Bioorganic & Medicinal Chemistry Letters 20 (2010) 3155–3157

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of anti-inflammatory clinical candidate E6201, inspired from resorcylic lactone LL-Z1640-2, III

Yongchun Shen [a], Roch Boivin [a], Naoki Yoneda [b], Hong Du [a], Shawn Schiller [a], Tomohiro Matsushima [b], Masaki Goto [b], Hiroshi Shirota [a], Fabian Gusovsky [a], Charles Lemelin [a], Yimin Jiang [a], Zhiyi Zhang [a], Robert Pelletier [a], Megumi Ikemori-Kawada [b], Yoshiyuki Kawakami [b], Atsushi Inoue [b], Matthew Schnaderbeck [a], Yuan Wang [a,*]

[a] Eisai Inc., 4 Corporate Drive, Andover, MA 01810, USA
[b] Eisai Tsukuba Research Laboratories, 1-3, Tokodai 5-chome, Tsukuba-shi, Ibaraki 300-2635, Japan

ARTICLE INFO

Article history:
Received 18 January 2010
Revised 20 March 2010
Accepted 26 March 2010
Available online 30 March 2010

Keywords:
LL-Z1640-2
f152A1
Anti-inflammatory
Resorcylic lactone

ABSTRACT

Inspired by natural product, LL-Z1640-2, clinical candidate, E6201 (**22**) was discovered in a medicinal chemistry effort through total synthesis. The modification on C14-position to *N*-alkyl substitution showed to be potent in vitro and orally active in vivo in anti-inflammatory assays.

In our effort of developing novel anti-inflammatory agent, inspired by natural product, f152A1 (**1**), we reported that the chemical modifications at C4 and C14 positions of resorcylic lactone lead to an in vitro and iv in vivo potent analog **2** (IC_{50}: 15 nM in vitro; ED_{50}: 6.5 mg/kg iv) (Fig. 1).[1–3] In the last letter we highlighted the structure activity relationship in C14–oxygen series.[3] However, the oral bioavailability of **2** in mouse was very low (4.1%), and was not suitable for exploring broader application outside of iv route. In this Letter, we report the successful discovery of benzimidazole **7** and C14–N substitution analogs including the clinical candidate E6201 (**22**).

Our previous investigations suggested that C14 region can tolerate substitutions. An imidazole group in **2** regained full potency of the natural product.[3] In addition, we found C13–halogen substitution resulted in modest reduction of potency. Based on this knowledge, we designed and synthesized analog **7**, *N*-methylimidazole-fusion at C13, C14 positions to explore changes of electronic characteristics on the aromatic ring.

The synthetic route for analog **7** is illustrated in Scheme 1. Starting with readily available aromatic compound **3** (synthesized from commercially available Methyl Orsellinate in two steps as described in Ref.[3]), a six-step sequence was employed to produce benzimidazole intermediate **4** in 43% overall yield. Compound **4** was deprotected with TFA and re-protected with TBDPS-Cl in 89% yield for two steps. The properly protected intermediate was brominated, displaced with thiophenol and followed by TBAF desilylation to afford phenol **5** in 78% yield over three steps. Phenol **5** went through the sequential MOM-Cl protection (87% yield),

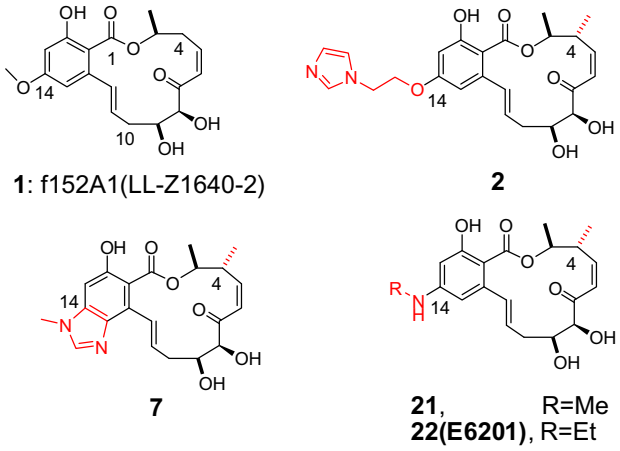
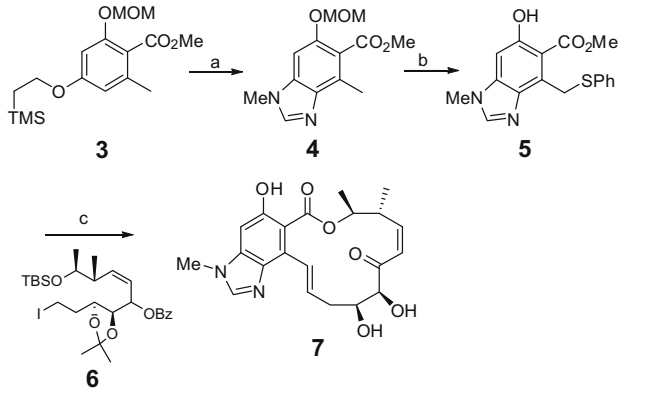


Figure 1. Structures of f152A1 and synthetic analogs **2**, **7**, **21** and **22** (E6201).

* Corresponding author. Tel.: +1 978 837 4859; fax: +1 978 837 4863.
E-mail address: yuan_wang@eisai.com (Y. Wang).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.03.087



Scheme 1. Synthesis of analog **7**. Reagents and conditions: (a) (i) TBAF, 95%; (ii) NO_2^+ BF_4^-, 80%; (iii) Tf_2O, 89%; (iv) BnNHMe, 75%; (v) H_2, 20% $Pd(OH)_2/C$; (iv) $HC(OEt)_3$, KSF clay (85% two steps); (b) (i) TFA; (ii) TBDPSCl (89% two steps); (iii) NBS; AIBN; (iv) PhSH, Cs_2CO_3; (v) TBAF (78% three steps); (c) (i) MOMCl, 87%; (ii) KOH, 74%; (iii) $TMS(CH_2)_2OH$, Ph_3P, DEAD, 100%; (iv) LiHMDS, 40%; (v) mCPBA; (vi) Et_3N; (vii) TBAF; (vii) 2-chloro-1-methylpyridinium iodode; (ix) NaOH, 55% five steps; (x) TFA, 50% two steps.

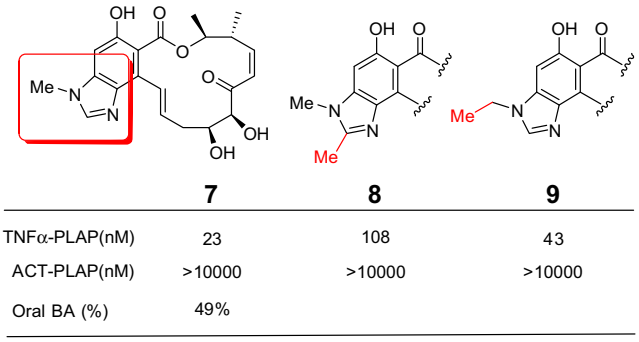


Figure 2. C13–14 Benzylimidazole series.

saponification (74% yield), Mitsunobu esterification (100% yield), coupling with acyclic iodide (40% yield), MCPBA oxidation, E_3N promoted elimination, TBAF deprotection, lactone cyclization under the influence of Mukaiyama's reagent,[4] basic debenzoylation (55% yield over five steps), PCC oxidation and acidic deprotection to furnish analog **7** in 50% yield over the final two steps.

Analog **7** was potent (IC_{50}: 23 nM in vitro) and had impressive oral bioavailability in mouse (49%). With this encouraging result, we further expanded to a limited set of substitution on benzimidazole. Analogs **8** and **9** showed slight loss of potency (IC_{50}: 108 and 43 nM, respectively, Fig. 2). Encouraged by the SAR of C14–*O* series, represented by **2**, we turned our focus to analogs with simple N-substituted side chains at C14 position. From advanced intermediate **10**, Buchwald chemistry was employed to transform O-substitution at C14 into N-substituent (Scheme 2).[3] From intermediates **11** and **12**, dozens of analogs bearing N-substitution at C14 were prepared.[5]

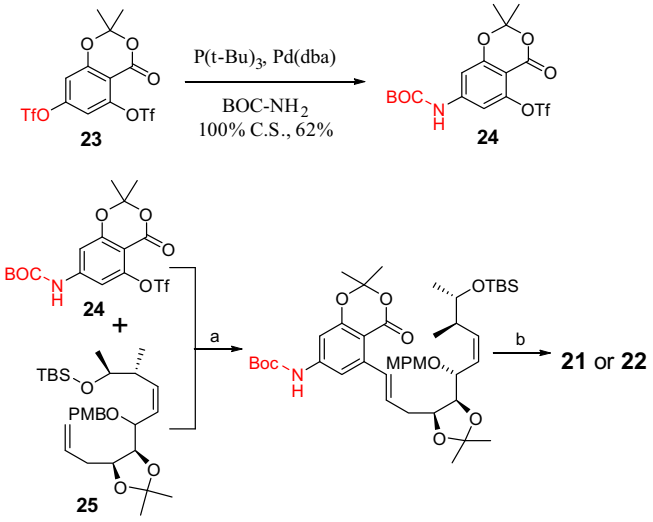
Table 1
Structures, activity and bioavailability data for selected analogs

Analog	R on C14	TNF PLAP IC50 (nM)	Mouse F% po
13	Et_2N–	2066	–
14	Benzyl-NH–	1251	–
15	*n*PrNH–	26	–
16	Me_2N–	437	21
17	H_2N–	360	49
18	Piperazine–	351	–
19	Morpholine–	129	48
20	HO–$(CH_2)_2$–NH–	71	–
21	MeNH–	85	29
22	EtNH–	56	12

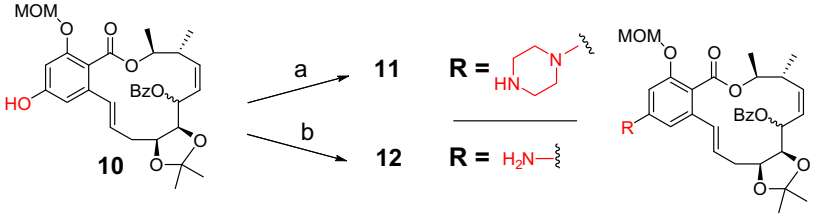
– Not tested.

Among the C14-substituted analogs, both the di-N-substituted compounds (**16** and **19**) and the unsubstituted amino analog (**17**) showed excellent oral bioavailability in mice but their potencies were lower than compound **7** (Table 1). Mono-substituted analogs with large R group (**14**, R = Benzyl) showed no improvement either. However, methyl substituted analog **21** demonstrated excellent bioavailability in mice (29%) and good in vitro potency (IC_{50} = 85 nM). The ethyl analog, **22**, showed similar potency as **21** and its oral bioavailability in mice was acceptable.

With this success, we decided to evaluate **21** and **22** in various inflammatory animal models. The synthetic route described above was flexible for diverse analog synthesis in small scale. However, the employment of selenium chemistry and long synthetic sequence (17 steps) presented significant challenge for larger scale synthesis, which was necessary for in vivo studies. In order to sup-



Scheme 3. Amination reaction and simplified synthetic route. Reagents: (a) $Pd_2(DBA)_3$, Cy_2NMe, 65%; (b) (i) LiHMDS, R–I (R = methyl of ethyl), 82%; (ii) TBAF, imidazole-HCL; (iii) KHMDS, 76% for two steps; (iv) TBDMSCl, imidazole, 99%; (v) DDQ, 98%; (vi) PCC, 81%; (vii) TFA, 96%.



Scheme 2. Introducing N-substitution at C14 position. Reagents: (a) (i) Tf_2O, Et_3N; (ii) piperazine, $Pd(OAc)_2$, BINAP, 21% for two steps; (b) (i) Tf_2O, Et_3N; (ii) benzophenone imine, $Pd(OAc)_2$, BINAP; (iii) $NH_2OH{\cdot}HCL$, NaOAc, 93% for three steps.

Table 2
In vivo effects of selected analogs and reference drugs on short-term arthritis

Analogs	Anti-arthritis (ED_{50}, mg/kg)	Tolerated dose (TD; mg/kg)	Ratio (ED_{50}/TD)
7	5	<5	1
8	10	<10	1
9	10	<10	1
16	15	~60	4
21	5	>30	6
22	5–10	>30	3–6
Indomethacin	1	3	3
Prednisolone	3	10	3

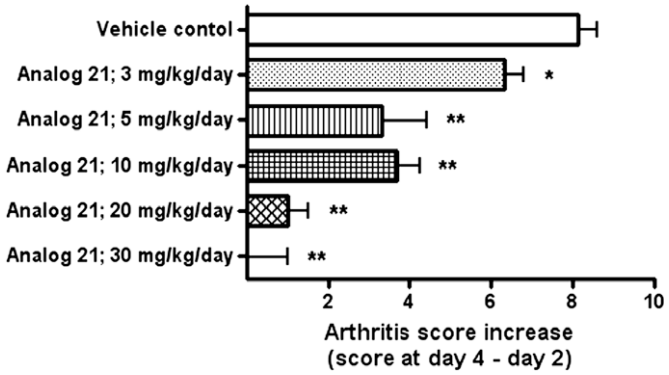


Figure 3. Analog **21** CAIA efficacy results by oral dosing.[9]

ply large amount of **21** and **22**, a simplified route featuring selective Buchwald–Hartwig amination reaction and Heck reaction were developed.[6,7] Careful survey of conditions for Buchwald–Hartwig amination reactions revealed that ditriflate **23** could be selectively aminated when P(*t*-Bu)$_3$ was used as the Pd ligand (Scheme 3). We found that all the amination took place at C14 position and no undesired regio-isomer was observed. Desired amide **24** was produced in 62% isolated yield from ditriflate **23**. The coupling of triflate **24** and acyclic olefin **25** proceeded smoothly under modified Heck conditions (65% yield after purification), which successfully replaced the selenium chemistry and the synthetic sequence was shortened to nine steps from ditriflate **23** with an overall yield of 19% (83% average yield for each step).

Using the new synthetic route, sufficient quantities of analogs **21, 22** and other analogs were produced for detailed biological evaluation. In a collagen antibody induced arthritis model (CAIA) in mice, a selected group of analogs were evaluated (Table 2). A representative dosing and responses in the CAIA model were shown in Figure 3. In this case, **21** showed potent effect with the ED_{50} of ~5 mg/kg by oral dosing, QD × 2. Overall, **21** and **22** showed good anti-inflammatory effects and wider therapeutic windows in in vivo model of arthritis with ED_{50} around 5–10 mg/kg (Table 2). Additional in vivo models will be reported separately.[8] Based on overall profile, **22** was chosen as our clinical candidate, and named E6201.[8,9]

In conclusion, our medicinal chemistry efforts have led to the discovery of E6201 (**22**), a fully synthetic analog of f152A1 (**1**) with desirable in vitro and in vivo pharmacological properties.[8–10] Development of practical synthetic routes afforded SAR investigation and detailed biological evaluation. E6201 entered clinical trial.

Acknowledgment

We thank Dr. Yoshito Kishi, chairman of Eisai Scientific Advisory Board on helpful discussion, scientific insight and encouragement over the years. We acknowledge contribution from researchers who supported this effort, Drs. Kenichi Chiba, Makoto Kotake, Yoshihito Eguchi, Hideki Sakurai, Hatsue Ito-Igarashi, Akifumi Kimura, Yoshikazu Kuboi, Kenzo Muramoto, Yoshiharu Mizui, Isao Tanaka, and Takatoshi Kawai. We thank Drs. Seiichi Kobayashi (Eisai, Japan), Ieharu Hishinuma and Nancy Wong (Eisai Inc.) for discussion and helpful advices.

References and notes

1. Ellestad, G. A.; Lovell, F. M.; Perkinson, N. A.; Hargreaves, R. T.; McGahren, W. J. *J. Org. Chem.* **1978**, *43*, 2339.
2. Du, H.; Matsushima, T.; Spyvee, M.; Goto, M.; Shirota, H.; Gusovsky, F.; Chiba, K.; Kotake, M.; Yoneda, N.; Eguchi, Y.; DiPietro, L.; Harmange, J-C.; Gilbert, S.; Li, X-Y.; Davis, H.; Jiang, Y.; Zhang, Z.; Pelletier, R.; Wong, N.; Sakurai, H.; Yang, H.; Ito-Igarashi, H.; Kimura, A.; Kuboi, Y.; Mizui, Y.; Tanaka, I.; Ikemori-Kawada, M.; Kawakami, Y.; Inoue, A.; Kawai, T.; Kishi, Y.; Wang, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6196.
3. A manuscript describing the SAR study on C14-oxygen substitution series has been submitted for publication in the same journal.
4. Mukaiyama's reagent as activating agent for carboxylic acids: (a) Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.* **1976**, 49; (b) Narasaka, K.; Maruyama, K.; Mukaiyama, T. *Chem. Lett.* **1978**, 885.
5. All routes for analog preparations were detailed in this publication: Boivin, R.; Chiba, K.; Chow, J.; Du, H.; Eguchi, Y.; Fujita, M.; Goto, M.; Gusovsky, F.; Harmange, J. C.; Inoue, A.; Jiang, Y.; Kawada, M.; Kawai, T.; Kawakami, Y.; Kimura, A.; Kotake, M.; Kuboi, Y.; Lemelin, C.; Li, X. Y.; Matsushima, T.; Mizui, Y.; Muramoto, K.; Sakurai, H.; Shen, Y.; Shirota, H.; Spyvee, M.; Tanaka, I.; Wang, Y. J.; Yamamoto, S.; Yoneda, N.; (Eisai Co. Ltd, Japan; et al.) PCT Int. Appl., 2003, PCT/US03/07377, WO 2003076424 A1 20030918.
6. Buchwald–Hartwig amination: (a) Wolfe, J. P.; Buchwald, S. L. *Org. Synth.* **2004**, *Coll. Vol. 10*, 423; (b) Louie, J.; Hartwig, J. F. *Tetrahedron Lett.* **1995**, *36*, 3609.
7. (a) Heck, R. F.; Nolley, J. P., Jr. *J. Org. Chem.* **1972**, *37*, 2320; (b) Heck, R. F. *Org. React.* **1982**, *27*, 345.
8. A manuscript describing the detailed in vivo anti-inflammatory studies of synthetic analogs is being prepared.
9. E6201, [(3*S*,4*R*,5*Z*,8*S*,9*S*,11*E*)-14-(ethylamino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9,19-tetrahydro-1*H*-2-benzoxzcyclotetradecine-1,7(8*H*)-dione], a Novel Kinase Inhibitor of Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase (MEK)-1 and MEK Kinase-1: In vitro characterization of its anti-inflammatory and anti-hyperproliferative activities: Goto, M.; Chow, J.; Muramoto, K.; Chiba, K.-i.; Yamamoto, S.; Fujita, M.; Obaishi, H.; Tai, K.; Mizui, Y.; Tanaka, I.; Young, D.; Yang, H.; Wang, Y. J.; Shirota, H.; Gusovsky, F. *J. Pharm. Exp. Ther.* **2009**, *331*, 485.
10. Detailed description on anti-arthritic effects of **21** on collagen antibody induced arthritis model in mice: The arthritis was induced in male BALB/c mice according to the protocol from Chondrex Inc. (Redmond, WA). The mice started to develop arthritis on day 2 after the injection of the antibody cocktail for type II collagen, then the mice were randomized to six groups according to the arthritis score (mean score; 1.5–1.7). Vehicle (0.5% methyl cellulose solution) or serial doses of ER-805940 (3, 5, 10, 20, 30 mg/kg) were orally administered therapeutically on day 2 and 3 (QD × 2). Further arthritis development in each mouse was evaluated on day 4, and the increased arthritis score from the score on day 2 was calculated. Each column represents the mean with SEM (n = 6–7) of the increased arthritis score in each treatment. $^{*}P$ <0.05, $^{**}P$ <0.01; statistically significant as compared with the vehicle control group in unpaired t-test.