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Studies on potential Stat-3 inhibitors: reactivity and behaviour of furazan derivatives

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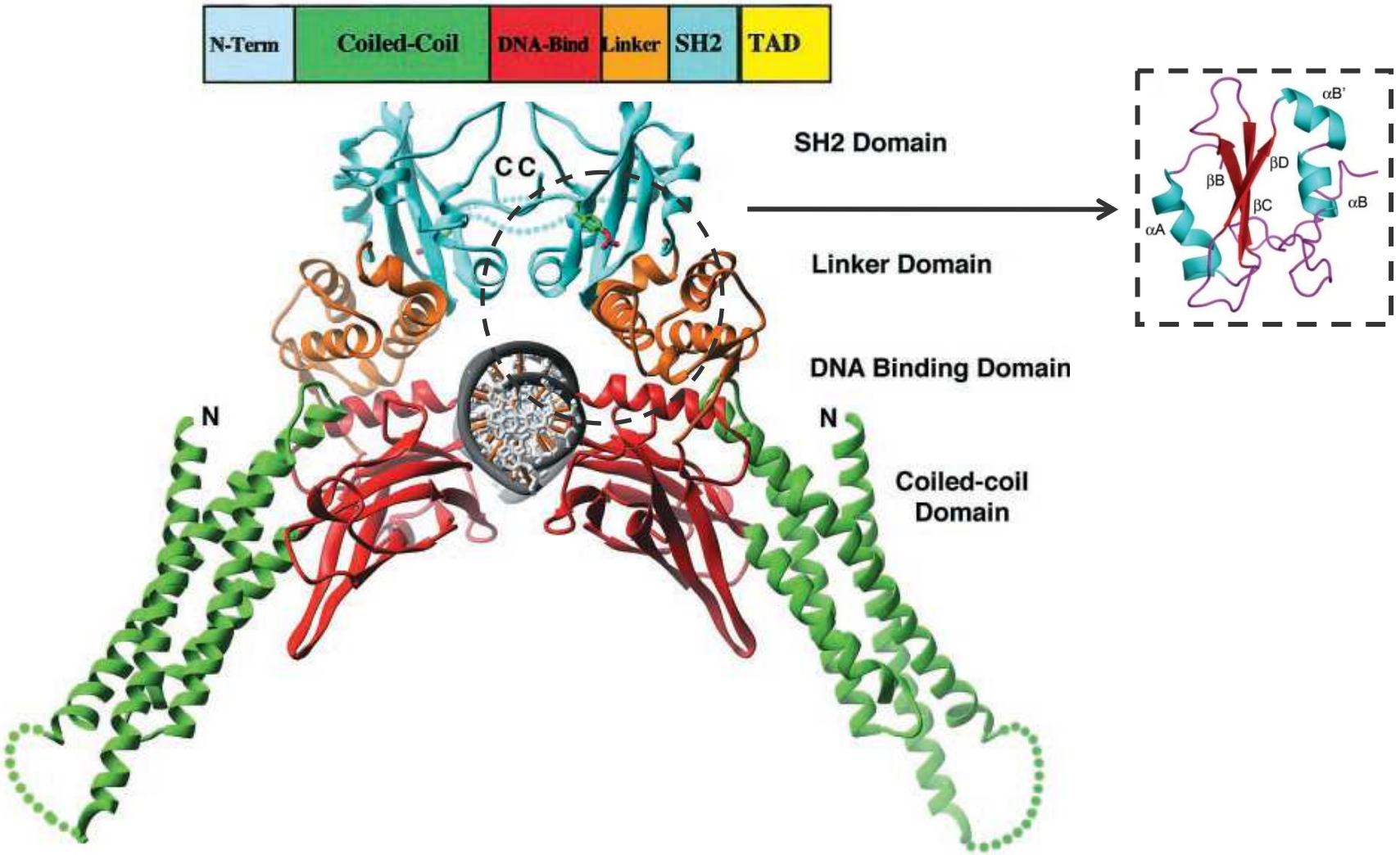
Introduction

Signal transduction and activator for transcription factor 3 (Stat-3) is a latent cytosolic protein member of Stat family. It is a transcription factor that transmits cytoplasmic signals (e.g. from growth factors, poly-peptide cytokines etc.) to the nucleous[1]. The mechanism of activation provides the recruitment of Stats to phosphorylated receptors via their SH2 domain and Stat-3 dimerization (**Figure 1**). Stat-3 is involved in cell growth and survival but Stat-3 signalling may contribute to malignancy by preventing apoptosis even if the molecular mechanism of oncogenesis by Stat-3 must be clearly defined. Stat-3 is constitutively activated by aberrant upstream tyrosine kinase activities in a broad spectrum of human solid and blood tumours. Numerous published reports have shown that blocking constitutively activated Stat-3 signalling leads to apoptosis of tumour cells[2-4] but does not affect normal cells[5-6] suggesting that its inhibition could be a leading target for cancer theraphy.

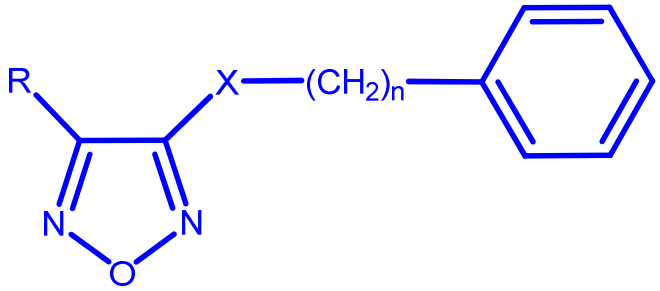
Figure 1. Stat-3 homodimer-DNA complex



Bromberg J. et all. *Oncogene*, **2000**, *19*, 2468-2473

Objectives

Our preliminary studies were focused on the discovery of new small molecules as potential Stat-3 inhibitors, with the aim of identifying the essential requirements for the development of novel lead compounds. On this basis we decided to explore the reactivity of a relatively little studied heterocycle, the furazan (1,2,5-oxadiazole) ring and we planned to synthesize a new series of compounds (**1a-e**, **2a-c**, **3a-c**) with the following general formula:



n = 0, 1

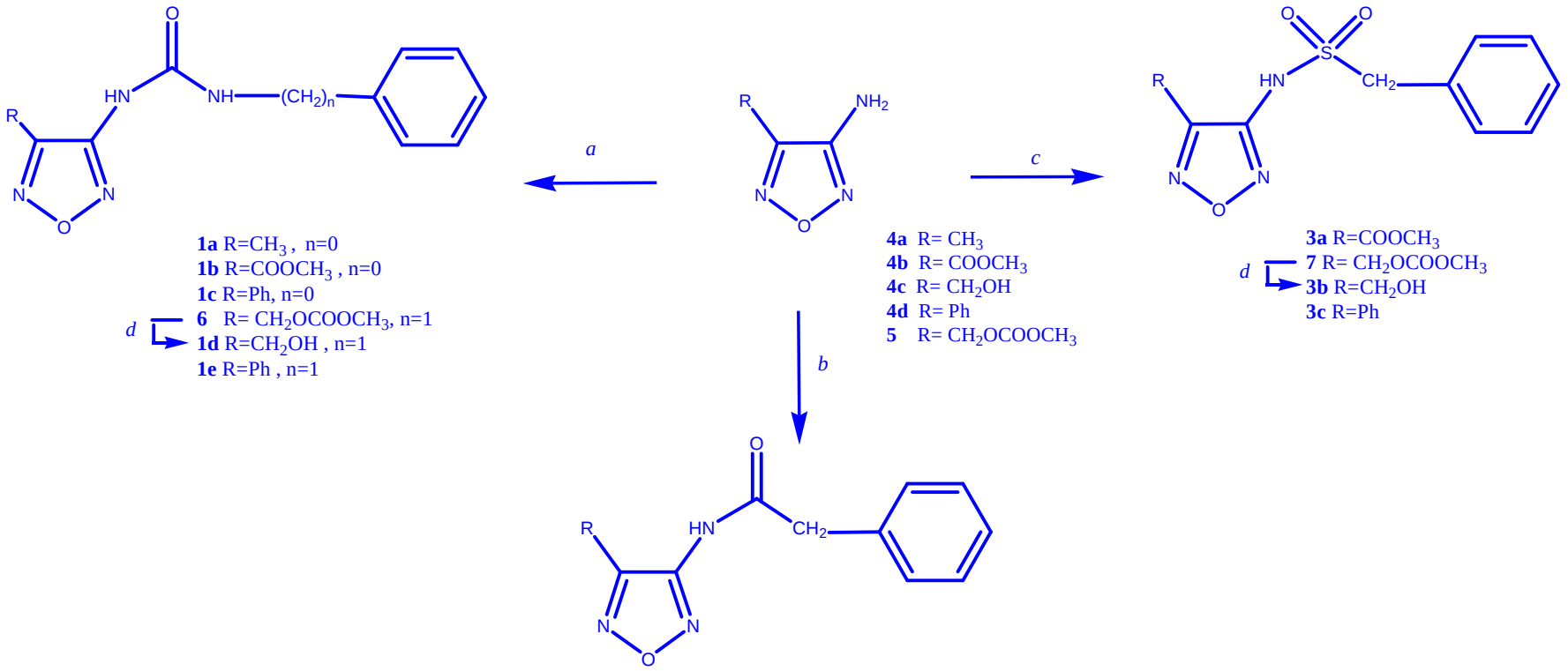
R = CH_3 , CH_2OH, $COOCH_3$, C_6H_5

X = NHCO, $NHSO_2$, NHCONH

In more detail the 4-substituted derivatives could be branched in three main classes: ureas (**1a-e),** amides (**2a-c**) and sulfonamides (**3a-c**) and were synthesized by the reported procedures (**Scheme 1**).

Chemistry

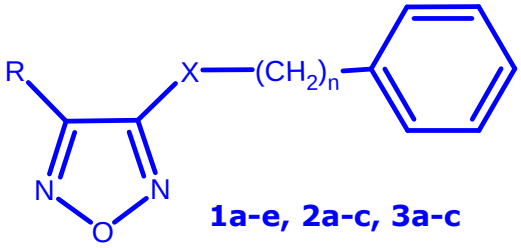
Scheme 1. Synthesis of ureas **1a-e**, amides **2a-c** and sulfonamides **3a-c**



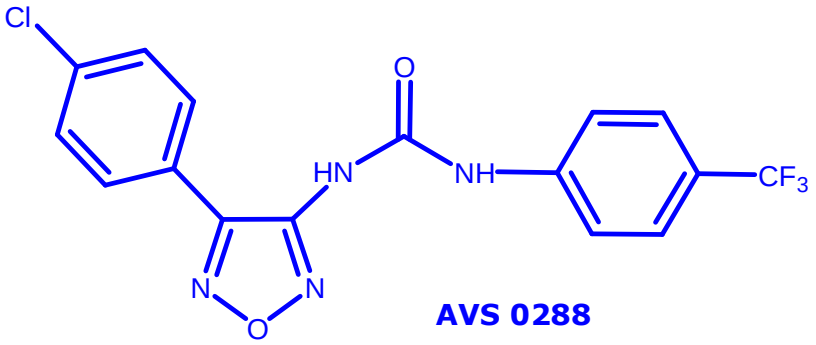
Reagents and conditions: *a*) $Ph(CH_2)_nNCO$, toluene, MW; *b*) $Ph\text{-}CH_2COCl$, $NaHCO_3$, r.t. or $Ph\text{-}CH_2\text{-}COCl$, pyridine, toluene/diethyl ether, r.t.; *c*) $Ph\text{-}CH_2\text{-}SO_2Cl$, Py, r.t.; *d*) 1% K_2CO_3 methanol, r.t.

All products were synthesized starting from the suitable key intermediate **4** or **5**. Compounds **4a**[7], **4b**[8], **4d**[9] were prepared as reported in literature, while **4c** was obtained by reduction of **4b** with $LiAlH_4$.

Results of biological tests



1a-e, 2a-c, 3a-c



AVS 0288

Compd	n	R	X	% Inhibition (5 μM)
1a	-	CH_3	NHCONH	10.48
1b	-	$COOCH_3$	NHCONH	-16.44
1c	-	C_6H_5	NHCONH	25.71
1d	1	CH_2OH	NHCONH	17.36
1e	1	C_6H_5	NHCONH	29.38
2a	1	CH_2OH	NHCO	6.51

Compd	n	R	X	% Inhibition (5 μM)
2b	1	$COOCH_3$	NHCO	25.77
2c	1	C_6H_5	NHCO	21.94
3a	1	CH_2OH	$NHSO_2$	-0.78
3b	1	$COOCH_3$	$NHSO_2$	-5.64
3c	1	C_6H_5	$NHSO_2$	14.15
AVS 0288		-	-	81.79

The inhibitory activity against Stat-3 was evaluated by a modified procedure of dual-luciferase assay[10] in human colorectal carcinoma cells (HCT-116), characterized by uncontrolled expression of Stat-3.
The activity was expressed as % of inhibition, after 24 h treatment with the tested compounds and **AVS 0288**[11], that was used as reference.

Crystallography

AVS 0288 and **1d** were solved by direct methods[12] and conventional Fourier synthesis.[13] The refinement of the structures was made by full matrix least-squares on F^2. All non-H-atoms were refined anisotropically and were introduced at calculated positions, in their described geometries and allowed to ride on the attached carbon atom with fixed isotropic thermal parameters (1.2Ueq of the parent carbon atom).

Table 1. Summary of the crystal data and refinement

Crystal system	Monoclinic	Monoclinic
Space group	P 2_1/a	P 2_1/n
Cell dimensions (Å)(°)	a=9.065(3 b=14.592(3) c=12.410(3) β=99.248(9)	a=8.135(5) b=12.855(5) c=11.815(5) β=109.13(1)
Volume (Å^3)	1620.1(7)	1104(1)
Z	4	4
Final R indices [I>2σ(I)]	R1= 0.049, wR2= 0.170	R1= 0.074, wR2= 0.195

Fig. 2. ORTEP of **AVS 0288**

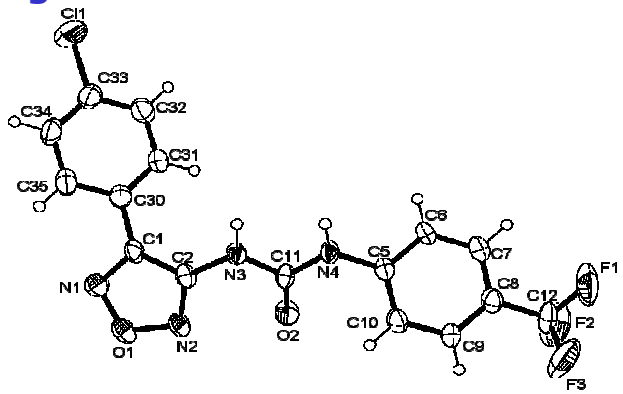


Fig. 3. ORTEP of **1d**

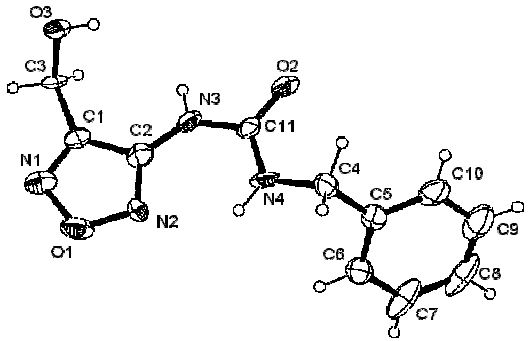
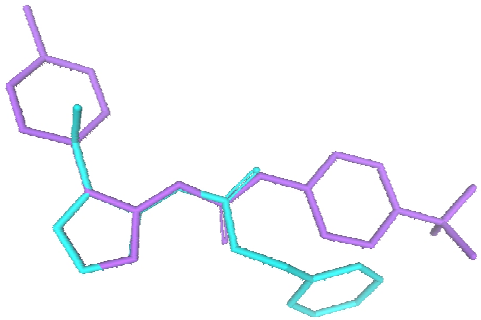


Fig. 4. Overlay of **AVS 0288** (purple) and **1d** (light blue)



The crystal structure of **AVS 0288** is characterized by the Cl-phenyl moiety almost coplanar to the furazane, which is inclined with respect to the CF_3-phyenyl group by a dihedral angle of 13(1)°. **1d** has three moieties nearly planar, the furazane (P1), the urea group (P2) and the phenyl ring (P3), oriented at dihedral angles of 130(1)°, 62(1)° and 57(1)° between P1/P2, P2/P3 and P1/P3 respectively. In **1d** the two amide bonds have a *cis/trans* conformation, differently with respect to the *trans* orientation of **AVS 0288**. The crystal packing of both molecules present a three-dimentional network of intermolecular interaction of type N-H…N, N-H…O, Cπ-H…n, and π-π stacking interaction.

Molecular modeling

The Stat-3 structure, co-crystallized with a DNA fragment[14], was downloaded from the Protein Data Bank[15] (PDB-ID 1BG1) and was optimized by NAMD[16] (30.000 steps, conjugate gradients). All considered compounds were built by VEGA ZZ[17] and docked to Stat-3 by GriDock[18], selecting the SH2 domain as target region.

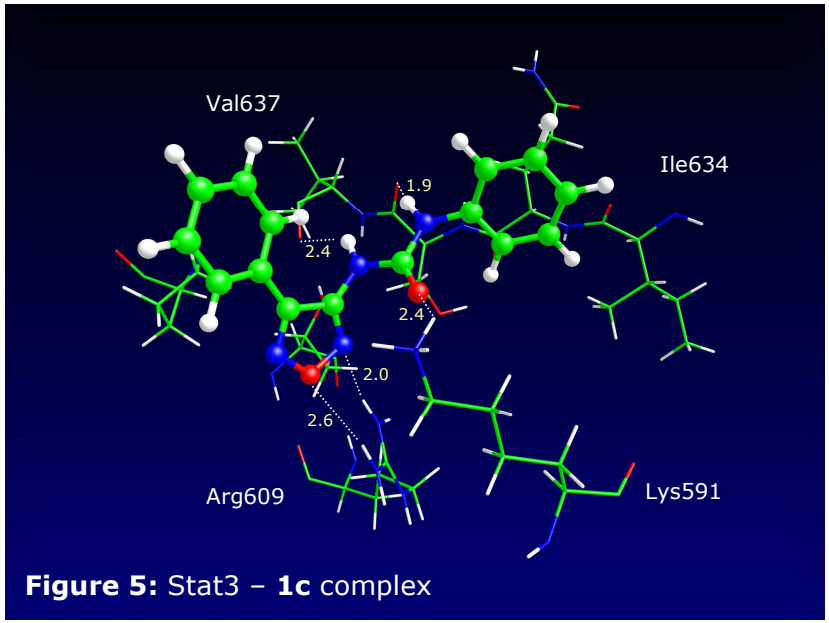


Figure 5: Stat3 – **1c** complex

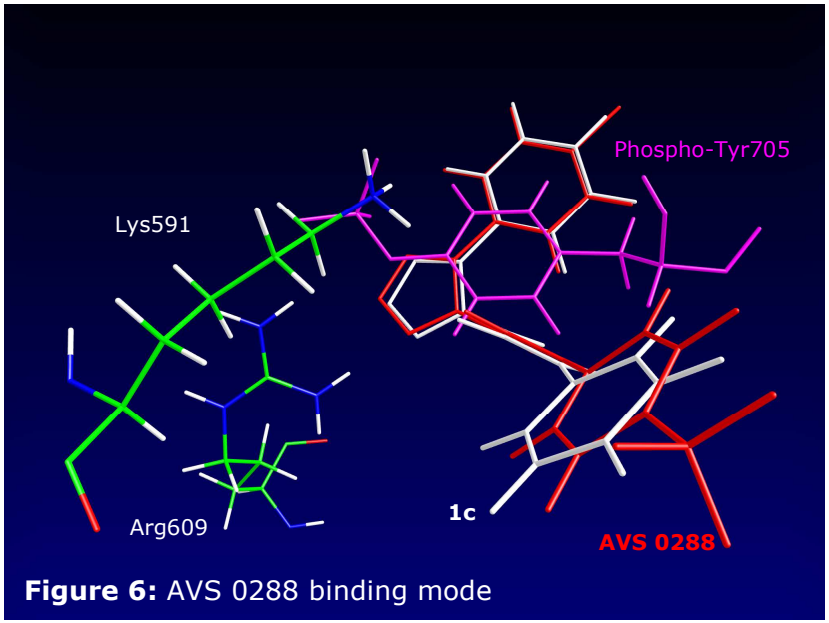


Figure 6: AVS 0288 binding mode

Product **1c**, most directly related to the reference compound, interacts with Stat-3 by a strong H-bond network (**Figure 5**). The binding mode can be compared to the complex with **AVS 0288** as shown in **Figure 6**, in which the ligands are placed in the positively charged pocket occupied by the phosphorylated tyrosine 705 when Stat-3 is dimerized.

Conclusions

We decided to perform crystallographic and molecular modeling studies with the aim of understanding the biological data obtained. Due to limited space, we presented only several data: we verified and explained that the lack of substituents on the phenyl rings (**1c**) such as the presence of a polar group linked to the furazan ring (**1d**) were not favourable for the activity.

The substitution of a ureidic bond (**1e**) by amidic function (**2c**) kept the % of inhibition unchanged while the sulfonamidic derivatives (**3c**) lost the interaction with Stat-3.

Although the results of our preliminary research were not satisfactory, just a few compounds (**1c**, **1e**, **2b** and **2c**) showed a slight interaction with Stat-3, they allowed us to obtain several indications useful to develop new derivatives.

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