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Evaluation of WO2017018805: 1,3,4-Oxadiazole sulfamide derivatives as selective HDAC6 inhibitors

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Abstract

Introduction: There are great potential in the development of selective HDAC6 inhibitors for the treatment of infectious diseases, neoplasms, endocrine diseases, and other diseases associated with HDAC6 activity.

Areas covered: The application claims 1,3,4-oxadiazole sulfamide derivatives as selective HDAC6 inhibitors for the treatment of infectious diseases, neoplasms, endocrine, nutritional and metabolic diseases; mental and behavioral disorders; neurological diseases; diseases of the eye and adnexa; cardiovascular diseases; respiratory diseases; digestive diseases; diseases of the skin and subcutaneous tissue; disease of the musculoskeletal system and connective tissue; or congenital malformations, deformations and chromosomal abnormalities. Many of the exemplified compounds showed nanomole potency against HDAC6 and were more than 5000-fold selectivity for HDAC6 over HDAC1.

Expert Opinion*:* These 1,3,4-oxadiazole sulfamide derivatives have a unique zinc-binding group (ZBG) that provide good leads for the discovery of potent selective HDAC6 inhibitors for the treatment of a variety of diseases associated with HDAC6 activity.

Keywords*:* HDAC6, inhibitors, 1,3,4-oxadiazole, sulfamide, zinc-binding group

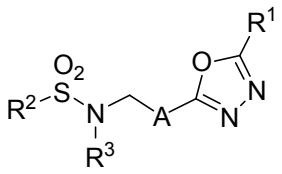
1. Introduction

Emerging evidence suggests that reversible acetylation of nucleosomal histone and the resultant changes in the chromatin structure are important process in gene transcription[1]. Most of these enzymes are large complexes and their activity, acetylation and deacetylation regulate the transcription by reducing the interaction between ribosomes and affecting the accumulation of ribosomes, resulting in chromatin openings required for transcription.[2]. Acetylation of the lysine tail of nucleosome-related histone neutralizes its positive charge, which causes the relaxed chromatin to induce transcriptional expression. In contrast, deacetylation of histone leads to the formation of condensed chromatin, which causes transcriptional repression[3,4]. The dysregulation of HDACs is implicated in many diseases, such as cancer, cellular metabolism disorders, neurological disorders, and inflammation, among many others[5-8]. In humans, 18 HDACs have been identified and are subdivided into four classes based on the homology to yeast HDACs. Among them, 11 HDACs use zinc as a cofactor and can be divided into three groups: Class Ⅰ (HDAC1, 2, 3 and 8), Class Ⅱ (Ⅱa: HDAC4, 5, 7 and 9; Ⅱb: HDAC6 and 10), Class Ⅳ (HDAC11). Additionally, 7 HDACs of class Ⅲ (SIRT 1-7) require NAD^+ instead of zinc as a cofactor[9,10]. So far, five HDAC inhibitors (HDACi) have been approved for the treatment of cutaneous T-cell lymphoma, T-cell lymphoma, and multiple myeloma, including vorinostat (SAHA), romidepsin (FK-228), belinostat (PXD-101) and panobinostat (LBH-589) and chidamide, for the treatment of cutaneous T-cell lymphoma, and multiple myeloma[11]. However, these nonselective（SAHA, LBH-589） or partially selective HDACi（FK-228, PXD-101） usually lead to undesirable side effects. For example, SAHA can cause fatigue, nausea/vomiting and gastrointestinal symptoms at a daily oral dose of 400 mg.[12-17]. Therefore, an increasing number of investigations are focusing on the development of isotype-selective HDAC inhibitors for the study of the complex interactions of these proteins central to transcription regulation as well as for the development of selective HDAC inhibitors as drugs in epigenetics[18-21]. In all 18 kinds of isoforms, HDAC6 is special with a number of non-histone proteins as substrates (such as α-tubulin and HSP90) and two homologous catalytic domains[22]. Each catalytic domain of HDAC6 has a complete biological function and can fully activate HDAC6 protein. The first domain is substantially free of catalytic activity, while the second catalytic domain is the major functional domain of HDAC6. Therefore, the inhibition of intact HDAC6 by small molecule inhibitors is only due to the simple interaction of these compounds with the second catalytic domain and is unaffected by domain-domain interactions[23]. Additionally, unlike broad-spectrum HDACi and those only targeting class I HDAC inhibitors, selective HDAC6 inhibitors are almost non-toxic to normal cells[24]. Compared to normal cells, tumor cells accumulate more misfolded proteins that can be broken down by proteasomes or aggregates[25], so tumor cells survive more than proteasomes and aggregates than normal cells. The main pathway for protein degradation is the ubiquitin-proteasome system. Under

the action of proteasome inhibitors, misfolded protein aggregation increases and cell biological activity decreases. The high binding of HDAC6 to ubiquitinated proteins activates the aggregator pathway, which increases the interpretation of misfolded proteins. Thus, selective HDAC6 inhibitors are less toxic to normal cells[26]. Therefore, selective HDAC6 inhibitors are an emerging class of pharmaceuticals due to the involvement of HDAC6 in different pathways related to cancer, neurodegenerative diseases, and immunology[27].

The active site of zinc-dependent HDACs include three or four prominent binding domains: the surface binding domain, the hydrophobic channel, the catalytic zinc binding domain, and/or the adjacent internal cavity. The pharmacophore model of HDACi can also be divided into three or four groups: a surface binding or cap group (Cap), a hydrocarbon linking motif (Linker), a zinc binding group (ZBG), and/or an internal cavity motif correspondingly[28-30]. The reported selective HDAC6 inhibitors mainly utilized hydroxamic acid and sulfur-containing moiety as ZBGs. 1,3,4-oxadiazole derivatives possessing sulfonamide moiety have been reported as potential antibacterial, anticancer agents, among many others. [31,32]. The sulfamides containing a trifluoromethyl or a difluoromethyl substituted 1,3,4-oxadiazole as novel ZBGs are first disclosed as selective HDAC6 inhibitors in the patent filings that form the subject of this evaluation.

2. Chemistry



1

A = phenyl or pyridyl; fluorine or chlorine substituted phenyl or pyridyl

R^1 = difluoromethyl, trifluoromethyl

R^2 = thiomorpholine 1,1-dioxide, piperazine and piperidine, in which the N atom is connected to the sulfonyl group to form sulfamides; The thiomorpholine 1,1-dioxide, piperazine and piperidine may be substituted on the ring or at the other N atom by a variety of alkyls, cycloalkyls, and heterocycloalkyls.

R^3 = phenyl or heteroaryl; fluorine substituted phenyl or heteroaryl

The claims of this application encompass substituted 1,3,4-oxadiazole sulfamide derivatives (**1**) including the stereoisomers and pharmaceutically acceptable salts and define a variety of potential substituents on the 1,3,4-oxadiazole sulfamide derivatives. A total of 62 compounds are specially claimed. In the majority of these, the 1,3,4-oxadiazole is connected to a phenyl or halogenated phenyl, or a pyridyl (represented by A in formula **1**). R^1 is a difluoromethyl or trifluoromethyl group that forms the ZBG portion together with the 1,3,4-oxadiazole moiety. The R^3 is usually a phenyl group substituted by at least one fluorine atom. Though broad claims are made for the R^2, the R^2 is generally a N-substituted piperazine. Further claims cover the capsule, injection, and tablet compositions containing them, and the processes for their preparation, and their applications in HDAC6-associated diseases.

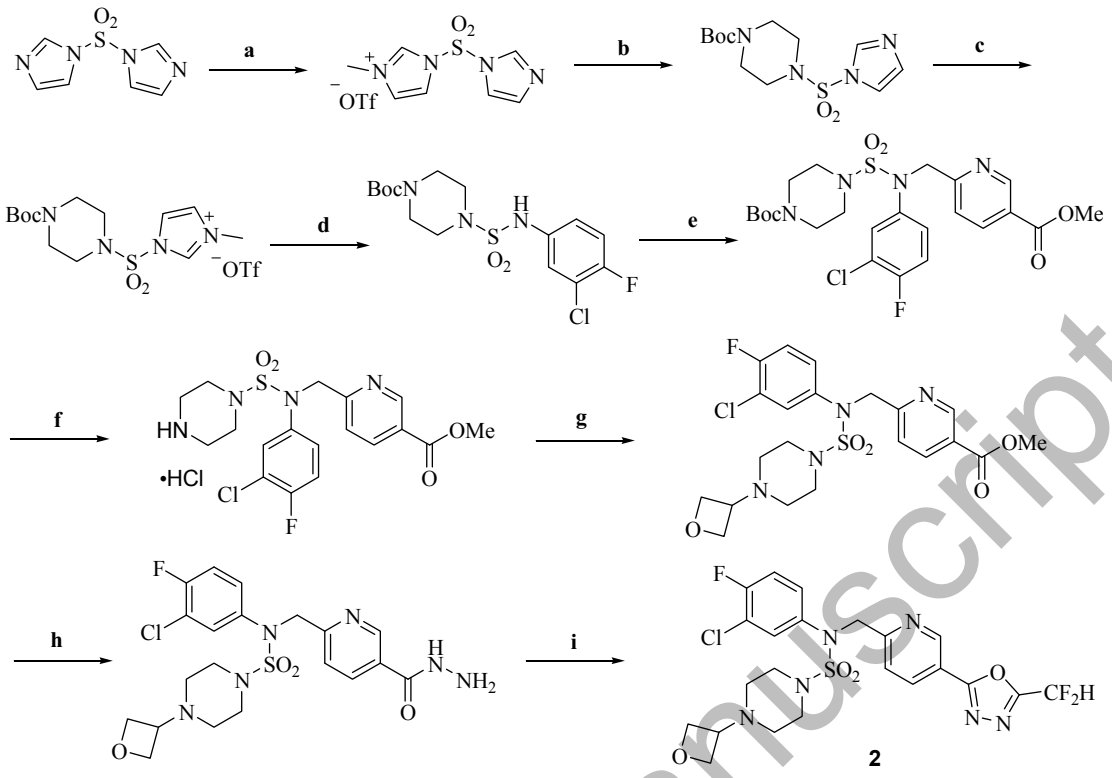


Figure 1. The preparation of compound **2** (example 56 in the applicant).

(a) MeOTf, CH_2Cl_2, 0°C, 3 h.
(b) *tert*-Butyl piperazine-1-carboxylate, MeCN, 16 h.
(c) MeOTf, CH_2Cl_2, 0°C–rt, 5 h.
(d) 3-Chloro-4-fluoroanline, MeCN, 80°C, 18 h.
(e) NaH, DMF, 10 min; methyl 6-bromomethylnicotinate, 50°C, 18 h.
(f) 4 M HCl/dioxane, CH_2Cl_2, 18 h.
(g) Oxetan-3-one, sodium triacetoxyborohydride, CH_2Cl_2, 18 h.
(h) $NH_2NH_2•H_2O$, EtOH/H_2O, 80°C, 18 h.
(i) $(CF_2HCO)_2O$, Et_3N, THF, 80°C, 1 h.

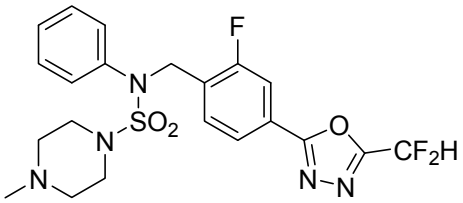
A detailed synthetic example illustrates the preparation of compound (**2**) is shown in Figure 1. The activation of *N*,*N*-sulfuryldiimidazole was readily achieved by treatment it with methyl triflate in an ice-cooled methylene chloride solution to give the resulting triflate salt as a white solid in 91.5% yield. The imidazolium group of the triflate salt was next displaced with *tert*-butyl piperazine-1-carboxylate in acetonitrile to afford the corresponding imidazoylsulfonylurea as a beige solid in moderate yield (56.9%). A second activation with methyl triflate then afforded the desired triflate salt almost quantitatively (95.6%). The activated imidazolium group of the triflate salt was next replaced by 3-chloro-4-fluoroanline to generate the corresponding sulfonylurea in 57.7% yield. The sulfonylurea was treated with NaH in *N*,*N*-dimethylformide (DMF) at room temperature for 10 min andreacted with methyl 6-bromomethylnicotinate at 50°C for 18 h tointroduc the substituted pyridine ring (45.9%). The Boc protective group was removed by the treatment with 4 M HCl in dioxane to

give the hydrochloride salt as a red solid (97.4%). The substitution of the piperazine amino group with an oxetan-3-yl group was carried out through the reaction of the corresponding hydrochloride salt with oxetan-3-one in methylene chloride in the presence of sodium triacetoxyborohydride in 98.6% yield. The methyl nicotinate derivative was further reacted with hydrazine monohydrate in ethanol/water (4/1) solution to give the nicotinohydrazide as a white solid (34.4%). Finally, the 1,3,4-oxadiazole sulfamide derivative **2** with a patent number of **11672** was obtained in 53.9% yield by the treatment of the nicotinohydrazide in tetrahydrofuran (THF) with 2,2-difluoroacetic anhydride in the presence of triethylamine at 80°C for 1 h.

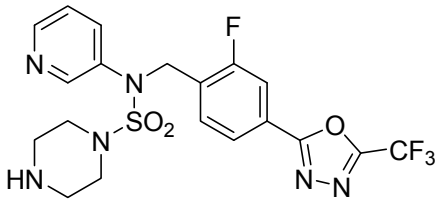
3. Biology

In order to examine the HDAC6 selectivity of the patented compounds (**1**), HDAC enzyme activity inhibition assay was performed using a HDAC Fluorimetric Drug Discovery Kit (BML-AK511). In the HDAC1 enzyme activity test, human recombinant HDAC1 (BML-SE456) was used as an enzyme source, and BNL-KI177 was used as a substrate. The tested compound was diluted in 5-fold and seeded into a 96-well plate. 0.3 μg of the enzyme and 10 μM of the substrate was added to each well and allowed to react for 30 or 60 min. The *Fluor* de Lys®-developer II (BML-KI176) was then added and reacted for 30 min. The fluorescence value (Ex 360, Em 460) was measured using a multi-plate reader (Flexstation 3, Molecular device). By the same protocol, the HDAC6 enzyme activity was measured using human recombinant HADC6 (382180) (Calbiochem) as the enzyme source. Based on the resulting values, each IC_{50} value was calculated using GrahPad Prism4.0 program. A total of 76 compounds of formula (**1**) were tested. These 1,3,4-oxadiazole sulfamide derivatives exhibited high potency against HDAC6 with their IC_{50} values in the ranges of 14 nM to 705 nM. Of importance, these novel HDAC6 inhibitors were about 142- to 7142-fold selective over the HDAC1 in general, the trifluoromethyl substituted oxadiazoles were less potent than their difluoromethyl counterparts. The representative compounds are shown in Figure 2.

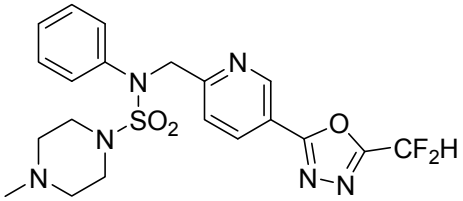
Tubulin is a HDAC6 major substrate of HDAC6. Inhibition of HDAC6 can increase the acetylation of tubulin and thereby improve the mitochondrial axonal transport velocity reduced by amyloid-beta treatment in neuronal axons. The HDAC6-specific inhibitors in this invention were examined for their effects on mitochondrial axonal transport. Hippocampal neurons from SD rat embryos at Embyonic day 17-18 (E17-18) were cultured in an extracellular matrix-coated dish for imaging for 7 days, and then treated with amyloid-beta peptides. After 24 h, the neurons were treated with compounds for 3 h on the 8^{th} day, and the mitochondria was stained with MitoTracker Red CMXRos (Life Technologies, NY, USA) at the last 5 min. Axonal transport of the stained mitochondria was imaged using a confocal microscope at 1-second intervals for 1 minute, and the transport velocity per second of each mitochondrion was determined using the IMARIS analysis software (BITPLANE, Zurich, Switherland). The results demonstrated that the patented 1,3,4-oxadiazole sulfamide derivatives could improve the velocity of mitochondrial axonal transport.



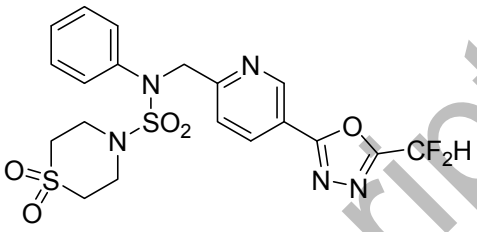
11306: IC_{50} = 23 nM (4363-fold)



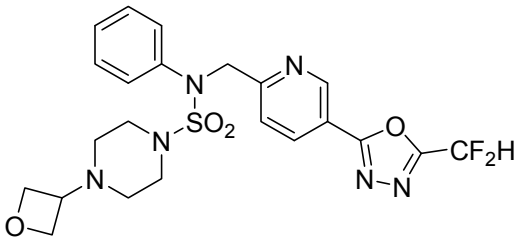
11321: IC_{50} = 44 nM (2249-fold)



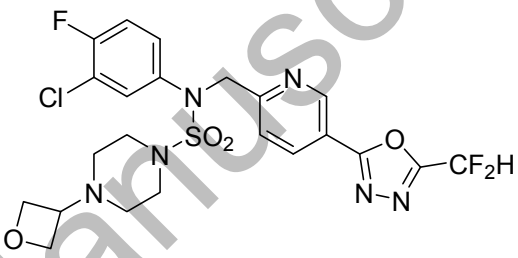
11363: IC_{50} = 15 nM (6863-fold)



11440: IC_{50} = 20 nM (5000-fold)



11574: IC_{50} = 16 nM (6250-fold)



11672: IC_{50} = 14 nM (7142-fold)

Figure 2. The representative compounds as HDAC6 inhibitors.

4. Expert opinion

The invention in this patent application relates to the substituted 1,3,4-oxadiazole sulfamide derivatives represented generally by formula (**1**) as selective HDAC6 inhibitors. Selective HDAC6 inhibitors have potential for a variety of therapeutic purposes, including cancer, neurodegenerative diseases and pathological autoimmune responses. At present, most of the reported HDAC6 inhibitors used hydroxamic acid as the ZBG. Though it is controversial on the genotoxicity of hydroxamic acids, some hydroxamic acid class of HDAC inhibitors has several problems with low solubility and poor pharmacokinetic properties[33]. A large number of sulfur containing ZBGs, including thiol, mercaptoamide, trithiocarbonate, sulfamide, 3-hydroxypyridin-2-thione and *N*-hydroxypyridin-2-thione, were investigated as HDAC6 selective inhibitors. However, the sulfur containing ZBG based HDAC6 inhibitors generally showed weak cellular activity. It is rather challenging to develop new ZBG based HDAC6 inhibitors. This patent provides good example of using the difluoromethyl or trifluoromethyl substituted oxadiazole as new ZBGs for the selective HDAC6 inhibition. These oxadiazole sulfamide derivatives may be good leads for the discovery of potent selective HDAC6 inhibitors as the drug in epigenetics for a variety of diseases associated HDAC6. However, the related biological information of these novel HDAC6 inhibitors is limited, the selectivity data over other HDAC subtypes except that for HDAC1 were not given, further investigation is needed, including the isoform selectivity over other HDACs, in vitro and in vivo evaluation as specific agent for cancer or neurological disease treatments.

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Declaration of interest
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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