# *In Vitro* Phototoxic Potential and Photochemical Properties of Imidazopyridine Derivative: A Novel 5-$HT_4$ Partial Agonist

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**ABSTRACT:** Drug-induced phototoxic skin responses have been recognized as undesirable side effects, and as we previously proposed the determination of reactive oxygen species (ROS) from photo-irradiated compounds can be effective for the prediction of phototoxic potential. In this investigation, we evaluated the photosensitizing properties of imidazopyridine derivative, a novel 5-$HT_4$ partial agonist, using ROS assay and several analytical/biochemical techniques. Exposure of the compound to simulated sunlight resulted in the significant production of singlet oxygen, which is indicative of its phototoxic potential. In practice, an imidazopyridine derivative under UVA/B light exposure also showed significant photodegradation and even photobiochemical events; peroxidation of fatty acid and genetic damage after DNA-binding, which are considered as causative agents for phototoxic dermatitis. Interestingly, both photodegradation and lipoperoxidation were dramatically attenuated by the addition of radical scavengers, especially singlet oxygen quenchers, suggesting the possible involvement of ROS generation in the phototoxic pathways. In the 3T3 neutral red uptake phototoxicity test, imidazopyridine derivative also showed the phototoxic effect on 3T3 mouse fibroblast cells. These results suggest the phototoxic risk of newly synthesized imidazopyridine derivative and also verify the usefulness of ROS assay for phototoxicity prediction.
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## INTRODUCTION

Several classes of drugs including antibacterials, thiazide diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs), quinolones and tricyclic antidepressants, even when not toxic by themselves, may

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become reactive under exposure to environmental light, inducing undesired side effects.[1] In many cases of drug-induced phototoxicity, skin reactions can be triggered by dose of sunlight, regarded as harmless most often in the UVA range (320–400 nm). There are at least three types of drug-induced phototoxic skin reactions; photo-irritant, photogenotoxic and photoallergic cascades, the mechanism and pathologic features of which are quite different.[2] New pharmaceutical or cosmetic compounds must have their phototoxic potential tested when they absorb wavelengths in the range of sunlight, composed of UVA and partial UVB (above 290 nm).[3,4] There is thus an obvious need for a phototoxic screening strategy based on complementary *in vitro* tests.[5]

A lot of photochemical experiments indicated that the primary event in any photosensitization process is the absorption of a photon energy, and the following generation of reactive oxygen species (ROS); superoxide through type I reaction and singlet oxygen through type II reaction by photo-excited drug molecules, appear to be the principal intermediate species in the phototoxic response.[6,7] In this context, we previously proposed that ROS assay is useful for understanding the type of drug-induced photochemical responses, and it can be indicative of phototoxic potential as well.[7] Actually, in some known phototoxic drugs, there appeared to be good relationship between ROS generation and induction of phototoxic skin responses. Thus, the usefulness of ROS assay for the risk assessment of phototoxicity was confirmed partly, and the present study is aimed to evaluate phototoxic potential of newly synthesized drug candidate using ROS assay and to verify the predictability of ROS assay.

Imidazopyridine derivative (compound A); 5-Amino-6-chloro-*N*-[(1-isobutylpiperidin-4-yl)methyl] 2-methylimidazo[1,2-a]pyridine-8-carboxamide (Fig. 1) was designed as 5-$HT_4$ receptor agonist for the clinical treatment of gastroesophageal reflux disease (GERD).[8] Compound A has a potent chromophore, imidazopyridine moiety, suggesting UVA-absorbing potential. Furthermore, previous works indicated that some imidazopyridine derivatives have an ability to bind with biomolecules and photosensitize them,[9,10] which may lead to phototoxic responses. In the present study, the phototoxic potential of compound A was assessed with the use of ROS assay, and the photochemical and photobiological behaviors of compound A were also evaluated by analytical and biochemical methodologies. To determine the photoreactivity, both solid and solution-state photostability studies on compound A were carried out, and we also investigated the inhibitory effect of various ROS scavengers on the photodegradation, for recognizing the possible degradation pathways. In addition, photobiological properties of compound A were examined, focusing on lipid peroxidation by TBA assay and DNA damage by capillary gel electrophoretic (CGE) study, as well as photochemical cytotoxicity by 3T3 neutral red uptake phototoxicity test (NRU PT). We also evaluated the DNA-binding activity of compound A by UV and CD spectral analyses, which might be important factor of the drug-induced photogenotoxicity.

## MATERIALS AND METHODS

### Chemicals

Quinine, sulisobenzone, and ROS scavengers, such as butylated hydroxyanisole (BHA), reduced gluthatione (GSH), sodium azide ($NaN_3$), DABCO (1,4-diazabicyclo[2,2,2]octane) and superoxide dismutase (SOD), were purchased from Sigma (St. Louis, MO) or Wako Pure Chemical Industries (Osaka, Japan). Salmon sperm DNA, plasmid pBR322 DNA, linoleic acid, thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), *p*-nitrosodimethylaniline, imidazole, nitroblue tetrazolium, 1,1,3,3-tetraethoxypropane, and Tween-20 were obtained from Wako Pure

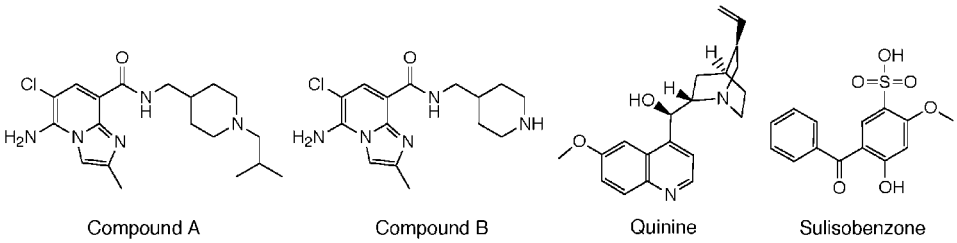


**Figure 1.** Structures of chemicals tested; compound A, an imidazopyridine derivative, compound B, a main metabolite of compound A, quinine and sulisobenzone.

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