RJC

RASĀYAN *J. Chem.*
Vol. 6 | No.3 | 230-237| July- September | 2013
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

NOVEL TRICYCLIC CYANOPYRROLIDINE DERIVATIVES AS DPP4 INHIBITORS: AN IMPROVED SYNTHESIS OF TRICYCLIC α-CYCNOPYRROLIDINE FROM CAMPHOR

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ABSTRACT
An improved multistep synthesis of tricyclic α-cyanopyrrolidine from camphor is presented. The synthesis was carried out by maintaining the cyano group in the form of an amide and its regeneration after the completion of the necessary chemical transformation of a particular functional group. The tricyclic α-cyanopyrrolidine prepared was used to synthesize two novel compounds of DPP4 (Dipeptidyl peptidase-4) inhibitors.
Keywords: Cyanopyrrolidine, amide, camphor, DPP4 inhibitors.
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INTRODUCTION

Conformationally restricted amino acids and cyclic amino acids, peptides and peptidomimetics[1] have attracted considerable interest particularly in the area of drug design and discovery. For example, conformationally restricted bicyclic and tricyclic proline analogues have been explored for the identification of DPP4 (Dipeptidyl peptidase-4) inhibitors[2], anti hypertensive agents[3] and ACE (angiotensin-converting-enzyme inhibitor) inhibitors[4]. Conformationally restricted amino acids are also used as chiral auxiliaries[5] in organic synthesis. In view of much success in using α-cyanopyrrolidine (either monocyclic or bicyclic) as a pharmacophore for the development of DPP4 inhibitors[6] e.g. Vildagliptin, Saxagliptin, etc (Figure-1) we became interested in exploring the tricyclic α-cyanopyrrolidine framework as an alternative pharmacophore. Accordingly, we designed and subsequently planned to synthesize the compound **1** and **2** as potential inhibitors of DPP4.

Vildagliptin Saxagliptin

Fig.-1: α-Cyanopyrrolidine based DPP4 inhibitors.

Based on the reported synthesis of Vildagliptin and Saxagliptin, a retro synthetic route was established for compound **1** and **2** (Figure-2) which identified compound **3** as a key intermediate for their synthesis.

EXPERIMENTAL

All reactions were performed in dried glassware under nitrogen atmosphere. All solvents used were commercially available and distilled before use. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing ultraviolet light (Camag 254 nm & 366 nm). Purifications via chromatography were performed on silica gel (100-200 mesh) using petroleum ether, ethyl acetate, dichloromethane, chloroform and methanol. ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer using $CDCl_3$ or CD_3OD as solvents. Proton chemical shifts (δ) are relative to

tetramethylsilane as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constant (*J*) are expressed in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were recorded on Polman melting point apparatus. MS spectra were obtained on a mass spectrometer. Specific optical rotations were recorded using Jasco P-2000 Polarimeter.

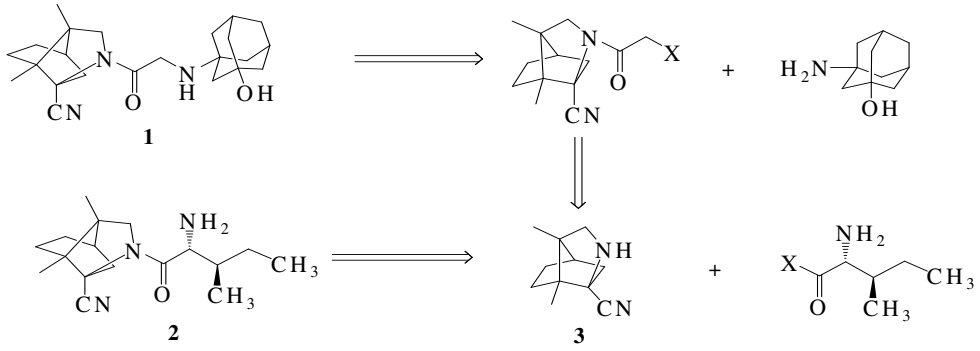


Fig.-2: Retro synthetic route for compound **1** and **2.**

Preparation of (+)-3-Bromocamphor (4)[7]

Bromine (8.9 ml, 0.17 mole) diluted with glacial acetic acid (200 ml) was added to (+)-camphor (25 g, 0.16 mol) in glacial acetic acid (50 ml) at 80 ^{o}C over a period of 3 h. After 24 h at 80 ^{o}C, starting material was absent by GC and the reaction mass was cooled to room temperature. Quenched in aqueous sodium bisulphite solution and the product was extracted with diisopropyl ether (2 x 100 ml). The combined organic layer was washed with aqueous sodium bicarbonate solution, dried over anhydrous Na_2SO_4 and concentrated to afford the desired product **4** (36.85 g, 97%); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.93 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 1.0.8 (s, 3H, CH_3), 1.38-1.47 (m, 1H), 1.65-1.74 (m, 1H), 1.86-1.90 (m, 1H), 2.04-2.13 (1H), 2.30 (t, *J* = 4.4 Hz, 1H, CH), 4.61 (d, *J* = 4.6 Hz, 1H, CH); MS (ESI Method) : 231 (M, 100%), 233 (M+2, 100%).

Preparation of (+)-3, 3-Dibromocamphor (5)[7]

To a round bottom flask covered with carbon sheet was taken (+)-3-bromocamphor **4** (15 g, 0.065 mol) followed by bromine (5 ml, 0.097 mole) and then heated to 55 ^{0}C. The reaction mass was stirred at 55 ^{o}C and monitored by GC. After 24 h at 55 ^{o}C, the reaction mass was quenched in saturated sodium bisulphite solution. The resulting mass was stirred for 1 h and precipitated solid product was filtered. The obtained solid was stirred in aqueous $NaHCO_3$ solution, filtered and dried under vacuum to yield (+)-3,3-dibromocamphor **5** (19.1g, 95%); mp 60-61 ^{o} C; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.01 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.24 (s, 3H, CH_3), 1.61-1.66 (m, 2H), 2.03-2.13 (m, 1H), 2.28-2.37 (m, 1H, CH), 2.82 (d, *J* = 4.1 Hz, 1H, CH); MS (ESI Method) : 310 (M), 312 (M+2); IR (cm^{-1}) : 2967, 2874, 1762, 1486, 1455, 1396, 1233,1020, 1001, 912, 815, 776, 703; $[\alpha]_D^{25}$: +38.2^{o} (c 1.67, 95% EtOH). .

Preparation of (+)-3, 3, 8-Tribromocamphor (6)[7]

(+)-3, 3-Dibromocamphor **5** (10 g, 0.032 mole) was added portion wise to a cooled (5 ^{0} C) solution of bromine (2.4 ml, 0.047 mole) in chlorosulphonic acid (17 ml, 0.255 mole). The reaction mass was stirred at RT for 4 h and then saturated $NaHSO_3$ solution was added to decompose excess bromine, HBr. The product was extracted with diisopropyl ether (2 x 100 ml), washed with aqueous $NaHCO_3$ solution and dried over anhydrous Na_2SO_4. The organic layer was concentrated at 40 ^{o}C under vacuum to afford the title compound **6** (9.0 g, 75%) with 51 % purity by GC. The crude product was taken for next step without purification. A small sample was purified by column chromatography for structure confirmation; mp 38 - 40 ^{o} C; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.04 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.76-1.81 (m, 2H),

1.98-2.10 (m, 1H), 2.33-2.42 (m, 1H), 3.1 (d, *J* = 3.9 Hz, 1H), 3.29 (d, *J* = 11.1 Hz, 1H, HCHBr), 3.73 (d, *J* = 11.0 Hz, 1H, HCHBr); IR (cm^{-1}) : 2969, 2877, 1765, 1485, 1455, 1380, 1261, 1230, 1016, 999,915, 818, 779, 758, 649; $[\alpha]_D^{25}$: +70.6° (c 0.72, $CHCl_3$).

Preparation of (+)-8-bromocamphor (7)[7]

Crude (+)-3,3,8-tribromocamphor **6** (24 g, 0.077 mole) was added to a pre-cooled glacial acetic acid (240 ml) in a round bottom flask. Zinc dust (12.1 g, 0.185 mole) was added as four portions with vigorous stirring at 5 - 15 °C and then stirred for 30 min at RT. The reaction mass was filtered through celite bed and the filtrate was diluted with diisopropyl ether. The organic layer was washed with aqueous $NaHCO_3$ solution, dried over anhydrous Na_2SO_4 and concentrated under vacuum to yield the desired 8-bromocamphor (13 g, 90%) with 40% purity by GC. The crude product was purified by HVD twice to yield the title product (4.3 g, yield 30%); mp 80 – 82 °C; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.93 (s, 3H, CH_3), 1.15 (s, 3H, CH_3), 1.35-1.43 (m, 1H), 1.52-1.61 (m, 1H), 1.77-1.99 (m, 3H), 2.33-2.47 (m, 2H), 3.09-3.20 (m, 2H, CH_2Br); MS (ESI Method) : 231 (M, 100%), 233 (M+2); $[\alpha]_D^{25}$: + 72 ° (c 1.17, 95% EtOH).

Preparation of 7-Bromomethyl-1,7-dimethyl-bicyclo[2.2.1]heptan-2-one oxime (11)[8]

$NH_2OH.HCl$ (4.5 g, 0.065 mole) in water was added to (+)-8-bromocamphor **7** (10 g, 0.043 mole) in ethanol (70 ml) at RT. Sodium acetate (5.5 g, 0.065 mole) was added and the reaction mass was heated to reflux temperature. After 6 h at reflux temperature, the starting material was absent by GC and the reaction mass was concentrated under vacuum. The residue was diluted with water and extracted with DCM. The combined organic layer was washed with water, dried over anhydrous sodium sulphate and concentrated under vacuum to yield oxime **11** (8.5 g, 80%); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.02 (s, 3H, CH_3), 1.11 (s, 3H, CH_3), 1.25-1.32 (m, 1H), 1.59-1.65 (m, 1H), 1.81-1.86 (m, 2H), 2.16 (d, *J* = 8.2 Hz, 1H), 2.29 (t, *J* = 4.2 Hz, 1H), 2.51-2.59 (m, 1H), 3.15 (d, *J* = 10.6 Hz, 1H, HCHBr), 3.23 (d, *J* = 9.7 Hz, 1H, HCHBr), 7.66 (s, 1H, NOH); MS (ESI Method) : 246.1 (M), 248.1 (M+2).

Preparation of 7-Bromomethyl-1,7-dimethyl-bicyclo[2.2.1]heptan-2-nitroimine (12)[8]

To a stirred solution of oxime **11** (10 g, 0.04 mole) in glacial acetic acid, was added sodium nitrite (4.32 g, 0.062 mole) portion wise at room temperature. After 2h at RT, the starting material was absent by TLC and the reaction mass was neutralized with aqueous $NaHCO_3$ solution. The product was extracted with dichloromethane, dried over anhydrous sodium sulphate and concentrated under vacuum. The crude product was purified by column chromatography to yield pure 7-bromomethyl-1,7-dimethyl-bicyclo[2.2.1]heptan-2-nitroimine **12** (7.8 g, 70%); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.09 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.35-1.42 (m, 1H), 1.72-1.79 (m, 1H), 1.90-2.03 (m, 2H), 2.25 (d, *J* = 18.9 Hz, 1H), 2.43 (t, *J* = 4.3 Hz, 1H), 2.69-2.77 (m, 1H), 3.16 (d, *J* = 10.3 Hz, 1H, HCHBr), 3.23 (d, *J* = 11 Hz, 1H, HCHBr); IR (KBr, cm^{-1}): 2960, 1650, 1567, 1458, 1434, 1315, 1294, 1256, 1095.

Preparation of 6,7-Dimethyl-4-nitro-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-3-carbonitrile (13)[8]

To a stirred solution of nitroimine **12** (7.78 g, 0.028 mole) in ethanol (78 ml) was added potassium cyanide (2.05 g, 0.031 mole) and the reaction mass was heated to reflux temperature. After 2 h at reflux temperature, the starting material was absent by TLC and the reaction mass was concentrated under vacuum. The residue was taken in water and extracted with dichloromethane. The combined organic was concentrated under vacuum. The crude product was purified by column chromatography to yield carbonitrile **13** (3.6 g, 56%); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.01 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.35-1.50 (m, 1H), 1.55-1.59 (m, 1H), 1.71 (m, 1H), 1.83-1.91 (m, 2H), 2.0-2.12 (m, 2H), 3.61 (d, *J* = 11.6 Hz, 1H, -HCH-N), 3.86 (d, *J* = 11.6 Hz, 1H, -HCH-N); MS (ESI Method) : 239.3 (M+NH_4, 100%), 244.3 (M+Na); IR (KBr, cm^{-1}): 2951, 2873, 2242 (C≡N), 1520, 1353, 1314, 1301, 1270, 1226, 1155, 1102, 769.

Preparation of 6,7-Dimethyl-4-nitro-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-3-carboxylic acid amide (16)

To a stirred solution of potassium hydroxide (2.4 g, 42.84 mmol) in water (15 ml) was added carbonitrile

13 (3 g, 13.56 mmol) and heated to reflux. After 2 h at reflux, the starting material was absent by TLC and the product was extracted with dichloromethane at RT. The combined organic layer was concentrated under vacuum to afford the title amide **16** (3 g, 93%); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 0.98 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.33-1.47 (m, 2H), 1.57-1.69 (m, 1H), 1.76-1.87 (m, 1H), 1.90-2.04 (m, 2H), 2.11 (d, *J* = 14 Hz, 1H), 3.65 (d, *J* = 11.8 Hz, 1H, -HCH-N), 3.96 (d, *J* = 11.8 Hz, 1H, -HCH-N), 5.46 - 5.66 (d (broad), 2H, $CONH_2$); MS (ESI Method) : 240.2 (M+1, 100%).

Preparation of 6,7-Dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-3-carboxylic acid amide (17)
To a stirred solution of amide **16** (3g, 12.6 mmol) in methanol (25 ml) was added 10% Pd/C (0.3 g) under nitrogen atmosphere. The resulting mass was stirred under hydrogen pressure (balloon) at RT and monitored by TLC. After 24 h, the catalyst was removed by filtration and the filtrate was concentrated to yield the desired amide **17** (2.41 g, 98%); ^{1}H-NMR (CD_3OD, 300 MHz): δ 0.88 (s, 3H, CH_3), 0.9 (s, 3H, CH_3), 1.28-1.39 (m, 2H), 1.57-1.63 (m, 1H), 1.78-1.84 (m, 3H), 1.96-2.02 (m, 1H), 2.70 (d, *J* = 10.4 Hz, 1H, -HCH-NH), 2.89 (d, *J* = 10.4 Hz, 1H, -HCH-NH); MS (ESI Method) : 195.3 (M+1, 100%).

Preparation of 6,7-Dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-3-carbonitrile hydrochloride (3)
Method A
A solution of carboxylic acid amide **17** (2 g, 10.26 mmol) in THF (10 ml) was cooled to 0 ^{o}C and trifluoroacetic anhydride (4.31 g, 20.53 mmol) was added at the same temperature. After 2h stirring at 0 ^{o} C, the staring material was absent by TLC and the reaction mass was concentrated at 40 ^{o} C under vacuum. The residue was diluted with dichloromethane and neutralized with aqueous sodium carbonate solution. The layers were separated and the aqueous layer was extracted with dichloromethane. HCl gas was passed through the combined organic layer for 5 min and concentrated. The residue was stirred with ether for 15 min., the precipitated product was filtered and dried to yield an off-white desired product **3** (1.9 g, 85%) as HCl salt; ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.00 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 1.25 (m, 1H), 1.41-1.52 (m, 1H), 1.60-1.71 (m, 1H), 1.89-1.99 (m, 3H), 2.05-2.13 (m, 2H), 3.44 (d, *J* = 3.06 Hz, 2H, -CH_2-NH); MS (ESI Method) : 177.1 (M+1, 100%); IR (KBr, cm^{-1}): 3436, 2962, 2875, 2247 (C≡N), 1742, 1666, 1449, 1423, 1387, 1296, 1262, 1167, 1140, 846.

Method B
To a stirred solution of carbonitrile **13** (2 g, 9 mmol) in glacial acetic acid (15 ml) was added zinc dust (10 mg) and heated to 60 ^{o}C. After 2 h, the starting material was absent by TLC and the catalyst was removed by filtration. Filtrate was concentrated under vacuum to remove the acetic acid and 5 ml of 6N HCl was added. Refluxed for additional 30 min and concentrated. The crude contains three products which was confirmed by mass. It was purified by column chromatography to yield the title compound (238 mg, 15%) with around 60% purity by TLC.

Preparation of 4-(2-Chloro-acetyl)-6,7-dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-3-carbonitrile (18)
6,7-Dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-3-carbonitrile.HCl **3** (1 g, 4.64 mmol) was neutralized with triethylamine (0.5 g, 4.94 mmol) and the free amine was extracted into DCM. The free amine in DCM was added to a flask containing chloroacetyl chloride (0.64 g, 5.66 mmol) in DCM (5 ml) at 0 ^{o} C over a period of 10 min. After 2 h stirring at 0 ^{o}C, the starting material was absent TLC. The reaction mass was quenched in ice water, the product was extracted with dichloromethane and concentrated to yield 4-(2-chloro-acetyl)-6,7-dimethyl-4-aza-tricyclo [4.3.0.0^{3,7}]nonane-3-carbonitrile **18** (0.83g, 70%); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.01 (s, 3H, CH_3), 1.08 (s, 3H, CH_3), 1.20-1.50 (m, 2H), 1.72 (d, *J* = 12.8 Hz, 1H), 1.82-2.09 (m, 4H), 3.41 (d, *J* = 9.6 Hz, 1H, -HCH-N), 3.55 (d, *J* = 9.6 Hz, 1H, -HCH-N), 3.93 (m, 2H, -NCH_2); MS (ESI Method) : 253.1 (M+1, 100%), 275.1 (M+Na).

Preparation of 4-[2-(3-Hydroxy-adamantan-1-ylamino)-acetyl]-6,7-dimethyl-4-azatricyclo [4.3.0.0^{3,7}]nonane-3-carbonitrile (1)
3-Amino-1-adamantanol (0.47 g, 2.78 mmol) was added to 4-(2-chloro-acetyl)-6,7-dimethyl-4-aza-

tricyclo[4.3.0.0^{3,7}]nonane-3-carbonitrile **18** (0.7 g, 2.78 mmol) in DMSO (8 ml) at 15 ^{o}C under nitrogen atmosphere. After 1 h at 15 ^{o} C, the starting material was absent by TLC. The reaction mass was quenched in ice water and the product was extracted with DCM. The combined organic layer was washed with water and then concentrated. It was subjected to column chromatography to yield pure 4-[2-(3-Hydroxy-adamantan-1-ylamino)-acetyl]-6,7-dimethyl-4-azatricyclo[4.3.0.0^{3,7}] nonane-3-carbonitrile **1** (0.52 g, 49%); ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.00 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 1.3-2.03 (m, 21H), 2.26 (s, 2H), 3.24-3.38 (m, 4H, CH_2); MS (ESI Method) : 384.3 (M+1, 100%); IR (cm $^{-1}$): 3435, 2921, 2852, 2239 (C≡N), 1663, 1451, 1411, 1333.

Preparation of [1-(3-Carbamoyl-6,7-dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-4-carbonyl)-2-methyl-butyl]-carbamic acid benzyl ester (19)

Triethylamine (0.73 g, 7.18 mmole) and methylchloroformate (0.49 g, 5.13 mmol) were added to a stirred solution of N-Cbz-isoleucine (1.43 g, 5.4 mmol) in N-methyl-2-pyrrolidone (4 ml) at 0 ^{o}C. Stirred for 30 min at 0 ^{o}C and then added 6,7-dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-3-carboxylic acid amide **17** (1.0 g, 5.16 mmole) in N-methyl-2-pyrrolidone (6 ml). The reaction mass was stirred at RT for overnight and quenched in water. The product was extracted with ethyl acetate and the ethyl acetate layer was washed thrice with water to remove N-methyl-2-pyrrolidone completely followed by $NaHCO_3$ solution. The organic layer was concentrated at 40 ^{o}C under vacuum to yield [1-(3-carbamoyl-6,7-dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-4-carbonyl)-2-methyl-butyl]-carbamic acid benzyl ester **19** (1.0 g, 40%). This crude as such taken for next step without any purification.

Preparation of [1-(3-Cyano-6,7-dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-4-carbonyl)-2-methyl -butyl]-carbamic acid benzyl ester (20)

To a cooled solution of [1-(3-carbamoyl-6,7-dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-4-carbonyl)-2-methyl-butyl]-carbamic acid benzyl ester **19** (0.7 mg, 1.6 mmol) in THF (5 ml) was added trifluoroacetic anhydride (0.88 ml, 6.2 mmol) at 0 ^{o} C. After 2 h stirring at 0 ^{o} C, the starting material was consumed completely. The excess acid was decomposed by aqueous $NaHCO_3$ solution and the product was extracted with ethyl acetate. Solvent was removed under vacuum to yield crude product (606 mg). It was subjected to column purification to yield pure desired product **20** (0.428 g, 63%); ^{1}H-NMR (CD_3OD, 300 MHz): δ 0.8-0.85 (m, 12H), 1.2-1.98 (m, 10H), 3.48 (d, *J* = 11Hz, 1H, CH_2), 3.63 (d, *J* = 11 Hz, 1H, CH_2), 3.92 (m, 1H), 5.01(s, 2H), 7.15-7.30 (m, 5H, Ar-H); MS (ESI Method) : 424.1 (M+1, 100%).

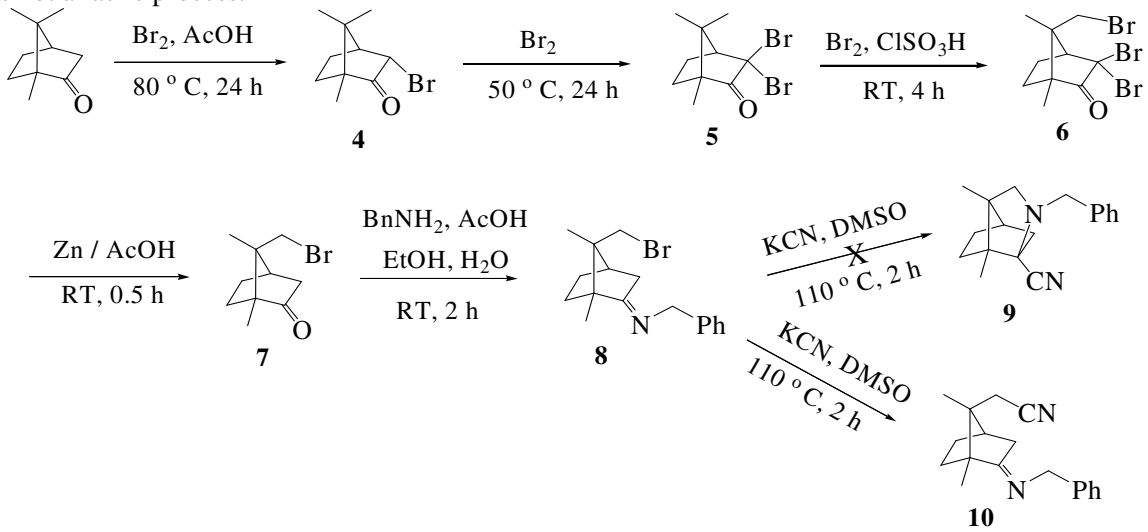
Preparation of 4-(2-Amino-3-methyl-pentanoyl)-6,7-dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-3-carbonitrile hydrochloride (2)

To a stirred solution of [1-(3-Cyano-6,7-dimethyl-4-aza-tricyclo[4.3.0.0^{3,7}]nonane-4-carbonyl)-2-methyl-butyl]-carbamic acid benzyl ester **20** (400 mg) in EtOAc : MeOH (5ml:1ml) was added 10% Pd/C (25 mg) under nitrogen atmosphere. The resulting mass was stirred under atmospheric hydrogen pressure for 2 h at RT and then the catalyst was removed by filtration. Dry HCl gas was passed through the filtrate for 2 min. and concentrated under vacuum. The obtained solid was washed with ethyl acetate and dried to yield target compound **2** as a HCl salt (186 mg, 66%); ^{1}H-NMR (CD_3OD, 300 MHz): δ 0.83(s, 3H, CH_3), 0.9-1.1 (m, 9H), 1.2-2.15 (m, 10H), 3.28 (d, *J* = 12 Hz, 1H, -HCH-N), 3.47 (d, *J* = 12 Hz, 1H, -HCH-N), 4.20 (d, *J* = 3 Hz, 1H); MS (ESI Method) : 290.1 (M+1, 100%).

RESULTS AND DISCUSSION

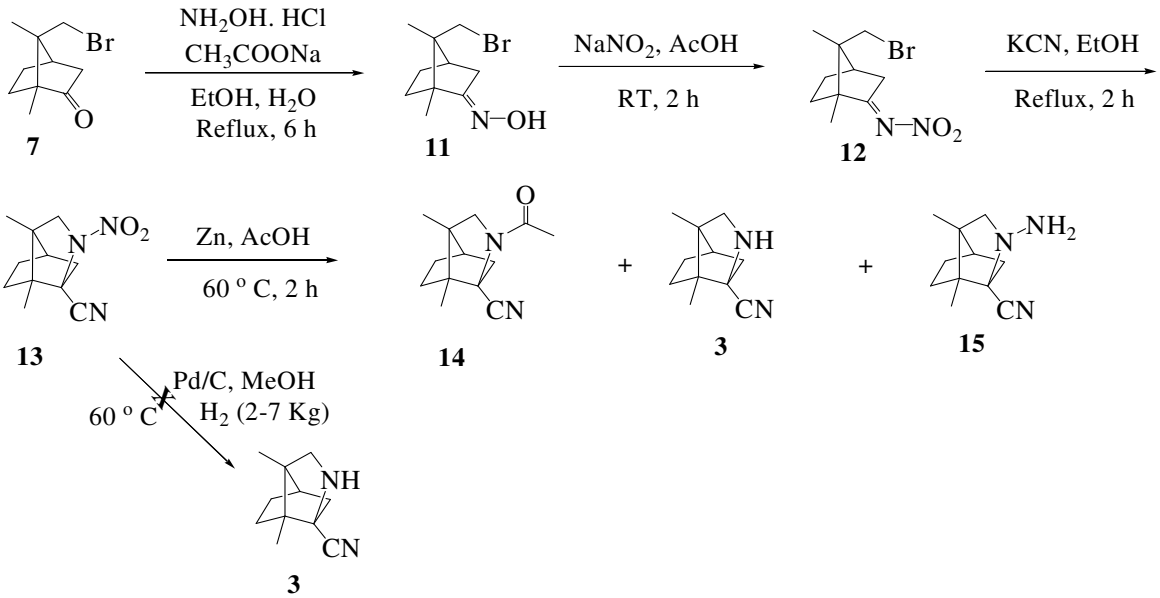
Initially, we planned to prepare the tricylic cyanopyrrolidine **3** from 8-bromocamphor **7** as shown in Scheme-1. The crucial 8-bromocamphor was prepared from camphor following a reported procedure[7] *via* step wise brominations (Scheme-1, steps 1-3) followed by selective alpha debromination (Scheme-1, step 4). The 8-bromocamphor **7** was converted to *N*-benzylimine **8** (Scheme-1, step 8) using benzyl amine without affecting the bromo group. However, when treated with KCN, compound **8** failed to undergo intramolecular domino type cyclization to give the desired product **9**. Instead, a simple cyano derivative **10** was isolated in this case suggesting that a nucleophilic attack on the imine carbon of **8** by a cyanide ion

was not a facile process.



Scheme-1: Attempt to synthesis tricyclic cyanopyrrolidine.

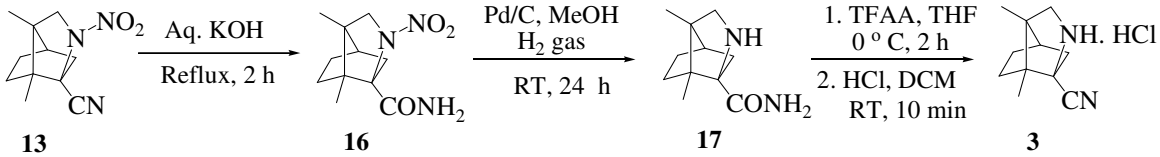
We presumed that an electron withdrawing group attached with the imine nitrogen would facilitate nucleophilic addition reaction. Accordingly, we decided to follow the procedure reported by Gorichko et al for the synthesis of tricyclic cyanopyrrolidine from camphor[8] as shown in Scheme-2. This multi-step procedure involves oxime formation, conversion of oxime to cyano derivative *via N*-nitro imine and finally removal of N-nitro group to give the key intermediate **3**.



Scheme-2: Reported method for the synthesis of tricyclic cyanopyrrolidine **3**.

While we were able to prepare the *N*-nitro amino cyano derivative **13** smoothly by using this synthetic route the major problem however was encountered in the conversion of compound **13** to the target intermediate **3**. The desired product was not formed when compound **13** was exposed to the Pd/C mediated hydrogenation reaction under 50 psi hydrogen pressure at 60 ºC according to the reported protocol[8]. This reaction was attempted in various solvents such as methanol, ethanol, acetic acid, 1,4-dioxane under hydrogen pressure ranging from 2 to 7 Kg at different temperatures but afforded a mixture

of unknown compounds along with major amount of unreacted starting material **13**. The use of Zn/ AcOH also provided a mixture of three products i.e. compound **3**, **14** and **15**. An attempt to isolate the pure product **3** *via* column chromatography was failed as the compound **3** was decomposed during the purification process. One of the reasons for this failure was thought to be due to the presence of sensitive cyano group which may have participated in side reactions thereby generating a number of major or minor impurities. We therefore adopted an alternative strategy where the CN group was maintained in the form of an amide moiety (Scheme-3).

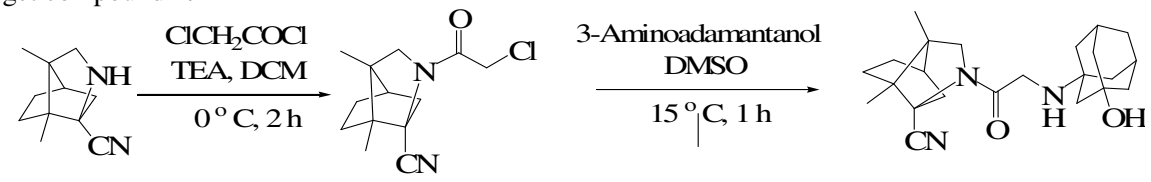


13 **16** **17** **3**

Scheme-3: Modified efficient method for the synthesis of tricyclic cyanopyrrolidine **3**.

Thus, the cyano derivative **13** was converted to the amide **16** (93% yield) which underwent smooth removal of *N*-nitro group under catalytic hydrogenation conditions to give the aminoamide derivative **17** in 98% yield. The cyano group was regenerated conveniently from the amide moiety of **17** to afford the desired compound **3** in 85% yield. After modifying the synthetic route, overall yield of compound **3** starting from **13** was increased from 15 to 80 %. It is worthy to mention that none of the steps in the conversion of compound **13** to **3** require any chromatographic purification and therefore the process is amenable for scale up.

Having prepared the key intermediate **3** successfully it was reacted with chloroacetyl chloride in the presence of triethylamine to yield the compound **18** (Scheme-4). The chloro derivative **18** was then reacted with commercially available 3-amino-1-adamantanol in dimethyl sulphoxide at 15 ºC to afford the target compound **1.**



3 **18** **1**

Scheme-4: Synthesis of target compound **1**.

In a separate study proline amide **17** was reacted with commercially available *N*-Cbz-L-leucine and the substituted amide was dehydrated in the presence of TFAA followed by catalytic hydrogenation to yield the desired product **2**.

DPP4 Activity

Assay buffer used for DPP4 inhibition studies was 25 mM TRIS-HCl, 140 mM NaCl, 10 mM KCl, 0.01% BSA. All working stocks of compounds **1**, **2** and DPP4 enzyme were prepared in assay buffer. Inhibition studies were performed by incubating different concentrations of compounds **1** and **2** with 10 ng/well of human DPP4 for 15 min at 30 ºC. Enzyme activity was initiated by 50 µM of substrate Gly-Pro-AMC. Fluorescence was measured after 30 min incubation at 30 ºC. Calculations for DPP4 enzyme percent inhibition were carried out using the formula shown below:

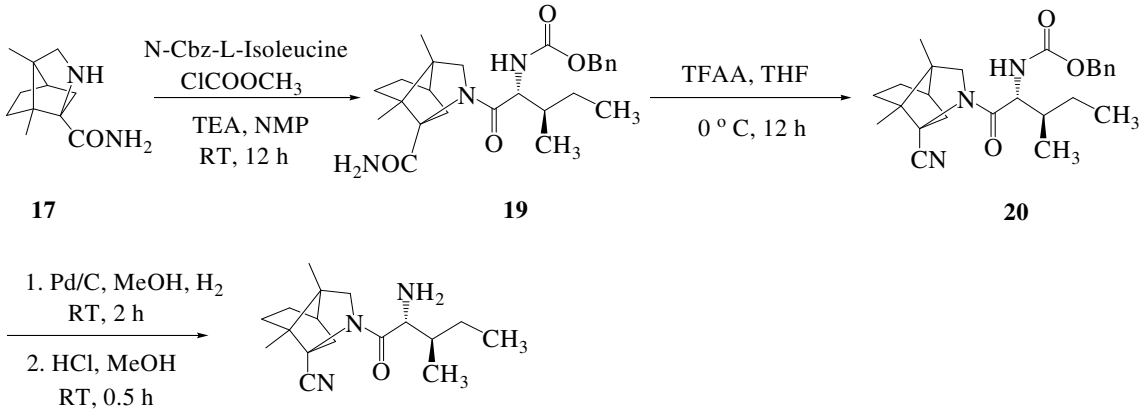
% DPP4 inhibition = 100-(RFU of test - RFU of blank) / (RFU of control - RFU of blank) X 100

(RFU: relative fluorescence units)

LAF-237 was used as a reference compound in this experiment and gave an IC_{50} of 5.79 ± 1.07 nM, which was close to the reported literature value of 3.5 ± 1.2 nM[9].

Compounds **1** and **2** were tested for their ability to inhibit DPP4 enzyme *in vitro* at three concentrations.

Compound **1** showed 50, 65 and 79% inhibition when tested at the concentration of 1, 5 and 10 µM respectively. Similarly, compound **2** showed 30, 55 and 63% inhibition at the concentration of 1, 5 and 10 µM respectively. While significant inhibition of DPP4 was observed at these concentrations, compounds **1** and **2** however were found to be less potent than Saxagliptin.



Scheme-5: Synthesis of target compound **2**

CONCLUSIONS

In conclusion, novel compounds **1** and **2** based on tricyclic α-cyanopyrrolidine were designed in search of potential inhibitors of DPP4. Synthesis of these compounds involved the preparation of a key intermediate i.e. tricyclic α-cyanopyrrolidine from camphor. After modifying the synthetic route for tricyclic α-cyanopyrrolidine from camphor, yield was drastically increased from 15 to 80%. The synthesis was carried out by maintaining the cyano group in the form of an amide and its regeneration after the completion of the necessary chemical transformation of a particular functional group. Both the target compounds synthesized showed significant inhibition of DPP4 when tested *in vitro*. The research presented here therefore would of interest to both organic and medicinal chemists.

ACKNOWLEDGEMENTS

The authors thank Dr. Manojit Pal and the management of Matrix Laboratories Limited, Hyderabad, India for continuous encouragement and support.

REFERENCES

1. Z. David, B. Gema, I.J. Ana, C. Jordi, H. Nurit, C. Carlos, C. David, N. Ruth and A. Carlos, *J. Chem. Inf. Model,* **49**, 1623(2009).
2. T.C. Vu, D.B. Brzozowski and R. Fox, *WO 2004052850*(2004).
3. R.E. Seamans and W.E. Behnke, *US 4490386*, (1984).
4. R. Henning, H. Urbach, V. Teetz and R. Becker, *US 4620012*, (1986).
5. P.H.Y. Cheong, K.N. Houk, J.S. Warrier and S. Hanessian, *Adv. Synth. Catal.*, **346***,* 1111(2004).
6. H. Shrikant and M. Pal, *Bioorg. Med. Chem.*, **17**, 1783(2009).
7. C.R. Eck, R.W. Mills and T. Money, *J. Chem. Soc. Chem. Commun.,* 911(1973).
8. M.V. Gorichko, O.O. Grygorenko and I.V. Komarov, *Tetrahedron Lett*, **43**, 9411(2002).
9. E.B. Villhauer, J.A. Brinkman, G.B. Naderi, B.F. Burkey, B.E. Dunning, K. Prasad, B.L. Mangold, M.E. Russel and T.E. Hughes, *J. Med. Chem.*, **46**, 2774(2003).

[RJC-1061/2013]