Asian Journal of Chemistry; Vol. 33, No. 1 (2021), 151-158

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2021.22954

Synthesis and Characterization of Novel Analogues of Lopinavir

PEKETI RAJESH REDDY[1,2,*], SIVANADH MUSUNURI[2], D. RAMASEKHARA REDDY[2], V. SUBRAHMANYAM CHITTALA[1], P.V.N.S. MURTHY[1] and K. KRISHNAMOHAN[1]

[1]Research and Development Department, Monvi Labs, 3rd Floor, Plot No 97, ALEAP Industrial Area, Gajularamaram, Hyderabad-500090, India
[2]Department of Chemistry, Krishna University, Machilipatnam-521001, India

*Corresponding author: E-mail: rajeshpeketi1979@gmail.com

Received: 19 August 2020; *Accepted*: 25 October 2020; *Published online*: 10 December 2020; AJC-20191

The present work describes the identification, origin, synthesis, characterization and control of four novel analogues of lopinavir *viz.* leucine analogue of lopinavir, isoleucine analogue of lopinavir, methyl analogue of lopinavir and dihydroxy analogue of lopinavir.

Keywords: Lopinavir, Leucine, Isoleucine, Analogues.

INTRODUCTION

Lopinavir (**1**) is known to have efficacy for the inhibition of HIV protease and the inhibition of HIV infection [1]. It is chemically known as (2*S*,3*S*,5*S*)-2-(2,6-dimethylphenoxy-acetyl)amino-3-hydroxy-5-[2-(1-tetrahydropyrimid-2-only)-3-methyl butanoyl]amino-1,6-diphenyl hexane and marketed by Abbott laboratories with the combination of ritonavir in the brand name of Kaletra®.

Lopinavir is unsuccessful for the treatment of HIV infection when administrated unaided. Lopinavir is more successful for the inhibition of HIV protease and for the inhibition of HIV infection when combined with ritonavir [2]. Lopinavir with combination of ritonavir, is particularly effective for the inhibition of HIV infection when used in grouping with one or more reverse transcriptase inhibitors and one or more other HIV protease inhibitors [2].

Impurities presence in an Active Pharmaceutical Ingredient (API) influences the drug effectiveness by changing the quality and safety. Impurities more than 0.1% [3] should be identified and characterized as per the International Conference on Harmonization (ICH) guidelines. Impurities are required to check the co-injection studies and analytical performance characteristic studies for example linearity, specificity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), system suitability testing and relative retention factor [4].

A number of impurities and analogues of lopinavir are reported in literature [5-12]. To our best of knowledge, the synthesis and characterization of the four novel analogues *viz.*, leucine, isoleucine, *N*-methyl and dihydroxy analogue of lopinavir are not reported yet. In view of regulatory importance of the related substances and analogues in the API, a study on all possible analogues in lopinavir was conducted. In the present work, the four novel analogues of lopinavir were identified, synthesized and characterized by various spectroscopic techniques.

EXPERIMENTAL

The solvents and reagents were obtained from commercial sources and were used without purification. ^{1}H & ^{13}C NMR spectral data were performed on Bruker-Avance 300 MHz, 500 MHz spectrometer in DMSO-d_6 and $CDCl_3$. The chemical shift values reported on the δ scale in parts per million, downfield from tetramethylsilane as an internal standard. IR spectra were recorded in the solid state using a Perkin-Elmer FT-IR spectrophotometer. Mass spectrum was recorded using a Perkin-Elmer PE SCIEX-API 2000, equipped with ESI source used online with a HPLC system after the ultraviolet (UV) detector.

Synthesis of leucine analogue of lopinavir

Synthesis of 2-(tetrahydro-2-oxopyrimidin-1(2*H*)-yl)-4-methylpentanoic acid (15): To a solution of KOH (25 g,

381.67 mmol) in demineralized water (100 mL) were added L-leucine (50 g, 381.67 mmol) and acrylonitrile (22.25 g, 419 mmol) at 0-5 ºC and the reaction mixture was stirred for 2 h to complete the reaction. Then, demineralized water (125 mL) was added to the reaction mixture at 0-5 °C. Methyl chloroformate (72.2 g, 763.2 mmol) was added to the reaction mixture by maintaining pH 9.5-10.5 with 30% w/w aqueous NaOH and the reaction mixture was stirred for 30 min for pH stabilization. Adjusted the pH of the reaction mixture to 1.3 with HCl (100 mL) and extracted the product in dichloromethane (200 mL). The resulting organic layer was concentrated under reduced pressure at 35-40 ºC yielding a 95 g residue. The resulting residue was dissolved in methanol (300 mL) and added NH_3 (300 mL) followed by Raney Ni (10 g). Hydrogenated the reaction mass with 5 kg/cm^2 hydrogen pressure at 50 ºC for 10 h to complete the reaction. Then, filtered the catalyst and resulting filtrate was concentrated under reduced pressure at 50-55 ºC to remove solvent. The resulting residue was dissolved in demineralized water (450 mL) and added aqueous NaOH solution (32 g dissolved in 450 mL water). The reaction mixture was heated to 98-100 ºC and maintained for 10 h to complete cylization. Then, adjusted pH of the reaction mass to 1.2 with concentrated HCl (30 mL). The precipitated product was filtered and washed with distilled water yielding 38 g of compound **15** (**Scheme-I**). HPLC Purity 98.40%; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.85-0.91 (m, 6H), 1.47-1.53 (m, 2H), 1.65-1.79 (m, 3H), 3.07-3.34 (m, 4H), 4.86-4.91 (m, 1H), 6.30 (s, 1H), 12.47 (brs, 1H). MS *m/z*: 215.1 [(M+H)$^+$].

Synthesis of leucine analogue of lopinavir amino alcohol (17): To a suspension of compound **15** (25 g, 116.8 mmol) in ethyl acetate (100 mL) was added carbonyldiimidazole (22 g, 135.7 mmol) at 15-20 ºC. The reaction mixture was stirred for 2 h at 15-20 ºC. This solution was added to a reaction mixture of compound **3** (50 g, 107.7 mmol) in ethyl acetate (200 mL) at 70-75 ºC. The reaction mixture was refluxed for 4 h at 70-75 ºC to complete the reaction. Then, the reaction mixture was cooled to 20-25 ºC and washed twice with demineralized water (250 mL) followed by 10% w/v aqueous NaCl solution (250 mL). The organic layer was concentrated under reduced pressure at 45-50 ºC results a 68 g of compound **16** as foamy solid (**Scheme-I**).

To a solution of compound **16** in methanol (300 mL), ammonium formate (16.4 g, 26.15 mmol) and 5% Pd on carbon (10.7 g, 21.5% w/w) at 25-30 ºC were added. The reaction mixture was heated to 50-55 ºC and stirred for 2 h to complete the reaction. Then, filtered the catalyst and the resulting filtrate was concentrated under reduced pressure at 45-50 ºC yielding 47 g of compound **17** as a foamy solid (**Scheme-I**). HPLC Purity 92.92%; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.796- 0.852 (2d, 6H), 1.176-1.284 (m, 1H), 1.303-1.325 (m, 3H), 1.546-1.686 (m, 5H), 1.945-1.966 (m, 3H), 1.990 (s, 1H), 2.016-2.128 (m, 6H), 2.204-2.262 (m, 3H), 2.532-2.578 (m, 2H), 2.673-2.818 (m, 7H), 2.966-3.023 (m, 3H), 3.270-3.319 (m, 2H), 3.462 (m, 1H), 3.583 (m, 1H), 3.892 (m, 3H), 4.009-4.108 (m, 2H), 4.673-4.694 (m, 1H), 6.273 (s, 1H), 7.113-7.665 (m, 18H), 7.662 (s, 1H). MS *m/z*: 481.3 [(M+H)$^+$].

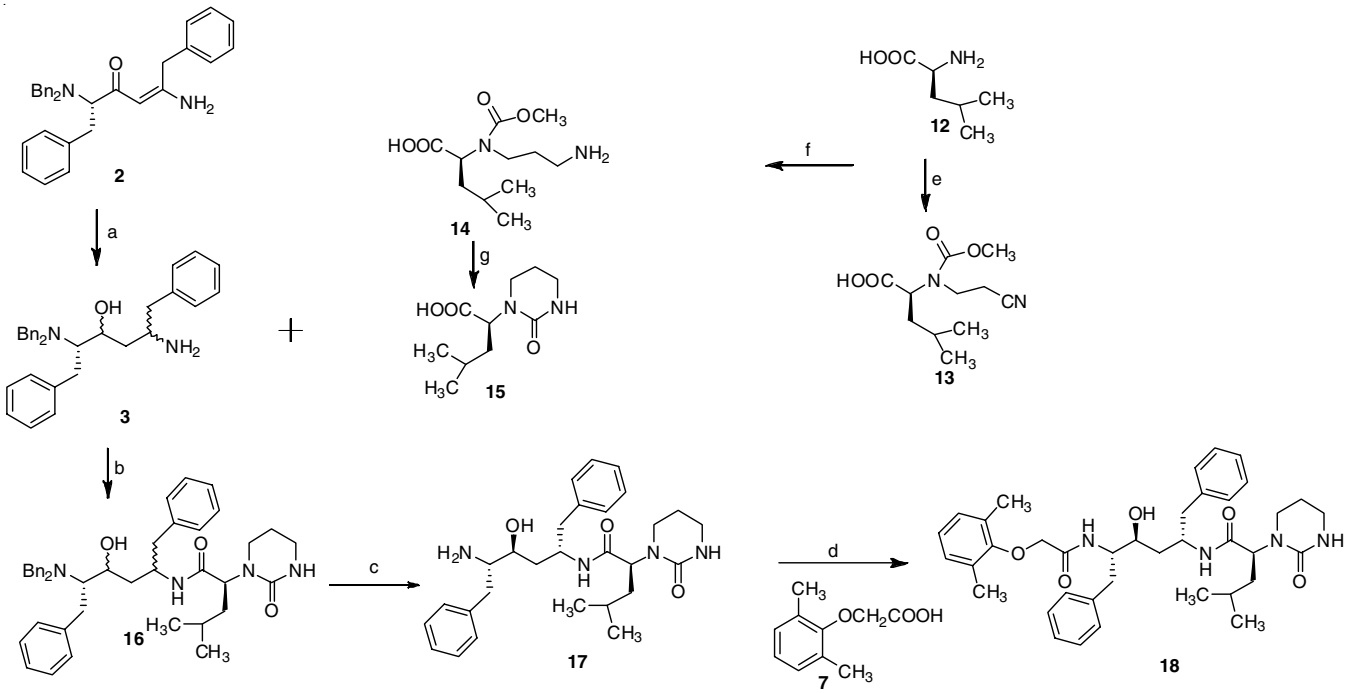
Synthesis of leucine analogue of lopinavir (18): To a suspension of 2,6-dimethylphenoxyacetic acid (2.75 g, 15.27 mmol) in ethyl acetate (10 mL) was added $SOCl_2$ (2.05 g, 17.24 mmol) at 20-25 ºC. The reaction mixture was stirred for 2 h at 50-55 °C and then cooled the reaction mixture to 20-25 ºC.

To a solution of compound **17** (10 g, 16.42 mmol), $NaHCO_3$ (8 g, 95.89 mmol) in ethyl acetate (75 mL), demineralized water (75 mL) and 2,6-dimethylphenoxyacetyl chloride solution at 10-15 ºC were added. The reaction mixture was stirred for 30 min to complete the reaction. The organic layer was separated and washed with demineralized water (50 mL) followed by brine solution (50 mL). The resulting organic layer was concentrated under reduced pressure 45-50 ºC yielding white coloured leucine analogue of lopinavir compound, which was crystallized from hexane give **18** (4.5 g, 34%) (**Scheme-I**). HPLC Purity 99.8%; IR (KBr pellet, cm^{-1}): 3411, 3320, 3061, 2954, 2868, 2333, 1947, 1651, 1516, 1477, 1385, 1366, 1346, 1264, 1231, 1195, 1125, 1092, 1057, 768, 752, 700; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.83-0.88 (m, 6H), 1.35-1.60 (m, 7H), 2.14 (s, 6H), 2.65-2.80 (2m, 4H), 2.81-3.04 (2m, 4H), 3.55 (m, 1H), 4.07-4.08 (ABq, 2H), 4.20 (m, 2H), 4.77 (m, 1H), 5.00 (d, 1H), 6.28 (s, 1H), 6.94-7.26 (m, 13H), 7.39-7.47 (m, 2H). MS *m/z*: 643.4 [(M+H)$^+$].

Synthesis of isoleucine analogue of lopinavir

Synthesis of 2-(tetrahydro-2-oxopyrimidin-1(2*H*)-yl)-3-methylpentanoic acid (22): To a solution of KOH (12.6 g, 190.8 mmol) in deminerlized water (50 mL) were added L-isoleucine (25 g, 190.8 mmol) and acrylonitrile (11.12 g, 209.8 mmol) at 0-5 ºC. The reaction mixture was stirred for 2 h at 0-5 ºC to complete the reaction. Then, demineralized water (62.5 mL) was added to the reaction mixture 0-5 ºC. Methyl chloroformate (36.06 g, 381.6 mmol) was added to the reaction mass by maintaining the pH 9.5-10.5 with 30% w/w aqueous NaOH at 0-5 ºC and then the reaction mixture was stirred for 30 min for pH stabilization. The pH of the reaction mixture was adjusted to 1.0 with HCl (50 mL) and the product was extracted in dichloromethane (200 mL). The organic layer was concentrated under reduced pressure at 35-40 ºC resulting a 32 g residue. The resulting residue was dissolved in methanol (150 mL), added NH_3 (150 mL) followed by Raney Ni (10 g). Hydrogenated the reaction mixture with 5 kg/cm^2 hydrogen pressure at 50 ºC for 10 h to complete the reaction. Then, filtered the catalyst and the resulting filtrate was concentrated under reduced pressure at 50-55 ºC to remove solvent. The resulting residue was dissolved in demineralized water (250 mL) and added aqueous NaOH solution (16 g dissolved in 250 mL distilled water). The reaction mixture was heated to 98-100 ºC and maintained for 16 h to complete cylization. The pH of the reaction mass was adjusted to 1.2 with conc. HCl (17 mL). The precipitated product was filtered and washed with water yielding **18** g of compound **22** (**Scheme-II**). HPLC Purity: 98.05%; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.81-0.89 (m, 6H), 1.03 (m, 1H), 1.29-1.37 (m, 1H), 1.74-2.20 (m, 3H), 3.09-3.34 (m, 4H), 4.52-4.55 (d, 1H), 6.35 (s, 1H), 12.52 (brs, 1H). MS *m/z*: 215.1 [(M+H)$^+$].

Synthesis of isoleucine analogue of lopinavir amino alcohol (24): To a suspension of compound **22** (15 g, 70 mmol) in ethyl acetate (90 mL) was add carbonyldiimidazole (13.2 g, 81.48 mmol) at 15-20 ºC. The reaction mixture was stirred for



Scheme-I: Synthetic scheme of leucine analogue of lopinaivr (**18**); **Reagents and conditions:** (a) sodium borohydride, methanesulfonic acid, trifluoroacetic acid (b) CDI, ethyl acetate (c) 5% Pd/C, ammonium formate, methanol, *L*-pyroglutamic acid (d) thionyl chloride, ethyl acetate, sodium bicarbonate, IPA, *n*-heptane (e) KOH, acrylonitrile, methyl chloroformate (f) methanol, ammonia, Raney Ni (g) sodium hydroxide; **Origin:** Presence of leucine in *L*-valine used during the preparation of compound **11** may produce Leucine analogue of lopinavir (**18**) in the subsequent steps of lopinavir; **Control:** Leucine analogue of lopinavir can be controlled in lopinavir by keeping limit of leucine in *L*-valine should be not more than 0.1% level

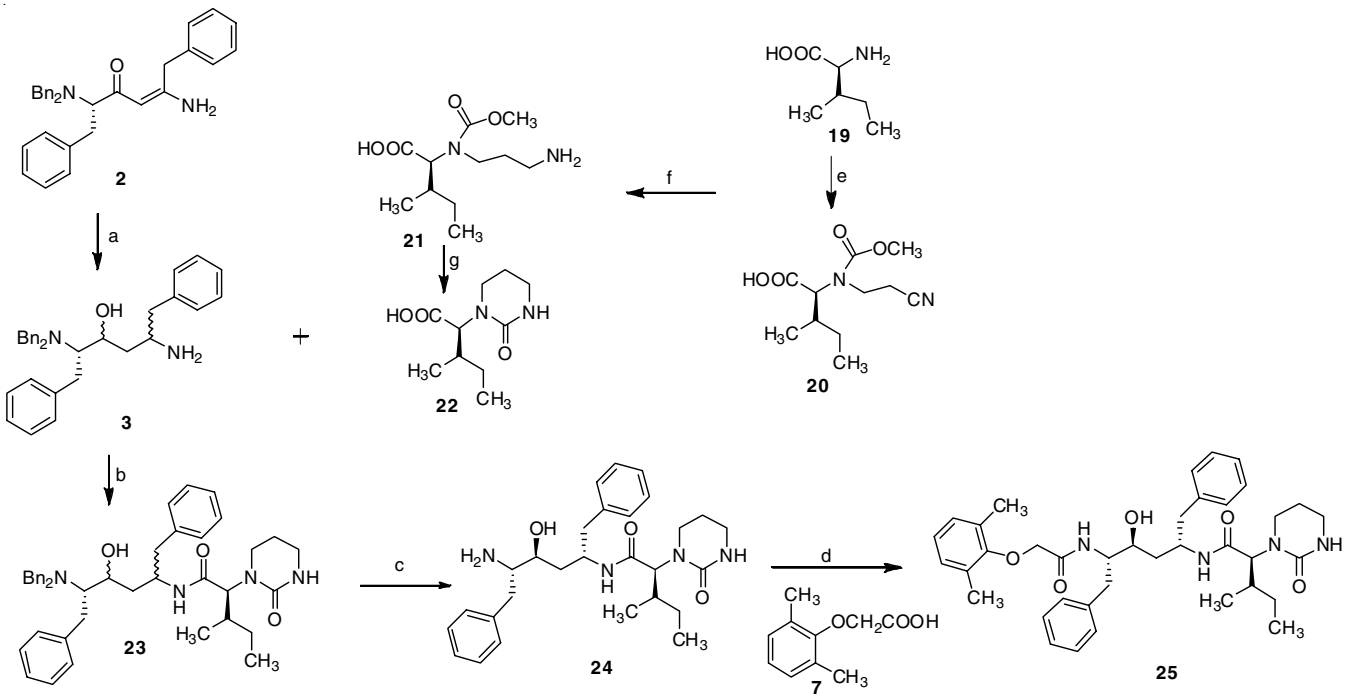
2 h 15-20 ºC. This solution was added to a reaction mixture of compound **3** (30 g, 64.65 mmol) in ethyl acetate (120 mL) at 70-75 ºC. The reaction mixture was refluxed for 4 h at 70-75 ºC to complete the reaction. Then, reaction mixture was cooled to room temperature and washed twice with demineralized water (100 mL) followed by 10% w/v aqueous NaCl solution (100 mL). The resulting organic layer was concentrated under reduced pressure at 45-50 ºC resulting a 47 g of compound **23** as foamy solid (**Scheme-II**).

To a solution of compound **23** in methanol (180 mL) , ammonium formate (12 g, 190.47 mmol) and 5% Pd on carbon (7.5 g, 21.5% w/w) 20-25 ºC were added. The reaction mixture was heated to 50-55 ºC and stir for 2 h to complete the reaction. Then, filtered the catalyst and the resulting filtrate was concentrated under reduced pressure at 45-50 ºC yielding 32 g of compound **24** as a foamy solid (**Scheme-II**). HPLC Purity 96.72%; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.653 (d, 3H), 0.776-0.791 (d, 3H), 0.877-0.908 (m, 1H), 1.257-1.273 (m, 1H), 1.421 (m, 1H), 1.550-1.682 (m, 3H), 1.787 (m, 1H), 1.925-1.962 (m, 1H), 2.061-2.081 (m, 2H), 2.190-2.265 (m, 1H), 2.472-2.517 (m, 1H), 2.631-2.674 (m, 1H), 2.727-2.754 (m, 2H), 2.874-2.890 (m, 2H), 3.016-3.074 (m, 3H), 3.496-3.511 (m, 1H), 3.887-3.905 (m, 1H), 4.037-4.051 (m, 1H), 4.359-4.382 (d, 1H), 6.283 (s, 1H), 7.109-7.548 (m, 10H), 7.567 (s, 1H), 7.653 (s, 1H). MS *m/z*: 481.3 [(M+H)$^+$].

Synthesis of isoleucine analogue of lopinavir (25): To a suspension of 2,6-dimethylphenoxyacetic acid (2.75 g, 15.27 mmol) in ethyl acetate (10 mL) was added $SOCl_2$ (2.05 g, 17.24 mmol) at 20-25 ºC. The reaction mixture was stirred for 2 h at 50-55 ºC to complete the reaction and then, cooled the reaction mass to 20-25 ºC. To a solution of compound **24** (10 g, 16.42 mmol), $NaHCO_3$ (8 g, 95.89 mmol) in ethyl acetate (75 mL), demineralized water (75 mL) and 2,6-dimethylphenoxy-acetyl chloride solution were added at 10-15 ºC. The reaction mixture was stirred for 30 min to complete the reaction. The organic layer was separated and washed with demineralized water (50 mL) followed by brine solution (50 mL). The resulting organic layer was concentrated under reduced pressure yielding white coloured isoleucine analogue of lopinavir (**25**) (7.2 g, 54%) (**Scheme-II**). HPLC Purity: 99.9%; IR (KBr pellet, cm^{-1}): 3411, 3320, 3061, 2961, 2929, 2873, 1946, 1647, 1513, 1477, 1378, 1366, 1349, 1265, 1230, 1195, 1156, 1092, 1056, 840, 762, 700; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.71 (d, 3H), 0.78-0.86 (m, 3H), 0.90-1.35 (m, 2H), 1.50 (m, 4H), 1.85 (m, 1H), 2.15 (s, 6H), 2.50-2.80 (m, 4H), 2.93-3.02 (m, 4H), 3.60 (m, 1H), 4.07-4.09 (ABq, 2H), 4.10-4.42 (m, 3H), 5.00 (d, 1H), 6.29 (s, 1H), 6.93-7.26 (m, 13H), 7.45-7.52 (2d, 2H). MS *m/z*: 643.4 [(M+H)$^+$].

Synthesis of *N*-methyl analogue of lopinavir

Synthesis of compound 26: To a solution of compound **3** (50 g, 107.75 mmol) in acetone (250 mL) were added K_2CO_3 (44.61 g, 323.26 mmol) and CH_3I (30.60 g, 215.5 mmol) at room temperature. The reaction mixture was stirred for 5 h at room temperature to complete the reaction. The resulting salts were removed by filtration and the filtrate was concentrate under



Scheme-II: Synthetic scheme of isoleucine analogue of lopinavir (**25**); **Reagents and conditions:** (a) sodium borohydride, methanesulfonic acid, trifluoroacetic acid (b) CDI, ethyl acetate (c) 5% Pd/C, ammonium formate, methanol, *L*-pyroglutamic acid (d) thionyl chloride, ethyl acetate, sodium bicarbonate, IPA, *n*-heptane (e) KOH, acrylonitrile, methyl chloroformate (f) methanol, ammonia, Raney Ni (g) sodium hydroxide; **Origin:** Presence of isoleucine in *L*-valine used during the preparation of compound **11** may produce isoleucine analogue of lopinavir (**25**) in the subsequent steps of lopinavir; **Control:** Isoleucine analogue of lopinavir can be controlled in lopinavir by keeping limit of isoleucine in *L*-valine should be not more than 0.1% level

reduced pressure at 40-45 ºC results a residue. The resulting residue was dissolved in ethyl acetate (100 mL), added demineralized water (50 mL) and the reaction mixture pH was adjusted to 7.0 with aqueous HCl solution. The organic layer was separated and washed with demineralized water (100 mL). The resulting organic layer was concentrated under reduced pressure at 45-50 ºC resulting a residue, which was further purified by column chromatography to obtain a pure compound **26** (9 g, 17%) (**Scheme-III**). HPLC Purity: 96.27%; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.87-0.92 (d, 1H), 1.80-1.88 (m, 1H), 2.23-2.26 (m, 1H), 2.47 (s, 3H), 2.48-2.54 (m, 1H), 2.67 (m, 1H), 2.81-3.1 (m, 4H), 3.19-3.37 (m, 3H), 4.04-4.08 (m, 2H), 7.08-7.39 (m, 20H). MS *m/z*: 479.3 [(M+H)$^+$]

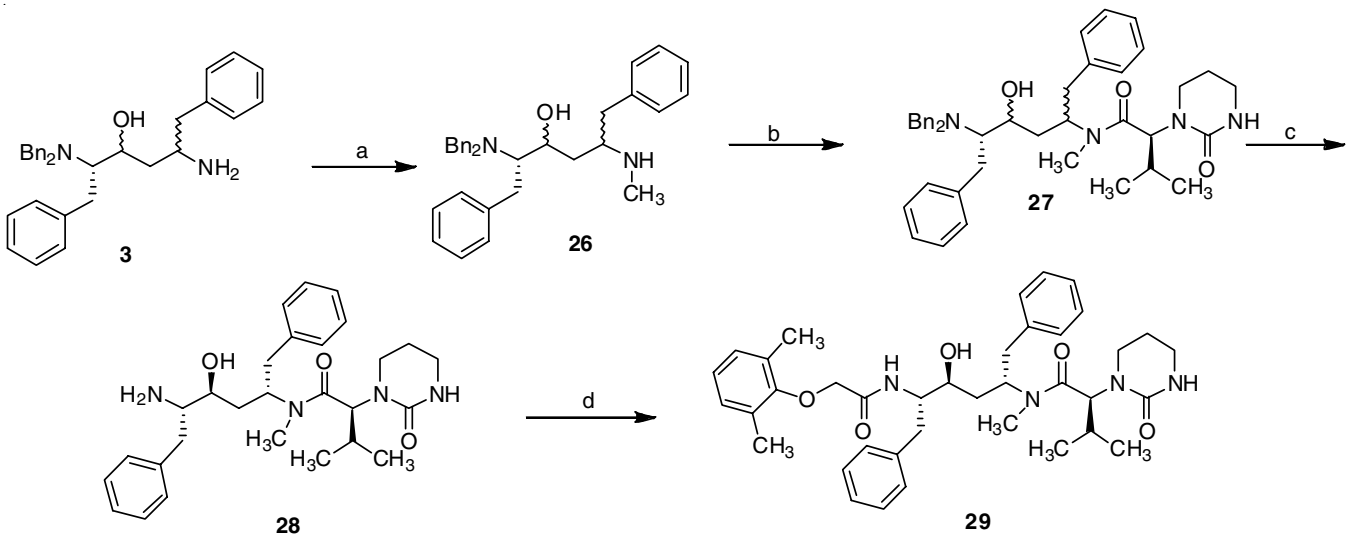
Synthesis of *N*-methyl analogue of lopinavir (29): To a suspension of compound **11** (2.4 g, 12 mmol) in ethyl acetate (20 mL) was added carbonyldiimidazole (2.11 g, 13 mmol) at 15-20 ºC. The reaction mixture was stirred for 2 h at the same temperature and this solution was added to a reaction mixture of compound **26** (5 g, 10.46 mmol) in ethyl acetate (20 mL) at 70-75 ºC. The reaction mixture was refluxed for 4 h at 70-75 ºC to complete the reaction and then, the reaction mixture was cooled to room temperature and washed twice with demineralized water (10 mL) followed by 10% w/v aqueous NaCl solution (10 mL). The resulting organic layer was concentrated under reduced pressure at 45-50 ºC resulting a 6.8 g of compound **27** as foamy solid (**Scheme-III**).

To a solution of compound **27** in methanol (30 mL) were added ammonium formate (1.64 g, 26.15 mmol) and 5% Pd on carbon (1.07 g, 21.5% w/w) at 20-25 ºC. The reaction mixture was heated to 50-55 ºC and stirred for 2 h to complete reaction. Then, filtered the catalyst and the resulting filtrate was concentrated under reduced pressure at 45-50 ºC yielding 4.4 g of compound **28** as a foamy solid (**Scheme-III**).

To a suspension of 2,6-dimethylphenoxyacetic acid (1.34 g, 7.75 mmol) in ethyl acetate (5 mL) was added $SOCl_2$ (0.99 g, 8.33 mmol) at 20-25 ºC. The reaction mixture was stirred for 2 h at 50-55 ºC and then the reaction was to cool at room temperature. To a solution of compound **28** (4 g, 8.33 mmol), $NaHCO_3$ (4 g, 48.66 mmol) in ethyl acetate (30 mL), demineralized water (30 mL) and 2,6-dimethylphenoxyacetyl chloride solution were added at 10-15 ºC and then the reaction mixture was stirred for 30 min to complete the reaction. The organic layer was separated and washed with demineralized water (100 mL) followed by brine solution (8 mL). The resulting organic layer was concentrated under reduced pressure 50-55 ºC yielding white coloured *N*-methyl analogue of lopinavir compound **29** (4.2 g, 78%) (**Scheme-III**). HPLC Purity: 96.40%; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.692-0.782 (2d, 6H), 1.234-1.553 (m, 4H), 1.664 (m, 1H), 2.10 (s, 6H), 2.503-2.683 (m, 4H), 2.821-2.861 (m, 3H), 2.91-2.99 (m, 4H), 3.480 (m, 1H), 4.038-4.182 (m, 3H), 4.469-4.675 (m, 1H), 4.890 (m, 1H), 6.906-6.934 (brs, 1H), 6.906-7.005 (m, 1H), 7.125-7.153 (m, 2H), 7.177-7.266 (m, 12H), 7.486-7.519 (d, 1H). MS *m/z*: 643.4 [(M+H)$^+$].

Synthesis of dihydroxy analogue of lopinavir (33)

Synthesis of (*2S,3S,5S*)-2-amino-3,4-hydroxy-5-[2*S*-(1-tetrahydropyrimid-2-onyl)-3-methylbutanoyl]amino-1,6-



Scheme-III: Synthetic scheme of *N*-methyl analogue of lopinavir (**29**); Reagents and conditions: (a) potassium carbonate, methyl iodide (b) CDI, ethyl acetate, **11** (c) 5% Pd/C, ammonium formate, methanol, *L*-pyroglutamic acid (d) **7**, thionyl chloride, ethyl acetate, sodium bicarbonate, IPA, *n*-heptane; **Origin:** *N*-Methylation is plausible during the preparation of lopinavir amino alcohol; **Control:** *N*-Methyl lopinavir amino alcohol should be controlled to not more than 0.3% level in lopinavir amino alcohol used during the preparation of lopinavir

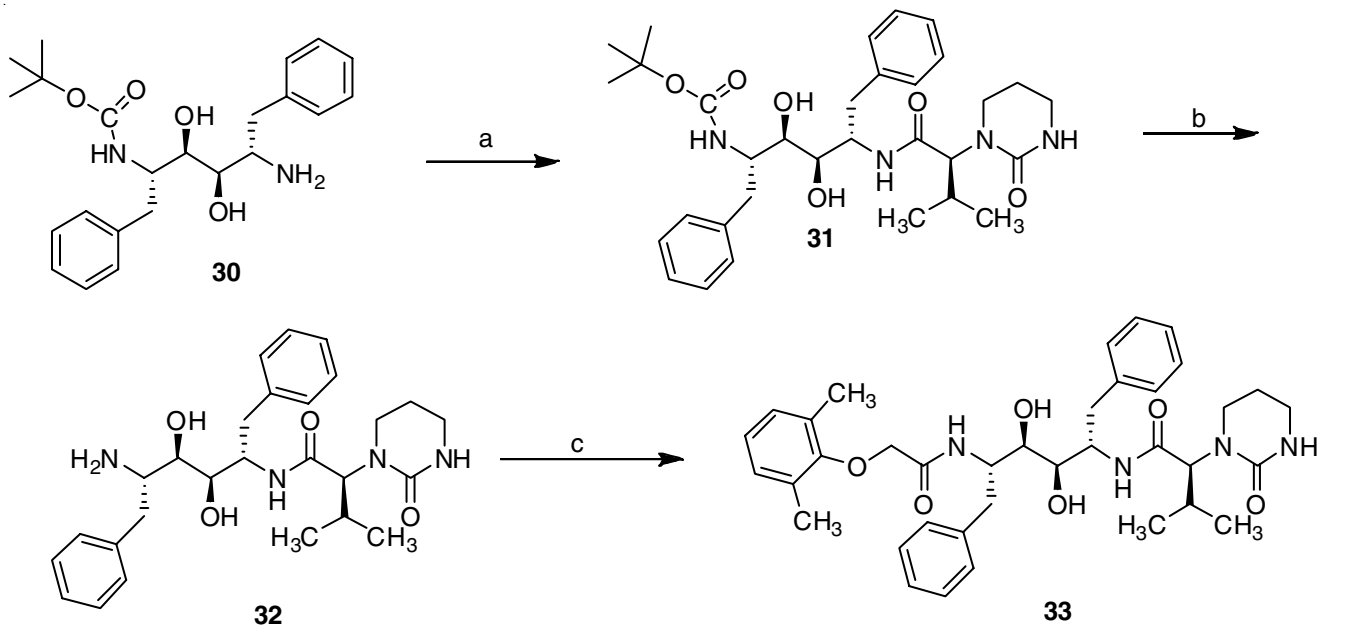
diphenylhexane (dihydroxy analogue of lopinavir amino alcohol) (32): To a suspension of compound **11** (0.575 g, 2.87 mmol) in ethyl acetate (10 mL) was added carbonyldiimidazole (0.506 g, 3.12 mmol) at 15-20 ºC. The reaction mixture was stirred for 2 h at 15-20 ºC. This solution was added to a reaction mixture of compound **30** (1 g, 2.5 mmol) in ethyl acetate (30 mL) at 70-75 ºC. The reaction mixture was refluxed for 4 h at 70-75 ºC to complete reaction and then, reaction mixture was cooled to room temperature and washed twice with demineralized water (10 mL) followed by 10% w/v aqueous NaCl solution (10 mL). The resulting organic layer was separated and concentrated under reduced pressure at 45-50 ºC resulting a 1.7 g of foamy solid. To this foamy solid was added methanolic HCl solution (30 mL, assay is 4% w/w) at 25-30 ºC and then the reaction mixture was stirred for 1 h at 35-40 ºC. The reaction mixture was concentrated under reduced pressure at 45-50 ºC results a solid material, which was dissolved in ethyl acetate (10 mL) and washed the reaction mass with 7% $NaHCO_3$ solution (10 mL). Finally, organic layer was washed with distilled water (10 mL) followed by 10% w/v aqueous NaCl solution (10 mL). The resulting organic layer was concentrated under reduced pressure 45-50 ºC yielding a 1.2 g of compound **32** as a foamy solid (**Scheme-IV**). HPLC Purity: 94.42%; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.65-0.69 (2d, 6H), 0.72 (m, 1H), 0.81-0.88 (m, 4H), 1.17-1.20 (m, 1H), 1.24-1.29 (m, 1H),1.96-1.99 (m, 2H), 2.60-2.65 (m, 3H), 2.83-2.92 (m, 2H), 3.00-3.16 (m, 8H), 3.62 (m, 1H), 3.91 (m, 1H), 4.29-4.33 (d, 1H), 4.48 (brs, 1H), 6.33 (brs, 1H), 7.14-7.28 (m, 12H), 7.76 (d, 1H). MS *m/z*: 483.3 [(M+H)$^+$].

Synthesis of dihydroxy analogue of lopinavir (33): To a suspension of 2,6-dimethylphenoxyacetic acid (0.186 g, 1.03 mmol) in ethyl acetate (2.5 mL) was added $SOCl_2$ (0.1296 g, 1.08 mmol) at 20-25 ºC. The reaction mixture was stirred for 2 h at 50-55 ºC. The reaction mixture was cooled to room temperature.

To a solution of compound **32** (0.5 g, 1.03 mmol), $NaHCO_3$ (1 g, 12.11 mmol) in ethyl acetate (3.75 mL), demineralized water (3.75 mL) and 2,6-dimethylphenoxyacetyl chloride solution were added at 10-15 ºC and then the reaction mixture was stirred for 30 min to complete the reaction. The organic layer was separated and washed with demineralized water (200 mL) followed by brine solution (2.5 mL). The organic layer was concentrated under reduced pressure yielding white coloured dihydroxy analogue of lopinavir compound **33** (0.5 g, 75%) (**Scheme-IV**). HPLC Purity: 90.20%; IR (KBr pellet, cm^{-1}): 3856, 3679, 3320, 3061, 2954, 2868, 2333, 1947, 1651, 1516, 1477, 1453, 1385, 1366, 1346, 1264, 1231, 1195, 1125, 768, 752, 700; ^{1}H NMR (DMSO-d_6, 300 MHz): 0.70-0.74 (2d, 6H), 1.33-1.53 (2m, 2H),1.96 (m, 1H), 2.16 (s, 6H), 2.74 (m, 4H), 2.89-2.98 (2m, 4H), 3.23-3.29 (2m, 2H), 4.12-4.16 (ABq, 2H), 4.26 (m, 1H), 4.45 (m, 2H), 4.91 & 5.65 (2brs, 2H), 6.32 (brs, 1H), 6.94-7.30 (m, 13H), 7.71 (d, 1H). MS *m/z*: 645.2 [(M+H)$^+$].

RESULTS AND DISCUSSION

Several reports [13-16] are available for the synthesis of lopinavir (**1**). The synthetic route for lopinavir (**1**), as mentioned in the literature (**Scheme-V**), which involves reaction of enaminone (**2**) with $NaBH_4$ and methanesulfonic acid to yield (2*S*,3*S*,5*S*)-2-*N*,*N*-dibenzylamino-3-hydroxy-5-amino-1,6-diphenylhexane (**3**). Activation of compound **3** with *N*,*N*-carbonyldiimidazole followed by condensation with *S*-tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetic acid (**11**) produces (2*S*,3*S*,5*S*)-2-*N*,*N*-dibenzylamino-3-hydroxy-5-[2*S*-(1-tetrahydropyrimid-2-onyl)-3-methylbutanoyl]amino-1,6-diphenylhexane (**4**). Compound **4** undergoes debenzylation with ammonium formate and palladium on charcoal produces (2*S*,3*S*,5*S*)-2-amino-3-hydroxy-5-[2*S*-(1-tetrahydropyrimid-2-



Scheme-IV: Synthetic scheme of dihydroxy analogue of lopinavir (**33**); **Reagents and conditions:** (a) CDI, ethyl acetate, **11** (b) methanolic HCl (c) **7**, thionyl chloride, ethyl acetate, sodium bicarbonate; **Control:** Dihydroxy lopinavir amino alcohol should be controlled to not more than 0.3% level in lopinavir amino alcohol used during the preparation of lopinavir

onyl)-3-methylbutanoyl]amino-1,6-diphenyl hexane (**5**), which is further purified by using *L*-pyroglutamic acid. Finally, compound **5** was condensed with 2,6-dimethylphenoxyacetic acid to produce lopinavir (**1**).

L-Valine is treated with acrylonitrile and methyl chloroformate in presence of KOH to give compound **9**. Compound **9**, when reduced with Raney nickel to yield compound **10**, which further cyclized to produce *S*-tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetic acid (**11**).

2,6-dimethylphenoxyacetic acid (**7**) is a key raw material for the synthesis of lopinavir, which is synthesized by reacting 2,6-dimethyl phenol (**6**) with chloroacetic acid in presence of sodium hydroxide base in water. Similarly, 2-(tetrahydro-2-oxopyrimidin-1(2*H*)-yl)-4-methylpentanoic acid (**15**) is one of the key raw materials for the synthesis of lopinavir, which was synthesized by using L-valine (**Scheme-V**). Since, L-Valine contains leucine and isoleucine analogues as related substances. Hence, there is a possibility of formation of corresponding related analogues (**18** and **25**) in lopinavir.

Origin, synthesis and control of lopinavir analogues

Leucine analogue of lopinavir (18): L-Leucine (**12**) is treated with acrylonitrile and methyl chloroformate in presence of KOH to provide compound **13**. Compound **13** was reduced with Raney nickel to produce compound **14**, which was further cyclized to produce 2-(tetrahydro-2-oxopyrimidin-1(2*H*)-yl)-4-methylpentanoic acid (**15**).

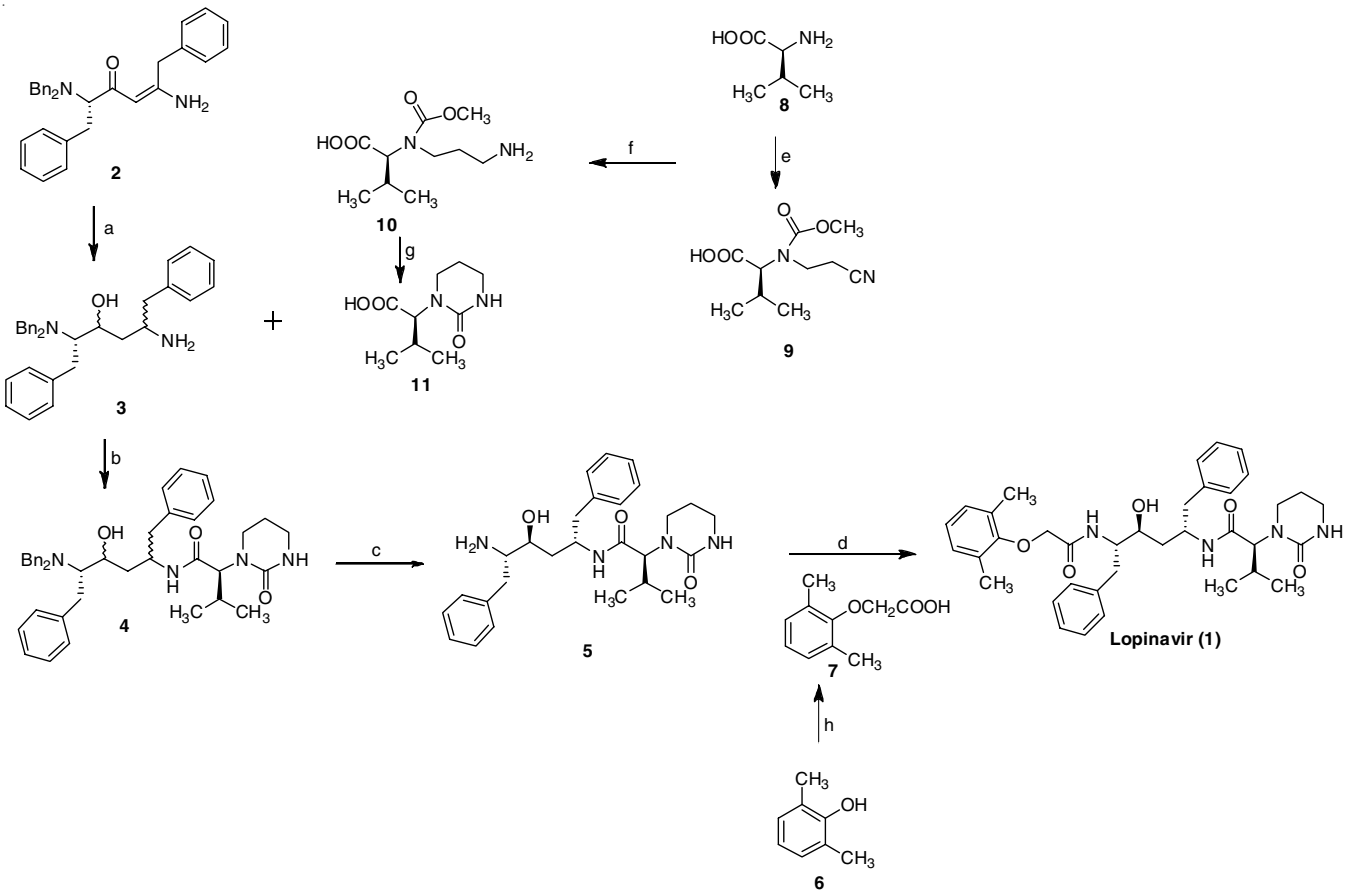
Activation of compound **3** with *N,N*-carbonyldiimidazole followed by condensation with 2-(tetrahydro-2-oxopyrimidin-1(2*H*)-yl)-4-methylpentanoic acid (**15**) yield compound **16**. Compound **16** undergoes debenzylation with ammonium formate and 5% palladium on charcoal produces compound **17**, which was further purified by using L-pyroglutamic acid. Compound **17** was finally condensed with 2,6-dimethylphenoxyacetic acid to give leucine analogue of lopinavir (**18**).

Isoleucine analogue of lopinavir (25): L-Isoleucine (**19**) was treated with acrylonitrile and methyl chloroformate in the presence of KOH to yield compound **20**. Compound **20** was then reduced with Raney nickel to give compound **21**, which was further cyclized to give 2-(tetrahydro-2-oxopyrimidin-1(2*H*)-yl)-3-methylpentanoic acid (**22**).

Activation of compound **3** with *N,N*-carbonyldiimidazole followed by condensation with 2-(tetrahydro-2-oxopyrimidin-1(2*H*)-yl)-3-methylpentanoic acid (**22**) yield compound **23**. Compound **23** undergoes debenzylation with ammonium formate and 5% palladium on carbon gives compound **24** which was further purified by using L-pyroglutamic acid. Compound **24** was finally condensed with 2,6-dimethylphenoxyacetic acid to give isoleucine analogue of lopinavir (**25**) (**Scheme-II**).

***N*-Methyl analogue of lopinavir (29):** (2*S*,3*S*,5*S*)-2-*N*,*N*-dibenzylamino-3-hydroxy-5-amino-1,6-diphenylhexane (**3**) is treated with CH_3I in presence of K_2CO_3 to yield compound **26**. Activation of compound **26** with *N,N*-carbonyldiimidazole followed by condensation with *S*-tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetic acid (**11**) gives compound **27**. Compound **27** undergoes debenzylation with ammonium formate and 5% palladium on carbon yield compound **28**, which was further purified by using L-pyroglutamic acid. Compound **28** was finally condensed with 2,6-dimethylphenoxyacetic acid to produce *N*-methyl analogue of lopinavir (**29**) (**Scheme-III**).

Dihydroxy analogue of lopinavir (33): (2*S*,3*S*,5*S*)-5-Amino-2[*N*-(*tert*-butyloxycarbonyl)amino]-3,4-dihydroxy-1,6-diphenyl hexane (**30**) was condensed with *S*-tetrahydro-α-(1-methylethyl)-2-oxo-1(2*H*)-pyrimidineacetic acid (**11**) to yield compound **31**. Compound **31** when treated with methanolic HCl yielded (2*S*,3*S*,5*S*)-2-amino-3,4-dihydroxy-5-[2*S*-(1-tetra-



Scheme-V: Synthetic scheme of lopinavir (**1**); **Reagents and conditions:** (a) sodium borohydride, methanesulfonic acid, trifluoroacetic acid (b) CDI, ethyl acetate (c) 5% Pd/C, ammonium formate, methanol, L-pyroglutamic acid (d) thionyl chloride, ethyl acetate, sodium bicarbonate, IPA, *n*-heptane (e) KOH, acrylonitrile, methyl chloroformate (f) methanol, ammonia, Raney Ni (g) sodium hydroxide (h) chloroacetic acid, sodium hydroxide, 2-(tetrahydro-2-oxopyrimidin-1(2*H*)-yl)-4-methylpentanoic acid (**15**) is one of the key raw material for the synthesis of lopinavir, which was prepared by using L-valine, as shown in **Scheme-I**. L-Valine contains leucine and isoleucine analogues as related substances. Hence, there is a possibility of formation of corresponding related analogues (**18** & **25**) in lopinavir

hydropyrimid-2-onyl)-3-methylbutanoyl]amino-1,6-diphenyl hexane (**32**) which was further purified by using L-pyroglutamic acid. Compound **32** was finally condensed with 2,6-dimethyl-phenoxyacetic acid gives dihydroxy analogue of lopinavir (**32**) (**Scheme-IV**).

All these novel analogues of lopinavir were assessed for potential mutagenicity by structure activity relationship (SAR) screening using the expert rule based software Derek Nexus and statistics based software leadscope and found to be non-mutagenic related substances.

Conclusion

A study on various analogues in lopinavir was conducted. Different process related analogues of lopinavir were identified, synthesized and characterized by using various spectroscopic techniques like liquid chromatography-mass (LC-MS), mass, ^{1}H NMR and FT-IR techniques. These efforts to synthesize and characterize the analogues of lopinavir effectively have proved to be beneficial.

ACKNOWLEDGEMENTS

The authors thank the Management of Monvi Labs for carrying out this work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. A. Chandwani and J. Shuter, *Ther. Clin. Risk Manag.*, **4**, 1023 (2008); https://doi.org/10.2147/tcrm.s3285
2. C. Flexner, *N. Engl. J. Med.*, **338**, 1281 (1998); https://doi.org/10.1056/NEJM199804303381808
3. International Conference on Harmonization (ICH) Guidelines. Q3A (R) Impurities in New Drug Substances, ICH Guidelines: Geneva, Switzerland (2002).
4. International Conference on Harmonization (ICH) Guidelines, Q2B Validation of Analytical Procedure: Methodology, ICH Guidelines: Geneva, Switzerland (1996).
5. H.L. Sham, D.A. Betebenner, T. Herrin, G. Kumar, S. Vasavanonda, A. Saldivar, A. Molla, D.J. Kempf, J.J. Plattner and W. Norbeck, *Bioorg. Med. Chem. Lett.*, **11**, 1351 (2001); https://doi.org/10.1016/S0960-894X(01)00243-8
6. S.R. Chitturi, Ch. Bharathi, A.V. Reddy, K.C. Reddy, H.K. Sharma, V.K. Handa, R. Dandala and V.H. Bindu, *J. Pharm. Biomed. Anal.*, **48**, 1430 (2008); https://doi.org/10.1016/j.jpba.2008.09.015
7. H.L. Sham, C. Zhao, L. Li, D.A. Betebenner, S. Vasavanonda, D.J. Kempf, A. Saldivar, J.J. Plattner and D.W. Norbeck, *Bioorg. Med. Chem. Lett.*, **12**, 3101 (2002); https://doi.org/10.1016/S0960-894X(02)00643-1

8. H.L. Sham, D.A. Betebenner, X. Chen, A. Saldivar, S. Vasavanonda, D.J. Kempf, J.J. Plattner and D.W. Norbeck, *Bioorg. Med. Chem. Lett.*, **12**, 1185 (2002); https://doi.org/10.1016/S0960-894X(02)00134-8
9. A.V. Raghava Reddy, *Sci. Pharm.*, **83**, 49 (2015); https://doi.org/10.3797/scipharm.1407-14
10. H.L. Sham, D.A. Betebenner, W. Rosenbrook, T. Herrin, A. Saldivar, S. Vasavanonda, J.J. Plattner and D.W. Norbeck, *Bioorg. Med. Chem. Lett.*, **14**, 2643 (2004); https://doi.org/10.1016/j.bmcl.2004.02.089
11. A.S. Lakshmi Devi, Y.S. Rao, Y. Suresh, M.Y. Reddy, G. Jyothi, B. Rajababu, V.S. Prasad and V.U. Rao, *Magn. Reson. Chem.*, **45**, 424 (2007); https://doi.org/10.1002/mrc.1968
12. U. Seshachalam, B. Haribabu and K. Chandrasekhar, *Biomed. Chromatogr.*, **21**, 716 (2007); https://doi.org/10.1002/bmc.810
13. V.R.R. Ambati, S. Garaga, R. Dandala and S. Meenakshisunderam, Process for the Preparation of Substantially Pure (2*S*,3*S*,5*S*)-5-Amino-2-*N*,*N*-dibenzylamino-3-hydroxy-1,6-diphenylhexane, US Patent No 8236991B (2007).
14. E.J. Stoner, A.J. Cooper, D.A. Dickman, L. Kolacz Kowski, J.E. Lallaman, J.H. Liu, P.A. Oliver-Shaffer, K.M. Patel, J.B. Paterson, D.J. Plata, D.A. Riley, H.L. Sham, P.J. Stengel and J.H. Tien, *Org. Process Res. Dev.*, **4**, 264 (2000); https://doi.org/10.1021/op990202j
15. H.L. Sham, D.W. Norbeck, X. Chen and D.A. Betebenner, Retroviral Protease Inhibiting Compounds, US Patent 5,914,332 (1995).
16. E. Ramu and B.V. Rao, *Tetrahedron Asymm.*, **20**, 2201 (2009); https://doi.org/10.1016/j.tetasy.2009.09.003