Bioorganic & Medicinal Chemistry Letters 20 (2010) 7029–7032

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# A novel small-molecule inhibitor of IL-6 signalling

Giovanna Zinzalla [a], Mohammad R. Haque [a], B. Piku Basu [b], John Anderson [b], Samantha L. Kaye [a], Shozeb Haider [c], Fyeza Hasan [b], Dyeison Antonow [a], Samantha Essex [a], Khondaker M. Rahman [a], Jonathan Palmer [a], Daniel Morgenstern [b], Andrew F. Wilderspin [a], Stephen Neidle [c], David E. Thurston [a,*]

[a] Cancer Research UK Protein–Protein Interactions Drug Discovery Research Group, The School of Pharmacy, University of London, 29-39 Brunswick Sq., London WC1N 1AX, United Kingdom
[b] UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, United Kingdom
[c] Cancer Research UK Biomolecular Structure Group, The School of Pharmacy, University of London, 29-39 Brunswick Sq., London WC1N 1AX, United Kingdom

ARTICLE INFO

*Article history:*
Received 11 August 2010
Revised 21 September 2010
Accepted 23 September 2010
Available online 29 September 2010

*Keywords:*
Small molecule
IL-6 signalling
Inhibitor
STAT3

ABSTRACT

A small library of pyrrolidinesulphonylaryl molecules has been synthesized via an efficient 4-step route, and members evaluated for their ability to inhibit IL-6 signalling. One molecule (**6a**) was found to have promising activity against IL-6/STAT3 signalling at the low micromolar level, and to selectively inhibit phosphorylation of STAT3 (but not STAT1) in IL-6 stimulated MDA-MB-231 breast cancer and HeLa cell lines. It was also selectively cytostatic in MDA-MB-231 (STAT3-dependent) versus A4 (STAT3-null) cells suggesting STAT3-specific inhibitory properties.



Interleukin-6 (IL-6) is a pleiotropic cytokine that regulates hematopoiesis, inflammation and the immune system. It is implicated in the pathogenesis of various inflammatory disorders such as rheumatoid arthritis, inflammatory bowel disease, osteoarthritis, psoriasis, endotoxemia and toxic shock syndrome.[1,2] IL-6 also plays a key role in the pathophysiology of several cancers, and its over-expression has been implicated in the tumourigenesis of multiple myeloma, ovarian, renal cell, prostate, cervical and breast carcinomas.[1,3–5] Therefore, the identification of inhibitory agents to target IL-6 signalling is currently of interest in both anti-inflammatory and anticancer drug discovery.

IL-6 signals mainly through activation of the STAT3 (signal transducer and activator of transcription-3) protein.[6,7] It initially complexes with its membrane-bound receptor, gp130, inducing dimerisation and activating bound JAK (Janus Associated Kinase) protein which autophosphorylates and in-turn phosphorylates STAT3 on its tyrosine 705 residue. This triggers homodimerisation of the STAT3 protein followed by movement to the nucleus where it binds to cognate DNA sequences and activates transcription in a range of genes associated with proliferation and survival. Although no selective small-molecule IL-6 inhibitors have been reported to date, there are examples of molecules in the patent literature that inhibit IL-6 signalling in combination with a number of other signalling pathways. Currently, several approaches are being investigated to selectively inhibit the IL-6 signalling pathway including targeting formation of the IL-6/gp130/JAK complex and inhibition of formation of the STAT3 homodimer.[8–10]

As part of a programme to discover novel inhibitors of signalling pathways known to be important for tumour cell growth and survival, we have identified the novel IL-6 pathway inhibitor **6a** through screening a small library of arylsulphonamidyl thiophene amides (Table 1) prepared in-house[11] based on preliminary in silico studies. The library was synthesised via a 4-step synthetic route (Scheme 1) starting with chlorosulfonation of commercially available **1**, followed by formation of the sulfonylamide derivatives **3a**,**b**. The next step involved attachment of the $R^1$ substituents (**4a**–**e**) via an amide bond using EDC/DMAP to provide intermediates **5a**–**f**. The final step involved Suzuki reaction with a range of boronic acids employing tetrakis(triphenylphosphine) palladium(0) as catalyst at 100 °C under microwave irradiation (Table 1). All reactions proceeded within a short time period (i.e., 5–20 min) and in good yields to afford the final products **6a**–**v**, all of which were typically >98% pure.[12]

The library members were initially evaluated in an MTS cell viability assay using both MDA-MB-231 breast (STAT3-dependent) and A4 (STAT3-null) cancer cells in which STAT3 signalling had been stimulated with IL-6. Both cell lines were treated with up to 125 μM of each library member, and then monitored for 24 h. Compound **6a** emerged as a potential STAT3-specific inhibitor,[13] leading to a reduction of ~40% viable cells in the MDA-MB-231 line compared to A4 (Fig. 1). An accompanying Trypan Blue exclusion

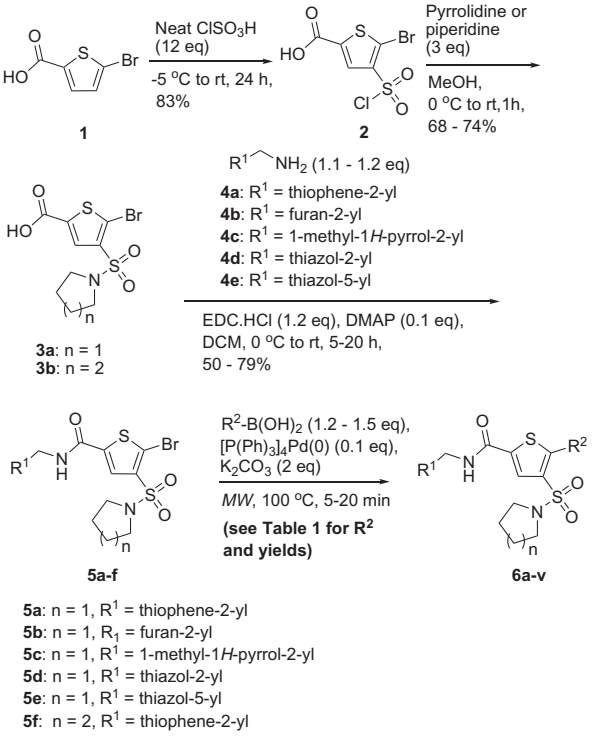
* Corresponding author. Tel.: +44 (0)207 753 5932; fax: +44 (0)207 753 5964.
*E-mail address:* david.thurston@pharmacy.ac.uk (D.E. Thurston).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.09.117

**Table 1**
Structures and yields of library members **6a–v**[12]

| SM | *n* | $R^1$ | $R^2$ | Product | Yield[a] (%) |
|---|---|---|---|---|---|
| **5a** | 1 | Thiophene-2-yl | Tolyl | **6a** | 75 |
| **5b** | 1 | Furan-2-yl | Tolyl | **6b** | 75 |
| **5c** | 1 | 1-Methyl-1*H*-pyrrol-2-yl | Tolyl | **6c** | 79 |
| **5d** | 1 | Thiazol-2-yl | Tolyl | **6d** | 73 |
| **5e** | 1 | Thiazol-5-yl | Tolyl | **6e** | 88 |
| **5a** | 1 | Thiophene-2-yl | 4-Methoxyphenyl | **6f** | 29 |
| **5a** | 1 | Thiophene-2-yl | 4-Fluorophenyl | **6g** | 44 |
| **5a** | 1 | Thiophene-2-yl | Pyridin-4-yl | **6h** | 70 |
| **5a** | 1 | Thiophene-2-yl | 4-Hydroxyphenyl | **6i** | 88 |
| **5a** | 1 | Thiophene-2-yl | 4-Mercaptophenyl | **6j** | 88 |
| **5a** | 1 | Thiophene-2-yl | 4-Chlorophenyl | **6k** | 86 |
| **5a** | 1 | Thiophene-2-yl | Thiophen-2-yl | **6l** | 55 |
| **5a** | 1 | Thiophene-2-yl | Furan-2-yl | **6m** | 75 |
| **5a** | 1 | Thiophene-2-yl | 4-(Trifluoromethyl) phenyl | **6n** | 95 |
| **5a** | 1 | Thiophene-2-yl | 4-Carboxyphenyl | **6o** | 64 |
| **5a** | 1 | Thiophene-2-yl | 4-Nitrophenyl | **6p** | 82 |
| **5a** | 1 | Thiophene-2-yl | 1*H*-Indol-5-yl | **6q** | 83 |
| **5a** | 1 | Thiophene-2-yl | 4-Alaninylphenyl[b] | **6r** | 78 |
| **5a** | 1 | Thiophene-2-yl | Naphthalen-1-yl | **6s** | 87 |
| **5a** | 1 | Thiophene-2-yl | 4-Aminophenyl[c] | **6t** | 65 |
| **5a** | 1 | Thiophene-2-yl | 5-Pyrimidinyl | **6u** | 49 |
| **5f** | 2 | Thiophene-2-yl | Tolyl | **6v** | 75 |

[a] >98% purity based on LC–MS and NMR analysis.
[b] Reaction carried out at 130 °C.
[c] As the pinacol ester.




**Scheme 1.** Synthesis of library members **6a–v**.

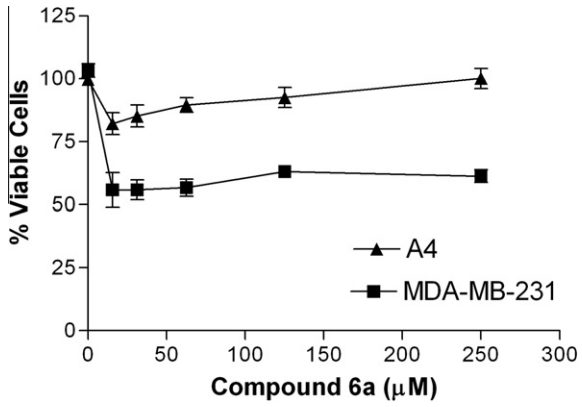



**Figure 1.** MTS cell viability assay profiles for **6a** in MDA-MB-231 (STAT3-dependent) and A4 (STAT3-null) cells after 24 h exposure.

assay using the same MDA-MB-231 cells and with identical exposure times to **6a** resulted in a high percentage of viable cells, thus confirming a cytostatic rather than a cytotoxic effect for this molecule (data not shown).[14]

Compound **6a** was progressed into a luciferase reporter assay based on HeLa cells containing plasmids constructed with either a STAT3 minimal promoter (stable line) or a SV40 control promoter (transient transfection) upstream from luciferase.[15] The cells were treated with Oncostatin M to activate STAT3 signalling via the IL-6/gp130 receptor prior to addition of **6a** which was shown to selectively inhibit STAT3 transcriptional activity with an $EC_{50}$ of 15 μM (Fig. 2). Interestingly, although some molecules of similar structure to **6a** (see Table 1) had a range of activities in the MTS assay in the MDA-MB-231 cell line (i.e., 5–100 μM) and modest activity in the STAT3-reporter assay, **6a** was the only molecule in the library with STAT3-selective properties.

Further confirmation of the specificity of **6a** for STAT3 inhibition was obtained by investigating its effect on phosphorylation of the tyrosine 705 residue within the STAT3 protein (i.e., to give P-STAT3) in serum-starved MDA-MB-231 and HeLa cells 20 min after the addition of IL-6 to induce phosphorylation.[16] Compound **6a** was found to inhibit STAT3 phosphorylation downstream from IL-6 in both HeLa and MDA-MB-231 cells at concentrations of 10–30 and 30–100 μM, respectively (Fig. 3). IL-6 did not induce phosphorylation of STAT1 in either cell line, although some phosphorylation was observed in HeLa cells in the presence of higher concentrations (30 and 100 μM) of **6a**. This could be due to diver-

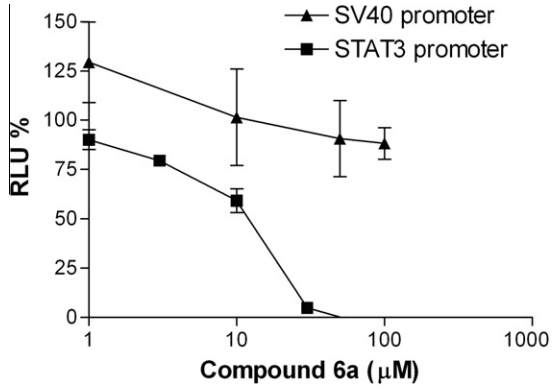



**Figure 2.** Effect of **6a** in a luciferase reporter assay in HeLa cells containing either STAT3 or SV40 promoters upstream of luciferase. Error bars indicate mean ± SEM from three independent experiments.

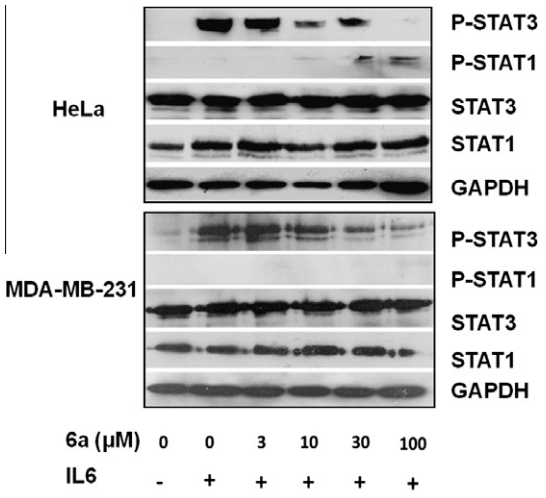



**Figure 3.** Western Blots showing the effect of **6a** on the expression of STAT3, P-STAT3, STAT1, P-STAT1 and GAPDH in IL-6-stimulated HeLa and MDA-MB-231 cells.

sion of IL-6 signalling through STAT1 due to STAT3 signalling blockade at these higher concentrations of **6a**, a phenomenon that has been previously described.[17]

There is no information as yet on a possible site of binding of **6a**, although there is a crystal structure available (PDB 1P9M) of part of the IL-6/α-receptor/gp130 complex.[18] We used this as a starting-point for an in silico modelling study,[19] which has so far located two possible low-energy sites. In one site (labelled B in Fig. 4), **6a** is shown binding in a cavity on the surface of the IL-6/α-receptor interface. We speculate that this could potentially prevent effective interaction of IL-6 with its receptors. In the other site (labelled A), **6a** is shown buried in a cavity at the IL-6/gp130 interface, and is thus less likely to interfere with receptor binding. The interaction energies for the dockings were calculated as −8.82 kcal/mol and −8.25 kcal/mol for sites A and B, respectively.

In summary, a novel synthetic IL-6 signalling inhibitor (**6a**) has been identified. Preliminary cellular studies indicate that, at low micromolar concentrations, the molecule is cytostatic and capable of selectively inhibiting STAT3 activation following IL-6 stimulation at both phosphorylation and transcriptional levels. Further studies are underway to identify the precise site of action of **6a** followed by the design of more-potent molecules in this series.

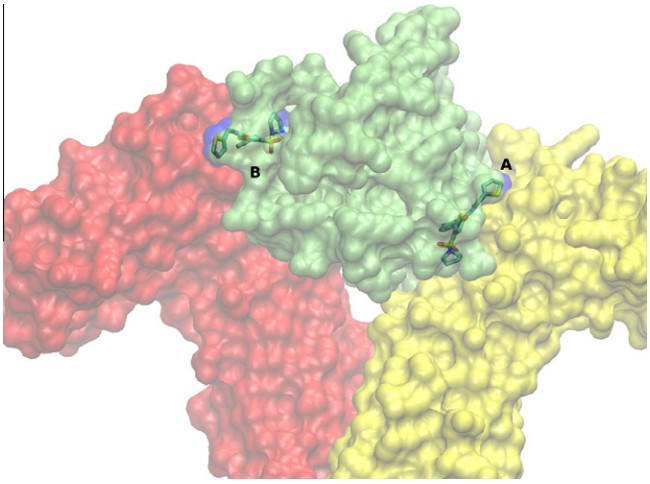



**Figure 4.** Potential binding sites of **6a** at the protein–protein interfaces of the IL-6/gp130 complex. Best docks of **6a** (represented as sticks) are shown at positions A and B. The model (PDB 1P9M) of the IL-6 α-receptor (red)/gp130 (yellow)/IL-6 (green) complex is coloured distinctly to highlight the protein–protein interfaces.

## Acknowledgments

Cancer Research UK is acknowledged for providing financial support for these studies (C180/A9327 to D.E.T. and C129/A4489 to S.N.), and the Commonwealth Commission is thanked for a Scholarship (BDCS-2007-44) for M.R.H.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.09.117.

## References and notes

1. Hodge, D. R.; Hurt, E. M.; Farrar, W. L. *Eur. J. Cancer* **2005**, *41*, 2502.
2. Scheller, J.; Ohnesorge, N. *Scand. J. Immun.* **2006**, *63*, 321.
3. Ara, T.; DeClerck, Y. A. *Eur. J. Cancer* **2010**, *46*, 1223.
4. Blanchard, F.; Duplomb, L.; Baud'huin, M.; Brounais, B. *Cytokine Growth Factor Rev.* **2009**, *20*, 19.
5. David, S. H.; Laura, S. A.; Razelle, K. *Cancer* **2007**, *110*, 1911.
6. Hirano, T.; Ishihara, K.; Hibi, M. *Oncogene* **2000**, *19*, 2548.
7. Yu, H.; Pardoll, D.; Jove, R. S. *Nat. Rev. Cancer* **2009**, *9*, 798.
8. Adachi, Y.; Yoshio-Hoshino, N.; Nishimoto, N. *Curr. Pharm. Des.* **2008**, *14*, 1217.
9. Mitsuyama, K.; Matsumoto, S.; Masuda, J.; Yamasaki, H.; Kuwaki, K.; Takedatsu, H.; Sata, M. *Anticancer Res.* **2007**, *27*, 3749.
10. Rose-John, S.; Waetzig, G. H.; Scheller, J.; Gratzinger, J.; Seegert, D. *Expert Opin. Ther. Targets* **2007**, *11*, 613.
11. *General procedure for library synthesis*. Preparation of **2**: Chlorosulfonation of **1** was carried out with an excess of chlorosulfonic acid (99%, 12 equiv) in neat conditions for 40 h at room temperature. The reaction mixture was poured into an ice bath and **2** (⩾98% pure) was collected as a white solid (83% yield). Preparation of **3a** and **3b**: Pyrrolidine or piperidine was added in excess (3 equiv) to a solution of **2** in methanol, and the mixture stirred for 1 h at room temperature. The reaction mixture was then extracted with water and acidified to pH ~1 to precipitation **3a** or **3b** as white solids (68–83% yields). Preparation of **5a–f**: Amide coupling was achieved by adding an excess (1.1–1.2 equiv) of one of the amines **4a–e** into a solution of **3a** or **3b**, EDC·HCl (1.2 equiv) and DMAP (0.1 equiv) in dichloromethane, followed by stirring for 16–20 h at room temperature. The resulting amides (**5a–f**) were isolated in 50–79% yields and >98% purity by column chromatography on silica gel. Preparation of **6a–v**: Suzuki cross-coupling between **5a–f** and the respective boronic acids (1.2–1.5 equiv) in $CH_3CN/H_2O$ (3:1) was achieved by addition of tetrakis(triphenylphosphine)palladium (0.1 equiv) and $K_2CO_3$ (2 equiv) followed by heating for 5–20 min at 100 °C under microwave irradiation. The products (**6a–v**) were isolated in 29–95% yields and >98% purity by column chromatography on silica gel.
12. Full characterisation data for **6a–v** are provided in Supplementary data.
13. *General procedure for the MTS assay*: 20,000 MDA-MB-231 or A4 cells were plated into a 96-well plate, and ~80% confluency achieved by incubating overnight. Inhibitors were added and the cells incubated for a further 24 h. MTS reagent (CellTiter 96® Aqueous One Solution Cell Proliferation Assay MTS

Solution; Promega) was added in accordance with the manufacturer's protocol. After 3 h, absorbance readings were taken at 490 nm and assumed to be directly proportional to the number of living cells. Absorbance values for cells treated with either vehicle or media alone were used as controls. The MTS data (calculated as percentage of control values) were calculated from triplicate measurements in at least two separate experiments to allow SDs to be calculated.

14. *General procedure for Trypan Blue assay*: MDA-MB-231 cells were plated into 24 well plates and allowed to incubate overnight to achieve 80% confluency. Inhibitors were then added, and the cells incubated for a further 24 h. After addition of Trypan Blue, unstained (viable) and stained (non-viable) cells were counted and calculated as a percentage of total cells using a haemocytometer.
15. *General procedure for the Luciferase Reporter assay*: HeLa cells containing the relevant plasmid were plated-out and incubated overnight to achieve 80% confluency. The medium was then changed to a serum-free type, and Oncostatin M (100 ng/ml) was added at the same time as an inhibitor. The cells were allowed to incubate for 8 h, and then cell lysates were prepared and luciferase activities measured using dual luciferase kits (Promega) in accordance with the manufacturer's protocol.
16. *General procedure for Western Blots*: $1 \times 10^6$cells were plated into 2% FCS media in 6-well plates and allowed to incubate overnight. The medium was then changed to a serum-free type, and IL-6 (20 ng/ml) was added at the same time as an inhibitor. Whole cell extracts were then prepared using RIPA buffer (Thermo Scientific) with protease and phosphatase inhibitors. Extracts were dissolved in SDS buffer and run on a 10% PAGE gel for 1 h at 100 V (4 °C). Blots were probed with the following antibodies: STAT3, P-STAT3, STAT1, P-STAT1 and GAPDH (Cell Signalling Inc.).
17. Regis, G.; Pensa, S.; Boselli, D.; Novelli, F.; Poli, V. *Semin. Cell Dev. Biol.* **2008**, *19*, 351.
18. Boulanger, M. J.; Chow, D. C.; Brevnova, E. E.; Garcia, K. C. *Science* **2003**, *300*, 2104.
19. *Molecular modeling methodology*: The ligand was generated via the PRODRG server (http://davapc1.bioch.dundee.ac.uk/prodrg/). The non-polar hydrogen atoms were merged, rotatable bonds assigned and partial atomic charges calculated as implemented in the AutoDockTools package (Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew R. K.; Olsen, A. J. *J. Comp. Chem.* **1998**, *19*, 1639). After removing IL-6, Grid maps were then generated on the receptor interfaces where the IL-6 α-receptor and gp130 should reside. The grid was large enough to encompass the entire IL-6 binding interface on the receptors. The grid points were thus chosen to be $118 \times 118 \times 118$ Å with a grid spacing of 0.375 Å. Automated docking was carried out using Autodock 4.0. A Lamarckian genetic algorithm (LGA) was applied to investigate plausible interactions between the ligand and receptor. The Solis and Wets algorithm (Solis, F. J.; Wets, J. B. *Math. Oper. Res.* **1981**, *6*, 19) was employed to carry out the local search. Values for all other docking parameters were kept as standard. Multiple docking runs can enhance the performance of docking programs (Wang, R.; Lu, Y.; Wang, S. *J. Med. Chem.* **2003**, *46*, 2287), and so 100 independent docking runs were carried out. The best docked conformation at each interface was then extracted based on interaction energies.