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Protein interactions are increasingly appreciated as targets in small-molecule drug discovery. The interaction between the adapter protein S100A10 and its binding partner annexin A2 is a potentially important drug target. To obtain small-molecule starting points for inhibitors of this interaction, a three-dimensional pharmacophore model was constructed from the X-ray crystal structure of the complex between S100A10 and annexin A2. The pharmacophore model represents the favourable hydrophobic and hydrogen bond interactions between the two partners, as well as spatial and receptor site constraints (excluded volume spheres). Using this pharmacophore model, UNITY flex searches were carried out on a 3D library of 0.7 million commercially available compounds. This resulted in 568 hit compounds. Subsequently, GOLD docking studies were performed on these hits, and a set of 190 compounds were purchased and tested biochemically for inhibition of the protein interaction. Three compounds of similar chemical structure were identified as genuine inhibitors of the binding of annexin A2 to S100A10. The binding modes predicted by GOLD were in good agreement with their UNITY-generated conformations. We synthesised a series of analogues revealing areas critical for binding. Thus computational predictions and biochemical screening can be used successfully to derive novel chemical classes of protein–protein interaction blockers.

Introduction

Protein–protein interactions are widely regarded as pivotal to cell regulation and are increasingly of interest as targets in small-molecule drug discovery.[1] Disruption of protein–protein interactions can be challenging; however, some progress has been made in this area. For example, the interaction between p53 and MDM2, which involves a comparatively small binding interface, can be blocked by several chemical classes of small molecules, some of which have progressed to the clinical investigation stage.[2] Similarly, small-molecule BH3 domain mimetics block the interaction of anti-apoptotic Bcl2 family members with BH3-domain-containing pro-apoptotic proteins.[3] These compounds show tumour growth inhibition in tumour models and have progressed to early clinical-stage research.[4] Finally, various investigational small-molecule blockers have been reported for the protein interaction between VLA4–VCAM,[5] B7.1–CD28,[6] oestrogen-related receptor-α and its co-activator,[6] and protein kinase C-iota and its effector PAR6.[7]

The interaction between S100 proteins and their targets has been implicated in numerous biological and pathological processes. The principle function of S100 proteins is regulation of the localisation and activity of other proteins by direct protein–protein interactions. Some of these interactions have potential therapeutic relevance. Recent genetic deletion studies have implicated both S100A10 and its binding partner annexin A2 in the process of neo-angiogenesis.[8] This parallel phenotype suggests these two partners may form a physical complex to regulate this process. Small-molecule blockers of the interaction would greatly aid the further investigation of this idea. At the molecular level, the interaction is very well characterised both by mutagenesis and crystallography, involving a small and largely hydrophobic binding area.[9] Two S100A10 molecules form a dimeric structure that yields two binding pockets, each of which accommodates the 14-residue N-terminal region of annexin A2. Using a structure-based virtual high-throughput docking approach, we previously identified several clusters of small molecules that dock into the annexin A2 binding pocket on S100A10.[10] Biochemical screening of these showed that substituted 4-aroyl-3-hydroxy-5-phenyl-1H-pyrrol-2(5H)-one analogues are capable of inhibiting the binding between S100A10 and annexin A2.[10] In the present study we sought to provide further evidence that this protein interaction can be targeted with small-molecule blockers. To do so, we used a ligand-guided method as an alternative to the above random docking approach. The binding pose of the cognate annexin A2 N terminus into the S100A10 dimer is very well...
characterised, and we used the topological arrangement of key chemical features in the annexin A2 N terminus known to be important for interacting with the S100A10 protein, to design a 3D pharmacophore. We screened a library of compounds against this 3D pharmacophore and identified molecules that match these features and their topology. These were tested for inhibition of the interaction between S100A10 and the annexin A2 N terminus. Thus we established that substituted 1,2,4-triazole analogues are able to compete with the binding of the annexin A2 N terminus to S100A10.

**Results**

**3D pharmacophore generation**

Taking into account the crystal structure model of the complex between S100A10 and the annexin A2 N terminus and the effect of modification of the annexin A2 N terminus on its interaction with S100A10, a pharmacophoric query was constructed. Within the annexin A2 N terminus, the valine residue at position 3 and the leucine residues at positions 7 and 10 are crucial for binding with S100A10 as is the N-acetyl group of the N-terminal serine residue. Contact surface analysis indicates that the acetyl group, as well as the Val3 and Leu7 residues point into the pocket (Figure 1A, represented as yellow sticks). The acetyl group occupies a relatively small hydrophilic pocket (acetyl pocket, Figure 1A) composed of Pro1, Ser2, His6, and Glu9 of molecule B of the S100A10 dimer. The isopropyl side chain of Val3 is accommodated in a hydrophobic pocket, which is also solvent accessible and is composed of Cys82 of S100A10 molecule A, and Met8, Met12, and Glu9 of S100A10 molecule B (valine pocket, Figure 1A). The side chain of Leu7 also partly occupies another hydrophobic cavity composed of Phe38, Pro39, Gly40, Phe41, and Leu78 of S100A10 molecule A.

**Figure 1.** A) Binding pose of the annexin A2 N-terminal peptide (represented as lines with the secondary helical structure superimposed) within the binding site of S100A10. The isopropyl side chain of Val3 (yellow sticks) is directed into the valine pocket. The side chain of Leu7 (yellow sticks) partly occupies the leucine pocket. The acetyl group at Ser1 (yellow sticks) occupies the acetyl pocket. B) Schematic representation of the pharmacophoric query. Two hydrophobic features (Hydrophobic1_Leu, Hydrophobic2_Val) are shown as red spheres, whilst the carbonyl group (C=O, Markush atom) is represented by a magenta sphere. Hydrogen bonds are defined as vectors (shown as yellow lines) from the donor atom of the peptide to the corresponding acceptor atom in the receptor and vice-versa. Asterisks indicate the centres of the spheres. Hydrogen bond donor features of the peptide (LD_16_Thr2, LD_11_Thr2, LD_255_Ser1, LD_31_His4) are shown as grey spheres. Hydrogen bond acceptor features of the receptor (S100A10) are shown in yellow (RA_805_Glu9B, RA_804_Glu9B, RA_773_Glu5B, RA_331_Phe41A). For Ser11 of the peptide, the side chain OH group acts as hydrogen bond donor as well as hydrogen bond acceptor (LD/LA_87_Ser11), represented as a green sphere. The hydrogen bond donor atom feature (RD_372_Gln45A) of the receptor is shown as an indigo sphere. For clarity, the receptor site constraints are not shown in the query (see also panel D). C) Overlay of the pharmacophore query generated under B and the annexin N-terminal peptide in its docked pose as shown under A. Amino acids associated with the pharmacophoric features are indicated in blue (receptor features) or black (ligand features). D) Representation of the pharmacophore query in the annexin N terminus binding groove of S100A10. The images in panels A and D were generated using SYBYL-X Molcad with the molecular surface of S100A10 coloured according to lipophilic potential. Images in panels B and C were generated with the SYBYL-X UNITY module.
and Glu5 and Met8 of S100A10 molecule B (leucine pocket, Figure 1 A).

The above hydrophobic contacts of Val3 and Leu7 are represented by hydrophobic features in the pharmacophore query (Figure 1 B–D) and include six- and five-membered rings, tert-butyl, cyclopropyl, isobutyl, and other aliphatic chains. Based on the size of the cavity available within the binding pocket, a spatial constraint with a tolerance of 1.5 Å was added to the Leu7 hydrophobic feature, and a spatial constraint with a tolerance of 1.25 Å was added to the Val3 hydrophobic feature. This resulted in spherical hydrophobic features (red, Figure 1 B–D) reflecting the tolerance in the 3D position. A carbonyl group, representing the N-acetyl group of annexin A2, was defined as a third pharmacophoric feature. Because the relevant pocket is relatively small, a spatial point constraint with a tolerance of 0.75 Å was added to the carbon atom (magenta sphere, Figure 1 B–D). This feature was further enhanced by marking Kush carbonyl atom definition, which allows compounds with specific functional groups at the acetyl pocket. These include -COCH3, -CONH2, -COCH3CH3, -COOCH3, -COOCH2CH3, -NHCOCH3, -NHCOCH2CH3, -NHCOOCH3, -NHCOOCH2CH3, -NHCONH2, -CH2COOH, and -NHCOOH.

Hydrogen bond interactions are represented in the query in the form of hydrogen bond donor and acceptor features (Figure 1 B–D). The side chain hydroxy group (LD_16_Thr2, grey sphere) and the backbone NH group (LD_11_Thr2, grey sphere) of Thr2 undergo hydrogen bond donor interactions with the carbonyl group (RA_804_Glu9B, yellow sphere) of the Glu9 residue in S100A10 molecule B. The backbone NH group of Ser1 (LD_255_Ser1, grey sphere) forms hydrogen bond donor interactions with the side-chain carbonyl (RA_805_Glu9B and RA_804_Glu9B yellow spheres) of Glu9 in S100A10 molecule B. The ring NH group of His4 (LD_31_His4, grey sphere) forms a hydrogen bond donor interaction with the carbonyl group (RA_773_Glu5B, yellow sphere) of Glu5 in S100A10 molecule B. Furthermore, the side-chain hydroxy group (LD_87_Ser11, green sphere) of Ser11 forms a hydrogen bond interaction with the backbone carbonyl oxygen (RA_331_Phe41A, yellow sphere) of Phe41 in S100A10 molecule A and hydrogen bond acceptor interaction (LA_87_Ser11, green sphere) with the side chain carbamido NH2, (RD_372_Gln45A, indigo sphere) of Gln45 in S100A10 molecule A. The backbone carbonyl oxygen (LA_78_Leu10, cyan sphere) of Leu10 undergoes a hydrogen bond donor interaction with the side-chain carbamido NH2, (RD_372_Gln45A, indigo sphere) of Gln45 in S100A10 molecule A. A spatial constraint with a tolerance of 1.0 Å was added to all hydrogen bond donor and acceptor features except for the hydrogen bond donor feature of Ser1, for which a tolerance of 0.25 Å was added in order to avoid the overlap of its spatial constraint with the adjacent carbonyl atom spatial constraint. A final significant feature included in the query was the receptor site constraint. This was added in the form of multiple excluded volume spheres with a van der Waals scaling factor of 0.5 Å (not shown for clarity). For a compound to be considered as a hit, it should match the query features without colliding with multiple excluded volume spheres that represent the protein surface.

Identification and analysis of inhibitory compounds

Hits were tested for the inhibition of the interaction between S100A10 and the annexin A2 N terminus in a competitive fluorescent binding assay. Each compound was tested in quadruplicate at a single concentration of 10 μM. Compounds showing inhibitory activity (defined as signal deviating by > 3 standard deviations from the no-compound control) were selected for repeat testing at the same concentration. Seven compounds showed consistent levels of inhibition, and these were taken forward for IC50 determination. Furthermore, these compounds were analysed in a counterscreen, which measures a nonrelated protein interaction using the same assay format.

Computational screening for pharmacophore matches

A 3D database containing 704511 structurally diverse compounds was generated from the Zinc database (Asinex and ChemBridge vendors) using the following five molecular filters: 1) M, range: 150–600 Da, 2) hydrogen bond donors: < 7, 3) hydrogen bond acceptors: < 14, 4) XLog P: < 5, and 5) rotatable bonds: < 12. For each molecule, a single conformation was stored in the 3D database. A UNITY flexible 3D search was then performed on the 3D pharmacophore model using the direct tweak algorithm, which adjusts the rotatable bonds of the molecules to match the 3D pharmacophoric model as closely as possible. The search was designed to match the most significant hydrophobic features derived from the leucine and valine pockets as well as the Markush carbonyl definition with a receptor site constraint in place. In addition, the search was refined to allow partial matching of hydrogen bonding donor or acceptor features, such that for a molecule to be considered a hit, a minimum of one and a maximum of five hydrogen bonding features were required to fit. The entire UNITY 3D database search of the 704511 compounds was performed directly on an Intel i7-based LINUX system. The time taken for the search was ~ 120 h. This search returned 568 hits.

These hits were ranked based on their best-fit values generated from the 3D search. The fit values reflect the agreement of the hit compounds with the pharmacophore model, with higher fit values indicating better mapping of the hit compound onto the pharmacophore model. The fit values ranged from 62 to 8. The hit compounds were also docked using the Genetic Optimisation for Ligand Docking (GOLD) program in standard parameter mode into the defined annexin A2 binding pocket of the S100A10 receptor. Docked compounds were ranked based on the GOLD score, which represents the sum of receptor–ligand hydrogen bonding energy, van der Waals energy, torsional energy, and hydrophobic interaction energies and ranked from 66 to 36. Higher GOLD scores indicate better binding interaction of the compounds with the S100A10 receptor. The docking procedure for the 568 hit compounds was carried out using the GOLD (V3.0.1) docking program; calculations required ~1 h running on 32 dual AMD Opteron 248 servers. A total of 190 hits that were selected based on best-fit values, higher GOLD fitness scores, and a good binding mode in accordance with our pharmacophore query were purchased.
Three compounds showed activity in the primary assay, but not in the counterscreen assay (Figure 2 A), whilst four compounds showed overlap in activity between the two assays (Figure 2 B for an example: a compound from the ChemBridge catalogue with vendor number 7832669 and Zinc database number ZINC02858054). The latter compounds could represent promiscuous protein interaction blockers, or compounds that in some way interfere with the fluorescence signal. The structures of three confirmed hit compounds are shown in Figure 3. Interestingly, all hit compounds featured the same acetamide side chain, suggesting a potentially significant contribution of this group to binding interactions. Analysis of the mapping of the pharmacophore onto these inhibitors indicates that the hydrophobic feature associated with the leucine pocket mapped onto one of the phenyl rings of the naphthyl ring system (1a), onto the phenyl moiety of the ortho-tolyl group (1b), or the phenyl moiety of the para-anisole group (1c) (Figure 4, top panels). The hydrophobic feature associated with the valine pocket mapped onto the N4-phenyl rings in compounds 1a and 1c, whereas in the case of compound 1b it overlapped with the ortho-ethyl group of the N4-phenyl ring. In the case of compounds 1a, 1b, and 1c the amide group matched the Markush atom definition with the NH$_2$ group locating to the hydrogen bond donor feature, within hydrogen bonding distance to the carboxyl group of Glu9 in S100A10 molecule B. Importantly, the UNITY-generated conformations of compounds 1a, 1b, and 1c mapped onto the 3D pharmacophoric query without clashing with the receptor site (multiple excluded volume spheres).

The binding modes predicted by GOLD for compounds 1a, 1b, and 1c are in good agreement with their UNITY-generated conformations (Figure 4, bottom panels). The predicted binding modes indicate that the substituted aromatic rings closely mimic Val3 and Leu7 in annexin A2, interacting with the hydrophobic pockets of S100A10. In case of compounds 1a and 1b the NH$_2$ group of the amide side chain at the acetyl pocket is in close proximity to Glu9 in S100A10 molecule B, suggesting the possibility of hydrogen bond interactions taking place. In compound 1c both the NH$_2$ group of the amide and the secondary amine group undergo hydrogen bond interactions with the carboxyl groups of S100A10 B-chain Glu9 and S100A10 B-chain Glu5, respectively. This appears to mimic the hydrogen bond interaction of Glu9 and Glu5 residues of the receptor with the annexin A2 N-terminal peptide in the crystal structure. Considering the inhibitory potential of these triazole compounds 1a (66 μM), 1b (24 μM), and 1c (90 μM) we synthesised a series of analogues and investigated their structure–activity relationships.

**Synthesis of substituted 1,2,4-triazoles**

The required disubstituted 1,2,4-triazoles 1b, 1c, and 7a–i were prepared as depicted in Scheme 1. The substituted acetic acid ethyl ester 3a (X=O) was prepared by treating ortho-cresol 2a with bromoethyl acetate in the presence of sodium hydride in N,N-dimethylformamide. The substituted acetic acid ethyl esters 3b–c (X=NH) were prepared by treating substituted anilines 2b–c with bromoethyl acetate in the presence of triethylamine in ethanol. Subsequent reaction of ethyl esters 3a–c (X=O or NH) with hydrazine monohydrate

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**Figure 2.** Inhibitory activity of A) compound 1b and B) compound 7832669 (ChemBridge reference number; also referred to as ZINC02858054). Both compounds were tested for inhibition of the interaction between S100A10 and the annexin A2 N terminus (• and △) or for inhibition of a non-target protein interaction (○ and ▲) as explained in the Results and Experimental Section. Data points represent the average ± SEM of four observations.

**Figure 3.** Structures and IC$_{50}$ values of hit compounds.
gave the corresponding acyl hydrazides \(4a-c\) in good to excellent yields.\(^{[14]}\) Condensation of the acyl hydrazides \(4a-c\) with substituted aromatic or aliphatic isothiocyanates \(5\), followed by base-catalysed cyclisation, resulted in 4-substituted 3-mercaptop-1,2,4-triazole analogues \(6a-k\). Treatment of these intermediates with 2-bromoacetamide in the presence of potassium carbonate resulted in the formation of disubstituted 1,2,4-triazole analogues \(1b-c\) and \(7a-i\).\(^{[15]}\)

Structure–activity relationships

The 1,2,4-triazole compounds synthesised were screened using an in vitro FRET-based competitive binding assay to evaluate their ability to inhibit the interaction between S100A10 and an annexin A2 N-terminal peptide.\(^{[13]}\) An IC\(_{50}\) value was obtained for each compound from nonlinear regression analysis (GraphPad Prism, variable slope) with top and bottom constraints of 100 and 0%, respectively.

To investigate the contribution of the phenyl ring at the N4 position of the 1,2,4-triazole ring system to the inhibition of binding, a set of analogues with \(X = O\) (\(7a-g\)) was prepared (Table 1). An unsubstituted phenyl ring at the N4-position (\(7a\), \(IC_{50} = 230\ \mu M\)) showed very low potency, suggesting that the aromatic ring alone is insufficient for inhibitory activity and that the substitution pattern is important. Replacement of the N4 ring system with a flexible aliphatic methoxyethyl group (yielding \(7c\)) abolished activity altogether, reinforcing the notion that a substituted aromatic ring system at this position is important for inhibition of binding. With reference to compound \(7a\) (230 \(\mu M\)), the effect of various substitutions on the phenyl ring was assessed. An ortho-ethyl substitution \(1b\) showed increased potency, possibly due to the introduction of a conformational restriction imposed on the phenyl group. However, introduction of a bulky chloro group at the ortho position \(7b\) (176 \(\mu M\)) was
less effective in terms of inhibitory activity. Introduction of a para-methyl group (7d, 88 μM) showed a moderate increase in inhibitory activity, but a chloro group at the para position (7e, 710 μM) resulted in a profound loss of activity. Introduction of a more polar methoxy substituent at the para position (7f, 122 μM) did not affect the inhibition of binding, but a compound with an isopropyl side chain at the para position (7g, 27 μM) was comparatively potent. This may be due to the fact that the phenyl ring projects towards the valine pocket, with the isopropyl group mimicking the valine side chain of the an- nexin peptide.

Because one of the original hit compounds (1c) contains X=NH, a small set of analogues with X=NH were prepared, and substituents at the N4 and R1 positions of the 1,2,4-triazole ring system were varied (Table 1). Compound 1c (90 μM) with a para-methylphenyl substituent at the N4 position and a para-methoxy substituent at the R1 position showed weak inhibitory activity. Replacement of both para-methyl and methoxy substituents in 1c with chloro and methyl substituents (7h) resulted in loss of activity. Replacement of the para-methyl group in 1c with a bulkier ortho-ethyl group (7i, 75 μM) largely retained activity. These patterns are, to an extent, reminiscent of those observed in the X=O analogues, with a para-chloro substituent being highly disfavoured (7e, 710 μM; 7h, inactive), and an ortho-ethyl group supporting inhibitory activity of these compounds (1b, 24 μM; 7i, 75 μM). The NH group may act as a hydrogen bond donor to the carbonyl oxygen of glutamic acid of the S100A10 protein. Reference to the modelled binding pose of 1c (Figure 4), in which the NH group is hydrogen bonded to the carbonyl oxygen atom of glutamic acid of S100A10, supports this notion. In this regard, the loss in activity observed for compound 7h was unexpected.

As mentioned above, a carbonyl group (representing the peptide N-acetyl) was defined as the third pharmacophoric feature, and a range of various functional groups were allowed at this part of the query. Rather strikingly, all three hit compounds contained the same acetamide side chain mapping onto the pharmacophore, suggesting a potentially significant contribution of this group for binding interactions. This was investigated further by truncating the sulfanylacetamide side chain to the corresponding thiol. This resulted in a sixfold decrease in binding activity of compound 1b, 24 μM (6e, 275 μM), a threefold decrease in binding activity of compound 7g, 27 μM (6k, 68 μM) and a twofold further decrease in binding activity of the already very weak blocker 7a, 230 μM (6d, 481 μM). For compounds 7d, 88 μM and 7f, 122 μM (yielding 6i and j, respectively), this modification resulted in conversion into partial blockers of the interaction, inhibiting <50% of the binding. Thus the acetamide side chain appears to make an important contribution to the activity of these compounds, possibly by acting as a hydrogen bond donor to its receptor. Consistent with this trend, truncation of the sulfanylacetamide side chain of compound 7i (75 μM) to the thiol 6a resulted in complete loss of activity. However, similar truncation compounds 7c and 7h (both inactive) resulted in moderately active compounds (6g, 117 μM and 6c, 115 μM respectively), whilst this modification is associated with a small increase in potency for compound 1c, 90 μM (6b, 35 μM) and a large gain in potency for 7e, 710 μM (6h, 23 μM).

### Discussion

We have presented a ligand-based virtual screening approach to identify inhibitors of the protein interaction between annexin A2 and S100A10. Based on the binding pose of the annexin A2 N terminus in S100A10, a Markush carbonyl atom, hydrophobic and hydrogen bond interactions, along with spatial and receptor constraints, were established as key pharmacophoric features that were translated into a 3D pharmacophore. Screening of >700 000 compounds against this pharmacophore resulted in the identification of 1,2,4-triazole compounds 1a-c as novel genuine inhibitors of the S100A10–annexin A2 interaction. The GOLD-predicted binding modes of these compounds were analysed, revealing close similarity to the UNITY-predicted binding modes. A set of 22 compounds were synthesised, and SARs were explored. Four compounds (1b, 7g, 6b, 6h) showed inhibitory activity <50 μM and an-

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**Table 1. Structure–activity relationships of 2-(4-substituted-5-substituted phenyl oxo/amino methyl-4H-[1,2,4]triazol-3-ylsulfonyl)acetamides.**

| Compd | Type | X | R2 | R4 | R1 | IC50 [μM][a] |
|-------|------|---|----|----|----|--------------|
| 7a    | I    | O | H  | H  | 2-Me| 230          |
| 1b    | I    | O | Et | H  | 2-Me| 24           |
| 7b    | I    | O | Cl | H  | 2-Me| 176          |
| 7c    | –    | – | –  | –  | –  | NA           |
| 7d    | I    | O | H  | Me | 2-Me| 88           |
| 7e    | I    | O | H  | Cl | 2-Me| 710          |
| 7f    | I    | O | H  | OMe| 2-Me| 122          |
| 7g    | I    | O | H  | Pr | 2-Me| 27           |
| 7h    | I    | NH | Cl | H  | 4-Me| NA           |
| 1c    | I    | NH | Me | 4-OMe| 90   |
| 7i    | I    | NH | Et | H  | 4-OMe| 75           |
| 6a    | II   | NH | Et | H  | 4-OMe| NA           |
| 6b    | II   | NH | Me | 4-OMe| 35   |
| 6c    | II   | NH | Cl | 4-Me| 115  |
| 6d    | II   | O | H  | H  | 2-Me| 481          |
| 6e    | II   | O | Et | H  | 2-Me| 275          |
| 6f    | II   | O | Cl | H  | 2-Me| 83           |
| 6g    | –    | – | –  | –  | –  | 117          |
| 6h    | II   | O | H  | Cl | 2-Me| 23           |
| 6i    | II   | O | H  | Me | 2-Me| partial      |
| 6j    | II   | O | H  | OMe| 2-Me| partial      |
| 6k    | II   | O | H  | Pr | 2-Me| 68           |

[a]: NA: no inhibition was observed; partial: compound behaved as partial blocker of binding.
Experimental Section

Computational techniques

S100A10–annexin A2 N-terminal peptide complex: The Tripos (SYBYL-X 1.0) Biopolymer module was used for the protein structure preparation process. Two chain termini (Lys91, Pro1) of each monomer of the S100A10 and Ser11 of each annexin A2 N-terminal peptide were charged. Hydrogen atoms were added, and Amber 7 FF99 charges were assigned to the complex. The side chain amides of Gln45 and Gln60 were re-oriented to maximise hydrogen bonding. The complex was then subjected to stepwise energy minimisation. The parameters used were Amber 7 FF99 force fields, Powell’s minimisation method, 0.5 kcal mol$^{-1}$ gradient, no initial optimisation was performed, and a maximum of 5000 iterations were used. A centroid was defined by a set of atoms within 5 Å of the annexin A2 peptide, and the peptide was then extracted from the complex. Water molecules were deleted before docking.

UNITY search: The UNITY module of SYBYL-X (V1.0) was used for the design of a highly focused pharmacophoric query. The query was directly built in UNITY from the energy-minimised co-crystal structure of S100A10–annexin A2 N-terminal peptide as described in the results section, using relevant features (hydrogen bond acceptor atom, hydrogen bond donor atom, acceptor site, and donor site to specify the directionality of the hydrogen bond, hydrophobic features) and constraints (distance, spatial point, and receptor site constraints). UNITY flex searches were performed on 3D compound databases with a single conformational representation of each compound, using the direct tweak algorithm to match the conformation of the molecule with the defined pharmacophoric query. Hits were generated based on how well the compounds matched the query.

GOLD (V3.0.1) docking studies: Ten docking runs were performed on each molecule and allowed the early termination of the docking runs if the top three solutions were within the 1.5 Å RMSD of each other. During the run, 100,000 genetic algorithm (GA) operations were performed on a single population of 100 individuals. Operator weights for the crossover, mutation, migration (95, 95, and 10 respectively) hydrogen bonding (4.0 Å), and van der Waals (2.5 Å) parameters were set as default value throughout the docking. An active site radius value of 12 Å was found to be optimum. Flipping was not allowed for those ligands that have ring-NHR and ring-NHR$'\text{R}^\prime$ groups in order to avoid the addition of large torsional energy penalties to the total fitness scores.

Biology

Fluorescence screening assay: Routine assessment of compound activity was performed as described by Li et al. Briefly, a Cy3-labelled S100A10 tracer was developed, and binding of a Cy3-labelled annexin A2(1–14) peptide ligand was assessed using a fluorescence resonance energy transfer (FRET) readout. Assays were carried out in Nunc black non-treated 384-well plates at 20 °C in 50 µl 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na$_2$HPO$_4$, 1.47 mM KH$_2$PO$_4$, pH 7.4 containing 2 mM 1,4-dithiothreitol. All incubations were performed in quadruplicate. Compounds, peptide, and buffer controls were added to the wells in a 10 µl volume in 5% DMSO. Cy5-labelled S100A10 tracer (407 nm) and Cy3-labelled annexin A2(1–14) peptide ligand (1.33 µM) were pre-incubated for 5 min at 20 °C, and 40 µl of the preformed complex was then added to the wells and mixed for 10 s to yield a final DMSO concentration of 1%. After 5 min incubation at 20 °C, readings were taken on a Perkin-Elmer Envision instrument by excitation at 488 nm and emission at
FRET was calculated by measuring the fluorescence emission of co-
incubated S100A10–Cy5 and annexin A21–14–Cy3 and subtract-
ing the same signal obtained in the presence of excess unlabelled
annexin A21–14 peptide. A counterscreen assay, which measures the
FRET signal from a Cy3-conjugated donkey anti-goat IgG
(4 μg/mL) onto a Cy5-labelled goat IgG (3 μg/mL), was used in
parallel to assess nonspecific interference with the fluorescence
readout. Compound binding was calculated as a percentage of un-
treated control, and data were analysed by nonlinear regression
(dose–response, variable slope) using GraphPad Prism Software
with top and bottom constraints at 100 and 0 %, respectively.

Chemistry

General: All reagents were purchased directly from commercial
sources and were used as supplied unless otherwise stated. Melt-
ing points were measured with a Gallenkamp melting point appa-
rat and are uncorrected. Accurate mass and nominal mass meas-
urements were performed using a Waters 2795-Micromass LCT
electrospray mass spectrometer. Infrared spectra were recorded on
an AVATAR 360 FTIR system. Samples were prepared as KBr discs
and scanned from 4000 to 500 cm⁻¹ (for compounds 7i and 6a).
All NMR spectra were recorded in [D6]DMSO, with trimethylsilane
as an internal standard, using a Bruker ACS-120 instrument at
400 MHz (1H NMR) and 100.6 MHz (13C NMR). Chemical shifts (δ)
are reported in ppm, and coupling constants (J) are given in Hz. Sig-
als are represented by s (singlet), d (doublet), t (triplet), q (quar-
tet), m (multiplet), bs (broad singlet), dd (double doublet), and td
triple doublet). Thin-layer chromatography was performed using
aluminum-backed silica gel 60 plates (0.20 mm layer), the ascend-
ing technique was used with a variety of solvents. Visualisation
was by UV light at either λ 254 or 365 nm.

o-Tolylacetic acid ethyl ester (3a): To a solution of o-cresol
(525 μL, 5.0 mmol, 1.0 equiv) in DMF (15 mL) under N2 was added
NaH (300 mg, 7.5 mmol, 1.5 equiv). The reaction was allowed to
stir for 60 min at RT under N2. To this was added ethyl bromoacet-
ate (566 μL, 5.0 mmol, 1.0 equiv), and the reaction mixture was
allowed to stir for 30 min. The reaction mixture was poured into
cold H2O (25 mL) and allowed to stand for 60 min. No precipitate
was observed. The aqueous phase was extracted with EtOAc, and
the EtOAc layer was dried (Na2SO4) and concentrated under re-
duced pressure to give product 3a as a pale-yellow oil (740 mg,
76 % yield): Rf = 0.44 (EtOAc/petroleum ether (PE), 1:6); 1H NMR
(400 MHz, [D6]DMSO): δ = 7.20–7.08 (2 H, m, Ar-H), 6.50 (2 H, d,
J = 8.9 Hz, Ar-H), 5.53 (1 H, t, J = 6.5 Hz, CH2), 4.10 (2 H, q, J = 7.1 Hz,
CH2CH3), 3.83 (2 H, d, J = 6.5 Hz, CH3), 2.12 (3 H, s, CH3), 1.19 ppm (3 H, t,
J = 7.1 Hz, CH2CH3); 13C NMR (100.6 MHz, [D6]DMSO): Cq = 171.4,
145.8, 124.7; CH2: 129.2, 112.2; CH3: 60.1, 45.0; CH: 20.0, 14.1 ppm;
HRMS (ES): m/z [M + H]+ calcd for C9H16NO3: 210.1052, found: 210.1320.

p-Tolylacetic acid hydrazide (4a): To a solution of p-tolyl-
acetic acid ethyl ester (3a, 657 mg, 3.38 mmol, 1.0 equiv) in EtOH
(20 mL), a large excess of hydrazine (939 μL, 20.3 mmol, 6.0 equiv) was
added, and the reaction mixture was heated at reflux overnight.
The reaction mixture was cooled and concentrated under re-
duced pressure. The white precipitate obtained was washed with
EtO/PE (1:3) to afford product 4a as a white crystalline solid
(367 mg, 60 % yield): Rf = 0.10 (EtOAc); mp: 114–116 ºC; 1H NMR
(400 MHz, [D6]DMSO): δ = 9.20 (1 H, s, NH), 7.18–7.06 (2 H, m, Ar-H),
6.89–6.79 (2 H, m, Ar-H), 4.49 (2 H, s, CH2), 3.58 (2 H, bs, NH2),
2.21 ppm (3 H, s, CH3); 13C NMR (100.6 MHz, [D6]DMSO): Cq = 166.8,
156.0, 126.2; CH2: 130.5, 126.8, 120.8, 111.3; CH: 66.5; CH3: 16.0,
16.1 ppm; HRMS (ES): m/z [M + H]+ calcd for C11H16N2O2: 210.0899,
found: 210.1532.

p-Tolylaminocarboxylic acid hydrazide (4b): The procedure was simi-
lar to the procedure for 4a except that p-tolylaminocarboxylic acid
ethyI ester (3b, 1.930 g, 10.0 mmol, 1.0 equiv) was used and the
reaction mixture and was heated at reflux for only 5 h. Product 4b
was isolated as a white-pale powder (1.34 g, 75 % yield): Rf = 0.23
(EtOAc/MeOH, 9:1); mp: 151–153 ºC; 1H NMR (400 MHz, [D6]DMSO):
δ = 9.00 (1 H, bs, NH-NH), 6.89 (2 H, d, J = 8.1 Hz, Ar-H), 6.46 (2 H, d,
J = 8.5 Hz, Ar-H), 5.59 (1 H, t, J = 6.1 Hz, NH), 4.21 (2 H, bs, NH2), 3.58
(2 H, d, J = 6.2 Hz, CH2), 2.14 ppm (3 H, s, CH3); 13C NMR (100.6 MHz,
[D6]DMSO): Cq = 169.5, 146.0, 124.7; CH: 129.2, 112.4; CH2: 45.7;
CH3: 20.0 ppm; HRMS (ES): m/z [M + H]+ calcd for C9H14N3O: 181.0899,
found: 181.1532.

(4-Methoxyphe\nylaminocarboxylic acid hydrazide (4c): The procedure was similar to that for 4a except that (4-methoxynaph-
linocarboxylic acid ethyl ester (3c, 5.0 g, 23.9 mmol, 1.0 equiv) was
used in the reaction mixture. Product 4c was isolated as a white

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crystalline solid (3.986 g, 86% yield); \( R_r = 0.20 \) (EtOAc/MeOH, 9:1); mp: 115–117 °C; \(^1^H\) NMR (400 MHz, \([D_2]DMSO\)): \( \delta = 9.0 \) (1H, bs, NH-NH), 7.67 (2H, d, \( J = 8.9 \) Hz, Ar-H), 6.51 (2H, d, \( J = 8.9 \) Hz, Ar-H), 5.41 (1H, t, \( J = 6.2 \) Hz, NH), 4.21 (2H, d, \( J = 3.3 \) Hz, NH), 3.63 (3H, s, OCH), 3.56 ppm (2H, d, \( J = 6.2 \) Hz, CH); \(^1^C\) NMR (100.6 MHz, \([D_2]DMSO\)): \( \delta = 170.1, 151.1, 142.9 \); CH: 115.0, 113.8; CH\(_2\): 46.7; CH\(_3\): 55.8 ppm; HRMS (ES): \( m/z \) [M + H\(^+\)]\(^+\) calculated for \( C_{11}H_{14}N_2O_3\): 196.1008, found: 196.1473.

4-(2-Ethylphenyl)-5-[(4-methoxyphenylamino)methyl]-4H-[1,2,4]triazole-3-thiol (6a): To a solution of (4-methoxyphenylamino)acetic acid hydrazide (4c) (4.0 g, 20.489 mmol, 1.0 equiv) in EtOH (150 mL) was added 2-ethylphenyl isothiocyanate (3.203 mL, 20.489 mmol, 1.0 equiv), and the reaction mixture was heated under reflux overnight. The reaction was cooled and concentrated under reduced pressure to result in a greasy yellow substance. This was added aqueous 1.5 M HCl and washed with EtOH/MeOH (1:1) to afford the pale-yellow solid was washed with EtOH/MeOH (1:1) to afford the product as a white powder (2.246 g, 32% yield): \( R_r = 0.65 \) (EtOAc); mp: 162–164 °C; \(^1^H\) NMR (400 MHz, \([D_2]DMSO\)): \( \delta = 13.82 \) (1H, s, SH or NH), 7.53–7.41 (2H, m, Ar-H), 7.36 (1H, td, \( J = 1.6/1.7, 7.2/7.7 \) Hz, Ar-H), 7.27 (1H, dd, \( J = 1.2, 7.8 \) Hz, Ar-H), 6.67 (2H, d, \( J = 8.9 \) Hz, Ar-H), 6.45 (2H, d, \( J = 8.9 \) Hz, Ar-H), 5.40 (1H, t, \( J = 5.9/6.0 \) Hz, NH), 4.1–3.8 (2H, m, CH\(_2\)), 3.61 (3H, s, OCH), 2.4–2.2 (2H, m, CH\(_2\)CH\(_2\)), 1.07 ppm (3H, t, \( J = 7.6 \) Hz, CH\(_2\)CH\(_3\)); \(^1^C\) NMR (100.6 MHz, \([D_2]DMSO\)): \( \delta = 168.1, 151.3, 150.7, 141.8, 146.1, 131.9; CH: 130.1, 128.9, 126.8, 126.9, 11.4; 113.4; CH\(_2\): 39.3, 23.1; CH\(_3\): 55.2, 13.3 ppm; \( R_{KBr} \): \( \nu = 3397 \) (NH), 3104, 3058 (CH of Ar), 2933, 2832 (CH from CH\(_2\)), 1514 (C–N), 1308 cm\(^{-1}\) (C–S); HRMS (ES): \( m/z \) [M + H\(^+\)]\(^+\) calculated for \( C_{18}H_{19}N_4OS\): 341.1358, found: 340.7935.

5-[(4-Methoxyphenylamino)ethyl]-4-p-tolyl-4H-[1,2,4]triazole-3-thiol (6b): To a solution of (4-methoxyphenylamino)acetic acid hydrazide (4c) (2.0 g, 10.244 mmol, 1.0 equiv) in EtOH (100 mL) was added 4-methylphenyl isothiocyanate (1.575 g, 10.244 mmol, 1.0 equiv), and the reaction mixture was heated under reflux for 90 min, and the formation of precipitate was observed. The reaction was cooled, and the white precipitate formed was filtered and washed with EtOH to result in a pale-white solid (2.187 g, 71% yield): \( R_r = 0.55 \) (EtOAc/PE, 1:3); mp: 232–234 °C; \(^1^H\) NMR (400 MHz, \([D_2]DMSO\)): \( \delta = 14.07 \) (1H, s, SH or NH), 7.60–7.49 (5H, m, Ar-H), 7.12–7.00 (2H, m, Ar-H), 6.88 (1H, d, \( J = 8.0 \) Hz, Ar-H), 6.82 (1H, td, \( J = 0.4/0.5, 7.3/7.4 \) Hz, Ar-H), 5.01 (2H, s, CH\(_2\)), 1.85 ppm (3H, s, CH\(_3\)); \(^1^C\) NMR (100.6 MHz, \([D_2]DMSO\)): \( \delta = 168.0, 151.1, 148.1, 133.5, 126.0; CH: 130.5, 129.5, 127.9, 126.8, 121.2, 111.5; CH\(_2\): 59.9; CH\(_3\): 15.6 ppm; HRMS (ES): \( m/z \) [M + H\(^+\)]\(^+\) calculated for \( C_{17}H_{19}N_4OS\): 317.0369, found: 317.0362.
5 N HCl. The white precipitate obtained was separated by filtration and washed further with H2O and freeze dried (3.076 g, 94% yield); Rf = 0.57 (EtOAc/PE, 1:1); mp: 156–158 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 14.16 (1H, s, SH or NH), 7.73–7.66 (1H, m, Ar-H), 7.62–7.24 (3H, m, Ar-H), 7.12–7.02 (2H, m, Ar-H), 6.89 (1H, d, J = 7.9 Hz, Ar-H), 6.82 (1H, td, J = 0.7, 7.4 Hz, Ar-H), 4.96 (2H, dd, J = 13.1, 29.7 Hz, Ar-H), 1.90 ppm (3H, s, CH3); 13C NMR (100.6 MHz, [D6]DMSO): δq: Cq = 168.7, 155.0, 148.1, 134.2, 132.5, 125.9; CH: 130.6, 129.9, 129.3, 126.8, 121.2, 111.6; CH2: 159.9; CH3: 15.5 ppm; HRMS (ES): m/z [M + H]+ calcld for C18H16N2O2S: 328.1045, found: 328.1055.

4-(2-Methoxyethyl)-5-o-tolyloxy-4H-[1,2,4]triazole-3-thiol (6g): To a solution of (4a) o-tolyloxyacetic acid hydrazide (2.138 g, 11.86 mmol, 1.0 equiv) in EtOH (100 mL) was added 4-methoxyphenyl isothiocyanate (1.690 mL, 11.095 mmol, 1.0 equiv), and the reaction mixture was heated under reflux for 30 min, then cooled. The reaction was neutralised with 5 N HCl. The white precipitate obtained was separated by filtration and washed further with EtOH and freeze dried (2.787 g, 90% yield): Rf = 0.13 (EtOAc/PE, 1:3); mp: 162–164 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 13.91 (1H, s, SH or NH), 7.22–7.24 (2H, m, Ar-H), 7.07 (1H, d, J = 8.0 Hz, Ar-H), 6.90 (1H, td, J = 0.8, 7.3 Hz, Ar-H), 5.21 (2H, s, CH2), 4.21 (2H, t, J = 5.5 Hz, CH2), 3.62 (2H, t, J = 5.5 Hz, CH2), 3.22 (3H, s, OCH3), 2.13 ppm (3H, s, CH3); 13C NMR (100.6 MHz, [D6]DMSO): Cq: δ = 167.4, 155.4, 147.8, 126.0; CH: 130.7, 127.0, 121.3, 111.8; CH2: 68.6, 60.1, 43.4; CH3: 58.4, 15.9 ppm; HRMS (ES): m/z [M + H]+ calcld for C19H22N3OS: 328.1041, found: 328.1116.

4-(4-Chlorophenyl)-5-o-tolyloxy-4H-[1,2,4]triazole-3-thiol (6h): To a solution of (4a) o-tolyloxyacetic acid hydrazide (2.5 g, 13.872 mmol, 1.0 equiv) in EtOH (100 mL) at 80 °C was added 4-chlorophenyl isothiocyanate (2.376 g, 13.872 mmol, 1.0 equiv), and almost immediately formation of the precipitate was observed. The reaction mixture was heated under reflux for 5 h. The reaction was cooled, and the white precipitate formed was filtered, washed with EtOH. The white precipitate was added aqueous 1.5 M KOH (30 mL) and heated at reflux for 30 min, then cooled. The reaction was neutralised with 5 N HCl. The white precipitate obtained was separated by filtration and washed further with H2O and freeze dried (2.737 g, 90% yield): Rf = 0.60 (EtOAc/PE, 1:1); mp: 168–170 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 14.04 (1H, s, SH or NH), 7.44–7.32 (4H, m, Ar-H), 7.11–7.00 (2H, m, Ar-H), 6.86 (1H, d, J = 7.9 Hz, Ar-H), 6.82 (1H, td, J = 0.7, 7.3 Hz, Ar-H), 5.03 (2H, s, CH2), 3.04–2.84 (1H, hept, CH of isopropyl), 1.81 (3H, s, CH3), 1.21 ppm (6H, d, J = 6.9 Hz, (CH3)2 of isopropyl); 13C NMR (100.6 MHz, [D6]DMSO): Cq: δ = 168.6, 155.1, 149.6, 148.3, 131.2, 126.1; CH: 130.5, 127.5, 127.1, 126.8, 121.2, 111.7, 33.2; CH2: 60.0; CH3: 23.7, 15.5 ppm; HRMS (ES): m/z [M + H]+ calcld for C19H19ClN2OS: 340.1405, found: 340.1575.

Library synthesis: The analogues of 1,2,4-triazole target compounds (1b–c, 7a–i except 7h) were synthesised using a 12-well Radley’s parallel synthesiser.

2-(4-Phenyl-5-o-tolyloxy-4H-[1,2,4]triazole-3-ylsulfanyl)acetamide (7a): A solution of product 6d (446 mg, 1.55 mmol, 1.0 equiv) in DMF (4 mL) and a solution of 2-bromoacetic acid (211 mg, 1.55 mmol, 1.0 equiv) in DMF (2 mL) were both added separately to a dry Radley’s reaction tube containing K2CO3 (249 mg, 1.8 mmol, 1.2 equiv). The reaction mixture was allowed to stir at 45 °C overnight in a Radley’s parallel synthesiser. The reaction mixture was poured onto crushed ice (50 mL) and allowed to stand for 3 h. The precipitate formed was collected by filtration, washed thoroughly with H2O to remove DMF then freeze dried, followed by recrystallisation from EtOH/EtO. Product 7a was obtained as a pale-yellow crystalline solid (232 mg, 44% yield): Rf = 0.28 (EtOAc/MeOH, 9:1); mp: 123–125 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 7.68 (1H, bs OH), 7.60–7.46 (5H, m, Ar-H), 7.25 (1H, bs, NH), 7.12–7.02 (2H, m, Ar-H), 6.96 (1H, d, J = 7.8 Hz, Ar-H), 6.81 (1H, td, J = 0.8, 7.3 Hz, Ar-H), 5.13 (2H, s, CH2), 3.95 (2H, s, CH2), 1.82 ppm (3H, s, CH3); 13C NMR (100.6 MHz, [D6]DMSO): Cq: δ = 168.5, 155.3, 151.8, 151.7, 132.8, 125.9; CH: 130.5, 130.0, 129.8, 126.8, 126.8, 3143 – 3157 © 2012 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim
7.21–7.10 (3 H, m, Ar-H), 6.92–6.86 (1 H, m, Ar-H), 5.27 (2 H, s, CH2), 3.95 (2 H, s, CH2), 2.96 (1 H, hep, CH of isopropyl), 1.80 (3 H, s, CH3), 1.72 (3 H, s, CH3) ppm; 13C NMR (100.6 MHz, [D6]DMSO): δ: 168.3, 155.3, 152.1, 151.7, 130.5, 130.0, 128.7, 126.8, 126.0, 111.1; CH3: 59.7, 36.3; 15 ppm; HRMS (ES): m/z [M+H]+ calcd for C19H21N4O3S: 389.0761, found: 389.0782.

7g: The procedure was similar to that for 7a except that product 6g (391 mg, 1.5 mmol, 1.0 equiv) was used and the recrystallisation was carried out from EtOH/THF. Product 7f was isolated as a white crystalline solid (300 mg, 76% yield): 8f = 0.30 (EtOAc/MeOH, 9:1); mp: 135–137 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 6.76 (1 H, bs, OH), 7.47–7.36 (4 H, m, Ar-H), 7.25 (1 H, bs, = NH), 7.02–7.12 (2 H, m, Ar-H), 6.91 (1 H, d, J = 7.6 Hz, Ar-H), 2.07 (3 H, s, CH3), 1.80 (3 H, s, CH3) ppm; 13C NMR (100.6 MHz, [D6]DMSO): δ: 168.5, 155.3, 152.0, 151.9, 152.9, 126.0; CH: 130.5, 127.0, 121.1, 111.9; CH2: 70.1, 60.1, 43.8, 36.9; CH3: 58.4, 16.0 ppm; HRMS (ES): m/z [M+H]+ calcd for C19H17N4O3S: 373.1256, found: 373.1235.

2-(4-Ethylphenyl)-5-o-tolyloxymethyl-4H-[1,2,4]triazol-3-ylsulfanyl]acetamide (7d): The procedure was similar to that for 7a except that product 6f (325.5 mg, 1.0 mmol, 1.0 equiv) was used and the recrystallisation was carried out from EtOH. Product 7d was isolated as a white needle-like crystalline solid (224 mg, 41% yield): 8d = 0.28 (EtOAc/MeOH, 9:1); mp: 136–138 °C; 1H NMR (400 MHz, [D6]DMSO): δ = 7.68 (1 H, bs, OH), 7.17–7.34 (4 H, m, Ar-H), 7.14–7.02 (2 H, m, Ar-H), 6.96 (1 H, d, J = 7.9 Hz, Ar-H), 6.82 (1 H, td, J = 0.8, 7.3 Hz, Ar-H), 5.09 (2 H, s, CH2), 3.94 (2 H, s, CH2), 2.36 (3 H, s, CH3), 1.88 ppm (3 H, s, CH3) ppm; 13C NMR (100.6 MHz, [D6]DMSO): δ: 168.5, 155.4, 152.0, 151.7, 139.9, 130.1, 125.9; CH: 130.3, 130.2, 126.9, 126.6, 121.0, 111.6; CH2: 59.6, 35.9; CH3: 20.7, 15.7 ppm; HRMS (ES): m/z [M+H]+ calcd for C18H17N4O2S: 348.1307, found: 348.1311.
CH₃), 3.88 (2H, s, CH₂), 2.12 ppm (3H, s, CH₃); ¹³C NMR (100.6 MHz, [D₆]DMSO): δ = 168.5, 154.0, 150.6, 145.6, 134.6, 131.8, 124.9; CH: 129.8, 125.2, 129.0, 112.4; CH₂: 38.4, 36.1; CH₃: 20.1 ppm; HRMS (ES): m/z [M+H]+ calcd for C₉H₇ClINO₃S: 388.0921, found: 388.1184.

2-[5-[(4-Methoxyphenylamino)methyl]-4-p-tolyl-4H-[1,2,4]triazolo-3-ylsulfanoyl]acetamide (1c): The procedure was similar to that for 7a except that product 6b (200 mg, 0.612 mmol, 1.0 equiv) was used and the recrystallisation was carried out from EtOH. Product 1c was isolated as a white poweder (147 mg, 63 % yield): Rf = 0.16 (EtOAc/MeOH, 9:1); mp: 157–159 °C; ¹H NMR (400 MHz, [D₆]DMSO): δ = 6.65 (1H, bs, OH), 6.66 (2H, d, J = 8.5 Hz, Ar-H), 7.33 (2H, d, J = 8.5 Hz, Ar-H), 7.21 (1H, bs, NH), 6.66 (2H, d, J = 9.0 Hz, Ar-H), 6.49 (2H, d, J = 9.0 Hz, Ar-H), 5.40 (1H, t, J = 5.7 Hz, NH), 4.13 (2H, d, J = 5.7 Hz, CH₂), 3.88 (2H, s, CH₂), 3.61 (3H, s, OCH₃), 2.39 ppm (3H, s, CH₃); ¹³C NMR (100.6 MHz, [D₆]DMSO): δ = 168.6, 154.0, 151.2, 150.7, 142.1, 139.7, 130.3; CH: 130.2, 126.8, 114.4, 113.5; CH₂: 38.9, 35.9; CH₃: 55.3, 20.8 ppm; HRMS (ES): m/z [M+H]+ calcd for C₁₇H₁₄N₂O₄S: 384.1416, found: 384.1509.

2-[4-[2-(Ethylphenyl)-5-[(4-methoxyphenylamino)methyl]-4H-[1,2,4]triazolo-3-ylsulfanoyl]acetamide (7b): The procedure was similar to that for 7a except that product 6a (340.5 mg, 1.0 mmol, 1.0 equiv) was used and the recrystallisation was carried out from EtOH. Product 7b was isolated as a white crystalline solid (222 mg, 56 % yield): Rf = 0.3 (EtOAc/MeOH, 9:1); mp: 134–136 °C; ¹H NMR (400 MHz, [D₆]DMSO): δ = 7.65 (1H, bs, OH), 7.57–7.46 (4H, m, Ar-H), 7.21 (1H, bs, NH), 6.65 (2H, d, J = 9.0 Hz, Ar-H), 6.48 (2H, d, J = 9.0 Hz, Ar-H), 5.40 (1H, t, J = 5.7–5.8 Hz, NH), 3.91 (2H, d, J = 1.9 Hz, CH₂), 3.61 (3H, s, OCH₃), 2.21 (2H, q, J = 7.6 Hz, -CH₂CH₃), 1.03 ppm (3H, t, J = 7.5 Hz, -CH₂CH₃); ¹³C NMR (100.6 MHz, [D₆]DMSO): δ = 168.5, 154.0, 151.2, 150.6, 142.1, 141.1, 130.6; CH: 130.6, 129.3, 128.1, 127.1, 114.4, 113.4; CH₂: 38.9, 35.7, 22.8; CH₃: 55.2, 13.6 ppm; IR (KBr): ν = 3384 (NH), 3154 (OH of Ar), 2977, 2831 (CH from CH₂), 1666 (C=C), 1517 cm⁻¹ (C=N); HRMS (ES): m/z [M+H]+ calcd for C₁₇H₁₄N₂O₄S: 398.1572, found: 398.1344.

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