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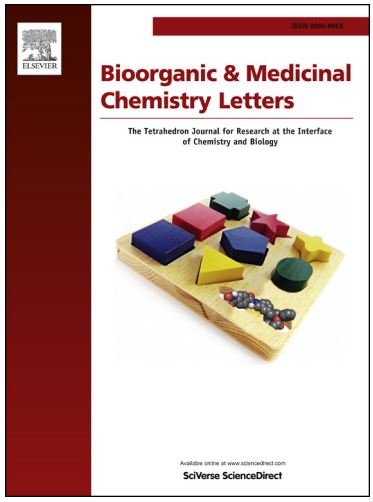

Synthesis and Biological Evaluation of Novel Orally Available 1-Phenyl-6-Aminouracils Containing Dimethyldihydrobenzofuranol Structure for the Treatment of Allergic Skin Diseases

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PII: S0960-894X(16)30019-1
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.01.019
Reference: BMCL 23484

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 7 September 2015
Revised Date: 30 December 2015
Accepted Date: 8 January 2016

Please cite this article as: Tobe, M., Tsuboi, K., Hasegawa, F., Fujiwara, N., Inoue, Y., Isobe, M., Isobe, Y., Synthesis and Biological Evaluation of Novel Orally Available 1-Phenyl-6-Aminouracils Containing Dimethyldihydrobenzofuranol Structure for the Treatment of Allergic Skin Diseases, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.01.019

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Synthesis and Biological Evaluation of Novel Orally Available 1-Phenyl-6-Aminouracils Containing Dimethyldihydrobenzofuranol Structure for the Treatment of Allergic Skin Diseases

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
CX-659S
1-phenyl-6-aminouracils
Dimethyldihydrobenzofuranol
Contact hypersensitivity reaction
Pruritus

ABSTRACT

We have designed and efficiently synthesized novel 1-phenyl-6-aminouracils by replacing the chroman moiety in CX-659S, a nonsteroidal dermatologic candidate, with dimethyldihydrobenzofuranol to cancel CX-659S asymmetric center. Medicinal chemistry effort culminated in the discovery of **13d** bearing a 3-methyl group at the 1-phenyl group as a promising compound. Compound **13d**, having good in vitro ADME profile and moderate oral bioavailability in mice, showed potent anti-inflammatory activity against hapten-induced contact hypersensitivity reaction in mice following topical and oral administration. The effects of **13d** were equipotent to that of tacrolimus or prednisolone. In addition, compound **13d**, having potent hydroxyl radical-scavenging activity, showed more potent suppressive effect on substance P-induced pruritus in mice than oxatomide.

Atopic dermatitis (AD) and allergic contact dermatitis are well-known allergic skin diseases that cause serious inflammation and pruritus, and subsequently lower patient quality of life. Especially, AD is a chronic disease with alternating remission and recurrence that affects both children and adults in industrialized countries.[1,2] Glucocorticoids are widely used in the treatment of allergic skin diseases and work by suppressing allergic responses. However, these steroids produce severe side effects, including steroid withdrawal syndrome, skin atrophia, and increased susceptibility to infection. As current treatment of allergic skin diseases relies mainly on the use of steroids, there is a great need for new dermatologic drugs that can alleviate both inflammation and pruritus without inducing undesirable side effects.

We have previously disclosed that CX-659S (**1**, Figure 1) inhibits hapten-induced acute and chronic contact hypersensitivity reaction (CHR) in mice.[3] In addition, we have shown that CX-659S acts as antioxidant in lipid peroxidation in rat brain homogenate and as scavenger of reactive oxygen species, which can contribute to skin inflammation.[4,5] As shown in Figure 1, CX-659S has an antioxidative chroman moiety that resembles the structure of vitamin E. This moiety plays an important role in CX-659S anti-inflammatory activity,[4] while generating an asymmetric center in the molecule. Development of chiral drug requires overcoming several hurdles, including separation of stereoisomers, determination of absolute stereochemistry and compliance with US Food and Drug Administration guidance for stereoisomeric drugs.[6]

To overcome these drawbacks, we aimed to cancel the asymmetric center of CX-659S, while maintaining its anti-inflammatory activity. Chugai (**2**) and Ono (**3**) scientists have independently reported that the dimethyldihydrobenzofuranol (DDB) acts as a potent antioxidant (Figure 1).[7] As the structure of DDB shows no asymmetry, we extracted it from compounds **2** and **3**, and designed a novel structural class of 1-phenyl-6-aminouracils by replacing CX-659S chroman moiety with DDB (Figure 1). In this report, we established the large-scale synthetic method of the DDB part and synthesized a series of novel 1-

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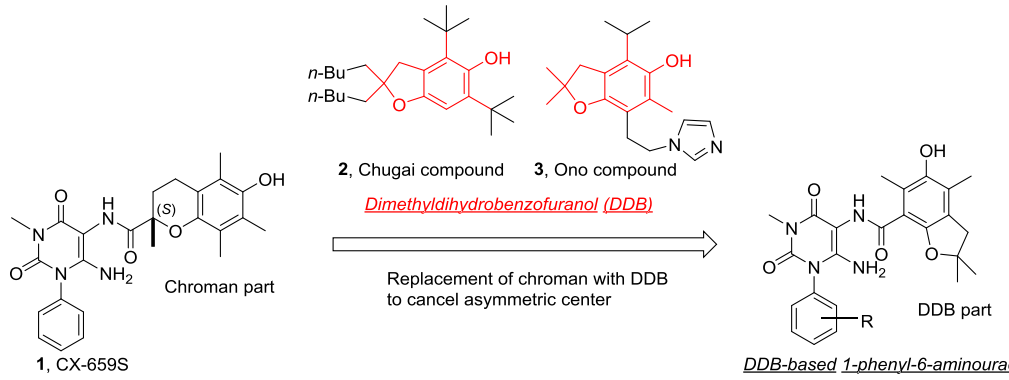
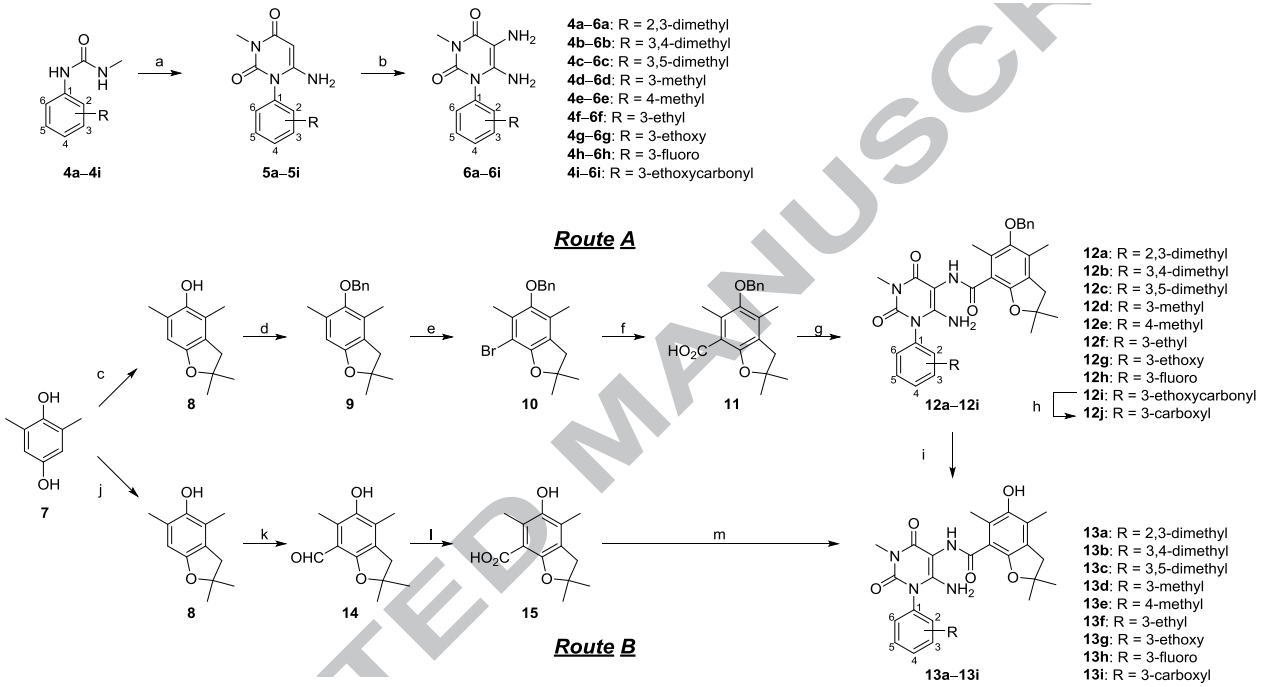


Figure 1. Design of DDB-based 1-phenyl-6-aminouracils using CX-659S



Scheme 1. Reagents and conditions: (a) (i) cyanoacetic acid, acetic anhydride, toluene, 80°C; (ii) 3 M NaOH or DBU, THF, 60–89% (2 steps); (b) (i) $NaNO_2$, 12 M HCl; (ii) 10% Pd/C, H_2, MeOH, 28–98% (2 steps); (c) β-methallyl alcohol, $ZnCl_2$, 12 M HCl, silica gel, 1,2-dichloroethane, 25%; (d) NaH, BnBr, DMF, 90%; (e) Br_2, CF_3CO_2Ag, CH_2Cl_2, 72%; (f) *n*-BuLi, Et_2O, −78°C then dry ice, 45%; (g) **6a–6i**, diphenylphosphoryl chloride, Et_3N, EtOAc; (h) 3 M NaOH, EtOH; (i) 10% Pd/C, H_2, MeOH, 32–89% (from **11**); (j) isobutyraldehyde, 6 M HCl, toluene, reflux, 74%; (k) Cl_2CHOMe, $TiCl_4$, chlorobenzene, 89%; (l) $NaClO_2$, NaH_2PO_4, 1,4-dioxane, *t*-BuOH, H_2O, 83%; (m) (i) **6a–6i**, diphenylphosphoryl chloride, Et_3N, EtOAc, 18–64%; (ii) 2 M NaOH, MeOH, 70% (only for the synthesis of **13i**).

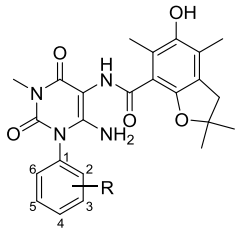
phenyl-6-aminouracils containing the DDB structure. In addition, we evaluated the anti-inflammatory activities of these compounds in mice hapten-induced CHR and assessed the suppressive effects of one of the selected compounds on pruritus in mice.

The general synthetic pathway to the 1-phenyl-6-aminouracils **13a–13i** is shown in Scheme 1. First, the 5,6-diaminouracils **6a–6i** were synthesized based on a previous method.[3,8] Next, as shown in Route A, 2,6-dimethylhydroquinone **7** was reacted with β-methallyl alcohol and zinc chloride under acidic conditions to afford the dimethyldihydrobenzofuranol **8**.[9] After the protection and bromination, the bromo group in **10** was converted to a carboxyl group via lithiation and dry ice trapping. Condensation of **11** with **6a–6i** followed by deprotection of the benzyl group led to **13a–13h**, respectively. As for synthesis of the carboxylic acid **13i**, alkaline hydrolysis of **12i**, followed by deprotection of the benzyl group gave **13i**.

Although we managed to synthesize this series of compounds according to Route A, it was necessary to improve the route for large-scale synthesis. Especially, the synthetic steps of both **8** and the carboxylic acid **11** were problematic due to their low yield (25% and 45%, respectively), troublesome work-up, and necessity of column chromatography. To improve these problems, we conducted an optimization and found the efficient scalable synthetic route shown as Route B in Scheme 1. Using isobutyraldehyde and hydrogen chloride instead of zinc chloride, the large-scale synthesis of **8** was successfully achieved (155.2 g, 74% yield). Next, formylation of **8** by Rieche reaction,[10] followed by Pinnick oxidation afforded the carboxylic acid **15** (98 g, 74% yield for two steps). These synthetic methods were easy to handle and applicable to the large-scale preparation of **15**.[11] Condensation of **15** with **6a–6i**, followed by hydrolysis (only for the synthesis of **13i**) gave **13a–13i**, respectively. This condensation step needed no protection of the phenolic hydroxy group in **15**. Thus, establishment of Route B for a facile synthesis of the 1-phenyl-6-aminouracils enabled us to shorten the synthetic route by two steps compared to Route A.

The 1-phenyl-6-aminouracils listed in Table 1 were evaluated for their topical anti-inflammatory activity against picryl chloride (PC)-induced CHR in mice.[3] Initially, we examined the effects of a dimethyl substitution on the phenyl group at the N(1)-position

Table 1. Effects of topical administration of compounds **13a**–**13i** on PC-induced CHR in mice



Compound	R	% inhibition[a] (0.1 mg/ear)
13a	2,3-dimethyl	ND[b]
13b	3,4-dimethyl	45
13c	3,5-dimethyl	15
13d	3-methyl	52
13e	4-methyl	30
13f	3-ethyl	14
13g	3-ethoxy	16
13h	3-fluoro	10
13i	3-carboxyl	9
Tacrolimus		53

[a] Percent inhibition was calculated from percent response in the drug-treated group as compared to the control group (n = 5). Results are expressed as the mean of 5 animals.
[b] ND: not determined; Compound **13a** could not be evaluated due to poor solubility in application solvent (acetone).

Table 2. In vitro ADME profile of compound **13d**

Solubility[a] (mg/mL)	MS[b] (mL/min/mg protein)	Membrane Permeability[c] (10^{-6} cm/s)
0.021	0.019 (human) / <0.01 (rat)	14

[a] Solubility was determined based on the amount of dissolved **13d** in phosphate buffer solution (pH 7.4).
[b] Metabolic stability (MS) was tested using human and/or rat liver microsomal fraction.
[c] Membrane permeability in Caco-2 cells was determined based on apparent permeability (P_{app}).

Table 3. Compound **13d** PK parameters in mice

Dose (mg/kg)	Route	CL (mL/min/kg)	V_{dss} (L/kg)	AUC (ng·h/mL)	C_{max} (ng/mL)	F (%)
1	iv	18	1.0	927	–	–
10	po	–	–	1096	511	12

Table 4. Hydroxyl radical-scavenging activities

Compound	IC_{50} (μM)[a]
L-Ascorbic acid	82
Trolox®	9.7
CX-659S (**1**)	12.1
13d	8.6

[a] The assay was performed in duplicate (n = 2).

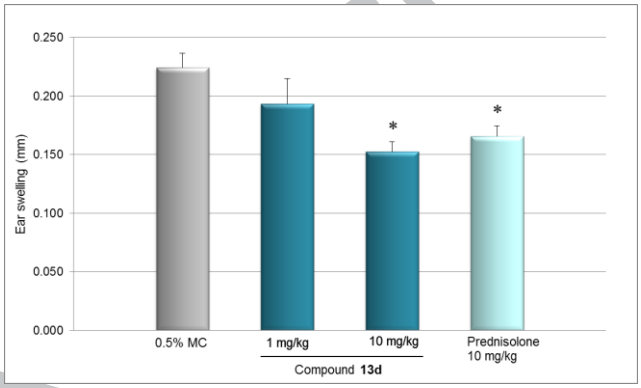


Figure 2. Effect of oral administration of compound **13d** on PC-induced CHR in mice. Mice were sensitized by PC one week before the study. Compound **13d** was orally administered 30 minutes before PC challenge. Data are given as mean ± SEM (n = 5/group). *: $p<0.05$ (Dunnett's test).

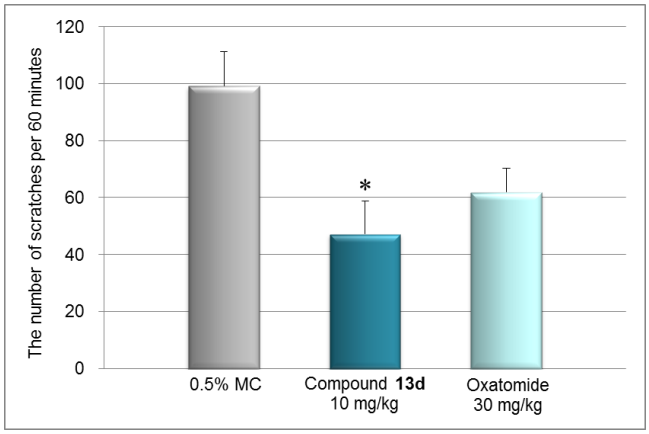


Figure 3. Effect of oral administration of compound **13d** on SP-induced pruritus in mice. Compound **13d** was administered 30 minutes before SP challenge. Bar graphs indicate the number of scratches per 60 minutes. Data are given as mean ± SEM (n = 7–8/group). *: $p<0.05$ (Dunnett's test).

of the uracil ring. Unexpectedly, the 2,3-dimethyl **13a** could not be evaluated for its anti-inflammatory activity due to poor solubility in acetone. In addition, compound **13a** seemed to have axial chirality at the N(1)-position of the uracil ring (data not shown). The 3,4-dimethyl **13b** showed potent anti-inflammatory activity, while the 3,5-dimethyl **13c** had lower activity than **13b**. The potent activity of **13b** urged us to investigate the effects of 3- or 4-methyl substitution on the phenyl group.

The anti-inflammatory activity of the 3-methyl **13d** was comparable to that of tacrolimus, a widely used dermatologic drug. On the other hand, the 4-methyl **13e** had weaker activity than **13d**. With **13d** potent inhibitory activity in mind, we next focused on 3-substitution on the phenyl group. Both the ethyl (**13f**) and ethoxy (**13g**) groups led to decreased activity compared to **13d**. From these results, we assumed that groups bulkier than the methyl group were unfavorable for better activity. However, a small fluoro group (**13h**) also resulted in loss of activity compared to **13d**. Moreover, a carboxyl group (**13i**), which is bulkier and more polar than **13d**, decreased the anti-inflammatory activity. These results suggested that a methyl group was the most suitable substituent at the 1-phenyl group.

On the basis of the above-mentioned results, we selected compound **13d** and evaluated its in vivo oral efficacy. First, we examined compound **13d** water solubility, metabolic stability, and membrane permeability in vitro. The profile of **13d** from these studies is shown in Table 2. Compound **13d** exhibited no serious issues related to ADME parameters. To investigate **13d** oral exposure, we conducted a mice pharmacokinetic (PK) study with this compound (Table 3). Intravenous injection of **13d** resulted in low clearance (CL) and low volume of distribution at steady state (V_{dss}). Given orally, the maximum concentration (C_{max}) of **13d** in plasma reached 511 ng/mL with an area under the concentration curve (AUC) of 1096 ng·h/mL. Compound **13d** bioavailability was moderate at 12%.

Based on **13d** PK profile, we next investigated this compound oral anti-inflammatory activity in mice PC-induced CHR. As shown in Figure 2, compound **13d** dose-dependently suppressed inflammation with significant effect at 10 mg/kg. The potency of **13d** at this dose was comparable to that of prednisolone. These good results prompted us to evaluate the suppressive effect of **13d** on pruritus using substance P (SP)-induced pruritus in mice.[12−15] The results of this evaluation are summarized in Figure 3. In our experiment, oxatomide, a well-known anti-allergic agent, showed a suppressive effect as described in the literatue.[15] Compound **13d** showed significant suppressive effect on pruritus in mice at 10 mg/kg. This effect was more potent than that of oxatomide. These findings indicate that compound **13d** has good potential as an orally active dermatologic drug with both anti-inflammatory and anti-pruritic effects.

Next, we evaluated a hydroxyl radical-scavenging activity of compound **13d** (Table 4).[16] As a result of the evaluation of the several reference compounds, both Trolox® and CX-659S showed more potent activity than L-ascorbic acid which is a well-known hydroxyl radical scavenger. In addition, the order of the activities among the three compounds was consistent with that of the previous report.[17] On the other hand, the hydroxyl radical-scavenging activity of compound **13d** was more potent than that of Trolox® or CX-659S. These results suggest that the hydroxyl radical-scavenging activity of **13d** is one of the contributing factors toward both its potent anti-inflammatory and its anti-pruritic effects.[4,18]

Finally, we set studies to investigate the target molecule of **13d**. Uchi *et al*. previously reported that CX-659S inhibits phosphorylation of mitogen-activated protein kinase/extracellular-signal regulated kinase (MEK) 1/2 in keratinocytes.[19] Based on this report, we examined the inhibitory activity of **13d** against MEK1/2 in an enzymatic assay. We found that compound **13d** weakly inhibits MEK1/2 (data not shown).[20]

In conclusion, we have designed and efficiently synthesized novel 1-phenyl-6-aminouracils by replacing the chroman moiety in CX-659S with DDB to cancel CX-659S asymmetric center. Medicinal chemistry effort on the phenyl group at the N(1)-position of the uracil ring led to the discovery of **13d** bearing a 3-methyl group as a suitable candidate for improved anti-inflammatory activity. Compound **13d** with good in vitro ADME profile and moderate oral bioavailability in mice showed potent anti-inflammatory activity in mice PC-induced CHR. The beneficial effects of **13d** were equipotent to that of tacrolimus or prednisolone. In addition, compound **13d**, having potent hydroxyl radical-scavenging activity, showed more potent suppressive effect on mice SP-induced pruritus than oxatomide. At present, we are conducting further chemical, pharmacological, and toxicological studies on the 1-phenyl-6-aminouracils and will report the findings in future publications.

Acknowledgments

We are thankful to Dr. Takayuki Fukaya, Dr. Tomoya Shiro, Mr. Takahiro Nagasaki and Mr. Masashi Tanaka for their great support throughout this research.

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Graphical Abstract

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