Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron
Tetrahedron 63 (2007) 12912–12916

Solution-phase synthesis of ICG-001, a β-turn peptidomimetic molecule inhibitor of β-catenin–Tcf-mediated transcription

Alessandro Piergentili,[a,*] Fabio Del Bello,[a] Francesco Gentili,[a] Mario Giannella,[a] Wilma Quaglia,[a] Cristian Vesprini,[a] Russell J. Thomas[b] and Graeme M. Robertson[b]

[a]*Dipartimento di Scienze Chimiche, Università di Camerino, Via S. Agostino, 1, 62032 Camerino, Italy*
[b]*Siena Biotech S.p.A., Via Fiorentina, 1, 53100 Siena, Italy*

Received 10 July 2007; revised 14 September 2007; accepted 11 October 2007
Available online 13 October 2007

Abstract—The solution-phase synthesis of the β-turn peptidomimetic ICG-001, a selective inhibitor of Wnt/β-catenin signalling, which has been found to be important for both initiation and progression of cancers of different tissues and has been exploited as an extremely useful chemogenomic tool, was developed. This route is particularly suitable for the multigram scale preparation of ICG-001.

1. Introduction

In a screen for regulators of β-catenin–T cell factor (Tcf)-mediated transcription in the Wnt pathway, the compound ICG-001 (Fig. 1), bearing the tetrahydro-1*H*-pyrazino[1,2-*a*]pyrimidine-4,7(6*H*,8*H*)-dione skeleton, a β-turn bicyclic peptidomimetic, proved to be one of the most potent inhibitors.[1]

The Wnt signalling cascade has important functions in tissue development, homeostasis and regeneration.[2] Wnts bind to members of the Frizzled family of cell-surface receptors and are known to activate at least three separate signalling pathways to effect gene expression.[3] One of these is the 'canonical' pathway, which regulates the ability of the β-catenin protein to drive activation of specific target genes.[2] Deregulation of Wnt/β-catenin signalling is frequently found in various human cancers and its activation has been shown to be important for both initiation and progression of cancers of different tissues, such as colorectal cancers, hepatocellular carcinomas or gastric cancers.[4] In the nucleus, β-catenin regulates gene expression promoting cell proliferation, migration and invasiveness. In fact, it acts as a transcriptional activator in conjunction with the DNA-binding T cell factor/lymphoid enhancer factor (Tcf/Lef) proteins.[5] Therefore, targeted inhibition of Wnt/β-catenin signalling has been a rational and promising new approach for the therapy of cancers of various origins and, though many efforts have been made, at present only a few small molecule inhibitors of β-catenin–Tcf have been identified by high-throughput screens of large synthetic compound collections.[6]

Among these, ICG-001 binds selectively cAMP response element-binding protein (CBP), which is an essential transcriptional co-activator protein, and prevents its interaction with β-catenin–Tcf-responsive gene.[7] ICG-001 selectively induces apoptosis in transformed cells but not in normal colonic epithelial cells by increasing caspase activity, reduces in vitro growth of colon carcinoma cells, and is efficacious in the Min mouse and nude mouse xenograft models of colon cancer.[7] Due to these positive effects, ICG-001 not only has potential therapeutic use in colon cancer, but also represents a lead compound for the development of new agents. Additionally, ICG-001 has been used as a chemogenomic tool compound to help tease apart the mechanisms behind *survivin* gene transcription,[8] and ready access to this molecule will undoubtedly facilitate similar studies in the future.

The published synthesis of ICG-001 uses a solid-phase synthetic protocol[9] as is the case for many such peptidomimetics. Though very useful in terms of rapid analogue

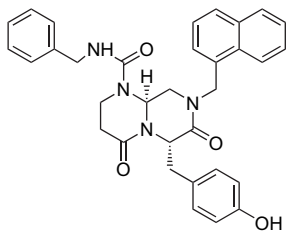
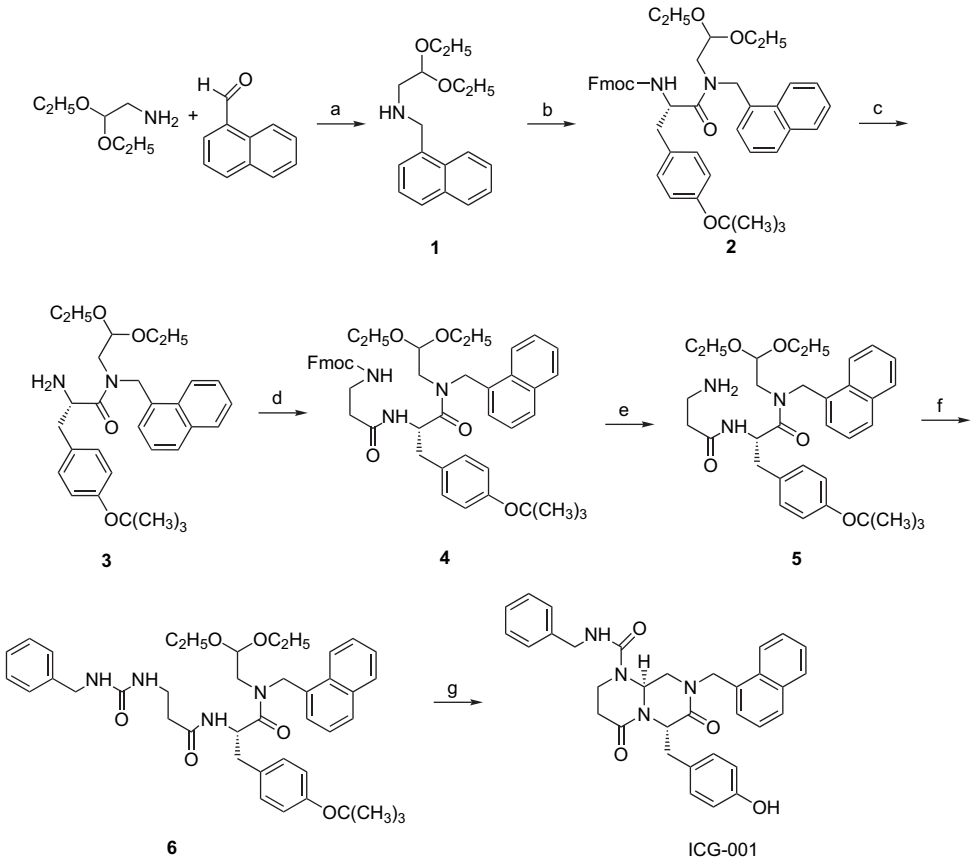


Figure 1. Structure of ICG-001.

Keywords: Wnt pathway; Solution-phase synthesis; Inhibitor; Peptidomimetic; ICG-001.
* Corresponding author. Tel.: +390737402237; fax: +390737637345; e-mail: alessandro.piergentili@unicam.it

0040–4020/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2007.10.048



Scheme 1. (a) $NaBH_4$, EtOH, 85%; (b) Fmoc-L-Tyr(*t*-Bu)-OH, HATU, DIEA, DMF, 78%; (c) DEA, DCM, 83%; (d) Fmoc-β-Ala-OH, HATU, DIEA, DMF, 77%; (e) DEA, DCM, 82%; (f) $C_6H_5CH_2NCO$, DCM, 76%; (g) HCOOH, 76%.

generation, this strategy is less efficient in terms of providing larger quantities of ICG-001. The use of multiple equivalents of costly raw materials required for this solid-phase route is largely responsible for this efficiency shortfall. Moreover, the solid-phase approach for small molecules can be limited by unfavourable heterogeneous reaction kinetics, longer development times and difficulties in analyzing resin-bound intermediates.[10] Therefore, the investigation of alternative synthetic methodologies is a matter of particular importance.

As for the preparation of ICG-001 the number of amino acid units to be combined was small, in the present paper an efficient method in solution-phase synthesis (Scheme 1), which is a method of choice for producing short peptides, was developed and is the subject of this paper. Scale-up of a solution-phase synthesis is more predictable and less expensive than a solid-phase synthesis and allows purification, isolation and characterization of the reaction intermediates to be possible after each reaction step.

2. Results and discussion

The synthetic approach to ICG-001 involved the solution-phase sequential amino acid addition to (2,2-diethoxyethyl)-naphthalen-1-ylmethyl-amine (**1**), prepared in 85% yield by condensation of 1-naphthaldehyde with 2,2-diethoxyethanamine, followed by the reduction of the Schiff base intermediate (Scheme 1). The amine compound **1** was coupled with Fmoc-L-Tyr(*t*-Bu)-OH. Fmoc was chosen as protecting group[11] as its cleavage is easily accomplished with diethylamine (DEA).[12] The reaction with triethylamine and ethyl chlorocarbonate in $CHCl_3$ gave compound **2** in moderate yield (30%). The yield of this synthetic step was improved to 78% by using *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and diisopropylethylamine (DIEA) as the base in dimethylformamide (DMF). The removal of the Fmoc group, to obtain compound **3** in 83% yield, was performed in dichloromethane (DCM) with DEA instead of piperidine, which was used in the solid-phase synthetic protocol.[9] Since the reaction workup foresaw the evaporation to dryness, the advantage lay in the fact that DEA has a boiling point lower than that of piperidine. The reaction of compound **3** with Fmoc-β-Ala-OH in the presence of HATU and DIEA in DMF afforded compound **4** in 77% yield. The cleavage of the Fmoc protection (yield 82%) and subsequent reaction with benzyl isocyanate gave compound **6** in 76% yield, which, finally, was cyclized upon treatment with formic acid for 12 h at rt to afford ICG-001 (76% yield). The final cyclization step produced only a single diastereomer, in which, as previously reported,[13] the hydrogen at the ring junction is trans to the hydrogen at the 6-position. In addition, on the basis of the ^1H NMR spectra the structure of the product here described is consistent with that previously reported.[9]

3. Conclusion

In conclusion we have described the solution-phase synthesis of ICG-001, a selective inhibitor of Wnt/β-catenin signalling, which has been found to be important for both initiation and progression of cancers of different tissues and is a very useful tool for chemogenomic analysis of this, and related pathways. Since in all steps good yields, ranging from 76% to 85%, were obtained, the new synthetic method has proven suitable for the multigram scale preparation of ICG-001.

4. Experimental

4.1. General

All commercially available reagents were used without further purification or were purified by standard methods prior to use. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and ^{1}H NMR spectra were recorded on Perkin–Elmer Spectrum 100 and Varian Mercury 400 instruments, respectively. Chemical shift values are reported in parts per million (ppm) relative to tetramethylsilane (TMS), used as an internal reference standard and spin multiplicities are given as s (singlet), br s (broad singlet), br t (broad triplet), d (doublet), dd (double doublet), t (triplet), q (quartet), or m (multiplet). Compounds **2–6** were observed as rotamers at 20 °C in ^{1}H and ^{13}C NMR: here only the major peaks are reported. Mass spectra were obtained using a Hewlett Packard 1100 MSD instrument utilizing electron-spray ionization (ESI). Elemental analyses were performed at the Microanalytical Laboratory of our department. All reactions were monitored by thin-layer chromatography (TLC) using silica gel plates (60 F_{254}; Merck), visualizing with ultraviolet light or iodine. Chromatographic separations were performed on silica gel columns (Kieselgel 40, 0.040–0.063 mm, Merck) by flash chromatography. Compounds were named following IUPAC rules as applied by Beilstein-Institut AutoNom, a software for systematic names in organic chemistry.

4.1.1. (2,2-Diethoxy-ethyl)-naphthalen-1-ylmethyl-amine (1). A mixture of 1-naphthaldehyde (15.6 g, 0.1 mol) and 2,2-diethoxyethanamine (13.3 g, 0.1 mol) was heated on a steam bath for 20 min, then cooled to rt and diluted with EtOH (75 mL). Sodium borohydride (3.8 g, 0.1 mol) was added in small portions and the mixture was stirred at rt for 24 h. After evaporation of the solvent under reduced pressure, the residue was partitioned between H_2O (50 mL) and EtOAc (200 mL). The organic layer was washed with H_2O (2×25 mL) and dried over Na_2SO_4. The solvent was concentrated in vacuo to give a residue, which was purified by column chromatography, eluting with petroleum ether/$CHCl_3$/CH_3OH/NH_4OH (10:10:0.2:0.01) to afford the product as an oil: 23.2 g (85% yield). R_f=0.51; IR (neat) 2974, 1597, 1217, 1120, 1057, 751 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.19 (t, J=7.0 Hz, 6H, CH_3), 1.64 (br s, 1H, NH, exchangeable with D_2O), 2.89 (d, J=5.0 Hz, 2H, CH_2N), 3.52 and 3.66 (two q, J_1=7 Hz, J_2=10 Hz, 4H, OCH_2), 4.28 (s, 2H, CH_2Ar), 4.62 (t, J=5.0 Hz, 1H, CH), 7.42–8.19 (m, 7H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=136.1, 134.1, 132.1, 128.9, 127.9, 126.2, 126.1, 125.8, 125.6, 123.9, 102.4, 62.5, 52.3, 51.7, 15.6; MS (ESI): m/z=274.1 (M+H)$^+$. Anal. Calcd for $C_{17}H_{23}NO_2$: C, 74.69; H, 8.48; N, 5.12. Found: C, 74.99; H, 8.41; N, 5.05.

4.1.2. {(*S*)-2-(4-*tert*-Butoxy-phenyl)-1-[(2,2-diethoxy-ethyl)-naphthalen-1-ylmethyl-carbamoyl]-ethyl}-carbamic acid 9*H*-fluoren-9-ylmethyl ester (2). (a) DIEA (5.84 g, 45.2 mmol) and HATU (17.18 g, 45.2 mmol) were added to a stirred solution of Fmoc-L-Tyr(*t*-Bu)-OH (18.84 g, 41.0 mmol) in DMF (150 mL) and the mixture was stirred at rt for 30 min. Compound **1** (12.24 g, 45.2 mmol) was then added and the reaction mixture was stirred at rt for 16 h. Removal of the solvent to dryness gave a residue, which was purified by column chromatography, eluting with cyclohexane/EtOAc (9:1) to give **2** as a white foam: 22.86 g (78% yield). R_f=0.10; IR (neat) 3285, 2975, 1713, 1634, 1236, 1044, 738 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.02–1.15 (m, 6H, CH_3CH_2), 1.15 and 1.40 (two s, 9H, $C(CH_3)_3$), 2.90 and 2.99 (two d, J=7.0, 9.2 Hz, 4H, NCH_2CH and Tyr-CH_2), 3.45 (m, 4H, OCH_2CH_3), 4.10–4.79 (m, 5H, Fmoc-CH, Fmoc-CH_2, Tyr-CH, CHO), 4.81–5.10 (m, 2H, Napht-CH_2), 5.79 (br d, 1H, NH, exchangeable with D_2O), 6.60–8.19 (m, 19H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.5, 155.7, 154.4, 144.1, 141.5, 134.1, 132.6, 132.0, 131.4, 130.0, 128.9, 128.2, 127.9, 127.3, 126.7, 126.2, 125.6, 125.4, 125.3, 124.4, 124.3, 120.1, 101.8, 78.5, 67.2, 63.7, 53.0, 50.0, 48.3, 47.2, 39.9, 28.9, 15.4; MS (ESI): m/z=753.4 (M+K)$^+$. Anal. Calcd for $C_{45}H_{50}N_2O_6$: C, 75.60; H, 7.05; N, 3.92. Found: C, 75.96; H, 7.14; N, 3.85.

(b) Ethyl chlorocarbonate (1.22 g, 11.3 mmol) was added dropwise to a stirred and cooled (0 °C) solution of Fmoc-L-Tyr(*t*-Bu)-OH (5.19 g, 11.3 mmol) and Et_3N (1.14 g, 11.3 mmol) in $CHCl_3$ (100 mL). After 30 min a solution of **1** (3.09 g, 11.3 mmol) in $CHCl_3$ (60 mL) was added. The resulting reaction mixture was stirred at rt overnight and then washed with 2 N NaOH (3×15 mL) and water (15 mL). Removal of solvents in vacuo gave an oil, which was purified by column chromatography to give **2** as a white foam 2.42 g (30% yield).

4.1.3. (*S*)-2-Amino-3-(4-*tert*-butoxy-phenyl)-*N*-(2,2-diethoxy-ethyl)-*N*-naphthalen-1-ylmethyl-propionamide (3). DEA (122.5 mL) was added to a solution of **2** (17.5 g, 24.5 mmol) in DCM (250 mL) at rt. The reaction, monitored by TLC, was complete in 3 h at rt. The solvent and DEA were removed and the residue was purified by column chromatography gradient eluent, eluting with cyclohexane/EtOAc (8:2) first and EtOAc next, to give **3** as an oil: 10.0 g (83% yield). R_f=0.08 (EtOAc); IR (neat) 3373, 2975, 1640, 1235, 1160, 1056, 751 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ=1.02–1.21 (m, 6H, CH_3CH_2), 1.25 and 1.35 (two s, 9H, $C(CH_3)_3$), 1.99 (br s, 2H, NH_2, exchangeable with D_2O), 2.66–3.21 (m, 4H, NCH_2CH and Tyr-CH_2), 3.45 (m, 4H, OCH_2CH_3), 3.71 (m, 1H, Tyr-CH), 3.91–4.72 (m, 1H, CHO), 4.81–5.22 (m, 2H, Napht-CH_2), 6.76–8.11 (m, 11H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=176.5, 154.2, 134.0, 133.1, 132.4, 129.9, 129.2, 128.8, 128.0, 127.6, 126.7, 126.2, 125.4, 124.3, 122.2, 101.6, 78.4, 63.6, 53.5, 50.5, 48.8, 42.0, 29.1, 15.4; MS (ESI): m/z=493.4 (M+H)$^+$. Anal.

Calcd for $C_{30}H_{40}N_2O_4$: C, 73.14; H, 8.18; N, 5.69. Found: C, 73.34; H, 8.19; N, 5.91.

4.1.4. (2-{(*S*)-2-(4-*tert*-Butoxy-phenyl)-1-[(2,2-diethoxy-ethyl)-naphthalen-1-ylmethyl-carbamoyl]-ethylcarbamoyl}-ethyl)-carbamic acid 9*H*-fluoren-9-ylmethyl ester (4). DIEA (4.7 g, 36.0 mmol) and HATU (13.6 g, 36.0 mmol) were added to a stirred solution of Fmoc-β-Ala-OH (10.2 g, 33.0 mmol) in DMF (120 mL) and the mixture was left at rt for 30 min. After the addition of compound **3** (18.0 g, 36.0 mmol), the reaction mixture was stirred at rt for 16 h. Removal of the solvent to dryness gave a residue, which was purified by column chromatography, eluting with cyclohexane/EtOAc (1:1), to give **4** as a white foam: 21.8 g (77% yield). R_f=0.23; IR (neat) 3300, 2975, 1721, 1631, 1236, 1056, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.03–1.21 (m, 6H, CH_3CH$_2$), 1.19 and 1.41 (two s, 9H, C(CH$_3$)$_3$), 2.30 and 2.40 (br s, 1H, Fmoc-NH, exchangeable with D_2O), 2.82–3.39 (m, 6H, NCH_2CH, Tyr-CH$_2$, CH$_2$CO), 3.52–3.79 (m, 8H, OCH_2CH$_3$, Fmoc-CH$_2$, CH$_2$CH_2NH), 4.21–5.52 (m, 5H, Fmoc-CH, Tyr-CH, CHO, Napht-CH$_2$), 6.21 and 6.41 (br d, 1H, Tyr-NH, exchangeable with D_2O), 6.62–8.15 (m, 19H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.5, 170.7, 156.6, 154.6, 144.2, 141.5, 134.1, 132.5, 132.0, 131.6, 129.9, 128.9, 128.3, 128.0, 127.2, 126.6, 126.2, 125.5, 125.4, 125.3, 124.4, 124.3, 120.1, 101.6, 78.3, 66.8, 63.7, 51.3, 50.0, 48.5, 47.5, 39.4, 37.4, 36.1, 28.9, 15.4; MS (ESI): m/z=786.9 $(M+H)^+$. Anal. Calcd for $C_{48}H_{55}N_3O_7$: C, 73.35; H, 7.05; N, 5.35. Found: C, 73.54; H, 7.15; N, 5.42.

4.1.5. (*S*)-2-(3-Amino-propionylamino)-3-(4-*tert*-butoxy-phenyl)-*N*-(2,2-diethoxy-ethyl)-*N*-naphthalen-1-ylmethyl-propionamide (5). DEA (125 mL) was added to a solution of **4** (20.0 g, 25.0 mmol) in DCM (250 mL). The reaction, monitored by TLC, was complete in 3 h at rt. The solvent and DEA were removed and the residue was purified by column chromatography gradient eluent, eluting with cyclohexane/EtOAc (1:1) first and with EtOAc/MeOH (8:2) next, to give **5** as an oil: 11.6 g (82% yield). R_f=0.20 (EtOAc/CH_3OH/NH_4OH 8:2:0.01); IR (neat) 3289, 2976, 1737, 1634, 1236, 1045, 792 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.07–1.18 (m, 6H, CH_3CH$_2$), 1.16 and 1.23 (two s, 9H, C(CH$_3$)$_3$), 2.41 (br t, 2H, NH_2, exchangeable with D_2O), 2.42–3.79 (m, 12H, NCH_2CH, Tyr-CH$_2$, OCH_2CH$_3$, CH$_2$CH$_2$CO), 4.51–5.61 (m, 4H, Tyr-CH, CHO, Napht-CH$_2$), 6.62–8.11 (m, 11H, ArH), 8.21 and 8.31 (br d, 1H, Tyr-NH, exchangeable with D_2O); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.7, 171.7, 154.4, 134.0, 132.6, 131.9, 129.9, 129.0, 128.8, 128.1, 127.9, 126.6, 126.1, 125.5, 124.3, 122.5, 102.0, 78.4, 63.9, 51.0, 50.0, 48.5, 39.1, 38.9, 38.7, 29.0, 15.4; MS (ESI): m/z=564.3 $(M+H)^+$. Anal. Calcd for $C_{33}H_{45}N_3O_5$: C, 70.31; H, 8.05; N, 7.45. Found: C, 70.52; H, 8.00; N, 7.31.

4.1.6. (*S*)-2-[3-(3-Benzyl-ureido)-propionylamino]-3-(4-*tert*-butoxy-phenyl)-*N*-(2,2-diethoxy-ethyl)-*N*-naphthalen-1-ylmethyl-propionamide (6). A solution of benzyl isocyanate (2.0 g, 15.0 mmol) in DCM (100 mL) was added to a solution of **5** (8.5 g, 15.0 mmol) in DCM (100 mL). The reaction, monitored by TLC, was complete in 14 h at rt. The mixture was then evaporated to dryness and the residue was purified by column chromatography, eluting with EtOAc, to yield **6** as a viscous oil: 7.9 g (76% yield). R_f=0.26; IR (neat) 3306, 2975, 1738, 1630, 1505, 1235, 1160, 1056, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=1.06–1.12 (m, 6H, CH_3CH$_2$), 1.20 and 1.28 (two s, 9H, C(CH$_3$)$_3$), 1.63 (br s, 1H, NH, exchangeable with D_2O), 2.21–2.38 (m, 2H, CH$_2$CO), 2.76–3.72 (m, 10H, NCH_2CH, Tyr-CH$_2$, OCH_2CH$_3$, CH_2CH$_2$CO), 4.22–5.48 (m, 7H, Tyr-CH, CHO, CH$_2$Ar, Napht-CH$_2$, NH, exchangeable with D_2O), 6.26 and 6.40 (br d, 1H, Tyr-NH, exchangeable with D_2O), 6.68–8.11 (m, 16H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$): δ=172.8, 171.7, 158.3, 154.4, 139.8, 134.1, 132.4, 131.9, 131.4, 130.0, 128.9, 128.6, 128.3, 127.6, 127.3, 126.7, 126.2, 125.6, 124.3, 124.0, 122.4, 101.7, 78.3, 63.9, 51.2, 49.9, 48.9, 47.9, 44.5, 38.8, 36.8, 28.9, 15.5; MS (ESI): m/z=719.5 $(M+Na)^+$. Anal. Calcd for $C_{41}H_{52}N_4O_6$: C, 70.66; H, 7.52; N, 8.04. Found: C, 70.76; H, 7.62; N, 8.20.

4.1.7. (6*S*,9a*S*)-6-(4-Hydroxy-benzyl)-8-naphthalen-1-ylmethyl-4,7-dioxo-hexahydro-pyrazino[1,2-*a*]pyrimidine-1-carboxylic acid benzylamide (ICG-001). Compound **6** (6.0 g, 8.55 mmol) was stirred in formic acid (60 mL) for 16 h at rt. Evaporation to dryness yielded 4.5 g of ICG-001 as a solid, which was recrystallized from EtOAc: 3.6 g (76% yield); mp 133–134 °C. R_f=0.48 (EtOAc/CH_3OH 9.5:0.5); IR (neat) 3321, 2928, 1627, 1515, 1260, 1101, 1056, 783 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ=2.21 (m, 1H, CH$_2$CO), 2.35 (m, 1H, CH$_2$CO), 2.90 (m, 1H, CH$_2$N), 3.05 (m, 1H, Tyr-CH$_2$), 3.11 (m, 1H, Tyr-CH$_2$), 3.28 (dd, J_1=5 Hz, J_2=14 Hz, 1H, CHCH_2N), 3.40 (dd, J_1=5 Hz, J_2=14 Hz, 1H, CHCH_2N), 3.82 (m, 1H, CH$_2$N), 4.19 (dd, J_1=5 Hz, J_2=14 Hz, 1H, CH_2NH), 4.31 (dd, J_1=5 Hz, J_2=14 Hz, 1H, CH_2Ar), 4.56 (br t, 1H, NH, exchangeable with D_2O), 4.98 (d, J=15 Hz, 1H, Napht-CH$_2$), 5.04 (dd, J_1=4 Hz, J_2=11 Hz, 1H, Tyr-CH), 5.23 (d, J=15 Hz, 1H, Napht-CH$_2$), 5.39 (t, J=6 Hz, 1H, NCHN), 6.58 (m, 2H, ArH), 7.05 (m, 2H, ArH), 7.13 (m, 2H, ArH), 7.30 (m, 4H, ArH), 7.37 (m, 1H, ArH), 7.55 (m, 2H, ArH), 7.83 (m, 1H, ArH), 7.88 (m, 1H, ArH), 8.08 (m, 1H, ArH); ^1H NMR (400 MHz, DMSO-d_6): δ=2.09 (m, 1H, CH$_2$CO), 2.99–3.39 (m, 5H, CH$_2$N, Tyr-CH$_2$, CHCH_2N), 3.59 (t, J=11 Hz, 1H, NH, exchangeable with D_2O), 3.92 (m, 1H, CH$_2$N), 4.19 (dd, J_1=6 Hz, J_2=15 Hz, 1H, CH$_2$Ar), 4.32 (dd, J_1=6 Hz, J_2=15 Hz, 1H, CH$_2$Ar), 4.86 (d, J=15 Hz, 1H, Napht-CH$_2$), 5.18 (m, 1H, Tyr-CH), 5.18 (d, J=5 Hz, 1H, Napht-CH$_2$), 5.79 (dd, J_1=4 Hz, J_2=11 Hz, 1H, NCHN), 6.52 (d, J=9 Hz, 2H, ArH), 6.89 (d, J=9 Hz, 2H, ArH), 7.19–8.18 (m, 12H, ArH), 9.19 (br s, 1H, OH, exchangeable with D_2O); ^{13}C NMR (100 MHz, CD_3OD): δ=167.3, 167.1, 157.1, 156.4, 139.7, 134.3, 131.7, 131.0, 130.5, 128.8, 128.7, 128.3, 127.1, 127.0, 126.9, 126.5, 126.0, 125.2, 123.3, 115.2, 61.2, 56.9, 48.3, 47.9, 44.2, 36.3, 35.6, 31.2; MS (ESI): m/z=549.2 $(M+H)^+$. Anal. Calcd for $C_{33}H_{32}N_4O_4$: C, 72.24; H, 5.88; N, 10.21. Found: C, 71.96; H, 5.90; N, 10.15.

Acknowledgements

This work was supported by the Monte dei Paschi di Siena Foundation Award.

References and notes

1. McMillan, M.; Kahn, M. *Drug Discov. Today* **2005**, *10*, 1467–1474.
2. Nusse, R. *Cell Res.* **2005**, *15*, 28–32.
3. Huelsken, J.; Behrens, J. *J. Cell Sci.* **2002**, *115*, 3977–3978.
4. Giles, R. H.; van Es, J. H.; Clevers, H. *Biochim. Biophys. Acta* **2003**, *1653*, 1–24.
5. Brantjes, H.; Barker, N.; van Es, J.; Clevers, H. *Biol. Chem.* **2002**, *383*, 255–261.
6. Barker, N.; Clevers, H. *Nat. Rev. Drug Discov.* **2006**, *5*, 997–1014.
7. Emami, K. H.; Nguyen, C.; Ma, H.; Kim, D. H.; Jeong, K. W.; Eguchi, M.; Moon, R. T.; Teo, J.-L.; Oh, S. W.; Kim, H. Y.; Moon, S. H.; Ha, J. R.; Kahn, M. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12682–12687.
8. Ma, H.; Nguyen, C.; Lee, K.-S.; Kahn, M. *Oncogene* **2005**, *24*, 3619–3631.
9. Eguchi, M.; Nguyen, C.; Lee, S. C.; Kahn, M. *Med. Chem.* **2005**, *1*, 467–472.
10. Zhang, W. *Curr. Opin. Drug Discov. Devel.* **2004**, *7*, 784–797.
11. Bodanszky, M.; Deshmane, S. S.; Martined, J. *J. Org. Chem.* **1979**, *44*, 1622–1625.
12. Tung, R. D.; Dhaon, M. K.; Rich, D. H. *J. Org. Chem.* **1986**, *51*, 3350–3354.
13. Eguchi, M.; Lee, M. S.; Nakanishi, H.; Stasiak, M.; Lovell, S.; Kahn, M. *J. Am. Chem. Soc.* **1999**, *121*, 12204–12205.