Bioorganic & Medicinal Chemistry Letters 24 (2014) 2123–2128

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis of strigolactones analogues by intramolecular [2+2] cycloaddition of ketene-iminium salts to olefins and their activity on *Orobanche cumana* seeds

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ARTICLE INFO

Article history:
Received 31 January 2014
Revised 12 March 2014
Accepted 14 March 2014
Available online 24 March 2014

Keywords:
Strigolactones
Cycloaddition
Ketene-iminium salts
Orobanche cumana
Germination inducer

ABSTRACT

Strigolactones have been the latest identified phytohormones. Among the strigolactones analogues described recently, GR-24 remains the most studied derivative which is used as standard in this field. In order to improve several properties of GR-24 for potential agronomical applications, we investigated the effect of substituents on the B and C-rings on the activity for seed germination induction. We report here the synthesis of 9 GR-24 analogues via a [2+2] intramolecular cycloaddition of ketene-iminium salts and a summary of their activity for the germination of *Orobanche cumana* (broomrape) seeds.

Strigolactones have been the latest identified phytohormones.[1] Very recently, major advances in the elucidation of the key roles played by strigolactones in seeds and in plants have been accomplished, including the identification of the molecular receptors involved in the signal transduction mechanism.[2] Strigolactones analogues are very attractive targets for potential agronomical applications, for example as seed germination stimulators, plant growth regulators, in particular under abiotic stress conditions.[2,3] Among the strigolactones analogues described recently, GR-24 remains the most studied derivative which is used as standard in this field.[4] In order to improve several properties of GR-24 for potential agronomical applications, we investigated the effect of substituents on the B, C-rings on the activity. Substitution of GR-24 has been shown to improve the activity on the germination of *Striga hermontica* and *Orobanche ramosa* seeds or on pea branching.[5] We report here the synthesis of 9 GR-24 analogues and for the first time their activity for the germination of *Orobanche cumana* (broomrape) seeds, a commercially very relevant parasitic weed species.

We have recently developed an efficient asymmetric synthesis of GR-24 using an intramolecular [2+2] cycloaddition of ketene and ketene-iminium salts to olefins.[6] We have now successfully extended this approach to synthesize GR-24 analogues carrying additional substituents at defined positions on the B and C-rings. The cyclobutanones are converted into the corresponding lactones by regioselective Baeyer–Villiger oxidation (Fig. 1).

From previous studies, we have shown that ketene-iminium salts are superior to the corresponding ketenes for the intramolecular [2+2] cycloaddition.[6] Therefore, we used in this present work exclusively ketene-iminium derivatives which give higher yield, especially under more concentrated conditions, compared to the corresponding ketenes. Amide **1**, the common starting material, was prepared from 2-iodophenyl acid and was coupled via Stille reaction with commercially available stannane **2** to afford allyl derivative **3a** in good yield (Scheme 1). When treated with trifluoromethylsulfonic anhydride in the presence of *sym*-collidine, the ketene-iminium salt was formed and subsequent intramolecular [2+2] cycloaddition gives the cyclobutanone **4a** with complete regioselectivity, as expected for a terminal olefin for steric and electronic reasons. This result contrasts with the mixture of regioisomers obtained with the corresponding unsubstituted allylic derivative.[6] Baeyer–Villiger oxidation was highly regioselective and the tricyclic lactone **5a** was isolated as single product.

We applied then the same approach to introduce a methyl substituent at C-8b (Scheme 2). Aryl iodide **1** was coupled to *n*-tributyl allylstannane and alkylated with methyl iodide to give compound **3b**. Surprisingly, under the standard conditions, the ketene-iminium formation and cycloaddition sequence was low yielding with only 10% of the desired cyclobutanone **4b** obtained, the starting compound being mostly recovered. Temperature had little effect

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http://dx.doi.org/10.1016/j.bmcl.2014.03.044
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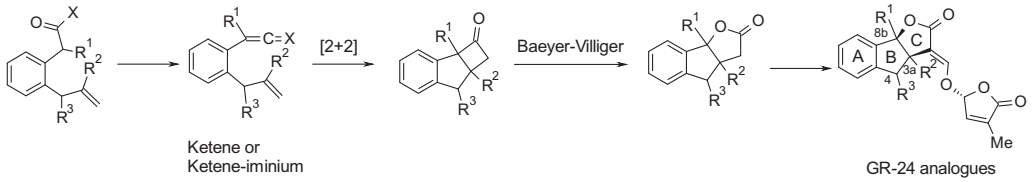
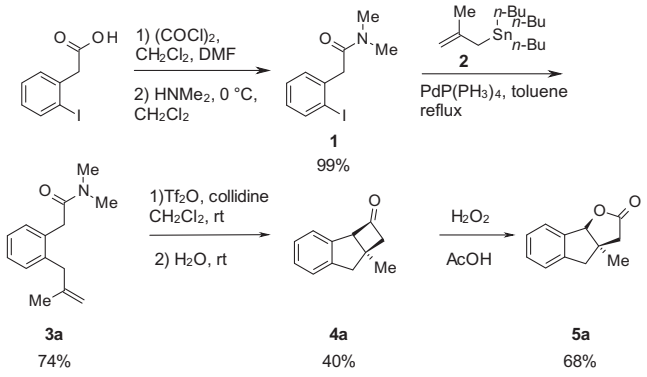
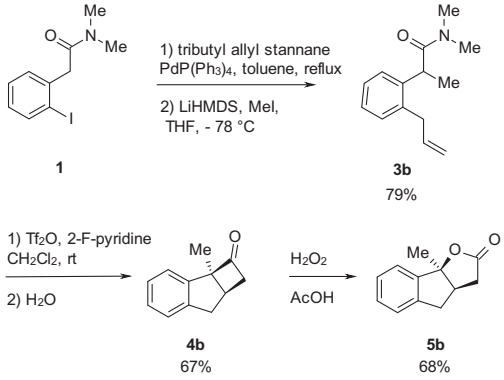


Figure 1. Synthesis of analogues of GR-24 substituted at C-3a, C-4 and C-8b.



Scheme 1. Synthesis substituted 4-methyl tricyclic ABC skeleton.



Scheme 2. Synthesis of tricyclic lactone substituted at C-8b.

on the outcome of the reaction but the addition to two equivalents of reagents and longer reaction time improved the conversion to 55% (entry 4). Different bases were then investigated (Table 1). Diisopropylethylamine (DIPEA) gave the desired product, albeit in only 26% yield. Triethylamine was too nucleophilic and decomposition was observed whereas the addition of DMAP inhibited completely the formation of the ketene-iminium (or quenched it after its formation). Recently, Maulide and co-workers have reported the use of 2-fluoropyridine to improve the formation of ketene-iminium salt.[7] This condition showed a great improvement in our system with 67% of the desired product isolated

Table 1
Optimization of the cycloaddition of **3b**

Entry	Base	Time and T (°C)	Yield **4b**
1	Collidine (1 equiv)	8 h at rt	10% (+85% 3b)
2	Collidine (1 equiv)	24 h at rt	8% (+85% 3b)
3	Collidine (1 equiv)	24 h at 40 °C	10% (+88% 3b)
4*	Collidine (2.4 equiv)	70 h at rt	50% (+45% 3b)
5	DIPEA (1.1 equiv)	8 h at rt	26%
6	DIPEA (5 equiv)	8 h at rt	Decomposition
7	DBU (1.1 equiv)	8 h at rt	Starting material
8	Triethylamine (1.1 equiv)	8 h at rt	Decomposition
9	Collidine, DMAP	8 h at rt	Starting material
10*	2-F-pyridine (2.4 equiv)	70 h at rt	67%

* 2.0 equiv of Tf_2O were used in the reaction.

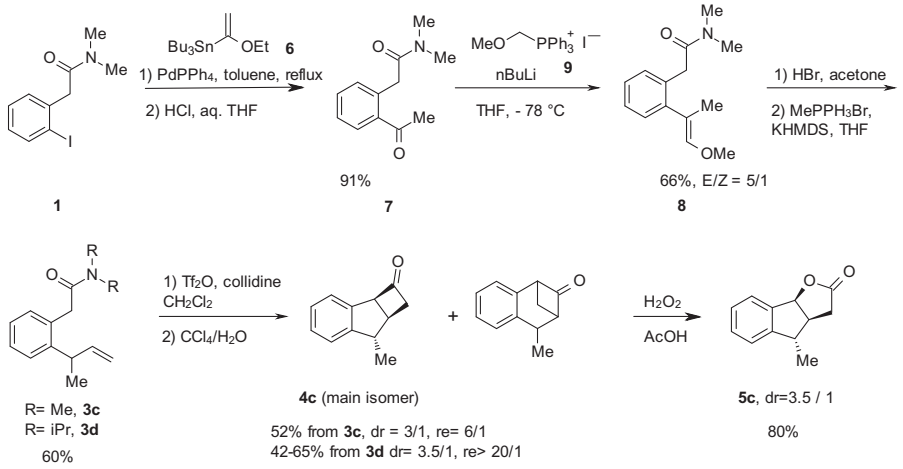
The low reactivity of our substrates was surprising as Ghosez and co-workers have shown that the formation of ketene-iminium salts and their cycloaddition tolerate two adjacent substituents on the amide.[8] In our case, in the preferred conformation of the *O*-trifluoromethylsulfonyl iminium intermediate, the benzylic proton suffered from steric hindrance and from reduced kinetic acidity due to its orthogonal orientation with the aryl ring. Both steric and electronic factors led to a slow formation of the ketene-iminium salt. The synthesis of the strigolactone analogue was carried on and tricyclic lactone **5b** was obtained (Scheme 2).

The synthesis of the C4-Me analogue required the introduction of an additional methyl group in the allylic position prior to the intramolecular [2+2] cycloaddition (Scheme 3). Stille coupling of aryl iodide **1** with stannane **6** followed by hydrolysis gave ketone **7**. Then, Wittig reaction between the ketone **7** and the phosphonium ylide **9** led to the compound **8** in good yield when *n*-BuLi was used as a base, to avoid the intramolecular Claisen condensation product. The hydrolysis with HBr was quantitative and another Wittig reaction afforded the olefin **3c** in 70%.

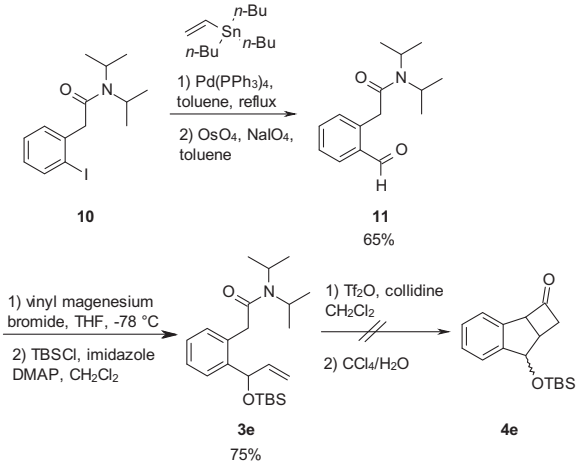
The cycloaddition was carried out under our standard conditions with the *N*,*N*-dimethylamide derivative **3c**, expecting that the additional methyl group in the allylic position could induce some stereocontrol during the intramolecular [2+2] cycloaddition. Cyclobutanone **4c** was isolated in good yield, however has a mixture of 2 regioisomers (6:1), each regioisomer being a mixture of diastereoisomers (3:1). We had found in our recent studies on GR-24 that the replacement of the *N*,*N*-dimethylamide by the *N*,*N*-diisopropylamide reduced the reactivity of the ketene-iminium and increased the regioselectivity of the reaction.[6] The *N*,*N*-diisopropylamide **3d** was prepared according to the same scheme. Indeed, the cycloaddition of **3d** gave 70% of the cyclobutanone **4c** as a single regioisomer and a 3.5:1 mixture of diastereoisomers. The stereochemistry of the 2 compounds was determined by ^{1}H NMR-NOE analysis. Baeyer–Villiger oxidation of the cyclobutanone **4c** afforded the tricyclic lactone **5c** (The major diastereoisomer is depicted in Scheme 3).

Finally, the incorporation of a hydroxy group at C-4 was investigated as a mimic of the natural products orobanchol and solanacol (Scheme 4). Aldehyde **11** was obtained in 2 steps by Stille coupling of aryl iodide **10** with vinyl stannane and oxidative cleavage with OsO_4 and $NaIO_4$. Then vinyl magnesium bromide was added and the resulting alcohol was protected with a TBS group. Unfortunately, the cycloaddition was disappointing, giving low yield of the desired cyclobutanone and with no diastereoselectivity. The allylic silylether deactivates the C=C bond for the intramolecular [2+2] cycloaddition reaction (lowering the level of the HOMO and the coefficient on the terminal carbon atom) and probably interferes through addition of one of the oxygen electron lone pairs to the highly electrophilic ketene-iminium.[9]

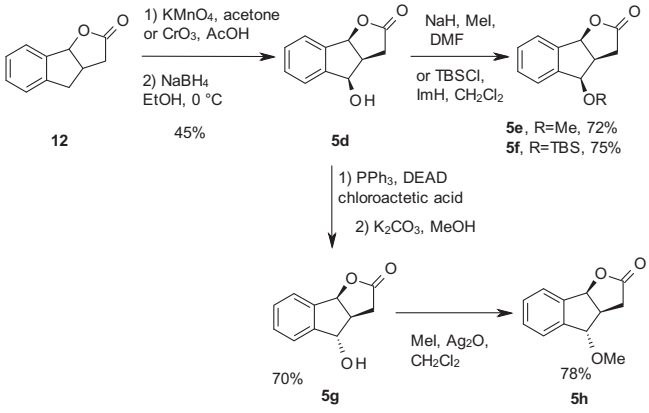
Consequently, we turned our strategy towards the direct oxidation of lactone **12**, in a similar manner as reported by Zwanenburg and co-workers.[5a] Treatment of lactone **12** with potassium



Scheme 3. Synthesis of analogues with substitution at C-4.



Scheme 4. Synthesis of solanacol analogue via cycloaddition.

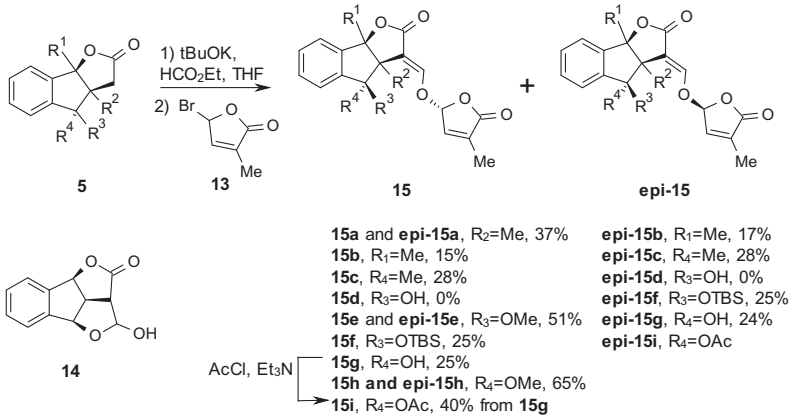


Scheme 5. Second approach to analogues of solanacol.

permanganate or chromium trioxide gave lactone **13** in good yield although with incomplete conversion.[5a] The ketone was reduced to the alcohol with sodium borohydride in methanol/THF at 0 °C and alcohol **5d** was obtained as a mixture of *cis* and *trans* isomer (5:1), which could be readily separated by chromatography. The hydroxy group of **5d** was methylated or protected with a TBS group to give tricylic lactone **5e** and **5f**. In order to obtain the hydroxy analogue with the natural configuration, the hydroxy group in **5d** was inverted by Mitsunobu reaction with chloroacetic acid followed by hydrolysis of the corresponding ester to give tricyclic lactone **5g**. The hydroxy group was methylated using silver oxide and iodomethane leading to lactone **5h** (Scheme 5).

With our eighth tricyclic ABC fragments in hand, the final steps were performed to access the different strigolactones analogues (Scheme 6). Formylation with *t*BuOK and ethyl formate gave the corresponding potassium salt which was either reacted directly in situ with the bromobutenolide **13** or after isolation of the enol followed by base treatment. The strigolactone analogues were obtained as a mixture of diastereoisomers **15** and **epi-15** which were easily separated by column chromatography. The strigolactone **15g** was also acetylated to give strigolactones analogues **15i**. To our surprise, reaction of the lactone **5d** having the unprotected β C4-OH with ethyl formate led to the formation of the hemiacetal **14**. The same was also obtained during deprotection of the TBS group of lactone **15f** with TBAF and acetic acid. We assumed that under these conditions cleavage of the central linkage between the A,B,C-rings and the butenolide occurred, leading to the corresponding formyl derivative which cyclized into the stable lactol **14** (Scheme 6).

The nine GR-24 analogues were then tested for their germination activity on *Orobanche cumana* seeds (Table 2), a commercially very relevant parasitic weed species (broomrape) causing significant yield losses in sunflower.[10] Standard protocol for broomrape seed germination induction was used.[11] All derivatives with the α-stereochemistry for ring D showed potent seed germination induction. For all compounds tested, the corresponding β-epimers for ring D displayed much weaker activity. The methyl group was very well tolerated at C-4 position (**15c**), but was otherwise detrimental to the activity when located at C-3a (**15a**) or at C-8b (**15b**). For the solanacol analogues, introduction of a C-4 β-OMe with the unnatural β-stereochemistry reduced the activity for **15e** compared to the parent compound GR-24. In sharp contrast, the C-4 hydroxy group with the 'natural' α-configuration had a beneficial effect on the germination activity (**15g**) compared to GR-24, as well as the corresponding acetylated alcohol **15i**, which could act as a procide of the alcohol. Compounds **15g** and **15i** are the most active germination inducers of *Orobanche cumana* seeds that we have identified, so far. In comparison to other parasitic weeds, *Orobanche cumana* seeds were highly sensitive to the stereochemistry of the butenolide whereas addition of C-4-*O* acetate was beneficial as observed with *Striga hermontica* and *Orobanche ramosa*.[5a] These results provide us very useful insights for the design of further im-



Scheme 6. Synthesis of GR-24 analogues.

Table 2
Germination of Orobanche Cumana induced by GR-24 analogues.

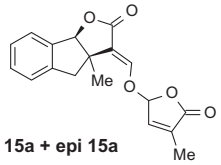
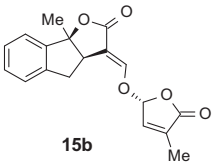
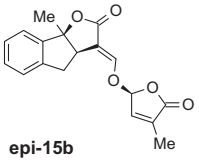
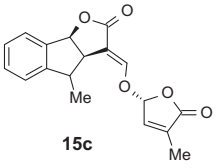
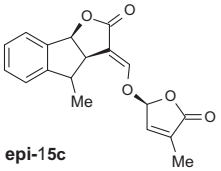
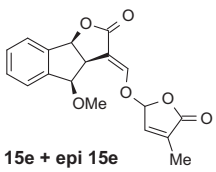
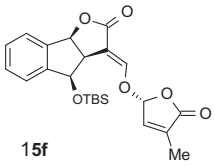
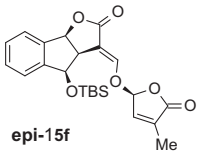
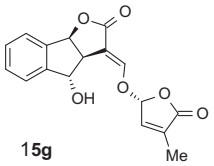
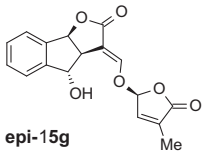
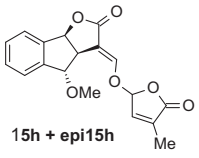
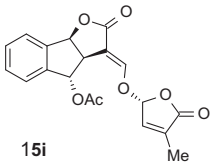
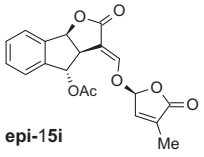
Compounds		Max. germination% at concentration of		
		0.1 mg l^{-1}	0.01 mg l^{-1}	0.001 mg l^{-1}
 **15a + epi 15a**	[a]	82.6	72.6	11.5
 **15b**	[b]	73.8	70.4	38.4
 **epi-15b**	[b]	72.4	23.8	0
 **15c**	[b]	75.2	79.4	70.0
 **epi-15c**	[b]	75.6	60.0	0.6
 **15e + epi 15e**	[c]	29.6	17.6	3.2

Table 2 (*continued*)

Compounds		Max. germination% at concentration of		
		0.1 mg l^{-1}	0.01 mg l^{-1}	0.001 mg l^{-1}
 **15f**	[c]	87.6	47.4	45.4
 **epi-15f**	[c]	35.4	6.6	1.2
 **15g**	[c]	85.4	88.6	79.2
 **epi-15g**	[c]	70.8	0.6	0
 **15h + epi15h**	[c]	93.8	74.0	22.4
 **15i**	[c]	94.8	95.4	75.8
 **epi-15i**	[c]	92.4	72.4	0.8

[a] Control = 0%, GR-24 (0.1 mg/L) = 88%; GR-24 (0.01 mg/L) = 78%.
[b] Control = 0.4%, GR-24 (0.1 mg/L) = 76%; GR-24 (0.01 mg/L) = 60%.
[c] Control = 0.4%, GR-24 (0.1 mg/L) = 60%; GR-24 (0.01 mg/L) = 70%.

proved GR-24 analogues. We have identified few positions in the reference compound GR-24 where substituents could be introduced, without compromising biological activity, which could be used for example to increase soil stability and bioavailability, resistance towards hydrolysis and selectivity.

In conclusion, our approach using intramolecular [2+2] cycloaddition of ketene-iminium salts to olefins is very efficient for the synthesis of substituted GR-24 analogues offering the possibility to introduce substituents able to modulate the activity and the properties of these derivatives in order to potentially use them for agronomical applications.

Acknowledgments

We thank Denise Baumann for her support for the *Orobanche cumana* test and Roman Staiger for essential experimental contribution.

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10. Parker, C. *Pest Manag. Sci.* **2009**, *65*, 453.
11. *Orobanche cumana germination assay:* Seeds of *Orobanche cumana* were collected from a sunflower field in Manzanilla (Seville, Spain), cleaned by sucrose floatation technique[12a], and disinfected 2 min in 1% sodium hypochlorite solution and 0.025% (v/v) Tween 20. Seeds were decanted onto two layers of cheesecloth, rinsed with sterile deionised water and re-suspended in sterile deionised water. Two ml seed suspension containing approximately 150–400 seeds were spread evenly on two layers of sterile wet glass fiber filter paper disc in Petri dishes (ø 9 mm). Seeds were incubated 10 days at 20 °C in the dark for seed conditioning. The upper disc with seeds was briefly dried, transferred to a petri dish lined with a dry GFFP disc, and wetted with 6 ml of the appropriate test solution. Compounds were tested at concentrations of 0.001, 0.01, and 0.1 mg l^{-1}. The strigolactone analogue GR24 was included as positive control and 0.01% DMSO as negative control. All treatments were tested in five replicates. Seeds were re-incubated at 20 °C in the dark and examined for germination 10 days later. The radicles of germinated seeds were stained for 5 min with blue ink (MIGROS, Switzerland) in 5% acetic acid.[12b] Seeds were photographed and germination of 100 seeds per replicate was evaluated on digital images. Seeds were considered germinated when the radicle protruded from the seed coat.
12. (a) Hartman, G. L.; Tanimonure, O. A. *Plant Dis.* **1991**, *75*, 494; (b) Long, S.; Lendzemo, V.; Kuyper, T. W.; Kang, Z.; Vierheilig, H.; Steinkellner, S. *Seed Sci. Res.* **2008**, *18*, 125.