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Metal-free quinoylation of the primary amino groups of amino acid derivatives and peptides with dihydrooxazolo[3,2-*a*]quinoliniums†

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The chemical modification of the primary amino groups of amino acid derivatives and peptides is an important process in the pharmaceutical industry and the field of chemical biology. However, suitable reactions that can be carried out under mild and environmentally friendly conditions are limited. We present a versatile method to selectively modify primary amino groups using novel dihydrooxazolo[3,2-*a*]quinoliniums in 1-butanol as solvent under mild and metal-free conditions. The application of this method to peptides with primary amino, secondary amino, amide, alcoholic hydroxyl, phenolic hydroxyl, disulfide bond, ester and cyano groups revealed that only the primary amino groups were selectively modified, suggesting that this method is compatible with other reactive moieties. We also demonstrated that the quinoylation of existing peptides affected peptide bioactivity and stability, indicating that the novel dihydrooxazolo[3,2-*a*]quinoliniums can be widely applied, especially in medicinal chemistry and chemical biology.

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Introduction

Peptide drugs interact with target proteins with highly specific binding capacity, high versatility, low immunogenicity and low toxicity, thereby attracting considerable interest in the pharmaceutical industry.^{1–5} The number of approved peptide drugs has been increasing in recent decades, and the drugs cover a broad range of therapeutic areas.^{6–8} Reported peptide drugs include atosiban (obstetrics),⁹ degarelix (oncology),¹⁰ liraglutide (metabolic disease),¹¹ tesamorelin (anti-infective),¹² peginesatide (hematology),¹³ linaclotide (gastroenterology),¹⁴ afamelanotide (dermatology),¹⁵ taltirelin (CNS)¹⁶ and teriparatide (osteoporosis).¹⁷ Despite their advantages, peptides have a few drawbacks such as low cell membrane permeability, metabolic

instability, poorly oral bioavailability and relatively short circulating half-life.^{18,19} Therefore, chemical modification strategies are essential to enhance the druggability of peptides. For instance, the angiotensin-converting enzyme (ACE) inhibitor captopril is obtained by optimizing a venom oligopeptide derived from the Brazilian viper.²⁰

Several strategies have been developed for the chemical modification of primary amino groups in amino acid derivatives and peptides, including acetylation,^{21–23} alkylation,²⁴ oximation,^{25–27} arylation²⁸ and quinoylation.²⁹ The quinoline skeleton is a prevalent structure in small-molecule drugs, including quinine sulfate (antimalarial),³⁰ montelukast sodium (asthma),³¹ and saquinavir (anti-HIV).³² Furthermore, the quinoylation of primary amino groups can also be used to generate peptide–drug conjugates (PDCs) and is thus important in the development of both peptide and PDC drugs. To the best of our knowledge, Buchwald–Hartwig amination is currently the only available quinolinylation method and requires metal catalysis, expensive ligands and high temperature (Fig. 1a).²⁸ Furthermore, no suitable method is available for the direct quinoylation of amino acid derivatives and peptides. Accordingly, new metal-free strategies with mild reaction conditions and good group compatibility are needed to selectively modify the primary amino groups of amino acid derivatives and peptides with the goal

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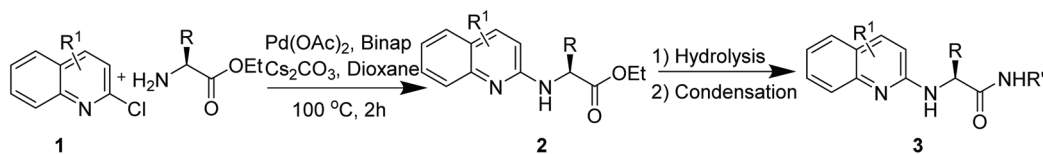
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a) N-quinolylation with palladium catalyst



b) N-quinolylation via a metal-free method (this work)

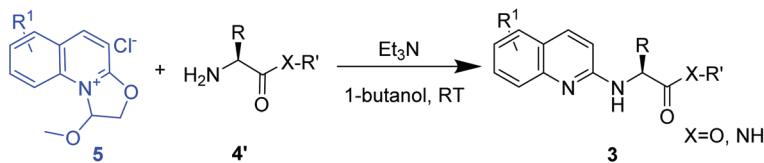


Fig. 1 N-Quinolylation of amino acid residues: (a) palladium-catalyzed Buchwald–Hartwig reaction; and (b) metal-free click reaction (this work).

of promoting the development of peptide drugs with green chemistry characteristics.

Previously, we disclosed a novel oxazoline[3,2-*a*]pyridinium that was treated with different nucleophiles for performing regioselective and metal-free C–O and C–N bond-cleaving to afford heterocyclic *N*-substituted pyridones and 2-substituted pyridines.³³ These results inspired us to investigate whether the oxazoline[3,2-*a*]pyridinium is suitable for coupling with amino acid residues. In this study, we found that the quinoline quaternary ammonium salt can be used to modify the primary amino group (Fig. 1b and S1†) under mild reaction conditions without the need for a heavy-metal catalyst. Therefore, we further optimized this reaction.

Results and discussion

Table 1 summarizes the optimization results of the reaction conditions. **5a** was prepared according to the reported methods,^{33,34} and the synthetic details are summarized in the ESI.† After preparation, **5a** was dissolved in different solvents, and 2-amino-*N*-methylacetamide was added to the reaction mixture to furnish the *N*-methyl-2-(quinolin-2-ylamino)acetamide **6**. To determine the ideal reaction conditions, different bases (Table 1, entries 1–7) were screened as additives, and Et₃N (Table 1, entry 7) was found to be the best. After exploration of different solvents, 1-butanol (Table 1, entries 7–18) was found to be ideal. Higher equivalents of 2-amino-*N*-methylacetamide led to an increased yield of **6** (Table 1, entries 7 and 19–21). Accordingly, two equivalents of 2-amino-*N*-methylacetamide were chosen for the reaction.

Using the optimized reaction conditions (Table 1, entry 20), we explored a variety of amino substrates bearing different substituents (Table 2). We found that α -amino amides with different substitutions at the α position gave the quinolyated products in 63%–92% yields over two steps (**6a**–**6d**, **6f**). Among these, (*S*)-2-amino-*N*-methylpropanamide afforded the (*S*)-*N*-methyl-2-(quinolin-2-ylamino)propanamide **6a** in 92% yield. By switching to 2-amino-*N*-3,3-trimethylbutanamide, which

Table 1 Optimization of reaction conditions^a

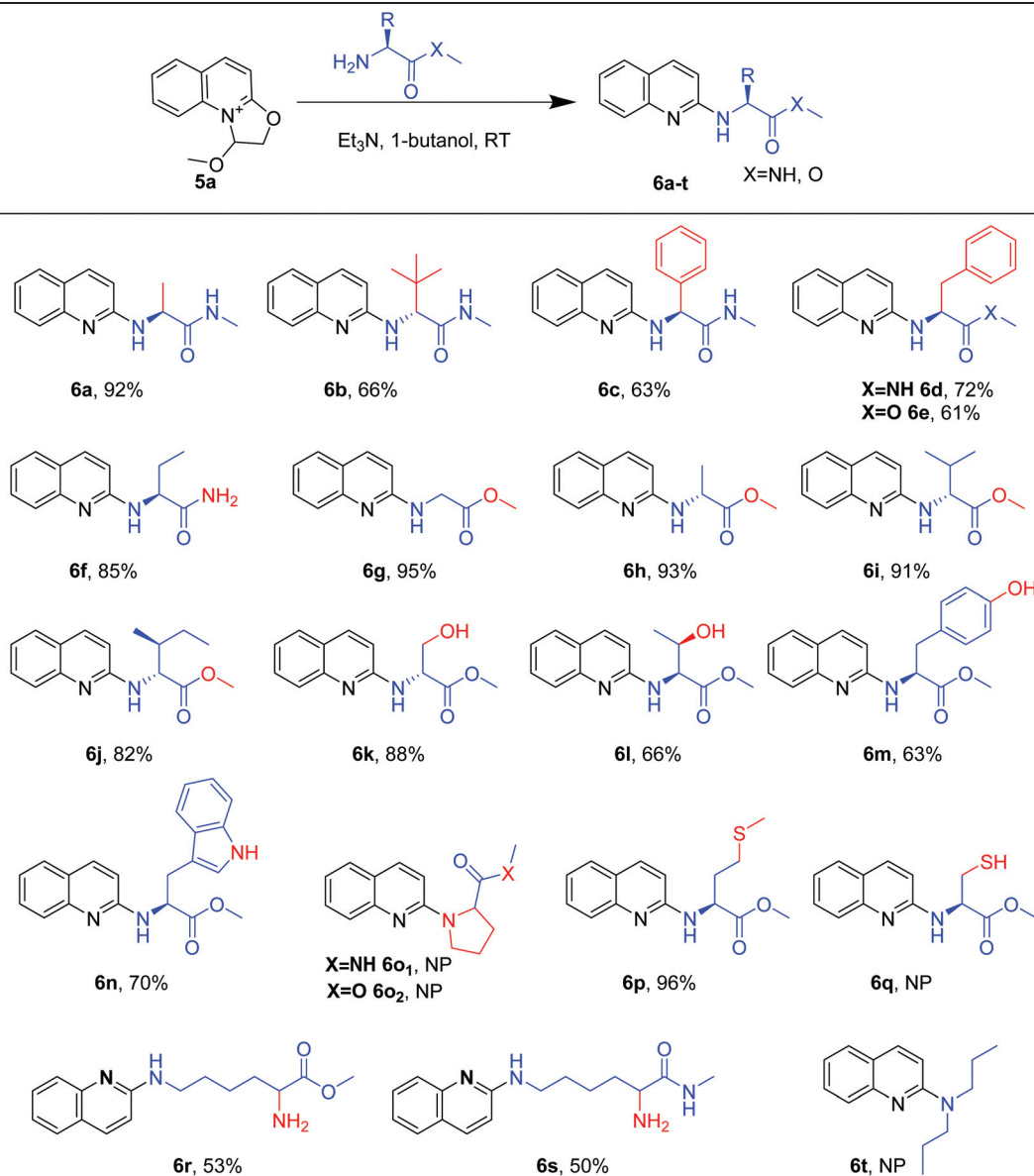
Entry	Base	Eq. of amino amide	Solvent	Yield ^b (%)
1	DABCO	1.0	1-Butanol	47
2	DMAP	1.0	1-Butanol	57
3	DBU	1.0	1-Butanol	47
4	CO(NH ₂) ₂	1.0	1-Butanol	11
5	DIPEA	1.0	1-Butanol	59
6	Arginine	1.0	1-Butanol	22
7	Et ₃ N	1.0	1-Butanol	62
8	Et ₃ N	1.0	DMSO	6
9	Et ₃ N	1.0	THF	6
10	Et ₃ N	1.0	1,4-Dioxane	9
11	Et ₃ N	1.0	Acetone	Trace
12	Et ₃ N	1.0	MeCN	10
13	Et ₃ N	1.0	PhMe	20
14	Et ₃ N	1.0	EtOH	20
15	Et ₃ N	1.0	DMF	17
16	Et ₃ N	1.0	CCl ₄	18
17	Et ₃ N	1.0	ClCH ₂ CH ₂ Cl	18
18	Et ₃ N	1.0	CH ₂ Cl ₂	26
19	Et ₃ N	1.5	1-Butanol	71
20	Et ₃ N	2.0	1-Butanol	95
21	Et ₃ N	3.0	1-Butanol	95

^a All reactions were performed at ambient temperature. **5a** was dissolved in different solvents and treated with 2-amino-*N*-methylacetamide and base. ^b The yields were determined by HPLC.

bears a hindered tertiary butyl at the α position of the amino group, the yield of **6b** decreased to 66%, suggesting that steric hindrance had an obvious effect on the reaction. Similarly, the *N*-quinolylation of (*S*)-2-amino-*N*-methyl-2-phenylacetamide and (*S*)-2-amino-*N*-methyl-3-phenylpropanamide proceeded smoothly to provide products **6c** and **6d** in 63% and 72% yields, respectively. Specially, the amino group of 2-aminobutanamide was preferentially *N*-quinolyated rather than the acyl-



Table 2 Scope of amino substrates

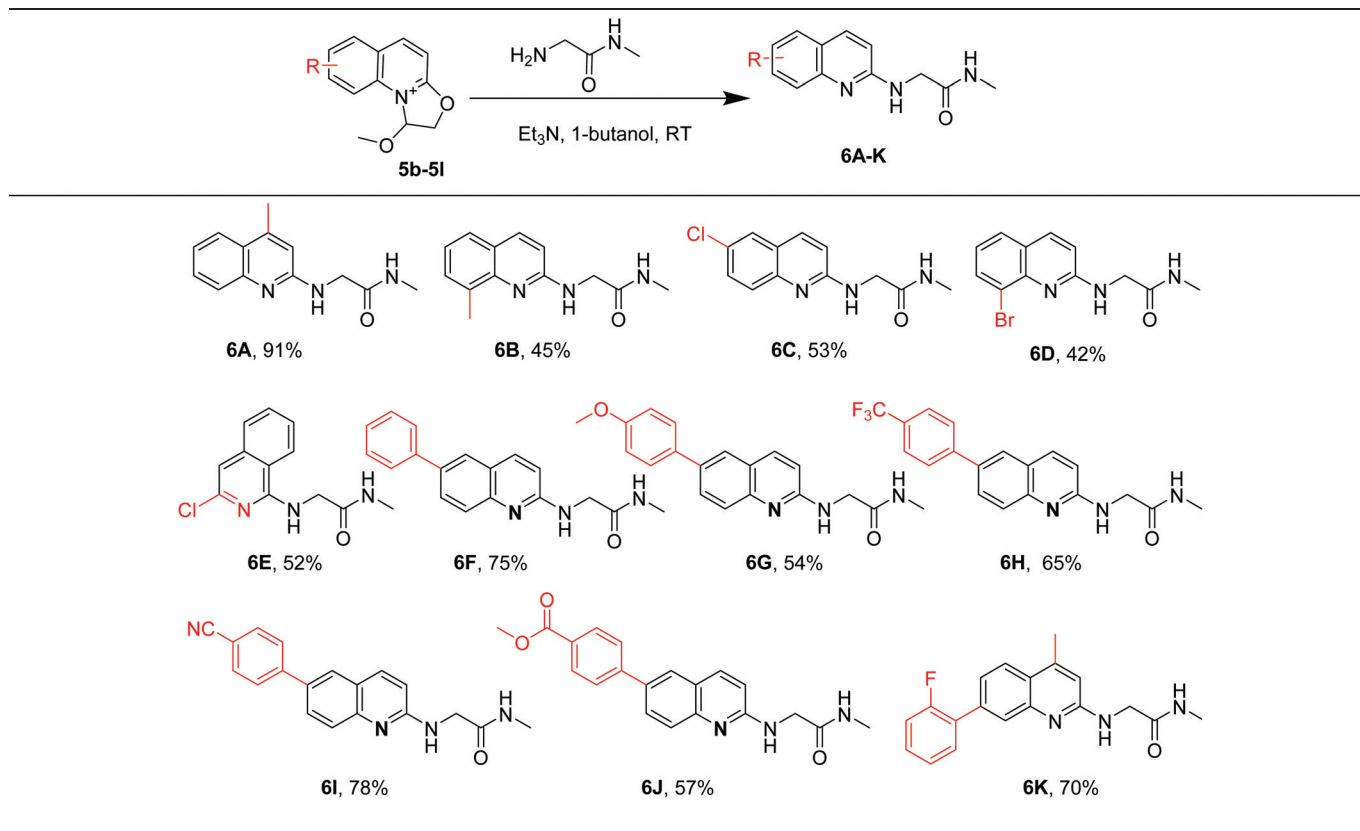


amino to afford **6f** in 85% yield. In addition, products **6g** and **6e** were obtained from glycine methyl ester and phenylalanine methyl ester in 95% and 61% yields, respectively. Accordingly, more amino acid esters were explored. Alanine methyl ester, valine methyl ester and isoleucine methyl ester gave the quinolyated products **6h**, **6i** and **6j** in high yields of 82%–93%. Serine methyl ester, threonine methyl ester and tyrosine methyl ester gave **6k**, **6l** and **6m** in moderate yields (63%–88%) without the interference of hydroxyl groups. Moreover, tryptophan methyl ester was converted into the quinolyated product **6n** in 70% yield, while the indole amino group was not *N*-quinolyated. Neither proline methyl ester nor formamide formed quinolyated products (**6o₁** and **6o₂**), and the di-*n*-propylamine did not form the quinolyated product (**6t**). These results indicate that the quinoline quaternary ammonium salt

selectively reacted with primary amino groups rather than secondary amino groups. Interestingly, methionine methyl ester furnished product **6p** in 96% yield, while quinolyated product **6p** was not observed with cysteine methyl ester, indicating that the thiol group needs to be protected before the quinoylation reaction. Particularly, *N*-quinoylation mainly occurred at the ϵ amino group rather than the α amino group of lysine methyl ester and amide (**6r** and **6s**).

To demonstrate the utility of this developed protocol, we investigated the *N*-quinoylation of 2-amino-*N*-methylacetamide with a variety of substituted 2-(2,2-dimethoxyethoxy)quinolines and isoquinolines (Table 3). Both electron-donating (Me-, phenyl) and withdrawing (Cl-, Br-) groups at different positions of the quinoline or isoquinoline moiety gave the quinolyated products **6A–6K** in 42%–91% yields. Among these,

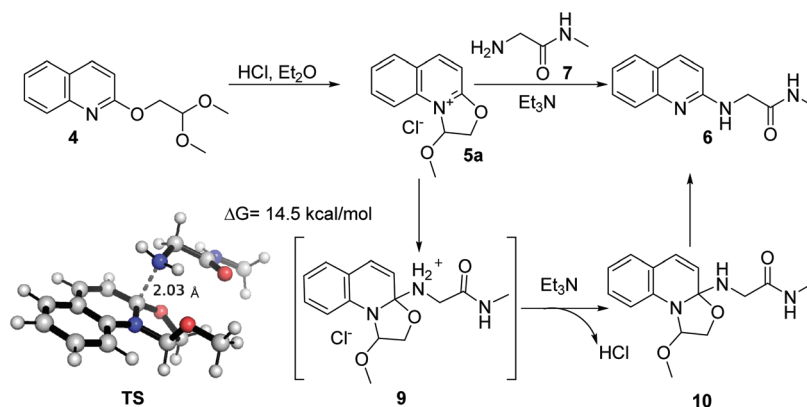


Table 3 Scope of dihydrooxazolo[3,2-*a*]quinoliniums

methyl group-substituted quinoline derivatives smoothly afforded **6A** and **6B** in 91% and 45% yields, respectively. The quinoline substrates substituted with chloro and bromo groups were also compatible under our experimental protocol, giving **6C** and **6D** in 53% and 42% yields, respectively. The halogens can be applied in further coupling reactions under Suzuki conditions.^{35,36} Moreover, when a 4-methoxyphenyl group was installed at the 6-position of the quinoline moiety, the substrate provided **6G** in 54% yield. Substrates in which phenyl groups were substituted by electron-withdraw-

ing groups (CF_3 -, CN -, CO_2Me -) also afforded the desired products **6H**, **6I** and **6J** in 65%, 78% and 57% yields, respectively. When the quinoline moiety was simultaneously substituted with 2-fluorophenyl and methyl, it provided **6K** in 70% yield.

The proposed mechanism of the reaction is shown in Scheme 1. First, dihydrooxazolo[3,2-*a*]quinolinium **5a** is generated from **4** after treatment with hydrogen chloride in diethyl ether. The nucleophilic attack of 2-amino-*N*-methylacetamide then affords the corresponding intermediate **9**. Quantum



Scheme 1 Proposed reaction mechanism.



chemistry calculation at the M06-2X/6-311+G(d) level shows that the activation energy of the transition state (TS) is 14.5 kcal mol⁻¹, indicating that the reaction occurs easily. The subsequent removal of hydrogen chloride facilitated by triethylamine provides the intermediate **10**, which ultimately undergoes aromatization to give product **6** with 2-butoxy-2-methoxyethan-1-ol as a possible byproduct.

As mentioned above, 2-(2,2-dimethoxyethoxy) quinoline was treated with hydrogen chloride in diethyl ether after distilling the solvent and without any complicated purification step to

give the 1-methoxy-1,2-dihydrooxazolo[3,2-*a*]quinolinium **5a** (Table 4). **5a** was reacted with 2-amino-*N*-methylacetamide to afford **6** in 95% yield. However, the *N*-quinolylation of other reported quaternary ammonium salts, *viz.*, 1-methylquinolin-1-ium (**11**), quinoline 1-oxide (**12**) and 1-acetylquinolin-1-ium (**13**), with 2-amino-*N*-methylacetamide did not occur, indicating that the novel dihydrooxazolo[3,2-*a*]quinolinium has unique reaction characteristics.

The established method was further used for the *N*-quinolylation of the primary amino groups of seven molecules (Table 5). Two drug molecules used to treat hypotension and type 2 diabetes, midodrine hydrochloride³⁷ and saxaglipitin,³⁸ were successfully coupled with quinoline quaternary ammonium salt to provide the quinolyated products **8a** and **8b** in 72% and 46% yields, respectively. Five peptide molecules, methyl tyrosyleutide (liver cancer),^{39,40} Val-Cit-PAB-OH (a linker for antibody-drug-conjugation),⁴¹⁻⁴³ oxytocin (improvement of uterine contractions),⁴⁴ dermorphin (a μ -opioid receptor agonist),⁴⁵ and octreotide (functional gastrointestinal pancreatic endocrine tumors),⁴⁶ were reacted to give

Table 4 Comparison of different quaternary ammonium salts for *N*-quinolylation

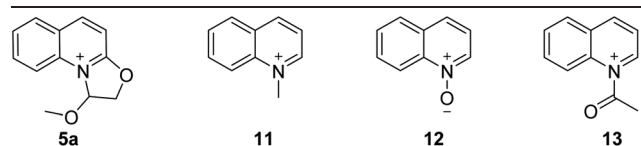
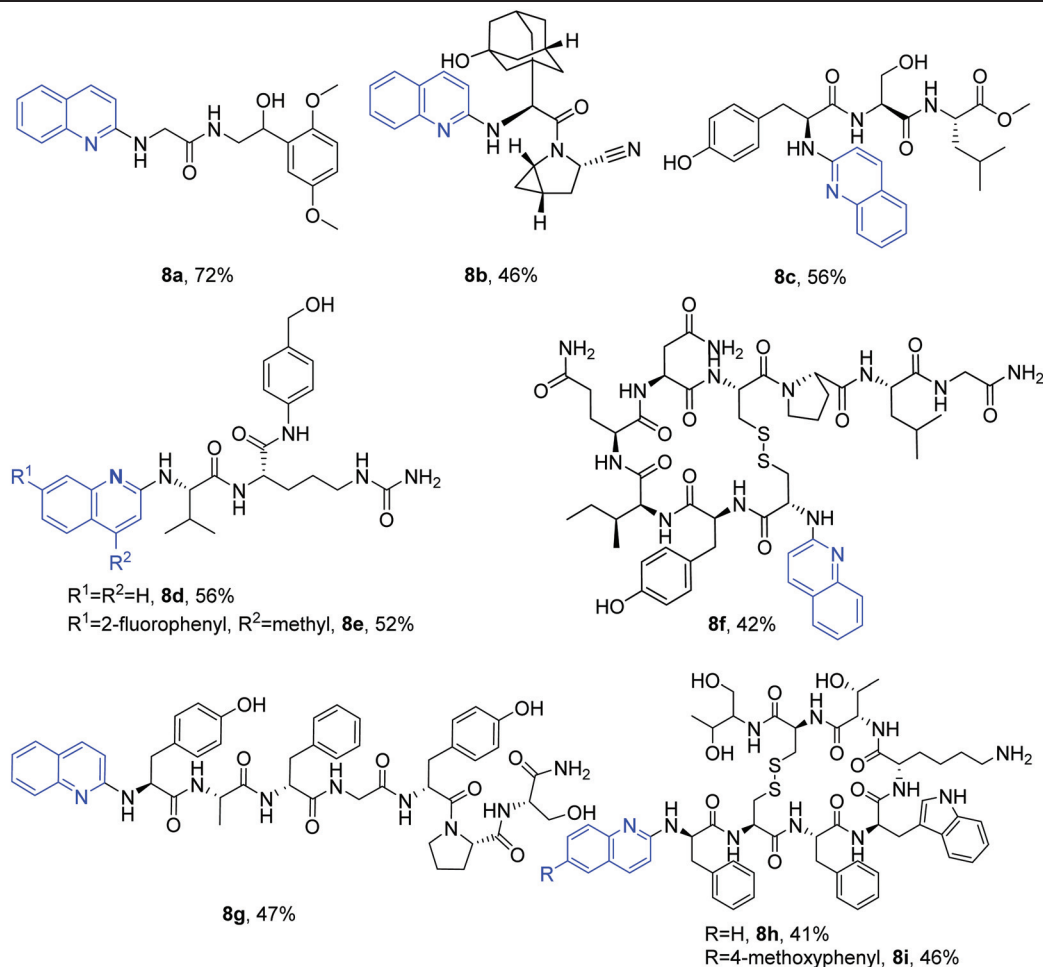


Table 5 *N*-Quinolylation of some complex molecules and oligopeptides^a



^a Used 2.0 equiv. quinoline quaternary ammonium salt and 1.0 equiv. peptide.



the desired site-selectively *N*-quinolyated peptides **8c–8i** under the same protocol.

These structural modifications of peptide drugs indicate that reactive or sensitive groups such as secondary amino groups (**6n**), amides (**6f**, **8d**, **8e**, **8f**, **8g**, **8h** and **8i**), alcoholic hydroxyls (**6k**, **6l**, **8a–8e**, **8g**, **8h** and **8i**), phenolic hydroxyls (**6m**, **8c**, **8f** and **8g**), disulfide bonds (**8f**, **8h** and **8i**), ester groups (**6e**, **6g–6n**, **6p**, **6r**, **8c**) and cyano groups (**8b**) are well tolerated, and the site-selective *N*-quinolylation occurs at the primary amino group position under metal-free reaction conditions.

Among these peptides, tyrosinerleutide (YSL) has been studied in phase III clinical trials for the treatment of liver cancer. Therefore, YSL, YSL-M (tyrosinerleutide methyl ester) and **8c** were evaluated for anticancer activity against hepatocarcinoma BEL-7402 and SMMC-7721 cells. Bioassay results revealed that **8c** exhibited the highest cytotoxicity against both BEL-7402 and SMMC-7721 cells (ESI Table S1[†]), indicating that the introduction of a quinolyl group into the peptide changed its bioactivity. Dermorphin is a clinically used μ -opioid receptor agonist. Its quinolyated compound **8g** exhibited a slightly decreased activity compared to dermorphin (ESI Table S2[†]), again suggesting that the modification of active peptides is of significance. 7-(2-Fluorophenyl)-4-methylquinolin-2(1*H*)-one was found to be a tankyrase inhibitor with an IC₅₀ value of 0.052 μ M.⁴⁷ This compound can be coupled with Val-Cit-PAB-OH to afford **8e** via our method. A preliminary *in vitro* liver stability assessment (ESI Fig. S2[†]) of **8e** indicated good metabolic stability, suggesting the potential to develop PDC drugs based on the target peptide using our new method. *N*-Quinolylation mainly occurred at the α amino group of octreotide with both lysine ϵ amino and α amino groups (**8h** and **8i**). Small amounts of the quinolylation products of both the α and ϵ amino groups were detected for this reaction, but no single ϵ amino group coupling product was observed.

Conclusions

In conclusion, we have developed a green method for the specific *N*-quinolyated modification of primary amino groups in complex molecules. This protocol was performed under room temperature and metal-free conditions in 1-butanol solvent. It has very good reactive moiety compatibility with secondary amino, amide, alcoholic hydroxyl, phenolic hydroxyl, disulfide bond, ester and cyano groups. Therefore, the developed method can be widely used, particularly in medicinal chemistry and chemical biology.

Data availability

Complete experimental procedures and compound characterization data are available within the article and its ESI,[†] or from the corresponding author upon request.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

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References

- 1 K. Fosgerau and T. Hoffmann, *Drug Discovery Today*, 2015, **20**, 122–128.
- 2 D. J. Craik, D. P. Fairlie, S. Liras and D. Price, *Chem. Biol. Drug Des.*, 2013, **81**, 136–147.
- 3 C. Katz, L. Levy-Beladev, S. Rotem-Bamberger, T. Rito, S. G. Rudiger and A. Friedler, *Chem. Soc. Rev.*, 2011, **40**, 2131–2145.
- 4 I. Avan, C. D. Hall and A. R. Katritzky, *Chem. Soc. Rev.*, 2014, **43**, 3575–3594.
- 5 B. J. Bruno, G. D. Miller and C. S. Lim, *Ther. Delivery*, 2013, **4**, 1443–1467.
- 6 S. S. Usmani, G. Bedi, J. S. Samuel, S. Singh, S. Kalra, P. Kumar, A. A. Ahuja, M. Sharma, A. Gautam and G. P. S. Raghava, *PLoS One*, 2017, **12**, e0181748.
- 7 L. Di, *AAPS J.*, 2015, **17**, 134–143.
- 8 A. Henninot, J. C. Collins and J. M. Nuss, *J. Med. Chem.*, 2018, **61**, 1382–1414.
- 9 S. H. Kim, O. Pohl, A. Chollet, J. P. Gotteland, A. D. Fairhurst, P. R. Bennett and V. Terzidou, *Mol. Pharmacol.*, 2017, **91**, 403–415.
- 10 M. Sakai, M. Elhilali and V. Papadopoulos, *Horm. Metab. Res.*, 2015, **47**, 925–931.
- 11 N. Matikainen, S. Soderlund, E. Bjornson, K. Pietilainen, A. Hakkarainen, N. Lundbom, M. R. Taskinen and J. Boren, *Diabetes, Obes. Metab.*, 2019, **21**, 84–94.
- 12 S. Dhillon, *Drugs*, 2011, **71**, 1071–1091.
- 13 S. Doss and B. Schiller, *Nephrol. Nurs. J.*, 2010, **37**, 617–626.
- 14 Y. Yang, J. Fang, X. Guo, N. Dai, X. Shen, Y. Yang, J. Sun, B. R. Bhandari, D. S. Reasner, J. A. Cronin, M. G. Currie, J. M. Johnston, P. Zeng, N. Montrewasuwat, G. Z. Chen and S. Lim, *J. Gastroenterol. Hepatol.*, 2018, **33**, 980–989.
- 15 T. Passeron, *JAMA Dermatol.*, 2015, **151**, 349–350.
- 16 K. Eto, S. K. Kim, J. Nabekura and H. Ishibashi, *Brain Res.*, 2011, **1414**, 50–57.
- 17 M. N. Michalski, A. L. Seydel, E. M. Siismets, L. E. Zweifler, A. J. Koh, B. P. Sinder, J. I. Aguirre, K. Atabai, H. Roca and L. K. McCauley, *FASEB J.*, 2018, **32**, 3730–3741.
- 18 N. Krall, F. P. da Cruz, O. Boutureira and G. J. Bernardes, *Nat. Chem.*, 2016, **8**, 103–113.



- 19 L. Otvos Jr. and J. D. Wade, *Front. Chem.*, 2014, **2**, 62.
- 20 C. Y. Koh and M. Kini, *Toxicol.*, 2012, **59**, 497–506.
- 21 S. Tsunasawa, J. W. Stewart and F. Sherman, *J. Biol. Chem.*, 1985, **260**, 5382–5391.
- 22 A. M. Wagner, M. W. Fegley, J. B. Warner, C. L. Grindley, N. P. Marotta and E. J. Petersson, *J. Am. Chem. Soc.*, 2011, **133**, 15139–15147.
- 23 W. K. Chan, C. M. Ho, M. K. Wong and C. M. Che, *J. Am. Chem. Soc.*, 2006, **128**, 14796–14797.
- 24 T. Chen, T. L. Muratore, C. E. Schaner-Tooley, J. Shabanowitz, D. F. Hunt and I. G. Macara, *Nat. Cell Biol.*, 2007, **9**, 596–603.
- 25 J. M. Gilmore, R. A. Scheck, A. P. Esser-Kahn, N. S. Joshi and M. B. Francis, *Angew. Chem., Int. Ed.*, 2006, **45**, 5307–5311.
- 26 L. S. Witus, C. Netirojjanakul, K. S. Palla, E. M. Muehl, C. H. Weng, A. T. Iavarone and M. B. Francis, *J. Am. Chem. Soc.*, 2013, **135**, 17223–17229.
- 27 R. A. Scheck, M. T. Dedeo, A. T. Iavarone and M. B. Francis, *J. Am. Chem. Soc.*, 2008, **130**, 11762–11770.
- 28 H. Hammoud, M. Schmitt, E. Blaise, F. Bihel and J. J. Bourguignon, *J. Org. Chem.*, 2013, **78**, 7930–7937.
- 29 A. C. Obermeyer, J. B. Jarman and M. B. Francis, *J. Am. Chem. Soc.*, 2014, **136**, 9572–9579.
- 30 D. Camp, *Drugs Future*, 2013, **38**, 245–256.
- 31 J. S. Barbosa, F. A. Almeida Paz and S. S. Braga, *Drug Delivery*, 2016, **23**, 3257–3265.
- 32 C. J. la Porte, *Expert Opin. Drug Metab. Toxicol.*, 2009, **5**, 1313–1322.
- 33 B. Li, S. Xue, Y. Yang, J. Feng, P. Liu, Y. Zhang, J. Zhu, Z. Xu, A. Hall, B. Zhao, J. Shi and W. Zhu, *Sci. Rep.*, 2017, **7**, 41287.
- 34 B. Li, G. Wang, Z. Xu, Y. Zhang, X. Huang, B. Zeng, K. Chen, J. Shi, H. Wang and W. Zhu, *Eur. J. Med. Chem.*, 2014, **77**, 204–210.
- 35 N. Miyaura and A. Suzuki, *J. Chem. Soc., Chem. Commun.*, 1979, 866–867.
- 36 N. Miyaura and A. Suzuki, *Chem. Rev.*, 1995, **95**, 2457–2483.
- 37 W. Smith, H. Wan, D. Much, A. G. Robinson and P. Martin, *Clin. Auton. Res.*, 2016, **26**, 269–277.
- 38 K. P. Garnock-Jones, *Drugs*, 2017, **77**, 319–330.
- 39 Z. Fu, L. Ren, H. Wei, J. Lv, X. Che, Z. Zhu, J. Jia, L. Wang, G. Lin, R. Lu and Z. Yao, *J. Drug Targeting*, 2014, **22**, 146–155.
- 40 C. Wang, S. Wang, R. Lu, L. Zhao, Z. F. Zhu, Q. Xu, J. Q. Lv, L. L. Wang, Z. Fu, G. Lin and Z. Yao, *Anticancer Drugs*, 2009, **20**, 534–542.
- 41 J. Grunewald, Y. Jin, J. Vance, J. Read, X. Wang, Y. Wan, H. Zhou, W. Ou, H. E. Klock, E. C. Peters, T. Uno, A. Brock and B. H. Geierstanger, *Bioconjugate Chem.*, 2017, **28**, 1906–1915.
- 42 Q. Zhou and J. Kim, *Anticancer Agents Med. Chem.*, 2015, **15**, 828–836.
- 43 L. J. Scott, *Drugs*, 2017, **77**, 435–445.
- 44 E. N. Erickson, C. S. Lee and C. L. Emeis, *J. Midwifery Womens Health*, 2017, **62**, 418–424.
- 45 H. Mizoguchi, G. Bagetta, T. Sakurada and S. Sakurada, *Peptides*, 2011, **32**, 421–427.
- 46 R. M. Borna, J. S. Jahr, S. Kmiecik, K. F. Mancuso and A. D. Kaye, *Anesthesiol. Clin.*, 2017, **35**, 327–339.
- 47 E. A. Larsson, A. Jansson, F. M. Ng, S. W. Then, R. Panicker, B. Liu, K. Sangthongpitag, V. Pendharkar, S. J. Tai, J. Hill, C. Dan, S. Y. Ho, W. W. Cheong, A. Poulsen, S. Blanchard, G. R. Lin, J. Alam, T. H. Keller and P. Nordlund, *J. Med. Chem.*, 2013, **56**, 4497–4508.

