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Discovery of Methyl 4'-Methyl-5-(7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)-[1,1'-biphenyl]-3-carboxylate, an Improved Small-Molecule Inhibitor of c-Myc–Max Dimerization

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Abstract

c-Myc is a bHLH-ZIP transcription factor that is responsible for the transcription of a wide range of target genes involved in many cancer-related cellular processes, such as proliferation, differentiation, apoptosis and metabolism. Over-expression of c-Myc has been observed in, and directly contributes to, a variety of human cancers including those of the hematopoietic system, lung, prostate and colon. To become transcriptionally active, c-Myc must first dimerize with Max via its own bHLH-ZIP domain. A proven strategy towards the inhibition of c-Myc oncogenic activity is to interfere with the structural integrity of the c-Myc–Max heterodimer. The small-molecule 10074-G5 is an inhibitor of c-Myc–Max dimerization ($IC_{50} = 146 \mu M$) that operates by binding and stabilizing c-Myc in its monomeric form. Herein, we report on our on-going efforts to optimize the c-Myc–Max inhibitory activity of 10074-G5-related molecules in vitro and in cancer cells that over-express c-Myc. Specifically, we have identified a congener of 10074-G5, termed 3jc48-3, that is about five times as potent ($IC_{50} = 34 \mu M$) at inhibiting c-Myc–Max dimerization as the parent compound. In addition, 3jc48-3 exhibited an approximate two-fold selectivity for c-Myc–Max heterodimers over Max–Max dimers, suggesting that, like its predecessor, its mode of action is through binding c-Myc. 3jc48-3 inhibited the proliferation of c-Myc-over-expressing HL60 and Daudi cells with single-digit micromolar IC_{50} values by causing growth arrest at the G_0/G_1 phase. Furthermore, co-immunoprecipitation studies indicated that 3jc48-3 inhibits c-Myc–Max dimerization in cells, which was further substantiated by the specific silencing of a c-Myc-driven luciferase reporter gene. Finally, unlike previously described 10074-G5 analogues, which are rapidly released and/or metabolized by cells following their uptake, 3jc48-3's intracellular half-life was >17 h. Collectively, these data demonstrate 3jc48-3 to be one of the most potent cellularly active c-Myc inhibitors reported to date.

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Keywords

10074-G5; JY-3-094; c-Myc; Max; protein–protein interaction

Introduction

c-Myc is an oncogenic member of the basic helix-loop-helixleucine zipper (bHLH-ZIP) transcription factor family.^[1-5] In its monomeric form, c-Myc's bHLH-ZIP domain is intrinsically disordered and the protein is transcriptionally inactive. However, upon dimerizing with its bHLH-ZIP relative Max, an obligatory event that is required for all of c-Myc's known biological activities,^[1-5] c-Myc and Max assume a rigid coiled coil structure that recognizes the canonical E-box sequence (5'-CACGTG-3') in DNA.^[6] Subsequently, various co-activators are recruited to modify transcription. Unlike c-Myc, Max forms homodimers, as well as heterodimers with other bHLH-ZIP family members that include Mad proteins and Mnt.^[7] These dimers retain the ability to bind E-boxes but repress transcription by competing with c-Myc–Max heterodimers, thus providing one means by which c-Myc's transcriptional activity is kept in check.^[7] c-Myc is important in the transcription of a myriad of genes involved in roles that include cell proliferation, apoptosis, differentiation and metabolism.^[1-5,6] The deregulation of c-Myc is associated with most^[8] human cancers including, but not limited to, leukaemias^[9] and lymphomas,^[10] as well as cancers of the breast,^[11] pancreas,^[12] lung^[13] and colon.^[14] Given its central role in many key cellular processes, there is concern that the successful design of non-toxic inhibitors would prove especially challenging.^[15] Recent reports, however, have demonstrated that, in the whole animal, the inhibition of c-Myc, inhibits tumorigenesis but exerts only mild and reversible effects on normal cells.^[8,16,17] Moreover, it now appears that c-Myc actually exerts an amplifying control on the transcription of its target genes,^[18,19] such that its inhibition would not result in the complete inactivation of transcription. Together, these findings support the idea that the inhibition of c-Myc is a viable strategy towards the development of targeted antineoplastics that might be useful in the clinic.

Since the heterodimerization of c-Myc is essential to its function, it follows that inhibiting its association with Max should be an effective way to abrogate its transcriptional activity. Towards this goal, two possible strategies with small-molecules can be envisaged: (i) direct binding to and stabilization of monomeric c-Myc, preventing its obligatory association with Max, and (ii) recognition and perturbation of the c-Myc–Max dimer to an extent that DNA recognition becomes compromised. In the former approach, the intrinsic disorder of c-Myc's bHLH-ZIP domain renders rational drug design a significant challenge, as the protein is bereft of any recognizable sites onto which molecules could bind or that computationally assisted docking programs could enlist in virtual screens.^[20-22] Moreover, there do not appear to be any obvious binding pockets on the structured c-Myc–Max heterodimer, which renders the latter approach also highly taxing.^[23] Unsurprisingly, therefore, the majority of small-molecule inhibitors of c-Myc–Max dimerization have been identified through screening large chemical libraries that span structurally diverse chemotypes.^[20-22] These small-molecules include rhodanine and thiazolidine-2,5-dione derivatives,^[24,25] peptidomimetics,^[26,27] “credit-card” compounds that are based on planar, hydrophobic

scaffolds designed to “slot” into the c-Myc–Max leucine zipper,^[28] and the pyrazolo[1,5-a]pyrimidine “Mycro” compounds.^[29,30] However, micromolar concentrations are required in order to elicit their biological effects. A select few of these small-molecules have been characterized more fully and shown to operate through the recognition and stabilization of transcriptionally inactive, monomeric c-Myc, preventing it from undergoing its obligatory dimerization with Max.^[32,33] Two such compounds are 10058-F4 ((Z)-5-(4-ethylbenzylidene)thiazolidine-2,4-dione (**1**), Figure 1) and 10074-G5 (*N*-([1,1'-biphenyl]-2-yl)-7-nitrobenzo[*c*][1,2,5]oxadiazol-4-amine (**2**)), both of which have been previously developed into more potent congeners.^[25,34,35] In contrast, indirect c-Myc inhibitors have also been developed that exhibit much more potent affinities for their target proteins whose globular structures allow for rational drug design.^[22] For example, the indirect c-Myc inhibitor “JQ-1”, a thieno-triazolobenzo-1,4-diazepine, inhibits bromodomain (BRD)-containing proteins in the nanomolar range in the case of BRD4.^[31] A recent publication has revealed that a number of kinase inhibitors exhibit polypharmacology, potentially inhibiting kinases and BRDs,^[36] which might establish a powerful strategy towards the development of next generation antineoplastics, or it may lead to other undesired side-effects. At the same time, the KAc-binding site of bromodomain, testis-specific (BRDT) is highly conserved with that of BRD4, so inhibition of BRD4 might lead to inadvertent male infertility.^[37] For these reasons, we believe the direct inhibition of c-Myc should remain a major goal in the quest for novel anti-cancer agents.

Recently, we determined the pharmacophore of 10074-G5.^[34] The electron rich nitro group and furazan ring were both found to be essential for maximal inhibition of c-Myc–Max dimerization, possibly interacting with Arg366, Arg367 and Arg372 of the HLH domain as previously proposed.^[32,33] At the same time, acylation of the reduced nitro group with electron-rich groups furnished compounds that approached the potency of the parent compound, thereby opening the door to the possible replacement of the potentially problematic nitro group – which is often susceptible to metabolism to inactive species – with suitable bioisosteres. Hydrophobic groups at the *ortho* position and a carboxylic acid at the *para* position of the aniline also furnished active compounds, possibly through interacting with, in the former case, a HLH hydrophobic pocket constructed from Phe375 and Ile381, while in the latter case, Arg378. Our experimental findings were in good agreement with the NMR-derived model of the 10074-G5 binding site (Figure 2).

In the course of our studies on 10074-G5, we developed small-molecule congener JY-3-094 (**3**), which is almost five times as potent at inhibiting the dimerization of purified c-Myc and Max proteins in electrophoretic mobility shift assays (EMSAs) (IC_{50} = 33 μ M vs 146 μ M). However, unlike 10074-G5, JY-3-094 was unable to inhibit the proliferation of c-Myc over-expressing human promyelocytic leukaemia (HL60) and Daudi Burkitt’s lymphoma cell lines, which we ascribed to its charged carboxylic acid that impeded cell entry. This was remedied by esterifying the carboxylic acid to generate a series of ester pro-drugs, all of which exhibited low micromolar IC_{50} values in the above cell lines, and which were converted by intracellular esterases to JY-3-094.^[35] Whereas this proved an effective strategy for enhancing the cellular uptake of JY-3-094, it represents an impasse since the activity of the ester pro-drug will always be limited by the activity of its carboxylic acid

metabolite. Herein, we report our on-going efforts to further optimize 10074-G5 through consideration of its recently disclosed pharmacophore.

Results and Discussion

Since a phenyl ring at the *ortho* position of the aniline in 10074-G5 improves c-Myc inhibitory activity as does a carboxylic acid at the *para* position of the aniline of JY-3-094, possibly through interacting with Phe375/Ile381 and Arg378, respectively, we considered that combining these features into a single molecule might furnish an especially potent c-Myc inhibitor through additive effects. To this end, a series of second generation analogues of JY-3-094 was prepared in which the aniline moiety was substituted with various aryl groups in the *ortho* position while retaining the carboxylic acid in the *para* position; the synthetic chemistry that was followed is shown in Scheme 1. Briefly, treatment of 3-bromo-4-amino-benzoic acid (**4**) with thionyl chloride (SOCl₂) in MeOH yielded the methyl ester **5** in quantitative yield. Suzuki cross-couplings of **5** with a range of boronic acids furnished biphenyls **6**. Attempted nucleophilic aromatic substitution (S_NAr) of anilines **6** with 4-chloro-7-nitrobenzo[c][1,2,5]oxadiazole were unsuccessful, affording no products. Instead, the more reactive fluoro-7-nitrobenzo[c][1,2,5]oxadiazole^[38] **13** was synthesized and then employed as the aryl halide coupling partner. In this event, S_NAr reactions proceeded in good to excellent yields to deliver methyl esters **14**. Finally, saponification of the methyl esters furnished the target molecules **15**, all of which carried a carboxylic acid in the *para* position of the aniline and various aryl groups in the *ortho* position. In addition, a small library of compounds based on **17** was prepared in which the aryl group was relocated to the *meta* position of the aniline to probe the requirement for the proper positioning of the biphenyl moiety.

The ability of small-molecules to interfere with c-Myc–Max dimerization is conveniently assessed by quantifying disruption of the ternary complex between c-Myc–Max and fluorescently-labeled DNA using EMSA.^[25] Accordingly, our target molecules **15** and **17** were tested in this assay along with the previously described c-Myc inhibitors 10074-G5 and JY-3-094, which were included as positive controls. Unfortunately, none of the “chimeric” molecules showed >50% disruption of c-Myc–Max when tested at 100 μM (Table 1), including **15a**, which projects an *ortho* phenyl ring and a *para* carboxylic acid and so is a direct amalgamation of 10074-G5 and JY-3-094. Compounds **15g**, **15h** and **15k** were the most potent of the series but exhibited activities comparable only to that of 10074-G5 (39% inhibition at 100 μM). In contrast, and as previously reported,^[34] JY-3-094 inhibited DNA binding by c-Myc–Max almost completely (94%). Although a lack of additivity is not unusual in drug design, it is still surprising that, in most cases, a complete loss of activity was observed with compounds that host both of the two key functionalities. Since the monomeric c-Myc bHLH-ZIP domain is unstructured^[6] and small-molecule inhibitors of c-Myc create their own binding sites,^[32,33] it is possible that JY-3-094 and 10074-G5 craft different binding pockets, neither of which can accommodate the additional functionality from the other small-molecule.

We also screened a range of the methyl ester precursors **14** by EMSA. Specifically, **14a**, **14d**, **14h**, **14o** were investigated, and we determined compound **14d** (methyl 4'-methyl-5-(7-

nitrobenzo[c][1,2,5]oxadiazol-4-yl)-[1,1'-biphenyl]-3-carboxylate), hereafter “3jc48-3”, to be a potent inhibitor (Figure 3A). Further evaluation revealed that 3jc48-3 disrupted c-Myc–Max heterodimers with an IC_{50} value of 34.8 μ M (Figure 3B). This represents an approximate five-fold improvement over the parent compound. 3jc48-3 also demonstrated an approximate two-fold selectivity for c-Myc–Max heterodimers over Max–Max homodimers (IC_{50} = 60.6 μ M, Figure 3B), thereby suggesting that, like its parent compound 10074-G5, it binds c-Myc directly. A co-immunoprecipitation assay of 3jc48-3 in HL60 cells demonstrated that the compound prevented and/or disrupted c-Myc–Max dimerization within cells in a concentration-dependent manner (Figure 3C). This correlated well with the demonstration that, at similar concentrations, the compound also inhibited the growth of these cells (as well as Daudi cells) (Figure 3D) and induced a proliferative arrest primarily in the G₀/G₁ phase of the cell cycle (Figure 3E). Finally, 3jc48-3 inhibited the expression of a c-Myc-dependent luciferase reporter, whereas an identical NF- κ B-responsive reporter was minimally affected (Figure 3F). Collectively, these data support the conclusion that the novel small-molecule 3jc48-3 causes a proliferative arrest and eventual cell death in c-Myc over-expressing cell lines as a result of its specific inhibition of c-Myc–Max dimerization and c-Myc transcriptional control.

We noted that the concentration of 3jc48-3 active in EMSA assays significantly exceeded that needed to achieve a similar degree of heterodimer disruption in HL60 cells and inhibit their proliferation. Taking advantage of the intrinsic fluorescence of 10074-G5 and its analogues, which previously allowed us to monitor their uptake and distribution by live cell confocal microscopy,^[35] we repeated these studies in HEK cells with 3jc48-3, which is also highly fluorescent. As seen in Figure 4, 3jc48-3 was concentrated intracellularly as rapidly as 10074-G5 and its highly cell permeable analogue 3jc91-2^[35] but also attained significantly higher levels than either.^[35] Moreover, unlike 10074-G5 and 3jc91-2, which were rapidly released and/or metabolized into non-fluorescent products when the compounds were removed, the majority of 3jc48-3 was retained intracellularly, with >60% still being detected 17 h after its removal. From these studies, we conclude that the apparent discrepancy between 3jc48-3's effect in EMSAs and in cells can likely be explained by its ability to achieve high and persistent intracellular concentrations.

Conclusions

Efforts to combine the *ortho*-phenyl ring of 10074-G5 and the *para*-carboxylic acid of JY-3-094 led to the discovery of 3jc48-3, which is the most potent, cellularly-active direct c-Myc inhibitor yet identified. Significantly, this represents a five-fold improvement over the parent inhibitor 10074-G5. Furthermore, unlike the equipotent in vitro inhibitor JY-3-094, 3jc48-3 inhibited the proliferation of HL60 and Daudi cells with single-digit micromolar IC_{50} values in a manner that correlated with the intracellular disruption of c-Myc–Max heterodimers and a pronounced G₀/G₁ arrest. 3jc48-3 also tended to be rapidly taken up and concentrated in cells and persisted intracellularly far longer than previously described 10074-G5 analogues,^[35] thus likely contributing to the discrepancy between its intracellular and in vitro IC_{50} s. Additional confirmation that 3jc48-3 is an inhibitor of c-Myc was provided by its ability to disrupt intracellular c-Myc–Max heterodimers and by its selective inhibition of a c-Myc-responsive luciferase reporter gene. Since none of the designed

compounds harbouring carboxylic acids in the *para* position of the aniline exhibited any c-Myc–Max inhibitory activities, further optimization of 3jc48-3 will require replacement of the methyl ester with amide isosteres that will be more resistant to cellular hydrolysis. Additionally, it seems probable that a suitable bioisostere for the nitro group of 3jc48-3 will need to be introduced, since this functional group is known to be critical to the activity of 10074-G5^[34] and is also responsible for its short half-life ($t_{1/2}$ = 37 min) in blood plasma.^[39]

Experimental Section

Chemistry

All chemicals were purchased from Sigma-Aldrich, VWR or Oakwood Chemicals. Anhydrous solvents were purchased from Sigma-Aldrich and used without further purification. 10074-G5 was purchased from Santa Cruz Biotech. All reactions were performed with oven-dried glassware and under an inert atmosphere of N₂. Crude material was purified using silica gel flash column chromatography. Purities of biologically active compounds were >90% as assessed by a Waters 2535 Quaternary Reverse Phase HPLC, using a λ = 214 nm and a gradient of 100% solvent A to 100% solvent B over 22 min followed by holding at 100% B for 8 min (solvent A = 100:0.1, H₂O/TFA; solvent B = 90:10:0.1, ACN/H₂O/TFA). Purities are stated after retention times, t_R . NMR spectra were calibrated to residual proton isotopic peaks (CDCl₃: δ_H = 7.26, δ_C = 77.23; *d*₆-DMSO: δ_H = 2.50, δ_C = 39.51).

Methyl 4-amino-3-bromobenzoate (5)—4-Amino-3-bromobenzoic acid (2 g, 9.30 mmol, 1 eq.) was dissolved in methanol (45 mL). SOCl₂ (2 mL, 27.9 mmol, 3 eq.) was carefully added at 0 °C, then the reaction was gently refluxed overnight. The reaction mixture was concentrated to dryness, then partitioned between EtOAc and sat. NaHCO₃. The organic layer was collected, dried (Na₂SO₄), filtered and concentrated to afford the title compound as an off-white solid (2.11 g, 99%). δ_H (DMSO-*d*₆, 400 MHz) 7.84 (s, 1 H, Ar), 7.61 (d, 1 H, *J* = 8.4, Ar), 6.76 (d, 1 H, *J* = 8.4, Ar), 6.17 (br s, 2 H, NH₂), 3.72 (s, 3 H, CO₂Me); δ_C (DMSO-*d*₆, 100 MHz) 160.6, 150.5, 134.1, 130.2, 118.0, 114.6, 106.4, 52.0.

General procedure for Suzuki-Miyaura coupling^[40]—Aryl bromide (2.00 mmol) and boronic acid (1.2 equiv., 2.40 mmol) were dissolved in toluene/ethanol (10/3, 20 mL). Aqueous Na₂CO₃ (2 M, 3 equiv.) was added and the resulting mixture was deoxygenated with a stream of argon. After 10 min, Pd(PPh₃)₄ (5 mol %) was added and the reaction mixture was heated to reflux for 6 h. After cooling, the reaction mixture was quenched with sat. NH₄Cl (3 mL) and partitioned between EtOAc (50 mL) and H₂O (20 mL). The aqueous phase was extracted with EtOAc (3 × 20 mL) and the organic portions combined, washed with H₂O (20 mL), sat. NaCl (20 mL), dried (Na₂SO₄) and reduced *in vacuo*. The residue was purified by column chromatography (hexane/EtOAc) to produce the desired compounds.

Methyl 6-amino-2'-methyl-[1,1'-biphenyl]-3-carboxylate (6b)—General Suzuki procedure gave the title compound as a white solid 147 mg, 80 %. δ_H (DMSO-*d*₆, 400 MHz)

7.68 (dd, 1 H, $J = 8.8, 1.6$, Ar), 7.44 (d, 1 H, $J = 1.6$, Ar), 7.33-7.30 (m, 3 H, Ar), 7.12 (d, 1 H, $J = 6.4$, Ar), 6.79 (d, 1 H, $J = 8.8$, Ar), 5.38 (br s, 2 H, NH_2), 3.74 (s, 3 H, CO_2Me), 2.09 (s, 3 H, Me); δ_{C} (DMSO- d_6 , 100 MHz) 166.7, 150.6, 138.1, 136.7, 132.0, 130.6, 130.4, 130.3, 128.2, 126.7, 125.2, 116.6, 113.9, 51.7, 19.6; m/z (APCI) 242 (MH $^+$).

Methyl 4-amino-2-bromobenzoate (8)—4-Amino-2-bromobenzoic acid (2 g, 9.30 mmol, 1 eq.) was dissolved in methanol (45 mL). SOCl_2 (2 mL, 27.9 mmol, 3 eq.) was carefully added at 0 °C, then the reaction was gently refluxed overnight. The reaction mixture was concentrated to dryness, then partitioned between EtOAc and sat. NaHCO_3 . The organic layer was collected, dried (Na_2SO_4), filtered and concentrated to afford the title compound as an off-white solid (2.10 g, 99%). δ_{H} (CDCl_3 , 400 MHz) 7.69 (d, 1 H, $J = 8.8$, Ar), 6.87 (s, 1 H, Ar), 6.50 (d, 1 H, $J = 8.8$, Ar), 4.19 (br s, 2 H, NH_2), 3.80 (s, 3 H, CO_2Me); δ_{C} (CDCl_3 , 100 MHz) 166.0, 150.8, 133.7, 124.1, 119.7, 119.2, 112.8, 51.9.

General procedure for $\text{S}_{\text{N}}\text{Ar}$ reaction—4-Fluoro-7-nitrobenzo[c][1,2,5]oxadiazole^[36] (“NDB-F”; 1.1 eq.) and the appropriate aniline (1.1 eq) were dissolved in MeCN (0.1 M). The reaction mixture was heated at 60 °C for 20 hours. The reaction mixture was cooled, reduced *in vacuo* and partitioned between EtOAc/ H_2O . The organic layer was washed twice with H_2O , sat. NaCl, dried over NaSO_4 and reduced *in vacuo*. The crude mixture was purified by column chromatography (hexane/EtOAc) to afford the target compound.

Methyl 6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14a)— $\text{S}_{\text{N}}\text{Ar}$ general procedure gave the title compound as a red solid (67 mg, 78 %). δ_{H} (DMSO- d_6 , 400 MHz) 11.05 (br s, 1 H, NH), 8.41 (d, 1 H, $J = 8.8$, Ar), 8.09 (d, 1 H, $J = 8.8$, Ar), 8.05 (s, 1 H, Ar), 7.69 (d, 1 H, $J = 8.8$, Ar), 7.45-7.36 (m, 2 H, Ar), 7.35-7.28 (m, 3 H, Ar), 6.25 (d, 1 H, $J = 8.8$, Ar), 3.92 (s, 3 H, CO_2Me); δ_{C} (DMSO- d_6 , 100 MHz) 165.9, 144.8, 144.4, 143.3, 139.8, 138.9, 137.9, 137.5, 132.4, 130.0, 129.5, 129.1, 128.8, 128.6, 128.4, 123.5, 103.5, 52.9; m/z (APCI) 391.1 [M+H] $^+$.

Methyl 2'-methyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14b)— $\text{S}_{\text{N}}\text{Ar}$ general procedure gave the title compound as a red solid (71 mg, 78 %). δ_{H} (DMSO- d_6 , 400 MHz) 10.83 (br s, 1 H, NH), 8.43 (d, 1 H, $J = 8.8$, Ar), 8.10 (d, 1 H, $J = 8.8$, Ar), 7.89 (d, 1 H, $J = 1.6$, Ar), 7.70 (d, 1 H, $J = 8.0$, Ar), 7.20-7.13 (m, 4 H, Ar), 6.35 (d, 1 H, $J = 8.8$, Ar), 3.90 (s, 3 H, CO_2Me), 2.10 (s, 3 H, Me); δ_{C} (DMSO- d_6 , 100 MHz) 166.0, 144.7, 144.4, 142.8, 140.7, 138.4, 137.3, 135.8, 132.9, 130.6, 130.2, 130.0, 128.8, 128.5, 127.8, 126.2, 123.8, 104.1, 52.8, 20.0; m/z (APCI) 405.1 [M+H] $^+$.

Methyl 3'-methyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14c)— $\text{S}_{\text{N}}\text{Ar}$ general procedure gave the title compound as a red solid (46 mg, 62 %). δ_{H} (DMSO- d_6 , 400 MHz) 11.02 (br s, 1 H, NH), 8.41 (d, 1 H, $J = 8.8$, Ar), 8.08 (d, 1 H, $J = 7.6$, Ar), 8.03 (d, 1 H, $J = 1.6$, Ar), 7.68 (d, 1 H, $J = 8.0$, Ar), 7.27 (s, 1 H, Ar), 7.20-7.09 (m, 3 H, Ar), 6.25 (d, 1 H, $J = 8.8$, Ar), 3.91 (s, 3 H, CO_2Me), 2.23 (s, 3 H, Me); δ_{C} (DMSO- d_6 , 100 MHz) 166.0, 144.8, 144.4, 143.4, 139.8, 138.9, 138.3, 137.8,

137.5, 132.4, 129.9, 129.5, 129.4, 129.0, 128.9, 128.5, 125.9, 123.5, 103.5, 52.9, 21.3; m/z (APCI) 405.1 $[M+H]^+$.

Methyl 4'-methyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14d)— S_NAr general procedure gave the title compound as a red solid (89 mg, 75 %). δ_H (DMSO- d_6 , 400 MHz) 11.02 (br s, 1 H, NH), 8.42 (d, 1 H, J = 8.8, Ar), 8.07 (d, 1 H, J = 7.6, Ar), 8.03 (s, 1 H, Ar), 7.68 (d, 1 H, J = 7.6, Ar), 7.34 (d, 2 H, J = 7.6, Ar), 7.16 (d, 2 H, J = 7.6, Ar), 6.24 (d, 1 H, J = 8.8, Ar), 3.91 (s, 3 H, CO₂Me), 2.25 (s, 3 H, Me); δ_C (DMSO- d_6 , 100 MHz) 167.3, 144.7, 143.5, 142.3, 140.4, 140.2, 136.8, 136.4, 136.3, 130.6, 128.3, 127.5, 127.3, 126.8, 123.8, 123.7, 120.5, 102.9, 51.4, 20.2; m/z (APCI) 405.1 $[M+H]^+$; t_R = 27.54 (94.30%).

Methyl 2'-phenyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14e)— S_NAr general procedure gave the title compound as a red solid (54 mg, 66 %). δ_H (DMSO- d_6 , 400 MHz) 10.39 (br s, 1 H, NH), 8.27 (d, 1 H, J = 8.4, Ar), 8.02 (s, 1 H, Ar), 7.99 (d, 1 H, J = 7.6, Ar), 7.54-7.47 (m, 3 H, Ar), 7.43-7.41 (m, 1 H, Ar), 7.32-7.29 (m, 1 H, Ar), 6.97-6.90 (m, 3 H, Ar), 6.83 (d, 2 H, J = 6.8, Ar), 6.00 (d, 1 H, J = 8.4, Ar), 3.89 (s, 3 H, CO₂Me); δ_C (DMSO- d_6 , 100 MHz) 165.9, 144.6, 144.3, 142.4, 140.9, 140.8, 140.0, 137.9, 137.4, 135.9, 133.8, 132.2, 130.6, 129.4, 129.2, 129.1, 128.4, 128.3, 128.2, 127.1, 126.8, 123.3, 102.8, 52.8; m/z (APCI) 467.1 $[M+H]^+$.

Methyl 3'-phenyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14f)— S_NAr general procedure gave the title compound as a red solid (70 mg, 79 %). δ_H (DMSO- d_6 , 400 MHz) 11.16 (br s, 1 H, NH), 8.40 (d, 1 H, J = 8.4, Ar), 8.14-8.12 (m, 2 H, Ar), 7.74-7.70 (m, 2 H, Ar), 7.55-7.35 (m, 8 H, Ar), 6.26 (d, 1 H, J = 8.4, Ar), 3.92 (s, 3 H, CO₂Me); δ_C (DMSO- d_6 , 100 MHz) 165.0, 143.7, 143.4, 141.5, 140.0, 139.9, 139.1, 137.0, 136.5, 135.0, 132.9, 131.3, 129.7, 128.5, 128.3, 128.1, 127.5, 127.3, 127.2, 126.2, 125.9, 122.4, 101.9, 51.8; m/z (APCI) 467.1 $[M+H]^+$.

Methyl 4'-phenyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14g)— S_NAr general procedure gave the title compound as a red solid (48 mg, 56 %). δ_H (DMSO- d_6 , 400 MHz) 11.08 (br s, 1 H, NH), 8.46 (d, 1 H, J = 8.8, Ar), 8.11-8.09 (m, 2 H, Ar), 7.74-7.61 (m, 5 H, Ar), 7.55-7.35 (m, 5 H, Ar), 6.34 (d, 1 H, J = 8.8, Ar), 3.93 (s, 3 H, CO₂Me); δ_C (DMSO- d_6 , 100 MHz) 166.5, 145.2, 144.9, 143.0, 141.5, 141.4, 140.6, 138.5, 138.0, 136.5, 134.4, 132.8, 131.2, 130.0, 129.8, 129.6, 129.0, 128.8, 128.7, 127.7, 127.4, 123.9, 103.4, 53.3; m/z (APCI) 467.1 $[M+H]^+$.

Methyl 2'-cyano-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14h)— S_NAr general procedure gave the title compound as a red solid (71 mg, 63 %). δ_H (DMSO- d_6 , 400 MHz) 11.35 (br s, 1 H, NH), 8.70 (d, 1 H, J = 8.4, Ar), 8.27 (d, 1 H, J = 8.4, Ar), 8.06 (d, 1 H, J = 6.8, Ar), 7.94-7.86 (m, 2 H, Ar), 7.77-7.66 (m, 3 H, Ar), 7.28 (d, 1 H, J = 8.4, Ar), 3.79 (s, 3 H, CO₂Me); δ_C (DMSO- d_6 , 100 MHz) 166.1, 145.9, 144.7, 144.5, 142.3, 141.1, 140.7, 137.5, 133.4, 132.9, 132.6, 130.0, 128.7, 126.0, 125.5, 124.9, 122.5, 118.4, 111.7, 104.7, 52.5; m/z (APCI) 416.1 $[M+H]^+$.

Methyl 3'-cyano-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14i)—S_NAr general procedure gave the title compound as a red solid (74 mg, 82 %). δ_{H} (DMSO-*d*₆, 400 MHz) 10.99 (br s, 1 H, NH), 8.43 (d, 1 H, *J* = 9.6, Ar), 8.13 (d, 1 H, *J* = 8.8, Ar), 8.10 (s, 1 H, Ar), 7.97 (s, 1 H, Ar), 7.78-7.72 (m, 3 H, Ar), 7.2 (t, 1 H, *J* = 7.6, Ar), 6.30 (d, 1 H, *J* = 9.6, Ar), 3.92 (s, 3 H, CO₂Me); δ_{C} (DMSO-*d*₆, 100 MHz) 165.8, 144.8, 144.4, 143.0, 139.9, 139.0, 137.4, 136.8, 133.8, 132.7, 132.5, 132.1, 130.8, 130.2, 129.6, 128.5, 124.0, 118.9, 112.1, 103.8, 52.9; *m/z* (APCI) 416.1 [M+H]⁺.

Methyl 4'-cyano-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14j)—S_NAr general procedure gave the title compound as a red solid (63 mg, 71 %). δ_{H} (DMSO-*d*₆, 400 MHz) 11.01 (br s, 1 H, NH), 8.43 (d, 1 H, *J* = 8.8, Ar), 8.14 (d, 1 H, *J* = 8.0, Ar), 8.07 (s, 1 H, Ar), 7.82 (d, 2 H, *J* = 8.8, Ar), 7.74 (d, 1 H, *J* = 8.0, Ar), 7.64 (d, 2 H, *J* = 8.8, Ar), 6.32 (d, 1 H, *J* = 8.8, Ar), 3.91 (s, 3 H, CO₂Me); δ_{C} (DMSO-*d*₆, 100 MHz) 166.1, 144.7, 144.4, 143.4, 141.2, 141.0, 140.0, 136.5, 131.4, 131.2, 128.7, 125.5, 123.9, 123.4, 121.1, 118.1, 109.6, 103.3, 51.4; *m/z* (APCI) 416.1 [M+H]⁺.

Methyl 4'-ethyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14k)—S_NAr general procedure gave the title compound as a red solid 42 mg, 67 %. δ_{H} (DMSO-*d*₆, 400 MHz) 11.02 (br s, 1 H, NH), 8.41 (d, 1 H, *J* = 8.8, Ar), 8.06 (d, 1 H, *J* = 8.0, Ar), 8.04 (s, 1 H, Ar), 7.68 (d, 1 H, *J* = 8.0, Ar), 7.34 (d, 2 H, *J* = 7.6, Ar), 7.17 (d, 2 H, *J* = 7.6, Ar), 6.26 (d, 1 H, *J* = 8.8, Ar), 3.91 (s, 3 H, CO₂Me), 2.54 (q, 2 H, *J* = 7.2, CH₂), 1.09 (t, 3 H, *J* = 7.2, CH₃); δ_{C} (DMSO-*d*₆, 100 MHz) 166.0, 144.8, 144.5, 144.1, 143.4, 139.8, 137.5, 135.2, 132.4, 129.7, 129.5, 128.8, 128.5, 128.4, 123.5, 103.6, 52.9, 28.2, 15.9; *m/z* (APCI) 419.2 [M+H]⁺.

Methyl 4'-isopropyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14l)—S_NAr general procedure gave the title compound as a red solid (89 mg, 49 %). δ_{H} (DMSO-*d*₆, 400 MHz) 11.02 (br s, 1 H, NH), 8.41 (d, 1 H, *J* = 8.8, Ar), 8.07-8.05 (m, 2 H, Ar), 7.68 (d, 1 H, *J* = 8.0, Ar), 7.35 (d, 2 H, *J* = 7.6, Ar), 7.19 (d, 2 H, *J* = 7.6, Ar), 6.28 (d, 1 H, *J* = 8.8, Ar), 3.91 (s, 3 H, CO₂Me), 2.82 (sept, 1 H, *J* = 7.2, CH), 1.11 (d, 6 H, *J* = 7.2, 2 × CH₃); δ_{C} (DMSO-*d*₆, 100 MHz) 166.0, 148.6, 144.8, 144.5, 143.4, 139.9, 138.6, 137.5, 135.4, 132.4, 129.7, 129.4, 128.9, 128.4, 127.0, 123.6, 103.8, 52.9, 33.5, 24.1; *m/z* (APCI) 433.2 [M+H]⁺.

Methyl 4'-(tert-butyl)-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14m)—S_NAr general procedure gave the title compound as a red solid (35 mg, 63 %). δ_{H} (DMSO-*d*₆, 400 MHz) 11.02 (br s, 1 H, NH), 8.42 (d, 1 H, *J* = 8.8, Ar), 8.08-8.04 (m, 2 H, Ar), 7.69 (d, 1 H, *J* = 8.0, Ar), 7.40-7.29 (m, 4 H, Ar), 6.30 (d, 1 H, *J* = 8.8, Ar), 3.91 (s, 3 H, CO₂Me), 1.20 (s, 9 H, 3 × CH₃); δ_{C} (DMSO-*d*₆, 100 MHz) 166.0, 150.8, 144.9, 144.5, 143.5, 138.4, 137.5, 135.0, 132.4, 129.7, 129.3, 128.6, 128.4, 125.8, 123.6, 104.0, 52.9, 35.0, 31.2; *m/z* (APCI) 447.2 [M+H]⁺.

Methyl 4'-methoxy-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14n)—S_NAr general procedure gave the title compound as a red solid (54 mg, 60 %). δ_{H} (DMSO-*d*₆, 400 MHz) 11.03 (br s, 1 H, NH), 8.41 (d, 1 H, *J* =

8.4, Ar), 8.05-8.02 (m, 2 H, Ar), 7.66 (d, 1 H, $J = 8.4$, Ar), 7.38 (d, 2 H, $J = 8.4$, Ar), 6.90 (d, 2 H, $J = 8.4$, Ar), 6.23 (d, 1 H, $J = 8.4$, Ar), 3.91 (s, 3 H, CO₂Me), 3.70 (s, 3 H, OMe); δ_C (DMSO-*d*₆, 100 MHz) 166.0, 159.5, 144.8, 144.5, 143.4, 139.6, 138.7, 137.6, 132.3, 130.1, 130.0, 129.5, 129.4, 128.6, 123.5, 114.5, 103.3, 55.5, 52.9; m/z (APCI) 421.1 [M+H]⁺.

Methyl 4'-carboxymethyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14p)—S_NAr general procedure gave the title compound as a red solid (81 mg, 54 %). δ_H (DMSO-*d*₆, 400 MHz) 11.04 (br s, 1 H, NH), 8.42 (d, 1 H, $J = 8.8$, Ar), 8.13 (d, 1 H, $J = 8.0$, Ar), 8.08 (s, 1 H, Ar), 7.92 (d, 2 H, $J = 8.0$, Ar), 7.73 (d, 1 H, $J = 8.0$, Ar), 7.59 (d, 2 H, $J = 8.0$, Ar), 6.30 (d, 1 H, $J = 8.8$, Ar), 3.92 (s, 3 H, CO₂Me), 3.82 (s, 3 H, CO₂Me); δ_C (DMSO-*d*₆, 100 MHz) 166.3, 165.8, 144.8, 144.5, 143.2, 142.6, 139.8, 137.7, 137.5, 132.4, 130.7, 129.9, 129.6, 129.3, 128.7, 123.9, 103.4, 52.9, 52.6; m/z (APCI) 449.1 [M+H]⁺.

Methyl 2'-hydroxy-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylate (14q)—Suzuki general procedure gave the title compound as a red solid (54 mg, 67 %). δ_H (DMSO-*d*₆, 400 MHz) 10.60 (br s, 1 H, NH), 10.17 (br s, 1 H, OH), 8.45 (d, 1 H, $J = 9.2$, Ar), 8.07 (d, 1 H, $J = 8.4$, Ar), 8.01 (s, 1 H, Ar), 7.74 (d, 1 H, $J = 8.4$, Ar), 7.21-7.14 (m, 2 H, Ar), 6.90 (d, 1 H, $J = 8.0$, Ar), 6.83 (d, 1 H, $J = 8.0$, Ar), 6.50 (d, 1 H, $J = 9.2$, Ar), 3.90 (s, 3 H, CO₂Me); δ_C (DMSO-*d*₆, 100 MHz) 166.0, 153.8, 144.7, 142.0, 137.1, 135.2, 133.1, 131.1, 129.7, 129.1, 127.9, 125.9, 124.2, 119.6, 115.8, 102.9, 79.2, 52.4; m/z (APCI) 407.1 [M+H]⁺.

General procedure for ester hydrolysis—The appropriate methyl ester (1 eq.) in a 1:1 mixture of THF/H₂O (0.02 M) was treated with 2 M LiOH.H₂O (2.2 eq.) at room temperature for 20 h. The reaction mixture was acidified with 1 M HCl (pH ~2) and extracted with EtOAc (× 2). The combined organic layers were washed with sat. NaCl, dried over NaSO₄, reduced *in vacuo* and lyophilized to give the carboxylic acid target compounds as red solids.

6-((7-Nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15a)—Ester hydrolysis general procedure gave the title compound as a red solid (47 mg, 77 %). δ_H (DMSO-*d*₆, 400 MHz) 13.20 (br s, 1 H, CO₂H), 11.04 (br s, 1 H, NH), 8.39 (d, $J = 8.8$, 1 H, Ar), 8.07 (d, $J = 8.0$, 1 H, Ar), 8.03 (s, 1 H, Ar), 7.66 (d, $J = 8.0$, 1 H, Ar), 7.46-7.23 (m, 5 H, Ar), 6.23 (d, $J = 8.8$, 1 H, Ar); δ_C (DMSO-*d*₆, 100 MHz) 171.7, 149.5, 149.2, 148.2, 144.0, 143.6, 142.7, 137.3, 135.6, 134.9, 133.8, 133.6, 133.2, 133.1, 128.1, 108.3; m/z (APCI) 377.1 [M+H]⁺.

2'-Methyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15b)—Ester hydrolysis general procedure gave the title compound as a red solid (30 mg, 48 %). δ_H (DMSO-*d*₆, 400 MHz) 13.10 (br s, 1 H, CO₂H), 10.83 (br s, 1 H, NH), 8.41 (d, $J = 8.8$, 1 H, Ar), 8.07 (d, $J = 7.8$, 1 H, Ar), 7.87 (s, 1 H, Ar), 7.65 (d, $J = 7.8$, 1 H, Ar), 7.20-7.18 (m, 4 H, Ar), 6.32 (d, $J = 8.8$, 1 H, Ar), 2.10 (s, 3 H, Me); δ_C (DMSO-*d*₆, 100 MHz) 171.8, 149.5, 149.1, 147.7, 143.1, 142.2, 140.5, 137.8, 135.3, 134.9, 134.8, 133.2, 132.4, 130.9, 128.2, 108.7, 24.8; m/z (APCI) 391.1 [M+H]⁺.

3'-Methyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15c)—Ester hydrolysis general procedure gave the title compound as a red solid (28 mg, 45 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.24 (br s, 1 H, CO₂H), 11.01 (br s, 1 H, NH), 8.39 (d, J = 8.4, 1 H, Ar), 8.07-8.05 (m, 2 H, Ar), 7.64 (d, J = 7.6, 1 H, Ar), 7.26 (s, 1 H, Ar), 7.23-7.15 (m, 2 H, Ar), 7.08 (s, 1 H, Ar), 6.22 (d, J = 8.4, 1 H, Ar), 2.21 (s, 3 H, Me); δ_{C} (DMSO- d_6 , 100 MHz) 171.7, 149.5, 149.2, 148.2, 144.1, 143.6, 143.0, 142.7, 142.3, 137.3, 135.5, 134.8, 134.3, 133.6, 133.5, 133.2, 130.6, 128.1, 108.1; m/z (APCI) 391.1 [M+H]⁺.

4'-Methyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15d)—Ester hydrolysis general procedure gave the title compound as a red solid (56 mg, 88 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.20 (br s, 1 H, CO₂H), 11.01 (br s, 1 H, NH), 8.40 (d, J = 8.4, 1 H, Ar), 8.05-8.00 (m, 2 H, Ar), 7.64 (d, J = 8.4, 1 H, Ar), 7.33 (d, J = 7.6, 2 H, Ar), 7.14 (d, J = 7.6, 2 H, Ar), 6.22 (d, J = 8.4, 1 H, Ar), 2.23 (s, 3 H, Me); δ_{C} (DMSO- d_6 , 100 MHz) 171.7, 149.5, 149.2, 148.3, 143.9, 143.6, 142.5, 139.8, 137.3, 135.6, 134.6, 134.4, 133.5, 133.3, 128.1, 107.8; m/z (APCI) 453.1 [M+H]⁺.

2'-Phenyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15e)—Ester hydrolysis general procedure gave the title compound as a red solid (40 mg, 55 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.14 (br s, 1 H, CO₂H), 10.41 (br s, 1 H, NH), 8.28 (d, J = 8.4, 1 H, Ar), 7.99-7.93 (m, 2 H, Ar), 7.50 (d, J = 8.4, 1 H, Ar), 7.48-7.38 (m, 3 H, Ar), 7.31-7.28 (m, 1 H, Ar), 6.97-6.90 (m, 3 H, Ar), 6.84 (dd, J = 7.6, 1.2, 1 H, Ar), 5.99 (d, J = 8.4, 1 H, Ar); δ_{C} (DMSO- d_6 , 100 MHz) 171.7, 149.4, 149.1, 147.4, 145.7, 145.6, 144.3, 142.5, 142.3, 140.8, 138.8, 136.9, 135.4, 134.5, 134.3, 133.8, 133.0, 132.9, 131.9, 131.5, 127.9, 107.3; m/z (APCI) 453.1 [M+H]⁺.

3'-Phenyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15f)—Ester hydrolysis general procedure gave the title compound as a red solid (36 mg, 49 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.27 (br s, 1 H, CO₂H), 11.15 (br s, 1 H, NH), 8.38 (d, J = 8.8, 1 H, Ar), 8.14-8.08 (m, 2 H, Ar), 7.71-7.66 (m, 2 H, Ar), 7.55-7.47 (m, 3 H, Ar), 7.42-7.30 (m, 5 H, Ar), 6.24 (d, J = 8.8, 1 H, Ar); δ_{C} (DMSO- d_6 , 100 MHz) 171.3, 149.5, 149.1, 148.2, 145.6, 144.8, 144.1, 143.5, 143.3, 142.3, 137.3, 135.7, 135.1, 134.4, 134.0, 133.4, 132.8, 132.6, 132.0, 131.8, 131.4, 128.1, 108.2; m/z (APCI) 453.1 [M+H]⁺.

4'-Phenyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15g)—Ester hydrolysis general procedure gave the title compound as a red solid (46 mg, 63 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.24 (br s, 1 H, CO₂H), 11.07 (br s, 1 H, NH), 8.44 (d, J = 8.8, 1 H, Ar), 8.10-8.06 (m, 2 H, Ar), 7.72-7.59 (m, 5 H, Ar), 7.54 (d, J = 8.0, 2 H, Ar), 7.43 (t, J = 7.2, 2 H, Ar), 7.34 (t, J = 7.2, 1 H, Ar), 6.32 (d, J = 8.8, 1 H, Ar); δ_{C} (DMSO- d_6 , 100 MHz) 171.7, 149.6, 149.3, 148.4, 144.7, 144.4, 144.1, 143.1, 141.8, 137.3, 135.6, 134.9, 134.3, 134.1, 133.3, 132.8, 132.0, 131.7, 128.3, 108.0; m/z (APCI) 453.1 [M+H]⁺.

2'-Cyano-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15h)—Ester hydrolysis general procedure gave the title compound as a red solid (37 mg, 57 %). δ_{H} (DMSO- d_6 , 400 MHz) 12.81 (br s, 1 H, CO₂H), 11.13 (br s, 1 H, NH), 8.48 (d, J = 8.8, 1 H, Ar), 8.07 (d, J = 8.0, 1 H, Ar), 7.83 (d, J = 8.0, 1 H, Ar), 7.69 (t, J = 8.0, 1 H, Ar), 7.63 (d, J = 8.0, 1 H, Ar), 7.51 (t, J = 8.0, 1 H, Ar), 7.48 (d, J = 8.0, 1 H, Ar), 7.40 (s, 1 H, Ar), 7.04 (d, J = 8.8, 1 H, Ar); δ_{C} (DMSO- d_6 , 100 MHz) 167.1, 145.9, 145.3, 144.5, 141.9, 141.1, 137.5, 133.3, 132.7, 130.0, 128.5, 127.4, 125.1, 125.0, 122.5, 118.6, 111.8, 104.4; m/z (APCI) 402.3 [M+H]⁺.

3'-Cyano-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15i)—Ester hydrolysis general procedure gave the title compound as a red solid (24 mg, 38 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.26 (br s, 1 H, CO₂H), 10.99 (br s, 1 H, NH), 8.40 (d, J = 8.4, 1 H, Ar), 8.11 (d, J = 8.0, 1 H, Ar), 8.08 (s, 1 H, Ar), 7.95 (s, 1 H, Ar), 7.77-7.67 (m, 3 H, Ar), 7.51 (t, J = 7.6, 1 H, Ar), 6.27 (d, J = 8.4, 1 H, Ar); δ_{C} (DMSO- d_6 , 100 MHz) 171.6, 149.6, 149.2, 147.9, 144.2, 143.9, 142.2, 141.5, 138.6, 137.4, 136.8, 135.7, 135.6, 134.9, 133.2, 128.6, 123.6, 116.9, 108.3; m/z (APCI) 402.1 [M+H]⁺.

4'-Cyano-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15j)—Ester hydrolysis general procedure gave the title compound as a red solid (40 mg, 62 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.29 (br s, 1 H, CO₂H), 11.01 (br s, 1 H, NH), 8.42 (d, J = 8.8, 1 H, Ar), 8.12 (dd, J = 8.4, 2.0, 1 H, Ar), 8.05 (d, J = 2.0, 1 H, Ar), 7.82 (dd, J = 6.4, 1.6, 2 H, Ar), 7.70 (d, J = 8.4, 1 H, Ar), 7.64 (dd, J = 6.4, 1.6, 2 H, Ar), 6.30 (d, J = 8.8, 1 H, Ar); δ_{C} (DMSO- d_6 , 100 MHz) 166.8, 144.9, 144.5, 144.3, 142.8, 139.5, 137.5, 137.0, 132.9, 132.5, 131.1, 130.8, 130.0, 128.5, 123.8, 119.0, 111.0, 103.4; m/z (APCI) 402.3 [M+H]⁺.

4'-Ethyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15k)—Ester hydrolysis general procedure gave the title compound as a red solid (42 mg, 64 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.17 (br s, 1 H, CO₂H), 10.98 (br s, 1 H, NH), 8.37 (d, J = 8.8, 1 H, Ar), 8.03-7.88 (m, 2 H, Ar), 7.61 (d, J = 7.6, 1 H, Ar), 7.30 (d, J = 8.0, 2 H, Ar), 7.12 (d, J = 8.0, 2 H, Ar), 6.20 (d, J = 8.8, 1 H, Ar), 2.54-2.44 (m, 2 H, CH₂), 1.04 (t, 3 H, CH₃); δ_{C} (DMSO- d_6 , 100 MHz) 171.7, 149.5, 149.2, 148.7, 148.3, 144.1, 143.4, 140.1, 137.3, 135.5, 134.6, 133.6, 133.2, 128.1, 108.1, 32.9, 20.6; m/z (APCI) 405.1 [M+H]⁺.

4'-Isopropyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15l)—Ester hydrolysis general procedure gave the title compound as a red solid (48 mg, 71 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.20 (br s, 1 H, CO₂H), 11.01 (br s, 1 H, NH), 8.40 (d, J = 8.8, 1 H, Ar), 8.06-8.00 (m, 2 H, Ar), 7.64 (d, J = 8.8, 1 H, Ar), 7.32 (d, J = 8.0, 2 H, Ar), 7.18 (d, J = 8.0, 2 H, Ar), 6.26 (d, J = 8.8, 1 H, Ar), 2.81 (hept, 1 H, CH), 1.10 (d, 6 H, 2 × CH₃); δ_{C} (DMSO- d_6 , 100 MHz) 171.7, 153.3, 149.6, 149.2, 148.3, 144.2, 143.2, 142.3, 140.3, 137.2, 135.4, 134.6, 133.6, 133.1, 131.7, 128.1, 108.4, 38.2, 28.8; m/z (APCI) 419.2 [M+H]⁺.

4'-(tert-Butyl)-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15m)—General procedure gave the title compound as a red solid (41 mg, 59 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.19 (br s, 1 H, CO₂H), 11.01 (br s, 1 H, NH), 8.40 (d, J = 8.4, 1 H, Ar), 8.06–8.00 (m, 2 H, Ar), 7.65 (d, J = 8.8, 1 H, Ar), 7.35–7.30 (m, 4 H, Ar), 6.27 (d, J = 8.4, 1 H, Ar), 1.19 (d, 9 H, 3 $\times$ CH₃); δ_{C} (DMSO- d_6 , 100 MHz) 171.7, 155.5, 149.6, 149.2, 148.4, 144.2, 143.1, 139.9, 137.2, 135.4, 134.6, 133.4, 133.1, 130.5, 128.2, 108.5, 39.4, 36.1; m/z (APCI) 433.2 [M+H]⁺.

4'-Methoxy-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15n)—Ester hydrolysis general procedure gave the title compound as a red solid (26 mg, 40 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.20 (br s, 1 H, CO₂H), 11.02 (br s, 1 H, NH), 8.40 (d, J = 8.8, 1 H, Ar), 8.05–7.98 (m, 2 H, Ar), 7.62 (d, J = 8.0, 1 H, Ar), 7.37 (d, J = 8.0, 2 H, Ar), 6.89 (d, J = 8.0, 2 H, Ar), 6.20 (d, J = 8.8, 1 H, Ar), 3.69 (s, 3 H, OCH₃); δ_{C} (DMSO- d_6 , 100 MHz) 167.0, 159.4, 144.8, 144.5, 143.6, 138.6, 137.6, 132.4, 130.8, 130.2, 130.1, 129.6, 128.5, 114.5, 103.1, 55.5; m/z (APCI) 407.3 [M+H]⁺.

4'-Trifluoromethyl-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15o)—Ester hydrolysis general procedure gave the title compound as a red solid (18 mg, 98 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.20 (br s, 1 H, CO₂H), 11.04 (br s, 1 H, NH), 8.43 (d, J = 8.8, 1 H, Ar), 8.11 (d, J = 8.0, 1 H, Ar), 8.07 (s, 1 H, Ar), 7.75–7.61 (m, 5 H, Ar), 6.35 (d, J = 8.8, 1 H, Ar); δ_{C} (DMSO- d_6 , 100 MHz) 166.4, 144.5, 144.1, 143.2, 141.8, 137.1, 136.7, 132.1, 130.5, 130.4, 129.5, 128.0, 125.5, 103.1; m/z (APCI) 445.1 [M+H]⁺.

4'-Carboxy-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15p)—Ester hydrolysis general procedure gave the title compound as a red solid (28 mg, 41 %). δ_{H} (DMSO- d_6 , 400 MHz) 13.12 (br s, 2 H, 2 $\times$ CO₂H), 11.02 (br s, 1 H, NH), 8.42 (d, J = 8.4, 1 H, Ar), 8.12–8.04 (m, 2 H, Ar), 7.89 (d, J = 8.0, 1 H, Ar), 7.75–7.66 (m, 1 H, Ar), 7.56 (d, J = 8.0, 2 H, Ar), 6.29 (d, J = 8.4, 1 H, Ar); δ_{C} (DMSO- d_6 , 100 MHz) 167.4, 166.9, 144.8, 144.5, 143.4, 142.4, 139.4, 137.8, 137.6, 132.5, 130.9, 130.7, 130.4, 130.0, 129.8, 129.3, 129.2, 128.6, 123.7, 103.3; m/z (APCI) 421.1 [M+H]⁺.

2'-Hydroxy-6-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-3-carboxylic acid (15q)—Ester hydrolysis general procedure gave the title compound as a red solid (22 mg, 35 %). δ_{H} (DMSO- d_6 , 400 MHz) 12.98 (br s, 1 H, CO₂H), 10.57 (br s, 1 H, NH), 10.15 (br s, 1 H, OH), 8.43 (d, J = 8.4, 1 H, Ar), 8.05 (d, J = 7.6, 1 H, Ar), 7.99 (s, 1 H, Ar), 7.71 (d, J = 8.0, 1 H, Ar), 7.22–7.10 (m, 2 H, Ar), 6.89 (d, J = 7.6, 1 H, Ar), 6.82 (d, J = 7.6, 1 H, Ar), 6.49 (d, J = 8.4, 1 H, Ar); δ_{C} (DMSO- d_6 , 100 MHz) 171.8, 159.0, 149.8, 149.2, 147.3, 144.8, 140.3, 138.4, 136.3, 134.8, 134.4, 131.0, 129.6, 128.3, 124.8, 124.7, 120.9, 107.9; m/z (APCI) 393.0 [M+H]⁺.

Methyl 5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylate (16a)—S_NAr general procedure gave the title compound as a red solid (98 mg, 78 %). δ_{H} (DMSO- d_6 , 400 MHz) 11.05 (br s, 1 H, NH), 8.40 (d, 1 H, J = 8.4, Ar), 8.09 (d, 1 H, J = 8.8, Ar), 8.05 (s, 1 H, Ar), 7.70 (d, 1 H, J = 8.8, Ar), 7.43–7.26 (m, 5 H, Ar),

6.25 (d, 1 H, $J = 8.4$, Ar), 3.91 (s, 3 H, CO₂Me); δ_C (DMSO- d_6 , 100 MHz) 165.9, 144.8, 144.4, 143.3, 139.7, 138.9, 137.8, 137.5, 132.4, 130.0, 129.5, 129.0, 128.8, 128.6, 128.4, 123.6, 103.4, 52.8; m/z (APCI) 391.1 [M+H]⁺.

Methyl 2'-methyl-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylate (16b)—S_NAr general procedure gave the title compound as a red solid (41 mg, 67 %). δ_H (DMSO- d_6 , 400 MHz) 11.13 (br s, 1 H, NH), 8.60 (d, 1 H, $J = 8.8$, Ar), 8.01 (d, 1 H, $J = 8.8$, Ar), 7.63 (dd, 1 H, $J = 8.8, 2.4$, Ar), 7.34 (d, 1 H, $J = 2.4$, Ar), 7.27-7.720 (m, 3 H, Ar), 7.09 (d, 1 H, $J = 8.0$, Ar), 7.03 (d, 1 H, $J = 8.8$, Ar), 3.56 (s, 3 H, CO₂Me), 2.08 (s, 3 H, Me); δ_C (DMSO- d_6 , 100 MHz) 166.8, 145.8, 144.6, 144.0, 141.7, 141.2, 140.8, 137.8, 135.1, 131.8, 129.9, 128.7, 127.8, 127.1, 125.8, 125.1, 124.8, 121.4, 104.3, 52.3, 20.1; m/z (APCI) 405.1 [M+H]⁺.

Methyl 3'-methyl-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylate (16c)—S_NAr general procedure gave the title compound as a red solid (56 mg, 74 %). δ_H (DMSO- d_6 , 400 MHz) 11.14 (br s, 1 H, NH), 8.59 (d, 1 H, $J = 8.4$, Ar), 7.87 (d, 1 H, $J = 8.8$, Ar), 7.59 (dd, 1 H, $J = 8.8, 2.4$, Ar), 7.52 (d, 1 H, $J = 2.4$, Ar), 7.33 (t, 1 H, $J = 7.6$, Ar), 7.23-7.7.12 (m, 3 H, Ar), 7.04 (d, 1 H, $J = 8.4$, Ar), 3.61 (s, 3 H, CO₂Me), 2.37 (s, 3 H, Me); δ_C (DMSO- d_6 , 100 MHz) 168.3, 145.8, 144.6, 143.4, 141.4, 141.3, 140.3, 137.9, 137.8, 131.5, 129.1, 128.7, 128.6, 127.9, 125.7, 124.9, 124.7, 121.6, 103.9, 52.4, 21.4; m/z (APCI) 405.1 [M+H]⁺.

Methyl 4'-methyl-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylate (16d)—S_NAr general procedure gave the title compound as a red solid (81 mg, 48 %). δ_H (DMSO- d_6 , 400 MHz) 11.12 (br s, 1 H, NH), 8.56 (d, 1 H, $J = 8.8$, Ar), 7.84 (d, 1 H, $J = 8.8$, Ar), 7.56 (d, 1 H, $J = 8.8$, Ar), 7.49 (s, 1 H, Ar), 7.31-7.20 (m, 4 H, Ar), 7.02 (d, 1 H, $J = 8.8$, Ar), 3.61 (s, 3 H, CO₂Me), 2.35 (s, 3 H, Me); δ_C (DMSO- d_6 , 100 MHz) 168.3, 145.7, 144.5, 143.3, 141.4, 141.2, 137.8, 137.4, 137.4, 131.6, 129.3, 128.5, 128.3, 127.8, 124.8, 124.7, 121.5, 103.9, 52.4, 21.2; m/z (APCI) 405.1 [M+H]⁺.

Methyl 2'-cyano-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylate (16e)—S_NAr general procedure gave the title compound as a red solid (69 mg, 63 %). δ_H (DMSO- d_6 , 400 MHz) 11.21 (br s, 1 H, NH), 8.57 (d, 1 H, $J = 8.8$, Ar), 8.14 (d, 1 H, $J = 8.8$, Ar), 7.93 (d, 1 H, $J = 8.8$, Ar), 7.80-7.72 (m, 2 H, Ar), 7.63-7.51 (m, 3 H, Ar), 7.15 (d, 1 H, $J = 8.8$, Ar), 3.65 (s, 3 H, CO₂Me); δ_C (DMSO- d_6 , 100 MHz) 166.1, 145.9, 144.7, 144.5, 142.3, 141.1, 140.8, 137.5, 133.4, 132.9, 132.6, 130.0, 128.7, 126.0, 125.5, 124.9, 122.5, 118.4, 111.6, 104.7, 52.5; m/z (APCI) 416.1 [M+H]⁺.

Methyl 3'-cyano-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylate (16f)—S_NAr general procedure gave the title compound as a red solid (38 mg, 66 %). δ_H (DMSO- d_6 , 400 MHz) 11.16 (br s, 1 H, NH), 8.58 (d, 1 H, $J = 8.4$, Ar), 8.01 (d, 1 H, $J = 8.0$, Ar), 7.88 (s, 2 H, Ar), 7.70-7.64 (m, 3 H, Ar), 7.56 (s, 1 H, Ar), 7.12 (d, 1 H, $J = 8.4$, Ar), 3.65 (s, 3 H, CO₂Me); δ_C (DMSO- d_6 , 100 MHz) 167.0, 145.8, 144.5, 142.0, 141.9, 141.8, 140.9, 137.7, 133.8, 132.4, 132.2, 131.8, 129.7, 126.5, 125.2, 124.8, 122.2, 119.1, 111.7, 104.5, 52.5; m/z (APCI) 416.1 [M+H]⁺.

Methyl 4'-cyano-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylate (16g)—S_NAr general procedure gave the title compound as a red solid (59 mg, 78 %). δ_{H} (DMSO-*d*₆, 400 MHz) 11.16 (br s, 1 H, NH), 8.56 (d, 1 H, *J* = 8.8, Ar), 8.00 (d, 1 H, *J* = 8.8, Ar), 7.92 (d, 2 H, *J* = 8.4, Ar), 7.66 (dd, 1 H, *J* = 8.4, 1.6, Ar), 7.58-7.52 (m, 3 H, Ar), 7.09 (d, 1 H, *J* = 8.8, Ar), 3.64 (s, 3 H, CO₂Me); δ_{C} (DMSO-*d*₆, 100 MHz) 167.2, 145.9, 145.5, 144.6, 142.4, 142.1, 141.2, 137.6, 132.5, 132.3, 129.8, 126.6, 125.0, 124.5, 122.2, 119.2, 110.8, 104.5, 52.5; *m/z* (APCI) 416.1 [M+H]⁺.

5-((7-Nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylic acid (17a)—Ester hydrolysis general procedure gave the title compound as a red solid (34 mg, 56 %). δ_{H} (DMSO-*d*₆, 400 MHz) 13.23 (br s, 1 H, CO₂H), 11.05 (br s, 1 H, NH), 8.39 (d, *J* = 8.4, 1 H, Ar), 8.07 (d, *J* = 8.0, 1 H, Ar), 8.03 (s, 1 H, Ar), 7.65 (d, *J* = 8.0, 1 H, Ar), 7.46-7.22 (m, 5 H, Ar), 6.22 (d, *J* = 8.4, 1 H, Ar); δ_{C} (DMSO-*d*₆, 100 MHz) 167.0, 144.8, 144.5, 144.3, 139.4, 138.7, 138.0, 137.5, 132.6, 130.8, 130.1, 129.0, 128.8, 128.4, 128.3, 123.3, 103.3; *m/z* (APCI) 377.1 [M+H]⁺.

2'-Methyl-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylic acid (17b)—Ester hydrolysis general procedure gave the title compound as a red solid (46 mg, 72 %). δ_{H} (DMSO-*d*₆, 400 MHz) 12.58 (br s, 1 H, CO₂H), 11.12 (br s, 1 H, NH), 8.58 (d, *J* = 8.8, 1 H, Ar), 7.99 (d, *J* = 8.4, 1 H, Ar), 7.59 (dd, *J* = 8.4, 2.4, 1 H, Ar), 7.27 (d, *J* = 2.4, 1 H, Ar), 7.26-7.15 (m, 3 H, Ar), 7.10 (d, *J* = 7.2, 1 H, Ar), 6.98 (d, *J* = 8.8, 1 H, Ar), 2.09 (s, 3 H, CH₃); δ_{C} (DMSO-*d*₆, 100 MHz) 167.6, 145.4, 144.2, 143.4, 141.1, 140.9, 140.8, 137.4, 134.8, 131.4, 129.4, 128.4, 128.2, 127.2, 125.3, 124.6, 124.3, 121.0, 103.6, 19.8; *m/z* (APCI) 391.1 [M+H]⁺.

3'-Methyl-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylic acid (17c)—Ester hydrolysis general procedure gave the title compound as a red solid (41 mg, 65 %). δ_{H} (DMSO-*d*₆, 400 MHz) 12.79 (br s, 1 H, CO₂H), 11.16 (br s, 1 H, NH), 8.56 (d, *J* = 8.4, 1 H, Ar), 7.85 (d, *J* = 8.4, 1 H, Ar), 7.54 (d, *J* = 8.0, 1 H, Ar), 7.45 (s, 1 H, Ar), 7.30 (t, *J* = 8.0, 1 H, Ar), 7.22-7.14 (m, 3 H, Ar), 6.97 (d, *J* = 8.4, 1 H, Ar), 2.35 (s, 3 H, CH₃); δ_{C} (DMSO-*d*₆, 100 MHz) 168.9, 145.4, 144.2, 142.8, 141.4, 140.5, 140.2, 137.4, 137.2, 130.9, 129.0, 128.9, 128.1, 128.0, 125.5, 124.5, 123.9, 121.2, 103.2; *m/z* (APCI) 391.1 [M+H]⁺.

4'-Methyl-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylic acid (17d)—Ester hydrolysis general procedure gave the title compound as a red solid (52 mg, 81 %). δ_{H} (DMSO-*d*₆, 400 MHz) 12.76 (br s, 1 H, CO₂H), 11.15 (br s, 1 H, NH), 8.55 (d, *J* = 8.8, 1 H, Ar), 7.84 (d, *J* = 8.8, 1 H, Ar), 7.51 (d, *J* = 8.0, 1 H, Ar), 7.43 (s, 1 H, Ar), 7.28 (d, *J* = 8.0, 2 H, Ar), 7.23 (d, *J* = 8.0, 2 H, Ar), 6.95 (d, *J* = 8.8, 1 H, Ar), 2.35 (s, 3 H, CH₃); δ_{C} (DMSO-*d*₆, 100 MHz) 169.4, 145.8, 144.7, 143.1, 137.8, 137.2, 131.4, 129.3, 129.2, 128.6, 124.9, 121.5, 103.7, 21.2; *m/z* (APCI) 391.1 [M+H]⁺.

2'-Cyano-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylic acid (17e)—Ester hydrolysis general procedure gave the title compound as a red solid (51 mg, 78 %). δ_{H} (DMSO-*d*₆, 400 MHz) 12.87 (br s, 1 H, CO₂H), 11.20 (br s, 1

H, NH), 8.54 (d, $J = 8.8$, 1 H, Ar), 8.13 (d, $J = 8.0$, 1 H, Ar), 7.89 (d, $J = 8.0$, 1 H, Ar), 7.75 (t, $J = 8.0$, 1 H, Ar), 7.70 (d, $J = 8.0$, 1 H, Ar), 7.57 (t, $J = 8.0$, 1 H, Ar), 7.52 (d, $J = 8.0$, 1 H, Ar), 7.46 (s, 1 H, Ar), 7.10 (d, $J = 8.8$, 1 H, Ar); δ_C (DMSO- d_6 , 100 MHz) 167.1, 145.9, 145.3, 144.5, 141.9, 141.1, 137.5, 133.3, 132.7, 130.0, 128.5, 127.4, 125.1, 125.0, 122.5, 118.6, 111.8, 104.4; m/z (APCI) 402.1 $[M+H]^+$.

3'-Cyano-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylic acid (17f)—Ester hydrolysis general procedure gave the title compound as a red solid (43 mg, 66 %). δ_H (DMSO- d_6 , 400 MHz) 12.92 (br s, 1 H, CO₂H), 11.14 (br s, 1 H, NH), 8.56 (d, $J = 8.4$, 1 H, Ar), 7.99 (d, $J = 8.8$, 1 H, Ar), 7.88-7.82 (m, 2 H, Ar), 7.71 (d, $J = 8.0$, 1 H, Ar), 7.63 (t, $J = 8.0$, 2 H, Ar), 7.51 (s, 1 H, Ar), 7.08 (d, $J = 8.4$, 1 H, Ar); δ_C (DMSO- d_6 , 100 MHz) 168.3, 145.7, 144.5, 142.2, 141.8, 141.4, 141.2, 137.8, 133.9, 132.3, 132.2, 131.6, 129.7, 128.2, 125.0, 124.9, 122.2, 119.2, 111.5, 104.2; m/z (APCI) 402.3 $[M+H]^+$.

4'-Cyano-5-((7-nitrobenzo[c][1,2,5]oxadiazol-4-yl)amino)-[1,1'-biphenyl]-2-carboxylic acid (17g)—Ester hydrolysis general procedure gave the title compound as a red solid (33 mg, 51 %). δ_H (DMSO- d_6 , 400 MHz) 12.91 (br s, 1 H, CO₂H), 11.22 (br s, 1 H, NH), 8.54 (d, $J = 8.8$, 1 H, Ar), 7.95 (d, $J = 8.4$, 1 H, Ar), 7.86 (d, $J = 8.8$, 2 H, Ar), 7.60 (d, $J = 8.4$, 1 H, Ar), 7.54 (d, $J = 8.8$, 2 H, Ar), 7.45 (s, 1 H, Ar), 7.04 (d, $J = 8.8$, 1 H, Ar); δ_C (DMSO- d_6 , 100 MHz) 168.2, 146.0, 144.7, 142.2, 137.6, 132.4, 132.1, 129.9, 124.7, 122.3, 119.3, 110.6, 104.4; m/z (APCI) 402.3 $[M+H]^+$.

Biology

c-Myc–Max electrophoretic mobility shift assays (EMSAs)—EMSAs were performed essentially as described utilizing bacterially-expressed recombinant proteins purified to >90% homogeneity.^[25] Experiments with Max homodimers used the His₆-tagged 160 amino acid isoform of the protein, termed Max(L), which homodimerizes and binds DNA well.^[25] For studies with Myc-Max heterodimers, the His₆-tagged 151 residue isoform of Max [Max(S)] was used, which binds DNA well as a heterodimer with Myc but poorly as a homodimer.^[25] Recombinant Myc protein, expressed in the pET151 vector, consisted of the His₆-TEV protease substrate-tagged 85 amino acid bHLH-ZIP domain^[23]. Each protein was purified by Ni-agarose affinity chromatography as previously described.^[25] The His₆-TEV tag was removed from the Myc protein by TEV protease cleavage followed by re-purification using Ni-agarose affinity chromatography to remove the His₆-TEV protease and the cleaved His₆-TEV tag as previously described.^[23] Binding assays were performed with 30 nM of each protein and 30 nM of a 6-carboxy-2',4,4',5',7,7'-hexachlorofluorescein (HEX)-tagged double-stranded oligonucleotide containing a consensus Myc binding site (IDT, Coralville, IA).^[25]

Growth inhibition assays—Daudi Burkitt lymphoma and HL60 promyelocytic leukemia cells were maintained in RPMI medium supplemented with 10% fetal bovine serum.^[25] For growth inhibition studies, 3×10^3 cells were seeded in 96 well plates in the presence of serial dilutions of each compound. A total of 10 dilutions, ranging from 0.1 to 50 μ M were performed for each compound. Standard MTT assays^[25] were used to quantify cell number

3-4 days later and compared to wells exposed to DMSO vehicle only. Each point was assayed in quadruplicate with the results being presented as the mean $\pm$ 1 standard error.

Co-immunoprecipitation (Co-IP) assays—Co-IP assays were performed essentially as described previously.^[25] Briefly, 5×10^6 HL60 cells (>90% viable) in log-phase growth were treated in suspension for 4-6 h with the stated concentration of 3jc48-3. As a negative control, samples exposed to DMSO vehicle only were included and processed in parallel. Treated cells were collected by centrifugation, washed twice in ice-cold PBS and lysed in IP Buffer. 300 μ g of cleared lysate was then precipitated with a 1:200 dilution of anti-Max antibody^[25] followed by subsequent precipitation with protein G-Sepharose using conditions recommended by the supplier (Santa Cruz Biotechnology, Inc. Santa Cruz, CA). The precipitate was washed three times in IP buffer, boiled in running buffer and resolved by 10% SDS-PAGE. After electrotransfer of the proteins to a PVDF membrane (Millipore Corp. Billerica, MA), the blot was probed with a 1:1000 dilution of anti-Myc monoclonal antibody (9E10, Santa Cruz Biotechnology, Santa Cruz, CA). Blots were developed using a Pierce ECL Plus enhanced chemiluminescence kit according to the directions of the supplier (Thermo-Fisher, Pittsburgh, PA).

Cell cycle analysis—Logarithmically-growing cells at >90% viability were plated into fresh medium at densities that allowed them to maintain log-phase growth for at least the ensuing 72 hr.. The next day, Myc inhibitors at the stated concentrations were added for 24-48 hr. The cells were then harvested, washed twice in PBS and stained with propidium iodide as previously described.^[25] Cell cycle analyses were performed on a BD FACSCalibur™ flow cytometer (Becton Dickinson, Inc. Franklin Lakes, NJ) and analyzed using ModFit LT 3.3 (Verity Software House, Topsham, ME).

Assay of Myc reporter gene expression—The generic reporter vector pGL4.28(luc2CP/minP/Hygro) (Promega Corp., Madison WI), which is specifically designed to reduce anomalous transcription, and which encodes a highly unstable luciferase reporter, was used to assess the effects of Myc inhibitors. A double-stranded palindromic oligonucleotide containing three tandem Myc-responsive E-box elements (5'-TCGAGCCACGTGGCCACGTGGCCACGTGGC-3') (E-boxes underlined) was self-annealed and cloned into an XhoI site adjacent to the minimal promoter element of this vector. A control vector containing this same oligonucleotide with mutant E-box sites (5'-TCGAGCCTCGAGGCCTCGAGGCCTCGAGGC-3') was used as a negative control. An additional vector was similarly constructed that contained a tandemly triplicated binding site for NF- κ B (5'-TCGAGGGACTTTCCATGGGACTTTCCATGGGACTTTCC-3'). After automated DNA sequencing to confirm the presence of its respective insert, each vector was then separately co-transfected into HeLa cells together with the vector pFR400, which encodes a mutant form of dihydrofolate reductase that facilitates the step-wise amplification of the plasmid and any co-transfected sequences in the presence of increasing concentrations of the folate antagonist methotrexate (MTX).^[41] Stably transfected, hygromycin-resistant clones were pooled and grown in serial four-fold increasing concentrations of MTX ranging from 0.25-16 μ M, thus allowing the luciferase signal originating from the Myc- and NF- κ B-responsive vectors to be easily detected above background. No luciferase signal over

background values could be obtained in unamplified cells or in amplified cells transfected with the mutant E-box-containing vector. For luciferase assays, 10^6 cells were plated in six well dishes and allowed to attach overnight. Fresh medium with the indicated amounts of c-Myc inhibitor was then added to triplicate wells for 6 hr. Cells were harvested, pelleted and resuspended in 70 μ l of cell lysis buffer from a One-Glo Luciferase Assay System Kit (Promega, Inc. Madison, WI). Assays were performed on 50 μ l of the lysate using the conditions recommended by the supplier. The remaining lysate was used to determine the total input protein which was used to normalize luciferase values. Differences in protein levels seldom exceeded 10%. All points were performed in triplicate.

Uptake and release of 3jc48-3 by live cell confocal imaging—The real-time uptake of 3jc48-3, 10074-G5 and 3jc91-2 was monitored essentially as described previously.^[35] Briefly, 35 mm glass bottom plates (MatTek Co., Ashland, MA) were seeded with human embryonic kidney (HEK) cells. The following day, fresh medium containing the indicated concentrations of compounds was added. Plates were then maintained on the microscope stage at 37°C and in a 5% CO₂ atmosphere. Real-time images were obtained with a LSM710 laser scanning confocal microscope (Carl Zeiss, Munich, Germany) using the following settings: objective: Plan-Apochromat 20 $\times$ /0.8 M27; Laser: Excitation 488 nm at 2%, Laser Pinhole 34 mm, pixel dwell 6.4 mm; Detection filter: 490-735, Digital Gain 1.0, Digital Offset 0.00, Master gain 740 (for c-Myc inhibitor) 276 (for DIC); Image size: 1024 $\times$ 1024; Z stack scan total: 20 μ m for 12 stacks. At the end of the uptake period, compound-containing medium was removed and fresh medium was added for the remainder of the experiment. Image analyses and quantification were performed with the histogram and mean of ROI functions using ZEN 2009 software (Carl Zeiss, Inc).

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Figure 1.
Small-molecule inhibitors of c-Myc–Max dimerization whose mode of inhibition is the stabilization of intrinsically disordered, monomeric c-Myc.

Figure 2. Computationally-generated probable binding mode of 10074-G5 to c-Myc. Reproduced with permission from reference [34].

Figure 3.

Characterization of 3jc48-3. **a)** Chemical structure of 3jc48-3. **b)** 3jc48-3 inhibits c-Myc–Max dimerization (left) and is about two-fold more selective for c-Myc–Max heterodimers over Max–Max homodimers (right), as analysed by EMSA. Either 30 nM of each of c-Myc and Max(S) or 60 nM of Max(L)^[25,35] were incubated with 20 nM 5'-hexachlorofluoresceine-CACCCGGTCACGTGGCCTACAC-3' and the indicated concentrations of 3jc48-3. EMSAs were then performed as previously described^[25,35] **c)** Co-immunoprecipitation. 3jc48-3 was incubated with 5×10^6 HL60 cells at the concentrations indicated. After 7 h, the cells were pelleted by centrifugation, washed and lysed. Total Max was precipitated with polyclonal rabbit anti-Max antibody, which in turn was precipitated with protein-G Sepharose. Max-bound c-Myc was then released by boiling and assessed by immunoblotting. Total Myc and Max input prior to immunoprecipitation were also assessed in parallel. **d)** Inhibition of HL60 and Daudi cell proliferation by 3jc48-3. Cells were incubated for 72 h with the indicated concentrations of 3jc48-3 at which time total viable cell number was assessed by an MTT assay. **e)** 3jc48-3 blocks cell cycle progression. Log-phase HL60 cells were exposed to the indicated concentrations of 3jc48-3 for 24 h at which time they were stained with propidium iodide (PI) and subjected to flow cytometric analysis to assess DNA content. The fraction of cells in Go/G1 (2n DNA content), G2/M (4n DNA content) and S-phase (2n<n<4n) is indicated alongside its respective peak. Approximately 10,000 cells were evaluated for each assay. Similar results were obtained on at least two additional occasions (not shown). **f)** 3jc48-3 selectively inhibits the expression of luciferase under the control of a c-Myc-responsive promoter. Separate wells of HeLa cells expressing a highly unstable form of luciferase under the control of either a c-Myc-dependent ("LUC-Myc") or NF-κB-dependent (LUC-NFκB)

minimal promoter were exposed to the indicated concentrations of 3jc48-3 for 6 h, assayed in quadruplicate for the expression of luciferase and adjusted to account for any differences in total protein content (generally <10%).

Figure 4.

Uptake and persistence of 3jc48-3. HEK cells were exposed to 10 μ M of 3jc48-3, 10074-G5 or 3JC-91-2, a highly cell-permeable analogue of 10074-G5.^[35] Uptake of each compound was monitored by live cell confocal fluorescence microscopy as previously described.^[35] 1.5-3 h after reaching equilibrium, monolayers were washed free of their respective compounds and monitoring was continued to obtain an estimate of the rate of compound release and/or metabolism to non-fluorescent metabolites. Insets show compounds within cells at 200 min after their addition

Scheme 1.

Reagents and conditions: (a) SOCl_2 , MeOH, 60 °C, 14 h, 99%; (b) ArB(OH)_2 , $\text{Pd(PPh}_3)_4$, toluene/EtOH (10/3), aq. Na_2CO_3 , 110 °C, 6 h, 55–87%; (c) *m*-CPBA, CH_2Cl_2 , 0 °C to RT, 2 h, 99%; (d) NaN_3 , DMSO, RT, 3 h; (e) HNO_3 , c. H_2SO_4 , H_2O , 0 °C, 5 min, 26% (over two steps); (f) CH_3CN , 60 °C, 16 h, 56–79%; (g) $\text{LiOH}\cdot\text{H}_2\text{O}$, THF- H_2O , 1:1, RT, 20 h, 35–88%.

Table 1

Target molecules were screened for their abilities to disrupt the c-Myc–Max/DNA ternary complex at 100 μ M. DMSO vehicle control regarded as 0% inhibition. Positive controls: 10074-G5 and JY-3-094 afforded 39% and 94% inhibition, respectively.

15			17		
Compound	Ar	Disruption of c-Myc-Max/DNA (%)	Compound	Ar	Disruption of c-Myc-Max/DNA (%)
15a		12	15m		5
15b		0	15n		7
15c		14	15o		17
15d		13	15p		8
15e		18	15q		0
15f		0	17a		5
15g		43	17b		1
15h		41	17c		2
15i		19	17d		0
15j		13	17e		0
15k		37	17f		17
15l		0	17g		32