氏　　名	ムハンマド　アスワド MUHAMMAD ASWAD
学位の種類	博士（薬科学）
学位記番号	富医薬博甲第 208 号
学位授与年月日	平成 28 年 6 月 22 日
学位授与の要件	富山大学学位規則第 3 条第 3 項該当
教育部名	富山大学大学院医学薬学教育部 博士後期課程 薬科学専攻
学位論文題目	Novel click-type reaction of thioamides with sulfonyl azides and its application for glucosidase inhibitor （チオアミドとスルホニルアジドの新規なクリック型カップリング反応とそのグルコシダーゼ阻害剤への展開）

論文審査委員

（主査）	教　授	井上　将彦
（副査）	教　授	森田　洋行
（副査）	教　授	友廣　岳則（指導教員）

論 文 内 容 の 要 旨

Introduction

Various click-type coupling reactions have been remarkably developed in fields of organic chemistry, material chemistry, and pharmaceutical sciences including drug discovery. Among them, in our research group, sulfo-click reaction has been used for the efficient derivatization of neuraminic acid to create neuraminidase inhibitors. However, thio acids, the sulfo-click reactants, frequently display fatal chemical instability that causes some trouble in sulfo-click reaction.

During searching of reactive groups to replace thio acids with other stable sulfur functional group, a thioamide was found to react with a sulfonyl azide to generate a sulfonyl amidine linkage. Since thioamides are easy to prepare and exhibit greater thermal stability than thio acids, this reaction could be useful as one of click-type reactions in biological usage. The aim of this doctoral research has been focused on development and application of altered sulfo-click reaction between thioamides and sulfonyl azides. In chapter 1, general scope of the coupling reaction and a plausible reaction mechanism are described, meanwhile reaction nature of the coupling reaction is explained according to both of the experimental and computational analyses in chapter 2. Subsequently, chapter 3 shows a bio-application that is a rational development of new glucosidase inhibitors by means of the coupling reaction.

Chapter 1: Development of a catalyst-free coupling reaction between thioamides and sulfonyl azides under mild conditions

Initially, a simple thioamide (thioacetamide) and a simple sulfonyl azide (mesyl azide) were coupled by stirring in various solvents at room temperature for 15 h. The reaction progressed in most solvents and yielded *N*-methanesulfonylacetamidine without side reaction. The reaction occurred in the absence of any activation additives such as metal ions or bases under mild conditions. Polar protic solvents such as MeOH, EtOH and water were preferred for the reaction and water gave the best yield of 63%. The preference for polar protic solvents is consistent with a plausible mechanism because such solvents would be expected to stabilize the polar and zwitterionic **T*** structure (resonance structure of thioamides) by hydrogen-bonding interactions.

Next, with the aim of optimizing the coupling reaction, the substituents on the thioamides and sulfonyl azides were varied systematically. The reactions were carried out in EtOH at room temperature for 15 h**.** Comparisons of methylsulfonyl and phenylsulfonyl azides did not show a significant effect on the reactivity against the same thioamide. Similarly, the substituents on the nitrogen atom of thioamides appeared to be relatively tolerant to the reactivity, although a single methyl group leads to diminished yields. Yields also decreased drastically when the substituent next to thiocarbonyl group of thioamide was a phenyl rather than a methyl group. The steric hindrance probably diminished the reaction efficiency. For the cyclic thioamides, ring size significantly affects the product yield when they were reacted with sulfonyl azides at room temperature. Overall, all of the substrates displayed excellent yields (90–99%) under reflux conditions.

In aqueous environment, the coupling reaction revealed the highest performance. All of the entries showed excellent isolated yields (83-99%) within 15 h at ambient temperature. *N*−Substituted, *N*,*N*−disubstituted, and cyclic thioamides proceeded the coupling reaction without difficulty in water. The combination of 2-thiopiperidone and phenyl sulfonylazide proved optimal, affording 99% yield of the product after only 1 h reaction at room temperature. These results suggest that this particular coupling reaction could be very useful not only for the production of sulfonyl amidines in organic synthesis but also for direct click-type ligations of biomacromolecules. In this context, the mild aqueous conditions and the absence of any additives are notable.

Chapter 2: Reaction nature of coupling reaction between thioamides and sulfonyl azides

In chapter 1, a similar reaction mechanism to sulfo-click reaction was suggested for the coupling reaction of thioamide and sulfonyl azide, in which ionic states mainly contributed to the reactivity. Based on the mechanism, electrophilicity of the azide group should be anticipated to play an important role for the coupling reaction. Thus, coupling reactions were performed between several substituted benzenesulfonyl azides and thioacetanilide to compare their reactivity. After stirring for 20 h at room temperature in EtOH, benzenesulfonyl azides substituted at para position of benzene ring with an electron-donating group, –Me or –OMe, gave the low isolated yields around 40%. In contrast, derivatives substituted with an electron-withdrawing group such as $–NO_2$ or $–CF_3$ afforded good yields, and 3,5-bisCF_3 substituted derivative showed the best performance in short reaction time. Density functional theory (DFT) calculation (RB3YLP//6-311+G(d,p)) of the azides revealed that the charge of N_3 as an approximate indicator of the electrophilicity displayed good correlation to the reactivity.

Next, a hypothesis for thioamide was set up that both of the nucleophilicity on sulfur (δ^-) and the electrophilicity on thiocarbonyl carbon (δ^+) would be important for the reactivity according to the predicted reaction pathway. In other word, dipole moment of thioamides would seemingly influence to the reactivity. Thus a series of thioacetanilides with various substituents on the phenyl ring were coupled with benzenesulfonyl azide to determine the reactivity due to their isolated yield. Meanwhile their dipole moment was estimated by DFT calculation. Since each thioamide derivative exhibits its dipole moment with different orientation, a decomposed-vector component of the dipole moment along with the C=S double-bond direction, $D_{C=S}$, is brought into for fair comparison toward the reactivity. 4-Methylthioacetanilide showed moderate reactivity with almost the same isolated yield and $D_{C=S}$ as those for thioacetanilide. By means of the introduction of MeO– group, 4-methoxyl derivative exhibited small $D_{C=S}$ with low reactivity. In contrast, 4-trifluoromethyl derivative displayed high $D_{C=S}$ with good reactivity in 81% yield. As a consequence, $D_{C=S}$ would be a good indicator for the reactivity. On the other hand, NO_2-tethered derivative displayed slightly large $D_{C=S}$ but low isolated yield, owing to the very large ΔE, energy difference between LUMO of azides and HOMO of thioamides, originated from the significant stabilization of the HOMO energy level by the introduction of a powerful electron-withdrawing nitro group. The similar tendency was observed for 3,5-bisCF_3 derivative possessing two electron-withdrawing groups and a large ΔE with a moderate $D_{C=S}$, providing low reactivity. These findings indicate that ΔE would be an important factor as well as $D_{C=S}$ for the reactivity. Fluorine-substituted derivative afforded moderate reactivity with slightly favorable $D_{C=S}$ and somewhat unfavorable ΔE, suggesting the importance of balance between ΔE and $D_{C=S}$ for the reactivity.

Efficiency prediction of the coupling reaction toward several thioamides and sulfonyl azides combination was performed by means of preliminary DFT estimation of charge of N_3, $D_{C=S}$, and ΔE.

Pentafluorobenzenesulfonyl azide and 3-trifluoromethylthioacetanilide were nominated for the most reactive combination. As expected, simple mixing of these compounds in EtOH at room temperature afforded the product amidine in a good isolated yield within 2 h.

Chapter 3: Promising new α− and β−glucosidase inhibitors derived from the coupling reaction between gluconothiolactam and sulfonyl azides

Inspiring from the structural similarity of iminosugars with 2-thiopiperidone, a well-reactive thioamide substrate in chapter 1, simple derivatization of nojirimycin was conducted to develop novel inhibitors for glucosidases. A cyclic thiaomide compound of nojirimycin, gluconothiolactam, could be obtained from gluconolactone derivatives. Coupling reaction of the gluconothiolactam with several sulfonyl azides successfully afforded gluconosulfonyl amidines as potential candidates for novel glucosidase inhibitors.

Inhibitory effect of these compounds was investigated toward α- and β-glucosidases by means of a conventional spectroscopic methodology with 4-nitrophenyl glucopyranosides (α- and β-NPG). Among them, a phenyl-substituted derivative exhibited stronger inhibition than 1-deoxynojirimycin against both α−glucosidase from *S. cereviceae* and β−glucosidase from almonds.

Conclusion

The mild, catalyst-free coupling of thioamides and sulfonyl azides to generate sulfonyl amidines had been described. This reaction was conducted by simply mixing the thioamides and sulfonyl azides at room temperature. Reactants bearing aromatic and aliphatic substituents are tolerated, as are cyclic skeletons in the case of the thioamides. Furthermore, the reaction is compatible with wide range of different solvents. Of those evaluated, water exhibits the highest performance with respect to efficiency. Importantly, toxic metal ions or other activation additives are not needed. Dipole moment of thioamides and electrophilicity of sulfonyl azides are likely to be significant factors for the reaction efficiency. Since sulfonyl amidines possesses a positive charge, this reaction could be listed as a unique method to create a potential compound library. For the application in drug discovery, novel α- and β-glucosidases derived from the coupling reaction were generated for promising compounds in medicinal chemistry.

[References]

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学 位 論 文 審 査 の 要 旨

小分子阻害物質の開発は生命科学研究あるいは創薬に必要不可欠にもかかわらず、最近の新規医薬品登録件数の大幅な減少は深刻である。その効果的な探索のために、有用な化合物ライブラリーの構築が重要視されており、新奇な骨格を有する既存化合物の収集や新しいカップリング反応の開発等が進められている。近年、多様な炭素–炭素結合反応や官能基選択的連結反応が開発され、後者は生体分子への応用面でも注目されている。本研究では、特に生体直交性の選択的反応特性を示す 1,3-双極子付加環化反応に着目し、その類似官能基を探索した結果、チオアミドとスルホニルアジドによるスルホニルアミジン形成反応を見出した。多様な誘導体合成を通して本反応の最適条件や汎用性を評価し、また計算化学を併用して反応機構を明らかにした。さらにその反応特性と生成物の物性を利用して、本反応をイミノ糖アナログ合成に展開し、高いグルコシダーゼ阻害活性を有する化合物作製に成功した。

１．チオアミド–スルホニルアジドのクリック型カップリング反応の確立

チオアセトアミドとメタンスルホニルアジドを用いて本カップリング反応を評価し、非極性溶媒に比べてプロトン性溶媒、特に水中で最も反応し易く、また、触媒など添加物を必要とせず室温で進行し、副反応を起こさない等、優れた特性を見出した。また、一連の誘導体を合成し、スルホンアジドではベンゼンなど芳香族誘導体が脂肪族誘導体に比べやや反応活性は高く、チオアミドでは逆に芳香族誘導体は反応活性が低下すること、チオノラクタム系では環のサイズで反応性が大きく変化し 6 員環で最大になること、その他 N 置換基の影響など、本反応の性質を詳細に評価した。以上、新規カップリング反応を発見し、その一般性および適用範囲を示した。

２．チオアミド–スルホニルアジドカップリング反応の制御因子に関する考察

反応機構や制御因子を明らかにすることは、本反応を応用する上で極めて重要である。本研究項目では、各種誘導体における反応性と、密度汎関数法（DFT）を用いた電子密度計算による考察から、反応初期過程における本反応の制御因子を明らかにした。DFT を用いたベンゼンスルホニルアジドとチオアセトアニリドのフロンティア軌道計算から、前者の LUMO と後者の HOMO の組合せが妥当であること、環化反応は協奏的ではなく、まずチオアミド基の硫黄原子によるアジド基への求核反応が起こることが示唆された。申請者はベンゼン環に多様な置換基を導入した各種反応基質を合成し、実際の反応活性を精査し

た結果、このカップリング反応性にはアジド基の求電子性とチオカルボニル基の双極子モーメント、及び HOMO-LUMO 間のエネルギー差が重要であることを明らかにした。実際、それら計算値に基づいて設計したフッ素置換化合物は高い反応性を示しており、理論の裏付けに成功した。

３．グルコシダーゼ阻害剤への展開

グルコノチオラクタムとスルホニルアジドとのカップリングにより、グルコシダーゼの糖加水分解反応に高い阻害効果を示す誘導体を合成した。この開発では、反応で形成されるスルホニルアミジン骨格の特性を効果的に利用することに成功している。即ち、本反応生成物は二重結合構造を持ち、遷移状態のオキソカルベニウムイオンを基本とする従来阻害剤の構造に近い。しかも、グアニジノ基は生理条件下で正電荷を持ち、これは同じように阻害効果を示す 1-デオキシノジリマイシンと同じ特性である。反応速度論的解析から混合阻害様式が示唆されたたが、α，β グルコシダーゼ双方に対して 1-デオキシノジリマイシン以上の阻害活性を示す化合物合成に成功した。カップリング反応では、グルコノチオラクタムのヒドロキシ基を保護することなくスルホニルアミジン誘導体に導いており、更なるバリエーションが可能であることを示している。

以上のように、温和な条件下、無触媒で進行し、副反応がなく、水中で最も高い反応性を示す優れたカップリング反応を見出し、多様な誘導体合成と電子密度計算によりその反応を制御する因子を明らかにした。さらに、この反応特性を十分に理解し、生成物構造の特徴を的確に捉えることで酵素阻害剤開発に展開した。新規反応の開発から応用に繋げた研究推進力と成果は十分に評価できる。この申請者の研究は、その反応特性と独特の物性の観点から、創薬面で高いポテンシャルを有する化合物ライブラリー構築の基盤技術として期待される。

主査及び副査は、申請者 Muhammad Aswad の論文内容について審査を行うと共に面接試験を行い、博士（薬科学）を授けるに値するものと判定した。

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