Comprehensive Synthesis of Photoactive Phenylthiourea Derivatives for the Photoaffinity Labeling


Abstract: Phenylthiourea (PTU) is well known as bioactive compound and one of the reversible inhibitors for tyrosinase. Tyrosinase plays very important roles for tyrosine metabolisms to maintain the skin homeostasis from sunlight with forming melanin. Synthesis of photoaffinity label reagents of PTU will be attractive thesis to elucidate functional analysis for tyrosinase.

Introduction

Phenylthiourea (PTU) and its synthetic derivatives are well-known as bioactive compounds that have high affinity for urea channels in human red cells,[1] bitter tastants[2] and tyrosinase inhibitors. [3] Tyrosinase is an enzyme that oxidizes the phenolic part of tyrosine, forming dihydroxyphenylalanine (DOPA) from tyrosine and further oxidizing of DOPA to dopaquinone. Dopquinone plays an important role in melanin formation in sunburns. Inhibition of melanin formation thus finds promising applications in skin-whitening (i.e., sun protection).[3] PTU derivatives are well-known reversible inhibitors for tyrosinase. The effects of substitution on aromatic rings inhibit tyrosinase have been reported.[3] Photoaffinity labeling[3] is a useful biochemical method for analysis biological interactions between low-molecular-weight bioactive compounds and biomolecules. The methodology may afford other information near the binding sites, which cannot obtain from structure-activity relationship studies. A few studies have elucidated biological activities on the synthesis of azide[6, 7] and benzoyl (benzophenone)[8] attached to the aromatic ring of PTU. However no study on typical photophores with their biological activities has been reported. In this paper, we describe the comprehensive synthesis of photoreactive PTU derivatives (Fig.1), which we then used in an inhibitory activity assay for tyrosinase.

Results and Discussion

Azide PTU derivatives: Lamotte et al. reported the synthesis of 4-azide PTU from the corresponding 4-nitro PTU derivative, but they did not give a detailed description of the synthesis.[8] Another study constructed the 3-azide-4-chloro PTU derivative by using ammonia and an isothiocyanate moiety. This route utilized thiophosgene to produce isothiocyanate.[7] Our synthetic strategies for both 3- and 4- isomers are identical and do not use highly toxic reagents. We reduced 3- (1a) or 4- (1b) nitro substituted PTU with iron under acidic conditions to form aniline derivatives (2a and b). The aniline derivatives were subjected to diazotization followed by azidation to form phenylazide derivatives (3a and 3b) at moderate yields (Scheme 1).

Scheme 1. Synthesis of phenylazide derivatives of phenylthioureas (3a and 3b)

Benzophenone PTU derivatives: Kamel et al. reported that 4- aminobenzophenone reacts with potassium thiocyanate, forming a PTU derivative.[8] In our preliminary experiments, however, m-toluidine did not react under the conditions they used. To establish general methods for the preparation of 3- (4a) or 4- (4b) aminobenzophenones were converted to benzoyl-protected thiourea derivatives by using ammonium thiocyanate and benzoyl chloride[9], and then deprotected in alkali to afford benzophenone derivatives of PTU (5a and 5b) at moderate yields (Scheme 2).

Scheme 2. Synthesis of benzophenone derivatives of phenylthioureas (5a and 5b)

3-trifluoromethyl diazirinyl (3-TFMD) derivatives: A few papers report the synthesis of 3-TFMD-aniline[10-12] which is a precursor for PTU derivatives. A common method using formamide-protected trifluoroacetophenone has been utilized to protect the aniline group.[10] After construction of TFMD, the formyl group was deprotected under acidic conditions, and the

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amine generated in situ was utilized for next reaction without further purifications.[11] Previous yields of diazirine from synthesis using four steps are very low (less than 30% each step, over all yield was 0.6%)[10], and the efficiency of deprotection of the formamide moiety is not established (less than 70%).[11a] Another method for generating the aniline skeleton is the reduction of nitrobenzene derivatives. The previously reported procedures contained several obscure points. (For example, the yields is over >100% chemical yields for 2 steps), and that of the a reduction of 3-nitrophenyl-TFMD to 3-aminophenyl-TFMD was less than 50%.[12]

On the basis of the reported procedures, we studied the synthesis of 3-aminophenyl-TFMD and attempted it to prepare PTU derivatives. We selected the nitro and NHBoc groups to compare the synthesis of 3-aminophenyl-TFMD. Trifluoroacetophenone 6 was nitrated to produce 3-nitro trifluoroacetophenone 7a and the minor product, its 2-nitro isomer (<15%). Reduction of 7a using iron followed by Boc protection led to the formation of 3-Boc-aminophenyl derivatives 7c. Compounds 7a and 7c were converted to oximes (8a and c) and O-tosylxime derivatives (9a and c). The O-Tosylximes were then subjected to stepwise conversion[12] to 10 and 11, and 9 was directly converted 11 via one-pot synthesis[14] to construct the TFMD moiety. One-pot conversions led to yields higher than those of the stepwise methods. Reductions of nitro group with iron or sodium bisulfide led to the formation of 3-nitrophenyl-TFMD (11a) and 3-aminophenyl-TFMD (12) with satisfactory yields. Treatment of Boc-aminophenyl-TFMD (11c) under acidic conditions produced 12, which was stable at 20°C for at least 1 month, at >90% yield. Our improvements thus promoted the synthesis of 3-aminophenyl-TFMD 12 at >60% yield from acetoephone derivatives (7a and 7c).

Compounds 12 were treated with ammonia thiocyanate and benzoyl chloride under reflux conditions to form benzoyl PTU derivatives. Alkaline hydrolysis of the reaction mixture produced 3-TFMD-modified PTU 13, which retains the TFMD moiety, at moderate yield (Scheme 3).

**Scheme 3.** Synthesis of 3-trifluoromethylaziridinyl derivative of phenylthiourea (13) from 2,2,2-trifluoroacetophenone derivatives 7a and 7c.
Scheme 4. Synthesis of 4-trifluoromethyl diazirinyl derivative of phenylthiourea (22) from 2,2,2-trifluoroacetophenone derivatives 16a–16e

Triphenylphosphine did not result in satisfactory yields because of the broken TFMD moiety during the reaction. Deprotection of the Boc group of 20e resulted in 4-aminophenyl- TFMD (21; >90% yield). Very recently, similar studies have been done to synthesize 21,[15] but the total yields of 21 from the common intermediates 16b via 16e (>40%) are more than twice those reported in these studies.

Compound 21 was used in thiourea formation. Refluxing with NH₄SCN and benzoyl chloride, which is identical to that used in the synthesis of 5 and 13, promoted decomposition of TFMD, converting the aniline moiety to urea during the reaction. Benzoyl isothiocyanate was used in the reaction to enable it to proceed at a lower temperature. The reaction at room temperature produced 4-TFMD-PTU 22 in moderate yield without decomposition of the TFMD moiety.

We also attempted to construct TFMD using PTU derivatives. 4-Trifluoroacetylaniline (23), which was easily prepared from phthalimide derivatives 16d, was converted to benzoylthiourea derivatives 24. Although the oximation of 24 occurred smoothly, tosylation of oxime 25 could not proceed in the general methods. Mesylation, which has reactivity greater than that of tosylation, also did not be afforded desired products.

Photoreactivity of synthesized PTU derivatives: The synthesized PTU azides (3a and 3b) derivatives were to photoirradiate in methanol using a mercury lamp. Similarly, the derivatives benzophenones (5a and 5b) and diazirines (13 and 22) were exposed using a black light. Each photolyzed mixture was analyzed by mass spectrometry. Methanol adduct signals for all samples were measured after 1 hour irradiation, and UV spectra for the diazirines were subjected to time-course analysis.
Photolysis of diazirine-based PTU derivatives 13 (a) and 22 (b) in methanol (1 mM) with black light (15W). UV spectra of the photolysis reaction were recorded every 1 min for 10 min, then every 10 min for 60 min. (Figure 2). Time-dependent decrease in maximum absorption at around 360 nm indicates that the synthetic compounds have enough photoreactivity for photoaffinity labeling. The azide derivatives (3a and 3b) are also same reactivity, but benzophenone derivatives (5a and 5c) are very low reactivity than these two photophores (SI). These results are consistent with previous reports.[6]

**Tyrosinase inhibitory activity assay:** The synthesized photoreactive PTU derivatives were used as inhibitors in the tyrosinase assay for tyrosine and DOPA as substrates. The PTU derivatives that contained photophores at 3-position (3a, 5a, and 13), presented high inhibitions for tyrosine oxidation as same as mother skeleton PTU. Compounds with photophores introduced into the 4-position were less inhibitory (Figure 3, A). On the other hand, azide substitutions (3a and 3b) resulted in inhibitory activity for DOPA oxidations that is higher than that achieved with the other two photophores (Figure 3, B). These results are consistent with substitution sites in the substrate were influenced for inhibitory activity of photoreactive PTU derivatives. On the basis of these results, a detailed analysis of the bioactivity of the photoreactive PTU derivatives was performed in addition to their functional analysis.
Figure 3. Inhibitory activities of synthetic photoreactive PTU derivatives (3a, 3b, 5a, 5b, 13 and 22) for tyrosinase. Details of the inhibitory activities for all compounds, along with standard deviations, are summarized in the Supporting Information.

Conclusions

The comprehensive synthesis of photoaffinity label reagents for PTU is firstly reported. Re-investigation for the synthesis of azide and benzophenone derivatives, establishments of the synthesis 3- and 4-aminophenyl-TFMD, and urea conversions from aniline derivatives based on the stabilities of the compounds are also reported. The photoreactive PTU derivatives have identical activities for mother compound PTU. These results indicate that the photoreactive PTU has enough affinity, allowing its use in the functional analysis of physiological conditions such as eating disorders. Such analysis ultimately provides an understanding of the underlying molecular mechanisms of biological processes.

Supporting Information Summary Paragraph

Experimental section, 1H and 13C NMR spectra, photolysis of azide- and benzophenone derivatives, and kinetic investigation of tyrosinase assay.

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Keywords: Benzophenone • Diazirine • Phenylazide • Phenylthiourea • Photoaffinity label

[17] Details of the inhibitory activities for all compounds along with standard deviation, are summarized in the Supporting Information.
Comprehensive synthesis of photoreactive phenylthiourea derivatives were achieved to elucidate photoaffinity labelling. The synthesized compounds were subjected to tyrosinae.

Keywords: Photoaffinity label / Phenylthiourea / Phenylazide / Benzophenone / Diazirine

(KeyTopics: Heterocycles)