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Patent Highlight

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Great Therapeutic Potential of Peptidylarginine Deiminase 4 (PAD4) Inhibitors: Treatment of Rheumatoid Arthritis, Epigenetic Tools, Regulation of Pluripotency in Stem Cells, and More

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Patent Application Title:	Benzomidazole Derivatives as PAD4 Inhibitors		
Patent Application Number:	WO 2016/185279 A1	**Publication date:**	24 November 2016
Priority Application:	US 62/164,906	**Priority date:**	21 May 2015
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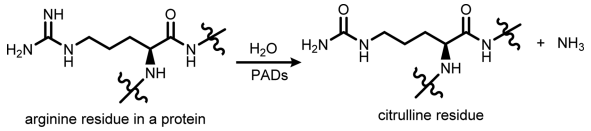
Applicant:	Glaxosmithkline Intellectual Property Development Limited; 980 Great West Road, Brentford, Middlesex TW8 9GS (GB)		
Disease Area:	Rheumatoid arthritis, vasculitis, systemic lupus erythematosus, ulcerative colitis, cancer, cystic fibrosis, asthma, cutaneous lupus erythematosis, and psoriasis.	**Biological Target:**	The peptidylarginine deiminase 4 (PAD4)

Summary:

The invention in this patent application relates to benzoimidazole derivatives represented generally by formula (I), which are inhibitors of PAD4. These compounds may be useful for the treatment of rheumatoid arthritis, vasculitis, systemic lupus erythematosus, ulcerative colitis, cancer, cystic fibrosis, asthma, cutaneous lupus erythematosis, and psoriasis.

Peptidylarginine deiminases (PADs) family include five members, PAD1, 2, 3, 4, and 6. They are calcium-dependent enzymes that catalyze the deimination (or citrullination) of post-translational arginine residues within proteins into citrulline residues. The process proceeds via the hydrolysis of the ketimine group (C═NH) of arginine into carbonyl group (C═O) and one molecule of ammonia as illustrated below. This conversion changes significantly the polarity and hydrogen bonding ability of the protein, which can affect its folding and functions.



Peptidylarginine deiminase 4 (PAD4) catalyzes the citrullination of a variety of proteins in vitro and in vivo, which causes diverse functional responses that may lead to a variety of diseases including rheumatoid arthritis (RA). RA is an autoimmune disease that affects approximately 1% of the population and is characterized by inflammation of articular joints leading to debilitating destruction of bone and cartilage. Citrullination by PAD4 may also lead to diseases with neutrophilic contributions to pathogenesis (for example, vasculitis, systemic lupus erythematosus, ulcerative colitis) in addition to oncology indications.

PAD4 and PAD2 have been detected in synovial tissue, and they are implicated in the citrullination of a variety of joint proteins. This action may contribute to the pathogenesis of rheumatoid arthritis in two different ways:

- Citrullination may trigger a response by the immune system to form anticitrullinated protein antibodies (ACPA). The ACPA act against citrullinated proteins in fibrinogen, vimentin, and collagen, which can contribute to pathogenesis of RA. The formation of ACPA can be used as a diagnostic test for RA.
- The increased citrullination affects the functions of several joint and inflammatory protein mediators such as fibrinogen, antithrombin, and multiple chemokines, which may contribute directly to the pathogenesis of RA. In a smaller subset of RA patients, anti-PAD4 antibodies are expressed and their level may correlate with a more erosive form of the disease.

Therefore, the inhibition of PAD4 is a viable therapeutic target that can potentially provide a treatment for rheumatoid arthritis.

The process of Neutrophil Extracellular Trap (NET) formation is an innate defense mechanism in which neutrophils immobilize and kill extracellular pathogens while minimizing damage to the host cells. Studies suggest that NET process is associated with histone citrullination; therefore, the inhibition of PAD4 may also provide therapy for diseases where NET formation in tissues contributes to local injury and disease pathology including, but not limited to, small vessel vasculitis, systemic lupus erythematosus, cystic fibrosis, asthma, deep vein thrombosis, periodontitis, sepsis, appendicitis, type 2 diabetes, and stroke. Studies also provide evidence that NETs may contribute to the pathology of some skin

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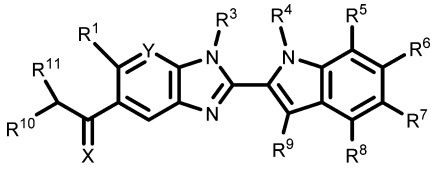
diseases such as cutaneous lupus erythematosis and psoriasis. Thus, PAD4 inhibitors may additionally treat these NET skin diseases through systemic or cutaneous administration. PAD4 inhibitors may affect additional functions within neutrophils and may potentially treat other neutrophilic diseases.

Studies have shown that PAD4 expression increases in the tissues of many malignant tumors in numerous cancers compared to cases of benign tumors and nontumor chronic inflammation diseases. Another finding was that PDA4 citrullinates arginine residues in histones at the promoters of p53-target genes such as p21, which are involved in cell cycle arrest and induction of apoptosis. Therefore, PAD4 inhibitors may be beneficial as antiproliferative agents.

PAD4 is the primary member of the PAD family that is expressed in the nucleus as well as the cytoplasm, which may be indicative of a larger and more general role in epigenetic regulation of gene expression. PAD4 can indirectly decrease histone arginine methylation (and hence epigenetic regulation associated with a particular mark) through the depletion of the available arginine residues by converting arginine residues into citrullines. Therefore, PAD4 inhibitors may also be useful as epigenetic tools or therapeutics to affect the expression of varied target genes in additional disease settings.

Recent studies show that PAD4-catalyzed citrullination of arginine residues on histone H1 promoter elements can promote localized chromatin decondensation in stem cells to regulate the pluripotent state. Therefore, PAD4 inhibitors may be able to perform the unique function of controlling citrullination levels and the switch between pluripotency and differentiation in stem cells. This function may thus be used therapeutically to affect the pluripotency status and differentiation potential of diverse stem cells including, but not limited to, embryonic stem cells, neural stem cells, hematopoietic stem cells, and cancer stem cells.

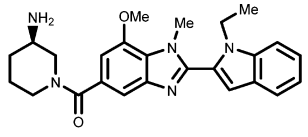
Important Compound Classes:



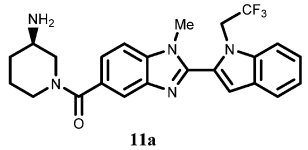
Formula (I)

Key Structures:

The inventors described the structures of 124 examples of formula (I) including the following representative compounds:



6a



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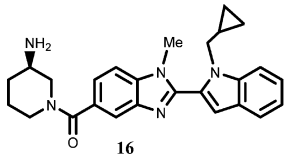

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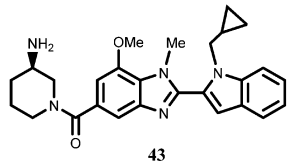

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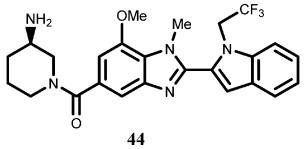

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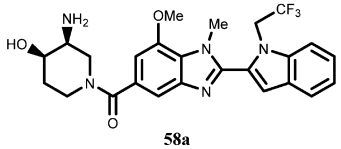

58a

Biological Assay:

- PAD4 Enzyme Assay
- PAD2 Enzyme Assay

Biological Data:

The mean pIC_{50} ($-\log IC_{50}$) values obtained from testing the above representative examples in the PAD4 and PAD2 enzyme assays are listed in the following table:

Compound	PAD4 enzyme assay mean pIC_{50}	PAD2 enzyme assay mean pIC_{50}
6	7.0	--
11a	6.8	--
16	6.9	<4.1 to 5
43	7.4	<4.1 to 5
44	7.3	<4.1 to 5
58a	7.1	<4.1 to 5

Recent Review Articles:

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Notes

The author declares no competing financial interest.

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