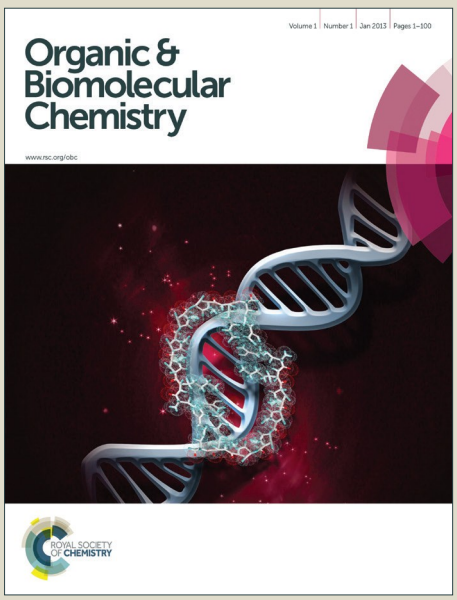
Organic & Biomolecular Chemistry

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

Organic and Biomolecular Chemistry RSCPublishing

Cite this: DOI: 10.1039/x0xx00000x

Thioimidazoline based compounds reverse glucocorticoid resistance in human acute lymphoblastic leukemia xenografts

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

Cara E. Toscan[1-3], Marwa Rahimi[2,3], Mohan Bhadbhade[2], Russell Pickford[4], Shelli R. McAlpine[2*] and Richard B. Lock[1,3*]

Glucocorticoids form a critical component of chemotherapy regimens for pediatric acute lymphoblastic leukemia (ALL) and the initial response to glucocorticoid therapy is a major prognostic factor, where resistance is predictive of poor outcome. A high-throughput screen identified four thioimidazoline-containing compounds that reversed dexamethasone resistance in an ALL xenograft derived from a chemoresistant pediatric ALL. The lead compound (1) was synergistic when used in combination with the glucocorticoids, dexamethasone or prednisolone. Synergy was observed in a range of dexamethasone-resistant xenografts representative of B-cell precursor ALL (BCP-ALL) and T-cell ALL. We describe here the synthesis of twenty compounds and biological evaluation of thirty two molecules that explore the structure-activity relationships (SAR) of this novel class of glucocorticoid sensitizing compounds. SAR analysis has identified that the most effective dexamethasone sensitizers contain a thioimidazoline acetamide substructure with a large hydrophobic moiety on the acetamide.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer.[1] Although five year survival rates are approaching 90%, pediatric ALL remains one of the most common causes of death from disease in children due to its high incidence.[2] Treatment of ALL consists of three phases of chemotherapy: remission induction, intensification, and continuation therapy, administered over 2 to 2.5 years.[3] Glucocorticoids, such as dexamethasone and prednisolone, are frequently used in all phases and are critical components of remission induction therapy protocols.[4] Glucocorticoids are also used as a prognostic indicator, where resistance to initial glucocorticoid therapy is predictive of poor outcome.[5] As resistance to glucocorticoids is common at relapse, its pharmacologic reversal may lead to improved outcomes for children with intrinsic/acquired glucocorticoid resistant ALL.[6]

Various strategies have been adopted to identify drugs that reverse glucocorticoid resistance. Using a gene expression profiling approach, the mTOR inhibitor, rapamycin, was identified as a glucocorticoid sensitizer.[7] The BCL-2 antagonist, obatoclax, was shown to overcome glucocorticoid resistance as the BCL-2 family plays an essential role in regulating glucocorticoid induced cell death.[8, 9] AKT inhibition was also shown to restore glucocorticoid receptor translocation to the nucleus in resistant T-cell ALL,[10] and the AKT inhibitor, MK2206, was identified as a glucocorticoid sensitizer. We have previously established primary biopsies from pediatric ALL patients as xenografts in immune deficient mice, and reported that their *in vivo* and *ex vivo* dexamethasone sensitivity correlated with patient outcome.[11, 12] Using this clinically relevant pediatric ALL xenograft model, we have shown that dexamethasone resistance can be partially reversed both *ex vivo* and *in vivo*, with the histone deacetylase inhibitor vorinostat, or the sunitinib analog and receptor tyrosine kinase inhibitor SU11657.[13, 14] However, none of these candidates were specifically developed as glucocorticoid sensitizers.

To identify a glucocorticoid sensitizer specifically designed to reverse dexamethasone resistance, a high-throughput screening (HTS) assay was performed using the pediatric ALL xenograft, ALL-19.[15] The xenograft, ALL-19, was derived from an aggressive and chemoresistant pediatric ALL that induced early fatality in the patient.[11, 12] ALL-19 exhibits dexamethasone resistance both *ex vivo* and *in vivo* and is representative of the most common pediatric ALL subtype, B-cell precursor ALL (BCP-ALL).[11, 12] A 40,000 compound HTS assay identified four thioimidazoline containing compounds (compounds **1**-**4**) that overcame dexamethasone resistance in ALL-19 cells (Figure 1).[15] However, not all thioimidazoline containing compounds are dexamethasone sensitizers, since an additional twenty four compounds with this substructure were included in the 40,000 compounds screened, but did not show any dexamethasone sensitizing effect (Supple. Figure 1). Described here is the synthesis of compounds **1** and **3** and twenty novel thioimidazoline containing compounds that were based on **1**-**4**. Biological evaluation of these twenty synthesized compounds as well as twelve purchased compounds produced a structure-activity relationship (SAR) of this novel class of glucocorticoid sensitizing compounds. Within these thirty two compounds, we identified three that are more potent dexamethasone sensitizers than compounds **1**-**4**.

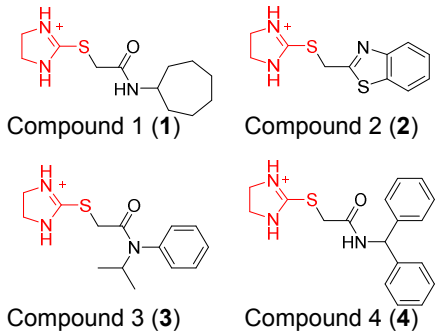


Figure 1. Novel dexamethasone sensitizers identified from HTS of human leukemia xenografts. Thioimidazoline substructure shown in red.

RESULTS AND DISCUSSION

ALL-19 xenograft cells are highly resistant to dexamethasone *ex vivo*, displaying minimal cell death when treated with 300 μM dexamethasone. To determine whether compounds were synergistic with dexamethasone the Bliss-Additivity model was used.[16] Compound **1** was selected as the lead candidate as it exerted the highest single level of synergism with dexamethasone of **1-4** (Supple. Figure 2 and Supple. Table 1). *Ex vivo* fixed-ratio combination cytotoxicity assays showed that the **1**/dexamethasone combination was synergistic against ALL-19 (Figure 2a and Supple. Table 1). As single agents, **1** has modest activity (IC_{50} = 19.8 μM) and dexamethasone has minimal affect (IC_{50} > 40 μM) on ALL-19 cells *ex vivo*, but when used in combination only 6.1 μM of each is required to inhibit 50% of cell survival relative to the control (Figure 2a). Almost identical results were observed when the same assay was repeated using flow cytometry, a direct measure of cell viability (Supple. Figure 3). Synergy was also observed with prednisolone (Figure 2b and Supple. Table 2), hence **1** is a broad glucocorticoid sensitizer.

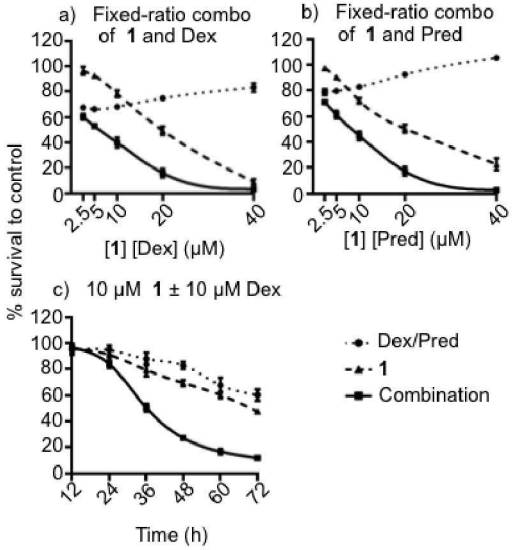


Figure 2. Synergistic antileukemic effects of 1 and glucocorticoids *ex vivo* against ALL xenograft cells. (a) ALL-19 cells were exposed to 1, Dex, or both in combination at a fixed-ratio of concentrations for 48 h. Cell viability was assessed by Alamar Blue assay. (b) ALL-19 cells were exposed to 1, Pred, or both in combination at a fixed-ratio of concentrations for 48 h. Cell viability was assessed by Alamar Blue assay. (c) ALL-19 cells were treated with 10 μM 1, 10 μM Dex, or both in combination. Cell viability was determined by flow cytometry at various time points up to 72 h. Each data point represents the mean ± SEM of three independent experiments.

Modifying the timing of compound addition confirmed that dexamethasone sensitization was maximal when ALL-19 cells were treated simultaneously with **1** and dexamethasone (Supple. Figure 4). A time course experiment was performed by flow cytometry with a fixed concentration of 10 μM **1** and dexamethasone, alone and in combination. The combination caused a marked decrease in cell viability compared to the single agents, with < 15% viable cells remaining after 72 h (Figure 2c).

To determine if **1** only sensitized ALL-19 cells to dexamethasone or whether it was broadly active, fixed-ratio combination cytotoxicity assays were performed against an additional five xenografts. The panel consisted of dexamethasone-sensitive and –resistant xenografts, representative of BCP-ALL, T-cell ALL, and *Mixed Lineage Leukemia*-rearranged ALL. Interestingly **1** was synergistic with dexamethasone in all dexamethasone-resistant xenografts, but did not further potentiate the effects of dexamethasone in dexamethasone-sensitive xenografts (Supple. Figure 5 and Supple. Table 3). Therefore, **1** is a glucocorticoid sensitizer that is specific to dexamethasone-resistant ALL.

Compounds **5-16**, (Figure 3) were designed based on **1.** Compounds **5, 6**, and **7** were chosen for testing as they represented the pharmacophores of **1**. Thus, testing these molecules will determine whether these were the active components for sensitizing the cells to dexamethasone. Compound **8** was a dimerized variation of **5**. Compounds **9-16** are analogs of **1**, with modifications to the acetamide side chain in order to evaluate the impact of changing that moiety. Compound **9** had the methylene between the thiazole and the amide bond removed, while **10** had two methylene units. Compounds **11-16** all had variation to the side chains on the acetamide moiety.

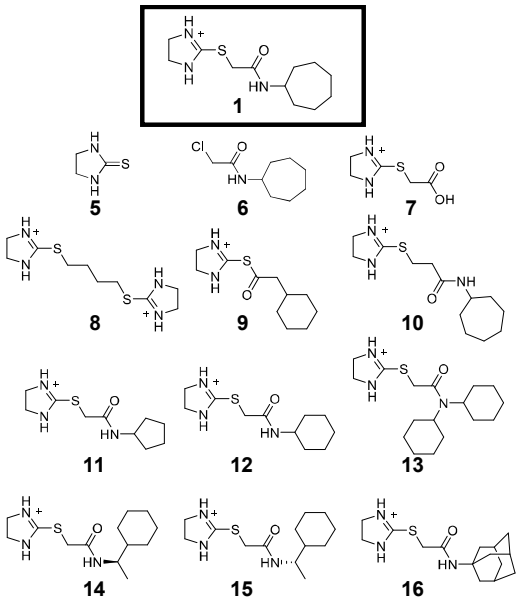
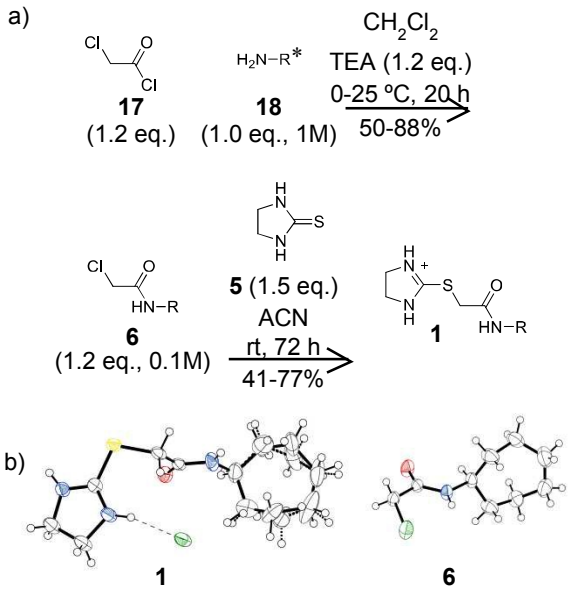


Figure 3. Compounds designed to investigate the SAR of **1**.

Compound **1** was synthesized in order to produce reasonable quantities of material for extensive testing. Chloroacetyl **6** was formed via amidation between **17** and **18** (Scheme 1a). Reacting **5** and the chloroacetyl **6** generated **1** in an overall reasonable yield (31%). The crystal structure of **1** shows the cycloheptane ring is distorted and adopts two conformations in the final compound (Scheme 1b). The conformation of the cycloheptane ring in the intermediate **6**, is similar to one of the conformations displayed in the final compound **1** (Supple. Figure 6). Synthesis of compounds **6-7**, and **10-15** was accomplished using the same approach as described for **1** (Scheme 1a), with overall final yields ranging from 18-36%. Compounds **2-5**, **8, 9,** and **16** were commercially available.

Scheme 1. (a) General synthesis of dexamethasone sensitizers with secondary acetamide. R = the amines shown in the final product in Table 1. **(b)** Crystal structure of compounds **1** and **6**.



*R= amine from Table 1 for compounds **1, 3, 4, 6, 10-15**

The *ex vivo* anti-leukemic activity of all sixteen compounds was tested alone and in combination with dexamethasone against ALL-19 (Supple. Figure 7). The IC_{50} values were calculated from the fixed-ratio combination cytotoxicity assays, where compounds were tested at a 1:1 ratio with dexamethasone on ALL-19 (Table 1). To determine whether compounds were synergistic, additive or antagonistic with dexamethasone, the Bliss-Additivity model was used.[16] Deviation from Bliss-Additivity (BA) was calculated at each tested dose, where synergy is defined as a positive deviation, additive effect as no deviation, and antagonism as a negative deviation (Table 1 and Supple. Table 4).[16] While a synergistic combination effect is required, the combination also needs to be potent to warrant further development. A synergistic and potent compound was defined as a compound that resulted in a combination IC_{50} less than compound **1** (combination IC_{50} < 6.1 μM) and was synergistic with dexamethasone.

Table 1. Antileukemic activity of compounds alone and in combination with dexamethasone *ex vivo* against ALL-19 xenograft cells.

Compound	Cmpd IC_{50} (μM)	Combo IC_{50} (μM)	Median BA	Combo effect	Active and synergistic
1	19.8 ± 1.2	6.1 ± 1.0	0.08	synergy	✔
2	15.4 ± 3.5	5.0 ± 1.6	0.08	synergy	✔
3	23.7 ± 1.4	10.2 ± 1.1	0.11	synergy	✔
4	12.9 ± 0.3	7.6 ± 0.3	0.07	synergy	✔
5	> 40	> 40	-0.03	antag.	
6	34.5 ± 1.0	> 40	0.00	additive	
7	> 40	> 40	0.05	Synergy	
8	> 40	> 40	0.04	Synergy	
9	> 40	> 40	-0.07	antag.	
10	> 40	> 40	-0.15	antag.	
11	27.5 ± 4.4	12.4 ± 1.6	0.11	Synergy	
12	32.1 ± 4.2	14.5 ± 1.7	0.07	Synergy	
13	25.1 ± 2.2	11.7 ± 0.6	0.15	synergy	
14	15.1 ± 1.3	7.6 ± 0.7	0.08	synergy	✔
15	15.9 ± 1.1	7.5 ± 0.4	0.11	synergy	✔
16	14.6 ± 0.8	7.5 ± 0.7	0.05	synergy	

The Compounds **5** and **6** had little effect as single agents and were not synergistic in combination with dexamethasone, which indicates that both the right and left hand sides of the molecule are required for activity. Furthermore, **1-4** all contain a thioimidazoline substructure, which alone has no dexamethasone sensitizing effect,

thus, molecules containing just this element are not necessarily going to be dexamethasone sensitizers. Removing the ring system at the R position (**7**) or addition of a second thioimidazoline group (**8**) results in the loss of single and combination activity, which indicates that the R group is important, although this differs considerably between **1**-**4**.

Eliminating the carbon between the sulfur and carbonyl groups (**9**) or the addition of an extra carbon (**10**) destroys single agent activity and dexamethasone sensitizing effects. Thus, one carbon between the sulfur and the carbonyl is required to sensitize ALL cells to dexamethasone. While the ring structures at the R position in structures **11**-**16** differ considerably, all the structures were synergistic, although none were as potent as compound **1**.

Comparing **12** and **13**, the addition of a second cyclohexane improves both the single agent and combination activity. Compounds **14** and **15** are enantiomers of each other and yet they have the same activity, which suggests that the biological target responsible for dexamethasone sensitization can accommodate a relatively large group at several orientations. These data are supported by the fact that **4** and **13** are both relatively potent while containing two large hydrophobic groups orientated at different angles. It appears that the pocket accommodating the hydrophobic side chain is quite large. Indeed, a large hydrophobic pocket can accommodate an adamantly moiety, as shown by compound **16**'s impressive single agent and combination activity, which is similar to that seen with **1**-**4**, **14** and **15**. Thus, these promising results suggest that increasing the hydrophobic nature at the R position has positive effects on the combination potency and combination effect.

The *ex vivo* half-lives of **1, 2**, **3, 4,** dexamethasone and the standard chemotherapy agent vincristine, were determined using mouse liver microsomes (Supple. Figure 8 and Supple. Table 5). While dexamethasone showed the greatest stability with a half-life > 90 min, the half-lives of **3** and vincristine were comparable (29.2 min and 25.4 min, respectively). The half-lives of **1**, **2**, and **4** were considerably poorer than **3** (2.0 min, 9 min, and 8.4 min, respectively), hence **3** shows the greatest stability of the analogues tested.

As compound **3** exerted the highest median synergy with dexamethasone of compounds **1**-**4** in ALL-19 and also displayed the longest half-life, a second generation of glucocorticoid sensitizers were developed based on compound **3** and the SAR developed from **1**-**16**. Compound **3** was synthesized in order to produce reasonable quantities of material for testing, and to verify its activity was repeatable. We designed and synthesized twelve molecules, where we varied the R_1 and R_2 positions on the acetamide (Figure 4). Variation of R_1 with R_2 = H generated 9 derivatives, compounds **19**-**27**. Incorporation of the isopropyl group based on **3** produced compounds **28**-**30** (Figure 4). Finally, inclusion of a large steric methylcyclohexyl moiety generated derivatives **31**-**34** (Figure 4).

The synthesis of **3** and derivatives **28**-**34** involved the addition of **18** refluxed with the appropriate alkyl bromide **35** (isopropyl or methylcyclohexyl) (Scheme 2). Chloroacetyl chloride **17**, is reacted with product **36**, generating acetamide **37**. Reaction between the acetamide and thioimidazoline **5** generates analogs **28**-**34** (**38**). The synthesis of compounds **19**-**22** and **27**, which contain a secondary acetamide, is shown in Scheme 1. Compounds **23**-**26** were commercially available.

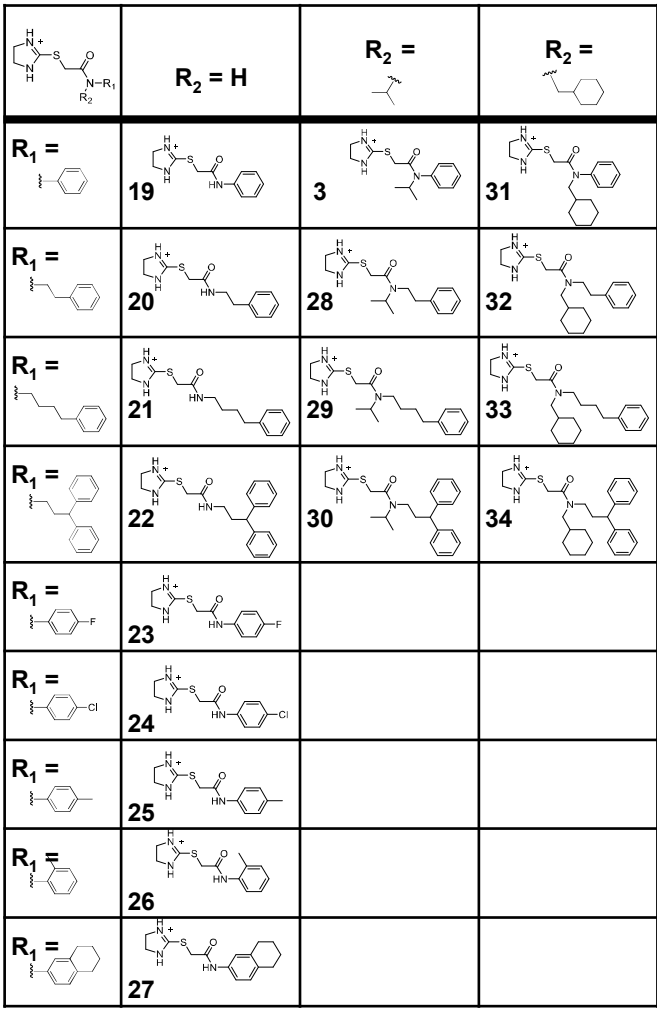
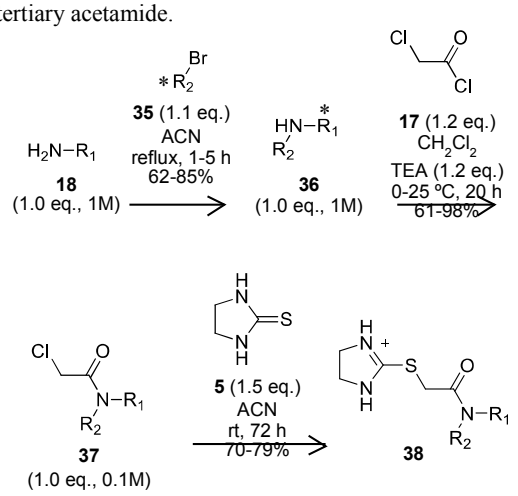


Figure 4. Compounds designed to investigate the SAR of 3.

The *ex vivo* anti-leukemic activity of all sixteen compounds and synthesized **3** were tested alone and in combination with dexamethasone against the xenograft, ALL-19, using fixed-ratio combination cytotoxicity assays (Suppl. Figure 9). The IC_{50} values were calculated from the fixed-ratio combination cytotoxicity assays, where compounds were tested at a 1:1 ratio with dexamethasone(Table 2). It is important to remember that in dexamethasone-resistant xenografts, including ALL-19, dexamethasone is completely inactive by itself. Thus, only the compounds or the combination will be active.

To determine whether compounds were synergistic, additive or antagonistic with dexamethasone, the deviation from Bliss-Additivity (BA) was calculated at each tested dose, where synergy is defined as a positive deviation, additive effect as no deviation, and antagonism as a negative deviation (Table 2 and Supple. Table 6).[16]

Scheme 2. General synthesis of dexamethasone sensitizers with tertiary acetamide.



*R_1 and R_2 are from the amine shown in Table 2 for compounds **3, 19-34**

Comparing analogs **19-27**, where R_2 = H, showed that molecules **19-24** and **27** were synergistic, while compounds **25** and **26** were antagonistic. However, none of the compounds met the potency criteria of a combination IC_{50} below that of compound **1** (IC_{50} < 6.1 μM). Compounds where R_2 = an isopropyl moiety (**28-30**) were more potent sensitizers than where R_2 = H (**20-22**). Specifically, **30** was synergistic and potent, with a combination IC_{50} = 5.0 μM. The exception to this is when the aromatic ring is placed 4 carbons from the acetamide moiety (**21** and **29**), where the addition of an isopropyl group reduces both potency and synergy with dexamethasone.

Evaluating compounds **31-34**, where R_2 = a methyl cyclohexyl moiety, showed that all compounds were synergistic and more potent sensitizers than where R_2 = H (**19-22**) or R_2 = an isopropyl moiety (**3**, **28-30**). Specifically, **32** and **34** where synergistic and potent, with combination IC_{50} values of 4.7 μM and 6.0 μM respectively. As observed in the isopropyl series, compound **33**, where R_2 = a methyl cyclohexyl moiety and the aromatic ring is placed 4 carbons from the acetamide moiety, was the least potent sensitizer out of **31-34**.

Analysis of the structure-activity relationships of these molecules showed that the optimal distance between the aromatic ring (R_1) and the acetamide moiety is 2 or 3 carbons, where **32** is 2 carbons, but **30** and **34** are both 3 carbons. Molecules possessing 0 or 4 carbons between the aromatic group and the amide nitrogen (**3**, **19**, **21**, **23-27**, **29**, **31** and **33**) all had higher combination IC_{50} values than their 2-3 carbon counterparts (**20**, **22**, **28**, **30**, **32**, **34**). These data suggest that the aromatic group is optimally interacting with the biological target when placed 2-3 carbons away from the acetamide. Furthermore, having a tertiary acetamide, compounds **28-34**, generated more potent compounds than molecules that were secondary acetamides. Interestingly, the secondary acetamides showed that the electronegative substituent F or the electron donating substituent methyl, do not enhance the potency of the molecule. However, a Cl substituent appears to play an important role in binding to the target.

Table 2. Antileukemic activity of compounds alone and in combination with dexamethasone *ex vivo* against ALL-19 xenograft cells.

Compound	Cmpd IC_{50} (μM)	Combo IC_{50} (μM)	Median BA	Combo effect
3	18.1 ± 2.0	9.2 ± 0.5	0.09	synergy
19	> 40	31.8 ± 4.8	0.04	synergy
20	37.9 ± 2.6	18.8 ± 2.2	0.10	synergy
21	29.0 ± 3.9	14.8 ± 1.0	0.07	synergy
22	20.1 ± 1.4	13.4 ± 0.8	0.04	synergy
23	> 40	21.5 ± 1.9	0.03	synergy
24	23.2 ± 2.4	9.7 ± 2.2	0.09	synergy
25	> 40	> 40	-0.02	antag.
26	> 40	> 40	-0.01	antag.
27	> 40	22.8 ± 4.2	0.03	synergy
28	18.1 ± 1.2	9.2 ± 0.9	0.05	synergy
29	22.4 ± 1.3	15.4 ± 0.1	0.00	additive
30	11.3 ± 1.4	5.0 ± 0.6	0.08	synergy
31	15.0 ± 0.7	6.6 ± 0.5	0.12	synergy
32	9.5 ± 1.3	4.7 ± 0.3	0.10	synergy
33	14.2 ± 0.4	7.5 ± 0.5	0.07	synergy
34	10.5 ± 1.0	6.0 ± 0.1	0.03	synergy

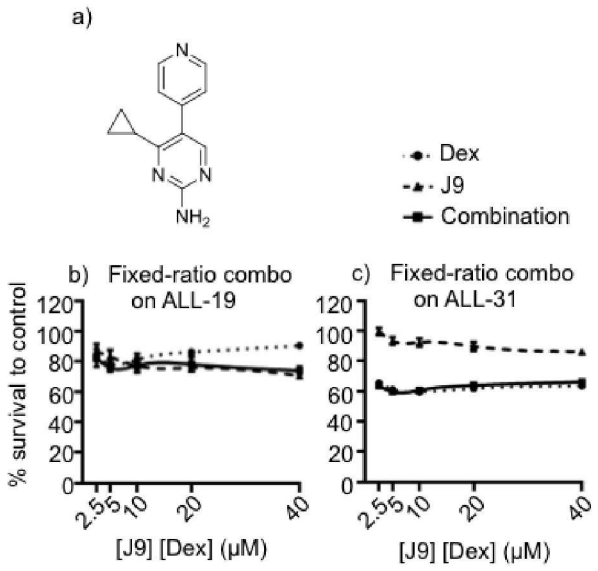


Figure 5. (a) Novel dexamethasone sensitizer identified from HTS of the T-cell ALL cell line, CUTLL1.[17] (b) ALL-19 xenograft cells were exposed to J9, Dex or both in combination at a fixed-ratio of concentrations for 48 h. Cell viability was assessed by Alamar Blue assay. (c) ALL-31 xenograft cells were exposed to J9, Dex, or both in combination at a fixed-ratio of concentrations for 48 h. Cell viability was assessed by Alamar Blue assay. Each data point represents the mean ± SEM of three independent experiments.

CONCLUSION

In summary, we have identified a novel class of dexamethasone sensitizing compounds that contain a thioimidazoline group. Compound **1** is a glucocorticoid sensitizer that acts specifically on glucocorticoid-resistant ALL. SAR analysis indicates that thioimidazoline alone does not have any dexamethasone sensitizing effect and the thioimidazoline acetamide substructure cannot be altered without losing dexamethasone sensitizing effect. Furthermore, we have identified three molecules from the series, **30**, **32** and **34**, that are synergistic and more potent than **1**. Our data suggest that increasing the hydrophobic bulk at positions R_1 and R_2 decreases the IC_{50} value when used in combination with dexamethasone. Future development of this novel class of dexamethasone sensitizers will focus on defining the mechanism by which these molecules function as glucocorticoid sensitizers.

EXPERIMENTAL SECTION

General Biological Procedures. Cell culture tested, water-insoluble dexamethasone and prednisolone were used in all experiments (Sigma-Aldrich, Castle Hill, Australia). Cell viability was determined using ViaCount (Merck Millipore, Billerica, MA) or Resazurin reagent (aka Alamar Blue). Flow cytometry was performed using a Guava easyCyte flow cytometer (Merck Millipore, Billerica, MA). Compounds **1** to **4** were identified from a high-throughput screen of a random selection of 40 000 compounds from the Australian Cancer Research Foundation Drug Discovery Centre for Childhood Cancer diversity library.[18]

***Ex vivo* cell culture.** The development and characterization of a series of pediatric ALL xenografts derived from patient biopsies have been previously described.[11] All assays were performed using mycoplasma-free and validated stocks of xenograft cells. For all experiments described in this manuscript, xenograft cells were retrieved from cryostorage and resuspended in RPMI-1640 medium (Invitrogen Life Technologies, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS; Invitrogen Life Technologies), penicillin (100 U/mL), streptomycin (100 μg/mL), and L-glutamine (2 mM) (complete RPMI). Cells were centrifuged at 490 × g for 5 minutes, aspirated, and washed with complete RPMI. The cells were resuspended in QBSF-60 medium (Quality Biological, Gaithersburg, MD) supplemented with Flt-3 ligand (20 ng/mL; Amgen, Thousand Oaks, CA), penicillin (100 U/mL), streptomycin (100 μg/mL), and L-glutamine (2 mM) (complete QBSF) at a cell concentration previously optimized for each xenograft (1-5 × 10^6 cells/mL). Viability was determined by the exclusion of 0.2% trypan blue (Sigma-Aldrich). For all experiments, cells were seeded and equilibrated at 37ºC, 5% CO_2, for 12-16 h prior to drug treatment.

Combination cytotoxicity assays. Xenograft cells were retrieved from cryostorage, washed with complete RPMI and resuspended in complete QBSF at a cell concentration previously optimized for each xenograft (1-5 × 10^6 cells/mL), and 100 μL was seeded in 96-well clear, U-bottom tissue culture treated plates (In Vitro Technologies, VIC, Australia). Plates were equilibrated at 37ºC, 5% CO_2, for 12-16 h prior to compound treatment. Compounds were serially diluted in complete QBSF medium and added in triplicate wells. Cells were treated with compound and dexamethasone/prednisolone simultaneously at a fixed-ratio of concentrations corresponding to ¼, ½, 1, 2 and 4 times the IC_{50} values for each drug independently and in combination. Where the IC_{50} values for each compound and dexamethasone were >10 μM, cells were treated with the following concentrations; 2.5 μM, 5 μM, 10 μM, 20 μM and 40 μM, independently and in combination. Following 48 h incubation at 37ºC, 5% CO_2, cell viability was assessed by mitochondrial activity assay (Resazurin cell viability assay, 6 h incubation) or by flow cytometry (ViaCount reagent). Cell viability was calculated as a percentage of untreated controls. Results presented are the mean ± standard error of the mean (SEM) of a minimum of 3 independent experiments. IC_{50} values were calculated from cumulative survival curves.

Calculation of combination effect. To determine whether compounds were synergistic, additive or antagonistic with dexamethasone/prednisolone, the Bliss-Additivity model was used.[16] The Bliss-Additivity model predicts the additive effect for two single compounds (A and B) at a single concentration as;

Bliss-Additive effect= (FaA + FaB) – (FaA × FaB)

Where, FaA is the fraction of cells affected by compound A alone at a single concentration, and FaB is the fraction of cells affected by compound B alone at a single concentration.

To determine the effect of the combination (C) at a single concentration, the deviation from the calculated Bliss-Additive effect is calculated as;

Deviation from Bliss-Additivity (BA)= (experimental FaC) – (calculated Bliss-Additive FaC)

Where, 'experimental FaC' is the fraction of cells affected by the combination of A and B at a single concentration, and 'calculated Bliss-Additive FaC' is the calculated additive effect of A and B at a single concentration.

Deviation from Bliss-Additivity (BA) was calculated at each tested dose, where synergy is defined as a positive deviation, additive effect as no deviation, and antagonism as a negative deviation.

Half-life assays. Half-life assays were performed to determine compound stability. Compounds were tested individually *ex vivo* in

liver microsomes. Compound (final concentration 1 μM) was equilibrated at 37ºC for 5 min in purified water (Gibco Ultrapure distilled water) supplemented with 20% potassium phosphate (0.5M, pH 7.4), 5% NADPH Regenerating System Solution A (BD Biosciences, catalogue number 451220), and 1% NADPH Regenerating System Solution B (BD Biosciences, catalogue number 451200). Liver microsomes (final concentration 0.5 mg/mL, BD Biosciences, catalogue number 452701) were added to the compound solution and a portion of the compound/microsome solution was immediately added to acetonitrile (1:1 dilution) and incubated at 0ºC (0 min time point).
The remaining compound/microsome solution was incubated at 37ºC and samples were taken, diluted with acetonitrile (1:1) and incubated at 0ºC at the following time points; 5 min, 15 min, 30 min, 60 min and 90 min. Each time point sample was centrifuged at 10,000 × g for 3 minutes at 0ºC and the supernatant was analyzed by mass spectroscopy. Compound stability in liver microsomes over time was calculated by plotting the area under the curve for each time point as a percentage of the 0 min time point. Results presented are the mean ± standard error of the mean (SEM) of a minimum of 3 independent experiments. Half-life values were calculated from one phase exponential decay curves.

General Chemical Procedures. All chemical reagents and purchased compounds were used without further purification (Sigma-Aldrich, Enamine Ltd, Princeton BioMolecular Research Inc.). All reactions were performed with anhydrous solvents under N_2 atmosphere. Reactions were monitored by thin-layer chromatography (TLC) using silica coated aluminium plates (250 μM Whatman®, 4861-820) and visualized with UV light (λ=254 nm) and the developing agents; potassium permanganate and ninhydrin. Solvent was removed in vacuo using a Buchi RE121 rotatory evaporator. Flash chromatography was performed using Davisil® silica gel (60Å, particle size 40-63 μM).
Reversed-phase HPLC purification was performed on a Shimadzu Prominence High-performance LCMS 2010EV system (Phenomenex® Jupiter C18 column, 4μm, 250x10mm). The Mobile phase was composed of deionised water (solvent A), and HPLC grade methanol (solvent B). The flow rate was 2 mL/min and the following elution gradient was employed; 0-10 min, 100% solvent A; 10-25 min, gradient increase from 0% solvent B to 100% solvent B; 25-35 min, 100% solvent B; 35-55 min, gradient decrease from 100% solvent B to 0% solvent B.

LC/MS analysis was performed on a Shimadzu Prominence High-performance LCMS 2010EV system (Waters Symmetry® C18 column, 3.5μm, 4.6 × 75mm) connected to a Shimadzu LCMS 2010EV mass spectrometer running in positive electrospray ionization (ESI+) mode. The Mobile phase was composed of deionised water with 0.1% (v/v) formic acid (solvent A), and HPLC grade acetonitrile with 0.1% (v/v) formic acid (solvent B). The flow rate was 0.5 mL/min and the following elution gradient was employed; 0-4 min, gradient increase from 30% solvent B to 100% solvent B; 4-12 min, 100% solvent B; 12-16 min, gradient decrease from 100% solvent B to 30% solvent B.

HRMS analysis was performed using a Thermo LTQ Orbitrap XL ESI/APCI with UPLC system at the Bioanalytical Mass Spectrometry Facility within the Mark Wainwright Analytical Centre at the University of New South Wales. NMR spectra were performed at ~298K on a Bruker Avance III 300MHz spectrometer. Chemical shifts (δ) were reported in ppm and were calibrated with the residual solvent resonance.
To perform X-ray crystallography, suitable single crystals of **1** and **6** were selected under a polarizing microscope (Leica M165Z), mounted on a MicroMount (MiTeGen, USA) consisting of a thin polymer tip with a wicking aperture. The X-ray diffraction measurements were performed on a Bruker kappa-II CCD diffractometer at 150 K, employing a IμS Incoatec Microfocus Source with Mo-Kα radiation (λ = 0.710723 Å) . A single crystal, mounted on the goniometer using cryo loops for intensity measurements, was coated with paraffin oil and then quickly transferred to the cold stream using an Oxford Cryo stream attachment. Symmetry related absorption corrections using the program SADABS (Bruker, AXS Inc., Wisconsin, USA, 2001) were applied and the data were corrected for Lorentz and polarisation effects using Bruker APEX2 software (Bruker, AXS Inc., Wisconsin, USA, 2007). The structure was solved by direct methods and the full-matrix least-square refinement was carried out using XL in Olex2.[19, 20] The non-hydrogen atoms were refined anisotropically. The molecular graphic was generated using program Olex2.[20]
MS analysis of half-life assay samples was performed using a ThermoFisher Scientific Quantum Access mass spectrometer and Accela UHPLC pump with a CTC Analytics PAL autosampler at the Bioanalytical Mass Spectrometry Facility within the Mark Wainwright Analytical Centre at the University of New South Wales. Compounds were quantified using ultra-high performance liquid chromatography coupled to mass spectrometry. Separation was performed on a Phenomenex Kinetix Column (2.1 x 50mm) held at 30 degrees C using a 5 minute gradient of 0.1% formic acid in water vs acetonitrile. Compounds were detected in the Selected Reaction Monitoring mode with transitions, polarity and instrument source conditions being optimized for each compound before analysis.

Purchased compounds 1-5, 8, 9, 16, 23-26 and J9. All purchased compounds were used without further purification.

***2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-cycloheptylacetamide* (1).** Purchased from Enamine Ltd, Ukraine, catalogue number T0511-1822. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.34-1.77 (m, 10H), 1.83-1.99 (m, 2H), 3.73-3.88 (m, 1H), 3.97 (s, 4H), 4.10 (s, 2H), 9.25 (d, 1H, *J*=7.68 Hz) 11.13 (s, 2H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 24.17, 28.00, 33.45, 34.15, 45.03, 52.06, 167.82, 172.16. **LCMS(ESI)**: calcd for $C_{12}H_{22}N_3OS$+ [M+] = 256.1478, found 256.30.

***2-(((4,5-dihydro-1H-imidazol-2-yl)thio)methyl)benzo[d]thiazole* (2).** Purchased from Enamine Ltd, Ukraine, catalogue number T0512-8364. **^{1}H NMR** (CD_3OD, 300 MHz) δ (ppm) 4.00 (s, 4H), 4.93 (s, 2H), 7.21 (d, 2H, *J*=8.02 Hz), 7.70 d, 2H, *J*=7.67 Hz) **^{13}C NMR** (CD_3OD, 300 MHz) δ (ppm) 13.02, 19.89, 22.29, 31.34, 31.74, 45.46, 121.76, 122.59, 125.54, 125.88, 126.53, 128.39, 140.24, 142.18, 152.41. **LCMS(ESI)**: calcd for $C_{11}H_{12}N_3S_2$+ [M+] = 250.0467, found 250.20.

***2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-isopropyl-N-phenylacetamide* (3).** Purchased from Princeton BioMolecular Research Inc., NJ, USA, catalogue number OSSK_003145. **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.11 (d, 6H, *J*=6.62 Hz), 3.60 (s, 2H), 3.99 (s, 4H), 4.87 (m, 1H, *J*=6.69 Hz), 7.14-7.24 (m, 2H), 7.46-7.55 (m, 3H), 11.07 (br, 2H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 20.64, 33.64, 45.85, 48.01, 129.65, 129.69, 130.22, 136.46, 167.45,

170.85. **LCMS(ESI)**: calcd for $C_{14}H_{20}N_3OS+$ [M+] = 278.1322, found 278.30.

***2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-benzhydrylacetamide* (4).** Purchased from Enamine Ltd, Ukraine, catalogue number T6256376. **LCMS(ESI)**: calcd for $C_{18}H_{20}N_3OS+$ [M+] = 326.1322, found 326.30.

***imidazolidine-2-thione* (5).** Purchased from Sigma-Aldrich, Castle Hill, Australia catalogue number 03940. **^{1}H NMR** ((CD_3)$_2$SO, 300 MHz) δ (ppm) 3.48 (s, 4H), 7.93 (br, 2H). **LCMS(ESI)**: calcd for $C_3H_6N_2S$ [M] = 102.0252, found 102.90.

***1,4-bis((4,5-dihydro-1H-imidazol-2-yl)thio)butane* (8).** Purchased from Sigma-Aldrich, Castle Hill, Australia, catalogue number CCA001895. **LCMS(ESI)**: calcd for $C_{10}H_{20}N_4S_2+$ [M+] = 260.1118, found [1/2 M+] 130.30.

S-(4,5-dihydro-1H-imidazol-2-yl)cyclohexylthiocarbamate (9). Purchased from Sigma-Aldrich, Castle Hill, Australia, catalogue number CCA002504. **LCMS(ESI)**: calcd for $C_{10}H_{17}N_3OS$ [M] = 227.1085, found 227.90.

***2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N((3s,5s,7s)adamantanyl)lacetamide* (16).** Purchased from Enamine Ltd, Ukraine, catalogue number Z48834237. **LCMS(ESI)**: calcd for $C_{11}H_{20}N_3OS+$ [M+] = 294.1635, found 294.00.

***2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(4-fluorophenyl)acetamide* (23).** Purchased from Enamine Ltd, Ukraine, catalogue number Z48834313. **LCMS(ESI)**: calculated for $C_{11}H_{13}FN_3OS+$ [M+] = 254.0758, found 254.30.

***N-(4-chlorophenyl)-2-((4,5-dihydro-1H-imidazol-2-yl)thio)acetamide* (24).** Purchased from Vitas-M Laboratory Ltd, the Netherlands, catalogue number STL112120). **LCMS(ESI)**: calculated for $C_{11}H_{13}ClN_3OS+$ [M+] = 270.0462, found 270.25.

***2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(p-tolyl)acetamide* (25).** Purchased from Chembridge, San Diego, CA, and catalogue number 9038095. **LCMS(ESI)**: calculated for $C_{12}H_{16}N_3OS+$ [M+] = 250.1009, found 250.35.

***2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(o-tolyl)acetamide* (26).** Purchased from Sigma-Aldrich, Castle Hill, Australia, catalogue number L120413. **LCMS(ESI)**: calculated for $C_{12}H_{16}N_3OS+$ [M+] = 250.1009, found 249.95.

J9. Purchased from Reagency Pty Ltd, Victoria, Australia, catalogue number RGNCY_0013. **^{1}H NMR** ((CD_3)$_2$SO, 300 MHz) δ (ppm) 0.88-0.96 (m, 2H), 1.04-1.10 (m, 2H), 1.87-1.98 (m, 1H), 6.67 (s, 1H), 7.43-7.47 (m, 2H), 8.08 (s, 1H), 8.59-8.64 (m, 2H). **^{13}C NMR** ((CD_3)$_2$SO, 300 MHz) δ (ppm) 11.05, 14.33, 39.99, 120.73, 124.14, 145.11, 150.18, 157.67, 163.55, 168.45. **LCMS(ESI)**: calcd for $C_{12}H_{12}N_4$ [M + H] = 213.1142, found 213.30. **HRMS(ESI)**: calcd for $C_{12}H_{12}N_4$ [M + H] = 213.1142, found 213.1134.

General procedure for synthesis of intermediate 6 (Scheme 1). The appropriate primary amine (**18**, 1 equiv, 7 mmol) was added drop wise to a mixture of triethylamine (1.2 equiv, 8.4 mmol) in dichloromethane (4.5 mL) under nitrogen atmosphere at 0 ºC. Chloroacetyl chloride (**17**, 1.2 equiv, 8.4 mmol) was added drop wise at 0 ºC, then the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was concentrated *in vacuo* and the residue washed with ice water (3 × 20 mL) and filtered. The solid residue was purified by flash chromatography on silica gel.

General procedure for synthesis of intermediate 36 (Scheme 2). The appropriate primary amine (**18**, 1 equiv, 30 mmol) was added drop wise to a mixture of the appropriate alkyl bromide (**35**, 1.1 equiv, 33 mmol) in acetonitrile (30 mL) under nitrogen atmosphere. The reaction mixture was refluxed for 1-5 h, then cooled to room temperature. The reaction mixture was washed with sodium hydrogen carbonate and extracted with dichloromethane (3 × 20 mL). The organic layers were combined and dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The crude product was used for the synthesis of intermediate **37** without further purification.

General procedure for synthesis of intermediate 37 (Scheme 2). The appropriate secondary amine (**36**, 1 equiv, 15 mmol) was added drop wise to a mixture of triethylamine (1.2 equiv, 18 mmol) in dichloromethane (15 mL) under nitrogen atmosphere at 0 ºC. Chloroacetyl chloride (**17**, 1.2 equiv, 18 mmol) was added drop wise at 0 ºC, then the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was concentrated *in vacuo* and the residue washed with ice water (3 × 20 mL) and filtered. The solid residue was purified by flash chromatography on silica gel.

General procedure for synthesis of final compounds (Schemes 1 and 2). The appropriate chloro acetamide (**6** or **37**, 1 equiv, 0.1 M) was stirred with *N,N'*-ethylenethiourea (**5**, 1.5 equiv) in acetonitrile under nitrogen atmosphere for 72 h. The precipitate was concentrated *in vacuo* and purified by either HPLC or recrystallization.

Synthesis of Compound 1:
***2-chloro-N-cycloheptylacetamide* (intermediate 1-6, compound 6).** Cycloheptylamine (**18**, 0.89 mL, 7 mmol), triethylamine (1.17 mL, 8.4 mmol), dichloromethane (4.5 mL), chloroacetyl chloride (**17**, 0.66 mL, 8.4 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **1-6** (pale yellow solid, 1163.4 mg, 88% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.40-1.72 (m, 10H), 1.86-2.02 (m, 2H), 3.90-4.04 (m, 1H), 4.02 (s, 2H), 6.50 (br, 1H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 23.97, 27.93, 34.82, 42.77, 50.88, 164.59.

***2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-cycloheptylacetamide* (1).** 2-chloro-*N*-cycloheptylacetamide (**1-6**, 409.8 mg, 2.2 mmol), *N,N'*-ethylenethiourea (**5**, 326.4 mg, 3.2 mmol), acetonitrile (21 mL). Purified by recrystallization (CH_2Cl_2 and hexane) to yield compound **1** (white solid, 322.2 mg, 58% isolated yield, 204.1 mg, 37% purified yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.34-1.80 (m, 10H), 1.84-1.99 (m, 2H), 3.74-3.88 (m, 1H), 3.97 (s, 4H), 4.11 (s, 2H), 9.26 (d, 1H, *J*=7.77 Hz) 11.15 (s, 2H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 24.19, 28.02, 33.45, 34.15, 45.04, 52.09, 167.85, 172.21. **HRMS(ESI)**: calcd for $C_{12}H_{22}N_3OS+$ [M + H] = 256.1478, found 256.1481.

Synthesis of Compound 3:
***N-isopropylaniline* (intermediate 3-36).** Aniline (**18**, 3 mL, 30 mmol), isopropyl bromide (**35**, 3.4 mL, 40 mmol), acetonitrile (30

mL). Crude yield 3.48 g, 85%. **LCMS:** calcd for $C_9H_{13}N$+ [M + H]= 135.10, found 134.90.

2-chloro-N-isopropyl-N-phenylacetamide **(intermediate 3-37).** *N*-isopropylaniline (**3-36**, 2 g, 15 mmol), triethylamine (1.8 g, 18 mmol), dichloromethane (9.9 mL), chloroacetyl chloride (**17**, 2g, 18 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **3-37** (pale yellow solid, 2.71 g, 85% yield). **LCMS:** calcd for $C_{11}H_{14}ClNO$+ [M + H]= 211.08, found: 211.10.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-isopropyl-N-phenylacetamide **(3).** 2-chloro-*N*-isopropyl-*N*-phenylacetamide (**3-37**, 1 g, 5.0 mmol), *N,N'*-ethylenethiourea (**5**, 0.58 g, 5.6 mmol), acetonitrile (50 mL). Purified by recrystallization (CH_2Cl_2 and hexane) to yield compound **3** (white solid, 73% purified yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.08 (s, 3H), 1.11 (s, 3H), 3.78 (s, 2H), 3.93 (s, 4H), 4.89 (5ry, 1H), 7.31 – 7.527 (m, $5H_{Ar}$). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 20.66, 34.41, 45.77, 47.91, 129.53, 129.80, 130.10, 136.52, 166.88, 170.56. **LCMS** calcd for $C_{14}H_{19}N_3OS$+ [M + H] = 278.12, found: 278.35.

Synthesis of Compound 7:

2-((4,5-dihydro-1H-imidazol-2-yl)thio)acetic acid **(7).** *2-((4,5-dihydro-1H-imidazol-2-yl)thio)acetic acid* was synthesized based on the method of Kushakova, et al.[21] Chloroacetic acid (720.1 mg, 7.6 mmol) and *N,N'*-ethylenethiourea (**5**, 516.2 mg, 5.1 mmol) were stirred at room temperature for 8 h in acetone (10 mL). The precipitate was concentrated *in vacuo* and purified by recrystallization (CH_2Cl_2 and hexane) to yield compound **7** (white solid, 228.4 mg, 28% purified yield). **^{1}H NMR** ($(CD_3)_2SO$, 300 MHz) δ (ppm) 3.82 (s, 4H), 4.32 (s, 2H), 10.64 (br, 2H). **^{13}C NMR** ($(CD_3)_2SO$, 300 MHz) δ (ppm) 33.79, 45.13, 168.31, 168.50. **HRMS(ESI)**: calcd for $C_5H_9N_2O_2S$+ [M + H] = 161.0379, found 161.0377.

Synthesis of Compound 10:

3-chloro-N-cycloheptylpropanamide **(intermediate 10-6).** Cycloheptylamine (**18**, 0.89 mL, 7 mmol), triethylamine (1.17 mL, 8.4 mmol), dichloromethane (4.1 mL), 3-chloropropionyl chloride (**17**, 0.80 mL, 8.4 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **10-6** (clear oil, 1012.5 mg, 71%). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.34-1.71 (m, 10H), 1.84-2.01 (m, 2H), 2.58 (t, 2H, *J*=6.50 Hz), 3.78 (t, 2H, *J*=6.50 Hz), 3.88-4.08 (m, 1H), 5.81 (br, 1H). **^{13}C NMR** (CD_3OD, 300 MHz) δ (ppm) 23.90, 27.88, 34.69, 38.6, 39.9, 50.49, 50.60, 124.92, 130.96, 165.33, 169.57. **HRMS(ESI)**: calcd for $C_{10}H_{18}ClNONa$ [M + Na] = 226.0975, found 226.0967.

3-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-cycloheptylpropanamide **(10).** 3-chloro-*N*-cycloheptylpropanamide (**10-6**, 300.0 mg, 1.5 mmol), *N,N'*-ethylenethiourea (**5**, 225.7 mg, 2.2 mmol), acetonitrile (15 mL). Purified by HPLC to yield compound **10** (white solid, 143.6 mg, 36% isolated yield, 12% purified yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.38-1.75 (m, 10H), 1.83-1.97 (m, 2H), 2.91 (t, 2H, *J*=6.74 Hz), 3.47 (t, 2H, *J*=6.47 Hz), 3.81-3.93 (m, 1H), 3.99 (s, 4H), 8.10 (d, 1H, *J*=7.84Hz). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 24.19, 27.56, 28.02, 34.66, 36.96, 45.85, 51.48, 169.95, 170.74. **HRMS(ESI)**: calcd for $C_{13}H_{24}N_3OS$+ [M + H] = 270.1635, found 270.1632

Synthesis of Compound 11:

2-chloro-N-cyclopentylacetamide **(intermediate 11-6).** Cyclopentylamine (**18**, 0.69 mL, 7 mmol), triethylamine (1.17 mL, 8.4 mmol), dichloromethane (4.5 mL), chloroacetyl chloride (**17**, 0.66 mL, 8.4 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **11-6** (pale orange solid, 562.3 mg, 50% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.35-1.49 (m, 2H), 1.54-1.77 (m, 4H), 1.93-2.08 (m, 2H), 4.01 (s, 2H), 4.14-4.27 (m, 1H), 6.48 (br, 1H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 23.67, 32.91, 42.68, 51.54, 165.30. **HRMS(ESI)**: calcd for $C_7H_{12}ClNONa$ [M + Na] = 184.0505, found 184.0500.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-cyclopentylacetamide **(11).** 2-chloro-*N*-cyclopentylacetamide (**11-6**, 200.0 mg, 1.2 mmol), *N,N'*-ethylenethiourea (**5**, 326.4 mg, 189.6 mg, 1.9 mmol), acetonitrile (12 mL). Purified by recrystallization (CH_2Cl_2 and hexane) to yield compound **11** (yellow crystals, 202.3 mg, 74% isolated yield, 180.4 mg, 66% purified yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.50-1.73 (m, 4H), 1.73-1.86 (m, 2H), 1.86-2.07 (m, 2H), 4..00 (s, 4H), 4.06-4.18 (m, 1H), 4.12 (s, 2H), 9.34 (d, 1H, *J*=7.26 Hz), 11.16 (s, 2H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 23.89, 32.39, 33.32, 45.84, 52.28, 168.58, 172.18. **HRMS(ESI)**: calcd for $C_{10}H_{18}N_3OS$+ [M + H] = 228.1165, found 228.1163.

Synthesis of Compound 12:

2-chloro-N-cyclohexylacetamide **(intermediate 12-6).** Cyclohexylamine (**18**, 0.80 mL, 7 mmol), triethylamine (1.17 mL, 8.4 mmol), dichloromethane (4.5 mL), chloroacetyl chloride (**17**, 0.66 mL, 8.4 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **12-6** (pale orange solid, 614.1 mg, 50% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.13-1.30 (m, 3H), 1.32-1.49 (m, 2H), 1.59-1.81 (m, 3H), 1.89-2.00 (m, 2H), 3.74-3.88 (m, 1H), 4.05 (s, 2H) 6.45 (br, 1H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 24.71, 25.41, 32.80, 42.74, 48.65, 164.81. **HRMS(ESI)**: calcd for $C_8H_{14}ClNONa$ [M + Na] = 198.0662, found 198.0655.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-cyclohexylacetamide **(12).** 2-chloro-*N*-cyclohexylacetamide (**12-6**, 300.0 mg, 1.7 mmol), *N,N'*-ethylenethiourea (**5**, 261.7 mg, 2.6 mmol), acetonitrile (17 mL). Purified by HPLC to yield compound **12** (white oil, 247.0 mg, 60% isolated yield, 52% purified yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.13-1.51 (m, 4H), 1.54-1.65 (m, 1H), 1.72-1.98 (m, 5H), 3.59-3.74 (m, 1H), 4.00 (s, 4H), 4.13, (s, 2H), 9.24 (d, 1H, *J*=7.95 Hz), 11.16 (s, 2H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 24.75, 25.30, 32.03, 33.46, 45.83, 49.79, 168.21, 172.19**. HRMS(ESI)**: calcd for $C_{11}H_{20}N_3OS$+ [M + H] = 242.1322, found 242.1319.

Synthesis of Compound 13:

2-chloro-N,N-dicyclohexylacetamide **(intermediate 13-6).** Dicyclohexylamine (**18**, 1.39 mL, 7 mmol), triethylamine (1.17 mL, 8.4 mmol), dichloromethane (4.5 mL), chloroacetyl chloride (**17**, 0.66 mL, 8.4 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **13-6** (brown solid, 912.0 mg, 51% yield). **^{1}H NMR** (CD_3OD, 300 MHz) δ (ppm) 1.09-1.96 (m, 18H), 2.41 (q, 2H, *J*=12.12 Hz), 3.00-3.19 (m, 1H), 3.51-3.66 (m, 1H), 4.19 (s, 2H). **^{13}C NMR** (CD_3OD, 300 MHz) δ (ppm) 26.20, 26.50, 26.76, 27.33, 30.55, 32.05, 44.09, 57.61, 60.35, 168.06. **HRMS(ESI)**: calcd for $C_{14}H_{24}ClNONa$ [M + Na] = 280.1444, found 280.1439.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N,N-dicyclohexylacetamide **(13).** 2-chloro-*N,N*-dicyclohexylacetamide

(**13-6**, 300.0 mg, 1.2 mmol), *N,N'*-ethylenethiourea (**5**, 178.3 mg, 1.8 mmol), acetonitrile (18 mL). Purified by HPLC to yield compound **13** (white solid, 288.1 mg, 77% isolated yield, 60% purified yield). **^{1}H NMR** (CD_3OD, 300 MHz) δ (ppm) 1.07-1.92 (m, 18H), 3.36 (q, 2H, *J*=11.61 Hz), 3.02-3.21 (m, 1H), 3.50-3.66 (m, 1H). 3.96 (s, 4H), 4.46 (s, 2H). **^{13}C NMR** (CD_3OD, 300 MHz) δ (ppm) 26.14, 26.48, 26.61, 27.31, 30.77, 31.90, 39.16, 46.66, 57.75, 60.26, 166.21, 171.96**. HRMS(ESI)**: calcd for $C_{17}H_{30}N_3OS$+ [M + H] = 324.2104, found 324.2103.

Synthesis of Compound 14:

(R)-2-chloro-N-(1-cyclohexylethyl)acetamide **(intermediate 14-6).** (S)-(+)-1-cyclohexylethylamine (**18**, 1.04 mL, 7 mmol), dichloromethane (4.5 mL), chloroacetyl chloride (**17**, 0.66 mL, 8.4 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **14-6** (pale orange solid, 828.2 mg, 58%). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 0.91-1.45 (m, 6H), 1.15 (d, 3H, *J*=6.56 Hz), 1.63-1.84 (m, 5H), 3.80-3.96 (m, 1H), 4.07 (s, 2H), 6.42 (br, 1H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 17.73, 26.10, 26.32, 28.91, 28.99, 42.89, 50.00, 164.96. **HRMS(ESI)**: calcd for $C_{10}H_{18}ClNONa$ [M + Na] = 226.0975, found 226.0971.

(R)-2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(1-cyclohexylethyl)acetamide **(14).** (*R)-2-chloro-N-(1-cyclohexylethyl)acetamide* (**14-6**, 400.0 mg, 2.0 mmol), *N,N'*-ethylenethiourea (**5**, 300.9 mg, 3.0 mmol), acetonitrile (20 mL). Purified by HPLC to yield compound **14** (white oil, 216.3 mg, 41% isolated yield, 32% purified yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 0.90-1.32 (m, 4H), 1.18 (d, 3H, *J*=7.00 Hz), 1.37-1.53 (m, 1H), 1.60-1.90 (6H), 3.66-3.81 (m, 1H), 4.00 (s, 4H), 4.16 (s, 2H), 9.13 (d, 1H, *J*=8.95 Hz), 11.16 (br, 2H) . **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 17.20, 26.09, 26.13, 26.34, 29.10, 29.27, 33.49, 42.66, 45.84, 51.17, 168.45, 172.17. **HRMS(ESI)**: calcd for $C_{13}H_{24}N_3OS$+ [M + H] = 270.1635, found 270.1632.

Synthesis of Compound 15:

(S)-2-chloro-N-(1-cyclohexylethyl)acetamide **(intermediate 15-6).** (R)-(-)-1-cyclohexylethylamine (**18**, 1.04 mL, 7 mmol), dichloromethane (4.5 mL), chloroacetyl chloride (**17**, 0.66 mL, 8.4 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **15-6** (pale orange solid, 1036.3 mg, 73%). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 0.91-1.45 (m, 6H), 1.15 (d, 3H, *J*=6.77 Hz), 1.64-1.84 (m, 5H), 3.81-3.96 (m, 1H), 4.07 (s, 2H), 6.42 (br, 1H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 17.73, 26.10, 26.32, 28.91, 28.99, 42.89, 50.00, 164.96. **HRMS(ESI)**: calcd for $C_{10}H_{18}ClNONa$ [M + Na] = 226.0975, found 226.0971 .

(S)-2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(1-cyclohexylethyl)acetamide **(15).** (*S)-2-chloro-N-(1-cyclohexylethyl)acetamide* (**15-6**, 400.0 mg, 2.0 mmol), *N,N'*-ethylenethiourea (**5**, 300.9 mg, 3.0 mmol), acetonitrile (20 mL). Purified by HPLC to yield compound **15** (white oil, 253.9 mg, 48% isolated yield, 36% purified yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 0.91-1.33 (m, 4H), 1.17 (d, 3H, *J*=6.80 Hz), 1.38-1.53 (m, 1H), 1.60-1.98 (6H), 3.65-3.80 (m, 1H), 3.99 (s, 4H), 4.16 (s, 2H), 9.12 (d, 1H, *J*=8.70 Hz), 11.15 (br, 2H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 17.20, 26.09, 26.12, 26.34, 29.10, 29.26, 33.49, 42.66, 45.84, 51.15, 168.42, 172.13. **HRMS(ESI)**: calcd for $C_{13}H_{24}N_3OS$+ [M + H] = 270.1635, found 270.1631.

Synthesis of Compound 19:

2-chloro-N-phenylacetamide **(intermediate 19-6).** Aniline (**18**, 0.64 mL, 7 mmol), triethylamine (1.17 mL, 8.4 mmol), dichloromethane (4.5 mL), chloroacetyl chloride (**17**, 0.66 mL, 8.4 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **19-6** (dark green solid, 996.5 mg, 84% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 4.18 (s, 2H), 7.17 (t, 1H, *J*=7.51 Hz), 7.36 (t, 2H, *J*=7.68 Hz), 7.55 (dd, 2H, *J*=8.02 Hz, *J*=1.02 Hz), 8.26 (br, 1H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 42.99, 120.25, 125.35, 129.23, 136.77, 163.94. **HRMS(ESI)**: calcd for C_8H_8ClNO [M + H] = 170.0374, found 170.0368.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-phenylacetamide **(19).** 2-chloro-*N*-phenylacetamide (**19-6**, 300.0 mg, 1.8 mmol), *N,N'*-ethylenethiourea (**5**, 271.0 mg, 2.7mmol), acetonitrile (18 mL). Purified by recrystallization (CH_2Cl_2 and hexane) to yield compound **19** (white solid, 312.2 mg, 75% isolated yield, 83.5 mg, 20% purified yield). **^{1}H NMR** ($(CD_3)_2SO$, 300 MHz) δ (ppm) 3.86 (s, 4H), 4.37 (s, 2H), 7.08 (t, 1H, *J*=7.33 Hz), 7.33 (t, 2H, *J*=7.33 Hz), 7.62 (d, 2H, *J*=7.89 Hz), 10.46 (br, 2H), 10.82 (br, 1H). **^{13}C NMR** ($(CD_3)_2SO$, 300 MHz) δ (ppm) 35.10, 45.16, 119.19, 123.79, 128.82, 138.61, 164.36, 168.92. **HRMS(ESI)**: calcd for $C_{11}H_{14}N_3OS$+ [M + H] = 236.0852, found 236.0851.

Synthesis of Compound 20:

2-chloro-N-phenethylacetamide **(intermediate 20-6).** 2-penylethan-1-amine (**18**, 1 mL, 8 mmol), triethylamine (1.33 mL, 10 mmol), dichloromethane (4.8 mL), chloroacetyl chloride (**17**, 0.8 mL, 10 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **20-6** (850.0 mg, 81% yield). **^{1}H NMR** (CD_3OD, 300 MHz) δ (ppm) 2.81 (t, 2H, *J*=7.43 Hz), 3.45 (t, 2H, *J*=8.21 Hz), 3.99 (s, 2H), 7.15-7.32 (m, 5H). **^{13}C NMR** (CD_3OD, 300 MHz) δ (ppm) 36.26, 42.34, 43.10, 127.40, 129.48, 129.78, 140.18, 169.14. **HRMS(ESI)**: calcd for $C_{10}H_{12}ClNONa$+ [M + Na] = 220.0505, found 220.0503.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-phenethylacetamide **(20).** 2-chloro-*N*-phenethylacetamide (**20-6**, 550.0 mg, 2.8 mmol), *N,N'*-ethylenethiourea (**5**, 430.0 mg, 4.2 mmol), acetonitrile (28 mL). Purified HPLC to yield compound **20** (white solid, 500.5 mg, 76%). **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) 2.87 (t, *J*=7.22Hz, 2H), 3.52ppm (t, *J*=7.22 Hz, 2H), 3.91 (s, 2H), 4.00 (s, 4H), 7.23 - 7.35(m, $5H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ (ppm) 32.81, 35.50, 43.81, 51.62, 126.52, 128.71, 137.93, 168.61, 171.14. **LCMS:** calcd for $C_{13}H_{17}N_3OS$+ [M + H] = 264.11, found 264.00.

Synthesis of Compound 21:

2-chloro-N-phenylbutylacetamide **(intermediate 21-6).** 4-phenylbutan-1-amine (**18**, 1 mL, 6 mmol), triethylamine (1 mL, 7.6 mmol), dichloromethane (3.4 mL), chloroacetyl chloride (**17**, 0.6 mL, 7.6 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 3:7) to yield pure **21-6** (990.0 mg, 87% yield). **LCMS:** calcd for $C_{12}H_{16}ClNO$+ [M + H] = 225.09, found 226.00.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(4-phenylbutyl)acetamide **(21).** 2-chloro-*N*-phenylbutylacetamide (**21-6**, 910.0 mg, 4.0 mmol), *N,N'*-ethylenethiourea (**5**, 610.0 mg, 6.0 mmol), acetonitrile (39 mL). Purified by recrystallization (CH_2Cl_2 and hexane) to yield compound **21** (white solid, 790.0 mg, 76%). **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) 1.65 (d, J=6.99, 4H), 2.60 – 2.64 (t, J=7.42, 2H), 3.26 – 3.28 (d, J=6.24, 2H), 3.94 (s, 4H), 4.08 (s, 2H), 7.15 – 7.28 (m, $5H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ (ppm) 28.50, 28.70, 33.45, 35.42, 40.10, 45.81, 125.74, 128.30, 128.40,

142.19, 168.98, 171.80. **LCMS:** calcd for $C_{15}H_{21}N_3OS$+ [M + H] = 292.14, found 292.35.

Synthesis of Compound 22:
2- chloro-N-(3,3-diphenylpropyl)acetamide **(intermediate 22-6).** Diphenylpropylamine (**18**, 3 g, 14 mmol), triethylamine (1.7 mL, 20 mmol), dichloromethane (7.5 mL), chloroacetyl chloride (**17**, 1.7 mL, 20 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:1) to yield pure **22-6** (2.94 g, 84% yield). **LCMS:** calcd for $C_{17}H_{18}ClNO$+ [M + H] = 287.11, found 288.10.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(3,3-diphenylpropyl)acetamide **(22).** 2- chloro-*N*-(3,3-diphenylpropyl)acetamide (**22-6**, 2.0 g, 7.0 mmol), *N,N'*-ethylenethiourea (**5**, 850.0 mg, 8.0 mmol), acetonitrile (70 mL). Purified by HPLC to yield compound **22** (75% yield). **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) 2.34 (q, *J*=7.87 Hz, 2H), 3.12 (t, *J*=7.14 Hz, 2H), 3.87 (s, 2H), 3.95 (s, 4H), 4.03 (t, *J*=7.82 Hz, 1H), 7.13 – 7.35 (m, $10H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ (ppm) 33.41, 34.40, 39.02, 45.79, 48.56, 126.32, 127.88, 128.53, 144.06, 169.02, 171.85. **LCMS:** calcd for $C_{20}H_{23}N_3OS$+ [M + H] = 354.16, found 354.45.

Synthesis of Compound 27:
2-chloro-N-(5,6,7,8-tetrahydronaphthalen-2-yl)acetamide **(intermediate 27-6).** 5,6,7,8-tetrahydro-2-naphthylamine (**18**, 1000 mg, 7.0 mmol), triethylamine (1.17 mL, 8.4 mmol), dichloromethane (4.5 mL), chloroacetyl chloride (**17**, 0.66 mL, 8.4 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **27-6** (black solid, 1406.3 mg, 90% yield). **^{1}H NMR** ($CDCl_3$, 300 MHz) δ (ppm) 1.81 (m, 4H), 2.77 (d, 4H, *J*=6.28 Hz), 4.19 (s, 2H), 7.06 (d, 1H, *J*=7.91 Hz), 7.22-7.30 (m, 2H), 8.17 (br, 1H). **^{13}C NMR** ($CDCl_3$, 300 MHz) δ (ppm) 23.02, 23.15, 28.93, 29.49, 42.19, 117.74, 120.75, 129.64, 133.98, 134.36, 138.02, 163.69. **HRMS(ESI)**: calculated for $C_{12}H_{14}ClNONa$+ [M + Na] = 246.0662, found 246.0655.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(5,6,7,8-tetrahydronaphthalen-2-yl)acetamide **(27).** 2-chloro-*N*-(*5,6,7,8-tetrahydro-2-naphthyl)*acetamide (**22-6**, 400.0 mg, 1.8 mmol), *N,N'*-ethylenethiourea (**5**, 274.0 mg, 2.7 mmol), acetonitrile (18 mL). Purified by HPLC to yield compound **27** (white solid, 420.3 mg, 81% isolated yield, 153.9 mg, 30% purified yield). **^{1}H NMR** ($(CD_3)_2SO$, 300 MHz) δ (ppm) 1.71 (m, 4H), 2.66 (d, 4H, *J*=5.37 Hz), 3.86 (s, 4H), 4.30 (s, 2H), 6.99 (d, 1H, *J*=8.80 Hz), 7.22-7.39 (m, 2H), 10.38 (br, 2H), 10.59 (s, 1H). **^{13}C NMR** ($(CD_3)_2SO$, 300 MHz) δ (ppm) 23.14, 23.25, 28.71, 29.43, 35.51, 45.69, 117.32, 119.89, 129.59, 132.62, 136.44, 137.32, 164.56, 169.38. **HRMS(ESI)**: calculated for $C_{15}H_{20}N_3OS$+ [M + H] = 290.1322, found 290.1322.

Synthesis of Compound 28:
N-phenethylpropan-2-amine **(intermediate 28-36).** Phenylethanamine (**18**, 3.0 mL, 24 mmol), isopropyl bromide (**35**, 2.5 mL, 26 mmol), acetonitrile (30 mL). Crude yield 2.69 g, 84%.
2-chloro-N-isopropyl-N-phenethylacetamide **(intermediate 28-37).** *N*-phenethylpropan-2-amine (**28-36**, 800.0 mg, 4.9 mmol), triethylamine (0.78 mL, 5.9 mmol), dichloromethane (3.0 mL), chloroacetyl chloride (**17**, 2 g, 18 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 2:3) to yield pure **28-37** (910 mg, 78% yield). **LCMS:** calcd for $C_{13}H_{16}ClNO$+ [M + H]= 239.11, found 240.14.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-isopropyl-N-phenethylacetamide **(28).** 2-chloro-*N*-isopropyl-*N*-phenethylacetamide (**28-37**, 600 mg, 2.5 mmol), *N,N'*-ethylenethiourea (**5**, 300 mg, 3.0 mmol), acetonitrile (25 mL). Purified by HPLC to yield compound **28** (white solid, 79% purified yield). **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) 1.24 – 1.28 (m, 6H), 2.81 – 2.90 (dt, *J*=7.98, *J*=8.12, 2H), 3.39 (t, *J*=7.15, 1H), 3.54 (t, *J*=7.14, 1H), 3.86 (s, 1H), 3.97 (s, 4H), 4.28 – 4.36 (5ry, *J*=6.17, 1H), 4.41 (s, 2H), 7.18 – 7.33 (m, $5H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ.(ppm) 20.27, 21.24, 35.20, 37.67, 43.56, 45.75, 49.91, 126.53, 128.60, 128.65, 137.79, 139.02, 165.80, 170.22. **LCMS** calcd for $C_{16}H_{23}N_3OS$+ [M + H] = 306.16, found: 306.35.

Synthesis of Compound 29:
N-isopropyl-4-phenylbutan-1-amine **(intermediate 29-36).** Phenylbutanamine (**18**, 2.0 mL, 13 mmol), isopropyl bromide (**35**, 1.3 mL, 14 mmol), acetonitrile (13 mL). Crude yield 3.76 g, 62%.
2-chloro-N-isopropyl-N-(4-phenylbutyl)acetamide **(intermediate 29-37).** *N*-isopropyl-4-phenylbutan-1-amine (**29-36**, 2.0 g, 10 mmol), triethylamine (1.0 mL, 12 mmol), dichloromethane (5.4 mL), chloroacetyl chloride (**17**, 1 mL, 12 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:1) to yield pure **29-37** (1.72 g, 85% yield). **LCMS:** calcd for $C_{15}H_{22}ClNO$+ [M + H]= 267.14, found 268.50.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-isopropyl-N-(4-phenylbutyl)acetamide **(29).** 2-chloro-*N*-isopropyl-*N*-(4-phenylbutyl)acetamide (**29-37**, 800 mg, 2.3 mmol), *N,N'*-ethylenethiourea (**5**, 370 mg, 3.6 mmol), acetonitrile (20 mL). Purified by HPLC to yield compound **29** (white solid, 70% purified yield). **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) 1.10 – 1.21 (m, 6H), 1.60 (s, 4H), 2.61 (d, *J*=7.22, 2H), 3.17 (s, 2H), 3.90 (s, 4H), 4.21 (s, 2H), 7.13 – 7.30 (m, $5H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ.(ppm) 20.12, 20.37, 21.26, 21.39, 29.16, 29.27, 35.61, 45.89, 125.72, 125.84, 125.99, 128.34, 166.67, 170.18. **LCMS** calcd for $C_{18}H_{27}N_3OS$+ [M + H] = 334.19, found 334.35.

Synthesis of Compound 30:
N-isopropyl-3,3-diphenylpropan-1-amine **(intermediate 30-36).** Diphenylpropylamine (**18**, 2.0 g, 9 mmol), isopropyl bromide (**35**, 1.0 mL, 10 mmol), acetonitrile (18 mL). Crude yield 2.2 g, 79%.
2-chloro-N-(3,3-diphenylpropyl)-N-isopropylacetamide **(intermediate 30-37).** *N*-isopropyl-3,3-diphenylpropan-1-amine (**30-36**, 1.8 g, 7.1 mmol), triethylamine (1.1 mL, 8.5 mmol), dichloromethane (3.5 mL), chloroacetyl chloride (**17**, 0.7 mL, 8.5 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 3:2) to yield pure **30-37** (1.94 g, 83% yield). **LCMS:** calcd for $C_{20}H_{24}ClNO$+ [M + H]= 392.15, found 393.20.

2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(3,3-diphenylpropyl)-N-isopropylacetamide **(30).** 2-chloro-*N*-(3,3-diphenylpropyl)-*N*-isopropylacetamide (**30-37**, 1000 mg, 3.0 mmol), *N,N'*-ethylenethiourea (**5**, 370 mg, 3.6 mmol), acetonitrile (20 mL). Purified by HPLC to yield compound **30** (white solid, 71% purified yield). **^{1}H NMR (300MHz, $CDCl_3$)** δ (ppm) 1.09 – 1.16 (dd, *J*=6.4, *J*=6.4, 6H), 2.35 (s, 2H), 3.09 – 3.28 (dt, *J*=7.9, *J*=8.1, 2H), 3.94 (s, 4H), 3.99 (s, 1H), 4.11 (t, *J*=8.5, 1H), 4.46 (s, 2H), 7.17 – 7.35 (m, $10H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ (ppm) 20.18, 21.20, 34.25, 41.02, 45.65, 45.74, 49.66, 50.02, 126.50, 127.65, 128.61, 143.63, 144.00, 165.93, 170.40. **LCMS** calcd for $C_{23}H_{29}N_3OS$+ [M + H] = 396.20, found 396.45.

Synthesis of Compound 31:
N-(cyclohexylmethyl)aniline **(intermediate 31-36).** Aniline (**18**, 3.1 g, 30 mmol), methylcyclohexane bromide (**35**, 5.0 mL, 36 mmol), acetonitrile (30 mL).

2-chloro-N-(cyclohexylmethyl)-N-phenylacetamide **(intermediate 31-37).** *N*-(cyclohexylmethyl)aniline (**31-36**, 2.0 g, 7.5 mmol), triethylamine (1.2 mL, 9.0 mmol), dichloromethane (3.6 mL), chloroacetyl chloride (**17**, 0.7 mL, 9.0 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 3:2) to yield pure **31-37** (2.51 g, 98% yield). **LCMS:** calcd for $C_{15}H_{20}ClNO+$ [M + H]= 265.12, found 266.10.

N-(cyclohexylmethyl)-2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-phenylacetamide **(31).** 2-chloro-*N*-(cyclohexylmethyl)-*N*-phenylacetamide (**31-37**, 1000 mg, 3.8 mmol), *N,N'*-ethylenethiourea (**5**, 460 mg, 4.5 mmol), acetonitrile (36 mL). Purified by HPLC to yield compound **31** (white solid, 71% purified yield). **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) 0.97 – 1.10 (m, 6H), 1.70 (d, *J*=10.9, 10H), 3.58 (s, 2H), 3.62 (d, *J*=7.45, 2H), 4.02 (s, 4H), 7.23 – 7.52 (m, $5H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ (ppm) 25.65, 26.25, 30.67, 33.09, 35.89, 45.85, 56.09, 127.66, 129.30, 130.63, 140.68, 168.34, 170.81. **LCMS** calcd for $C_{18}H_{25}N_3OS+$ [M + H] = 332.17, found 332.50.

Synthesis of Compound 32:
N-(cyclohexylmethyl)-2-phenylethan-1-amine **(intermediate 32-36).** Phenylethanamine (**18**, 2.0 mL, 16 mmol), methylcyclohexane bromide (**35**, 2.4 mL, 17 mmol), acetonitrile (36 mL).

2-chloro-N-(cyclohexylmethyl)-N-phenethylacetamide **(intermediate 32-37).** *N*-(cyclohexylmethyl)-2-phenylethan-1-amine (**32-36**, 0.5 g, 2.3 mmol), triethylamine (0.4 mL, 2.8 mmol), dichloromethane (1.2 mL), chloroacetyl chloride (**17**, 0.2 mL, 2.8 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:4) to yield pure **32-37** (330 mg, 61% yield). **LCMS:** calcd for $C_{17}H_{24}ClNO+$ [M + H]= 293.15, found 294.30.

N-(cyclohexylmethyl)-2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-phenethylacetamide **(32).** 2-chloro-*N*-(cyclohexylmethyl)-*N*-phenethylacetamide (**32-37**, 200 mg, 6.8 mmol), *N,N'*-ethylenethiourea (**5**, 83 mg, 0.8 mmol), acetonitrile (4.5 mL). Purified by HPLC to yield compound **32** (white solid, 77% purified yield). **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) 0.91 – 1.66 (m, 6H), 2.86 – 2.89 (m, 2H), 3.15 3.20 (dd, *J*=6.88, *J*=6.35, 2H), 3.75 (d, *J*=7.32, 2H), 3.97 (d, *J*=6.44, 4H), 7.15 – 7.30 (m, $5H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ (ppm) 25.76, 30.49, 37.43, 45.74, 126.55, 127.01, 128.84, 129.22, 137.66, 138.41, 167.27, 170.46, 170.57 **LCMS** calcd for $C_{20}H_{29}N_3OS+$ [M + H] = 360.20, found 360.40.

Synthesis of Compound 33:
N-(cyclohexylmethyl)-4-phenylbutan-1-amine **(intermediate 33-36).** Phenylbutylanamine (**18**, 2.0 mL, 10 mmol), methylcyclohexane bromide (**35**, 1.9 mL, 14 mmol), acetonitrile (6 mL).

2-chloro-N-(cyclohexylmethyl)-N-(4-phenylbutyl)acetamide **(intermediate 33-37).** *N*-(cyclohexylmethyl)- 4-phenylbutan-1-amine (**33-36**, 2.6 g, 16 mmol), triethylamine (1.7 mL, 13 mmol), dichloromethane (5.3 mL), chloroacetyl chloride (**17**, 1.4 mL, 13 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:3) to yield pure **33-37** (1.35 g, 80% yield). **LCMS:** calcd for $C_{19}H_{28}ClNO+$ [M + H]= 321.19, found 320.55.

N-(cyclohexylmethyl)-2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(4-phenylbutyl)acetamide **(33).** 2-chloro-*N*-(cyclohexylmethyl)-*N*-(4-phenylbutyl)acetamide (**33-37**, 1000 mg, 3.1 mmol), *N,N'*-ethylenethiourea (**5**, 380 mg, 3.7 mmol), acetonitrile (18 mL). Purified by HPLC to yield compound **33** (white solid, 77% purified yield). **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) 0.89 – 1.19 (m, 6H), 2.63 (dt, *J*=7.42, 2H), 3.14 3.26 (dd, *J*=6.8, 2H), 3.26 3.44 (dt, *J*=7.3, 2H), 3.92 (s, 2H), 3.97 (s, 2H), 4,25 (s, 1H), 4.28 (s, 1H), 7.14 – 7.30 (m, $5H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ (ppm) 25.84, 28.73, 30.83, 35.53, 45.84, 125.90, 128.40, 128.43, 141.92, 167.17, 167.28, 170.34. **LCMS** calcd for $C_{22}H_{33}N_3OS+$ [M + H] = 388.23, found 388.15.

Synthesis of Compound 34:
N-(cyclohexylmethyl)-3,3-diphenylpropan-1-amine **(intermediate 34-36).** 3,3-diphenylpropan-1-amine (**18**, 2.0 mL, 10 mmol), methylcyclohexane bromide (**35**, 1.9 mL, 14 mmol), acetonitrile (6 mL).

2-chloro-N-(cyclohexylmethyl)-N-(3,3-diphenylpropyl)acetamide **(intermediate 34-37).** *N*-(cyclohexylmethyl)-4-phenylbutan-1-amine (**34-36**, 2.0 g, 6.5 mmol), triethylamine (1.0 mL, 7.8 mmol), dichloromethane (21.3 mL), chloroacetyl chloride (**17**, 0.6 mL, 7.8 mmol). Purified by flash chromatography on silica gel (EtOAc:Hexane, 1:3) to yield pure **34-37** (1.35 g, 80% yield). **LCMS:** calcd for $C_{24}H_{30}ClNO+$ [M + H]= 384.96, found 385.22.

N-(cyclohexylmethyl)-2-((4,5-dihydro-1H-imidazol-2-yl)thio)-N-(3,3-diphenylpropyl)acetamide **(34).** 2-chloro-*N*-(cyclohexylmethyl)-*N*-(3,3-diphenylpropyl)acetamide (**34-37**, 1000 mg, 2.6 mmol), *N,N'*-ethylenethiourea (**5**, 220 mg, 2.2 mmol), acetonitrile (11 mL). Purified by HPLC to yield compound **34** (white solid, 77% purified yield). **^{1}H NMR** (300MHz, $CDCl_3$) δ (ppm) 0.67 – 1.20 (m, 6H), 1.32 – 1.72 (m, 6H), 2.19 – 2.37 (m, 3H), 3.10 (d, *J*=7.14, 1H), 3.24 (t, *J*=7.14, 2H), 3.38 (dt, *J* = 7.33, *J* = 7.91, 1H), 3.85 (dt, *J* = 7.75, *J*=7.75, 1H), 3.90 (s, 4H), 4.08 (t, *J* = 7.75, 1H), 4.20 (s, 1H), 4.39 (s, 1H), 7.16 – 7.29 (m, $10H_{Ar}$). **^{13}C NMR** (300MHz, $CDCl_3$) δ (ppm) 25.6, 25.8, 26.1, 30.4, 30.7, 32.5, 35.7, 35.9, 36.6, 37.1, 45.7, 48.1, 49.1, 51.7, 54.9, 126.5, 126.6, 127.6, 127.7, 128.6, 128.8, 143.6, 143.9, 166.8, 167.1, 170.3. **LCMS** calcd for $C_{27}H_{35}N_3OS+$ [M + H] = 449.66, found 450.66.

ASSOCIATED CONTENT
Supporting Information. Additional biological data and characterization data are available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION
Corresponding Authors
*s.mcalpine@unsw.edu.au and **rlock@ccia.unsw.edu.au
Author Contributions
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.
Funding Sources
This research was funded by an NHMRC grant. R.B.L. is funded by an NHMRC fellowship. C.E.T. is funded by a Research Excellence Award from UNSW.
Notes
The authors declare no competing financial interests.

ACKNOWLEDGMENT

Children's Cancer Institute Australia is affiliated with UNSW Australia and the Sydney Children's Hospitals Network.

ABBREVIATIONS

ALL, acute lymphoblastic leukemia; BCP-ALL, B-cell precursor ALL; Dex, dexamethasone; eq, equivalent; HTS, high throughput screening; Pred, prednisolone; TEA, triethyl amine; TLC, thin layer chromatography.

REFERENCES

1. R. Siegel, D. Naishadham and A. Jemal, *CA: A Cancer Journal for Clinicians*, 2012, **62**, 10-29.
2. C.-H. Pui and W. E. Evans, *Seminars in Hematology*, 2013, **50**, 185-196.
3. H. Inaba, M. Greaves and C. G. Mullighan, *The Lancet*, 2013, **381**, 1943-1955.
4. H. Inaba and C.-H. Pui, *The Lancet Oncology*, 2010, **11**, 1096-1106.
5. G. Gaipa, G. Basso, A. Biondi and D. Campana, *Cytometry Part B: Clinical Cytometry*, 2013, **84**, 359-369.
6. P. S. Gaynon, *Br J Haematol*, 2005, **131**, 579-587.
7. G. Wei, D. Twomey, J. Lamb, K. Schlis, J. Agarwal, R. W. Stam, J. T. Opferman, S. E. Sallan, M. L. den Boer, R. Pieters, T. R. Golub and S. A. Armstrong, *Cancer Cell*, 2006, **10**, 331-342.
8. L. Bonapace, B. C. Bornhauser, M. Schmitz, G. Cario, U. Ziegler, F. K. Niggli, Sch, xE, B. W. fer, M. Schrappe, M. Stanulla and J.-P. Bourquin, *The Journal of Clinical Investigation*, 2010, **120**, 1310-1323.
9. N. Heidari, M. A. Hicks and H. Harada, *Cell Death and Disease*, 2010, **1**, e76.
10. E. Piovan, J. Yu, V. Tosello, D. Herranz, A. Ambesi-Impiombato, AnaÂ C. DaÂ Silva, M. Sanchez-Martin, A. Perez-Garcia, I. Rigo, M. Castillo, S. Indraccolo, JustinÂ R. Cross, E. deÂ Stanchina, E. Paietta, J. Racevskis, JacobÂ M. Rowe, MartinÂ S. Tallman, G. Basso, JulesÂ P. Meijerink, C. Cordon-Cardo, A. Califano and AdolfoÂ A. Ferrando, *Cancer Cell*, 2013, **24**, 766-776.
11. N. L. M. Liem, R. A. Papa, C. G. Milross, M. A. Schmid, M. Tajbakhsh, S. Choi, C. D. Ramirez, A. M. Rice, M. Haber, M. D. Norris, K. L. MacKenzie and R. B. Lock, *Blood*, 2004, **103**, 3905-3914.
12. R. B. Lock, N. Liem, M. L. Farnsworth, C. G. Milross, C. Xue, M. Tajbakhsh, M. Haber, M. D. Norris, G. M. Marshall and A. M. Rice, *Blood*, 2002, **99**, 4100-4108.
13. P. S. Bachmann, R. Gorman, R. A. Papa, J. E. Bardell, J. Ford, U. R. Kees, G. M. Marshall and R. B. Lock, *Cancer Res*, 2007, **67**, 4482-4490.
14. P. S. Bachmann, R. G. Piazza, M. E. Janes, N. C. Wong, C. Davies, A. Mogavero, V. A. Bhadri, B. Szymanska, G. Geninson, V. Magistroni, G. Cazzaniga, A. Biondi, D. Miranda-Saavedra, B. GÃ¶ttgens, R. Saffery, J. M. Craig, G. M. Marshall, C. Gambacorti-Passerini, J. E. Pimanda and R. B. Lock, *Blood*, 2010, **116**, 3013-3022.
15. C. E. Toscan, T. Failes, G. M. Arndt and R. B. Lock, *Journal of Biomolecular Screening*, **19,** 1391-1401
16. C. I. Bliss and B. L. Bartels, *Federation Proceedings*, 1946, **5**.
17. A. M. Cantley, M. Welsch, A. Ambesi-Impiombato, M. Sanchez-Martin, M.-Y. Kim, A. Bauer, A. Ferrando and B. R. Stockwell, *ACS Medicinal Chemistry Letters*, 2014, **5**, 754-759.
18. C. E. Toscan, T. Failes, G. M. Arndt and R. B. Lock, *Journal of Biomolecular Screening*, 2014, **19**, 1391-1401.
19. G. Sheldrick, *Acta Crystallographica Section A*, 2008, **64**, 112-122.
20. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A. K. Howard and H. Puschmann, *Journal of Applied Crystallography*, 2009, **42**, 339-341.
21. P. M. Kushakova, S. M. Ramsh and A. V. Garabadgiu, *Chem Heterocycl Compd*, 2006, **42**, 221-226.