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Identification of novel small molecule TGF-β antagonists using structure-based drug design

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Abstract Aberrant transforming growth factor-β (TGF-β) signalling has been associated with a number of disease pathologies, such as the development of fibrosis in the heart, lung and liver, cardiovascular disease and cancer, hence the TGF-β pathway represents a promising target for a variety of diseases. However, highly specific ways to inhibit TGF-β signalling need to be developed to prevent cross-talk with related receptors and minimise unwanted side effects. We have used used virtual screening and molecular docking to identify small molecule inhibitors of TGF-β binding to TßRII. The crystal structure of TGF-β3 in complex with the extracellular domain of the type II TGF-β receptor was taken as a starting point for molecular docking and we developed a structure-based pharmacophore model to identify compounds that competitively inhibit the binding of TGF-β to TβRII and antogonize TGF-β signalling. We have experimentally tested 67 molecules suggested by in silico screening and similarity searching for their ability to inhibit TGF-β signalling in TGF-β-dependent luciferase assays in vitro and the molecule with the strongest inhibition had an IC_{50} of 18 μM. These compounds were selected to bind to the SS1 subsite (composed of F30, C31, D32, I50, T51 S52, I53, C54 and E55) of TßRII and all share the general property of being aromatic and fairly flat. Molecular dynamics simulations confirmed that this was the most likely binding mode. The computational methods used and the hits identified in this study provide an excellent guide to medicinal chemistry efforts to design tighter binding molecules to disrupt the TGF-β/TßRII interaction.

Keywords TGF-β · Molecular docking · Molecular dynamics simulations · Drug discovery

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Introduction

TGF-β is a ubiquitously expressed growth factor that plays a major role in embryonic development and in the maintenance of adult tissue homeostasis by regulating a diverse array of cellular processes, such as proliferation, differentiation, epithelial to mesenchymal transition and apoptosis [1]. Aberrant TGF-β signalling has been associated with a number of disease pathologies, such as the development of fibrosis in the heart, lung and liver, cardiovascular disease and cancer [2, 3]. Consequently, a number of strategies to target the TGF-β signalling pathway therapeutically are

being developed and these include ligand traps and receptor inhibitors [3]. Small molecule inhibitors that target the ATP binding site of the receptors have been developed but lack specificity and new ways to inhibit receptor signalling are required to circumvent these problems. TGF-ß signal transduction is initiated following binding of the active ligand to the type II TGF-ß receptor (TßR-II) and, after heterodimerisation and transphosphorylation of TßR-I, signal propagation is initiated following phosphorylation of the receptor-specific (R) Smads (Smad2 and Smad3). The phosphorylated R-Smads then oligomerise with Smad4, translocate to the nucleus and regulate the transcription of target genes [4]. By designing molecules capable of binding to TβR-II, recruitment of TGF-β may be disrupted, thereby providing a specific way to competitively inhibit TGF-β signalling.

In the present study, we used virtual screening and molecular docking to identify small molecule inhibitors of TGF-β binding to TßRII. The crystal structure of TGF-β3 in complex with the extracellular domain of the type II TGF-β receptor (Protein Data Bank accession number 1KTZ) [5] was taken as a starting point for molecular docking and we developed a structure-based pharmacophore model to identify compounds that competitively inhibit the binding of TGF-β to TβRII. We have experimentally screened 67 molecules for their ability to inhibit TGF-β signalling in in vitro assays in a dose-dependent manner and the strongest inhibition was shown by molecule NCI-48455, with an IC_{50} of 18 μM.

Methods and materials

Selection of docking libraries

The "clean and drug-like" subset of the ZINC database (v 8; 2008) [6] of commercially available compounds containing around 8 M compounds and was chosen for virtual screening. The National Cancer Institute offers free samples for academic research, hence the NCI-2003 library (260,071 compounds) was chosen for a separate screening and docking experiment. The 3D conformations of all the molecules were generated using the Sybyl (v 7.3; Tripos) virtual screening tools.

Identification of the binding site

The crystal structure of TGF-β3 in complex with the extracellular domain of TßRII (Protein Data Bank accession number 1KTZ) [5] was taken as a starting point for molecular docking. Hydrogen atoms were added using Sybyl consistent with pH 7 and all water molecules were removed. The residues of TGF-β3 that interact with the receptor were used as a reference to indict the binding site for potential small-molecule TGF-β antagonists.

Virtual screening

The Unity module within Sybyl was used to create a 3D-pharmacophore model representing the interactions in the TGF-β complex. This model included two H-bonds corresponding to those formed between Arg94 on TGF-β3 and Asp32 on the receptor, and the excluded volumes defined by the binding site on TßRII. The ZINC was submitted to virtual screening using the Unity flexible search protocol.

Molecular docking

The whole NCI 2003 library and those molecules found by Unity virtual screening were docked into the TGF-β3 binding site on TßRII using two docking programs, FRED and GOLD.

FRED docking

FRED (v 2.2.3; Fast Rigid Exhaustive Docking) is a protein–ligand docking program released by Openeye. Multi-conformer libraries were generated with the Omega2 (v 2.4.3) module in the Openeye package using the default settings and saving 200 conformations per compound, These libraries were docked to TßRII using FRED with default settings. Seven different scoring functions: chemgauss2, chemgauss3, shapegauss [7], PLP [8], chemscore [9], oechemscore and screenscore [10], were applied to rank the hit list. Within each scoring function, the top 1,000 compounds were recorded, and only those molecules that appeared in more than four of the seven ranking lists were considered for experimental testing.

GOLD docking

GOLD (v 4.0.1; Genetic Optimization for Ligand Docking) [11] is a well-known protein–ligand docking program released by the Cambridge Crystallographic Data Centre (CCDC). In this study, GOLD was used to dock each ligand 10 times, starting each time from a different random population of ligand orientations and using the default automatic genetic algorithm parameter settings. All torsion angles in each compound were allowed to rotate freely and the results of the different docking runs were ranked using GoldScore. The top 500 molecules were considered for experimental testing.

Similarity searches

Similarity searches were performed using ROCS (v 3.1.2; Rapid Overlay of Chemical Structures) and followed by EON (v 2.1.0; electrostatic similarity) in the Openeye package. Default parameters for ROCS and EON were

employed. The best 5,000 hits and a maximum of 3 conformations for each hit returned by ROCS were saved and used as the input database for EON. The top 500 hits identified by EON were recorded.

In-vitro screening for inhibition of TGF-β signalling

The ability of selected compounds to inhibit TGF-β signalling was assayed using mink lung epithelial cells stably transfected with a TGF-β-responsive plasminogen activator inhibitor-1 promoter-luciferase construct [12]. Briefly, cells were seeded in 48 well plates at a density of 1.6×104 cells well^{-1} in DMEM (Dulbecco's Modified Eagle Medium) with 10 % FCS (Foetal Calf Serum), and allowed to adhere for a period of 5 h. The medium was removed and the cells subsequently treated with 0.2 ml DMEM containing 1 % FCS and 0.1 ng/ml TGF-β1 (R&D systems) or TGF-β1 vehicle plus the compound to be assayed. For each compound, 0.001 g compound was dissolved in 100 µl DMSO and then diluted 200 times (final DMSO concentration 0.5 %) with media. Following incubation for 18 h, the medium was removed, the cells were washed with PBS, removing all the unbound ligand, and incubated with 0.1 ml Steady-Glo luciferase assay reagent (Promega). The lysate was then transferred to a 96 well plate for measurement on a luminometer. IC_{50} values were determined for any compounds shown to cause a reduction in luminescence in the presence of TGF-β1 by repeating the assay using serial dilutions of the compound. As a further control, another set of assays were performed that included the active compounds during the luciferase readings. After the PBS washing step, the luminescence assay was performed in the presence of ¼ maximum compound concentration. There were no statistical differences (Dunnett's two sided test) between the luminescence measurements in wells containing the compound and controls (TGF-β1 alone), except for compound 31 which reduced the luminescence readings by approximately 30 % (data not shown). As unbound ligand was removed prior to our screening assay, we believe it is extremely unlikely that there was sufficient residual compound left after the washing step to affect the results with compound 31. The luciferase measurements obtained when testing the remaining compounds could not have been affected.

An assessment of cell number and viability was performed in parallel plates, using a combination of the tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium, innersalt (MTS, Promega, UK) and the electron coupling reagent phenazine methosulphate (PMS, Sigma). MTS and PMS were prepared separately in pre-warmed (37 °C) phenol red free DMEM/F12, allowed to dissolve and then combined so that 1 ml of a 1 mg/ml solution of PMS was combined to 19 ml of a 2 mg/ml solution of MTS. The resultant mixture was passed through a 0.2 mm filter. After the media was removed, the cells in each well were treated with 0.5 ml new media and 0.1 ml of the MTS/PMS reagent mixture. The same treatment was applied to two blank wells as control groups. Plates were left for 1 h under conventional culturing conditions. Once incubated, samples (0.1 ml) from each well were dispensed onto a 96-well microtitre plate and the absorbance at 490 nm determined using a multiplate reader. Plates were staggered to ensure that all samples were recovered within 5 min to minimise any error introduced during the formation of further formazan product by the cell monolayer.

Molecular dynamics simulations

Preparation of the systems for simulation

Molecular mechanics calculations were performed using the AMBER 10 [13] package. For compatibility with standard AMBER parameterization protocols, the ab initio QM calculations were performed on compounds with low IC_{50}s using the Hartree–Fock 6-31G* basis set within Gaussian03 [14]. The molecular geometries were optimised and the atom-centered point charges were calculated to fit the electrostatic potential using RESP [15].

The complexes in PDB format obtained from the docking were used as the input structures for molecular dynamics simulations. Using the *leap* module in AMBER10 with Amber-03 and gaff force fields, the solute was immersed in a truncated octahedral periodic box of TIP3P water (minimum distance from solute to the box edge was 10 Å), and sufficient sodium ions were added to achieve charge neutrality.

Equilibration protocol

Our standard equilibration protocol is as follows:

1. The solvent is energy minimised while the solute is held fixed. The minimisation need not be exhaustive—a target RMS gradient of 0.1 kcal/mol/Å is sufficient.
2. The entire system is energy minimised to the same target gradient.
3. The solvent is subjected to a short (10 ps) MD simulation at a temperature of 100 K, under constant pressure conditions. The purpose of this is to remove voids in the solvent. The success of this stage is easily monitored by observing the change in the reported density of the system. For this and all following MD simulations, the Particle Mesh Ewald (PME) method is used to model long-range electrostatic interactions.
4. Over 10 ps, the solvent temperature is raised to 300 K. During both this phase and the last, position restraints

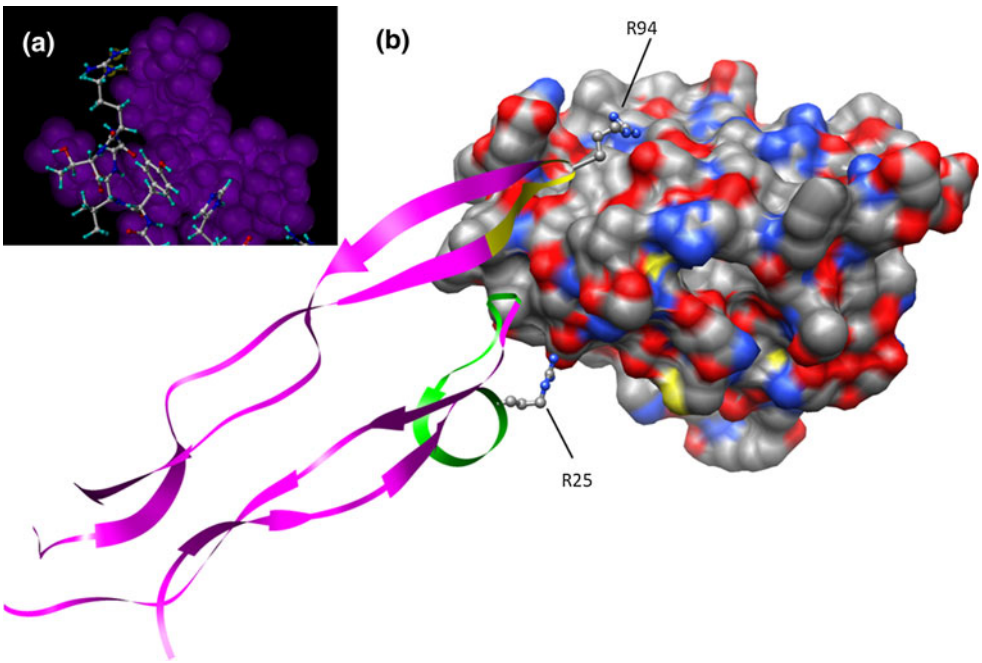


Fig. 1 **a** The 3D pharmacophore designed with Unity. A fragment of TGF-β3 is shown as sticks, the excluded volume of the pharmacophore is in *purple spheres* and the hydrogen-bond acceptor site is in *yellow spheres*. **b** The binding of TGF-β3 and TßRII. TGF-β3 is shown as a ribbon and the receptor is shown with a surface. The loop in contact with the receptor surface SS1 (containing R94) is coloured *yellow* and the loop in contact with SS2 (containing R25) is *green*

on every solute atom (force constant 100 kcal/mol/Å^2) maintain it in its energy-minimised conformation.

5. Over a series of 10 ps constant-pressure simulations at 300 K, the restraints on the solute are gradually relaxed (50, 25, 10, 5, 2 and then 1 kcal/mol/Å^2 respectively).

Production MD

After equilibration, the position restraints were removed and the production MD simulations were performed. Structures of the systems were saved every 10 ps, and simulations were run out to 40 ns. We used the Principal Component Analysis (PCA) [16] approach, from the collaborative computational project for biomolecular simulation (CCPB, www.ccpb.ac.uk), to check for equilibration and sampling.

Results and discussion

The interruption and modulation of protein–protein interactions at the cell surface by exogenous compounds is a promising approach to influence cell signalling networks to therapeutic advantage [17]. Such compounds are not required to penetrate the cell, hence may require lower dosing or lower affinity. They will utilise the normal cellular control systems rather than interfering with the complex and interdependent signalling networks within the cell where overall outcome may be hard to predict. The major difficulty of this approach is the drugability of the surfaces typical of those found at protein–protein interfaces [18]. Compounds that bind to these surfaces are typically not very drug-like in the conventional sense, often flout Lipinski;'s rules [19] and tight binding is typically hard to achieve. Nevertheless, further research in this area will eventually clarify these issues and will lead to a better understanding of possibilities and limitations of the approach [20].

Computer modelling

The binding site on TßRII can be divided into two parts, each interacting with different loops on TGF-β3, as shown in Fig. 1b. Sub-site one (SS1) interacts with residues Y90, Y91, V92, G93, R94, T95 (yellow ribbon) and sub-site two (SS2) interacts with R25, K31, W32, V33, H34 (green ribbon) of TGF-β3. Amongst these sets of TGF-β3 residues the positively charged residues R25 (SS2) and R94 (SS1), which are located on opposite peripheries of the binding site, form two hydrogen-bonded ion pairs with the negatively charged TßRII residues E119 and D32 respectively [5]. If a compound can bind either SS1 or SS2 it will inhibit the binding of TGF-β3 to the receptor and if it is large enough to occupy both sub-sites a higher binding affinity might be achieved. However, a compound large enough to span both sites will exceed the molecular weight limit of 500 suggested for drug-like molecules by Lipinski's rule of five [17]. It is not straightforward to predict which sub-site provides the major contribution to the binding free energy of the TGF-β complex, since both possess a hydrophobic region and hydrogen-bond donors and acceptors. However, inspection of the structure shows that the surface of SS1,

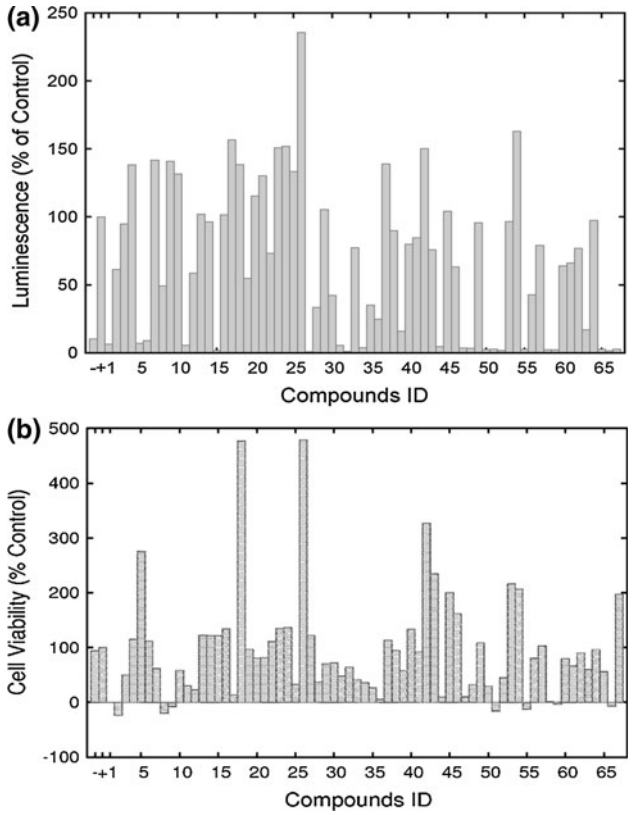


Fig. 2 **a** In-vitro screening for inhibition of TGF-β signalling using the luciferase assay. **b** The assessment of cell number and viability using the MTS assay

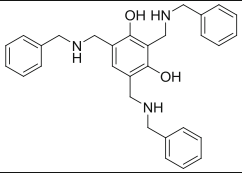
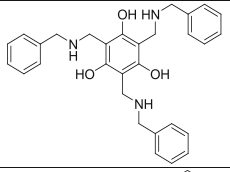
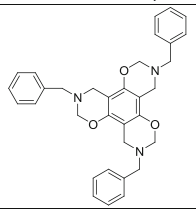
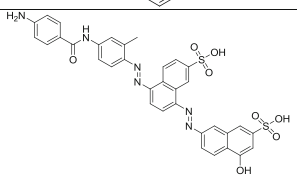
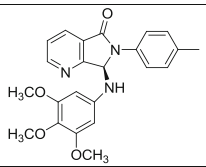
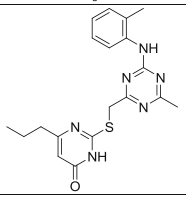
composed of F30, C31, D32, I50, T51 S52, I53, C54 and E55, forms a hydrophobic cavity while the surface of SS2 is flatter, suggesting that SS1 will be a better binding site for small molecules. To explore this idea, FRED was used to dock the Maybridge compound library (58,854 molecules in total and 58,796 successfully docked) into the whole binding site. The chemgauss3 scoring function was used and a strong preference for binding to SS1 was observed.

A 3D-pharmacophore representing the interactions in the TGF-β complex was constructed as shown Fig. 1a and virtual screening performed with Unity. This procedure identified 11,796 compounds in the ZINC drug-like library, in turn, these molecules were docked into the TßRII binding site with FRED. The scoring algorithm described in Methods was applied, giving 362 hits. Docking the whole NCI library into the TßRII binding site with FRED gave 70 hits (top 10 hits given by each scoring function) and with GOLD gave 500 hits.

Inhibition of TGF-β signalling

After removal of duplicates, around 100 compounds were chosen from the final hit list, by inspection, with the

Table 1 Active compounds lacking cytotoxicity

Compound 6 (NCI-48454)	
Compound 67*	
Compound 67(NCI-48455)	
Compound 31 (NCI-79727)	
Compound 65 (ChemDiv C999-1174)	
Compound 63 (Enamine T5440096)	

combined aims of covering the maximum amount of chemical space while avoiding non drug-like function groups. Of these, 55 compounds were actually available to assay for their ability to inhibit TGF-β signalling. The effect of the compounds on TGF-β-induced luciferase activity is shown in Fig. 2a. To determine whether effects on TGF-β signalling were due to cytotoxicity, we performed cell viability assays using parallel MTS assays (Fig. 2b). Three compounds (Table 1), whose Compound ID in Fig. 2 are 6 (NCI 48454), 67* (NCI 48455) and 31 (NCI 79727), caused a significant reduction in TGF-β-induced luciferase activity without causing significant cell death and were selected for further study.

Among these three compounds, 31 (IC_{50} around 40 μM) is not particularly drug-like and shows no clear route to further chemical elaboration. Hence we regard 31 as a tool compound rather than a potential lead into drug discovery.

The IC_{50} of compounds 6 and 67* are tighter binders with the IC_{50} values of 26 and 18 μM respectively. The mass spectra and NMR (^{13}C and ^{1}H) were measured for compounds 6 and 67* to confirm their structures, but neither were the correct compounds. We were unable to determine the structure for NCI 48454, but could show that NCI 48455 was the cyclised formaldehyde adduct, compound 67. Similarity searches based on the structures of compounds 6 and 67 were applied to screen the ZINC library for similar compounds. After repeating the docking step and evaluating the IC_{50}s of the best candidate molecules, another two of twelve compounds were identified with IC_{50}s of 105 μM (65, ChemDiv C999-1174, ChemDiv Inc) and 77 μM (63, Enamine T5440096, Enamine Ltd) shown in Table 1.

Molecular dynamics simulation

The molecular dynamics simulations were performed on the complex of receptor II and compounds 6, 67* and 67 for up to 40 ns as described in Methods with the AMBER package. The stability of each simulation was checked using PCA. Analysis of the molecular dynamics trajectories indicates that all three compounds remain bound to SS1 on TßRII for the whole period of the simulations, despite conformational fluctuations of both protein and ligand in the complexes. All three compounds form dynamic hydrogen-bonds with the receptor. The final structures of the docked poses collected from the dynamics trajectories are shown for all three complexes in Fig. 3, illustrating hydrogen bonding interactions. Compound 6 (Fig. 3a) forms 4 hydrogen-bonds with S52, I53 and E55, and 67* (Fig. 3c) forms 3 hydrogen-bonds with S52 and E55, but can form a fourth hydrogen bond with the backbone carbonyl of I53 since there is free rotation around the C–N single bonds. Despite compound 67* having one more hydroxyl group compared with compound 1, it can only form same number of hydrogen-bonds due to the pattern of hydrogen-bond donors and acceptors on the receptor. Compound 67 (Fig. 3b) lacks hydroxyl groups and only forms two hydrogen-bonds with E55 and S52, but it contains a larger hydrophobic centre than the other two. The

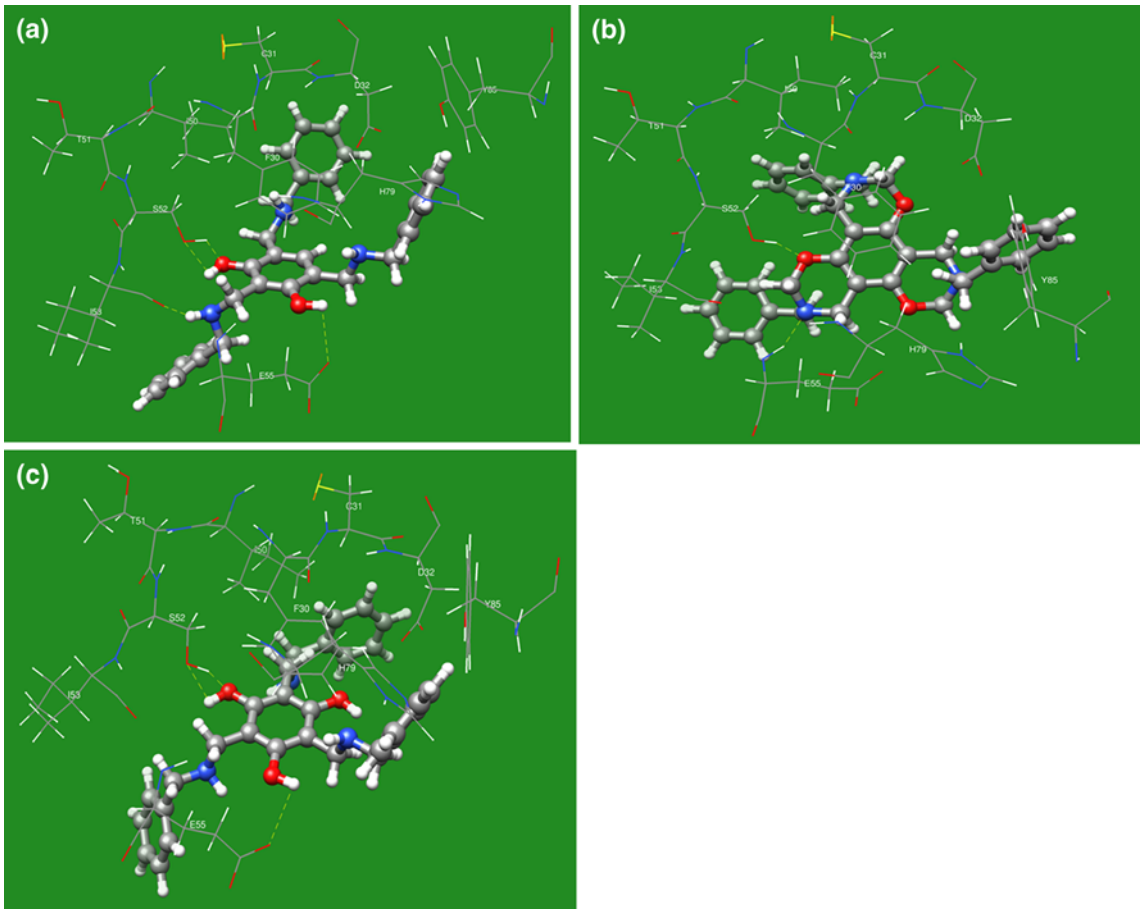


Fig. 3 Persistent hydrogen bonds in the molecular dynamics simulations, indicated by *yellow dashed lines*: Four hydrogen-bonds are formed between compound 6 and TßRII **a**. Two are formed between compound 67 and TßRII **b** and three are formed between compound 67* and TßRII **c**

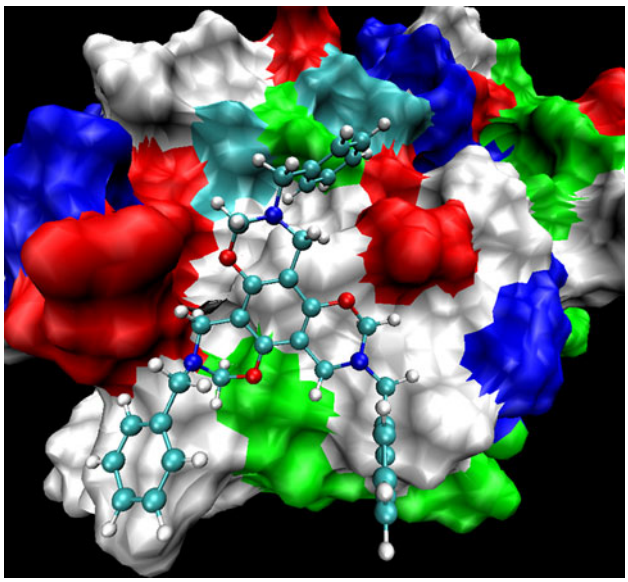


Fig. 4 The binding of compounds 67 to the surface of TßRII. The receptor surface is coloured: non-polar residues (*white*), basic residues (*blue*), acidic residues (*red*), polar residues (*green*) and histidine with hydrogen on the epsilon nitrogen (*cyan*). The hydrophobic surface patch on the receptor is triangular, matching the binding pose of the ligand

modelling suggests that F30 on the receptor is an important residue for ligand binding, since it forms π–π interactions with all three compounds.

Conclusions

Using in silico methods, we have identified a set of small molecules that both antagonise the TGF-β3/TßRII interaction and are tolerated in a cellular assay of toxicity. These compounds were selected to bind to the SS1 subsite of TßRII and all share the general property of being aromatic and fairly flat. Confirmation that this is the likely binding mode was afforded by 40 ns molecular dynamics simulations in explicit water under periodic boundary conditions. In all cases the proposed binding poses were essentially maintained. At least one of the molecules provides an appropriate start point for further chemical elaboration guided by molecular modelling. It is noteworthy that the most active binding compound 67 has a good geometric match between its three legged geometry and the three cornered non-polar surface patch in SS1 (Fig. 4).

Disrupting the TGF-β signalling pathway represents a promising therapeutic approach for a number of disease pathologies including fibrosis, cardiovascular disease and cancer [2]. However, highly specific approaches to inhibit TGF-β signalling that do not interfere with the activity of related signalling receptors need to be developed before the full potential of this approach can be achieved clinically. We consider that the computational methods used and the hits identified in this study provide an excellent guide to medicinal chemistry efforts to design tighter binding molecules to disrupt the TGF-β/TβRII interaction.

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References

1. Heldin CH, Landstrom M, Moustakas A (2009) Mechanism of TGF-beta signaling to growth arrest, apoptosis, and epithelial-mesenchymal transition. Curr Opin Cell Biol 21:166–176
2. Meulmeester E, ten Dijke P (2011) The dynamic roles of TGF-β in cancer. J Pathol 223:205–218
3. Santibanez JF, Quintanilla M, Bernabeu C (2011) TGF-β/TGF-β receptor system and its role in physiological and pathological conditions. Clin Sci 121:233–251
4. Shi Y, Massague J (2003) Mechanisms of TGF-β signalling from cell membrane to nucleus through Smad proteins. Cell 113: 685–700
5. Hart PJ, Deep S, Taylor AB, Shu Z, Hinck CS, Hinck AP (2002) Crystal structure of the human TGF-beta type II receptor extracellular domain in complex with TGF-beta3. Nat Struct Biol 9:203–208
6. Irwin JJ, Sterling T, Mysinger MM, Bolstad ES, Coleman RG (2012) ZINC: a free tool to discover chemistry for biology. J Chem Inf Model 52(7):1757–1768. doi:10.1021/ci3001277
7. McGann MR, Almond HR, Nicholls A, Grant JA, Brown FK (2003) Gaussian docking functions. Biopolymers 68:76–90
8. Verkivker GM, Bouzida D, Gehlaar DK, Rejto PA, Arthurs S, Colson AB, Freer ST, Larson V, Luty BA, Marrone T, Rose PW (2000) Deciphering common failures in molecular docking of ligand–protein complexes. J Comp Aided Mol Des 14:731–751
9. Eldridge MD, Murray CW, Auton TR, Paolini GV R, Mee RP (1997) Empirical scoring functions. I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. Comp Aided Mol Des 11:425–445
10. Stahl M, Rarey M (2001) Detailed analysis of scoring functions for virtual screening. J Medicinal Chem 44:1035–1042
11. Jones G, Willett P, Glen RC, Leach AR, Taylor R (1997) Development and validation of a genetic algorithm for flexible docking. J Mol Biol 267:727–748
12. Abe M, Harpel JG, Metz CN, Nunes I, Loskutoff DJ, Rifkin DB (1994) An assay for transforming growth factor-β using cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct. Anal Biochem 216:276–284

13. Case DA, Darden TA, Cheatham TE III, Simmerling CL, Wang J, Duke RE, Luo R, Crowley M, Walker RC, Zhang W, Merz KM, Wang B, Hayik S, Roitberg A, Seabra G, Kolossváry I, Wong KF, Paesani F, Vanicek J, Wu X, Brozell SR, Steinbrecher T, Gohlke H, Yang L, Tan C, Mongan J, Hornak V, Cui G, Mathews DH, Seetin MG, Sagui C, Babin V, Kollman PA (2008) AMBER 10. University of California, San Francisco
14. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA Jr, Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA (2004) Gaussian 03. Gaussian, Inc., Wallingford
15. Bayly CI, Cieplak P, Cornell WD, Kollman PA (1993) A well-behaved electrostatic potential based method using charge restraints for deriving atomic charges: the RESP model. J Phys Chem 97:10269–10280
16. Sherer EC, Harris SA, Soliva R, Orozco M, Laughton CA CA (1999) Molecular dynamics studies of DNA A-tract structure and flexibility. J Am Chem Soc 121:5981–5991
17. Bakheet TM, Doig AJ (2008) Properties and identification of human protein drug targets. Bioinformatics 25:451–457
18. Arkin MA, Wells JA (2004) Small molecule inhibitors of protein–protein interactions: progressing towards the dream. Nat Rev Drug Disc 3:301–317
19. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Del Rev 23:3–25
20. Wells JA, McClendon CL (2007) Reaching for the high-hanging fruit in drug discovery at protein–protein interfaces. Nature 450:1001–1009