J. Chem. Sci. Vol. 129, No. 2, February 2017, pp. 249–258. © Indian Academy of Sciences.
DOI 10.1007/s12039-017-1223-4

REGULAR ARTICLE

A novel synthesis and preliminary *in vitro* cytotoxic evaluation of dihydropyrimidine-2,4(1*H*,3*H*)-dione derivatives

V UDAYAKUMAR[a,*], J GOWSIKA[b] and A PANDURANGAN[a]
[a]Department of Chemistry, Anna University, Guindy, Chennai 600 025, India
[b]Department of Chemistry, Pachaiyappa's College, University of Madras, Chennai 600 030, India
Email: udaya_taurus@yahoo.co.in

MS received 8 September 2016; revised 1 October 2016; accepted 25 December 2016

Abstract. We report the synthesis and characterization of four new compounds: 6-(2-(1H-inden-2-yl) phenyl)-3-(2-morpholinoethyl)dihydropyrimidine-2,4(1H,3H)-dione (**13a**), 6-(2-(1H-inden-2-yl)phenyl)-3-(2-(pyrrolidin-1-yl)ethyl)dihydropyrimidine-2,4(1H,3H)-dione (**13b**), 6-(2-(1H-inden-2-yl)phenyl)-3-phenethyl-dihydropyrimidine-2,4(1H,3H)-dione (**13c**) and 3-(3-(1H-imidazol-1-yl)propyl)-6-(2-(1H-inden-2-yl)phenyl) dihydropyrimidine-2,4(1H,3H)-dione (**13d**). A series of dihydropyrimidine-2,4(1*H*, 3*H*)-dione moieties was derived from methyl 3-amino-3-(2-chlorophenyl)propanoate precursor in a multi-step synthesis. Acid-amine coupling of 3-(2-(1*H*-inden-2-yl)phenyl)-3-((*tert*-butoxycarbonyl)amino)propanoic acid with a series of amine derivatives under mild reaction conditions led to form the corresponding dihydropyrimidine-2,4(1*H*, 3*H*)-dione derivatives *via* de-Boc and cyclization reaction in modest yield. Spectroscopic (^{1}H, ^{13}C NMR, and Mass) and analytical techniques have been used to identify and confirm the structure of the products.

Keywords. Triflic anhydride; Boc anhydride; Negishi coupling; acid-amine coupling; cyclization reaction; cytotoxicity; MCF-7.

1. Introduction

Synthesis of organic scaffolds from a mere precursor through a multi-step reaction is a challenge in synthetic organic chemistry. Heterocyclic Scaffolds play a vital role in biological and pharmacologic activities in medicinal chemistry.[1] Pyrimidine-fused heterocycles has found a wide range of biological applications as anticancer, anti-inflammatory, antibacterial, antifungal, anthelmintic, antitopoisomerase, antitumour, antiviral and antioxidant agents.[2] Nucleoside and non-nucleoside[3] reverse transcriptase inhibitors have gained a significant role in HIV infection. In particular, compounds like 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio) thymine (HEPT), 6-benzyl-1-(ethoxymethyl)-5-isopropyl pyrimidine-2,4-dione (MKC-442) and 6-benzyl-1-(benzyloxymethyl)-5-isopropylpyrimidine-2,4-dione (TNK-651) have high activity against HIV-1. N-heterocyclic compounds have gained interest over the past few years in pharmaceutical industries. Recently, pyrimidine derivatives like 4-(2-chlorophenyl)-6-(2, 4-dichlorophenyl)pyrimidin-2-amine,[4] 4-indazolyl-N-phenylpyrimidin-2-amines, N-phenyl-4-pyrazolo[3,4-b] pyridin-pyrimidin-2-amines[4] and 2-Anilino-4-(benzimidazol-2-yl)-pyrimidines derivatives[4] have shown anticancer activity against various cancer cell lines (NCI 60, HCT-116, MCF-7, Aurora B, PLK1, FAK and VEGF-R2). Since the diverse applications in pyrimidine contain heterocycles, we proceeded to synthesize novel 6-(2-(1*H*-inden-2-yl)phenyl)-3-(2-morpholinoethyl)dihydropyrimidine-2,4(1*H*, 3*H*)-dione and substituted derivatives in a stepwise way. The synthesized products shown in Figure 1 have been tested in few cancer cell lines.

2. Experimental

2.1 *Materials and methods*

^{1}H and ^{13}C NMR spectra were recorded in deuterated solvents on Bruker 500, 400 MHz and 125, 100 MHz spectrometer, respectively. LC-MS were recorded on Agilent Technology LCMS-1200 mass spectrometer. Reaction progress was monitored by using thin-layer chromatography (TLC) on 60 F_{254} silica gel plates and visualized by UV light (254 nm) or $KMnO_4$. Column chromatography was performed using 60–120 μm and 230–400 μm silica gel. All the reactions were carried out under argon atmosphere and distilled solvents were used.

*For correspondence

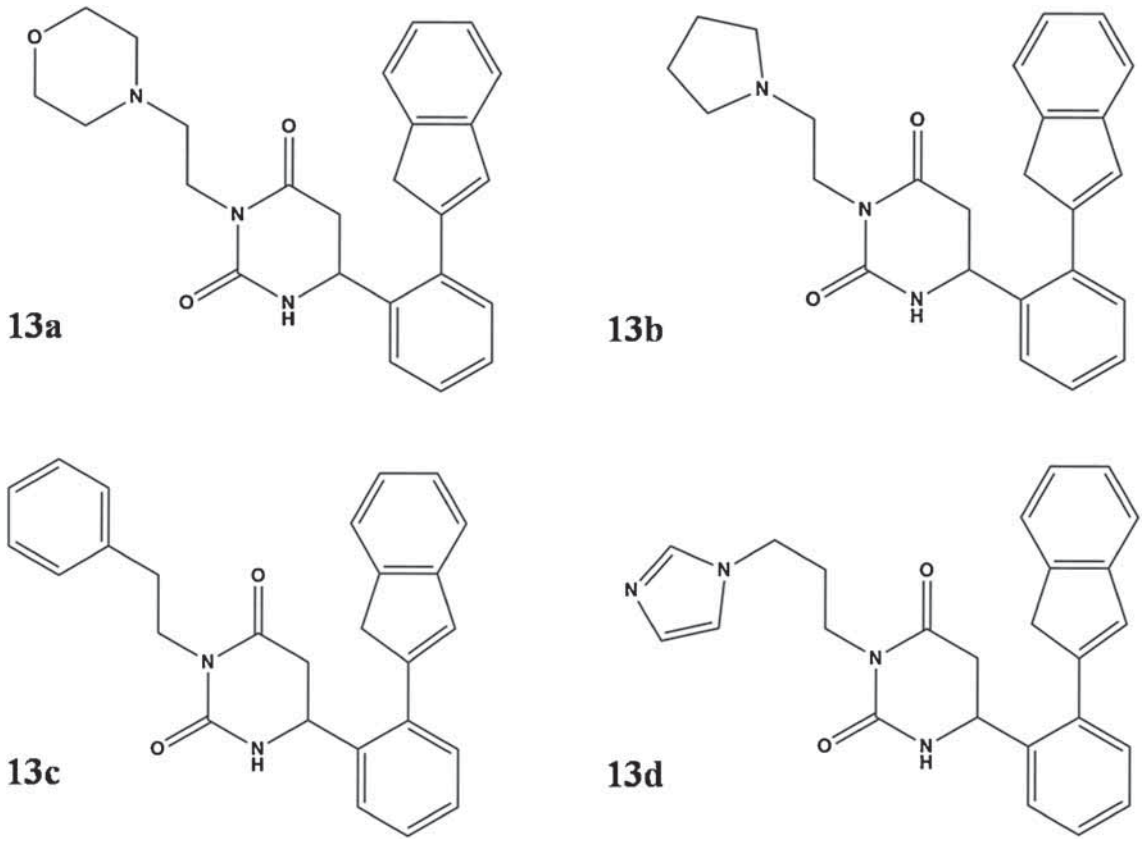


Figure 1. Biological active dihydropyrimidine-2,4(1*H*, 3*H*)-dione derivatives **13a–d**.

2.2 *1H-inden-2-yl trifluoromethanesulfonate (**2**)*

Stirred solution of 1*H*-inden-2(3*H*)-one (**1**; 10 g, 75.6 mmol) in DCM (50 mL) was added to *N*, *N*-Diisopropylethylamine (11.73 g, 90.7 mmol, 1.2 equiv.). Triflic anhydride (25.6 g, 90.7 mmol, 1.2 equiv.) was then treated dropwise at 40°C for 1 h, until the consumpion of (**1**) as shown by TLC and the residual mass was washed with water and extracted with DCM. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude was then purified by column chromatography over silica gel using 7–10% EtOAc in hexane as eluent to give the target compound **2** (13 g, 65% yield) as yellow oil (R_f-0.57, 3:7 ethylacetate/hexane, visualized by UV light (254 nm)); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.37 (d, 1H, ArH), 7.26 (d, 1H, ArH), 7.24 (m, 2H, ArH), 5.81 (s, 1H, = CH), 3.21 (s, 2H, CH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 174.1, 144.1, 142.9, 127.6, 126.1, 125.8, 125.4, 118.6, 98.5; LCMS 265.21 $(M+H)^+$. Anal. Calcd. (%) for $C_{10}H_7F_3O_3S$: C, 45.46; H, 2.67; S, 12.14. Found (%): C, 45.44; H, 2.65; S, 12.13.

2.3 *Methyl 3-((tert-butoxycarbonyl)amino)-3-(2-chlorophenyl) propanoate (**4**)*

To a stirred mixture of methyl 3-amino-3-(2-chlorophenyl) propanoate (**3**; 10 g, 46.8 mmol), DMAP (5.17 g, 46.8 mmol, 1 equiv.) in THF (50 mL) was added Boc anhydride (11.22 g, 51.4 mmol, 1.1 equiv.). The mixture was heated at 60°C for 3 h. After checking TLC, the resulting solution was then washed with water and aqueous layer was extracted with ethyl acetate, dried (Na_2SO_4) and concentrated under reduced pressure. The crude was then purified by column chromatography over silica gel (60–120 mesh) using 10–15% EtOAC in hexane as eluent to give the target compound **4** (12 g, 82% yield) as brown solid (R_f-0.38, 3:7 ethylacetate/hexane, visualized by UV light (254 nm)); M.p. 179–181°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.91 (s, 1H, NH), 7.69 (d, 1H, ArH), 7.21–7.28 (m, 3H, ArH), 5.58 (m, 1H, CH), 3.70 (s, 3H, CH_3), 2.62–2.83 (d, 2H, CH_2), 1.42 (s, 9H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.2, 155.4, 143.7, 132.5, 128.4, 126.1, 79.6, 51.3, 49.6, 37.1, 28.4; LCMS 314.75 $(M+H)^+$. Anal. Calcd. (%) for $C_{15}H_{20}ClNO_4$: C, 57.42; H, 6.42; N, 4.46. Found (%) C, 57.45; H, 6.39; N, 4.43.

2.4 *Methyl 3-(2-(1H-inden-2-yl)phenyl)-3-((tert-butoxycarbonyl)amino) propanoate (**5**)*

Zinc dust (5.17 g, 79.6 mmol, 2.5 equiv.) is activated by heating with a hot air gun under vacuum over 10 min, cooled to room temperature, then dried DMF (20 mL) and iodine (0.485 g, 1.9 mmol, 0.06 equiv.) were added which decolorized within few minutes. *N*-Boc protected amine (**4**; 10 g, 31.8 mmol) was added, followed by a pinch of iodine (0.485 g, 1.9 mmol, 0.06 equiv.). The resulting mixture

was stirred at room temperature for 2 h. After checking TLC, starting material was consumed (**4**) and zinc insertion was formed (R_f-0.17, 3:7 ethylacetate/hexane, visualized by UV light (254 nm)). Into this mixture $Pd_2(dba)_3$ (0.583 g, 0.6 mmol, 0.02 equiv.), SPhos (0.523 g, 1.2 mmol, 0.04 equiv.) were charged and inden-*O*-triflate (**2**; 10.1 g, 38.2 mmol, 1.2 equiv.) in dry DMF (20 mL) was added dropwise through a cannula. After complete addition, the reaction mixture was allowed to stir at room temperature for 12 h. After checking TLC, intermediate was consumed and the reaction mixture was then filtered through celite bed. The filtrate was then washed with water and extracted with dichloromethane, the organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The crude was then purified by column chromatography over silica gel (60–120 mesh) using 7–12% EtOAC in hexane as eluent to give the target compound **5** (8.2 g, 66% yield) as off-white solid (R_f-0.43, 3:7 ethylacetate/hexane, visualized by UV light (254 nm)). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.95 (s, 1H, NH), 7.21–7.47 (m, 8H, ArH), 6.81 (s, 1H, =CH), 5.44 (t, 1H, CH), 3.71 (s, 3H, CH_3), 3.24 (s, 1H, CH), 2.65–2.89 (d, 2H, CH_2), 1.42 (s, 9H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 171.9, 156.1, 146.5, 144.2, 137.2, 130.1, 129.2, 128.6, 126.3, 125.2, 122.3, 79.6, 52.6, 40.1, 39.6, 38.4, 28.5. LCMS 394.51 $(M+H)^+$. Anal. Calcd. (%) for $C_{24}H_{27}NO_4$ C, 73.26; H, 6.92; N, 3.56. Found (%) C, 73.22; H, 6.89; N, 3.54.

2.5 *3-(2-(1H-inden-2-yl)phenyl)-3-((tert-butoxycarbonyl)amino) propanoicacid (**6**)*

Starting material (**5**; 8 g, 20.3 mmol), H_2O/dioxane (240 mL) in 1:1 ratio and then LiOH.H_2O (1.7 g, 40.6 mmol, 2 equiv.) was added. The reaction mixture was stirred at room temperature for 3 h. After checking TLC, the reaction mixture was concentrated, and water (100 mL) and ethyl acetate (100 mL) was added. The aqueous layer was then acidified with 1 M HCl under ice and then extracted with ethyl acetate (100 mL). The organic layers were combined, washed with brine, then dried with Na_2SO_4 and concentrated under reduced pressure. The crude was co-evaporated with hexane to afford acid **6** (6.5 g, 84% yield) as a pale white solid (R_f-0.12, 1:9 methanol/chloroform, visualized by UV light (254 nm) and $KMnO_4$ activity on heating). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.5 (s, 1H, OH), 8.94 (s, 1H, NH), 7.21–7.49 (m, 8H, ArH), 6.81 (s, 1H, =CH), 5.44 (t, 1H, CH), 3.24 (s, 1H, CH), 2.65–2.89 (d, 2H, CH_2), 1.42 (s, 9H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.1, 156.1, 146.5, 145.8, 137.2, 130.1, 129.2, 128.6, 126.3, 125.2, 122.3, 79.6, 53.3, 39.6, 28.5. LCMS 380.32 $(M+H)^+$. Anal. Calcd. (%) for $C_{23}H_{25}NO_4$ C, 72.80; H, 6.64; N, 3.69. Found (%) C, 72.69; H, 6.61; N, 3.65.

2.6 *Tert-butyl (1-(2-(1H-inden-2-yl)phenyl)-3-((2-morpholinoethyl)amino)-3-oxopropyl) carbamate (**11a**)*

3-(2-(1*H*-inden-2-yl)phenyl)-3-((*tert*-butoxycarbonyl)amino) propanoic acid (**6**; 1.25 g, 3.2 mmol) was dissolved in DCM (20 mL), the mixture was further treated with 1-[Bis (dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, N-[(Dimethylamino)-1H-1,2,3-triazolo-[4,5-b]pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide (1.5 g, 3.9 mmol, 1.2 equiv.), 2-morpholinoethanamine (**7**; 0.85 g, 6.5 mmol) and *N*-ethylmorpholine (1.1 g, 9.8 mmol, 3 equiv.), and stirred at room temperature for 12 h. The resulting mixture was diluted with water and extracted with DCM, and the organic layers were washed with brine, dried over Na_2SO_4 and solvent was removed by rotary evaporation. The crude was then purified by column chromatography over silica gel (60–120 mesh) using 2–10% MeOH in chloroform as eluent to give the target compound **11a** (1.2 g, 74% yield) as a pale white solid (R_f-0.66, 1:9 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (s, 2H, NH), 7.21–7.47 (m, 8H, ArH), 6.82 (s, 1H, =CH), 5.42 (t, 1H, CH), 3.68 (t, 4H, CH_2), 3.12–3.22 (m, 4H, CH_2), 2.38–2.62 (m, 8H, CH_2), 1.42 (s, 9H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 156.1, 146.5, 141.1, 137.2, 130.2, 129.3, 128.6, 126.3, 125.2, 122.3, 80.1, 67.1, 58.4, 56.2, 53.4, 41.1, 40.1, 28.6. LCMS 492.72 $(M+H)^+$. Anal. Calcd. (%) for $C_{29}H_{37}N_3O_4$ C, 70.85; H, 7.59; N, 8.55. Found (%) C, 70.74; H, 7.61; N, 8.52.

2.7 *Tert-butyl (1-(2-(1H-inden-2-yl)phenyl)-3-oxo-3-((2-pyrrolidin-1-yl)ethyl)amino)propyl) carbamate (**11b**)*

Adopting the procedure described for the synthesis of **11a**, **6** (1.25 g, 3.2 mmol) was coupled with **8** (750 mg, 6.5 mmol, 2 equiv.). The crude was then purified by column chromatography over silica gel (60–120 mesh) using 2–10% MeOH in chloroform as eluent to give the target compound **11b** (1.2 g, 76% yield) as a pale white solid (R_f-0.69, 1:9 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 2H, NH), 7.22–7.54 (m, 8H, ArH), 6.82 (s, 1H, =CH), 5.42 (t, 1H, CH), 3.28 (s, 2H, CH_2), 3.14 (m, 2H, CH_2), 2.43–2.84 (m, 8H, CH_2), 1.64 (m, 4H, CH_2), 1.41 (s, 9H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 156.2, 146.2, 141.3, 137.4, 130.4, 129.3, 128.6, 126.3, 125.2, 121.8, 80.4, 58.4, 56.4, 53.4, 41.1, 40.1, 28.6, 23.4. LCMS 476.53 $(M+H)^+$. Anal. Calcd. (%) for $C_{29}H_{37}N_3O_3C$, 73.23; H, 7.84; N, 8.83. Found (%)C, 73.18; H, 7.84; N, 8.79.

2.8 *Tert-butyl (1-(2-(1H-inden-2-yl)phenyl)-3-oxo-3-(phenethylamino)propyl) carbamate (**11c**)*

Adoptng the procedure described for the synthesis of **11a**, **6** (1.25 g, 3.2 mmol) was coupled with **9** (790 mg, 6.5 mmol, 2 equiv.). The crude was then purified by column chromatography over silica gel (60–120 mesh) using 2–10% MeOH in chloroform as eluent to give the target compound **11c** (1.25 g, 79% yield) as a pale white solid (R_f-0.71, 1:9 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (s, 2H, NH), 7.22–7.49 (m, 13H, ArH), 6.79 (s, 1H,

=CH), 5.41 (t, 1H, CH), 3.37 (m, 2H, CH_2), 3.25 (s, 2H, CH_2), 2.61–2.87 (m, 4H, CH_2), 1.41 (s, 9H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 156.2, 146.2, 144.3, 139.4, 136.4, 129.2, 128.8, 127.4, 125.2, 124.2, 121.8, 80.4, 53.4, 41.1, 40.1, 35.7, 28.6. LCMS 483.32 $(M+H)^+$. Anal. Calcd. (%) for $C_{31}H_{34}N_2O_3C$, 77.15; H, 7.10; N, 5.80. Found (%)C, 77.27; H, 7.08; N, 5.78.

2.9 *Tert-butyl (3-((3-(1H-imidazol-1-yl)propyl)amino) -1-(2-(1H-inden-2-yl)phenyl)-3-oxopropyl) carbamate (**11d**)*

Adopting the procedure described for the synthesis of **11a**, **6** (1.25 g, 3.2 mmol) was coupled with **10** (820 mg, 6.5 mmol, 2 equiv.). The crude was then purified by column chromatography over silica gel (60–120 mesh) using 2–15% MeOH in chloroform as eluent to give the target compound **11d** (0.93 g, 72% yield) as a pale white solid (R_f-0.57, 1:9 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 2H, NH), 8.02 (s, 1H, CH), 7.22–7.55 (m, 8H, ArH), 6.85 (s, 2H, =CH), 5.41 (t, 1H, CH), 4.53 (t, 2H, CH_2), 3.62 (t, 2H, CH_2), 3.28 (s, 2H, CH_2), 2.53–2.84 (m, 4H, CH_2), 1.41 (s, 9H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 156.2, 146.2, 141.3, 138.4, 136.2, 130.4, 129.3, 128.6, 126.3, 125.2, 121.5, 120.7, 80.4, 53.4, 47.3, 41.1, 40.1, 38.4, 32.8, 28.6. LCMS 487.71 $(M+H)^+$. Anal. Calcd. (%) for $C_{29}H_{34}N_4O_3C$, 71.58; H, 7.04; N, 11.51. Found (%)C, 71.57; H, 7.06; N, 11.48.

2.10 *3-(2-(1H-inden-2-yl)phenyl)-3-amino-N-(2-morpholinoethyl)propanamide hydrochloride (**12a**)*

Removal of Boc group was done by bubbling HCl gas into a stirred solution of **11a** (0.9 g, 1.83 mmol) in dichloromethane (18 mL) at room temperature for 3 h, until completion of the reaction as shown by TLC. The solvent was vaporized under reduced pressure and the crude was then purified by column chromatography over neutral alumina (70–290 mesh) using 5–25% MeOH in chloroform as eluent to give **12a** (0.5 g, 70% yield) as a white solid (R_f-0.48, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (s, 1H, NH), 7.21–7.47 (m, 8H, ArH), 6.82 (s, 1H, =CH), 5.24 (s, 2H, NH_2), 4.38 (t, 1H, CH), 3.68 (t, 4H, CH_2), 3.12–3.22 (m, 4H, CH_2), 2.91–2.62 (m, 4H, CH_2), 2.35 (m, 4H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 146.5, 141.1, 137.2, 130.2, 129.3, 128.6, 126.3, 125.2, 122.3, 67.1, 58.4, 56.2, 48.6, 43.2, 40.1. LCMS 392.62 $(M+H)^+$. Anal. Calcd. (%) for $C_{24}H_{29}N_3O_2C$, 73.63; H, 7.47; N, 10.73. Found (%) C, 73.59; H, 7.46; N, 10.71.

2.11 *3-(2-(1H-inden-2-yl)phenyl)-3-amino-N-(2-(pyrrolidin-1-yl)ethyl)propanamide hydrochloride (**12b**)*

Following the procedure for the synthesis of **12a**, using **11b** (0.9 g, 1.89 mmol) and dichloromethane (18 mL) gave **12b** (0.51 g, 70% yield) as a white solid (R_f-0.51, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 1H, NH), 7.22–7.54 (m, 8H, ArH), 6.82 (s, 1H, =CH), 5.25 (s, 2H, NH_2), 4.35 (t, 1H, CH), 3.28 (s, 2H, CH_2), 3.14 (m, 2H, CH_2), 2.53–2.84 (m, 8H, CH_2), 1.64 (m, 4H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 146.2, 141.3, 137.4, 130.4, 129.3, 128.6, 126.3, 125.2, 121.8, 58.4, 56.4, 48.2, 42.1, 40.1, 23.4. LCMS 376.42 $(M+H)^+$. Anal. Calcd. (%) for $C_{24}H_{29}N_3O$ C, 76.76; H, 7.78; N, 11.19. Found (%) C, 76.75; H, 7.75; N, 11.20.

2.12 *3-(2-(1H-inden-2-yl)phenyl)-3-amino-N-phenethylpropanamide hydrochloride (**12c**)*

Following the procedure for the synthesis of **12a**, using **11c** (0.9 g, 1.86 mmol) and dichloromethane (18 mL) yielded **12c** (0.51 g, 71% yield) as a white solid (R_f-0.53, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (s, 1H, NH), 7.22–7.44 (m, 13H, ArH), 6.79 (s, 1H, =CH), 5.24 (s, 2H, NH_2), 4.31 (t, 1H, CH), 3.37 (m, 2H, CH_2), 3.25 (s, 2H, CH_2), 2.61–2.87 (m, 4H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 146.2, 144.3, 139.4, 136.4, 129.7, 128.1, 127.5, 125.4, 124.2, 121.1, 48.4, 44.8, 40.1, 35.7. LCMS 383.52 $(M+H)^+$. Anal. Calcd. (%) for $C_{26}H_{26}N_2O$ C, 81.64; H, 6.85; N, 7.32. Found (%) C, 81.61; H, 6.82; N, 7.31.

2.13 *N-(3-(1H-imidazol-1-yl)propyl)-3-(2-(1H-inden-2-yl)phenyl)-3-aminopropanamide hydrochloride (**12d**)*

Following the procedure for the synthesis of **12a**, using **11d** (0.7 g, 1.43 mmol) and dichloromethane (18 mL) yielded **12d** (0.39 g, 70% yield) as a white solid (R_f-0.43, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 1H, NH), 8.02 (s, 1H, CH), 7.22–7.63 (m, 8H, ArH), 6.85 (s, 2H, =CH), 5.26 (s, 2H, NH_2), 4.34 (m, 1H, CH), 4.19 (m, 2H, CH_2), 3.62 (t, 2H, CH_2), 3.28 (s, 2H, CH_2), 2.53–2.84 (m, 4H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.6, 146.2, 144.3, 138.4, 136.2, 130.4, 129.3, 128.6, 126.3, 125.2, 122.3, 121.5, 48.8, 47.1, 45.3, 40.1, 38.4, 32.8. LCMS 387.49 $(M+H)^+$. Anal. Calcd. (%) for $C_{24}H_{26}N_4O$ C, 74.58; H, 6.78; N, 14.50. Found (%) C, 74.63; H, 6.75; N, 14.29.

2.14 *6-(2-(1H-inden-2-yl)phenyl)-3-(2-morpholinoethyl)dihydropyrimidine-2,4(1H,3H)-dione (**13a**)*

12a (300 mg, 0.76 mmol) was added to ethyl chloroformate (4.5 mL), heated and stirred at reflux for 2 h. The intermediate was confirmed by TLC analysis (R_f-0.60, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). The reaction mixture was then cooled to room temperature and solid was collected by filtration, washed with ethanol and the residue was charged with ethanol (6 mL), anhydrous K_2CO_3 for 3 h at reflux. The volatiles were removed under reduced pressure, and the crude was dissolved in water and adjusted the pH (6-7) using acetic acid. The white precipitate was collected by filtration, washed with ethanol to afford the cyclic compound **13a** (281 mg, 90% yield) as a

white solid (R_f-0.53, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H, NH), 7.21–7.49 (m, 8H, ArH), 6.82 (s, 1H, =CH), 5.15 (t, 1H, CH), 3.68 (t, 4H, CH_2), 3.12–3.22 (m, 4H, CH_2), 2.87–2.62 (m, 4H, CH_2), 2.35 (m, 4H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.9, 153.3, 146.5, 144.1, 137.2, 130.2, 129.3, 128.6, 126.3, 125.2, 122.3, 67.1, 56.2, 47.3, 43.2, 40.1, 38.2. LCMS 418.63 $(M+H)^+$. Anal. Calcd. (%) for $C_{25}H_{27}N_3O_3C$, 71.92; H, 6.52; N, 10.06. Found (%) C, 71.86; H, 6.50; N, 10.09.

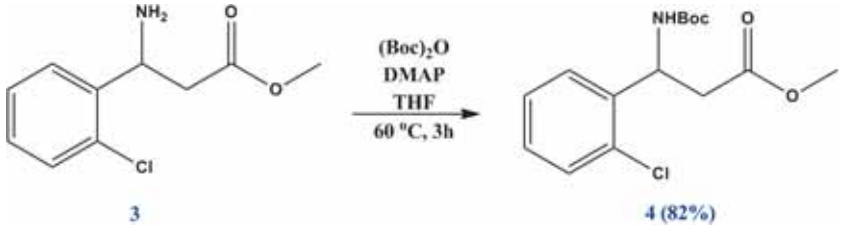
2.15 *6-(2-(1H-inden-2-yl)phenyl)-3-(2-(pyrrolidin-1-yl)ethyl)dihydropyrimidine-2,4(1H,3H)-dione (**13b**)*

12b (300 mg, 0.79 mmol) was added to ethyl chloroformate (4.5 mL), heated and stirred at reflux for 2 h. The intermediate was confirmed by TLC analysis (R_f-0.66, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). The reaction mixture was then cooled to room temperature and solid was collected by filtration, washed with ethanol and the residue was charged with ethanol (6 mL) and anhydrous K_2CO_3 for 3 h at reflux. The volatiles were removed under reduced pressure, and the crude was dissolved in water and adjusts the pH (6–7) using acetic acid. The white precipitate was collected by filtration, washed with ethanol to afford cyclic compound **13b** (290 mg, 90% yield) as a white solid (R_f-0.57, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (s, 1H, NH), 7.22–7.50 (m, 8H, ArH), 6.82 (s, 1H, =CH), 5.15 (t, 1H, CH), 3.22–3.28 (m, 4H, CH_2), 2.53–2.84 (m, 8H, CH_2), 1.64 (m, 4H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.1, 153.2, 146.5, 144.7, 137.4, 130.4, 129.3, 128.6, 126.3, 125.2, 121.8, 56.7, 52.3, 46.9, 40.1, 39.3, 23.4. LCMS 402.45 $(M+H)^+$. Anal. Calcd. (%) for $C_{25}H_{27}N_3O_2C$, 74.79; H, 6.78; N, 10.47. Found (%) C, 74.77; H, 6.56; N, 10.44.

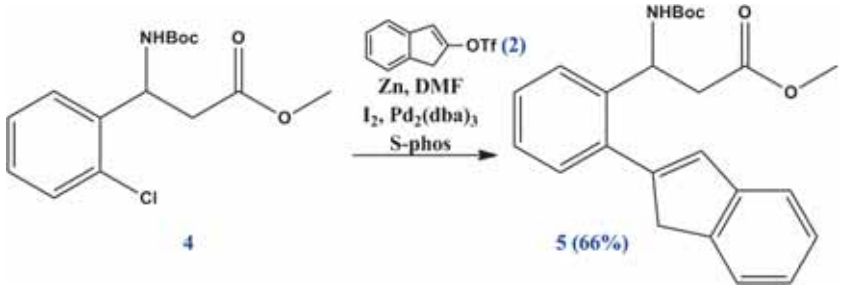
2.16 *6-(2-(1H-inden-2-yl)phenyl)-3-phenethyldihydropyrimidine-2,4(1H,3H)-dione (**13c**)*

12c (300 mg, 0.78 mmol) was added to ethyl chloroformate (4.5 mL), heated and stirred at reflux for 2 h. The intermediate was confirmed by TLC analysis (R_f-0.70, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). The reaction mixture was then cooled to room temperature and the solid was collected by filtration, washed with ethanol

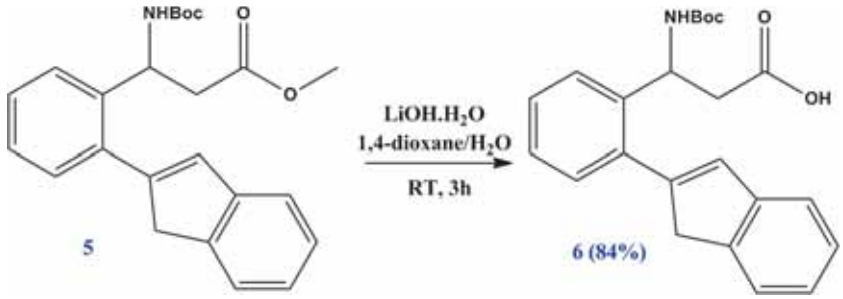
Scheme 1. Synthesis of 1*H*-inden-2-yl trifluoromethanesulfonate (**2**).



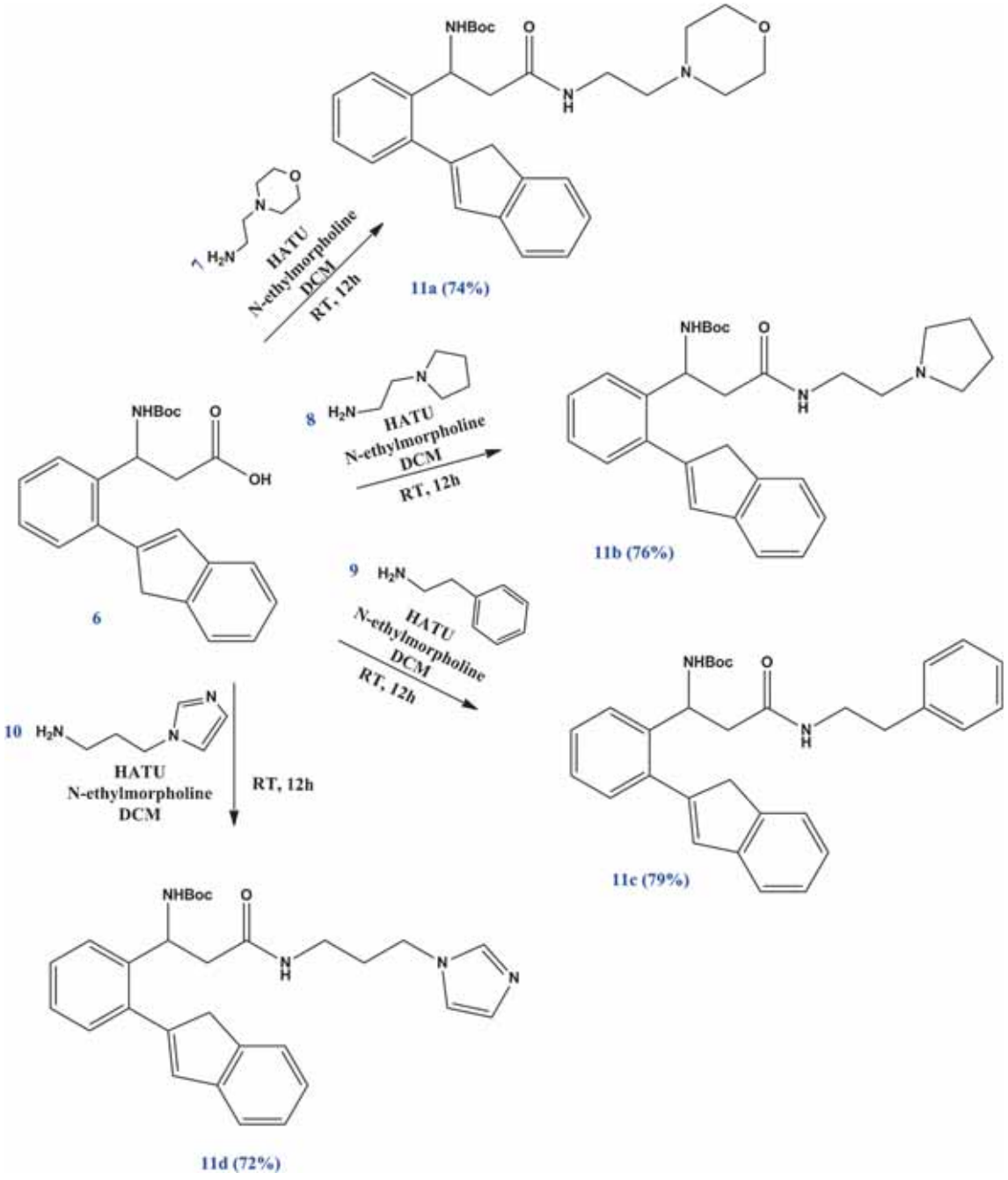
Scheme 2. Protecting Boc group in methyl 3-amino-3-(2-chlorophenyl)propanoate.



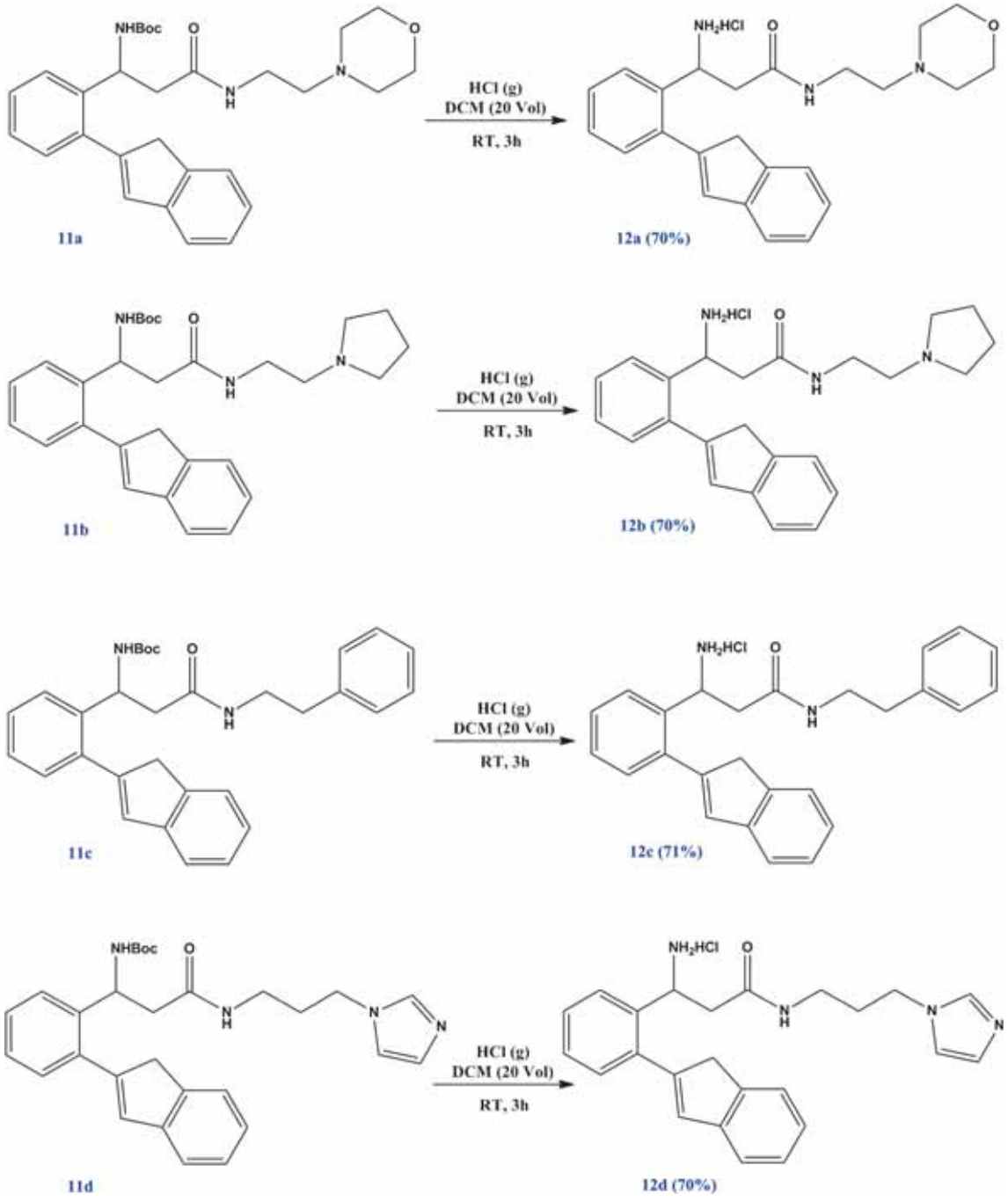
Scheme 3. Coupling of **4** with **2** and synthesis of methyl 3-(2-(1*H*-inden-2-yl)phenyl)-3-((*tert*-butoxycarbonyl)amino)propanoate.



Scheme 4. Synthesis of 3-(2-(1*H*-inden-2-yl)phenyl)-3-((*tert*-butoxycarbonyl)amino)propanoic acid.



Scheme 5. Synthesis of amide derivatives by coupling of acid **6** with series of amines **7–10**.



Scheme 6. Removal of Boc group to make primary amines.

and the residue was charged with ethanol (6 mL), anhydrous K_2CO_3 for 3 h at reflux. The volatiles were removed under reduced pressure, and the crude was dissolved in water and The volatiles were removed the pH (6–7) The volatiles were removed using acetic acid. The white precipitate was collected by filtration, washed with ethanol to afford cyclic compound **13c** (301 mg, 94% yield) as a white solid (R_f-0.58, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (s, 1H, NH), 7.22–7.52 (m, 13H, ArH), 6.79 (s, 1H, =CH), 5.15 (t, 1H, CH), 3.37 (m, 2H, CH_2), 3.25 (s, 2H, CH_2), 2.61–2.87 (m, 4H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 153.2, 147.1, 144.3, 139.4, 136.4, 128.8, 124.2, 121.8, 47.5, 44.8, 40.1, 39.2. LCMS 409.51 $(M+H)^+$. Anal. Calcd. (%) for $C_{27}H_{24}N_2O_2C$, 79.39; H, 5.92; N, 6.86. Found (%) C, 79.35; H, 5.94; N, 6.82.

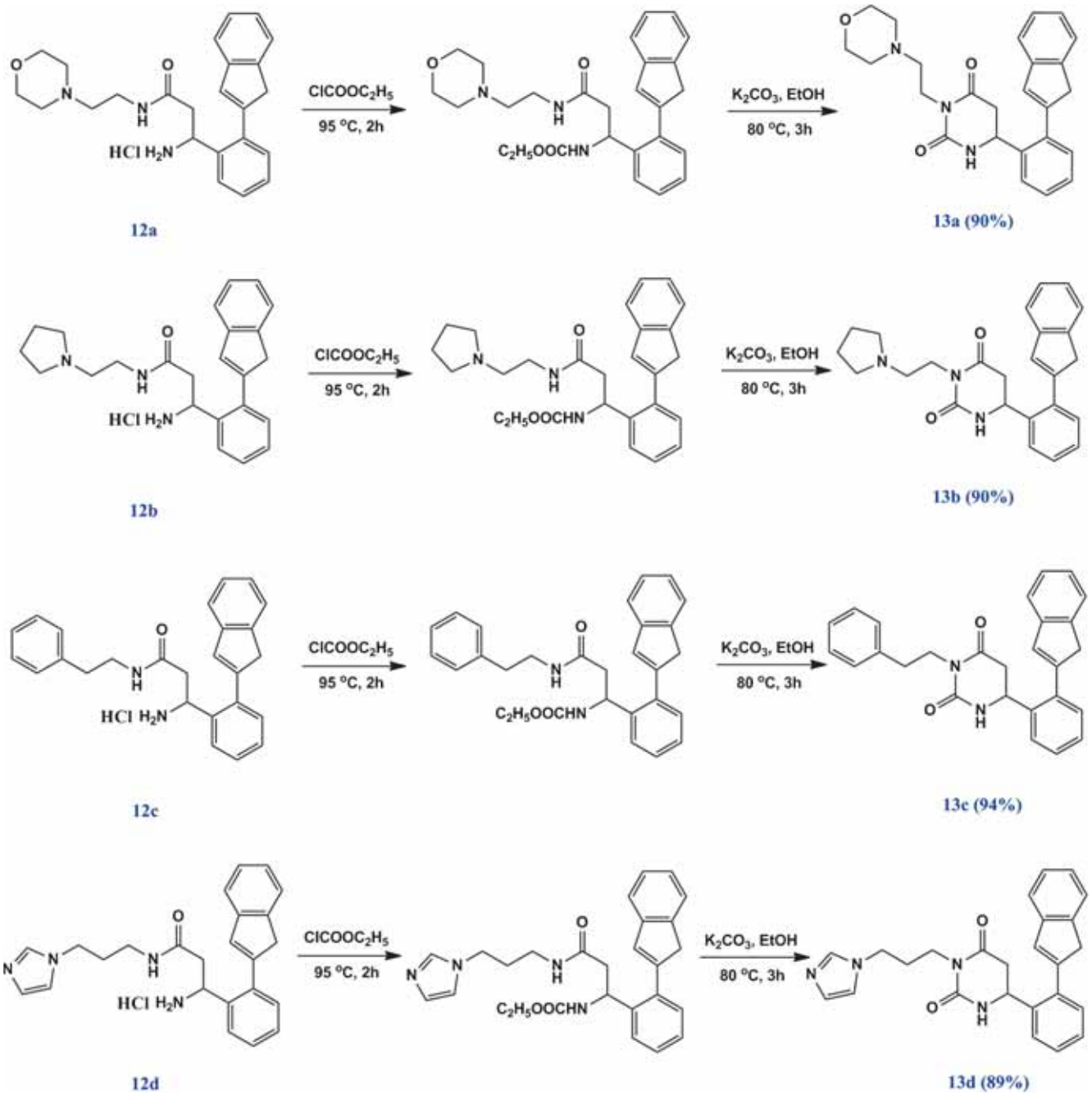
2.17 *3-(3-(1H-imidazol-1-yl)propyl)-6-(2-(1H-inden-2-yl)phenyl)dihydropyrimidine-2,4(1H,3H)-dione (**13d**)*

12d (200 mg, 0.51 mmol) was added to ethyl chloroformate (3 mL), heated and stirred at reflux for 2 h. The intermediate was confirmed by TLC analysis (R_f-0.58, 2:8

MeOH/chloroform, visualized by UV light (254 nm)). The reaction mixture was then cooled to room temperature and the solid was collected by filtration, washed with ethanol. and the residue was charge with ethanol (4 mL), anhydrous K_2CO_3 for 3 h at reflux. The volatiles were removed under reduced pressure, and the crude was dissolved in water and adjusted the pH (6–7) using acetic acid. The white precipitate was collected by filtration and washed with ethanol to afford cyclic compound **13d** (191 mg, 89% yield) as a white solid (R_f-0.48, 2:8 MeOH/chloroform, visualized by UV light (254 nm)). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.13 (s, 1H, NH), 8.02 (s, 1H, CH), 7.22–7.73 (m, 8H, ArH), 6.85 (d, 2H, =CH), 5.15 (t, 1H, CH), 4.11 (m, 4H, CH_2), 3.28 (s, 2H, CH_2), 2.53–2.84 (m, 4H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.6, 153.2, 146.2, 144.3, 138.4, 136.2, 130.4, 129.3, 128.6, 126.3, 125.2, 121.5, 47.3, 46.2, 41.7, 40.1, 39.8, 28.3. LCMS 413.64 $(M+H)^+$. Anal. Calcd. (%) for $C_{25}H_{24}N_4O_2C$, 72.80; H, 5.86; N, 13.58. Found (%) C, 72.74; H, 5.83; N, 13.56.

3. Results and Discussion

Treatment of 2-indanone (**1**) with potassium bis(trimethylsilyl)amide in dichloromethane followed by enolate



Scheme 7. Synthesis of dihydropyrimidine-2,4(1*H*, 3*H*)-dione.

with $(t\text{Bu})_2\text{Si}(\text{OTf})_2$[5] gave poor yield, but the desired product 1*H*-inden-2-yl trifluoromethanesulfonate (**2**; Scheme 1) was obtained in moderate yield (65%) using *N*, *N*-Diisopropylethylamine and trifluoromethanesulfonic anhydride.

Methyl 3-amino-3-(2-chlorophenyl)propanoate (3) was added with Boc anhydride and 4-dimethylaminopyridine (DMAP) to give *N*-Boc protected amine (4; Scheme 2) in 82% yield.[6]

Negishi coupling reaction was carried out with methyl 3-((*tert*-butoxycarbonyl)amino)-3-(2-chlorophenyl)propanoate (**4**) and activated zinc dust (Scheme 3).[7] *N*-Boc protected amine (**4**) was converted into corresponding PhZnCl intermediate which was coupled with 1H-inden-2-yl trifluoromethanesulfonate (**2**) using $Pd_2(dba)_3$ catalyst, to give methyl 3-(2-(1*H*-inden-2-yl)phenyl)-3-((*tert*-butoxycarbonyl)amino)propanoate (**5**) in a yield of 66%. 3-*N*-Boc-propanoic acid (**6**, Scheme 4) was prepared from 3-*N*-Boc-methylpropanoate (**5**) by reacting with $\text{LiOH}.\text{H}_2\text{O}$.[8]

Amide derivatives (**11 a–d**, Scheme 5)[8] were synthesized by the coupling reaction of acid (**6**) on treatment with different amines (**7–10**) in the presence of HATU and *N*-ethylmorpholine which gave a good yield (75–80%) when compared with the reaction proceeded with HATU and DIEA. Elimination of Boc group in amide derivatives (**11 a–d**) with HCl in dichloromethane gave the corresponding amine hydrochloride (**12 a–d**, Scheme 6).[8]

Cyclic compounds (**13 a–d**, Scheme 7)[9] gave 90% yield by cyclization of corresponding unprotected amines (**12 a–d**) on heating with ethyl chloroformate and ethanol.

The anticancer efficacy of the synthesized dihydropyrimidine-2,4(1*H*,3*H*)-dione derivatives (**13a–d**) were tested against a few cancer cell lines including MCF-7 (breast), U87 (glioma), HeLa (cervix), A549 (lung), A431 (vulvar) using MTT assay. The obtained inhibitory activity (IC_{50}) of the synthesized compounds (**12a–d** and **13a–d**) using 5-fluorouracil as standard are tabulated in Table S1 (in Supplementray Information).

The incubation of organic molecules on cancer cell lines was taken in 24 well plates for 24 h. The viability of the cells were calculated by seeding the organic molecules in different concentrations; normal concentration has 100% of cell spreading. After incubation, all the synthesized molecules (**13a–d**) exhibited moderate cytotoxic activity against cancer cell lines at the concentration of 62.5 μg/L. Cytotoxicity results exhibited that compound **13d** in A431 cell line showed IC_{50} value of 1.07 μM, indicating better activity. Besides, we also investigated the bioactivity of some of the intermediates (**12a–d**) where the IC_{50} value was found to be above 2 μM. These results suggested that the synthesized compounds (**13a–d**) will work as good scaffolds for cancer therapy research.

4. Conclusions

In summary, we have developed a new method for the synthesis of dihydropyrimidine-2,4(1*H*, 3*H*)-dione derivatives through a multi-step reaction with modest to high yields. NMR spectroscopy confirmed the structures of the synthesized compounds in each step. The biological evaluation of the synthesized compounds against A431 cell line exhibited an active cytotoxic effect compared with other cell lines. When compared among the series of compounds, we found that compound **13d** has highest cytotoxic effect with the IC_{50} value of 1.07 μM. We anticipate that this route can be useful towards the synthesis of dihydropyrimidine-2,4(1*H*, 3*H*)-dione scaffolds.

Supporting Information (SI)

^{1}H, ^{13}C NMR spectra of all compounds are available at www.ias.ac.in/chemsci.

Acknowledgements

Theauthors would like to thank the Department of Chemistry, Pachaiyappa's College, University of Madras, Chennai for the instrumental facilities provided under the DST-FIST programme. We also like to thank Mr. R. Balamurali, Senior Research Associate for his contribution to the LC-MS analysis.

References

1. (a) Clercq E D and Bernaerts R 1987 Specific phosphorylation of 5-ethyl-2′-deoxyuridine by herpes simplex virus-infected cells and incorporation into viral DNA *J. Biol. Chem.* **262** 14905; (b) Gready J E, McKinlay C and Gebauer M G 2003 Synthesis of quaternised 2-aminopyrimido[4,5-*d*]pyrimidin-4(3*H*)-ones and their biological activity with dihydrofolate reductase *Eur. J. Med. Chem.* **38** 719; (c) Gangjee A, Adair O O and Queener S F 1999 *Pneumocystis carinii* and *Toxoplasma gondii* Dihydrofolate Reductase Inhibitors and Antitumor Agents: Synthesis and Biological Activities of 2,4-Diamino-5-methyl-6-[(monosubstituted anilino) methyl]- pyrido[2,3-*d*]pyrimidines *J. Med. Chem.* **42** 2447; (d) Gangjee A, Adair O O and Queener S F 2008 N9-Substituted 2,4-Diaminoquinazolines: Synthesis and Biological Evaluation of Lipophilic Inhibitors of *Pneumocystis carinii* and *Toxoplasma gondii* Dihydrofolate Reductase *J. Med. Chem.* **51** 6195; (e) Chan D C M, Fu H, Forsch R A, Queener S F and Rosowsky A 2005 Design, Synthesis, and Antifolate Activity of

New Analogues of Piritrexim and Other Diaminopyrimidine Dihydrofolate Reductase Inhibitors with ω-Carboxyalkoxy or ω-Carboxy-1-alkynyl Substitution in the Side Chain *J. Med. Chem.* **48** 4420; (f) Radi M, Schenone S and Botta M 2009 Recent highlights in the synthesis of highly functionalized pyrimidines *Org. Biomol. Chem.* **7** 2841

2. (a) Gibson C L, Huggan J K, Kennedy A, Kiefer L, Lee J H, Suckling C J, Clements C, Harvey A L, Hunter W N and Tulloch L B 2009 Diversity oriented syntheses of fused pyrimidines designed as potential antifolates *Org. Biomol. Chem.* **7** 1829; (b) Sakata K I, Someya M, Matsumoto Y, Tauchi H, Kai M, Toyota M, Takagi M, Hareyama M and Fukushima M 2011 Gimeracil, an inhibitor of dihydropyrimidine dehydrogenase, inhibits the early step in homologous recombination *Cancer Sci.* **102** 1712; (c) Ramesh B and Bhalgat C M 2011 Novel dihydropyrimidines and its pyrazole derivatives: Synthesis and pharmacological screening *Eur. J. Med. Chem.* **46** 1882; (d) Zhu L, Cheng P, Lei N, Yao J, Sheng C, Zhuang C, Guo W, Liu W, Zhang Y, Dong G, Wang S, Miao Z and Zhang W 2011 Synthesis and Biological Evaluation of Novel Homocamptothecins Conjugating with Dihydropyrimidine Derivatives as Potent Topoisomerase I Inhibitors *Arch. Pharm. Chem. Life Sci.* **344** 726; (e) Gangjee A, Zhu Y and Queener S F 1998 6-Substituted 2,4-Diaminopyrido[3,2-*d*]pyrimidine Analogues of Piritrexim as Inhibitors of Dihydrofolate Reductase from Rat Liver, *Pneumocystis carinii*, and *Toxoplasma gondii* and as Antitumor Agents *J. Med. Chem.* **41** 4533
3. (a) Wang X, Lou Q, Guo Y, Xu Y, Zhang Z and Liu J 2006 The design and synthesis of 9-phenylcyclohepta[*d*]pyrimidine-2,4-dione derivatives as potent non-nucleoside inhibitorsof HIV reverse transcriptase *Org. Biomol. Chem.* **4** 3252; (b) Yachoan R, Mitsuya H, Thomas R V, Pioda J M, Hartman N R, Perno C F, Marczky K S, Allain J P, Johns D G and Broder S 1989 In Vivo Activity Against HIV and Favorable Toxicity Profile of 2′,3′-Dideoxyinosine *Science* **245** 412; (c) Mellors J W, Im G J, Tramontano E, Winkler S R, Medina D J, Dutschman G E, Bazmi H Z, Piras G, Gonzalez C J and Cheng Y C 1993 A single conservative amino acid substitution in the reverse transcriptase of human immunodeficiency virus-1 confers resistance to (+)-(5S)-4,5,6,7-tetrahydro-5-methyl-6-(3-methyl-2-butenyl)imidazo[4,5, 1-jk][1,4]benzodiazepin-2(1H)-thione (TIBO R82150) *Mol. Pharmacol.* **43** 11
4. (a) Rashid M, Husain A, Shaharyar M, Mishra R, Hussain A and Afzal O 2014 Design and synthesis of pyrimidine molecules endowed with thiazolidin-4-one as new anticancer agents *Eur. J. Med. Chem.* **83** 630; (b) Lukasik P M, Elabar S, Lam F, Shao H, Liu X, Abbas A Y and Wang S 2012 Synthesis and biological evaluation of imidazo[4,5-*b*]pyridine and 4-heteroarylpyrimidine derivatives as anti-cancer agents *Eur. J. Med. Chem.* **57** 311; (c) Determann R, Dreher J, Baumann K, Preu L, Jones P G, Totzke F, Schachtele C, Kubbutat M H and Kunick C 2012 2-Anilino-4-(benzimidazol-2-yl)pyrimidines – A multikinase inhibitor scaffold with antiproliferative activity toward cancer cell lines *Eur. J. Med. Chem.* **53** 254
5. (a) Van den Berg R J B H N, Korevaar C G N, Van der Marel G A, Overkleeft H S and Van Boom J H 2002 A simple and low cost synthesis of d-*erythro*-sphingosine and d-*erythro*-azidosphingosine from d-*ribo*-phytosphingosine: Glycosphingolipid precursors *Tetrahedron Lett.* **43** 8409; (b) Mangfei Y, Qian Z, Jia W, Peng H, Pengfei Y, Rui Z and Dewen D 2016 Triflic Anhydride-Mediated Beckmann Rearrangement Reaction of β-Oximyl Amides: Access to 5-Iminooxazolines *J. Chem. Sci.* **128** 951
6. (a) Darnbrough S, Mervic M, Condon S M and Burns C J 2001 An improved synthesis of *N*-Boc protected aryl amines *Synth. Commun.* **31** 3273; (b) Helgen C and Bochet C G 2003 Photochemical Protection of Amines with Cbz and Fmoc Groups *J. Org. Chem.* **68** 2483
7. (a) Knappke C E I and Von Wangelin A J 2011 35 years of palladium-catalyzed cross-coupling with Grignard reagents: How far have we come? *Chem. Soc. Rev.* **40** 4948; (b) Suzuki A 1999 Preparation of Cyclobutarenes *via* the Steam Pyrolysis of Aromatic Derivatives *J. Org. Chem.* **576** 147; (c) Olsen J A, Severinsen R, Rasmussen T B, Hentzer M, Givskov M and Nielsen J 2002 Synthesis of new 3- and 4-substituted analogues of acyl homoserine lactone quorum sensing autoinducers *Bioorg. Med. Chem. Lett.* **12** 325
8. (a) Kaul R, Surprenant S and Lubell W D 2005 Systematic Study of the Synthesis of Macrocyclic Dipeptide β-Turn Mimics Possessing 8-, 9-, and 10-Membered Rings by Ring-Closing Metathesis *J. Org. Chem.* **70** 3838; (b) Atmuri N D P and Lubell W D 2015 Insight into Transannular Cyclization Reactions To Synthesize Azabicyclo[*X.Y.Z*]alkanone Amino Acid Derivatives from 8-, 9-, and 10-Membered Macrocyclic Dipeptide Lactams *J. Org. Chem.* **80** 4904
9. El-Sherief H A H, El-Naggar G M, Hozien Z A and El-Sawaisi S M 2008 Studies on the reaction of *N*-(3-carbethoxy-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)-*N*′-phenylthiourea with hydrazine hydrate (Part 1) *J. Heterocycl. Chem.* **45** 467