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Original article

The novel phosphoramidate derivatives of NSAID 3-hydroxypropylamides: Synthesis, cytostatic and antiviral activity evaluations

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Abstract

The target phosphoramidates **5a–e** were prepared in one step from 3-hydroxypropyl derivatives **3a–e** of nonsteroidal anti-inflammatory drugs (fenoprofen, ketoprofen, ibuprofen, indomethacin, diclofenac). The products **3a–e** and **5a–e** were evaluated for their cytostatic and antiviral activity against malignant tumour cell lines and normal human fibroblasts (WI 38). All phosphoramidate derivatives **5a–e** possess significantly greater inhibitory activities than the corresponding 3-hydroxypropyl derivatives **3a–e**, whereby compound **5a** showed the most potent inhibitory activities against cervical, pancreatic and colon carcinoma cell lines ($IC_{50} = 5 - 7$ μM).

Keywords: Nonsteroidal anti-inflammatory drugs (NSAID); 3-Hydroxypropylamides; Phosphoramidates; Cytostatic activity

1. Introduction

The phosphoramidate approach has been conceived as a means to improve cellular penetration of antiviral nucleotides and to bypass the first step of kinase-mediated activation of nucleosides [1]. Alkyl and aryloxy phosphoramidate and specially substituted aryl phosphoramidate derivatives of the anti-HIV drugs stavudine and zidovudine demonstrated an elevation of in vitro potency relative to that of the parent nucleosides [2]. Exhaustive modifications to the amino acid moiety in phosphoramidate derivatives established L-alanine as the moiety for optimal antiviral activity. In addition, WHI-05 and WHI-07, bromo-methoxy-substituted aryl phosphate derivatives of zidovudine proved to be potent dual-action contraceptive agents [3,4], while the aryloxy phosphoramidate prodrugs of antineoplastic agents showed a substantial enhancement of potency versus colon and prostate cancer cell lines [5].

In this paper the phosphoramidate strategy has been applied to nonsteroidal anti-inflammatory drugs (NSAID). Numerous experimental, epidemiologic and clinical studies provide evidence that NSAIDs are promising anticancer drugs [6]. Thus, ibuprofen, indomethacin and some other NSAIDs are effective chemopreventive agents against carcinogen-induced and genetically manipulated animal models of colon carcinogenesis [6–9]. NSAIDs may also be associated with reduced risk of cancers of bladder, breast, oesophagus, lung, ovary, prostate, stomach, liver, pancreas, tongue and glioblastoma multiforme [10].

Furthermore, it has been demonstrated that modification of the NSAID by amidation provides COX-2 selective inhibitors [11], while fenoprofen and ketoprofen amidation significantly enhance antiproliferative activity of the parent compounds [12]. On the other hand, diclofenac with bisphosphonic moiety

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is convenient prodrug for bone-specific delivery [13,14]. This led us to synthesize a series of new phosphoramidate derivatives (**5a–e**) of NSAID 3-hydroxypropylamides (fenoprofen, ketoprofen, ibuprofen, indomethacin and diclofenac) (**3a–e**). Here we report their synthesis and evaluation of their cytostatic and antiviral activity potency.

2. Materials and methods

2.1. Synthesis

2.1.1. Materials and general methods

Melting points were determined on a Stuart SMP 3 melting point apparatus (Barloworld Scientific, UK) and were uncorrected. IR spectra were recorded on a FTIR Perkin Elmer Paragon 500 spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer, operating at 300, 75.5 and 243 MHz for the ^{1}H, ^{13}C and ^{31}P nuclei, respectively. Samples were measured in DMSO-d_6 solutions at 20 °C in 5 mm NMR tubes. Chemical shifts (δ) in ppm were referred to TMS. Precoated Merck silica gel 60 F_{254} plates were used for thin-layer chromatography. Solvent systems were cyclohexane/ethyl acetate (1:1), ethyl acetate, dichloromethane/methanol (50:1). Spots were visualized by shortwave UV light and iodine vapour. Column chromatography was performed on silica gel (0.063–0.200 mm), with dichloromethane/methanol (50:1) as eluent.

Ketoprofen and indomethacin were purchased from Belupo (Croatia), fenoprofen from Eli Lilly Company (USA), diclofenac from Pliva (Croatia), benzotriazole and 3-hydroxypropylamine from Merck (Germany), whereas *N*-methylimidazole, L-alanine methyl ester hydrochloride and *p*-chlorophenyl phosphochloridate were purchased from Sigma Aldrich (Germany). All solvents were of analytical grade purity and dry.

2.1.2. Fenoprofen 3-hydroxypropylamide (*3a*) and ketoprofen 3-hydroxypropylamide (*3b*)

Compounds **3a** and **3b** were prepared following the published procedures [15,16].

2.1.3. Ibuprofen 3-hydroxypropylamide (*3c*)

A solution of ibuprofen benzotriazolide **2c** (0.615 g, 2.00 mmol), 3-hydroxypropylamine (0.165 g, 2.20 mol) and triethylamine (0.202 g, 2.00 mmol) in ethyl acetate (10 ml) was stirred for 1 h at room temperature. The reaction mixture was extracted several times with sodium hydroxide solution (pH 8). The organic layer was washed with water, dried over anhydrous sodium sulphate and evaporated under reduced pressure. The obtained crude product was triturated three times with petrol ether. Yield: 0.450 g (85%); oil; IR (film, ν/cm^{-1}) 3299, 3088, 2954, 2932, 2869, 1652, 1548, 1512, 1065, 849; ^{1}H NMR (DMSO-d_6) δ 7.88 (t, 1H, NH), 7.22–7.19 (d, 2H, 5, 9), 7.08–7.06 (d, 2H, 6, 8), 4.39 (t, 1H, OH), 3.57–3.50 (q, 1H, 2), 3.38–3.32 (q, 2H, 3″), 3.13–3.01 (m, 2H, 1″), 2.41–2.39 (d, 2H, 10), 1.87–1.74 (m, 1H, 11), 1.56–1.47 (m, 2H, 2″), 1.31–1.29 (d, 3H, 3), 0.87–0.85 (d, 6H, 12, 13). Anal. ($C_{16}H_{25}NO_2$) C, H, N.

2.1.4. Indomethacin 3-hydroxypropylamide (*3d*)

The synthesis of compound **3d** was previously published but no spectral data were available [17]. Mp 130–131 °C; IR (KBr, ν/cm^{-1}) 3428, 3314, 3093, 2928, 2884, 1673, 1620, 1561, 1478, 1317, 1071, 835, 755; ^{1}H NMR (DMSO-d_6) δ 8.02 (t, 1H, NH), 7.71–7.63 (q, 4H, 13, 14, 16, 17), 7.12–7.11 (s, 1H, 9), 6.96–6.93 (d, 1H, 7), 6.72–6.69 (dd, 1H, 6), 4.43 (t, 1H, OH), 3.77 (s, 3H, 2′), 3.49 (s, 2H, 2), 3.42–3.37 (q, 2H, 3″), 3.15–3.08 (q, 2H, 1″), 2.23 (s, 3H, 1′), 1.60–1.51 (m, 2H, 2″).

2.1.5. Diclofenac 3-hydroxypropylamide (*3e*)

A solution of diclofenac benzotriazolide **2e** (0.795 g, 2.00 mmol), 3-hydroxypropylmine (0.165 g, 2.20 mmol), triethylamine (0.202 g, 2.00 mmol) and sodium dithionite (0.010 g) in ethyl acetate (10 ml) was stirred for 1 h at room temperature. The solvent was evaporated under reduced pressure. The residue was dissolved in acetone/water (1:1) mixture and acidified with HCl to pH 1. Acetone was evaporated under reduced pressure. The precipitated product was filtered off, several times washed with water and triturated with petrol ether. Yield: 0.676 g (96%); mp 130–133 °C; IR (KBr, ν/cm^{-1}) 3495, 34323, 3297, 3253, 3082, 2947, 2882, 1618, 1565, 1508, 1453,1286, 1070, 775, 747; ^{1}H NMR (DMSO-d_6) δ 8.45 (s, 1H, 9), 8.35 (t, 1H, NH), 7.52–7.7.49 (d, 2H, 12, 14), 7.20–7.12 (m, 2H, 4, 6), 7.04 (t, 1H, 5), 6.85 (t, 1H, 13), 6.30–6.28 (d, 1H, 7), 4.45 (t, 1H, OH), 3.57 (s, 1H, 2), 3.45–3.39 (q, 2H, 3″), 3.17–3.10 (q, 2H, 1″), 1.62–1.53 (m, 2H, 2″). Anal. ($C_{17}H_{18}Cl_2N_2O_2$) C, H, N.

2.1.6. p-Chlorophenyl (methoxy-L-alaninyl)phosphochloridate (*4*)

Compound **4** was prepared in accord with a procedure given in the literature [5].

2.1.7. Phosphoramidate derivatives (*5a–e*): general procedure

N-methylimidazole (NMI) (5.00 mmol) was added dropwise with vigorous stirring to a cold solution of NSAID 3-hydroxypropylamide **3** (1.00 mmol) and *p*-chlorophenyl (methoxyalaninyl)phosphochloridate **4** (5.00 – 6.00 mmol) in anhydrous THF (3 – 4 ml) at −70 °C. After 15 min the temperature was left to rise to ambient temperature with stirring over a period of 12 h. The solvent was removed under reduced pressure. The obtained oil was dissolved in dichloromethane and washed with 0.5 M HCl and water. The organic layer was dried over $MgSO_4$, filtered, evaporated to dryness and purified by column chromatography.

2.1.8. Fenoprofen 3-hydroxypropylamide (p-chlorophenyl (methoxy-L-alaninyl)phosphate) (*5a*)

Compound **5a** was prepared according to general procedure using fenoprofen 3-hydroxypropylamide (**3a**) (0.170 g, 0.57 mmol), phosphochloridate **4** (1 g, 3.20 mmol) and *N*-methylimidazole (271 µl, 3.42 mmol). Yield: 0.130 g (40%); oil; ^{31}P NMR (DMSO-d_6) δ 4.65, 4.40; ^{1}H NMR ($CDCl_3$) δ 7.37–6.86 (m, 13H, 5, 7–9, 2′-6′, 5″, 6″, 8″, 9″),

4.05–3.92 (m, 4H, 2, 3″, Ala–CH), 3.71 (s, 3H, Ala–OCH_3), 3.32–3.16 (m, 2H, 1″), 1.83–1.69 (m, 2H, 2″), 1.49 (d, 3H, 3), 1.38 (d, 3H, Ala–CH_3). Anal. ($C_{28}H_{32}ClN_2O_7P$) C, H, N.

2.1.9. Ketoprofen 3-hydroxypropylamide (p-chlorophenyl (methoxy-L-alaninyl)phosphate) (5b)

Compound **5b** was prepared according to general procedure using ketoprofen 3-hydroxypropylamide (**3b**) (0.144 g, 0.46 mmol), phosphochloridate **4** (0.720 g, 2.31 mmol) and *N*-methylimidazole (220 μl, 2.78 mmol). Yield: 0.150 g (55%); oil; ^{31}P NMR (DMSO-d_6) δ 4.64, 4.39; ^1H NMR (DMSO-d_6) δ 8.11 (C═O–NH) 7.73–7.15 (m, 13H, 5, 7–9, 2′-6′, 5″, 6″, 8″, 9″), 4.02–3.91 (m, 3H, 2, 3″), 3.69 (d, 2H, Ala–NH), 3.57 (s, 3H, Ala–OCH_3), 3.14–3.04 (m, 2H, 1″), 1.77–1.65 (m, 2H, 2″), 1.35 (d, 3H, 3), 1.20 (d, 3H, Ala–CH_3). Anal. ($C_{29}H_{32}ClN_2O_7P$) C, H, N.

2.1.10. Ibuprofen 3-hydroxypropylamide (p-chlorophenyl (methoxy-L-alaninyl)phosphate) (5c)

Compound **5c** was prepared according to general procedure using ibuprofen 3-hydroxypropylamide (**3c**) (0.150 g, 0.57 mmol), phosphochloridate **4** (0.900 g, 2.85 mmol) and *N*-methylimidazole (271 μl, 3.42 mmol). Yield: 0.131 g (43%); oil; ^{31}P NMR δ 3.87, 3.63; ^1H NMR δ 7.95 (t, 1H, 1′), 7.43–7.37 (d, 2H, 5, 9), 7.20–7.18 (m, 4H, 8′, 9′, 10′, 11′), 7.07–7.04 (d, 2H, 6, 8), 4.02–3.93 (q, 1H, 2′), 3.86–3.80 (m, 2H, Ala–CH, Ala–NH), 3.59 (t, 2H, 4′), 3.58 (s, 3H, Ala–OCH_3), 3.14–3.03 (m, 2H, 2′), 2.38 (d, 2H, 10), 1.80–1.67 (m, 3H, 11, 3′), 1.29 (d, 3H, 3), 1.22 (d, 3H, Ala–CH_3), 0.85–0.83 (d, 6H, 12, 13). Anal. ($C_{26}H_{36}ClN_2O_6P$) C, H, N.

2.1.11. Indomethacin 3-hydroxypropylamide (p-chlorophenyl (methoxy-L-alaninyl)phosphate) (5d)

Compound **5d** was prepared according to general procedure using indomethacin 3-hydroxypropylamide (**3d**) (0.159 g, 0.38 mmol), phosphorchloridate **4** (0.600 g, 1.92 mmol) and *N*-methylimidazole (183 μl, 2.30 mmol). Yield: 0.112 g (41%); oil; ^{31}P NMR (DMSO-d_6) δ 4.70, 4.46; ^1H NMR (DMSO-d_6): δ 8.07 (t, 1H, 1′), 7.70–7.62 (q, 4H, 13, 14, 16, 17), 7.42–7.39 (d, 2H, 9′, 13′), 7.21–7.18 (d, 2H, 10′, 12′), 7.10 (s, 1H, 9), 6.95–6.93 (d, 1H, 7), 6.72–6.69 (dd., 1H, 6), 4.04–3.96 (m, 2H, Ala–CH, Ala–NH), 3.75 (s, 3H, 2″), 3.57 (s, 3H, Ala–OCH_3), 3.50 (s, 2H, 2), 3.31 (s, 2H, 4′), 3.16–3.11 (q, 2H, 2′), 2.22 (s, 3H, 1″), 1.79–1.71 (m, 2H, 3′), 1.20 (d, 3H, Ala–CH_3). Anal. ($C_{32}H_{34}Cl_2N_3O_8P$) C, H, N.

2.1.12. Diclofenac 3-hydroxypropylamide (p-chlorophenyl (methoxy-L-alaninyl)phosphate) (5e)

Compound **5e** was prepared according to general procedure using diclofenac 3-hydroxypropylamide (**3e**) (0.150 g, 0.43 mmol), phosphochloridate **4** (0.530 g, 1.70 mmol) and *N*-methylimidazole (202 μl, 2.55 mmol). Yield: 0.159 g (60%); oil; ^{31}P NMR (DMSO-d_6) δ 4.64, 4.39; ^1H NMR (DMSO-d_6) δ 8.42 (s, 1H, NH-9), 8.38 (t, 1H, 1′), 7.52–7.49 (d, 2H, 12, 14), 7.45–7.42 (d, 2H, 9, 9′), 7.23–7.13 (m, 4H, 4, 6, 10, 10′), 7.04 (t, 1H, 5) 6.83 (t, 1H, 13), 6.29 (d, 1H, 7), 4.04 (m, 2H, 4′), 3.81 (m, 1H, Ala–CH), 3.58 (m, 5H, 2, Ala–CH, Ala–OCH_3), 3.16 (m, 2H, 2′), 1.78 (m, 2H, 3′), 1.21 (d, 3H, Ala–CH_3). Anal. ($C_{27}H_{29}Cl_3N_3O_6P$) C, H, N.

2.2. Biological tests

2.2.1. Cytostatic activity assays

The cytostatic experiments were carried out on nine human cell lines, eight of which are derived from eight cancer types and one normal, fibroblast cell line. The following cell lines were used: murine leukaemia (L1210), human T-lymphocytes (Molt4/C8, CEM), cervical carcinoma (HeLa), pancreatic carcinoma (MiaPaCa-2), colon carcinoma (SW 620), breast carcinoma (MCF-7), lung carcinoma (H 460) and diploid fibroblasts (WI 38).

Cytostatic activity against L1210, Molt4/C8 and CEM cell lines were measured essentially as originally described [18]. After 48 (L1210) or 72 (CEM, Molt4/C8) hours, the tumour cell number was counted by a Coulter counter.

The cytostatic activity against HeLa, MiaPaCa-2, SW 620, MCF-7, H 460 and WI 38 cell lines was assessed as described previously, according to the slightly modified procedure of the National Cancer Institute, Developmental Therapeutics Program [12,19]. Briefly, the cells were cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. The cells were inoculated onto a series of standard 96-well microtiter plates on day 0, at 1×10^4 to 3×10^4 cells/ml, depending on the doubling times of specific cell line. Test agents were then added in five 10-fold dilutions (10^{-8} to 10^{-4} M) and incubated for a further 72 h. Working dilutions were freshly prepared on the day of testing. The solvent was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in the working concentrations. The cell growth rate was evaluated by performing the MTT assay after 72 h of incubation, which detects mitochondrial dehydrogenase activity in viable cells.

Each test point was performed in quadruplicate in three individual experiments, except for WI 38 cells, whereby only one experiment was performed, and L1210, CEM and Molt4/C8 cells for which two independent experiments were performed. The results were expressed as IC_{50}, a compound concentration necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from dose–response curves using linear regression analysis by fitting the test concentrations that give percentage-of-growth values above and below the reference value (i.e., 50%). If, however, for a given cell line all of the tested concentrations produce PGs exceeding the respective reference level of effect (e.g., PG value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a sign >.

2.2.2. Antiviral activity assays

Antiviral activity against HSV-1, HSV-2, vaccinia virus, vesicular stomatitis virus (VSV) in human embryonic lung

(HEL) cell cultures, Coxsackie virus B4, Sindbis virus, Punta Toro virus, parainfluenza-3 virus and reovirus-1 in Vero cell cultures, and respiratory syncytial virus in HeLa cell cultures was determined. After a 2-h incubation period with 100 $CCID_{50}$ of the respective viruses, residual virus was removed and the infected cells were further incubated with the medium containing different concentrations of the tested compounds. After incubation for 3 days at 37 °C, virus-induced cytopathogenicity was monitored microscopically. Antiviral activity was expressed as the concentration required to reduce virus-induced cytopathogenicity by 50% (EC_{50}).

For the CMV assays, confluent HEL fibroblasts were grown in 96-well microtiter plates and infected with the human cytomegalovirus strains Davis and AD-169 at 100 PFU per well. After a 2-h incubation period, residual virus was removed and the infected cells were further incubated with medium containing different concentrations of the test compounds (in duplicate). After incubation for 7 days at 37 °C, virus-induced cytopathogenicity was monitored microscopically after ethanol fixation and staining with Giemsa. Antiviral activity was expressed as the EC_{50} or compound concentration required to reduce virus-induced cytopathogenicity by 50%.

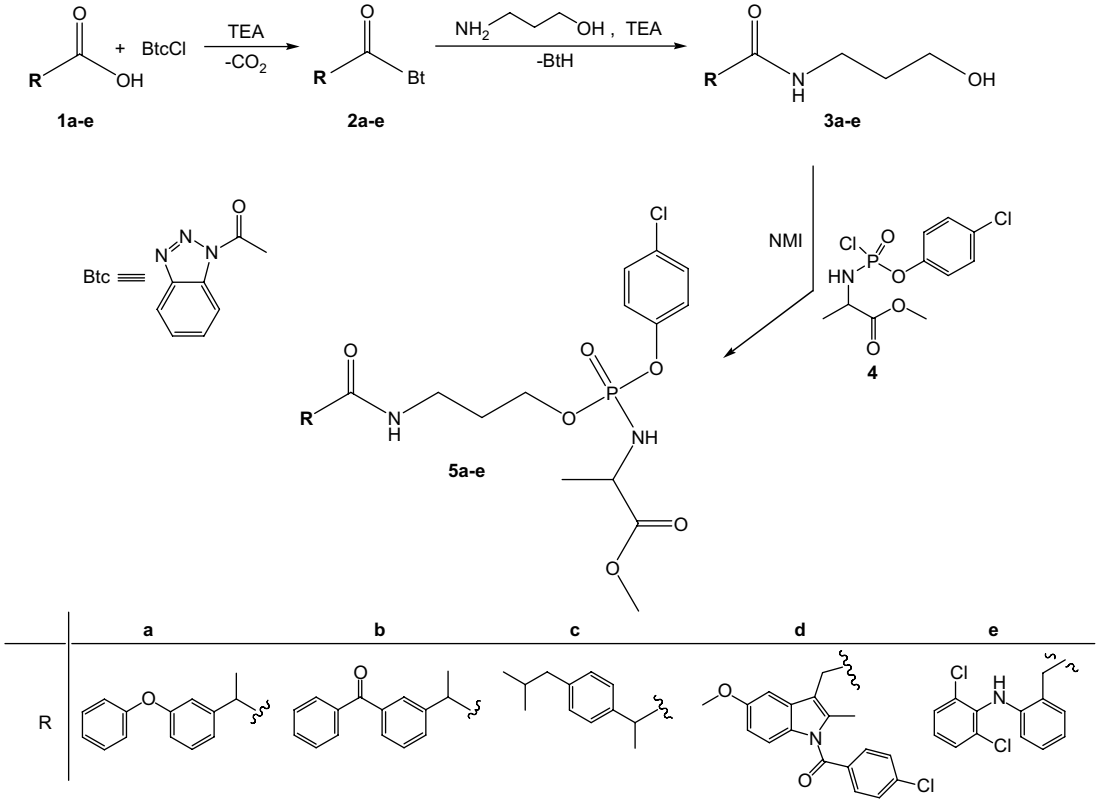
For the VZV assays, the laboratory wild-type VZV strain Oka and the thymidine kinase-deficient VZV strain 07/1 were used. Confluent HEL cells grown in 96-well microtiter plates were inoculated with VZV at an input of 20 PFU per well. After a 2-h incubation period, residual virus was removed and varying concentrations of the test compounds were added (in duplicate). Antiviral activity was expressed as EC_{50}, the compound concentration required to reduce viral plaque formation after 5 days by 50% as compared with untreated controls.

For the HIV-1(III_B) and HIV-2(ROD) assays, virus-induced cytopathicity was recorded in CEM cell cultures as described [18].

3. Results and discussion

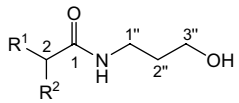
3.1. Chemistry

NSAID amides **3a–e** were synthesized by method developed by us from 3-hydroxypropylamine and NSAID benzotriazolides **2a–e** [15,16]. Compounds **2a–e** were prepared by reaction of the corresponding NSAID drugs **1a–e** (fenoprofen, ketoprofen, ibuprofen, indomethacin, diclofenac) and *N*-1-benzotriazole carboxylic acid chloride (BtcCl). Phosphochloridate **4** was synthesized from *p*-chlorophenyl phosphorodichloridate (CPD) and L-alanine amino acid ester hydrochloride [5]. The target phosphoramidates **5a–e** were prepared in one step from the corresponding NSAID 3-hydroxypropylamides **3a–e** and compound **4** (Scheme 1). The reaction was performed in THF in the presence of *N*-methylimidazole at low temperature, rising to room temperature. Evaporation, extraction and column chromatography on silica gave the desired compounds **5a–e** in 40–60% yields. The products were isolated as roughly 50:50 mixtures of diastereoisomers at the phosphate centre. These isomers do not easily

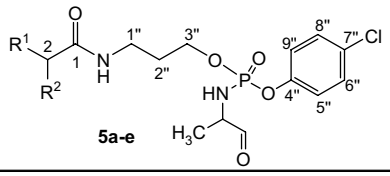


Scheme 1. Synthesis of NSAID 3-hydroxypropylamides **3a–e** and their phosphoramidate derivatives **5a–e**.

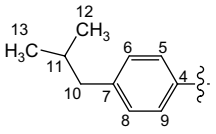
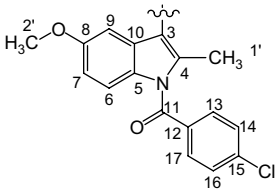
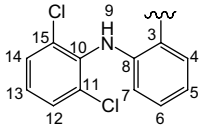
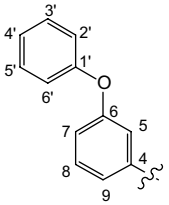
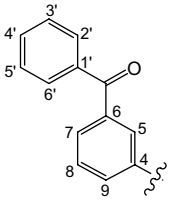
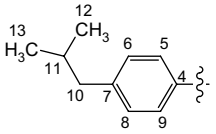
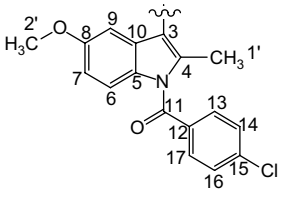
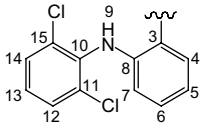
Table 1
^{13}C NMR data and atom enumeration for compounds **3c–e** and **5a–e**



3c-e



5a-e

Compd.	R^1	R^2	^{13}C NMR (DMSO-d_6, δ ppm)[a]
3c	$^3CH_3$		173.52 (1), 139.79 (4), 139.25 (7), 128.85 (5, 9), 127.03 (6, 8), 58.46 (3″), 44.86 (2), 44.37 (10), 35.87 (1″), 32.50 (2″), 29.75 (11), 22.32 (12, 13), 18.74 (3)
3d	H		169.82 (1), 168.31 (11), 156.01 (8), 138.01 (15), 135.56 (4), 134.74 (5), 131.61 (13, 17), 131.35 (12), 130.74 (10), 129.49 (14, 16), 115.02 (9), 114.90 (3), 111.75 (7), 102.25 (6), 58.85 (3″), 55.87 (2′), 36.41 (1″), 32.89 (2″), 31.66 (2), 13.84 (1′)
3e	H		172.03 (1), 143.43 (8), 137.65 (10), 130.82 (4), 129.83 (11, 15), 129.65 (12, 14), 127.63 (6), 126.03 (3), 125.44 (13), 121.11 (7), 116.40 (5), 58.77 (3″), 40.04 (1″), 36.45 (2″), 32.67 (2)
5a	$^3CH_3$		174.15 (C═O 1), 174.02 (C═O Ala), 157.56 (1′), 156.96 (6), 149.27 (4), 143.80 (4″), 143.63 (7″), 130.02 (6′), 129.78 (5″, 9″), 129.67 (8), 123.39 (6″, 8″), 122.34 (4′), 121.54 (9), 118.88 (3′, 5′), 118.13 (5), 117.25 (7), 64.40 (3″), 52.54 (CH_3O–Ala), 50.14 (CH–Ala), 46.94 (2), 35.43 (1″), 29.60 (2″), 20.88 (CH_3–Ala), 18.34 (3)
5b	$^3CH_3$		196.19 (C═O), 174.15 (C═O 1), 174.02 (C═O Ala), 150.13 (1′), 150.03 (6), 143.19 (4), 137.51 (4″), 137.32 (7″), 132.14 (6′), 132.05 (5″, 9″), 130.27 (8), 129.91 (6″, 8″), 129.14 (4′), 128.56 (9), 122.53 (3′, 5′), 122.49 (5), 122.42 (7), 64.57 (3″), 52.27 (CH_3O–Ala), 50.14 (CH–Ala), 45.36 (2), 35.66 (1″), 30.29 (2″), 20.08 (CH_3–Ala), 19.01 (3)
5c	$^3CH_3$		174.08 (C═O Ala), 173.92 (C═O 1), 150.06 (4″), 140.01 (4), 139.62 (7), 129.90 (5″, 9″), 129.20 (5, 9), 128.90 (7″), 127.34 (6, 8), 122.43 (6″, 8″), 64.62 (3″), 52.29 (CH_3O–Ala), 50.15 (CH–Ala), 45.23 (2), 44.70 (10), 35.61 (1″), 30.38 (2″), 30.07 (11), 22.63 (12, 13), 20.12 (CH_3–Ala), 19.06 (3)
5d	H		174.10 (C═O Ala), 169.91 (1), 168.30 (11), 156.02 (8), 150.03 (8′), 138.01 (15), 135.60 (4), 134.71 (5), 131.69 (13, 17), 131.62 (12), 131.34 (10), 129.93 (14, 16), 129.53 (9′, 13′), 128.91 (11′), 122.55 (10′, 12′), 115.04 (9), 114.74 (3), 111.74 (7), 102.21 (6), 64.61 (3″), 55.87 (2′), 52.30 (CH_3O–Ala), 50.13 (CH–Ala), 35.72 (1″), 31.63 (2″), 30.43 (2), 20.05 (CH_3–Ala), 13.82 (1′)
5e	H		174.10 (C═O Ala), 172.13 (1), 150.07 (4″), 143.42 (8), 137.62 (10), 130.82 (4), 129.91 (12, 14), 129.84 (11, 15), 129.65 (5″, 9″), 128.92 (7″), 127.68 (6), 125.90 (3), 125.49 (13), 122.48 (6″, 8″), 121.14 (7), 116.42 (5), 64.53 (3″), 52.31 (CH_3O–Ala), 50.15 (CH–Ala), 40.07 (1″), 35.82 (2″), 30.16 (2), 20.08 (CH_3–Ala)

[a] The spectrum of **5a** was recorded in $CDCl_3$ whereas the spectra of **5b–e** in DMSO-d_6.

Table 2
Inhibitory effects of NSAID 3-hydroxypropylamides **3a–e** (cf. Scheme 1) and their phosphoramidate derivatives **5a–e** on the growth of malignant tumour cell lines in comparison with their effects on the growth of normal human diploid fibroblast (WI 38)

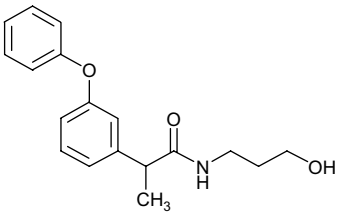
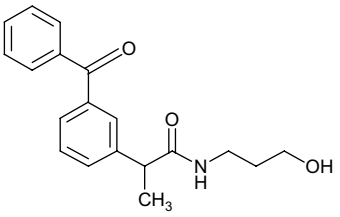
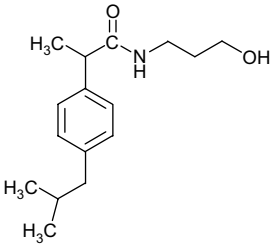
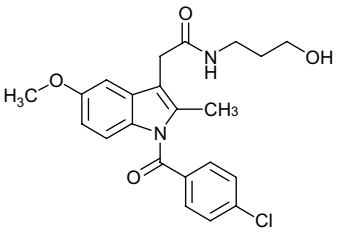
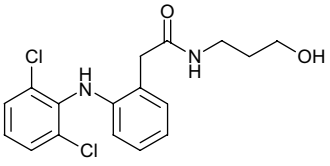
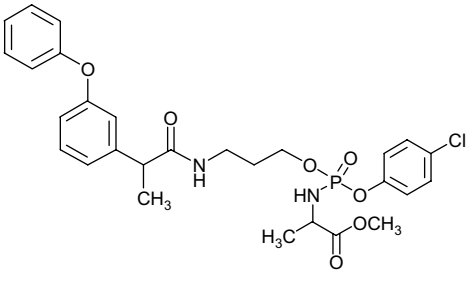
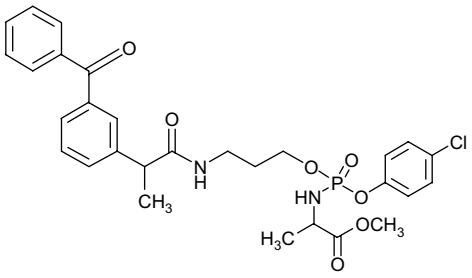
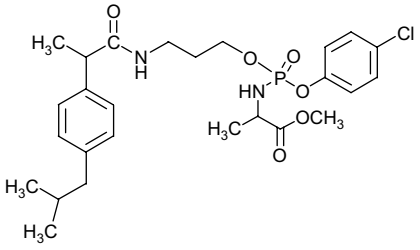
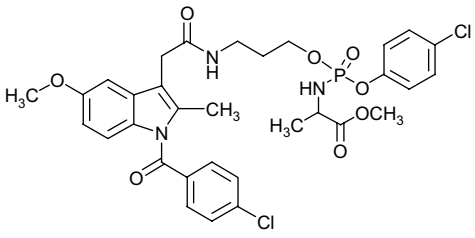
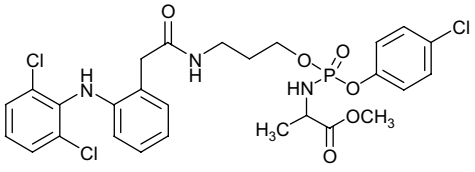
Compd.	Tumour cell growth [IC_{50}[a] (μM)]								
	L1210	Molt4/C8	CEM	HeLa	MIA PaCa-2	SW 620	MCF-7	H 460	WI 38
3a 	230 ± 6	99 ± 18	71 ± 1	>100	>100	>100	>100	>100	N.T.[b]
3b 	273 ± 14	97 ± 18	71 ± 9	>100	>100	>100	>100	>100	N.T.
3c 	213 ± 16	93 ± 7	55 ± 3	>100	≥100	>100	>100	>100	N.T.
3d 	179 ± 93	113 ± 68	78 ± 26	26 ± 16	44 ± 20	47 ± 2	22 ± 14	>100	N.T.
3e 	200 ± 35	215 ± 12	212 ± 23	46 ± 10	54 ± 25	36 ± 3	39 ± 29	68 ± 34	N.T.
5a 	24 ± 17	39 ± 4	38 ± 5	6 ± 5	5 ± 3	7 ± 6	12 ± 2	13 ± 5	20

Table 2 (*continued*)

Compd.	Tumour cell growth [IC_{50}[a] (μM)]								
	L1210	Molt4/C8	CEM	HeLa	MIA PaCa-2	SW 620	MCF-7	H 460	WI 38
5b 	44 ± 7	40 ± 2	40 ± 2	19 ± 2	14 ± 2	17 ± 2	14 ± 3	18 ± 1	30
5c 	64 ± 59	48 ± 7	90 ± 67	15 ± 0.4	13 ± 4	21 ± 7	14 ± 2	19 ± 0.3	21
5d 	24 ± 7	28 ± 16	11 ± 4	17 ± 1	8 ± 7	15 ± 2	16 ± 1	18 ± 0.5	16
5e 	13 ± 8	9.7 ± 0.8	22 ± 17	16 ± 1	16 ± 2	16 ± 0.6	9 ± 7	17 ± 1	18

[a] IC_{50} – the concentration that causes 50% growth inhibition.
[b] NT – not tested.

separate by column chromatography, but were readily distinguished by ^{31}P NMR.

Structures of new compounds were deduced from their ^{1}H, ^{13}C and ^{31}P NMR as well as IR spectra and confirmed by elemental analysis. The spectral data are given in experimental part and in Table 1.

3.2. Biological evaluations

3.2.1. Cytostatic activity

Phosphoroamidate derivatives **5a–e** and NSAID 3-hydroxypropylamides **3a–e** were evaluated in vitro on their cytostatic effects against malignant tumour cell lines: murine leukaemia (L1210) cells, human T-lymphocytes (Molt4/C8 and CEM), cervical carcinoma (HeLa), pancreatic carcinoma (MIA PaCa-2), colon carcinoma (SW 620), breast carcinoma (MCF-7), H 460 (lung carcinoma) and compared with their effects on the growth of human normal fibroblasts (WI 38) (Table 2 and Fig. 1).

The results of the in vitro cytostatic activity evaluations showed that virtually all phosphoramidate derivatives **5a–e** possess significantly greater inhibitory activities (IC_{50} = 5–90 μM) than the corresponding parent NSAID 3-hydroxypropylamides (**3a–e**), whose IC_{50} concentrations mostly exceed 100 μM (Table 2). The increased antiproliferative activity was up to >20-fold depending on the nature of the drug and the tumour cell line evaluated. Among all investigated compounds, compound **5a** showed the most pronounced inhibitory activity against the cervical carcinoma (HeLa), pancreatic carcinoma (MIA PaCa-2) and colon carcinoma (SW 620) cell lines (IC_{50} = 5–7 μM). Additionally, compounds **5a–c** showed the most pronounced antiproliferative effect against the solid tumour cell lines HeLa, MIA PaCa-2, SW 620, MCF-7, and H 460. Instead, the increase of cytostatic

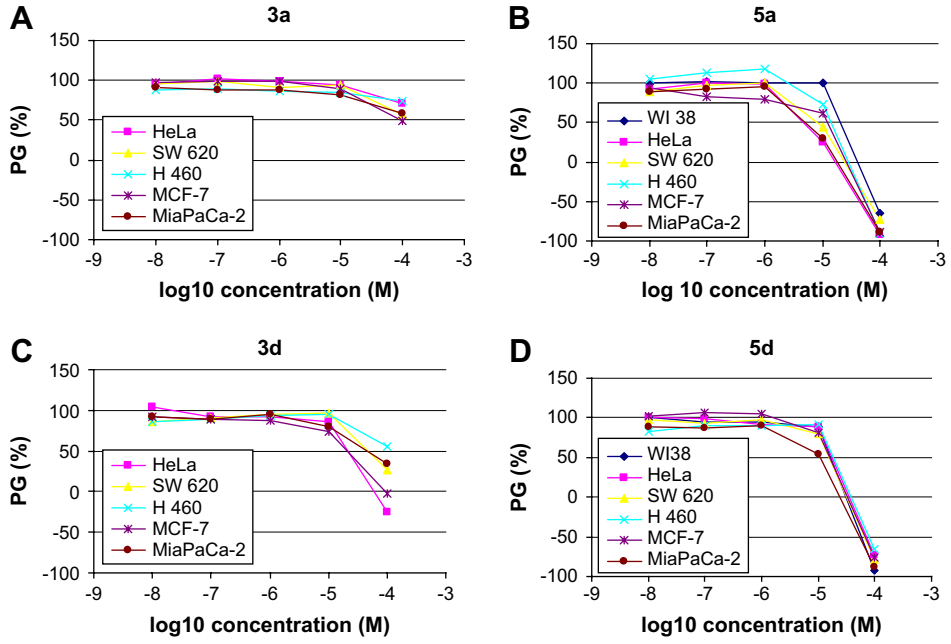


Fig. 1. Dose–response profiles for fenoprofen 3-hydroxypropylamide **3a** (A), indomethacin 3-hydroxypropylamide **3d** (C) and the corresponding phosphoramidate derivatives **5a** (B) and **5d** (D) tested on various human cell lines in vitro. The cells were treated with the compounds at different concentrations, and percentage-of-growth (PG) was calculated.

activity of compounds **5a–c** compared to **3a–e** was much less pronounced against the human T-lymphocyte Molt4/C8 and CEM cells (at most 2-fold, and in case of **5c** even a ~2-fold lower cytostatic activity). It is currently unclear, however, why the lymphocytic suspension cells do behave somewhat different from the solid (monolayer) tumour cells. Different levels of uptake and/or hydrolyzing enzymes in the solid versus suspension tumour cells may be likely. The preferential effect of **5a–c** for solid tumour cells versus suspension tumour cells is not observed for compounds **5d** and **5e**. Still, it should be additionally evaluated whether the more pronounced cytostatic activity of the phosphoramidate derivatives is due to an increased uptake (increased intracellular delivery) of the compounds and/or to the release of the phosphorylated parent compound. It would therefore be interesting to synthesize the phosphorylated derivatives of the compounds for testing against the tumour cell lines.

3.2.2. Antiviral activity

Compounds **3a–e** and **5a–e** were evaluated for their inhibitory activity against human immunodeficiency virus type 1(IIIB) and type 2(ROD) in CEM cell cultures, herpes simplex virus type 1 and 2, vaccinia virus, varicella-zoster virus, cytomegalovirus (CMV) and vesicular stomatitis virus (VZV) in HEL cell cultures; parainfluenza-3 virus, reovirus-1, sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell cultures and respiratory syncytial virus (RSV), VSV and Coxsackie virus B4 in HeLa cell cultures. No specific antiviral effects (i.e. the minimal antiviral effective concentration less than 5-fold lower than the minimal cytotoxic concentration) were noted for any of the compounds against any of the viruses that were evaluated (data not shown), except for **3d** and **5b** that showed some slight inhibitory activity against HCMV and VZV at 30–77 μM.

4. Conclusions

This study is a continuation of our research and synthetic optimisation of novel NSAID derivatives as potential prodrugs for anticancer therapy or chemopreventive applications with less toxic side effects. In this paper we have proved that phosphoramidate derivatives of fenoprofen, ketoprofen, ibuprofen, indomethacine and diclofenac (**5a–e**) possess significantly higher antiproliferative activities than the corresponding NSAID 3-hydroxypropylamides, probably due to a better cell uptake. The most evident increase in the cytostatic activity was demonstrated for fenoprofen phosphoroamidate derivative. Evaluation of NSAID phosphoramidate derivatives as osteotropic drug delivery systems for bone-located inflammatory and malignant diseases is planned.

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