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A Potent and Highly Efficacious Bcl-2/Bcl-xL Inhibitor

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Abstract

Our previously reported Bcl-2/Bcl-xL inhibitor, **4**, effectively inhibited tumor growth but failed to achieve complete regression *in vivo.* We have now performed extensive modifications on its pyrrole core structure, which has culminated in the discovery of **32** (BM-1074). Compound **32** binds to Bcl-2 and Bcl-xL proteins with K_i values of < 1 nM and inhibits cancer cell growth with IC_{50} values of 1-2 nM in four small-cell lung cancer cell lines sensitive to potent and specific Bcl-2/Bcl-xL inhibitors. Compound **32** is capable of achieving rapid, complete and durable tumor regression *in vivo* at a well-tolerated dose-schedule. Compound **32** is the most potent and efficacious Bcl-2/Bcl-xL inhibitor reported to date.

INTRODUCTION

A common feature in many different types of human tumors is overexpression of the prosurvival Bcl-2 family members Bcl-2 and Bcl-xL,[1-4] which make tumor cells resistant to conventional cancer therapeutic agents. Therefore, it has been proposed that small-molecule inhibitors of Bcl-2 and Bcl-xL may have a promising therapeutic potential for the treatment of human cancer.[3]

Compounds **1**[5] and **2**[6] represent two highly potent and specific Bcl-2/Bcl-xL inhibitors. Preclinical studies have shown that **1** and **2** are effective as single agents against lymphomas, chronic lymphoid leukemia (CLL) and a subset of small-cell lung cancer (SCLC) models, and can enhance the antitumor activity of conventional anticancer drugs and γ-irradiation in preclinical models of diverse tumor types.[3] Compound **2** is currently in Phase I/II clinical trials, where it has shown promising single-agent activity in patients with CLL and B-cell lymphomas.

Because design of Bcl-2 and Bcl-xL inhibitors involves targeting the interaction of Bcl-2/Bcl-xL proteins with their pro-apoptotic binding partners such as BAD and BIM proteins, a challenging task in drug discovery, very few new, potent, specific and *bona fide* small-molecule inhibitors of this interaction have been reported, even after the discovery of **1** and

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2. Recently, we reported the structure-based design of a family of new, highly potent and specific Bcl-2/Bcl-xL inhibitors (Figure 1).[7-9] Our initial lead compound **3** binds to Bcl-2 and Bcl-xL with high affinities and potently inhibits cell growth in cancer cell lines that are sensitive to **1** and **2**, but it lacks chemical stability and fails to achieve significant *in vivo* antitumor activity.[7] Subsequent structure-based design and optimization of **3** led to compounds **4** and **5**, which have excellent chemical stability, bind to Bcl-2 and Bcl-xL with K_i values of <1 nM and inhibit cancer cell growth with low nanomolar activity.[8] While **5** effectively inhibits tumor growth and in fact induces tumor regression in the H146 small-cell lung cancer model at its maximum tolerated dose (MTD), the tumor regression it caused was transient,[8] suggesting further optimization was needed toward our goal of developing a new class of Bcl-2/Bcl-xL inhibitors for cancer treatment. Very recently, we have reported further structure-based optimization of compound **5**, with a focus on two regions in the molecule, which led to the successful discovery of a superior compound, **6** (**BM-957**).[9] Compound **6** binds to Bcl-2 and Bcl-xL with K_i values < 1 nM and inhibits tumor cell growth with IC_{50} values of 21-22 nM against H146 and H1417 small-cell cancer cell lines.[9] Significantly, **6** achieved tumor regression in an animal model of human cancer.[9]

In the previous study, which yielded compound **6**, we focused our modifications on the nitro group and the soluble "tail" containing the *N,N*-dimethylamino group in compound **4**. In the present study, we report our further optimization of **4**, with a focus on its 1*H*-pyrrole-3-carboxylic acid core structure. Our efforts have culminated in the discovery of **32** (BM-1074), which, based upon its cellular activity and *in vivo* efficacy, is arguably the most potent and efficacious Bcl-2/Bcl-xL inhibitor discovered to date.

Results and Discussion

Previously, we have shown that removal of the acid group from the pyrrole carboxylic acid of **4**, yielding compound **7**, resulted in a >50-fold decrease in binding affinity to Bcl-2 and a modest decrease in binding affinity to Bcl-xL.[8] Compound **7**, at concentrations as high as 10 μM, was found to be completely inactive in inhibition of cell growth in the H146 cancer cell line (Table 1), suggesting that very high binding affinity to Bcl-2/Bcl-xL is clearly needed in order for small-molecule inhibitors to effectively inhibit cancer cell growth.[8] Converting this acid group into a methylamide (compound **8**) has a modest negative effect on binding to Bcl-2 but has no effect on binding to Bcl-xL (Table 1). Interestingly, compound **8** has an IC_{50} value of 36 nM in the H146 cell line (Table 1), and is thus slightly more potent than compound **4**, suggesting that compound **8** has superior cell permeability compared to compound **4**. These binding and cellular data showed that modifications of the acid group of **4** can have a significant negative or positive effect on binding to Bcl-2/Bcl-xL and on cellular activity. Accordingly, we have made additional modifications at this position in order to further explore the structure-activity relationships and to identify promising new compounds. All the designed and synthesized new compounds were tested with our standard fluorescence-polarization (FP) assays[7] for their binding affinities to Bcl-2 and Bcl-xL proteins and for their cell growth inhibitory activity in the H146 small-cell lung cancer cell line, which is sensitive to potent and *bona fide* Bcl-2/Bcl-xL inhibitors such as compounds **1**-**6**,[7] and the results are presented in Table 1.

First, changing the *N*-methylamide in compound **8** to an amide group resulted in compound **9**, which is 4- and 2-times less potent than **8**, respectively, in binding to Bcl-2 and Bcl-xL. Compound **9** has an IC_{50} value of 245 nM in the H146 cell line and is thus 6-times less potent than **8** in this cell line. Replacement of the methyl group in the *N*-methylamide of compound **8** with a *cis*-3-hydroxy-3-methylcyclobutyl group or a 3-methylazetidine-3-ol group resulted in compounds **10** and **11**, respectively. Compounds **10** and **11** bind to Bcl-2 with affinities similar to that of compound **8** but both compounds are slightly less potent

than **8** in binding to Bcl-xL. While compounds **10** and **11** have very similar affinities to Bcl-2 and Bcl-xL proteins, their activity in inhibition of cell growth in the H146 cell line differs by a factor of 6, further indicating that modifications of this site can have a significantly different effect on binding affinities to Bcl-2/Bcl-xL and cellular activity for this class of compounds.

In the co-crystal structure of **4** in complex with Bcl-xL (Figure 2), the acid group in **4** forms a hydrogen bond/salt bridge with Arg 132 in Bcl-xL,[8] and we synthesized compound **12** to test if the methylsulfonylamide, a bioisostere of the carboxylic acid, can replace the acid group in **4** and achieve high binding affinities to Bcl-2 and Bcl-xL and potent cellular activity in the H146 cell line. Compound **12** is as potent as **4** in binding to both Bcl-2 and Bcl-xL proteins, and has an IC_{50} value of 38 nM in the H146 cell line, thus twice as potent as **4**. These results show that the methylsulfonylamide group can indeed effectively replace the acid group, achieving not only high binding affinities to Bcl-2/Bcl-xL, but also potent cellular activity.

In addition to their ability to form hydrogen bonds and salt bridges, acid and methylsulfonylamide groups have strong electron withdrawing properties. To further define the contributions of these groups for binding to Bcl-2 and Bcl-xL and cellular activity in compounds **4** and **12**, we synthesized compounds **13**-**15** to investigate if other electron withdrawing groups, such as Cl, CF_3 and CN, can effectively replace the acid group in compound **4** to achieve high binding affinities to Bcl-2 and Bcl-xL and potent cellular activity. Although compound **13** with Cl and compound **14** with CF_3 have similar potencies to Bcl-xL as compared to **4**, both compounds are 100-times less potent than **4** in binding to Bcl-2. Compounds **13** and **14** are also >100-times less potent than **4** in inhibition of cell growth in the H146 cancer cell line. Compound **15**, in which the acid group in **4** has been replaced by a nitrile, is >10-times less potent than **4** in binding to Bcl-2 but has a similar binding affinity to Bcl-xL, as compared to **4**. Compound **15** has an IC_{50} value of 496 nM in the H146 cell line, and is thus 8 times less potent than **4**.

In our co-crystal structure of **4** in complex with Bcl-xL (Figure 2), the methyl group in **4** ($R_1 = CH_3$) is in close contact with the hydrophobic portion of the Glu129 side chain in Bcl-xL. We thus synthesized compounds **16** and **17** to determine whether the methyl group can be replaced by other small hydrophobic groups such as CF_3 and Cl groups to achieve high binding affinities to Bcl-2/Bcl-xL. As suggested by the co-crystal structure for compound **4**, both **16** and **17** bind to Bcl-2 and Bcl-xL with the same high affinities as compared to compound **4** (Table 1). In the H146 cell line, compound **17** is slightly less potent than **4**, but **16** is 8-times less potent than **4**.

In all the synthesized compounds described here, the pyrrole was retained as the core structure. We next designed and synthesized 5 classes of compounds to investigate if other 5-membered heteroaromatic groups can effectively replace the pyrrole moiety in compound **4** and achieve high binding affinities to Bcl-2/Bcl-xL and potent cellular activity. Since our data showed that removal of the carboxylic acid in **4** greatly decreases its binding to Bcl-2 and cellular activity while replacement of the carboxylic acid with a methylsulfonylamide group can effectively maintain both high binding affinities to Bcl-2/Bcl-xL and potent cellular activity, we have synthesized several new compounds possessing or lacking an acid or a methylsulfonylamide group in each class of compounds. The binding and cellular data for these compounds are summarized in Table 2.

For each of these five different classes, compounds **23**-**27**, containing either an acid or a methylsulfonylamide group, show high binding affinities to both Bcl-2 and Bcl-xL proteins with IC_{50} values of 3-7 nM. However, these compounds have a significantly weaker activity

in cellular assays than compounds **4** and **12**. While compounds **26** and **27** have IC_{50} values of 541 nM and 199 nM, respectively, compounds **23**, **24** and **25** have minimal activity at 1 μM.

In comparison, analogues (**18**-**22**) without either an acid or a methylsulfonylamide group show at least a 10-fold weaker binding affinity to Bcl-2 than their corresponding analogues **23**-**27**. Compounds **18**-**22** are also 5-10 times less potent in their binding to Bcl-xL than compounds **23**-**27**. Consistent with their weaker affinities to both Bcl-2 and Bcl-xL, compounds **18**-**22** have IC_{50} values of >10 μM in the H146 cell line in the cell growth inhibition assay, further emphasizing that very high binding affinities are needed in order for small molecule inhibitors of Bcl-2 and Bcl-xL to achieve potent cellular activity.

Judged by the binding affinities to Bcl-2 and Bcl-xL and the cellular activity in the H146 cell line, compounds **4** and **12** are two of the most potent and promising compounds and, accordingly, we focused on these two compounds in our further optimization.

Based upon the co-crystal structure of compound **4** complexed with Bcl-xL (Figure 2), the *N*-methyl group in **4** inserts into a hydrophobic pocket in Bcl-xL, which can, however, accommodate a hydrophobic group larger than methyl. Indeed, our previous study showed that replacement of the *N*-methyl group in **4** ($R_2 = CH_3$, Table 3) with either an *N*-ethyl or *N*-isopropyl group yielded analogues **5** and **28** with high binding affinities to Bcl-2/Bcl-xL and significantly improved cellular activity (Table 3).[8] In fact, compounds **5** and **28** are 8- and 20-times more potent than compound **4** in inhibition of cell growth in the H146 cell line.

To probe this site further and to identify the optimal group for binding to Bcl-2/Bcl-xL and cellular activity, we designed and synthesized compounds **29** and **30** in which the *N*-methyl group in compound **4** was replaced by either an *N*-n-propyl or *N*-n-butyl group, respectively. Although both **29** and **30** have high binding affinities to Bcl-2 and Bcl-xL, they are several times less potent than compounds **5** and **28** in their binding to Bcl-2. Consistent with their weaker affinities to Bcl-2, compounds **29** and **30** are >5- and >10-times less potent than compounds **5** and **28**, respectively, in inhibition of cell growth in the H146 cell line. Our data therefore showed that the *N*-ethyl and *N*-isopropyl groups at this site in compounds **5** and **28** are most optimal for achieving high binding affinities to Bcl-2 and Bcl-xL and potent cell growth inhibition of the H146 cell line.

Since the methylsulfonylamide group can effectively replace the acid group in compound **4** and retain high binding affinities to Bcl-2/Bcl-xL and potent cellular activity, we next synthesized compounds **31** and **32** in which the acid group in compounds **5** and **28** was replaced by a methylsulfonylamide group (Table 3). As shown in Table 3, compounds **31** and **32** show high binding affinities to both Bcl-2 and Bcl-xL proteins and are very potent in inhibition of cell growth in the H146 cell line, and achieve IC_{50} values of 4.8 nM and 1.3 nM, respectively. To assess their binding specificity, we tested compounds **31** and **32** for their binding affinity to Mcl-1 in our optimized FP assay (Table 3).[7] Our data showed that compounds **31** and **32** have no appreciable binding to Mcl-1 at concentrations as high as 2 μM. Hence, compounds **31** and **32** are potent and specific Bcl-2 and Bcl-xL inhibitors.

Based upon their high binding affinities to Bcl-2 and Bcl-xL and potent cell growth inhibitory activity in the H146 cell line, **31** and **32** represent promising new lead compounds for further *in vivo* evaluation for their therapeutic potential.

First, we evaluated the maximum tolerated dose (MTD) of both compounds in severe combined immunodeficient (SCID) mice. Both compounds administered intravenously (*i.v.*) in mice at 15 mg/kg, daily, 5 days a week for 2 weeks, were found to be well tolerated and

did not cause significant weight loss (<5%) or other signs of toxicity during and after the treatment. However, at 25 mg/kg, both compounds caused significant weight loss (~10%) and, therefore, we concluded that 15 mg/kg dosed intravenously is the MTD for both drugs in SCID mice.

We next tested compounds **31** and **32** for their ability to induce apoptosis at the MTD in H146 xenograft tumors in SCID mice. A single dose of either compound at 15 mg/kg was administered *i.v.* to SCID mice bearing the H146 xenograft tumors. Animals were sacrificed at 3 h, 6 h and 24 h time points, and tumor tissues were removed for western blot analysis for cleavage of Poly ADP ribose polymerase (PARP) and caspase-3, two critical biochemical apoptosis markers. The data in Figure 3 showed that both compounds induce robust cleavage of PARP and caspase-3 at both 3 and 6-hr time-points in H146 tumor tissues, indicative of strong apoptosis induction *in vivo*.

Based upon the strong apoptosis induction *in vivo* observed for both **31** and **32**, we evaluated their antitumor efficacy in the H-146 xenograft tumor model, and the results are shown in Figure 4. Both compounds **31** and **32** show strong antitumor activity. While compound **31** achieves only partial tumor regression, **32** is capable of achieving rapid, complete and persistent tumor regression. At day 62, 18 days after the treatment was ended, none of the 8 mice treated with **32** had measurable tumors, and at day 117, 74 days after the end of the treatment with **32**, four mice (50%) did not have measurable tumors. The average tumor size for the 8 mice was 47 mm^3 at day 117, as compared to 130 mm^3 at the start of the treatment (day 33). Furthermore, all the mice treated with compound **32** suffered no significant weight loss (<5%) and did not show other signs of toxicity during or after the treatment. In our previous study, we had tested compound **5** for its antitumor activity in the H146 xenograft model and found that while **5** effectively inhibited tumor growth at 25 mg/kg, *i.v.*, 5 days *per* week for 2 weeks, it failed to achieve long-lasting tumor regression.[8] Hence, compound **32** at 15 mg/kg is considerably more effective than compound **5** at 25 mg/kg in inducing complete and persistent tumor regression in the H146 xenograft model.

To further define their anticancer activity, we next tested compounds **31** and **32** in three additional small-cell lung cancer cell lines, known to be sensitive to **1** and **2**, and the data are summarized in Table 4. While all four compounds effectively inhibit cell growth in these three cancer cell lines, compound **32** is the most potent of the compounds. Compound **32** has IC_{50} values of 1.0 nM, 1.4 nM and 2.3 nM in these three cancer cell lines and is >10-times more potent than **2**, and >50-times more potent than **1**, against each of these three cell lines, based upon their IC_{50} values.

Chemistry

Initially, we employed a convergent synthesis[7] (**Method A**) for the preparation of the target molecules. The piperazine **38**, embedded in our target molecules, was prepared in three steps starting with the reaction of the aniline (**33**) and 4-fluoro-3-nitrobenzene-1-sulfonyl chloride (**34**), forming the sulfonamide (**35**).[7] Displacement of the activated fluorine in **35** with (*R*)-N^1,N^1-dimethyl-4-(phenylthio)butane-1,3-diamine (**36**) and acid removal of the Boc protecting group generated **38**. The second fragment was designed as a variable scaffold, in which diversity could be built upon to develop compounds for SAR. These scaffolds consisted of various 5-membered hetero-aromatic rings with a 4-chlorophenyl and as a synthetic handle, an adjacent 3-iodophenyl group, to which **38** could be attached. Accordingly, in this methodology, **38** was subjected to Buchwald-Hartwig coupling[7, 10] with the synthesized scaffolds **9a**, and **18a-21a** furnishing **9** and **18-21**, respectively. However, this synthetic route was complicated by poor yields of the target molecules, especially when

the scaffolds contained a carboxylic acid or bioisosteres and, ultimately, this precluded further use of this methodology for further studies.

Consequently, a stepwise synthesis[7, 8] (**Method B**), applicable to all scaffolds was carried out. In this methodology, (4-nitrophenyl)piperazine (**39**) was subjected to Ullman coupling[11] with the synthetic scaffolds 4b, 5b, 12a, 13a, 14a, 15a, 16b, 17a, 22a, 23b, 24b, 27a, 28b, 29a, 30a, 31a, and **32a** producing intermediates 4c, 5c, 12b, 13b, 14b, 15b, 16c, 17b, 22b, 23c, 24c, 27b, 28c, 29b, 30b, 31b, and **32b**, respectively. Intermediates **25a** and **26a** were obtained using the method described in Scheme 6. Base hydrolysis of ethyl ester intermediates **17b** and **27b**, or low temperature acid hydrolysis of the *tert*-butyl ester intermediates **29b** and **30b**, gave the corresponding carboxylic acids. Reduction of the nitro groups in these intermediates (17b-carboxylic acid, 27b-carboxylic acid, 29b-carboxylic acid, 30b-carboxylic acid, 4c, 5c, 12b, 13b, 14b, 15b, 16c, 22b, 23c, 24c, 25a, 26a, 28c, 31b, and **32b**) by catalytic hydrogenation led to aniline intermediates that were subjected to the same synthetic strategy used for the preparation of **37**, generating the target compounds **4, 5, 12-17** and **22-32**. Decarboxylation of **4** by acid treatment generated target compound **7**. Target compounds **8, 10**, and **11** were obtained by EDCI-mediated coupling to carboxylic acid **4** of methylamine, (1*s*,3*s*)-3-amino-1-methylcyclobutanol, and 3-methylazetidine-3-ol, respectively.

Preparation of the 2-methylfuran scaffold **27a**, and the variously substituted 1*H*-pyrrole scaffolds 4b, 5b, 9a, 12a, 13a, 14a, 15a, 16b, 28b, 29a, 30a, 31a, 32a is outlined in Scheme 2. Intermediates **44a-d** were prepared using an established synthetic strategy[8] in which condensation of the β-ketoesters **40a-c** with 3-iodobenzaldehyde (**41a**) produced intermediates **42a-c**, respectively, and the bromo-analogue (**42d**) was prepared by the condensation of β-ketoester (**40a**) with 3-bromobenzaldehyde (**41b**). Compounds **42a-d** were subjected to a Stetter reaction with 4-chlorobenzaldehyde furnishing **44a-d**.[8, 12] The furan intermediate (**27a**) was prepared by heating **44a** with HCl, and AcOH in EtOH. The 2-trifluoromethyl-1*H*-pyrrole intermediate (**16a**) was prepared by condensation of intermediate (**44b**) with methylamine. Paal-Knorr cyclization of **44a** with methylamine, of **44d** with methylamine, and **44c** with ethylamine, *n*-propylamine, *n*-butylamine, or *iso*-propylamine furnished pyrroles **4a, 46, 5a, 28a, 29a**, and **30a**, respectively.[13] Base hydrolysis of the ethyl ester intermediates **4a, 16a** or low temperature acid hydrolysis of the *tert*-butyl ester intermediates **5a**, **28a** furnished the corresponding 1*H*-pyrrole-3-carboxylic acid scaffolds **4b, 16b** and **5b, 28b**, respectively.[8]

EDCI-catalyzed coupling of ammonia to the carboxylic acid **4b** furnished the 1*H*-pyrrole-3-carboxamide scaffold **9a**, but the same conditions failed to produce the N-(methylsulfonyl)-carboxamide (**12a**) which was instead prepared by formation of the acid chloride of **4b** and heating it with excess methanesulfonamide. In a similar manner used for the preparation of **12a**, **31a** and **32a** were prepared from **5b** and **28b**, respectively. Scaffold **13a** was prepared in two steps starting with the acid-mediated decarboxylation of **4b** followed by chlorination at the 3-position with *N*-chlorosuccinamide (NCS). The 3-trifluoromethyl-1*H*-pyrrole scaffold **14a** was prepared from **46** in three steps. Acid-mediated decarboxylation of **46** generated intermediate **47** which was iodinated at the 3-position with *N*-iodosuccinamide (NIS), producing **48**. A trifluoromethyl group was selectively installed at the 3-position of the iodopyrrole by Cu(I) catalyzed coupling with methyl 2,2-difluoro-2-(fluorosulfonyl)acetate, producing **14a**.[14] The 1*H*-pyrrole-3-carbonitrile scaffold **15a** was prepared by dehydration of pyrrole-3-carboxamide **9a**.[15]

Imidazole scaffolds **18a** and **23b** were prepared as outlined in Scheme 3. The imidazole **18a** was synthesized by a two-step 1,3-dipolar addition of toluenesulfonylmethyl isocyanide (TosMIC) with the imine **50**.[16] Compound **50** was prepared by reaction of 4-chloroaniline

(**49**) with **41a** under Dean-Stark conditions.[16] The imidazole (**23b**) was prepared using a procedure described by Yang, et al., for the construction of imidazol[1,5-a][1,4]benzodiazapines.[17] EDCI coupling of **49** with 3-iodobenzoic acid (**51**) generated the amide **52**, which was treated with diethyl chlorophosphate to generate the unstable iminophosphate which was condensed under basic conditions with ethyl isocyanoacetate to produce the imidazole **23a**.[17] Base hydrolysis of the ethyl ester (**23a**) produced its corresponding acid that was converted to its acid chloride and reacted with methanesulfonamide to generate the imidazole scaffold (**23b**).

The pyrazole scaffolds (**19a** and **24b**) were prepared using a previously established method.[18] Treatment of **53a** or **53b** with *N,N*-dimethylformamide dimethylacetal (**54**) generated the enaminones (**55a** and **55b**) which, condensed with 4-chlorophenylhydrazine (**56**), produced the pyrazoles (**19a** and **24a**), respectively.[18] Base hydrolysis of the ethyl ester (**24a**) produced the corresponding carboxylic acid that was converted to its acid chloride and reacted with methanesulfonamide to produce the pyrazole scaffold **24b**.

The pyrazoles **20a** and **21a** and the isoxazole **22a** were prepared from a common intermediate, the enaminone (**59**). 3-Iodophenylacetic acid (**57**) was converted to its acid chloride by refluxing in thionyl chloride. Friedel-Craft acylation of chlorobenzene with 3-iodophenylacetyl chloride, using a standard method[19], resulted in removal of the iodine atom from **58** and, to avoid this problem, the reaction was diluted and carried out at 0°C, producing compound **58** as the major product. Treatment of ketone **58** with **54** produced the enaminone (**59**). Condensation of methyl hydrazine with **59** produced a mixture of 2 regio-isomers that were separated to give the pyrazoles (**20a** and **21a**).[20] Condensation of **59** with hydroxylamine produced only one isoxazole (**22a**).[21]

The 2-chloropyrrole (**17a**) was prepared in four steps starting from intermediate **58**. Compound **58** was treated with Br_2 in AcOH to produce the α-bromoketone (**60**), reacted with ethyl 2-cyanoacetate under basic conditions to produce compound **61**,[22] treatment of which with 4M HCl in 1,4-dioxane formed the 2-chloropyrrole (**62**), which was methylated with CH_3I under basic conditions to give the ethyl ester (**17a**).[22]

The pyrazole acids (**25a** and **26a**) were synthesized following the procedure outlined in Scheme 6. Condensation of **63** with methyl hydrazine produced two regio-isomers, (**64a** and **64b**),[23] which were separated and were treated separately with NIS, producing **65a** and **65b**.[24] In parallel, the pinacol boronic ester (**68**) was prepared by displacement of the activated fluorine in 4-fluoronitrobenzene (**67**) with the piperazine (**66**). This boronic ester was subjected to Suzuki coupling with **65a** or **65b**.[24] The Suzuki coupling condition also resulted in hydrolysis of the methyl ester and furnished the pyrazole acids **25a** and **26a**.

Summary

In the present study, we have optimized compound **4**, which is a potent Bcl-2/Bcl-xL inhibitor but was unable to achieve tumor regression in animal models of human cancer. In contrast to our previous study[9], in which our modifications were focused on the nitro group and the soluble thiophenyl-containing “tail” group in compound **4**, the present study was centered on the pyrrole core structure, which has a significant effect on binding affinities to Bcl-2/Bcl-xL and cellular activity in cancer cells. Our optimization efforts have yielded compound **32** which binds to Bcl-2 and Bcl-xL with K_i values <1 nM. Similar to other initial lead compounds, compound **32** does not show any appreciable binding to Mcl-1 protein at concentrations as high as 2 μM. Compound **32** potently inhibits cancer cell growth in the H146 small-cell lung cancer cell line and achieves an IC_{50} value of 1.3 nM. Compound **32** also potently inhibits cell growth inhibition, with IC_{50} values of 1.0-2.3 nM,

in three other small-cell lung cancer cell lines which were known to be sensitive to **1** and **2**. In direct comparison, compound **32** is >10- and >50-times more potent than **2** and **1**, respectively, against these cancer cell lines. Significantly, compound **32** achieves rapid, complete and persistent tumor regression in the H146 xenograft tumor model in mice at a well-tolerated dose-schedule. Taken together, these data show that compound **32** is arguably the most potent and efficacious Bcl-2/Bcl-xL inhibitor reported to date and warrants extensive evaluation as a potential clinical development candidate for the treatment of human cancer.

Experimental

General Information—Unless otherwise stated, all reactions were performed under a nitrogen atmosphere in dry solvents under anhydrous conditions and all commercial reagents were used as supplied without further purification. NMR spectra were obtained on a Bruker 300 UltraShield spectrometer at a ^{1}H frequency of 300 MHz and ^{13}C frequency of 75 MHz. Chemical shifts (δ) are reported in parts per million (ppm) relative to an internal standard. The final products were purified by a C18 reverse phase semi-preparative HPLC column with solvent A (0.1% of TFA in water) and solvent B (0.1% of TFA in CH_3CN) as eluents. All final compounds have purity ≥ 95% as determined by Waters ACQUITY UPLC. Synthesis of compounds **4**, **5**, **7**, **8**, **28** and their intermediates were reported previously.[8]

Ethyl 2-acetyl-4-(4-chlorophenyl)-3-(3-iodophenyl)-4-oxobutanoate (44a)[8]: Ethyl acetoacetate **44a** (2.27g, 17.4 mmol), 3-iodobenzaldehyde **41a** (4.04g, 17.4 mmol), piperidine (70 μL), and acetic acid (200 μL) were dissolved in toluene (10 ml) and refluxed with azeotropic removal of water using a Dean-Stark apparatus. After reaction overnight, the solution was cooled, diluted with EtOAc, washed sequentially with 1.0M HCl, saturated sodium bicarbonate, and brine, then dried over sodium sulfate. Purification by column chromatography (9:1 hexane:EtOAc) provided 5.3 g of the product **42a** as a mixture of isomers. Triethylamine (1.55 mL) was added to a slurry of **42a** (5.3 g, 15.4 mmol), 4-chlorobenzaldehyde **43** (2.16 g, 15.4 mmol), and 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (0.583 g, 2.3 mmol) and the mixture was stirred with heating at 70 °C until **42a** was consumed. After cooling to room temperature, the mixture was diluted with EtOAc, washed sequentially with 1.0M HCl, saturated sodium bicarbonate, and brine, and dried over sodium sulfate. The EtOAc was removed *in vacuo* and provided 7.5 g of crude **44a** which was used without further purification.

Ethyl 5-(4-chlorophenyl)-4-(3-iodophenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxylate (4a)[8]: A 2.0M solution of methylamine in MeOH (35 mL, 70 mmol) was added to compound **44a** (7.5 g, 15.4 mmol) dissolved in 5 mL of MeOH. After 24 hours the solution was acidified with 1.0 M HCl and the compound was extracted with EtOAc. The EtOAc solution was washed with brine, dried over sodium sulfate and concentrated *in vacuo* to provide crude **4a**. Purification by column chromatography (5:1 hexane:EtOAc) provided 4.9 g (59 % after 3 steps) of **4a**.

Ethyl 5-(4-chlorophenyl)-4-(3-iodophenyl)-2-methylfuran-3-carboxylate (27a): Concentrated HCl (25 mL), and glacial acetic acid (5 mL) were added to a solution of **44a** (1.0 g, 2.06 mmol) in EtOH (25 mL) and the mixture was heated to 70 °C. After 4 hours, the reaction was quenched with saturated sodium bicarbonate, extracted with EtOAc, washed with brine, dried over sodium sulfate, filtered, and the EtOAc was removed *in vacuo* to produce crude **27a**. Purification by column chromatography provided 834 mg (96% yield) of **27a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.74-7.65 (m, 2H), 7.29-7.17 (m, 5H), 7.11 (t, J = 7.74 Hz, 1H), 4.09 (q, J = 7.13 Hz, 2H), 2.68 (s, 3H), 1.06 (t, J = 7.15 Hz, 3H); ^{13}C-NMR

(75 MHz, $CDCl_3$) δ ppm 163.83, 159.06, 146.84, 139.22, 136.78, 135.95, 133.65, 130.11, 129.63, 128.88(2C), 128.59, 126.93(2C), 121.23, 115.78, 94.03, 60.22, 14.39, 14.08.

Ethyl 2-(2-(4-chlorophenyl)-1-(3-iodophenyl)-2-oxoethyl)-4,4,4-trifluoro-3-oxobutanoate (44b): Starting with the ethyl trifluoroacetoacetate (**40b**), compound **44b** was prepared according to the procedure described for the preparation of compound **44a**.

Ethyl 5-(4-chlorophenyl)-4-(3-iodophenyl)-1-methyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylate (16a): Starting with **44b**, compound **16a** was prepared in a similar manner described for the preparation of compound **4a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.54-7.45 (m, 2H), 7.35 (d, J = 8.51 Hz, 2H), 7.10 (d, J = 8.50 Hz, 2H), 7.01-6.85 (m, 2H), 4.22 (q, J = 7.14 Hz, 2H), 3.58 (s, 3H), 1.18 (t, J = 7.13 Hz, 3H); ESI-MS m/z 533.83 $(M+H)^+$.

***tert*-Butyl 2-acetyl-4-(4-chlorophenyl)-3-(3-iodophenyl)-4-oxobutanoate (44c):** Starting with *tert*-butyl acetoacetate (**40c**), compound **44c** was prepared according to the procedure described for the preparation of compound **44a**.

***tert*-Butyl 5-(4-chlorophenyl)-1-ethyl-4-(3-iodophenyl)-2-methyl-1*H*-pyrrole-3-carboxylate (5a):** Ethylamine (11.7 mL, 2.0 M in MeOH, 23.40 mmol) was added to a solution of compound **44c** (3.0 g, 5.85 mmol) in MeOH (20 mL). After 24 hours, the solution was acidified with 1.0 M HCl, stirred briefly and the compound was extracted with EtOAc. The EtOAc solution was washed sequentially with saturated $NaHCO_3$, brine, then dried over Na_2SO_4 and concentrated *in vacuo* to provide crude **5a**. Purification by column chromatography (using a gradient of hexane and DCM, the product eluted at 100% DCM) provided 1.30 g (43% yield) of **5a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.53 (t, J = 1.63 Hz, 1H), 7.44 (dt, J = 1.39, 7.76 Hz, 1H), 7.26 (d, J = 8.50 Hz, 2H), 7.07 (d, J = 8.49 Hz, 2H), 6.93 (dt, J = 1.34, 7.66 Hz, 1H), 6.84 (t, J = 7.72 Hz, 1H), 3.80 (q, J = 7.18 Hz, 2H), 2.61 (s, 3H), 1.27 (s, 9H), 1.14 (t, J = 7.18 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 165.26, 140.07, 138.85, 135.37, 134.70, 134.12, 132.78(2C), 130.50, 129.73, 129.66, 129.16, 128.75(2C), 122.64, 113.05, 93.37, 79.83, 39.18, 28.26(3C), 16.25, 11.47.

***tert*-Butyl 5-(4-chlorophenyl)-4-(3-iodophenyl)-1-isopropyl-2-methyl-1*H*-pyrrole-3-carboxylate (28a):** Starting with *iso*-propylamine and **44c**, compound **28a** was prepared according to the procedure described for the preparation of compound **5a**. In this case the reaction was heated in a sealed tube. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.50 (t, J =1.39 Hz, 1H), 7.42 (dt, J = 1.30, 7.67 Hz, 1H), 7.25 (d, J = 8.41 Hz, 2H), 7.05 (d, J = 8.39 Hz, 2H), 6.92-6.87 (m, 1H), 6.82 (t, J = 7.69 Hz, 1H), 4.33 (sept, J = 7.07 Hz, 1H), 2.69 (s, 3H), 1.41 (d, J = 7.09 Hz, 6H), 1.25 (s, 9H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 165.35, 139.97, 139.05, 135.17, 134.57, 134.14, 133.16(2C), 131.21, 130.26, 129.53, 129.11, 128.56(2C), 122.48, 113.91, 93.34, 79.83, 48.81, 28.18(3C), 22.48(2C), 13.06.

***tert*-Butyl 5-(4-chlorophenyl)-4-(3-iodophenyl)-2-methyl-1-propyl-1*H*-pyrrole-3-carboxylate (29a):** Starting with *n*-propylamine and **44c**, compound **29a** was prepared according to the procedure described for the preparation of compound **5a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.53 (t, J = 1.56 Hz, 1H), 7.46-7.41 (m, 1H), 7.25 (d, J = 8.48 Hz, 2H), 7.05 (d, J = 8.49 Hz, 2H), 6.94-6.89 (m, 1H), 6.84 (t, J = 7.69 Hz, 1H), 3.75-3.66 (m, 2H), 2.59 (s, 3H), 1.51 (sex, J = 7.69 Hz, 2H), 1.27 (s, 9H), 0.75 (t, J = 7.42 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 165.28, 140.07, 138.86, 135.71, 134.66, 133.99, 132.76(2C), 130.55, 130.00, 129.65, 129.15, 128.71(2C), 122.53, 112.94, 93.37, 79.81, 45.96, 28.23(3C), 24.24, 11.67, 11.28.

***tert*-Butyl 1-butyl-5-(4-chlorophenyl)-4-(3-iodophenyl)-2-methyl-1*H*-pyrrole-3-carboxylate (30a):** Starting with *n*-butylamine and **44c**, compound **30a** was prepared according to the procedure described for the preparation of compound **5a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.53 (s, 1H), 7.43 (d, J = 7.75 Hz, 1H), 7.25 (d, J = 8.38 Hz, 2H), 7.05 (d, J = 8.40 Hz, 2H), 6.92 (d, J = 7.60 Hz, 1H), 6.84 (t, J = 7.70 Hz, 1H), 3.79-3.69 (m, 2H), 2.60 (s, 3H), 1.47 (quin, J = 7.68 Hz, 2H), 1.27 (s, 9H), 1.14 (sex, J = 7.28 Hz, 2H), 0.78 (t, J = 7.31 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 165.27, 140.07, 138.87, 135.66. 134.65, 133.98, 132.78(2C), 130.53, 129.97, 129.65, 129.13, 128.68(2C), 122.53, 112.95, 93.35, 79.79, 44.15, 32.98, 28.23(3C), 19.97, 13.71, 11.66.

Ethyl 2-acetyl-3-(3-bromophenyl)-4-(4-chlorophenyl)-4-oxobutanoate (44d): Starting with 3-bromobenzaldehyde (**41b**) and ethyl acetoacetate (**40a**), compound **44d** was prepared according to the procedure described for the preparation of compound **44a**.

Ethyl 4-(3-bromophenyl)-5-(4-chlorophenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxylate (46): Starting with **44d**, compound **46** was prepared in the manner described for the preparation of compound **4a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.31 (s, 1H), 7.29-7.20 (m, 3H), 7.07-6.90 (m, 4H), 4.07 (q, J = 7.1 Hz, 2H), 3.39 (s, 3H), 2.61 (s, 3H), 1.03 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 165.55, 138.25, 136.80, 134.06, 133.84, 132.45(2C), 130.55, 129.96, 129.39, 128.93, 128.80, 128.68(2C), 122.51, 121.17, 111.21, 59.39, 31.92, 14.02, 11.87.

5-(4-Chlorophenyl)-4-(3-iodophenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxylic acid (4b)[8]: NaOH (8.2 g, 204.3 mmol) was added to a solution of **4a** (4.9 g, 10.2 mmol) in 300 ml of 1:1:1 dioxane, EtOH, and H_2O, and the solution was heated at reflux until no compound **4a** was observed by TLC. After cooling, the reaction was slowly neutralized with 1M HCl and the compound was extracted with EtOAc. The EtOAc solution was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to produce compound **4b** as a pale solid which was not further purified.

5-(4-Chlorophenyl)-4-(3-iodophenyl)-1-methyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (16b): Compound **16b** was prepared according to the procedure described for the preparation of **4b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.55-7.41 (m, 2H), 7.35 (d, J = 8.46 Hz, 2H), 7.09 (d, J = 8.42 Hz, 2H), 7.06-6.99 (m, 1H), 6.92 (t, J = 7.76 Hz, 1H), 3.59 (s, 3H). ESI-MS m/z 505.83 $(M+H)^+$.

5-(4-Chlorophenyl)-4-(3-iodophenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxamide (9a): Ammonia (1.5 mL, 0.5M in 1,4-dioxane, 0.73 mmol) was added to a solution of **4b** (164 mg, 0.36 mmol), EDCI (104 mg, 0.54 mmol), HOBt (70 mg, 0.54 mmol), and DIEA (188 μL, 1.08 mmol) in 4 mL of DCM. After stirring overnight, the solvent was removed *in vacuo* and the crude was purified by column chromatography (the compound eluted at a 5:1 to 1:1 ratio of DCM:EtOAc) to give 145 mg (90 % yield) of **9a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.60-7.51 (m, 2H), 7.27 (d, J = 8.49 Hz, 2H), 7.13-7.07 (m, 1H), 7.03 (d, J = 8.50 Hz, 2H), 6.96 (t, J = 7.72 Hz, 1H), 5.40-4.91 (m, 2H), 3.40 (s, 3H), 2.63 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 167.64, 139.85, 137.42, 136.19, 135.65, 134.10, 132.47(2C), 130.37, 130.24, 130.20, 129.98, 128.84(2C), 120.07, 113.60, 94.38, 31.89, 11.82. ESI-MS m/z 451.25 $(M+H)^+$.

5-(4-Chlorophenyl)-4-(3-iodophenyl)-1,2-dimethyl-*N*-(methylsulfonyl)-1*H*-pyrrole-3-carboxamide (12a): Oxalyl chloride (40 μL, 0.44 mmol) followed by DMF (catalytic) was added to a solution of the carboxylic acid **4b** (100 mg, 0.22 mmol) in DCM (4 mL) and heated under reflux for 30 min. After cooling, DCM and excess oxalyl chloride were

removed *in vacuo* to produce the corresponding acid chloride. The resulting solid was re-dissolved in 1,2-DCE (5 mL), then methanesulfonamide (104 mg, 1.1 mmol) and DMAP (13 mg, 0.11 mmol) were added, and the solution heated at reflux overnight. Then the solvent was removed *in vacuo* and the crude was purified by column chromatography. The compound eluted between a 1:1 ratio of hexanes:EtOAc to 100% EtOAc) to give 91 mg (79 % yield) of **12a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.63 (dt, J = 1.44, 7.73 Hz, 1H), 7.54 (t, J = 1.57 Hz, 1H), 7.30 (d, J = 8.49 Hz, 2H), 7.20 (bs, 1H), 7.12 (dt, J = 1.41, 7.62 Hz, 1H), 7.09-7.00 (m, 3H), 3.42 (s, 3H), 3.27 (s, 3H), 2.64 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.09, 139.80, 138.67, 137.28, 135.82, 134.65, 132.38(2C), 131.16, 130.83, 130.06, 129.11, 129.03(2C), 119.86, 111.60, 94.91, 42.03, 32.10, 12.04. ESI-MS m/z 528.92 $(M+H)^{+}$.

5-(4-Chlorophenyl)-1-ethyl-4-(3-iodophenyl)-2-methyl-*N*-(methylsulfonyl)-1*H*-pyrrole-3-carboxamide (31a): Concentrated H_2SO_4 (2 mL) was added to a cooled (0 °C) solution of **5a** (620 mg, 1.19 mmol) in a mixture of DCM (5 mL) and THF (2 mL). After 10 min, the reaction was slowly quenched with saturated $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to produce 569 mg of its carboxylic acid **5b** as a white solid. Oxalyl chloride (222 μL, 2.44 mmol) followed by DMF (catalytic amount, ~ 10 drops) were added to a solution of the carboxylic acid (569 mg, 1.22 mmol) in DCM (7 mL) and heated to reflux for 30 min. After cooling, DCM and excess oxalyl chloride were removed *in vacuo* to produce the corresponding acid chloride. The resulting solid was redissolved in 1,2-DCE (10 mL), then methanesulfonamide (580 mg, 6.1 mmol), DMAP (75 mg, 0.61 mmol) were added and the solution heated under reflux overnight. After this time, the solvent was removed *in vacuo* and the crude was purified by column chromatography (the compound eluted between a 1:1 ratio of hexanes:EtOAc to 100% EtOAc) to give 526 mg (82% yield, 3 steps) of **31a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.60 (dt, J = 1.42, 7.82 Hz, 1H), 7.55 (t, J = 1.52 Hz, 1H), 7.31 (d, J = 8.44 Hz, 2H), 7.22 (bs, 1H), 7.14-6.99 (m, 4H), 3.83 (q, J = 7.18 Hz, 2H), 3.27 (s, 3H), 2.65 (s, 3H), 1.17 (t, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.17, 139.74, 137.64, 137.13, 135.74, 134.78, 132.59(2C), 130.72, 130.61, 129.96, 129.37, 128.99(2C), 120.20, 111.67, 94.81, 41.99, 39.44, 16.08, 11.77.

5-(4-Chlorophenyl)-4-(3-iodophenyl)-1-isopropyl-2-methyl-*N*-(methylsulfonyl)-1*H*-pyrrole-3-carboxamide (32a): Starting with **28a**, compound **32a** was prepared according to the procedure described for the preparation of compound **31a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.58 (d, J = 7.70 Hz, 1H), 7.52 (s, 1H), 7.29 (d, J = 8.37 Hz, 2H), 7.18 (bs, 1H), 7.11-7.04 (m, 3H), 7.01 (t, J = 7.68 Hz, 1H), 4.35 (sept, J = 7.02, 1H), 3.25 (s, 3H), 2.73 (s, 3H), 1.44 (d, J = 7.09 Hz, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.28, 139.66, 137.34, 136.93, 135.87, 134.76, 132.94(2C), 131.08, 130.62, 130.13, 129.83, 128.79(2C), 120.16, 112.50, 94.69, 49.26, 41.87, 22.33(2C), 13.29.

2-(4-Chlorophenyl)-3-(3-iodophenyl)-1,5-dimethyl-1*H*-pyrrole (45): Trifluoroacetic acid (3 mL) was added to a solution of **4b** (500 mg, 1.11 mmol) in DCM (2 mL). After standing overnight at room temperature, the reaction was slowly quenched with saturated $NaHCO_3$ and extracted with EtOAc. The combined organic layers was dried over Na_2SO_4, filtered and concentrated in vacuo to give crude **45**. Purification by column chromatography (using a gradient of hexanes: EtOAc) produced 223 mg (49% yield) of **45**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.56 (t, J = 1.6 Hz, 1H), 7.40-7.30 (m, 3H), 7.16 (d, J = 8.5 Hz, 2H), 6.99-6.93 (m, 1H), 6.82 (t, J = 7.8 Hz, 1H), 6.12 (s, 1H), 3.34 (s, 3H), 2.29 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 139.02, 136.75, 134.04, 133.76, 132.52(2C), 131.61, 130.18, 129.90, 129.05(2C), 127.02, 120.59, 106.85, 94.51, 31.60, 12.68.

3-Chloro-5-(4-chlorophenyl)-4-(3-iodophenyl)-1,2-dimethyl-1*H*-pyrrole (13a): *N*-chlorosuccinamide (91 mg, 0.68 mmol) was added to a solution of **45** (213 mg, 0.52 mmol) in DMF (3 mL). After overnight at room temperature, water was added and the mixture was extracted with EtOAc. The combined EtOAc layer was washed with brine, dried over Na_2SO_4, filtered and the solvent was removed *in vacuo* to give crude **13a**. Purification by column chromatography (using a gradient of hexanes: EtOAc) produced 145 mg (63% yield) of **13a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.58 (s, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.29 (d, J = 8.5 Hz, 2H), 7.11-7.01 (m, 3H), 6.91 (t, J = 7.8 Hz, 1H), 3.39 (s, 3H), 2.31 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 139.05, 136.16, 135.09, 133.92, 132.41(2C), 130.28, 129.77, 129.47, 129.01, 128.96(2C), 126.35, 118.83, 108.99, 93.98, 32.35, 10.28.

3-(3-Bromophenyl)-2-(4-chlorophenyl)-1,5-dimethyl-1*H*-pyrrole (47): Starting with **46** (300 mg, 0.69 mmol), 181 mg (72%) of compound **47** was obtained according to the procedure described for the preparation of **45**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.37-7.28 (m, 3H), 7.20-7.11 (m, 3H), 6.98-6.90 (m, 2H), 6.12 (s, 1H), 3.32 (s, 3H), 2.28 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 138.94, 133.75, 132.51(2C), 131.60, 130.64, 130.18, 129.72, 129.05(2C), 128.03, 126.38, 122.44, 120.69, 106.87, 31.56, 12.66.

3-(3-Bromophenyl)-2-(4-chlorophenyl)-4-iodo-1,5-dimethyl-1*H*-pyrrole (48): Starting with NIS (129 mg, 0.58 mmol) and **47** (173 mg, 0.48 mmol), 174 mg (75% yield) of compound **48** was obtained according to the procedure described for the preparation of **13a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.37-7.22 (m, 4H), 7.11-6.95 (m, 4H), 3.46 (s, 3H), 2.40 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 138.17, 133.83, 133.55, 132.27(2C), 132.02, 130.91, 130.49, 129.51, 129.39, 129.37, 128.85(2C), 124.02, 121.90, 33.27, 14.00.

3-(3-Bromophenyl)-2-(4-chlorophenyl)-1,5-dimethyl-4-(trifluoromethyl)-1*H*-pyrrole (14a): Methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (785 μL, 6.2 mmol) and copper (I) iodide (142 mg, 0.74 mmol) were added to a solution of **48** (300 mg, 0.62 mmol) in DMF (3 mL). The solution was placed under vacuum and flushed with nitrogen three times, then heated to 100 °C overnight. After cooling to room temperature, the reaction was slowly quenched with saturated ammonium chloride, then extracted with diethyl ether, and the extracted solution was washed with water, brine, dried over Na_2SO_4, filtered and concentrated to give crude **14a**. Purification by column chromatography (using a gradient of hexanes:EtOAc) provided 152 mg (57% yield) of **14a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.32-7.22 (m, 4H), 7.08-6.97(m, 4H), 3.40 (s, 3H), 2.43 (d, J = 1.4 Hz, 3H).

5-(4-Chlorophenyl)-4-(3-iodophenyl)-1,2-dimethyl-1*H*-pyrrole-3-carbonitrile (15a): To a solution of **9a** (86 mg, 0.19 mmol) and pyridine (31 μL, 0.38 mmol) in 1,4-dioxane (2 mL) at 0 °C was added trifluoroacetic anhydride (TFAA) (30 μL, 0.21 mmol) dropwise. After 3 h at room temperature, the reaction was quenched with water and extracted with EtOAc. The EtOAc solution was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to provide crude **15a**. Purification by column chromatography (gradient of hexanes:EtOAc) provided 65 mg (79%) of **15a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.56-7.46 (m, 2H), 7.36 (d, J = 8.39 Hz, 2H), 7.17-7.07 (m, 3H), 6.95 (t, J = 7.91 Hz, 1H), 3.40 (s, 3H), 2.47 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 139.18, 137.82, 135.83, 135.21, 134.87, 132.43, 130.42, 130.22, 129.32, 129.20, 128.28, 122.29, 116.88, 94.36, 91.91, 32.43, 12.09.

4-Chloro-*N*-(3-iodobenzylidene)aniline (50): A solution of 3-iodobenzaldehyde (4.1 g, 17.7 mmol), and 4-chloroaniline (2.25 g, 17.7 mmol) in toluene (70 mL) was heated to reflux in a Dean-Stark apparatus. After reaction overnight, the solvent was removed *in vacuo* and the crude product was used in the following reaction. ^{1}H-NMR (300 MHz,

$CDCl_3$) δ ppm 8.31 (d, J = 16.03 Hz, 2H), 7.81 (d, J = 7.71 Hz, 2H), 7.36 (d, J = 7.08 Hz, 2H), 7.24-7.09 (m, 3H).

1-(4-Chlorophenyl)-5-(3-iodophenyl)-1*H*-imidazole (18a): A solution of **50** (6.05 g, 17.7 mmol), toluenesulfonylmethyl isocyanide (5.2 g, 26.6 mmol), potassium carbonate (4.9 g, 35.4 mmol) in 120 mL of MeOH and 50 mL of DME was refluxed for two hours. After cooling to room temperature, the reaction was quenched with water and extracted with EtOAc. The EtOAc solution was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to provide crude **18a**. Purification by column chromatography (the compound eluted between 1:1 and 1:2 hexanes:EtOAc) provided 3.95 g (59%, 2 steps) of **18a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.68 (d, J = 1.04 Hz, 1H), 7.63-7.58 (m, 2H), 7.40 (d, J = 8.80 Hz, 2H), 7.28 (d, J = 1.06 Hz, 1H), 7.13 (d, J = 8.80 Hz, 2H), 7.01-6.96 (m, 2H).

***N*-(4-Chlorophenyl)-3-iodobenzamide (52):** 4-chloroaniline (2.06 g, 16.13 mmol) was added to a solution of 3-iodobenzoic acid **51** (2.0 g, 8.06 mmol), EDCI (2.32 g, 12.09 mmol), HOBt (1.56 g, 12.09 mmol), and DIEA (4.2 mL, 24.18 mmol) in 130 mL of DCM. After stirring overnight, the solvent was removed *in vacuo* and the crude was purified by column chromatography to give 2.4 g (83%) of **52**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.69 (s, 1H), 7.65 (d, J = 8.00 Hz, 1H), 7.40-7.24 (m, 5H), 7.07 (d, J = 7.74 Hz, 1H), 6.83 (s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 206.32, 144.03, 137.70, 137.59, 135.22, 134.86, 130.52, 129.26, 128.13, 127.69, 94.74.

Ethyl 1-(4-chlorophenyl)-5-(3-iodophenyl)-1*H*-imidazole-4-carboxylate (23a): Potassium *tert*-butoxide (0.516 g, 4.61 mmol) was added to a solution of **52** (1.5 g, 4.19 mmol), at 0 °C, in THF. After 20 min at 0 °C, the reaction was cooled to −35 °C and diethyl chlorophosphate (783 μL, 5.45 mmol) was added slowly. After 30 min at 0 °C, the reaction was cooled to −35 °C, then ethyl isocyanoacetate (504 μL, 4.61 mmol) and *t*-BuOK (0.516, 4.61 mmol) were added and the reaction was allowed to warm to room temperature. After 4 h, the reaction was quenched with saturated sodium bicarbonate and extracted with EtOAc. The EtOAc solution was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to provide crude **23a**. Purification by column chromatography (the compound eluted between 1:1 and 1:2 hexanes:EtOAc) provided 1.15 g (61%) of **23a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.76 (s, 1H), 7.69-7.63 (m, 2H), 7.34 (d, J = 8.68 Hz, 2H) 7.15 (d, J = 7.83 Hz, 1H), 7.10-6.98 (m, 3H), 4.28 (q, J = 7.12 Hz, 2H), 1.28(t, J = 7.13 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 162.37, 139.53, 137.81, 137.73, 135.86, 134.77, 133.65, 131.09, 129.97, 129.80, 129.74(2C), 129.48, 126.98(2C), 93.33, 60.56, 14.17. ESI-MS m/z 453.50 $(M+H)^+$.

1-(4-Chlorophenyl)-5-(3-iodophenyl)-*N*-(methylsulfonyl)-1*H*-imidazole-4-carboxamide (23b): Compound **23a** was converted to its corresponding carboxylic acid in the manner described for the preparation of **4b**. A solution of this carboxylic acid (300 mg, 0.71 mmol) in 3 mL of thionyl chloride was heated at reflux for 2 h. After cooling, thionyl chloride was removed in vacuo to produce the corresponding acid chloride. The resulting solid was re-dissolved in 10 mL of 1,2-DCE, followed by the addition of methane sulfonamide (337 mg, 3.55 mmol), and DMAP (43 mg, 0.355 mmol), and the solution was heated at reflux overnight. After this time, the reaction was cooled, water was added and extracted with EtOAc. The EtOAc solution was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to provide crude **23b**. Purification by column chromatography provided 195 mg (55% yield) of **23b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 9.57 (s, 1H), 7.69 (d, J = 7.92 Hz, 1H), 7.66 (s, 1H), 7.61 (s, 1H), 7.39 (d, J = 8.70 Hz, 2H), 7.11-6.99 (m, 3H), 3.35 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 160.19, 139.39, 138.67, 137.39, 136.27, 135.61, 133.53,

130.69, 130.23(2C), 130.17, 129.97, 128.81, 127.19(2C), 93.75, 42.00. ESI-MS m/z 502.67 $(M+H)^+$.

1-(4-Chlorophenyl)-5-(3-iodophenyl)-1*H*-pyrazole (19a): A solution of 3-iodoacetophenone **53a** (5.0 g, 20.3 mmol), and *N,N*-dimethylformamide dimethylacetal (41 mL, 304.8 mmol) in 200 mL of toluene was refluxed with azeotropic removal of water using a Dean-Stark apparatus. After 3 h, the solvent was removed *in vacuo* to give **55a** as an oil. A solution of crude **55a** (20.3 mmol), and 4-chlorophenylhydrazine hydrochloride (**56**) (4.0 g, 22.3 mmol) in ethanol was heated to reflux for 4 h. After cooling, EtOH was removed and the residue re-dissolved in EtOAc. The EtOAc solution was washed with H_2O, then brine, dried over Na_2SO_4, filtered and concentrated to give crude **19a**. Purification by column chromatography (hexanes:EtOAc) provided 6.48 g (84%, 2 steps) of **19a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.75-7.60 (m, 3H), 7.33 (d, J = 8.57 Hz, 2H), 7.23 (d, J = 8.61 Hz, 2H), 7.12-6.97 (m, 2H), 6.51 (d, J = 1.28 Hz, 1H).

Ethyl 5-(3-bromophenyl)-1-(4-chlorophenyl)-1*H*-pyrazole-4-carboxylate (24a): Starting with ethyl 3-(3-bromophenyl)-3-oxopropanoate **53b** (1.0 g, 3.69 mmol), 0.67 g (44%, 2 steps) of compound **24a** was prepared according to the procedure described for the preparation of **19a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.18 (s, 1H), 7.56-7.50 (m, 2H), 7.29 (d, J = 8.84 Hz, 2H), 7.22 (t, J = 7.68 Hz, 1H), 7.18-7.10 (m, 3H), 4.22 (q, J = 7.14 Hz, 2H), 1.23 (t, J = 7.14 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 162.70, 143.66, 142.89, 137.56, 134.18, 133.65, 132.56, 130.80, 129.82, 129.38, 129.19, 126.53, 122.21, 114.78, 60.51, 14.28.

5-(3-Bromophenyl)-1-(4-chlorophenyl)-*N*-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (24b): Starting with **24a**, compound **24b** was obtained according to the procedure described for the preparation of **23b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.17 (s, 1H), 7.64 (ddd, J = 1.24, 1.85, 7.91 Hz, 1H), 7.48 (t, J = 1.68 Hz, 1H), 7.38-7.27 (m, 4H), 7.14 (d, J = 8.88 Hz, 2H), 3.34 (s, 3H).

1-(4-Chlorophenyl)-2-(3-iodophenyl)ethanone (58): A solution of 3-iodophenylacetic acid (**57**) (2.0 g, 7.63 mmol) and thionyl chloride (3 mL) was refluxed for 2 h. After cooling to room temperature, excess thionyl chloride was removed *in vacuo*, producing 3-iodophenylacetyl chloride as a brown solid. The crude 3-iodophenylacetyl chloride was dissolved in chlorobenzene (10 mL) and added to a 0 °C solution of aluminum chloride (1.73 g, 12.97 mmol) and chlorobenzene (50 mL). After 2 h at 0 °C, the reaction was poured into ice and extracted with DCM. The DCM solution was washed with brine, dried over sodium sulfate and concentrated *in vacuo* to provide crude **58**. Purification by column chromatography (the compound eluted at 19:1 hexanes:EtOAc) provided 2.03 g (75%) of **58**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.93 (d, J = 8.68 Hz, 2H), 7.66-7.56 (m, 2H), 7.45 (d, J = 8.68 Hz, 2H), 7.21 (d, J = 7.82 Hz, 1H), 7.07 (t, J = 8.23 Hz, 1H), 4.20 (s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 195.82, 140.14, 138.55, 136.57, 136.39, 134.81, 130.57, 130.15, 129.31, 128.99, 94.82, 44.94.

1-(4-Chlorophenyl)-3-(dimethylamino)-2-(3-iodophenyl)prop-2-en-1-one (59): A solution of **58** (1.5 g, 4.21 mmol), and *N,N*-dimethylformamide dimethylacetal (**54**) (8.4 mL, 63.1 mmol) in 70 mL of toluene was heated to reflux with azeotropic removal of water using a Dean-Stark apparatus. After 3 h, the solvent was removed *in vacuo* to give **59** as an oil that was used without purification in the following reactions.

3-(4-Chlorophenyl)-4-(3-iodophenyl)-1-methyl-1*H*-pyrazole (21a) and 5-(4-chlorophenyl)-4-(3-iodophenyl)-1-methyl-1*H*-pyrazole (20a): A solution of **59** (1.1 g,

2.67 mmol), methylhydrazine (210 μL, 4.01 mmol) and EtOH (80 mL) was refluxed for 4 h. After cooling, EtOH was removed and the residue redissolved in EtOAc. The EtOAc solution was washed with water, then brine, dried over Na_2SO_4, filtered and concentrated to give a crude mixture of **21a** and **20a**. Purification by column chromatography (the compounds eluted between a solvent mixture of 4:4:0.1 and 4:4:0.2 DCM:hexanes:ethyl ether) eluted **21a** first, followed by **20a**. **21a** ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.66 (s, 1H), 7.59 (d, J = 7.87 Hz, 1H), 7.46 (s, 1H), 7.41 (d, J = 8.36 Hz, 2H), 7.29 (d, J = 8.48 Hz, 2H), 7.16 (d, J = 7.80 Hz, 1H), 7.01 (t, J = 7.74 Hz, 1H), 3.97 (s, 3H); **20a** ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.70 (s, 1H), 7.58 (s, 1H), 7.49 (d, J = 7.74 Hz, 1H), 7.44 (d, J = 8.33 Hz, 2H), 7.23 (d, J = 8.44 Hz, 2H), 7.01 (d, J = 7.82 Hz, 1H), 6.92 (t, J = 7.75 Hz, 1H), 3.77 (s, 3H).

5-(4-Chlorophenyl)-4-(3-iodophenyl)isoxazole (22a): A solution of **59** (300 mg, 0.73 mmol), hydroxylamine hydrochloride (56 mg, 0.801 mmol), sodium carbonate (108 mg, 1.02 mmol), MeOH (50 mL), H_2O (25 mL) and AcOH (1 mL) was heated to reflux for 4 h. After cooling to room temperature, H_2O was added and the mixture was extracted with EtOAc. The EtOAc solution was washed with sodium bicarbonate, then brine, dried over Na_2SO_4, filtered and concentrated to give crude **22a**. Purification by column chromatography (hexanes:EtOAc) provided 247 mg (89%) of **22a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.33 (s, 1H), 7.79-7.67 (m, 2H), 7.54 (d, J = 8.58 Hz, 2H), 7.36 (d, J = 8.59 Hz, 2H), 7.32 (d, J = 7.78 Hz, 1H), 7.13 (t, J = 7.77 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.43, 151.71, 137.44, 137.42, 136.59, 132.10, 130.84, 129.40, 128.63, 128.01, 125.75, 115.13, 94.98.

2-Bromo-1-(4-chlorophenyl)-2-(3-iodophenyl)ethanone (60): A solution of bromine (86 μL, 1.68 mmol) in acetic acid (4 mL) was added to a solution of **58** (500 mg, 1.40 mmol) in DCM (4 mL). After 3 h at room temperature, H_2O was added and the mixture was extracted with DCM. The extracted solution was washed sequentially with sodium bicarbonate, H_2O, and brine, then dried over Na_2SO_4, filtered and concentrated to give crude **60**. Purification by column chromatography (hexanes:EtOAc) provided 220 mg (36%) of **60**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.91 (d, J = 8.55 Hz, 2H), 7.87 (s, 1H), 7.63 (d, J = 7.91 Hz, 1H), 7.48 (d, J = 7.80 Hz, 1H), 7.40 (d, J = 8.53 Hz, 2H), 7.08 (t, J = 7.85 Hz, 1H), 6.21 (s, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 189.53, 140.55, 138.32, 137.96, 137.60, 132.17, 130.66, 130.61, 129.33, 128.66, 94.62, 48.74.

Ethyl 4-(4-chlorophenyl)-2-cyano-3-(3-iodophenyl)-4-oxobutanoate (61): A mixture of potassium carbonate (636 mg, 4.60 mmol) and ethyl cyanoacetate (1.71 mL, 16.07 mmol) was heated at 45 °C. After 45 min, the mixture was allowed to cool to room temperature, then a solution of **60** (1.0g, 2.30 mmol) in 5 mL of Me_2CO was added dropwise. After stirring overnight, H_2O was added and the solution was extracted with EtOAc. The extracted solution was sequentially washed with H_2O, and brine, then dried over Na_2SO_4, filtered and concentrated to give crude **61**. Purification by column chromatography (hexanes:EtOAc) provided 1.01 g (94%) of **61**.

Ethyl 2-chloro-5-(4-chlorophenyl)-4-(3-iodophenyl)-1*H*-pyrrole-3-carboxylate (62): To **61** (500 mg, 1.07 mmol) was added 6 mL of 4M HCl in 1,4-dioxane. After four hours, the reaction was slowly quenched with saturated sodium bicarbonate and the solution extracted with EtOAc. The extracted solution was washed with brine, dried over Na_2SO_4, filtered and concentrated to give crude **62**. Purification by column chromatography (the compound eluted with 100% DCM) provided 323 mg (62%) of **62**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 9.10 (s, 1H), 7.67-7.53 (m, 2H), 7.18 (d, J = 8.43 Hz, 2H), 7.13 (d, J = 7.67 Hz, 1H), 7.06-6.93 (m, 3H), 4.11 (q, 7.10 Hz, 2H), 1.08 (t, J = 7.11 Hz, 3H); ^{13}C-NMR (75 MHz,

$CDCl_3$) δ ppm 163.72, 139.73, 136.97, 136.18, 133.57, 130.06, 129.72, 129.27, 129.09, 128.51, 127.64, 122.19, 121.89, 112.37, 93.73, 60.40, 14.11.

Ethyl 2-chloro-5-(4-chlorophenyl)-4-(3-iodophenyl)-1-methyl-1*H*-pyrrole-3-carboxylate (17a): Potassium carbonate (274 mg, 1.98 mmol) was added to a solution of **62** (323 mg, 0.66 mmol) in DMF (3 mL). After 10 min at room temperature, iodomethane (86μL, 1.33 mmol) was added and the mixture stirred overnight. The reaction was slowly quenched with saturated ammonium chloride and extracted with EtOAc. The extracted solution was washed with brine, dried over Na_2SO_4, filtered and concentrated to give crude **17a**. Purification by column chromatography (the compound eluted at a solvent mixture between 1:1 to 3:1 of DCM:hexanes) provided a quantitative yield of **17a**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.58-7.45 (m, 2H), 7.28 (d, J = 8.44 Hz, 2H), 7.05 (d, J = 8.42 Hz, 2H), 6.98 (d, J = 7.70 Hz, 1H), 6.88 (t, J = 7.67 Hz, 1H), 4.13 (q, J = 7.12 Hz, 2H), 3.49 (s, 3H), 1.08 (t, J = 7.13 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.43, 139.87, 136.91, 135.49, 134.53, 132.38, 130.84, 129.94, 129.20, 129.02, 128.93, 123.51, 122.76, 110.84, 93.24, 60.05, 32.64, 14.14.

Methyl 3-(4-chlorophenyl)-1-methyl-1*H*-pyrazole-5-carboxylate (64b) and methyl 5-(4-chlorophenyl)-1-methyl-1*H*-pyrazole-3-carboxylate (64a): Methyl hydrazine (0.656 mL, 12.47 mmol) was added, dropwise, to a solution of methyl 4-(4-chlorophenyl)-2,4-dioxobutanoate **63** (2.0 g, 8.31 mmol), AcOH (2.4 mL), and EtOH (40 mL). After 3 h of stirring at room temperature, H_2O was added and the mixture extracted with EtOAc. The extracted solution was washed with saturated sodium bicarbonate, then brine, dried over Na_2SO_4, filtered and concentrated to give a crude mixture of **64a** and **64b**. Purification by column chromatography (using a gradient of hexanes:EtOAc where **64b** eluted at 9:1 and **64a** eluted between 4:1 to 1:1) provided 0.824 g (40%) of **64b** and 0.974 g (47%) of **64a**. **64b**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.71 (d, J = 8.57 Hz, 2H), 7.36 (d, J = 8.56 Hz, 2H), 7.07 (s, 1H), 4.21 (s, 3H), 3.90 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 160.32, 148.88, 133.96, 133.77, 131.24, 129.06(2C), 126.91(2C), 108.03, 52.17, 39.83. **64a**: 7.46 (d, J = 8.58 Hz, 2H), 7.37 (d, J = 8.59 Hz, 2H), 6.85 (s, 1H), 3.95 (s, 3H), 3.94 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 162.52, 143.87, 142.35, 135.23, 130.01(2C), 129.11(2C), 127.90, 108.92, 51.95, 38.27.

Methyl 3-(4-chlorophenyl)-4-iodo-1-methyl-1*H*-pyrazole-5-carboxylate (65b): *N*-iodosuccinamide (903 mg, 4.01 mmol), followed by ceric ammonium nitrate (17 mg, 0.0308 mmol) was added to a solution of **64b** (774 mg, 3.08 mmol) in MeCN (30 mL), and the mixture was heated at 70 °C overnight. After cooling to room temperature, the solvent was removed *in vacuo* and the residue re-dissolved in EtOAc. The EtOAc solution was washed with H_2O, then brine, dried over Na_2SO_4, filtered and concentrated to give crude **65b**. Purification by column chromatography (using a gradient of hexanes:EtOAc) provided 1.03 g (89% yield) of **65b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.71 (d, J = 8.61 Hz, 2H), 7.41 (d, J = 8.61 Hz, 2H), 4.23 (s, 3H), 3.97 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 159.73, 151.89, 135.04, 134.73, 131.05, 130.23(2C), 128.64(2C), 64.17, 52.31, 41.57.

Methyl 5-(4-chlorophenyl)-4-iodo-1-methyl-1*H*-pyrazole-3-carboxylate (65a): Starting with **64a** (974 mg, 3.89 mmol), compound **65a** (337 mg, 23% yield) was obtained according to the procedure described for the preparation of compound **65b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 7.52 (d, J = 8.34 Hz, 2H), 7.33 (d, J = 8.39 Hz, 2H), 3.97 (s, 3H), 3.89 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 161.80, 146.46, 142.35, 136.30, 131.68(2C), 129.41(2C), 127.37, 63.65, 52.31, 39.28.

1-(4-Nitrophenyl)-4-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)piperazine (68): Potassium carbonate (361 mg, 2.61 mmol) was added to a solution of 3-

piperazinylphenylboronic acid pinacol ester **66** (500 mg, 1.74 mmol) and 1-fluoro-4-nitrobenzene **67** (269 mg, 1.91 mmol) in DMSO (5 mL). After stirring overnight, the reaction was quenched with 2N HCl and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to produce crude **68**. Column chromatography, using a gradient of DCM:EtOAc, produced 238 mg (33% yield) of pure **68** as an orange oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.11 (d, J = 9.35 Hz, 2H), 7.45-7.27 (m, 3H), 7.09-7.02 (m, 1H), 6.82 (d, J = 9.41 Hz, 2H), 3.60-3.50 (m, 4H), 3.41-3.30 (m, 4H), 1.35 (s, 12H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 154.75, 150.17, 138.54, 128.82, 126.98, 126.00(2C), 122.44, 119.45, 112.76(2C), 83.90(2C), 48.97(2C), 47.06(2C), 24.95(4C). ESI-MS m/z 410.42 $(M+H)^+$.

5-(4-Chlorophenyl)-1-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-pyrazole-3-carboxylic acid (25a): $Pd(PPh_3)_4$ (10 mg) was added to a solution of **65a** (30 mg, 0.08 mmol), boronic ester **68** (65 mg, 0.16 mmol), and potassium carbonate (44 mg, 0.32 mmol) in DME/H_2O (9 mL, 2:1). The mixture was placed under vacuum, flushed with nitrogen twice, and heated to 90 °C overnight. After cooling to room temperature, saturated NH_4Cl was added and the mixture was extracted with EtOAc. The combined EtOAc layers were washed with brine, dried over Na_2SO_4, filtered through a plug of celite and the solvent was removed *in vacuo* to give crude **25a**. Purification by reverse phase HPLC provided pure **25a**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.15 (d, J = 9.17 Hz, 2H), 7.38 (d, J = 8.32 Hz, 2H), 7.22-7.12 (m, 3H), 6.96-6.82 (m, 4H), 6.78 (d, J = 7.54 Hz, 1H), 3.89 (s, 3H), 3.64-3.51 (m, 4H), 3.30-3.19 (m, 4H). ESI-MS m/z 518.50 $(M+H)^+$.

3-(4-Chlorophenyl)-1-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-pyrazole-5-carboxylic acid (26a): Starting with **65b**, compound **26a** was prepared according to the procedure described for the preparation of compound **25a**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.14 (d, J = 9.35 Hz, 2H), 7.33-7.16 (m, 5H), 6.97-6.77 (m, 5H), 4.23 (s, 3H), 3.62-3.50 (m, 4H), 3.32-3.21 (m, 4H). ESI-MS m/z 518.33 $(M+H)^+$.

5-(4-Chlorophenyl)-1,2-dimethyl-*N*-(methylsulfonyl)-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-pyrrole-3-carboxamide (12b): A mixture of **12a** (585 mg, 1.1 mmol), 1-(4-nitrophenyl)piperazine (916 mg, 4.43 mmol), copper (I) iodide (105 mg, 0.55 mmol), L-proline (127 mg, 1.1 mmol), and potassium carbonate (612 mg, 4.43 mmol) was placed under vacuum and then flushed with N_2 three times. To this mixture was added DMSO (13 mL), the solution was placed under vacuum and flushed with N_2 three times, then heated to 100 °C overnight. After cooling to room temperature, the reaction was slowly quenched with saturated ammonium chloride and stirred for 10 min. The solution was then extracted with DCM and the extracted solution was washed with saturated ammonium chloride, 2M hydrochloric acid, brine, dried over Na_2SO_4, filtered and concentrated to give crude **12b**. Purification by column chromatography (using a gradient of DCM:EtOAc) provided 491 mg (74%) of **12b** as a yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.15 (d, J = 9.36 Hz, 2H), 7.53 (bs, 1H), 7.32-7.21 (m, 3H), 7.07 (d, J = 8.40 Hz, 2H), 6.93 (dd, J = 2.21, 8.16 Hz, 1H), 6.88-6.78 (m, 3H), 6.70 (d, J = 7.49 Hz, 1H), 3.62-3.52 (m, 4H), 3.45 (s, 3H), 3.39-3.29 (m, 4H), 3.24 (s, 3H), 2.67 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.39, 154.76, 150.58, 139.05, 139.02, 134.66, 134.36, 132.38(2C), 130.91, 130.42, 129.64, 128.91(2C), 126.19(2C), 123.20, 121.84, 119.16, 116.34, 113.05(2C), 111.38, 48.96(2C), 46.83(2C), 42.24, 32.13, 12.20. ESI-MS m/z 608.50 $(M+H)^+$.

1-(3-(4-Chloro-2-(4-chlorophenyl)-1,5-dimethyl-1*H*-pyrrol-3-yl)phenyl)-4-(4-nitrophenyl)piperazine (13b): Starting with **13a** (123 mg, 0.28 mmol), 95 mg (65% yield) of compound **13b** was obtained according to the procedure described for the preparation of

12b. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.11 (d, J = 9.3 Hz, 2H), 7.28 (d, J = 8.4 Hz, 2H), 7.18-7.07 (m, 3H), 6.81 (d, J = 9.4 Hz, 2H), 6.77-6.68 (m, 3H), 3.54-3.44 (m, 4H), 3.42 (s, 3H), 3.23-3.11 (m, 4H), 2.32 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 154.86, 150.18, 138.67, 134.69, 133.55, 132.52(2C), 130.92, 128.90, 128.80(2C), 128.70, 126.22, 126.10(2C), 122.32, 120.36, 118.60, 114.18, 112.81(2C), 108.96, 48.86(2C), 46.98(2C), 32.29, 10.25. ESI-MS m/z 521.67 $(M+H)^+$.

1-(3-(2-(4-Chlorophenyl)-1,5-dimethyl-4-(trifluoromethyl)-1*H*-pyrrol-3-yl)phenyl)-4-(4-nitrophenyl)piperazine (14b): Starting with **14a** (152 mg, 0.36 mmol), 68 mg (34% yield) of compound **14b** was obtained according to the procedure described for the preparation of **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.14 (d, J = 9.3 Hz, 2H), 7.30-7.20 (m, 2H), 7.16-7.02 (m, 3H), 6.84 (d, J = 9.3 Hz, 2H), 6.76 (d, J = 8.4 Hz, 1H), 6.73-6.63 (m, 2H), 3.56-3.46 (m, 4H), 3.43 (s, 3H), 3.25-3.13 (m, 4H), 2.45 (s, 3H). ESI-MS m/z 555.17 $(M+H)^+$.

5-(4-Chlorophenyl)-1,2-dimethyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-pyrrole-3-carbonitrile (15b): Starting with **15a** (216 mg, 0.50 mmol), 150 mg (59%) of compound **15b** was obtained according to the procedure described for the preparation of **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.14 (d, J = 9.36 Hz, 2H), 7.37 (d, J = 8.42 Hz, 2H), 7.22-7.08 (m, 3H), 6.91-6.79 (m, 3H), 7.76 (dd, J = 2.00, 8.14 Hz, 1H), 6.66 (d, J = 7.67 Hz, 1H), 3.58-3.47 (m, 4H), 3.42 (s, 3H), 3.29-3.17 (m, 4H), 2.49 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 154.89, 150.60, 139.03, 138.80, 134.67, 133.87, 132.67(2C), 130.07, 130.03, 129.45, 129.28(2C), 126.17(2C), 124.18, 120.96, 117.46, 117.23, 114.74, 112.88(2C), 91.98, 48.82(2C), 47.06(2C), 32.36, 12.08. ESI-MS m/z 512.33 $(M+H)^+$.

5-(4-Chlorophenyl)-1-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (16c): Starting with **16b** (484 mg, 0.96 mmol), 150 mg (27%) of compound **16c** was obtained according to the procedure described for the preparation of **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.10 (d, J = 9.36 Hz, 2H), 7.34 (d, J = 8.42 Hz, 2H), 7.13 (d, J = 8.43 Hz, 2H), 7.06 (t, J = 7.83 Hz, 1H), 6.88-6.68 (m, 4H), 6.55 (d, J = 7.65 Hz, 1H), 3.60 (s, 3H), 3.51-3.41 (m, 4H), 3.24-3.07 (m, 4H). ESI-MS m/z 585.42 $(M+H)^+$.

Ethyl 2-chloro-5-(4-chlorophenyl)-1-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-pyrrole-3-carboxylate (17b): Starting with **17a** (353 mg, 0.71 mmol), 199 mg (51%, 2 steps) of compound **17b** was obtained according to the procedure described for the preparation of **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.12 (d, J = 9.33 Hz, 2H), 7.27 (d, J = 8.46 Hz, 2H), 7.16-7.04 (m, 3H), 6.84 (d, J = 9.41 Hz, 2H), 6.77 (dd, J = 1.76, 8.18 Hz, 1H), 6.71-6.60 (m, 2H), 4.13 (q, J = 7.17 Hz, 2H), 3.51 (s, 3H), 3.58-3.43 (m, 4H), 3.22-3.10 (m, 4H), 1.10 (t, J = 7.11 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.74, 154.81, 149.83, 138.62, 135.31, 134.17, 132.41(2C), 130.58, 129.65, 128.77(2C), 128.32, 126.06(2C), 124.57, 123.25, 122.91, 119.24, 114.72, 112.80(2C), 111.01, 59.98, 48.99(2C), 46.96(2C), 32.61, 14.13.

5-(4-Chlorophenyl)-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)isoxazole (22b): Starting with **22a** (100 mg, 0.24 mmol), 43 mg (39%) of compound **22b** was obtained according to a modified procedure described for the preparation of **12b**. For this scaffold the reaction is not heated; instead it is run at room temperature. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.35 (s, 1H), 8.15 (d, J = 9.33 Hz, 2H), 7.61 (d, J = 8.57 Hz, 2H), 7.41-7.29 (m, 3H), 7.03-6.80 (m, 5H), 3.65-3.52 (m, 4H), 3.41-3.30 (m, 4H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.07, 154.79, 152.18, 151.36, 139.10, 136.36, 131.11, 130.33, 129.31(2C),

128.72(2C), 126.32, 126.19(2C), 120.63, 117.02, 116.29, 116.07, 113.05(2C), 48.67(2C), 47.16(2C). ESI-MS m/z 461.83 $(M+H)^+$.

1-(4-Chlorophenyl)-*N*-(methylsulfonyl)-5-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-imidazole-4-carboxamide (23c): Starting with **23b**, compound **23c** was obtained according to the procedure described for the preparation of **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.15 (d, J = 9.28 Hz, 2H), 7.38 (d, J = 7.05 Hz, 2H), 7.22 (t, J = 7.63 Hz, 1H), 7.16-7.01 (m, 2H), 7.01-6.89 (m, 2H), 6.86 (d, J = 9.32 Hz, 2H), 6.70 (d, J = 7.04 Hz, 1H), 3.61-3.50 (m, 4H), 3.35 (s, 3H), 3.32-3.22 (m, 4H). ESI-MS m/z 581.92 $(M+H)^+$.

1-(4-Chlorophenyl)-*N*-(methylsulfonyl)-5-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-pyrazole-4-carboxamide (24c): Starting with **24b**, compound **24c** was obtained according to the procedure described for the preparation of **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.26 (s, 1H), 8.13 (d, J = 9.19 Hz, 2H), 8.02 (bs, 1H), 7.37 (t, J = 7.94 Hz, 1H), 7.29 (d, J = 8.72 Hz, 2H), 7.18 (d, J = 8.68 Hz, 2H), 7.04 (d, J = 8.41 Hz, 1H), 6.93-6.78 (m, 3H), 6.74 (d, J = 7.39 Hz, 1H), 3.66-3.53 (m, 4H), 3.45-3.35 (m, 4H), 3.31 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 160.36, 154.62, 151.32, 143.95, 142.48, 138.93, 137.47, 134.41, 130.97, 129.40(2C), 128.34, 126.28(2C), 126.16(2C), 120.87, 117.81, 116.90, 115.42, 112.88(2C), 47.92(2C), 46.72(2C), 42.30. ESI-MS m/z 581.58 $(M+H)^+$.

Ethyl 5-(4-chlorophenyl)-2-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)furan-3-carboxylate (27b): Starting with **27a** (400 mg, 0.95 mmol), 132 mg (27%, 2 steps) of compound **27b** was obtained according to the procedure described for the preparation of **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.10 (d, J = 9.38 Hz, 2H), 7.36-7.24 (m, 3H), 7.16 (d, J = 8.73 Hz, 2H), 6.98 (dd, J = 2.00, 8.19 Hz, 1H), 6.92-6.77 (m, 4H), 4.10 (q, J = 7.12 Hz, 2H), 3.63-3.48 (m, 4H), 3.39-3.26 (m, 4H), 2.68 (s, 3H), 1.07 (t, J = 7.13 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.86, 158.48, 154.74, 150.63, 146.43, 138.73, 134.53, 133.14, 129.34, 128.90, 128.63(2C), 126.70(2C), 126.05(2C), 123.11, 122.40, 118.17, 115.96, 115.67, 112.84(2C), 60.01, 49.02(2C), 47.02(2C), 14.39, 14.06. ESI-MS m/z 546.42 $(M+H)^+$.

***tert*-Butyl 5-(4-chlorophenyl)-2-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1-propyl-1*H*-pyrrole-3-carboxylate (29b):** Starting with **29a** (390 mg, 0.81 mmol), 242 mg (53%) of compound **29b** was obtained according to the procedure described for the preparation of **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.14 (d, J = 9.36 Hz, 2H), 7.24 (d, J = 8.42 Hz, 2H), 7.13-7.03 (m, 3H), 6.85 (d, J = 9.42 Hz, 2H), 6.77-6.60 (m, 3H), 3.79-3.68 (m, 2H), 3.55-3.43 (m, 4H), 3.22-3.09 (m, 4H), 2.59 (s, 3H), 1.53 (sex, J = 7.66 Hz, 2H), 1.28 (s, 9H), 0.76 (t, J =3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 165.53, 154.96, 149.82, 138.80, 137.20, 135.10, 133.66, 132.79(2C), 131.16, 129.77, 128.56(2C), 128.18, 126.14(2C), 124.35, 123.53, 119.27, 114.25, 113.16, 112.92(2C), 79.57, 49.25(2C), 47.11(2C), 45.99, 28.22(3C), 24.28, 11.70, 11.29. ESI-MS m/z 615.00 $(M+H)^+$.

***tert*-Butyl 1-butyl-5-(4-chlorophenyl)-2-methyl-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-pyrrole-3-carboxylate (30b):** Starting with **30a** (400 mg, 0.81 mmol), 227 mg (49%) of compound **30b** was obtained according to the procedure described for the preparation of **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.14 (d, J = 9.36 Hz, 2H), 7.24 (d, J = 8.42 Hz, 2H), 7.14-7.04 (m, 3H), 6.85 (d, J = 9.36 Hz, 2H), 6.76-6.58 (m, 3H), 3.83-3.70 (m, 2H), 3.55-3.44 (m, 4H), 3.23-3.12 (m, 4H), 2.59 (s, 3H), 1.49 (p, J = 7.94 Hz, 2H), 1.28 (s, 9H), 1.16 (sex, J = 7.45 Hz, 2H), 0.79 (t, J = 7.31 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 165.53, 154.96, 149.83, 138.82, 137.22, 135.06, 133.67, 132.82(2C), 131.16, 129.75, 128.55(2C), 128.18, 126.14(2C), 124.36, 123.55, 119.29, 114.25, 113.17, 112.93(2C),

79.57, 49.26(2C), 47.12(2C), 44.16, 33.04, 28.23(3C), 19.99, 13.72, 11.71. ESI-MS m/z 629.42 $(M+H)^+$.

5-(4-Chlorophenyl)-1-ethyl-2-methyl-*N*-(methylsulfonyl)-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-pyrrole-3-carboxamide (31b): Starting with **31a**, compound **31b** was prepared according to the procedure described for the preparation of compound **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.14 (d, J = 9.34 Hz, 2H), 7.59 (bs, 1H), 7.32-7.18 (m, 3H), 7.11 (d, J = 8.40 Hz, 2H), 6.88-6.79 (m, 3H), 6.73 (s, 1H), 6.65 (d, J = 7.33 Hz, 1H), 3.86 (q, J = 7.02 Hz, 2H), 3.58-3.47 (m, 4H), 3.36-3.26 (m, 4H), 3.23 (s, 3H), 2.69 (s, 3H), 1.19 (t, J = 7.12 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.46, 154.54, 151.10, 138.84, 138.00, 134.46, 132.60(2C), 130.29, 130.21, 130.00, 128.86(2C), 126.17(2C), 122.40, 122.35, 118.75, 115.87, 112.89(2C), 111.44, 48.51, 46.90, 42.18, 39.42, 16.13, 11.99. ESI-MS m/z 622.17 $(M+H)^+$.

5-(4-Chlorophenyl)-1-isopropyl-2-methyl-*N*-(methylsulfonyl)-4-(3-(4-(4-nitrophenyl)piperazin-1-yl)phenyl)-1*H*-pyrrole-3-carboxamide (32b): Starting with **32a**, compound **32b** was prepared according to the procedure described for the preparation of compound **12b**. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.12 (d, J = 9.34 Hz, 2H), 7.55 (bs, 1H), 7.30-7.14 (m, 3H), 7.10 (d, J = 8.38 Hz, 2H), 6.88-6.78 (m, 3H), 6.70 (s, 1H), 6.63 (d, J = 7.52 Hz, 1H), 4.39 (sept, J = 6.96 Hz, 1H), 3.60-3.47 (m, 4H), 3.33-3.24 (m, 4H), 3.23 (s, 3H), 2.77 (s, 3H), 1.46 (d, J = 7.08 Hz, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 163.63, 154.81, 150.99, 138.69, 137.87, 134.57, 134.44, 132.96(2C), 130.85, 130.71, 130.08, 128.66(2C), 126.14(2C), 122.31, 121.03, 118.68, 115.74, 112.82(2C), 112.08, 49.23, 48.45, 46.81, 42.05, 22.37, 13.54.

(*R*)-*tert*-Butyl 4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazine-1-carboxylate (37): To a cooled (0 °C) solution of *tert*-butyl 4-(4-aminophenyl)piperazine-1-carboxylate **33** (2 g, 7.21 mmol) in pyridine (30 mL) was added 4-fluoro-3-nitrobenzene-1-sulfonyl chloride **34** (2.25 g, 9.37 mmol). After 30 minutes, pyridine was removed *in vacuo* and the oil was purified by column chromatography to give 1.82 g (53% yield) of intermediate **35**. Intermediate **35** (1.82 g, 3.79 mmol) was re-dissolved in DMF (10 mL), then (*R*)-N^1,N^1-dimethyl-4-(phenylthio)butane-1,3-diamine **36** (1.02 g, 4.55 mmol) and DIEA (2 mL, 11.37 mmol) were added. After stirring overnight, the solvent was removed *in vacuo* and the crude was purified by column chromatography (using a gradient of DCM: EtOAc) to produce 2.14 g (82% yield) of **37** as a yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ ppm 8.99 (d, J = 8.24 Hz, 1H), 8.49 (d, J = 2.21 Hz, 1H), 7.48 (dd, J = 1.83, 9.14 Hz, 1H), 7.35-7.28 (m, 2H), 7.25-7.16 (m, 3H), 7.02 (d, J = 8.82 Hz, 2H), 6.80 (d, J = 8.93 Hz, 2H), 6.61 (d, J = 9.34 Hz, 1H), 4.03-3.88 (m, 1H), 3.60-3.48 (m, 4H), 3.11 (d, J = 6.07 Hz, 2H), 3.09-3.01 (m, 4H), 2.53-2.40 (m, 1H), 2.35-2.24 (m, 1H), 2.20 (s, 6H), 2.11-1.94 (m, 1H), 1.90-1.74 (m, 1H), 1.47 (s, 9H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ ppm 154.86, 149.70, 146.82, 134.95, 133.56, 131.11(2C), 130.93, 129.32(2C), 128.42, 127.84, 127.37, 125.34, 124.89(2C), 117.18(2C), 114.52, 80.18, 55.34, 51.55, 49.38(2C), 45.54(2C), 38.57, 30.59, 28.59(3C). ESI-MS m/z 685.33 $(M+H)^+$.

(*R*)-4-(4-(Dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitro-*N*-(4-(piperazin-1-yl)phenyl)benzenesulfonamide (38): Trifluoroacetic acid (5 mL) was added to a solution of **37** (2.14g, 3.12 mmol) in DCM (3 mL). After 5 h at room temperature, the reaction was slowly quenched with saturated $NaHCO_3$ extracted with EtOAc, and solid NaCl was added to salt out the product. The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo to give quantitative yield of **38** as an orange solid. ^{1}H-NMR (300 MHz, CD_3OD) δ ppm 8.30 (d, J = 2.11 Hz, 1H), 7.53 (dd, J = 2.09, 9.16 Hz, 1H), 7.24-7.15

(m, 2H), 7.10-6.97 (m, 5H), 6.92-6.82 (m, 3H), 4.13-3.99 (m, 1H), 3.37-3.31 (m, 1H), 3.18 (d, J = 5.87 Hz, 1H), 3.15-3.07 (m, 4H), 3.07-2.95 (m, 4H), 2.57-2.33 (m, 2H), 2.25 (s, 6H), 2.13-1.78 (m, 2H); ^{13}C-NMR (75 MHz, CD_3OD) δ ppm 150.43, 148.03, 136.52, 134.31, 131.70, 131.59(2C), 131.05, 130.04(2C), 128.04, 127.74, 126.90, 124.80(2C), 118.12(2C), 116.18, 56.62, 52.77, 50.11(2C), 45.90(2C), 45.32(2C), 39.47, 32.18. ESI-MS m/z 585.92 $(M+H)^+$.

(*R*)-*N*-(4-(4-(3-(1-(4-Chlorophenyl)-1*H*-imidazol-5-yl)phenyl)piperazin-1-yl)phenyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide (18): **(Method A)** A round bottom flask containing a solution of imidazole **18a** (20 mg, 0.051 mmol), piperazine **38** (60 mg, 0.103 mmol), $Pd(dba)_2$ (5.8 mg, 0.01 mmol), P(*t*Bu)$_3$ (50 μL), sodium *tert*-butoxide (10 mg, 0.103 mmol), toluene (2 mL) and DMF (1 mL) was placed under vacuum briefly, then flushed with N_2 three times. The solution, under nitrogen, was heated to 90 °C overnight. After cooling, the reaction was filtered through celite, then through a plug of silica. The crude was dissolved in MeOH, 4.0 M HCl in dioxane was added and after 5 minutes the solvent was removed. The residue was re-dissolved in 3:1 MeOH/water and purified by reverse phase HPLC to give 12 mg (28% yield) of **18** as a yellow powder. ^{1}H-NMR (300 MHz, CD_3OD) δ ppm 9.23 (s, 1H), 8.33 (d, J = 2.0 Hz, 1H), 7.83 (s, 1H), 7.60 (dd, J = 2.0, 9.0 Hz, 1H), 7.55 (d, J = 8.8 Hz, 2H), 7.43 (d, J = 8.8 Hz, 2H), 7.30- 7.15 (m, 3H), 7.12-7.00 (m, 6H), 7.00-6.90 (m, 3H), 6.80-6.71 (m, 2H), 4.19-4.04 (m, 1H), 3.28-3.12 (m, 12H), 2.87 (s, 6H), 2.35-2.09 (m, 2H). ESI-MS m/z 837.50 $(M+H)^+$.

(*R*)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxamide (9): Starting with **9a**, compound **9** was obtained according to the procedure described for the preparation of **18**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 2.2 Hz, 1H), 7.63 (dd, J = 2.3, 9.1 Hz, 1H), 7.29-7.21 (m, 4H), 7.20-7.11 (m, 4H), 7.10-7.03 (m, 4H), 6.97-6.85 (m, 3H), 6.80-6.74 (m, 3H), 4.13-4.05 (m, 1H), 3.43 (s, 3H), 3.31-3.20 (m, 9H), 3.19-3.09 (m, 3H), 2.82 (s, 6H), 2.62 (s, 3H), 2.40-2.05 (m, 2H). ESI-MS m/z 907.67 $(M+H)^+$.

(*R*)-*N*-(4-(4-(3-(1-(4-Chlorophenyl)-1*H*-pyrazol-5-yl)phenyl)piperazin-1-yl)phenyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide (19): Starting with **19a**, compound **19** was obtained according to the procedure described for the preparation of **18**. ^{1}H-NMR (300 MHz, CD_3OD) δ ppm 8.35 (d, J = 2.0 Hz, 1H), 7.73 (d, J = 1.8 Hz, 1H), 7.59 (dd, J = 2.0, 9.0 Hz, 1H), 7.40 (d, J = 8.8 Hz, 2H), 7.32-7.23 (m, 3H), 7.22-7.16 (m, 2H), 7.12-6.87 (m, 9H), 6.80-6.73 (m, 2H), 6.60 (d, J = 1.9 Hz, 1H), 4.16-4.02 (m, 1H), 3.29-3.12 (m, 11H), 2.87 (s, 6H), 2.31-2.09 (m, 2H). ESI-MS m/z 837.50 $(M+H)^+$.

(*R*)-*N*-(4-(4-(3-(5-(4-Chlorophenyl)-1-methyl-1*H*-pyrazol-4-yl)phenyl)piperazin-1-yl)phenyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzene-sulfonamide (20): Starting with **20a**, compound **20** was obtained according to the procedure described for the preparation of **18**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.44 (d, J = 1.9 Hz, 1H), 7.74 (s, 1H), 7.64 (dd, J = 1.8, 9.1 Hz, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.31-7.23 (m, 4H), 7.23-7.09 (m, 6H), 7.08-7.00 (m, 2H), 6.94 (d, J = 8.30 Hz, 1H), 6.89-6.74 (m, 3H), 4.19-4.04 (m, 1H), 3.78 (s, 3H), 3.49-3.09 (m, 12H), 2.82 (s, 6H), 2.40-2.07 (m, 2H). ESI-MS m/z 851.33 $(M+H)^+$.

(*R*)-*N*-(4-(4-(3-(3-(4-Chlorophenyl)-1-methyl-1*H*-pyrazol-4-yl)phenyl)piperazin-1-yl)phenyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide (21): Starting with **21a**, compound **21** was obtained according to the procedure described for the preparation of **18**. ^{1}H-NMR (300 MHz, 10:1

$CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 2.2 Hz, 1H), 7.62 (dd, J = 2.2, 9.1 Hz, 1H), 7.52 (s, 1H), 7.42 (d, J = 8.5 Hz, 2H), 7.31-7.19 (m, 5H), 7.18-7.10 (m, 3H), 7.07 (d, J = 8.9 Hz, 2H), 6.94-6.71 (m, 6H), 4.11-4.02 (m, 1H), 3.97 (s, 3H), 3.31-3.06 (m, 12H), 2.82 (s, 6H), 2.38-2.08 (m, 2H). ESI-MS m/z 851.58 $(M+H)^+$.

(*R*)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-*N*,1,2-trimethyl-1*H*-pyrrole-3-carboxamide (8)[8]: To a solution of **4** (50 mg, 0.055 mmol), EDCI (16 mg, 0.083 mmol), HOBt (11 mg, 0.083 mmol), and DIEA (28 μL, 0.165 mmol) in 5 mL of DCM, was added methyl amine (138 μL of 2M in THF, 0.275 mmol). After stirring overnight, the solvent was removed *in vacuo* and the crude was re-dissolved in MeOH, 4.0M HCl in dioxane was added and after 5 min the solvent was removed. The residue was re-dissolved in 3:1 MeOH/H_2O and purified by reverse phase HPLC to give 34 mg (68%) of **8** as a yellow powder.

5-(4-Chlorophenyl)-4-(3-(4-(4-(4-((*R*)-4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-*N*-((1*s*,3*s*)-3-hydroxy-3-methylcyclobutyl)-1,2-dimethyl-1*H*-pyrrole-3-carboxamide (10): Starting with (1*s*,3*s*)-3-amino-1-methylcyclobutanol and **4**, compound **10** was obtained according to the procedure described for the preparation of **8**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.37 (d, J = 2.1 Hz, 1H), 7.55 (dd, J = 2.1, 9.1 Hz, 1H), 7.21-7.15 (m, 4H), 7.14-7.05 (m, 4H), 7.03-6.95 (m, 4H), 6.86-6.77 (m, 3H), 6.71-6.59 (m, 3H), 4.06-3.96 (m, 1H), 3.80 (p, 7.9 Hz, 1H), 3.34 (s, 3H), 3.20-3.00 (m, 12H), 2.73 (s, 6H), 2.49 (s, 3H), 2.30-2.00 (m, 4H), 1.48-1.37 (m, 2H), 1.18 (s, 3H). ESI-MS m/z 991.42 $(M+H)^+$.

(*R*)-*N*-(4-(4-(3-(2-(4-Chlorophenyl)-4-(3-hydroxy-3-methylazetidine-1-carbonyl)-1,5-dimethyl-1*H*-pyrrol-3-yl)phenyl)piperazin-1-yl)phenyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide (11): Starting with 3-methylazetidin-3-ol and **4**, compound **11** was obtained according to the procedure described for the preparation of **8**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J =2.2 Hz, 1H), 7.63 (dd, J = 2.2, 9.1 Hz, 1H), 7.34-7.22 (m, 4H), 7.19-7.03 (m, 8H), 6.89 (d, J = 9.0 Hz, 2H), 6.83-6.73 (m, 2H), 6.67 (br.s., 1H), 6.61 (d, J = 7.6 Hz, 1H), 4.13-4.05 (m, 1H), 3.83 (q, 10Hz, 2H), 3.40 (s, 3H), 3.30-3.10 (m, 13H), 2.82 (s, 6H), 2.39 (s, 3H), 2.36-2.10 (m, 2H), 1.11 (s, 3H). ESI-MS m/z 977.17 $(M+H)^+$.

(*R*)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1,2-dimethyl-*N*-(methylsulfonyl)-1*H*-pyrrole-3-carboxamide (12): A round bottom flask containing a solution of compound **12b** (100 mg, 0.16 mmol) in MeOH (10 mL) was placed under vacuum briefly, then flushed with N_2 atmosphere three times. 10 % Pd-C (50 mg) was added to this solution, which was then purged again. The N_2 was removed in vacuum and a balloon of H_2 gas was connected to the flask. After 15 min, the reaction was filtered through celite, and the solvent was removed *in vacuo* to produce the aniline intermediate that was used without further purification. To a cooled (0 °C) solution of the aniline intermediate in pyridine (6 mL) was added 4-fluoro-3-nitrobenzene-1-sulfonyl chloride **34** (50 mg, 0.21 mmol). After 30 min, pyridine was removed *in vacuo* and the oil was purified by column chromatography to give 79 mg of an oily red intermediate. The resulting oil (79 mg, 0.10 mmol) was re-dissolved in DMF (2 mL), then (*R*)-N^1,N^1-dimethyl-4-(phenylthio)butane-1,3-diamine **36** (45 mg, 0.20 mmol) and DIEA (0.1 mL) were added. After standing overnight, the solvent was removed *in vacuo* and the crude was purified by column chromatography to produce **12** as a yellow solid. The solid was dissolved in MeOH, 4.0 M HCl in dioxane was added and after 5 minutes the solvent was removed. The residue

was re-dissolved in 3:1 MeOH/H_2O and purified by reverse phase HPLC to give 92 mg (58 %, 3 steps) of **12** as a yellow powder. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 1.81 Hz, 1H), 7.61 (dd, J = 1.61, 8.84 Hz, 1H), 7.32-7.00(m, 13H), 6.94-6.82 (m, 3H), 6.79-6.63 (m, 3H), 4.14-4.00 (m, 1H), 3.45 (s, 3H), 3.31-3.07 (m, 15H), 2.82 (s, 6H), 2.65 (s, 3H), 2.37-2.05 (m, 2H). ESI-MS m/z 985.67 $(M+H)^+$.

(*R*)-*N*-(4-(4-(3-(4-Chloro-2-(4-chlorophenyl)-1,5-dimethyl-1*H*-pyrrol-3-yl)phenyl)piperazin-1-yl)phenyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide (13): Starting with **13b**, compound **13** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 2.2 Hz, 1H), 7.62 (dd, J = 2.3, 9.1 Hz, 1H), 7.31-7.23 (m, 4H), 7.18-7.02 (m, 8H), 6.90-6.72 (m, 6H), 4.17-4.07 (m, 1H), 3.43 (s, 3H), 3.29-3.06 (m, 12H), 2.81 (s, 6H), 2.33 (s, 3H), 2.40-2.10 (m, 2H). ESI-MS m/z 900.17 $(M+H)^+$.

(*R*)-*N*-(4-(4-(3-(2-(4-Chlorophenyl)-1,5-dimethyl-4-(trifluoromethyl)-1*H*-pyrrol-3-yl)phenyl)piperazin-1-yl)phenyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide (14): Starting with **14b**, compound **14** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.46 (d, J = 2.2 Hz, 1H), 7.61 (dd, J = 2.2, 9.1 Hz, 1H), 7.29-7.23 (m, 4H), 7.18-7.10 (m, 4H), 7.09-7.02 (m, 4H), 6.90-6.67 (m, 6H), 4.12-4.04 (m, 1H), 3.44 (s, 3H), 3.28-3.08 (m, 12H), 2.82 (s, 6H), 2.45 (d, J = 1.4 Hz, 3H), 2.37-2.08 (m, 2H). ESI-MS m/z 932.42 $(M+H)^+$.

(*R*)-*N*-(4-(4-(3-(2-(4-Chlorophenyl)-4-cyano-1,5-dimethyl-1*H*-pyrrol-3-yl)phenyl)piperazin-1-yl)phenyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide (15): Starting with **15b**, compound **15** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.46 (d, J = 2.2 Hz, 1H), 7.61 (dd, J = 2.2, 9.1 Hz, 1H), 7.36 (d, J = 9.0 Hz, 2H), 7.28-7.22 (m, 2H), 7.20-7.08 (m, 6H), 7.05 (d, H = 8.9 Hz, 2H), 6.87 (d, J = 9.0 Hz, 2H), 6.83-6.67 (m, 4H), 4.12-4.02 (m, 1H), 3.43 (s, 3H), 3.30-3.08 (m, 12H), 2.82 (s, 6H), 2.49 (s, 3H), 2.39-2.09 (m, 2H). ESI-MS m/z 889.33 $(M+H)^+$.

(*R*)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1-methyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid (16): Starting with **16c**, compound **16** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 1.7 Hz, 1H), 7.61 (d, J = 9.1 Hz, 1H), 7.38-7.29 (m, 2H), 7.28-7.23 (m, 2H), 7.20-7.01 (m, 8H), 6.91-6.57 (m, 6H), 4.12-4.04 (m, 1H), 3.58 (s, 3H), 3.27-3.02 (m, 12H), 2.81 (s, 6H), 2.36-2.07 (m, 2H). ESI-MS m/z 962.58 $(M+H)^+$.

(*R*)-*N*-(4-(4-(3-(5-(4-Chlorophenyl)isoxazol-4-yl)phenyl)piperazin-1-yl)phenyl)-4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrobenzenesulfonamide (22): Starting with **22b**, compound **22** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 2.2Hz, 1H), 8.38 (s, 1H), 7.64 (dd, J = 2.3, 9.1 Hz, 1H), 7.61-7.58 (m, 2H), 7.39-7.31 (m, 3H), 7.28-7.23 (m, 2H), 7.17-7.09 (m, 5H), 7.52-7.00 (m, 3H), 6.99-6.90 (m, 2H), 6.78 (d, J = 9.4 Hz, 1H), 4.14-4.07 (m, 1H), 3.43-3.09 (m, 12H) 2.82 (s, 6H), 2.37-2.09 (m, 2H). ESI-MS m/z 838.33 $(M+H)^+$.

(*R*)-1-(4-Chlorophenyl)-5-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-*N*-

(methylsulfonyl)-1*H*-imidazole-4-carboxamide (23): Starting with **23c**, compound **23** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 2.2 Hz, 1H), 7.71 (s, 1H), 7.63 (dd, J = 2.1, 9.1 Hz, 1H), 7.36 (d, J = 9.0, 2H), 7.29-7.05 (m, 10H), 7.00-6.91 (m, 4H), 6.77 (d, J = 9.3 Hz, 1H), 6.71 (d, J = 7.6 Hz, 1H), 4.14-4.05 (m, 1H), 3.32 (s, 3H), 3.30-3.05 (m, 12H), 2.81 (s, 6H), 2.36-2.08 (m, 2H). ESI-MS m/z 959.42 $(M+H)^+$.

(*R*)-1-(4-Chlorophenyl)-5-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-*N*-(methylsulfonyl)-1*H*-pyrazole-4-carboxamide (24): Starting with **24c**, compound **24** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 2.1 Hz, 1H), 8.24 (s, 1H), 7.63 (dd, J = 2.0, 9.1 Hz, 1H), 7.32-7.22 (m, 5H), 7.20-7.00 (m, 8H), 6.99-6.89 (m, 3H), 6.79-6.70 (m, 2H), 4.15-4.05 (m, 1H), 3.35-3.04 (m, 15H), 2.81 (s, 6H), 2.37-2.08 (m, 2H). ESI-MS m/z 958.42 $(M+H)^+$.

(*R*)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1-methyl-1*H*-pyrazole-3-carboxylic acid (25): Starting with **25a**, compound **25** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, CD_3OD) δ ppm 8.31 (d, J = 2.2 Hz, 1H), 7.60 (dd, J = 2.3, 9.2 Hz, 1H), 7.38-7.35 (m, 2H), 7.26-6.90 (m, 15H), 6.85 (d, J = 7.6 Hz, 1H), 4.11-4.07 (m, 1H), 3.82 (s, 3H), 3.45-3.33 (m, 9H), 3.21-3.14 (m, 3H), 2.84 (s, 6H), 2.25-2.15 (m, 2H). ESI-MS m/z 895.75 $(M+H)^+$.

(*R*)-3-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1-methyl-1*H*-pyrazole-5-carboxylic acid (26): Starting with **26a**, compound **26** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, CD_3OD) δ ppm 8.32 (d, J = 2.3 Hz, 1H), 7.58 (dd, J = 2.3, 9.2 Hz, 1H), 7.30-7.25 (m, 3H), 7.20-7.14 (m, 4H), 7.09-6.96 (m, 9H), 6.90 (d, J = 10.2 Hz, 1H), 6.85 (d, J = 7.6 Hz, 1H), 4.18 (s, 3H), 4.10-4.06 (m, 1H), 3.37-3.31 (m, 9H), 3.21-3.15 (m, 3H), 2.84 (s, 6H), 2.24-2.13 (m, 2H). ESI-MS m/z 895.50 $(M+H)^+$.

(*R*)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-2-methylfuran-3-carboxylic acid (27): To a solution of **27b** (260 mg, 0.48 mmol) in 60 mL of 1:1:1 dioxane, EtOH, and H_2O, was added NaOH (190 mg, 4.8 mmol), and the solution was refluxed until no compound **27b** was observed by TLC. After cooling, the reaction was slowly neutralized with 1M HCl and the compound was extracted with EtOAc. The EtOAc solution was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to produce 97 mg of the acid intermediate as a yellow solid. Starting with this acid, compound **27** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, CD_3OD) δ ppm 8.32 (d, J = 2.2 Hz, 1H), 7.58 (dd, J = 2.3, 9.2 Hz, 1H), 7.35-6.97 (m, 16H), 6.91-6.88 (m, 2H), 4.09-4.07 (m, 1H), 3.34-3.31 (m, 9H), 3.21-3.15 (m, 3H), 2.84 (s, 6H), 2.65 (s, 3H), 2.25-2.15 (m, 2H). ESI-MS m/z 895.92 $(M+H)^+$.

(*R*)-2-Chloro-5-(4-chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1-methyl-1*H*-pyrrole-3-carboxylic acid (17): Starting with **17b**, compound **17** was obtained according to the procedure described for the preparation of **27**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 2.3 Hz, 1H), 7.62 (dd, J = 2.3, 9.1 Hz, 1H), 7.31-7.23 (m, 4H), 7.19-7.11 (m, 4H), 7.10-7.02 (m, 4H), 6.94-6.85 (m, 3H), 6.84-6.80 (m,

1H), 6.76 (d, J = 9.2 Hz, 2H), 4.13-4.04 (m, 1H), 3.51 (s, 3H), 3.31-3.07 (m, 12H), 2.81 (s, 6H), 2.35-2.08 (m, 2H). ESI-MS m/z 928.42 $(M+H)^+$.

(*R*)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1-propyl-1*H*-pyrrole-3-carboxylic acid (29): Concentrated H_2SO_4 (2 mL) was added to a cooled (0 °C) solution of **29b** (92 mg, 0.15 mmol) in a mixture of DCM (2 mL) and THF (0.5 mL). After 10 minutes the reaction was slowly quenched with saturated $NaHCO_3$ and extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated *in vacuo* to produce 68 mg of its carboxylic acid as a yellow solid. Starting with this acid, compound **29** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 2.17 Hz, 1H), 7.62 (dd, J = 2.16, 9.10 Hz, 1H), 7.30-7.22 (m, 4H), 7.18-7.10 (m, 4H), 7.10-7.03 (m, 4H), 6.94-6.73 (m, 6H), 4.14-4.02 (m, 1H), 3.79-3.69 (m, 2H), 3.34-3.19 (m, 9H), 3.19-3.07 (m, 3H), 2.81 (s, 6H), 2.62 (s, 3H), 2.37-2.06 (m, 2H), 1.54 (sex, J = 7.62 Hz, 2H), 0.77 (t, J = 7.39 Hz, 3H). ESI-MS m/z 936.83 $(M+H)^+$.

(*R*)-1-Butyl-5-(4-chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-2-methyl-1*H*-pyrrole-3-carboxylic acid (30): Starting with **30b**, compound **30** was obtained according to the procedure described for the preparation of **29**. ^{1}H-NMR (300 MHz, 10:1 $CDCl_3$:CD_3OD) δ ppm 8.45 (d, J = 2.11 Hz, 1H), 7.63 (dd, J = 1.92, 9.10 Hz, 1H), 7.31-7.20 (m, 4H), 7.20-7.04 (m, 8H), 7.04-6.85 (m, 5H), 6.78 (d, J = 9.24 Hz, 1H), 4.16-4.01 (m, 1H), 3.85-3.73 (m, 2H), 3.44-3.07 (m, 12H), 2.81 (s, 6H), 2.63 (s, 3H), 2.38-2.03 (m, 2H), 1.48 (sex, J = 6.87 Hz, 2H), 1.29-1.08 (m, 2H), 0.79 (t, J = 7.31 Hz, 3H). ESI-MS m/z 950.67 $(M+H)^+$.

(*R*)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1-ethyl-2-methyl-*N*-(methylsulfonyl)-1*H*-pyrrole-3-carboxamide (31): Starting with **31b**, compound **31** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300MHz, CD_3OD) δ ppm 8.35 (d, J = 2.2 Hz, 1H), 7.62 (dd, J = 2.1, 9.1 Hz, 1H), 7.35 (d, J = 8.5 Hz, 2H), 7.23-6.93 (m, 15H), 6.80 (d, J = 7.6 Hz, 1H), 4.19-4.08 (m, 1H), 3.89 (q, J = 7.0 Hz, 2H), 3.46-3.33 (m, 9H), 3.26-3.16 (m, 7H), 2.87 (s, 6H), 2.70 (s, 1H), 2.55 (s, 3H), 2.34-2.11 (m, 2H), 1.13 (t, J = 7.1 Hz, 3H). ESI-MS m/z 999.83 $(M+H)^+$.

(*R*)-5-(4-Chlorophenyl)-4-(3-(4-(4-(4-(4-(dimethylamino)-1-(phenylthio)butan-2-ylamino)-3-nitrophenylsulfonamido)phenyl)piperazin-1-yl)phenyl)-1-isopropyl-2-methyl-*N*-(methylsulfonyl)-1*H*-pyrrole-3-carboxamide (32): Starting with **32b**, compound **32** was obtained according to the procedure described for the preparation of **12**. ^{1}H-NMR (300MHz, CD_3OD) δ ppm 8.34 (d, J = 2.2 Hz, 1H), 7.65 (dd, J = 2.1, 9.1 Hz, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.27-6.98 (m, 15H), 6.90 (d, J = 7.3 Hz, 1H), 4.40 (h, J = 7.0 Hz, 1H), 4.21-4.10 (m, 1H), 3.62-3.34 (m, 9H), 3.27-3.15 (m, 6H), 2.99 (s, 1H), 2.87 (s, 7H), 2.61 (s, 3H), 2.34-2.10 (m, 2H), 1.42 (d, J = 7.0 Hz, 6H). ESI-MS m/z 1013.83 $(M+H)^+$.

Fluorescence Polarization based (FP) binding assays: Binding affinities of our synthesized compounds to Bcl-2, Bcl-xL and Mcl-1 were determined using an FP based competitive binding assay described previously.[7] Briefly, pre-incubated protein/probe complex (1.5 and 1 nM for the Bcl-2 assay, 10 and 2 nM for the Bcl-xL assay, and 20 and 2 nM for the Mcl-1 assay, respectively) and serial dilutions of the inhibitors were incubated at room temperature for 2 h with gentle shaking. Millipolarization (mP) values were measured

and plotted over total compound concentrations, nonlinear regression fitting to the binding curves generated the IC_{50} values, and the K_i values were calculated using the equation described previously.[25]

Cell growth inhibition assay: This assay was performed as previously reported.[7] Human small cell lung cancer cell lines H146, H1963, H187, and H1417 were purchased from American Type Culture Collection (ATCC) and were maintained in RPMI-1640 medium containing 10% FBS. Various concentrations of compounds were incubated for 4 days in 96-well flat bottom cell culture plates that were seeded at a density of 1×10^{14} cells/well. At the end of incubation, the effect of compounds on cell growth was evaluated by WST-assay. In this assay, 20 μL of WST-8 dye was added to each well and incubated for an additional 1–2 h, and then the absorbance was measured, at 450 nm, in a microplate reader (Molecular Devices). Using the GraphPad Prism software (GraphPad Software, La Jolla), the IC_{50} values were calculated by comparing absorbance in the untreated cells and the cells treated with the compounds. The standard deviation for each compound was obtained by performing a minimum of three independent experiments in each cell line.

***In Vivo* Pharmacodynamic (PD) and Efficacy Studies in the H146 Xenograft Model—**We employed a similar procedure to that used in our previous studies.[7, 8] An H146 small-cell lung cancer xenograft model was used for our *in vivo* PD and efficacy studies. Xenograft tumors (one tumor per mouse) were developed by injection of 5×10^6 H146 cancer cells with Matrigel, subcutaneously, on the dorsal side of the SCID mice (from Charles River).

For the PD studies, mice bearing tumors approximately 100 mm^3 in volume were given a single dose of **31**, **32** (15 mg/kg) or vehicle. The tumor tissues were harvested at 3h, 6h or 24h, followed by Western blot analysis to determine levels of PARP and caspase-3, as well as cleaved PARP and caspase-3.

For the efficacy studies, mice with tumor volumes between 100 and 150 mm^3 were randomized into different groups (8 mice per group) with mean tumor volume of 126 mm^3. Each group of mice was treated intravenously with either vehicle control or **31** or **32** at 15 mg/kg, daily, 5 days/week for 2 weeks. Tumor sizes and animal weights were measured 3 times per week during the treatment and twice per week after the treatment. Data are presented as mean tumor volumes ± SEM. Statistical analyses were performed using two-way ANOVA and unpaired two-tailed *t* test, using Prism (version 4.0, GraphPad, La Jolla, CA). $P < 0.05$ was considered statistically significant. The efficacy experiment was performed under the guidelines of the University of Michigan Committee for Use and Care of Animals.

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ABBREVIATIONS USED

AcOH	acetic acid
DIEA	*N,N*-diisopropylethylamine

EDCI	1-ethyl-(3-dimethylaminopropyl)carbodiimide
Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
FBS	fetal bovine serum
FP	fluorescence-polarization
HOBt	hydroxybenzotriazole
MeOH	methanol
mP	millipolarization
NIS	*N*-iodosuccinamide
PARP	poly ADP ribose polymerase
$Pd(dba)_2$	Bis(dibenzylidene-acetone)palladium(0)
SCID	Severe combined immunodeficient
TosMIC	Toluenesulfonylmethyl isocyanide

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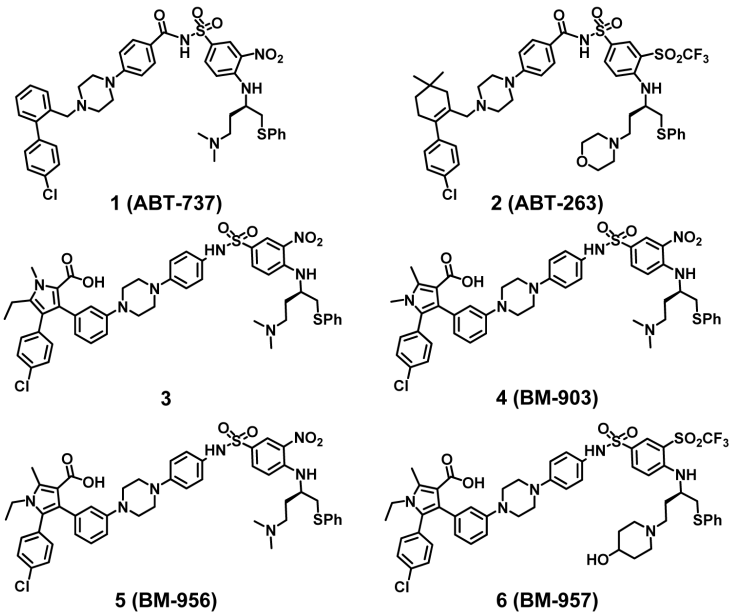


Figure 1.
Chemical structures of **1** (ABT-737)[5], **2** (ABT-263)[6] and our recently reported potent and specific Bcl-2/Bcl-xL inhibitors.

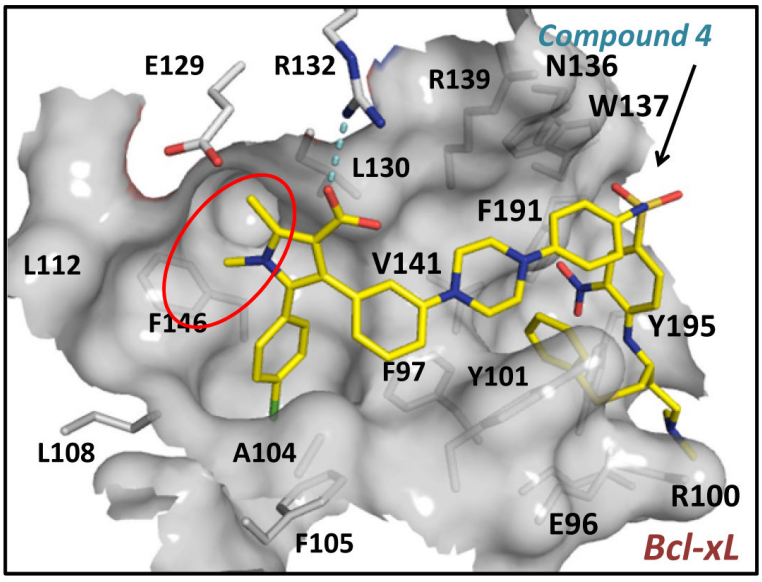


Figure 2.

Crystal structure of **4** (yellow) in a complex with Bcl-xL (gray) (PDB entry: 3SP7).[8] The red oval highlights space available for further modifications around the pyrrole ring. The hydrogen bond/salt bridge of the acid group to Arg 132 is indicated by a dashed cyan line.

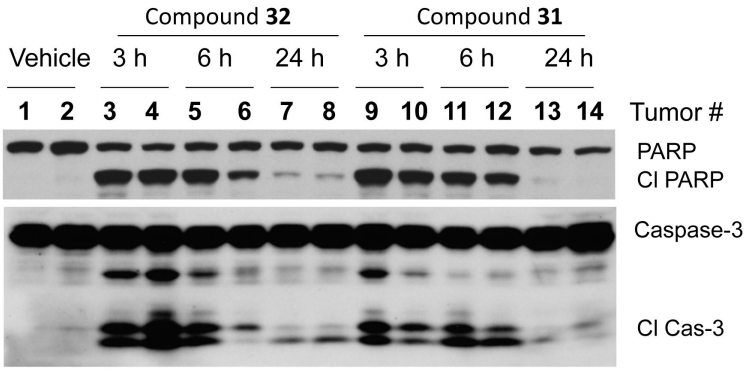


Figure 3.

Western blot analysis of tumor tissues for cleavage of PARP and caspase-3. Mice were treated with a single dose of compound **31** or **32** (15 mg/kg, *i.v.*), were sacrificed at 3, 6 and 24-h time points and tumors were removed for western blot analysis. Cleavage of Cl PARP, cleaved PARP; Cl Cas-3, cleaved caspase-3.

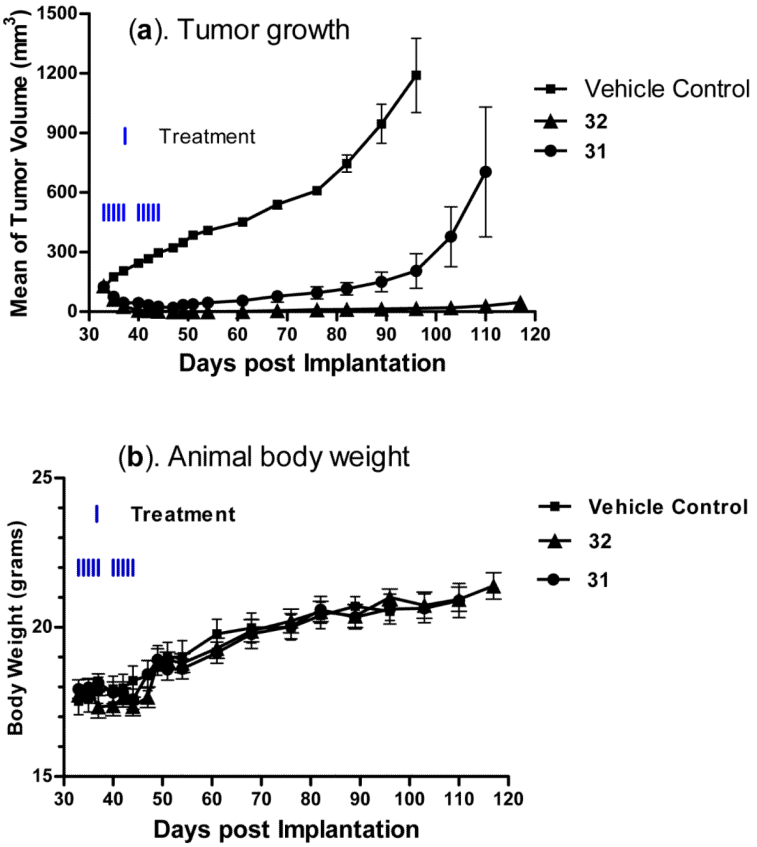
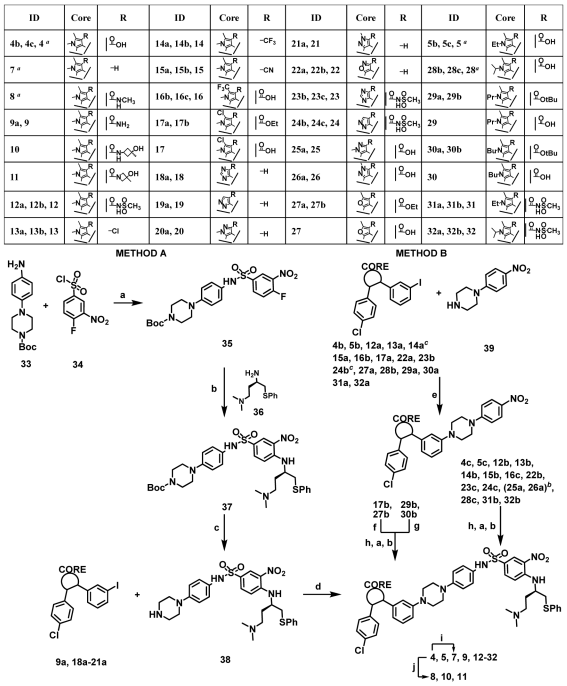


Figure 4.

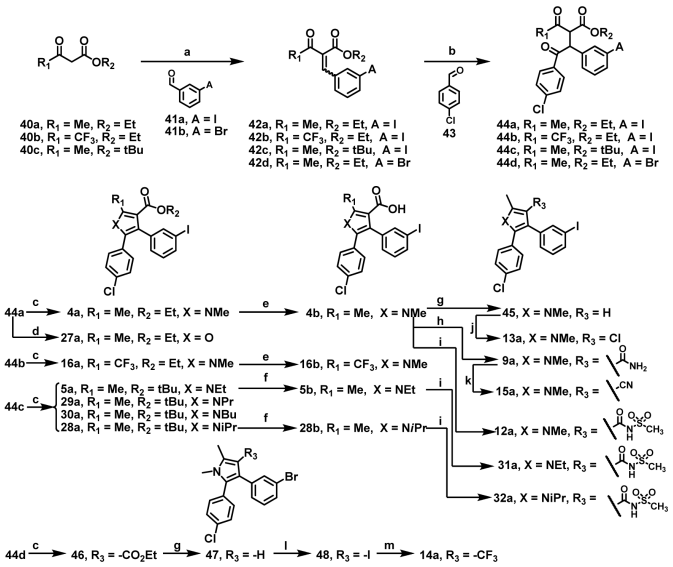
Antitumor activity of compounds **31** and **32** in the H146 small-cell lung cancer xenograft model in SCID mice. Tumors were grown to an average size of 126 mm^3 and compound **31** or **32** was administered at 15 mg/kg intravenously, daily, 5 days a week for 2 weeks. (**a**). Tumor growth. (**b**). Animal body weight.



Reagents and conditions: (a) Pyridine, **34**, 0°C, 15 min.; (b) DMF, **36**, DIEA, overnight; (c) DCM, TFA, rt; (d) 2:1 Toluene:DMF, $Pd(dba)_2$, NaO*t*Bu, P(*t*Bu)$_3$; (e) CuI, L-proline, K_2CO_3, DMSO, 90°C overnight; (f) NaOH, 1:1:1 1,4-dioxane:EtOH:H_2O, reflux (g) (1) DCM, H_2SO_4 (conc.), 0°C 10 min, (2) saturated $NaHCO_3$; (h) 10% Pd-C/H_2, MeOH, 1 atm, 15 min; (i) TFA, 10 min; (j) amine, EDCI, HOBt, DIEA, DCM, rt overnight. ([a]previously reported[8], [b]synthesis described in **Scheme 6**, [c]the I = Br)

Scheme 1.

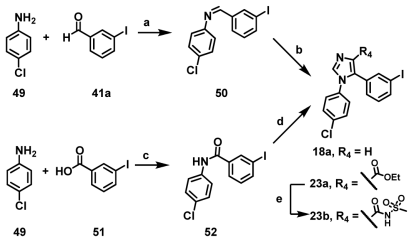
The convergent Method A and stepwise Method B for the preparation of **4**, **5**, and **7-32**



Reagents and conditions: (a) piperidine, **41a** or **41b**, AcOH, toluene reflux; (b) **43**, thiazolium catalyst, Et_3N, 70°C, 5h; (c) (i) primary amine, MeOH, overnight (ii) 2N HCl, 5 min; (d) AcOH, HCl (conc.), EtOH, 70°C 4h; (e) NaOH, 1:1:1 1,4-dioxane:EtOH:H_2O, reflux; (f) (1) DCM, H_2SO_4 (conc.), 0°C 10 min, (2) saturated $NaHCO_3$; (g) TFA, DCM, rt overnight; (h) ammonia (0.5 M in 1,4-dioxane), EDCI, HOBt, DIEA, DCM, rt overnight; (i) (1) $(COCl)_2$, DCM, cat. DMF, reflux 1h (2) methanesulfonamide, DMAP, $ClCH_2CH_2Cl$, reflux overnight; (j) NCS, DMF, rt overnight; (k) pyridine, TFAA, 1,4-dioxane, 0°C, 3h rt; (l) NIS, DMF, rt overnight; (m) Methyl 2,2-difluoro-2-(fluorosulfonyl)acetate, CuI, DMF, 100°C overnight.

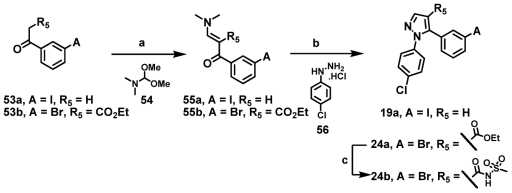
Scheme 2.

Synthesis of variously substituted 1*H*-pyrroles and 2-methyl furan scaffolds



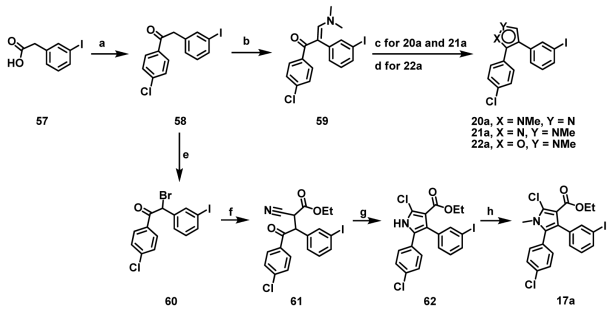
Reagents and conditions: (a) toluene, reflux; (b) TosMIC, K_2CO_3, MeOH, DME; (c) EDCI, HOBt, DIEA, DCM, rt overnight; (d) (i) KO*t*-Bu, THF, $ClPO(OEt)_2$, (ii) ethyl isocyanoacetate, KO*t*-Bu; (e) (i) NaOH, 1:1:1 1,4-dioxane:EtOH:H_2O, reflux, (ii) (1) $SOCl_2$, reflux 3h (2) methanesulfonamide, DMAP, $ClCH_2CH_2Cl$, reflux overnight.

Scheme 3.
Synthesis of imidazole scaffolds 18a and 23b



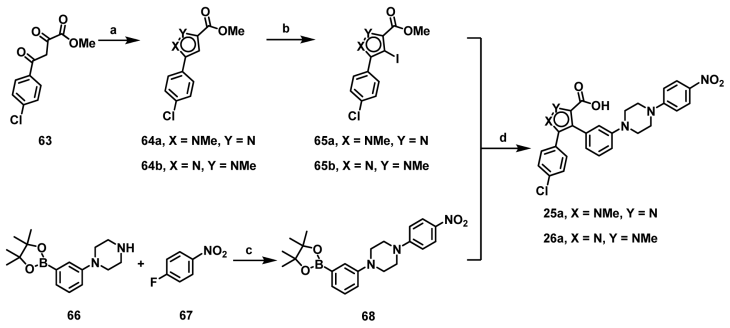
Reagents and conditions: (a) toluene, **54**, reflux; (b) **56**, EtOH, reflux; (c) (i) NaOH, 1,4-dioxane:EtOH:H_2O (1:1:1 v/v/v), reflux, (ii) (1) $SOCl_2$, reflux 3h (2) methanesulfonamide, DMAP, $ClCH_2CH_2Cl$, reflux overnight

Scheme 4.
Synthesis of pyrazole scaffolds 19a and 24b



Reagents and conditions: (a) (i) $SOCl_2$, reflux 2h, (ii) $AlCl_3$, chlorobenzene, 0°C, 2h; (b) **54**, toluene, reflux 2h; (c) NH_2NHCH_3, EtOH, reflux; (d) $NH_2OH.HCl$, Na_2CO_3, 2:1 MeOH:H_2O, AcOH, reflux; (e) AcOH, Br_2, DCM; (f) 2-cyanoacetate, K_2CO_3, acetone; (g) 4M HCl in 1,4-dioxane; (h) MeI, K_2CO_3, DMF.

Scheme 5.
Synthesis of pyrazoles 20a and 21a, isoxazole 22a, and 2-chloropyrrole 17a

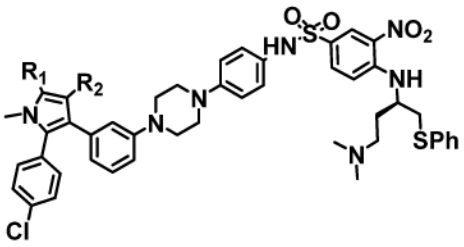


Reagents and conditions: (a) NH_2NHCH_3, AcOH, EtOH; (b) NIS, CAN, CH_3CN, 70°C; (c) K_2CO_3, DMSO, rt overnight; (d) $Pd(PPh_3)_4$, K_2CO_3, 2:1 DME/H_2O reflux, 2h.

Scheme 6.
Synthesis of pyrazole acid intermediates 25a and 26a

Table 1

Structure-activity relationship studies on the pyrrole ring of lead compound **4**



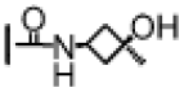
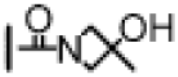
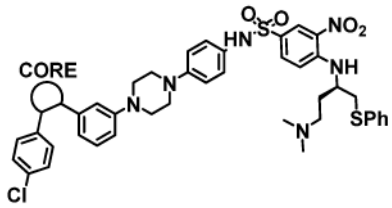
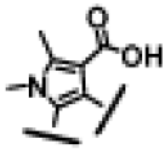
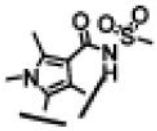
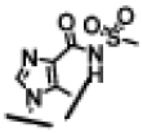
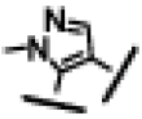
Compound ID	R_1	R_2	Binding Affinities (IC_{50} ± SD, nM)		Cell growth inhibition (IC_{50} ± SD, nM)
			Bcl-2	Bcl-X_L	H-146
4	$-CH_3$	$-CO_2H$	1.3 ± 0.2	6 ± 1	61 ± 39
7	$-CH_3$	−H	99 ±5	11 ± 6	>1,000
8	$-CH_3$	$-CONHCH_3$	5 ± 1	6 ± 3	36 ± 26
9	$-CH_3$	$-CONH_2$	19 ± 5	14 ± 5	245 ± 17
10	$-CH_3$		7.5 ± 1.2	15 ± 1	12 ± 2
11	$-CH_3$		5.6 ± 0.6	9.4 ± 0.6	66 ± 4
12	$-CH_3$	$-CONHSO_2CH_3$	0.9 ± 0.2	5 ± 1	38 ± 22
13	$-CH_3$	−Cl	260 ± 93	12 ± 8	>1,000
14	$-CH_3$	$-CF_3$	271 ± 74	13 ± 2	>1,000
15	$-CH_3$	−CN	17 ± 6	4.5 ± 1.3	491 ± 160
16	$-CF_3$	$-CO_2H$	1.1 ± 0.6	6 ± 2	496 ± 59
17	−Cl	$-CO_2H$	1.5 ± 0.9	4.7 ± 1.5	100 ± 17

Table 2

Structure-activity relationships for compounds in which other 5-membered hetero-aromatic rings were used to replace the pyrrole in the initial lead compound **4**



Compound ID	Core	Binding Affinities (IC_{50} ± SD, nM)		Cell growth inhibition (IC_{50} ± SD, nM)	Compound ID	Core	Binding Affinities (IC_{50} ± SD, nM)		Cell growth inhibition (IC_{50} ± SD, nM)
		Bcl-2	Bcl-X_L	H-146			Bcl-2	Bcl-X_L	H-146
					4		1.3 ± 0.2	6 ± 1	61 ± 39
7		99 ± 5	11 ± 6	>10,000					
					12		0.9 ± 0.2	5 ± 1	38 ± 22
18		46 ± 10	18 ± 1	>10,000	**23**		3.8 ± 1.1	8.3 ± 0.7	>1,000
19		110 ± 4	32 ± 9	>10,000	**24**		5.4 ± 1.1	5 ± 2	>1,000

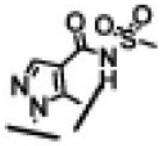
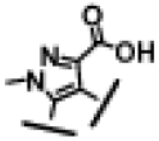
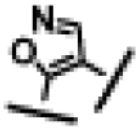
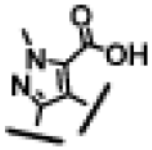
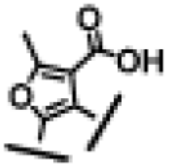
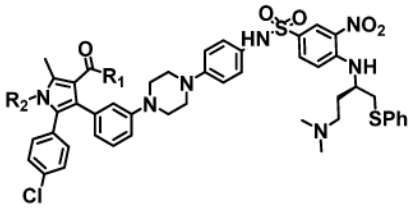
Compound ID	Core	Binding Affinities (IC_{50} ± SD, nM)		Cell growth inhibition (IC_{50} ± SD, nM)	Compound ID	Core	Binding Affinities (IC_{50} ± SD, nM)		Cell growth inhibition (IC_{50} ± SD, nM)
		Bcl-2	Bcl-X_L	H-146			Bcl-2	Bcl-X_L	H-146
20		210 ± 28	15 ± 1	>10,000	**25**		7.1 ± 1.6	6.1 ± 2.2	>1,000
21		140 ± 53	26 ± 3	>10,000	**26**		3.7 ± 0.6	5.9 ± 1.7	541 ± 76
22		350 ± 146	22 ± 7	>10,000	**27**		3.8 ± 1.0	4.7 ± 0.2	199 ± 46

Table 3

Structure-activity relationship of analogues of compounds **4** and **12**



COMPOUND	R_1	R_2	Binding Affinities (IC_{50} ± SD, nM)			Cell Growth Inhibition (IC_{50} ± SD, nM)
			Bcl-2	Bcl-X_L	Mcl-1	H-146
5	−OH	Et-	2 ± 1.6	6.6 ± 2.3	NT	8.1 ± 3.5
28	−OH	*i*Pr-	1.4 ± 0.5	4.8 ± 0.1	NT	3 ± 2.4
29	−OH	Pr-	10 ±4	8.2 ± 2.2	NT	42 ± 5
30	−OH	Bu-	23 ± 2	16 ± 2	NT	67 ± 5
31(BM-1075)	$-NHSO_2CH_3$	Et-	1.8 ± 0.3	7.0 ± 1.8	> 2000	4.8 ± 0.9
32 (BM-1074)	$-NHSO_2CH_3$	*i*Pr-	1.8 ± 0.2	6.9 ± 1.8	> 2000	1.3 ± 0.3

NT = not tested

Table 4

Cell growth inhibitory activity of compounds **31, 32, 1** and **2** in three small-cell lung cancer cell lines

Cell Lines	IC_{50} ± SD (nM)			
	1	**2**	**31**	**32**
H1963	54.0±28.2	26.6±7.9	8.2±4.9	1.0±0.5
H187	137.7±71.3	38.4±26.8	7.9±3.9	1.4±1.3
H1417	173.4±122.1	54.2±11.1	11.1±2.0	2.3±0.2