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Design, Synthesis, and Biological Activity of Diiminoisoindolines as Complement Component 3a Antagonists

Eugene B. Grant,* Deodialsingh Guiadeen, Monica Singer, Dennis Argentieri, Dennis J. Hlasta and Michael Wachter

Inflammation Research, The R. W. Johnson Pharmaceutical Research Institute, Route 202, PO Box 300, Raritan, NJ 08869, USA

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Abstract—The failure to fully regulate the inflammation response has been linked to diseases such as rheumatoid arthritis, septic shock syndrome, and asthma. The human complement system initiates and regulates the inflammation response through a cascade of regulatory factors. Complement Component 3a (C3a) is an essential regulatory factor and inhibiting its binding to a C3a receptor will diminish the inflammation response by disrupting the cascade. We report the design, synthesis, in vitro and in vivo activity of diiminoisoindolines as C3a antagonists. © 2001 Elsevier Science Ltd. All rights reserved.

Chronic diseases such as rheumatoid arthritis, Alzheimer syndrome, bronchial asthma, and myocardial infarction are associated with excessive complement activity.[1] The human complement system is complex, with approximately 30 plasma and membrane components, factors, regulators, and receptors working together to target invading microorganisms and antigens. It also defends the body by recruiting phagocytes and destroying invading cells. The complement system is activated by the classical immune complex and C1 activators or by antigens specific to microorganisms. In either method of activation, the third component (C3) is cleaved to release C3a and this activator promotes the cleavage of C5 into C5a and C5b. Component C5b binds with components C6–C9 to form the membrane attack complex (MAC), leading to cell lysis, and if unchecked, the resulting excessive complement system activity will lead to several chronic immunological diseases.

The pivotal position and role of C3a in promotion of complement cascade makes it an ideal drug target. Inhibiting C3a binding with a small molecule is a significant challenge and, to date, no clinically useful small molecule C3a antagonists have been described in the literature.[2] The C3a receptor is a 7-transmembrane domain protein, while C3a is constructed from 77 amino acids and has a molecular weight of 9000 Daltons.[3] While C3a has many possible points of contact with its receptor, the *C*-terminus arginine is essential.[4] Removal of this arginine rendered the C3a inactive. We believe binding a small molecule to this essential contact point in the receptor could disrupt C3a binding and prevent the propagation of the complement cascade. In this communication, we report our efforts to develop a small molecule that competes for the C3a receptor.

Based on the key role played by the terminal arginine in C3a, the 1,3-diiminoisoindoline nucleus (**1**) was envisioned as a possible competitor for the C3a receptor. This heterocyclic system is the foundation for selective metal chelators,[5] but it has not been explored as the basis for novel therapeutic agents. An exception is a brief report by Hall on the in vivo activity of compounds similar to **1** in a carrageenan induced edema assay in mice.[6] Surprisingly, these heterocycles showed good oral bioavailability in this model. In addition, the 1,3-diiminoisoindoline ring system is easily modified and can incorporate a variety of alkyl and aryl functionalities. These desirable characteristics make this class of heterocycles ideal for pursuing a potential antagonist (Fig. 1).

Synthetically, the preparation of **1** is straightforward and is sufficiently flexible for the rapid preparation of diverse analogues (Scheme 1). Typically, the commercially

*Corresponding author. Tel.: +1-908-218-6589; fax: +1-908-203-8109; e-mail: egrant@prius.jnj.com

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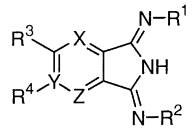

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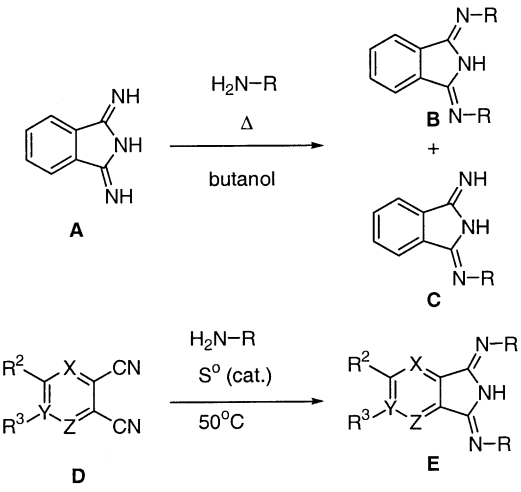
Figure 1.

available 1,3-diiminoisoindoline (**A**) is treated with 2.5 equiv of an amine in butanol.[7] The reaction is allowed to warm to 80 °C, and stirred for 12 h. The products (**B** and **C**) are isolated either by recrystallization of the crude reaction isolate or by flash chromatography. Typically, poor to modest yields are observed. Unsymmetrical analogues can be prepared by treating 1,3-diiminoisoindoline (**A**) with 1.0 equiv of an amine in refluxing butanol for 12 h, and concentrating the reaction mixture to afford a mixture of products that is predominantly **C**. Treating **C** with 1.0 equiv of a second amine in refluxing butanol for an additional 12 h afforded unsymmetrical **B**. The desired, unsymmetrical product could be separated from the undesired symmetrical product by flash chromatography.

In an alternate route, **1** can be prepared by treating appropriately substituted 1,2-dinitriles (**D**) with 4 equiv of an amine along with a catalytic amount of elemental sulfur.[8] Typically, the reaction proceeds to completion within 1 h at 50 °C. Purification of the desired products is achieved by recrystallization or flash chromatography.

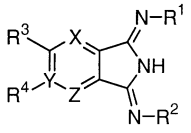


Scheme 1.

The ease of synthesis and wide variety of starting materials allowed us to prepare several analogues with different aryl and alkyl substituents (Table 1). Analogues were tested in a human neutrophil based C3a binding assay[9] and in a secondary C3a-induced calcium mobilization assay.[10] The results are described in Table 2.

For compounds **2**–**10**, optimum potency in these assays was attained when both 1 and 3 imino groups were substituted with a phenalkyl group. Potency was only slightly affected by the length of the alkyl tether (cf. **5** vs **9**). Binding affinity to the C3a receptor was affected

Table 1. Diiminoisoindoline analogues



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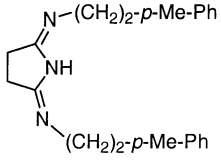

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Compd	R^1	R^2	R^3	R^4	X	Y	Z	Yield (%)
2[b]	Isopropyl	$(CH_2)_2$-*p*-Cl-Ph	H	H	C	C	C	10
3[a]	Isopropyl	Isopropyl	H	H	C	C	C	68
4[a]	$(CH_2)_2$-*p*-F-Ph	$(CH_2)_2$-*p*-F-Ph	H	H	C	C	C	29
5[a]	$(CH_2)_2$-*p*-Me-Ph	$(CH_2)_2$-*p*-Me-Ph	H	H	C	C	C	45
6[a]	CH_2-CH(Me)-*p*-Cl-Ph	CH_2-CH(Me)-*p*-Cl-Ph	H	H	C	C	C	23
7[a]	$(CH_2)_2$-*p*-Cl-Ph	$(CH_2)_2$-*p*-Cl-Ph	H	H	C	C	C	52
8[a]	$(CH_2)_2$-*m*-Cl-Ph	$(CH_2)_2$-*m*-Cl-Ph	H	H	C	C	C	42
9[a]	CH_2-*p*-Me-Ph	CH_2-*p*-Me-Ph	H	H	C	C	C	81
10[b]	$(CH_2)_2$-*p*-Cl-Ph	H	H	H	C	C	C	5
11[c]	$(CH_2)_2$-*p*-F-Ph	$(CH_2)_2$-*p*-F-Ph	H	O–*n*Pr	C	C	C	67
12[c]	$(CH_2)_2$-*p*-F-Ph	$(CH_2)_2$-*p*-F-Ph	H	$O(CH_2)_2NMe_2$	C	C	C	26
13[c]	$(CH_2)_2$-*p*-Me-Ph	$(CH_2)_2$-*p*-Me-Ph	H	OMe	C	C	C	5
14[c]	CH_2-*p*-Me-Ph	CH_2-*p*-Me-Ph	H	NO_2	C	C	C	25
15[c]	CH_2-*p*-Me-Ph	CH_2-*p*-Me-Ph	H	NHCOMe	C	C	C	71
16[c]	$(CH_2)_2$-*p*-Me-Ph	$(CH_2)_2$-*p*-Me-Ph	Cl	Cl	C	C	C	11
17[c]	$(CH_2)_2$-*p*-F-Ph	$(CH_2)_2$-*p*-F-Ph	H	—	C	N	C	33
18[c]	$(CH_2)_2$-*p*-F-Ph	$(CH_2)_2$-*p*-F-Ph	H	H	N	C	N	32
19[c]	$(CH_2)_2$-*p*-Me-Ph	$(CH_2)_2$-*p*-Me-Ph	—	—	—	—	—	6

[a]Prepared according to ref 7.
[b]Prepared according to ref 7 using the modification described in the text.
[c]Prepared according to ref 8.

Table 2. In vitro activity of diiminoisoindoline analogues

Compd	C3a binding IC_{50} (μM)	Calcium mobilization IC_{50} (μM)	C3a induced chemotaxis IC_{50} (μM)
2	8.45	—	—
3	IA[b]	—	—
4	11.41	3.1	6.5
5	6.14	3.3	6.5
6	5.9	(69)[a]	—
7	7.16	8.2	10.2
8	7.48	5.4	IA[b]
9	1.7	2.0	—
10	IA[b]	—	—
11	5.97	(79)[a]	6.1
12	5.36	—	7.8
13	12.55	3.8	(37)[a]
14	IA[b]	—	—
15	(32)[a]	—	—
16	(48)[a]	—	—
17	13.4	—	—
18	IA[b]	—	—
19	IA[b]	—	—

— Not tested.
[a]()% inhibition at 25 μM.
[b]IA = Not active at 25 μM.

Table 3. In vivo activity of selected diiminoisoindolines

Compd	Route of delivery	Dose (mg/kg)	% inhibition
4	po	15	44
	po	30	72
	ip	30	73
5	po	30	67
	ip	30	38
7	po	30	63
	ip	30	26
8	po	15	28
	po	30	55
	ip	30	72
9	po	15	30
	po	30	61
	ip	30	64

po, oral; ip, intraperitoneal

little by the nature of the substituent on the pendant phenyl ring(s) (**2**, **4**, **5**, **7**, **8** and **9**) or by branching in the alkyl chain (**6**). Branching did appear to reduce efficacy in the calcium mobilization functional assay in which all analogues uniformly behaved as C3a antagonists. Finally, substitution on the aryl ring in **1** provided mixed results. Nitro analogue **14**, aniline **15**, pyridine **17**, pyrimidine **18,** and diiminosuccinimide **19** were less efficacious than the parent compounds **4** and **5**. Alkoxy-substituted analogues **11** and **12** had slightly improved binding affinities compared to the corresponding parent compound **4**, but **11** appeared to be less efficacious in the functional assay. In contrast, alkoxy-substituted analogue **13** was slightly less potent than parent compound **5** in the binding assay, but equipotent in the calcium mobilization assay.

Several of the more potent analogues were tested in a C3a induced chemotaxis assay using purified human eosinophils.[11] Analogues **4**, **5**, **7**, **11**, and **12** had comparable potency in this assay while **8** was inactive at 25 μM.

Five diiminoisoindolines (**4**, **5**, **7**, **8** and **9**) were chosen for further evaluation in a C3a induced wheal formation assay in the rat (Table 3).[12] All five analogues showed comparable levels of efficacy when administered orally at 30 mg/kg; however, compounds **5** and **7** were less efficacious when administered intraperitoneally.

In conclusion, the rapid synthesis of modified diiminoisoindoline analogues led to the discovery of several antagonists with micromolar potency in C3a binding and functional assays. The di-*p*-methylbenzyl analogue **9** was the most potent compound in vitro with an IC_{50} of 1.7 μM in the binding assay and an IC_{50} of 2.0 μM in the calcium mobilization functional assay. Several compounds, including **9**, showed activity in vivo after po and ip dosing, suggesting that the inhibition of the C3a-mediated inflammatory response may be a useful approach to the treatment of chronic and acute inflammatory diseases.

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6. Butner, L.; Huang, Y.; Tse, E.; Hall, I. H. *Biomed. Pharmacother.* **1996**, *50*, 290.
7. Elvidge, J. A.; Linstead, R. P. *J Chem. Soc.* **1955**, 3536. Representative experimental (**9**): A slurry of 1,3-diiminoisoindoline (1.0 g, 6.9 mmol) in *tert*-butanol (25 mL) was treated with 2-(4-methylphenyl)ethylamine (1.9 mL, 15 mmol) via a syringe and the reaction mixture was warmed to 80 °C. After 16 h, the reaction mixture was allowed to cool and was concentrated in vacuo to afford a white solid. Purification by recrystallization (CH_2Cl_2/hexanes) afforded **9** (1.98g, 81%) as a white solid.
8. Sato, R.; Senzaki, T.; Shikazaki, Y.; Goto, T.; Saito, M. *Chem. Lett.* **1984**, 1423. Representative experimental (**17**): a mixture of 1,2-dicyanopyrazine (0.39 g, 3.05 mmol), 2-(4-fluorophenyl)ethylamine (0.9 mL, 6.89 mmol) and elemental sulfur (0.097 g, 3.05 mmol) was heated to 50 °C. After 1 h, the reaction mixture was dissolved in hot ethanol (25 mL) and filtered. The filtrate was allowed to cool and concentrated in vacuo. Purification by flash chromatography (0–50% EtOAc/CH_2Cl_2) afforded **17** (0.395 g, 34%) as a white solid.
9. C3a binding assay was conducted in a flash plate by the addition of ^{125}I-labeled C3a (20 μL) to the binding buffer (50 μL, RPMI 1640; 10 mg/mL BSA). After the addition was complete, the test compound (5 μL in 30% DMSO, 50 mM

HEPES buffer) was added. Freshly purified human neutrophils (125 μL, 5×10^5 cells/well) were added and the buffer solution was allowed to incubate for 1 h at 37 °C then aspirated to remove the resulting mixture, sealed and counted.

10. Calcium mobilization assay was conducted by adding 5×10^6 Fluo-3-labeled human neutrophils and the test compound to the wells of a 96-well plate. Following a 1 h incubation, 1 mM C3a (final concentration) was added and the fluorescence measured on a fluorescent imaging plate reader (FLIPR). Minimum values were subtracted from maximum values to determine the signal. Drug treated wells were compared to vehicle treated wells for percent inhibition of calcium mobilization. The chemotactic peptide fMLP (0.3 mM final concentration) served as the standard agonist.

11. Human eosinophils were purified using positive selection with anti-CD16 antibody and a Vario MACS System (Miltenyi Biotec). Chemotaxis assay was initiated by adding purified eosinophils and the test drug to the top chamber of the Transwell® plate and buffer or C3a (0.05 mg/mL) to the bottom reservoir. The plates were covered, and incubated at 37 °C for 3 h. A sample of the lower chamber (containing migrated cells) was counted and compared to vehicle treated wells for inhibition of chemotaxis. The decrease in the number of cells migrating as compared to vehicle treated wells afforded percent inhibition.

12. 30–60 min following oral administration of test compound or vehicle, 1.0 mL of a 0.5% solution of Evan's Blue dye was injected into the tail vein of male Sprague–Dawley rats (ca. 200 g). Immediately following Evan's Blue injection, C3a was injected intradermally into the clipped skin of the back. After 15 min, the rats were sacrificed and the blue areas (wheals) resulting from dye leakage into the injection sites were measured and compared to vehicle controls for efficacy of the anti-inflammatory compounds. Typically, tripelennamine (10 mg/kg) produced approximately 70% inhibition of C3a induced wheals.