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RESEARCH ARTICLE

Antiviral Activity of Benzotriazole Based Derivatives

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Abstract:

Background:

For the last thirty years, the benzotriazole scaffold has been the object of our group interest and we have already presented some results on the antiviral activity of our compounds.

Objective:

In this article, we conclude the exploration of *N*-(4-(*R*-2*H*-benzo[d][1,2,3]triazol-2-yl)phenyl)-4-*R*'-benzamides and 1-(4-(*R*-2*H*-benzo[d][1,2,3]triazol-2-yl)phenyl)-3-*R*'-ureas by synthesizing further modified derivatives, in order to have more elements for SARs evaluation.

Methods:

Here, we reported the synthesis and the antiviral screening results of 38 newly synthesized benzotriazole derivatives against a panel of DNA and RNA viruses. We also analyse SARs in comparing these compounds with previously published benzotriazole analogues, taking stock of the situation.

Results:

Among the newly presented derivatives, compounds **17** and **18** were the most active with EC₅₀ 6.9 and 5.5 μM, respectively against Coxsackievirus B5 (CV-B5) and 20.5 and 17.5 μM against Poliovirus (Sb-1).

Conclusion:

We can conclude that *N*-(4-(2*H*-benzo[d][1,2,3]triazol-2-yl)phenyl)-*R*-amide is a good chemical scaffold for the development of new antiviral molecules.

Keywords: Benzotriazole, Enteroviruses, CV-B5, Poliovirus, SARs, Antiviral.

Article History

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1. INTRODUCTION

Benzotriazole and its derivatives have a great importance in the biomedical research. The chemical activities of this scaffold are manifold; it can be used as intermediate [1 - 3], leaving group [4], or inserted into other chemical structures [5]. The antimicrobial activity of benzotriazole derivatives has been extensively investigated since the late 1980s and several authors disclosed the biological evaluation of imidazole derivatives and benzotriazole analogues as antibacterial, antimycotic, antitubercular, and antiviral agents [6]. The

number of viruses and the viral infections are continuously increasing. Poverty, rapid urbanization, evolving human migration patterns, re-emerging viruses, all contribute to the expanding impact of viral diseases. Environmental variations and derangements caused by changes in the climate and alterations to the ecology caused by man have increased the possibility for new contacts between the various factors involved, giving rise conditions for the emergence of new infections. This is reflected by the recent emergence of viral outbreaks caused by West Nile virus (WNV), severe acute respiratory syndrome (SARS) virus, Enterovirus A71 and D68, influenza virus, measles virus (MV) [7 - 9] and coronavirus (SARS-CoV-2) nowadays [10]. While vaccine efforts have been proven successful for preventing and eradicating some viral infections, for many viruses there is not any vaccine,

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efficacious drugs for prophylaxis or therapeutic treatments available. Thus, more attempts in developing antiviral therapies are needed to protect public health against a range of important viral pathogens. Progress has been made recently on azole derivatives as antibacterial, antifungal, antitubercular and antiviral agents, including mono-nitrogen azoles (oxazoles, thiazoles and carbazoles), bis-nitrogen azoles (imidazoles, pyrazoles and benzimidazoles) and tri-nitrogen azoles (triazoles and benzotriazoles) [11 - 14]. Starting from these considerations and among a multi-annual antiviral research program, in the last years, we described the chemical characterization and antiviral activity of several series of azole derivatives as benzimidazole, benzotriazoles [15 - 17], and a large series of both benzimidazole and benzotriazole derivatives condensed with a pyridine ring [18, 19]. Among these azoles, benzotriazoles derivatives bearing one or two chlorine atoms on the benzene moiety of benzotriazole, in positions 5 and 6, have proved to be of particular interest. From an extensive screening against both RNA and DNA viruses, these derivatives were found to be active against BVDV and hRSV [16], while the elimination of a chlorine atom on the

same scaffold resulted in the loss of activity on all viral strains tested [16].

This project aimed to evaluate how changes on the benzotriazole scaffold, or in the amide portion, could affect the antiviral activity of previously reported derivatives. Starting from compounds with general structure depicted in Fig. (1), which turned out active towards Coxsackieviruses, BVDV and hRSV [16], we designed and synthesised a series of derivatives **4-7c**, **8a,b,c-15a,b,c**, **16c-26c** (underline-labelled in Fig. (2)). and compared their antiviral activity with previously published compounds (**4a,b-26a,b**, **16d-18d**, **20d-26d**; not underline-labelled in Fig. (2)). describing Structure-activity Relationships (SARs).

In all these derivatives, we evaluated the role of the substitution of the benzotriazole ring with hydrogen, fluorine, chlorine atoms, or methyl groups. Similarly, the replacement of the amide moiety with an ureidic function on all series of new benzotriazole derivatives was assessed, since previously synthesised similar compounds (**16a,b-26a,b**, **16d-18d**, **20d-26d**) had shown interesting antiviral activity [16, 17], modification of the chemical scaffold are outlined in Fig. (3).

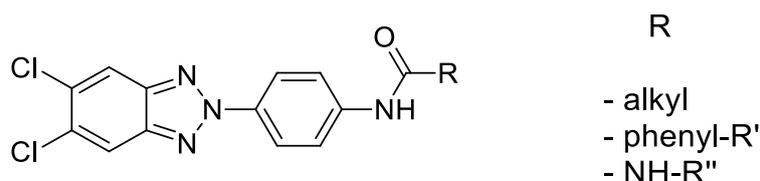


Fig. (1). General structure of previously reported compounds.

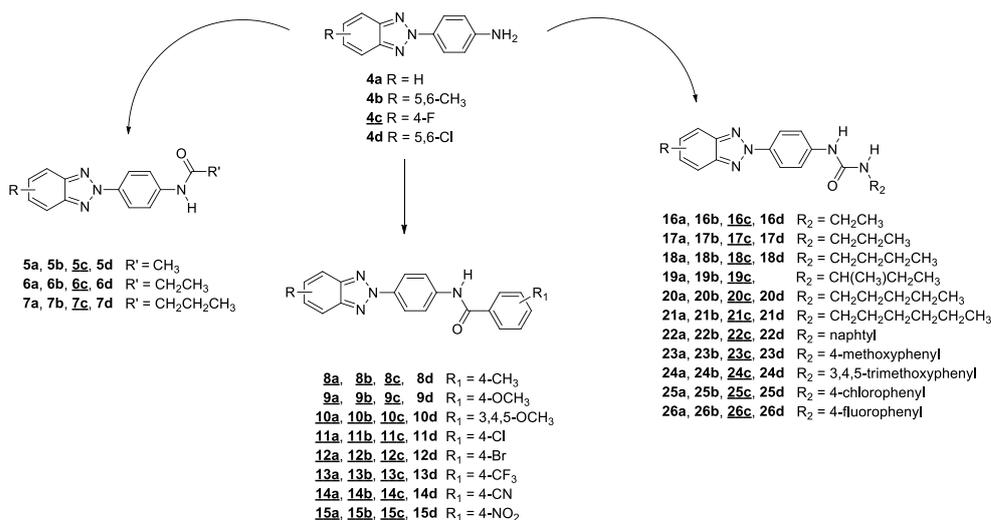


Fig. (2). Panel of all molecules analysed for SARs.

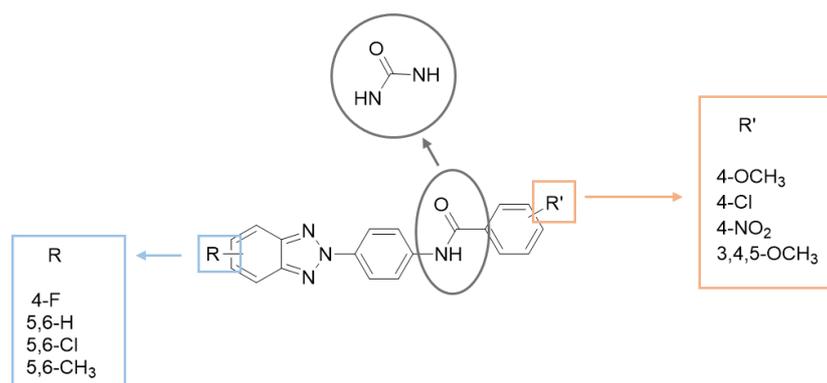


Fig. (3). Chemical modification on benzotriazole scaffold to obtain intended derivatives.

All newly prepared derivatives were assayed against representatives positive- and negative- sense single-stranded RNA, double-stranded RNA viruses and DNA viruses. In order to establish whether tested compounds worked selectively as antiviral agents, their cytotoxicity against cell monolayers (in stationary growth) analogous to those supporting the replication of the above viruses, but left uninfected, was evaluated in parallel with their antiviral activity.

2. MATERIALS AND METHODS

2.1. Synthetic Methods

Melting points (m.p.) were carried out with a Kofler hot stage or Digital Electrothermal melting point apparatus. Nuclear magnetic resonance ($^1\text{H-NMR}$ and $^{13}\text{C-NMR}$) spectra were determined in CDCl_3 or DMSO-d_6 and recorded with a Bruker Avance III 400 NanoBay. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) used as internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; quin, quintet; sext, sextet; sept, septet; m, multiplet; br s, broad singlet; dd, double doublet. Mass spectra (MS) were performed on the combined Liquid Chromatograph-Agilent 1100 series Mass Selective Detector (MSD). Analytical thin-layer Chromatography (TLC) was performed on Merck silica gel F-254 plates. Pure compounds showed a single spot in TLC. For flash chromatography, Merck silica gel 60 was used with a particle size of 0.040-0.063mm (230-400 mesh ASTM). Elemental analyses were performed on a Perkin-Elmer 2400 instrument and the results were within $\pm 0.4\%$ of theoretical values.

2.2. Starting Material and Known Intermediates

Anhydrides, benzoyl derivatives, 1-chloro-4-nitrobenzene, and inorganic reagent were commercially available. The intermediate 3-fluorobenzene-1,2-diamine, common to the whole series, was prepared according to the procedures described in the literature [20, 21]. Details of the synthesis of each compound are provided as follows.

2.3. Cells and Viruses

Cell lines were purchased from American Type Culture

Collection (ATCC). Cell lines supporting the multiplication of virus were following: Monkey kidney (Vero-76) [ATCC CRL 1587 *Cercopithecus Aethiops*]. Viruses were purchased from the American Type Culture Collection (ATCC). Viruses representative of positive-sense, single-stranded RNAs (ssRNA⁺) were: *Picornaviridae*: human enterovirus B [coxsackie type B5 (CV-B5), strain Faulkner (ATCC VR-185)], and human enterovirus C [poliovirus type-1 (Sb-1), Sabin strain Chat (ATCC VR-1562)]. Cell cultures were checked periodically for the absence of mycoplasma contamination with MycoTect Kit (Gibco). Viruses were maintained in our laboratory and propagated in appropriate cell lines. The viruses were stored in small aliquots at $-80\text{ }^\circ\text{C}$ until use.

2.4. Cytotoxicity Assays

Vero-76 cells were seeded in 96-well plates at an initial density of 5×10^5 cells/mL, in Dulbecco's Modified Eagle Medium (D-MEM) with L-glutamine and 25 mg/L kanamycin, supplemented with 10% FBS. Cell cultures were then incubated at $37\text{ }^\circ\text{C}$ in a humidified, 5% CO_2 atmosphere, in the absence or presence of serial dilutions of test compounds. The test medium used for the cytotoxic assay as well as for antiviral assay contained 1% of the appropriate serum. Cell viability was determined after 72 hrs at $37\text{ }^\circ\text{C}$ by the MTT method [22].

2.5. Antiviral Assay

Compound's activity against CV-B5 and Sb-1 was determined by plaque reduction assays in infected cell monolayers. Briefly, a monolayer of Vero-76 cells was grown overnight on 24-well plate. The cells were then infected for 2 hrs with 250 μL of proper virus dilutions to give 50-100 PFU/well. Following the removal of unadsorbed virus, 500 μL of the medium [D-MEM with L-glutamine and 4500 mg/L D-glucose, supplemented with 1% inactivated FBS] containing 0.75% methylcellulose, with serial dilutions of test compounds, were added. The overlaid medium was also added to not treated wells as non-infection controls. Cultures were incubated at $37\text{ }^\circ\text{C}$ for 2 (Sb-1), 3 (CV-B5) days and then fixed with PBS containing 50% ethanol and 0.8% crystal violet, washed and air-dried. Plaques in the control (no inhibitor) and experimental wells were then counted.

2.6. Linear Regression Analysis

The extent of cell growth/viability and viral multiplication, at each drug concentration tested, were expressed as a percentage of untreated controls. Concentrations resulting in 50% inhibition (CC₅₀ or EC₅₀) were determined by linear regression analysis.

2.7. Experimental Section

2.7.1. General procedure for the preparation of 7-fluoro-1-(4-nitrophenyl)-1H-benzo[d][1-3]triazole (1), 4-fluoro-2-(4-nitrophenyl)-2H-benzo[d][1-3]triazole (2) and 4-fluoro-1-(4-nitrophenyl)-1H-benzo[d][1-3]triazole (3).

To a solution of 2 g (14.6 mmol) of 4-fluoro-1H-benzo[d][1-3]triazole in 14 mL of dimethyl acetamide (DMA), 1-chloro-4-nitrobenzene (2.3 g, 14.6 mmol) and Cs₂CO₃ (9.51 g, 29.2 mmol) were added. The mixture was stirred at 75°C for 36 h. The precipitate was filtered by washing a little amount of water to remove Cs₂CO₃, and the filtrate was poured on ice. The obtained light-yellow precipitate was the mixture of the three isomers and was filtered under vacuum. The three isomers were separated by flash chromatography using petroleum ether/ethyl acetate in different ratio as eluting system. The assignment of the correct structure to the isomers (1), (2) and (3) was achieved from NOESY experiment as discussed in paragraph 2.1 of this article.

2.7.1.1. 7-fluoro-1-(4-nitrophenyl)-1H-benzo[d][1-3]triazole (1)

Compound (1) was purified by silica gel column chromatography eluting with a 9:1 mixture of petroleum ether/ethyl acetate. Light-yellow solid, m.p.: 188-191°C. Yield, 1%. TLC (petroleum ether/ethyl acetate 9:1): R_f 0.22. ¹H-NMR (DMSO, 400 MHz) δ: 8.51 (2H, d, J = 8.4 Hz, H-3',5'), 8.13 (2H, d, J = 8.4 Hz, H-2',6'), 8.091 (1H, d, J = 8 Hz, H-4), 7.59-7.55 (2H, m, H-5,6). ¹³C-NMR (CDCl₃, 100 MHz) δ: 149.5 (C, C-7), 148.4 (C, C-3a), 147.3 (C, C-7a), 145.7 (C, C-4'), 140.9 (C, C-1'), 126.0 (CH, C-5), 125.49 (CH, C-2', C-6'), 124.91 (CH, C-3', 5'), 116.20 (CH, C-4), 114.23 (CH, C-6). LC/MS: m/z 259 (M+H). Anal. Calcd. (%) C₁₂H₇FN₄O₂: C, 55.82; H, 2.73; N, 21.70. Found: C, 55.94; H, 2.77; N, 21.84.

2.7.1.2. 4-fluoro-2-(4-nitrophenyl)-2H-benzo[d][1-3]triazole (2)

Compound (2) was purified by silica gel column chromatography eluting with a 9.8:0.2 mixture of petroleum ether/ethyl acetate. Light-yellow solid, m.p.: 234-237°C. Yield, 37%. TLC (petroleum ether/ethyl acetate 9:1): R_f 0.46. ¹H-NMR (DMSO, 400 MHz) δ: 8.59 (2H, d, J = 9.2 Hz, H-2,6), 8.51 (2H, d, J = 9.2 Hz, H-3,5), 7.93 (1H, d, J = 8.8 Hz, H-7), 7.60-7.54 (1H, m, H-6), 7.41 (1H, dd, J = 7.2 and 10.8 Hz, H-5). ¹³C-NMR (DMSO, 100 MHz) δ: 152.8 (C, C-4), 147.5 (C, C-7a), 147.3 (C, C-1'), 136.2 (C, C-3a), 134.1 (C, C-4'), 125.6 (CH, C-6), 124.4 (CH, C-7), 121.5 (CH, C-2', C-6'), 115.1 (CH, C-3', 5'), 111.6 (CH, C-5). LC/MS: m/z 259 (M+H). Anal. Calcd. (%) C₁₂H₇FN₄O₂: C, 55.82; H, 2.73; N, 21.70. Found: C, 55.92; H, 2.78; N, 21.85.

2.7.1.3. 4-fluoro-1-(4-nitrophenyl)-1H-benzo[d][1-3]triazole (3)

Compound (3) was purified by silica gel column chromatography eluting with a 8.5:1.5 mixture of petroleum ether/ethyl acetate. Light-yellow solid, m.p.: 233-236°C. Yield, 2%. TLC (petroleum ether/ethyl acetate 9:1): R_f 0.16. ¹H-NMR (DMSO, 400 MHz) δ: 8.52 (2H, d, J = 8.8 Hz, H-3', 5'), 8.25 (2H, d, J = 8.8 Hz, H-2', 6'), 7.92 (1H, d, J = 8.4 Hz, H-7), 7.61 (1H, m, H-6), 7.44 (1H, t, H-5). ¹³C-NMR (DMSO, 100 MHz) δ: 152.4 (C, C-4), 146.9 (C, C-7a), 140.8 (C, C-1'), 136.0 (C, C-3a), 134.4 (C, C-4'), 125.6 (CH, C-6), 123.4 (CH, C-2', C-6'), 110.0 (CH, C-7), 109.9 (CH, C-3', 5'), 107.9 (CH, C-5). LC/MS: m/z 259 (M+H). Anal. Calcd. (%) C₁₂H₇FN₄O₂: C, 55.82; H, 2.73; N, 21.70. Found: C, 55.90; H, 2.80; N, 21.83.

2.7.2. 4-(4-fluoro-2H-benzo[d][1-3]triazol-2-yl)aniline (4c)

Compound (4c) was obtained by dissolving 2g of derivative 2 (7.74 mmol) in 269 mL of ethanol followed by the addition of 10% Palladium on activated charcoal and hydrazine hydrate (3.47 g, 108.08 mmol). The mixture was heated at 110°C for 20 h, after cooling the catalyst was filtered and washed with ethanol, the filtrate was evaporated under reduced pressure. The crude product was purified by flash chromatography using petroleum ether/ethyl acetate 7:3 as eluting system. 933 mg of 4c was obtained as a light-yellow solid, m.p. 167-169°C (from EtOH). Yield: 53%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.41. ¹H-NMR (CDCl₃, 400 MHz) δ: 8.05 (2H, d, J = 7.6 Hz, H-2, 6), 7.62 (1H, d, J = 8.4 Hz, H-7), 7.26-7.21 (1H, m, H-6), 6.96 (1H, t, H-5), 6.73 (2H, d, J = 7.6 Hz, H-3', 5'), 3.89 (2H, br s, NH₂). ¹³C-NMR (CDCl₃, 100 MHz) δ: 152.5 (C, C-4), 147.8 (C, C-7a), 147.7 (C, C-1'), 135.9 (C, C-3a), 131.8 (C, C-4'), 126.5 (CH, C-6), 122.2 (CH, C-3', C-5'), 115.0 (CH, C-2', C-6'), 114.0 (CH, C-7), 109.8 (CH, C-5). LC/MS: m/z 229 (M+H). Anal. Calcd. (%) C₁₂H₉FN₃: C, 63.15; H, 3.97; N, 24.55. Found: C, 63.24; H, 4.06; N, 24.65.

2.7.3. General procedure for the preparation of N-(4-(4-fluoro-2H-benzo[d][1-3]triazol-2-yl)phenyl)amide derivatives (5c-7c).

To 4-(4-fluoro-2H-benzo[d][1-3]triazol-2-yl)aniline (4c) (200 mg, 0.87 mmol) was added an excess (26.1 mmol) of either required anhydride (acetic anhydride, propionic anhydride and butyric anhydride). The resulting mixture was stirred at 100°C for different times as reported below, then it was cooled to room temperature and crushed ice was added. The solid obtained in each reaction was filtered under vacuum. Purification by flash chromatography using as eluting system, a mixture of petroleum ether/ethyl acetate in different ratios as reported below, furnished the amide derivatives 5c-7c.

2.7.3.1. N-(4-(4-fluoro-2H-benzo[d][1-3]triazol-2-yl)phenyl)acetamide (5c)

Compound (5c) was obtained as described in the general procedure starting from 4c and acetic anhydride for 6h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 191-194°C (from

EtOH). Yield, 28%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.45. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 10.13 (1H, br s, NH), 8.52 (2H, d, $J=8.4$ Hz, H-3,5), 7.65 (1H, d, $J=8.8$ Hz, H-7), 7.38 (2H, d, $J=8.8$ Hz, H-2,6), 7.40-7.27 (1H, m, H-6), 7.09 (1H, t, H-5), 2.35 (3H, s, CH_3). $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 172.6 (C, CO), 152.6 (C, C-4), 147.6 (C, C-7a), 140.2 (C, C-1'), 140.1 (C, C-4'), 136.7 (C, C-3a), 131.0 (CH, C-3', C-5'), 127. (CH, C-6), 122.0 (CH, C-2', C-6'), 114.6 (CH, C-7), 110.7 (CH, C-5), 25.8 (CH_3). LC/MS: m/z 271 (M+H). Anal. Calcd. (%) $\text{C}_{14}\text{H}_{11}\text{FN}_4\text{O}$: C, 62.22; H, 4.10; N, 20.73. Found: C, 62.33; H, 4.15; N, 20.80.

2.7.3.2. N-(4-(4-fluoro-2H-benzod[1,3] triazol-2-yl) phenyl) propionamide (6c)

Compound (6c) was obtained as described in the general procedure starting from 4c and propionic anhydride for 72h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Ocre yellow solid, m.p.: 179-182°C (from EtOH). Yield, 26%. TLC (petroleum ether/ethyl acetate 6:4): R_f 0.51. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ : 8.319 (2H, d, $J=8.8$ Hz, H-3,5), 7.76 (1H, d, $J=8.4$ Hz, H-7), 7.70 (2H, d, $J=8.8$ Hz, H-2,6), 7.48 (1H, br s, NH), 7.36-7.26 (1H, m, H-6), 7.07-7.05 (1H, t, H-5), 2.45 (2H, d, CH_2), 1.28 (3H, t, CH_3). $^{13}\text{C-NMR}$ (CDCl_3 , 100 MHz) δ : 172.2 (C, CO), 152.5 (C, C-4), 147.4 (C, C-7a), 139.1 (C, C-1'), 136.2 (C, C-4'), 135.9 (C, C-3a), 127.0 (CH, C-6), 121.4 (CH, C-3', C-5'), 118.2 (CH, C-2', C-6'), 114.3 (CH, C-7), 110.2 (CH, C-5), 30.8 (CH_2 , CH_2CH_3), 9.55 (CH_3 , CH_2CH_3). LC/MS: m/z 285 (M+H). Anal. Calcd. (%) $\text{C}_{15}\text{H}_{13}\text{FN}_4\text{O}$: C, 63.37; H, 4.61; N, 19.71. Found: C, 64.07; H, 4.69; N, 19.75.

2.7.3.3. N-(4-(4-fluoro-2H-benzod[1,3] triazol-2-yl) phenyl) butyramide (7c)

Compound (7c) was obtained as described in the general procedure starting from 4c and butyric anhydride for 120h. The crude product was purified by silica gel column chromatography eluting with a 9:1 mixture of petroleum ether/ethyl acetate. Wight solid, m.p.: 208-209°C (from EtOH). Yield, 31%. TLC (petroleum ether/ethyl acetate 8:2): R_f 0.48. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.25 (1H, br s, NH), 8.26 (2H, d, $J=8.8$ Hz, H-3,5), 7.89 (2H, d, $J=8.8$ Hz, H-2,6), 7.88 (1H, d, $J=8.0$ Hz, H-7), 7.52-7.47 (1H, m, H-6), 7.35-7.30 (1H, m, H-5), 2.35 (2H, t, CH_2), 1.65 (2H, sext., CH_2), 0.94 (3H, t, CH_3). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 171.8 (C, CO), 151.4 (C, C-4), 146.8 (C, C-7a), 140.5 (C, C-1'), 135.2 (C, C-3a), 134.2 (C, C-4'), 127.6 (CH, C-6), 120.9 (CH, C-3', C-5'), 119.7 (CH, C-2', C-6'), 112.6 (CH, C-7), 110.7 (CH, C-5), 18.4 (CH_2 , $\text{CH}_2\text{-CH}_2\text{CH}_3$), 13.5 (CH_3 , $\text{CH}_2\text{-CH}_2\text{CH}_3$). LC/MS: m/z 299 (M+H). Anal. Calcd. (%) $\text{C}_{16}\text{H}_{15}\text{FN}_4\text{O}$: C, 64.42; H, 5.07; N, 18.78. Found: C, 64.51; H, 4.75; N, 19.82.

2.7.4. General procedure for the preparation of N-(4-(4-fluoro-2H-benzod[1,3]triazol-2-yl)phenyl)benzamide derivatives (8a,b,c-15a,b,c).

The respective amines 4a-c (166 mmol) were solubilized in 8-10 ml of DMF (compounds a,b) or DMA (compounds c) and

an equimolar amount (compounds c) or an equimolar amount with 20% in excess (compounds a,b) of the required benzoyl chloride derivatives was added. The solution was stirred at 80°C for different times as reported below, then it was cooled to room temperature and crushed ice was added. The reaction products were solid or a gummy oil; The solid obtained was filtered under vacuum, while the gummy oil was treated with ether. Purification by flash chromatography using as eluting system, a mixture of petroleum ether/ethyl acetate in different ratios as reported below, furnished the benzamide derivatives 8a,b,c-15a,b,c.

2.7.4.1. N-(4-(2H-benzod[1,3] triazol-2-yl) phenyl) -4-methylbenzamide (8a)

Compound (8a) was obtained as described in the general procedure starting from 4a and 4-methylbenzoyl chloride for 24h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 266-268°C (from EtOH). Yield, 80%. TLC (petroleum ether/ethyl acetate 1:1): R_f 0.90. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.49 (1H, s, NHCO), 8.32 (2H, d, $J=8.4$ Hz, H-2', 6'), 8.09 (2H, d, $J=8.4$ Hz, H-3', 5'), 8.05-8.00 (2H, m, H-4, 7), 7.92 (2H, d, $J=7.6$ Hz, H-2'', 6''), 7.53-7.50 (2H, m, H-5, 6), 7.37 (2H, d, $J=7.6$ Hz, H-3'', 5''). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 165.6 (C, CO), 144.3 (C, C-4'), 141.9 (C, C-3a, C-7a), 140.4 (C, C-4'), 134.9 (C, C-1'), 131.7 (C, C-1''), 128.9 (CH, C-3'', C-5''), 127.8 (CH, C-2'', C-6''), 127.4 (CH, C-5, C-6), 120.9 (CH, C-2', C-6'), 120.7 (CH, C-3', C-5'), 118.0 (CH, C-4, C-7), 21.0 (CH_3). LC/MS: m/z : 329 (M+H). Anal. Calcd (%) $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}$: C, 73.15; H, 4.91; N, 17.06. Found: C, 73.41; H, 4.98; N, 16.91.

2.7.4.2. N-(4-(2H-benzod[1,3]triazol-2-yl)phenyl)-4-methoxy benzamide (9a)

Compound (9a) was obtained as described in the general procedure starting from 4a and 4-methoxybenzoyl chloride for 48h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 217-218°C (from EtOH). Yield, 90%. TLC (petroleum ether/ethyl acetate 1:1): R_f 0.81. $^1\text{H-NMR}$ (DMSO, 400MHz) δ : 10.42 (1H, s, NHCO), 8.31 (2H, d, $J=8.4$ Hz, H-2'', 6''), 8.08 (2H, d, $J=8.0$ Hz, H-3'', 5''), 8.18-7.98 (4H, m, H-2', 6', 3', 5'), 7.53-7.49 (2H, m, H-4, 7), 7.15-7.03 (2H, m, H-5, 6). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 165.1 (C, CO), 162.1 (C, O-C), 144.3 (C, C-3a, C-7a), 140.5 (C, C-4'), 134.8 (C, C-1'), 129.7 (CH, C-2'', C-6''), 127.4 (CH, C-5, C-6), 126.6 (C, C-1''), 120.8 (CH, C-2', C-6'), 120.7 (CH, C-3', C-5'), 118.1 (CH, C-4, C-7), 113.7 (CH, C-3'', C-5''), 55.4 (OCH_3). LC/MS: m/z : 345 (M+H). Anal. Calcd (%) $\text{C}_{20}\text{H}_{16}\text{N}_4\text{O}_2$: C, 69.76; H, 4.68; N, 16.27. Found: C, 69.41; H, 4.43; N, 16.50.

2.7.4.3. N-(4-(2H-benzod[1,3] triazol-2-yl) phenyl)-3,4,5-trimethoxybenzamide (10a)

Compound (10a) was obtained as described in the general procedure starting from 4a and 3,4,5-trimethoxybenzoyl chloride for 2 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of

petroleum ether/ethyl acetate. Light yellow solid, m.p.: 217-229°C (from EtOH). Yield, 30%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.29. ^1H NMR (DMSO, 400 MHz) δ : 10.44 (1H, s, NHCO), 8.34 (2H, d, $J=8.4$ Hz, H-2', 6'), 8.04-8.00 (4H, m, H-4, 7, 3', 5'), 7.53-7.50 (2H, m, H-5, 6), 7.33-7.28 (2H, m, H-2'', 6''). ^{13}C -NMR (DMSO, 100 MHz) δ : 165.2 (C, CO), 152.6 (C, C-3'', C-5''), 144.4 (C, C-3a, C-7a), 140.4 (C, C-4''), 135.0 (C, C-1''), 129.7 (C, C-1', C-4'), 127.5 (CH, C-5, C-6), 121.2 (CH, C-2', C-6'), 120.7 (CH, C-3', C-5'), 118.0 (CH, C-4, C-7), 105.4 (CH, C-2'', C-6''), 60.1 (OCH₃-C4''), 56.1 (OCH₃-C3'', OCH₃-C5''). LC/MS: m/z : 405 (M+H). Anal. Calcd (%) C₂₂H₂₀N₄O₄: C, 65.34; H, 4.98; N, 13.85. Found: C, 64.98; H, 4.63; N, 14.20.

2.7.4.4. *N*-(4-(2H-benzof[d][1-3] triazol-2-yl) phenyl) -4-chlorobenzamide (11a)

Compound (11a) was obtained as described in the general procedure starting from **4a** and 4-chlorobenzoyl chloride for 2 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 280-281°C (from EtOH). Yield, 68%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.83. ^1H -NMR (DMSO, 400 MHz) δ : 10.64 (1H, s, NHCO), 8.33 (2H, d, $J=8.4$ Hz, H-2', 6'), 8.08 (2H, d, $J=8.8$ Hz, H-2'', 6''), 8.08-8.00 (4H, m, H-3', 5', 3'', 5''), 7.69-7.63 (2H, m, H-4, 7), 7.57-7.50 (2H, m, H-5, 6). ^{13}C -NMR (DMSO, 100 MHz) δ : 164.7 (C, CO), 144.4 (C, C-3a, C-7a), 140.0 (C, C-4''), 136.6 (C, C-4'), 135.1 (C, C-1'), 133.3 (C, C-1''), 129.7 (CH, C-2'', C-6''), 128.5 (CH, C-3'', C-5''), 127.5 (CH, C-5, C-6), 121.0 (CH, C-2', C-6'), 120.7 (CH, C-3', C-5'), 118.0 (CH, C-4, C-7). LC/MS: m/z : 349 (M+H). Anal. Calcd (%) C₁₉H₁₃ClN₄O: C, 65.43; H, 3.76; N, 16.06. Found: C, 65.66; H, 4.04; N, 16.00.

2.7.4.5. *N*-(4-(2H-benzof[d][1-3] triazol-2-yl) phenyl) -4-bromobenzamide (12a)

Compound (12a) was obtained as described in the general procedure starting from **4a** and 4-bromobenzoyl chloride for 2 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 248-249°C (from EtOH). Yield, 79%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.72. ^1H -NMR (DMSO, 400 MHz) δ : 10.64 (1H, s, NHCO), 8.33 (2H, d, $J=8.4$ Hz, H-2', 6'), 8.07 (2H, d, $J=8.4$ Hz, H-3', 5'), 8.03-8.01 (2H, m, H-4, 7), 7.96 (2H, d, $J=8.0$ Hz, H-2'', 6''), 7.79 (2H, d, $J=8.4$ Hz, H-3'', 5''), 7.53-7.49 (2H, m, H-5, 6). ^{13}C -NMR (DMSO, 100 MHz) δ : 164.8 (C, CO), 144.4 (C, C-3a, C-7a), 140.0 (C, C-4'), 135.1 (C, C-1'), 133.7 (C, C-1''), 131.5 (CH, C-3'', C-5''), 129.9 (CH, C-2'', C-6''), 127.5 (CH, C-5, C-6), 125.6 (C, C-4''), 121.0 (CH, C-2', C-6'), 120.8 (CH, C-3', C-5'), 118.1 (CH, C-4, C-7). LC/MS: m/z : 395 (M+H). Anal. Calcd (%) C₁₉H₁₃BrN₄O: C, 58.03; H, 3.33; N, 14.25. Found: C, 58.23; H, 3.54; N, 14.00.

2.7.4.6. *N*-(4-(2H-benzof[d][1-3] triazol-2-yl)phenyl)-4-(trifluoromethyl)benzamide (13a)

Compound (13a) was obtained as described in the general procedure starting from **4a** and 4-trifluorobenzoyl chloride for

2 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 277-278°C (from EtOH). Yield, 72%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.79. ^1H -NMR (DMSO, 400 MHz) δ : 10.79 (1H, s, NHCO), 8.35 (2H, d, $J=8.8$ Hz, H-2', 6'), 8.20 (2H, d, $J=7.6$ Hz, H-2'', 6''), 8.09 (2H, d, $J=8.4$ Hz, H-3', 5'), 8.03-8.00 (2H, m, H-4, 7), 7.96 (2H, d, $J=8.0$ Hz, H-3'', 5''), 7.54-7.50 (2H, m, H-5, 6). ^{13}C -NMR (DMSO, 100 MHz) δ : 164.6 (C, CO), 144.4 (C, C-3a, C-7a), 139.9 (C, C-4', C-1''), 138.4 (C, C-1'), 135.3 (C, C-4''), 131.5 (C, CF₃), 128.7 (CH, C-2'', C-6''), 127.5 (CH, C-5, C-6), 125.4 (CH, C-3'', C-5''), 121.1 (CH, C-2', C-6'), 120.8 (CH, C-3', C-5'), 118.1 (CH, C-4, C-7). LC/MS: m/z : 383 (M+H). Anal. Calcd (%) C₂₀H₁₃F₃N₄O: C, 62.83; H, 3.43; N, 14.65. Found: C, 62.52; H, 3.23; N, 14.86.

2.7.4.7. *N*-(4-(2H-benzof[d][1-3] triazol-2-yl)phenyl)-4-cyanobenzamide (14a)

Compound (14a) was obtained as described in the general procedure starting from **4a** and 4-cyanobenzoyl chloride for 48h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 243-245°C (from EtOH). Yield, 22%. TLC (petroleum ether/ethyl acetate 75:25): R_f 0.13. ^1H -NMR (DMSO, 400 MHz) δ : 10.59 (1H, s, NHCO), 8.40 (2H, d, $J=13.6$ Hz, H-4, 7), 8.34 (2H, d, $J=8.4$ Hz, H-2', 6'), 8.14-8.06 (3H, m, H-5, 2'', 6''), 7.95 (2H, d, $J=20.4$ Hz, H-3', 5'), 7.58-7.51 (3H, m, H-6, 3'', 5''). ^{13}C -NMR (DMSO, 100 MHz) δ : 165.0 (C, CO), 145.0 (C, C-3a, C-7a), 138.9 (C, C-1''), 137.7 (C, C-4'), 134.9 (C, C-1'), 132.2 (CH, C-3'', C-5''), 128.7 (CH, C-2'', C-6''), 126.9 (CH, C-5, C-6), 121.1 (CH, C-2', C-6'), 120.9 (CH, C-3', C-5'), 120.2 (CH, C-4, C-7), 118.4 (C, CN), 116.6 (C, C-4''). LC/MS: m/z : 340 (M+H). Anal. Calcd (%) C₂₀H₁₃N₅O: C, 70.79; H, 3.86; N, 20.64. Found C, 70.49; H, 3.52; N, 20.74.

2.7.4.8. *N*-(4-(2H-benzof[d][1-3] triazol-2-yl)phenyl)-4-nitrobenzamide (15a)

Compound (15a) was obtained as described in the general procedure starting from **4a** and 4-nitrobenzoyl chloride for 2h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: > 300°C (from EtOH). Yield, 15%. TLC (petroleum ether/ethyl acetate 1:1): R_f 0.91. ^1H -NMR (DMSO, 400 MHz) δ : 10.90 (1H, s, NHCO), 8.41 (2H, d, $J=8.0$ Hz, H-2', 6'), 8.35 (2H, d, $J=8.4$ Hz, H-2'', 6''), 8.24 (2H, d, $J=8.4$ Hz, H-3', 5'), 8.09 (2H, d, $J=8.8$ Hz, H-3'', 5''), 8.08-8.00 (2H, m, H-4, 7), 7.58-7.50 (2H, m, H-5, 6). ^{13}C -NMR (DMSO, 100 MHz) δ : 164.2 (C, CO), 149.3 (C, C-4''), 144.4 (C, C-3a, C-7a), 140.3 (C, C-4'), 139.8 (C, C-1''), 135.4 (C, C-1'), 129.3 (CH, C-2'', C-6''), 127.5 (CH, C-5, C-6), 123.6 (CH, C-3'', C-5''), 121.2 (CH, C-2', C-6'), 120.8 (CH, C-3', C-5'), 118.1 (CH, C-4, C-7). LC/MS: m/z : 360 (M+H). Anal. Calcd (%) C₁₉H₁₃N₅O₃: C, 63.51; H, 3.65; N, 19.49. Found: C, 63.40; H, 3.35; N, 19.60.

2.7.4.9. *N*-(4-(5,6-dimethyl-2H-benzof[d][1-3] triazol-2-yl)phenyl)-4-methylbenzamide (8b)

Compound (**8b**) was obtained as described in the general procedure starting from **4b** and 4-methylbenzoyl chloride for 24 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. white solid, m.p.: 295-296°C (from EtOH). Yield, 46%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.53. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.46 (1H, s, NH), 8.25 (2H, d, $J=9.2$ Hz, H-2', 6'), 8.05 (2H, d, $J=9.2$ Hz, H-3', 5'), 7.91 (2H, d, $J=8.4$ Hz, H-2'', 6''), 7.76 (2H, s, H-4, 7), 7.37 (2H, d, $J=8.4$ Hz, H-3'', 5''), 2.51 (3H, s, CH₃), 2.40 (6H, s, 2CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 165.6 (C, CO), 143.8 (C, C-3a, C-7a), 141.9 (C, C-4''), 139.9 (C, C-4'), 137.9 (C, C-5, C-6), 135.1 (C, C-1'), 131.7 (C, C-1''), 128.9 (CH, C-3'', C-5''), 127.7 (CH, C-2'', C-6''), 121.4 (CH, C-4, C-7), 120.9 (CH, C-2', C-6'), 116.2 (CH, C-3', C-5'), 29.9 (CH₃, CH₃C4''), 21.0 (CH₃, CH₃-C5, CH₃-C6). LC/MS: m/z : 357 (M+H) Anal. Calcd (%) C₂₂H₂₀N₄O: C, 74.14; H, 5.66; N, 15.72. Found: C, 74.24; H, 5.80; N, 16.02.

2.7.4.10. *N*-(4-(5,6-dimethyl-2H-benzof[d][1-3]triazol-2-yl)phenyl)-4-methoxybenzamide (9b)

Compound (**9b**) was obtained as described in the general procedure starting from **4b** and 4-methoxybenzoyl chloride for 24 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Cream solid, m.p.: 285-287°C (from EtOH). Yield, 32%. TLC (petroleum ether/ethyl acetate 6:4): R_f 0.59. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.38 (1H, s, NH), 8.25 (2H, d, $J=8.0$ Hz, H-2', 6'), 8.05 (2H, d, $J=8.4$ Hz, H-3', 5'), 8.01 (2H, d, $J=7.6$ Hz, H-2'', 6''), 7.76 (2H, s, H-4, 7), 7.09 (2H, d, $J=8.0$ Hz, H-3'', 5''), 3.86 (3H, s, OCH₃), 2.40 (6H, s, 2CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 165.1 (C, CO), 162.1 (C, C-4''), 143.8 (C, C-3a, C-7a), 140.0 (C, C-4'), 137.8 (C, C-5, C-6), 134.9 (C, C-1'), 129.7 (CH, C-2'', C-6''), 126.6 (C, C-1''), 120.8 (CH, C-4, C-7), 120.3 (CH, C-2', C-6'), 116.2 (CH, C-3', C-5'), 113.6 (CH, C-3'', C-5''), 55.4 (OCH₃), 20.4 (CH₃, CH₃-C5, CH₃-C6). LC/MS: m/z : 373 (M+H) Anal. Calcd (%) C₂₂H₂₀N₄O₂: C, 70.95; H, 5.41; N, 15.04. Found: C, 70.85; H, 5.40; N, 15.44.

2.7.4.11. *N*-(4-(5,6-dimethyl-2H-benzof[d][1-3] triazol-2-yl)phenyl)-3,4,5-trimethoxybenzamide (10b)

Compound (**10b**) was obtained as described in the general procedure starting from **4b** and 3,4,5-trimethoxybenzoyl chloride for 24 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. beige solid, m.p.: 272-273°C (from EtOH). Yield, 13%. TLC (petroleum ether/ethyl acetate 6:4): R_f 0.34. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.40 (1H, s, NH), 8.28 (2H, d, $J=8.8$ Hz, H-2', 6'), 8.02 (2H, d, $J=9.2$ Hz, H-3', 5'), 7.76 (2H, s, H-2'', 6''), 7.32 (2H, s, H-4, 7), 3.90 (6H, s, 2OCH₃), 3.75 (3H, s, OCH₃), 2.41 (6H, s, 2CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 165.1 (C, CO), 152.6 (C, C-3'', C-5''), 143.8 (C, C-3a, C-7a), 140.5 (C, C-4''), 139.7 (C, C-4'), 137.9 (C, C-5, C-6), 135.2 (C, C-1'), 129.7 (C, C-1''), 121.1 (CH, C-4, C-7), 120.3 (CH, C-2', C-6'), 116.3 (CH, C-3', C-5'),

105.4 (CH, C-2'', C-6''), 60.1 (OCH₃-C4''), 56.1 (OCH₃-C3'', OCH₃-C5''), 20.4 (CH₃, CH₃-C5, CH₃-C6). LC/MS: m/z : 433 (M+H). Anal. Calcd (%) C₂₄H₂₄N₄O₄: C, 66.65; H, 5.59; N, 12.96. Found: C, 66.35; H, 5.42; N, 13.06.

2.7.4.12. 4-chloro-*N*-(4-(5,6-dimethyl-2H-benzof[d][1-3] triazol-2-yl)phenyl)benzamide (11b)

Compound (**11b**) was obtained as described in the general procedure starting from **4b** and 4-chlorobenzoyl chloride for 2 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Whight solid, m.p.: >300°C (from EtOH). Yield, 37%. TLC (petroleum ether/ethyl acetate 6:4): R_f 0.25. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.61 (1H, s, NH), 8.27 (2H, d, $J=8.4$ Hz, H-2', 6'), 8.06-8.02 (4H, m, H-2'', 3'', 5'', 6''), 7.77 (2H, s, H-4, 7), 7.64 (2H, d, $J=8.4$ Hz, H-3', 5'), 2.41 (6H, s, 2CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 153.6 (C, CO), 143.9 (C, C-3a, C-7a), 143.8 (C, C-4'), 138.8 (C, C-4''), 138.3 (C, C-5, C-6), 138.0 (C, C-1'), 137.9 (C, C-1''), 121.1 (CH, C-2'', C-6''), 120.1 (CH, C-3'', C-5''), 119.2 (CH, C-4, C-7), 116.3 (CH, C-2', C-3', C-5', C-6'), 20.42 (CH₃, CH₃-C5, CH₃-C6). LC/MS: m/z : 377 (M+H) Anal. Calcd (%) C₂₁H₁₇ClN₄O: C, 66.93; H, 4.55; N, 14.87. Found: C, 66.83; H, 4.55; N, 15.00.

2.7.4.13. 4-bromo-*N*-(4-(5,6-dimethyl-2H-benzof[d][1-3] triazol-2-yl)phenyl)benzamide (12b)

Compound (**12b**) was obtained as described in the general procedure starting from **4b** and 4-bromobenzoyl chloride for 24 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Whight solid, m.p.: >300°C (from EtOH). Yield, 28%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.58. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.43 (1H, s, NH), 8.36 (2H, s, H-4, 7), 8.23 (2H, d, $J=8.4$ Hz, H-2', 6'), 7.82 (2H, d, $J=8.0$ Hz, H-3', 5'), 7.76 (4H, s, H-2'', 3'', 5'', 6''), 2.40 (6H, s, 2-CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 164.8 (C, CO), 143.8 (C, C-3a, C-7a), 139.6 (C, C-4'), 137.9 (C, C-5, C-6), 133.7 (C, C-4''), 131.5 (CH, C-3'', C-5''), 129.9 (CH, C-2'', C-6''), 125.6 (C, C-1', C-1''), 121.0 (CH, C-4, C-7), 120.4 (CH, C-2', C-6'), 116.3 (CH, C-3', C-5'), 20.4 (CH₃, CH₃-C5, CH₃-C6). LC/MS: m/z : 422 (M+H) Anal. Calcd (%) C₂₁H₁₇BrN₄O: C, 59.87; H, 4.07; N, 13.30. Found: C, 60.87; H, 4.37; N, 13.55.

2.7.4.14. *N*-(4-(5,6-dimethyl-2H-benzof[d][1-3] triazol-2-yl)phenyl)-4-(trifluoromethyl)benzamide (13b)

Compound (**13b**) was obtained as described in the general procedure starting from **4b** and 4-(trifluoromethyl)benzoyl chloride for 24 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. white solid, m.p.: 295-297°C (from EtOH). Yield, 20%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.66. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.76 (1H, s, NH), 8.27 (2H, s, H-4, 7), 8.19 (2H, d, $J=8.0$ Hz, H-2', 6'), 8.06 (2H, t, H-3'', 5''), 7.94 (2H, d, $J=8.0$, H-3', 5'), 7.76 (2H, d, $J=10.0$ Hz, H-2'', 6''), 2.40 (6H, s, 2CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 164.6 (C, CO), 143.8 (C, C-3a, C-7a), 139.9 (C, C-4'), 139.4 (C, C-1''), 138.9 (C, C-1'), 137.9 (C, C-5, C-6), 135.0 (C, C-4''), 131.5 (C, CF₃), 128.7 (CH, C-2'',

C-6"), 125.4 (CH, C-3", C-5"), 121.0 (CH, C-4, C-7), 120.4 (CH, C-2', C-6'), 116.3 (CH, C-3', C-5'), 20.4 (CH₃, CH₃-C5, CH₃-C6). LC/MS: *m/z*: 411 (M+H). Anal. Calcd (%) C₂₂H₁₇F₃N₄O: C, 64.39; H, 4.18; N, 13.65. Found: C, 64.19; H, 4.20; N, 13.84.

2.7.4.15. 4-cyano-N-(4-(5,6-dimethyl-2H-benzof[d][1-3]triazol-2-yl)phenyl)benzamide (14b)

Compound (14b) was obtained as described in the general procedure starting from 4b and 4-cyanobenzoyl chloride for 24 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 294-297°C (from EtOH). Yield, 44%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.40. ¹H-NMR (DMSO, 400 MHz) δ: 10.76 (1H, s, NH), 8.28 (2H, d, *J* = 9.2 Hz, H-2', 6'), 8.14 (2H, d, *J* = 8.4 Hz, H-2", 6"), 8.06-8.04 (4H, m, H-3', 5', 3", 5"), 7.76 (2H, s, H-4, 7), 2.40 (6H, s, 2CH₃). ¹³C-NMR (DMSO, 100 MHz) δ: 164.3 (C, CO), 143.8 (C, C-3a, C-7a), 139.3 (C, C-1"), 138.6 (C, C-4'), 137.9 (C, C-5, C-6), 135.5 (C, C-1'), 132.5 (CH, C-3", C-5") 128.6 (CH, C-2", C-6"), 121.1 (CH, C-4, C-7), 120.4 (CH, C-2', C-6'), 118.2 (C, CN), 116.3 (CH, C-3', C-5'), 114.0 (C, C-4"), 20.41 (CH₃, CH₃-C5, CH₃-C6). LC/MS: *m/z*: 368 (M+H). Anal. Calcd (%) C₂₂H₁₇N₅O: C, 71.92; H, 4.66; N, 19.06. Found: C, 72.00; H, 4.76; N, 19.36.

2.7.4.16. N-(4-(5,6-dimethyl-2H-benzof[d][1-3] triazol-2-yl)phenyl)-4-nitrobenzamide (15b)

Compound (15b) was obtained as described in the general procedure starting from 4b and 4-nitrobenzoyl chloride for 2 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 297-298°C (from EtOH). Yield, 49%. TLC (petroleum ether/ethyl acetate 6:4): R_f 0.75. ¹H-NMR (DMSO, 400 MHz) δ: 10.86 (1H, s, NH), 8.40 (2H, d, *J* = 9.2 Hz, H-3", 5"), 8.30 (2H, d, *J* = 7.6 Hz, H-2', 6'), 8.23 (2H, d, *J* = 7.6 Hz, H-3', 5'), 8.06 (2H, d, *J* = 9.2 Hz, H-2", 6"), 7.77 (2H, s, H-4, 7), 2.41 (6H, s, 2-CH₃). ¹³C-NMR (DMSO, 100 MHz) δ: 165.8 (C, CO), 150.0 (C, C4"), 143.9 (C, C-3a, C-7a), 143.8 (C, C-4'), 138.2 (C, C-5, C-6), 138.0 (C, C-1"), 136.4 (C, C-1'), 130.7 (CH, C-2", C-6"), 129.4 (CH, C-4, C-7), 123.7 (CH, C-3", C-5"), 121.0 (CH, C-2', C-6'), 116.3 (CH, C-3', C-5'), 20.4 (CH₃, CH₃-C5, CH₃-C6). LC/MS: *m/z*: 388 (M+H). Anal. Calcd (%) C₂₁H₁₇N₅O₃: C, 65.11; H, 4.42; N, 18.08. Found: C, 65.00; H, 4.12; N, 18.28.

2.7.4.17. N-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)-4-methylbenzamide (8c)

Compound (8c) was obtained as described in the general procedure starting from 4c and 4-methylbenzoyl chloride for 72 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 249-250°C (from EtOH). Yield, 17%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.59. ¹H-NMR (DMSO, 400 MHz) δ: 10.50 (1H, br s, NH), 8.32 (2H, d, *J* = 8 Hz, H-2", 6"), 8.10 (2H, d, *J* = 8.8 Hz, H-2'-6'), 7.93-7.83 (3H, m, H-3'-5' e H-7), 7.53-7.48 (1H, m, H-6), 7.48-7.29 (3H, m, H-3", 5" and H-5), 2.40 (3H, s, CH₃).

¹³C-NMR (DMSO, 100 MHz) δ: 165.8 (C, CO), 151.3 (C, C-4), 146.9 (C, C-7a), 146.3 (C, C-4"), 142.1 (C, C-4'), 140.5 (C, C-1'), 134.6 (C, C-3a), 131.4 (C, C-1"), 129.0 (CH, C-3", C-5"), 127.7 (CH, C-2", C-6"), 121.3 (CH, C-6), 121.0 (CH, C-2', C-3', C-5', C-6'), 120.7 (CH, C-7), 115.2 (CH, C-5), 21.0 (CH₃). LC/MS: *m/z* 347 (M+H). Anal. Calcd. (%) C₂₀H₁₅FN₄O: C, 69.35; H, 4.37; N, 16.18. Found: C, 69.41; H, 4.43; N, 16.21.

2.7.4.18. N-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)-4-methoxybenzamide (9c)

Compound (9c) was obtained as described in the general procedure starting from 4c and 4-methoxybenzoyl chloride for 168 h. The crude product was purified by silica gel column chromatography eluting with a 8:2 mixture of petroleum ether/ethyl acetate. Cream solid, m.p.: 223-225°C (from EtOH). Yield, 15%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.42. ¹H-NMR (DMSO, 400 MHz): 10.42 (1H, br s, NH), 8.32 (2H, d, *J* = 8.4 Hz, H-2", 6"), 8.06 (2H, d, *J* = 8.4 Hz, H-2', 6'), 8.01 (2H, d, *J* = 8 Hz, H-3'-5'), 7.88 (1H, d, *J* = 8.4 Hz, H-7), 7.53-7.48 (1H, m, H-6), 7.34 (1H, t, H-5), 7.10 (2H, d, *J* = 8 Hz, H-3", 5"), 2.56 (3H, s, OCH₃). ¹³C-NMR (DMSO, 100 MHz) δ: 165.1 (C, CO), 162.1 (C, C-4), 151.5 (C, C-4"), 146.9 (C, C-7a), 144.3 (C, C-1'), 140.9 (C, C-1"), 134.4 (C, C-3a), 129.7 (CH, C-2", C-6"), 127.7 (CH, C-6), 126.5 (C, C-4'), 121.0 (CH, C-2', C-6'), 120.8 (CH, C-3', C-5'), 118.0 (CH, C-7), 114.6 (CH, C-3", C-5"), 112.6 (CH, C-5), 55.4 (OCH₃). LC/MS: *m/z* 363 (M+H). Anal. Calcd. (%) C₂₀H₁₅FN₄O₂: C, 66.29; H, 4.17; N, 15.46. Found: C, 66.38; H, 4.25; N, 15.54.

2.7.4.19. N-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)-3,4,5-trimethoxybenzamide (10c)

Compound (10c) was obtained as described in the general procedure starting from 4c and 3,4,5-trimethoxybenzoyl chloride for 72 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Orange solid, m.p.: 216-219°C (from EtOH). Yield, 26%. TLC (petroleum ether/ethyl acetate 6:4): R_f 0.46. ¹H-NMR (DMSO, 400 MHz) δ: 10.45 (1H, br s, NH), 8.35 (2H, d, *J* = 8.4 Hz, H-3', 5'), 8.07 (2H, d, *J* = 8.4 Hz, H-2', 6'), 7.89 (1H, d, *J* = 8.8 Hz, H-7), 7.54-7.48 (1H, m, H-6), 7.37-7.34 (1H, m, H-5), 7.32 (2H, s, H-2", 6"), 3.92 (6H, s, CH₃), 3.755 (3H, s, CH₃). ¹³C-NMR (DMSO, 100 MHz) δ: 165.2 (C, CO), 152.6 (C, C-4), 151.5 (C, C-3", C-5"), 146.9 (C, C-7a), 140.5 (C, C-4"), 135.0 (C, C-3a), 134.7 (C, C-1'), 129.6 (C, C-4'), 127.7 (CH, C-6), 121.1 (CH, C-2', C-3', C-5', C-6'), 114.6 (CH, C-7), 110.8 (CH, C-5), 105.4 (CH, C-2", C-6"), 60.1 (CH₃, OCH₃-C4"), 56.1 (CH₃, OCH₃-C3", OCH₃-C5"). LC/MS: *m/z* 423 (M+H). Anal. Calcd. (%) C₂₂H₁₉FN₄O₄: C, 62.55; H, 4.53; N, 13.26. Found: C, 62.61; H, 4.60; N, 13.30.

2.7.4.20. 4-chloro-N-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)benzamide (11c)

Compound (11c) was obtained as described in the general procedure starting from 4c and 4-chlorobenzoyl chloride for 192 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum

ether/ethyl acetate. Wight solid, m.p.: 281-283°C (from EtOH). Yield, 69%. TLC (petroleum ether/ethyl acetate 8:2): R_f 0.43. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.63 (1H, br s, NH), 8.33 (2H, d, $J = 8.8$ Hz, H-2'', 6''), 8.08 (2H, d, $J = 8.8$ Hz, H-3'', 5''), 8.03 (2H, d, $J = 8$ Hz, H-3', 5'), 7.92 (1H, s, H-7), 7.63 (2H, d, $J = 8$ Hz, H-2', 6'), 7.54-7.51 (2H, m, H-5,6). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 164.7 (C, CO), 145.2 (C, C-4), 144.4 (C, C-7a), 140.0 (C, C-1'), 137.6 (C, C-4''), 136.6 (C, C-1''), 135.1 (C, C-3a), 133.3 (C, C-4'), 129.7 (CH, C-3'', C-5''), 128.5 (CH, C-2'', C-6''), 127.5 (CH, C-6), 121.1 (CH, C-7), 121.0 (CH, C-2', C-6'), 120.7 (CH, C-3', C-5'), 118.1 (CH, C-5). LC/MS: m/z 369 (M+H). Anal. Calcd. (%) $\text{C}_{19}\text{H}_{12}\text{ClFN}_4\text{O}$: C, 62.22; H, 3.30; N, 15.28. Found: C, 62.30; H, 3.38; N, 15.34.

2.7.4.21. 4-bromo-N-(4-(4-fluoro-2H-benzo[d][1-3] triazol-2-yl)phenyl)benzamide (12c)

Compound (12c) was obtained as described in the general procedure starting from **4c** and 4-bromobenzoyl chloride for 168 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Wight solid, m.p.: 285-286°C (from EtOH). Yield, 9%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.60. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.64 (1H, br s, NH), 8.32 (2H, d, $J = 7.6$ Hz, H-2'', 6''), 8.07 (2H, d, $J = 7.6$ Hz, H-3'', 5''), 8.03 (1H, d, $J = 7.4$ Hz, H-7), 7.96 (2H, d, $J = 7.6$ Hz, H-3', 5'), 7.78 (2H, d, $J = 7.6$ Hz, H-2'-6'), 7.53-7.51 (1H, m, H-6), 7.34 (1H, t, H-5). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 164.9 (C, CO), 151.5 (C, C-4), 144.3 (C, C-7a), 140.0 (C, C-1'), 139.8 (C, C-1''), 135.2 (C, C-3a), 133.5 (C, C-4'), 131.5 (CH, C-3'', C-5''), 129.8 (CH, C-2'', C-6''), 127.6 (CH, C-6), 125.6 (C, C-4''), 121.1 (CH, C-7), 121.0 (CH, C-2', C-6'), 120.8 (CH, C-3', 5'), 118.0 (CH, C-5). LC/MS: m/z 412 (M+H). Anal. Calcd. (%) $\text{C}_{19}\text{H}_{12}\text{BrFN}_4\text{O}$: C, 55.49; H, 2.94; N, 13.62. Found: C, 55.53; H, 2.98; N, 13.68.

2.7.4.22. N-(4-(4-fluoro-2H-benzo[d][1-3] triazol-2-yl)phenyl)-4-(trifluoromethyl)benzamide (13c)

Compound (13c) was obtained as described in the general procedure starting from **4c** and 4-(trifluoromethyl)benzoyl chloride for 72 h. The crude product was purified by silica gel column chromatography eluting with a 8:2 mixture of petroleum ether/ethyl acetate. Beige solid, m.p.: 218-220°C (from EtOH). Yield, 22%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.15. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.79 (1H, 1, br s, NH), 8.32 (2H, d, $J = 8.8$ Hz, H-2'', 6''), 8.15 (2H, d, $J = 7.6$ Hz, H-3'-5'), 8.07-7.10 (3H, m, H-3'', 5'', H-7), 7.92 (2H, d, $J = 7.6$ Hz, H-2', 6'), 7.52-7.47 (1H, m, H-6), 7.32 (1H, t, H-5). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 164.8 (C, CO), 151.4 (C, C-4), 144.3 (C, C-7a), 139.8 (C, C-1'), 138.3 (C, C-1''), 135.1 (C, C-3a), 131.6 (C, C-4''), 128.6 (CH, C-2'', C-6''), 127.6 (CH, C-6), 125.4 (CH, C-7), 125.2 (C, C-1''), 122.5 (C, CF₃), 121.1 (CH, C-3'', C-5''), 120.8 (CH, C-2', C-6'), 118.0 (CH, C-3', C-5'), 112.6 (CH, C-5). LC/MS: m/z 401 (M+H). Anal. Calcd. (%) $\text{C}_{20}\text{H}_{12}\text{F}_3\text{N}_4\text{O}$: C, 60.00; H, 3.02; N, 14.00. Found: C, 60.09; H, 3.08; N, 14.10.

2.7.4.23. 4-cyano-N-(4-(4-fluoro-2H-benzo[d][1-3] triazol-2-yl)phenyl)benzamide (14c)

Compound (14c) was obtained as described in the general procedure starting from **4c** and 4-cyanobenzoyl chloride for 144 h. The crude product was purified by silica gel column chromatography eluting with a 6:4 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 234-235°C (from EtOH). Yield, 21%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.20. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.57 (1H, br s, NH), 8.34 (1H, s, H-7), 8.26 (2H, d, $J = 8.8$ Hz, H-3'', 5''), 7.98 (2H, d, $J = 6.8$ Hz, H-2', 6'), 7.84 (2H, d, $J = 8.8$ Hz, H-2''-6''), 7.50 (2H, d, $J = 6.8$ Hz, H-3'-5'), 7.32-7.28 (1H, m, H-6), 7.30 (1H, t, H-5). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 151.5 (C, CO), 146.9 (C, C-4), 144.3 (C, C-7a), 139.6 (C, C-1'), 139.5 (C, C-1''), 139.2 (C, C-4'), 135.2 (C, C-3a), 135.0 (C, CN), 134.6 (C, C-4''), 127.5 (CH, C-3'', C-5''), 121.5 (CH, C-6), 121.3 (CH, C-7), 121.0 (CH, C-2'', C-6''), 119.9 (CH, C-2', C-6'), 118.0 (CH, C-3', C-5'), 118.0 (CH, C-5). LC/MS: m/z 358 (M+H). Anal. Calcd. (%) $\text{C}_{20}\text{H}_{12}\text{FN}_4\text{O}$: C, 67.22; H, 3.38; N, 19.60. Found: C, 67.31; H, 3.43; N, 19.70.

2.7.4.24. N-(4-(4-fluoro-2H-benzo[d][1-3] triazol-2-yl)phenyl)-4-nitrobenzamide (15c)

Compound (15c) was obtained as described in the general procedure starting from **4c** and 4-nitrobenzoyl chloride for 144 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Light yellow solid, m.p.: 315-317°C (from EtOH). Yield, 79%. TLC (petroleum ether/ethyl acetate 6:4): R_f 0.63. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 10.88 (1H, br s, NH), 8.39 (2H, d, $J = 8.4$ Hz, H-2'', 6''), 8.36-8.33 (2H, m, H-2', 6'), 8.22 (2H, d, $J = 8.4$ Hz, H-3'', 5''), 8.11-8.01 (2H, m, H-3', 5'), 7.88 (1H, d, $J = 8.4$ Hz, H-7), 7.53-7.48 (1H, m, H-6), 7.34 (1H, t, H-5). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 164.2 (C, CO), 151.5 (C, C-4), 146.9 (C, C-7a), 144.4 (C, C-NO₂), 140.2 (C, C-4'), 139.8 (C, C-1''), 135.3 (C, C-3a), 135.0 (C, C-1'), 129.0 (CH, C-2'', C-6''), 127.7 (CH, C-6), 123.6 (CH, C-3'', C-5''), 121.1 (CH, C-2', C-6'), 121.1 (CH, C-7), 120.8 (CH, C-3', C-5'), 110.8 (CH, C-5). LC/MS: m/z 378 (M+H). Anal. Calcd. (%) $\text{C}_{19}\text{H}_{12}\text{FN}_5\text{O}_3$: C, 60.48; H, 3.21; N, 18.56. Found: C, 60.52; H, 3.28; N, 18.62.

2.7.5. General procedure for the preparation of 3-(4-(4-fluoro-2H-benzo[d][1-3] triazol-2-yl)phenyl)urea derivatives (16c-26c)

To a solution of 4-(4-fluoro-2H-benzo[d][1-3]triazol-2-yl)aniline (**4c**) (200 mg, 0.87 mmol) in N,N-dimethylacetamide (DMA, 7 ml) or toluene (7 ml) an excess (2.61 mmol) of the required isocyanate derivatives was added. The resulting solution was stirred at 110°C for different times as reported below, then it was cooled to room temperature and crushed ice was added. The solid obtained in each reaction was filtered under vacuum. Purification by flash chromatography using as eluting system, a mixture of petroleum ether/ethyl acetate or dichloromethane/ethyl acetate in different ratios as reported below, furnished the urea derivatives **16c-26c**.

2.7.5.1. 1-ethyl-3-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)urea (16c)

Compound (16c) was obtained as described in the general procedure starting from **4c** and ethyl isocyanate in DMA for 120 h. The crude product was purified by silica gel column chromatography eluting with a 1:1 mixture of petroleum ether/ethyl acetate. White solid, m.p.: 345-348°C (from EtOH). Yield, 38%. TLC (petroleum ether/ethyl acetate 1:1): R_f 0.38. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 8.86 (1H, br s, NH), 8.19 (2H, d, $J = 7.6$ Hz, H-2',6'), 7.86 (1H, d, $J = 7.4$ Hz, H-7), 7.68 (2H, d, $J = 8.8$ Hz, H-3',5'), 7.47-7.51 (1H, m, H-6), 7.32 (1H, t, H-5), 6.27 (1H, br t, NH), 3.15 (2H, m, CH₂), 1.08 (3H, t, CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 154.8 (C, CO), 151.4 (C, C-4), 146.8 (C, C-7a), 142.1 (C, C-1'), 135.2 (C, C-3a), 132.6 (C, C-4'), 127.4 (CH, C-6), 121.0 (CH, C-3', C-5'), 118.4 (CH, C-2', C-6'), 114.4 (CH, C-7), 110.5 (CH, C-5), 34.0 (CH₂, CH₂CH₃), 15.3 (CH₃, CH₂CH₃). LC/MS: m/z 300 (M+H). Anal. Calcd. (%) C₁₅H₁₄FN₅O: C, 60.19; H, 4.71; N, 23.40. Found: C, 60.32; H, 4.81; N, 23.51.

2.7.5.2. 1-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)-3-propylurea (17c)

Compound (17c) was obtained as described in the general procedure starting from **4c** and 1-propyl isocyanate in DMA for 72 h. The crude product was purified by silica gel column chromatography eluting with a 6:4 mixture of petroleum ether/ethyl acetate. Cream solid, m.p.: 321-324°C (from EtOH). Yield, 46%. TLC (petroleum ether/ethyl acetate 6:4): R_f 0.32. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 8.84 (1H, br s, NH), 8.19 (2H, d, $J = 7.6$ Hz, H-2',6'), 7.86 (1H, d, $J = 7.4$ Hz, H-7), 7.68 (2H, d, $J = 8.8$ Hz, H-3',5'), 7.50-7.45 (1H, m, H-6), 7.31 (1H, t, H-5), 6.30 (1H, br t, NH), 3.08 (2H, q, CH₂), 1.47 (2H, sext, CH₂), 0.89 (3H, t, CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 155.0 (C, CO), 151.4 (C, C-4), 146.8 (C, C-7a), 142.2 (C, C-1'), 135.1 (C, C-3a), 133.5 (C, C-4'), 127.5 (CH, C-6), 121.2 (CH, C-3', C-5'), 118.2 (CH, C-2', C-6'), 114.4 (CH, C-7), 110.5 (CH, C-5), 40.9 (CH₂, COCH₂), 22.8 (CH₂, CH₂CH₃), 11.2 (CH₃, CH₂CH₃). LC/MS: m/z 314 (M+H). Anal. Calcd. (%) C₁₆H₁₆FN₅O: C, 61.33; H, 5.15; N, 22.35. Found: C, 61.42; H, 5.20; N, 22.42.

2.7.5.3. 1-butyl-3-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)urea (18c)

Compound (18c) was obtained as described in the general procedure starting from **4c** and 1-butyl isocyanate in DMA for 96 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Brown solid, m.p.: 243-245°C (from EtOH). Yield, 51%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.42. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 8.84 (1H, br s, NH), 8.18 (2H, d, $J = 8.8$ Hz, H-3', 5'), 7.85 (1H, d, $J = 8.4$ Hz, H-7), 7.67 (2H, d, $J = 8.8$ Hz, H-2', 6'), 7.50-7.45 (1H, m, H-6), 7.31 (1H, t, H-5), 6.28 (1H, br t, NH), 3.12 (2H, d, CH₂), 1.44 (2H, q, CH₂), 1.33 (2H, sext, CH₂), 0.90 (3H, t, CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 154.9 (C, CO), 151.3 (C, C-4), 146.7 (C, C-7a), 142.0 (C, C-1'), 135.1 (C, C-3a), 132.6 (C, C-4'), 127.5 (CH, C-6), 121.1 (CH, C-3', C-5'), 118.0 (CH, C-2', C-6'), 116.3 (CH, C-7),

112.4 (CH, C-5), 39.2 (CH₂, COCH₂), 31.6 (CH₂, CH₂CH₂CH₂), 19.4 (CH₂, CH₂CH₃), 13.6 (CH₃, CH₂CH₃). LC/MS: m/z 328 (M+H). Anal. Calcd. (%) C₁₇H₁₈FN₅O: C, 62.37; H, 5.54; N, 21.39. Found: C, 62.41; H, 5.67; N, 21.43.

2.7.5.4. 1-(sec-butyl)-3-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)urea (19c)

Compound (19c) was obtained as described in the general procedure starting from **4c** and sec-butyl isocyanate in DMA for 144 h. The crude product was purified by silica gel column chromatography eluting with a 75:25 mixture of petroleum ether/ethyl acetate. White solid, m.p.: 252-255°C (from EtOH). Yield, 42%. TLC (petroleum ether/ethyl acetate 75:25): R_f 0.32. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 8.71 (1H, br s, NH), 8.17 (2H, d, $J = 8.8$ Hz, H-3', 5'), 7.86 (1H, d, $J = 8.4$ Hz, H-7), 7.42 (2H, d, $J = 8.8$ Hz, H-2', 6'), 7.34-7.30 (1H, m, H-6), 7.31 (1H, t, H-5), 6.128 (1H, br t, NH), 3.63 (1H, sept, CH), 1.45 (2H, quin, CH₂), 1.10 (3H, d, CH₃), 0.89 (3H, t, CH₃). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 152.7 (C, CO), 148.5 (C, C-4), 146.8 (C, C-7a), 144.2 (C, C-1'), 142.0 (C, C-3a), 132.7 (C, C-4'), 127.3 (CH, C-6), 121.2 (CH, C-3', 5'), 120.9 (CH, C-7), 117.9 (CH, C-2', C-6'), 114.4 (CH, C-5), 46.2 (CH, CH-CH₃), 29.2 (CH₂, CH₂-CH₃), 20.6 (CH₃, CH-CH₃), 10.3 (CH₃, CH₂-CH₃). LC/MS: m/z 328 (M+H). Anal. Calcd. (%) C₁₇H₁₈FN₅O: C, 62.37; H, 5.54; N, 21.39. Found: C, 62.42; H, 5.60; N, 21.44.

2.7.5.5. 1-cyclopentyl-3-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)urea (20c)

Compound (20c) was obtained as described in the general procedure starting from **4c** and cyclopentyl isocyanate in DMA for 168 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Cream solid, m.p.: 339-341°C (from EtOH). Yield, 23%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.27. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 8.71 (1H, br s, NH), 8.17 (2H, d, $J = 8.8$ Hz, H-3', 5'), 8.01 (1H, s, H-7), 7.65 (2H, d, $J = 8.8$ Hz, 2', 6'), 7.50-7.49 (2H, m, H-5), 6.32 (1H, br d, NH), 3.00-3.97 (1H, m, CH cyclopentyl), 1.87-1.82 (2H, m, CH₂ cyclopentyl), 1.65-1.55 (4H, m, CH₂, cyclopentyl), 1.40-1.39 (2H, m, CH₂ cyclopentyl). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 154.6 (C, CO), 144.7 (C, C-4), 144.2 (C, C-7a), 142.0 (C, C-1'), 139.4 (C, C-4'), 133.0 (C, C-3a), 127.2 (CH, C-6), 121.0 (CH, C-3', C-5'), 117.9 (CH, C-2', C-6'), 114.4 (CH, C-7), 110.6 (CH, C-5), 50.9 (CH, CH-NH), 32.7 (CH₂CH₂-CH), 23.1 (CH₂, CH₂-CH), 22.5 (CH₂, CH₂-CH₂), 21.2 (CH₂, CH₂-CH₂). LC/MS: m/z 340 (M+H). Anal. Calcd. (%) C₁₈H₁₈FN₅O: C, 63.70; H, 5.35; N, 20.64. Found: C, 63.81; H, 5.43; N, 20.70.

2.7.5.6. 1-cyclohexyl-3-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)urea (21c)

Compound (21c) was obtained as described in the general procedure starting from **4c** and cyclohexyl isocyanate in DMA for 72 h. The crude product was purified by silica gel column chromatography eluting with a 7:3 mixture of petroleum ether/ethyl acetate. Cream solid, m.p.: 212-214°C (from EtOH). Yield, 86%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.46. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 8.73 (1H, br s, NH), 8.28 (2H, d, $J = 8.4$ Hz, H-3', 5'), 7.86 (1H, d, $J = 8.8$ Hz, H-7), 7.66 (2H,

d, $J = 8.4$, H-2', 6'), 7.51-7.46 (1H, m, H-6), 7.36- 7.29 (1H, m, H-5), 6.23 (1H, br d, NH), 1.84-1.49 (6H, m, H-cyclohexyl), 1.37-1.11 (5H, m, H- cyclohexyl). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 156.6 (C, CO), 154.1 (C, C-4), 151.4 (C, C-1'), 146.8 (C, C-7a), 142.2 (C, C-4'), 135.2 (C, C-3a), 127.4 (CH, C-6), 121.2 (CH, C-3', C-5'), 117.8 (CH, C-2', C-6'), 114.4 (CH, C-7), 110.5 (CH, C-5), 47.6 (CH, $\underline{\text{CH-NH}}$), 33.3 (CH_2 , $\underline{\text{CH}_2\text{-CH}}$), 32.8 (CH_2 , $\underline{\text{CH}_2\text{-CH}}$), 25.2 (CH_2), 24.3 (CH_2), 22.4 (CH_2). LC/MS: m/z 354 (M+H). Anal. Calcd. (%) $\text{C}_{19}\text{H}_{20}\text{FN}_5\text{O}$: C, 64.57; H, 5.70; N, 19.82. Found: C, 64.61; H, 5.79; N, 19.88.

2.7.5.7. 1-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)-3-(naphthalen-1-yl)urea (22c)

Compound (22c) was obtained, pure, as described in the general procedure starting from 4c and 1-naphthylisocyanate in DMA for 24 h. White solid, m.p.: $>350^\circ\text{C}$ (from EtOH). Yield, 40%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.36. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 9.47 (1H, br s, NH), 8.90 (1H, br s, NH), 8.28 (2H, d, $J = 8.8$ Hz, H-2', 6'), 8.14 (1H, d, $J = 8.4$ Hz, H-naphthalene), 8.02-7.95 (3H, m, H-6, 2H-naphthalene), 7.88 (1H, d, $J = 8.4$ Hz, H-7), 7.80 (2H, d, $J = 8.8$ Hz, H-3', 5'), 7.70-7.48 (4H, m, H-naphthalene), 7.33 (1H, t, H-5). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 152.9 (C, CO), 152.7 (C, C-4), 150.1 (C, C-7a), 144.2 (C, C-1'), 141.2 (C, C-4'), 135.2 (C, C-3a), 133.7 (C, C-4a'), 133.3 (C, C-1'), 128.4 (CH, C-5', 7'), 127.6 (CH, C-6), 126.3 (C, C-8a'), 126.0 (CH, C-3', C-6'), 123.6 (CH, C-2', C-6'), 123.6 (CH, C-4'), 118.7 (CH, C-3', C-5'), 117.9 (CH, C-8'), 118.4 (CH, C-7), 114.4 (CH, C-5), 110.6 (CH, C-2'). LC/MS: m/z 398 (M+H). Anal. Calcd. (%) $\text{C}_{23}\text{H}_{16}\text{FN}_5\text{O}$: C, 69.51; H, 4.06; N, 17.62. Found: C, 69.82; H, 4.25; N, 17.71.

2.7.5.8. 1-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)-3-(4-methoxyphenyl)urea (23c)

Compound (23c) was obtained as described in the general procedure starting from 4c and 4-methoxyphenyl isocyanate in toluene for 96 h. The crude product was purified by silica gel column chromatography eluting with a 9.5:0.5 mixture of dichloromethane/ethyl acetate. Light pink solid, m.p.: $274\text{-}276^\circ\text{C}$ (from EtOH). Yield, 33%. TLC (dichloromethane/ethyl acetate 9.5:0.5): R_f 0.36. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 9.08 (1H, br s, NH), 8.67 (1H, br s, NH), 8.25 (2H, d, $J = 8.8$ Hz, H-2', 6'), 7.88 (1H, d, $J = 8.8$ Hz, H-7), 7.74 (2H, d, $J = 9.2$ Hz, H-2'', 6''), 7.53-7.47 (1H, m, H-6), 7.40 (2H, d, $J = 8.8$ Hz, H-3', 5'), 7.36- 7.31 (1H, m, H-5), 6.90 (2H, d, $J = 9.2$ Hz, H-3'', 5''), 3.73 (3H, s, OCH_3). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 156.0 (C, CO), 154.8 (C, C-4), 152.6 (C, C-4'), 152.4 (C, C-7a), 141.3 (C, C-1'), 136.0 (C, C-3a), 132.2 (C, C-1'), 132.0 (C, C-4'), 127.7 (CH, C-6), 121.3 (CH, C-2', C-6'), 120.4 (CH, C-3', C-5'), 118.6 (CH, C-2', C-6'), 114.5 (CH, C-3', C-5'), 114.0 (CH, C-7), 110.6 (CH, C-5), 55.2 (CH_3 , OCH_3). LC/MS: m/z 378 (M+H). Anal. Calcd. (%) $\text{C}_{20}\text{H}_{16}\text{FN}_5\text{O}_2$: C, 63.65; H, 4.27; N, 18.56. Found: C, 63.71; H, 4.34; N, 18.65.

2.7.5.9. 1-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)-3-(3,4,5-trimethoxyphenyl)urea (24c)

Compound (24c) was obtained, pure, as described in the

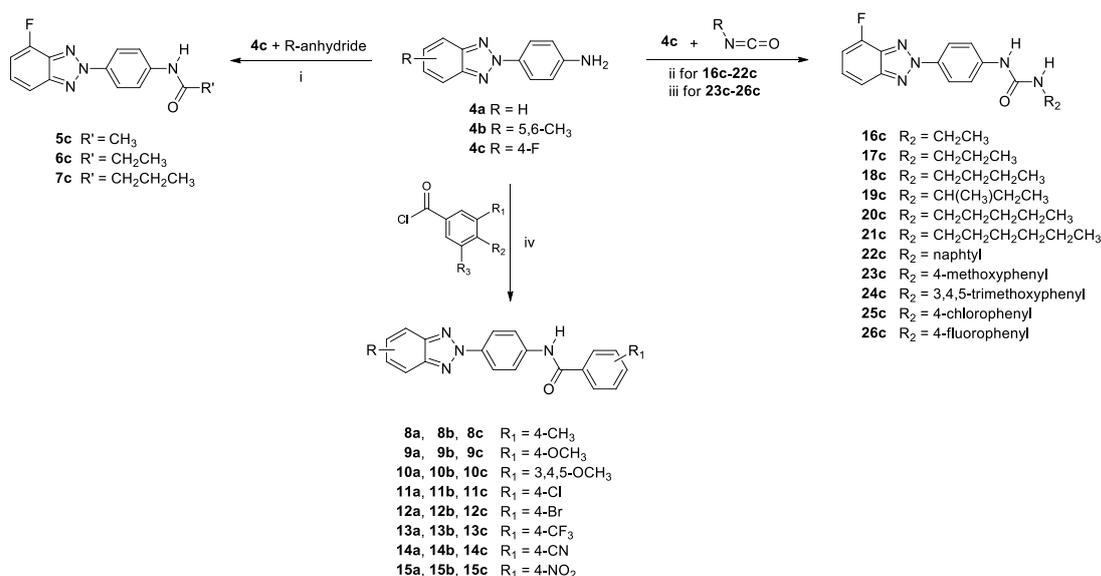
general procedure starting from 4c and 3, 4, 5-trimethoxyphenyl isocyanate in toluene for 20 h. White solid, m.p.: $272\text{-}274^\circ\text{C}$ (from EtOH). Yield, 73%. TLC (petroleum ether/ethyl acetate 1:1): R_f 0.32. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 9.06 (1H, br s, NH), 8.77 (1H, br s, NH), 8.20 (2H, d, $J = 8.8$ Hz, H-2', 6'), 7.83 (1H, d, $J = 8.4$ Hz, H-7), 7.70 (2H, d, $J = 8.8$ Hz, H-3', 5'), 7.51- 7.46 (1H, m, H-6), 7.29 (1H, m, H-5), 6.80 (2H, s, H-2'', 6''), 3.77 (6H, s, OCH_3), 3.60 (3H, s, OCH_3). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 152.8 (C, CO), 152.6 (C, C-4), 150.1 (C, C-3', C-5'), 146.8 (C, C-7a), 140.9 (C, C-1'), 140.5 (C, C-1'), 140.2 (C, C-4'), 135.1 (C, C-3a), 133.4 (C, C-4'), 121.3 (CH, C-6), 119.0 (CH, C-3', C-5'), 117.8 (CH, C-7), 114.4 (CH, C-2', C-6'), 110.7 (CH, C-2', C-6'), 96.3 (CH, C-5), 60.2 (CH_3 , $\text{OCH}_3\text{-C4''}$), 55.7 (CH_3 , $\text{OCH}_3\text{-C3''}$, $\text{OCH}_3\text{-C5''}$). LC/MS: m/z 438 (M+H). Anal. Calcd. (%) $\text{C}_{22}\text{H}_{20}\text{FN}_5\text{O}_4$: C, 60.41; H, 4.61; N, 16.01. Found: C, 60.51; H, 4.67; N, 16.10.

2.7.5.10. 1-(4-chlorophenyl)-3-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)urea (25c)

Compound (25c) was obtained, pure, as described in the general procedure starting from 4c and 4-chlorophenyl isocyanate in toluene for 3.5 h. White solid, m.p.: $241\text{-}244^\circ\text{C}$ (from EtOH). Yield, 63%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.20. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 9.06 (1H, br s, NH), 8.91 (1H, br s, NH), 8.23 (2H, d, $J = 8.8$ Hz, H-6'', 5''), 8.09-8.06 (1H, m, H-6), 7.94 (1H, d, $J = 8.4$ Hz, H-7), 7.81 (2H, d, $J = 8.8$ Hz, H-2'', 6''), 7.58 (2H, d, $J = 8.8$ Hz, H-2', 6'), 7.41 (2H, d, $J = 8.8$ Hz, H-3', 5'), 7.29 (1H, t, H-5). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 153.1 (C, CO), 151.6 (C, C-4), 147.3 (C, C-7a), 144.7 (C, C-1'), 141.4 (C, C-1'), 138.5 (C, C-3a), 133.9 (C, C-4'), 129.1 (CH, C-3', C-5'), 126.31 (C, C-4'), 121.8 (CH, C-6), 120.5 (CH, C-2', C-6'), 119.3 (CH, C-3', C-5'), 118.4 (CH, C-2', C-6'), 114.9 (CH, C-7), 111.2 (CH, C-5). LC/MS: m/z 384 (M+H). Anal. Calcd. (%) $\text{C}_{19}\text{H}_{13}\text{ClFN}_5\text{O}$: C, 59.77; H, 3.43; N, 18.34. Found: C, 59.86; H, 3.62; N, 18.42.

2.7.5.11. 1-(4-(4-fluoro-2H-benzof[d][1-3] triazol-2-yl)phenyl)-3-(4-fluorophenyl)urea (26c)

Compound (26c) was obtained, pure, as described in the general procedure starting from 4c and 4-fluorophenyl isocyanate in toluene for 48 h. White solid, m.p.: $346\text{-}347^\circ\text{C}$ (from EtOH). Yield, 38%. TLC (petroleum ether/ethyl acetate 7:3): R_f 0.47. $^1\text{H-NMR}$ (DMSO, 400 MHz) δ : 9.05 (1H, br s, NH), 8.80 (1H, br s, NH), 8.22 (2H, d, $J = 8.0$ Hz, H-2', 6'), 7.84 (1H, d, $J = 8.8$ Hz, H-7), 7.72 (2H, d, $J = 8.0$ Hz, H-3', 5'), 7.49- 7.46 (3H, m, H-6, 2'', 6''), 7.29 (1H, t, H-5), 7.13 (2H, t, H-3'', 5''). $^{13}\text{C-NMR}$ (DMSO, 100 MHz) δ : 158.0 (C, CO), 152.9 (C, C-4), 151.9 (C, C-4'), 147.3 (C, C-7a), 144.7 (C, C-1'), 144.8 (C, C-1'), 136.2 (C, C-3a), 133.8 (C, C-4'), 121.7 (CH, C-2', C-6'), 120.7 (CH, C-6), 119.2 (CH, C-3', C-5'), 115.9 (CH, C-2', 6'), 115.6 (CH, C-3', C-5'), 115.0 (CH, C-7), 111.1 (CH, C-5). LC/MS: m/z 366 (M+H). Anal. Calcd. (%) $\text{C}_{19}\text{H}_{13}\text{F}_2\text{N}_5\text{O}$: C, 62.46; H, 3.59; N, 19.17. Found: C, 62.53; H, 3.65; N, 19.26.



Scheme 2. Synthetic routes for synthesis of derivatives **5c-7c**, **8a,b,c-15a,b,c**, **16c-26c**. i: 100°C, 6h-120h; ii: DMA, 100°C, 24h-168h; iii: toluene, 100°C, 3.5h-96h; iv: DMF, 80°C, 2-48h (**8a,b-15a,b**); DMA, 100°C, 72-192h (**8c-15c**).

Results are presented and divided into three groups based on chemical similarities. As far as the antiviral activity is concerned, compounds belonging to *N*-(4-(4-fluoro-2*H*-benzo[*d*] [1 - 3]triazol-2-yl)phenyl)amide derivatives (**5c-7c**) proved to be endowed with selective anti-enterovirus activity, as well as the 4-(4-fluoro-2*H*-benzo[*d*] [1 - 3]triazol-2-yl)aniline parent (**4c**), EC₅₀ ranged between 3.8 and 50 μM, as shown in Table 1. These antiviral activity results turned out not far from the previously studied parental compounds either with two methyl groups in positions 5 and 6 (**5b-7b**) or without any substituent (**5a-7a**), on the benzotriazole moiety [15], which were reported in the same Table 1 to better compare the activities. Of the last compounds, we were able to show the

compound/Polio helicase complexes, by *in silico* assays and we provided an insight into the interactions occurring in the active site [15]. We have also observed that the substitution of the methyl groups with chlorine atoms, in position 5 and 6 on the benzotriazole scaffold (**5d-7d**), causes a completely loss of activity, with both EC₅₀ and CC₅₀ values higher than 100 μM [16]. The newly synthesised derivatives with a fluorine atom in position 4 showed an increase in the antiviral activity; they have more widespread activities against CV-B5 and Sb-1 if compared to previously reported derivatives and turned out with lower EC₅₀ values, combined with a low or no cytotoxicity.

Table 1. Cytotoxicity and antiviral activity of derivatives **4a,b,c,d-7a,b,c,d** against CV-B5 and Sb-1. Data represent mean values of three independent determinations. Pleconaril was used as a positive control.

Compounds	Vero76	CV-B5	Sb-1	Ref.
-	aCC50	bEC50	-	-
4a	60	>60	42	[15]
4b	>100	>100	>100	[15]
4c	>100	42	19	-
4d	>100	>100	>100	[16]
5a	71	28	>71	[15]
5b	>100	>100	>100	[15]
5c	>100	41	50	-
5d	>100	>100	>100	[16]
6a	>100	30	47	[15]
6b	>100	10	>100	[15]
6c	>100	11	37	-
6d	>100	>100	>100	[16]
7a	>100	>100	>100	[15]
7b	>100	30	53	[15]

Compounds	Vero76	CV-B5	Sb-1	Ref.
-	aCC50	bEC50	-	-
7c	>100	10.2	3.8	-
7d	>100	>100	>100	[16]
Ref. compounds	-	-	-	-
Pleconaril	77±6.8	0.005±0.002	2±0.6	-

^aCompound concentration (μM) required to reduce the viability of mock-infected Vero76 monolayers by 50%. ^bCompound concentration (μM) required to reduce the plaque number of the indicated virus by 50% in Vero76 monolayers.

As shown in Table 1, the antiviral activity against the CV-B5 and Sb-1 for derivatives **5c-7c**, increases with the size of the side chain, with compound **7c** being the most active against both viruses.

Moreover, the substitution of the aliphatic side chain with an aromatic moiety on the amide nitrogen (compounds **8a,b,c-15a,b,c**) dramatically cancels the antiviral activity (not listed in a table); on the contrary, the corresponding chlorine-derivatives have shown extensive antiviral activity against CV-B5, BVDV, hRSV [16] and orthohantavirus (HTNV) [25] with EC₅₀ values ranged between 3 and 27 μM .

Emboldened by the high anti-hRSV activity of 5,6-dichloro-2-phenyl-benzotriazole urea derivatives [16], we have synthesised and tested a series of ureidic derivatives bearing the fluorine atom in position 4 (**16c-26c**). Surprisingly, none of the newly synthesised compounds **16c-26c** showed antiviral activity against hRSV. On the other hand, they have once again

proved to be active against CV-B5 and Sb-1, as shown in Table 2. Further, only derivatives bearing an aliphatic side chain were proved active against the virus replication. The potency increases as the chain length up to a maximum of 4 linear carbon atoms (compound **18c** showed an EC₅₀ value of 5.5 μM). Among urea derivatives already published for their antiviral activity against orthohantavirus (**16d-18d**, **20d-26d**) [25], hRSV (**16d-18d**, **20d-26d**) [16] and CV-B5 (**16a,b-26a,b**) [17], compounds **16d-18d**, **20d-26d** had turned out completely inactive when tested against CV-B5 and Sb-1, while compounds **16a,b-26a,b** were active against CV-B5 and Sb-1 but inactive when tested against hRSV. In this series, as previously seen with benzamide derivative, the insertion of an aromatic moiety on the urea nitrogen leads to total loss of activity. The newly synthesised 4-F urea derivatives showed an additional anti-Sb-1 activity, while none of the previously tested compounds were found to be active.

Table 2. Cytotoxicity and antiviral activity of derivatives 16c-26c against CV-B5 and Sb-1. Data represent mean values of three independent determinations. Pleconaril was used as a positive control.

Compounds	Vero76	CV-B5	Sb-1
-	aCC50	bEC50	-
16c	>100	53	>100
17c	>100	6.9	20.5
18c	>100	5.5	17.5
19c	>100	>100	>100
20c	87	>87	>87
21c	>100	>100	>100
22c	>100	>100	>100
23c	>100	>100	>100
24c	>100	>100	>100
25c	>100	>100	>100
26c	>100	>100	>100
Ref. compds	-	-	-
Pleconaril	77±6.8	0.005±0.002	2±0.6

^aCompound concentration (μM) required to reduce the viability of mock-infected Vero76 monolayers by 50%. ^bCompound concentration (μM) required to reduce the plaque number of the indicated virus by 50% in Vero76 monolayers.

Table 3. Comparison of the antiviral activity of urea derivatives 16a,b,c-19a,b,c, 16d-18d against CV-B5, Sb-1. Data represent mean values of three independent determinations. Pleconaril was used as a positive control.

Compounds	Vero76	CV-B5	Sb-1	Ref
-	aCC50	bEC50	-	-
16a	>100	10	>100	[17]
16b	>100	>100	>100	[17]
16c	>100	53	>100	-
16d	>100	>100	>100	[16,25]

Compounds	Vero76	CV-B5	Sb-1	Ref
-	aCC50	bEC50	-	-
17a	100	>100	>100	[17]
17b	>100	>100	>100	[17]
17c	>100	6.9	20.5	-
17d	30	>30	>30	[16,25]
18a	>100	>100	>100	[17]
18b	>100	>100	>100	[17]
18c	>100	5.5	17.5	-
18d	90	>95	>95	[16,25]
19a	>100	16	>100	[17]
19b	>100	53	>100	[17]
19c	>100	>100	>100	-
Ref. compds	-	-	-	-
Pleconaril	77±6.8	0.005±0.002	2±0.6	-

^aCompound concentration (μM) required to reduce the viability of mock-infected Vero76 monolayers by 50%. ^bCompound concentration (μM) required to reduce the plaque number of the indicated virus by 50% in Vero76 monolayers.

The antiviral activity profile of these newly designed and synthesised compounds was compared with others that we previously reported, to extend the SARs analysis. In the series of *N*-(4-(*R*-2*H*-benzo[*d*] [1-3]triazol-2-yl)phenyl)alkylamides, the introduction of a fluorine atom in position 4, resulted in a power increase on both viral strains if compared to the parental compounds without any substituent on the benzotriazole scaffold or with methyl groups which were found only slightly active, while derivatives with two chlorine atoms were totally inactive (Table 3). Concerning the series of *N*-(4-(*R*-2*H*-benzo[*d*] [1-3]triazol-2-yl)phenyl)-4-aryl-benzamides, the introduction of a functionalized aromatic substituent was brought to a total loss of activity. The only exception was represented by already known derivatives with two chlorine atoms, which were active towards hRSV, CV-B5, BVDV and HNTV. 3-(4-(*R*-2*H*-benzo[*d*] [1-3]triazol-2-yl)phenyl)urea derivatives series showing a peculiar trend; only compounds with alkyl chains linked to urea nitrogen were proven active. New derivatives with fluorine atom in position 4 were detected as the best when compared with parental published compounds, both for the EC₅₀ values and for the wider spectrum of activity, since they are also active towards Sb-1. The replacement with two chlorine atoms completely changed the activity spectrum as they were found inactive against enteroviruses, but very active when tested against hRSV and HTNV.

CONCLUSION

Presently, we can conclude that *N*-(4-(2*H*-benzo[*d*] [1 - 3] triazol-2-yl)phenyl)-*R*-amide is a good chemical scaffold for the development of new antiviral molecules. To gain the best anti-enterovirus profile, the substituent on benzotriazole moiety should be small as a fluorine atom, and in position 4; the substitution on the amide nitrogen must be with an alkyl short chain. The replacement of the amide group with alkyl-urea groups was beneficial for the antiviral activity. On the contrary, in both series (amide derivatives and urea compounds), the presence of aromatic substituents caused a total loss of activity. According to the high selectivity, lack of cytotoxicity and interesting potency, we can select **7c**, **17c** and **18c** as lead compounds. Future aims will be to extend the screen and to

involve in our research additional enteroviruses with enormous medical impacts such as enterovirus A71 and enterovirus D68.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The author declares no conflict of interest, financial or otherwise.

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SUPPLEMENTARY MATERIAL

Supplementary material is available on the publishers website along with the published article.

REFERENCES

- [1] Kale, R.R.; Prasad, V.; Mohapatra, P.P.; Tiwari, V.K. Recent developments in benzotriazole methodology for construction of pharmacologically important heterocyclic skeletons. *Monatshefte für Chemie - Chem. Mon.*, **2010**, *141*(11), 1159-1182. [http://dx.doi.org/10.1007/s00706-010-0378-1]
- [2] Katritzky, A.R.; Henderson, S.A.; Yang, B. Applications of benzotriazole methodology in heterocycle ring synthesis and substituent introduction and modification. *J. Heterocycl. Chem.*, **1998**, *35*(5), 1123-1159. [http://dx.doi.org/10.1002/jhet.5570350511]
- [3] Katritzky, A.R.; Lan, X.; Yang, J.Z.; Denisko, O.V. Properties and synthetic utility of n-substituted benzotriazoles. *Chem. Rev.*, **1998**, *98*(2), 409-548. [http://dx.doi.org/10.1021/cr941170v] [PMID: 11848906]
- [4] Katritzky, A.R.; Rogovoy, B.V. Benzotriazole: An ideal synthetic auxiliary. *Chemistry*, **2003**, *9*(19), 4586-4593. [http://dx.doi.org/10.1002/chem.200304990] [PMID: 14566863]
- [5] Wang, X.; Liu, Y. Z. Elimination of benzotriazolyl group in n-(α -benzotriazol-1-ylalkyl)amides and n-(α -benzotriazol-1-ylalkyl)sulfonamides: Their self-coupling and cross-coupling reactions with carbonyl compounds. *Tetrahedron*, **2003**, *59*, 8257-8263. [http://dx.doi.org/10.1016/j.tet.2003.08.039]
- [6] Briguglio, I.; Piras, S.; Corona, P.; Gavini, E.; Nieddu, M.; Boatto, G.; Carta, A. Benzotriazole: An overview on its versatile biological behavior. *Eur. J. Med. Chem.*, **2015**, *97*(1), 612-648. [http://dx.doi.org/10.1016/j.ejmech.2014.09.089] [PMID: 25293580]
- [7] Christou, L. The global burden of bacterial and viral zoonotic infections. *Clinical Microbiology and Infection*, **2011**, *17*(3), 326-30.
- [8] Wolfe, N. D.; Dunavan, C. P.; Diamond, J. Origins of major human infectious diseases. *Nature*, **2007**, 279-283. Nature Publishing Group May 17
- [9] Grais, R. F.; Strebel, P.; Mala, P.; Watson, J.; Nandy, R.; Gayer, M. Measles vaccination in humanitarian emergencies: A review of recent practice. *Conflict Health. BioMed Central*, **2011**, 21.
- [10] Coronavirus, Available at: https://www.who.int/health-topics/coronavirus#tab=tab_1
- [11] Zhang, H-Z.; Gan, L-L.; Wang, H.; Zhou, C-H. New progress in azole compounds as antimicrobial agents. *Mini Rev. Med. Chem.*, **2017**, *17*(2), 122-166. [http://dx.doi.org/10.2174/1389557516666160630120725] [PMID: 27484625]
- [12] Carta, A.; Sanna, G.; Briguglio, I.; Madeddu, S.; Vitale, G.; Piras, S.; Corona, P.; Peana, A.T.; Laurini, E.; Fermeglia, M.; Pricl, S.; Serra, A.; Carta, E.; Loddo, R.; Giliberti, G. Quinoxaline derivatives as new inhibitors of coxsackievirus B5. *Eur. J. Med. Chem.*, **2018**, *145*, 559-569. [http://dx.doi.org/10.1016/j.ejmech.2017.12.083] [PMID: 29339251]
- [13] Loddo, R.; Briguglio, I.; Corona, P.; Piras, S.; Loriga, M.; Paglietti, G.; Carta, A.; Sanna, G.; Giliberti, G.; Ibba, C.; Farci, P.; La Colla, P. Synthesis and antiviral activity of new phenylimidazopyridines and N-benzylidenequinolinamines derived by molecular simplification of phenylimidazo [4,5-g]quinolines. *Eur. J. Med. Chem.*, **2014**, *84*, 8-16. [http://dx.doi.org/10.1016/j.ejmech.2014.07.011] [PMID: 25014745]
- [14] Sanna, G.; Madeddu, S.; Giliberti, G.; Piras, S.; Struga, M.; Wrzosek, M.; Kubiak-Tomaszewska, G.; Koziol, A.E.; Savchenko, O.; Lis, T.; Stefanska, J.; Tomaszewski, P.; Skrzycki, M.; Szulczyk, D. Synthesis and biological evaluation of novel indole-derived thioureas. *Molecules*, **2018**, *23*(10), 2554. [http://dx.doi.org/10.3390/molecules23102554] [PMID: 30301264]
- [15] Carta, A.; Loriga, G.; Piras, S.; Paglietti, G.; Ferrone, M.; Fermeglia, M.; Pricl, S.; La Colla, P.; Secci, B.; Collu, G.; Loddo, R. Synthesis and in vitro evaluation of the anti-viral activity of N-[4-(1H(2H)-benzotriazol-1(2)-yl)phenyl]alkylcarboxamides. *Med. Chem.*, **2006**, *2*(6), 577-589. [http://dx.doi.org/10.2174/1573406410602060577] [PMID: 17105439]
- [16] Piras, S.; Sanna, G.; Carta, A.; Corona, P.; Ibba, R.; Loddo, R.; Madeddu, S.; Caria, P.; Aulic, S.; Laurini, E.; Fermeglia, M.; Pricl, S. Dichloro-phenyl-benzotriazoles: A new selective class of human respiratory syncytial virus entry inhibitors. *Front Chem.*, **2019**, *7*(MAR), 247. [http://dx.doi.org/10.3389/fchem.2019.00247] [PMID: 31041309]
- [17] Piras, S.; Corona, P.; Ibba, R.; Riu, F.; Murineddu, G.; Sanna, G.; Madeddu, S.; Delogu, I.; Loddo, R.; Carta, A. Preliminary anti-coxsackie activity of novel 1-[4-(5,6-dimethyl(h)-1h(2h)-benzotriazol-1(2)-yl)phenyl]-3-alkyl(aryl)ureas. *Med. Chem.*, **2020**, *16*(5), 677-688. [http://dx.doi.org/10.2174/1573406416666191226142744] [PMID: 31878859]
- [18] Briguglio, I.; Loddo, R.; Laurini, E.; Fermeglia, M.; Piras, S.; Corona, P.; Giunchedi, P.; Gavini, E.; Sanna, G.; Giliberti, G.; Ibba, C.; Farci, P.; La Colla, P.; Pricl, S.; Carta, A. Synthesis, cytotoxicity and antiviral evaluation of new series of imidazo[4,5-g]quinoline and pyrido[2,3-g]quinoxaline derivatives. *Eur. J. Med. Chem.*, **2015**, *105*, 63-79. [http://dx.doi.org/10.1016/j.ejmech.2015.10.002] [PMID: 26479028]
- [19] Carta, A.; Briguglio, I.; Piras, S.; Corona, P.; Ibba, R.; Laurini, E.; Fermeglia, M.; Pricl, S.; Desideri, N.; Atzori, E.M.M.; La Colla, P.; Collu, G.; Delogu, I.; Loddo, R. A combined in silico/in vitro approach unveils common molecular requirements for efficient BVDV RdRp binding of linear aromatic N-polycyclic systems. *Eur. J. Med. Chem.*, **2016**, *117*, 321-334. [http://dx.doi.org/10.1016/j.ejmech.2016.03.080] [PMID: 27161176]
- [20] Kirk, K.L.; Cohen, L.A. The synthesis of some fluoronitrobenzimidazoles and their reactivities toward peptide nucleophiles. *J. Org. Chem.*, **1969**, *34*(2), 384-389. [http://dx.doi.org/10.1021/jo01254a027] [PMID: 5786166]
- [21] Liedholm, B.; Nyberg, I.; Svanholm, U.; Duffield, A.M.; Balaban, A.T.; Craig, J.C. Copper(I) catalysed replacement of bromine by chloride ion in halonitrobenzenes. *Acta Chem. Scand.*, **1969**, *23*, 3175-3186. [http://dx.doi.org/10.3891/acta.chem.scand.23-3175]
- [22] Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J. Virol. Methods*, **1988**, *20*(4), 309-321. [http://dx.doi.org/10.1016/0166-0934(88)90134-6] [PMID: 2460479]
- [23] Carta, A.; Bua, A.; Corona, P.; Piras, S.; Briguglio, I.; Mollicotti, P.; Zanetti, S.; Laurini, E.; Aulic, S.; Fermeglia, M.; Pricl, S. Design, synthesis and antitubercular activity of 4-alkoxy-triazoloquinolones able to inhibit the M. tuberculosis DNA gyrase. *Eur. J. Med. Chem.*, **2019**, *161*, 399-415. [http://dx.doi.org/10.1016/j.ejmech.2018.10.031] [PMID: 30384044]
- [24] Carta, A.; Sanna, P.; Paglietti, G.; Sparatore, F. ¹³C-NMR as useful tool for unambiguous identification of ring substituted n1(2)(3)-alkylbenzotriazole isomers. *Heterocycles*, **2001**, *55*(6), 1133. [http://dx.doi.org/10.3987/COM-01-9189]
- [25] Sanna, G.; Piras, S.; Madeddu, S.; Busonera, B.; Klempa, B.; Corona, P.; Ibba, R.; Murineddu, G.; Carta, A.; Loddo, R. 5,6-Dichloro-2-phenyl-benzotriazoles: New potent inhibitors of orthohantavirus. *Viruses*, **2020**, *12*(1), 122. [http://dx.doi.org/10.3390/v12010122] [PMID: 31968537]