127.25), C-5 (δ 139.79), C-30 (δ 132.29), C-34 (δ 132.17), C-31 (δ 115.70) and C-33 (δ 115.41). The relative orientation of **12** was analyzed by correlations in a Nuclear Overhauser effect spectroscopy (NOESY) experiment. The NOESY correlation between CH_3-19/H-4b and H-3/H-4a revealed the β-orientation of CH_3-19, H-4b and α-orientation of H-3, H-4a respectively. It confirmed the bulky ester linkage has β-orientation as shown by arrow in Fig. 2.

Crystal structure of compound 12

Crystal structure determination

Needle shaped crystals of compound **12** were obtained by slow evaporation of solvent hexane: ethyl acetate at room temperature, and were in the monoclinic space group $P2_1$. The packing is stabilized by C-H…O and C-H… π intermolecular interactions. These interactions lend stability to the molecules in the crystal structure. The molecules are arranged to form layers along b axis and the pair of molecules are oriented over each other. An ORTEP view of the **12** indicating atomic labeling is shown in Fig. 3. ORTEP view of **12** with displacement ellipsoids drawn at 40%. H atoms of Ring A adopts the chair conformation with rotational axis bisecting the bonds C5-C6 and C2-C3 with the asymmetry parameter ΔC_2 = 0.86 and mirror plane passing through C2 and C5 with asymmetry parameter ΔC_s = 1.732. It is confirming that rotational symmetry is dominant over mirror symmetry. The shortening in the C2–C3 bond length [1.508(7) Å] can be because of the presence of a substituent at C-3[18]. The bond lengths of O1-C28 and C28-O2 are 1.343(7) and 1.206(7) Å, confirming the conversion of –OH group to ester group present at C-3. The bond distances 1.383(7), 1.346(8) and 1.376(7) Å of C34=C33, C32=C31 and C30=C29, respectively, confirm the presence of aromatic ring.

In ring B, location of double bond at C5-C6 is confirmed by its bond length which is equal to 1.312(6) Å, and the environment at C5 atom was found to be planar. Ring B adopts half chair conformation with asymmetry parameter ΔC_2 = 1.20. Ring C adopts the chair conformation with rotational axis bisecting the bonds C8-C9 and C12-C13 with the asymmetry parameter ΔC_2 =4.03 and mirror plane passing through C9 and C13 with asymmetry parameter ΔC_s = 1.9, indicating that mirror symmetry is dominant over rotational symmetry. Ring D adopts envelope conformation with rotational axis passing through atom C14 and bisecting the bond C16-C17 with asymmetry parameter ΔC_s = 17.01. Ring E adopts envelope conformation with rotational axis passing through atom O3 and bisecting the bond C17-C20 with asymmetry parameters ΔC_s = 3.65. Ring F adopts chair conformation with rotation axis bisecting

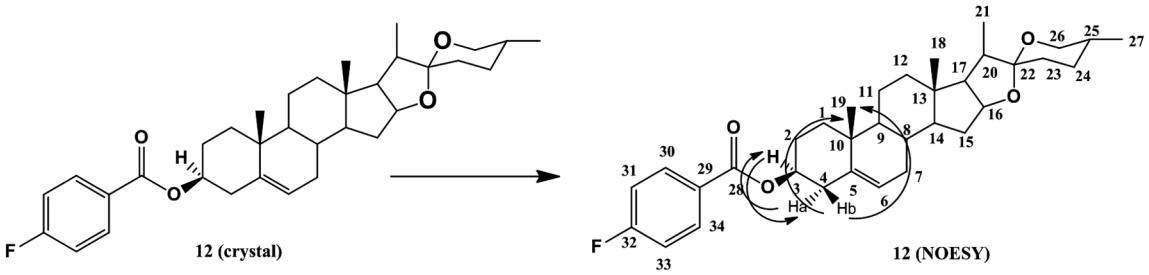


Fig. 2 — Nuclear Overhauser effect spectroscopy (NOESY) correlation of compound **12**.

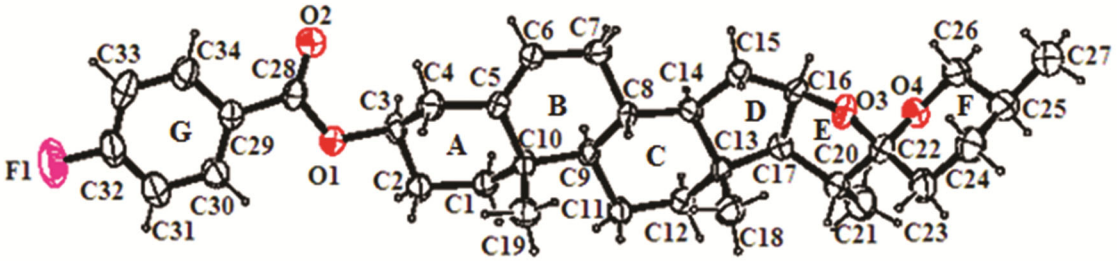


Fig. 3 — ORTEP view of compound **12** with displacement ellipsoids drawn at 40% H atoms

bonds C23-C24 and C26-O4 with asymmetry parameters ΔC_2 = 1.27 and mirror plane passing through C24 and O4 with asymmetry parameter ΔC_s = 1.88 showing that rotational symmetry is predominant.

Endocyclic torsional angles confirm that ring junction A/B is quasi trans while ring junctions B/C & C/D are trans and D/E is cis. Stereochemistry at C5-C6 double bond is cis (Z). From the crystal structure it is proved that angular methyl group CH_3-18, CH_3-19 and bulky ester group is β oriented (Fig. 4). There are 11 stereogenic centers and with the help of crystal structure following configuration has been assigned to them: C3: S, C8: S, C9: S, C10: R, C13: S, C14: S. C16: S, C17: R, C20: S, C22: R and C25: R. Endocyclic torsion angles (°) about the ring junctions of compound **12** are given in Table 2.

Computational simulations

Global reactivity descriptors

The chemical reactivity of the synthesized compounds has been determined by the conceptual DFT[19]. On the basis of Koopmans's theorem[20], electronegativity (χ), chemical potential (μ), global hardness (η), global softness (S) and electrophilicity index (ω) are the global reactivity descriptors which are useful in predicting global reactivity trends[21]. Electrophilic (acceptor) or nucleophilic (donor) nature of compound depends upon the value of electrophilicity index (ω). Higher the value of the ω better is the electrophilic character. The low values of global electrophilicity index (ω= 2.230, 2.226, 2.63 and 2.67) for the reactants **1**, **4**, **7** and **10** and higher value of global electrophilicity index (ω= 2.847, 2.691, 2.848, 2.693, 3.01, 2.95, 2.83 and 2.70) for the synthesized compounds **2, 3, 5, 6, 8, 9, 11** and **12** indicate that the reactants behave as a good nucleophile and it is for this reason that they readily undergo esterification on reaction with 2- and 4-fluoro benzoic acid to yield the corresponding products. Amongst them compound **8** acts as the best electrophile since the molecule shows high values for three global reactivity parameters, namely chemical potential (μ) = -3.75, global electrophilicity index (ω) = 3.01, and softness (*S*) = 0.21 along with the lowest HOMO-LUMO energy gap (-4.67 eV), as compared to other synthesized compounds- "as the number of fluorine atom in the molecule increases HOMO-

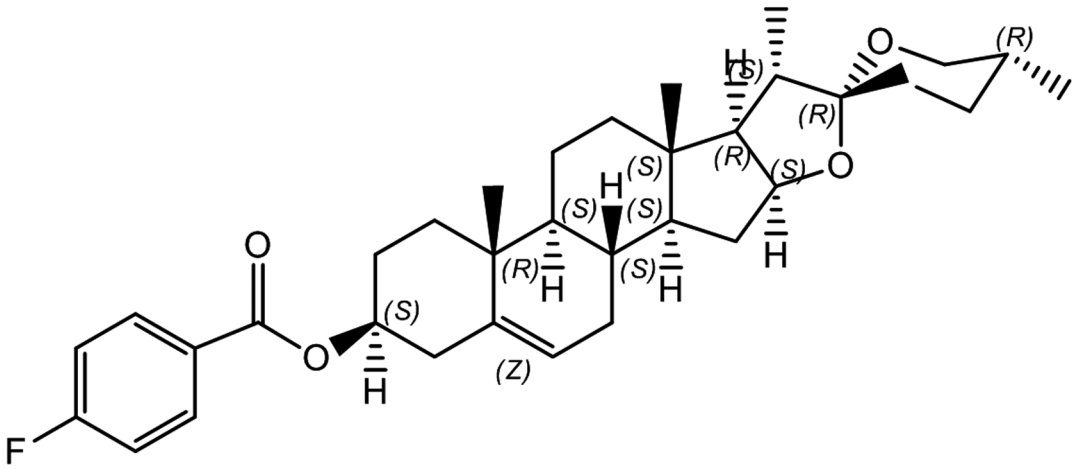


Fig. 4 — Representation of stereochemistry in **12**

Table 2 — Endocyclic torsion angles (°) about the ring junctions of compound **12**

Junction	Atoms	Torsion angle	Characteristics
A/B	C4–C5–C10–C1	50.6(6)	Quasi-trans
	C6–C5–C10–C9	-12.4(7)	
B/C	C7–C8–C9–C10	-63.1(6)	Trans
	C14–C8–C9–C11	46.8(6)	
C/D	C12–C13–C14–C8	59.0(6)	Trans
	C17–C13–C14–C15	-46.6(5)	
D/E	C13–C17–C16–C15	-14.2(6)	Cis
	C20–C17–C16–O3	-20.6(5)	

LUMO energy gap decreases''[22]. The non fluorinated compound (**1**) exhibits high HOMO-LUMO gap as compared to fluorinated compound (**2**).

NLO analysis

Molecules with nonlinear optical (NLO) property have vast applications in the telecommunications, optical switching, information storage, optical fibers and signal processing[23,24]. Hence, we also investigated the NLO properties of all the synthesized compounds. 'F atom enhances the NLO property. A non fluorinated compound (**1**) exhibits low hyperpolarizability as compare to fluorinated compound **2**. As we increase the number of fluorine atoms in the molecule HOMO-LUMO energy gap become decrease and show better non linear optical properties'[22]. The total static dipole moment (μ_o), mean polarizability ($|\alpha_0|$) and the mean first hyperpolarizability β_o can be calculated by following formula:

$$\mu_0 = (\mu_x^2 + \mu_y^2 + \mu_z^2)^{1/2}$$

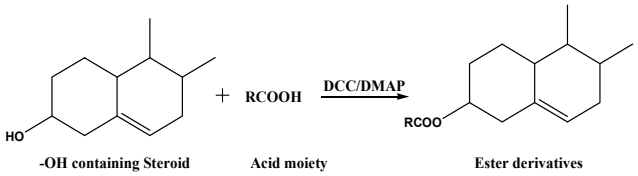
$$|\alpha_0| = 1/3\ (\alpha_{xx} + \alpha_{yy} + \alpha_{zz})$$

$$\beta_0 = [(\beta_{xxx} + \beta_{xyy} + \beta_{xzz})^2 + (\beta_{yyy} + \beta_{xxy} + \beta_{yzz})^2 + (\beta_{zzz} + \beta_{xxz} + \beta_{yyz})^2]^{1/2}$$

The value of the polarizability ($|\alpha_0|$), first hyperpolarizability (β_o) of Gaussian 09 output are reported in atomic unit (a.u.) therefore these values are converted into electrostatic unit (esu) as (for ($|\alpha_0|$): 1 a.u. = 0.1482×10^{-24} esu; for β_o: 1 a.u = 0.008639×10^{-30} esu). The calculated values of β_o for compound 2, 3, 5, 6, 8, 9, 11 and 12 by DFT method was 1.528×10^{-30}, 4.322×10^{-30}, 1.16×10^{-30}, 4.0×10^{-30}, 2.292×10^{-30}, 2.437×10^{-30}, 1.292×10^{-30} and 4.513×10^{-30} esu, respectively, which was found to be greater than those of urea (β_o of urea being 0.3728×10^{-30} esu by DFT method) so it may be concluded that the newly synthesized compounds may find use as non linear optical material.

Mathematical model

In this section, we develop a mathematical model for the reaction. Even the simplest chemical reactions can be highly complex and difficult to model[25-27]. Physical parameters such as temperature, pressure, and mixing, for example, are ignored in this text but effect of moisture is considered in the reaction and non linear differential equations with holling type II functional response are constructed that are dependent on the concentrations of the chemicals involved in the reaction. This is potentially a very difficult subject and some assumptions have to be made to make progress. Consider the simple chemical reaction



Let R be the density of -OH containing steroid, S be the density of acid moiety and P be the density of ester derivative. It is assumed that density of -OH containing steroid and acid moiety are increased by constant rate A_1 and A_2, respectively. In modelling process, the effect of catalyst (DMAP, $FeCl_3$, H_2SO_4) in this reacation is represented by h_c, which increase the rate of reaction and density of product. Let α be the interaction rate between reacants, a is half satuation constant which act in the model as effect of moisture on the chemical reaction and β is the growth rate of the product. We also assumed that density of reacants and product is decreased by different factors like oxidation etc, this effect is represented by μ, μ_1 and β_0 respectively. Thus keeping in view of these considerations, the non-linear model is proposed as follows

$$\frac{dR}{dt} = A_1 - \frac{\alpha R S}{a+R} - \mu R - h_c,$$
$$\frac{dS}{dt} = A_2 - \frac{\alpha R S}{a+R} - \mu_1 S - h_c, \qquad \ldots (1)$$
$$\frac{dP}{dt} = \frac{\beta R S}{a+R} + h_c - \beta_0 P.$$

where $R(0) \geq 0, S(0) \geq 0, P(0) \geq 0.$

The stability of the non-linear model system (Eqn. 1), in the positive octant, is investigated numerically by using the following set of parameters. $A_1 = 2$, $A_2 = 2$, $\alpha = 0.02$, $\mu = 0.0002$, $h_c = 0.001$, $\mu_1 = 0.0003$, $\beta = 0.01$, $\beta_0 = 0.018$ & $a = 0.5$

The interior equilibrium point of the model system (1) corresponding to the above parameters values is $E(148.2, 98.8002, 54.76)$. The characteristic

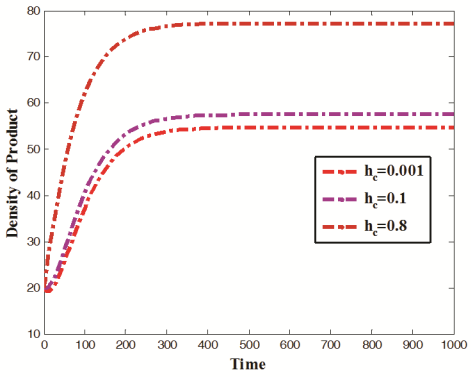


Fig. 5 — Effect of Catalyst on the reaction

polynomial and characteristic roots of the model system corresponding to the interior equilibrium point are given as:

$$\lambda^3 + 0.0384774\,\lambda^2 + 0.000372654\,\lambda + 7.30792\times10^{-8} = 0. \quad \ldots (2)$$

$$\lambda_1 = -0.0202772, \lambda_2 = -0.018, \lambda_3 = -0.0002.00223 \quad \ldots (3)$$

From Eqn. (3), it is clear that all characteristic roots of the characteristic polynomial (Eqn. 2) are negative. So, the interior equilibrium of the model system (Eqn. 1) is locally asymptotically stable.

Fig. S1 and Fig. S2 (Supplementary Data) shows the local and global stable behaviour of the system. Fig. 5 shows that effect of catalyst on the product generation. If we use three different catalysts with different rates, for example H_2SO_4 (A), $FeCl_3$ (B), DMAP (C) with rates 0.001, 0.1 and 0.8, respectively, then the product generation is highest for catalyst DMAP and lowest for catalyst H_2SO_4. Fig. S3 shows the effect of moisture on the reaction, when the value of parameter 'a' increases then the density of product decreases. The 3D view of stability behaviour of the system is shown in Fig. S4.

Conclusion

Cholesterol, stigmasterol and hydrocortisone acetate isolated from the flowers of *Allamanda violacea* were converted into their prodrugs **2, 3, 5, 6, 8,** and **9** whereas prodrugs **11** and **12** were synthesized from diosgenin by Steglich esterification and characterized by different spectroscopic techniques. The high value of global electrophilicity index (ω) for the products (**2, 3, 5, 6, 8, 9, 11** and **12**) and low value of ω for reactants (**1**, **4** & **7**) revealed that the synthesized compounds behave as good electrophiles as compared to the reactants. The compounds thus obtained can further undergo different reactions by reacting with different nucleophiles. NLO study showed that synthesized compounds might behave as good non linear optical material with comparison to the reactants. The proposed mathematical model was analyzed by the stability theory of differential equations. The conditions of existence of equilibrium point and its stability in both local and global cases was obtained. The condition, under which the system persists, by using differential inequality, has been found. By using numerical simulation, the effects of different catalyst on the system, it has been proved that catalyst DMAP gives highest productivity in comparison to catalyst H_2SO_4. From the mathematical modeling we also find the effect of moisture on the reaction, when the value of parameter 'a' increases then the density of product decreases.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/58776.

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