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An accelerated Rauhut-Currier dimerization enabled synthesis of (±)-Incarvilleatone and anticancer studies

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Abstract:

The total synthesis of racemic incarvilleatone has been achieved by utilizing unexplored accelerated Rauhut-Currier (RC) dimerization. The other key steps of the synthesis are *oxa*-Michael and aldol reactions in a tandem sequence. Racemic incarvilleatone was separated by chiral HPLC and the configuration of each enantiomer was determined by single-crystal X-ray analysis. In addition, one-pot synthesis of (±)-incarviditone has been achieved from *rac*-rengyolone by using KHMDS as a base. We have also assessed the anti-cancer activity of all the synthesized compounds in breast cancer cells nonetheless, they exhibited very limited growth suppression activity.

Keywords: oxa-Michael • Rauhut-Currier • dimerization • incarvilleatone • incarviditone

Introduction

(±)-Incarvilleatone **1** is a dimeric cyclohexylethanoid isolated by Zhang and co-workers [1] in racemic form from the Chinese plant, *Incarvillea younghusbandii* (**Figure 1**). This plant is used in Chinese folk medicine to treat dizziness and anemia [1]. Zhang and co-workers [1] separated the racemic incarvilleatone in two individual enantiomers, (-)-incarvilleatone (-)-**1**

and (+)-incarvilleatone (+)-**1** by performing chiral HPLC. The structure of *rac*-incarvilleatone **1** was determined by spectroscopic methods and single crystal X-ray analysis. However, they are unable to obtain single crystals of either of enantiomers (-)-incarvilleatone (-)-1 and (+)-incarvilleatone (+)-**1**. (±)-Incarviditone **2**, a novel benzofuranone dimer was isolated from *I. delavayi* along with known (±)-rengyolone **3** [2]. (±)-Incarviditone **2** is the first benzofuranone dimer connected by a C-C bond, which presents a new carbon-skeleton. The cytotoxicity of (±)-incarviditone **2** has been assayed against cell lines A549, LOVO, HL-60, 6TCEM, and HepG2, respectively. (±)-Incarviditone **2** exhibited cytotoxicity only against HL-60 and 6T-CEM cell lines with IC_{50} values of 14.8 and 22.2 mg/mL, respectively.

Lawrence et al [3] and Tang et al [4] have reported the synthesis of (±)-incarvilleatone **1** and (±)-incarviditone **2** *via* biomimetic dimerization of (±)-rengyolone **3**. Despite of these syntheses, the synthesis of dimeric natural product (±)-incarvilleatone **1**, whose monomeric unit (±)-rengyolone **3** was connected by accelerated intermolecular Rauhut Currier (RC) reaction not reported in literature. Herein we present the total synthesis of (±)-incarvilleatone **1** by utilizing unexplored accelerated Rauhut-Currier (RC) dimerization as a key step. In addition to that chiral HPLC separation of (±)-incarvilleatone **1** into its enantiomers (-)-incarvilleatone (-)-**1** and (+)-incarvilleatone (+)-**1** and their X-ray crystallographic analysis which has not yet been reported in the literature.

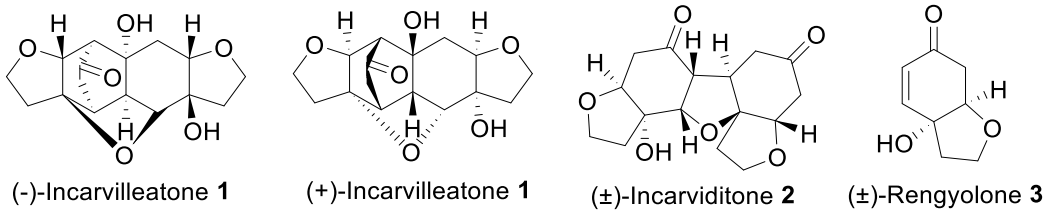
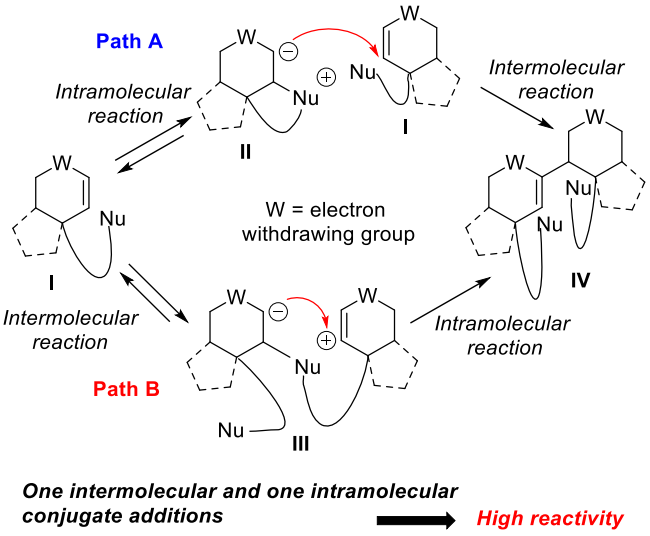


Figure 1. Structures of (±)-incarvilleatone **1**, (±)-incarviditone **2,** and (±)-rengyolone **3**.

The reaction was discovered by Rauhut and Currier [5] in the year 1963. It is a nucleophile catalyzed C-C bond-forming reaction between two Michael acceptors. This reaction provides

access to diverse classes of densely functionalized molecules. Rauhut-Currier (RC) dimerization has some limitations such as its controlling selectivity for intermolecular reactions in differently activated alkenes, and low reactivity. Han and co-workers [6] addressed the latter one by designing a substrate in which nucleophile functionality is also present in the Michael acceptor to accelerate the reaction. In the conventional intermolecular RC reactions, reaction proceeds by the intermolecular addition of nucleophilic catalyst to the *enone* substrate to generate enolate in the first step. In the second step enolate ion attacks to the other Michael acceptor at β-position in an intermolecular fashion to form a C-C bond between two Michael acceptors.



Scheme 1. Possible modes of accelerated intermolecular RC reaction.

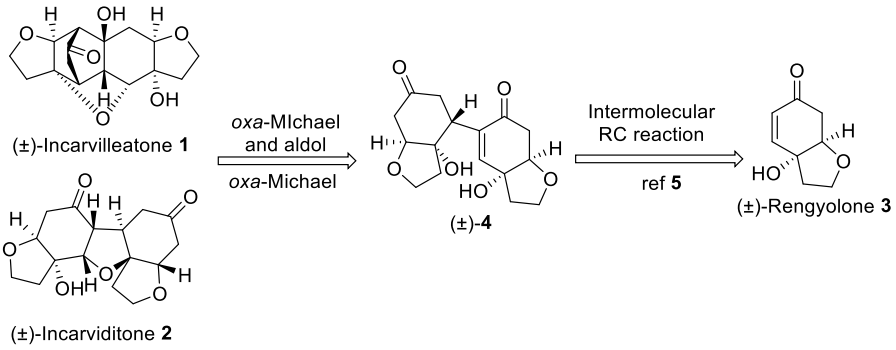
This whole process involves two intermolecular conjugate additions, which leads to low reactivity. In case of intramolecular RC reactions, high reactivity is observed. This is due to one intermolecular and one intramolecular conjugate addition reactions being involved. The low reactivity of intermolecular RC reactions can be improved by incorporating the nucleophilic functionality within the molecule like **I** (Scheme 1). This nucleophilic functionality present within the *enone* system first undergoes intramolecular conjugate addition and is followed by intermolecular conjugate addition to forming a C-C bond (Path

A). In an alternative approach (Path B) first **I** undergo nucleophilic conjugate addition in intermolecular fashion to give intermediate **III** and followed by intramolecular addition to give compound **IV**. In both the paths, involvement of one intramolecular and one intermolecular conjugate addition reaction leads to notable high acceleration in RC reactions.

Based on the accelerated intermolecular Rauhut Currier reaction reported in the literature [7-9] and our interest in the synthesis of dimeric complex natural products [10], we designed a synthetic scheme for the synthesis of (±)-incarvilleatone **1** and (±)-incarviditone **2** starting from (±)-rengyolone **3**. We anticipated that (±)-rengyolone **3** will be a good substrate to test the accelerated intermolecular Rauhut Currier reaction. The presence of nucleophilic functionality (hydroxyl group) and *enone* system within the same molecule exactly needed to accelerate the Intermolecular RC reaction.

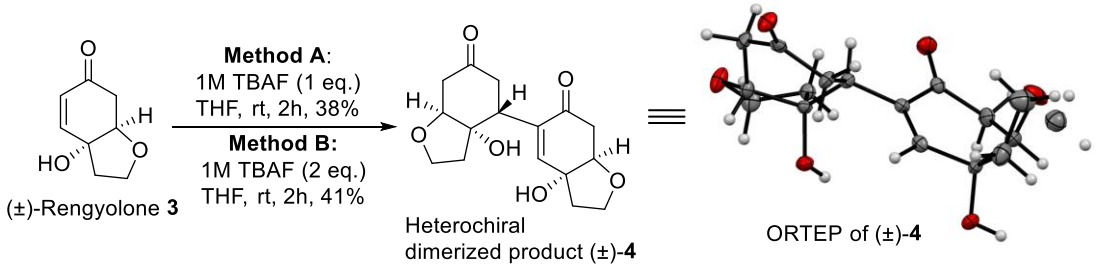
Results and discussion

A retrosynthetic plan for the synthesis of (±)-incarvilleatone **1** and (±)-incarviditone **2** is delineated in Scheme 2. We envisaged that both the natural products (±)-incarvilleatone **1** and (±)-incarviditone **2** could be obtained from the RC product **4** by using *oxa*-Michael and aldol reactions. The RC product **4** in turn can be obtained from the monomeric Michael acceptor, *i.e*. (±)-rengyolone **3.**



Scheme 2. Retrosynthetic plan for the synthesis of (±)-incarvilleatone **1** and (±)-incarviditone **2**.

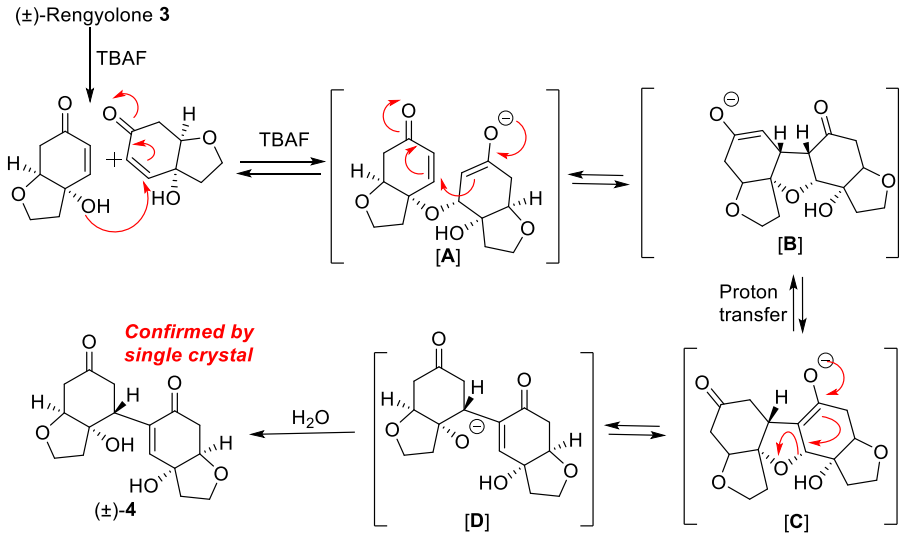
Michael acceptor for the intermolecular Rauhut–Currier (RC) reaction, (±)-rengyolone **3** was synthesized by following the literature procedure [4]. With the (±)-rengyolone **3**, *i.e.* monomeric Michael acceptor in hand, we attempted for Rauhut–Currier dimerization. We found out that treatment of (±)-rengyolone **3** with 1.0 M TBAF [11-15] (1 equiv.) in THF at room temperature resulted in the formation of heterodimerized product (±)-**4** in 38% as a pale-yellow solid (Scheme 3, Method A).



Scheme 3. Synthesis of RC dimerized product (±)-**4**.

The yield of this reaction could not be improved even by prolonging the reaction time (up to 24 h). However, when we treated (±)-rengyolone **3** with 1.0 M TBAF (2 equiv.) in THF at room temperature, we obtained the dimeric product (±)-**4** in 41% yield. The formation of dihydroxy dimerized RC product (±)-**4** was confirmed with NMR spectra. In the ^{1}H NMR spectrum, the olefin proton was observed at δ 6.75 as a singlet, and the two hydroxyl protons were observed at δ 5.60 (s, 1H) and 5.03 (s, 1H), respectively. In the ^{13}C NMR, the two carbonyl groups appeared at δ 197.4 and 209.4, and the corresponding two olefinic carbons were observed at δ 135.7 and 148.5. The formation of the dihydroxy compound (±)-**4** was also confirmed with D_2O shake experiment. When we added a drop of D_2O to the ^{1}H NMR sample, the peaks corresponding to two hydroxyl groups were completely absent at δ 5.60 (s, 1H) and 5.03 (s, 1H) (**Figure S1**, **see SI**). The formation of the dihydroxy dimeric compound (±)-**4** was further confirmed by HRMS, which showed a peak at 331.1150 corresponding to the $C_{16}H_{20}O_6Na$ $[M+Na]^+$. After some efforts, to our delight, we could obtain single crystals

of (±)-**4** using EtOAc as a solvent. Finally, the formation of heterodimerized dihydroxy RC product (±)-**4** was confirmed by its single-crystal X-ray analysis [16]. It is pertinent to mention here that in this reaction we obtained a heterochiral dimerized product **4**. We did not observe any homochiral dimerized product formation in under TBAF reaction conditions. A plausible mechanism for the formation of heterochiral dimerized dihydroxy RC product (±)-**4** through accelerated RC [6] is outlined in Scheme 4.



Scheme 4. Proposed reaction mechanism for the formation of compound (±)-4 under TBAF-mediated Rauhut-Currier reaction.

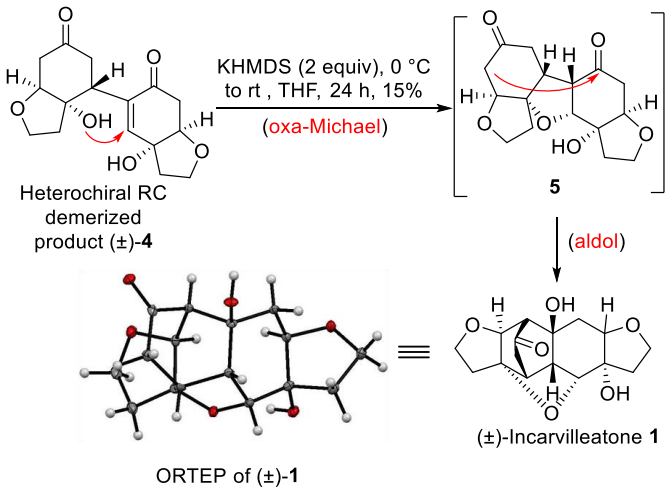
We propose that the one of the rengyolone **3** undergoes a hydroxyl-directed intermolecular conjugate addition to another rengyolone **3** (*enone* system) to afford intermediate [A] with high selectivity. The enolate intermediate [A] may undergo rapid intramolecular Michael addition to give tetrahydrofuran intermediate [B] with *cis*-fusion ring systems by literature precedents [7]. The enolate moiety [B] will then trigger a proton transfer to yield another enolate [C] followed by the β-alkoxy elimination [17] of intermediate [C] to form intermediate [D]. The intermediate [D] on protonation leads to the dihydroxy RC product (±)-**4**. Synthesis of dihydroxy product (±)-**4** was carried out at gram-scale utilizing the key accelerated RC dimerization. In order to synthesize (±)-incarvilleatone **1** first, we needed to

perform oxa-Michael addition reaction. For this, we screened various bases [11-15] such as NaHMDS, DBU, NaH, DABCO, ^{t}BuOK, aq. NaOH but none of them gave the desired product. Instead, either complex mixture was formed, or the starting material was recovered as such (Table 1).

Table 1. Conditions screened for the formation of the (±)-incarvilleatone **1** from (±)-**4**

Entry	Base	Solvent	Yield (%)
1	DBU (1 equiv.)	DCM	No reaction
2	DABCO (1 equiv.)	Dioxane/H_2O	No reaction
3	NaH (2 equiv.)	DCM	No reaction
4	Et_3N (2 equiv.)	DCM	No reaction
5	Et_3N (2 equiv.)	THF	No reaction
6	NaHMDS (2 equiv.)	THF	No reaction
7	KHMDS (2 equiv.)	THF	15
8	1 M aq. NaOH (few drops)	THF	Complex mixture

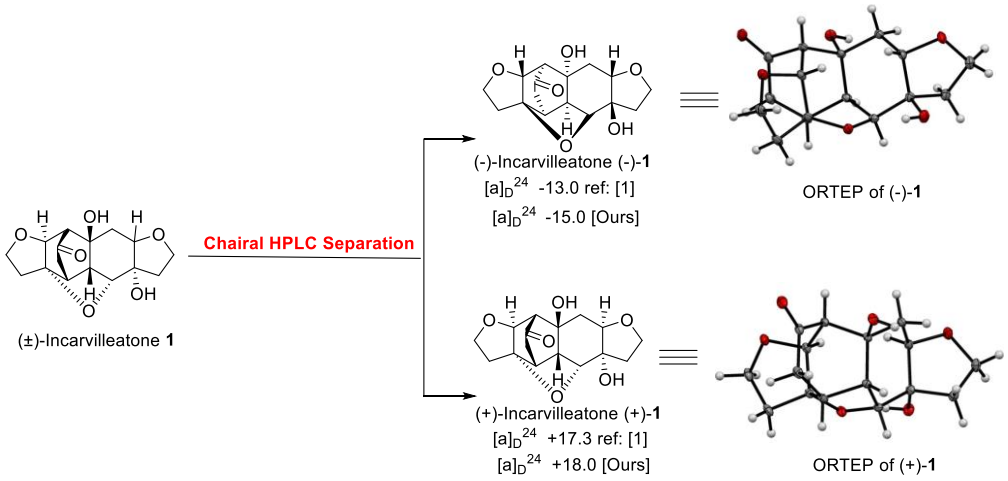
However, when we treated compound (±)-4 with a strong base such as KHMDS (2 equiv.) in THF at 0 °C, (**Table 1**, entry 7) we obtained the product as a colorless solid (15% yield) after 24 h stirring at room temperature (Scheme 5).



Scheme 5. Synthesis of (±)-incarvilleatone 1 from RC dimerized product (±)-4.

The product was characterized as (±)-incarvilleatone **1** by comparison of its ^{1}H NMR and ^{13}C NMR with the reported [1] natural (±)-incarvilleatone **1**. The formation of (±)-incarvilleatone **1** perhaps due to RC dimerized product (±)-**4** first undergoes *oxa*-Michael followed by aldol reaction in one-pot. The aldol reaction occurs in the intermediate **5** in the basic medium due to the close proximity of two carbonyl groups. Finally, the structure of (±)-incarvilleatone **1** was confirmed by its single crystal X-ray analysis [16]. We then undertook chiral separation of both the enantiomers of (±)-incarvilleatone **1** (40 mg, Scheme 6) by using Chiralpak IA analytical column with mobile phase MeCN:H_2O (70:30). The chiral HPLC resulted in the separation of enantiomers, (-)-incarvilleatone [(-)-**1**, 15 mg] and (+)-incarvilleatone [(+)-**1**, 14 mg]. The optical rotations of the individual enantiomers were recorded and compared with Zhang and co workers [1] reported data and were found to be nearly same *i.e*. optical rotation of -13.0 (*c* 0.30, MeOH) (isolated by Zhang and co workers [1]) and -15.0 (*c* 0.30, MeOH) (chiral separation by us) for (-)-incarvilleatone (-)-**1**; and +17.3 (*c* 0.30, MeOH) (isolated by Zhang and co workers [1]) and + 18.0 (*c* 0.30, MeOH) (chiral separation by us) for (+)-incarvilleatone (+)-**1**. We tried to crystallize both the enantiomers and after some efforts we could crystallize both the enantiomers using EtOAc as a solvent. The absolute configurations were assigned using single crystal X-ray analysis for (-)-incarvilleatone (-)-**1** as 4*R*,5*S*,8*S*,9*R*,4'*R*,5'*S*,6'*R*,7'*R*,9'*S* and for (+)-incarvilleatone (+)-**1** as 4*S*,5*R*,8*R*,9*S*,4'*S*,5'*R*,6'*S*,7'*S*, 9'*R* (Scheme 6).

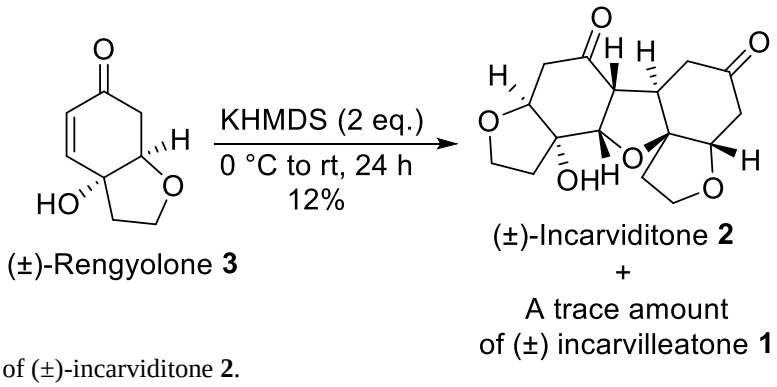
After chiral HPLC separation of individual enantiomers, we recorded the CD spectra of both enantiomers in MeOH. (-)-Incarvilleatone (-)-**1** shows negative optical rotation and negative Cotton effect in the CD spectrum whereas the other enantiomer (+)-incarvilleatone (+)-**1** showed positive optical rotation and positive Cotton effect in the CD spectrum (**Figure S2, see SI**).



Scheme 6. Chiral separation of rac-incarvilleatone 1 and determination of absolute configurations of both the enantiomers using single crystal X-ray analysis.

Synthesis of (±)-incarviditone 2

When we treated (±)-rengyolone **3** with the same base *i.e*. KHMDS (2 equiv.) in THF at 0 °C to rt for 24 h, resulted in formation of a white solid (12% yield) which was identified as (±)-incarviditone **2** by comparison of its NMR spectra with reported in the literature [2]. In this reaction we detected a trace amount of (±)-incarvilleatone **1** along with the (±)-incarviditone **2** (Scheme 7).



Scheme 7. Synthesis of (±)-incarviditone **2**.

Previous study [1] showed that (±)-incarviditone has limited anticancer activity. Anticancer activity is closely correlated with the growth suppression. MTT assay is widely used to examine the growth suppression. It is a colorimetric assay. In this assay, viable cells reduce MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to its insoluble formazan by oxidoreductase enzymes in nicotinamide adenine dinucleotide phosphate (NADPH) dependent manner. Thus, reduction of MTT is closely related to the viability of the

cells. Therefore, we have examined the growth suppressive effect of all the synthesised compounds using MTT assay [18-20].

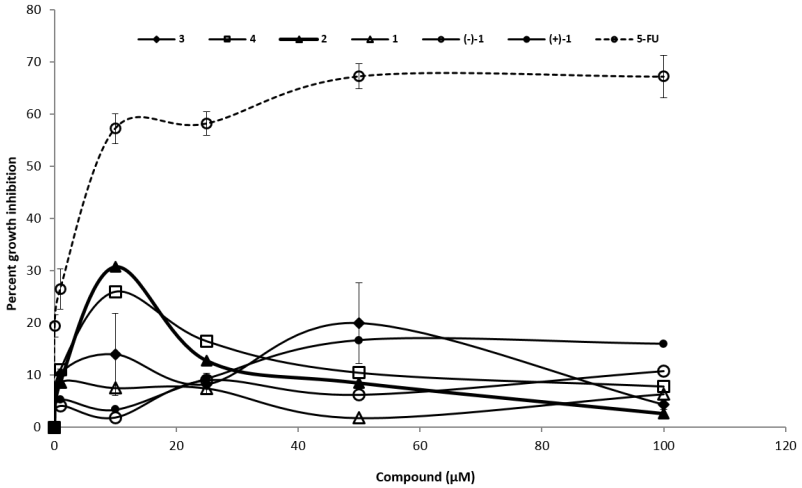


Figure 2. Growth suppression activity of the synthesized compounds in the breast cancer cell line MCF-7.

Results data revealed that these compounds have very minimal effect on the growth of breast cancer cell line MCF7. For instance, we did not observe the 50% growth inhibition event at 100 µM. In contrast, 5-fluorouracil potently inhibited the growth of MCF7 cells with 50% growth inhibition at 7.1±0.62 µM (Figure 2).

Conclusion

In summary, we have successfully achieved the total synthesis of (±)-incarvilleatone **1** starting from *rac*-rengyolone **3** through accelerated RC intermolecular dimerization catalyzed by TBAF to synthesize a heterochiral dimerized product (±)-**4** followed by one-pot oxa-Michael and aldol reaction sequences using KHMDS as a base. The synthesized (±)-incarvilleatone **1** was separated into its individual enantiomers by using chiral HPLC (analytical Chiralpak IA column). The absolute configurations of both the enantiomers were determined by their single crystal X-ray analysis.[12] We have also synthesized (±)-incarviditone **2** starting from *rac*-rengyolone **3** by using KHMDS as a base. Anti-proliferative

activity of these compounds was tested using MTT assay and results revealed that these compounds are less efficient in inhibiting the growth of breast cancer cells.

Declaration of competing Interest

The authors have no conflict of interest to declare.

Acknowledgements

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Supporting Information

Supporting Information File 1
Biological Protocols, ^{1}H & ^{13}C NMR and HRMS, Figure S1 and S2 and HPLC chromatograms.

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16. Crystallographic data (excluding structure factors): CCDC-1818615−1818618 contains the supplementary crystallographic data for this paper. These data can be obtained free

of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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