Article type : Research Article

A field-based disparity analysis of new 1,2,5-oxadiazole derivatives endowed with antiproliferative activity

SAR of antiproliferative 1,2,5-oxadiazoles

Federica Porta^a, Arianna Gelain^a, Daniela Barlocco^a, Nicola Ferri^b, Silvia Marchianò^c, Valentina Cappello^d, Livia Basile^d, Salvatore Guccione^{d,*}, Fiorella Meneghetti^{a,*} and Stefania Villa^a

^a Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, via L. Mangiagalli 25, I-20133 Milan, Italy

^b Dipartimento di Scienze del Farmaco, Università degli Studi di Padova, Largo E. Meneghetti 2, I-35131 Padua, Italy

^c Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Via Balzaretti 9, I-20131 Milan, Italy

^d Dipartimento di Scienze del Farmaco, Università degli Studi di Catania, V.le Andrea Doria 6, I-95125 Catania, Italy

*Corresponding authors. Tel.: +39-02-503-19306; fax: +39-02-503-19359; e-mail: fiorella.meneghetti@unimi.it (FM); Tel. +39 095-738-4020; e-mail: guccione@unict.it (SG).

ABSTRACT

A series of 1,2,5-oxadiazoles was synthesized as new potential antiproliferative agents. The *in vitro* cytotoxic activity evaluation of title compounds through MTT-assay revealed that some of them showed significant activity against the HCT-116 cancer cell line. The field-based disparity analysis provided indications about the electrostatic, hydrophobic and shape features underlying the cytotoxicity, suggesting that increasing the negative electrostatic field on the heterocyclic core of the structure has positive effects on the activity. The structure-activity relationships (SAR) around a particular compound can be explained allowing for a structural rationale for the differences in activity. The SAR provided by this series of compounds can be exploited to carry out further lead optimization.

Keywords: Anticancer agents; Heteroaryl amide; Activity cliff; SAR; X-ray

^{*} Corresponding authors. Tel.: +39-02-503-19306 ; fax: +39-02-503-19359; e-mail: fiorella.meneghetti@unimi.it (FM); Tel. +39 095-738-4020; e-mail: guccione@unict.it (SG).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cbdd.13003

1,2,5-Oxadiazoles have received considerable attention in recent years from our research group due to the interesting results they disclosed as potential antitumor agents.^[1-5] On this respect, we explored their antiproliferative potential by synthesizing several compounds differently substituted at positions 3 and 4 of the heterocycle.^[1] Of note, the derivative bearing in 3 the amide function (MD77) emerged for its significant biological results,^[3] when submitted to a panel of 58 human tumor cell lines, derived from 9 cancer cell types (NCI, Bethesda, USA). The dose-response curves showed that it inhibited the growth of different cell lines (e.g. HCT-116, DU145, MDAMB-231) at low micromolar concentrations. Since MD77 showed a noteworthy cytotoxic effect on the HCT-116 cell line, we aimed at deepening our understanding of the structural characteristics responsible for its antiproliferative properties. To address this issue, we designed and synthesized some compounds strictly related to **MD77**, performing iterative modifications in order to recognize hits inducing a similar phenotypic effect on HCT-116. As we previously assessed the role of the linker structure,^[1] we maintained the amidic linkage throughout this lead optimization. Firstly, we investigated the impact of the substituents by changing their electronic characteristics or their position, and retaining the 1,2,5-oxadiazole scaffold (compounds 1-24). Then, we explored the role of the heterocycle by its replacement with 1,3,4-oxadiazole isomer^[6] (25) or with other aromatic systems, e.g. isoxazole (26 and 27), imidazole (28), furan (29) and phenyl ring (30) (Figure 1).

All compounds were submitted to molecular modeling studies by means of the innovative Activity Miner module as implemented in the Cresset Forge software ^[7] to provide comprehensive structure-activity relationships (SAR). This methodology allows the assessment of a structure-activity landscape for a series of 3D aligned structures based on the 'activity cliff' concept.^[8] The local SAR information, obtained from individual compound pairs associated with activity cliffs across the series, was then summarized into a global model through the Activity Atlas methodology.^[9] This technique provides 3D visually interpretable maps that can be used to inform new molecule design. In support of the *in silico* methodology, compounds **5** and **8**, having the two phenyl rings alternatively monosubstituted with the trifluoromethyl and chlorine moiety respectively, were selected for crystal structure determination. This analysis was carried out to verify if the two molecular skeletons shared a similar conformation, independently from the intermolecular interactions made by the phenyl substituents. Here, we present the synthesis and the characterization of these new compounds together with the evaluation of their cytotoxic activity. In addition, the activity cliffs between molecules are discussed, in order to derive a computational model useful for lead optimization.

1. Materials and Methods

1.1 Chemistry

Reagents and solvents were purchased from Sigma-Aldrich and used without further purification. Some reactions involving air-sensitive reagents were performed under nitrogen atmosphere and anhydrous solvents were used when necessary. The Biotage Initiator microwave synthesizer was used. Reactions were monitored by thin layer chromatography analysis on aluminum-backed Silica Gel 60 plates (70-230 mesh, Merck), using an ultraviolet fluorescent lamp at 254 nm and 365 nm. Visualization was aided by opportune staining reagents. Purification of intermediates and final compounds was performed by flash chromatography using Geduran® Si 60 (40-63 μ m, Merck). The melting points were determined in open capillary tubes on a Buchi Melting Point B540 instrument. ¹H and ¹³C NMR spectra were recorded in CDCl₃, CD₃OD, acetone-d₆ or DMSO-d₆ on a Variant 300 MHz Oxford instrument at 25° C. Chemical shifts are expressed as δ (ppm). Multiplicity is reported as *s* (singlet), *br s* (broad singlet), *d* (doublet), *t* (triplet), *q* (quartet), *m* (multiplet), *dd* (doublet of doublets), *dt* (doublet of triplets). The coupling constants (J-values) are given in Hertz (Hz). All spectroscopic data match the assigned structures (see Supporting Information). The mass spectrometry analyses were carried out on an LTQ Orbitrap XL mass

spectrometer (Thermo Fisher Scientific, Milan, Italy) equipped with a FinniganIonMax Electrospray interface.

1.1.1. General procedures for the synthesis of 4-phenyl-1,2,5-oxadiazol-3-amines (31a-g)

Procedure a. A one-pot reaction ^[1] on ethyl benzoylacetate afforded 4-phenyl-1,2,5-oxadiazol-3-amine (**31a**).

Procedure b. The key intermediates **31b-g** were obtained as previously reported ^[3] starting from the appropriate benzaldehyde.

1.1.1.1. 4-phenyl-1,2,5-oxadiazol-3-amine (**31a**). Procedure a: yield 34 % as white solid (m.p. 99-100 °C). ¹H NMR (300 MHz, CDCl₃): δ 7.70-7.81 (m, 2H, ArH), 7.51-7.59 (m, 3H, ArH), 4.20 (br s, 2H, NH₂ exchanged with D₂O) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 154.30, 146.94, 130.58, 129.42, 127.57, 125.49 ppm.

1.1.1.2. 4-(2-chlorophenyl)-1,2,5-oxadiazol-3-amine (**31b**). Procedure b: yield 60 % (starting from 15 mmol of 2-chlorobenzaldehyde, 9 mmol of **31b** were obtained) as light yellow solid (m.p. 59-60 °C). ¹H NMR (300 MHz, CDCl₃): δ 7.33-7.57 (m, 4H, ArH), 4.22 (br s, 2H, NH₂ exchanged with D₂O) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 154.99, 146.46, 133.48, 132.29, 132.22, 130.54, 127.77, 124.78 ppm.

1.1.1.3. 4-(3-chlorophenyl)-1,2,5-oxadiazol-3-amine (**31c**). Procedure b: yield 41 % (starting from 15 mmol of 3-chlorobenzaldehyde, 6.15 mmol of **31c** were obtained) as light yellow solid (m.p. 132.6-132.9 °C). ¹H NMR (300 MHz, CDCl₃): δ 7.74 (t, J = 1.6 Hz, 1H, ArH), 7.63 (dt, $J_I = 1.6$ Hz, $J_2 = 6.9$ Hz, 1H, ArH), 7.45-7.54 (m, 2H, ArH), 4.22 (br s, 2H, NH₂ exchanged with D₂O) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 155.41, 146.24, 134.74, 131.12, 130.44, 128.36, 127.74, 126.55 ppm.

1.1.1.4. 4-(4-chlorophenyl)-1,2,5-oxadiazol-3-amine (**31d**). Procedure b: yield 19 % (starting from 15 mmol of 4-chlorobenzaldehyde, 2.85 mmol of **31d** were obtained) as white solid (m.p. 140.3-143.8 °C). ¹H NMR (300 MHz, CDCl₃): δ 7.69 (d, J = 8.1 Hz, 2H, ArH), 7.52 (d, J = 8.1 Hz, 2H, ArH), 4.21 (br s, 2H, NH₂ exchanged with D₂O) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 155.10, 146.20, 135.77, 129.44, 129.31, 124.95 ppm.

1.1.1.5. 4-(4-trifluoromethylphenyl)-1,2,5-oxadiazol-3-amine (**31e**). Procedure b: yield 25 % (starting from 15 mmol of 4-trifluoromethylbenzaldehyde, 3.75 mmol of **31e** were obtained) as light brown solid (m.p. 107.2-109.3 °C). ¹H NMR (300 MHz, acetone-d₆): δ 8.04 (d, J = 8.7 Hz, 2H, ArH), 7.91 (d, J = 8.7 Hz, 2H, ArH), 5.74 (s, 2H, NH₂ exchanged with D₂O) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 155.27, 146.11, 131. 53, 130.18, 128.58, 125.97 (q, J = 2.9 Hz), 122.28 ppm. ¹⁹F NMR (282 MHz, acetone-d₆): δ 63.47 (s, CF₃) ppm.

1.1.1.6. 4-(4-benzyloxyphenyl)-1,2,5-oxadiazol-3-amine (**31***f*). Procedure b: yield 21 % (starting from 15 mmol of 4-benzozyloxoxybenzaldehyde, 5.91 mmol of **31f** were obtained) as white solid (m.p. 144.5-145.5 °C). ¹H NMR (300 MHz, CDCl₃): δ 7.66 (d, J = 8.8 Hz, 2H, ArH), 7.32-7.48 (m, 5H, ArH), 7.11 (d, J = 8.8 Hz, 2H, ArH), 5.14 (s, 2H, CH₂), 4.19 (br s, 2H, NH₂ exchanged with D₂O) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 160.67, 154.21, 146.79, 136.49, 129.31, 128.95, 128.47, 127.69, 118.24, 116.01, 70.37 ppm.

1.1.1.7. 4-(4-nitrophenyl)-1,2,5-oxadiazol-3-amine (**31g**). Procedure b: yield 12 % (starting from 15 mmol of 4-nitrobenzaldehyde, 1.80 mmol of **31g** were obtained) as brown-red solid (m.p. 135.4-137.6 °C). ¹H NMR (300 MHz, CDCl₃): δ 8.40 (d, J = 8.9 Hz, 2H, ArH), 7.98 (d, J = 8.9 Hz, 2H, ArH), 4.27 (br s, 2H, NH₂ exchanged with D₂O) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 155.28, 148.87, 145.76, 132.43, 129.09, 124.09 ppm.

1.1.2. Synthesis of 4-(4-chlorophenyl)-3-aminoisoxazole (35)

1.1.2.1. Synthesis of ethyl 4-(4-chlorophenyl)isoxazole-3-carboxylate (32). Ethyl 5-amino-4-(4-chlorophenyl)isoxazole-3-carboxylate (0.750 mmol) was dissolved in a mixture of acetic acid (1.5 mL), H₂O (1.5 mL) and THF (2 mL). NaNO₂ (7.5 mmol) was slowly added and the reaction mixture was stirred at room temperature for 1 hour. After evaporating solvents, the residue was diluted with water and extracted with dichloromethane (3×2 mL). The collected organic layers were firstly washed with a saturated aqueous solution of sodium bicarbonate and then with water. The organic phase was dried over anhydrous Na₂SO₄, filtered and then concentrated under reduced pressure. The crude product was purified by flash chromatography (eluent: petroleum ether/ethyl acetate from 95:5 to 9:1) to afford compound **32**. Yield 71 % as colorless oil. TLC: petroleum ether/ethyl acetate (95:5) - R_f: 0.26. ¹H NMR (300 MHz, acetone-d₆): δ 9.12 (s, 1H, ArH), 7.57 (d, *J* = 8.6 Hz, 2H, ArH), 7.46 (d, *J* = 8.6 Hz, 2H, ArH), 4.38 (q, *J* = 7.1 Hz, 2H, CH₂), 1.30 (t, *J* = 7.1 Hz, 3H, CH₃) ppm.

1.1.2.2. Synthesis of 4-(4-chorophenyl)isoxazole-3-carbohydrazide (**33**). NH₂NH₂ · H₂O (1.596 mmol) was added portion-wise at 0 °C to a stirred solution of intermediate **32** (0.397 mmol) in ethanol (1.3 mL). The reaction mixture was warmed to 40 °C for 90 minutes and then the solvent was evaporated under reduced pressure. The residue was extracted with water (6 mL) and ethyl acetate (3×3 mL) and the organic layers were collected, dried over anhydrous Na₂SO₄, filtered and concentrated under *vacuum*. The intermediate **33** was used in the next step without any further purification. Yield 85 % as white foam. TLC: dichloromethane/methanol (95:5) - R_f: 0.45. ¹H NMR (300 MHz, acetone-d₆): δ 10.11 (br s, 1H, NH exchanged with D₂O), 9.17 (s, 1H, ArH), 7.65 (d, *J* = 8.6 Hz, 2H, ArH), 3.02 (br s, 2H, NH₂ exchanged with D₂O) ppm.

1.1.2.3. Synthesis of 4-(4-chorophenyl)isoxazole-3-carbonyl azide (**34**). Acyl hydrazine **33** (0.420 mmol) was dissolved in a 1:1 mixture of acetone/10 % HCl (1.8 mL). After cooling at 0 °C, a solution of NaNO₂ (0.63 mmol) in water (1.8 mL) was added dropwise. The mixture was stirred for 40 minutes at room temperature and then quenched by slowly adding water (1.80 mL). The resulting mixture was cooled and filtered obtaining compound **34** which was used without any further purification. Yield 86 % as white foam. TLC: cyclohexane/ethyl acetate (9:1) - R_f: 0.38. ¹H NMR (300 MHz, acetone-d₆): δ 9.20 (s, 1H, ArH), 7.61 (d, *J* = 8.6 Hz, 2H, ArH), 7.48 (d, *J* = 8.6 Hz, 2H, ArH) ppm.

1.1.2.4. Synthesis of 4-(4-chlorophenyl)-3-aminoisoxazole (35). Acyl azide 34 (0.403 mmol) was suspended in a mixture of water/acetic acid (1:1) (6.4 mL) and the reaction was refluxed for 1 hour. Then the solvents were removed under *vacuum* and the residue was extracted with H₂O and ethyl acetate (3×2 mL), dried with anhydrous Na₂SO₄ and concentrated under reduced pressure obtaining intermediate 35. Quantitative yield as red foam. TLC: cyclohexane/ethyl acetate (7:3) - R_f: 0.25. ¹H NMR (300 MHz, acetone-d₆): δ 8.58 (s, 1H, ArH), 7.57 (d, *J* = 8.5 Hz, 2H, ArH), 7.46 (d, *J* = 8.5 Hz, 2H, ArH), 5.10 (br s, 2H, NH₂ exchanged with D₂O) ppm. MS (ESI) *m/z* 194.9 [M + H]⁺.

1.1.3. Synthesis of 3-(4-chlorophenyl)-4-aminoisoxazole (41)

1.1.3.1. Synthesis of 4-chlorobenzoylchloride oxime (36). α-Chlorobenzaldoxime 36, an intermediate in the synthesis of compound 31d, was prepared according to literature procedure^[3]: yield 87 % (starting from 10 mmol of 4-chlorobenzaldehyde, 8.7 mmol of 36 were obtained) as light-yellow solid (m.p. 48-49 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f : 0.60. ¹H NMR (300 MHz, CDCl₃): δ 7.74 (d, J = 8.7 Hz, 2H), 7.32 (d, J = 8.7 Hz, 2H), 2.88 (s, 1H, OH, exchanged with D₂O) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 135.80, 135.71, 131.87, 128.66, 128.31 ppm.

1.1.3.2. Synthesis of ethyl (E)-3-pyrrolidin-1-yl acrylate (37). A solution of pyrrolidine (14.06 mmol) in toluene (2.4 mL) was added dropwise over 10 minutes to a solution of ethyl propiolate (14.06 mmol) in toluene (9.7 mL). The resulting mixture was stirred at room temperature for 16 hours and then the solvent was evaporated under reduced pressure. The residue was diluted with water (9 mL) and extracted with ethyl acetate (3×3 mL). The collected organic layers were dried

over Na₂SO₄ and concentrated *in vacuo* to give pure compound **37**. Quantitative yield as orange oil. TLC: cyclohexane/ethyl acetate (9:1) - R_f: 0.11. ¹H NMR (300 MHz, CDCl₃): δ 7.65 (d, J = 12.9 Hz, 1H, CH), 4.48 (d, J = 12.9 Hz, 1H, CH), 4.13 (q, J = 7.1 Hz, 2H, CH₂), 3.27 (br s, 4H, CH₂), 1.93 (br s, 4H, CH₂), 1.26 (t, J = 7.1, 3H, CH₃) ppm.

1.1.3.3. Synthesis of ethyl 3-(4-chlorophenyl)isoxazole-4-carboxylate (38). To a stirred solution of intermediate **36** (6.74 mmol) in anhydrous diethyl ether (13.4 mL) and triethylamine (1.11 mL), a solution of compound **37** (6.74 mmol) in anhydrous diethyl ether was dripped at 0 °C and then the resulting mixture was stirred at room temperature for 18 hours under nitrogen atmosphere. The orange suspension was partitioned between water (8 mL) and diethyl ether (6 mL) and the aqueous phase was extracted with further diethyl ether (2×4 mL). The organic layers were combined, washed with 1M HCl (4 mL), dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude intermediate was purified by flash chromatography on silica gel (eluent: cyclohexane/ethyl acetate 95:5) to afford intermediate **38**. Yield 74 % as colorless oil. TLC: cyclohexane/ethyl acetate (9:1) - R_f: 0.37. ¹H NMR (300 MHz, CDCl₃): δ 8.96 (s, 1H, ArH), 7.70 (d, *J* = 8.7 Hz, 2H, ArH), 7.36 (d, *J* = 8.7 Hz, 2H, ArH), 4.22 (q, *J* = 7.5 Hz, 2H, CH₂), 1.24 (t, *J* = 7.5 Hz, 3H, CH₃) ppm.

1.1.3.4. Synthesis of 3-(4-chlorophenyl)isoxazole-4-carboxilic acid (**39**). A stirred solution of ester **38** (5.01 mmol) in acetic acid (21 mL) was treated with 6N HCl (35 mL) at reflux for 6 hours. After cooling, the reaction mixture was extracted with ethyl acetate (3×10 mL). The concentrated organic layer was treated with sat. NaHCO₃ (10 mL) and the aqueous solution was separated, cooled and acidified with 6N HCl. Intermediate **39** was extracted with ethyl acetate (3x10 mL) and the combined organic phases were dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. Acid **39** was used in the next step without any further purification. Yield 96 % as white foam. TLC: dichloromethane/methanol/acetic acid (98:2:0.02) - R_f: 0.25. ¹H NMR (300 MHz, CDCl₃): δ 9.12 (s, 1H, ArH), 7.69 (d, *J* = 9 Hz, 2H, ArH), 7.49 (d, *J* = 9 Hz, 2H, ArH) ppm.

1.1.3.5. Synthesis of [3-(4-chlorophenyl)isoxazol-4-yl]-carbamic acid tert-butyl ester (40). A mixture of intermediate **39** (2.94 mmol), diphenylphosphorylazide (2.94 mmol) and triethylamine (0.41 mL) in dry *tert*-butanol was refluxed for 16 hours under nitrogen atmosphere. Compound **40** was extracted with ethyl acetate (3×4 mL). The combined organic phases were washed with 1M HCl and brine, then dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography on silica gel (eluent: cyclohexane/ethyl acetate from 8:2 to 7:3) to afford intermediate **40**. Yield 53 % as brown solid (m.p. 160.5-163.2 °C). TLC: cyclohexane/ethyl acetate (7:3) - R_f: 0.28. ¹H NMR (300 MHz, CDCl₃): δ 8.85 (s, 1H, ArH), 7.52 (d, *J* = 8.5 Hz, 2H, ArH), 7.45 (d, *J* = 8.6 Hz, 2H, ArH), 6.26 (br s, 1H, NH exchanged with D₂O), 1.46 (s, 9H, C(CH₃)₃) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 152.62, 148.62, 136.27, 129.59, 129.18, 126.14, 118.21, 81.71, 28.13 ppm.

1.1.3.6. Synthesis of 3-(4-chlorophenyl)-4-aminoisoxazole (41). To a stirred solution of compound 40 (1.076 mmol) in anhydrous dichloromethane (0.5 mL), trifluoracetic acid (0.5 mL) was slowly added and the reaction mixture was stirred at room temperature for 16 hours. The resulting mixture was extracted with water (3×2 mL) and the collected acidic aqueous solutions were basified with a 4N NaOH. Amine 41 was extracted with ethyl acetate (3×4 mL) and the organic layers were combined, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The crude was purified by flash chromatography on silica gel (eluent: cyclohexane/ethyl acetate 7:3) to provide compound 41. Yield 84 % as yellow foam. TLC: cyclohexane/ethyl acetate (7:3) - R_f: 0.25. ¹H NMR (300 MHz, acetone-d₆): δ 8.30 (s, 1H, ArH), 7.89 (d, *J* = 8.5 Hz, 2H, ArH), 7.53 (d, *J* = 8.5 Hz, 2H, ArH), 3.99 (s, 2H, NH₂ exchanged with D₂O) ppm.

1.1.4. Synthesis of 5-(4-chlorophenyl)-1-methyl-4-amino-imidazole (43)

1.1.4.1. Synthesis of 5-(4-chlorophenyl)-1-methyl-4-nitro-imidazole (42). A mixture of 5-chloro-1-methyl-4-nitroimidazole (6.19 mmol), 4-chlorophenylboronic acid (6.19 mmol), Pd(PPh₃)₂Cl₂ (0.19 mmol), K₂CO₃ (15.48 mmol) and TBAB (6.19 mmol) in water (10 mL) was stirred at 80 °C for 16 hours, under nitrogen atmosphere. The resulting precipitate was filtered to obtain pure intermediate 42. Yield 92 % as yellow foam. TLC: dichloromethane/methanol (95:5) - R_f: 0.50. ¹H NMR (300 MHz, CDCl₃): δ 7.48-7.55 (m, 3H, ArH), 7.34 (d, *J* = 8.5 Hz, 2H, ArH), 3.54 (s, 3H, CH₃) ppm. MS (ESI) *m/z* 238.4 [M + H]⁺.

1.1.4.2. Synthesis of 5-(4-chlorophenyl)-1-methyl-4-amino-imidazole (43). Compound 42 (0.42 mmol) was dissolved in dichloromethane/methanol (9:1, 3 mL) and Pd(OH)₂ (0.07 mmol) was added. The mixture was stirred under hydrogen atmosphere at room temperature overnight and then filtered on Celite®. The organic solvents were removed under *vacuum* to give the pure amine 43. Yield 91 % as brownish oil. TLC: dichloromethane/methanol (9:1) - R_f: 0.44. ¹H NMR (300 MHz, CDCl₃): δ 7.41 (d, J = 8.5 Hz, 2H, ArH), 7.27 (d, J = 8.5 Hz, 2H, ArH), 3.53 (s, 3H, CH₃), 3.41 (br s, 2H, NH₂ exchanged with D₂O) ppm. MS (ESI) m/z 208.8 [M + H]⁺.

1.1.5. Synthesis of ethyl 3-amino-4-(4-chlorophenyl)furan-2-carboxylate (45)

1.1.5.1. Synthesis of 4-chlorophenyl-acrylonitrile sodium salt (44). Sodium (6.612 mmol) was suspended in dry ethanol (8 mL) and refluxed under nitrogen atmosphere. A solution of 4-chlorophenylacetonitrile (3.306 mmol) and ethyl formate (4.98 mmol) in ethanol (2 mL) was added dropwise and the reaction mixture was stirred at reflux for 16 hours. After cooling, diethyl ether was added and the resulting precipitate was collected by filtration, washed with diethyl ether and dried to give 4-chlorophenylacrylonitrile as sodium salt (44). Yield 78 % as white solid. TLC: dichloromethane/methanol (95:5) - R_f: 0.51. ¹H NMR (300 MHz, DMSO-d₆): δ 7.73 (d, *J* = 8.8 Hz, 2H, ArH), 7.06 (d, *J* = 8.8 Hz, 2H, ArH), 7.04 (s, 1H, CH) ppm.

1.1.5.2. Synthesis of ethyl 3-amino-4-(4-chlorophenyl)furan-2-carboxylate (45). Intermediate 44 (2.48 mmol) was dissolved in dry DMF (3.97 mL) and diethyl chloromalonate (2.73 mmol) was added. The reaction mixture was stirred for 5 hours at room temperature under nitrogen atmosphere and then the solvent was removed under reduced pressure. The obtained orange solid was diluted with water (10 mL) and extracted with dichloromethane (3×5 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and evaporated under *vacuum*. The resulting intermediate (diethyl 2-((2-(4-chlorophenyl)-2-cyanovinyl)oxy)malonate) was dissolved in dry ethanol (2.6 mL) and 1,5diazabicyclo-[4.3.0]-non-5-ene (2.73 mmol) was added under nitrogen atmosphere. After refluxing for 16 hours, the solution was concentrated and the residue was diluted with water (5 mL) and extracted with dichloromethane (3×3 mL). The organic layers were dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude was purified by silica gel chromatography (eluent: cyclohexane/ethyl acetate 9:1) to obtain compound 45. Yield 41 % as brownish foam. TLC: cyclohexane/ethyl acetate (8:2) - R_f : 0.51. ¹H NMR (300MHz, DMSO-d₆): δ 7.87 (s, 1H, ArH), 7.41-7.56 (m, 4H, ArH), 5.54 (br s, 2H, NH₂ exchanged with D_2O), 4.22 (q, J =7.1 Hz, 2H, CH₂), 1.25 (t, J = 7.1 Hz, 3H, CH₃) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 160.35, 142.29, 141.35, 133.85, 129.42, 128.82, 128.46, 119.44, 60.13, 14.56 ppm.

1.1.6. Synthesis of 4'-chloro-[1,1-biphenyl]-2-amine (48)

In a two-necked flask, 2-iodoaniline (1.14 mmol) was dissolved in dry toluene/ethanol (20:1, 20 mL) under nitrogen atmosphere. Then 4-chlorophenylboronic acid (1.256 mmol), 2M Na₂CO₃ (3.43 mmol) and Pd(PPh₃)₄ (0.923 mmol) were added. The mixture was stirred at 70 °C for 16 hours. After solvent evaporation, water was added (4 mL) and the residue was extracted with ethyl acetate (2×2 mL). The organic layers were collected, dried over anhydrous Na₂SO₄, filtered and concentrated under *vacuum*. The crude was purified by flash chromatography on silica gel (eluent: cyclohexane/ethyl acetate, 9:1) to afford compound **48**. Yield 81 % as orange oil. TLC:

cyclohexane/ethyl acetate (9:1) - R_f : 0.27. ¹H NMR (300 MHz, CDCl₃): δ 7.34-7.46 (m, 4H, ArH), 7.06-7.21 (m, 2H, ArH), 6.72-6.89 (m, 2H, ArH), 3.75 (br s, 2H, NH₂ exchanged with D₂O) ppm.

1.1.7. General procedures for the synthesis of N-aryl amides (1-13,16,18,20,22,24-30)

Procedure a. In a microwave vessel, to a solution of the suitable 4-phenyl-1,2,5-oxadiazol-3amine (0.255 mmol) and 4-dimethylaminopyridine (0.306 mmol) in dry dichloroethane (1.5 mL), the opportune benzoyl chloride (0.306 mmol) was added at room temperature under nitrogen atmosphere. The reaction mixture was irradiated in a microwave synthesizer at 300 Watts and 120 °C for 45 minutes. Upon completion, the solution was treated with water (3 mL) and extracted with dichloromethane (3×3 mL) and the collected organic phases were washed with 1N HCl (2 mL). The organic layer was dried over Na₂SO₄, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography.

Procedure b. In a two-necked flame-dried flask under nitrogen atmosphere, the suspension of 60 % NaH mineral oil (0.3 mmol) in dry DMF (3 mL) was cooled at 0 °C and the appropriate aryl amine (0.25 mmol) was added. The mixture was stirred for 30 minutes at 0 °C. Then, the suitable commercially available acyl chloride (0.3 mmol) was added dropwise and the mixture was stirred at 60 °C for 12 hours. The reaction was quenched with water (3 mL), and DMF was removed under *vacuum*. The residue was extracted with ethyl acetate (3×2 mL); the collected organic layers were dried over Na₂SO₄ and evaporated under reduced pressure. The crude product was purified by column chromatography to obtain the desired adduct.

Procedure c. In a two-necked flame-dried flask, the proper aryl amine (0.373 mmol) was dissolved in dry pyridine (0.54 mL) under nitrogen atmosphere and cooled at 0 °C. After 10 minutes, the suitable acyl chloride (0.341 mmol) was dripped and the mixture was stirred at room temperature until completion. The solvent was concentrated under reduced pressure and the residue was diluted with water (4 mL) and extracted with ethyl acetate (3×2 mL). The organic layers were collected, dried over anhydrous Na₂SO₄, filtered and concentrated *in vacuo*. The pure 1,3,4-oxadiazol-2-yl-benzamide was isolated after flash chromatography.

Procedure d. In a two-necked flame-dried flask, the opportune aryl amine (0.48 mmol) and triethylamine (0.96 mmol) were dissolved in dry dichloromethane (3 mL) under nitrogen atmosphere. The solution was cooled on an ice-bath and after 30 minutes the suitable acyl chloride (0.53 mmol) was added dropwise. The reaction was stirred for 90 minutes at different temperatures for each substrate. After cooling, the reaction was quenched with water (3 mL) and extracted with dichloromethane (2×3 mL). The collected organic layers were firstly washed with 1M HCl (2 mL) and then with brine (2 mL). The resulting solution was dried over anhydrous Na₂SO₄, filtered and concentrated under *vacuum*. The final benzamide was obtained as pure product by flash chromatography or precipitation as hydrochloride salt.

Procedure e. In a microwave vial, the aryl amine (0.15 mmol) was dissolved in dry dichloromethane (4.29 mL) under nitrogen atmosphere. Then triethylamine (0.378 mmol) and benzoyl chloride (0.15 mmol) were added. The reaction mixture was irradiated at 300 Watts and at 40 °C for 80 minutes in a microwave synthesizer. The crude was washed with 1M HCl, saturated bicarbonate solution and brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography.

1.1.7.1. 4-(trifluoromethyl)-N-(4-phenyl-1,2,5-oxadiazol-3-yl) benzamide (8). Starting compounds: **31a** and 4-(trifluoromethyl)benzoyl chloride. Procedure a: yield 9 %. Procedure b: yield 34 %. White solid (m.p. 198-199 °C). TLC: cyclohexane/ethyl acetate (7:3) - R_f: 0.50. Eluent for chromatography: cyclohexane/ethyl acetate (7:3). ¹H NMR (300 MHz, acetone-d₆): δ 10.53 (br s, 1H, NH exchanged with D₂O), 8.26 (d, J = 8.1 Hz, 2H, ArH), 7.92 (d, J = 8.1 Hz, 2H, ArH), 7.83-7.86 (m, 2H, ArH), 7.50-7.54 (m, 3H, ArH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 165.20,

151.59, 149.74, 136.25, 133.64, 133.21, 130.63, 129.04, 128.87, 127.51, 125.72 (q, J = 3.7 Hz), 122.11 ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.58 (s, CF₃) ppm.

1.1.7.2. *N*-(4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl)benzamide (5). Starting compounds: **31d** and benzoyl chloride. Procedure a: yield 12 %. Procedure b: yield 32 %. Brown solid (m.p. 177.4-178.7 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.28. Eluent for chromatography: cyclohexane/ethyl acetate (8:2). ¹H NMR (300 MHz, CDCl₃): δ 8.20 (br s, 1H, NH exchanged with D₂O), 7.81 (d, *J* = 7.6 Hz, 2H, ArH), 7.62-7.52 (m, 3H, ArH), 7.42-7.50 (m, 2H, ArH), 7.39 (d, *J* = 8.5 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 166.49, 151.07, 150.34, 136.37, 133.06, 132.74, 129.45, 129.42, 128.96, 128.27, 125.10 ppm.

1.1.7.3. 4-chloro-N-(4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl) benzamide (10). Starting compounds: **31d** and 4-chlorobenzoyl chloride. Procedure a: yield 15 % as white solid (m.p. 155-160 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.44. Eluent for chromatography: cyclohexane/ethyl acetate (8:2). ¹H NMR (300 MHz, CDCl₃): δ 8.07 (br s, 1H, NH exchanged with D₂O), 7.81 (d, *J* = 8.2 Hz, 2H, ArH), 7.64 (d, *J* = 8.2 Hz, 2H, ArH), 7.51 (d, *J* = 8.7 Hz, 2H, ArH), 7.46 (d, *J* = 8.7 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 166.69, 140.26, 139.97, 135.43, 130.07, 129.59, 129.49, 129.02, 128.86, 128.79, 126.27 ppm.

1.1.7.4. 4-chloro-N-(4-(4-trifluoromethylphenyl)-1,2,5-oxadiazol-3-yl)benzamide (12). Starting compounds: **31e** and 4-chlorobenzoyl chloride. Procedure b: yield 8 % as yellow solid (m.p. 200-201 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.42. Eluent for chromatography: cyclohexane/ethyl acetate (8:2). ¹H NMR (300 MHz, CDCl₃): δ 8.11 (br s, 1H, NH exchanged with D₂O), 7.81-7.85 (m, 4H, ArH), 7.74 (d, *J* = 8.4 Hz, 2H, ArH), 7.51-7.54 (m, 2H, ArH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 165.09, 149.15, 140.56, 132.69, 130.17, 129.71, 129.46, 129.17, 128.22, 126.27 (q, *J* = 3.7 Hz), 125.64, 121.92 ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.08 (s, CF₃) ppm.

1.1.7.5. N-(*4-phenyl-1,2,5-oxadiazol-3-yl)benzamide* (**9**). Starting compounds: **31a** and benzoyl chloride. Procedure a: yield 13 % as white solid (m.p. 139-140 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f : 0.28. Eluent for chromatography: cyclohexane/ethyl acetate (from 9:1 to 6:4). ¹H NMR (300 MHz, CDCl₃): δ 8.00-8.22 (m, 4H, ArH), 7.56-7.66 (m, 2H, ArH), 7.39-7.53 (m, 4H, ArH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 172.56, 133.82, 133.12, 130.76, 130.21, 129.34, 129.24, 128.97, 128.47, 127.76, 127.57 ppm.

1.1.7.6. *N*-(4-(2-chlorophenyl)-1,2,5-oxadiazol-3-yl)benzamide (**6**). Starting compounds: **31b** and benzoyl chloride. Procedure b: yield 7 % as white solid (m.p. 101-104 °C). TLC: cyclohexane/ethyl acetate/dichloromethane (8:1:1) - R_f : 0.28. Eluent for chromatography: cyclohexane/ethyl acetate (from 9:1 to 6:4). ¹H NMR (300 MHz, CDCl₃): δ 8.23 (br s, 1H, NH exchanged with D₂O), 7.78-7.84 (m, 2H, ArH), 7.55-7.63 (m, 2H, ArH), 7.41-7.53 (m, 5H, ArH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 156.98, 134.46, 133.80, 130.20, 129.91, 129.85, 129.33, 128.47, 128.05, 126.00 ppm.

1.1.7.7. 4-(*trifluoromethyl*)-*N*-(4-(2-*chlorophenyl*)-1,2,5-*oxadiazol*-3-*yl*)*benzamide* (1). Starting compounds: **31b** and 4-(trifluoromethyl)benzoyl chloride. Procedure b: yield 24 % as white solid (m.p. 122-123 °C). TLC: petroleum ether/ethyl acetate (9:1) - R_f: 0.21. Eluent for chromatography: petroleum ether/ethyl acetate (8:2). ¹H NMR (300 MHz, CDCl₃): δ 8.37 (br s, 1H, NH exchanged with D₂O), 7.94 (d, *J* = 8.4 Hz, 2H, ArH), 7.76 (d, *J* = 8.1 Hz, 2H, ArH), 7.60-7.63 (m, 1H, ArH), 7.42-7.52 (m, 3H, ArH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 163.73, 149.33, 148.92, 135.60, 135.10, 134.66, 132.94, 132.42, 132.18, 130.23, 128.33, 127.90, 126.33 (q, *J* = 3.7 Hz), 125.30 ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.83 (s, CF₃) ppm.

1.1.7.8. N-(*4*-(*3-chlorophenyl*)-*1,2,5-oxadiazol-3-yl*)-*benzamide* (7). Starting compounds: **31c** and benzoyl chloride. Procedure b: yield 7 % as white solid (m.p. 119-121 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f : 0.20. Eluent for chromatography: cyclohexane/dichloromethane/ethyl acetate (8:1:1) and 1 % acetic acid. ¹H NMR (300 MHz, CDCl₃): δ 8.16 (br s, 1H, NH exchanged with D₂O), 7.88 (d, *J* = 7.3 Hz, 2H, ArH), 7.74 (t, *J* = 1.6 Hz, 1H, ArH), 7.68-7.37 (m, 6H, ArH) ppm.

¹³C NMR (75 MHz, CDCl₃): δ 166.02, 149.42, 148.98, 135.16, 133.80, 133.27, 130.81, 130.41, 130.17, 128.99, 128.48, 127.73, 125.50 ppm.

1.1.7.9. 4-(*trifluoromethyl*)-*N*-(4-(3-*chlorophenyl*)-*1*,2,5-*oxadiazol*-3-*yl*)*benzamide* (**2**). Starting compounds: **31c** and 4-(trifluoromethyl)benzoyl chloride. Procedure b: yield 32 % as white solid (m.p. 196-197 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.35. Eluent for chromatography: cyclohexane/ethyl acetate (75:25). ¹H NMR (300 MHz, acetone-d₆): δ 8.25 (d, *J* = 8.1 Hz, 2H, ArH), 7.94 (d, *J* = 8.4 Hz, 2H, ArH), 7.86 (s, 1H, ArH), 7.80 (d, *J* = 6.6 Hz, 1H, ArH), 7.52-7.62 (m, 2H, ArH) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 165.45, 150.76, 149.90, 136.41, 134.58, 133.93, 133.49, 131.06, 130.80, 129.13, 128.06, 127.63, 126.35, 125.88 (q, *J* = 3.8 Hz) ppm. ¹⁹F NMR (282 MHz, acetone-d₆): δ -63.43 (s, CF₃) ppm.

1.1.7.10. 2-(*trifluoromethyl*)-*N*-(4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl)benzamide (**3**). Starting compounds: **31d** and 2-(trifluoromethyl)benzoyl chloride. Procedure b: yield 56 % as light gray solid (m.p. 197-199 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.27. Eluent for chromatography: cyclohexane/ethyl acetate (8:2). ¹H NMR (300 MHz, CD₃OD): δ 7.67-7.85 (m, 6H, ArH), 7.55 (d, *J* = 8.5 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, CD₃OD): δ 149.07, 136.79, 132.38, 130.90, 129.67, 129.39, 129.14, 128.44, 127.69, 127.26, 126.65 (q, *J* = 5.1 Hz), 125.57, 124.31, 121.96 ppm. ¹⁹F NMR (282 MHz, CD₃OD): δ -60.30 (s, CF₃) ppm.

1.1.7.11. 3-(trifluoromethyl)-N-(4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl)benzamide (4). Starting compounds: **31d** and 3-(trifluoromethyl)benzoyl chloride. Procedure b: yield 60 % as light gray solid (m.p. 113-115 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.36. Eluent for chromatography: cyclohexane/ethyl acetate (8:2). ¹H NMR (300 MHz, CDCl₃): δ 8.54 (br s, 1H, NH exchanged with D₂O), 8.14 (s, 1H, ArH), 8.06 (d, *J* = 7.9 Hz, 1H, ArH), 7.89 (d, *J* = 7.9 Hz, 1H, ArH), 7.60-7.71 (m, 3H, ArH), 7.40-7.49 (m, 2H, ArH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 164.54, 149.88, 148.85, 137.44, 132.74, 132.25, 131.81, 131.07, 130.17, 130.08, 129.82, 129.03, 124.96 (q, *J* = 3.7 Hz), 124.03 ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -62.27 (s, CF₃) ppm.

1.1.7.12. 4-methoxy-N-(4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl) benzamide (13). Starting compounds: **31d** and 4-methoxybenzoyl chloride. Procedure b: yield 70 % as white solid (m.p. 161-163 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.20. Eluent for chromatography: cyclohexane/dichloromethane/ethyl acetate (7:2:1). ¹H NMR (300 MHz, acetone-d₆): δ 10.23 (br s, 1H, NH exchanged with D₂O), 8.02 (d, J = 8.7 Hz, 2H, ArH), 7.83 (d, J = 8.4 Hz, 2H, ArH), 7.53 (d, J = 8.4 Hz, 2H, ArH), 7.06 (d, J = 8.7 Hz, 2H, ArH), 3.89 (s, 3H, CH₃) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 165.65, 163.46, 150.84, 150.28, 136.05, 130.11, 129.15, 129.13, 124.97, 124.55, 113.91, 55.09 ppm.

1.1.7.13. 4-nitro-N-(4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl) benzamide (16). Starting compounds: **31d** and 4-nitrobenzoyl chloride. Procedure b: yield 46 % as yellow solid (m.p. 230-231 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.21. Eluent for chromatography: cyclohexane/ethyl acetate from (98:2) to (7:3). ¹H NMR (300 MHz, acetone-d₆): δ 10.79 (br s, NH, 1H exchanged with D₂O), 8.41 (d, J = 8.7 Hz, 2H, ArH), 8.30 (d, J = 8.7 Hz, 2H, ArH), 7.88 (d, J = 8.4 Hz, 2H, ArH), 7.57 (d, J = 8.4 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 164.87, 150.85, 150.56, 149.79, 138.06, 136.37, 129.56, 129.31, 129.22, 124.74, 123.73 ppm.

1.1.7.14. 4-cyano-N-(4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl) benzamide (18). Starting compounds: **31d** and 4-cyanobenzoyl chloride. Procedure b: yield 60 % as white solid (m.p. 212-213 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.27. Eluent for chromatography: dichloromethane/cyclohexane (98:2). ¹H NMR (300 MHz, acetone-d₆): δ 10.64 (br s, NH, 1H exchanged with D₂O), 8.21 (d, *J* = 9.0 Hz, 2H, ArH), 8.00 (d, *J* = 9.0 Hz, 2H, ArH), 7.86 (d, *J* = 7.8 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 165.22, 150.95, 149.87, 136.58, 136.46, 132.85, 129.52, 129.47, 129.12, 124.89, 117.87, 116.32 ppm.

1.1.7.15. 4-trifluoromethyl-N-(4-(4-trifluoromethylphenyl)-1,2,5-oxadiazol-3-yl)benzamide (11). Starting compounds: **31e** and 4-(trifluoromethyl)benzoyl chloride. Procedure a: yield 6 % as off-white solid (m.p. 211-212 °C). TLC: cyclohexane/ethyl acetate (7:3) - R_f: 0.60. Eluent for chromatography: cyclohexane/ dichloromethane (3:7). ¹H NMR (300 MHz, CD₃OD): δ 8.12 (d, *J* = 8.4 Hz, 2H, ArH), 7.95 (d, *J* = 8.4 Hz, 2H, ArH), 7.86 (d, *J* = 8.1 Hz, 2H, ArH), 7.80 (d, *J* = 8.1 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 169.85, 149.20, 142.35, 130.75, 128.65 (q, *J* = 3.6 Hz), 128.27 (q, *J* = 3.6 Hz), 128.06, 126.32, 126.27, 126.22, 126.10, 125.91, 125.86 ppm. ¹⁹F NMR (282 MHz, CD₃OD): δ -64.90 (s, CF₃), -65.43 (s, CF₃) ppm.

1.1.7.16. 4-trifluoromethyl-N-(4-(4-nitrophenyl)-1,2,5-oxadiazol-3-yl)benzamide (22). Starting compounds: **31g** and 4-(trifluoromethyl)benzoyl chloride. *Procedure b*: yield 23 % as yellow-white solid (m.p. 102-104 °C). TLC: cyclohexane/ ethyl acetate (8:2) - R_f: 0.37. Eluent for chromatography: cyclohexane/ethyl acetate (8:2). ¹H NMR (300 MHz, CD₃OD): δ 8.35 (d, *J* = 8.9 Hz, 2H, ArH), 8.12 (d, *J* = 8.2 Hz, 2H, ArH), 8.00 (d, *J* = 8.9 Hz, 2H, ArH), 7.86 (d, *J* = 8.2 Hz, 2H, ArH). ¹³C NMR (75 MHz, CD₃OD): δ 180.32, 166.46, 150.20, 149.92, 149.27, 135.98, 132.45, 128.80, 128.70, 128.13, 125.70 (q, *J* = 3.7 Hz), 123.90 ppm. ¹⁹F NMR (282 MHz, CD₃OD): δ - 65.10 (s, CF₃) ppm.

1.1.7.17. N-(*4*-(*4*-chlorophenyl)-*1*,2,5-oxadiazol-3-yl)-butyramide (**24**). Starting compounds: **31d** and *n*-butyryl chloride. Procedure b: yield 98 % as white solid (m.p. 139-140 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f : 0.26. Eluent for chromatography: cyclohexane/dichloromethane/ethyl acetate (4:5:1). ¹H NMR (300 MHz, CDCl₃): δ 7.53 (d, 2H, *J* = 8.1 Hz, ArH), 7.42 (d, 2H, *J* = 8.1 Hz, ArH), 7.31 (br s, 1H, NH exchanged with D₂O), 2.42 (t, 2H, *J* = 7.5 Hz, CH₂), 1.61-1.73 (m, 2H, CH₂), 0.93 (t, 3H, *J* = 7.4 Hz, CH₃) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 172.17, 149.17, 148.68, 137.42, 129.81, 129.26, 123.81, 38.32, 18.60, 13.66 ppm.

1.1.7.18. 4-trifluoromethyl-N-(4-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl)benzamide (**25**). Starting compounds: 2-amino-5-(4-chloropheny)-1,3,4-oxadiazole and 4-(trifluoromethyl)benzoyl chloride. Procedure c: reaction time 16 hours at room temperature, yield 33 % as red solid (m.p. 234-235 °C). TLC: dichloromethane/methanol/ethyl acetate (95:2.5:2.5) - R_f : 0.34. Eluent for chromatography: dichloromethane/methanol/ethyl acetate (95:2.5:2.5 to 90:5:5). ¹H NMR (300 MHz, CDCl₃): δ 8.33 (d, *J* = 7.0 Hz, 2H, ArH), 7.97 (d, *J* = 8.0 Hz, 2H, ArH), 7.73 (d, *J* = 7.0 Hz, 2H, ArH), 7.52 (d, *J* = 8.0 Hz, 2H, ArH) ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.08 (s, CF₃) ppm.

1.1.7.19. 4-trifluoromethyl-N-(4-(4-chlorophenyl)-isoxazol-3-yl) benzamide (26). Starting compounds: **35** and 4-(trifluoromethyl)benzoyl chloride. Procedure d: at 40 °C, yield 10 % as light yellow solid (m.p. 171-173 °C). TLC: cyclohexane/ethyl acetate (7:3) - R_f: 0.45. Eluent for chromatography: cyclohexane/ethyl acetate (7:3). ¹H NMR (300 MHz, acetone-d₆): δ 10.17 (br s, 1H, NH exchanged with D₂O), 9.08 (s, 1H, ArH), 8.22 (d, J = 8.4 Hz, 2H, ArH), 7.90 (d, J = 8.4 Hz, 2H, ArH), 7.59 (d, J = 8.6 Hz, 2H, ArH), 7.41 (d, J = 8.6 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 165.01, 158.22, 155.66, 136.93, 133.19, 132.84, 128.85, 128.78, 128.70, 127.80, 125.59 (q, J = 3.8 Hz), 122.17, 117.33 ppm. ¹⁹F NMR (282 MHz, acetone-d₆): δ -63.56 (s, CF₃) ppm. MS (ESI) m/z 367.0 [M + H]⁺.

1.1.7.20. 4-trifluoromethyl-N-(3-(4-chlorophenyl)-isoxazol-4-yl) benzamide (27). Starting compounds: **41** and 4-(trifluoromethyl)benzoyl chloride. Procedure b: yield 53 % as off-white solid (m.p. 162-163 °C). TLC: cyclohexane/ ethyl acetate (7:3) - R_f : 0.58. Eluent for chromatography: cyclohexane/ethyl acetate (75:25). ¹H NMR (300 MHz, acetone-d₆): δ 9.57 (br s, 1H, NH exchanged with D₂O), 9.31 (s, 1H, ArH), 8.19 (d, J = 8.2 Hz, 2H, ArH), 7.87 (d, J = 8.2 Hz, 2H, ArH), 7.82 (d, J = 8.5 Hz, 2H, ArH), 7.57 (d, J = 8.5 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 164.64, 155.70, 152.68, 135.46, 132.96, 132.54, 129.78, 129.18, 128.56, 127.07, 125.46 (q, J = 3.8 Hz), 122.18, 117.69 ppm. ¹⁹F NMR (282 MHz, acetone-d₆): δ -63.49 (s, CF₃) ppm.

1.1.7.21. 4-trifluoromethyl-N-(5-(4-chlorophenyl)-1-methylimidazol-4-yl)benzamide (28). Starting compounds: **43** and 4-(trifluoromethyl)benzoyl chloride. Procedure c: at 0 °C, yield 28 % as brown oil. TLC: dichloromethane/methanol (9:1) – R_f: 0.55. Purified by precipitation as hydrochloride salt with 2M HCl in Et₂O. ¹H NMR (300 MHz, CD₃OD): δ 8.96 (s, 1H, ArH), 8.04 (d, J = 8.2 Hz, 2H, ArH), 7.83 (d, J = 8.2 Hz, 2H, ArH), 7.60 (d, J = 8.6 Hz, 2H, ArH), 7.55 (d, J = 8.6 Hz, 2H, ArH), 3.84 (s, 3H, CH₃) ppm. ¹³C NMR (75 MHz, CD₃OD): δ 167.29, 136.53, 135.79, 135.77, 133.57, 133.51, 131.55, 129.28, 128.41, 126.67, 125.39 (q, J = 3.7 Hz), 125.14, 122.91, 34.12 ppm. ¹⁹F NMR (282 MHz, CD₃OD): δ -64.52 (s, CF₃) ppm.

1.1.7.22. 4-trifluoromethyl-N-(4-chloro-[1,1-biphenyl]-2-yl) benzamide (**30**). Starting compounds: **48** and 4-(trifluoromethyl)benzoyl chloride. *Procedure b*: yield 40 % as brown solid (m.p. 169-170 °C). TLC: petroleum ether/ethyl acetate (9:1) - R_f : 0.28. Eluent for chromatography: cyclohexane/ethyl acetate (9:1). ¹H NMR (300 MHz, CDCl₃): δ 8.39-8.47 (m, 1H, ArH), 7.82 (br s, 1H, NH exchanged with D₂O), 7.66-7.76 (m, 4H, ArH), 7.42-7.52 (m, 3H, ArH), 7.35-7.41 (m, 2H, ArH), 7.26-7.29 (m, 2H, ArH) ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.09 (s, CF₃) ppm.

1.1.7.23. 4-trifluoromethyl-N-(4-(4-benzyloxyphenyl)-1,2,5-oxadiazol-3-yl)benzamide (20). Starting compounds: **31f** and 4-(trifluoromethyl)benzoyl chloride. Procedure b: yield 30 % as gray solid (m.p. 218-220 °C). TLC: cyclohexane/ethyl acetate (7:3) - R_f: 0.54. Eluent for chromatography: cyclohexane/ethyl acetate (8:2). ¹H NMR (300 MHz, CDCl₃): δ 8.11 (br s, 1H, NH exchanged with D₂O), 7.99 (d, J = 8.7 Hz, 2H, ArH), 7.79 (d, J = 8.7 Hz, 2H, ArH), 7.64 (d, J = 9.0 Hz, 2H, ArH), 7.35-7.44 (m, 5H, ArH), 7.09 (d, J = 9.0 Hz, 2H, ArH), 5.12 (s, 2H, CH₂) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 165.23, 160.76, 151.23, 149.56, 136.92, 136.28, 133.60, 133.17, 129.08, 128.88, 128.43, 127.90, 127.63, 125.73 (q, J = 3.9 Hz), 117.90, 115.34, 69.68 ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.58 (s, CF₃) ppm.

1.1.7.24. Ethyl 3-N-(4-trifluoromethylbenzamide)-4-(4-chlorophenyl)furan-2-carboxylate (**46**). Starting compounds: **45** and 4-(trifluoromethyl)benzoyl chloride. Procedure d: yield 46 % as brownish solid (m.p. 166-168 °C). TLC: petroleum ether/ ethyl acetate (9:1) - R_f: 0.16. Eluent of chromatography: petroleum ether/ethyl acetate (9:1). ¹H NMR (300 MHz, CDCl₃): δ 9.20 (s, 1H, NH exchanged with D₂O), 7.99 (d, J = 8.2 Hz, 2H, ArH), 7.74 (d, J = 8.2 Hz, 2H, ArH), 7.59 (s, 1H, ArH), 7.30-7.35 (m, 4H, ArH), 4.42 (q, J = 7.1 Hz, 2H, CH₂), 1.41 (t, J = 7.1 Hz, 3H, CH₃) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 162.77, 158.89, 141.96, 135.43, 133.31, 132.87, 132.71, 131.10, 128.95, 127.79, 127.25, 126.99, 124.89 (q, J = 3.7 Hz), 123.16, 120.69, 60.46, 13.31 ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.11 (s, CF₃) ppm.

1.1.8. Synthesis of 4-hydroxy-N-(4-(4-chlorophenyl)-1,2,5-oxadiazol-3yl)benzamide (14)

A solution of 1M BBr₃ in dry dichloromethane (2.26 mmol) was added dropwise to a solution of compound **13** (0.430 mmol) in dry dichloromethane (5 mL) at room temperature, under nitrogen atmosphere. The mixture was stirred for 12 hours. Upon completion, the solution was treated with water (4 mL) and the organic layer was separated. Then the aqueous phase was extracted with ethyl acetate (3×10 mL) and the collected organic phase was washed with brine. The organic layer was dried over Na₂SO₄, filtered and evaporate under reduced pressure giving a crude product, which was purified by flash chromatography (eluent: cyclohexane/ethyl acetate 7:3) to afford intermediate **14**. Yield 35 % as white solid (m.p. 225-226 °C). (TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.20). ¹H-NMR (300 MHz, acetone-d₆): δ 10.16 (br s, 1H, NH exchanged with D₂O), 9.23 (br s, 1H, OH exchanged with D₂O), 7.94 (d, *J* = 8.7 Hz, 2H, ArH), 7.83 (d, *J* = 8.5 Hz, 2H, ArH), 7.54 (d, *J* = 8.5 Hz, 2H, ArH), 6.96 (d, *J* = 8.7 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 167.77, 162.38, 150.75, 150.07, 136.58, 130.30, 129.12, 128.87, 124.89, 123.05, 115.31 ppm.

1.1.9. Synthesis of 4-((4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl)carbamoyl)phenyl dihydrogenphosphate (15)

In a two-necked flask, compound 14 (0.143 mmol) was dissolved in dry acetonitrile (1 mL) under nitrogen atmosphere and the mixture was cooled to -10 °C with an ice-acetone bath. CCl₄ (0.07 mL) was added followed by N,N-diisopropylethylamine (0.301 mmol) and N,N-dimethylaminopyridine (0.014 mmol). After dropping dibenzylphosphite (0.280 mmol), the mixture was stirred for 3 hours at -10 °C. When the reaction was complete, an aqueous solution of 0.5M KH₂PO₄ (2 mL) was added and the system was allowed to warm at room temperature. The mixture was extracted with ethyl acetate $(3 \times 10 \text{ mL})$ and the combined organic phases were washed with water $(1 \times 5 \text{ mL})$ and brine $(1 \times 5 \text{ mL})$, dried over Na₂SO₄, filtered and concentrated *in vacuo*. Dibenzyl (4-((4-(h-chlorophenyl)-1,2,5-oxadiazol-3-yl)carbamoyl)phenyl) phosphate was purified by flash chromatography (eluent: methanol Yield dichloromethane/ 98:2). 45 % as brown foamy solid. TLC: dichloromethane/methanol (98:2) - R_f : 0.3. ¹H NMR (300 MHz, CD₃OD) δ 7.95 (d, J = 8.8 Hz, 2H, ArH), 7.74 (d, J = 8.7 Hz, 2H, ArH), 7.50 (d, J = 8.8 Hz, 2H, ArH), 7.34-7.39 (m, 5H, ArH), 7.27 (d, J = 8.7 Hz, 2H, ArH), 5.21 (s, 2H, CH₂), 5.18 (s, 2H, CH₂) ppm. A suspension of the dibenzyl phenylphosphate (0.044 mmol) and palladium on carbon (10 % w/w, 2.54 mg) in methanol (5 mL) was hydrogenated at room temperature for 3 hours. After completion of the reaction, the catalyst was filtered off on Celite® and the filtrate was concentrated under reduced pressure to afford the final compound 15. The product was purified on semipreparative RP-HPLC (Kinetex® 2.6 µm C18 column, 100 Å, 30 x 2.1 mm, 80 % H₂O/ 20 % acetonitrile and 1/1000 of trifluoroacetic acid, 0.8 mL/min flow rate and UV detector). Yield 69 % as light brown oil. ¹H NMR (300 MHz, CD₃OD): δ 7.84 (d, J = 10.3 Hz, 2H, ArH), 7.63 (d, J = 9.7 Hz, 2H, ArH), 7.39 (d, J = 9.7 Hz, 2H, ArH), 7.26 (d, J = 10.3 Hz, 2H, ArH) ppm. MS (ESI) m/z 396.1 [M + H]⁺.

1.1.10. Synthesis of 4-aminophenyl derivatives (17,23)

To a solution of the suitable nitrophenyl derivative (0.062 mmol) in ethyl acetate (4 mL), tin (II) chloride (0.310 mmol) was added and the mixture was refluxed for 7 hours. After quenching by addition of a saturated aqueous NaHCO₃ until pH=7-8, the precipitated tin salts were eliminated by filtration and the aqueous phase was extracted with ethyl acetate (3×10 mL). The organic layer was dried over Na₂SO₄, filtered and evaporated under *vacuum*. The residue was purified on silica gel column chromatography (eluent: cyclohexane/ethyl acetate 6:4) to give the final compound.

1.1.10.1 4-amino-N-(4-(4-chlorophenyl)-1,2,5-oxadiazol-3-yl)benzamide (17). Starting compound: **16**. Yield 70 % as yellow solid (m.p. 153-154 °C). TLC: cyclohexane/ethyl acetate (6:4) - R_f : 0.22. Eluent for flash chromatography cyclohexane/ethyl acetate 6:4. ¹H NMR (300 MHz, acetone-d₆): δ 9.82 (br s, NH, 1H exchanged with D₂O), 7.64-7.70 (m, 4H, ArH), 7.40 (d, J = 8.7 Hz, 2H, ArH), 6.60 (d, J = 8.4 Hz, 2H, ArH), 5.35 (br s, 2H, NH₂ exchanged with D₂O) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 166.09, 153.41, 151.15, 150.88, 136.17, 130.26, 129.36, 129.30, 125.46, 119.84, 113.35 ppm.

1.1.10.2. 4-trifluoromethyl-N-(4-(4-aminophenyl)-1,2,5-oxadiazol-3-yl)benzamide (23). Starting compound: 22. Yield: 78 % as brown foamy solid. TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.30. Eluent for flash chromatography: from cyclohexane/ethyl acetate 8:2 to 6:4. ¹H NMR (300 MHz, acetone-d₆): δ 10.15 (br s, 1H, NH exchanged with D₂O), 8.14 (d, *J* = 8.2 Hz, 2H, ArH), 7.80 (d, *J* = 8.2 Hz, 2H, ArH), 7.43 (d, *J* = 8.8 Hz, 2H, ArH), 6.60 (d, *J* = 8.8 Hz, 2H, ArH), 5.03 (br s, 2H, NH₂ exchanged with D₂O) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 165.21, 151.60, 151.02, 149.25, 136.49, 128.81, 128.64, 128.34, 125.72 (q, *J* = 3.5 Hz), 119.99, 114.09, 112.63 ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.99 (s, CF₃) ppm.

1.1.11. Synthesis of 4-((4-(4-chlorophenyl)-1,2,5-oxadiazol-3yl)carbamoyl)benzoic acid (19)

To a solution of compound **18** (0.066 mmol) in ethanol (5 mL), 3N NaOH (2 mL) was added at room temperature. The reaction mixture was heated at reflux for 3 hours and, after cooling to room temperature, it was concentrated under *vacuum*. The residue was diluted with water and extracted with ethyl acetate (3×10 mL). The aqueous phase, which contained the sodium salt of **19**, was

acidified with a solution of 3N HCl until pH = 2 and extracted with ethyl acetate (3×10 mL). The organic layer was dried over Na₂SO₄, filtered and evaporated under reduced pressure. Yield 81 % as white solid (m.p. 297-298 °C). TLC: dichloromethane/methanol (9:1) - R_f : 0.21. ¹H NMR (300 MHz, DMSO-d₆): δ 8.02-8.09 (m, 4H, ArH), 7.78 (d, *J* = 8.4 Hz, 2H, ArH), 7.59 (d, *J* = 8.7 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, DMSO-d₆): δ 167.26, 166.52, 151.32, 150.66, 136.37, 136.31, 135.23, 130.20, 130.01, 129.85, 129.10, 124.78 ppm.

1.1.12. Synthesis of 4-((4-(4-hydroxyphenyl)-1,2,5-oxadiazol-3-yl)-4trifluoromethylbenzamide (21)

A suspension of compound **20** (0.54 mmol) and catalytic amount of palladium on carbon (10 % w/w) in ethyl acetate (9 mL) and methanol (1 mL) was hydrogenated at room temperature for 24 hours. The resulting mixture was filtered and extracted with diethyl ether (3×10 mL). the organic layer was dried over Na₂SO₄, filtered and evaporated *in vacuo*. The crude oil was purified by flash chromatography eluting with cyclohexane/ethyl acetate (7:3) to afford **21**. Yield: 91.5 % as white foamy solid. TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.13. ¹H NMR (300 MHz, acetone-d₆): δ 10.43 (br s, 1H, NH exchanged with D₂O), 8.98 (br s, 1H, OH exchanged with D₂O), 8.26 (d, *J* = 8.1 Hz, 2H, ArH), 7.93 (d, *J* = 8.1 Hz, 2H, ArH), 7.71 (d, *J* = 8.7 Hz, 2H, ArH), 6.95 (d, *J* = 8.7 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, acetone-d₆): δ 165.52, 159.90, 151.61, 149.69, 136.60, 129.44, 129.08, 125.95 (q, *J* = 3.7 Hz), 116.78, 116.20 ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.97 (s, CF₃) ppm.

1.1.13. Synthesis of 3-[4-(trifluoromethyl) benzamino]-4-(chlorophenyl)furan-2carboxylic acid (47)

Intermediate **46** (0.27 mmol) was dissolved in a mixture of tetrahydrofuran and ethanol (1:1, 4 mL) and 1N NaOH (1 mL) was added dropwise. The reaction was refluxed for 1 hour and the solvents were removed under *vacuum*. In order to purify the reaction mixture from the unreacted starting materials, the mixture was diluted with water (4 mL) and washed with dichloromethane (4 mL). The aqueous layer that contained the sodium salt of the final compound was acidified with 1N HCl (2 mL) and extracted with ethyl acetate (2×2 mL). The organic layers were dried and concentrated under *vacuum*, affording the pure carboxylic acid (**47**). Yield: 71 % as yellow-brown solid (m.p. 188-191 °C). TLC: dichloromethane/ methanol (8:2) - R_f = 0.26. ¹H NMR (300 MHz, CD₃OD): δ 8.09 (d, *J* = 8.0 Hz, 2H, ArH), 7.79 (d, *J* = 8.0 Hz, 2H, ArH), 7.72 (s, 1H, ArH), 7.44 (d, *J* = 8.5 Hz, 2H, ArH), 7.31 (d, *J* = 8.5 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, CD₃OD): δ 166.53, 165.74, 141.43, 140.42, 137.04, 132.62, 130.55, 128.13, 128.11, 128.09, 125.84, 125.57, 125.14 (q, *J* = 3.5 Hz), 124.17, 121.97 ppm. ¹⁹F NMR (282 MHz, CD₃OD): δ -64.49 (s, CF₃) ppm.

1.1.14. Synthesis of N-(4-(4-chlorophenyl)-furan-3-yl)-trifluoromethylbenzamide (29)

A mixture of compound **47** (0.293 mmol), Ag₂CO₃ (0.0293 mmol), acetic acid (0.015 mmol) in dry DMSO (0.29 mL) was stirred at 120 °C under nitrogen atmosphere for 16 hours. After the completion, the reaction was cooled to room temperature and ethyl acetate (4 mL) was added. The organic layer was washed with saturated sodium bicarbonate solution (2 mL), water (2 mL) and brine (2 mL), and dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude was purified by flash chromatography (eluent: cyclohexane/ethyl acetate - 8:2) to give the final compound. Yield 25 % as yellow-orange solid (m.p. 115-116 °C). TLC: cyclohexane/ethyl acetate (8:2) - R_f: 0.58. ¹H NMR (300 MHz, CDCl₃): δ 8.28 (s, 1H, ArH), 7.79 (d, *J* = 8.1 Hz, 2H, ArH), 7.66 (d, *J* = 8.3 Hz, 2H, ArH), 7.62 (br s, 1H, NH exchanged with D₂O), 7.36-7.44 (m, 3H, ArH), 7.27 (d, *J* = 8.3 Hz, 2H, ArH) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 163.18, 138.63, 136.75, 134.48, 134.31, 133.54, 129.83, 129.34, 128.83, 127.86, 127.35, 125.98 (q, *J* = 3.7 Hz), 122.06, 119.01 ppm. ¹⁹F NMR (282 MHz, CDCl₃): δ -63.11 (s, CF₃) ppm.

1.2. Biological evaluation

The MTT-assay was performed as previously described.^[10,11] The HCT-116 cell line (colorectal cancer) was cultured in McCoy's media. The media was supplemented with penicillin (10,000 U/mL), streptomycin (10 mg/mL), nonessential amino acid and 10 % Fetal Calf Serum (FCS). The same media was utilized for the experiments where cells were incubated with newly synthesized compounds dissolved in DMSO. The same volume of solvent was added to control conditions and did not exceed 0.5 % v/v.

1.3. Computational methodologies

Compounds were drawn by using ChemDraw^[12] program and VEGA ZZ suite^[13,14]. The eXtended Electron Distribution (XED) force field implemented in Cresset's Forge v10.4.2 software was used to generate conformations and molecular fields.^[15] Conformations were optimized until the gradient was smaller than 0.1 kcal mol⁻¹ Å; duplicates were filtered setting a 0.5 Å heavy atom RMSD threshold. Only conformations 3.0 kcal mol⁻¹ energy window from the global minimum were retained, up to a maximum of 1000 for each molecule. Compounds were aligned by maximum common substructure against the crystallographic structure of **MD77**.^[3] The functional groups which were not part of the maximum common substructure were aligned based on a combination of three-dimensional shape and molecular interaction fields, each weighted at 50 %. A disparity matrix was generated by the same 3D similarity function used for the alignment. The disparity matrix was used to carry out the analysis of activity cliff pairs through the Activity Miner methodology.^[8] The information obtained from individual activity cliffs was then conveyed into a qualitative Activity Atlas model using the recommended default settings.^[9]

1.4. Crystallography

The crystals of **5** and **8** were obtained by crystallization from methanol as colorless prisms. They were mounted on a Bruker-Axs CCD-based three circle diffractometer (**5**) and on an Enraf-Nonius CAD-4 diffractometer (**8**), both working at ambient temperature with graphite-monochromatized using MoK α (λ =0.71073Å) radiation. The structures were solved by direct methods^[16] and the refinements were carried out by full-matrix least-squares. All non-H-atoms were refined anisotropically. Hydrogen atoms in both molecules were introduced at calculated positions, in their described geometries and allowed to ride on the attached carbon atom with fixed isotropic thermal parameters (1.2Ueq of the parent carbon atom). Refinements were carried out with SHELX-97. ^[17] Geometrical calculations were carried out with the program PARST.^[18]

CCDC-1496338 (5) and CCDC-1496339 (8) numbers contain the supplementary crystallographic data for this paper (excluding structure factors). These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; fax: (+44)1223-336-033; or deposit@ccdc.cam.ac.uk).

Crystal data of 5: C15 H10 Cl N3 O2, $M_r = 299.71$ g/mol, orthorhombic, Space group *Pbc21*; a = 13.768 (3) Å, b = 5.074 (2) Å, c = 19.460 (3) Å, V = 1382.5(5) Å³, Z = 4, $D_{calc} = 1.44$ Mg/m³, F(000) = 616, R = 0.028 (reflections collected = 10460), wR2 = 0.059, T = 293(2)K, *GOF* = 0.929. The reflections were collected in the range $2.08^{\circ} \le \theta \le 27.12^{\circ}$ (limiting indices = $-17 \le h \le 17$, $-6 \le k \le 6$, $-25 \le l \le 25$) employing a 0.55 x 0.22 x 0.02 mm³ crystal. The residual positive and negative electron densities in the final map were 0.129 and -0.159 eÅ⁻³.

Crystal data of 8: C16 H10 F3 N3 O2, $M_r = 333.27$ g/mol, orthorhombic, Space group *Pbc21*; a = 4.982(2) Å, b = 14.446(3) Å, c = 20.630(5) Å, V = 1484.6(8) Å³, Z = 4, $D_{calc} = 1.491$ Mg/m³, F(000) = 680, R = 0.0985 (reflections collected = 1163), wR2 = 0.229, T = 293(2)K, GOF = 1.219. The reflections were collected in the range $2.82^{\circ} \le \theta \le 25.97^{\circ}$ (limiting indices = $-1 \le h \le 6$, $-1 \le k \le 17$,

 $-1 \le 1 \le 25$) employing a 0.65 x 0.52 x 0.42 mm³ crystal. The residual positive and negative electron densities in the final map were 0.386 and -0.244 eÅ⁻³.

2. Results and discussion

2.1. Chemistry

Compounds 1-24 were prepared following the general procedures reported in Scheme 1.

Key intermediates **31a** and **31b-g** were synthesized by a one pot reaction^[1] or following a multi-step procedure^[3], respectively.

The coupling reaction between the proper 1,2,5-oxadiazol-3-amines (**31a-g**) and the suitable acyl chloride was performed in presence of sodium hydride as base, affording compounds **1-13,16,18,20,22,24**.

Compound 13 was demethylated (14), the hydroxyl group was phosphorylated with dibenzylphosphite and subsequently debenzylated by catalytic hydrogenation over Pd/C leading to derivative 15. The nitro group of compounds 16 and 22 was reduced to the corresponding amine (17 and 23, respectively), while the hydrolysis of nitrile 18 proceeded under basic conditions to achieve carboxylic acid 19. The cleavage of benzyl ether (20) was performed by palladium-catalyzed hydrogenation, to give compound 21.

The other *N*-heteroaryl-benzamides (25-30) were prepared starting from the corresponding heteroaryl amines which were coupled with 4-(trifluoromethyl)benzoyl chloride in presence of a proper base (pyridine, trimethylamine or 60 % sodium hydride).

In details, compounds **25** was prepared starting from the commercially available 2-amino-5-(4-chlorophenyl)-1,3,4-oxadiazole which was acylated in pyridine as shown in **Scheme 2**.

The key intermediate 4-(4-chlorophenyl)-3-aminoisoxazole (**35**) was synthesized starting from the commercially available ethyl 5-amino-4-(4-chlorophenyl) isoxazol-3-carboxylate by a Sandmeyer reaction, performed in aqueous solution.^[19] The obtained compound **32** was treated with hydrazine monohydrate (**33**) and then with sodium nitrite in presence of hydrochloric acid giving after Curtius rearrangement the corresponding acyl azide (**34**). The addition of water led to 4-(4-chlorophenyl)-3-aminoisoxazole (**35**),^[20] which was acylated in presence of triethylamine, providing compound **26** (**Scheme 3**).

The intermediate 4-amino-3-(4-chlorophenyl) isoxazole (**41**) was prepared starting from the commercially available 4-chlorophenyl benzaldehyde, which was firstly treated with hydroxylamine hydrochloride and then with *N*-chlorosuccinimide.^[3] The chloroxime (**36**) was reacted with compound **37**, previously synthesized following a literature procedure.^[21] The resulting ethyl 3-(4-chlorophenyl)-4-isoxazolcarboxylate (**38**) was hydrolyzed under acidic conditions (**39**) and the acyl azide was formed by direct reaction of the carboxylic acid with diphenyl phosphoryl azide (DPPA). According to Curtius rearrangement, acyl azide group decomposed to isocyanate, which underwent nucleophilic attack to yield the corresponding carbamate (**40**). Subsequently, the *N*-Boc protecting group was removed with trifluoroacetic acid and the amine **41** was finally condensed with 4-(trifluoromethyl)benzoyl chloride in the presence of 60 % sodium hydride, leading to compound **27** (**Scheme 4**).

The synthesis of the final product **28** started from the commercially available 5-chloro-1-methyl-4nitroimidazole, which underwent Suzuki coupling reaction (**42**),^[22] followed by catalytic hydrogenation with $Pd(OH)_2$, to afford the amine **43**.^[23,24] This latter was condensed with the acyl chloride in the presence of trimethylamine, affording the compound **28** (Scheme 5).

The synthesis of the final product **29** started from (4-chlorophenyl)acetonitrile, which was treated with metallic sodium, ethyl formate and dry ethanol for 16 hours, obtaining the sodium salt intermediate **44**. The latter was cyclized with ethyl chloroformate in a one pot reaction^[25] to intermediate **45**, which was condensed with 4-(trifluoromethyl)benzoyl chloride in the presence of

triethylamine, by microwave assisted reaction (46). The ester group of intermediate 46 was firstly hydrolyzed (47) and then decarboxylated^[26] to give compound 29 (Scheme 6).

The starting reagent for the synthesis of **30** was 2-iodoaniline, which was arylated by Suzuki reaction (**48**) $^{[27]}$ and condensed with the acyl chloride in the presence of triethylamine (**Scheme 7**).

2.2 Biological evaluation

The compounds were evaluated by MTT-assay on the HCT-116 cancer cell line and the results were reported as half maximal inhibitory concentration (IC₅₀, μ M) in **Table 1**.

The SAR study on 1,2,5-oxadiazole series started evaluating the effects of the displacement of the chlorine atom from *para*- to *ortho*- and *meta*- positions in the phenyl ring, keeping the *p*-CF₃ group on the benzamide. Isomers bearing the chlorine atom in *ortho* (**1**, $IC_{50} = 56.7 \mu M$) and *meta* (**2**, $IC_{50} = 15.4 \mu M$) positions were endowed with lower antiproliferative activity than the parent compound. In particular, **1** showed moderate growth inhibition properties, while **2** exhibited a more interesting activity, although the *para* substitution remained the preferred.

Similarly, the effects of moving CF₃ group to different positions on the benzoyl ring was considered: the isomers **3** (n.a) and **4** (IC₅₀ = 7.4 μ M) indicated the *ortho* position as detrimental, because compound **3** completely lost the activity, whereas the *meta* isomer retained almost the same biological properties of the lead.

Once evaluated the activity of **MD77** isomers, also partial (5-8) or total elimination (9) of substituents on aromatic rings was taken into account in order to ascertain their biological role.

The double *p*-Cl (10) and *p*-CF₃ (11) substitution as well as the exchange between the *p*-Cl and *p*-CF₃ groups (12), were also designed. If the phenyl rings were both substituted with chlorine atoms (10) or with CF₃ groups (11) a different behavior was observed: the first one was completely inactive, while the second showed a moderate activity (IC₅₀ = 24.1 μ M).

On the other hand, compound **12**, characterized by the same substituents of **MD77** but in inverted position, emerged as the most active among the analogues bearing the 1,2,5-oxadiazole scaffold, being slightly more potent than the reference (IC₅₀ = 0.95 *versus* 1.3 μ M, respectively).

Finally, fixing the *p*-Cl phenyl moiety, the substituent in *para*- position of the benzamide group was modulated (**13-19**). When the CF₃ group in *para* position of the benzoyl ring was substituted by a NO₂ (**16**, IC₅₀ = 46.7 μ M) or CN (**18**, IC₅₀ = 19.4 μ M) group, a partial cytotoxic activity was retained.

Analogously, fixing the CF₃ in *para*- position of the benzamide, the substituent in *para*- position of the phenyl moiety was investigated (**20-23**). Among these latter, **22**, bearing the NO₂ substituent instead of the *p*-chlorine atom on the 4-phenyl, showed modest growth inhibitory properties (IC₅₀ = 58.2μ M).

As for **MD77** bioisosteric analogues, only **25** and **28** presented a modest and similar potency (IC₅₀ about 50 μ M). This drop in biological activity supported the importance of the 1,2,5-oxadiazole scaffold for cytotoxicity.

2.3 Computational studies

To get an overview of the SAR landscape, focusing on the prevalent SAR signals, the whole pool of molecules was analyzed by using Activity Miner and Activity Atlas modules as implemented in Cresset's Forge software.^[7] Pairs of compounds can be characterized by their "disparity", namely the difference in their activity divided by the distance between them, where this latter is obtained from their 2D or 3D similarity.

Molecule pairs that have a low disparity and high similarity define bioisosteres or flat regions in the SAR. These regions can be useful to modify physicochemical properties without losing activity. Conversely, pairs with high disparity lie in the steepest regions of the SAR landscape and feature small structural variations that are crucial for the biological activity. By comparing all pairs of molecules, Activity Miner identifies the "activity cliffs", i.e. those pairs where small structural

differences cause large changes in activity. The wealth of information gathered from the analysis of each individual activity cliff can be summarized into a global, interpretable Activity Atlas model. The Activity Atlas method analyzes activity and alignment data via a Bayesian approach: each activity cliff provides evidence that a specific change in 3D properties causes a significant change in biological activity: the larger the disparity, the stronger the evidence. An Activity Atlas model consists of three different 3D visual maps: the 'Activity cliff summary' which reports the SAR gathered from the analysis of individual activity cliffs; the 'average of actives', which highlights the features that active molecules have in common, and the "regions explored" map, which shows how thoroughly the SAR have been explored in a series and helps prioritizing molecules which bring novel SAR insight over trivial analogues. As in this work the disparity matrix, on which the Activity Miner and Activity Atlas analyses are based, was computed using a 3D similarity metric, it is important that a reasonable alignment of the molecules is generated. This task is made easier by the availability of the crystallographic coordinates of the lead MD77^[3] (Figure 2a: for sake of clarity the three rings were named A, B and C), which was used as a template to align the rest of the series. Its molecular arrangement in the solid state was found to largely correspond to the most stable conformation as calculated *in vacuo*.^[3] To verify the reliability of this approximation, we determined the crystal structures of two monosubstituted analogues bearing either the Cl atom on system A (5) or the CF₃ group on ring C (8), which confirmed to have almost the same conformation of the reference (see below). As the compounds share a fair degree of structural similarity across the series, they were aligned by superimposing their maximum common substructure, while the functional groups which were not part of it were aligned based on a combination of 3D shape and molecular interaction fields. Firstly, the disparity matrix across all aligned ligands was generated by Activity Miner (Figure 3). The analysis of this matrix led to identify a number of compound pairs that constituted activity cliffs, and were further studied through their 3D field difference maps (**Figure 4**). Then, an Activity Atlas model was computed to enable a global view at the SAR of the series.^[28,29] The results of the activity cliff summary analysis were discussed considering MD77 as reference and built up from representative low energy conformations of the aligned compounds of the dataset (Figure 2b). These outcomes were shown as 3D maps:

- Average electrostatics/hydrophobics of actives (Figure 2c,e) was calculated considering only the active molecules and pointed out the essential structural features for the activity.
- Activity cliff summary of electrostatic/hydrophobic fields (Figure 2d,f) displayed the effects on the activity of the various structural modulations explored throughout the series, taking into account both cytotoxic and inactive compounds.

The models provided indications about the electrostatic, hydrophobic and shape features underlying the cytotoxic activity. An increase in negative electrostatic field on core *B* of the structure could have positive effects on the activity (in cyan in **Figure 2c**), while an enhanced π electrostatic potential on system *C* is not suitable (in red in **Figure 2c**). Electron withdrawing substituents which generate a more positive (or less negative) electrostatic field in *meta* position on ring *A* (in red in **Figure 2d**) are beneficial for activity, whereas opposite characteristics are required for *para* position.

Regarding the hydrophobic and steric aspects, **Figure 2e** highlighted the regions (in yellow) where active molecules could make hydrophobic interactions. As suitable changes, **Figure 2f** showed that steric hindrance on ring A was well tolerated in *para* position (green area), while to *ortho* or *meta* (magenta area) it was detrimental for activity. Moreover, steric bulk was unfavorable on system C (magenta area).

As for the disparity matrix (**Figure 3**), the similarity value for each pair of compounds was computed as the average of their 3D field and shape similarity. The color of each cell indicates the direction the activity is going, moving from one member of the pair to the other: red means the activity is decreasing; green means the activity is increasing between the pair. The intensity of the shading increases with the magnitude of the disparity, which in turn relates to how steep the activity

cliff is. The result is a focused view of the SAR around a particular compound which can help understand subtle molecule-to-molecule structure-activity changes and identify potential outliers. Plotting the field difference maps (**Figure 4**) for selected pairs helps finding a structural rationale for the differences in activity.

Matching **MD77** with representative compounds in the dataset with high, mid, and low potencies, we evidenced that the lead and the most active derivative **12** shared an equivalent electrostatic potential profile (**Figure 4a**), in line with their comparable biological activity. This observation is almost conserved taking into account the active derivative **2**, which displayed a reasonable electrostatic potential overlay with **MD77** (**Figure 4b**). Regarding the cytotoxic properties of this derivative, the effect of the *meta*-Cl substitution of ring *A* was remarkable, which aligned well with the average electrostatics. In this context, the lower potency of compound **1**, characterized by the shift of the chlorine substituent in *ortho* position, could be correlated to the negative electrostatic field difference present on ring *A* (**Figure 4c**). The mid-range cytotoxicity of compound **16** (**Figure 4d**), bearing the NO₂ on ring *C*, could be associated to a more extended negative electrostatic potential region on this group. In the inactive compound **3**, this key field difference on region *C* was more evident, as the π -cloud spanned over a larger surface comprising the *ortho* position. Moreover, a positivity associated to the *para* position of *C* was seen (**Figure 4e**).

Most striking were the differences between **MD77** and compound **25**, having in *B* an 1,3,4-oxadiazole (**Figure 4f**). Despite the substantial changes of electrostatic potential around ring *A*, **25** retained a modest activity, explained by the electron density over the 1,3,4-oxadiazole, which was suggested as important for activity by the average electrostatics analysis (**Figure 2C**).

2.4 Crystallographic studies

We supported the above modeling studies by means of a crystallographic analysis on two representative terms **5** and **8**, for understanding the influence of the phenyl substituents on the conformation (**Figure 5**).

A comparison of crystal structures **5** and **8** with that of **MD77** previously described ^[3] showed an almost perfect superimposition. As underlined in the previously published results, this molecular arrangement corresponded to the most stable conformation calculated *in vacuo*. For both derivatives, the analysis of the crystal packing evidenced that adjacent molecules were connected by NH^{$\cdot\cdot$}O, CH^{$\cdot\cdot$}N, and CH^{$\cdot\cdot$}Cl (**5**), C=O^{$\cdot\cdot$}F (**8**), type contacts, stabilized by stacking interactions. Despite the increased number of interactions that could be forwarded by the presence of the substituents in both phenyl groups, we pointed out that in the new two crystal structures the amidic groups arrange in the same way and the respective quite perpendicular orientation of the benzene rings does not change. This finding supports the hypothesis that the 3D electrostatic environment could have a greater influence on the activity over other types of geometry.

Conclusions

We designed and synthesized a new series of 1,3,5-oxadiazole derivatives, as potential anticancer agents. We described the synthesis of compounds having different substituents at position 4 of the heterocycle, various functional groups on the phenyl ring of the benzamide moiety and bearing an aliphatic chain substituting the latter, with the aim of building a diverse library for molecular modeling studies.

The MTT-assay found out promising antiproliferative agents, highlighting the substantial potency of some compounds. In particular, the most significant candidate, derivative **12**, exhibited IC₅₀ value of 0.95 μ M against HCT-116 cancer cell line, showing a slightly better profile of activity with respect to the lead compound.

The focused SAR study by field-based disparity analysis revealed that an increasing of negative electrostatic field on the heterocyclic core of the structure could have positive effects on the activity, while an enhanced π electrostatic potential on the benzamide group is not suitable. Electron

withdrawing substituents which generated a more positive (or less negative) electrostatic field in *meta* position on phenyl ring *A* were beneficial for activity, while opposite characteristics are required for *para* position.

Steric hindrance on the phenyl ring was well tolerated in *para* position, while in *ortho* or *meta* was detrimental for activity. Moreover, steric bulk was unfavorable on the benzamide system.

The solid state conformation determined for two representative terms showed an almost perfect superimposition with respect to the reference, supporting the hypothesis that the 3D electrostatic environment could have a greater influence on the activity over changes in geometry.

Concluding, these new insights, obtained using a purely ligand-based strategy based on the analysis of three-dimensional shape and molecular fields, will help driving further lead optimization efforts on this scaffold.

Acknowledgments

The authors acknowledge the financial support of the University of Milan and the Italian MIUR (PRIN 2010-11).

The authors thank Cresset (New Cambridge House, Bassingbourn Road, Litlington, Cambridgeshire, SG8 0SS, UK; www.cresset-group.com), for the software Forge 10.4.2. Dr. P. Tosco (Cresset) is gratefully acknowledged for providing technical support and helpful discussions for FP's PhD thesis and VC's graduation thesis, which report part of this work. Prof. G. Marverti (Dipartimento di Scienze Biomediche, Metaboliche e Neuroscienze, Università degli Studi di Modena e Reggio Emilia) is gratefully acknowledged for pharmacological support.

Conflict of Interest: All authors declare no financial/commercial conflicts of interest.

Figure legends

Figure 1. Database of MD77 derivatives submitted to Forge analysis.

Figure 2. Activity Atlas visual maps. **a) MD77** X-ray structure.^[3] **b)** Three-dimensional alignment of the dataset. **c)** Average electrostatic fields of active molecules. **d)** Activity cliff summary of electrostatic field. **e)** Average hydrophobic fields of active molecules. **f)** Activity cliff summary of hydrophobic region.</sup>

Figure 3. Disparity matrix computed by Activity Miner. Each cell of the matrix reports the disparity value between a specific pair of compounds; the similarity was calculated as the average of 3D molecular fields and shape, each weighted at 50 %. Red and green boxes indicate respectively a decrease and an increase in activity moving from one compound to the other, while the intensity of the shading increases with the sharpness of the activity cliff associated to that pair.

Figure 4. Comparison of field differences for a selection of molecules: compound **1** (a), **2** (b), **3** (c), **12** (d), **16** (e), **25** (f) with respect to **MD77**. The field difference map displays the surfaces as relative values between pairs of molecules. The regions where the surface is displayed have stronger electrostatic fields than the corresponding regions in the compared molecule.

Figure 5. a. ORTEP ^[30] views of **5** (above) and **8** (below), showing the arbitrary atom-labeling scheme. Atomic displacement parameters for non-H atoms are at 40% probability level. **b.** Superimposition between **5** (above in green) and **8** (below in blue) onto **MD77** (red).

References

[1] D-S. Shin, D. Masciocchi, A. Gelain, S. Villa, D. Barlocco, F. Meneghetti, A. Pedretti, Y-M. Han, D.C. Han, B-M. Kwon, L. Legnani, L. Toma, *Med. Chem. Commun.* **2010**, *1*, 156.

- [2] S. Villa, D. Masciocchi, A. Gelain, F. Meneghetti, *Chem. & Biodiv.* 2012, 9, 1240.
- [3] D. Masciocchi, S. Villa, F. Meneghetti, A. Pedretti, D. Barlocco, L. Legnani, L. Toma, B-M. Kwon, S. Nakano, A. Asai, A. Gelain, *Med. Chem. Commun.* 2012, *3*, 592.
- [4] E. Gabriele, F. Porta, G. Facchetti, C. Galli, A. Gelain, F. Meneghetti, I. Rimoldi, S. Romeo, S. Villa, C. Ricci, N. Ferri, A. Asai, D. Barlocco, A. Sparatore, *Arkivoc*, **2017**, *ii*, 235.
- [5] D. Masciocchi, A. Gelain, S. Villa, F. Meneghetti, D. Barlocco, *Future Med. Chem.* **2011**, *3*, 367.
- [6] G. A. Pinna, G. Murineddu, C. Murruzzu, V. Zuco, F. Zunino, G. Cappelletti, R. Artali, G. Cignarella, L. Solano, S. Villa, *ChemMedChem*, **2009**, 4, 998.
- [7] T. Cheeseright, R. Lawrence, M. Mackey, Slater, M. 246th ACS National Meeting & Exposition, 8-12 September **2013**, Indianapolis, IN. COMP 346; http://www.cresset-group.com/products/activityminer.
- [8] M. Cruz-Monteagudo, J. L. Medina-Franco, Y. Perez-Castillo, O. Nicolotti, M.N.D.S. Cordeiro, F. Borges, *Drug. Disc. Today* **2014**, *19*, 1069.
- [9] M. Mackey, 250th ACS National Meeting & Exposition, 16–20 August **2015**, Boston, MA; http://www.cresset-group.com/activity-atlas/.
- [10] N. Ferri, S. Cazzaniga, L. Mazzarella, G. Curigliano, G. Lucchini, D. Zerla, R. Gandolfi, G. Facchetti, M. Pellizzoni, I. Rimoldi, *Bioorg. Med. Chem.* **2013**, *21*, 2379.
- [11] N. Ferri, G. Facchetti, S. Pellegrino, E. Pini, C. Ricci, G. Curigliano, I. Rimoldi, *Bioorg. Med. Chem.* **2015**, *23*, 2538.
- [12] https://www.cambridgesoft.com/
- [13] A. Pedretti, L. Villa, G. Vistoli, J. Mol. Graph. Model. 2002, 21, 47.
- [14] http://nova.disfarm.unimi.it/manual/pages/cite.htm
- [15] T. Cheeseright, M. Mackey, S. Rose, A. Vinter, J. Chem. Inf. Model. 2006, 46, 665.
- [16] A. Altomare, M. C. Burla, M. Camalli, G. Cascarano, C. Giacovazzo, A. Gagliardi, G. Polidori, *J. Appl. Cryst.* **1994**, *27*, 435.
- [17] G. M. Sheldrick, SHELX-97. Program for the Refinement of Crystal Structure, University of Göttingen, Germany, **1997**.
- [18] M. Nardelli, J. Appl. Cryst. **1995**, 28, 659.
- [19] A. B. Sheremetev, Y.L. Shamshina, D.E. Dmitriev, Russ. Chem. Bull. Int. Ed. 2005, 54, 1032.
- [20] M. E. Beccalli, A. Manfredi, A. Marchesini J. Org. Chem. 1985, 50, 2372.
- [21] A. P. Kozikowski, M. Adamcz, J. Org. Chem. 1983, 48, 366.
- [22] E. J. Hanan, A. Abbema, K. Barrett, W. S. Blair, J. Blaney, C. Chang, C. Eigenbrot, S. Flynn, P. Gibbons, C. A. Hurley, J. R. Kenny, J. Kulagowski, L. Lee, S. R. Magnuson, C. Morris, J. Murray, R. M. Pastor, T. Rawson, M. Siu, M. Ultsch, A. Zhou, D. Sampath, J. P. Lyssikatos, *J. Med. Chem.* 2012, 55, 10090.
- [23] H.A. Saadeh, M. I. Mosleh, M. M. El-Abadelah, *Molecules*, 2009, 14, 2758.
- [24] Q.Su, S. Ioannidis, C. Chuaqui, L. Almeida, M. Alimzhanov, G. Bebernitz, K. Bell, M. Block, T. Howard, S. Huang, D. Huszar, J. A. Read, C. Rivard Costa, J. Shi, M. Su, M. Ye, M. Zinda, *J. Med. Chem.* **2014**, *57*, 144.
- [25] V. Lisowski, D.N. Vu, X. Feng, S. Rault, Synthesis. 2002, 6, 753.
- [26] P. Lu, C. Sanchez, J. Cornella, I. Larrosa, Org. Lett. 2009, 11, 5710.
- [27] J. Xi, Q-L. Dong, G-S. Liu, S. Wang, L. Chen, Z-J. Yao, Syn. Lett. 2010, 11, 1674.
- [28] S. Merlo, L. Basile, L. M. Giuffrida, M. A. Sortino, S. Guccione, A. Copani, *J. Nat. Prod.*, **2015**, *78*, 2704.
- [29] M. Tuyishime, L. Lawrence, S. Cocklin, Bioorg. Med. Chem. Lett. 2016, 26, 228.
- [30] L. J. Farrugia, J. Appl. Cryst. 1999, 32, 837



Scheme 1. Reagents and conditions: (a) R₁-PhCOC1, DMAP, dry C1CH₂CH₂C1, N₂, MW, 45 min, 120 °C; (b) i. 60 % NaH, dry DMF, 30 min, 0 °C, N₂; ii. R₁-PhCOC1, 12 h, 60 °C; (c) BBr₃, dry DCM, 12 h, rt, N₂; (d) i: dibenzyl phosphite, CC1₄, DIPEA, DMAP, dry CH₃CN, -10 °C, 3 h, N₂; ii: H₂, Pd/C, MeOH, 3 h, rt; (e) SnC1₂, AcOEt, 7 h, reflux; (f) NaOH, EtOH, 3 h, reflux; (g) H₂, Pd/C, AcOEt/MeOH (9:1), 24 h, rt.



Scheme 2. Reagents and conditions: (a) 4-CF₃PhCOC1, dry Py, 16 h, rt, N₂.



Scheme 3. Reagents and conditions: (a) NaNO₂, AcOH/H₂O (1:1), THF, 1 h, rt; (b) NH₂NH₂·· H₂O, EtOH, 90 min, rt; (c) NaNO₂, CO(CH₃)₂/ 10 % HCl (1:1), 40 min, rt; (d) AcOH, H₂O, 1 h, reflux; (e) i: NEt₃, dry DCM, 30 min, 0 °C, N₂; ii: 4-CF₃-PhCOCl, 90 min, 40 °C.



Scheme 4. Reagents and conditions: (a) i: NH₂OHHCl, NaHCO₃, MeOH/H₂O (2:1), 2 h, rt; ii: NCS, DMF, 16 h, rt; (b) toluene, 16 h, rt; (c) NEt₃, dry Et₂O, 18 h, rt, N₂; (d) 6N HCl, CH₃COOH, 6 h, reflux; (e) DPPA, NEt₃, dry *t*-BuOH, 16 h, reflux, N₂; (f) i: TFA, dry DCM, 16 h, rt, N₂; ii: 1N NaOH, dry DCM, 16 h, rt, N₂; (g) i: 60 % NaH, dry DMF, 30 min, 0 °C, N₂; ii: 4-CF₃-PhCOCl, 12 h, 60 °C.



Scheme 5. Reagents and conditions: (a) 4-ClPhB(OH)₂, K₂CO₃, TBAB, Pd(PPh₃)₂Cl₂, H₂O, 16 h, 75-85 °C, N₂; (b) H₂ (30 atm), Pd(OH)₂, DCM/MeOH (9:1), 16 h, rt; (c) i: NEt₃, dry DCM, 30 min, 0 °C, N₂; ii: 4-CF₃-PhCOCl, 90 min, 0 °C.





Scheme 7. Reagents and conditions: (a) 4-CIPhB(OH)₂, sat. Na₂CO₃, Pd(PPh₃)₄, dry toluene/EtOH (20:1), 16 h, 70 °C, N₂; (b) i. 60 % NaH, dry DMF, 30 min, 0 °C, N₂; ii. 4-CF₃-PhCOC1, 12 h, 60 °C.

-		Стр	R	R ₁	IC ₅₀ µM
_		MD77	p-Cl	<i>p</i> -CF ₃	1.3±0.2
		1	o-Cl	<i>p</i> -CF ₃	56.7±3.1
		2	<i>m</i> -Cl	<i>p</i> -CF ₃	15.4 ± 2.8
		3	p-Cl	o-CF ₃	>100
		4	p-Cl	<i>m</i> -CF ₃	7.4±1.15
		5	p-Cl	Н	>100
		6	o-Cl	Н	76.3±12.8
		7	<i>m</i> -Cl	Н	73.4±8.3
		8	Н	<i>p</i> -CF ₃	>100
		9	Н	Н	>100
		10	p-Cl	p-Cl	>100
		11	<i>p</i> -CF ₃	<i>p</i> -CF ₃	24.1±2.3
		12	<i>p</i> -CF ₃	p-Cl	0.95 ± 0.02
		13	p-Cl	<i>p</i> -OCH ₃	>100
	$\overline{\mathbb{R}}_{1}$	14	p-Cl	<i>p</i> -OH	>100
	R A	15	p-Cl	p-OPO ₃ H ₂	>100
		16	p-Cl	p-NO ₂	46.7±14.5
	N O N	17	p-Cl	p-NH ₂	>100
		18	<i>p</i> -Cl	<i>p</i> -CN	19.4±2.4
		19	p-Cl	<i>p</i> -COOH	>100
		20	<i>p</i> -OBn	<i>p</i> -CF ₃	>100
		21	<i>р</i> -ОН	<i>p</i> -CF ₃	>100
		22	p-NO ₂	<i>p</i> -CF ₃	58.2±5.2
		23	<i>p</i> -NH ₂	<i>p</i> -CF ₃	>100
		24			>100
	-	25	1,2,4-oxadiazole	CF ₃	53.9±5.8
	CI,	26	1,2-isoxazole	CF ₃	>100
		27	1,5-isoxazole	CF ₃	>100
		28	N-Me-Imidazole	CF ₃	52.3±8.8
	(n)	29	Furan	CF ₃	>100
		30	Phenyl	CF ₃	>100

Table 1. Antiproliferative activity on HCT-116 cancer cell line.



MD77 27 11 12 10 23 21 5 13 14 22 6 9 7 17 18 16 29 30 26 4 28 3 19 24 15 20 25 0.0 0.0 10.0 18.9 0.0 0.0 0.0 0.0 0.0 0.0 5.0 2.6 0.0 2.8 0.0 9.4 4.6 0.0 0.0 0.8 3 3.0 0.0 0.0 0.0 0.0 1.4 -5.5 3.4 11.9 -3.8 -6.4 -5.4 -2.7 -3.2 -2.5 -0.1 -1.5 -3.6 -0.9 -2.3 3.4 0.5 -3.4 -2.7 -3.4 4.5 0.2 -1.9 -2.3 -1.8 -2.0 -2.4 0.3 10.3 -5.7 -8.6 19.8 -10.3 19.8 - 17.9 - 2.5 10.7 - 11.9 - 17.9 - 19.8 - 7.5 - 9.4 - 6.9 - 8.2 - 4.9 - 8.0 - 9.3 - 5.9 - 1.0 - 4.1 - 9.2 - 6.9 - 9.1 1.9 - 3.2 - 4.9 - 6.0 - 4.8 - 5.1 - 6.7 - 1.2 0.0 16.0 34.7 0.0 0.0 0.0 0.0 0.0 8.4 1.9 0.0 2.4 0.0 15.9 7.3 0.0 0.0 0.0 11.1 3.9 0.0 0.0 0.0 0.0 1.3 0.0 5.7 0.0 0.0 0.0 0.0 0.0 0.0 7.7 1.9 0.0 2.3 0.0 11.8 5.9 0.0 0.0 0.0 9.7 3.3 0.0 0.0 0.0 0.0 1.3 5.5 17.9 0.0 16.0 27.7 0.0 0.0 280 -12.5 -8.2 -8.8 -7.3 -9.6 -6.8 -5.8 -2.6 -4.9 -3.0 -5.5 1.1 -3.0 -8.3 -5.6 -8.5 3.6 -2.2 -4.7 -5.7 -4.6 -4.6 -5.7 -0.7 -10.0 -34 2.5 -16.0 -16.0 -16.4 -18.1 -29.6 -31.3 -29.8 -18.0 -11.7 -16.8 -13.5 -22.4 -15.3 -17.5 -15.9 -12.1 -17.2 -7.7 -8.9 -14.7 -17.9 -14.2 -12.3 -13.2 -3.8 0.0 0.0 0.0 5.1 2.5 0.0 3.2 0.0 16.9 7.9 0.0 0.0 0.0 0.0 0.0 14.3 2.8 0.0 0.0 0.0 0.0 0.0 1.3 0.0 12.5 0.0 0.0 0.0 0.0 3.5 2.1 0.0 2.5 0.0 8.9 4.4 0.0 0.0 0.0 8.0 2.8 0.0 0.0 0.0 0.0 1.3 0.0 0.0 8.2 0.0 0.0 0.0 0.0 4.9 1.9 0.0 2.6 0.0 9.3 4.6 0.0 0.0 0.0 8.2 2.7 0.0 0.0 0.0 0.0 0.0 1.3 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 3.3 3.6 0.0 5.2 0.0 11.7 6.1 0.0 0.0 0.0 17.0 2.3 0.0 0.0 0.0 0.0 0.0 1.1 0.0 0.0 0.0 0.0 4.1 2.1 0.0 2.6 0.0 15.0 8.5 0.0 0.0 12.3 2.7 0.0 0.0 0.0 0.0 1.2 0.0 3.2 0.0 0.0 0.0 9.6 31.3 3.0 2.2 0.0 2.9 0.0 11.5 4.8 0.0 0.0 0.0 10.8 2.2 0.0 0.0 0.0 0.0 0.0 1.1 0.0 0.0 0.0 0.0 0.0 0.0 2.5 6.9 0.0 0.0 6.8 29.8 -0.6 -2.6 -0.6 -2.6 4.7 0.8 -4.1 -2.9 -4.0 5.4 0.2 -2.1 -2.7 -2.1 -2.2 -3.1 0.3 -5.0 -7.7 5.8 18.0 -5.1 -3.5 -4.9 -3.3 -4.1 -3.0 0.1 8.2 -2.1 -1.9 -3.6 -2.1 -2.2 0.6 -6.0 0.1 -2.2 3.9 1.3 -1.4 -1.2 -1.5 6.6 0.6 -1.9 -1.5 -1.7 -1.2 -1.2 0.5 2.6 11.7 -2.5 0.0 4.9 0.0 0.0 0.0 0.0 0.0 0.0 2.6 6.0 6.2 0.0 7.4 4.0 0.0 0.0 0.0 10.7 1.9 0.0 0.0 0.0 0.0 0.0 1.1 0.0 8.0 0.0 93 -24 -23 30 135 -32 -25 -26 -52 -26 -29 06 -01 -62 -27 43 1.5 -1.5 -1.3 -1.6 7.5 0.6 -22 -1.8 -20 -1.4 -1.4 0.5 -2.8 0.9 6.7 3.3 0.0 0.0 0.0 12.4 2.2 0.0 0.0 0.0 0.0 0.0 1.1 5.9 0.0 0.0 5.5 22.4 0.0 0.0 0.0 0.0 0.0 0.0 2.6 2.2 0.0 2.7 1.0 -15.9 -11.8 -1.1 15.3 -16.9 -8.9 -9.3 -11.7 -15.0 -11.5 -4.7 -3.9 -7.4 -4.3 -6.7 -7.6 -7.8 -5.8 -8.0 3.4 -2.7 -5.6 -9.3 -5.9 -5.8 -4.9 -0.8 -4.6 -0.5 4.1 -7.3 -5.9 3.0 17.5 -7.9 -4.4 -4.6 -6.1 -8.5 -4.8 -0.8 -1.3 -4.0 -1.5 -3.3 7.6 -3.9 -3.0 -3.8 6.4 -0.3 -3.2 -6.0 -3.5 -2.9 -2.6 0.0 0.0 9.2 0.0 0.0 8.3 15.9 0.0 0.0 0.0 0.0 0.0 0.0 4.1 1.4 0.0 1.5 0.0 7.8 3.9 0.0 0.0 7.0 5.7 0.0 0.0 0.0 0.0 0.0 1.3 6.9 0.0 0.0 5.6 12.1 0.0 0.0 0.0 0.0 0.0 0.0 2.9 1.2 0.0 1.3 0.0 5.8 3.0 0.0 0.0 6.1 5.3 0.0 0.0 0.0 0.0 0.0 1.3 9.1 0.0 0.0 8.5 17.2 0.0 0.0 0.0 0.0 0.0 0.0 4.0 1.5 0.0 1.6 0.0 8.0 3.8 0.0 0.0 7.4 4.9 0.0 0.0 0.0 0.0 0.0 1.2 -1.9 -11.1 -9.7 -3.6 7.7 -14.3 -8.0 -8.2 -17.0 -12.3 -10.8 -5.4 -6.6 -10.7 -7.5 -12.4 -3.4 -6.4 -7.0 -6.1 -7.4 -3.8 -9.8 -7.8 -8.5 -6.0 -5.7 -1.6 -0.2 3.2 -3.9 -3.3 2.2 8.9 -2.8 -2.8 -2.7 -2.3 -2.7 -2.2 -0.2 -0.8 -1.9 -0.6 -2.2 2.7 0.3 -5.7 -5.3 -4.9 3.8 -1.8 -2.3 -1.7 -1.9 -2.2 0.2 4.9 0.0 0.0 4.7 14.7 0.0 0.0 0.0 0.0 0.0 0.0 2.1 1.9 0.0 2.2 0.0 5.6 3.2 0.0 0.0 0.0 9.8 1.8 0.0 0.0 0.0 0.0 1.0 6.0 0.0 0.0 5.7 17.9 0.0 0.0 0.0 0.0 0.0 2.7 1.5 0.0 1.8 0.0 9.3 6.0 0.0 0.0 7.8 2.3 0.0 0.0 0.0 0.0 1.1 0.0 2.3 1.8 4.8 0.0 0.0 4.6 14.2 0.0 0.0 0.0 0.0 0.0 0.0 2.1 1.7 0.0 2.0 0.0 5.9 3.5 0.0 0.0 0.0 8.5 1.7 0.0 0.0 0.0 0.0 0.9 0.0 2.0 5.1 0.0 0.0 4.6 12.3 0.0 0.0 0.0 0.0 0.0 0.0 2.2 1.2 0.0 1.4 0.0 5.8 2.9 0.0 0.0 0.0 6.0 1.9 0.0 0.0 0.0 0.0 0.0 1.0 67 0.0 0.0 5.7 13.2 0.0 0.0 0.0 0.0 0.0 0.0 0.0 3.1 1.2 0.0 1.4 0.0 4.9 2.6 0.0 0.0 5.7 2.2 0.0 0.0 0.0 0.0 0.0 1.2 2.4 -1.4 -0.3 1.2 -1.3 -1.3 0.7 3.8 -1.3 -1.3 -1.3 -1.1 -1.2 -1.1 -0.3 -0.5 -1.1 -0.5 -1.1 0.8 -0.0 -1.3 -1.3 -1.2 1.6 -0.2 -1.0 -1.1 -0.9 -1.0 -1.2

a. d.









C.

