Bioorganic & Medicinal Chemistry Letters 26 (2016) 3581–3585

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Optimization of pyrazole-containing 1,2,4-triazolo-[3,4-*b*] thiadiazines, a new class of STAT3 pathway inhibitors

Matthew G. LaPorte [a,*], Zhuzhu Wang [a,b], Raffaele Colombo [a], Atefeh Garzan [a], Vsevolod A. Peshkov [a], Mary Liang [a,b], Paul A. Johnston [b,c], Mark E. Schurdak [c,d], Malabika Sen [e], Daniel P. Camarco [b], Yun Hua [b], Netanya I. Pollock [e], John S. Lazo [f], Jennifer R. Grandis [g], Peter Wipf [a,b,c,d,*], Donna M. Huryn [a,b,*]

[a] University of Pittsburgh Chemical Diversity Center, University of Pittsburgh, Pittsburgh, PA 15260, USA
[b] Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA 15261, USA
[c] University of Pittsburgh Cancer Institute, Pittsburgh, PA 15232, USA
[d] University of Pittsburgh Drug Discovery Institute, University of Pittsburgh, Pittsburgh, PA 15261, USA
[e] Department of Otolaryngology, University of Pittsburgh, Pittsburgh, PA 15213, USA
[f] Department of Pharmacology, University of Virginia, Charlottesville, VA 22908, USA
[g] Department of Otolaryngology-Head and Neck Surgery, University of California, San Francisco, CA 94158, USA

ARTICLE INFO

Article history:
Received 15 April 2016
Revised 2 June 2016
Accepted 8 June 2016
Available online 9 June 2016

Keywords:
STAT3 inhibitor
Triazolo-thiadiazines
Anti-cancer agents
STAT1

ABSTRACT

Structure–activity relationship studies of a 1,2,4-triazolo-[3,4-*b*]thiadiazine scaffold, identified in an HTS campaign for selective STAT3 pathway inhibitors, determined that a pyrazole group and specific aryl substitution on the thiadiazine were necessary for activity. Improvements in potency and metabolic stability were accomplished by the introduction of an α-methyl group on the thiadiazine. Optimized compounds exhibited anti-proliferative activity, reduction of phosphorylated STAT3 levels and effects on STAT3 target genes. These compounds represent a starting point for further drug discovery efforts targeting the STAT3 pathway.

While advancements in detection and treatment have aided in the longstanding campaign against cancer, new annual U.S. cancer cases and related deaths still exceed 1.5 million and 580,000, respectively.[1] Recently, new therapies have targeted signaling pathways that are aberrantly activated in transformed cells. These pathways promote cancer cell survival and proliferation, but they play a less important role in normal cell survival.[2] As a part of our interest in the development of mechanism-based anticancer agents,[3,4] we have been pursuing novel small molecule inhibitors of the signal transducer and activation of transcription 3 (STAT3) pathway.[5–7] STAT3 is a transcription factor that influences many of the acquired capabilities of cancer tumorigenesis, thereby making it an attractive target for the development of oncolytics.[8–12]

There is mounting evidence for its role in cancer such as increased levels of activated STAT3 (*p*STAT3-Y705) observed in many cancers including head and neck squamous cell carcinomas (HNSCC).[4,5] We previously reported the use of a high content phenotypic screen to identify selective inhibitors of the STAT3 activation pathway compared to STAT1 which served as an important selectivity control since the latter is a tumor-suppressive transcription factor.[6,7] We identified several scaffolds that met this criteria. Herein, we describe the optimization and structure activity relationship for a series of pyrazole-containing 1,2,4-triazolo-[3,4-*b*] thiadiazines with selective STAT3 pathway inhibition.

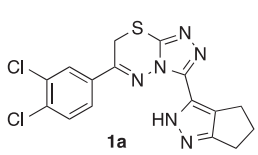
The high content phenotypic screen, which utilized an interleukin-6 (IL-6)-induced STAT3 activation assay in Cal33 head and neck tumor cells, identified several triazolo-thiadiazines as selective STAT3 pathway inhibitors (e.g., **1a** and **2b**, Table 1).[7] The biological activities of these structurally similar analogs were confirmed through resynthesis and re-assay (*vide infra*). These HTS/HCS hits had no effect on interferon-γ (IFN-γ)-induced STAT1 pathway activation at concentrations up to 50 μM. This selectivity is not observed for many other STAT3 pathway inhibitors reported in the literature including the pan-Janus kinase (JAK) inhibitor, pyridone 6.[6] Furthermore, this series exhibited acceptable drug-like properties: low molecular weight (<400), clog*P* values between 3 and 4,[13] and anti-proliferative activities with several HNSCC cell lines (GI_{50} 14–45 μM with 686LN, Cal33, FaDu, and OSC19).

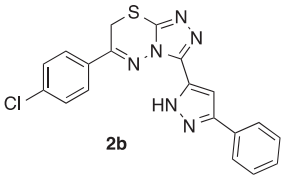
Triazolothiadiazines have been reported to exhibit an array of pharmacological effects including anti-proliferative activities.[14] However, the HTS library included a number of inactive analogs of **1a** and **2b** where the pyrazole was replaced with an alkyl, aryl, or

* Corresponding authors.

http://dx.doi.org/10.1016/j.bmcl.2016.06.017
0960-894X/

Table 1
Triazolothiadiazines **1a** and **2b** and summary of biological activities and physicochemical properties





Cmpd #	1a	2b
STAT3 IC_{50} (μM)	6.8 ± 3.7	9.6 ± 7.8
STAT1 IC_{50} (μM)	>50	>50
GI_{50} HNSCC[a] (μM)	14–26	27–44
MW	391.2	392.8
*c*Log*P*	3.2	3.7
HBD/HBA	1/4	1/4
tPSA	71.7	71.7
LE	0.29	0.26

[a] Cell lines: 686LN, FaDu, Cal33, OSC19.

alternative heterocyclic substituent (Fig. 1). This suggested that we were not observing broadly promiscuous effects with this scaffold. These observations together with the desirable biological selectivity profile and favorable drug-like physical properties encouraged us to pursue a medicinal chemistry optimization effort for this series.

The synthesis of **1a** and **2b** required key amino-triazole intermediates **3a** and **3b** that were readily assembled according to literature procedures.[15–19] Alkylations with the appropriate α-halo ketone and microwave-assisted cyclodehydrations afforded the original hits **1a** and **2b** (Scheme 1).

In addition to **1a** and **2b**, two sub-libraries of triazolothiadiazines that maintained either the fused cyclopentyl-pyrazole group (‘**a**’) or the pendant phenyl-pyrazole group (‘**b**’) with diversified R- group modifications on the triazolothiadiazine were synthesized. These compounds were prepared according to Scheme 1 with yields ranging from 24–90% by using a variety of α-halo ketones in the microwave-assisted cyclodehydration reaction. A protected aldehyde derivative was used to prepare **9a** and **9b**.

We selected substituents with diverse steric, polar, and electronic characteristics. Table 2 illustrates a representative subset of these analogs and their activities in STAT3 and STAT1 assays. The R-substitution on the thiadiazine influenced activities by a factor of greater than 10 with many modifications leading to a loss of STAT3 potency. The importance of the chlorine substitution on the arene groups was evident by the significant drop in activity seen with the removal of the halogen regardless of the pyrazole scaffold (**5a** vs. **1a**; **5b** vs. **2b**). As a general trend, hydrogen (**9**), aliphatic (**10**, **11**), and heterocyclic (**7**) R-groups were inactive regardless of the pyrazole substructure. One notable exception was the chlorothiophene analog **8a** that maintained comparable potency to the initial hit. However, when the chlorothiophene was combined with the phenyl-pyrazole (‘**b**’) scaffold, the loss of activity of analog **8b** was consistent with other heterocyclic derivatives.

A few SAR trends diverged between the two pyrazole scaffolds. For example, exchanging the R-substituents of **1a** and **2b** provided analogs **1b** and **2a** that were 3–4 times less active. The reduced

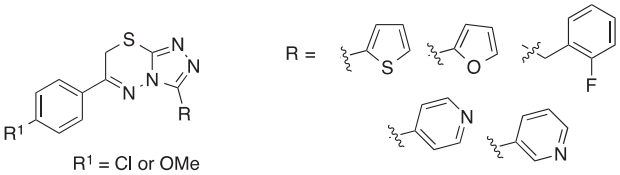
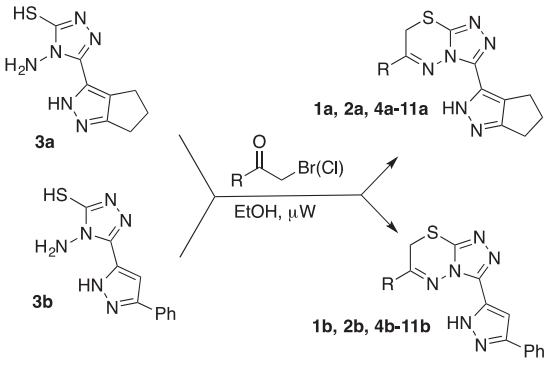
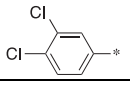
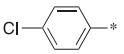
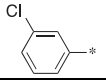
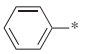
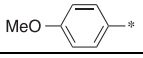
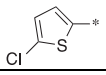
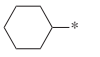


Figure 1. Inactive triazolothiadiazines (STAT3 IC_{50} > 50 μM).



Scheme 1. Preparation of 1,2,4-triazolo-[3,4-*b*]thiadiazines.

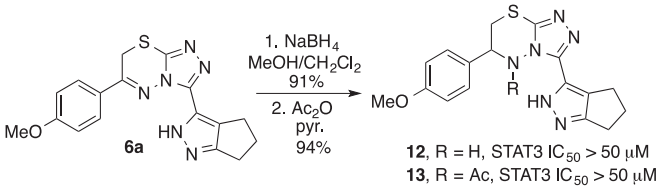
Table 2
STAT3 and STAT1 activities of triazolothiadiazines

R	Cmpd #	STAT3 IC_{50} (μM)	STAT1IC_{50} (μM)
	**1a** **1b**	6.8 ± 3.7 31.2 ± 20.0	>50 >50
	**2a** **2b**	26.8 ± 22.3 9.6 ± 7.8	>50 >50
	**4a** **4b**	21.2 ± 5.9 5.6 ± 4.0	>50 45.9 ± 8.3
	**5a** **5b**	21.2 ± 23.5 27.0 ± 17.4	>50 >50
	**6a** **6b**	17.9 ± 22.9 3.7 ± 2.5	40.6 ± 7.5 >50
	**7a** **7b**	>50 >50	>50 >50
	**8a** **8b**	11.0 ± 5.5 >50	>50 >50
H–*	**9a** **9b**	>50 >50	>50 >50
Me–*	**10a** **10b**	>50 >50	>50 >50
	**11a** **11b**	>30 >50	>50 >50

a: cyclopentyl-pyrazole series; **b**: 3-phenyl-pyrazole series.

STAT3 potency of compounds **2a** (27 μM) and **4a** (21 μM) established the importance of the *ortho*- and *para*-chlorine atoms on the phenyl ring in **1a**. Interestingly, the phenyl-pyrazole with the same *meta*-chlorophenyl R-substituent (**4b**) retained, or perhaps, improved the STAT3 potency compared to the *para*-chlorophenyl **2a**, suggesting that either lipophilicity or electronic effects were important. However, the potent activity of the *para*-methoxyphenyl analog **6b** did not support a purely electronic contribution. The selective inhibition of STAT3 over STAT1 activation was maintained within this entire subseries.

To evaluate the effect of thiadiazine modifications, hydrazone **6a** was converted to dihydrothiadiazines **12** and **13** (Scheme 2).



Scheme 2. Synthesis of dihydrothiadiazine analogs.