Bioorganic & Medicinal Chemistry Letters 20 (2010) 7283–7287

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel TNF-α converting enzyme (TACE) inhibitors as potential treatment for inflammatory diseases

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ARTICLE INFO

Article history:
Received 2 September 2010
Revised 14 October 2010
Accepted 15 October 2010
Available online 23 October 2010

Keywords:
Hydantoin
TACE inhibitor
Rheumatoid arthritis

ABSTRACT

Our research on hydantoin based TNF-α converting enzyme (TACE) inhibitors has led to an acetylene containing series that demonstrates sub-nanomolar potency (K_i) as well as excellent activity in human whole blood. These studies led to the discovery of highly potent TACE inhibitors with good DMPK profiles.
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Rheumatoid arthritis is a chronic autoimmune inflammatory disorder that attacks the joints, often progressing to the destruction of the particular cartilage and alkylosis of the joints. Biologics, such as Remicade® and Enbrel®, have had success in treating the disease by controlling the levels of tumor necrosis factor-α (TNF-α), a major immunomodulatory and proinflammatory cytokine.[1] A small molecule drug discovery approach could entail controlling the level of TNF-α by inhibiting the *T*NF-α *c*onverting *e*nzyme (TACE/ADAM 17), a metalloproteinase (MMP) known to cleave the 26-kD membrane-bound form of TNF-α to its 17 kDa soluble component.[2,3] Since musculoskeletal side effects have been seen with the use of broad spectrum MMPs, our goal was to achieve a good selectivity profile for any small molecule metalloproteinase inhibitor.[4–7] Our early research efforts on small molecule TACE inhibitors led to a series of hydantoin compounds.[8,9] After extensive efforts to optimize the series, a lead biaryl series was identified which provided selective sub-nanomolar inhibitors (K_i). However, these compounds exhibited a significant shift in human whole blood activity (*h*WBA) (Fig. 1).[10] In an effort to optimize the *h*WBA of the biaryl series, several modifications were made to the distal ring 2, which is known to project into the solvent exposed S1 pocket of the enzyme.[11,9a] Most of these modifications did not enhance the *h*WBA, therefore, focus was then shifted to modifications of Ring 1. As shown in Figure 1, an acetylene could be an intriguing choice as a linker since it projects Ring 2 in a similar linear orientation as in the biaryl series and it could also provide access to other types of functionalities as aryl replacements.

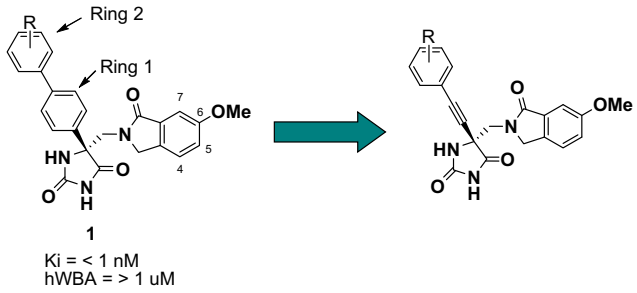

Figure 1. Development of an acetylene series of inhibitors.

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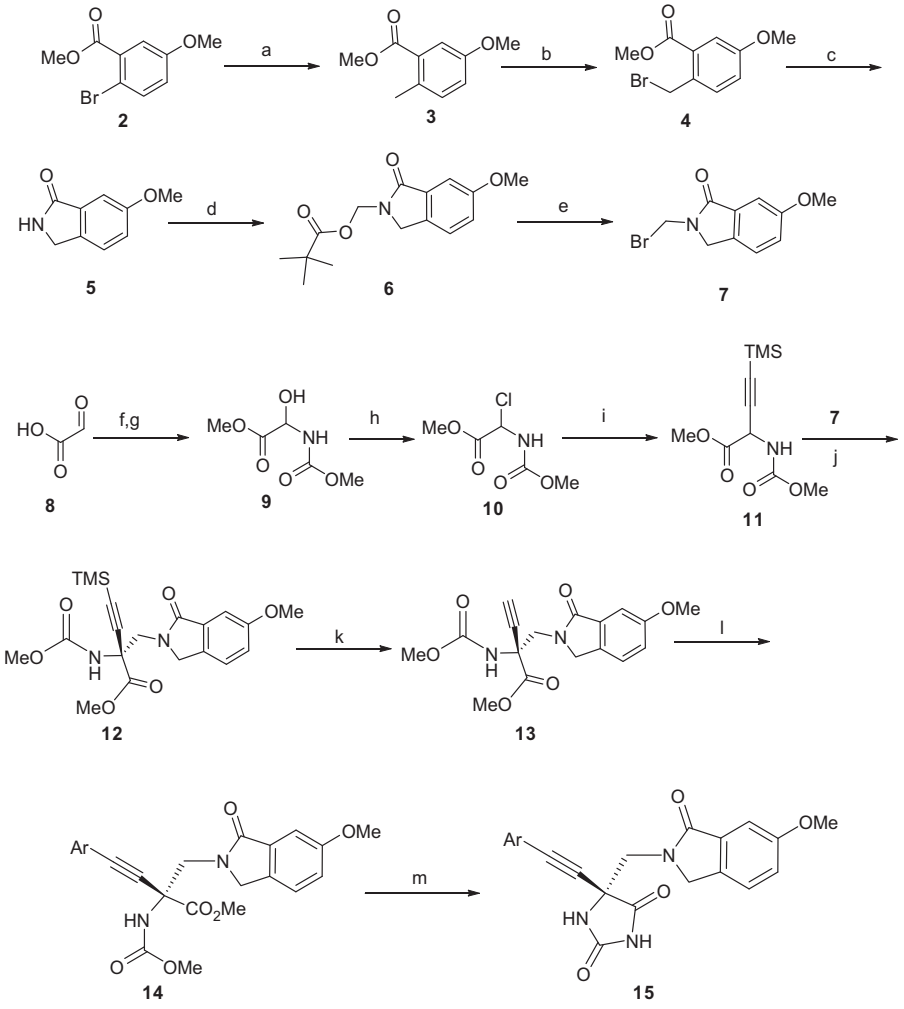
0960-894X/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.10.081



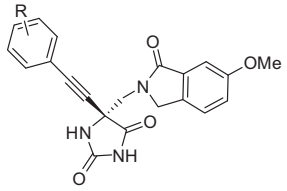
Scheme 1. Reagents and conditions: (a) trimethylboroxine, $Pd(dppf)Cl_2$,Cs_2CO_3, dioxane, H_2O, 80%; (b) NBS, benzoylperoxide, 98%; (c) 7 M NH_3/MeOH, 60 °C, 67%; (d)NaO*t*-Bu, THF, DMPU, chloromethylpivalate, 91%; (e) bromotrimethylsilane, DCM, 95%; (f) methyl carbamate, Et_2O, 98%; (g) H_2SO_4, MeOH, 71%; (h) PCl_5, CCl_4, 96%; (i) bis-trimethylsilylacetylene, $AlCl_3$, DCM, 0–25 °C, 69%; (j)LiHMDS, THF, **7**, −78 °C, 63% then chiral chromatography, Chiralcel OD column; (k) TBAF, THF 95%; (l) Ar-X, CuI, $Pd(PPh_3)_2Cl_2$, DIEA, DMF, 80 °C, 60–90%; (m) 7 M NH_3/MeOH, 80 °C, 95%.

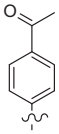
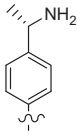
The first goal was to develop a route that could be used to synthesize numerous acetylene analogs in a convergent manner. The 6-OMe lactam which was found to be the optimal piece with regards to K_i in compound **1** was retained for this study. The synthesis began with the methylation of methyl 2-bromo-5-methoxybenzoate **2** using trimethylboroxine under palladium cross coupling conditions to provide compound **3** (Scheme 1).[12] The benzylic position was brominated using NBS and benzoyl peroxide and then cyclized to the lactam **5** using ammonia in methanol. Alkylation of the lactam with chloromethylpivalate provided compound **6**, which was then treated with bromotrimethylsilane to provide compound **7**. The synthesis of the amino acid derivative began with the treatment of glyoxylic acid with methyl carbamate followed by esterification with sulfuric acid/methanol to provide compound **9**.[13] Compound **9** was treated with phosphorus pentachloride to produce the 2-chloroglycinate intermediate **10**, a stable white powder that could be stored at room temperature. Reaction of **10** with bis-trimethylsilyl acetylene and aluminum trichloride provided the trimethylsilyl-acetylene **11** which was alkylated by treatment with LiHMDS followed by the addition of the bromomethyl lactam **7** to provide compound **12**. The two enantiomers could be separated by HPLC on large scale using a Chiralcel OD column. The desired enantiomer was subsequently treated with TBAF to remove the trimethylsilyl group.

As part of the synthetic strategy, the key intermediate **13** could be coupled to various different aryl halides using standard Sonagashira reaction conditions to provide compounds of type **14**. The final step in the synthesis was the cyclization of the amino acid derivative to the desired hydantoin using ammonia to provide target compounds of type **15**. This robust synthesis could be used to synthesize multigram quantities of the desired final products and provided a versatile method of attaching different aryl/heteroaryl groups to the hydantoin series late in the synthesis.

Table 1 displays the TACE K_i/*h*WBA activities of aryl-acetylene compounds. Despite their subnanomolar K_i values, compounds **16**–**23** did not have *h*WBA in the desired range (Table 1). Since this part of the molecule projects into the solvent exposed S1 pocket, the incorporation of polar functionality could potentially improve the *h*WBA.[14,9a] When a nitrile was placed on the 3-position of the ring (**24**) we discovered an improvement in both K_i and *h*WBA. When the nitrile was converted to the primary amide (**25**) the K_i decreased, but the *h*WBA was similar. Movement of the primary amide to the other positions of the phenyl ring (**26 and 27**) demonstrated that the 4- and 3-position were optimal for *h*WBA. Though there was an improvement in *h*WBA in compounds **25 and 26**, the K_i was slightly higher than found in compounds **16–23** which had demonstrated poor *h*WBA. This divergence in SAR would prove to be a trend for the program and therefore, *h*WBA

Table 1
TACE K_i/*h*WBA of aryl acetylene analogs



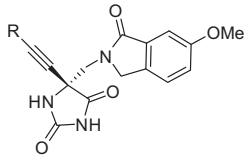
Compound	R	K_i (nM)	*h*WBA (nM)
16	H	0.14	784
17	4-F	0.26	1675
18	3-F	0.2	873
19	2-F	0.13	617
20	4-OMe	0.41	1332
21	3-*i*Pr	0.5	19000
22	3-Me	0.72	5148
23	2-Me	2.0	3142
24	3-CN	0.06	273
25	3-$CONH_2$	0.85	302
26	4-$CONH_2$	0.56	165
27	2-$CONH_2$	2.78	5311
28	4-CONH-cyclopropyl	2.55	487
29	4-CH_2CONH_2	0.42	139
30		0.5	352
31		2.35	621

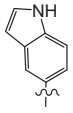
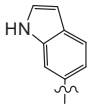
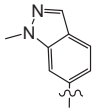
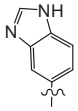
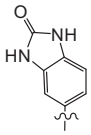
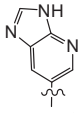
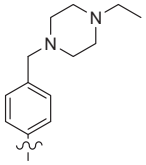
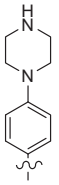
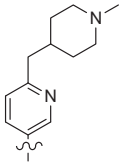
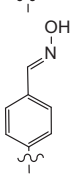
was used as an important criterion for the advancement of compounds.[15] N-Alkylation of the amide (**28**) diminished the *h*WBA, but homologation of the primary amide (**29**) provided a slight improvement in *h*WBA.

In order to optimize for *h*WBA, it was important to examine the factors that contributed to the improved *h*WBA of the amides. The primary amides demonstrated that polar functionality at the 3- or 4-position of the phenyl ring could provide improvements to *h*WBA. Given that a primary amide group can act as both a hydrogen bond donor and acceptor, it was important to determine which of these characteristics influenced the *h*WBA most. When the primary amide was replaced by a methyl ketone (**30**) which only has the ability to accept hydrogen bonds, the *h*WBA was slightly diminished. Replacement of the primary amide with the primary amine **31** displayed an even greater loss in potency. These results prompted a continuous effort to find alternative polar functional groups that could lead to enhanced *h*WBA. One strategy was to replace the phenyl/amide functionalities with heteroaryl rings as well as aryl rings containing polar functionalities. These functionalities could also be used to further probe whether *h*WBA is dependent on hydrogen bonding ability and also if basicity is tolerated in this portion of the molecule.

As shown in Table 2, the hydrogen bond donating indoles **32 and 33** both demonstrated a loss in *h*WBA. When the hydrogen bonding ability was completely removed as in indazole **34** there was a large decrease in *h*WBA. However, *h*WBA was improved in the case of benzimidazole **35** which has the ability to accept hydrogen bonds. It was uncertain whether the improvement in *h*WBA was due to the hydrogen bond ability of the benzimidazole, thus, the 2-hydroxybenzimidazole (**36**) was synthesized and confirmed

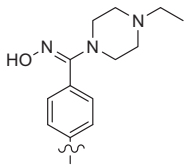
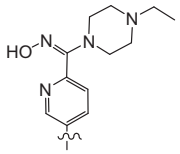
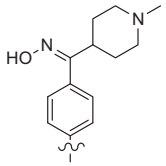
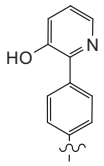
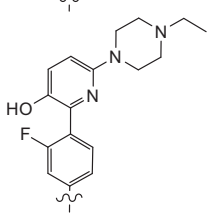
Table 2
TACE K_i/*h*WBA of acetylene analogs



Compound	R	K_i (nM)	*h*WBA (nM)
32		0.17	751
33		0.19	2965
34		0.52	10400
35		0.6	211
36		0.53	240
37		0.62	220
38		0.92	193
39		0.29	239
40		0.61	205
41		0.47	195
42		0.16	167

(*continued on next page*)

Table 2 (*continued*)

Compound	R	K_i (nM)	*h*WBA (nM)
43		0.16	20
44		0.18	87
45		0.22	33
46		0.07	117
47		0.1	40

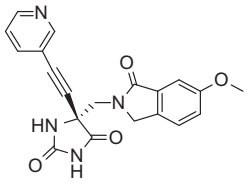
that hydrogen bonding accepting ability in the area very close to the 3- or 4-position of the phenyl ring is beneficial for *h*WBA. Since hydrogen bond accepting groups were tolerated the next step was to determine if basic functionalities could be added to this portion of the molecule. When basic heterocycles such as **37** and **38** were incorporated into the molecules the *h*WBA was retained. Accordingly, other non-aromatic basic amines were incorporated and it was found that amines such as **39**–**41** also provided good *h*WBA. These compounds suggested that basic functionality could be placed further from the ring and retain *h*WBA.

At this point it was evident that we could consistently achieve *h*WBA levels of ~200 nM by adding functionalities with hydrogen bond accepting potential in close proximity to the 3- or 4-position of the phenyl ring with the optional presence of basic functionalities further from the phenyl ring. However, in desiring to improve the *h*WBA even further we decided to return to our most potent compounds and make adjustments to the hydrogen bonding ability as well as basicity of functional groups. Since only the 4-position primary amide displayed *h*WBA less than 200 nM it was important to retain that substitution pattern. When the amide was replaced by the oxime **42** the *h*WBA was retained. Further introduction of a basic piperazine component to the structure of oxime **42**, which is capable of hydrogen bond accepting, resulted in the novel amidoxime **43** in which a significant improvement in *h*WBA was observed. Incorporation of a heteroatom, such as in **44**, resulted in only a slight reduction in activity relative to **43**.

Replacement of the amidoxime in compound **43** by the analogous oxime **45** demonstrated retention of potency as well. The potent compound **46** was designed as a constrained analog of **43** and possesses both the basic pyridine nitrogen and the hydrogen bond accepting group on opposing sides of the same ring. Compound **47** incorporates the piperazine found in **39** to further improve the *h*WBA to less than 100 nM.

Of all the compounds synthesized, compound **37** displayed the best overall profile. It had an encouraging PK in rat, monkey, and dog and had a reasonable selectivity profile (Fig. 2).

In summary, we have developed novel acetylene-based hydantoin inhibitors of the TACE enzyme that have excellent K_i and *h*WBA. Vast improvements to the *h*WBA were discovered by adding hydrogen bonding and basic functionalities in the solvent exposed S1 region of the enzyme. These modifications led to the discovery of compound **37** which has a good selectivity profile and demonstrates PK in multiple animal species. Further optimization of the human whole blood activity and PK profiles of our lead hydantoin TACE inhibitors will be reported in future publications.



37

TACE K_i = 0.62 nM
*h*WBA = 220 nM
Enzyme IC50 (nM)

MMP1	5200	MMP2	431
MMP3	5800	MMP7	>40000
MMP9	1010	MMP13	2920
ADAM10	22		

Rat PK (Na^+ salt):
iv Dose (mg/kg) 3
AUC (uM.h) = 7.3
T1/2(h) = 2.2
Cl(L/h/kg) = 19

po Dose (mg/kg) = 10
AUC (uM.h) = 4.0
Tmax(h) =2 .2
F% = 15

Dog PK (Na^+ salt):
iv Dose (mg/kg) 0.5
AUC (uM.h) = 1.3
T1/2 (h) = 0.9
Cl (L/h/kg) = 19

po Dose (mg/kg) = 5
AUC (uM.h) = 1.1
Tmax (h) = 0.1
F% = 9

Monkey PK (Na^+ salt):
iv Dose (mg/kg) 1
AUC (uM.h) = 3.8
T1/2 (h) = 2.4
Cl (L/h/kg) = 13

po Dose (mg/kg) = 3
AUC (uM.h) = 2.6
Tmax (h) = 1.2
F% = 23

Figure 2. Overall profile of compound **37**.

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10. *Human whole blood procedure*: Human whole blood is diluted 1:1 with serum free medium (RPMI, L-glutamine, Pen-Strep, HEPES) and incubated with a compound in a final volume of 360 L for 1 h at 37 °C. Forty microliters of LPS (10 μg/mL) is then added. Supernatant is collected after 3.5 h incubation and the concentration of TNF-α is determined by ELISA (R&D Systems).
11. X-ray structures can be found in the following papers: (a) Yu, W.; Guo, Z.; Orth, P.; Madison, V.; Dai, C.; Feltz, R. J.; Girijavallabhan, V. M.; Kim, S. H.; Kozlowski, J. A.; Lavey, B. J.; Li, D.; Lundell, D.; Niu, X.; Piwinski, J. J.; Popovici-Muller, J.; Rizvi, R.; Rosner, K. E.; Shankar, B. B.; Shih, N. Y.; Siddiqui, M. A.; Sun, J.; Tong, L.; Umland, S.; Wong, M. K.; Yang, D. Y.; Zhou, G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1877.
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15. Attempts were made to make correlations between *h*WBA and protein binding but no trends were apparent. It became clear through our studies that *h*WBA optimization was empirical.