Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/d0ob00336k

Received 14th February 2020,
Accepted 23rd April 2020

DOI: 10.1039/d0ob00336k

rsc.li/obc

Late-stage synthesis and application of photoreactive probes derived from direct benzoylation of heteroaromatic C–H bonds†

Kevin D. Hesp, * Jun Xiao and Graham M. West

A C–H functionalization strategy for the expedient access to photoreactive chemical probes of commonly found heterocyclic fragments or drug molecules of pharmaceutical relevance is described. A series of aryl glyoxylic acid reagents featuring pendant alkyne or azide clickable handles have been developed for application in the radical-mediated appendage of benzoyl fragments onto simple heteroaromatic fragments, as well as more complex drug-like compounds. This unprecedented strategy of chemical probe synthesis allows for direct access to photoreactive chemical probes without any requirement of fragment pre-functionalization or significant synthetic re-evaluation.

Introduction

Benzophenone-derived photoaffinity probes have a longstanding history for interrogating specific ligand–protein interactions along complex biochemical pathways in the field of chemical biology.[1] In particular, such probes have been routinely leveraged for the identification of new targets for known biologically active compounds, as well as for elucidating the binding site or mechanistic subtleties of a specific ligand within a known target. The popularity of benzophenone is in part related to its high chemical stability and ready availability when compared to related azide and diazirine photophores,[1b,c,2] but is also heavily rooted in its unique ability to reversibly generate reactive radical intermediates without probe destruction at non-protein damaging wavelengths.

Despite these advantages, the inherent lipophilicity and steric profile of the benzophenone moiety persist as limitations from the perspective of increased non-specific protein binding, as well as sterically-driven binding differences when compared to unmodified parent ligands or fragments. Often the additional synthetic evaluation required to introduce suitable synthetic handles onto target ligands to facilitate incorporation of benzophenone without disrupting key binding features can present challenges for photoreactive probe design. In addition to the application of minimalist photoreactive probe designs (Fig. 1A),[3] the advent of fully-functionalized fragment probes, which imbed the photoreactive group into the ligand of interest, have presented an opportu-

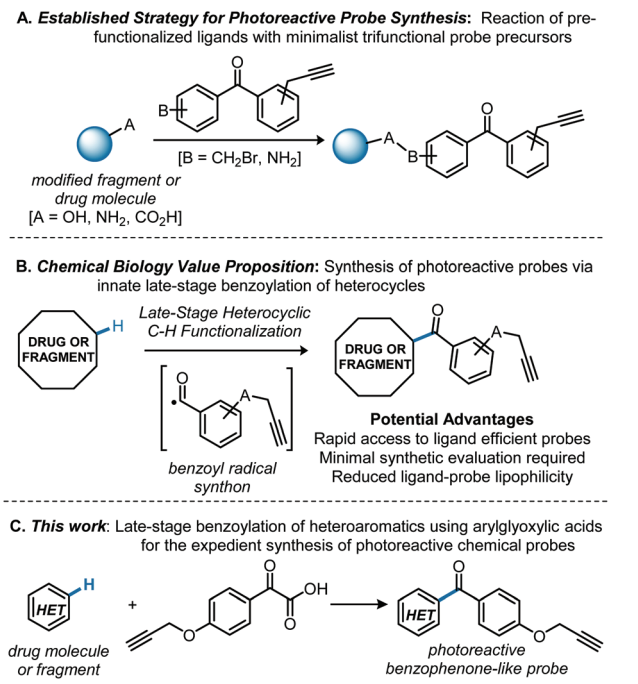


Fig. 1 (A) Current strategy for benzophenone photoreactive probe synthesis from pre-functionalized fragments; (B) proposed investigation of streamlined probe synthesis by late-stage C–H functionalization on unmodified parent ligands or fragments; (C) use of arylglyoxylic acid reagents with pendant alkynes for the synthesis of heterocyclic benzophenone probes *via* radical benzoylation of heteroaromatics in biologically active molecules.

Pfizer, Inc., Medicine Design, Eastern Point Road, Groton, Connecticut, 06340, USA. E-mail: kevin.hesp@pfizer.com

† Electronic supplementary information (ESI) available: Experimental details and supporting characterization. See DOI: 10.1039/d0ob00336k

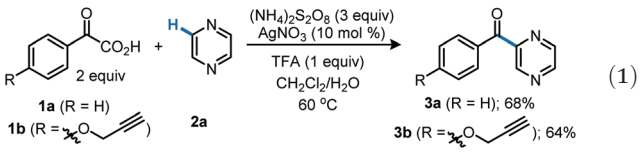
nity to alleviate aspects of these limitations in the context integrated phenotypic screening and target identification.[4] In order to expand the synthetic accessibility and structural diversity of this concept, we became interested in the identification of synthetic options for the late stage benzoylation of unmodified fragments and parent ligands (Fig. 1B). Ideally this strategy would rely solely on the innate reactivity of commonly encountered heterocycles, which are pervasive in fragments and bioactive molecules of pharmaceutical interest. If successful, this strategy could have significant impact on the ability to rapidly access photoreactive probe fragment libraries with minimal synthetic investment from large substrate diversity to expedite new biological target identification efforts.

The use of radical chemistry has been established as a practical and selective strategy for the functionalization of complex molecules in a predictable manner.[5] The most appealing attributes of radical-based chemistry in the context of photoreactive probe synthesis are the opportunities to alleviate heightened synthetic costs and time for preparing complex molecules featuring minor modifications to the parent compounds, as well as the innate tolerance of molecular complexity and reactive functional groups. Given these clear benefits, we focused our efforts on the identification of suitable benzoyl radical precursors for the development of an efficient late-stage synthesis of benzophenone-like photoreactive probes.

Based on these considerations, we report the identification and reactivity of readily accessible alkyne-substituted, aryl glyoxylic acid reagents for application in the mild C–H benzoylation of both simple heteroarene fragments, as well as complex biologically active molecules, to provide streamlined access to benzophenone-like photoreactive probes (Fig. 1C). Of importance, this strategy offers potential advantages over more classical benzophenone in the context of developing ligand-efficient and less lipophilic photoreactive chemical probes that are rapidly accessible with minimal synthetic assessment.

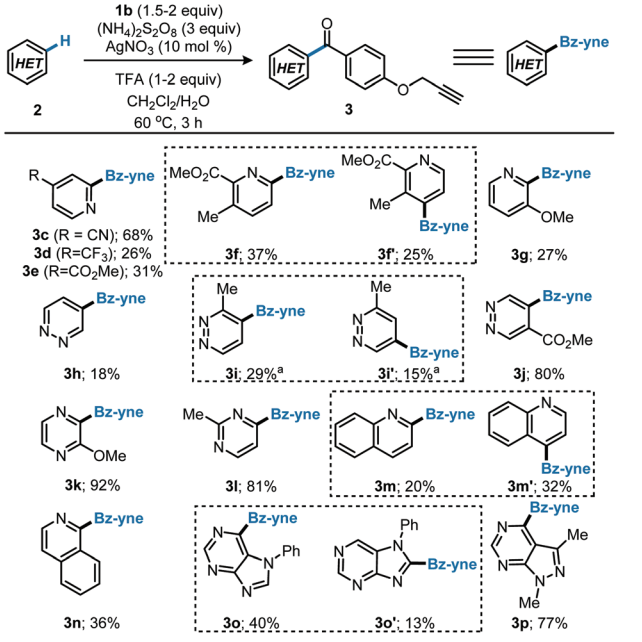
Results and discussion

Building on the chemistry pioneered by Minisci,[6] and in line with the goals of this study, we prepared a series of substituted aryl glyoxylic acids that featured clickable alkyne or azide functional handles.[7] Optimization of the aryl glyoxylic acid substitution led to the identification of propargyl ether **1b**, which provided the 2-benzoyl pyrazine product **3b** in a comparable yield to that observed in our initial test reaction employing unsubstituted phenyl glyoxylic acid **1a** (eqn (1)).[6a] Of importance, these initial results established that the alkyne clickable handle is unreactive under the radical forming conditions and, thus, provided the impetus for continued pursuit of a heteroaromatic C–H benzoylation strategy for photoreactive probe synthesis.



(1)

Following the identification of **1b** as an optimal reagent, the scope of heteroaromatic benzoylation was initiated with a focus on defining the reactivity and regioselectivity trends on simple heteroarene fragments to guide its use for more elaborate photoreactive probe synthesis on complex drug-like structures (Scheme 1).[8] In general, most classes of heterocycles were reactive using the standard conditions, but those that were either inherently electron-deficient or featured electron-withdrawing substituents typically proceeded with superior conversions to the C–H benzoylation products. Indeed, simple pyridine derivatives featuring electron-withdrawing groups in the 4-position (**2c–2e**) were readily benzoylated at the 2-position to give the corresponding 2-benzoyl pyridine products in good yields. Whereas benzoylation of methyl 3-methyl picolinate (**2f**) gave a mixture of regioisomers slightly favouring the C6-substituted product (**3f** : **3f'** = 1.5 : 1), the use 3-methoxypyridine selectively reacted at the 2-position to give **3g** in 27% isolated yield. The methodology was successfully extended to include substituted diazines that are commonly found in drug-like molecules, such as pyridazine (**2h–2j**), pyrazine (**2k**), and pyrimidine (**2l**), with good yields and high regioselectivity in most examples. In addition to monocyclic heterocycles, the use of [6,6]- and [5,6]-ring systems, such as quinoline (**2m**), isoquinoline (**2n**), imidazopyrimidine (**2o**), and pyrazolopyrimidine (**2p**), were also readily benzoylated providing access to the



Scheme 1 Substrate scope for benzoylation of heteroaromatic fragments using aryl glyoxylic acid **1b**. [a] The corresponding bis addition product was isolated in a 18% yield.

benzophenone-like compounds **3m–3p**. Notably, several substrates that featured multiple potential reaction sites exhibited complete regioselectivity, which resulted in only a single observable regioisomer with C–H benzoylation proceeding at electron-deficient positions.

Building on the observed reactivity trends presented in Scheme 1, we evaluated the methodology using more complex chemical structures that would be more commonly encountered in drug discovery programs. Using our standard conditions, a range of biologically active compounds were readily functionalized using the C–H benzoylation protocol to provide rapid access to benzophenone-like photoreactive probes (Fig. 2). In line with the reactivity trends identified in Scheme 1, the pyrimidine-containing PDE9A inhibitor, serotonin 5-HT_{1A} receptor partial agonist, and BACE inhibitor were selectively functionalized to provide access to the benzophenone-like products in 12% (**3q**), 9% (**3r**), and 11% (**3s**) yields, respectively.[9] Additionally, an mGluR5 modulator and the smoking cessation drug Chantix,[9] which feature highly substituted pyrazine and quinoxaline cores, were readily benzoylated in modest yields when employing aryl glyoxylic acid **1b** under the standard radical forming conditions (**3t**; 39% and **3u**; 32%, respectively). Notably, the C–H functionalization chemistry proceeds in the presence of potentially reactive functional groups, such as the pyrimidinone and thioamidine groups or basic secondary amines. Although low to moderate isolated yields are often observed, the most powerful feature of this methodology is the ability to rapidly access photoreactive probes for assessment in key chemical biology applications without the requirement of additional synthetic assessment or functional group installation.

There are few examples of heterocyclic benzoyl derivatives behaving as ketyl radical precursors[10] and scarce precedent for their use in interrogating protein–ligand interactions *via* a photo-initiated crosslinking event.[4b] In this regard, it was critical to exemplify the use of a representative ligand-benzoyl structural motif as a competent source of a ketyl-like radical in order to validate the synthetic strategy for more broad application in the chemical biology community. In light of Pfizer's recent campaign in search of a novel PDE9A inhibitor for treatment of cognitive disorders,[11] we sought to showcase and validate this strategy for the expedient identification of a photoreactive chemical probe of the clinical candidate **2q** for PDE9A binding site interrogation.

In addition to the facile introduction of the photoreactive group in a single step from unmodified parent ligand **2q**, the resulting probe **3q**, as well as the direct benzophenone comparator **4**, were observed to maintain comparable potency against PDE9A (Fig. 3). In line with the overarching goals of this study, the resulting minimalist probe **3q** favourably offset the overall probe lipophilicity when compared to the benzophenone derived probe **4**, as judged by cLogP and the moderate impact on the lipophilic efficiency (LipE) (Fig. 3).[12] Moderation of this key physicochemical property presents opportunities for the design of chemical probes with favourable cell permeability and the potential mitigation of unproductive non-specific protein binding – two important factors when considering their use in cell-based phenotypic screening or photoaffinity studies. The measured reduction of lipophilic character between probes **3q** and **4** was also manifest following investigation into the off-target pharmacology within the PDE protein family. Indeed, the more lipophilic probe **4** had a marked increase in potency against related PDE proteins, while **3q** maintained selectivity for PDE9A (Fig. 3).[7,13]

In light of the conserved potency of **3q** and **4** against PDE9A, as well as the proximity of the binding pocket to a potentially reactive methionine residue,[11,14] we reasoned that photoactivation of **3q** and **4** in the presence of PDE9A were ideal experiments to test the relative propensity of a heterocyclic benzophenone to participate as a photo-crosslinking functional group. Incubation of recombinant PDE9A protein with a 10-fold excess of **3q** under photoirradiation using 365 nm UV-light for 30 minutes, followed by deconvulated mass spectroscopic analysis, revealed that a successful covalent modification of the protein had occurred with 34% photolabeling efficiency (M + 553 observed; based on relative

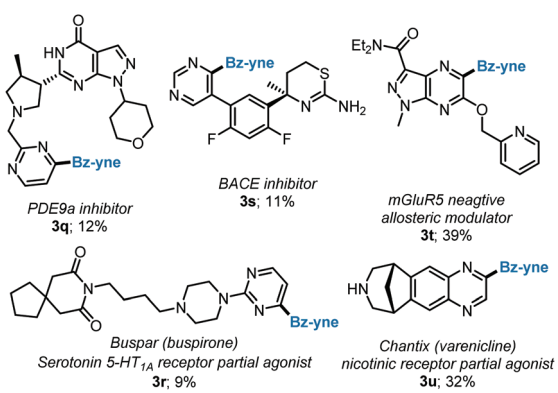


Fig. 2 Application of late-stage photoreactive probe synthesis to drug molecules.

	Potency/Properties				Selected Off-Target PDE Activity (nM)		
	MW	clogP	IC_{50}	LipE	PDE5A	PDE6A	PDE7B
parent PDE9A inhibitor (**2q**)	395	-1.50	8.3 nM	9.58	–	–	–
PDE9A hetBz-yne probe (**3q**)	553	1.09	2.0 nM	7.60	10494	2561	16048
PDE9A PhBz-yne probe (**4**)	551	2.83	0.4 nM	6.57	141	10.2	168.2

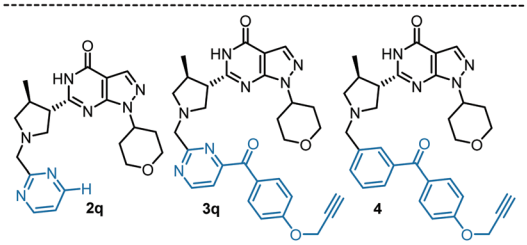


Fig. 3 Comparison of potency, properties, and selected off-target pharmacology for **2q**, **3q**, and **4**.

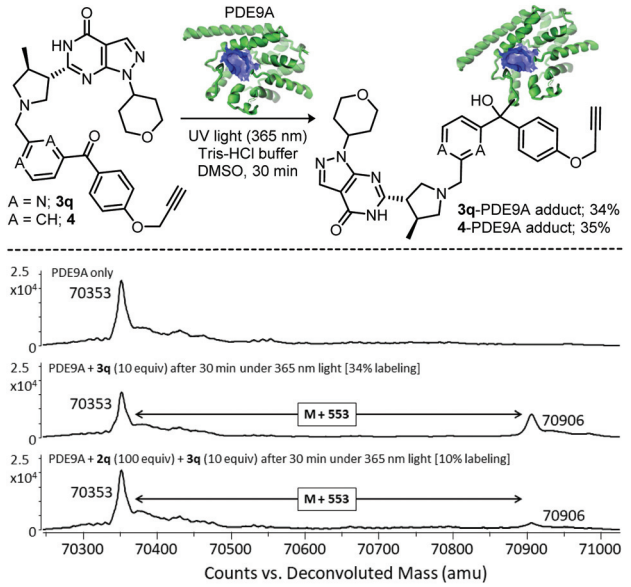


Fig. 4 PDE9A photolabeling experiments with **3q** and **4**.

ion intensities) (Fig. 4).[7,15] When the same experiment was conducted in the presence of the probe **4**, which featured the more classical benzophenone photophore, a similar photolabeling efficiency of 35% was observed (M + 551 observed; based on relative ion intensities).[7] The results of this direct PDE9A photolabeling comparison support that heterocyclic variants of benzophenone represent comparable photoreactive functional groups to benzophenone for chemical biology applications.

As a means of providing confidence that the photoinitiated cross-linking event had indeed proceeded in the binding pocket of PDE9A and was not the result of a non-specific labelling event, a competition experiment between the parent ligand **2q** (100-fold excess over PDE9A) and probe **3q** (10-fold excess over PDE9A) was performed. The outcome of this experiment showed significantly diminished photolabeling efficiency (*cf.* 34% *versus* 10%), which is consistent with competitive binding of **2q** and **3q** for a distinct pocket (Fig. 4). This result, in conjunction with the conserved potency against PDE9A, is highly suggestive that probe **3q** retains the same PDE9A binding site as the parent ligand and has covalently labelled the protein primarily at this site, as opposed to an unproductive non-specific modification.[14]

Conclusions

In summary, we have demonstrated a synthetically driven strategy for the streamlined preparation of heterocyclic alternatives to more classical benzophenone photoreactive probes through the direct benzoylation of heteroaromatic C–H bonds in fragments and drug molecules. As a critical proof of principle experiment, the "minimalist" heterocyclic benzophenone probe of a known PDE9A inhibitor, derived from this methodology, was shown to be effective in the key photoaffinity labeling of the PDE9A protein while maintaining favorable drug-like physicochemical properties. Given the operational simplicity and broad scope of compatible heteroarenes, this powerful strategy holds promise to complement more classical photoreactive probe identification and is poised to rapidly impact the interrogation of protein–ligand interactions, among other chemical biology applications.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors thank Vincent Mascitti, Christopher am Ende, Whitney Nolte, and Brian Raymer for helpful discussions, Steven Jenkinson for potency determination of **3q** and **4**, Ingrid Stock for assistance in acquiring PDE9A protein, Lise Hoth and Thomas McLellan was mass spectrometry assistance, and Matthew Teague and Thomas O'Connell for HRMS acquisition. All noted individuals are Pfizer colleagues.

Notes and references

1 (*a*) G. Dormán, H. Nakamura, A. Pulsipher and G. D. Prestwich, *Chem. Rev.*, 2016, **116**, 15284; (*b*) E. Smith and I. Collins, *Future Med. Chem.*, 2015, **7**, 159; (*c*) D. J. Lapinsky and D. S. Johnson, *Future Med. Chem.*, 2015, **7**, 2143; (*d*) S. A. Fleming, *Tetrahedron*, 1995, **51**, 12479; (*e*) G. Dormán and G. D. Prestwich, *Biochemistry*, 1994, **33**, 5661.

2 L. Dubinsky, B. P. Krom and M. M. Meijler, *Bioorg. Med. Chem.*, 2012, **20**, 554.

3 For selected examples, see: (*a*) C. J. Crump, H. E. Murrey, T. E. Ballard, C. W. am Ende, X. Wu, N. Gertsik, D. S. Johnson and Y.-M. Li, *ACS Chem. Neurosci.*, 2016, **7**, 1166; (*b*) Z. Li, P. Hao, L. Li, C. Y. J. Tan, X. Cheng, G. Y. J. Chen, S. Kwan Sze, H.-M. Shen and S. Q. Yao, *Angew. Chem., Int. Ed.*, 2013, **52**, 8551.

4 (*a*) C. G. Parker, A. Galmozzi, Y. Wang, B. E. Correia, K. Sasaki, C. M. Joslyn, A. S. Kim, C. L. Cavallaro, R. M. Lawrence, S. R. Johnson, I. Narvaiza, E. Saez and B. F. Cravatt, *Cell*, 2017, **168**, 527; (*b*) J. S. Cisar and B. F. Cravatt, *J. Am. Chem. Soc.*, 2012, **134**, 10385.

5 M. Yan, J. C. Lo, J. T. Edwards and P. S. Baran, *J. Am. Chem. Soc.*, 2016, **138**, 12692.

6 (*a*) F. Fontana, F. Minisci, M. C. N. Barbosa and E. Vismara, *J. Org. Chem.*, 1991, **56**, 2866. For recent representative applications of aryl glyoxylic acids in radical-based benzoylation reactions, see: (*b*) J. K. Laha, M. K. Hunjan, S. Hegde and A. Gupta, *Org. Lett.*, 2020, **22**, 1442; (*c*) M. T. Westwood, C. J. C. Lamb, D. R. Sutherland and A.-L. Lee, *Org. Lett.*,

2019, **21**, 7119; (*d*) S. Manna and K. R. Prabhu, *J. Org. Chem.*, 2019, **84**, 5067; (*e*) G. Bogonda, H. Y. Kin and K. Oh, *Org. Lett.*, 2018, **20**, 2711; (*f*) Q.-Q. Wang, K. Xu, Y.-Y. Jiang, Y.-G. Liu, B.-G. Sun and C.-C. Zeng, *Org. Lett.*, 2017, **19**, 5517. For general reviews of Minisci chemistry applications, see: (*g*) R. S. J. Proctor and R. J. Phipps, *Angew. Chem., Int. Ed.*, 2019, **58**, 13666; (*h*) M. A. J. Duncton, *Med. Chem. Commun.*, 2011, **2**, 1135.

7 See ESI† for additional details.

8 For a comprehensive study on the regiochemistry and predictive power of radical-based C–H functionalization of heteroarenes, see: F. O'Hara, D. G. Blackmond and P. S. Baran, *J. Am. Chem. Soc.*, 2013, **135**, 12122.

9 For complete reference citations for these known biologically active compounds, please refer to the ESI.†

10 (*a*) J. Pérez-Prieto, M. C. Morant-Miñana, R. E. Gallan and M. A. Miranda, *Photochem. Photobiol.*, 2006, **82**, 231; (*b*) A. Albini, P. Bortolus, E. Fasani, S. Monti, F. Negri and G. Orlandi, *J. Chem. Soc., Perkin Trans. 2*, 1993, 691; (*c*) C. R. Hurt and N. Filipescu, *J. Am. Chem. Soc.*, 1972, **94**, 3649; (*d*) E. V. Brown and M. B. Shambhu, *J. Heterocycl. Chem.*, 1971, **8**, 967; (*e*) F. L. Minn, C. L. Trichilo, C. R. Hurt and N. Filipescu, *J. Am. Chem. Soc.*, 1970, **92**, 3600.

11 P. R. Verhoest, K. R. Fonseca, X. Hou, C. Proulx-LaFrance, M. Corman, C. J. Helal, M. M. Claffey, J. B. Tuttle, K. J. Coffman, S. Liu, F. Nelson, R. J. Kleiman, F. S. Menniti, C. J. Schmidt, M. Vanase-Frawley and S. Liras, *J. Med. Chem.*, 2012, **55**, 9045.

12 M. P. Edwards and D. A. Price, *Annu. Rep. Med. Chem.*, 2010, **45**, 380.

13 The cLogP values of the minimalist probes **3r** (4.60) and **3s** (5.45) were also favourably offset when compared to the corresponding benzophenone derivatives (5.89 and 7.20, respectively).

14 (*a*) A. Wittelsberger, B. E. Thomas, D. F. Mierke and M. Rosenblatt, *FEBS Lett.*, 2006, **580**, 1872; (*b*) L. Rihakova, M. Deraet, M. Auger-Messier, J. Perodin, A. A. Boucard, G. Guillemette, R. Leduc, P. Lavigne and E. Escher, *J. Recept. Signal Transduction Res.*, 2002, **22**, 297.

15 Although digestion of the PDE9A-**3q** covalent adduct with trypsin and AspN proteases provided a total sequence coverage of 69%, peptides modified with **3q** were not identified.