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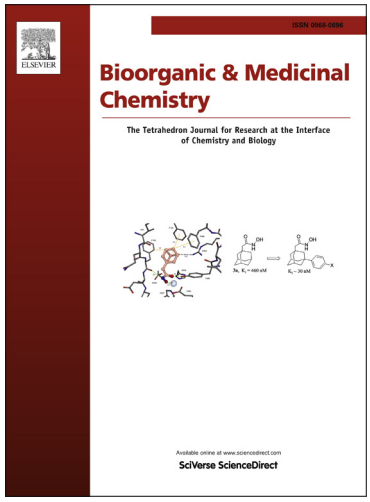

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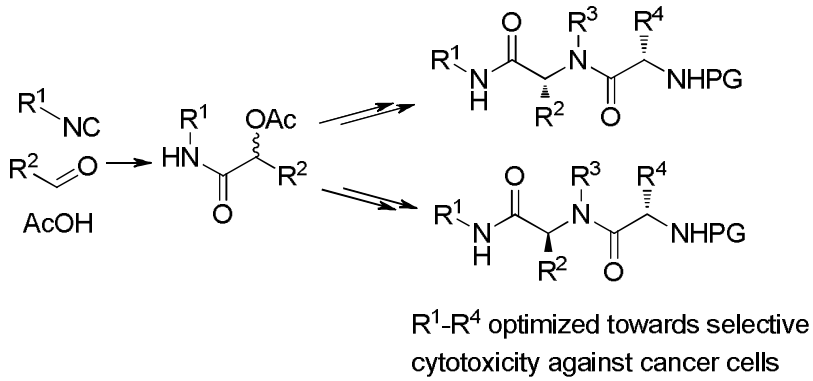
Graphical abstract:



Synthesis of Novel, Peptidic Kinase Inhibitors with Cytostatic/Cytotoxic Activity

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Abstract: The utility of a novel, chemoenzymatic procedure for the stereocontrolled synthesis of small peptides is presented in the preparation and structure optimisation of dipeptides with cytostatic/cytotoxic activity. The method uses Passerini multicomponent reaction for the preparation of racemic scaffold which is then enantioselectivelly hydrolysed by hydrolytic enzymes. Products of these transformations are further functionalised towards title compounds. Both activity and selectivity towards tumor cells is optimised. Final compound is shown to be an inhibitor of the protein kinase signaling pathway.

Keywords: peptidomimetics, biotransformations, cytostatic/cytotoxic effect, kinase inhibitors

1. Introduction

The promising use of small peptides as therapeutics suffers seriously from the problems with pharmacokinetics of these compounds. These problems include proteolytic instability and high polarity, which prevents small peptides from crossing cell membranes. The synthesis of peptidomimetics aims at the solution of these problems. The changes, which are usually introduced into the structure of the peptide are: *N*-alkylation of peptide bond,[1] the incorporation of non-coded amino acids[2] and the introduction of special functional groups at the termini of the peptide.[3]

A novel method for the preparation of peptidomimetics of structure **7** has been recently developed in our group (**Scheme 1**).[4] It uses Passerini multicomponent reaction for the preparation of racemic scaffolds ***rac*-4** which is then enantioselectivelly hydrolysed by hydrolytic enzymes to the enantiomerically enriched alcohols **5**, leaving the enriched esters **4**. A method for the conversion of compounds **4** and **5** into each other, with the inversion of configuration, has also been established,[5] allowing a more efficient synthesis of a chosen enantiomer. Alcohols **5** are then transformed into amines **6** (by an S_N2 type reaction of sodium azide with methanesulfonic ester of **5**, followed by the reduction of the azide), which are subsequently coupled with *N*-protected amino acids to yield the title compounds **7** (R^3 = H). An alternative route leads to *N*-methylamines **6** (R^3 = Me), by reacting methanesulfonic ester of **5** with aqueous methylamine. These compounds are further coupled with *N*-protected amino acids to yield title compounds **7** (R^3 = Me), derivatives of *N*-alkylated amino acids.

Mitogen-Activated Protein Kinase (MAPK) pathway is essential for induction of cell proliferation and may be hyperactivated in various tumor cells [6,7]. This pathway consists of small, guanine nucleoside-binding proteins, RAS, that regulate multiple signal transduction pathways, including the activation of downstream RAF-MEK1/2-ERK1/2

protein kinase cascade[7,8]. Inhibition of this cascade is known to block activation and subsequent cell proliferation, and at least two inhibitors of the pathway were shown to have promising antitumor effects *in vitro* and *in vivo*.[9,10]

In this paper we describe our studies on the use of above-described methodology for the preparation and optimization of a group of dipeptides with selective effect on MAPK kinase pathway that would exert cytostatic/cytotoxic effects towards selected murine tumor cell line displaying Ras overactivation (RAS-3T3 cells). This work was inspired by the observation, that for protein kinase inhibitors, the amide bond is essential for maintain the activity,[11] which is consistent with the natural substrate scope of the kinases.

2. Results and discussion

2.1 The optimisation of R^1 fragment derived from isocyanide

An analysis of the ideal structure of MAPK pathway inhibitor, reported by Yingchun et all,[11] reveals that the peptide bond should have two hydrophobic substituents.[11] Therefore, in this part of the study, the R^2 substituent was fixed as *n*-heptyl chain, and a set of hydrophobic groups was tested as R^1.(**Scheme 2)**

Racemic compounds **7**, bearing a glycine fragment (R^4 = H) and no *N*-modification (R^3 = H) were synthesized (**Scheme 2**) according to the previously established procedures.[1] Isocyanides, the precursors of R^1 fragment were either commercially available or were synthesized by the dehydration of corresponding *N*-formylamines by $POCl_3/Et_3N$. Yields of key transformations are given in **Table 1**, entries **a**-**e**. Racemic α-hydroxyamides **5** were synthesized in good overall yields, as both the Passerini reaction and the ester hydrolysis step gave good results. The yield of Passerni reaction was slightly lowered only in the case of cyclohexyl isocyanide (**1a**), probably due to the steric hindrance on the α carbon. The three-step transformation of hydroxy group into an amino group was performed with overall yields exceeding 75% and the coupling steps gave products with excellent yields (>85%).

Results of the bioactivity study for the compounds **7a-e** are presented in **Table 2**. All the compounds displayed cytotoxic/cytostatic activity against both tumor (RAS-3T3) and non-tumor (NIH-3T3) cell lines, however with different selectivity. Compounds **7d** and **7e** showed no selectivity as their IC50 was similar for both cell lines. Compounds **7a-c** were more selective towards RAS-3T3 cell line, which might suggests possible interaction with the MAP kinase pathway. The best result was obtained for compound **7c**, possessing the phenethyl moiety in the optimized fragment.

2.2 The optimisation of R^2 fragment derived from the aldehyde

The same methodology was used for the synthesis of a series of amides **7f-k** (**Table 1**), bearing a glycine fragment (R^4 = H) and no *N*-modification (R^3 = H). R^1 fragment was set as *p*-methoxybenzyl, due to the ease of synthesis and low volatility of respective isocyanide.

All the compounds were obtained in good yields (**Table 1**), and only the synthesis of a compound **7i** with cyclohexyl group proved to be problematic, as the S_N2 reaction with sodium azide is very sensitive to the bulkiness of R^2 substituent and gave product in only 15% yield.

Results of the bioactivity study for the compounds **7d** and **7f-k** (which all have the same R^1 fragment) are presented in **Table 3**. Out of all the studied structures, only those with aliphatic R^2 groups showed any cytostatic/cytotoxic effect. Significant selectivity was reached only in compound **7k** indicating that *n*-nonyl group is the best out of the studied fragments.

2.3 The influence of *N*-methylation

The influence of the *N*-methylation of peptide bond (R^3, **Scheme 1**) was also investigated. *N*-methylated analog of **7k** was prepared (**Scheme 3**) by reaction of respective methansulfonic ester with aqueous methylamine. Thus obtained α-methylaminoamide

was coupled with N-protected glycine to give compound **7l**. IC50 values for compound **7l** were 30 μM both for NIH-3T3 and RAS-3T3 cell lines. This indicates, that the studied modification decreases the activity and selectivity and should be avoided in further study.

2.4 The optimisation of the amino acid-derived fragment (R^4)

The influence of the type of *N*-terminal amino acid (R^4, **Scheme 1**) was studied on four different small peptides containing: glycine (**7k**), L-alanine (**7m**),), L-leucine (**7n**) and L-phenylalanine (**7o**). Synthesis of these compounds was achieved by the coupling of respective amino acid to the α-aminoamide **6k**.

Results of the bioactivity study for the compounds **7k** and **7m-o** are presented in **Table 4**. They indicate, that the introduction of R^4 substituent results in higher selectivity toward tumor cell lines. However, this introduction resulted in lowering the activity of the compounds.

2.5 Determination of the active enantiomer

Enantiomerically enriched α-hydroxyamides **5k**, precursors of **7k**, were obtained in Lipase from porcine pancreas (PPL)-catalyzed hydrolysis of racemic ester **4k** (**Scheme 5**). Further modifications (according to **Scheme 2**) enabled the synthesis of enantiomerically enriched compounds **(*R*)-7k** and **(*S*)-7k**. Absolute configuration of these compounds was assigned using CD spectroscopy (see supporting information for further details).

The effect of these compounds on the activity of MAP kinase cascade was evaluated by means of *in vitro* MEK/ERK1 cascade assay using myelin basic protein (MBP) as a substrate. As shown in **Figure 1**, compound **7k** reduced, in a dose-dependent manner, the level of BMP phosphorylation, thus suggesting that it inhibited MEK/ERK1 activity. The inhibitory effect of compound **(*R*)-7k** was less than that of compound **7k**. On the other hand, compound **(*S*)-7k** was more active than **7k** alone in reduction of MEK/ERK1 cascade activity suggesting that this form is more potent in kinase activity inhibition. The

difference was, however, not strongly pronounced, and therefore, compound**7k** in racemic form was used for further experiments.

2.6 Inhibitory properties of the optimised compound

Basing on the gathered information, an optimised structure **7p** was deduced (**Scheme 4**), which possesses the best of the studied R^1-R^4 substituents. The results of the synthesis of racemic compound **7p** (according to **Scheme 2**) are collected in **Table 1**.

The results of evaluation of MEK/ERK1 kinase activity (Figure 1) strongly implied that compound 7k inhibited either MEK or ERK1 or both. Thus, for characterization of inhibitory activity of optimized compound **7p** were performed *in vitro* experiments to identify which MAP kinase is a potential target for the studied compound.

The effect of racemic compound **7p** on MEK-mediated ERK phosphorylation was tested on RAS-3T3 cells by means of Western-blot and specific quantitative Enzyme-Linked Immunosorbent Assay (ELISA). U0216, a commercial inhibitor of MEK[12] served as control. Western-blot analysis (**Figure 2A)** has revealed that treatment of RAS-3T3 cells with compound **7p** almost completely inhibited phosphorylation of ERK1/2 and this effect was comparable to the effect of U0216. Neither tested compound affected the level of unphosphorylated ERK. Almost complete (90%) reduction of the pERK1/2 level by treatment with compound **7p** was also revealed by specific ELISA (**Figure 2B**). Similar effect was also seen with U0126. These results clearly indicate that the compound **7p** is able to enter the cytoplasm of living cells where it affects ERK phosphorylation, possibly by inhibition of MEK activity.

These results, however, do not exclude the possibility that compound **7p** may also inhibit activity of activated pERK1/2. Thus, we performed *in vitro* experiments with purified activated ERK1 and MBP, as substrate. As shown in **Figure 3A**, the compound **7p** inhibited ERK1 activity in a dose-dependent manner. To reveal whether this compound inhibitory activity was related to blocking of substrate binding site of EKR1 we

performed experiments with addition of increasing amounts of the substrate (MBP) as a putative competitor. As shown in Figure 3B, the compound **7p**-mediated ERK1 inhibition was almost completely reversed by the maximal amount of MBP. This strongly suggests that compound **7p** is also a substrate-competitive inhibitor ofERK1.

Finally, we evaluated the activity of described **7p** on other kinases. We performed the activity test on thirteen enzymes: AurA, ALK, IGF1R, PIM1, FLT3, FGFR2, JAK2, JAK3, BTK, TNIK, KDR, PDGFR-β, EGFR. (Figure 4). Compound **7p** was tested with U0126 as a reference at 10 μM concentration. Remarkably, we did not observed the significant inhibition of studied kinases at this concentration. This indicates that, in studied kinome, the obtained inhibitor is selective against ERK1.

3. Conclusion

We have presented the utility of a methodology, established in our group, for the synthesis of nonnatural dipetide analogues. It enables a convenient introduction of various substituents and peptide bond modifications, also allowing the stereocontrolled synthesis of title compounds.

The cytostatix/cytotoxic effect of the synthesised compounds was evaluated on tumor cell line (RAS-3T3) displaying overactivation of the MAP kinase RAS/MEK/ERK pathway in comparison to the parental non-tumorigenic cells. This enabled selection of compounds with the highest specificity against tumor cells. Final optimized compound was found to be an inhibitor of both MEK and ERK protein kinases being key elements of MAP kinase cascade pathway responsible for proliferation of tumor cells.

4. Experimental

4.1 Materials and Methods

Analytics:

Optical rotations were measured with a JASCO DIP-360 polarimeter. NMR spectra were measured with a Varian 200 GEMINI and Varian 400 GEMINI spectrometer, with TMS used as internal standard. TLCs were performed with silica gel 60 (230–400 mesh, Merck) and silica gel 60 PF254 (Merck), respectively. MS spectra were recorded on an API - 365 (SCIEX) apparatus.

Reagents:

U0126 (Promega Madison, WI) was maintained according to manufacturers instructions. All synthesized substances were dissolved in DMSO as 10 mM stocks and stored at – 20^0C.

Cell lines:

Experiments were performed on murine NIH-3T3 fibroblasts (ATCC), stably transfected with *Ha-Ras* oncogene (RAS-3T3 cells).[14] Parental NIH-3T3 cells servedas control. The cells were routinely cultured in DMEM supplemented with 2 mM 2-mercapthomethanol, 10% fetal bovine serum (FBS) and 1% antibiotic-antymycotic solution (all from Gibco) at 37^{o}C in humified atmosphere of 5% CO_2 in air.

Growth retardation/cytotoxicity assay:

The cytostatic/cytotoxic effect of the studied compounds on RAS-3T3 and NIH-3T3 cells was evaluated by crystal violet assay, as described in detail previously.[14]

Western-blot analysis of ERK1/2:

For examination of phosphorylation status of ERK1/2 RAS-3T3, cells were starved overnight in the medium containing 2% FBS. Then the medium was replaced with a new one containing 10% FBS and different concentrations of the tested compounds. Following 4 hrs of incubation, 4 x 10^6 cells were lysed in lysis buffer (0.1% Triton X-100, 20 mM Tris-HCl (pH 7.5), 10 mM EDTA, 100 mM NaCl, supplemented with 1 mM phenylmethylsulfonyl fluoride, 2 µg/ml pepstatin, 2 µg/ml leupeptin and 2 µg/mL apoprotinin). Next, cell lysates were cleared by centrifugation (15 minutes, 14000 rpm at 4°C). Protein concentrations were determined by colorimetric bicinchoninic acid method

(Pierce). Lysate proteins (50 µg) were separated on 10% SDS-PAGE and transferred to nitrocellulose membranes. Equal loading was confirmed by staining with 1% Ponceau Red. Blots were blocked in 10% horse serum/5% (w/v) non-fat dry milk/0.4% Tween 20 in PBS for 1h and then labeled with rabbit polyclonal anti pERK antibody and goat anti total ERK antibody (Santa Cruz, CA, USA) diluted in 2% dry milk/0.1% Tween 20 in PBS. The immunoblots were visualized with horseradish peroxidase-conjugated secondary antibodies (Jackson Immunoresearch, West Grove, PA) and enhanced by cheminluminescence (Roche, Indianapolis, Indiana).

Enzyme-linked immunosorbent assay (ELISA) of pERK1/2:

Phosphorylated ERK1/2 (pERK1/2) was evaluated using commercial DuoSet ELISA specific for pERK1/2 (R&D Systems, Abingdon, UK). For this purpose the cells were pre-starved and treated with the tested compounds as described above. Then, cell lysates were prepared using the reagents attached reagents and the assay was performed according to the detailed description provided by the manufacturer. The extinction was read at 405 nm using the FLUOstar Omega plate reader (BMG Labtech, Ortenberg, Germany).

In vitro *evaluation of MEK/ERK-mediated phosphorylation activity (MEK/ERK cascade assay):*

To characterize the effect of compound **7k** on the activity of MEK/ERK cascade we used MAP Kinase/Erk Sampler Kit provided by Upstate, Lake Placid, NY and the assay was performed according to the detailed protocol provided by the manufacturer. In brief, 20 ng of each active phosphorylated MEK (Biaffin, Kassel, Germany) and inactive nonphosphorylated ERK1, 20 µg of myelin basic protein (MBP) as a substrate and the tested compound at concentrations ranging from 10-150 µM were mixed in a reaction vial in a final volume of 50 µL of assay dilution buffer (20 mM MOPS (pH 7.2), 25 mM sodium-beta-glycerophosphate, 5 mM EGTA, 1 mM sodium orthovanadate, 1 mM dithithreitol in 50% glycerol). Reaction vials without the tested compound served as control. As a phosphate donor [γ-^{32}P]ATP cocktail (specific activity 3000 Ci/mmol, Hartman Analytic, Braunschweig, Germany) was added to the reaction mixture at the

amount of 10 μCi/reaction and the assay was performed at 30ºC for 10 min. The phosphorylated substrate was then separated form residual [γ-^{32}P]ATP using P81 phosphocellulose paper and quantitated by Wallac MicroBeta 1450 scintillation counter (Perkin-Elmer, WalthamMassachusetts). The assay was performed in quadruplicate and the results were calculated as percent of control without the tested compound.

In vitro evaluation of pERK1 phosphorylation activity:

To characterize the effect of optimized compound **7p** on *in vitro* activity of activated pEKR1 we used MAP Kinase/Erk Sampler Kit provided by Upstate, Lake Placid. The assay was performed according to the manufacturers description as described above with the following modifications. Reaction mixture contained 20 ng pERK1 and 20 μg BMP and the optimized compound **7p** which was added at the final concentrations ranging from 0.05-5 μM. Instead of radioactive ATP, non-radioactive ATP was used (Sigma-Aldrich, St. Louis, MO) was added to the reaction mixture at concentration 0.2 μM. Following the assay, the remaining ATP was determined with the ATPLite luciferase/luciferin luminescennt assay system (Perkin-Elmer, Waltham Massachusetts) and Fluostar Omega plate reader (BMG Labtech) as described in detail in an attached protocol. The relative activity of pERK was then estimated on a basis of ATP consumption rate during the assay (decrease of ATP concentration in reaction mixture following the reaction).

To examine whether inhibitory effect of **7p** on pERK1 was related to its competitive inhibition of substrate binding, we performed a competitive assay with 20 ng pERK1, 5 μM concentration of **7p** and different concentrations of MBP ranging from 0.2 to 200 μg/reaction vial.

Evaluation of selectivity of 7p – the studies on various kinases:

To characterize the effect of optimized compound **7p** on activity of other kinases, the ADP-Glo Kinase Assay provided by Promega was employed. The assay was performed according to the manufacturers description. The solution of an inhibitor was prepared in buffer A (50 μM). The 5 μL of the solution was preincubated with 5 μL the solution of a kinase (1 ng) at 25^{o}C at 100 rpm for 10 min. Then, to the reaction mixture was added,

containing the substrate (10 – 60 μM), ATP (25μM), buffer A and water. The mixture was incubated for 1 h at 24°C. Then the ADP-Glo Reagent (25 μL) was added. The reaction was further incubated for 40 min at 25°C. The Kinase Detection Reagent was added (25 μL) and the reaction was incubated for additional 30 min. at 25°C and luminescence was measured.

4.2 Chemistry

Protocol for the enzymatic resolution of ***rac*-4k:**

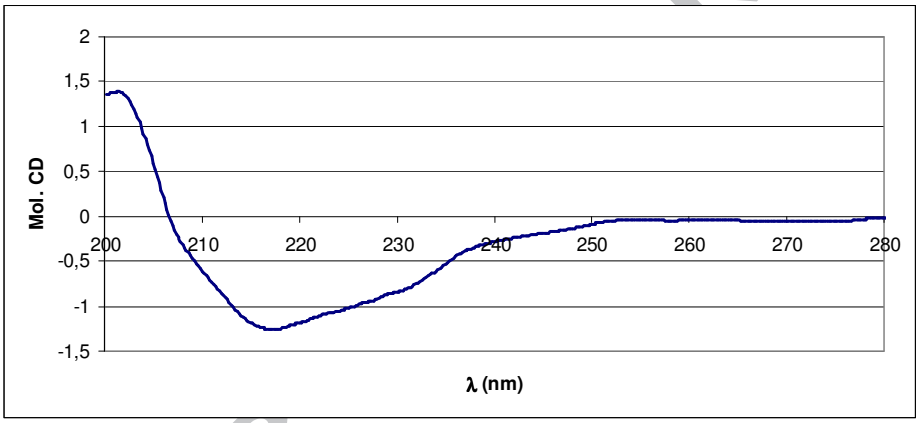
Ester ***rac*-4k** (300 mg) was dissolved in 30 ml of solvent (water / Et_2O, 8:2, v/v). Porcine Pancreas Lipase (50.7 mg) was added in one portion and the reaction mixture was stirred on a shaker at 300 rpm at room temperature for 7 days. Extraction with ethyl acetate, concentration *in vacuo* and purification of the resulting residue by flash chromatography (silicagel, hexane:ethyl acetate) afforded ester **(*R*)-4k** and alcohol **(*S*)-5k**.

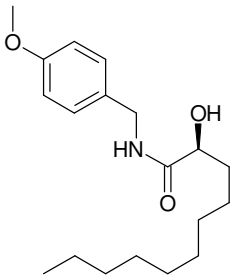
Ester (*R*)-4k: Yield 52 %. e.e.$_R$(HPLC): 59 %. R_f = 0.21 (hexane/AcOEt; 8:2); mp. 63-64 °C (hexane/AcOEt); $[\alpha]_D^{25} = 2.3$ (c 1.0; $CHCl_3$, 59% e.e.); Anal. Calc for $C_{21}H_{33}NO_4$: C, 69.39%; H, 9.15%; N, 3.85%; found: C, 69.33%; H, 9.27%; N, 3.82%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.88-0.92 (t, J = 7.2 Hz, 3H, $CH_2C\mathbf{H_3}$), 1.20-1.40 (m, 18H, $(C\mathbf{H_2})_9CH_3$), 2.11 (s, 3H, $C\mathbf{H_3}CO$) 3.78 (s, 3H, $C\mathbf{H_3}O$), 5.16-5.22 (m, 2H, $ArC\mathbf{H_2}$), 6.20-6.30 (m, 1H, N**H**), 6.80-7.30 (m, 4H, Ar**H**). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 14.1, 21.0, 22.6, 24.7, 29.2, 29.2, 29.4, 29.4, 31.8, 91.9, 42.6, 55.3, 74.2, 114.1, 129.0, 129.9, 159.1, 169.7, 169.7; HPLC: T_1 = 7.88, T_2 = 11.35 (DAICEL CHIRACEL OD-H, eluent: hexane/*iso*-propanol 9:1 (v/v), 1 ml/min).

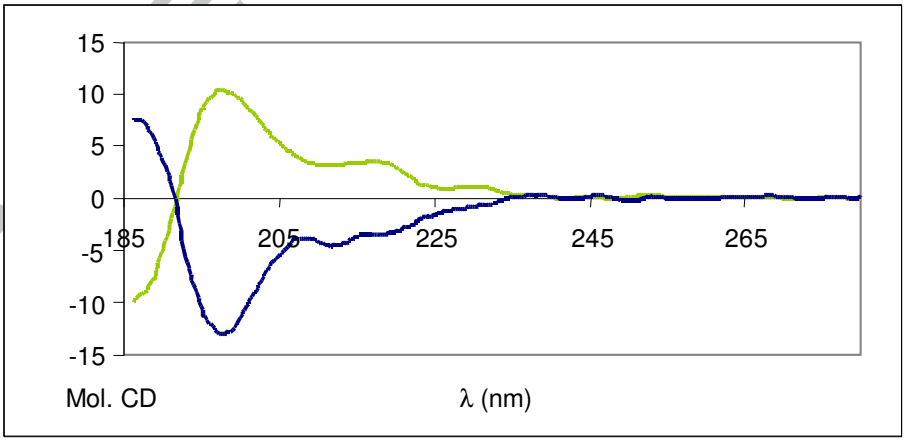
Alcohol (*S*)-5k: Yield 35 %. e.e.$_S$ (HPLC): 99 %; White crystalls; R_f = 0.60 (hexane/AcOEt; 4:6); mp. 112-113 °C (hexane/AcOEt) $[\alpha]_D^{25} = -13.1$ (c 1.0; $CHCl_3$, 99% e.e.) ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.85 (t, 3H, J = 6.8 Hz, $C\mathbf{H_3}CH_2$)**,** 1.20-1.40 (m, 16H, $CH_2(C\mathbf{H_2})_8CH_3$), 1.59-1.70 (m, 1H, $CHC\mathbf{H}_2(CH_2)_8$), 1.79-1.90 (m, 1H, $CHC\mathbf{H}_2(CH_2)_8$),

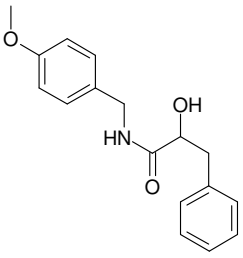
2.80-2.90 (m, 1H, O**H**), 3.79 (s, 3H, C**H**$_3$O), 4.10-4.14 (m, 1H, C**H**(OH)CH$_2$), 4.36-4.40 (m, 2H, ArC**H**$_2$NH), 6.75-6.82 (m, 1H, N**H**), 6.83-7.21 (m, 4H, Ar**H**). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 14.1, 22.7, 25.0, 29.3, 29.4, 29.5, 31.8, 34.9, 42.6, 55.3, 72.2, 114.0, 129.1, 130.0, 159.0, 173.7. HPLC: T_R = 6.27 min., T_S = 8.11 min (DAICEL CHIRACEL OD-H, eluent: hexane/*iso*-propanol 9:1 (v/v), 1 ml/min.).

Absolute configuration for alcohol **5k** was assigned by comparison of the CD spectrum obtained it with the spectra of similar α-hydroxyamides obtained by EDC-madiated coupling of α-hydroxyacids with amines. α-Hydroxyacids were prepared from optically pure, comercially availiable α-aminoacids via diazo compounds. CD spactra are shown below:

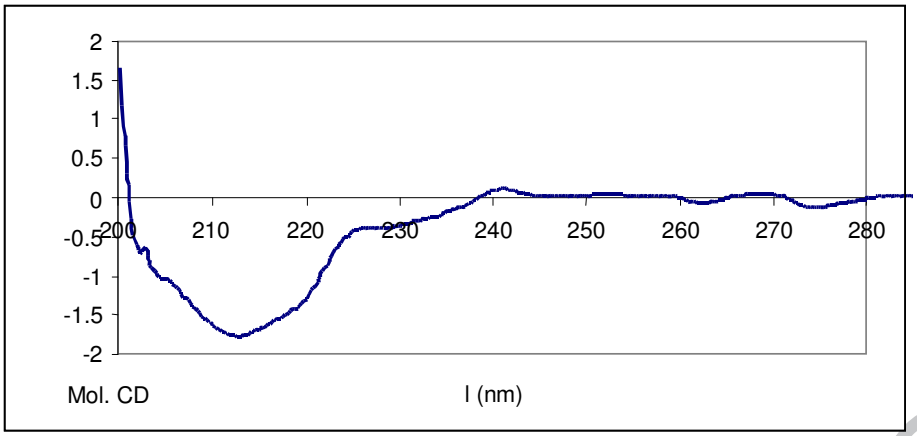


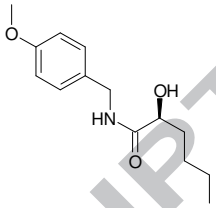






Green line – (*R*), blue line – (*S*)





All the compounds **7** were synthesised using the protocole published by us elswhere.[4] The analytical data for all the new compounds is listed below:

7a; White crystals; R_f = 0.42 (hexane/AcOEt; 4:6); mp. 137-138 °C (AcOEt/hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J* = 7.0 Hz, C**H**$_3$CH$_2$), 1.10-1.90 (m, 22H, (C**H**$_2$)$_6$, ***c-Hex***), 3.71-3.76 (m, 1H, C**H**NH), 3.88 (d, 2H, *J* = 5.4 Hz, gly C**H**$_2$), 4.31-4.38 (m, 1H, C**H**C$_7$H$_{15}$), 5.12 (s, 2H, CBz C**H**$_2$), 5.71 (t, *J* = 5.6 Hz, 1H, N**H**CBz), 6.33 (d, *J* = 8.0 Hz, 1H, N**H**), 6.97 (d, *J* = 8.0 Hz, 1H, N**H**), 7.32-7.34 (m, 5H, Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.6, 24.7, 25.4, 25.5, 29.1, 29.2, 31.7, 32.7, 32.9, 44.4, 48.4, 53.4, 67.1, 128.0, 128.2, 128.5, 136.1, 156.6, 168.9, 170.4; HRMS (ESI, $[M+Na]^+$) 468.2815 ($C_{25}H_{39}N_3O_4Na$: 468.2833).

7b; White crystalls; R_f = 0.33 (hexane/AcOEt; 4:6); mp. 104-106 °C (AcOEt/ hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J* = 6.8 Hz, C**H**$_3$CH$_2$), 1.10-1.38 (m, 10H, (C**H**$_2$)$_5$), 1.52-1.65 (m, 1H, C**H**$_2$CHNH), 1.75-1.82 (m, 1H, C**H**$_2$CHNH), 3.77-3.90 (m, 2H, gly C**H**$_2$), 4.25-4.52 (m, 3H, PhC**H**$_2$, C**H**C$_7$H$_{15}$), 5.02 (s, 2H, PhC**H**$_2$O), 5.80 (br s, 1H, N**H**CBz), 7.06-7.42 (m, 11H, Ar**H,** N**H**); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.6, 25.5, 29.1, 29.2, 31.7, 32.5, 43.4, 44.4, 53.4, 67.1, 127.4, 127.6, 128.0, 128.2, 128.5, 128.6, 136.1, 137.8, 156.6, 169.3, 171.6; HRMS (ESI, $[M+Na]^+$) 476.2523 ($C_{26}H_{35}N_3O_4Na$: 476.2520).

7c; White crystalls; R_f = 0.38 (hexane/AcOEt; 4:6); mp. 118-119 °C (AcOEt/hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J* = 7.0 Hz, C**H$_3$**CH$_2$), 1.21-1.30 (m, 10H, (C**H$_2$**)$_5$), 1.53-1.72 (m, 2H, CH$_2$C**H$_2$**CHNH), 2.77 (t, 2H, *J* = 7.2 Hz, PhC**H$_2$**), 3.38-3.56 (m, 2H, gly C**H$_2$**), 3.85 (d, 2H, *J* = 5.3 Hz, gly C**H$_2$**), 4.32-4.38 (m, 1H, NHC**H**), 5.08 (s, 2H, CBz C**H$_2$**), 5.80 (s, 1H, N**H**), 6.61 (s, 1H, N**H**), 7.17-7.33 (m, 11H, Ar**H**, N**H**); ^{13}C NMR (100 MHz, DMSO-d6 + $CDCl_3$): δ 14.0, 22.6, 25.5, 29.1, 29.2, 31.7, 32.5, 35.5, 40.6, 44.3, 53.3, 67.0, 126.5, 128.0, 128.2, 128.5, 128.5, 128.7, 136.1, 138.6, 156.6, 169.0, 171.5; HRMS (ESI, $[M+Na]^+$) 490.2663 ($C_{26}H_{33}N_3O_5Na$: 490.2676).

7d; White crystalls; R_f = 0.58 (hexane/AcOEt; 4:6); mp. 129-130 °C (AcOEt/ hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J* = 6.8 Hz, C**H$_3$**CH$_2$), 1.21-1.71 (m, 12H, (C**H$_2$**)$_6$), 3.72 (s, 3H, C**H$_3$**O), 3.83-3.87 (m, 2H, gly C**H$_2$**), 4.18-4.48 (m, 3H, C**H$_2$**NH, C**H**NH), 5.01 (s, 2H, PhC**H$_2$**O), 6.78-7.31 (m, 9H, 2Ar**H**), 7.03 (s, 1H, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.6, 25.5, 29.1, 29.2, 31.7, 32.6, 42.9, 44.4, 53.3, 55.2, 67.0, 113.9, 128.0, 128.1, 128.5, 129.0, 129.9, 136.1, 156.6, 158.9, 169.2, 171.4; HRMS (ESI, $[M+Na]^+$) 506.2619 ($C_{27}H_{37}N_3O_5Na$: 506.2625).

7e; White crystalls; R_f = 0.33 (hexane/AcOEt; 4:6); mp. 90-92 °C (AcOEt/hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J* = 6.8 Hz, C**H$_3$**CH$_2$), 1.10-1.40 (m, 10H, (C**H$_2$**)$_5$), 1.52-1.62 (m, 1H, C**H**$_2$CHNH), 1.72-1.81 (m, 1H, C**H**$_2$CHNH), 3.76 (s, 3H, C**H$_3$**O), 3.79 (s, 3H, C**H$_3$**O), 3.80-3.91 (m, 2H, gly C**H$_2$**), 4.25-4.42 (m, 3H, ArC**H$_2$**, C**H**C$_7$H$_{15}$), 5.07 (s, 2H, PhC**H$_2$**O), 5.63 (br s, 1H, N**H**CBz), 6.38-6.42 (m, 3H, Ar**H**), 6.57 (br s, 1H, N**H**), 6.91 (br s, 1H, N**H**), 7.11 (d, *J* = 8.4 Hz, 1H, Ar**H**), 7.30-7.40 (m, 5H, Ar**H**); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 22.6, 25.3, 29.1, 29.2, 31.7, 32.8, 39.1, 44.3, 53.2, 55.3, 55.3, 67.1, 98.5, 103.8, 118.2, 128.1, 128.2, 128.5, 130.3, 136.2, 156.5, 158.5, 160.6, 168.7, 171.0; HRMS (ESI, $[M+Na]^+$) 536.2753 ($C_{28}H_{39}N_3O_6Na$: 536.2731).

7g; White crystalls; R_f = 0.29 (hexane/AcOEt; 4:6); mp. 163-163 °C (AcOEt/hexane); ^{1}H NMR (400 MHz, DMSO-d6 + $CDCl_3$): δ 2.76 (dd, 1H, *J* = 8.4 Hz, *J* = 13.6 Hz, C**H**$_2$CH), 2.92 (dd, 1H, *J* = 5.6 Hz, *J* = 14.0 Hz, C**H**$_2$CH), 3.31 (s, 2H, gly C**H$_2$**), 3.68 (s, 3H,

C**H**$_3$O), 3.69 (s, 3H, C**H**$_3$O), 4.15-4.19 (m, 2H, PMBC**H**$_2$NH), 4.38-4.49 (m, 1H, C**H**CH$_2$), 4.97 (s, 2H, PhCH$_2$O), 6.70-7.30 (m, 13H, Ar**H**), 7.92-7.93 (m, 1H, NH), 8.23-8.26 (m, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d6 + $CDCl_3$): δ 36.9, 41.5, 43.5, 54.1, 54.6, 54.7, 65.5, 113.3, 127.4, 127.5, 128.0, 128.3, 129.2, 130.0, 130.6, 136.6, 156.3, 157.6, 158.0, 168.6, 170.4; HRMS (ESI, $[M+Na]^+$) 528.2129 ($C_{28}H_{31}N_3O_6Na$: 528.2105).

7h; Yellow oil; R_f = 0.20 (hexane/AcOEt; 4:6); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24-1.35 (m, 6H, (C**H**$_2$)$_3$), 1.41 (s, 9H, CH(C**H**$_3$)$_3$), 1.57-1.82 (m, 2H, CH$_2$C**H**$_2$CH(NH$_2$)), 3.04-3.06 (m, 2H, C**H**$_2$NH), 3.70-3.75 (m, 1H, C**H**NH$_2$), 3.76 (s, 3H, C**H**$_3$O), 4.15-4.45 (m, 4H, C**H**$_2$Ph, gly C**H**$_2$), 4.70 (br s, 1H, N**H**), 5.02 (s, 2H, PhCH$_2$O), 6.81-7.20 (m, 4H, Ar**H**), 6.85-6.89 (m, 1H, N**H**); ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.7, 25.9, 28.4, 29.5, 32.2, 40.0, 42.9, 44.4, 53.1, 55.2, 67.0, 79.1, 113.9, 121.5, 126.8, 128.0, 128.1, 128.5, 129.0, 129.9, 136.1, 156,7, 158.9, 169.4, 171.4; HRMS (ESI, $[M+Na]^+$) 593.2924 ($C_{30}H_{42}N_4O_7Na$: 593.2955).

7i; White crystalls; R_f = 0.22 (hexane/AcOEt; 4:6); mp. 198-199 °C (AcOEt/hexane) ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.91-1.66 (m, 11H, *c-Hex*), 3.76 (s, 3H, C**H**$_3$O), 3.87 (s, 2H, gly CH$_2$), 4.26-4.34 (m, 2H, C**H**$_2$Ph), 5.06 (s, 2H, PhC**H**$_2$O), 5.52 (s, 1H, N**H**), 6.49 (s, 1H, N**H**), 6.76 (s, 1H, N**H**), 6.82-7.33 (m, 4H, Ar**H**); HRMS (ESI, $[M+Na]^+$) 490.2305 ($C_{26}H_{33}N_3O_5Na$: 490.2312).

7k; White crystals; R_f = 0.40 (hexane/AcOEt; 4:6); mp. 141-144 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 0.82-0.92 (m, 3H, CH$_3$(CH$_2$)$_8$), 1.16-1.86 (m, 16H, CH$_3$(C**H**$_2$)$_8$), 3.74 (s, 3H, C**H**$_3$O), 3.80-3.88 (m, 2H, gly C**H**$_2$), 4.20-4.50 (m, 3H, ArC**H**$_2$NH, COC**H**(NH)CH$_2$), 4.98-5.10 (m, 2H, OC**H**$_2$Ar), 5.56-5.66 (m, 1H, N**H**), 6.66-6.74 (m, 1H, N**H**), 6.74-6.90 (m, 3H, Ar**H**, N**H**), 7.10-7.20 (m, 2H, Ar**H**), 7.22-7.40 (m, 5H, Ar**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) *δ*: 14.1, 22.6, 25.5, 29.3, 29.4, 29.5, 31.8, 32.5, 43.0, 53.3, 55.2, 67.2, 114.0, 128.1, 128.2, 128.5, 129.0, 129.9, 136.0, 159.0, 168.9, 171.1; HRMS (ESI, $[M + Na]^+$) 534.2914 ($C_{29}H_{41}N_3O_5Na$: 534.2938). Compounds obtained from the products of enzymatic reaction: (*R*) - $[\alpha]_D^{25} = +7.6$ (c 1.0; $CHCl_3$); (*S*) - $[\alpha]_D^{25} = -8.0$ (c 1.0; $CHCl_3$).

7l; Clear oil; R_f = 0.59 (hexane/AcOEt; 4:6); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.80-0.92 (m, 3H, C**H$_3$**CH$_2$), 1.10-1.95 (m, 16H, (C**H$_2$**)$_8$), 2.84-2.92 (m, 3H, C**H$_3$**N), 3.74-3.80 (m, 3H, C**H$_3$**O), 4.20-4.40 (m, 4H, ArC**H$_2$**NH, COC**H$_2$**NH), 4.90-5.40 (m, 1H, COC**H**(N)CH$_2$), 5.08-5.14 (m, 2H, OC**H$_2$**Ph), 6.30-6.42 (m, 1H, N**H**), 6.48-6.56 (m, 1H, N**H**), 6.80-6.86 (m, 2H, Ar**H**), 7.10-7.16 (m, 2H, Ar**H**), 7.24-7.38 (m, 5H, Ar**H**); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 14.1, 22.0, 22.6, 26.0, 22.4, 31.3, 33.8, 42.7, 42.9, 55.2, 56.0, 56.8, 67.0, 114.0, 114.0, 128.0, 128.2, 128.5, 128.9, 128.9, 159.0, 169.7, 170.4, 172.0; HRMS (ESI, [M + Na]$^+$) 548.3117 ($C_{30}H_{43}N_3O_5Na$: 548.3095).

7m; White crystals; R_f = 0.22 (hexane/AcOEt; 4:6); mp. 131-134 °C (AcOEt/hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J* = 7.0 Hz, C**H$_3$**CH$_2$), 1.10-1.35 (m, 19H, (C**H$_2$**)$_8$, C**H$_3$**CH), 1.52-1.62 (m, 1H, C**H**$_2$CHNH), 1.72-1.81 (m, 1H, C**H**$_2$CHNH), 3.73 (s, 3H, C**H$_3$**O), 4.20-4.44 (m, 3H, gly CH$_3$C**H**, ArC**H$_2$**NH), 4.84-5.05 (m, 2H, PhC**H$_2$**O), 5.60 (d, 1H, *J* = 6.6 Hz, N**H**CBz), 6.79-7.38 (m, 11H, 2Ar**H,** 2N**H**); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 18.6, 22.6, 25.5, 29.3, 29.3, 29.4, 29.5, 29.5, 31.8, 32.2, 32.5, 42.9, 42.9, 50.7, 53.3, 55.2, 67.0, 114.0, 128.0, 128.2, 128.5, 128.9, 129.0, 130.0, 136.0, 156.0, 158.9, 171.2, 171.3, 172.4; HRMS (ESI, [M+Na]$^+$) 548.3081 ($C_{30}H_{43}N_3O_5Na$: 548.3095).

7n; White crystalls; R_f = 0.46 (hexane/AcOEt; 4:6); mp. 119-122 °C (AcOEt/hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.85-0.92 (m, 18H, 2 x C**H$_3$**CH$_2$, 2 x (C**H$_3$**)$_2$CH), 1.22-1.90 (m, 38H, 2 x (C**H$_2$**)$_8$, 2 x (CH$_3$)$_2$C**H**C**H$_2$**), 3.71 (s, 3H, C**H$_3$**O), 3.73 (s, 3H, C**H$_3$**O), 4.18-4.47 (m, 8H, 2 x leu C**H**, 2 x C$_9$H$_{19}$C**H**, 2 x C**H$_2$**NH), 4.83-5.05 (m, 4H, 2 x PhC**H$_2$**O), 5.54 (d, 1H, *J* = 8.0 Hz, N**H**CBz), 5.60 (d, 1H, *J* = 6.4 Hz, N**H**CBz), 6.78-7.32 (m, 22H, 2 x 2Ar**H**, 2 x 2N**H**); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 21.9, 22.1, 22.6, 22.7, 22.8, 24.6, 24.7, 25.6, 25.6, 29.2, 29.3, 29.3, 29.4, 29.5, 29.5, 31.8, 32.0, 32.4, 41.4, 41.5, 42.8, 42.9, 53.2, 54.0, 55.1, 67.0, 113.9, 127.9, 128.1, 128.4, 129.0, 130.1, 136.0, 136.1, 156.2, 158.8, 158.9, 171.3, 171.3, 172.4, 172.5; HRMS (ESI, [M+Na]$^+$) 590.3543 ($C_{33}H_{49}N_3O_5Na$: 590.3564). This compound was obtained as a ca 1:1 mixture of two diastereoisomers, as judged by the integration of NMR singlets for methoxy group at 3.71 ppm and 3.73 ppm.

7o; White crystals; R_f = 0.42 (hexane/AcOEt; 4:6); mp. 135-140 °C (AcOEt/hexane); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J* = 7.0 Hz, C**H$_3$**CH$_2$), 0.88 (t, 3H, *J* = 7.0 Hz, C**H$_3$**CH$_2$), 1.01-1.82 (m, 32H, 2 x (C**H$_2$**)$_8$), 2.99 (d, 4H, 2 x PhC**H$_2$**), 3.69 (s, 3H, C**H$_3$**O), 3.72 (s, 3H, C**H$_3$**O), 4.18-4.53 (m, 8H, 2 x phe C**H**, 2 x C$_9$H$_{19}$C**H**, 2 x C**H$_2$**NH), 4.81-5.00 (m, 4H, 2 x PhC**H$_2$**O), 5.51 (d, 1H, *J* = 6.6 Hz, N**H**CBz), 5.65 (d, 1H, *J* = 6.6 Hz, N**H**CBz), 6.59-7.31 (m, 32H, 2 x 3Ar**H,** 2 x 2N**H**); ^{13}C NMR (100 MHz, $CDCl_3$): δ14.1, 22.6, 25.4, 25.5, 29.3, 29.4, 29.5, 29.5, 29.5, 31.8, 31.9, 32.0, 32.3, 38.3, 38.5, 42.9, 53.4, 55.1, 55.2, 56.1, 56.8, 67.0, 67.1, 113.9, 114.0, 127.0, 127.1, 127.9, 128.0, 128.1, 128.2, 128.5, 128.5, 128.6, 128.9, 129.0, 129.2, 129.2, 130.1, 136.0, 136.0, 136.0, 136.1, 156.0, 158.8, 158.9, 170.9, 171.0, 171.0, 171.1; HRMS (ESI, $[M+Na]^+$) 624.3391 ($C_{36}H_{47}N_3O_5Na$: 624.3407). This compound was obtained as a ca 1:1 mixture of two diastereoisomers, as judged by the integration of NMR singlets for methoxy group at 3.69 ppm and 3.72 ppm.

7p; White crystals; R_f = 0.35 (hexane/AcOEt; 5:5); mp. 96-97 °C (AcOEt/hexane); ^{1}H NMR (200 MHz, $CDCl_3$): δ 0.87 (t, 3H, *J* = 6.8 Hz, C**H$_3$**CH$_2$), 1.21-1.30 (m, 14H, (C**H$_2$**)$_7$), 1.40-1.82 (m, 2H, CH$_2$C**H$_2$**CHNH), 2.78 (t, 2H, *J* = 5.0 Hz, PhC**H$_2$**), 3.38-3.64 (m, 2H, CH$_2$C**H$_2$**NH), 3.65-4.00 (m, 2H, gly C**H$_2$**), 4.22-4.40 (m, 1H, NHC**H**), 5.10 (s, 2H, CBz C**H$_2$**), 5.68 (s, 1H, N**H**), 6.49 (s, 1H, N**H**), 7.13-7.44 (m, 11H, Ar**H**, N**H**); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.4, 23.0, 25.8, 29.6, 29.8, 29.9, 32.2, 32.6, 35.7, 41.0, 53.8, 67.6, 126.8, 127.9, 128.3, 128.6, 128.9, 128.9, 129.0, 157.1, 169.5, 172.0; HRMS (ESI, $[M+Na]^+$) 518.2978 ($C_{29}H_{41}N_3O_4Na$: 518.2989).

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References and Notes

1. Aurelio, L.; Brownlee, R.T.C.; Hughes, A.B. *Chem. Rev.* **2004**, *104*, 5823.

2. Huang, Y.; Hang, Y.-B.; Chen, Z.C.; Xu, P.F. *Tetrahedron: Asymmetry* **2006**, *17*, 3152.

3. Marastoni, M.; Baldisserotto, A.; Canella, A.; Gavioli, R.; De Risi, C.; Pollini, G.P.; Tomatis, R. *J. Med. Chem.* **2004**, *47*, 1587.

4. Szymanski, W.; Zwolinska, M.; Ostaszewski, R. *Tetrahedron* **2007**, *63*, 7647.

5. Szymanski, W.; Ostaszewski, R. *Tetrahedron: Asymmetry* **2006**, *17*, 2667.

6. Campbell, S.L.; Khosravi-Far, R.; Rossman, K.L.; Clark G.J.; Der, C.J. *Oncogene* **1998**, *17*, 1395.

7. Chang, F.; Steelman, L.S.; Lee, J.T.; Shelton, J.G.; Navolanic, P.M.; Blalock, W.L.; Franklin, R.A.; McCubrey, J.A.; *Leukemia* **2003**, *17*, 1263.

8. Gupta, S.; Plattner, R.; Der, C.J.; Stanbridge, *E.J. Mol. Cell. Biol* **2000**, *20*, 9294

9. Adjei, A. A., Cohen, R.B., Franklin, W, Morris, C., Wilson, D., Molina J.R., Hanson, L.J., Gore, L., Chow, L., Leong, S., Maloney, L., Gordon, G., Simmons, H., Marlow, A., Litwiler, K., Brown, S., Poch, G., Kane, K., Haney, J., Eckhardt, S.G.,.; *J. Clin. Oncol* **2008**, *26*, 2139.

10. Lorusso P.M., Adjei, A.A., Varterasian, M., Gadgeel, S., Reid, J., Mitchell, D.Y., Hanson, L., DeLuca, P., Bruzek, L., Piens, J., Asbury, P., Van Becelaere, K., Herrera, R., Sebolt-Leopold, J., Meyer, M.B.,.; *J. Clin. Oncol* **2005**, *23*, 5281.

11. Yingchun, L.; Sukumar, S.; Chen, Q.Z.; Keng, Y.F.; Khazak, V.; Illgen, K.; Shabbert, S.; Weber, L.; Menon, S.R.; *Bioorg. Med. Chem. Lett* **2004**; *14;* 3957.

12. Klein, P.J., Schmidt, C.M., Wiesenauer, C.A., Choi, J.N., Gage, E.A., Yip-Schneider, M.T., Wiebke, E.A., Wang, Y., Omer, C., Sebolt-Leopold, J.S.,.*Neoplasia* **2006**, 8, 1.

13. Maruta, H.; Holden, J.; Sizeland, A.; D'Abaco, G.; *J. Biol. Chem* **1991,** *226*, 11661.

14. Młynarczuk-Biały, I.; Roeckmann, H.; Kuckelkorn, U.; Schmidt, B.; Umbreen, S.; Gołab, J.; Ludwig, A.; Montag, C.; Wiebusch, L.; Hagemeier, C.; Schadendorf D.; Kloetzel, P.M.; Seifert, U.; *Cancer Research* **2006**, *66*, 7598.

Tables

Table 1 Optimisation of R^1 and R^2 fragments (Yields)

No.	R^1	R^2	*rac*-4	4→5	5→6[a]	6→7
a	*c*-hex	$CH_3(CH_2)_6$	65%	95%	75%	97%
b	$PhCH_2$	$CH_3(CH_2)_6$	87%	99%	81%	96%
c	$PhCH_2CH_2$	$CH_3(CH_2)_6$	81%	96%	72%	95%
d	4-(MeO)-$PhCH_2$	$CH_3(CH_2)_6$	83%	94%	79%	84%
e	2,4-di-(MeO)-$PhCH_2$	$CH_3(CH_2)_6$	86%	94%	81%	90%
f	4-(MeO)-$PhCH_2$	$PhCH_2$	89%	99%	96%	73%
g	4-(MeO)-$PhCH_2$	4-(MeO)-$PhCH_2$	99%	92%	86%	75%
h	4-(MeO)-$PhCH_2$	$(CH_2)_5NHBoc$	96%	99%	84%	93%
i	4-(MeO)-$PhCH_2$	*c*-Hex	99%	93%	15%	65%
j	4-(MeO)-$PhCH_2$	$(CH_3)_2CHCH_2$	73%	97%	84%	80%
k	4-(MeO)-$PhCH_2$	$(CH_2)_8CH_3$	80%	99%	93%	83%
p	$PhCH_2CH_2$	$(CH_2)_8CH_3$	96%	98%	81%	99%

[a] Summary yield over 3 steps;

Table 2 Biological activity of compounds **7a-e***

Cell line	7a	7b	7c	7d	7e
RAS-3T3	30	33	20	40	40
NIH-3T3	40	38	33	40	50

*** Results are shown as IC50 (μM)**

Table 3 Biological activity of compounds **7f-k***

Cell line	7d	7f	7g	7h	7i	7j	7k
RAS-3T3	40	>100	>100	>100	40	>100	19
NIH-3T3	40	>100	>100	>100	50	>100	55

*** Results are shown as IC50 (μM)**

Table 4 Biological activity of compounds **7m-o***

Cell line	7k	7m	7n	7o
RAS-3T3	19	55	50	68

NIH-3T3	55	150	>200	>200

*** Results are shown as IC50 (μM)**

Figures

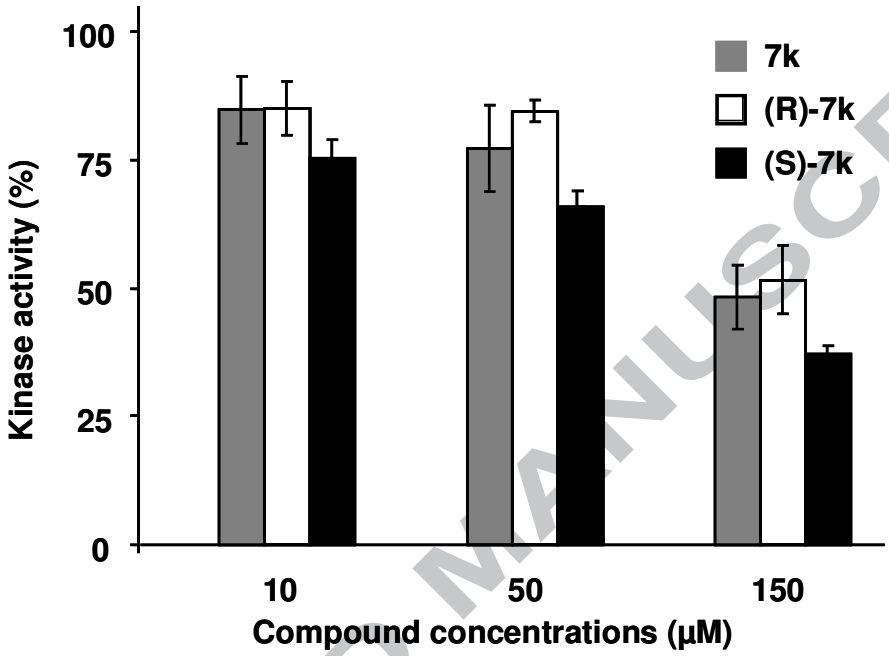


Fig 1. *In vitro* effect of compund 7k racemate and its enantiomers on activity of MEK/ERK1 kinase cascade. Results are shown as percent MEK/ERK1 activity in the absence of the tested agents. Each bar shows mean ± SD from 3 independent experiments.

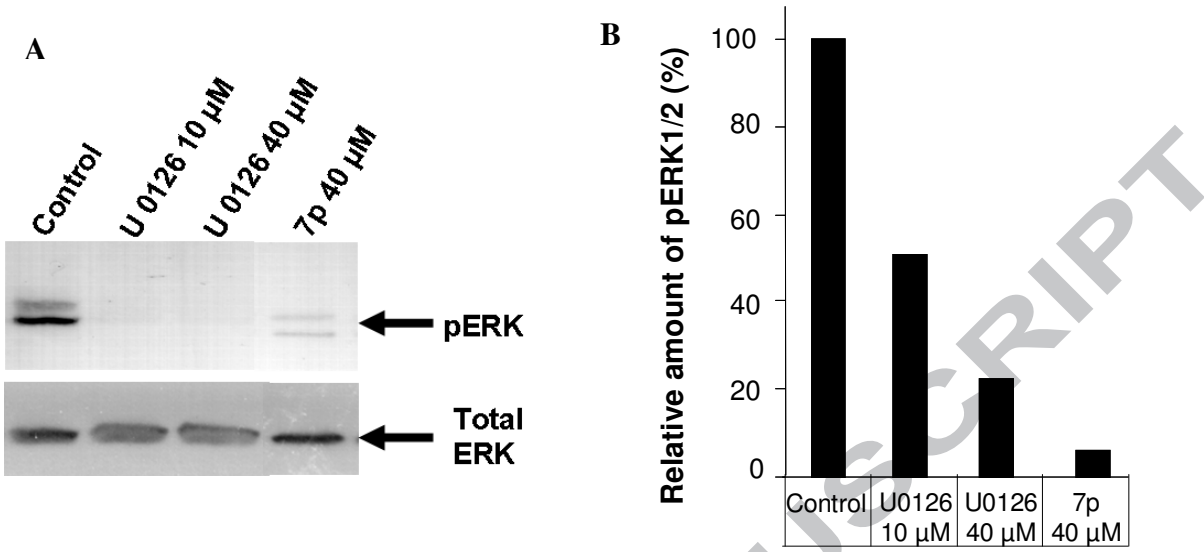


Fig 2.(A) Effect of compound 7p and U0126 on ERK1/2 phosphorylation in RAS-3T3 cells as revealed by Western blot analysis (A) and evaluation by specific ELISA (B). Western blot analysis and ELISA were performed as described in detail in Material and Methods. Untreated RAS-3T3 cells served as controls. Figure show results of a representative experiment.

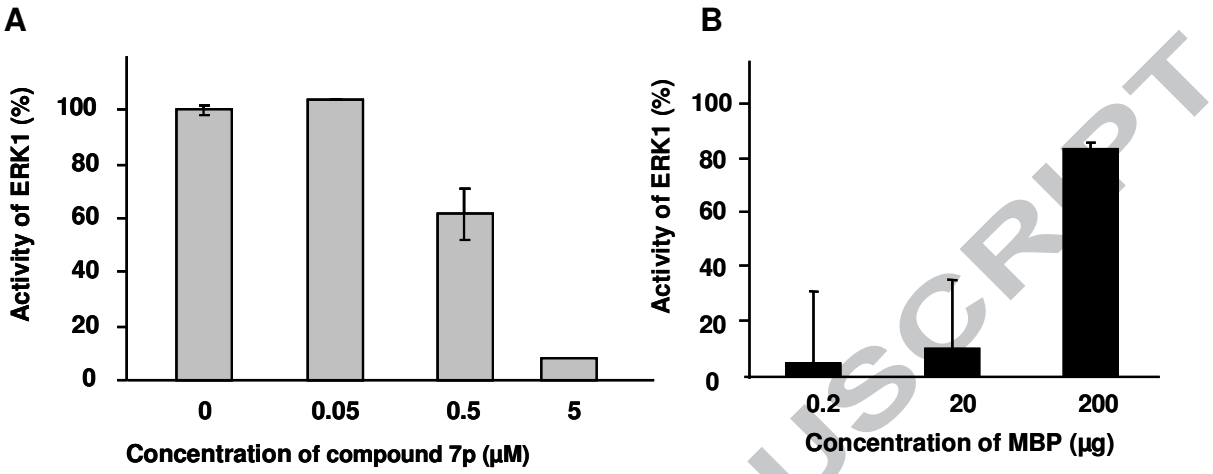


Fig. 3. *In vitro* effect of compound 7p on activity of ERK1. (A) Effect of different doses of compound 7p on ERK1-mediated phosphorylation of BMP. Reactions were performed at the presence of 20 μg of BMP. Samples without 7p served as control. (B) Evaluation of ERK1 activity at 5 μM dose of 7p and different concentrations of BMP. As control served samples without 7p and different corresponding amounts of BMP. Each bar shows mean ± SD from 3 independent experiments.

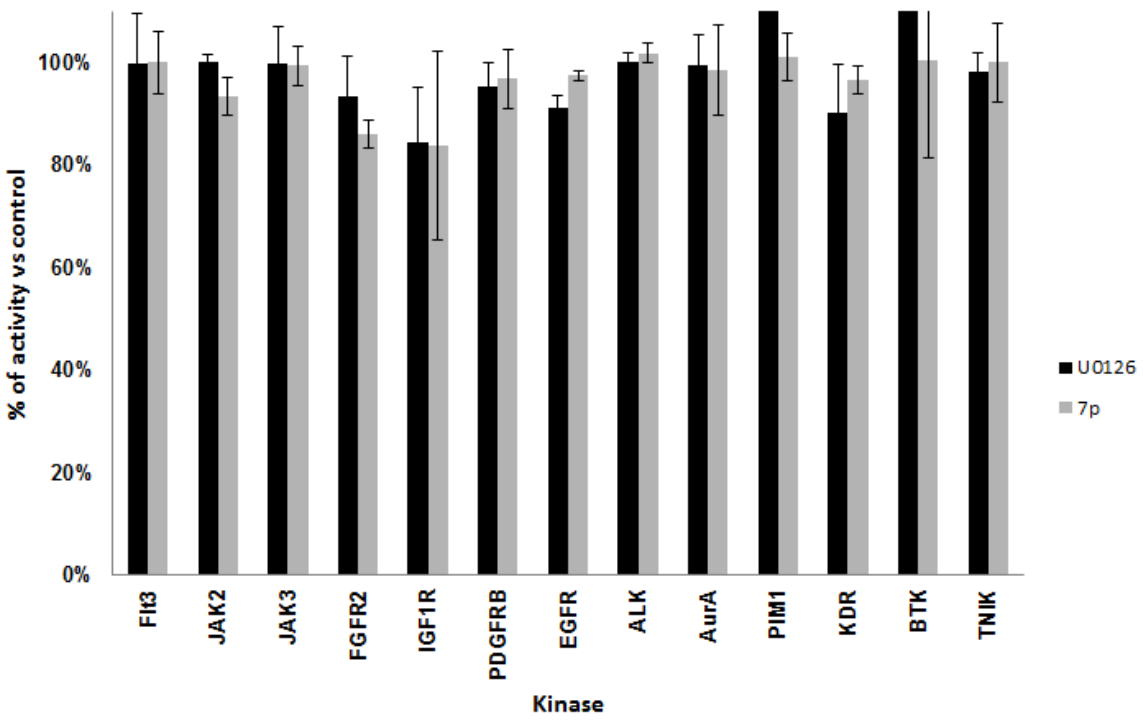
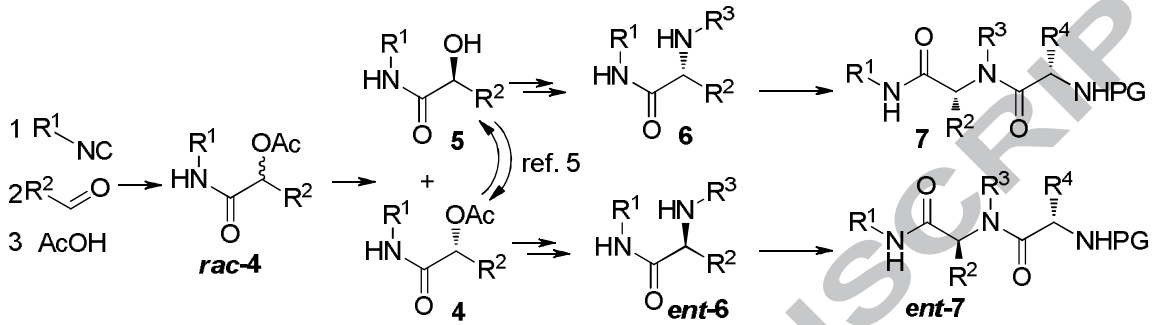


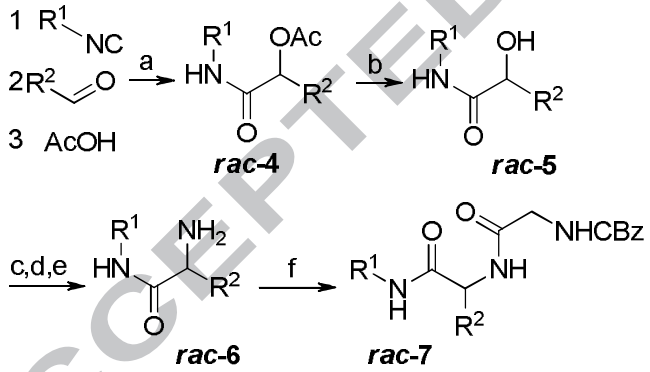
Fig 4: The effect of **7p** and U0126 on activity of various kinases. The % of activity of a kinase was measured versus the activity without an inhibitor. Evaluation of the kinases activities was performed at 10 μM dose of an inhibitor. Bars show mean ± SD from 2 independent experiments

Schemes

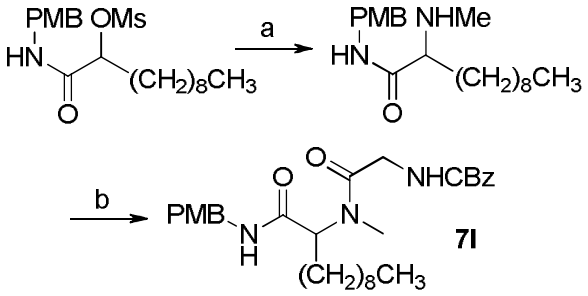
Scheme 1. Synthetic methodology.



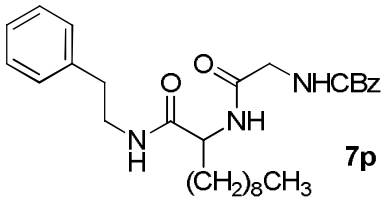
Scheme 2. Optimisation of R^1 and R^2 fragments; a) CH_2Cl_2, rt; b) NaOH, H_2O/MeOH; c) MsCl, Et_3N, DMAP, CH_2Cl_2, 20 °C, 30 min; d) NaN_3, DABCO, DMAP, benzo-15-crown-5, CH_2Cl_2, 40 °C, 24 h; e) H_2, Pd/C, methanol, 4h. f) CBz-glycine, EDC, HOBt, CH_2Cl_2, 20 °C, 20 h.



Scheme 3. Optimisation of R^3; a) MeNH2, H2O/DMF, 50 °C; b) CBz-glycine, EDC, HOBt, CH_2Cl_2, 20 °C, 20 h.



Scheme 4. Optimised structure **7p**.



Scheme 5. Synthesis of enantiomerically enriched compounds 5k; a) PPL, Phosph. Buff. pH 7.0 / Et_2O, 8:2 (v/v); b) NaOH, MeOH, H_2O.