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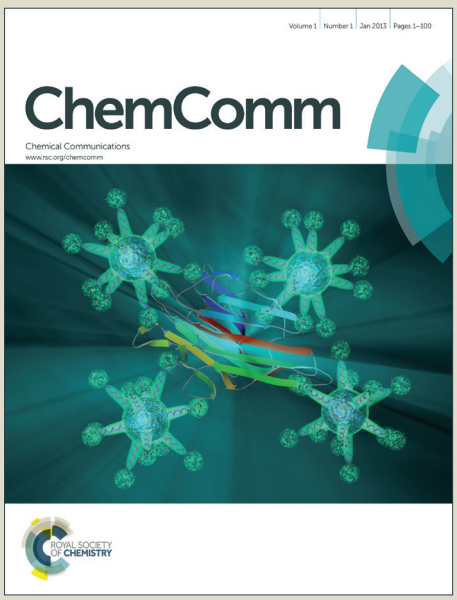

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Synthesis and Biological Evaluation of Pharbinilic Acid and Derivatives as NF-κB Pathway Inhibitors

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A 7-step synthesis of pharbinilic acid, a member of the gibberellin family of natural products and the first naturally occurring allogibberic acid, is reported. An efficient decarboxylative aromatization reaction enables the synthesis of pharbinilic acid and related analogs for evaluation as modulators of NF-κB activity. Remarkably, one analog displays a 2 μM IC_{50} in an NF-κB activity assay and inhibits an endogenous NF-κB-regulated pathway.

Pharbinilic acid (**1**) is a member of the rare class of allogibberic acids, isolated from the seeds of morning glory (*Pharbitis nil*) used in Korea, China and Japan as a medicinal agent.[1] Allogibberic acids were originally reported as laboratory-generated decomposition products[2] of gibberellic acid (**2**), a phytohormone responsible for the regulation of growth and developmental processes in higher plants.[3] Pharbinilic acid (**1**) represents the first naturally occurring allogibberic acid reported to date.

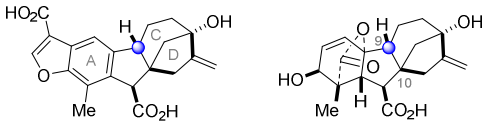


Figure 1. Pharbinilic acid (**1**) isolated from the seeds of morning glory (*Pharbitis nil*), and gibberellic acid (**2**). The natural products share the same stereochemistry at the 5-7 ring juncture (blue circle).

Pharbinilic acid (**1**) was evaluated for anticancer cytotoxicity and displayed activity against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines[1]. Recently, gibberellic acid (**2**) and allogibberic acid (**5**) were identified by A. Koehler as modulators of the NF-κB signaling pathway.[4] Aberrant activity of the transcriptional activator NF-κB has been shown to play an important role in various cancers, inflammatory diseases and autoimmune diseases.[5] In an effort to synthesize and

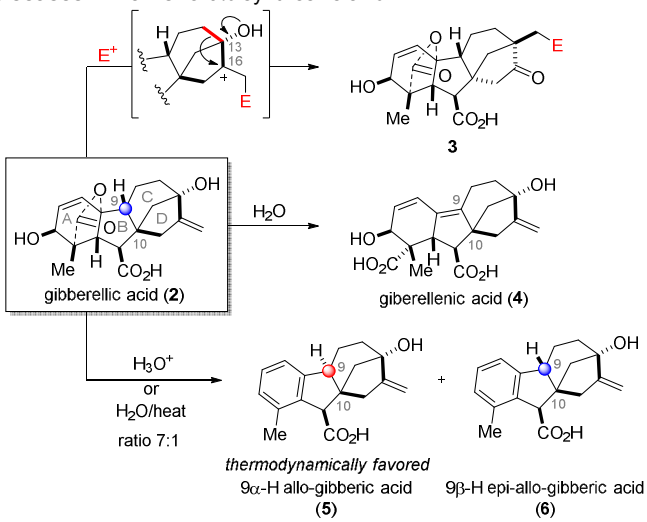
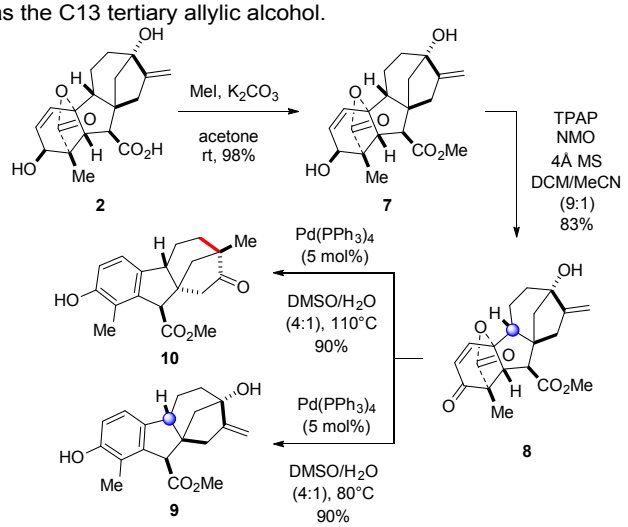


Figure 2. Synthetic challenges associated with the inherent reactivity of gibberellic acid (**1**).

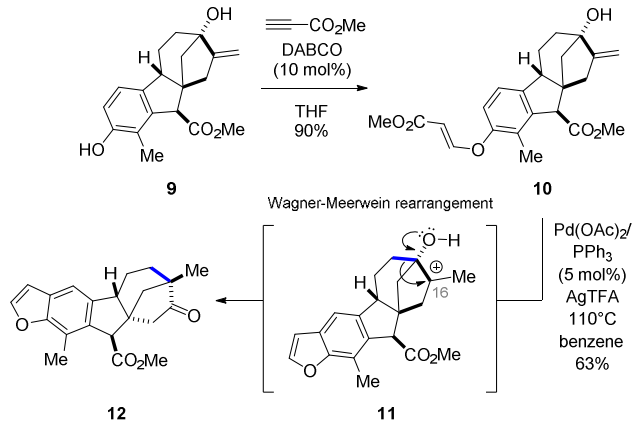
identify compounds that modulate the NF-κB pathway, we became interested in evaluating the efficacy of pharbinilic acid (**1**) and analogs to modulate NF-κB activity. Herein we report the culmination of these efforts in an efficient 7-step synthesis of pharbinilic acid (**1**) from gibberellic acid (**2**). Our 7-step synthetic sequence relies on commercially available gibberellic acid (**2**)[6] ($1.56 per 1g) which is produced industrially (50 tons per year) by the fermentation of the fungus *Gibberella fujikuroi*.[7] In comparison, only 6 mg of pharbinilic acid (**1**) were isolated from 2 kg of dried seeds of *Pharbitis nil*.[1] Extensive studies towards the synthesis of gibberellic acid (**2**) in the 1970s revealed specific challenges associated with its highly functionalized tetracyclic core, especially the B,C,D-ring junction also present in pharbinilic acid (**1**).[8] The inherent reactivity of gibberellic acid (**2**) posed very specific constraints on the development of a reliable synthetic route to pharbinilic acid (**1**). Specifically, the C9-C10 cis-fused

tricyclic ring system present in gibberellic acid (**2**) is known to be considerably more strained than the corresponding C9-C10 trans-fused system found in allogibberic acid **5**.[8b] As a result, gibberellic acid (**2**) was found to undergo trans elimination of the lactone subunit to form gibberellenic acid (**4**) even at neutral pH. Thermal decomposition or treatment of **2** with mineral acids results in a mixture of 9α-H (**5**) and 9β-H *epi*-allogibberic acid **6** (5/6 = 7:1). The observed epimerization at C9 in the major product **5** was shown to result as a consequence of the intermediacy of **4** and subsequent protonation to yield the thermodynamically favored C9-C10 trans- fused 9α-H allogibberic acid **5**.[9] Moreover, the latent reactivity of the tertiary allylic alcohol of the C and D rings is known to be difficult to control. Exposure to various electrophiles (E^+ in Figure 2) capable of reacting with the terminal alkene of 2 creates an electron-deficient C16 carbon center.[2a] Hydroxy-assisted Wagner-Meerwein rearrangement of the C12-C13 bond results in the formation of a new bicyclo[3.2.1]octanone **3**. Although this rearrangement poses a potential issue in designing our synthetic strategy, we also recognized an opportunity to prepare additional analogs based on this observed reactivity. The initial isolation of pharbinilic acid (**1**) not only provided evidence that allogibberic acids do exist as genuine natural products, and not merely isolation artifacts, but more importantly determined that **1** is of the same absolute configuration[10] as 9β-H allogibberic acid (**6**), the minor product obtained upon thermal decomposition of gibberellic acid (**2**) bearing the strained C9-C10 cis-fused tricyclic core. As a result, a successful synthetic strategy towards pharbinilic acid (**1**) is faced with two major challenges and has to result in control of both the inherent reactivity of the 9β-H as well as the C13 tertiary allylic alcohol.



Scheme 1. Synthesis of the hydroxyl-allogibberic methyl ester (**9**).

Our synthetic strategy to pharbinilic acid (**1**) requires a mild aromatization protocol to form phenol **9**[11] under non-acidic conditions to avoid both epimerization of the C9β-H present in the *epi*-allogibberic acid core as well as the undesired C12-C13 rearrangement of the C and D rings. Gibberellic acid (**2**) was initially converted to its corresponding methyl ester (**7**) using methyl iodide[12] in acetone in 98% yield. Griffith-Ley oxidation[13] (TPAP/NMO) of the secondary alcohol proved superior over other oxidation conditions investigated (e.g. DMP, IBX, PDC) which resulted in either low yields or a complex product mixture, leading to the formation of enone **8** in 83% yield. Enone **8** was found to be very sensitive to Brønsted acids, both in aqueous and anhydrous environments. Treatment of **8** with dilute mineral acids (e.g. HCl, H_2SO_4) resulted in the formation of **9** along with the undesired C9α-H epimer. Changing to anhydrous organic acids (e.g. acetic acid, formic acid, *p*TsOH) circumvented the issue of epimerization; however, only the product of Wagner-Meerwein rearrangement of the C12-C13 bond without A-ring aromatization was observed. We next investigated selective transformations of the allylic lactone in **8** using transition metal catalysis. The desired rearomatization of the A ring was accomplished using $Pd(PPh_3)_4$ (5 mol%)[11] in aqueous DMSO at 110°C, however concomitant Wagner-Meerwein (WM) rearrangement was also observed to form ketone **10** as the sole product. Careful investigation of the reaction conditions revealed that the Wagner-Meerwein rearrangement was highly sensitive to the reaction temperature. As a result, conducting the aromatization at 80°C provided the desired phenol **9** in 90% yield

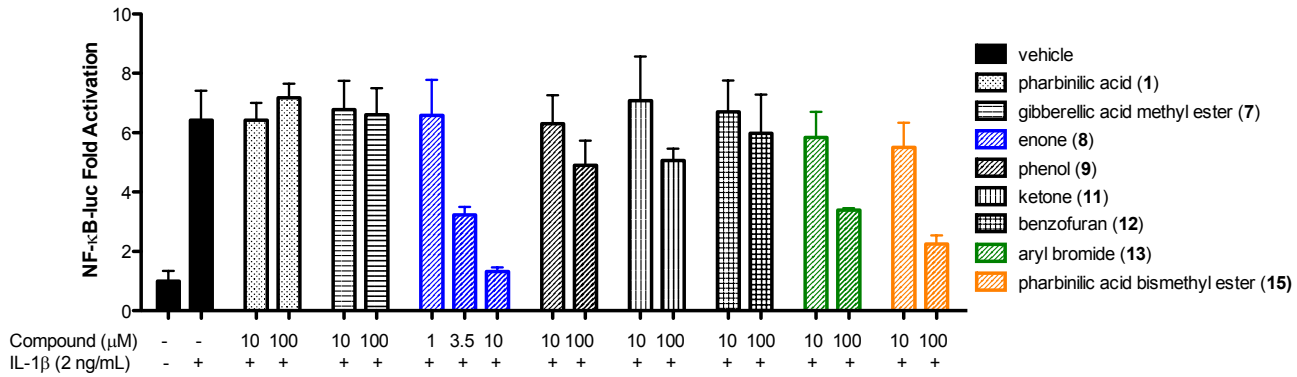


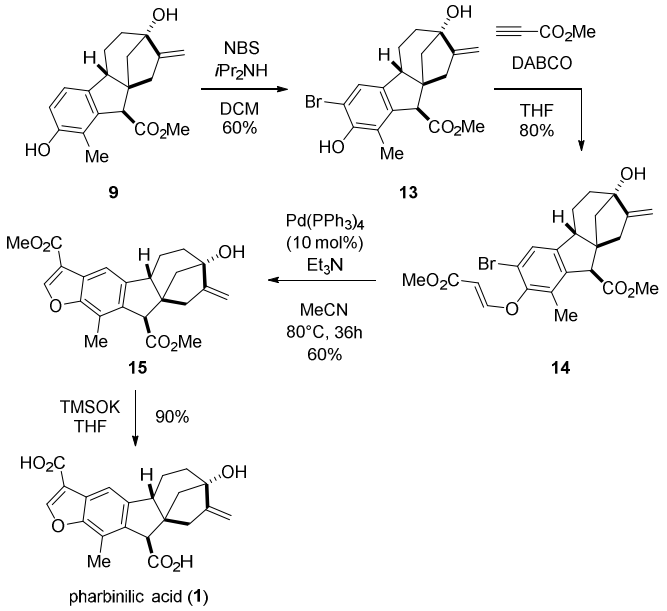
Scheme 2. Synthesis of derivatives of pharbinilic acid (**1**) via Wagner-Meerwein rearrangement of the C,D-bicyclic ring system in **10** leading to ketone **12**.

With a route to phenol **9**[14] secured, the viability of a palladium-catalyzed oxidative cyclization approach to construct the A-ring benzofuran moiety was explored. The required 3-phenoxyacrylate **11** was readily prepared as the corresponding conjugate addition product of methyl propiolate and phenol **9** in 90% yield.[15] Treatment of **11** with $Pd(OAc)_2/PPh_3$ and $AgCO_2CF_3$[16] in benzene at 110°C resulted in the formation of benzofuran **12** (63% yield) as the sole product of a Wagner-Meerwein rearrangement-decarboxylation sequence. Subsequent attempts to avoid decarboxylation during oxidative benzofuran formation centered around changing the palladium source ($Pd(OAc)_2$, $Pd(PPh_3)_4$, $PdCl_2$) as well as the corresponding oxidant ($AgCO_2CF_3$, $PhI(OAc)_2$, O_2) while varying the temperature from ambient temperature to 110ºC. However, none of these conditions led to the formation of the desired benzofuran methyl ester and the sole product isolated upon reaction of phenoxyacrylate **11** remained benzofuran **12.** As we were unable to circumvent decarboxylation and/or WM

rearrangement from **10**, we revised our strategy to rely on bromophenol **13** to enable an intramolecular Heck reaction to form the benzofuran, avoiding the use of Lewis acidic additives that may have contributed to the undesired Wagner-Meerwein rearrangement and/or decarboxylation. Bromination[17] of **10** (NBS, *i*Pr$_2$NH, 60% yield) followed by conjugate addition with methyl propiolate (DABCO, THF)[15] provided **14** in 80%

Figure 3. Synthetic analogs of 1 and 2 inhibit NF-κB-driven transcription in cells. HeLa cells transfected with a NF-κB-driven luciferase reported plasmid and a CMV-driven β-galactosidase reporter were dosed with compounds **1**, **7**-**9**, **11**-**13**, and **15**. After 1 h, th addition of IL-1β stimulated NF-κB activity, in accordance with standard protocols (see SI for details). The fold activation shown is the luciferase activity of each experiment normalized to β-galactosidase activity. All data is represented as the mean and S.D. of 4 technical replicates.





Scheme 3. Completion of the synthesis of pharbinilic acid (**1**) in seven synthetic transformations.

yield. Heck annulation[18] of phenoxyacrylate **14** proceeded at 80ºC, preventing the undesired rearrangement/decarboxylation side products, and afforded the pharbinilic bismethyl ester **15** in 60% overall yield. Saponification was best carried out under anhydrous conditions (TMSOK, THF) to provide the desired natural product, **1**, in 19% overall yield in seven total synthetic transformations starting from commercially available gibberellic acid **2**.

Due to the report of gibberellic acid and related structures as NF-κB inhibitors,[1,4] we examined the activity of **1** and analogs (**7**-**9**, **11**-**13**, **15**) in an NF-κB reporter gene assay. In this experiment, HeLa cells bearing a luciferase reporter plasmid driven by NF-κB were treated with each compound for 1 h followed by IL-1β stimulation of NF-κB activity. Two results are of particular note. While pharbilinic acid (**1**) was not active in this assay, conversion of the two carboxylic acid moieties to methyl esters as in **15** substantially enhanced activity (Table 1). Additionally, enone **8** was the most active of the group, with significant inhibition observed even at 2 μM concentrations. The efficacy of **8** as a modulator of endogenous NF-κB was further assessed through examination of a native NF-κB-regulated gene, MIP3α. As shown in Figure 4, analogous levels of inhibition were observed. Further cellular studies are currently underway to characterize the mode of action of these molecules and their effects in other cellular models of NF-κB pathways.[20]

Table 1. IC_{50} values of compounds **8**, **13** and **15** against NF-κB-luc activity.

compound	IC_{50} (μM)*
enone (**8**)	1.9 ± 0.4
aryl bromide (**13**)	102 ± 34
pharbinilic acid bismethyl ester (**15**)	69 ± 22

* all values are presented as the mean and S.E. of 4 replicates.

Conclusions

In summary, we have developed a robust and concise synthesis to the first naturally occurring allogibberic acid, pharbinilic acid (**1**), which is tailored to the inherent reactivity associated with the gibberellins to proceed under mild reaction conditions without observing any epimerization of the C9β-H or Wagner-Meerwein rearrangement of the C12-C13 bond. Additionally, we have evaluated the inhibitory effects of these compounds against NF-

κB activity, identifying compound **8** as a potent inhibitor. The difference in biological activity reported in luciferase reporter gene assays for 9α-H allo-gibberic acid (**5**)[4] and pharbinilic acid (**1**), a 9β-H allo-gibberic acid bearing opposite configuration at the C-9 stereocenter compared to **5**, are particularly intriguing. Further studies to elucidate the importance of the C-9 stereocenter in the biological activity are underway. The flexible and concise synthetic strategy will enable a full structure-activity relationship study of this class of NF-κB inhibitors, leading to chemical probes against this centrally important transcription factor.

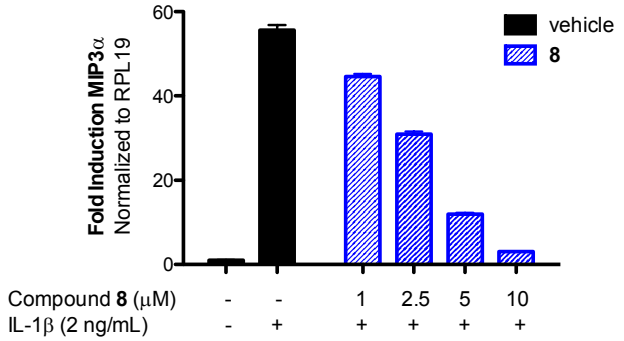


Figure 4. Inhibition of MIP3α gene expression. Compound **8** was tested for its ability to inhibit IL-1β stimulated MIP3α gene expression in HeLa cells. All data is presented as the mean C_T and S.D. of 3 replicates.

Notes and references

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Electronic Supplementary Information (ESI) available: [Methods, ^{1}H NMR, ^{13}C NMR, and selected 2D NMR available]. See DOI: 10.1039/c000000x/

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