UNIVERSITÀ DEGLI STUDI DI MILANO
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DEVELOPEMENTS ON FURAZAN DERIVATIVES AS POTENTIAL STAT-3 INHIBITORS

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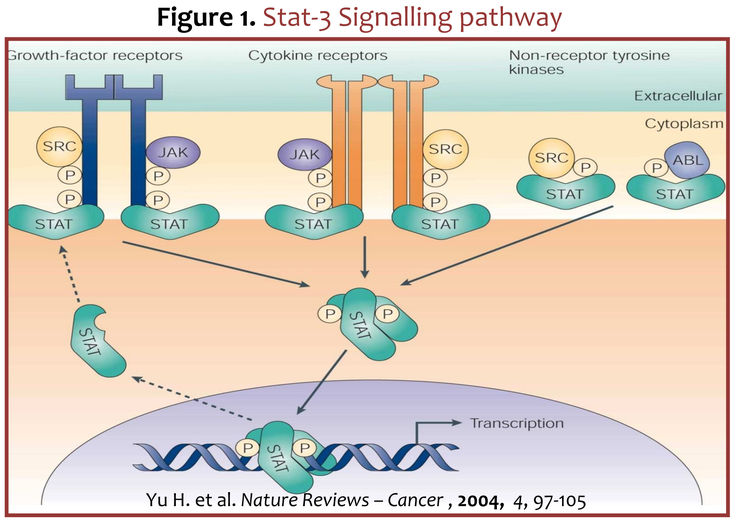
Background

Stat-3 (signal transduction and activator for transciption factor 3) is a latent cytosolic protein that, in its activated form, directly relates extracellular signals (e.g. growth factors, poly-peptide, cytokines) from the plasma membrane to the nucleus[1] (**Figure 1**). It is involved in cell growth and survival. Stat-3 is the member of Stat family most closely linked to tumour genesis[2] as its signalling might 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. Since Stat-3 inhibition leads to apoptosis in tumour cells[2-4] but has no effect in normal cells[5-6], it represents a promising target for cancer theraphy.

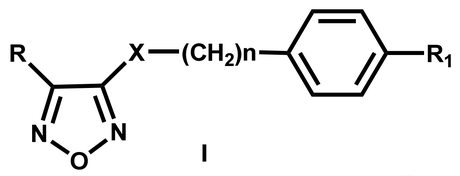
Figure 1. Stat-3 Signalling pathway



Yu H. et al. *Nature Reviews – Cancer* , **2004,** 4, 97-105

Research project

As a part of our ongoing studies, focused on the discovery of new small molecules as potential Stat-3 inhibitors, we synthesized a series of new furazan derivatives[7] (compounds **1a-d**, **2a-c**, **3a-c**) closely related to the reference compound (**AVS 0288**)[8]. We have now prepared compounds **1e-i**, **2d,e** and **3d,e** in order to better analyze the features required for the inhibition of Stat-3.



1a-d, 2a-c, 3a-c
- n = 0, 1
- X = NHCONH, NHCO, $NHSO_2$
- R = CH_3 , CH_2OH, $COOCH_3$, C_6H_5
- R_1 = H

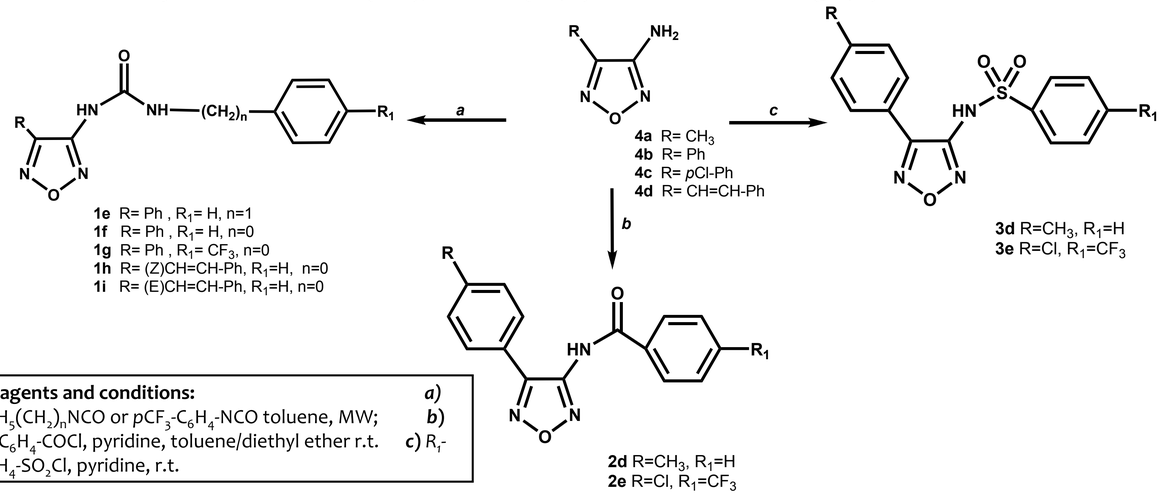
⇒ **1e-i, 2d,e, 3d,e**
- n = 0, 1
- X = NHCONH, NHCO, $NHSO_2$
- R = CH_3, C_6H_5, *p*Cl-C_6H_4, CH=CH-C_6H_5
- R_1 = H, CF_3

The derivatives **1e-i, 2d,e** and **3d,e** were synthesized according to **Scheme 1.**

Synthesis

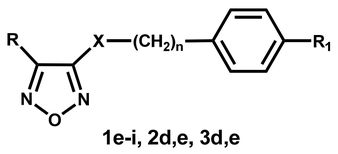
All products were synthesized starting from the suitable intermediate **4.** Compounds **4a**[9], **4b**[10], **4c**[11] were prepared according to literature data.

Scheme 1. Synthesis of urea- (**1e-i**), amide- (**2d,e**) and sulfonamide- (**3d,e**) derivatives

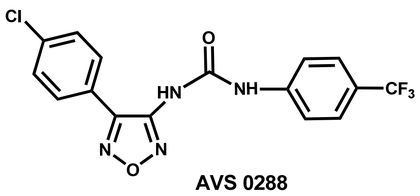


Reagents and conditions: *a)* $C_6H_5(CH_2)_nNCO$ or *p*CF_3-C_6H_4-NCO toluene, MW; *b)* R_1-C_6H_4-COCl, pyridine, toluene/diethyl ether r.t. *c)* R_1-C_6H_4-SO_2Cl, pyridine, r.t.

Biological activity



1e-i, 2d,e, 3d,e



AVS 0288

Compd.	n	R	R_1	X	% Inh. (5 µM)
1e	1	C_6H_5	H	NHCONH	29.38
1f	-	C_6H_5	H	NHCONH	25.71
1g	-	C_6H_5	CF_3	NHCONH	-4.39
1h	-	(*Z*)CH=CH-Ph	H	NHCONH	27.27
1i	-	(*E*)CH=CH-Ph	H	NHCONH	17.86
2d	-	CH_3	H	NHCO	-10.14
2e	-	pCl-C_6H_5	CF_3	NHCO	19.37
3d	-	CH_3	H	$NHSO_2$	-14.2
3e	-	pCl-C_6H_5	CF_3	$NHSO_2$	1.05
AVS 0288	-	-	-	-	81.79

The Stat-3 inhibitory activity was evaluated by a modified procedure of dual-luciferase assay[12] 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 vs AVS 0288[8], which was used as reference.

Molecular modeling

The Stat-3 structure, co-crystallized with a DNA fragment[15], was downloaded from the Protein Data Bank[16] (PDB-ID 1BG1) and was optimized by NAMD[17] (30.000 steps, conjugate gradients). All considered compounds were built by VEGA ZZ[18] and docked to Stat-3 by GriDock[19], selecting the SH2 domain as target region.

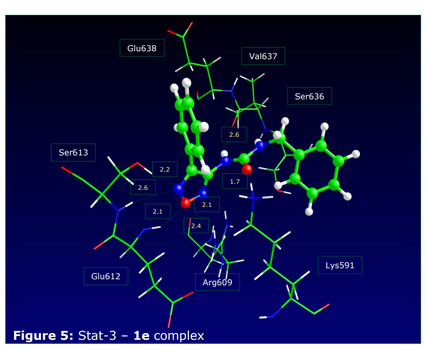


Figure 5: Stat-3 – **1e** complex

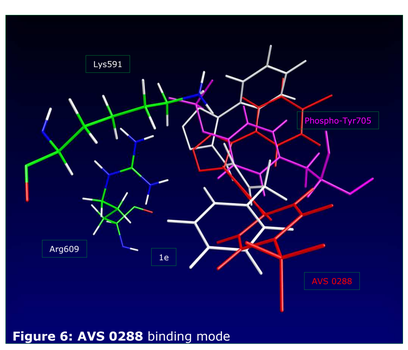


Figure 6: AVS 0288 binding mode

The synthesized compound with the best inhibition activity (**1e**) interacts with Stat-3 by a strong H-bond network (**Figure 5**). The binding mode is quite similar if compared to the complex with **AVS 0288** (**Figure 6**), although the introduction of the methylene group pushes away the heterocyclic ring from Arg609, that plays a pivotal role in the Stat-3 dimerization.

Crystallography

2d and **3d** were solved by direct methods[13] and conventional Fourier synthesis.[14] 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.

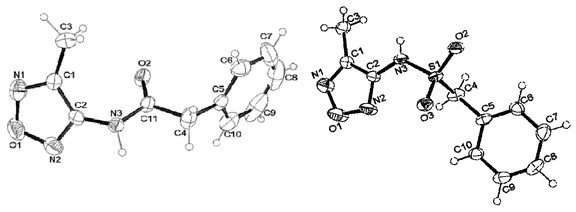


Fig. 2. ORTEP of **2d**

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

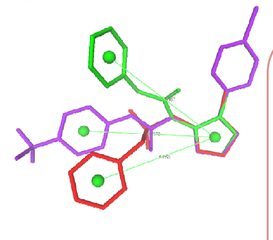


Fig. 4. Overlay of **AVS-0288** (purple)[7], **2d** (green) and **3d** (red)

Summary of the crystal data and refinement

	2d	3d
Cryst.system	Monoclinic	Triclinic
Space group	P $2_1/n$	P -1
Unit cell dim. (Å) (°)	a=6.619(2) b=20.540(3) β=103.72(3) c=8.364(5)	a= 5.589(2) α= 70.09(1) b= 10.096(2) β= 86.17(1) c= 10.981(2) γ= 74.03(1)
Volume (Å^3)	1104.7(8)	559.9(9)
Z	4	2
Final R ind. [I>2σ(I)]	R1 = 0.0637 WR2 = 0.1816	R1= 0.0542 wR2= 0.1300

The crystal structure of **2d** presents a more extended chain conformation than **3d**, as shown by the torsional angles C2-N3-C11-C4 of 178(1)° and C2-N3-S1-C4 of 73(1)° respectively. The dihedral angle between the mean plane of the oxadiazole and the phenyl ring is 63(1)° in **2d** and 13(1)° in **3d**, leading in the latter to a short centroid distance with respect to **2d** and **AVS-0288**.
The crystal packing of both molecules present a three-dimentional network of intermolecular interactions of type, N-H…O, Cπ-H…n, and π-π stacking interaction.

Discussion

The results from the biological assays clearly show that:

- substitution of the ureidic moiety of the model either with a benzamido- (**2d,e**) or a sulfonamido- (**3d,e**) group caused a significant decrease in activity;
- lack of the chloro group on the phenyl ring linked to the heterocycle brought about complete loss of activity (see **1g** vs the model).

According to the principle of vinilogy, the cinnamyl derivatives (**1h,i**) are comparable to their analogous **1f**, though stereochemistry seems also to play a role.

Finally, the lack of activity of compounds **2d** and **3d** could be interpreted on the basis of molecular modeling and crystallographic studies, which distinctly show a poor superimposition of these two derivatives with the model.

References

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