Tetrahedron Letters 55 (2014) 3308-3311

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

A rapid, chromatography-free route to substituted acridine-isoalloxazine conjugates under microwave irradiation

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ARTICLE INFO

Article history: Received 29 October 2013 Revised 2 April 2014 Accepted 10 April 2014 Available online 21 April 2014

Keywords: Acridine Isoalloxazine Flavin Microwave

ABSTRACT

Microwave irradiation was applied to a sequence of condensation reactions from readily available 9-chloroacridines to provide a range of novel acridine-isoalloxazine conjugates. The combination of these two moieties, both of biological interest, was achieved by a chromatography-free route. © 2014 Elsevier Ltd. All rights reserved.

Acridine moieties have been of interest to medicinal chemists for many years, often appearing as central scaffolds in many biologically active compounds.^{1–3} Indeed acridines exhibit significant pharmaceutical importance due to their potent photodynamic biological activities.⁴ However this goes hand-in-hand with the well established toxicity profile⁵ of the molecule itself, which represents a considerable obstacle to overcome for progression into mainstream healthcare. In contrast, 7,8-dimethyl-10-ribityl isoalloxazine (riboflavin or vitamin B₂) is an essential nutrient in humans, and is also a photosensitiser which absorbs in the visible region. It shows impressive activity both as an antibacterial⁶ and antiviral agent through both Type I and Type II photosensitisations.⁷

Inspired by the biological activity of both, herein we present a short and efficient method for the rapid generation of hybrid acridine-isoalloxazine conjugates (Fig. 1). The isoalloxazine moiety also contains an acidic N-3 proton which may prove useful for attachment of either a solubilising or a targeting moiety.⁸

Our initial attempts to form the isoalloxazine-acridines followed a three-step route from a range of 9-aminoacridines (route A, Scheme 1); firstly condensation with 2-chloronitrobenzene gave the desired N-substituted 2-nitroaniline in low to moderate yields after chromatography. However, attempted reduction of the nitro group using standard conditions (Zn/H⁺) and subsequent condensation with alloxan monohydrate gave a mixture of products from which the desired compounds could not be isolated. We hypothesised there were three major problems with this route; firstly, the 9-aminoacridines were poor nucleophiles, due to electron withdrawal from the ring nitrogen, resulting in poor yields for the initial S_NAr reaction;⁹ secondly, the zinc reduction was not completely selective resulting in numerous by-products, and finally that the intermediates and products were likely to be poorly soluble in most common solvents making column chromatography challenging.¹⁰ Our second route (route B, Scheme 1) utilised 9chloroacridines as the electrophilic component using the electron-withdrawing acridine to our advantage. By using phenylene diamines as the nucleophiles the reduction step was avoided entirely, albeit at the cost of perhaps limiting our isoalloxazines to containing the same group at both the C7 and C8 positions.¹¹

In order to optimise the reaction conditions, commercially available 6-chloro-2-methoxyacridine (4a) was chosen as a model substrate. There have been several reports of 9-chloroacridines reacting with anilines to form substituted N(9)-anilinoacridine hydrochloride salts by heating under reflux in methanol. Following the procedure of Plater et al.,¹² heating **4a** and aniline in methanol under reflux gave the desired product 6a in moderate yield (Table 1, entry 1), with the only purification required being precipitation and filtration. The use of microwave acceleration of organic reactions has found widespread use.¹³ Two of the major advantages of microwave heating are the ability to heat solvents rapidly in sealed tubes past their boiling points, and the uniformity of





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Figure 1. Structures of isoalloxazine and acridine.



Scheme 1. Routes to isoalloxazine–acridine conjugates. Route A: previously attempted three-step route. Route B: a new two-step route.

heating, potentially resulting in fewer by-products resulting from localised hotspots.¹⁴ Performing the reaction under conditions of microwave irradiation (110 °C, 10 min) resulted in a much faster, and pleasingly, a higher yielding reaction (Table 1, entry 2), although heating the reaction at higher temperature (140 °C) did result in decreased purity. During this work, Staderini et al. reported a solvent-free microwave route for amination of π -electron deficient heterocycles including acridines; in their cases the reactions were promoted by the addition of two equivalents of phenol, giving excellent yields.¹⁵ Conventional conditions for condensation of 2-aminoanilines with alloxan monohydrate generally require prolonged (≥ 12 h) stirring at room temperature with ace-

Table 1

Synthesis of acridine-isoalloxazines 7a-g and intermediate diaryl amines 6a-g derived from 6,9-dichloroacridines 4a-g

Entry	Starting material	\mathbb{R}^2	R ³	R ⁴	R ⁵	Yield 6 ª (%)	Yield 7 ^b
1	4a	Н	OMe	Н	Н	6a :53 ^c	N/D ^d
2	4a	Н	OMe	Н	Н	6a :93	7a:95%
3	4a	Н	OMe	Н	Me	6a ':91	7a ':86%
4	4a	Н	OMe	Н	Cl	6a ":90	7a ":92%
5	4b	Н	Н	OMe	Н	6b :83	7b :(76%) ^e
6	4c	Н	OMe	OMe	Н	6c :69	7c :(68%) ^e
7	4d	OMe	Н	OMe	Н	6d:83	7d :(84%) ^e
8	4d	OMe	Н	OMe	Cl	6d":84	7d ":(38%) ^e
9	4e	Н	Me	Н	Н	6e :95	7e :86%
10	4f	Н	Н	Me	Н	6f :96	7f :(61%) ^e
11	4g	Н	Н	Н	Н	6g :82	7g :83%

 $^{\rm a}$ Isolated yields after precipitation from $Et_2O,$ filtration, washing and drying (1 mmol scale).

 $^{\rm b}$ Isolated yields after precipitation from Et_2O, filtration, washing and drying (0.5 mmol scale).

^c Heating under reflux for 1.5 h.

^d Not determined, see entry 2.

^e *Pro rata* after high temperature microwave extraction/recrystallisation of a 150 mg portion of the crude product (see Supplementary information).

tic and boric acid giving moderate to high yields (most often 40-70%) of isoalloxazines.¹⁶ In order to increase the rate of this reaction, we again attempted this reaction under microwave irradiation. Condensation of alloxan monohydrate with the acridine salt 6a in the absence of boric acid, even at elevated temperatures (140 °C) did not occur in MeOH or AcOH to any measurable extent. The reaction was successful in AcOH with one equivalent of boric acid giving the product 7a in excellent yield (95%, 140 °C, 10 min, Table 1, entry 2). Chauhan et al. have reported the condensation of some alkyl and aryl substituted 2-aminoanilines with alloxan monohydrate under microwave irradiation in a domestic microwave oven to give 10-substituted isoalloxazines in good yield.¹ The use of our own conditions gave the product isoalloxazine 7a rapidly in high yield and purity, after precipitation, filtration and washing (including water washes to remove boric acid), without recourse to column chromatography (85% over 2 steps from **4a**).

Preparation of a range of 9-chloroacridines was performed following reported methods (Scheme 2)¹⁸ via reaction of anilines **2** with several 2-chlorobenzoic acids **1**.¹⁹ The formed crude *N*-aryl anthranillic acids **3** were then ring-closed with POCl₃ under microwave irradiation.²⁰ The desired 9-chloroacridines **4** could be isolated by a variety of methods including vacuum sublimation, vacuum filtration through a pad of silica gel or recrystallisation to give single compounds. Several greener methods have been reported in the literature for the synthesis of *N*-aryl anthranilic acids including solvent-free conditions with microwave irradiation²¹ and the use of ultrasound in aqueous solution.²²

The optimised conditions were applied to a range of acridines 4a-g derived from 2,4-dichlorobenzoic acid (Scheme 3, Table 1). Several different phenylene diamines **5a-c** were used to vary the electronics of the isoalloxazine system and to make flavin derivatives, giving the corresponding products 6, 6' or 6" in moderate to high yields as the corresponding hydrochloride salts.²³ The lowest yield was obtained from 2,4-dimethoxyacridine 4c, which has the most electron-rich C9 position (69%, Table 1, entry 6). In all cases the reaction was highly regioselective with no evidence of any reaction with the 6-chloro group. The isoalloxazines 7, 7', 7" were formed in all cases.²⁴ however, in several examples, the final compounds were contaminated with by-products, often from residual phenylene diamines present in the intermediate hydrochloride salts. Attempted purification of several products that were of unacceptable purity by column chromatography or recrystallisation was particularly difficult due to the insolubility of the acridine-isoalloxazines 7. It was discovered that the majority of impurities could be removed by high temperature extraction/ recrystallisation in methanol under microwave irradiation (see Supplementary information), and the purified yields are reported in parentheses. Extraction in a sealed system at high temperature



Scheme 2. Preparation of the 9-chloroacridine starting materials 4.



Scheme 3. Microwave route to acridine-isoalloxazines 7a-g from 6,9-dichloroacridines.

is both faster, uses a much smaller volume of solvent than traditional methods such as Soxhlet extraction, and is often used in the extraction of organic molecules from environmental²⁵ or cellular matrices.²⁶ As, in this case, the products **7** were observed to be sparingly soluble in methanol, the use of lower volumes of solvent results in less product being lost in the extraction solvent. With the 1,4-dimethoxy substituted intermediate **6d**", ¹H NMR of the product **7d**["] after precipitation showed residual starting material, which was also successfully removed by high temperature extraction. We suggest that the increased steric hindrance in this case (and presumably to a lesser extent in the case of **6d**) resulted in slower conversion and the reaction would benefit from a prolonged reaction time. Scale-up $(4\times)$ of the reactions to form **7a** and **7a**' gave the desired products in similar yields, although it should be noted that stirring of the reaction mixture must be efficient to ensure complete conversion of the starting material.

Similar reactions were attempted with acridines 4h-l and 4m,n derived from 2-chlorobenzoic acid and 2-chloro-4-nitrobenzoic acid, respectively (Scheme 4, Table 2). With substrate 4i, which was particularly deactivated by the 4-methoxy group, only a moderate yield of acridine salt 6i was obtained on reacting with o-phenylene diamine (5a) (67%), however extending the reaction time to 20 minutes increased the yield such that it was comparable with the others (86%, Table 2, entry 3). Substrate 41 had the opposite problem, activation by the 4-nitro group made the substrate too reactive, and the reaction produced a complex mixture of products as a black tar (Table 2, entry 10). Indeed nucleophilic displacement on 9-chloro-4-nitroacridine **41** has been reported in the literature only once with an aniline electrophile, and in this case, was performed at 0 °C.²⁷ Pleasingly, 6-nitro substituted acridines (where the nitro group does not withdraw electrons from the 9-position by resonance) did not suffer from this problem giving the desired products (6m and 6n, Table 2, entries 11 and 12). Conversion into the acridine-isoalloxazines gave the products 7, in most cases without further purification being required, and where this was not the case, high temperature microwave extraction was performed to remove unwanted impurities. The products containing 6-nitro-substituted acridines **7m** and **7n** were particularly insoluTable 2

Synthesis of acridine-isoalloxazines **7h-n** and intermediate diaryl amines **6h-n** derived from acridines **4h-n** without a 6-chloro group

Entry	Starting material	R ¹	R ³	\mathbb{R}^4	R ⁵	Yield 6 ª (%)	Yield 7 ^b
1	4h	Н	OMe	Н	Н	6h :85	7h :73%
2	4h	Н	OMe	Н	Me	6h ′:97	7h ′:77%
3	4i	Н	Н	OMe	Н	6i :86 ^c	7i :(64%) ^d
4	4i	Н	Н	OMe	Me	6i ′:93 ^e	7i ′:73%
5	4i	Н	Н	OMe	Cl	6i ":82	7i ":82%
6	4j	Н	Me	Н	Н	6j :83	7j :70%
7	4k	Н	Н	Н	Н	6k :97	7k :88%
8	4k	Н	Н	Н	Me	6k ′:85	7k ′:89%
9	4k	Н	Н	Н	Cl	6k ":87	7k ":94%
10	41	Н	Н	NO_2	Н	-	_
11	4m	NO_2	OMe	Н	Н	6m :86	7m :79% ^f
12	4n	NO_2	Me	Н	Н	6n :90	7n :76% ^f

 $^{\rm a}$ Isolated yields after precipitation from $Et_2O,$ filtration, washing and drying (1 mmol scale).

 $^{\rm b}$ Isolated yields after precipitation from Et_2O, filtration, washing and drying (0.5 mmol scale).

^c After heating for 20 min (67% with only 10 min heating).

^d *Pro rata* after high temperature microwave extraction/recrystallisation of a 150 mg portion of the crude product (see Supplementary information).

e After 20 min heating.

^f Isolated as a \sim 1:1 mixture with AcOH.

ble in acetic acid and crystallised in the reaction vessel as a \sim 1:1 mixture with acetic acid (by ¹H NMR) and were isolated as such.

The use of substrate **40**, containing an extra contiguous ring proved challenging (Scheme 5), and the normal optimised conditions failed to give any product, whilst raising the temperature significantly resulted in the formation of several by-products; in this case a slower one-hour reaction at a slightly elevated temperature (120 °C) gave the best compromise between rate and purity. The isoalloxazine **70** was formed from the HCl salt **60** using the optimised conditions. In this case two purifications, including chromatography and a high temperature microwave extraction, were required to give the product in high purity and the significantly lower yield can be attributed to this.



Scheme 4. Microwave route to acridine-isoalloxazines 7h-n from 6-hydro or 6-nitro-9-chloroacridines 4h-n.



Scheme 5. Extension of the method to form an isoalloxazine conjugate of a 3,4-benzacridine.

The products were characterised initially by ¹H NMR spectroscopy and exhibited some interesting features. Signal broadening was often observed in the protons between the two tricvclic systems. Steric clashes along these sides will prevent the molecules from adopting a planar structure. The two ring systems must be offset with rotation around the N10–C9' bond being restricted, resulting in the products existing as two enantiomeric atropisomers. Separation of axially chiral N-phenyl isoalloxazines has been reported,²⁸ and planar chiral flavin derivatives have been used as asymmetric catalysts in sulfide²⁹ and Baever–Villiger³⁰ oxidations. The twist in the structure will also result in decreased conjugation between the two ring systems. In the case of product 7d one of the acridine methoxy groups points directly towards the isoalloxazine ring system, and the chemical shift of the methyl group is particularly low (3.23 ppm in 7d compared to 4.03 ppm in 6d); the shielding in this case is due to the magnetic anisotropy of the isoalloxazine system.

In summary, we have reported a rapid, chromatography-free method for the preparation of acridine–isoalloxazine conjugates from substituted 9-chloroacridines. We are currently undertaking biological and photophysical testing of the compounds to determine their applicability for photodynamic antimicrobial chemotherapy.

Acknowledgments

We are pleased to acknowledge the financial support from the Centre for Materials Sciences, School of Forensic and Investigative Sciences and the EPSRC national mass spectrometry service centre (NMSSC), Swansea for the recording of HRMS.

Supplementary data

Supplementary data (experimental procedures and analytical data for the compounds **6a–o** and **7a–o**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.04.035.

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- 23. For example: A mixture of 6,9-dichloro-2-methoxyacridine (4a) (278 mg, 1.0 mmol, 1 equiv) and phenylenediamine (5a) (238 mg, 2.2 mmol, 2.2 equiv) was suspended in MeOH (5 mL) and subjected to microwave irradiation (110 °C, 10 min, CEM Discover[®] microwave). The mixture was allowed to cool to room temperature giving a red liquid with a red/orange precipitate. The mixture was then transferred into rapidly stirred Et₂O. After stirring for 5 min, the solid was isolated by vacuum filtration, washed with Et₂O and dried giving the product hydrochloride salt 6a (359 mg, 93%) as a red/orange solid.
- 24. For example: A mixture of diamine intermediate **6a** (193 mg, 0.50 mmol, 1.0 equiv), alloxan monohydrate (96.0 mg, 0.60 mmol, 1.2 equiv) and boric acid (30.9 mg, 0.50 mmol, 1.0 equiv) were suspended in AcOH (5 mL) and subjected to microwave irradiation (140 °C, 10 min, CEM Discover[®] microwave). The mixture was allowed to cool to room temperature forming a yellow/orange precipitate. The mixture was then transferred into rapidly stirred Et₂O. After stirring for 5 min, the solid was isolated by vacuum filtration, washed with Et₂O and H₂O and dried giving the product isoalloxazine–acridine conjugate **7a** (217 mg, 95%) as a yellow solid.
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