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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Novel derivatives of substituted 6-fluorobenzothiazole diamides: synthesis, antifungal activity and cytotoxicity

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Abstract A new series of 1-[(1*R*)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]-3-substituted phenyl diamides were synthesized in vitro as potential antifungal agents. Chemical structures of the synthesised compounds were substantiated by IR, ^{1}H, ^{13}C, ^{19}F nuclear magnetic resonance spectra, high resolution mass spectrometry, elemental analysis and also by X-ray diffraction. In addition, the cytotoxicity of the most active compounds was investigated against cancer cell line (Jurkat) and one type of normal lung fibroblast cells (MRC-5) by (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) tetrazolium salt reduction assay, propidium iodide flow cytometry assay and xCELLigence system allowing a label-free assessment of the cells proliferation. Compounds indicated as **11e**, **11g**, **11j**, **11n** and **11o**, were the best of the series, showing minimum inhibitory concentration values of 6.25–50 μg/mL against pathogenic strains *Candida albicans* HE 169, *Candida tropicalis* 31/HK and *Candida parapsilosis* p69. Moreover compounds **11e**, **11g**, **11j** and **11o** did not show any cytotoxic effect against human Jurkat and MRC-5 cells.

Keywords Benzothiazole derivatives · Diamide · *Candida* · Antifungal activity · Cytotoxicity

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Introduction

Fungal infections cause a spectrum of diseases in humans. These range in order from relatively innocuous infections of the outer layers of the stratum corneum of the skin to deeply invasive life-threatening infections affecting the brain, heart, liver, lungs, kidneys and spleen. Although systemic infections caused by fungi are rarely serious unless the immune system is weakened, the incidence has increased in recent years. The rise of infections caused by fungi became important because of the AIDS epidemic, ageing of population, increase of number of immunocompromised patients, easily available drugs and excessive treatment of common deceases. Fungal diseases are difficult to treat since fungi are eukaryotes just like us humans and offer few pathogen-specific targets. Moreover, there have been increasing reports of antifungal resistance, which could have negative implications for patient outcomes (Pfaller 2012). Thus, new antifungal agents with enhanced activity and low toxicity are needed.

Generally, benzothiazoles reveal interesting biocide activities against a wide range of bacteria (Bondock et al. 2010; Amnerkar and Bhusari 2011), viruses (Nagarajan et al. 2003), helminths (Sarkar et al. 2008; Amnerkar and Bhusari 2011), fungi (Bujdakova et al. 1993; Bujdakova and Muckova 1994; Mittal et al. 2007; Amnerkar and Bhusari 2011) and last but not least some tumour cell lines (Lion et al. 2006; Sekar et al. 2010). Molecular skeleton of these compounds can serve as a unique and versatile playground for further synthetic modification and thus also for an experimental drug design. The study of structure–activity relationships interestingly reveals that a slight variation of the structure of substituent group at C-2 position commonly results in the significant change of its biological activity (Pejchal et al. 2011a, 2011b; Imramovsky et al. 2013;

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Pejchal et al. 2015; Pejchal et al. 2016). (*R*)-1-(6-fluorobenzothiazol-2-yl)ethanamine is a basic scaffold for antimicrobials (Bondock et al. 2010), herbicides, plant desiccants and defoliant compounds (Menges et al. 1999). Isopropyl [(*S*)-1-[(*R*)-1-(6-fluorobenzothiazole-2-yl)ethylcarbamoyl]-2-methylpropyl] carbamate, also known with common name benthiavalicarb-isopropyl is a commercially used fungicide against the oomycete fungal plant pathogen *Plasmopara viticola* (Reuveni 2003).

In the past, our research group was interested in the synthesis, structural characterisation and microbiological evaluation of a series of 6-fluorobenzothiazole amides, some of which exhibited interesting antifungal properties. In a search for new leads toward potent antimicrobial agents, following our previous work (Pejchal et al. 2015), we synthesised a series of novel substituted 6-fluorobenzothiazole diamides, and have investigated their antifungal activity and cytotoxicity.

Materials and methods

Chemistry

All reagents and solvents were purchased from commercial sources (Sigma-Aldrich, Merck, Acros Organics). Phosgene was purchased from Synthesia a. s. (Pardubice, Czech Republic). Reactions were monitored by thin layer chromatography (TLC) plates coated with 0.2 mm silica gel 60 F_{254} (Merck, Germany). TLC plates were visualised by the ultraviolet (UV) irradiation (254 nm). All the melting points were determined on Melting Point B-545 apparatus (Buchi, Germany) and are uncorrected. Infrared spectra (ZnSe ATR experiments) were recorded on a FT-IR spectrometer (Perkin Elmer, USA) in the range of 600–4000 cm^{-1}. The nuclear magnetic resonance (NMR) spectra were measured in dimethyl sulfoxide-d_6 (DMSO-d_6) solutions at ambient temperature on a Bruker Avance III 400 (400.13 MHz for ^1H, 100.62 MHz for ^{13}C and 376.46 MHz for ^{19}F). Coupling constants are given in Hz. Proton chemical shifts in DMSO-d_6 are related to the middle of the residual multiplet ($\delta =$ 2.50). ^{13}C NMR spectra were measured using APT pulse sequence optimised to $^1J(^{13}C, ^1H) = 145$ Hz. Carbon chemical shifts are referenced to the signal of the solvent ($\delta =$ 39.5 in DMSO-d_6). ^{19}F-NMR spectra were measured using waltz-16 proton decoupling and were standardised against fluorobenzene as the secondary external standard ($\delta = -113.1$) against $CFCl_3$ as the primary standard. Elemental analysis (C, H, N) were performed on an automatic microanalyser CE instruments EA 1110 CHN elemental analyser (Fisons instruments, UK). Mass spectra were measured using high resolution MALDI mass spectrometer LTQ Orbitrap XL (Thermo Fisher Scientific, Germany) via "dried droplet" method. The LTQ Orbitrap instrument equipped with nitrogen UV laser (337 nm, 60 Hz) was operated in positive-ion or negative-ion mode over a normal mass range (m/z 50–2000) with resolution 100,000 at $m/z = 400$. Predefined spiral plate motion patterns were set for the choice of laser shot position. The used matrices were 0.2 M solutions of 2,5-dihydroxybenzoic acid in MeCN:H_2O (95:5) or 2-[(2*E*)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) in MeCN. The matrix:sample molar ratio was approx. 40:1. For all measured samples, the mass spectra were averaged over the whole MS record.

(4R)-4-methyl-1,3-oxazolidine-2,5-dione **1** and (1*R*)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethanamine p-toluenesulfonic salt **2** were synthesised by the reported method (Pejchal et al. 2011a). The structures of the intermediates were confirmed by ^1H, ^{13}C, ^{19}F NMR, melting point and in the case of compound **5** by elemental analysis (C, H, N, S).

General experimental procedure and characterisation of synthesised compounds 2, 4 and 5

*(R)-4-methyloxazolidine-2,5-dione (**2**)*

This compound was obtained by the reaction of D-alanine **1** with phosgene. The mixture of 150 mL dry tetrahydrofuran and 100 mmol finely milled D-alanine was placed under nitrogen into 250 mL three-neck flask. Phosgene (250 mmol) then was bubbled into rapidly stirring reaction mixture. The reaction mixture was stirred at 40–45 °C for 2 h to afford homogeneous solution. The solution was cooled down to 20 °C and purged of excess phosgene by bubbling N_2 through the reaction mixture, and passing the exhaust gases through aqueous sodium hydroxide solution (15%). The solvent was removed in vacuum to afford a crude solid, which was recrystallised from hexan to afford **2** as a white crystalline solid; yield: 83%; m.p. 89–90 °C ^1H NMR (DMSO-d_6, 400.13 MHz,): δ_H 9.01 (s, 1H, N*H*), 4.47 (q, 1H, $^3J_{H-H}$ 7.2 Hz, C*H*), 1.33 (d, 3H, $^3J_{H-H}$ 7,2 Hz, CH_3); ^{13}C NMR (DMSO-d_6, 100.62 MHz,) δ_C 172.5 (COO), 151.8 (CONH), 52.9 (CH), 16.8 (CH_3).

*2-amino-5-fluorobenzenethiol potassium salt (**4**)*

This compound was obtained by reaction of 2-amino-6-fluorobenzothiazole with potassium hydroxide. To the 48% water solution of potassium hydroxide (370 mmol), 70 mmol of 2-amino-6-fluorobenzothiazole was added under nitrogen. The reaction mixture was stirred and refluxed for 5 h to afford a homogeneous solution. Thereafter, the solution was cooled down to 50 °C. Toluene (30 mL) was added to the solution and stirred at 50 °C for 30 min The water layer was separated and used to next step.

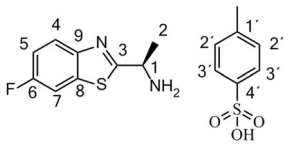


Fig. 1 Atom numbering for assignment of ^{1}H and ^{13}C NMR shifts (compound **5**)

*(1R)-1-(6-Fluoro-1,3-benzothiazol-2-yl)ethanamine 4-toluenesulfonate (**5**)*

This compound was obtained by reaction of (*R*)-4-methyloxazolidine-2,5-dione with 2-amino-6-fluorobenzothiazole (Fig. 1). The mixture of 53 mL water and 39 mL of 36% hydrochloric acid was cooled to 0 °C by stirring. To this was added dropwise at 0 to 5 °C by stirring (70 mmol) an aqueous solution of 2-amino-5-fluorobenzenethiol potassium salt **4**. In the next step, the solution of *(R)*-4-methyloxazolidine-2,5-dione **2** in 35 mL tetrahydrofuran was added at 0–5 °C. The reaction mixture was stirred at 50 °C for 5 h. Subsequently, 50 mL of toluene was added and the reaction mixture was stirred at 45–50 °C for 30 min The aqueous layer was separated and cooled to 20 °C. The 70 mmol of *p*-toluenesulfonic acid was added. The precipitate product was filtrated and washed by 3 × 30 mL of water. It was obtained as white solid; yield: 81%, m.p. 241–242 °C (from hexane). ^{1}H NMR (DMSO-d_6,400.13 MHz,): δ_H 8.74 (s, 2H, NH_2), 8.12 (dd, 1H, $^{4}J_{H–H}$ 2.4 Hz, $^{3}J_{F–H}$ 8.4 Hz, H-7), 8.09 (dd, 1H, $^{3}J_{H–H}$ 9.2 Hz, $^{4}J_{F–H}$ 4.8 Hz, H-4), 7.48 (d, 2H, $^{3}J_{H–H}$ 8.0 Hz, H-2′), 7.37 (dt, 1H, $^{4}J_{H–H}$ 2.4 Hz, $^{3}J_{H–H}$ 9.2 Hz, $^{3}J_{F–H}$ 9.2 Hz, H-5), 7.10 (d, 2H, $^{3}J_{H–H}$ 8.0 Hz, H-3′), 5.01 (quin, 1H, $^{3}J_{H–H}$ 6.8 Hz, H-3), 2.28 (s, 3H, CH_3), 1.66 (d, 3H, $^{3}J_{H–H}$ 6.8 Hz, H-2); ^{13}C NMR (DMSO-d_6,100.62 MHz,): δ_C 169.1 (C, d, $^{4}J_{F–C}$ 3.4 Hz, C-9), 159.9 (C, d, $^{1}J_{F–C}$ 243.5 Hz, C-6), 148.8 (C, C-3), 144.9 (C, C-4′), 138.5 (C, C-1′), 136.4 (C, d, $^{3}J_{F–C}$ 11.9 Hz, C-8), 128.5 (CH, C-2′), 125.7 (CH, C-3′), 124.3 (CH, d, $^{3}J_{F–C}$ 9.6 Hz, C-4), 115.5 (CH, d, $^{2}J_{F–C}$ 24.9 Hz, C-5), 109.0 (CH, d, $^{2}J_{F–C}$ 27.4 Hz, C-7), 48.4 (CH, C-1), 20.9 (CH_3), 19.9 (CH_3, C-2); ^{19}F NMR (DMSO-d_6, 376.46 MHz,): δ_F -115.21. Anal. calcd. for $C_{16}H_{17}FN_2O_3S_2$ (368.44): C, 52.16; H, 4.65; N, 7.60; S,17.41%. Found: C, 52.00; H, 4.82; N, 17.51; S, 17.29%.

General experimental procedure and characterisation of synthesised compounds 11a-11q

Amino acids **6a–q** (8.62 mmol) were dissolved in 10 mL of distilled water and 4.8 g of NaOH (23% aqueous solution) was added. The mixture was stirred for 30 min and during this time cooled to a lower temperature than 10 °C. Substituted benzoyl chloride **7** (8.63 mmol) dissolved in 20 mL of toluene was subsequently added to the prepared solution

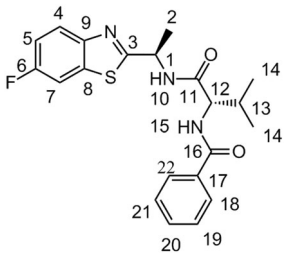


Fig. 2 Atom numbering for assignment of ^{1}H and ^{13}C NMR shifts (compound **11a–j**)

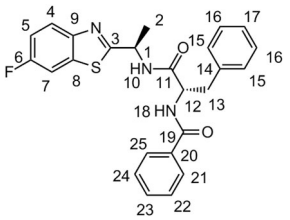


Fig. 3 Atom numbering for assignment of ^{1}H and^{13}C NMR shifts (compound **11k–q**)

of amino acid sodium salt during 15 min The reaction mixture was then stirred for 45 min at 10 °C. After this time, the water layer was separated and pH was adjusted with HCl (approx. 2.4 g of 10% water solution) to 7–8. By described procedures, **8a–q** was formed. Afterwards, toluene (20 mL) and *N*,*N*-dimethylbenzylamine (1.55×10^{-4}mol) were added to the reaction mixture at a temperature lower than 10 °C along with *iso*-butyl chloroformate **9** (8.60 mmol) during 15 min. After warming the mixture to 25 °C, the distilled water (35 mL) was added and the organic layer was separated. The toluene (20 mL) solution of an equivalent of (*R*)-1-(6-fluorobenzo[*d*]thiazol-2-yl)ethaneammonium *p*-toluene sulphonate (PTS) **5** (8.60 mmol) was added to the separated organic layer **10a–q**. Solution of sodium hydroxide was added dropwise to the reaction mixture in order to change the pH to 9–10 (approx. 4.5 g of 10% solution). The reaction mixture was stirred for additional 5 h at room temperature. In order to separate the product, which was formed as a light precipitate, the reaction mixture was heated to 70 °C and the toluene layer containing dissolved product **11a–q** separated (Figs. 2 and 3). The solution was concentrated in vacuo and the residue was cooled down to 0–5 °C, and the precipitate formed was collected by filtration and dried.

*N-[(2S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl] amino}-3-methyl-1-oxobutan-2-yl]benzamide (**11a**)*

White solid; yield 82.0%; m.p. 187–188 °C (from toluene); IR (ν_{max}, cm^{-1}): 3262, 1531 (NH of CONH), 1631 (CO of CONH), 1456 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.00 (1H, d, ^{3}J 7.7 Hz, NH-H-15), 8.34 (1H, d, $^{3}J_{H–H}$ 8.8 Hz, NH-H-10), 7.98 (1H, dd, $^{4}J_{H–H}$ 2.4 Hz, $^{3}J_{F–H}$ 8.8 Hz, H-7),

7.96 (1H, dd, $^{3}J_{H–H}$ 8.9 Hz, $^{4}J_{F–H}$ 5.0 Hz, H-4), 7.90 (2 H, d, $^{3}J_{H–H}$ 7.6 Hz, H-17, H-22), 7.54 (1H, t, $^{3}J_{H–H}$ 7.6 Hz, H-20), 7.47 (2H, t, $^{3}J_{H–H}$ 7.6 Hz, H-19, H-21), 7.36 (1H, dt, $^{4}J_{H–H}$ 2.7 Hz, $^{3}J_{H–H}$ 9.1 Hz, $^{3}J_{F–H}$ 9.1 Hz, H-5), 5.31 (1H, quin, $^{3}J_{H–H}$ 7.2 Hz, H-1), 4.40 (1H, t, $^{3}J_{H–H}$ 8.6 Hz, H-12), 2.17 (1H, m, H-13), 1.58 (3H, d, $^{3}J_{H–H}$ 7.2 Hz, H-2), 0.97 (6H, d,$^{3}J_{H–H}$ 6.7 Hz, H-14); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^{4}J_{F–C}$ 3.2 Hz, C-9), 171.3 (C-11), 166.5 (C-16), 159.5 (d, $^{1}J_{F–C}$ 241.8 Hz, C-6), 149.6 (C-3), 135.9 (d, $^{3}J_{F–C}$ 11.6 Hz, C-8), 134.3 (C-17), 131.3 (C-20), 128.2 (C-19, C-21), 127.6 (C-18, C-22), 123.7 (d, $^{3}J_{F–C}$ 9.6 Hz, C-4), 114.5 (d, $^{2}J_{F–C}$ 24.8 Hz, C-5), 108.6 (d, $^{2}J_{F–C}$ 26.9 Hz, C-7), 59.1 (C-12), 47.3 (C-1), 30.2 (C-13), 20.2 (C-2), 19.4 (C-14), 19.0 (C-14); ^{19}F NMR (376,46 MHz, DMSO-d_6): δ_F −116.5. Anal. calcd. for $C_{21}H_{22}FN_3O_2S$ (399.48): C, 63.14; H, 5.55; N, 10.52; S, 8.03%. Found C, 64.08; H, 5.48; N, 10.60; S, 8.12%. HR-MS: for $C_{21}H_{22}FN_3O_2S$ [M + H$^+$] calcd. 400.14895 *m/z*, found 400.14915 *m/z*.

*2-chloro-N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]-benzamide (**11b**)*

White solid; yield 85.0%, m.p. 223–224 (from toluene); IR (ν_{max}, cm^{-1}): 3255, 1544 (NH of CONH), 1631 (CO of CONH), 1451 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.03 (1H, d, $^{3}J_{H–H}$ 7.9 Hz, NH-H-10), 8.65 (1H, d, $^{3}J_{H–H}$ 9.0 Hz, NH-H-15), 8.00 (1H, dd, $^{4}J_{H–H}$ 2.3 Hz, $^{3}J_{F–H}$ 8.6 Hz, H-7), 7.96 (1H, dd, $^{3}J_{H–H}$ 8.8 Hz, $^{4}J_{F–H}$ 4.7 Hz, H-4), 7.49–7.39 (4H, m, H-19, H-20, H-21, H-22), 7.36 (1H, dt, $^{4}J_{H–H}$ 2.5 Hz, $^{3}J_{H–H}$ 9.0 Hz, $^{3}J_{F–C}$ 9.0 Hz, H-5), 5.32 (1H, quin, $^{3}J_{H–H}$ 7.2 Hz, H-1), 4.38 (1H, t, $^{3}J_{H–H}$ 8.4 Hz, H-12), 2.11 (1H, m, H-13), 1.59 (3H, d, $^{3}J_{H–H}$ 7.1Hz, H-2), 0.98 (6H, d,$^{3}J_{H–H}$ 6.8 Hz, H-14); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^{4}J_{F–C}$ 3.0 Hz, C-9), 170.8 (C-11), 168.4 (C-16), 159.5 (d, $^{1}J_{F–C}$ 242.3 Hz C-6), 149.6 (d, $^{5}J_{F–C}$ 1.5 Hz, C-3), 136.8 (C-18), 135.9 (d, $^{3}J_{F–C}$ 11.7 Hz, C-8), 130.7 (C-17), 129.9 (C-20), 129.5 (C-19), 129.1 (C-22), 127.0 (C-21), 123.7 (d, $^{3}J_{F–C}$ 9.5 Hz, C-4), 114.6 (d, $^{2}J_{F–C}$ 24.7 Hz, C-5), 108.6 (d, $^{2}J_{F–C}$ 27.1 Hz, C-7), 58.9 (C-12), 47.3 (C-1), 30.3 (C-13), 20.1 (C-2), 19.4 (C-14), 18.7 (C-14); ^{19}F NMR (376,46 MHz, DMSO-d_6): δ_F −116.5; Anal. calcd. for $C_{21}H_{21}ClFN_3O_2S$ (433.93): C, 58.13; H, 4.88; N, 9.68; S, 7.39%. Found C, 58.33; H, 5.00; N, 9.46; S, 7.19%. HR-MS: for $C_{21}H_{21}ClFN_3O_2S$ [M + H$^+$] calcd. 434.10998 *m/z*, found 434.11011 *m/z*.

*3-chloro-N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]-benzamide (**11c**)*

White solid; yield 84.0%; m.p. 213–214 °C (from toluene); IR (ν_{max}, cm^{-1}): 3252, 1533 (NH of CONH), 1629 (CO of CONH), 1457 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.02 (1H, d, $^{3}J_{H–H}$ 7.6 Hz, NH-H-10), 8.55 (1H, d, $^{3}J_{H–H}$ 8.6 Hz, NH-H-15), 7.96 (3H, m, H-4, H-7, H-18), 7.87 (1H, d, $^{3}J_{H–H}$ 7.6 Hz, H-20), 7.61 (1H, d, $^{3}J_{H–H}$ 7.6 Hz, H-22), 7.51 (1H, t, $^{3}J_{H–H}$ 7.6 Hz, H-21), 7.36 (1H, dt, $^{4}J_{H–H}$ 2.4 Hz, $^{3}J_{H–H}$ 9.0 Hz, $^{3}J_{F–C}$ 9.0 Hz, H-5), 5.31 (1H, quin, $^{3}J_{H–H}$ 7.2 Hz, H-1), 4.38 (1H, t, $^{3}J_{H–H}$ 8.6 Hz, H-12), 2.17 (1H, m, H-13), 1.58 (3H, d,$^{3}J_{H–H}$ 7.2 Hz, H-2), 0.97 (6H, d, $^{3}J_{H–H}$ 6.5 Hz, H-14); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.4 (d, $^{4}J_{F–C}$ 3.3 Hz, C-9), 171.1 (C-11), 165.2 (C-16), 159.5 (d, $^{1}J_{F–C}$ 242.4 Hz, C-6), 149.6 (C-3), 136.2 (C-17), 135.9 (d, $^{3}J_{F–C}$ 11.8 Hz, C-8), 133.1 (C-19), 131.1 (C-20), 130.2 (C-21), 127.4 (C-22), 126.4 (C-18), 123.7 (d, $^{3}J_{F–C}$ 9.7 Hz, C-4), 114.5 (d, $^{2}J_{F–C}$ 24.8 Hz, C-5), 108.6 (d, $^{2}J_{F–C}$ 27.1 Hz, C-7), 59.3 (C-12), 47.3 (C-1), 30.1 (C-13), 20.1 (C-2), 19.3 (C-14), 19.1 (C-14); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −116.5. Anal. calcd. for $C_{21}H_{21}ClFN_3O_2S$ (433.93): C, 58.13; H, 4.88; N, 9.68; S, 7.39%. Found C, 58.08; H, 4.78; N, 9.79; S, 7.48%. HR-MS: for $C_{21}H_{21}ClFN_3O_2S$ [M + H$^+$] calcd. 434.10998 *m/z*, found 434.11017 *m/z*.

*4-chloro-N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]- benzamide (**11d**)*

White solid; yield 82.0%; m.p. 226–227 °C (from toluene); IR (ν_{max}, cm^{-1}): 3270, 1539 (NH of CONH), 1635 (CO of CONH), 1458 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.03 (1H, d, $^{3}J_{H–H}$ 7.7 Hz, NH-H-10), 8.48 (1H, d, $^{3}J_{H–H}$ 8.6 Hz, NH-H-15), 7.97–7.92 (4H, m, H-4, H-7, H-19, H-21), 7.53 (2H, d, $^{3}J_{H–H}$ 8.6 Hz, H-18, H-22), 7.35 (1H,dt, $^{4}J_{H–H}$ 2.7 Hz, $^{3}J_{H–H}$ 9.2 Hz, $^{3}J_{F–H}$ 9.2 Hz, H-5), 5.31 (1H, quin, $^{3}J_{H–H}$ 7.2 Hz, H-1), 4.39 (1H, t, $^{3}J_{H–H}$ 8.6 Hz, H-12), 2.17 (1H, m, H-13), 1.58 (3H, d,$^{3}J_{H–H}$ 7.1 Hz, H-2), 0.97 (6H, d,$^{3}J_{H–H}$ 6.7 Hz, H-14); ^{13}C NMR (100,62 MHz, DMSO-d_6): δ_C 175.4 (d, $^{4}J_{F–C}$ 3.0 Hz, C-9), 171.2 (C-11), 165.6 (C-16), 159.5 (d, $^{1}J_{F–C}$ 242.0 Hz, C-6), 149.6 (C-3), 136.1 (C-20), 135.9 (d, $^{3}J_{F–C}$ 11.8 Hz, C-8), 133.0 (C-17), 129.6 (C-18, C22), 128.3 (C-19, C-21), 123.7 (d, $^{3}J_{F–C}$ 9.7 Hz, C-4), 114.5 (d, $^{2}J_{F–C}$ 24.9 Hz, C-5), 108.6 (d, $^{2}J_{F–C}$ 27.1 Hz, C-7), 59.2 (C-12), 47.3 (C-1), 30.1 (C-13), 20.1 (C-2), 19.3 (C-14), 19.0 (C-14); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F -116.5. Anal. calcd. for $C_{21}H_{21}ClFN_3O_2S$ (433.93): C, 58.13; H, 4.88; N, 9.68; S, 7.39%. Found C, 58.06; H, 4.75; N, 9.77; S, 7.51%. HR-MS: for $C_{21}H_{21}ClFN_3O_2S$ [M + H$^+$] calcd. 434.10998 *m/z*, found 434.11020 *m/z*.

*3-fluoro-N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]-benzamide (**11e**)*

White solid; yield 85.0%; m.p. 195–196 °C (from toluene); IR (ν_{max}, cm^{-1}): 3278, 1540 (NH of CONH), 1634 (CO of CONH), 1458 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.05 (1H, d, $^{3}J_{H–H}$ 7.8 Hz, NH-H-10), 8.53 (1H, d, $^{3}J_{H–H}$ 8.7 Hz, NH-H-15), 7.96 (2H, m, H-4, H-7), 7.76 (2H, m, H-18, H-22), 7.52 (1H, m, H-21), 7.39 (1H, dt, $^{4}J_{H–H}$ 2.4 Hz,

$^{3}J_{H–H}$ 8.7 Hz, $^{3}J_{F–H}$ 8.7 Hz, H-20), 7.36 (1H, dt, $^{4}J_{H–H}$ 2.7 Hz, $^{3}J_{H–H}$ 8.9 Hz, $^{3}J_{F–H}$ 8.9 Hz, H-5), 5.32 (1H, quin, $^{3}J_{H–H}$ 7.2 Hz, H-1), 4.39 (1H, t, $^{3}J_{H–H}$ 8.7 Hz, H-12), 2.18 (1H, m, H-13), 1.58 (3H, d,$^{3}J_{H–H}$ 7.2 Hz, H-2), 0.97 (6H, d,$^{3}J_{H–H}$ 6.7 Hz, H-14); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^{4}J_{F–C}$ 3.0 Hz, C-9), 171.1 (C-11), 165.2 (d, $^{4}J_{F–C}$ 3.0 Hz, C-16), 161.9 (d, $^{1}J_{F–C}$ 244.1 Hz, C19), 159.5 (d, $^{1}J_{F–C}$ 241.7 Hz, C-6), 149.6 (d, $^{5}J_{F–C}$ 1.4 Hz, C-3), 136.5 (d, $^{3}J_{F–C}$ 6.7 Hz, C-17), 135.9 (d, $^{3}J_{F–C}$ 11.7 Hz, C-8), 130.3 (d, $^{3}J_{F–C}$ 7.9 Hz, C-21), 123.9 (d, $^{4}J_{F–C}$ 2.7 Hz, C-22), 123.7 (d, $^{3}J_{F–C}$ 9.7 Hz, C-4), 118.1 (d, $^{2}J_{F–C}$ 21.1 Hz, C-20), 114.6 (d, $^{2}J_{F–C}$ 24.8 Hz, C-5), 114.5 (d, $^{2}J_{F–C}$ 22.8 Hz, C-18), 108.6 (d, $^{2}J_{F–C}$ 27.2 Hz, C-7), 59.3 (C-12), 47.3 (C-1), 30.1 (C-13), 20.1 (C-2), 19.3 (C-14), 19.1 (C-14); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −113.0, −116.5. Anal. calcd. for $C_{21}H_{21}F_2N_3O_2S$ (417.47): C, 60.42; H, 5.07; N, 10.07; S, 7.68%. Found C, 60.51; H, 5.11; N, 10.15; S, 7.54%. HR-MS: for $C_{21}H_{21}F_2N_3O_2S$ [M + H$^+$] calcd. 418.13953 *m/z*, found 418.13922 *m/z*.

*4-fluoro-N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]-benzamide (**11f**)*

White solid; yield 81.0%; m.p. 220–221 °C (from toluene); IR (ν_{max}, cm^{-1}): 3255, 1542 (NH of CONH), 1635 (CO of CONH), 1458 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.01 (1H, d, $^{3}J_{H–H}$ 7.7 Hz, NH-H-10), 8.40 (1H, d, $^{3}J_{H–H}$ 8.5 Hz, NH-H-15), 7.96 (4H, m, H-4, H-7, H-18, H-22), 7.35 (1H, dt, $^{4}J_{H–H}$ 2.4 Hz, $^{3}J_{H–H}$ 9.1 Hz, $^{3}J_{F–H}$ 9.1 Hz, H-5), 7.29 (2H, m, H-19, H-21), 5.30 (1H, quin, $^{3}J_{H–H}$ 7.2 Hz, H-1), 4.37 (1H, t, $^{3}J_{H–H}$ 8.6 Hz, H-12), 2.15 (1H, m, H-13), 1.56 (3H, d,$^{3}J_{H–H}$ 7.2 Hz, H-2), 0.96 (6H, d,$^{3}J_{H–H}$ 6.5 Hz, H-14); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^{4}J_{F–C}$ 3.2 Hz, C-9), 171.2 (C-11), 165.5 (C-16), 163.9 (d, $^{1}J_{F–C}$ 248.6 Hz, C-20), 159.5 (d, $^{1}J_{F–C}$ 242.2 Hz, C-6), 149.6 (d, $^{5}J_{F–C}$ 1.0 Hz, C-3), 135.9 (d, $^{3}J_{F–C}$ 11.7 Hz, C-8), 130.7 (d, $^{4}J_{F–C}$ 2.8 Hz, C-17), 130.3 (d, $^{3}J_{F–C}$ 8.8 Hz, C-18, C-22), 123.7 (d, $^{3}J_{F–C}$ 9.7 Hz, C-4), 115.1 (d, $^{2}J_{F–C}$ 21.3 Hz, C-19, C-21), 114.6 (d, $^{2}J_{F–C}$ 25.0 Hz, C-5), 108.6 (d, $^{2}J_{F–C}$ 27.3 Hz, C-7), 59.2 (C-12), 47.3 (C-1), 30.1 (C-13), 20.1 (C-2), 19.3 (C-14), 19.0 (C-14); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −109.4, −116.5. Anal. calcd. for $C_{21}H_{21}F_2N_3O_2S$ (417.47): C, 60.42; H, 5.07; N, 10.07; S, 7.68%. Found C, 60.55; H, 5.15; N, 10.00; S, 7.50%. HR-MS: for $C_{21}H_{21}F_2N_3O_2S$ [M + H$^+$] calcd. 418.13953 *m/z*, found 418.13917 *m/z*.

*2-methyl-N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]-benzamide (**11g**)*

White solid; yield 81.0%; m.p. 236–237 °C (from toluene); IR (ν_{max}, cm^{-1}): 3272, 1541 (NH of CONH), 1636 (CO of CONH), 1456 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 8.96 (1H, d, $^{3}J_{H–H}$ 7.6 Hz, NH-H-10), 8.27 (1H, d, $^{3}J_{H–H}$ 8.7 Hz, NH-H-15), 7.99 (1H, dd, $^{4}J_{H–H}$ 2.3 Hz, $^{3}J_{F–H}$ 8.7 Hz, H-7), 7.97 (1H, dd, $^{3}J_{H–H}$ 8.8 Hz, $^{4}J_{F–H}$ 4.6 Hz, H-4), 7.37 (1H, dt, $^{4}J_{H–H}$ 2.7 Hz, $^{3}J_{H–H}$ 9.0 Hz, $^{3}J_{F–H}$ 9.0 Hz, H-5), 7.33–7.21 (4H, m, H-19, H-20, H-21, H-22), 5.31 (1H, quin, $^{3}J_{H–H}$ 7.2 Hz, H-1), 4.34 (1H, t, $^{3}J_{H–H}$ 8.5 Hz, H-12), 2.32 (3H, s, CH_3), 2.09 (1H, m, H-13), 1.59 (3H, d,$^{3}J_{H–H}$ 7.2 Hz, H-2), 0.97 (6H, d,$^{3}J_{H–H}$ 6.2 Hz, H-14); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.6 (d, $^{4}J_{F–C}$ 3.1 Hz, C-9), 171.2 (C-11), 169.2 (C-16), 159.6 (d, $^{1}J_{F–C}$ 241.8 Hz, C-6), 149.6 (d, $^{5}J_{F–C}$ 1.5 Hz, C-3), 137.1 (C-17), 135.8 (d, $^{3}J_{F–C}$ 11.5 Hz, C-8), 135.1 (C-18), 130.3 (C-20), 129.2 (C-19), 127.2 (C-21), 125.4 (C-22), 123.7 (d, $^{3}J_{F–C}$ 9.8 Hz, C-4), 114.5 (d, $^{2}J_{F–C}$ 25.0 Hz, C-5), 108.5 (d, $^{2}J_{F–C}$ 27.3 Hz, C-7), 58.8 (C-12), 47.3 (C-1), 30.0 (C-13), 20.1 (C-2), 19.4 (C-14), 19.3 (C-14), 18.9 (CH_3); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −116.5; anal. calcd. for $C_{22}H_{24}FN_3O_2S$ (413.51): C, 63.90; H, 5.85; N, 10.16; S, 7.75%. Found C, 64.08; H, 5.78; N, 10.30; S, 7.62%. HR-MS: for $C_{22}H_{24}FN_3O_2S$ [M + H$^+$] calcd. 414.16460 *m/z*, found 414.16477 *m/z*.

*4-methyl-N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]-benzamide (**11h**)*

White solid; yield 78.0%; m.p. 191–192 °C (from toluene); IR (ν_{max}, cm^{-1}): 3266, 1535 (NH of CONH), 1630 (CO of CONH), 1456 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 8.99 (1H, d, $^{3}J_{H–H}$ 7.7 Hz, NH-H-10), 8.23 (1H, d, $^{3}J_{H–H}$ 8.8 Hz, NH-H-15), 7.96 (2H, m, H-4, H-7), 7.80 (2H, d, $^{3}J_{H–H}$ 8.1 Hz, H-18, H-22), 7.35 (1H, dt, $^{4}J_{H–H}$ 2.6 Hz, $^{3}J_{H–H}$ 9.0 Hz, $^{3}J_{F–H}$ 9.0 Hz, H-5), 7.26 (2H, d, $^{3}J_{H–H}$ 8.1 Hz, H-19, H-21), 5.29 (1H, quin, $^{3}J_{H–H}$ 7.2 Hz, H-1), 4.37 (1H, t, $^{3}J_{H–H}$ 8.5 Hz, H-12), 2.35 (3H, s, CH_3), 2.15 (1H, m, H-13), 1.56 (3H, d,$^{3}J_{H–H}$ 7.2 Hz, H-2), 0.95 (3H, d,$^{3}J_{H–H}$ 6.6 Hz, H-14), 0.94 (3H, d,$^{3}J_{H–H}$ 6.6 Hz, H-14); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^{4}J_{F–C}$ 3.2 Hz, C-9), 171.3 (C-11), 169.3 (C-16), 159.6 (d, $^{1}J_{F–C}$ 242.0 Hz, C-6), 149.6 (d, $^{5}J_{F–C}$ 1.4 Hz, C-3), 141.2 (C-20), 135.8 (d, $^{3}J_{F–C}$ 11.3 Hz, C-8), 131.4 (C-17), 128.7 (C-19, C-21), 127.6 (C-18, C-22), 123.7 (d, $^{3}J_{F–C}$ 9.5 Hz, C-4), 114.6 (d, $^{2}J_{F–C}$ 24.7 Hz, C-5), 108.6 (d, $^{2}J_{F–C}$ 26.8 Hz, C-7), 59.0 (C-12), 47.3 (C-1), 30.2 (C-13), 21.0 (CH_3), 20.2 (C-2), 19.4 (C-14), 19.0 (C-14); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F -116.5; anal. calcd. for $C_{22}H_{24}FN_3O_2S$ (413.51): C, 63.90; H, 5.85; N, 10.16; S, 7.75%. Found C, 63.78; H, 5.76; N, 10.30; S, 7.88%. HR-MS: for $C_{22}H_{24}FN_3O_2S$ [M + H$^+$] calcd. 414.16460 *m/z*, found 414.16488 *m/z*.

*4-nitro-N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]-benzamide (**11i**)*

White solid; yield 84.0%; m.p. 229–230 °C (from toluene); IR (ν_{max}, cm^{-1}): 3273, 1543 (NH of CONH), 1635 (CO of

CONH), 1454 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.08 (1H, d, $^{3}J_{H-H}$ 7.8 Hz, NH-H-10), 8.79 (1H, d, $^{3}J_{H-H}$ 8.6 Hz, NH-H-15), 8.31 (1H, d, $^{3}J_{H-H}$ 8.7 Hz, H-19, H-21), 8.12 (2H, d, $^{3}J_{H-H}$ 9.0 Hz, H-18, H-22), 7.98 (1H, dd, $^{4}J_{H-H}$ 2.7 Hz, $^{3}J_{F-H}$ 9.1 Hz, H-7), 7.96 (1H, dd, $^{3}J_{H-H}$ 9.0 Hz, $^{4}J_{F-H}$ 5.0 Hz, H-4), 7.35 (1H, dt, $^{4}J_{H-H}$ 2.7 Hz, $^{3}J_{H-H}$ 9.1 Hz, $^{3}J_{F-H}$ 9.1 Hz, H-5), 5.31 (1H, quin, $^{3}J_{H-H}$ 7.2 Hz, H-1), 4.40 (1H, t, $^{3}J_{H-H}$ 8.5 Hz, H-12), 2.17 (1H, m, H-13), 1.57 (3H, d,$^{3}J_{H-H}$ 7.2 Hz, H-2), 0.97 (6H, d,$^{3}J_{H-H}$ 6.7 Hz, H-14); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.4 (d, $^{4}J_{F-C}$ 3.4 Hz, C-9), 170.9 (C-11), 165.0 (C-16), 159.6 (d, $^{1}J_{F-C}$ 242.2 Hz, C-6), 149.6 (C-3), 149.0 (C-20), 139.9 (C-17), 135.9 (d, $^{3}J_{F-C}$ 11.2 Hz, C-8), 129.2 (C-19, C-21), 123.7 (d, $^{3}J_{F-C}$ 9.3 Hz, C-4), 123.4 (C-18, C-22), 114.6 (d, $^{2}J_{F-C}$ 24.4 Hz, C-5), 108.6 (d, $^{2}J_{F-C}$ 26.9 Hz, C-7), 59.4 (C-12), 47.3 (C-1), 30.0 (C-13), 20.1 (C-2), 19.3 (C-14), 19.0 (C-14); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −116.5; anal. calcd. for $C_{21}H_{21}FN_4O_4S$ (444.48): C, 56.75; H, 4.76; N, 12.61; S, 7.21%. Found C, 56.88; H, 4.67; N, 12.50; S, 7.12%. HR-MS: for $C_{21}H_{21}FN_4O_4S$ [M + H$^+$] calcd. 445.13403 *m/z*, found 445.13427 *m/z*.

*4-chloro-3-nitro-N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylpropyl]-benzamide (**11j**)*

White solid; yield 83.0%; m.p. 247–248 °C (from toluene); IR (ν_{max}, cm^{-1}): 3247, 1535 (NH of CONH), 1641 (CO of CONH), 1458 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.07 (1H, d, $^{3}J_{H-H}$ 7.6 Hz, NH-H-10), 8.82 (1H, d, $^{3}J_{H-H}$ 8.4 Hz, NH-H-15), 8.59 (1H, d, $^{4}J_{H-H}$ 1.9 Hz, H-18), 8.20 (1H, dd, $^{4}J_{H-H}$ 1.9 Hz, $^{3}J_{H-H}$ 8.3 Hz, H-22), 7.95 (2H, m H-4, H-7), 7.89 (1H, d, $^{3}J_{H-H}$ 8.5 Hz, H-21), 7.35 (1H, dt, $^{4}J_{H-H}$ 2.7 Hz, $^{3}J_{H-H}$ 9.2 Hz, $^{3}J_{F-H}$ 9.2 Hz, H-5), 5.30 (1H, quin, $^{3}J_{H-H}$ 7.2 Hz, H-1), 4.39 (1H, t, $^{3}J_{H-H}$ 8.5 Hz, H-12), 2.16 (1H, m, H-13), 1.56 (3H, d,$^{3}J_{H-H}$ 7.2 Hz, H-2), 0.97 (3H, d,$^{3}J_{H-H}$ 6.4 Hz, H-14), 0.96 (3H, d,$^{3}J_{H-H}$ 6.4 Hz, H-14); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.4 (d, $^{4}J_{F-C}$ 3.3 Hz, C-9), 170.8 (C-11), 163.7 (C-16), 159.6 (d, $^{1}J_{F-C}$ 242.2 Hz, C-6), 149.6 (C-3), 147.3 (C-19), 135.8 (d, $^{3}J_{F-C}$ 11.8 Hz, C-8), 134.1 (C-17), 132.9 (C-22), 131.8 (C-21), 127.9 (C-20), 124.8 (C-18), 123.7 (d, $^{3}J_{F-C}$ 9.6 Hz, C-4), 114.6 (d, $^{2}J_{F-C}$ 25.2 Hz, C-5), 108.6 (d, $^{2}J_{F-C}$ 27.1 Hz, C-7), 59.4 (C-12), 47.3 (C-1), 30.1 (C-13), 20.1 (C-2), 19.3 (C-14), 19.0 (C-14); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F -116.5; anal. calcd. for $C_{21}H_{20}ClFN_4O_4S$ (478.92): C, 52.66; H, 4.21; N, 11.70; S, 6.70%. Found C, 52.78; H, 4.17; N, 11.56; S, 6.52%. HR-MS: for $C_{21}H_{20}ClFN_4O_4S$ [M + H$^+$] calcd. 479.09506 *m/z*, found 479.09490 *m/z*.

*N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylphenyl]benzamide (**11k**)*

White solid; yield 82.0%; m.p. 180–181 °C (from toluene; IR (ν_{max}, cm^{-1}): 3274, 1523 (NH of CONH), 1628 (CO of CONH), 1455 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H9.06 (1H, d, $^{3}J_{H-H}$ 7.4 Hz, NH-H-10), 8.66 (1H, d, $^{3}J_{H-H}$ 8.4 Hz, NH-H-18), 7.98 (2H, m, H-4, H-7), 7.82 (2H, d, $^{3}J_{H-H}$ 7.2 Hz, H-21, H-25), 7.52–7.38 (5H, m, H-15, H-22, H-23, H-24), 7.36 (1H,dt,$^{4}J_{H-H}$ 2.8 Hz, $^{3}J_{H-H}$ 9.2 Hz, $^{3}J_{F-H}$ 9.2 Hz, H-5), 7.28 (2H, t, $^{3}J_{H-H}$ 7.7 Hz, H-15), 7.18 (1H, t, $^{3}J_{H-H}$ 7.3 Hz, H-17), 5.26 (1H, quin, $^{3}J_{H-H}$ 7.2 Hz, H-1), 4.82 (1H, m, H-12), 3.09 (2H, m, H-13), 1.54 (3H, d,$^{3}J_{H-H}$ 7.2 Hz, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.8 (d, $^{4}J_{F-C}$ 3.2 Hz, C-9), 171.8 (C-11), 166.5 (C-19), 159.5 (d, $^{1}J_{F-C}$ 241.9 Hz, C-6), 149.6 (d, $^{5}J_{F-C}$ 1.9 Hz, C-3), 138.4 (C14), 136.2, (d, $^{3}J_{F-C}$ 11.7 Hz, C-8), 134.3 (C-20), 131.6 (C-23), 129.5 (C-22, C-24), 128.5 (C-16), 128.4 (C-15), 128.2 (C-21, C-25), 126.6 (C-17), 123.9 (d, $^{3}J_{F-C}$ 9.8 Hz C-4), 114.8 (d, $^{2}J_{F-C}$ 24.9 Hz, C-5), 108.9 (d, ^{2}J(^{19}F, ^{13}C) 27.4 Hz, C-7), 55.2 (C-12), 47.8 (C-1), 37.6 (C-13), 20.3 (C-2); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F -116.5; anal. calcd. for $C_{25}H_{22}FN_3O_2S$ (447.52): C, 67.10; H, 4.95; N, 9.39; S, 7.16%. Found C, 67.28; H, 4.88; N, 9.53; S, 7.07%. HR-MS: for $C_{25}H_{22}FN_3O_2S$ [M + H$^+$] calcd. 448.14895 *m/z*, found 448.14880 *m/z*.

*N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]carbamoyl}-2-methylphenyl]-3-chloro-benzamide (**11l**)*

White solid; yield 87.0%; m.p. 210–211 °C (from toluene); IR (ν_{max}, cm^{-1}): 3288, 1532 (NH of CONH), 1636 (CO of CONH), 1455 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.07 (1H, d, $^{3}J_{H-H}$ 7.6 Hz, NH-H-10), 8.82 (1H, d, $^{3}J_{H-H}$ 8.3 Hz, NH-H-18), 7.97 (2H, m, H-4, H-7), 7.88 (1H, t, $^{4}J_{H-H}$ 1.8 Hz, H-21), 7.77 (1H, d, $^{3}J_{H-H}$ 7.8 Hz, H-25), 7.58 (1H, d, $^{3}J_{H-H}$ 7.8 Hz, H-23), 7.47 (1H, t, $^{3}J_{H-H}$ 7.8 Hz, H-22), 7.38 (2H, d, $^{3}J_{H-H}$ 8.8 Hz, H-15), 7.36 (1H, dt,$^{4}J_{H-H}$ 2.7 Hz, $^{3}J_{H-H}$ 9.1 Hz, $^{3}J_{F-H}$ 9.1 Hz, H-5), 7.27 (2H, t, $^{3}J_{H-H}$ 7.5 Hz, H-16), 7.18 (1H, t, $^{3}J_{H-H}$ 7.3 Hz, H-17), 5.26 (1H, quin, $^{3}J_{H-H}$ 7.2 Hz, H-1), 4.80 (1H, m, H-12), 3.10 (1H, dd, $^{2}J_{H-H}$ 10.5 Hz, $^{3}J_{H-H}$ 5.0 Hz, H-13), 3.09 (1H, dd, $^{2}J_{H-H}$ 10.5 Hz, $^{3}J_{H-H}$ 4.8 Hz, H-13), 1.52 (3H, d,$^{3}J_{H-H}$ 7.2 Hz, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^{4}J_{F-C}$ 3.2 Hz, C-9), 171.3 (C-11), 164.8 (C-19), 159.5 (d, $^{1}J_{F-C}$ 241.7 Hz, C-6), 149.6 (d, $^{5}J_{F-C}$ 1.5 Hz, C-3), 138.1 (C-14), 136.0 (C-20), 135.9, (d, $^{3}J_{F-C}$ 11,7 Hz, C-8), 133.1 (C-22), 131.2 (C-23), 130.3 (C-24), 129.2 (C-25), 128.1 (C-16), 127.3 (C-15), 126.4 (C-21), 126.3 (C-17), 123.6 (d, $^{3}J_{F-C}$ 9.8 Hz, C-4), 114.6 (d, $^{2}J_{F-C}$ 24.4 Hz, C-5), 108.6 (d, ^{2}J (^{19}F, ^{13}C) 26.9 Hz, C-7), 55.0 (C-12), 47.5 (C-1), 37.3 (C-13), 20.0 (C-2); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F

−116.5; anal. calcd. for $C_{25}H_{21}ClFN_3O_2S$ (481.97): C, 62.30; H, 4.39; N, 8.72; S, 6.65%. Found C, 62.38; H, 4.48; N, 8.63; S, 6.57%. HR-MS: for $C_{25}H_{21}ClFN_3O_2S$ [M + H^+] calcd. 482.10998 *m/z*, found 482.11019 *m/z*.

*N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl] carbamoyl}-2-methylphenyl]-4-chloro-benzamide (**11m**)*

White solid; yield 82.0%; m.p. 211–212 °C (from toluene); IR (ν_{max}, cm^{-1}): 3284, 1535 (NH of CONH), 1632 (CO of CONH), 1460 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.07 (1H, d, $^3J_{H–H}$ 7.5 Hz, NH-H-10), 8.77 (1H, d, $^3J_{H–H}$ 8.4 Hz, NH-H-18), 7.99 (1H, dd, $^4J_{H–H}$ 2.6 Hz, $^3J_{F–H}$ 9.0 Hz, H-7), 7.96 (1H, dd, $^3J_{H–H}$ 9.0 Hz, $^4J_{F–H}$ 4.8 Hz, H-4), 7.84 (2H, d, $^3J_{H–H}$ 8.6 Hz, H-21, H-25), 7.52 (2H, d, $^3J_{H–H}$ 8.6 Hz, H-22, H-24), 7.38 (2H, d, $^3J_{H–H}$ 7.3 Hz, H-15), 7.36 (1H, dt, $^4J_{H–H}$ 2.7 Hz, $^3J_{H–H}$ 9.2 Hz, $^3J_{F–H}$ 9.2 Hz, H-5), 7.27 (2H, t, $^3J_{H–H}$ 7.3 Hz, H-16), 7.18 (1H, t, $^3J_{H–H}$ 7.3 Hz, H-17), 5.25 (1H, quin, $^3J_{H–H}$ 7.2 Hz, H-1), 4.80 (1H, m, H-12), 3.09 (2H, m, H-13), 1.52 (3H, d,$^3J_{H–H}$ 7.2 Hz, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^4J_{F–C}$ 3.2 Hz, C-9), 171.4 (C-11), 166.2 (C-19), 159.5 (d, $^1J_{F–C}$ 241.9 Hz, C-6), 149.6 (d, $^5J_{F–C}$ 1.4 Hz, C-3), 138.1 (C-23), 136.2 (C-14), 135.9 (d, $^3J_{F–C}$ 11,4 Hz, C-8), 132.7 (C-20, C-25), 129.4 (C-22, C-24), 129.2 (C-16), 128.3 (C-15), 126.4 (C-17), 123.7 (d, $^3J_{F–C}$ 9.6 Hz, C-4), 114.5 (d, $^2J_{F–C}$ 25.1 Hz, C-5), 108.6 (d, $^2J_{F–C}$ 26.9 Hz, C-7), 55.0 (C-12), 47.5 (C-1), 37.3 (C-13), 20.0 (C-2); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −116.5; anal. calcd. for $C_{25}H_{21}ClFN_3O_2S$ (481.97): C, 62.30; H, 4.39; N, 8.72; S, 6.65%. Found C, 62.18; H, 4.48; N, 8,63; S, 6.77%. HR-MS: for $C_{25}H_{21}ClFN_3O_2S$ [M + H^+] calcd. 482.10998 *m/z*, found 482.11025 *m/z*.

*N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl] carbamoyl}-2-methylphenyl]-3-fluoro-benzamide (**11n**)*

White solid; yield 84.0%; m.p. 166–167 °C (from toluene); IR (ν_{max}, cm^{-1}): 3285, 1533 (NH of CONH), 1636 (CO of CONH), 1456 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.09 (1H, d, $^3J_{H–H}$ 7.4 Hz, NH-H-10), 8.78 (1H, d, $^3J_{H–H}$ 8.5 Hz, NH-H-18), 7.98 (2H, m, H-4, H-7), 7.66 (2H, m, H-21, H-25), 7.50 (1H, m, H-24), 7.38 (3H, m, H-15, H-23), 7.36 (1H, dt,$^4J_{H–H}$ 2.6 Hz, $^3J_{H–H}$ 9.0 Hz, $^3J_{F–H}$ 9.0 Hz, H-5), 7.27 (2H, t, $^3J_{H–H}$ 7.7 Hz, H-16), 7.18 (1H, t, $^3J_{H–H}$ 7.3 Hz, H-17), 5.26 (1H, quin, $^3J_{H–H}$ 7.2 Hz, H-1), 4.82 (1H, m, H-12); 3.11 (1H, dd, $^2J_{H–H}$ 10.4 Hz, $^3J_{H–H}$ 4.9 Hz, H-13), 3.09 (1H, dd, $^2J_{H–H}$ 10.4 Hz, $^3J_{H–H}$ 4.7 Hz, H-13), 1.53 (3H, d,$^3J_{H–H}$ 7.2 Hz, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^4J_{F–C}$ 2.9 Hz, C-9), 171.3 (C-11), 164.9 (d, $^4J_{F–C}$ 2.6 Hz, C-19), 161.8 (d, $^1J_{F–C}$ 243.7 Hz, C-22), 159.5 (d, $^1J_{F–C}$ 242.1 Hz, C-6), 149.6 (d, $^5J_{F–C}$ 1.4 Hz, C-3), 138.1 (C-14), 136.3 (d, $^3J_{F–C}$ 6.8 Hz, C-20), 135.9 (d, $^3J_{F–C}$ 11.7 Hz, C-8), 130.3 (d, $^3J_{F–C}$ 7.8 Hz, C-24), 129.3 (C-16), 128.1 (C-15), 126.4 (C-17), 123.8 (d, $^4J_{F–C}$ 2.6 Hz, C-25), 123.7 (d, $^3J_{F–C}$ 9.7 Hz, C-4), 118.1 (d, $^2J_{F–C}$ 21.2 Hz, C-23), 114.6 (d, $^2J_{F–C}$ 24.9 Hz, C-5), 114.2 (d, $^2J_{F–C}$ 22.7 Hz, C-21), 108.6 (d, $^2J_{F–C}$ 27.1 Hz, C-7), 55.0 (C-12), 47.5 (C-1), 37.4 (C-13), 20.0 (C-2); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −112.9, −116.5; anal. calcd. for $C_{25}H_{21}F_2N_3O_2S$ (465.51): C, 64.50; H, 4.55; N, 9.03; S, 6.89%. Found C, 64.61; H, 4.61; N, 8.95; S, 6.74%. HR-MS: for $C_{25}H_{21}F_2N_3O_2S$ [M + H^+] calcd. 466.13953 *m/z*, found 466.13924 *m/z*.

*N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl] carbamoyl}-2-methylphenyl]-2-methyl-benzamide (**11o**)*

White solid; yield 80%; m.p. 218–219 °C (from toluene); IR (ν_{max}, cm^{-1}): 3278, 1536 (NH of CONH), 1636 (CO of CONH), 1456 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 8.99 (1H, d, $^3J_{H–H}$ 7.8 Hz, NH-H-10), 8.47 (1H, d, $^3J_{H–H}$ 8.4 Hz, NH-H-18), 7.99 (1H, dd, $^4J_{H–H}$ 2.6 Hz, $^3J_{F–H}$ 8.8 Hz, H-7), 7.97 (1H, dd, $^3J_{H–H}$ 8.7 Hz, $^4J_{F–H}$ 5.0 Hz, H-4), 7.39–7.16 (10H, m, H-5, H-15, H-16, H-17, H21, H-22, H-23, H-24, H-25), 5.26 (1H, quin, $^3J_{H–H}$ 7.2 Hz, H-1), 4.80 (1H, m, H-12), 3.02 (1H, dd, $^2J_{H–H}$ 10.5 Hz, $^3J_{H–H}$ 4.9 Hz, H-13), 3.00 (1H, dd, $^2J_{H–H}$ 10.5 Hz, $^3J_{H–H}$ 4.9 Hz, H-13), 2.11 (3 H, s, CH_3), 1.54 (3H, d,$^3J_{H–H}$ 7.2 Hz, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^4J_{F–C}$ 3.6 Hz, C-9), 171.3 (C-11), 168.9 (C-19), 159.6 (d, $^1J_{F–C}$ 242.1 Hz, C-6), 149.6 (C-3), 138.0 (C-20), 136.7 (C-21), 135.9 (d, $^3J_{F–C}$ 12.6 Hz, C-8), 135.3 (C-14), 130.2 (C-23), 129.2 (C-22), 128.1 (C-24), 127.0 (C-16), 126.3 (C-25), 126.4 (C-15), 125.3 (C-17), 123.7 (d, $^3J_{F–C}$ 9.6 Hz, C-4), 114.5 (d, $^2J_{F–C}$ 24.2 Hz, C-5), 108.5 (d, $^2J_{F–C}$ 27.0 Hz, C-7), 54.3 (C-12), 47.4 (C-1), 37.2 (C-13), 20.0 (C-2), 19.1 (CH_3); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −116.5; anal. calcd. for $C_{26}H_{24}FN_3O_2S$ (461.55): C, 67.66; H, 5.24; N, 9.10; S, 6.95%. Found: C, 67.78; H, 5.33; N, 9.03; S, 6.87%. HR-MS: for $C_{26}H_{24}FN_3O_2S$ [M + H^+] calcd. 462.16460 *m/z*, found 462.16487 *m/z*.

*N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl] carbamoyl}-2-methylphenyl]-4-methyl-benzamide (**11p**)*

White solid; yield 82.0%; m.p. 177–178 °C (from toluene); IR (ν_{max}, cm^{-1}): 3265, 1533 (NH of CONH), 1627 (CO of CONH), 1458 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.02 (1H, d, $^3J_{H–H}$ 7.8 Hz, NH-H-10), 8.52 (1H, d, $^3J_{H–H}$ 8.4 Hz, NH-H-18), 7.99 (1H, dd, $^4J_{H–H}$ 2.6 Hz, $^3J_{F–H}$ 8.9 Hz, H-7), 7.96 (1H, dd, $^3J_{H–H}$ 8.9 Hz, $^4J_{F–H}$ 5.0 Hz, H-4), 7.73 (2H, d, $^3J_{H–H}$ 8.2 Hz, H-21, H-25), 7.38–7.14 (8H, m, H-5, H15, H16, H17, H-22, H-24), 5.24 (1H, quin, $^3J_{H–H}$ 7.2 Hz, H-1), 4.80 (1H, m, H-12), 3.08 (1H, dd, $^2J_{H–H}$ 10.2 Hz, $^3J_{H–H}$ 5.2 Hz, H-13), 3.06 (1H, dd, $^2J_{H–H}$ 10.2 Hz, $^3J_{H–H}$ 5.2 Hz, H-13), 2.33 (3H, s, CH_3), 1.52 (3H, d,$^3J_{H–H}$

7.2 Hz, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.5 (d, $^4J_{F-C}$ 3.6 Hz, C-9), 171.5 (C-11), 166.0 (C-19), 159.5 (d, $^1J_{F-C}$ 242.2 Hz, C-6), 149.6 (d, $^5J_{F-C}$ 1.4 Hz, C-3), 141.2 (C-23), 138.2 (C-14), 135.9 (d, $^3J_{F-C}$ 11.9 Hz, C-8), 131.2 (C-20), 129.2 (C-22, C-24), 128.7 (C-16), 128.1 (C-21, C-25), 127.5 (C-15), 126.3 (C-17), 123.6 (d, $^3J_{F-C}$ 9.4 Hz, C-4), 114.5 (d, $^2J_{F-C}$ 24.6 Hz, C-5), 108.6 (d, $^2J_{F-C}$ 27.4 Hz, C-7), 54.8 (C-12), 47.4 (C-1), 37.4 (C-13), 21.0 (CH_3), 20.0 (C-2); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −116.5; anal. calcd. for $C_{25}H_{21}ClFN_3O_2S$ (461.55): C, 67.66; H, 5.24; N, 9.10; S, 6.95%. Found C, 67.48; H, 5.28; N, 9.23; S, 7.17%. HR-MS: for $C_{26}H_{24}FN_3O_2S$ [M + H$^+$] calcd. 462.16460 *m/z*, found 462.16479 *m/z*.

N-[(1S)-1-{[(1R)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl] carbamoyl}-2-methylphenyl]-4-chloro-3-nitrobenzamide ***(11q)***

White solid; yield 84.0%; m.p. 198–199 °C (from toluene); IR (ν_{max}, cm^{-1}): 3267, 1529 (NH of CONH), 1637 (CO of CONH), 1458 (C=N); ^{1}H NMR (400.13 MHz, DMSO-d_6): δ_H 9.11 (1H, d, $^3J_{H-H}$ 7.5 Hz, NH-H-18), 9.09 (1H, d, $^3J_{H-H}$ 8.3 Hz, NH-H-10), 8.50 (1H, d, $^4J_{H-H}$ 2.0 Hz, H-21), 8.12 (1H, dd, $^4J_{H-H}$ 2.0 Hz, $^3J_{H-H}$ 8.5 Hz, H-25), 7.97 (2H, m H-4, H-7), 7.88 (1H, d, $^3J_{H-H}$ 8.5 Hz, H-24), 7.35 (3H, m, H-5, H-16), 7.27 (2H, t, $^3J_{H-H}$ 7.5 Hz, H-15), 7.18 (1H, t, $^3J_{H-H}$ 7.2 Hz, H-17), 5.26 (1H, quin, $^3J_{H-H}$ 7.2 Hz, H-1), 4.84 (1H, m, H-12); 3.09 (1H, dd, $^2J_{H-H}$ 10.2 Hz, $^3J_{H-H}$ 4.8 Hz, H-13), 3.09 (1H, dd, $^2J_{H-H}$ 10.2 Hz, $^3J_{H-H}$ 4.8 Hz, H-13), 1.51 (3H, d,$^3J_{H-H}$ 7.2 Hz, H-2); ^{13}C NMR (100.62 MHz, DMSO-d_6): δ_C 175.3 (d, $^4J_{F-C}$ 3.1 Hz, C-9), 171.0 (C-11), 163.3 (C-19), 159.6 (d, $^1J_{F-C}$ 242.3 Hz, C-6), 149.6 (d, $^5J_{F-C}$ 1.4 Hz, C-3), 147.2 (C-22), 137.8 (C-14), 135.9 (d, $^3J_{F-C}$ 11.8 Hz, C-8), 133.8 (C-20), 132.6 (C-21), 131.9 (C-25), 129.2 (C-16), 128.1 (C-15), 128.0 (C-23), 126.4 (C-24), 124.7 (C-17), 123.7 (d, $^3J_{F-C}$ 9.6 Hz, C-4), 114.6 (d, $^2J_{F-C}$ 25.0 Hz, C-5), 108.6 (d, $^2J_{F-C}$ 27.2 Hz, C-7), 55.1 (C-12), 47.5 (C-1), 37.4 (C-13), 19.9 (C-2); ^{19}F NMR (376.46 MHz, DMSO-d_6): δ_F −116.5; anal. calcd. for $C_{25}H_{20}FN_4O_4S$ (526.97): C, 56.98; H, 3.83; N, 10.63; S, 6.08%. Found C, 56.81; H, 3.91; N, 10.75; S, 6.18%. HR-MS: for $C_{25}H_{20}FN_4O_4S$ [M + H$^+$] calcd. 527.09506 *m/z*, found 527.09478 *m/z*.

Crystallographic details

The X-Ray data for colourless crystal of compound **11l**, were obtained at 150 K using Oxford Cryostream low-temperature device on a Nonius Kappa CCD diffractometer with MoKα radiation ($\lambda = 0.71073$ Å), a graphite monochromator and the φ and χ scan mode. Data reductions were performed with DENZO-SMN (Otwinowski and Minor 1997). The absorption was corrected by integration methods (Ahmed et al. 1970). Structures were solved by direct methods (Sir92) (Altomare et al. 1993) and refined by full matrix least-square based on *F2* (SHELXL97) (Sheldrick 1997). Hydrogen atoms were mostly localised on a difference Fourier map, however to ensure uniformity of the treatment of the crystal, all hydrogen atoms were recalculated into idealised positions (riding model) and assigned temperature factors Hiso(H) = 1.2 Ueq(pivot atom) or of 1.5 Ueq for the methyl moiety with C–H = 0.96, 0.98 and 0.93 Å for methyl, methine and hydrogen atoms in the aromatic rings, respectively. Crystallographic data for structural analysis have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 1025821). Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge CB2 1EY, UK (fax:+44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

Crystallographic data for **11l**: $C_{25}H_{21}ClFN_3O_2S$, M = 481.96, triclinic, P 1, $a = 5.0840(3)$, $b = 9.2029(6)$, $c = 11.9901(5)$ Å, $\alpha = 92.770(3)$ $\beta = 98.043(4)$, $\gamma = 92.084(4)$ °, $Z = 1$, $V = 554.29(5)$ Å^3, $D_c = 1.444$ g cm^{-3}, $\mu = 0.304$ mm^{-1}, $T_{min}/T_{max} = 0.942/ 0.968$; $-6 \le h \le 6$, $-11 \le k \le 11$, $-15 \le l \le 15$; 11184 reflections measured ($\theta_{max} = 27.5$ °), 4738 independent ($R_{int} = 0.0494$), 3917 with $I > 2\sigma(I)$, 298 parameters, $S = 1.207$, $R1$(obs. data) = 0.0475, $wR2$(all data) = 0.0908; max., min. residual electron density = 0.240, −0.319 eÅ^{-3}.

Antifungal assay

The antifungal assay was carried out by using agar dilution method. This method was modified from the standard Clinical and Laboratory Standards Institute (CLSI) M07-A9 (CLSI 2012) using *Candida albicans* (CCM 8311), *C. albicans* HE 169, *Candida glabrata* (CCM 8270), *C. glabrata* 196/98, *C. glabrata* 71/97, *C. krusei* S1, *Candida krusei* 802/97, *Candida tropicalis* 31/HK, *C. tropicalis* 14/HK and *Candida parapsilosis* p69 in Sabouraud's dextrose agar medium. Nutrient broth was prepared using 9 mL of Sabouraud's dextrose agar (Sigma-Aldrich, Germany) and 1 mL of each dilution tested compounds prepared in sterile dry test tubes. The mixture was immediately poured into a sterile petri dish with a diameter of 10 cm. A twofold serial dilution of the compounds and the reference drug were dissolved in DMSO. Tested compounds were taken at different concentrations (400, 200, 100, 50, 25, 12.5 and 6.25 μg/mL) for minimum inhibitory concentration (MIC). One hundred microlitres microbial suspension of 3×10^6 cfu/mL density was streaked on the nutrient agar medium after solidification. The petri dishes were incubated at 30 °C for 48 h. The MIC was the lowest concentration of the tested compound that resulted in no visible growth of the organisms. To ensure that the solvent had no effect on bacterial

growth, a control test was also performed with test medium supplemented with DMSO at same dilutions as used in the experiment.

In vitro cytotoxicity assay

Cell lines

The experiments were carried out with the MRC-5 (the human primary human lung fibroblast) and Jurkat (the human T-cell acute lymphoblastic leukaemia) cell lines from the European Collection of Cell Cultures (Salisbury, UK). MRC-5 cells were cultured in Eagle's minimum essential medium (MEM) with L-glutamine and sodium bicarbonate (Sigma-Aldrich, St. Louis, MO, USA) in the presence of 10% foetal calf serum, 2 mM L-glutamine, MEM non-essential amino acids 10 µl/mL, 50 µg/mL penicillin and 50 µg/mL streptomycin (all reagents from Life Technologies, Grand Island, NY, USA). Jurkat cells were cultured in RPMI 1640 medium supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 1 mM pyruvate, 10 mM HEPES, MEM non-essential amino acids 10 µl/mL, 50 µg/mL penicillin and 50 µg/mL streptomycin (all reagents from Life Technologies, Grand Island, NY, USA). The cell cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37 °C.

Real-time cytotoxicity assay

The cytotoxicity of the most active compounds **11e**, **11g**, **11j**, **11n** and **11o** was assessed against human foetal lung fibroblast (MRC-5) cells using the xCELLigence RTCA (Real-Time Cell Analysis) SP (Single plate) system (Roche Diagnostic, Germany), allowing label-free, dynamic monitoring of cell events in real-time. The principle of the system is to monitor the changes in electrode impedance induced by the interaction between testing cells and electrodes (Xing et al. 2005). Briefly, the xCELLigence system was connected and tested by Resistor Plate Verification before the RTCA SP station was placed inside the incubator at 37 °C and 5% CO_2. Background measurements were taken by adding 100 µl of appropriate medium to the wells of the E-Plate 96. Cell suspension (90 µl) at cell density of 17,000 cells per well was added to each well of the E-plate 96 in triplicate. The MRC-5 cell proliferation was dynamically monitored at 30 min interval. When the cells entered logarithmic growth phase, they were treated with 10 µL of tested compounds dissolved in DMSO at concentrations ranging from 25–400 µg/mL for compounds **11e**, **11j**, **11n** and **11o**, and 25–200 µg/mL for compound **11g**. Cells treated with 0.2% of DMSO was used as vehicle control, while cells treated with 5% DMSO were used as positive control. After 72 h of incubation with tested compounds, the cell status and the cytotoxic effect were plotted using characteristic cell index-time profile. Growth curves were normalised to the time point of treatment. Evaluations were performed using the RTCA 1.2.1 software.

Propidium iodide cell viability assay

The Jurkat cells from 0.1% DMSO vehicle control, 5 µM of cisplatin (Sigma-Aldrich, St. Louis, MO, USA)-treated cells, used as a positive control and experimental cultures treated with **11e**, **11g**, **11j**, **11n** and **11o** at 100 and 200 µg/mL were collected, and washed in Dulbecco's phosphate-buffered saline (Sigma-Aldrich, St. Louis, MO, USA). Washed samples were stained with 5 µl (250 µg/mL) of propidium iodide (PI), membrane impermeable nucleic acid stain with excitation/emission wavelength at 488 nm/617 nm, for 5 min at room temperature to assess dead cells. This dye cannot pass through intact cell membranes, but may freely enter cells with compromised cell membranes. Stained samples were analysed with a CyAn flow cytometer and the data were plotted using Summit v 4.3 software (both from Beckman Coulter, Miami, FL, USA). Fluorescence intensity of 10,000 cells was analysed.

(2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) cell proliferation and viability assay

The effects of the **11e**, **11g**, **11j**, **11n** and **11o** on the proliferation and viability of Jurkat cells were quantified with the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay, a colorimetric assay of the activity of mitochondrial dehydrogenases, which correlates with the number of living cells. The cells were seeded at previously established optimal density in a 96-well plate. After 48 h incubation, cell viability was determined using Cell Proliferation Kit II (XTT, Roche, Germany) according to manufacturer's instructions. XTT-assay was conducted using 200 µL of volume and 100 µL of XTT-labelling mixture. Absorbance was then measured at 480 nm using a 96-multiwell microplate reader Tecan Infinite M200 (Tecan Group Ltd., Männedorf, Switzerland). Viability was calculated as described in the paper by Havelek and colleagues using the following formula: (%) viability = (A480sample − A480blank)/(A480control − A480blank) × 100, where A480 is the absorbance of utilised XTT formazan measured at 480 nm (Havelek et al. 2012). Data were analysed with GraphPad Prism 5 biostatistics (GraphPad Software, La Jolla, CA, USA) statistical software. Each value is the mean of four independent replicates of each condition. The viability of the treated cells was normalised to the 0.1% DMSO vehicle-treated control cells. Cells treated with 5% DMSO were used for positive control in this assay.

Statistical analysis

The descriptive statistics of the results were calculated and the charts were made in Microsoft Office Excel 2010 (Microsoft, Redmond, WA, USA) or GraphPad Prism 5 biostatistics (GraphPad Software, La Jolla, CA, USA). In this study all the values were expressed as arithmetic means with SD of triplicates, unless otherwise noted. The significant differences between the groups were analysed using the Student's *t*-test.

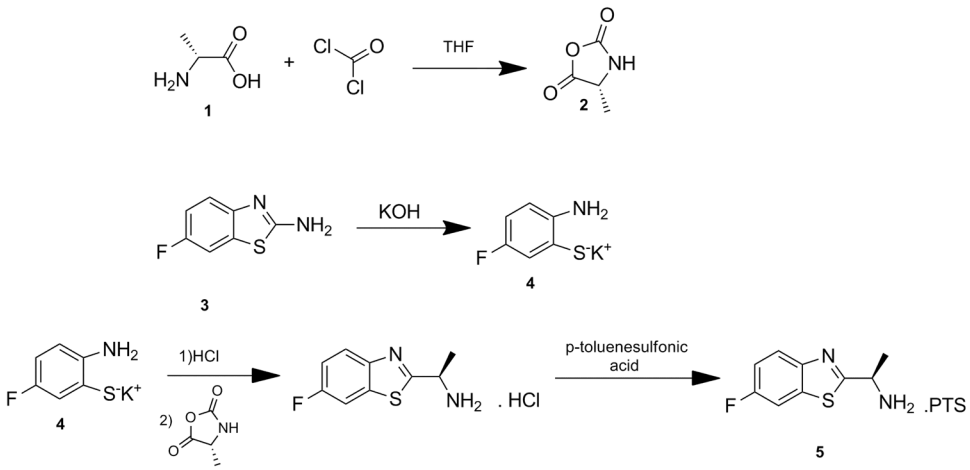
Results and discussion

Chemistry

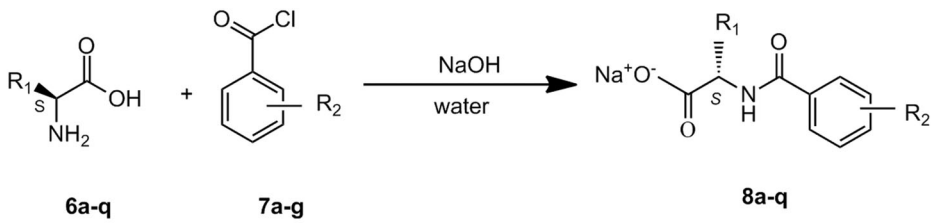
The starting compound (*R*)-1-(6-fluorobenzothiazol-2-yl) ethanamine **5** was prepared in the form of PTS salt according to the procedures described elsewhere (Pejchal et al. 2011a). (4*R*)-4-Methyl-1,3-oxazolidine-2,5-dione **2** was prepared by reaction of D-alanine **1** with phosgene in tetrahydrofuran (Pejchal et al. 2011a). (1*R*)-1-(6-Fluoro-1,3-benzothiazol-2-yl)ethanamine *p*-toluenesulfonic salt **5** was prepared by a three-step process described in Scheme 1. 2-amino-6-fluorobenzothiazole **3** reacted with aqueous solution of potassium hydroxide in the first step to give 2-amino-5-fluorobenzenethiol potassium salt **4**, which reacted with hydrochloric acid and compound **2** in the second step to give hydrochloride of **5**. Product **5** *p*-toluenesulfonic salt was prepared by reaction of hydrochloride of **5** with *p*-toluenesulfonic acid in water.

The synthesis of desired compounds can be described as a step by step synthesis (Schemes 2 and 3). In general, three subsequent condensation reactions were performed to form targeted molecules.

Scheme 1 Synthetic route to compound **5**



Scheme 2 Condensation of amino acids and substituted benzoyl chloride

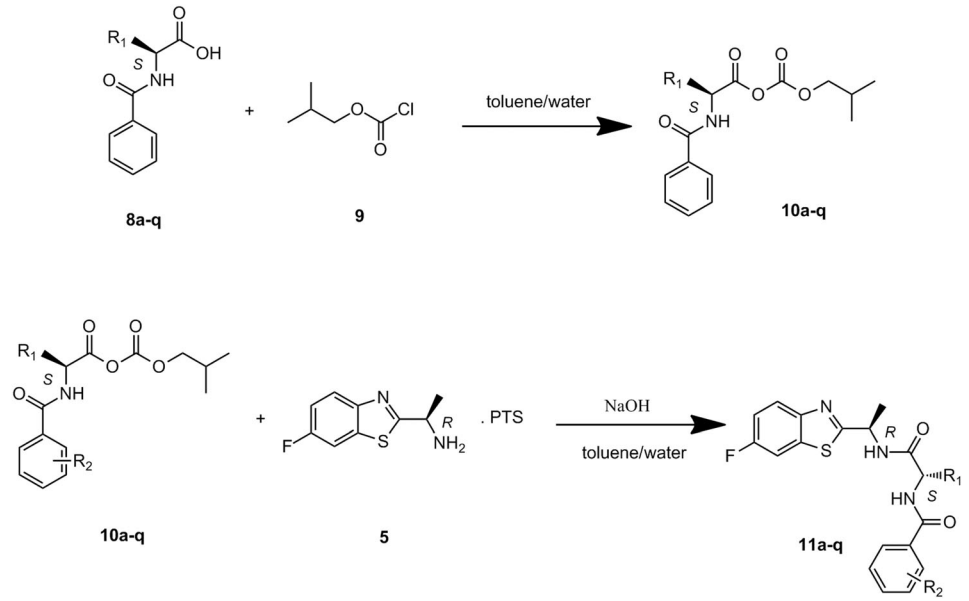


6a-q **7a-g** **8a-q**

a) R_1= *S*-isopropyl, R_2 = H
b) R_1= *S*-isopropyl, R_2 = 2-Cl
c) R_1= *S*-isopropyl, R_2 = 3-Cl
d) R_1= *S*-isopropyl, R_2 = 4-Cl
e) R_1= *S*-isopropyl, R_2 = 3-F
f) R_1= *S*-isopropyl, R_2 = 4-F
g) R_1= *S*-isopropyl, R_2 = 2-CH_3
h) R_1= S-isopropyl, R_2 = 4-CH_3
i) R_1= *S*-isopropyl, R_2 = 4-NO_2
j) R_1= *S*-isopropyl, R_2 = 4-Cl-3-NO_2

k) R_1= *S*-benzyl, R_2 = H
l) R_1= *S*-benzyl, R_2 = 3-Cl
m) R_1= *S*-benzyl, R_2 = 4-Cl
n) R_1= *S*-benzyl, R_2 = 3-F
o) R_1= *S*-benzyl, R_2 = 2-CH_3
p) R_1= *S*-benzyl, R_2 = 4-CH_3
q) R_1= *S*-benzyl, R_2 = 4-Cl-3-NO_2

Scheme 3 Activation of carboxylic group and subsequent amide formation



a) R_1= *S*-isopropyl, R_2 = H
b) R_1= *S*-isopropyl, R_2 = 2-Cl
c) R_1= *S*-isopropyl, R_2 = 3-Cl
d) R_1= *S*-isopropyl, R_2 = 4-Cl
e) R_1= *S*-isopropyl, R_2 = 3-F
f) R_1= *S*-isopropyl, R_2 = 4-F
g) R_1= *S*-isopropyl, R_2 = 2-CH_3
h) R_1= S-isopropyl, R_2 = 4-CH_3
i) R_1= *S*-isopropyl, R_2 = 4-NO_2
j) R_1= *S*-isopropyl, R_2 = 4-Cl-3-NO_2

k) R_1= *S*-benzyl, R_2 = H
l) R_1= *S*-benzyl, R_2 = 3-Cl
m) R_1= *S*-benzyl, R_2 = 4-Cl
n) R_1= *S*-benzyl, R_2 = 3-F
o) R_1= *S*-benzyl, R_2 = 2-CH_3
p) R_1= *S*-benzyl, R_2 = 4-CH_3
q) R_1= *S*-benzyl, R_2 = 4-Cl-3-NO_2

Condensation of substituted benzoyl chlorides with amino acids

The synthetic pathway begins with the condensation of appropriate L-amino acid **6** and substituted benzoyl chlorides **7a–q** dissolved in toluene (Scheme 2). Substituted benzoyl chlorides and amino acids are buildings blocks for the side chain and determine the properties of the targeted molecule (Leone-Bay et al. 1995). Unreacted substituted benzoyl chloride was removed by separation of toluene layer. Water solution of intermediates **8a–q** were used for the next procedure after the separation of organic layer.

Activation of carboxylic group and formation of target molecules 8

The second synthetic step is the activation of the carboxylic acid group of corresponding compounds **8a–q** using isobutyl chloroformate **9** to form intermediate **10a–q**. Intermediates **10a–q** were used for the next step in toluene solution without any isolation. The final step is the condensation with (*R*)-1-(6-fluorobenzothiazol-2-yl)ethanamine liberated from its PTS salt **5** directly by the reaction with an aqueous solution of sodium hydroxide. Reactions of series of intermediates **10a–q** with **5** gave target molecules **11a–q** (Scheme 3). Afterwards, the toluene layer was warmed in order to dissolve product formed. Products were precipitated by cooling of the separated and concentrated toluene solution. Products were separated by filtration in high yields 80–90%. For the detailed description of experimental procedure, see Materials and methods.

Products **11a–q** were characterised by melting points, IR, ^{1}H, ^{13}C, ^{19}F NMR spectra, high resolution mass spectrometry and elemental analysis (CHN). The most significant peaks recorded in IR spectra of compounds **11a–q** were attributed to the characteristic vibrations of C=O from CONH group at 1627–1641 cm^{-1}; NH of CONH at 1523–1544 and at 3247–3308 cm^{-1} and of C=N at 1451–1460 cm^{-1}. The presence two different CONH amide groups is well proven by the presence of two doublets in all ^{1}H NMR spectra of compounds **11a–q**, where the signal attributed to CH (H-1) group is split to a quintet by hydrogen atoms of CH_3 (H-2) and CONH (H-10) groups. In the cases of compounds **11a-j**, the second CH (H-12) group is split to a triplet by CH (H-13) and CONH (H-15) amide groups with the same coupling constant values. In cases of **11k–q**, the same CH (H-12) group appears as a multiplet and the CH_2 group resonates as two doublets of doublets. The rest of the signals observed in the ^{1}H NMR spectra of all compounds reveal signals of remaining hydrogen atoms

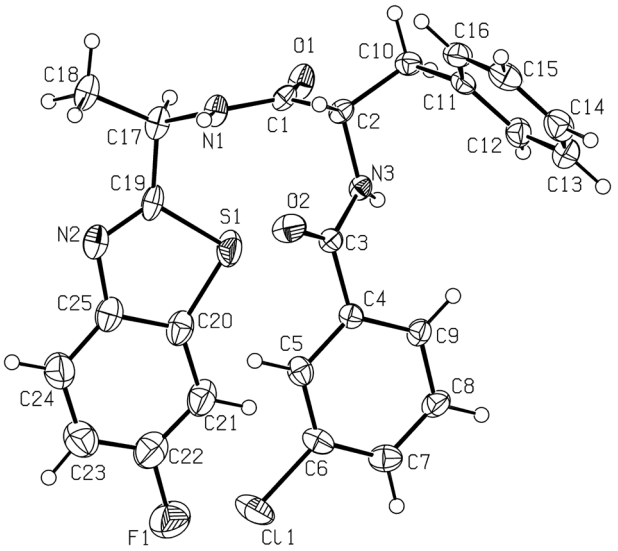


Fig. 4 The molecular structure (ORTEP 40% probability level) of **11l** selected interatomic distances [Å] and angles [°]: N1 C1 1.341(3), N1 C17 1.465(4), C17 C18 1.520(4), C19 C17 1.503(5), N2 C19 1.282 (4), S1 C19 1.760(3), O1 C1 1.227(3), C1 C2 1.526(4), N3 C2 1.462 (3), C3 N3 1.335(3), O2 C3 1.230(3), C3 C4 1.494(4), C2 C10 1.526 (4), C11 C10 1.510(4), N1 C1 C2 114.5(2), O1 C1 N1 123.4(3), O1 C1 C2 121.8(2), N1 C17 C18 110.3(2), N1 C17 C19 109.4(2), C17 C19 S1 119.3(2), N2 C19 C17 125.2(3), N2 C19 S1 115.5(3), C10 C2 C1 114.1(2), C3 N3 C2 122.7(2), N3 C3 C4 115.6(2), O2 C3 N3 122.8 (3), O2 C3 C4 121.5(2), C11 C10 C2 110.5(2)

Table 1 Hydrogen-bond geometry (Å, °)

D-H···*A*	d(*D*···*A*)	angle *D*-H···*A*	symm. transformation
N1-H1···O1	2.970(3)	160	$x+1, y, z$
N3-H3···O2	2.969(3)	165	$x-1, y, z$

at predictable positions, and of characteristic integral intensity and multiplicity. For all compounds, two peaks in the alkyl region caused by CH_3–CH– group were found in the ^{13}C NMR spectra. Moreover, the ^{13}C NMR spectra of **11a–j** reveal also additional four signals indicating the presence of the $(CH_3)_2$–CH–CH-chain, and in cases of **11k–q** only two adjacent signals due to –CH–CH_2– group. Other seven peaks appearing as doublets (split by an interaction with ^{19}F nuclei) were found in the aromatic part of spectra and are assigned to substituted benzothiazole group. The rest of signals in the aromatic part are due to substituted phenyl groups.

Crystallography

The compound **11l** (Fig. 4) crystallises in the triclinic crystal system with P_1 space group with one molecule in the unit cell. The intermolecular contacts via N1-H1···C=O1 and N3-H3···C=O2 bridges are present (Table 1), these H-bonds made available the formation of doubly connected chain structure (Fig. 5). To the best of our knowledge, a plethora of diamide structures is known in the literature, but in none of those contains the benzothiazole group. Moreover the benzothiazole unit is interconnected to the diamide core by the chiral CH bridge. Both amides as well as the benzothiazole parts of the molecule reveal usual conjugation of the π-electron density known for peptide type of bonding as well as for the S, N-heterocyclic moieties (Kello et al. 1986; Allen et al. 1987; Brandenburg et al. 1987; Pindinelli et al. 2007; Zhang and Zhao 2009; Karagiannidis et al. 2011; Pejchal et al. 2011b; Pejchal et al. 2015; Pejchal et al. 2016). Although the orientation of the halogenated

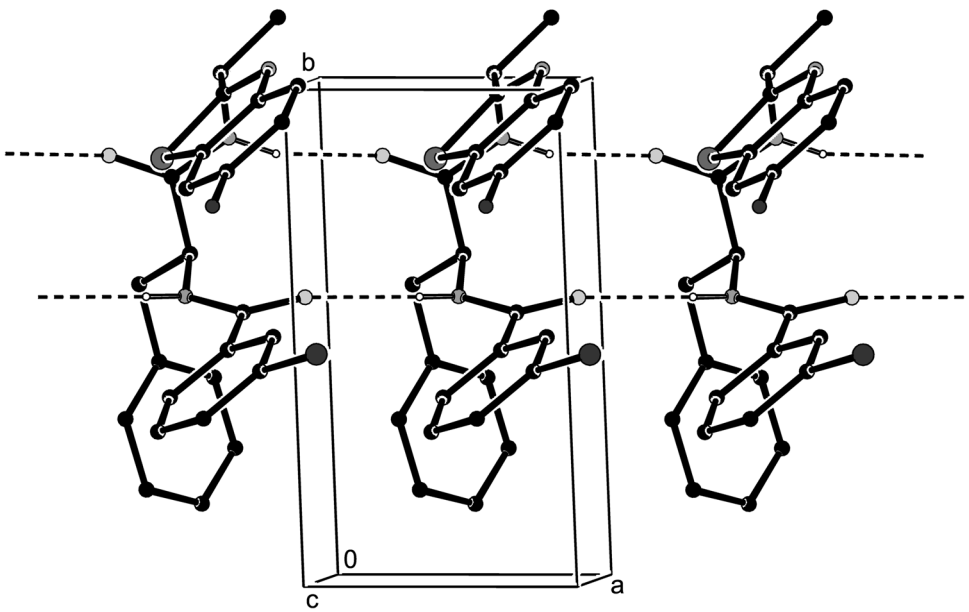


Fig. 5 Crystal packing of **11l** with intermolecular H interactions view along axis *z*

Table 2 Antifungal activities of the compound **11a–q**

Compound	MIC (μg/mL)				
	C. albicans CCM 8311	*C. albicans* HE 169	*C. krusei* S1	*C. krusei* 802/97	*C. glabrata* CCM 8270
11a	200	>400	>400	200	>400
11b	200	>400	>400	>400	>400
11c	200	>400	>400	200	>400
11d	200	200	>400	>400	200
11e	200	25	>400	>400	200
11f	200	>400	>400	>400	>400
11g	200	12.50	>400	>400	200
11h	200	50	>400	>400	200
11i	>400	>400	>400	>400	>400
11j	200	12.5	>400	>400	>400
11k	200	200	200	200	>400
11l	200	>400	>400	>400	>400
11m	>400	>400	>400	>400	>400
11n	200	50	>400	200	200
11o	200	50	200	200	200
11p	200	100	200	200	200
11q	200	50	200	200	200
Amphotericin B	25	50	200	100	6.25

aromatic rings is mutually syn, any noncovalent interaction of those rings via for example a π–π stacking is observed. Only short contacts, responsible for a supramolecular architecture of this compound in the solid state, are of F-HC and N-HC types, respectively.

Antifungal assay

All the compounds have been screened for antifungal activities using agar dilution method (for results, see Tables 2 and 3). Amphotericin B was used as comparative standard drug under the same protocol. All compounds were screened for antifungal activity against *C. albicans* (CCM 8311), *C. albicans* HE 169, *C. glabrata* (CCM 8270), *C. glabrata* 196/98, *C. glabrata* 71/97, *C. krusei* S1, *C. krusei* 802/97, *C. tropicalis* 31/HK, *C. tropicalis* 14/HK, and *C. parapsilosis* p69 in Sabouraud's dextrose agar medium (for results, see Tables 2 and 3). These present clinical isolates of patients were obtained from the Faculty of Medicine and Dentistry Palacky Univesity of Olomouc, Czech Republic. *Candida* strains bearing CCM originated from the Czech Collection of Microorganisms (CCM), Masaryk University of Brno, Czech republic. Compounds **11e**, **11g**, **11h**, **11j**, **11n**, **11o**, **11p** and **11q** exhibited satisfactory antifungal activity against four *Candida* genuses. As the number of immunologically weakened patients increase, opportunistic infections have become a widely recognised public health problem (Diekema et al. 2012). In

Table 3 Antifungal activities of the compound **11a–q** (continued)

Compound	MIC (μg/mL)				
	C. glabrata 196/98	*C. glabrata* 71/97	*C. tropicalis* 31/HK	*C. tropicalis* 14/HK	*C. parapsilosis* p69
11a	200	>400	>400	>400	>400
11b	200	>400	200	>400	>400
11c	200	>400	100	200	>400
11d	>400	200	200	>400	>400
11e	200	200	6.25	100	50
11f	>400	>400	>400	>400	>400
11g	>400	200	12.50	100	6.25
11h	200	200	25	100	25
11i	>400	>400	>400	>400	>400
11j	200	200	12.5	100	6.25
11k	200	>400	200	>400	>400
11l	200	>400	200	>400	>400
11m	>400	200	200	200	>400
11n	200	200	25	100	12.5
11o	200	200	25	100	12.5
11p	200	200	50	100	50
11q	200	200	25	100	25
Amphotericin B	100	50	6.25	100	6.25

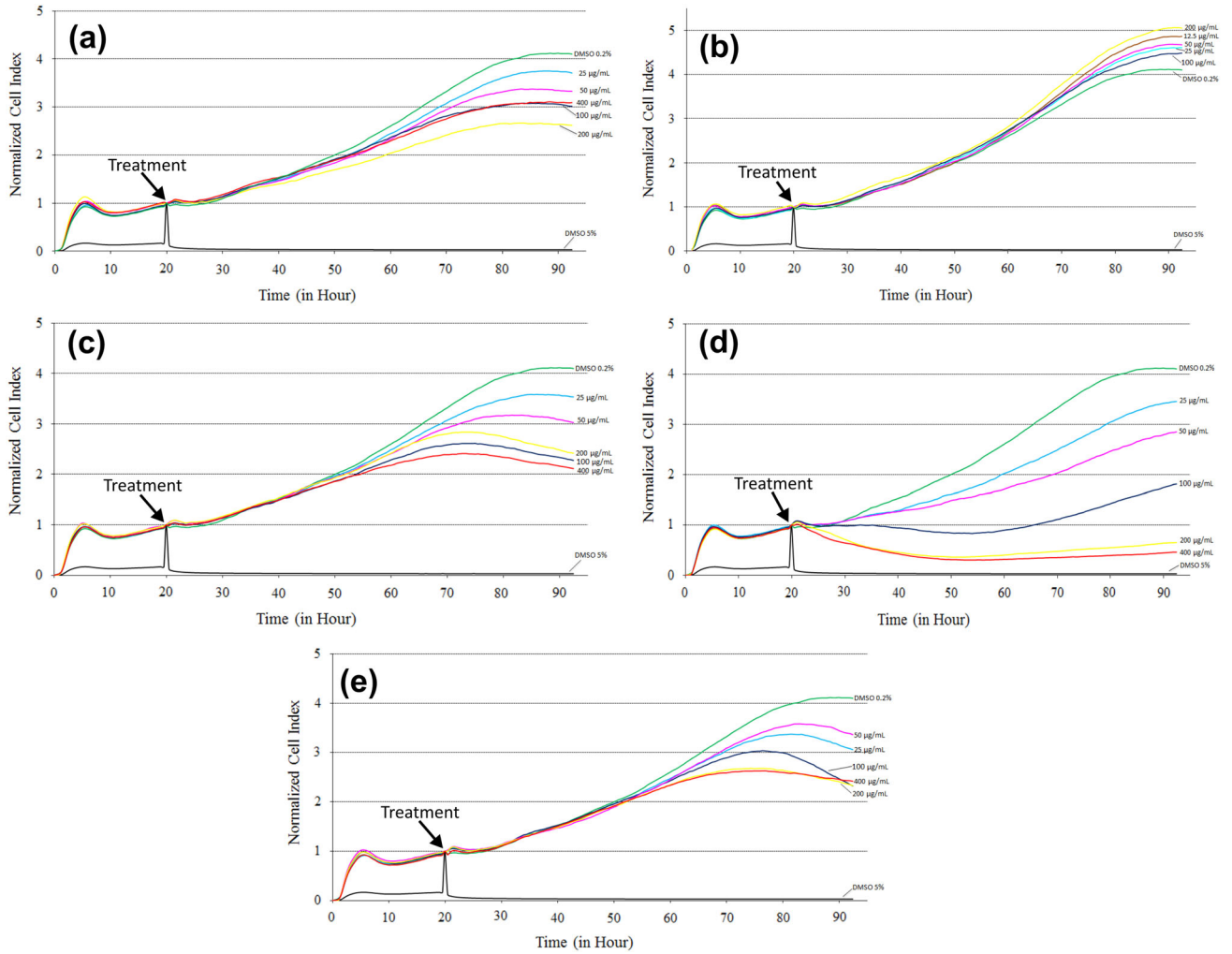


Fig. 6 Dynamic monitoring of cytotoxic response to different concentrations of the compound **11e** (**a**), **11g** (**b**), **11j** (**c**), **11n** (**d**) and **11o** (**e**). Normalised CI measured for 72 h on human lung fibroblast (MRC-5) cells. Cells treated with 0.2% of DMSO was used as vehicle control and 5% DMSO treated cells were used as positive control. Plotted CI values were normalised to the to the time point of treatment

that respect, compound **11e** (MIC = 6.25 µg/mL) exhibited comparable antifungal activity against *C. albicans* HE 169, *C. tropicalis* 31/HK, *C. tropicalis* 14/HK when amphotericin B is taken as a standard drug in use. Compounds **11g** and **11j** exhibited high activity against to *C. albicans* HE 169, *C. tropicalis* 31/HK, *C. tropicalis* 14/HK and particularly against *C. parapsilosis* p69. Compounds **11h**, **11n** and **11o** were active against *C. albicans* HE 169, *C. tropicalis* 31/HK, *C. tropicalis* 14/HK and *C. parapsilosis* p69 too.

The investigation of structure–activities relationships in the series of these species, based on current results, indicated that some of synthesised derivatives exhibited significant antifungal activity: (i) the most active compounds in particular antifungal screening seem to be **11e**, **11g**, **11j**, **11n, 11o** and **11q**, which is most probably caused by the presence of electron withdrawing fluoro and nitro substituents in *meta* positions (**11e**, **11j**, **11o** and **11q**) or electron donating methyl group in *ortho* positions of the phenyl substituent, (ii) compounds having electron withdrawing substituent in respective *ortho* or *para* positions exhibited much lower or negligible antibacterial activities. The compounds **11a** and **11k** containing non-substituted phenyl group exhibited low antimicrobial and lack of antifungal activity was observed.

In vitro cytotoxicity assay

The cytotoxicity of the **11e**, **11g**, **11j**, **11n** and **11o** was analysed using xCELLigence system on the human foetal lung fibroblast (MRC-5) cells. It was observed that MRC-5 cells treated with 25–400 µg/mL of **11e** (Fig. 6a), **11g** (Fig. 6b), **11j** (Fig. 6c) and **11o** (Fig. 6e) were proliferating in parallel to cells treated with 0.1% DMSO vehicle control,

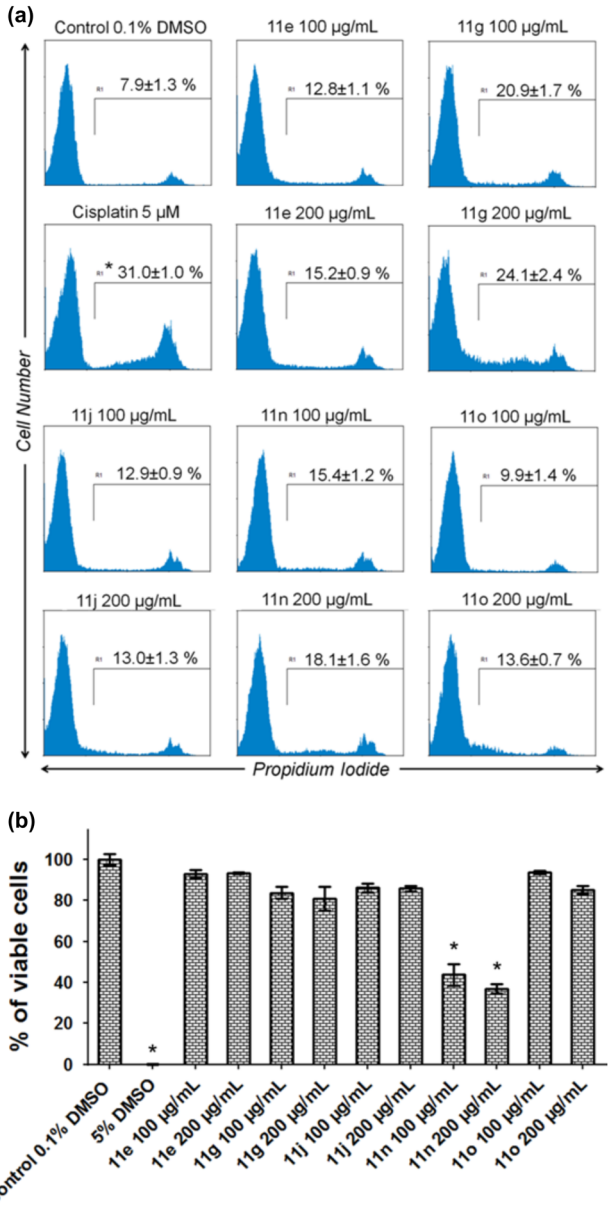


Fig. 7 Cytotoxic and antiproliferative activity of **11e**, **11g**, **11j**, **11n** and **11o** in Jurkat cells. **a** Viability assessment by PI in Jurkat cells following 48-h exposure to evaluated compounds, 0.1% DMSO (mock treated negative control) and 5 µM cisplatin (positive control). Figure shows flow cytometric histograms depicting PI positive populations vs. cell number. The flow cytometric histograms are representative of three independent experiments with mean values ± SD, $n = 3$. *significantly different to control ($P \leq 0.001$). **b** Cell proliferation and viability of Jurkat cells measured by using XTT assay 48 h after the treatment. Viability is referred to cells treated with 0.1% DMSO (control DMSO). Five percent DMSO was used as a positive control in this assay. Data are shown as mean values ± SD, $n = 4$. *significantly different to control ($P \leq 0.001$)

although treatment with 100 µg/mL, 200 µg/mL and 400 µg/mL of **11e**, **11j** and **11o** caused a slight reduction in Cell Index (CI) value after 48 h of treatment. In contrast, treatment of MRC-5 cells with 25, 50 and 100 µg/mL of **11n** resulted in decreased cell proliferation compared to control. The **11n** treatment, at 200 and 400 µg/mL, resulted in complete inhibition of cell proliferation (Fig. 6d).

In the second set of experiments, the **11e**, **11g**, **11j**, **11n** and **11o** were tested on the viability of acute T cell leukaemia cells Jurkat using PI staining. Propidium iodide readily enters and stains nonviable cells, but cannot cross the membrane of viable cells. Viable and dead cells can be therefore easily distinguished from their fluorescence intensity (viable cells exhibiting low vs. dead cells with high fluorescence intensity). The Jurkat cells were exposed to 100 and 200 µg/mL concentrations of these compounds for 48 h. There were no significant changes in the viability of Jurkat cells, leading to significant increase in population with high PI fluorescence intensity, as compared to cisplatin treatment at 5 µM (Fig. 7a).

In order to determine the number of viable Jurkat cells in proliferation, the XTT assay was performed in the presence or absence of evaluated compounds **11e**, **11g**, **11j**, **11n** and **11o** at 100 and 200 µg/mL, using as controls cells exposed to 5% DMSO vehicle (positive control) and 0.1% DMSO vehicle (negative control). The conversion of XTT tetrazolium salt into the aqueous soluble formazan product is accomplished by dehydrogenase enzymes found in metabolically active cells. The results show that treatment with **11n** at both evaluated concentrations resulted in dose-dependent decrease in the proliferation of viable cells compared to vehicle 0.1% DMSO exposure ($P \leq 0.001$; Fig. 7b).

Conclusion

The set of 1-[(1*R*)-1-(6-fluoro-1,3-benzothiazol-2-yl)ethyl]-3-substituted phenyl diamides was synthesised, structurally evaluated and screened for antifungal activity against a variety of *Candida* strains. Compounds **11e**, **11g**, **11h**, **11j**, **11n**, **11o**, **11p** and **11q** exhibited satisfactory antifungal activity against pathogenic *C. albicans* HE 169, *C. tropicalis* 31/HK, *C. tropicalis* 14/HK and *C. parapsilosis* p69 comparable or higher than amphotericin B as standard drug used. It seems that the methyl group in *ortho* or fluorine atom in the *meta* position are very significant for enhancing activity against *Candida* genus. The cytotoxicity of the most active compounds (**11e, 11g, 11j, 11n** and **11o**) was determined in vitro using human lung fibroblasts and human cancer cell line. The three different methods, of proliferation and viability analysis showed that compouds **11e, 11g, 11j** and **11o** possess low cytotoxicity at concentrations substantially higher than corresponding MICs evaluated for tested compounds. Thus, these compounds deserve further investigation due to their satisfactory antifungal activity and low cytotoxicity against mammalian cells.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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