Tetrahedron xxx (2015) 1–12

Contents lists available at ScienceDirect

Tetrahedron

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

Synthesis and fluorescence properties of 4-diarylmethylene analogues of the green fluorescent protein chromophore

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ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form 20 May 2015
Accepted 21 May 2015
Available online xxx

Keywords:
Fluorescence
Chromophore
Imidazolinones
Green fluorescent protein

ABSTRACT

New green fluorescent protein (GFP) chromophore analogues, namely 4-(diarylmethylene)imidazolinones (DAINs), were readily synthesized under weakly acidic conditions using a novel condensation reaction between methyl imidate (or thioimidate) and ethyl *N*-(diarylmethylene)glycinate. DAINs showed notable fluorescence properties. Although they were nearly non-fluorescent in the solution, visible emissions were detected in most of their frozen solution states and crystalline powder states. Therefore, control of the molecular motions significantly affected emissions by DAINs. Comparison of the fluorescence properties of DAIN **5a** with those of the corresponding GFP-chromophore analogues **8** revealed that **5a** possessed superior solid-state fluorescence properties.

1. Introduction

Green fluorescent protein (GFP) is a well-known fluorescent protein, and is widely employed as a useful imaging tool because it can help visualise various biological events through highly sensitive fluorescence detection.[1] The chromophore within GFP is formed from a Ser-Tyr-Gly tripeptide in the primary protein structure by post-translational cyclodehydration and subsequent oxidation to yield the 4-(*p*-hydroxybenzylidene)imidazolinone (HO-BDI) moiety. The conformation of the HO-BDI chromophore is restricted to the *Z*-form within the GFP β-barrel tertiary structure, which enables emission by GFP.[2] However, outside the protein, fluorescence quenching of the chromophore is induced mainly by molecular motions such as the free rotation of the aryl–alkene single bond and the double bond isomerization of the *Z*-4-hydroxybenzylidene moiety ('bright' form) to the corresponding *E*-isomer,[3,4] which is generally known to exhibit extremely weak fluorescence ('dark' form),[5] although exceptions are known[6] (Fig. 1A).

In an effort to prepare new small-molecule fluorescent materials, we designed a series of diarylmethylene-based compounds, namely 4-(diarylmethylene)imidazolinones (DAINs), as novel analogues of the GFP chromophore (Fig. 1B). Our design comprised three

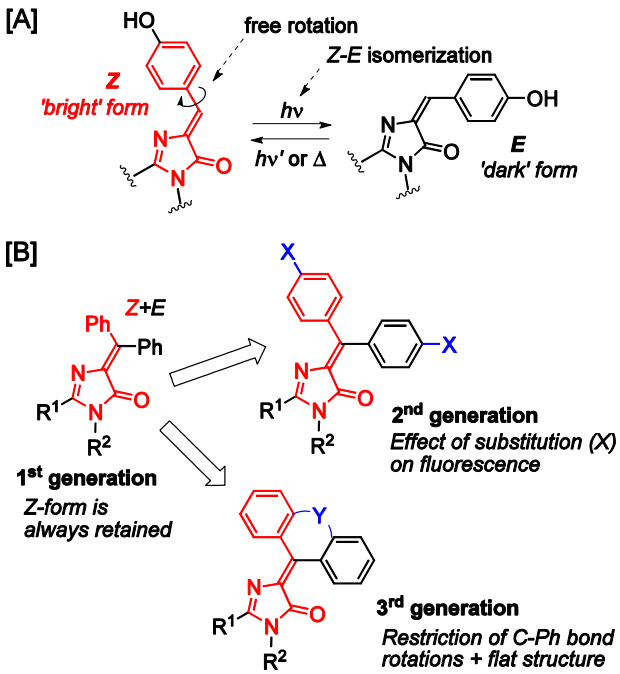

Fig. 1. A. Structure of the GFP chromophore (HO-BDI). B. Design of the model compound, 4-(diarylmethylene)imidazolinone (DAIN).

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http://dx.doi.org/10.1016/j.tet.2015.05.073
0040-4020/

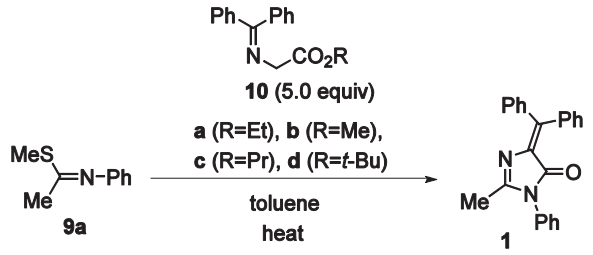
structural generations. The first generation contained a diphenylmethylene unit on the imidazolinone ring. Because this moiety is symmetrical, the structure is the same after the double-bond isomerization. Therefore, the fluorescence (attributed to the *Z*-form) could be maintained regardless of the isomerization. The second- and third-generation structures were designed by focusing on the diarylmethylene moiety. As a common imidazolinone moiety of these two generations, we selected a 1,2-pentanoimidazolinone structure based on the fluorescence properties of the first-generation DAINs **1**–**7** (Fig. 2), whose details are described in Section 2.2.1.1. The second-generation structures were designed to elucidate substituent effects (electron donating and electron withdrawing) on the diphenyl moiety of the first-generation compounds (DAINs **5b** and **5c**). In the third generation, the diphenyl rings were designed to be in nearly the same plane as the imidazolinone ring by installing a bridge between the diphenyl rings (DAINs **5d** and **5e**). Such installation would prevent C–Ph free rotation. We expected that this third generation would exhibit the strongest emission because of the overall structural advantages described above. Although several types of 4-benzylideneimidazolinone (BDI) analogues with substantial fluorescence have been reported so far,[7–10] to the best of our knowledge, there has been no report on such diarylmethylene analogues of the GFP chromophore. In this study, we report the synthesis of the DAIN series (Section 2.1) and their fluorescence properties, including a comparison of DAIN **5a** with analogous BDI compounds, *Z*- and *E*-**8** (Section 2.2).[11]

2. Results and discussion

2.1. Synthesis of the DAIN series

2.1.1. Optimization of reaction conditions. Although several synthetic approaches towards BDI-based structures have been reported,[10,12,13] no effective approaches towards DAIN structures are known. Notably, the syntheses used for BDI[10,12] were ineffective in the synthesis of DAIN **1**. Thus, we endeavoured to develop a novel synthetic method to obtain DAIN **1**, and eventually identified a suitable concise condensation reaction between methyl thioimidate **9a** and ethyl *N*-(diphenylmethylene)glycinate **10a**, as shown in Table 1. Although the product, **1**, was not yielded by mixing **9a** and **10a** at room temperature, it was obtained in 52%–60% yield in hot solvents like toluene and 1,2-dichloroethane, although a long reaction time was required (Table 1, runs 1–4). However, contrary to our expectations, the product was not

Table 1
Reaction of thioimidate **9a** with *N*-(diphenylmethylene)glycinates **10a**–**d**



Run	**10**	Temp (°C)	Additive [equiv]	Time (days)	Yield (%)
1	**a**	rt	None	7.0	0
2	**a**	70	None	7.0	55
3	**a**	80	None	4.0	60
4	**a**	80	None	3.0	52[a]
5	**a**	Reflux	None	7.0	Trace[b]
6	**a**	80	EtOH [5.0]	2.5	43
7	**a**	80	BuSH [1.0]	3.0	58
8	**a**	80	AcOH [1.0]	1.0	64
9	**a**	Reflux	AcOH [2.0]	1.0	78
10	**a**	rt to reflux	MsOH [2.0]	1.0	0[c]
11	**a**	Reflux	$ZnCl_2$ [2.0]	0.1	0[c]
12	**a**	Reflux	LiCl [2.0]	1.0	0[b]
13	**b**	Reflux	AcOH [2.0]	1.0	7[d]
14	**c**	Reflux	AcOH [2.0]	1.0	12[d]
15	**d**	Reflux	AcOH [2.0]	1.0	13[d]

[a] $ClCH_2CH_2Cl$ was used as the solvent.
[b] Almost no reaction.
[c] Complex mixture.
[d] The reaction was not complete within 24 h.

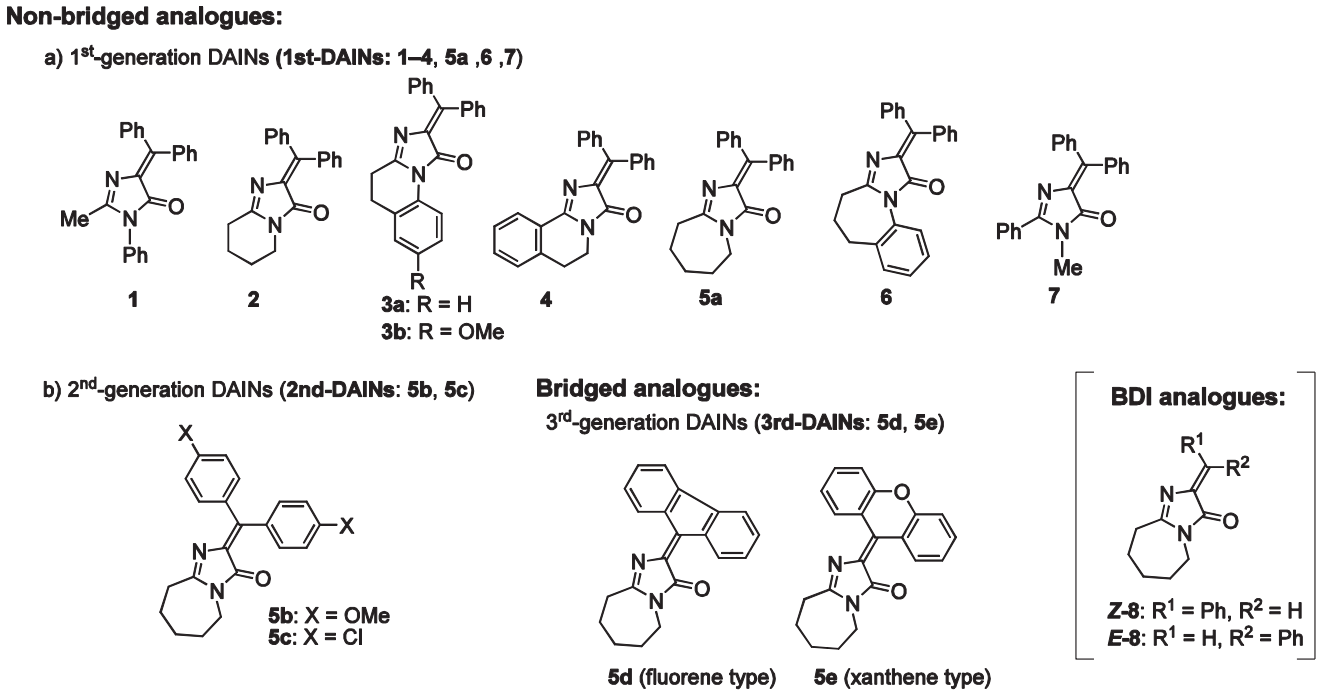


Fig. 2. Structures of DAIN series and BDI analogues.