Send Orders for Reprints to reprints@benthamscience.net

RESEARCH ARTICLE

Design, Synthesis, and Evaluation of Novel Pyruvate Dehydrogenase Kinase Inhibitors

Deniz Arslan[1], Matthieu Schoumacher[1], Sébastien Dilly[1], Benaïssa Elmoualij[2], Danièle Zorzi[2], Pascale Quatresooz[2], Vincent Lambert[3], Agnès Noël[3], Bernard Pirotte[1,#,*] and Pascal de Tullio[1,#]

[1]Laboratory of Medicinal Chemistry, Center for Interdisciplinary Research on Medicines (CIRM) – Université de Liège, Avenue Hippocrate, 15, B-4000 Liège, Belgium; [2]Centre de Recherche sur les Protéines Prions (CRPP) – Université de Liège, Avenue Hippocrate, 15, B-4000 Liège, Belgium; [3]Laboratory of Tumor and Development Biology, GIGA, Université de Liège, Avenue Hippocrate, 11, B-4000 Liège, Belgium

ARTICLE HISTORY

Received: April 19, 2022
Revised: June 02, 2022
Accepted: June 10, 2022

DOI:

10.2174/1573406418666220819102627

Abstract: ***Aims:*** The present work describes the synthesis and the biological evaluation of novel compounds acting as pyruvate dehydrogenase kinase (PDK) inhibitors. These drugs should become a new therapeutic approach for the treatment of pathologies improved by the control of the blood lactate level.

Methods: Four series of compounds belonging to *N*-(4-(*N*-alkyl/aralkylsulfamoyl)phenyl)-2-methylpropanamides and 1,2,4-benzothiadiazine 1,1-dioxides were prepared and evaluated as PDK inhibitors.

Results: The newly synthesized *N*-(4-(*N*-alkyl/aralkylsulfamoyl)phenyl)-2-methylpropanamides structurally related to previously reported reference compounds **4** and **5** were found to be potent PDK inhibitors (i.e. **10d**: IC_{50} = 41 nM). 1,2,4-Benzothiadiazine 1,1-dioxides carrying a (methyl/trifluoromethyl)-propanamide moiety at the 6-position were also designed as conformationally restricted ring-closed analogues of *N*-(4-(*N*-alkyl/aralkylsulfamoyl)phenyl)-2-hydroxy-2-methylpropanamides. Most of them were found to be less potent than their ring-opened analogues. Interestingly, the best choice of hydrocarbon side chain at the 4-position was the benzyl chain, providing **11c** (IC_{50} = 3.6 μM) belonging to "unsaturated" 1,2,4-benzothiadiazine 1,1-dioxides, and **12c** (IC_{50} = 0.5 μM) belonging to "saturated' 1,2,4-benzothiadiazine 1,1-dioxides.

Conclusion: This work showed that ring-closed analogues of *N*-(4-(*N*-alkyl/aralkylsulfamoyl) phenyl)-2-hydroxy-2-methylpropanamides were less active as PDK inhibitors than their corresponding ring-opened analogues. However, the introduction of a bulkier substituent at the 4-position of the 1,2,4-benzothiadiazine 1,1-dioxide core structure, such as a benzyl or a phenethyl side chain, was allowed, opening the way to the design of new inhibitors with improved PDK inhibitory activity.

Keywords: Pyruvate dehydrogenase kinase inhibitor, pyruvate dehydrogenase complex, lactate, conformationally restricted analogues, benzothiadiazine dioxides, 2-hydroxy-2-(methyl/trifluoromethyl)propanamides.

1. INTRODUCTION

Numerous previous works have highlighted the relation between deficiency of pyruvate dehydrogenase complex (PDC) activity and several physiopathological processes. Overproduction of PDKs has been evidenced in many diseases, including lactic acidosis, diabetes and other insulin-resistant states, age-related macular degeneration, cerebrovascular and cardiovascular diseases, cancer, pulmonary arterial hypertension, late-onset neurodegenerative diseases, and ageing [1-9]. Therefore, the regulation or the disruption of the PDKs activity has become an attractive approach for the development of medicines listed in the treatment of diseases that involved dysregulation of glucose metabolism [10]. According to the possible binding sites existing on PDK which are the pyruvate, the nucleotide, the lipoamide, and the allosteric binding sites, four classes of PDK modulators have been developed [11,12]. Dichloroacetic acid (DCA) is probably the best known and most studied inhibitor. Two mechanisms are generally described to explain the inactivation of PDKs by DCA. The dominant idea is that DCA binds to the pyruvate binding pocket [13-15]. Despite promising results, its applications to medicine, and more

*Address correspondence to this author at the Laboratory of Medicinal Chemistry, Center for Interdisciplinary Research on Medicines (CIRM) – Université de Liège, Avenue Hippocrate, 15, B-4000 Liège, Belgium; Tel: + 32 4 366 43 65; Fax: + 32 4 366 43 62. E-mail: b.pirotte@uliege.be

[#]*These authors contributed equally.*

1573-4064/23 $65.00+.00

precisely for the treatment of cancer, are limited by its relatively low-potency and its numerous side effects, especially after chronic treatment [16-25]. Several series of molecules targeting the different binding sites have been developed. The most promising results have been obtained with compounds that bind to the lipoamide site and especially with derivatives of the 3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid, such as Nov3r (**1**), AZD7545 (**2**), AZ12 (**3**), **4** and **5** (Fig. **1**) [26-32].

Thus, to discover novel drugs targeting PDK, we aim to develop a new series of PDK inhibitors based on anilide derivatives of (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid. A dual rational approach was used for this purpose. The first one consists of a classical modulation of some structural key positions based on the rational drug design to continue the exploration of the chemical space of AZD7545 (**2**) and its *N*-alkyl-sulfonamide analogues **4** and **5** (Fig. **1**). The second one, more innovative, aims to modify and rigidify the benzenesulfonamide core. Indeed, a free rotation about unhindered single bonds allows access to the molecule at many conformations which, in some cases, can be responsible for the loss of selectivity and/or can induce severe side effects. The rigidification restricts the conformations that the molecule can adopt by keeping it in a specific form, eliminating the alternative conformations, thus in some cases improving activity, selectivity, binding site interactions and/or minimizing side effects. Rigidification can be easily achieved by locking bonds within a ring. Based on the laboratory experience in the chemistry of ring-fused thiadiazine dioxides, we chose to design various series of 1,2,4-benzothiadiazine 1,1-dioxides onto which we grafted the several structural elements expected to improve the inhibitory activity on PDK. A particular attention was paid to the design of 1,2,4-benzothiadiazine 1,1-dioxides resulting from the combination of two reported PDK inhibitors (compounds **6** and **7** [28]) to explore the impact of the introduction of a bulky substituent at the 4-position of the heterocycle (Fig. **2**).

Thanks to the knowledge of the 3D structure of PDK1 co-crystallized with AZD7545 [13], a molecular modelling approach (molecular docking) was performed prior to the synthesis of the new compounds to justify the rationale of the exploration of rigidified ring-closed compounds bearing bulky groups in unusual positions. Moreover, examples of compounds resulting from the ring closure of N-alkyl-benzenesulfonamides and bearing the bulky group at the 2-position of the thiadiazine ring were also envisaged.

The general formulas of the compounds described in this work are reported in Fig. (**3**). The PDKs inhibitory potentials of the newly synthesized molecules were assessed indirectly by measuring residual PDC activity after kinase reaction, using a model that utilizes the commercially available pig PDC preparation containing intrinsic kinase activity.

2. EXPERIMENTAL SECTION

2.1. Molecular Modelling

The cocrystal structure of AZD7545 with PDK1 (PDB code 2Q8G) was used in the docking study. The target pro-

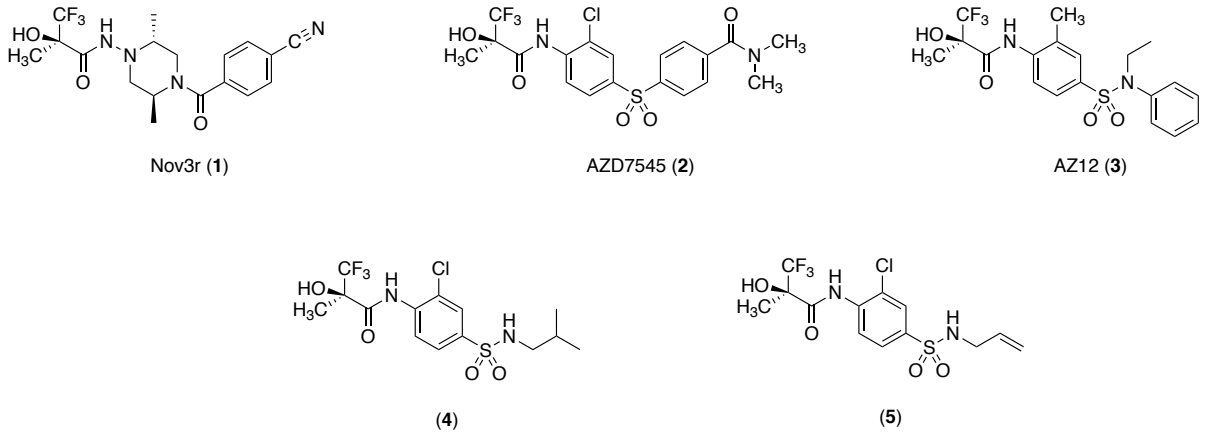


Fig. (1). Examples of pyruvate dehydrogenase kinase inhibitors belonging to 3,3,3-trifluoro-2-hydroxy-2-methylpropanamide derivatives.

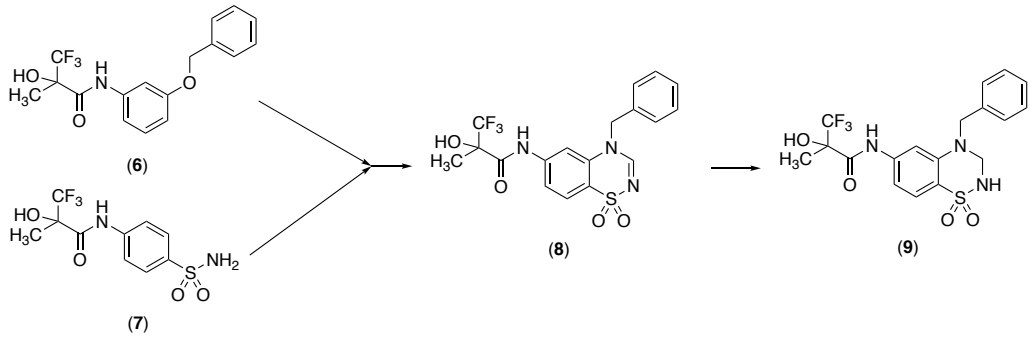
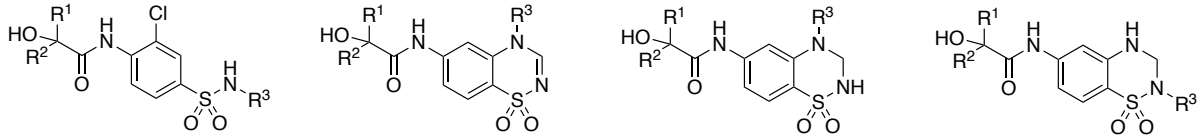


Fig. (2). Design of new PDK inhibitors belonging to 4-alkyl/aralkyl-substituted 1,2,4-benzothiadiazine 1,1-dioxides as conformationally restricted analogues of 3,3,3-trifluoro-2-hydroxy-2-methyl-*N*-(4-sulfamoylphenyl)propanamide derivatives.



(**10**) (**11**) (**12**) (**13**)

Fig. (3). General formulas of the newly synthesized compounds.

tein was prepared with Discovery Studio version 2020 (Biovia, Dassault Systems, France), while the docking procedure was carried out with the automated GOLD 5.3.0 program [33]. The binding site was defined as a 10 Å sphere allowing the incorporation of the tested compounds as well as the amino acids interacting with them. Compounds were prepared and optimized as mol2 molecules using Discovery Studio version 2020. For each ligand, the number of genetic algorithm (GA) runs was set at 100. The default ChemPLP score function was used, and the search efficiency was fixed at 200%. For the output, we asked GOLD to keep the 20 best solutions for each ligand. From these solutions, clusters based on the orientation adopted by the docking poses were identified. From the cluster having the highest occurrence, we kept the best representative (higher PLP fitness score). This representative was then used for the minimization in situ and free binding energy calculation. The in situ minimization, free binding energy calculations, and interactions visualization were performed with Discovery Studio version 2020 (DS). The in situ minimization and free binding energy calculation was launched in the same DS protocol. In this protocol, the ligand conformational entropy [34,35] was also considered (conformers generated with the BEST algorithm) and generalized Born with molecular volume (GBMV) was used as an implicit solvent model. Globally, the binding mode of ligands in the enzyme lipoamide binding site was assessed in a three-step protocol; (1) docking to find the best pose for each ligand given by scoring functions; (2) molecular dynamics simulations to optimize interactions from the pose found by the docking process; (3) Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) free binding energy calculation method to evaluate the post dynamic drug-target interactions [36,37]. From a methodological point of view, the first two steps were to validate the docking and molecular dynamics protocol by comparing the model results with those referenced by the literature for AZD7545 already characterized [13].

2.2. Chemistry

2.2.1. General

All commercial chemicals and solvents were reagent grade and used without further purification unless otherwise stated. All reactions were followed by thin-layer chromatography (Silicagel $60F_{24}$ Merck®), and visualization was accomplished after elution with UV light (254 nm and 366 nm). The ^{1}H NMR and ^{13}C, as well as 2D COSY, HSQC, and HMBC spectra were taken on a Bruker® AVANCE NEO 500 (500 MHz) using DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed in d (ppm) downfield from TMS. For the ^{1}H NMR spectra the abbreviation s = singlet, d = doublet, t = triplet, q = quadruplet, quint = quintuplet, m = multiplet, and br = broad is used throughout. The purification steps were carried out with a Buchi Reveleris® X2 flash chromatography system incorporating two detections methods, a UV detector, and an Evaporative Light Scattering Detector (ELSD). Elemental analyses (C, H, N, S) were determined on a FlashEA® 1112 (Thermo Fischer Scientific) and expected to be within ± 0.4% of the theoretical values. This analytical method certified a purity of ≥ 95% for each tested compound. Melting points were determined on a Stuart smp3 capillary apparatus and are uncorrected. Most products are dried in a ventilated oven at 30°C, without other special precautions.

2.2.2. General procedure for the synthesis of N-substituted 3-chloro-4-nitrobenzenesulfonamides

3-Chloro-4-nitroaniline (**17**) (10 g, 57.9 mmol) was suspended in 120 mL of an acetic and hydrochloric acid mixture (3:1). The solution was maintained at a temperature below 0-5°C. Aqueous sodium nitrite solution of 5 mL (1.5 eq) was gradually added to form the diazonium salt. This mixture containing the diazonium salt was added to a saturated acetic acid solution of sulphur dioxide (50 mL) in the presence of $CuCl_2$ (0.33 eq). The resulting sulphonyl chloride derivative (18) precipitated in the medium after the addition of ice and was recovered on a paper filter after filtration. The sulphonyl chloride intermediate was added to a stirred solution of appropriate amine (R^3-NH_2) (1.2 eq), and triethylamine (TEA) (1.2 eq) in dioxane (100 mL) maintained at 0°C. The solution was stirred at room temperature for 1-2 hrs. When the reaction was completed, the solvent was evaporated under reduced pressure, and the residue was suspended in water. The suspension was extracted thrice with ethyl acetate (100 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure, and the resulting solid of the final compound (**19a-d**) was recrystallized in a mixture of ethyl acetate/hexane.

2.2.3. 3-Chloro-N-isobutyl-4-nitrobenzenesulfonamide (19a)

The title compound was obtained as described in the general procedure using isobutylamine (Yield: 58%). White solid; Mp: 96.5-97.9°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.30 (d, *J* = 8.5 Hz, 1H, 5-*H*), 8.09 (d, *J* = 1.9 Hz, 1H, 2-*H*), 7.96 (dd, *J* = 8.5, 1.9 Hz, 1H, 6-*H*), 7.94 (bs, 1H, N*H*-CH_2-CH-$(CH_3)_2$), 2.64 (d, *J* = 6.8 Hz, 2H, NH-C*H*$_2$-CH-$(CH_3)_2$), 1.63 (m, *J* = 6.8, 6.7 Hz, 1H, NH-CH_2-C*H*-$(CH_3)_2$), 0.83 (d, *J* = 6.7 Hz, 6H, NH-CH_2-CH-(C*H*$_3$)$_2$). Anal. ($C_{10}H_{13}ClN_2O_4S$) theoretical: C, 41.03; H, 4.48; N, 9.57. Found: C, 41.08; H, 4.52; N, 9.72.

2.2.4. N-Allyl-3-chloro-4-nitrobenzenesulfonamide (19b)

The title compound was obtained as described in the general procedure using allylamine (Yield: 60%). White solid; Mp: 80.3-82.5°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.30 (d,

J = 8.4 Hz, 1H, 5-*H*), 8.24 (t, *J* = 5.9 Hz, 1H,[320] SO_2-N*H*-CH_2-), 8.10 (d, *J* = 1.8 Hz, 1H, 2-*H*), 7.97 (dd, *J* = 8.4, 1.8 Hz, 1H, 6-*H*), 5.74 – 5.62 (m, 1H, NH-CH_2-C*H*=C(H)$_2$), 5.16 (dd, J_{trans} = 17.1, 1.7 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 5.05 (dd, J_{cis} = 10.3, 1.7 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 3.53 (t, *J* = 5.7 Hz, 2H, NH-C*H*$_2$-CH=C(H)$_2$). Anal. ($C_9H_9ClN_2O_4S$) theoretical: C, 39.07; H, 3.28; N, 10.12. Found: C, 39.13; H, 3.37; N, 10.16.

2.2.5. N-Benzyl-3-chloro-4-nitrobenzenesulfonamide (19c)

The title compound was obtained as described in the general procedure using benzylamine (Yield: 75%). White solid; Mp: 110.6-112.4°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.62 (bs, 1H, N*H*-CH_2-C_6H_5), 8.20 (d, *J* = 8.5 Hz, 1H, 5-*H*), 7.94 (d, *J* = 1.8 Hz, 1H, 2-*H*), 7.90 (dd, *J* = 8.5, 1.9 Hz, 1H, 2-*H*), 7.28 – 7.16 (m, 5H, NH-CH_2-C_6H_5), 4.12 (d, 2H, NH-C*H*$_2$-C_6H_5). Anal. ($C_{13}H_{11}ClN_2O_4S$) theoretical: C, 47.79; H, 3.39; N, 8.57. Found: C, 47.99; H, 3.59; N, 8.67.

2.2.6. 3-Chloro-4-nitro-N-phenethylbenzenesulfonamide (19d)

The title compound was obtained as described in the general procedure using 2-phenethylamine (Yield: 45%). White solid; Mp: 83.3-84.5°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.23 (d, *J* = 8.4 Hz, 1H, 5-*H*), 8.15 (t, *J* = 5.7 Hz, 1H, N*H*-CH_2-CH_2-C_6H_5), 7.99 (d, *J* = 1.9 Hz, 1H, 2-*H*), 7.90 (dd, *J* = 8.4, 1.9 Hz, 1H, 6-*H*), 7.26 – 7.13 (m, 5H, NH-CH_2-CH_2-C_6H_5), 3.11 (td, *J* = 7.2, 5.7 Hz, 2H, NH-C*H*$_2$-CH_2-C_6H_5), 2.70 (t, *J* = 7.2 Hz, 2H, NH-CH_2-C*H*$_2$-C_6H_5). Anal. ($C_{14}H_{13}ClN_2O_4S$) theoretical: C, 49.34; H, 3.85; N, 8.22. Found: C, 49.37; H, 3.84; N, 8.26.

2.2.7. General procedure for synthesis of N-substituted 4-amino-3-chlorobenzenesulfonamides

The appropriate *N*-substituted 3-chloro-4-nitrobenzenesulfonamide (1.5 g) was dissolved in a mixture of acetic acid (20 mL) and water (5 mL). The solution was refluxed on an oil bath, and iron powder (3.2 eq) was added. The suspension was stirred for 2-4 hrs. When the reaction was completed, the suspension was filtered through Celite®. The pH value of the filtrate was adjusted to neutrality with sodium carbonate powder, and the suspension was extracted thrice with ethyl acetate (100 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the final compound (**20a-d**) obtained as an oil was used without further purification.

2.2.8. 4-Amino-3-chloro-N-isobutylbenzenesulfonamide (20a)

The title compound was obtained as described in the general procedure starting from 3-chloro-*N*-isobutyl-4-nitrobenzenesulfonamide (**19a**) (Yield: 78%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.54 (d, *J* = 2.1 Hz, 1H, 2-*H*), 7.40 (dd, *J* = 8.6, 2.1 Hz, 1H, 6-*H*), 7.27 (t, *J* = 6.2 Hz, 1H, SO_2-N*H*-CH_2-), 6.84 (d, *J* = 8.6 Hz, 1H, 5-*H*), 6.17 (s, 2H, N*H*$_2$), 2.47 (dd, *J* = 6.2 Hz, 2H, NH-C*H*$_2$-CH-(CH_3)$_2$), 1.64 – 1.54 (m, 1H, NH-CH_2-C*H*-(CH_3)$_2$), 0.80 (d, *J* = 6.7 Hz, 6H, NH-CH_2-CH-(C*H*$_3$)$_2$).

2.2.9. N-Allyl-4-amino-3-chlorobenzenesulfonamide (20b)

The title compound was obtained as described in the general procedure starting from *N*-allyl-3-chloro-4-nitrobenzenesulfonamide (**19b**) (Yield: 85%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.55 (d, *J* = 2.1 Hz, 1H, 2-*H*), 7.47 (t, *J* = 5.8 Hz, 1H, SO_2-N*H*-CH_2-), 7.41 (dd, *J* = 8.6, 2.1 Hz, 1H, 6-*H*), 6.85 (d, *J* = 8.6 Hz, 1H, 5-*H*), 6.20 (s, 2H, N*H*$_2$), 5.67 (ddt, *J* = 17.2, 10.3, 5.8 Hz, 1H, NH-CH_2-C*H*=C(H)$_2$), 5.14 (dd, J_{trans} = 17.2, 1.8 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 5.03 (dd, J_{cis}= 10.3, 1.8 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 3.35 (dd, *J* = 5.8 Hz, 2H, NH-C*H*$_2$-CH=C(H)$_2$).

2.2.10. 4-Amino-N-benzyl-3-chlorobenzenesulfonamide (20c)

The title compound was obtained as described in the general procedure starting from *N*-benzyl-3-chloro-4-nitrobenzenesulfonamide (**19c**) (Yield: 87%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.83 (t, *J* = 6.4 Hz, 1H, N*H*-CH_2-C_6H_5), 7.54 (d, *J* = 2.2 Hz, 1H, 2-*H*), 7.42 (dd, *J* = 8.6, 2.2 Hz, 1H, 6-*H*), 7.32 – 7.19 (m, 5H, NH-CH_2-C_6H_5), 6.83 (d, *J* = 8.6 Hz, 1H, 5-*H*), 6.20 (s, 2H, N*H*$_2$), 3.91 (d, *J* = 6.4 Hz, 2H, NH-C*H*$_2$-C_6H_5).

2.2.11. 4-Amino-3-chloro-N-phenethylbenzenesulfonamide (20d)

The title compound was obtained as described in the general procedure starting from 3-chloro-4-nitro-*N*-phenethylbenzenesulfonamide (**19d**) (Yield: 77%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.53 (d, *J* = 2.1 Hz, 1H, 2-*H*), 7.40 (dd, *J* = 8.6, 2.1 Hz, 1H, 6-*H*), 7.37 (t, *J* = 5.9 Hz, 1H, N*H*-CH_2-CH_2-C_6H_5), 7.29 – 7.11 (m, 5H, NH-CH_2-CH_2-C_6H_5), 6.84 (d, *J* = 8.6 Hz, 1H, 5-*H*), 6.19 (s, 2H, N*H*$_2$), 2.90 (td, *J* = 7.5, 5.9 Hz, 2H, NH-C*H*$_2$-CH_2-C_6H_5), 2.66 (t, *J* = 7.5 Hz, 2H, NH-CH_2-C*H*$_2$-C_6H_5).

2.2.12. General procedure for the synthesis of 2-acetoxyalkanoic acids

The solution of the appropriate α-hydroxycarboxylic acid (**14a-f**) (5 g) in dry dichloromethane (DCM) (20 mL) was cooled on ice. Acetyl chloride (2.2 eq) was slowly added, and the reaction mixture was refluxed in an oil bath. After 1 hr, the solution was cooled to room temperature and evaporated under reduced pressure. The residue was suspended in water and extracted thrice with DCM (50 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the compound (**15a-f**) was recrystallized in a mixture of DCM/hexane.

2.2.13. (R,S)-2-Acetoxy-3,3,3-trifluoro-2-methylpropanoic acid (15a)

The title compound was obtained as described in the general procedure starting from (*R,S*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanoic acid (**14a**) (Yield: 96%). White solid; Mp: 58.4-61.5°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.25 (bs, 1H, COO*H*), 2.13 (s, 3H, C*H*$_3$-COO), 1.71 (s, 3H, C*H*$_3$). Anal. ($C_6H_7F_3O_4$) theoretical: C, 36.01; H, 3.53. Found: C, 36.15; H, 3.63.

2.2.14. (R)-2-Acetoxy-3,3,3-trifluoro-2-methylpropanoic acid (15b)

The title compound was obtained as described in the general procedure starting from (*R*)-3,3,3-trifluoro-2-hydroxy-2-methylpropanoic acid (**14b**) (Yield: 93%). White solid; Mp: 58.9-61.4°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.25 (bs,

1H, COO*H*), 2.13 (s, 3H, C*H*$_3$-COO), 1.71 (s, 3H, C*H*$_3$). Anal. ($C_6H_7F_3O_4$) theoretical: C, 36.01; H, 3.53. Found: C, 36.10; H, 3.58.

2.2.15. (R,S)-2-Acetoxy-2-phenylacetic acid (15d)

The title compound was obtained as described in the general procedure starting from *R,S*-mandelic acid (**14d**) (Yield: 97%). White solid; Mp: 78.9-81.3°C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 13.22 (bs, 1H, COO*H*), 7.54 – 7.31 (m, 5H, C$_6$*H*$_5$), 5.80 (s, 1H, C*H*), 2.12 (s, 3H, C*H*$_3$-COO). Anal. ($C_{10}H_{10}O_4$) theoretical: C, 61.85; H, 5.19. Found: C, 62.10; H, 5.35.

2.2.16. (R)-2-Acetoxy-2-phenylacetic acid (15e)

The title compound was obtained as described in the general procedure starting from (*R*)-mandelic acid (**14e**) (Yield: 96%). White solid; Mp: 80.1-81.6°C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 13.22 (bs, 1H, COO*H*), 7.54 – 7.31 (m, 5H, C$_6$*H*$_5$), 5.80 (s, 1H, C*H*), 2.12 (s, 3H, C*H*$_3$-COO). Anal. ($C_{10}H_{10}O_4$) theoretical: C, 61.85; H, 5.19. Found: C, 61.95; H, 5.25.

2.2.17. (S)-2-Acetoxy-2-phenylacetic acid (15f)

The title compound was obtained as described in the general procedure starting from (*S*)-mandelic acid (**14f**) (Yield: 97%). White solid; Mp: 79.3-80.8°C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 13.22 (bs, 1H, COO*H*), 7.54 – 7.31 (m, 5H, C$_6$*H*$_5$), 5.80 (s, 1H, C*H*), 2.12 (s, 3H, C*H*$_3$-COO). Anal. ($C_{10}H_{10}O_4$) theoretical: C, 61.85; H, 5.19. Found: C, 61.80; H, 5.15.

2.2.18. General procedure for the synthesis of 2-acetoxyalkanoyl chlorides

The appropriate 2-acetoxyalkanoic acid (**15a-f**) (1 g) was dissolved in dry DCM (10 mL) and supplemented with one drop of anhydrous DMF. The solution was put in a closed vial with a septum and cooled on an ice bath. Oxalyl chloride (1 eq) was added dropwise to the solution. The mixture was then stirred overnight at room temperature and used without further purification.

2.2.19. (R,S)-2-Acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (16a)

The title compound was obtained as described in the general procedure starting from (*R-S*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoic acid (**15a**).

2.2.20. (R)-2-Acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (16b)

The title compound was obtained as described in the general procedure starting from (*R*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoic acid (**15b**).

2.2.21. (R,S)-2-Acetoxy-2-phenylethylacetyl chloride (16d)

The title compound was obtained as described in the general procedure starting from (*R,S*)-2-acetoxy-2-phenylacetic acid (**15d**).

2.2.22. (R)-2-Acetoxy-2-phenylethylacetyl chloride (16e)

The title compound was obtained as described in the general procedure starting from (*R*)-2-acetoxy-2-phenylacetic acid (**15e**).

2.2.23. (S)-2-Acetoxy-2-phenylethylacetyl chloride (16f)

The title compound was obtained as described in the general procedure starting from (*S*)-2-acetoxy-2-phenylacetic acid (**15f**).

2.2.24. (R)-N-(2-Chloro-4-(N-isobutylsulfamoyl)phenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (10a)

(*R*)-2-Acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16b**) (1.5 eq) was rapidly added to a solution of 4-amino-3-chloro-*N*-isobutylbenzenesulfonamide (**20a**) (130 mg, 0.49 mmol) and pyridine (2.5 eq) in dry chloroform cooled on an ice bath. The flask was immediately hermetically closed, and after stirring for 2 hrs at room temperature, the solvent was evaporated under reduced pressure. The residue was taken up in the water and extracted thrice with ethyl acetate (50 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the obtained compound (**21a**) was solubilized in methanol (10 mL). A cold aqueous NaOH solution (2.5 N, 10 g/mL) was added dropwise up to alkaline pH. After 30 minutes, the mixture was neutralized by the addition of an aqueous 2 N HCl solution. Methanol was evaporated under reduced pressure, and the resulting precipitate of the final compound (**10a**) suspended in water was collected by filtration (Yield: 44 %). White solid; Mp: 139.6-141.5°C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 9.90 (s, 1H, CO-N*H*-), 8.25 (d, *J* = 8.6 Hz, 1H, 11-*H*), 7.99 (s, 1H, C-O*H*), 7.90 (d, *J* = 2.0 Hz, 1H, 8-*H*), 7.79 (dd, *J* = 8.6, 2.0 Hz, 1H, 10-*H*), 7.71 (t, *J* = 6.1 Hz, 1H, SO$_2$-N*H*-CH$_2$-), 2.56 (dd, *J* = 6.1 Hz, 2H, NH-C*H*$_2$-CH-(CH$_3$)$_2$), 1.68 – 1.56 (m, 1H, NH-CH$_2$-C*H*-(CH$_3$)$_2$), 1.62 (s, 3H, C-C*H*$_3$), 0.82 (d, *J* = 6.7 Hz, 6H, NH-CH$_2$-CH-(C*H*$_3$)$_2$). Anal. ($C_{14}H_{18}ClF_3N_2O_4S$) theoretical: C, 41.74; H, 4.50; N, 6.95. Found: C, 41.83; H, 4.62; N, 7.37.

2.2.25. (R)-N-(4-(N-Allylsulfamoyl)-2-chlorophenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (10b)

The title compound was obtained as described for (**10a**) starting from *N*-allyl-4-amino-3-chlorobenzenesulfonamide (**20b**) (140 mg, 0.57 mmol) and (*R*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16b**) (1.5 eq) (Yield: 40 %). White solid; Mp: 114.6-116.3°C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 9.90 (s, 1H, CO-N*H*-), 8.26 (d, *J* = 8.6 Hz, 1H, 13-*H*), 7.99 (br, 1H, C-O*H*), 7.95 (br, 1H, SO$_2$-N*H*-CH$_2$-) 7.91 (d, *J* = 2.1 Hz, 1H, 10-*H*), 7.80 (dd, *J* = 8.6, 2.1 Hz, 1H, 12-*H*), 5.74 – 5.62 (m, 1H, NH-CH$_2$-C*H*=C(H)$_2$), 5.16 (dd, J_{trans} = 17.1, 1.6 Hz, 1H, NH-CH$_2$-CH=C(*H*)$_2$), 5.04 (dd, J_{cis}= 10.3, 1.6 Hz, 1H, NH-CH$_2$-CH=C(*H*)$_2$), 3.45 (dd, *J* = 4.5 Hz, 2H, NH-C*H*$_2$-CH=C(H)$_2$), 1.62 (s, 3H, C-C*H*$_3$). Anal. ($C_{13}H_{14}ClF_3N_2O_4S$) theoretical: C, 40.37; H, 3.65; N, 7.24. Found: C, 40.38; H, 3.93; N, 7.58.

2.2.26. (R,S)-N-(2-Chloro-4-(N-isobutylsulfamoyl)phenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (10c)

The title compound was obtained as described for (**10a**) starting from 4-amino-3-chloro-*N*-isobutylbenzenesulfonamide (**20a**) (180 mg, 0.68 mmol) and (*R,S*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16a**) (1.5 eq) (Yield: 75 %). White solid; Mp: 139.7-141.9°C; ^{1}H NMR (500 MHz, DMSO-d$_6$): δ 9.87 (br, 1H, CO-N*H*-), 8.25 (d, *J* = 8.6 Hz, 1H, 12-*H*), 8.01 (br, 1H, C-O*H*), 7.90 (d, *J* = 1.9 Hz, 1H,

9-*H*), 7.79 (dd, *J* = 8.6, 1.9 Hz, 1H, 11-*H*), 7.72 (t, *J* = 6.1 Hz, 1H, SO_2-N*H*-CH_2-), 2.56 (dd, *J* = 6.1 Hz, 2H, NH-C*H*$_2$-CH-$(CH_3)_2$), 1.68 – 1.56 (m, 1H, NH-CH_2-C*H*-$(CH_3)_2$), 1.62 (s, 3H, C-C*H*$_3$), 0.82 (d, *J* = 6.8 Hz, 6H, NH-CH_2-CH-$(CH_3)_2$). Anal. ($C_{14}H_{18}ClF_3N_2O_4S$) theoretical: C, 41.74; H, 4.50; N, 6.95. Found: C, 41.99; H, 4.40; N, 7.14.

2.2.27. (R,S)-N-(4-(N-Benzylsulfamoyl)-2-chlorophenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (10d)

The title compound was obtained as described for (**10a**) starting from 4-amino-*N*-benzyl-3-chlorobenzenesulfonamide (**20c**) (200 mg, 0.68 mmol) and (*R,S*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16a**) (1.5 eq) (Yield: 71 %). White solid; Mp: 184.6-185.3°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.87 (br, 1H, CO-N*H*-), 8.27 (t, *J* = 2.9 Hz, 1H, SO_2-N*H*-CH_2-), 8.24 (d, *J* = 8.6 Hz, 1H, 11-*H*), 8.04 (br, 1H, C-O*H*) ,7.85 (d, *J* = 2.2 Hz, 1H, 8-*H*), 7.79 (dd, *J* = 8.6, 2.2 Hz, 1H, 10-*H*), 7.31 – 7.18 (m, 5H, NH-CH_2-C_6H_5), 4.02 (d, *J* = 2.9 Hz, 2H, NH-C*H*$_2$-C_6H_5), 1.63 (s, 3H, C-C*H*$_3$). Anal. ($C_{17}H_{16}ClF_3N_2O_4S$) theoretical: C, 46.74; H, 3.69; N, 6.41. Found: C, 46.64; H, 3.56; N, 6.80.

2.2.28. (R,S)-N-(2-Chloro-4-(N-phenethylsulfamoyl) phenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (10e)

The title compound was obtained as described for (**10a**) starting from 4-amino-3-chloro-*N*-phenethylbenzenesulfonamide (**20d**) (300 mg, 0.97 mmol) and (*R,S*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16a**) (1.5 eq) (Yield: 65%). White solid; Mp: 156.5-157.2°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.89 (s, 1H, CO-N*H*), 8.24 (d, *J* = 8.6 Hz, 1H, 11-*H*), 8.00 (s, 1H, C-O*H*), 7.86 (d, *J* = 2.1 Hz, 1H, 8-*H*), 7.83 (t, *J* = 5.8 Hz, 1H, SO_2-N*H*-CH_2-CH_2-C_6H_5), 7.77 (dd, *J* = 8.6, 2.1 Hz, 1H, 10-*H*), 7.29 – 7.12 (m, 5H, NH-CH_2-CH_2-C_6H_5), 3.01 (td, *J* = 7.4, 5.8 Hz, 2H, NH-C*H*$_2$-CH_2-C_6H_5), 2.68 (t, *J* = 7.4 Hz, 2H, NH-CH_2-C*H*$_2$-C_6H_5), 1.62 (s, 3H, C-C*H*$_3$). Anal. ($C_{18}H_{18}ClF_3N_2O_4S$) theoretical: C, 47.95; H, 4.02; N, 6.21. Found: C, 47.94; H, 4.05; N, 6.25.

2.2.29. N-(2-Chloro-4-(N-isobutylsulfamoyl)phenyl)-2-hydroxy-2-methylpropanamide (10f)

The title compound was obtained as described for (**10a**) starting from 4-amino-3-chloro-*N*-isobutylbenzenesulfonamide (**20a**) (180 mg, 0.68 mmol) and 2-acetoxy-2-methylpropanoyl chloride (**16c**) (1.5 eq) (Yield: 68%). White solid; Mp: 129.4-130.3°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.76 (s, 1H, CO-N*H*), 8.50 (d, *J* = 8.7 Hz, 1H, 12-*H*), 7.88 (d, *J* = 2.1 Hz, 1H, 9-*H*), 7.76 (dd, *J* = 8.7, 2.1 Hz, 1H, 11-*H*), 7.66 (br, 1H, SO_2-N*H*-CH_2-CH-$(CH_3)_2$), 6.30 (s, 1H, C-O*H*), 2.55 (dd, *J* = 6.8 Hz, 2H, NH-C*H*$_2$-CH-$(CH_3)_2$), 1.62 (m, 1H, NH-CH_2-C*H*-$(CH_3)_2$), 1.39 (s, 6H, C-(C*H*$_3$)$_2$), 0.81 (d, *J* = 6.7 Hz, 6H, NH-CH_2-CH-(C*H*$_3$)$_2$). Anal. ($C_{14}H_{21}ClN_2O_4S$) theoretical: C, 48.20; H, 6.07; N, 8.03. Found: C, 48.55; H, 6.18; N, 8.32.

2.2.30. N-(4-(N-allylsulfamoyl)-2-chlorophenyl)-2-hydroxy-2-methylpropanamide (10g)

The title compound was obtained as described for (**10a**) starting from *N*-allyl-4-amino-3-chlorobenzenesulfonamide (**20b**) (140 mg, 0.57 mmol) and 2-acetoxy-2-methylpropanoyl chloride (**16c**) (1.5 eq) (Yield: 65 %). White solid; Mp: 113.6-114.4°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.77 (s, 1H, CO-N*H*), 8.51 (d, *J* = 8.5 Hz, 1H, 13-*H*), 7.89 (d, *J* = 2.2 Hz, 1H- 10-*H*), 7.87 (br, 1H, SO_2-N*H*-CH_2-), 7.77 (dd, *J* = 8.5, 2.2 Hz, 1H, 12-*H*), 6.30 (s, 1H, C-O*H*), 5.67 (ddt, *J* = 17.0, 10.2, 5.6 Hz, 1H, NH-CH_2-C*H*=C(H)$_2$), 5.15 (dd, J_{trans} = 17.0, 1.7 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 5.04 (dd, J_{cis}= 10.2, 1.7 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 3.44 (dd, 2H, NH-C*H*$_2$-CH=C(H)$_2$), 1.39 (s, 6H, C-(C*H*$_3$)$_2$). Anal. ($C_{13}H_{17}ClN_2O_4S$) theoretical: C, 46.92; H, 5.15; N, 8.42. Found: C, 47.11; H, 5.21; N, 8.58.

2.2.31. *N*-(4-(*N*-benzylsulfamoyl)-2-chlorophenyl)-2-hydroxy-2-methylpropanamide (10h)

The title compound was obtained as described for (**10a**) starting from 4-amino-*N*-benzyl-3-chlorobenzenesulfonamide (**20c**) (200 mg, 0.68 mmol) and 2-acetoxy-2-methylpropanoyl chloride (**16c**) (1.5 eq) (Yield: 71 %). White solid; Mp: 189.7-190.8°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.76 (s, 1H, CO-N*H*-), 8.48 (d, *J* = 8.7 Hz, 1H, 12-*H*), 8.24 (t, *J* = 6.0 Hz, 1H, SO_2-N*H*-CH_2-), 7.83 (d, *J* = 2.1 Hz, 1H, 9-*H*), 7.77 (dd, *J* = 8.7, 2.1 Hz, 1H, 11-*H*), 7.31 – 7.18 (m, 5H, NH-CH_2-C_6H_5), 6.32 (s, 1H, C-O*H*), 4.00 (d, *J* = 6.0 Hz, 2H, SO_2-NH-C*H*$_2$-C_6H_5), 1.39 (s, 6H, C-(C*H*$_3$)$_2$). Anal. ($C_{17}H_{19}ClN_2O_4S$) theoretical: C, 53.33; H, 5.00; N, 7.32. Found: C, 53.61; H, 5.10; N, 7.54.

2.2.32. *N*-(2-chloro-4-(*N*-phenethylsulfamoyl)phenyl)-2-hydroxy-2-methylpropanamide (10i)

The title compound was obtained as described for (**10a**) starting from 4-amino-3-chloro-*N*-phenethylbenzenesulfonamide (**20d**) (250 mg, 0.81 mmol) and 2-acetoxy-2-methylpropanoyl chloride (**16c**) (1.5 eq) (Yield: 69 %). White solid; Mp: 125.4-126.8°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.77 (s, 1H, -CO-N*H*-), 8.48 (d, *J* = 8.6 Hz, 1H, 13-*H*), 7.84 (d, *J* = 2.1 Hz, 1H, 10-*H*), 7.78 (s, 1H, SO_2-N*H*-CH_2-), 7.75 (dd, *J* = 8.6, 2.1 Hz, 1H, 12-*H*), 7.28 – 7.13 (m, 5H, NH-CH_2-CH_2-C_6H_5), 6.30 (s, 1H, C-O*H*), 2.99 (t, *J* = 7.4 Hz, 2H, NH-C*H*$_2$-CH_2-C_6H_5), 2.68 (t, *J* = 7.4 Hz, 2H, NH-C*H*$_2$-CH_2-C_6H_5), 1.39 (s, 6H, C-$(CH_3)_2$). Anal. ($C_{18}H_{21}ClN_2O_4S$) theoretical: C, 54.47; H, 5.33; N, 7.06. Found: C, 54.51; H, 5.72; N, 7.33.

2.2.33. (R,S)-N-(2-Chloro-4-(N-isobutylsulfamoyl)phenyl)-2-hydroxy-2-phenylacetamide (10j)

The title compound was obtained as described for (**10a**) starting from 4-amino-3-chloro-*N*-isobutylbenzenesulfonamide (**20a**) (130 mg, 0.50 mmol) and (*R,S*)-2-acetoxy-2-phenylethylacetyl chloride (**16d**) (1.5 eq) (Yield: 57 %). White solid; Mp: 124.9-126.8°C; ^{1}H NMR (DMSO-d_6): δ 9.82 (s, 1H, -CO-N*H*-), 8.33 (d, *J* = 8.7 Hz, 1H, 17-*H*), 7.88 (d, *J* = 1.9 Hz, 1H, 14-*H*), 7.74 (dd, *J* = 8.7, 1.9 Hz, 1H, 16-*H*), 7.66 (s, 1H, SO_2-N*H*-CH_2-), 7.51 – 7.30 (m, 5H, -CH-C_6H_5), 7.10 (s, 1H, CH-O*H*), 5.23 (s, 1H, -C*H*-), 2.54 (d, *J* = 6.8 Hz, 2H, NH-C*H*$_2$-CH-$(CH_3)_2$), 1.64 (m, *J* = 6.8, 6.7 Hz, 1H, NH-CH_2-C*H*-$(CH_3)_2$), 0.80 (d, *J* = 6.7 Hz, 6H, NH-CH_2-CH-(C*H*$_3$)$_2$). Anal. ($C_{18}H_{21}ClN_2O_4S$) theoretical: C, 54.47; H, 5.33; N, 7.06. Found: C, 54.74; H, 5.40; N, 7.34.

2.2.34. (R,S)-N-(4-(N-Allylsulfamoyl)-2-chlorophenyl)-2-hydroxy-2-phenylacetamide (10k)

The title compound was obtained as described for (**10a**) starting from *N*-allyl-4-amino-3-chlorobenzenesulfonamide (**20b**) (126 mg, 0.51 mmol) and (*R,S*)-2-acetoxy-2-

phenylethylacetyl chloride (**16d**) (1.5 eq) (Yield: 72 %). White solid; Mp: 126.7-128.2°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.84 (s, 1H, -CO-N*H*-), 8.34 (d, *J* = 8.7 Hz, 1H, 17-*H*), 7.89 (d, *J* = 2.1 Hz, 1H, 14-*H*), 7.88 (s, 1H, SO_2-N*H*-CH_2-), 7.75 (dd, *J* = 8.7, 2.1 Hz, 1H, 16-*H*), 7.51 – 7.30 (m, 5H, -CH-C_6H_5), 7.08 (s, 1H, CH-O*H*), 5.66 (ddt, *J* = 17.1, 10.3, 5.5 Hz, 1H, NH-CH_2-C*H*=$C(H)_2$), 5.24 (s, 1H, -C*H*-), 5.14 (dd, *J trans* = 17.1, 1.7 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 5.03 (dd, J_{cis} = 10.3, 1.6 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 3.43 (dt, *J* = 5.7, 1.9 Hz, 2H, NH-C*H*$_2$-CH=$C(H)_2$). Anal. ($C_{17}H_{17}ClN_2O_4S$) theoretical: C, 53.61; H, 4.50; N, 7.36. Found: C, 53.92; H, 4.69; N, 7.55.

2.2.35. (R,S)-N-(4-(N-Benzylsulfamoyl)-2-chlorophenyl)-2-hydroxy-2-phenylacetamide (10l)

The title compound was obtained as described for (**10a**) starting from 4-amino-*N*-benzyl-3-chlorobenzenesulfonamide (**20c**) (100 mg, 0.34 mmol) and (*R,S*)-2-acetoxy-2-phenylethylacetyl chloride (**16d**) (1.5 eq) (Yield: 73 %). White solid; Mp: 145.7-147.9°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.83 (s, 1H, -CO-N*H*-), 8.31 (d, *J* = 8.6 Hz, 1H, 17-*H*), 8.23 (t, *J* = 6.3 Hz, 1H, SO_2-N*H*-CH_2-), 7.83 (d, *J* = 2.2 Hz, 1H, 14-*H*), 7.74 (dd, *J* = 8.6, 2.2 Hz, 1H, 16-*H*), 7.53 – 7.16 (m, 11H, -CH-C_6H_5, NH-CH_2-C_6H_5), 7.09 (d, *J* = 4.3 Hz, 1H, CH-O*H*), 5.24 (d, *J* = 4.3 Hz, 1H, C*H*-OH), 4.00 (d, *J* = 6.3 Hz, 2H, NH-C*H*$_2$-C_6H_5). Anal. ($C_{21}H_{19}ClN_2O_4S$) theoretical: C, 58.54; H, 4.44; N, 6.50. Found: C, 58.70; H, 4.48; N, 6.62.

2.2.36. (R)-N-(4-(N-Benzylsulfamoyl)-2-chlorophenyl)-2-hydroxy-2-phenylacetamide (10m)

The title compound was obtained as described for (**10a**) starting from 4-amino-*N*-benzyl-3-chlorobenzenesulfonamide (**20c**) (150 mg, 0.51 mmol) and (*R*)-2-acetoxy-2-phenylethylacetyl chloride (**16e**) (Yield: 80 %). White solid; Mp: 146.1-148.2°C; ^{1}H NMR (500 MHz, DMSO-d_6):

δ 9.83 (s, 1H, -CO-N*H*-), 8.31 (d, *J* = 8.6 Hz, 1H, 17-*H*), 8.23 (t, *J* = 6.3 Hz, 1H, SO_2-N*H*-CH_2-), 7.83 (d, *J* = 2.2 Hz, 1H, 14-*H*), 7.74 (dd, *J* = 8.6, 2.2 Hz, 1H, 16-*H*), 7.53 – 7.16 (m, 11H, -CH-C_6H_5, NH-CH_2-C_6H_5), 7.09 (d, *J* = 4.3 Hz, 1H, CH-O*H*), 5.24 (d, *J* = 4.3 Hz, 1H, C*H*-OH), 4.00 (d, *J* = 6.3 Hz, 2H, NH-C*H*$_2$-C_6H_5). Anal. ($C_{21}H_{19}ClN_2O_4S$) theoretical: C, 58.54; H, 4.44; N, 6.50. Found: C, 58.60; H, 4.49; N, 6.58.

2.2.37. (S)-N-(4-(N-Benzylsulfamoyl)-2-chlorophenyl)-2-hydroxy-2-phenylacetamide (10n)

The title compound was obtained as described for (**10a**) starting from 4-amino-*N*-benzyl-3-chlorobenzenesulfonamide (**20c**) (180 mg, 0.61 mmol) and (*S*)-2-acetoxy-2-phenylethylacetyl chloride (**16f**) (1.5 eq) (Yield: 72 %). White solid; Mp: 145.9-148.0°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.83 (s, 1H, -CO-N*H*-), 8.31 (d, *J* = 8.6 Hz, 1H, 17-*H*), 8.23 (t, *J* = 6.3 Hz, 1H, SO_2-N*H*-CH_2-), 7.83 (d, *J* = 2.2 Hz, 1H, 14-*H*), 7.74 (dd, *J* = 8.6, 2.2 Hz, 1H, 16-*H*), 7.53 – 7.16 (m, 11H, -CH-C_6H_5, NH-CH_2-C_6H_5), 7.09 (d, *J* = 4.3 Hz, 1H, CH-O*H*), 5.24 (d, *J* = 4.3 Hz, 1H, C*H*-OH), 4.00 (d, *J* = 6.3 Hz, 2H, NH-C*H*$_2$-C_6H_5). Anal. ($C_{21}H_{19}ClN_2O_4S$) theoretical: C, 58.54; H, 4.44; N, 6.50. Found: C, 58.60; H, 4.49; N, 6.58.

2.2.38. 2-Fluoro-4-nitrobenzenesulfonamide (24)

2-Fluoro-4-nitroaniline (**22**) (15 g, 96,10 mmol) was suspended in an acetic acid and hydrochloric acid mixture (3:1) (120 mL). The solution was maintained at a temperature below 0-5°C. An aqueous sodium nitrite solution (5 mL, 1.5 eq) was gradually added to form the diazonium salt. This mixture containing the diazonium salt was added to a saturated acetic acid solution of sulphur dioxide (70 mL) in the presence of $CuCl_2$ (0.33 eq). The resulting sulphonyl chloride derivative (**23**) precipitated in the medium after the addition of ice. It was recovered on a paper filter after filtration. The sulphonyl chloride intermediate (**23**) was added into a cooled solution of 1,4-dioxane (30 mL) and ammonia solution (30 mL). After stirring for 45 minutes, the mixture was concentrated to dryness under reduced pressure. The residue was taken up in water (100 mL), and the pH was adjusted to 1 by the addition of an aqueous solution of concentrated hydrochloric acid. The precipitate was collected by filtration, washed with water and dried (Yield: 72 %). White solid; Mp: 151.3-152.4°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.35 (dd, *J* = 9.8, 2.2 Hz, 1H, 5-*H*), 8.23 (dd, *J* = 8.6, 2.2 Hz, 1H, 6-*H*), 8.06 (dd, *J* = 8.6, 7.3 Hz, 1H, 3-*H*), 7.82 (s, 2H, SO_2-N*H*$_2$). Anal. ($C_6H_5FN_2O_4S$) theoretical: C, 32.73; H, 2.29; N, 12.72. Found: C, 32.83; H, 2.19; N, 13.00.

2.2.39. 2-(Isobutylamino)-4-nitrobenzenesulfonamide (25a)

In an appropriate microwave vial, a solution of 2-fluoro-4-nitrobenzenesulfonamide (**24**) (2g, 9.10 mmol), TEA (2.2 eq) and isobutylamine (1.2 eq) in 1,4-dioxane (10 mL) was heated at 130°C during 45 minutes. After cooling to room temperature, the media was poured onto ice, and the pH of the aqueous mixture was adjusted to neutrality by means of an aqueous concentrated hydrochloric acid solution. The title compound precipitated was collected by filtration (Yield: 80 %). White solid; Mp: 161.0-162.0°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.84 (d, *J* = 8.5 Hz, 1H, 6-*H*), 7.74 (s, 2H, SO_2-N*H*$_2$), 7.45 (d, *J* = 2.3 Hz, 1H, 3-*H*), 7.43 (dd, *J* = 8.5, 2.3 Hz, 1H, 5-*H*), 6.29 (t, *J* = 5.5 Hz, 1H, N*H*-CH_2-CH-$(CH_3)_2$), 3.10 (dd, *J* = 6.9, 5.5 Hz, 2H, NH-C*H*$_2$-CH-$(CH_3)_2$), 1.95 (m, *J* = 6.9, 6.6 Hz, 1H, NH-CH_2-C*H*-$(CH_3)_2$), 0.97 (d, *J* = 6.6 Hz, 6H, NH-CH_2-CH-(C*H*$_3$)$_2$). Anal. ($C_{10}H_{15}N_3O_4S$) theoretical: C, 43.95; H, 5.53; N, 15.38. Found: C, 43.95; H, 5.29; N, 15.41.

2.2.40. 2-(Allylamino)-4-nitrobenzenesulfonamide (25b)

The title compound was obtained as described for (**25a**) using allylamine instead of isobutylamine (Yield: 86 %). White solid; Mp: 136.5-137.9°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.86 (d, *J* = 8.6 Hz, 1H, 6-*H*), 7.73 (s, 2H, SO_2-N*H*$_2$), 7.46 (dd, *J* = 8.6, 2.2 Hz, 1H, 5-*H*), 7.43 (d, *J* = 2.2 Hz, 1H, 3-*H*), 6.46 (t, *J* = 5.8 Hz, 1H, N*H*-CH_2-CH=$C(H)_2$), 5.93 (ddt, *J* = 17.3, 10.4, 4.8 Hz, 1H, NH-CH_2-C*H*=$C(H)_2$), 5.25 (dd, J_{trans} = 17.3, 1.9 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 5.19 (dd, J_{cis} = 10.4, 1.9 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 4.01 (dd, *J* = 5.8, 4.8 Hz, 2H, NH-C*H*$_2$-CH=$C(H)_2$). Anal. ($C_9H_{11}N_3O_4S$) theoretical: C, 42.02; H, 4.31; N, 16.33. Found: C, 41.89; H, 4.28; N, 16.14.

2.2.41. 2-(Benzylamino)-4-nitrobenzenesulfonamide (25c)

The title compound was obtained as described for (**25a**) using benzylamine instead of isobutylamine (Yield: 76 %).

White solid; Mp: 122.5-123.3°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.87 (d, *J* = 8.6 Hz, 1H, 6-*H*), 7.80 (s, 2H, SO_2-NH_2), 7.44 (dd, *J* = 8.6, 2.2 Hz, 1H, 5-*H*), 7.38 (d, *J* = 2.2 Hz, 1H, 3-*H*), 7.44 – 7.24 (m, 5H, NH-CH_2-C_6H_5), 6.86 (t, *J* = 5.8 Hz, 1H, N*H*-CH_2-C_6H_5), 4.59 (d, *J* = 5.8 Hz, 2H, NH-CH_2-C_6H_5). Anal. ($C_{13}H_{13}N_3O_4S$) theoretical: C, 50.81; H, 4.26; N, 13.67. Found: C, 50.88; H, 4.31; N, 13.54.

2.2.42. 4-Nitro-2-(phenethylamino)benzenesulfonamide (25d)

The title compound was obtained as described for (**25a**) using phenethylamine instead of isobutylamine (Yield: 88 %). White solid; Mp: 138-139.2°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.84 (d, *J* = 8.6 Hz, 1H, 6-*H*), 7.68 (s, 2H, SO_2-NH_2), 7.48 (d, *J* = 2.3 Hz, 1H, 3-*H*), 7.44 (dd, *J* = 8.6, 2.3 Hz, 1H, 5-*H*), 7.33 – 7.19 (m, 5H, NH-CH_2-CH_2-C_6H_5), 6.30 (t, *J* = 5.4 Hz, 1H, N*H*-CH_2-CH_2-C_6H_5), 3.53 (dt, *J* = 7.3, 5.4 Hz, 2H, NH-CH_2-CH_2-C_6H_5), 2.94 (t, *J* = 7.3 Hz, 2H, NH-CH_2-CH_2-C_6H_5). Anal. ($C_{14}H_{15}N_3O_4S$) theoretical: C, 52.33; H, 4.71; N, 13.08. Found: C, 51.99; H, 5.59; N, 12.91.

2.2.43. 4-Amino-2-(isobutylamino)benzenesulfonamide (26a)

The suspension of 2-(isobutylamino)-4-nitrobenzenesulfonamide (**25a**) (1 g, 3.66 mmol) in a 2:1 ethanol/water mixture (90 mL) was heated until the product dissolved. Then, ammonium chloride (3.2 eq) and powdered iron (3.2 eq) were added. After the mixture was refluxed for 45 min, the insoluble material was removed by filtration through Celite® and rinsed with a small amount of hot ethanol. The filtrate was concentrated under reduced pressure. The residue was suspended in water (40 mL) and extracted thrice with ethyl acetate (50 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure. The title compound was obtained after purification by chromatography on silica gel (ethyl acetate/hexane 15:5) (Yield: 95 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.25 (d, *J* = 9.1 Hz, 1H, 6-*H*), 6.84 (s, 2H, SO_2-NH_2), 5.84 (d, *J* = 2.1 Hz, 1H, 3-*H*), 5.83 (dd, *J* = 9.1, 2.1 Hz, 1H, 5-*H*), 5.83 (t, *J* = 5.3 Hz, 1H, , N*H*-CH_2-CH-$(CH_3)_2$), 5.55 (s, 2H, -NH_2), 2.85 (dd, *J* = 6.8, 5.3 Hz, 2H, NH-CH_2-CH-$(CH_3)_2$), 1.89 (hept, *J* = 6.8, 6.6 Hz, 1H, NH-CH_2-C*H*-$(CH_3)_2$), 0.95 (d, *J* = 6.6 Hz, 6H, NH-CH_2-CH-$(CH_3)_2$). Anal. ($C_{10}H_{17}N_3O_4S$) theoretical: C, 49.36; H, 7.04; N, 17.27. Found: C, 49.39; H, 7.06; N, 17.29.

2.2.44. 2-(Allylamino)-4-aminobenzenesulfonamide (26b)

The title compound was obtained as described for (**26a**) starting from 2-(allylamino)-4-nitrobenzenesulfonamide (**25b**) (1 g, 3,90 mmol) (Yield: 77 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.27 (d, *J* = 8.5 Hz, 1H, 6-*H*), 6.87 (s, 2H, SO_2-NH_2), 5.97 – 5.92 (ddt, *J* = 17.2, 10.4, 4.8 Hz, 1H, CH_2-C*H*=C(H)$_2$), 5.90 (t, *J* = 5.3 Hz, 1H, N*H*-CH_2-CH=C(H)$_2$), 5.86 (dd, *J* = 8.5, 1.9 Hz, 1H, 5-*H*), 5.81 (d, *J* = 1.9 Hz, 1H, 3-*H*), 5.55 (s, 2H, -NH_2), 5.26 (dd, J_{trans} = 17.2, 1.7 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 5.15 (dd, J_{cis} = 10.4, 1.7 Hz, 1H, NH-CH_2-CH=C(*H*)$_2$), 3.73 (dd, *J* = 5.3, 4.8 Hz, 2H, NH-CH_2-CH=C(H)$_2$). Anal. ($C_9H_{13}N_3O_4S$) theoretical: C, 47.56; H, 5.77; N, 18.49. Found: C, 47.63; H, 5.74; N, 18.65.

2.2.45. 4-Amino-2-(benzylamino)benzenesulfonamide (26c)

The title compound was obtained as described for (**26a**) starting from 2-(benzylamino)-4-nitrobenzenesulfonamide (**25c**) (1 g, 3.30 mmol) (Yield: 76 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.40 – 7.20 (m, 5H, NH-CH_2-C_6H_5), 7.28 (d, *J* = 8.6 Hz, 1H, 6-*H*), 6.93 (s, 2H, SO_2-NH_2), 6.27 (t, *J* = 5.8 Hz, 1H, N*H*-CH_2-C_6H_5), 5.85 (dd, *J* = 8.6, 2.0 Hz, 1H, 5-*H*), 5.77 (d, *J* = 2.0 Hz, 1H, 3-*H*), 5.52 (s, 2H, -NH_2), 4.34 (d, *J* = 5.8 Hz, 2H, NH-CH_2-C_6H_5). Anal. ($C_{13}H_{15}N_3O_4S$) theoretical: C, 56.30; H, 5.45; N, 15.15. Found: C, 56.21; H, 5.31; N, 15.05.

2.2.46. 4-Amino-2-(phenethylamino)benzenesulfonamide (26d)

The title compound was obtained as described for (**26a**) starting from 4-nitro-2-(phenethylamino)benzenesulfonamide (**25d**) (1 g, 3.10 mmol) (Yield: 83 %). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.32 – 7.20 (m, 5H, NH-CH_2-CH_2-C_6H_5), 7.27 (d, *J* = 8.5 Hz, 1H, 6-*H*), 6.80 (s, 2H, 10), 5.94 (d, *J* = 2.0 Hz, 1H, 3-*H*), 5.86 (dd, *J* = 8.5, 2.0 Hz, 1H, 5-*H*), 5.83 (t, *J* = 5.4 Hz, 1H, N*H*-CH_2-CH_2-C_6H_5), 5.59 (s, 2H, NH_2), 3.26 (dt, *J* = 6.5, 5.4 Hz 2H, NH-CH_2-CH_2-C_6H_5), 2.88 (t, *J* = 6.5 Hz, 2H, NH-CH_2-CH_2-C_6H_5). Anal. ($C_{14}H_{17}N_3O_4S$) theoretical: C, 57.71; H, 5.88; N, 14.42. Found: C, 58.01; H, 5.88; N, 14.08.

2.2.47. (R,S)-2-Acetoxy-N-(4-isobutyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-methylpropanamide (28a)

To a solution of 4-amino-2-(isobutylamino)benzenesulfonamide (**26a**) (426 mg, 1.75 mmol) and TEA (2.2 eq) in acetonitrile (20 mL) cooled in an ice bath, (*R,S*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16a**) (1.5 eq) was added. The flask was immediately hermetically closed. After stirring for 1 hr at room temperature, the solvent was evaporated under reduced pressure. The residue was taken up with water and extracted thrice with ethyl acetate (50 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure. To the resulting oil (compound **27a**) was added triethyl orthoformate (30 mL). The mixture was heated at 130°C for 24 hrs. After cooling to room temperature, the title compound, which precipitated, was collected by filtration, washed with diethyl ether, and dried (Yield: 27 %). White solid; Mp: 175.2-176.7°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.47 (s, 1H, -CO-N*H*-), 8.12 (s, 1H, 3-*H*), 7.89 (d, *J* = 8.7 Hz, 1H, 8*H*), 7.83 (d, *J* = 2.1 Hz 1H, 5-*H*), 7.77 (dd, *J* = 8.8, 2.1 Hz, 1H, 7-*H*), 3.85 (d, *J* = 7.5 Hz, 2H, N-CH_2-CH-$(CH_3)_2$), 2.22 (s, 3H, O-CO-CH_3), 2.11 (hept, *J* = 7.5, 6.6 Hz, 1H, N-CH_2-C*H*-$(CH_3)_2$), 1.84 (s, 3H, C-CH_3), 0.91 (d, *J* = 6.6 Hz, 6H, N-CH_2-CH-$(CH_3)_2$). Anal. ($C_{17}H_{20}F_3N_3O_5S$) theoretical: C, 46.89; H, 4.63; N, 9.65. Found: C, 46.80; H, 4.66; N, 9.39.

2.2.48. (R,S)-2-Acetoxy-N-(4-allyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-methylpropanamide (28b)

The title compound was obtained as described for (**28a**) starting from 2-(allylamino)-4-aminobenzenesulfonamide

(**26b**) (411 mg, 1.81 mmol) and (*R,S*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16a**) (Yield : 24 %). White solid ; Mp :123.1-126.8°C ; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.45 (s, 1H, -CO-N*H*-), 8.15 (s, 1H, 3-*H*), 7.89 (d, *J* = 8.7 Hz, 1H, 8-*H*), 7.84 (d, *J* = 1.9 Hz 1H, 5-*H*), 7.76 (dd, *J* = 8.7, 1.9 Hz, 1H, 7-*H*), 6.04 (ddt, *J* = 17.4, 10.5, 5.0 Hz, 1H, , N-CH_2-C*H*=C(H)$_2$), 5.31 (dd, J_{cis}= 10.5, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 5.21 (d, J_{trans} = 17.4, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 4.68 (d, *J* = 5.0 Hz, 2H, N-C*H*$_2$-CH=C(H)$_2$), 2.22 (s, 3H, O-CO-C*H*$_3$), 1.83 (s, 3H, C-C*H*$_3$). Anal. ($C_{16}H_{16}F_3N_3O_5S$) theoretical: C, 45.82 ; H, 3.85 ; N, 10.02. Found : C, 45.94 ; H, 3.80 ; N, 10.00.

2.2.49. (R,S)-2-Acetoxy-N-(4-benzyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-methylpropanamide (28c)

The title compound was obtained as described for (**28a**) starting from 4-amino-2-(benzylamino)benzenesulfonamide (**26c**) (430 mg, 1.55 mmol) and (*R,S*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16a**) (Yield: 35 %). White solid; Mp: 245.5-246.7°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.38 (s, 1H, -CO-N*H*-), 8.39 (s, 1H, 3-*H*), 7.89 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.85 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.71 (dd, *J* = 8.6, 1.9 Hz, 1H, 7-*H*), 7.40 – 7.29 (m, 5H, N-CH_2-C_6H_5), 5.28 (s, 2H, N-C*H*$_2$-C_6H_5), 2.19 (s, 3H, O-CO-C*H*$_3$), 1.79 (s, 3H, C-C*H*$_3$). Anal. ($C_{20}H_{18}F_3N_3O_5S$) theoretical: C, 51.17; H, 3.87; N, 8.95. Found: C, 51.50; H, 4.06; N, 8.91.

2.2.50. (R,S)-2-Acetoxy-N-(4-phenethyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-methylpropanamide (28d)

The title compound was obtained as described for (**28a**) starting from 4-amino-2-(phenethylamino)benzenesulfonamide (**26d**) (364 mg, 1.25 mmol) and (*R,S*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16a**) (Yield: 30 %). White solid; Mp:186.2-188.0°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.53 (s, 1H, -CO-N*H*-), 7.97 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.91 (s, 1H, 3-*H*), 7.91 (d, *J* = 8.7 Hz, 1H, 8-*H*), 7.77 (dd, *J* = 8.7, 1.9 Hz, 1H, 7-*H*), 7.35–7.23 (m, 5H, N-CH_2-CH_2-C_6H_5), 4.24 (d, *J* = 7.7 Hz, 2H, N-C*H*$_2$-CH_2-C_6H_5), 3.06 (d, *J* = 7.7 Hz, 2H, N-CH_2-C*H*$_2$-C_6H_5), 2.25 (s, 3H, O-CO-C*H*$_3$), 1.87 (s, 3H, C-C*H*$_3$). Anal. ($C_{21}H_{20}F_3N_3O_5S$) theoretical: C, 52.17; H, 4.17; N, 8.69. Found: C, 52.10; H, 4.00; N, 8.59.

2.2.51. 2-Acetoxy-N-(4-isobutyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-2-methylpropanamide (28e)

The title compound was obtained as described for (**28a**) starting from 4-amino-2-(isobutylamino)benzenesulfonamide (**26a**) (300 mg, 1.24 mmol) and 2-acetoxy-2-methylpropanoyl chloride (**16c**) (Yield: 41 %). White solid; Mp: 233.3-234.8°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.05 (s, 1H, -CO-N*H*-), 8.10 (s, 1H, 3-*H*), 7.90 (d, *J* = 1.8 Hz, 1H, 5-*H*), 7.83 (d, *J* = 8.8 Hz, 1H, 8-*H*), 7.78 (dd, *J* = 8.8, 1.8 Hz, 1H, 7-*H*), 3.84 (d, *J* = 7.5 Hz, 2H, N-C*H*$_2$-CH-(CH_3)$_2$), 2.18 – 2.10 (m, 7.5, 6.6 Hz 1H, N-CH_2-C*H*-(CH_3)$_2$), 2.09 (s, 3H, O-CO-C*H*$_3$), 1.57 (s, 6H, C-(C*H*$_3$)$_2$), 0.91 (d, *J* = 6.6 Hz, 6H, N-CH_2-CH-(C*H*$_3$)$_2$). Anal. ($C_{17}H_{23}N_3O_5S$) theoretical: C, 53.53; H, 6.08; N, 11.02. Found: C, 53.75; H, 6.32; N, 10.85.

2.2.52. 2-Acetoxy-N-(4-allyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-2-methylpropanamide (28f)

The title compound was obtained as described for (**28a**) starting from 2-(allylamino)-4-aminobenzenesulfonamide (**26b**) (340 mg, 1.43 mmol) and 2-acetoxy-2-methylpropanoyl chloride (**16c**) (Yield: 66 %). White solid; Mp: 168.4-170.8°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.03 (s, 1H, -CO-N*H*), 8.13 (s, 1H, 3-*H*), 7.90 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.83 (d, *J* = 8.7 Hz, 1H, 8-*H*), 7.76 (dd, *J* = 8.7, 1.9 Hz, 1H, 7-*H*), 6.04 (ddt, *J* = 17.3, 10.4, 5.0 Hz, 1H, N-CH_2-C*H*=C(H)$_2$), 5.31 (dd, J_{cis}= 10.4, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 5.22 (dd, J_{trans} = 17.3, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 4.66 (d, *J* = 5.0 Hz, 2H, N-C*H*$_2$-CH=C(H)$_2$), 2.08 (s, 3H, O-CO-C*H*$_3$), 1.56 (s, 6H, C-(C*H*$_3$)$_2$). Anal. ($C_{16}H_{19}N_3O_5S$) theoretical: C, 52.59; H, 5.24; N, 11.50. Found: C, 52.32; H, 5.22; N, 11.10.

2.2.53. 2-Acetoxy-N-(4-benzyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-2-methylpropanamide (28g)

The title compound was obtained as described for (**28a**) starting from 4-amino-2-(benzylamino)benzenesulfonamide (**26c**) (300 mg, 1.10 mmol) and 2-acetoxy-2-methylpropanoyl chloride (**16c**) (Yield: 58 %). White solid; Mp: 145.6-148.2°C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.07 (s, 1H, -CO-N*H*-), 8.35 (s, 1H, 3-*H*), 8.04 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.95 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.85 (dd, *J* = 8.6, 1.9 Hz, 1H, 7-*H*), 7.42 – 7.29 (m, 5H, N-CH_2-C_6H_5), 5.75 (s, 2H, N-C*H*$_2$-C_6H_5), 2.19 (s, 3H, O-CO-C*H*$_3$), 1.33 (s, 6H, C-(C*H*$_3$)$_2$). Anal. ($C_{20}H_{21}N_3O_5S$) theoretical: C, 57.82; H, 5.10; N, 10.11. Found: C, 57.82; H, 5.11; N, 9.98.

2.2.54. 2-Acetoxy-N-(4-phenethyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-2-methylpropanamide (28h)

The title compound was obtained as described for (**28a**) starting from 4-amino-2-(phenethylamino)benzenesulfonamide (**26d**) (330 mg, 1.13 mmol) and 2-acetoxy-2-methylpropanoyl chloride (**16c**) (Yield: 36 %). White solid; Mp: 182.6-184.9°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.11 (s, 1H, -CO-N*H*-), 8.05 (s, 1H, 3-*H*), 7.88 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.85 (d, *J* = 8.7 Hz, 1H, 8-*H*), 7.78 (dd, *J* = 8.7, 1.9 Hz, 1H, 7-*H*), 7.36 – 7.25 (m, 5H, N-CH_2-CH_2-C_6H_5), 4.23 (t, *J* = 7.6 Hz, 2H, N-C*H*$_2$-CH_2-C_6H_5), 3.07 (t, *J* = 7.6 Hz, 2H, N-CH_2-CH_2-C_6H_5), 2.11 (s, 3H, O-CO-C*H*$_3$), 1.60 (s, 6H, C-(C*H*$_3$)$_2$). Anal. ($C_{21}H_{23}N_3O_5S$) theoretical: C, 58.73; H, 5.40; N, 9.78. Found: C, 58.61; H, 5.25; N, 9.85.

2.2.55. (R,S)-N-(4-Isobutyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (11a)

To a solution of (*R,S*)-2-acetoxy-*N*-(4-isobutyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-methylpropanamide (**28a**) (115 mg, 0.26 mmol) in methanol, was added an aqueous solution of Na_2CO_3 (1.2 eq in 1mL). After 2 hrs of stirring at room temperature, the solvent was removed by distillation under reduced pressure, and the residue was suspended in water. The mixture was neutralized by HCl 0.1N and extracted thrice with ethyl acetate (50 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under

reduced pressure, and the title compound was recrystallized in ethyl acetate/hexane (1:2) (Yield: 70 %). White solid; Mp: 183-193°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.50 (s, 1H, -CO-N*H*-), 8.11 (s, 1H, 3-*H*), 8.09 (dd, *J* = 8.8, 1.9 Hz, 1H, 7-*H*), 7.93 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.86 (d, *J* = 8.8 Hz, 1H, 8-*H*), 7.68 (s, 1H, C-O*H*), 3.84 (d, *J* = 7.6 Hz, 2H, N-CH_2-CH-(CH_3)$_2$), 2.14 (hept, *J* = 7.6, 6.6 Hz, 1H, N-CH_2-C*H*-(CH_3)$_2$), 1.61 (s, 3H, C-CH_3), 0.91 (d, *J* = 6.6 Hz, 6H, N-CH_2-CH-(CH_3)$_2$). Anal. ($C_{15}H_{18}F_3N_3O_4S$) theoretical: C, 45.80; H, 4.61; N, 10.68. Found: C, 46.13; H, 4.29; N, 10.34.

2.2.56. (R,S)-N-(4-Allyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (11b)

The title compound was obtained as described for (**11a**) starting from (*R,S*)-2-acetoxy-*N*-(4-allyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-methylpropanamide (**28b**) (176 mg, 0.42 mmol) (Yield: 50 %). White solid; Mp: 100-107.5°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.47 (s, 1H, -CO-N*H*-), 8.14 (s, 1H, 3-*H*), 8.02 (d, *J* = 1.8 Hz, 1H, 5-*H*), 8.00 (d, *J* = 8.7 Hz, 1H, 8-*H*), 7.85 (dd, *J* = 8.7, 1.8 Hz, 1H, 7-*H*), 7.64 (s, 1H, C-O*H*), 6.04 (ddt, *J* = 17.4, 10.5, 5.1 Hz, 1H, N-CH_2-C*H*=C(H)$_2$), 5.30 (dd, J_{cis} = 10.5, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 5.23 (dd, J_{trans}= 17.3, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 4.67 (d, *J* = 5.1 Hz, 2H, N-CH_2-CH=C(H)$_2$), 1.59 (s, 3H, C-CH_3). Anal. ($C_{14}H_{14}F_3N_3O_4S$) theoretical: C, 44.56; H, 3.74; N, 11.14. Found: C, 44.41; H, 3.55; N, 10.98.

2.2.57. (R,S)-N-(4-Benzyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (11c)

The title compound was obtained as described for (**11a**) starting from (*R,S*)-2-acetoxy-*N*-(4-benzyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-methylpropanamide (**28c**) (255 mg, 0.55 mmol) (Yield: 70 %). White solid; Mp: 228.3-229.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.40 (s, 1H, -CO-N*H*-), 8.36 (s, 1H, 3-*H*), 8.00 (d, *J* = 1.8 Hz, 1H, 5-*H*), 7.97 (dd, *J* = 8.7, 1.8 Hz, 1H, 7-*H*), 7.86 (d, *J* = 8.7 Hz, 1H, 8-*H*), 7.58 (s, 1H, C-O*H*), 7.41 – 7.30 (m, 5H, 21, N-CH_2-C_6H_5), 5.28 (s, 2H, N-CH_2-C_6H_5), 1.56 (s, 3H, C-CH_3). Anal. ($C_{18}H_{16}F_3N_3O_4S$) theoretical: C, 50.58; H, 3.77; N, 9.83. Found: C, 50.33; H, 3.87; N, 10.04.

2.2.58. (R,S)-N-(4-Phenethyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-hydroxy-2-methylpropanamide (11d)

The title compound was obtained as described for (**11a**) starting from (*R,S*)-2-acetoxy-*N*-(4-phenethyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-3,3,3-trifluoro-2-methylpropanamide (**28d**) 198 mg, 0.41 mmol) (Yield: 78 %). White solid; Mp: 210-216°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.57 (s, 1H, -CO-N*H*-), 8.09 (d, *J* = 1.9 Hz, 1H, 5-*H*), 8.06 (dd, *J* = 8.6, 1.9 Hz, 1H, 7-*H*), 7.87 (s, 1H, 3-*H*), 7.87 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.68 (s, 1H, C-O*H*), 7.34 – 7.24 (m, 5H, N-CH_2-CH_2-C_6H_5), 4.25 (t, *J* = 7.5 Hz, 2H, N-CH_2-CH_2-C_6H_5), 3.07 (t, *J* = 7.5 Hz, 2H, N-CH_2-CH_2-C_6H_5), 1.63 (s, 3H, C-CH_3). Anal. ($C_{19}H_{18}F_3N_3O_4S$) theoretical: C, 51.70; H, 4.11; N, 9.52. Found: C, 51.49; H, 4.23; N, 9.41.

2.2.59. N-(4-Isobutyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropanamide (11e)

The title compound was obtained as described for (**11a**) starting from 2-acetoxy-*N*-(4-isobutyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-methylpropanamide (**28e**) (100 mg, 0.26 mmol) (Yield: 65 %). White solid; Mp: 224.1-225.8°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.19 (s, 1H, -CO-N*H*-), 8.09 (s, 1H, 3-*H*), 8.08 (dd, *J* = 8.8, 1.9 Hz, 1H, 7-*H*), 7.98 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.82 (d, *J* = 8.8 Hz, 1H, 7-*H*), 5.91 (s, 1H, C-O*H*), 3.83 (d, *J* = 7.6 Hz, 2H, N-CH_2-CH-(CH_3)$_2$), 2.14 (hept, *J* = 7.6, 6.6 Hz, 1H, N-CH_2-C*H*-(CH_3)$_2$), 1.37 (s, 6H, C-(CH_3)$_2$), 0.91 (d, *J* = 6.6 Hz, 6H, N-CH_2-CH-(CH_3)$_2$). Anal. ($C_{15}H_{21}N_3O_4S$) theoretical: C, 53.08; H, 6.24; N, 12.38. Found: C, 53.42; H, 6.20; N, 12.04.

2.2.60. N-(4-Allyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropanamide (11f)

The title compound was obtained as described for (**11a**) starting from 2-acetoxy-*N*-(4-allyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-methylpropanamide (**28f**) (360 mg, 0.99 mmol) (Yield: 56 %). White solid; Mp: 198.1-201.5°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.13 (s, 1H, -CO-N*H*-), 8.12 (s, 1H, 3-*H*), 8.03 (d, *J* = 1.8 Hz, 1H, 5-*H*), 7.98 (dd, *J* = 8.7, 1.8 Hz, 1H, 7-*H*), 7.81 (d, *J* = 8.7 Hz, 1H, 8-*H*), 6.03 (ddt, *J* = 17.3, 10.5, 4.8 Hz, 1H, N-CH_2-C*H*=C(H)$_2$), 5.88 (s, 1H, C-O*H*), 5.29 (dd, J_{cis} = 10.5, 1.4 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 5.23 (dd, J_{trans} = 17.3, 1.4 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 4.66 (d, *J* = 4.8 Hz, 2H, N-CH_2-CH=C(H)$_2$), 1.36 (s, 6H, C-(CH_3)$_2$). Anal. ($C_{14}H_{17}N_3O_4S$) theoretical: C, 52.00; H, 5.30; N, 12.99. Found: C, 51.84; H, 5.32; N, 12.86.

2.2.61. N-(4-Benzyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropanamide (11g)

The title compound was obtained as described for (**11a**) starting from 2-acetoxy-*N*-(4-benzyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-methylpropanamide (**28g**) (240 mg, 0.58 mmol) (Yield: 81 %). White solid; Mp: 133.0-135.8°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.07 (s, 1H, -CO-N*H*-),), 8.35 (s, 1H, 3-*H*), 8.04 (d, *J* = 1.8 Hz, 1H, 5-*H*), 7.95 (dd, *J* = 8.6, 1.8 Hz, 1H, 7-*H*), 7.82 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.42 – 7.29 (m, 5H, N-CH_2-C_6H_5), 5.85 (s, 1H, C-O*H*), 5.28 (s, 2H, N-CH_2-C_6H_5), 1.33 (s, 6H, C-(CH_3)$_2$). Anal. ($C_{18}H_{19}N_3O_4S$) theoretical: C, 57.89; H, 5.13; N, 11.25. Found: C, 57.73; H, 5.24; N, 10.87.

2.2.61. N-(4-Phenethyl-1,1-dioxo-4H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropanamide (11h)

The title compound was obtained as described for (**11a**) starting from 2-acetoxy-*N*-(4-phenethyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-methylpropanamide (**28h**) (100 mg, 0.24 mmol) (Yield: 76 %). White solid; Mp: 168.1-170.7°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.25 (s, 1H, -CO-N*H*-), 8.16 (d, *J* = 1.7 Hz, 1H, 5-*H*), 8.04 (dd, *J* = 8.7, 1.7 Hz, 1H, 7-*H*), 7.86 (s, 1H, 3-*H*), 7.83 (d, *J* = 8.7 Hz, 1H, 8-*H*), 7.36 – 7.24 (m, 5H, N-CH_2-CH_2-C_6H_5), 5.92 (s, 1H, C-O*H*), 4.24 (t, *J* = 7.5 Hz, 2H, N-CH_2-CH_2-C_6H_5), 3.07 (t, *J* = 7.5 Hz, 2H, N-CH_2-CH_2-C_6H_5), 1.40 (s, 6H, C-(CH_3)$_2$). Anal. ($C_{19}H_{21}N_3O_4S$) theoretical: C, 58.90; H, 5.46; N, 10.85. Found: C, 58.72; H, 5.49; N, 10.80.

2.2.62. (R,S)-N-(4-Allyl-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-3,3,3-trifluoro-2-methylpropionamide (12b)

A solution of (*R,S*)-*N*-(4-allyl-1,1-dioxo-4*H*-1,2,4- benzothiadiazin-6-yl)-2-hydroxy-3,3,3-trifluoro-2-methylpropionamide (**11b**) (100 mg, 0.27 mmol) in 2-propanol (30 mL) heated at 60°C was supplemented under stirring with sodium borohydride (4 eq). After stirring for 5 min., the solvent was removed by distillation under reduced pressure, and the residue was suspended in water (10 mL). The alkaline suspension was adjusted to pH 7 with 0.1 N HCl and extracted thrice with DCM (15 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure, and the residue of the title compound was recrystallized in DCM/hexane (1:2) (Yield: 56 %). White solid; Mp: 170.9-172.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.01 (s, 1H, -CO-N*H*-), 8.00 (t, *J* = 8.1 Hz, 1H, SO_2-N*H*-CH_2), 7.51 (s, 1H, C-O*H*), 7.47 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.32 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.28 (dd, *J* = 8.6, 1.9 Hz, 1H, 7-*H*), 5.86 (ddt, *J* = 17.2, 10.3, 4.8 Hz, 1H, N-CH_2-C*H*=$C(H)_2$), 5.23 (dd, J_{trans} = 17.2, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 5.17 (dd, J_{cis} = 10.3, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 4.68 (d, *J* = 8.1 Hz, 2H, 3-C*H*$_2$), 3.95 (d, *J* = 4.8 Hz, 2H, N-C*H*$_2$-CH=$C(H)_2$), 1.56 (s, 3H, C-C*H*$_3$). Anal. ($C_{14}H_{16}F_3N_3O_4S$) theoretical: C, 44.33; H, 4.25; N, 11.08. Found: C, 44.52; H, 4.47; N, 11.00.

2.2.63. (R,S)-N-(4-Benzyl-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-3,3,3-trifluoro-2-methylpropionamide (12c)

The title compound was obtained as described for (**12b**) starting from (*R,S*)-*N*-(4-benzyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-3,3,3-trifluoro-2-methylpropionamide (**11c**) (120 mg, 0.28 mmol) (Yield: 58 %). White solid; Mp: 143.7-145.2°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.98 (bs, 1H, -CO-N*H*-), 8.13 (bs, 1H, SO_2-N*H*-CH_2), 7.50 (d, *J* = 9.1 Hz, 1H, 8-*H*), 7.48 - 7.30 (m, 7H, 6-*H*, 7-*H,* N-CH_2-C_6H_4-H, C-O*H*), 7.27 (t, J = 6.7 Hz, 1H, N-CH_2-C_6H_4-*H*), 4.80 (s, 2H, N-C*H*$_2$-C_6H_5), 4.58 (s, 2H, 3-C*H*$_2$), 1.52 (s, 3H, C-C*H*$_3$). Anal. ($C_{18}H_{18}F_3N_3O_4S$) theoretical: C, 50.35; H, 4.23; N, 9.79. Found: C, 50.49; H, 4.52; N, 9.67.

2.2.64. (R,S)-N-(4-Phenethyl-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-3,3,3-trifluoro-2-methylpropionamide (12d)

The title compound was obtained as described for (**12b**) starting from (*R,S*)-*N*-(4-phenethyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-3,3,3-trifluoro-2-methylpropionamide (**11d**) (112 mg, 0.25 mmol) (Yield: 71 %). White solid; Mp: 165.3-167.4°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.12 (s, 1H, -CO-N*H*-), 7.88 (t, *J* = 8.1 Hz, 1H, SO_2-N*H*-CH_2), 7.54 (s, 1H, C-O*H*), 7.48 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.44 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.30 (dd, *J* = 8.6, 1.9 Hz, 1H, 7-*H*), 7.37 – 7.20 (m, 5H, N-*C*H_2-CH_2-C_6H_5), 4.62 (d, *J* = 8.1 Hz, 2H, 3-C*H*$_2$), 3.53 (t, *J* = 7.9 Hz, 2H, N-*CH*$_2$-CH_2-C_6H_5), 2.86 (t, *J* = 7.9 Hz, 2H, N-*C*H_2-C*H*$_2$-C_6H_5), 1.60 (s, 3H, C-C*H*$_3$). Anal. ($C_{19}H_{20}F_3N_3O_4S$) theoretical: C, 51.46; H, 4.55; N, 9.48. Found: C, 51.51; H, 4.90; N, 9.66.

2.2.65. N-(4-Allyl-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropionamide (12f)

The title compound was obtained as described for (**12b**) starting from *N*-(4-allyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropionamide (**11f**) (101.4 mg, 0.31 mmol) (Yield: 63 %). White solid; Mp: 187.5-190.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.65 (s, 1H, -CO-N*H*-), 7.96 (t, *J* = 8.1 Hz, 1H, SO_2-N*H*-CH_2), 7.44 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.34 (d, *J* = 1.8 Hz, 1H, 5-*H*), 7.24 (dd, *J* = 8.6, 1.8 Hz, 1H, 7-*H*), 5.85 (ddt, *J* = 17.2, 10.3, 4.9 Hz, 1H, N-CH_2-C*H*=$C(H)_2$), 5.78 (s, 1H, C-O*H*), 5.23 (dd, J_{trans} = 17.2, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 5.17 (dd, J_{cis} = 10.3, 1.6 Hz, 1H, N-CH_2-CH=C(*H*)$_2$), 4.67 (d, *J* = 8.1 Hz, 2H, 3-C*H*$_2$), 3.95 (d, *J* = 4.9 Hz, 2H, N-C*H*$_2$-CH=$C(H)_2$), 1.33 (s, 6H, C-(CH_3)$_2$). Anal. ($C_{14}H_{19}N_3O_4S$) theoretical: C, 51.68; H, 5.89; N, 12.91. Found: C, 51.42; H, 5.71; N, 12.29.

2.2.66. N-(4-Benzyl-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropionamide (12g)

The title compound was obtained as described for (**12b**) starting from *N*-(4-benzyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropionamide (**11g**) (130 mg, 0.35 mmol) (Yield: 82 %). White solid; Mp: 168.1-169.7°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.61 (s, 1H, -CO-N*H*-), 8.09 (t, *J* = 7.7 Hz, 1H, SO_2-N*H*-CH_2), 7.47 (d, *J* = 8.6 Hz, 1H, 8-*H*), 7.38 – 7.31 (m, 5H, N-CH_2-C_6H_5), 7.28 (dd, *J* = 8.6, 1.9 Hz, 1H, 7-*H*), 7.26 (d, *J* = 1.9 Hz, 1H, 5-*H*), 5.73 (s, 1H, C-O*H*), 4.79 (d, *J* = 7.7 Hz, 2H, 3-C*H*$_2$), 4.58 (s, 2H, N-C*H*$_2$-C_6H_5), 1.29 (s, 6H, C-(CH_3)$_2$). Anal. ($C_{18}H_{21}N_3O_4S$) theoretical: C, 57.58; H, 5.64; N, 11.19. Found: C, 57.22; H, 5.65; N, 11.16.

2.2.67. N-(4-Phenethyl-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropionamide (12h)

The title compound was obtained as described for (**12b**) starting from *N*-(4-phenethyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropanamide (**11h**) (150 mg, 0.38 mmol) (Yield: 82 %). White solid; Mp: 176.6-178.2°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.76 (s, 1H, -CO-N*H*-), 7.79 (t, *J* = 8.1 Hz, 1H, SO_2-N*H*-CH_2), 7.50 (d, *J* = 2.9 Hz, 1H, 5-*H*), 7.44 (dd, *J* = 8.5, 2.9 Hz, 1H, 7-*H*), 7.36 – 7.30 (m, 5H, N-*C*H_2-CH_2-C_6H_5), 7.25 (d, *J* = 8.5 Hz, 1H, 8-*H*), 5.81 (s, 1H, C-O*H*), 4.61 (d, *J* = 8.1 Hz, 2H, 3-C*H*$_2$), 3.52 (t, *J* = 8.0 Hz, 2H, N-*CH*$_2$-CH_2-C_6H_5), 2.86 (t, *J* = 8.0 Hz, 2H, N-*C*H_2-C*H*$_2$-C_6H_5), 1.37 (s, 6H, C-(CH_3)$_2$). Anal. ($C_{19}H_{23}N_3O_4S$) theoretical: C, 58.59; H, 5.95; N, 10.79. Found: C, 58.43; H, 5.94; N, 10.68.

2.2.68. N-Benzyl-2-fluoro-4-nitrobenzenesulfonamide (29)

2-Fluoro-4-nitroaniline (**22**) (5 g, 32,10 mmol) was suspended in 40 mL of an acetic acid and hydrochloric acid mixture (3:1). The solution was maintained at a temperature below 0-5°C. An aqueous sodium nitrite solution (5 mL; 1.5 eq) was gradually added to obtain the diazonium salt. This mixture was added to a saturated acetic acid solution of

sulphur dioxide 50 mL in the presence of $CuCl_2$ (0.33 eq). The resulting sulphonyl chloride (**23**) precipitated in the medium after the addition of ice. It was collected by filtration and then poured into a cooled solution of benzylamine (1.2 eq) and TEA (1.2 eq) in 1,4-dioxane (30 mL). After stirring for 45 minutes, the mixture was concentrated to dryness under reduced pressure. The residue was taken up in water (30 mL) and extracted thrice with ethyl acetate. The combined organic layers were treated with charcoal and filtered through Celite®. The filtrate was dried over $MgSO_4$ and evaporated under reduced pressure. The title compound was recrystallized in ethyl acetate-hexane (1:2) (Yield: 47 %). White solid; Mp: 113.1-114.2°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.91 (t, *J* = 6.2 Hz, 1H, SO_2-N*H*-CH_2, 8.23 (dd, *J* = 9.8, 2.2 Hz, 1H, 3-*H*), 8.13 (dd, *J* = 8.7, 2.3 Hz, 1H, 5-*H*), 7.97 (dd, *J* = 8.6, 7.3 Hz, 1H, 6-*H*), 7.25 – 7.15 (m, 5H, NH-CH_2-C_6H_5), 4.17 (d, *J* = 6.2 Hz, 2H, NH-CH_2-C_6H_5). Anal. ($C_{13}H_{11}FN_2O_4S$) theoretical: C, 50.32; H, 3.57; N, 9.03. Found: C, 50.31; H, 3.58; N, 8.99.

2.2.69. 2-Amino-N-benzyl-4-nitrobenzenesulfonamide (30)

In an appropriate microwave vial, a solution of *N*-benzyl-2-fluoro-4-nitrobenzenesulfonamide (**29**) (2g, 6.20 mmol) and ammonia solution (2.2 eq) in 1,4-dioxane (10 mL) was heated at 120°C for 30 minutes. After cooling to room temperature, the media was poured onto ice, and the pH was adjusted to neutrality by means of concentrated hydrochloric acid. The title compound precipitated and was collected by filtration (Yield: 95 %). White solid; Mp: 138.4-140.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.43 (t, *J* = 6.2 Hz, 1H, SO_2-N*H*-CH_2), 7.70 (d, *J* = 8.7 Hz, 1H, 6-*H*), 7.64 (d, *J* = 2.4 Hz, 1H, 3-*H*), 7.31 (dd, *J* = 8.7, 2.4 Hz, 1H, 5-*H*), 7.27 – 7.18 (m, 5H, NH-CH_2-C_6H_5), 6.46 (s, 2H, -NH_2), 4.01 (d, *J* = 6.2 Hz, 2H, NH-CH_2-C_6H_5). Anal. ($C_{13}H_{13}N_3O_4S$) theoretical: C, 50.81; H, 4.26; N, 13.67. Found: C, 50.89; H, 4.26; N, 13.70.

2.2.70. 2-Benzyl-6-nitro-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (31)

In an appropriate microwave vial, a solution of 2-amino-*N*-benzyl-4-nitrobenzenesulfonamide (**30**) (1g, 3.1 mmol) in acetonitrile was supplemented with ten drops of sodium isopropanoate and paraformaldehyde (1 eq). The mixture was heated for 20 minutes at 100°C. After cooling to room temperature, the media was evaporated under reduced pressure. The residue was suspended in water (15 mL) and extracted thrice with ethyl acetate (50 mL). The combined organic layers were treated with charcoal and filtered through Celite®. The filtrate was dried over $MgSO_4$ and concentrated to dryness under reduced pressure. The title compound was obtained after a column purification on silica gel (DCM/hexane: 18:2) (Yield: 58 %). White solid; Mp: 243.8-245.6°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.90 (t, *J* = 3.2 Hz, 1H, -N*H*-), 7.85 (d, *J* = 8.7 Hz, 1H, 8-*H*), 7.73 (d, *J* = 2.2 Hz, 1H, 5-*H*), 7.51 (dd, *J* = 8.7, 2.2 Hz, 1H, 7-*H*), 7.41 – 7.33 (m, 5H, N-CH_2-C_6H_5), 4.75 (d, *J* = 3.2 Hz, 2H, 3-CH_2), 4.10 (s, 2H, NH-CH_2-C_6H_5). Anal. ($C_{14}H_{13}N_3O_4S$) theoretical: C, 52.66; H, 4.10; N, 13.16. Found: C, 52.61; H, 4.10; N, 13.12.

2.2.71. 6-Amino-2-benzyl-3,4-dihydro-2H-1,2,4-benzothiadiazine 1,1-dioxide (32)

6-Nitro-2-benzyl-2,3-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxyde (**31**) (0.6 g, 1.80 mmol) was dispersed in a 2:1 ethanol/water mixture (90 mL). The suspension was heated until the product dissolved. Then, ammonium chloride (3.2 eq) and powdered iron (3.2 eq) were added. After the mixture was refluxed for 60 min, the insoluble material was removed by filtration through Celite® and rinsed with a small amount of hot ethanol. The filtrate was concentrated under reduced pressure. The residue was suspended in water (40 mL) and extracted thrice with ethyl acetate (50 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated to dryness under reduced pressure. The title compound was recrystallized in ethyl acetate-hexane (1:2) (Yield: 89 %). White solid; Mp: 182.9-184.1°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.41–7.30 (m, 5H, N-CH_2-C_6H_5), 7.17 (d, *J* = 8.6 Hz, 1H, 8-*H*), 6.83 (t, *J* = 3.5 Hz, 1H, -N*H*-), 6.04 (dd, *J* = 8.6, 2.1 Hz, 1H, 7-*H*), 5.89 (d, *J* = 2.1 Hz, 1H, 5-*H*), 5.67 (s, 2H, -NH_2), 4.50 (d, *J* = 3.5 Hz, 2H, 3-CH_2), 4.01 (s, 2H, N-CH_2-C_6H_5). Anal. ($C_{14}H_{15}N_3O_2S$) theoretical: C, 58.11; H, 5.23; N, 14.52. Found: C, 58.10; H, 5.28; N, 14.55.

2.2.72. (R,S)-N-(2-Benzyl-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-3,3,3-trifluoro-2-methylpropionamide (13c)

To a solution of 6-amino-2-benzyl-2,3-dihydro-2*H*-1,2,4-benzothiadiazine 1,1-dioxyde (**32**) (340 mg, 1.12 mmol) and TEA (1.5 eq) in acetonitrile (15 mL) cooled in an ice bath, was added (*R,S*)-2-acetoxy-3,3,3-trifluoro-2-methylpropanoyl chloride (**16a**) (1.2 eq). The flask was immediately hermetically closed. After stirring for 1 hr at room temperature, the solvent was evaporated under reduced pressure. The residue was dissolved in water and extracted thrice with ethyl acetate (50 mL). The combined organic layers were dried over $MgSO_4$ and filtered. The filtrate was concentrated under reduced pressure and the residue of compound (**33**) was solubilized in methanol (10 mL). A cool aqueous NaOH solution (2.5 N, 10 g/mL) was added dropwise up to alkaline pH. After stirring for 30 minutes, the reaction mixture was neutralized by means of 2N HCl. The solvent was evaporated under reduced pressure, and the resulting precipitate was recrystallized in methanol-water (2:1) (Yield: 63 %). White solid; Mp: 205.5-206.8°C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.05 (s, 1H, -CO-N*H*-), 7.53 (d, *J* = 1.9 Hz, 1H, 5-*H*), 7.50 (s, 1H, C-O*H*), 7.49 (d, *J* = 8.7 Hz, 1H, 8-*H*), 7.42 – 7.33 (m, 5H, N-CH_2-C_6H_5), 7.31 (t, *J* = 3.0 Hz, 1H, -N*H*-), 7.08 (dd, *J* = 8.7, 1.9 Hz, 1H, 7-*H*), 4.59 (d, *J* = 3.0 Hz, 2H, 3-CH_2), 4.03 (s, 2H, N-CH_2-C_6H_5), 1.58 (s, 3H, C-CH_3). Anal. ($C_{18}H_{18}F_3N_3O_4S$) theoretical: C, 50.35; H, 4.23; N, 9.79. Found: C, 50.32; H, 4.23; N, 9.74.

2.3. Biological Assays

In vitro determination of the inhibitory activity of the new compounds on the pyruvate dehydrogenase kinase complex. Each compound was dissolved in pure DMSO. The initial concentrations were 1.0 10^{-2} M. For the evaluation, the stock

solutions were diluted using the same solvent to obtain a range of reduced concentrations. The protocol was carried out according to the modification of the method by Jackson *et al.* [38] which describes a high throughput screening for PDK inhibitors by measuring the residual activity of the PDH complex after kinases reaction. This assay consisted of three steps.

Step 1: preincubation/acetylation: Pig PDC containing intrinsic kinase (Sigma Aldrich) was incubated for 40 min at 37 °C in buffer A [40 mM Mops (pH 7.20, 0.5 mM EDTA, 30 mM KCl, 1.5 mM $MgCl_2$, 0.25 mM acetyl-CoA, 0.05 mM NADH, 2 mM dithiothreitol, 10mM NaF] at a concentration of approximatively 100 µg/ml.

Step 2: PDK reaction: The PDK reaction was initiated by adding 36 µL of the PDC mixture to a vial containing 1µL of DMSO or 1µL of tested drug solution at varying final concentration between 10^{-4} and 10^{-9} M supplemented with 64 µL of buffer B [buffer A + (55 µM ADP, 100 µM ATP)] at 37°C. After 5 min the PDK reaction was terminated by the addition of 10 µL of stopping buffer (55 mM ADP, 55 mM pyruvate).

Steps 3: residual PDH complex activity: The PDH complex activity remaining was assayed by the addition of 100 µL of buffer C [120mM Tris (pH 7.8), 0.61mM EDTA, 0.73mM $MgCl_2$, 2.2mM thiamine pyrophosphate, 11 mM 2-mercaptoethanol, 2.2 mM NAD+, 2.2mM pyruvate, 1.1mM CoA] to each tube which are then briefly vortexed and incubated for 10 minutes at 37°C. At the end of incubation, they were transferred to a 96-well plate, and the production of NADH was measured at 340 nm.

The compound inhibitory effect can be expressed as:

$$\%_{inh} = 100 \times (A\text{-}A_0/A_{max} - A_0)$$

In this relationship, A_0 represents the basal production of NADH (sample without a compound of interest). The PDH complex is completely inhibited. While A_{max} is the maximum amount of NADH produced, there is no ATP or compound of interest in the sample.

Results were expressed as the mean ± standard deviation from at least three determinations ($n \geq 3$). The substance concentration preventing 50% of the PDH complex inhibition (IC_{50}) was calculated by non-linear regression analysis (GraphPad Prism software) from at least three dose-response curves.

3. RESULTS AND DISCUSSION

3.1. Molecular Modelling

The X-ray structure of PDK1 co-crystallized with AZD7545 [13] extracted from the Protein Data Bank (PDB code: 2Q8G) was used (Fig. **4**) to perform docking experiments with a set of proposed molecules belonging to the different series of compounds. The structure of the PDK1-AZD7545 complex showed that the inhibitor binds to the lipoamide-binding pocket in the N-terminal domain of PDK1 [13]. Moreover, X-ray data clearly indicated that the N-

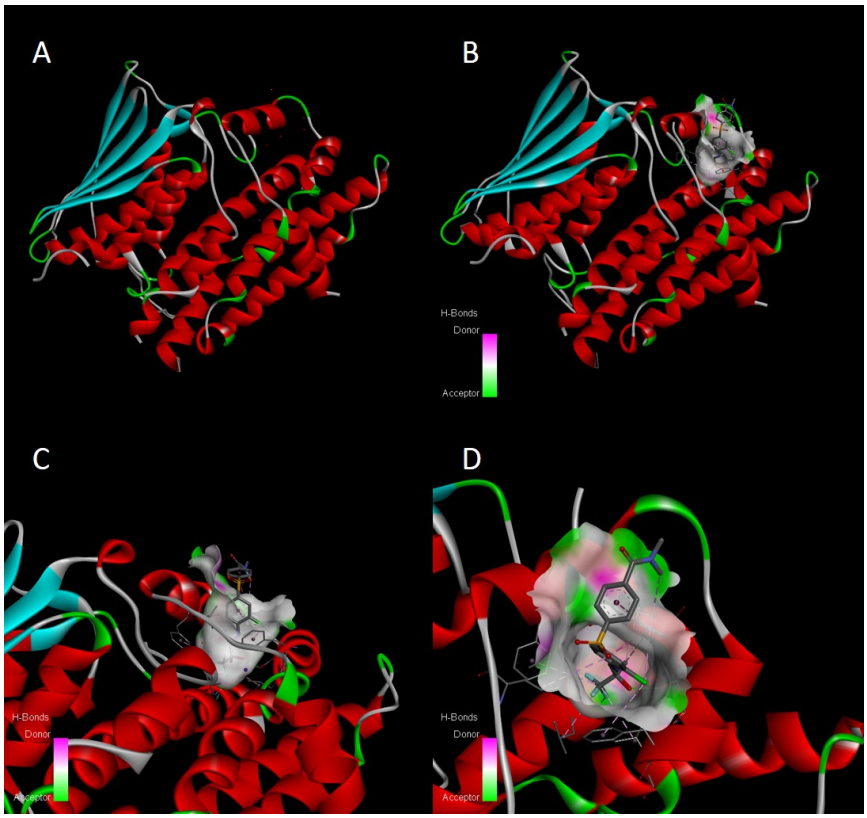


Fig. (4). (**A**) The 1.9 Å crystal structure of human PDK1. (**B**, **C**) Structure of human PDK1 associated to AZD7545. (**D**) The close-up view of the interaction between the lipoamide-binding pocket and AZD7545. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

phenyl-2-hydroxy-3,3,3-trifluoro-2-methylpropionamide moiety of the inhibitor was the critical fragment interacting intimately with the binding site. On the contrary, the 'sulfonyl' side of the molecule, which is located outside the binding site, seemed to offer great possibilities of modulation by the introduction of bulky groups without causing apparent steric hindrance.

According to this observation, the docking study was performed with an example of the compound from each series **10**, **11**, **12** and **13** (Fig. **3**) bearing the required 2-hydroxy-3,3,3-trifluoro-2-methylpropionamide moiety (R^1 = methyl and R^2 = trifluoromethyl) on one side, and a benzyl moiety (R^3) on the other side (compounds **10d**, **11c**, **12c** and **13c**; Fig. **5**).

The 2D diagram of the PDK1-AZD7545 complex interaction shown in Fig. (**6**) indicates that the lipoamide-binding pocket interior is lined with highly conserved hydrophobic residues (Leu57, Phe62, Phe65, Phe78, Leu79, Leu201, and Phe202), which form a hydrophobic interface with the compound. The phenylalanine residues (Phe65 and Phe78) sandwich the 2-chlorophenyl group required for the interaction. The Ser75 forms an H-bond with the ligand's hydroxyl group, which promotes AZD7545 binding. Moreover, a water molecule is trapped inside the lipoyl-binding pocket, which coordinates an H-bonding network involving Phe62, Gln197, and AZD7545 amide oxygen atoms.

Comparable to those already available for AZD7545, these results allowed us to validate this protocol for our investigation. The most significant aspect of molecular docking is the calculation of the free binding energy to fit a ligand into a binding site. It makes it possible to estimate the drug's affinity toward the target. In general, the more negative the energy, and more favorable the interaction and the better the

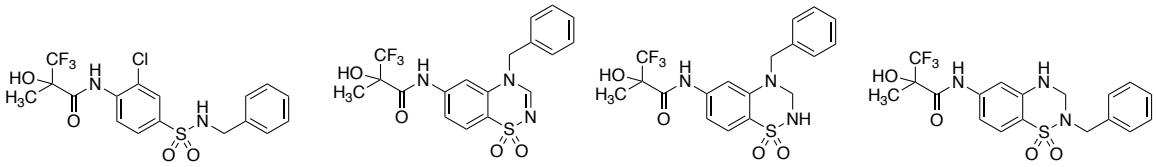


Fig. (5). Molecules examined in the docking experiments.

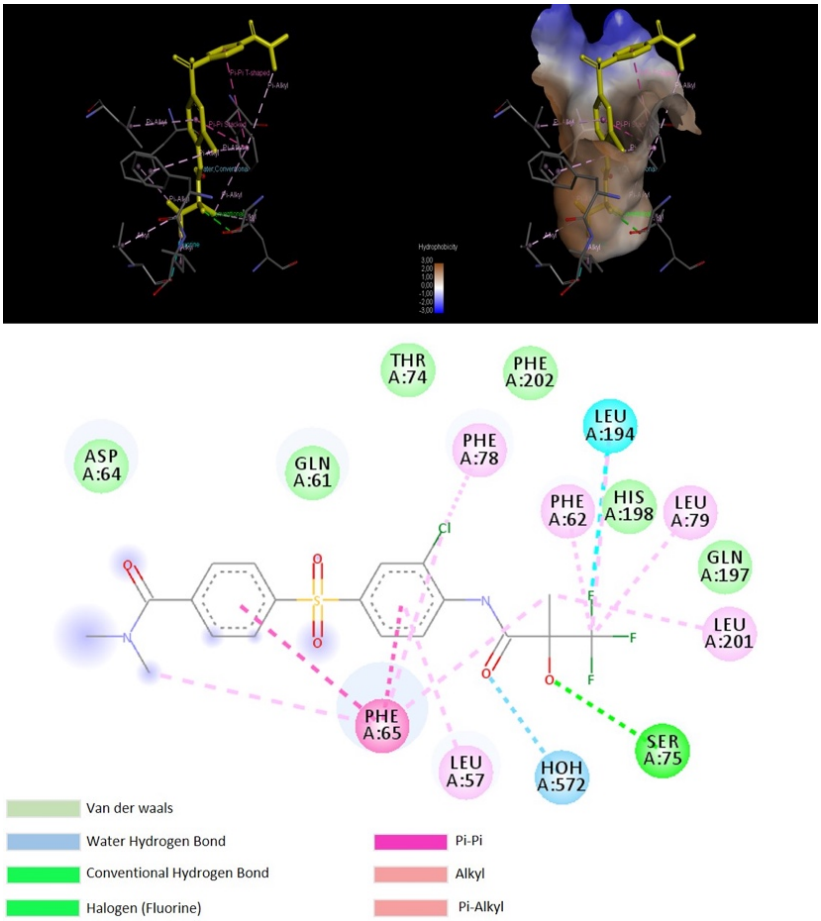


Fig. (6). The 2D diagram of the PDK1-AZD7545 complex interaction. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

adjustment. The Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) free binding energy calculation method [36,37] was used in these experiments. The MM-PBSA results obtained for AZD7545 and the four selected protein-ligand complexes are shown in Table **1**.

Table 1. Lipoamide binding site-ligands free binding energy evaluated by MM-PBSA method

Complexes	PBSA (Kcal/mol)
PDK1-AZD7545	-47.28
PDK1-**10d**	-45.32
PDK1-**11c**	-37.44
PDK1-**12c**	-40.26
PDK1-**13c**	-33.96

Although the free binding energy calculated indicated that the reference compound AZD7545 appeared to express the best affinity for the lipoyl-binding site of PDK1, the docking studies clearly showed that our compounds favorably interacted with this binding site, thus confirming the interest in synthesizing such kind of rigidified molecules as putative novel PDK inhibitors. Interestingly, it was also found that the saturation of the double bond of the 1,2,4-benzothiadiazine 1,1-dioxide ring (**11c** -> **12c**) seemed to increase the affinity for the binding site.

3.2. Chemistry

The synthesis of the new compounds **10**, **11**, **12** and **13** is described in schemes (**1-5**). The first synthetic pathway (Scheme **1**) reports the preparation of the acid chlorides of the diversely R^1,R^2-substituted 2-acetoxyacetic acids (**16**). The hydroxyl function of the appropriate carboxylic acids **14** was protected by acetylation after reaction with acetyl chloride. In the next step, the corresponding acid chlorides **16** were obtained by using oxalyl chloride in the presence of a catalytic amount of DMF in dichloromethane.

Scheme **2** describes the access to the *N*-alkyl/aralkyl-substituted 4-amino-3-chlorobenzenesulfonamides **20**, starting from the commercially available 3-chloro-4-nitroaniline **17**. Diazotization of **17** in the presence of sulfur dioxide and cuprous chloride (cupric chloride reduced by SO_2 into cuprous chloride) provided the corresponding sulfonyl chlorides **18**. The latter was converted into the corresponding *N*-alkyl/aralkylsubstituted sulfonamides **19** after reaction with the appropriate alkyl/aralkylamines. The reduction of the nitro function of **19** by means of iron powder in acetic acid provided the expected *para*-aminobenzenesulfonamides **20**.

The final compounds **10** were obtained after reaction of the appropriate acid chloride **16** with the appropriate *para*-aminobenzenesulfonamide **20** followed by the deprotection of the acetyl group of the intermediate **21** in aqueous alkaline hydrolytic conditions (Scheme **3**).

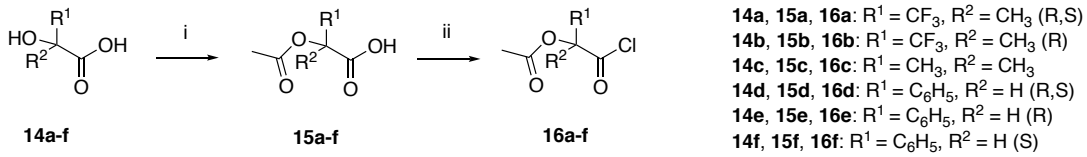
The access to the 4-alkyl/aralkylsubstituted 1,2,4-benzothiadiazine 1,1-dioxides **11** and **12** is reported in Scheme (**4**). Starting from 2-fluoro-4-nitroaniline **22**, the diazotization reaction conducted in the presence of sulfur dioxide and cuprous chloride (see above for **17**) provided the corresponding sulfonyl chloride **23**, which was immediately converted into the sulfonamide **24** after reaction with aqueous ammonia. Nucleophilic substitution of the fluorine atom at the *ortho*-position of the sulfonamide function with the appropriate alkyl/aralkylamine gave access to the expected *ortho*-alkyl/aralkylaminobenzenesulfonamides **25**. The primary amine function at the 4-position of compounds **26** (rather than the secondary bulky alkyl/aralkylamine function at the 2-position), due to more favorable steric conditions, reacted with the appropriate acid chlorides **16** to provide the 2-acetoxy-methylpropanamide intermediates **27**. Ring closure reaction in the presence of triethyl orthoformate gave access to the "unsaturated" 1,2,4-benzothiadiazine 1,1-dioxides **28**, which were deacetylated in mild alkaline conditions to provide the final compounds **11**. The latter were converted into their "saturated" analogues **12** after reaction with sodium borohydride in isopropanol.

Lastly, one example of "saturated" 1,2,4-benzothiadiazine 1,1-dioxide bearing the benzyl group at the 2-position of the heterocycle instead of the 4-position was prepared according to Scheme (**5**). The sulfonyl chloride **23** (see scheme 4) reacted with benzylamine to provide the *N*-benzylsulfonamide **29**. The reaction between **29** and aqueous ammonia produced the corresponding *ortho*-aminobenzenesulfonamide **30**, which was engaged in a ring closure reaction with paraformaldehyde in the presence of a strong base to provide the corresponding 3,4-dihydro-2*H*-1,2,4-benzothiadiazine 11-dioxide **31** bearing a nitro function at the 6-position. Reduction of the nitro function of **31** into the amino function giving intermediate **32**, followed by acylation of **32** with the acid chloride **16a** and deprotection of the acetoxy group of the resulting intermediate **33** provided the final compound **13c**.

3.3. Biological Results

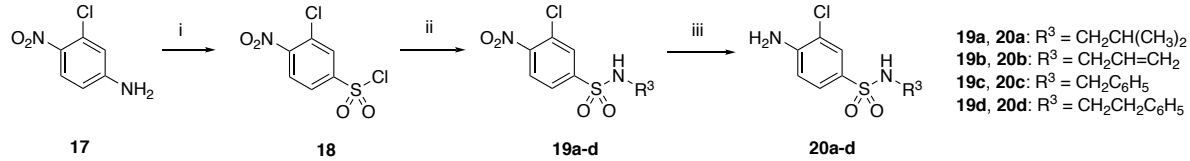
Table **2** reports the inhibitory activity on the pyruvate dehydrogenase kinase (PDK) complex of the newly synthesized *N*-(2-chloro-4-(*N*-alkyl/aralkylsulfamoyl)phenyl)-2-hydroxy-2-methylpropanamides (**10c-n**) compared to the reference compounds **10a** (or **4**) and **10b** (or **5**). The table indicates that the racemic mixture **10c** was logically found to be less active than the reference compound **10a** (or **4**), which is the R-enantiomer [compare the IC_{50} values of **10c** (41 nM) versus **10a** (7 nM)]. Interestingly, the introduction of a benzyl chain instead of an isobutyl chain at the level of the sulfonamide nitrogen atom provided equipotent compounds (compare **10d** versus **10c**). However, the introduction of a longer side chain (see the *N*-phenethyl-substituted analogue **10e** with an IC_{50} value of 75 nM) was found to be less favorable.

Replacement of the trifluoromethyl group by a simple methyl group on the propanamide moiety located at the 4-position of the benzenesulfonamide core structure resulted in the suppression of the chiral carbon atom. However, this modification was also responsible for a strong decrease of the inhibitory activity on the PDK complex (see for example **10c** and **10d** compared to **10f** and **10h**).



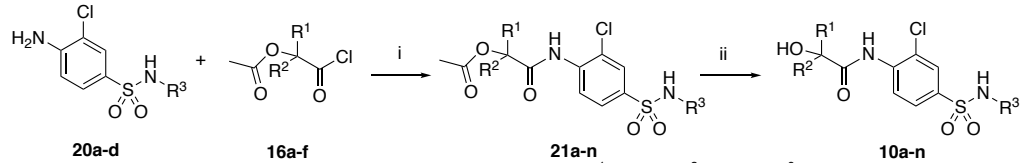
14a, **15a**, **16a**: $R^1 = CF_3$, $R^2 = CH_3$ (R,S)
14b, **15b**, **16b**: $R^1 = CF_3$, $R^2 = CH_3$ (R)
14c, **15c**, **16c**: $R^1 = CH_3$, $R^2 = CH_3$
14d, **15d**, **16d**: $R^1 = C_6H_5$, $R^2 = H$ (R,S)
14e, **15e**, **16e**: $R^1 = C_6H_5$, $R^2 = H$ (R)
14f, **15f**, **16f**: $R^1 = C_6H_5$, $R^2 = H$ (S)

Scheme 1. Synthetic pathway to compounds **16a-f**. Reagents: i: CH_3COCl, CH_2Cl_2; ii: oxalyl chloride, DMF, CH_2Cl_2.



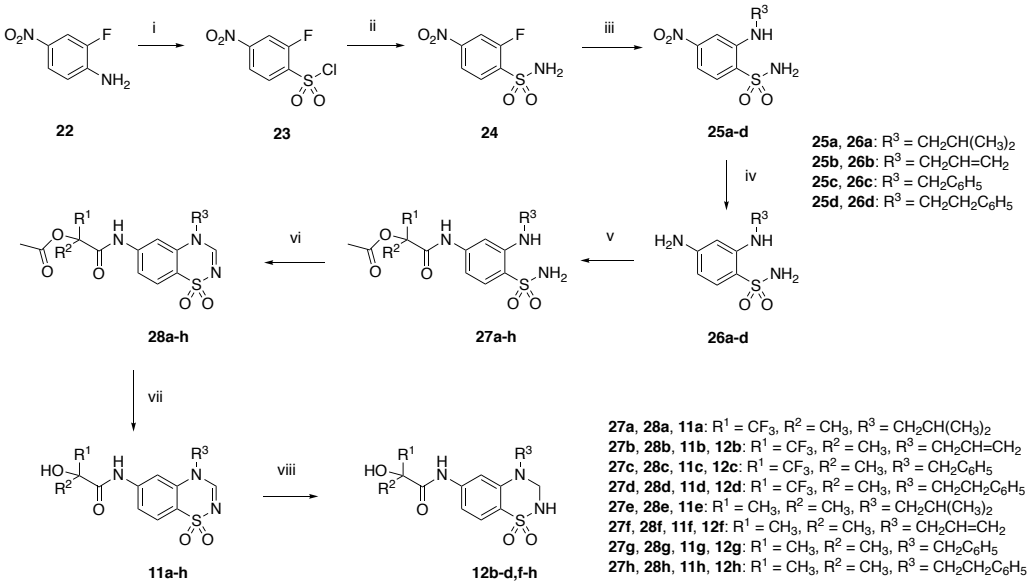
19a, **20a**: $R^3 = CH_2CH(CH_3)_2$
19b, **20b**: $R^3 = CH_2CH{=}CH_2$
19c, **20c**: $R^3 = CH_2C_6H_5$
19d, **20d**: $R^3 = CH_2CH_2C_6H_5$

Scheme 2. Synthetic pathway to compounds **20a-d**. Reagents: i: 1. $NaNO_2$, HCl, HOAc; 2. SO_2, $CuCl_2$, HOAc; ii: R^3-NH_2, TEA, dioxane; iii: Fe, HOAc, H_2O.



21a, **10a** (= **4**): $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = CH_2CH(CH_3)_2$ (R)
21b, **10b** (= **5**): $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = CH_2CH{=}CH_2$ (R)
21c, **10c**: $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = CH_2CH(CH_3)_2$ (R,S)
21d, **10d**: $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = CH_2C_6H_5$ (R,S)
21e, **10e**: $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = CH_2CH_2C_6H_5$ (R,S)
21f, **10f**: $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = CH_2CH(CH_3)_2$
21g, **10g**: $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = CH_2CH{=}CH_2$
21h, **10h**: $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = CH_2C_6H_5$
21i, **10i**: $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = CH_2CH_2C_6H_5$
21j, **10j**: $R^1 = C_6H_5$, $R^2 = H$, $R^3 = CH_2CH(CH_3)_2$ (R,S)
21k, **10k**: $R^1 = C_6H_5$, $R^2 = H$, $R^3 = CH_2CH{=}CH_2$ (R,S)
21l, **10l**: $R^1 = C_6H_5$, $R^2 = H$, $R^3 = CH_2C_6H_5$ (R,S)
21m, **10m**: $R^1 = C_6H_5$, $R^2 = H$, $R^3 = CH_2C_6H_5$ (R)
21n, **10n**: $R^1 = C_6H_5$, $R^2 = H$, $R^3 = CH_2C_6H_5$ (S)

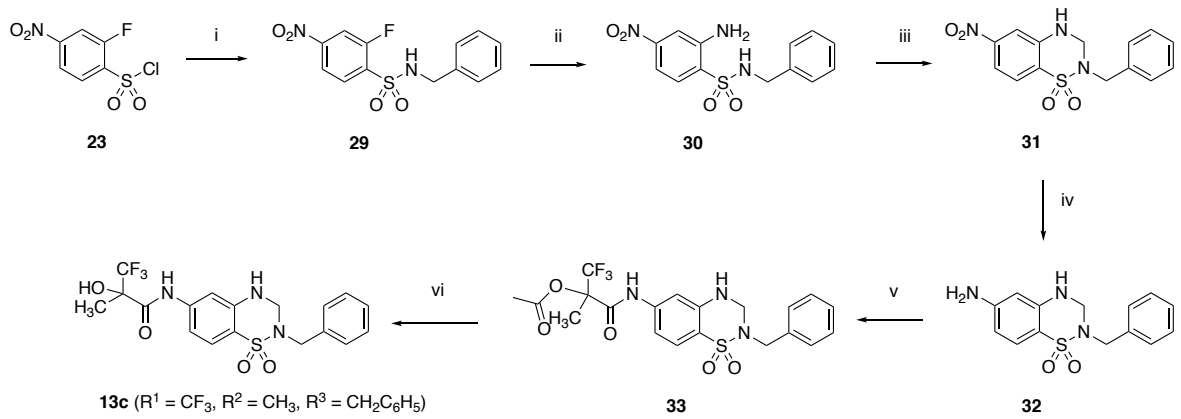
Scheme 3. Synthetic pathway to compounds **10a-n**. Reagents: i: pyridine, CH_2Cl_2; ii: NaOH, H_2O.



25a, **26a**: $R^3 = CH_2CH(CH_3)_2$
25b, **26b**: $R^3 = CH_2CH{=}CH_2$
25c, **26c**: $R^3 = CH_2C_6H_5$
25d, **26d**: $R^3 = CH_2CH_2C_6H_5$

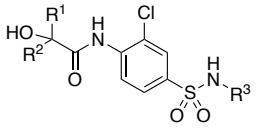
27a, **28a**, **11a**: $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = CH_2CH(CH_3)_2$
27b, **28b**, **11b**, **12b**: $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = CH_2CH{=}CH_2$
27c, **28c**, **11c**, **12c**: $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = CH_2C_6H_5$
27d, **28d**, **11d**, **12d**: $R^1 = CF_3$, $R^2 = CH_3$, $R^3 = CH_2CH_2C_6H_5$
27e, **28e**, **11e**: $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = CH_2CH(CH_3)_2$
27f, **28f**, **11f**, **12f**: $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = CH_2CH{=}CH_2$
27g, **28g**, **11g**, **12g**: $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = CH_2C_6H_5$
27h, **28h**, **11h**, **12h**: $R^1 = CH_3$, $R^2 = CH_3$, $R^3 = CH_2CH_2C_6H_5$

Scheme 4. Synthetic pathway to compounds **11a-h** and **12b-d,f-h**. Reagents: i: 1. $NaNO_2$, HCl, HOAc; 2. SO_2, $CuCl_2$, HOAc; ii: NH_3, H_2O; iii: R^3-NH_2; iv: Fe, NH_4Cl, H_2O, EtOH; v: **16a-d**, TEA, CH_3CN; vi: $H(COEt)_3$; vii: $NaHCO_3$, H_2O, MeOH; viii: $NaBH_4$, isopropanol.



Scheme 5. Synthetic pathway to compound **13c.** Reagents: i: benzylamine; ii: NH_3, H_2O; iii: paraformaldehyde, sodium isopropanoate; iv: Fe, NH_4Cl, H_2O, EtOH; v: **16a**, TEA, CH_3CN; vi: NaOH, MeOH.

Table 2. Inhibitory activity on the pyruvate dehydrogenase kinase (PDK) complex of *N*-(2-chloro-4-(*N*-alkyl/aralkylsulfamoyl) phenyl)-2-hydroxy-2-methylpropanamides (10).



(**10**)

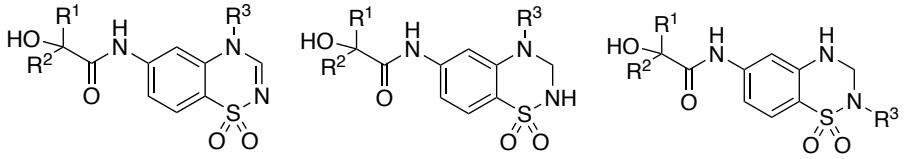
Compounds	Conf.	-R^1	-R^2	-R^3	IC_{50} (nM)[a]
10a (**4**)	R	$-CF_3$	$-CH_3$	$-CH_2CH(CH_3)_2$	7.3 ± 1.5
10b (**5**)	R	$-CF_3$	$-CH_3$	$-CH_2CH=CH_2$	10.1 ± 2.2
10c	R,S	$-CF_3$	$-CH_3$	$-CH_2CH(CH_3)_2$	40.8 ± 11.4
10d	R,S	$-CF_3$	$-CH_3$	$-CH_2C_6H_5$	40.1 ± 8.2
10e	R,S	$-CF_3$	$-CH_3$	$-CH_2CH_2CH(CH_3)_2$	75.0 ± 15.5
10f	-	$-CH_3$	$-CH_3$	$-CH_2CH(CH_3)_2$	114.9 ± 24.6
10g	-	$-CH_3$	$-CH_3$	$-CH_2CH=CH_2$	197.5 ± 51.1
10h	-	$-CH_3$	$-CH_3$	$-CH_2C_6H_5$	175.1 ± 46.8
10i	-	$-CH_3$	$-CH_3$	$-CH_2CH_2C_6H_5$	136.9 ± 25.1
10j	R,S	$-C_6H_5$	-H	$-CH_2CH(CH_3)_2$	n.a.[b]
10k	R,S	$-C_6H_5$	-H	$-CH_2CH=CH_2$	n.a.
10l	R,S	$-C_6H_5$	-H	$-CH_2C_6H_5$	n.a.
10m	R	$-C_6H_5$	-H	$-CH_2C_6H_5$	n.a.
10n	S	$-C_6H_5$	-H	$-CH_2C_6H_5$	n.a.

[a] Estimated IC_{50} values for the inhibition of PDKs. Results are expressed as mean ± standard deviation of at least three determinations (n≥3). [b] n.a.: not active (IC_{50} > 100 μM).

The introduction of a bulkier phenyl group instead of the two methyl or the trifluoromethyl/methyl groups on the α-position of the acetamide moiety, providing the mandelic acid derivatives **10j-n**, resulted in a complete loss of inhibitory activity on the PDK complex.

According to this first set of biological results, the next series of compounds (1,2,4-benzothiadiazine 1,1-dioxides **11**, **12** and **13** designed as conformationally restricted ring-closed analogues of N-alkyl/aralkyl-substituted benzenesulfonamides) carried only a (methyl/trifluoromethyl) propanamide moiety at the 6-position of the heterocycle.

Table 3. Inhibitory activity on the pyruvate dehydrogenase kinase (PDK) complex of *N*-(4-alkyl/aralkyl-1,1-dioxo-4*H*-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropanamides (11), *N*-(4-alkyl/aralkyl-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropanamides (12) and *N*-(2-alkyl/aralkyl-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazin-6-yl)-2-hydroxy-2-methylpropionamide (13).



(**11**) (**12**) (**13**)

Compounds	Conf.	-R^1	-R^2	-R^3	IC_{50} (μM)[a]
11a	R,S	$-CF_3$	$-CH_3$	$-CH_2CH(CH_3)_2$	33.5 ± 8.2
11b	R,S	$-CF_3$	$-CH_3$	$-CH_2CH=CH_2$	22.8 ± 8.8
11c	R,S	$-CF_3$	$-CH_3$	$-CH_2C_6H_5$	3.6 ± 0.9
11d	R,S	$-CF_3$	$-CH_3$	$-CH_2CH_2C_6H_5$	8.2 ± 1.5
11e	-	$-CH_3$	$-CH_3$	$-CH_2CH(CH_3)_2$	86.1 ± 13.9
11f	-	$-CH_3$	$-CH_3$	$-CH_2CH=CH_2$	64.4 ± 14.2
11g	-	$-CH_3$	$-CH_3$	$-CH_2C_6H_5$	80.8 ± 12.4
11h	-	$-CH_3$	$-CH_3$	$-CH_2CH_2C_6H_5$	24.8 ± 10.8
12b	R,S	$-CF_3$	$-CH_3$	$-CH_2CH=CH_2$	4.6 ± 1.2
12c	R,S	$-CF_3$	$-CH_3$	$-CH_2C_6H_5$	0.5 ± 0.2
12d	R,S	$-CF_3$	$-CH_3$	$-CH_2CH_2C_6H_5$	1.2 ± 0.5
12f	-	$-CH_3$	$-CH_3$	$-CH_2CH=CH_2$	12.9 ± 4.4
12g	-	$-CH_3$	$-CH_3$	$-CH_2C_6H_5$	13.2 ± 4.2
12h	-	$-CH_3$	$-CH_3$	$-CH_2CH_2C_6H_5$	3.6 ± 1.3
13c	R,S	$-CF_3$	$-CH_3$	$-CH_2C_6H_5$	1.36 ± 0.34

[a] Estimated IC_{50} values for the inhibition of PDKs. Results are expressed as mean ± standard deviation of at least three determinations (n≥3).

Table **3** reports the biological results obtained with the novel 1,2,4-benzothiadiazine 1,1-dioxides **11**, **12** and **13**. As previously observed with the *N*-(2-chloro-4-(*N*-alkyl/aralkylsulfamoyl)phenyl)-2-hydroxy-2-methylpropanamides **10**, the presence of a 2-trifluoromethylpropanamide side chain was always preferred to that of a 2-methylpropanamide moiety at the 6-position of the 4-alkyl/aralkyl-substituted 4*H*-1,2,4-benzothiadiazine 1,1-dioxides ("unsaturated" 1,2,4-benzothiadiazine 1,1-dioxides; compare **11a-d** with **11e-h**). Interestingly, the best choice of hydrocarbon side chain at the 4-position was found to be the benzyl chain, providing **11c**, the most active compound (IC_{50} = 3.6 μM) belonging to the 4-alkyl/aralkyl-substituted "unsaturated" 1,2,4-benzothiadiazine 1,1-dioxides. Another attractive observation was also the quite marked inhibitory activity of the phenethyl-substituted compounds **11d** and **11h** compared to the other representatives of this series of drugs. Such a result supports the view that bulky substituents could be tolerated at the 4-position of the heterocycle.

Saturation of the C(2)=N(3) double bound of compounds **11**, providing the corresponding "saturated" analogues **12** ("saturated" 1,2,4-benzothiadiazine 1,1-dioxides), was responsible for a clear increase of the inhibitory activity on the PDK complex (see Table **3**; compare **12b-c** with **11b-c** and **12f-h** with **11f-h**). Once again, the most active compound of this series was the benzyl-substituted trifluoromethylpropanamide derivative **12c** with an IC_{50} value below the micromolar concentration (IC_{50} = 0.5 μM). It was concluded that the saturation of the C=N double bound favorably modified the 3D-conformation of the molecules so that a better recognition by the biological target was observed. In this series of saturated compounds, the 4-phenethyl-substituted derivatives **12d** and **12h** were also found to express a marked activity.

Lastly, compound **13c** was prepared to examine the impact of the introduction of the benzyl side chain at the 2-position instead of the 4-position of the saturated heterocycle. Such a compound could also be viewed as a tightly related ring-closed analogue of compound **10d**. According to

Table **2**, compound **13c** was found to be quite active as an inhibitor of the PDK complex (IC_{50} = 1.36 μM), but less active than compound **12c**. It is concluded that conformationally restricted analogues of *N*-(4-(*N*-alkyl/aralkylsulfamoyl)phenyl)-2-hydroxy-2-methylpropanamides belonging to "saturated" 1,2,4-benzothiadiazine 1,1-dioxides can advantageously bear a bulky aralkyl side chain at the 2- or the 4-position of the heterocycle, but that the 4-position appeared to be preferred.

CONCLUSION

The present work reported the synthesis and the biological evaluation of novel pyruvate dehydrogenase kinase (PDK) inhibitors belonging to *N*-(4-(*N*-alkyl/aralkylsulfamoyl)phenyl)-2-methylpropanamides and to 1,2,4-benzothiadiazine 1,1-dioxides carrying a (methyl/trifluoromethyl)propanamide moiety at the 6-position designed as conformationally restricted ring-closed analogues of *N*-(4-(*N*-alkyl/aralkylsulfamoyl)phenyl)- 2-methyl-propanamides.

The newly synthesized *N*-(4-(*N*-alkyl/aralkylsulfamoyl) phenyl)-2-methylpropanamides structurally related to the reference compounds **4** and **5** were found to be potent inhibitors of the PDK complex. The introduction of a benzyl side chain instead of an isobutyl or an allyl chain on the sulfonamide nitrogen atom was found to preserve potent inhibitory activity. Replacement of the trifluoromethyl group by a simple methyl group on the propanamide moiety located at the 4-position of the benzenesulfonamide core structure resulted in a strong decrease in the inhibitory activity of the PDK complex.

Most of the 1,2,4-benzothiadiazine 1,1-dioxides designed as conformationally restricted ring-closed analogues of *N*-(4-(*N*-alkyl/aralkylsulfamoyl)phenyl)-2-hydroxy-2-methylpropanamides were found to be less potent than their rind-opened analogues. However, the best choice of hydrocarbon side chain at the 4-position was again the benzyl chain. Saturated 1,2,4-benzothiadiazine 1,1-dioxides were always found to be more potent than the corresponding unsaturated analogues. Another observation was also the rather marked inhibitory activity of the phenethyl-substituted compounds supporting the view that bulky substituents could be tolerated at the 4-position of the heterocycle.

In the near future, the *in vivo* effect in animal models of a selection of these new PDK inhibitors as modulators of the lactate plasma levels has to be performed in order to examine a possible beneficial effect of these drugs in the treatment of pathologies requiring the control of excessive lactate production.

LIST OF ABBREVIATIONS

DCA	=	Dichloroacetic acid
DCM	=	Dichloromethane
DS	=	Discovery studio
EDTA	=	Ethylenediaminetetraacetic acid
ELSD	=	Evaporative light scattering detector
GA	=	Genetic algorithm
GBMV	=	Generalized born with molecular volume
MM-PBSA	=	Molecular mechanics Poisson-Boltzmann surface area
NAD+/NADH	=	Nicotinamide adenine dinucleotide
PDB	=	Protein data bank
PDC	=	Pyruvate dehydrogenase complex
PDK	=	Pyruvate dehydrogenase kinase
TEA	=	Triethylamine

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

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

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

This work was supported by Grants from the Belgian National Fund for Scientific Research (F.R.S. – F.N.R.S.), from which Dr. P. de Tullio is Research Director at the University of Liège (Belgium).

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests, financial or otherwise.

ACKNOWLEDGEMENTS

The assistance of Stéphane Counerotte and Eric Goffin is gratefully acknowledged.

REFERENCES

[1] Lambert, V.; Hansen, S.; Schoumacher, M.; Lecomte, J.; Leenders, J.; Hubert, P.; Herfs, M.; Blacher, S.; Carnet, O.; Yip, C.; Blaise, P.; Duchateau, E.; Locht, B.; Thys, M.; Cavalier, E.; Gothot, A.; Govaerts, B.; Rakic, J.M.; Noel, A.; de Tullio, P. Pyruvate dehydrogenase kinase/lactate axis: A therapeutic target for neovascular age-related macular degeneration identified by metabolomics. *J. Mol. Med. (Berl.),* **2020**, *98*(12), 1737-1751. http://dx.doi.org/10.1007/s00109-020-01994-9 PMID: 33079232

[2] Porporato, P.E.; Payen, V.L.; De Saedeleer, C.J.; Préat, V.; Thissen, J.-P.; Feron, O.; Sonveaux, P. Lactate stimulates angiogenesis and accelerates the healing of superficial and ischemic wounds in mice. *Angiogenesis,* **2012**, *15*(4), 581-592. http://dx.doi.org/10.1007/s10456-012-9282-0 PMID: 22660894

[3] Kersten, E.; Paun, C.C.; Schellevis, R.L.; Hoyng, C.B.; Delcourt, C.; Lengyel, I.; Peto, T.; Ueffing, M.; Klaver, C.C.W.; Dammeier, S.; den Hollander, A.I.; de Jong, E.K. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. *Surv. Ophthalmol.,* **2018**, *63*(1), 9-39.

http://dx.doi.org/10.1016/j.survophthal.2017.05.003 PMID: 28522341

[4] Korotchkina, L.G.; Patel, M.S. Probing the mechanism of inactivation of human pyruvate dehydrogenase by phosphorylation of three sites. *J. Biol. Chem.,* **2001**, *276*(8), 5731-5738. http://dx.doi.org/10.1074/jbc.M007558200 PMID: 11092882

[5] Roche, T.E.; Hiromasa, Y. Pyruvate dehydrogenase kinase regulatory mechanisms and inhibition in treating diabetes, heart ischemia, and cancer. *Cell. Mol. Life Sci.,* **2007**, *64*(7-8), 830-849. http://dx.doi.org/10.1007/s00018-007-6380-z PMID: 17310282

[6] Gray, L.R.; Tompkins, S.C.; Taylor, E.B. Regulation of pyruvate metabolism and human disease. *Cell. Mol. Life Sci.,* **2014**, *71*(14), 2577-2604. http://dx.doi.org/10.1007/s00018-013-1539-2 PMID: 24363178

[7] Stacpoole, P.W. Therapeutic targeting of the pyruvate dehydrogenase complex/pyruvate dehydrogenase kinase (PDC/PDK) axis in cancer. *J. Natl. Cancer Inst.,* **2017**, *109*(11), djx071. http://dx.doi.org/10.1093/jnci/djx071 PMID: 29059435

[8] Bhandary, S.; Aguan, K. Pyruvate dehydrogenase complex deficiency and its relationship with epilepsy frequency-An overview. *Epilepsy Res.,* **2015**, *116*, 40-52. http://dx.doi.org/10.1016/j.eplepsyres.2015.07.002 PMID: 26354166

[9] Patel, K.P.; O'Brien, T.W.; Subramony, S.H.; Shuster, J.; Stacpoole, P.W. The spectrum of pyruvate dehydrogenase complex deficiency: Clinical, biochemical and genetic features in 371 patients. *Mol. Genet. Metab.,* **2012**, *106*(3), 385-394. http://dx.doi.org/10.1016/j.ymgme.2012.03.017 PMID: 22896851

[10] Jeoung, N.H. Pyruvate dehydrogenase kinases: Therapeutic targets for diabetes and cancers. *Diabetes Metab. J.,* **2015**, *39*(3), 188-197. http://dx.doi.org/10.4093/dmj.2015.39.3.188 PMID: 26124988

[11] Zhang, S.L.; Hu, X.; Zhang, W.; Yao, H.; Tam, K.Y. Development of pyruvate dehydrogenase kinase inhibitors in medicinal chemistry with particular emphasis as anticancer agents. *Drug Discov. Today,* **2015**, *20*(9), 1112-1119. http://dx.doi.org/10.1016/j.drudis.2015.03.012 PMID: 25842042

[12] Saunier, E.; Benelli, C.; Bortoli, S. The pyruvate dehydrogenase complex in cancer: An old metabolic gatekeeper regulated by new pathways and pharmacological agents. *Int. J. Cancer,* **2016**, *138*(4), 809-817. http://dx.doi.org/10.1002/ijc.29564 PMID: 25868605

[13] Kato, M.; Li, J.; Chuang, J.L.; Chuang, D.T. Distinct structural mechanisms for inhibition of pyruvate dehydrogenase kinase isoforms by AZD7545, dichloroacetate, and radicicol. *Structure,* **2007**, *15*(8), 992-1004. http://dx.doi.org/10.1016/j.str.2007.07.001 PMID: 17683942

[14] Knoechel, T.R.; Tucker, A.D.; Robinson, C.M.; Phillips, C.; Taylor, W.; Bungay, P.J.; Kasten, S.A.; Roche, T.E.; Brown, D.G. Regulatory roles of the N-terminal domain based on crystal structures of human pyruvate dehydrogenase kinase 2 containing physiological and synthetic ligands. *Biochemistry,* **2006**, *45*(2), 402-415. http://dx.doi.org/10.1021/bi051402s PMID: 16401071

[15] Li, J.; Kato, M.; Chuang, D.T. Pivotal role of the C-terminal DW-motif in mediating inhibition of pyruvate dehydrogenase kinase 2 by dichloroacetate. *J. Biol. Chem.,* **2009**, *284*(49), 34458-34467. http://dx.doi.org/10.1074/jbc.M109.065557 PMID: 19833728

[16] Kankotia, S.; Stacpoole, P.W. Dichloroacetate and cancer: New home for an orphan drug? *Biochim. Biophys. Acta,* **2014**, *1846*(2), 617-629. http://dx.doi.org/10.1016/j.bbcan.2014.08.005 PMID: 25157892

[17] Chu, Q.S-C.; Sangha, R.; Spratlin, J.; Vos, L.J.; Mackey, J.R.; McEwan, A.J.; Venner, P.; Michelakis, E.D. A phase I open-labeled, single-arm, dose-escalation, study of dichloroacetate (DCA) in patients with advanced solid tumors. *Invest. New Drugs,* **2015**, *33*(3), 603-610. http://dx.doi.org/10.1007/s10637-015-0221-y PMID: 25762000

[18] Stacpoole, P.W. Lactic acidosis. *Endocrinol. Metab. Clin. North Am.,* **1993**, *22*(2), 221-245. http://dx.doi.org/10.1016/S0889-8529(18)30163-4 PMID: 8325284

[19] Bersin, R.M.; Stacpoole, P.W. Dichloroacetate as metabolic therapy for myocardial ischemia and failure. *Am. Heart J.,* **1997**, *134*(5 Pt 1), 841-855. http://dx.doi.org/10.1016/S0002-8703(97)80007-5 PMID: 9398096

[20] Duncan, G.E.; Perkins, L.A.; Theriaque, D.W.; Neiberger, R.E.; Stacpoole, P.W. Dichloroacetate therapy attenuates the blood lactate response to submaximal exercise in patients with defects in mitochondrial energy metabolism. *J. Clin. Endocrinol. Metab.,* **2004**, *89*(4), 1733-1738. http://dx.doi.org/10.1210/jc.2003-031684 PMID: 15070938

[21] Stacpoole, P.W.; Harman, E.M.; Curry, S.H.; Baumgartner, T.G.; Misbin, R.I. Treatment of lactic acidosis with dichloroacetate. *N. Engl. J. Med.,* **1983**, *309*(7), 390-396. http://dx.doi.org/10.1056/NEJM198308183090702 PMID: 6877297

[22] Michelakis, E.D.; Webster, L.; Mackey, J.R. Dichloroacetate (DCA) as a potential metabolic-targeting therapy for cancer. *Br. J. Cancer,* **2008**, *99*(7), 989-994. http://dx.doi.org/10.1038/sj.bjc.6604554 PMID: 18766181

[23] Thai, S.F.; Allen, J.W.; DeAngelo, A.B.; George, M.H.; Fuscoe, J.C. Altered gene expression in mouse livers after dichloroacetic acid exposure. *Mutat. Res.,* **2003**, *543*(2), 167-180. http://dx.doi.org/10.1016/S1383-5742(03)00014-0 PMID: 12644186

[24] Stacpoole, P.W.; Gonzalez, M.G.; Vlasak, J.; Oshiro, Y.; Bodor, N. Dichloroacetate derivatives. Metabolic effects and pharmacodynamics in normal rats. *Life Sci.,* **1987**, *41*(18), 2167-2176. http://dx.doi.org/10.1016/0024-3205(87)90535-2 PMID: 3669916

[25] Stacpoole, P.W.; Henderson, G.N.; Yan, Z.; James, M.O. Clinical pharmacology and toxicology of dichloroacetate. *Environ. Health Perspect.,* **1998**, *106*(Suppl. 4), 989-994. http://dx.doi.org/10.1289/ehp.98106s4989 PMID: 9703483

[26] Aicher, T.D.; Anderson, R.C.; Bebernitz, G.R.; Coppola, G.M.; Jewell, C.F.; Knorr, D.C.; Liu, C.; Sperbeck, D.M.; Brand, L.J.; Strohschein, R.J.; Gao, J.; Vinluan, C.C.; Shetty, S.S.; Dragland, C.; Kaplan, E.L.; DelGrande, D.; Islam, A.; Liu, X.; Lozito, R.J.; Maniara, W.M.; Walter, R.E.; Mann, W.R. (R)-3,3,3-Trifluoro-2-hydroxy-2-methylpropionamides are orally active inhibitors of pyruvate dehydrogenase kinase. *J. Med. Chem.,* **1999**, *42*(15), 2741-2746. http://dx.doi.org/10.1021/jm9902584 PMID: 10425084

[27] Aicher, T.D.; Anderson, R.C.; Gao, J.; Shetty, S.S.; Coppola, G.M.; Stanton, J.L.; Knorr, D.C.; Sperbeck, D.M.; Brand, L.J.; Vinluan, C.C.; Kaplan, E.L.; Dragland, C.J.; Tomaselli, H.C.; Islam, A.; Lozito, R.J.; Liu, X.; Maniara, W.M.; Fillers, W.S.; DelGrande, D.; Walter, R.E.; Mann, W.R. Secondary amides of (R)-3,3,3-trifluoro-2-hydroxy-2-methylpropionic acid as inhibitors of pyruvate dehydrogenase kinase. *J. Med. Chem.,* **2000**, *43*(2), 236-249. http://dx.doi.org/10.1021/jm990358+ PMID: 10649979

[28] Bebernitz, G.R.; Aicher, T.D.; Stanton, J.L.; Gao, J.; Shetty, S.S.; Knorr, D.C.; Strohschein, R.J.; Tan, J.; Brand, L.J.; Liu, C.; Wang, W.H.; Vinluan, C.C.; Kaplan, E.L.; Dragland, C.J.; DelGrande, D.; Islam, A.; Lozito, R.J.; Liu, X.; Maniara, W.M.; Mann, W.R. Anilides of (R)-trifluoro-2-hydroxy-2-methylpropionic acid as inhibitors of pyruvate dehydrogenase kinase. *J. Med. Chem.,* **2000**, *43*(11), 2248-2257. http://dx.doi.org/10.1021/jm0000923 PMID: 10841803

[29] Mann, W.R.; Dragland, C.J.; Vinluan, C.C.; Vedananda, T.R.; Bell, P.A.; Aicher, T.D. Diverse mechanisms of inhibition of pyruvate dehydrogenase kinase by structurally distinct inhibitors. *Biochim. Biophys. Acta,* **2000**, *1480*(1-2), 283-292. http://dx.doi.org/10.1016/S0167-4838(00)00079-0 PMID: 11004568

[30] Mayers, R.M.; Butlin, R.J.; Kilgour, E.; Leighton, B.; Martin, D.; Myatt, J.; Orme, J.P.; Holloway, B.R. AZD7545, a novel inhibitor of pyruvate dehydrogenase kinase 2 (PDHK2), activates pyruvate dehydrogenase *in vivo* and improves blood glucose control in obese (fa/fa) Zucker rats. *Biochem. Soc. Trans.,* **2003**, *31*(Pt 6), 1165-1167. http://dx.doi.org/10.1042/bst0311165 PMID: 14641018

[31] Morrell, J.A.; Orme, J.; Butlin, R.J.; Roche, T.E.; Mayers, R.M.; Kilgour, E. AZD7545 is a selective inhibitor of pyruvate dehydrogenase kinase 2. *Biochem. Soc. Trans.,* **2003**, *31*(Pt 6), 1168-1170. http://dx.doi.org/10.1042/bst0311168 PMID: 14641019

[32] Kakkar, R.; Arora, R.; Gahlot, P.; Gupta, D. An insight into pyruvate dehydrogenase kinase (PDHK) inhibition through pharmacophore modeling and QSAR studies. *J. Comput. Sci.,* **2014**, *5*(4), 558-567.

http://dx.doi.org/10.1016/j.jocs.2014.04.006

[33] Jones, G.; Willett, P.; Glen, R.C.; Leach, A.R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.,* **1997**, *267*(3), 727-748. http://dx.doi.org/10.1006/jmbi.1996.0897 PMID: 9126849

[34] Sakkiah, S.; Arooj, M.; Kumar, M.R.; Eom, S.H.; Lee, K.W. Identification of inhibitor binding site in human sirtuin 2 using molecular docking and dynamics simulations. *PLoS One,* **2013**, *8*(1), e51429. http://dx.doi.org/10.1371/journal.pone.0051429 PMID: 23382805

[35] Tirado-Rives, J.; Jorgensen, W.L. Contribution of conformer focusing to the uncertainty in predicting free energies for protein-ligand binding. *J. Med. Chem.,* **2006**, *49*(20), 5880-5884. http://dx.doi.org/10.1021/jm060763i PMID: 17004703

[36] Huang, K.; Luo, S.; Cong, Y.; Zhong, S.; Zhang, J.Z.H.; Duan, L. An accurate free energy estimator: Based on MM/PBSA combined with interaction entropy for protein-ligand binding affinity. *Nanoscale,* **2020**, *12*(19), 10737-10750. http://dx.doi.org/10.1039/C9NR10638C PMID: 32388542

[37] Wang, E.; Sun, H.; Wang, J.; Wang, Z.; Liu, H.; Zhang, J.Z.H.; Hou, T. End-point binding free energy calculation with MM/PBSA and MM/GBSA: Strategies and applications in drug design. *Chem. Rev.,* **2019**, *119*(16), 9478-9508. http://dx.doi.org/10.1021/acs.chemrev.9b00055 PMID: 31244000

[38] Jackson, J.C.; Vinluan, C.C.; Dragland, C.J.; Sundararajan, V.; Yan, B.; Gounarides, J.S.; Nirmala, N.R.; Topiol, S.; Ramage, P.; Blume, J.E.; Aicher, T.D.; Bell, P.A.; Mann, W.R. Heterologously expressed inner lipoyl domain of dihydrolipoyl acetyltransferase inhibits ATP-dependent inactivation of pyruvate dehydrogenase complex. Identification of important amino acid residues. *Biochem. J.,* **1998**, *334*(Pt 3), 703-711. http://dx.doi.org/10.1042/bj3340703 PMID: 9729480