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Synthesis of Bis-pyrrolizidine-Fused Dispiro-oxindole Analogues of Curcumin via One-Pot Azomethine Ylide Cycloaddition: Experimental and Computational Approach toward Regio- and Diastereoselection

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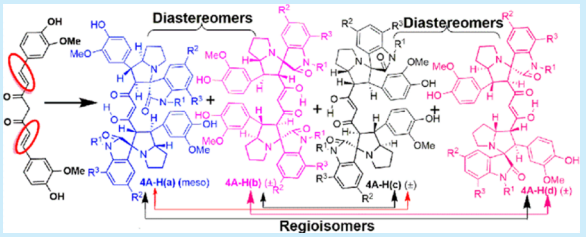
Supporting Information

ABSTRACT: Curcumin has been transformed to racemic curcuminoids via an azomethine ylide cycloaddition reaction using isatin/acenaphthoquinone and proline as the reagents. The products were characterized by extensive 1D/2D NMR analysis and single-crystal X-ray crystallographic studies. The enantiomers of one racemic product were separated by HPLC on a Chiralcel OD-H column and were indeed confirmed by the CD spectra of the separated enantiomers.



Curcumin, obtained from turmeric, has been one of the most explored molecules during the last century. It has shown a plethora of biological activities, such as antioxidant,[1] anticancer,[2] antimalarial,[3] antihepatotoxic,[4] etc. A recent review outlined 728 natural, semisynthetic, and synthetic analogues of curcumin with a structure−activity relationship (SAR).[5] This indicated that various groups have used curcumin as a lead compound to develop numerous bioactive analogues, but there appeared to be some conflicting reports on SAR studies. Some studies revealed that the phenolic ring or the $-CH_2$ group of the β-diketone moiety may be essential for antioxidant activity,[5,6] whereas others reported that unsaturation and $-CH_2$ group do not seem to play any significant role.[7] Although an unavailability of reports on resistance development against curcumin has been its most characteristic feature,[8] poor aqueous solubility, low bioavailability, unsatisfactory pharmaco-kinetics, and intense staining color have been its major drawbacks.[9] This led to a new concept of a "super curcumin" to be classified under two broad categories, namely, (1) synthetic analogues or derivatives and (2) formulations,[6] which will be free from these problems and with efficacy equal to or better than that of curcumin. This concept of "super curcumin" attracted our attention, and we opted for the first category, i.e., synthetic analogues or derivatives to overcome the prevailing drawbacks with curcumin. It was observed that compounds such as pyrazole/isoxazole,[10] metal conjugates,[11] and PEGylated/ferrocenylated[12] curcuminoids, etc. have been synthesized using the diketone functionality, phenolic OH group, and the active methylene group of curcumin, but no report was found that used electron-deficient double bonds. In continuation of our molecular diversity program, very recently we have reported a number of dispiro analogues of andrographolide and withanolide via azomethine ylide cycloaddition[13] utilizing the 12/13 exocyclic double bond of the former and the endocyclic double bond in ring A of the latter.[14] The very promising biological activity of these dispiro analogues[15] prompted us to explore the electron-deficient double bonds of curcumin in order to construct the imaginary "super curcumin" skeleton. The motive behind the selection of the pyrrolidine-2-spiro-3′-oxindole ring system as the attachment motif is its biological importance[16] and also the likelihood of enhancing the absorption characteristics, namely, aqueous solubility and permeability (log S and log P). It has been observed that use of metabolically cleavable groups increases the aqueous solubility of the parent molecule.[17] In addition, enhanced water solubility increases a drug's bioactivity/bioavailability and greatly affects the routes of administration and ADME (absorption, distribution, metabolism, and elimination) properties. The solubility characteristics of a molecule are dependent upon the hydrogen-bond-forming and ionization potential of the functional groups present. Thus, in our presumed embodiments, the presence of spiro-oxindoles would be useful for strong hydrogen bonding via donor/acceptor groups such as −NH, −C═O, and pyrrolizidine nitrogen. This was expected to improve the aqueous solubility of the molecules relative to curcumin. To our satisfaction, we could build up the planned curcumin analogues having both spiro-oxindole and pyrrolizidine rings assimilated into its skeleton. In this paper, we report the synthesis and characterization of the products and

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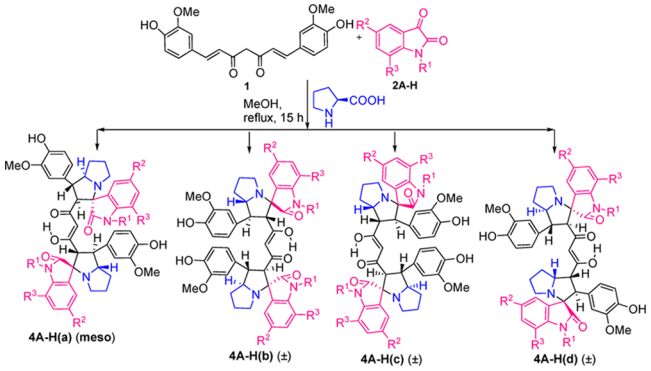
attempts to correlate the selectivities achieved using experimental and computational techniques. We believe that, due to the structural novelty of the products having more donor/acceptor groups, these might qualify as "super curcumins", although the required physicochemical parameters are yet to be studied.

The cycloaddition reaction of curcumin (**1**), isolated from *Curcuma longa*, was accomplished using isatin (**2A**) and proline in a 1:2:2 mol ratio in refluxing methanol (65 °C) via in situ generation of azomethine ylides. It took approximately 14−16 h to complete the reactions, and four bis-pyrrolizidine fused-dispiro-oxindole products **4Aa**−**Ad** were isolated in total yields of 85−90% (Scheme 1).

Scheme 1. Preparation of Bis-pyrrolizidine-Fused Dispiro-oxindolo Curcuminoids



The products were characterized mainly from spectral studies. All four products gave the same *m*/*z* peak at 769 $[M + H]^+$/791 $[M + Na]^+$ in the ESI-Q-TOF MS spectrum, indicating them to be isomeric. Detailed NMR studies showed that compounds **4Aa** and **4Ab** have a close spectral resemblance with 23 unique carbon signals, while **4Ac** and **4Ad** displayed 45 carbon signals each. This confirmed that **4Aa** and **4Ab** are symmetrical type diastereomers and **4Ac** and **4Ad** are unsymmetrical diastereomers. As would be expected, the signals for the aromatic ring fragments of curcumin remained virtually unaltered in the spectra of the products, but the chemical shifts for the nuclei belonging to the α,β-unsaturated-diketone part of **1** were distinctly perturbed, with C3/25 and C4/26 suffering profound alteration from downfield to upfield resonance position. Isatin, which contributes as a substituents in the product to the neighboring carbon, appeared likely to add from the C3/25 end in the **a**/**b** series but from the C3/26 end in the **c**/**d** series. This was also borne out by the 2D NMR spectral correlations for the representative compounds **4Aa**, **4Ab**, **4Bc**, and **4Ad**. The crucial evidence in support of this came from the observed HMBC correlation in the spectrum of **4Aa**/**Ab** between both signals of C-10/32, i.e., the oxindole carbonyls (δ 181.4/181.6) and H-3/25 (δ 4.46/4.50) and C-5/27 (i.e., the point of attachment with proline δ 74.0/74.2) and C-6/28 (δ 31.5/31.8) with H-4/26 (δ 3.71/3.67) respectively. Further, the COSY relationship between H-4/26 (δ 3.71/3.67) and H-5/27 (δ 4.24/4.16), coupled with medium to low NOESY cross peaks, strongly supports the mode of additions. The support for the operation of opposite regiochemistry (**c**/**d** series) came from the observed HMBC correlation in the spectrum of **4Bc** between both the signals of C-10/32, i.e., the oxindole carbonyls (δ 181.6/179.5) and H-3/26 (δ 4.62/4.29) and of C-5/31 (i.e., the point of attachment with proline δ 74.0/65.9) and C-6/30 (δ 31.3/29.2) with H-4/25 (δ 3.99/5.05), respectively. Further, the COSY relationship between H-4 (δ 3.99) and H-5 (δ 4.29) along with H-25 (δ 5.05) and H-31 (δ 4.89) strongly support the reverse mode of additions. Weak NOESY cross peaks between H-4 (δ 3.99) and H-5 (δ 4.29) indicate an anti relationship, but strong NOESY cross peaks between H-25 (δ 5.05) and H-31 (δ 4.89) indicate a syn relationship (Figure 1,

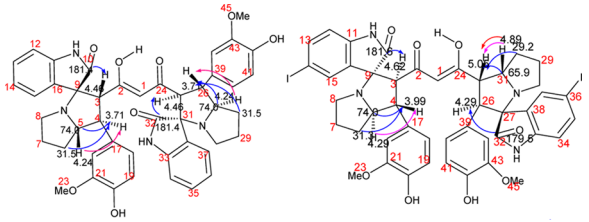


Figure 1. Important correlations of **4Aa**/**4Bc** [HMBC (blue arrow), COSY (magenta arrow), NOESY (red arrow)].

Figure S1 and Tables S1 and S2, Supporting Information). From similar observed 2D NMR correlations, the structure of **4Ad** was also suggested (Figure S2 and Table S3, Supporting Information).

This was further confirmed by single-crystal X-ray diffraction study in the case of **4Aa**, **4Bc** (Figure 2), **4Ea**, **4Fb**, **4Gb**, and **4Gc** (Figures S3 and S4, Supporting Information).

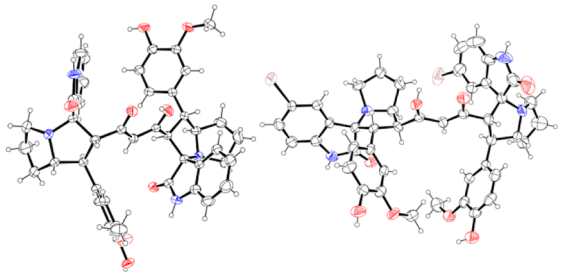


Figure 2. ORTEP representation of **4Aa** and **4Bc**.

From the X-ray data, it is worth mentioning that the adjacent hydrogens on both side of the diketone functionality are *anti* to each other, whereas they remain in a *syn* orientation with the spirocyclic oxindole bond of the respective sides.

The reaction appears to proceed through the formation of mono cycloaddition regioisomers **3Aa** and **3Ab**, which after further reaction produce products **4Aa**−**Ad**. This was in fact proved by the isolation of **3Aa** (85%) and **3Ab** (5%) via 1:1:1 mol reaction of curcumin, isatin, and proline followed by their reaction separately with another 1 mol of proline and isatin to isolate the bis addition products (Scheme 2). The small difference in the yields of **4Ac**−**Ad** (49%) against **4Aa**−**Ab** (41%) can be easily explained by the fact that the former products can be derived from both intermediates while the latter can originate only from **3Aa**. Following the successful synthesis of spiro-pyrrolizidino oxindole adducts of curcumin using isatin, we performed the reaction using other substituted isatins and good yields of the products were obtained (85−90%); the individual yields of the isomers **a**−**d** are given in Table 1.

The presence of the second diastereotopic double bond too far away from the chiral centers generated in the first step is the main reason for the low diastereoselectivity. We also performed the reaction with proline and acenaphthoquinone (**5**) as the 1,2-diketone compound. In this case also, we obtained the bis-pyrrolizidine fused-dispiro-oxindole adducts **6a** and **6b** in around

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Scheme 2. Probable Reaction Pathway for Dispiro-oxindolo Curcuminoids

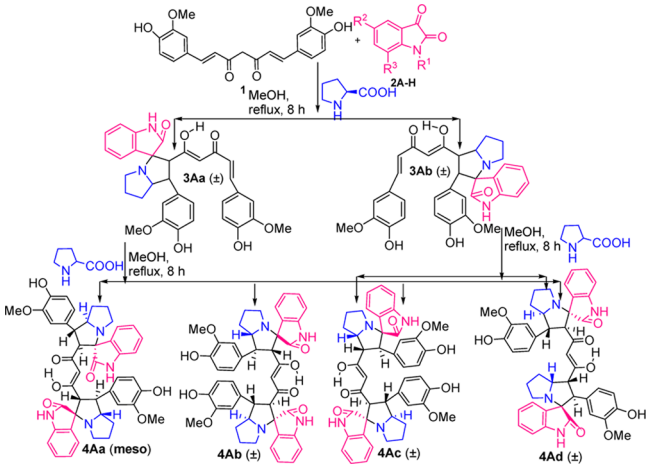


Table 1. Yields of 4(A−H)(a−d) Derived from Curcumin

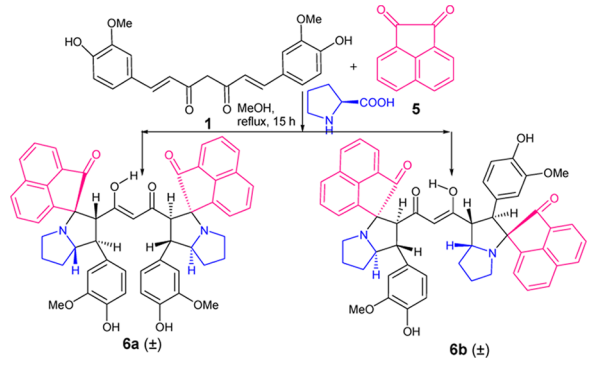
entry	R^1	R^2	R^3	product	yield[a] (%)
1	H	H	H	**4Aa**	18
2	H	H	H	**4Ab**	23
3	H	H	H	**4Ac**	21
4	H	H	H	**4Ad**	28
5	H	I	H	**4Ba**	19
6	H	I	H	**4Bb**	23
7	H	I	H	**4Bc**	21
8	H	I	H	**4Bd**	27
9	H	F	H	**4Ca**	17
10	H	F	H	**4Cb**	21
11	H	F	H	**4Cc**	20
12	H	F	H	**4Cd**	28
13	H	Cl	H	**4 Da**	17
14	H	Cl	H	**4Db**	23
15	H	Cl	H	**4Dc**	21
16	H	Cl	H	**4Dd**	28
17	H	Me	H	**4Ea**	18
18	H	Me	H	**4Eb**	22
19	H	Me	H	**4Ec**	22
20	H	Me	H	**4Ed**	28
21	H	Me	Me	**4Fa**	18
22	H	Me	Me	**4Fb**	23
23	H	Me	Me	**4Fc**	21
24	H	Me	Me	**4Fd**	27
25	Me	H	H	**4Ga**	19
26	Me	H	H	**4Gb**	23
27	Me	H	H	**4Gc**	22
28	Me	H	H	**4Gd**	27
29	H	OMe	H	**4Ha**	16
30	H	OMe	H	**4Hb**	22
31	H	OMe	H	**4Hc**	21
32	H	OMe	H	**4Hd**	26

[a]Isolated yield.

76% yield in total (Scheme 3) and confirmed the structures by 2D NMR analysis (Figure S5, Table S4, Supporting Information) followed by single-crystal X-ray diffraction analysis (Figure S6, Supporting Information).

The competitive pathways of the isomer formation were investigated with DFT methods using the Jaguar module of the Schrodinger software package.[18] The geometry optimization and

Scheme 3. Preparation of Dispiro-acenaphthylen-2-one Curcuminoids



energy calculation of all starting materials, products, intermediates, and transition states for one model reaction were performed using the DFT method with M06-2X/6-31G** functional and basis sets (Figure 3) in the gas phase.

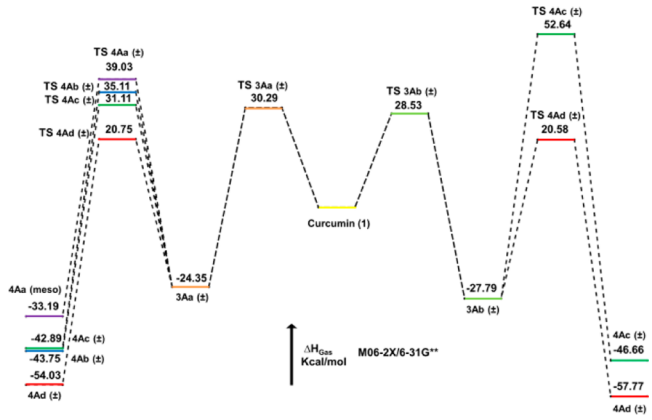


Figure 3. Energy calculation for cycloaddition of azomethine ylide with curcumin computed via the M06-2X/6-31G** level of theory in the gas phase.

In this cycloaddition reaction, two possible pathways (via **3Aa** and **3Ab**) were investigated, and it can be easily seen that the calculated energies of **4Ac** and **4Ad** are almost same in both pathways. In addition, the yields of the individual diastereomers were comparable to their respective energy profile. As expected, all of the products are racemic, indicated by the chiral HPLC profile of **3Aa**, **3Ab**, **4Bc**, and **4Ad** (Figures S7−S10, Supporting Information). Compound **4Aa** does not give any chiral HPLC separation due to its mesolike configuration. The enantiomers of racemic **4Ad** were indeed separated by HPLC on a Chiralcel OD-H column, and the CD spectra of the separated enantiomers confirm this (Figure 4). Compounds (+)-**4Ad** and (−)-**4Ad** represent the enantiomers with retention times of 8.571 and 14.514 min, respectively, in hexane/ethanol (80:20) solvent system.

In conclusion, curcumin has been successfully used in a 1,3-dipolar cycloaddition reaction to produce novel bis-pyrrolizidine fused dispiro-oxindolo curcuminoids with slight diastereoselectivity. The structures of the products were determined by 1D/2D NMR analysis and unequivocally confirmed by X-ray crystallography.[19] Chiral HPLC separation of enantiomers followed by CD spectroscopy and optical rotation measurement were performed for **4Ad**. The unaltered pharmacophores of curcumin along with

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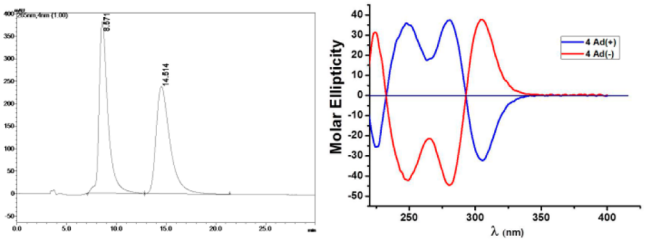

Figure 4. Chiral HPLC profile of **4Ad** (±); CD spectra of **4Ad** (+) and **4Ad** (−).

the newly generated spiro-oxindolo pyrrolizidine moiety might add to the biological effectiveness of the scaffold with increased solubility and bioavailability.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02085.

^{1}H, ^{13}C NMR and HRMS data and NMR spectra of all compounds (PDF)
X-ray crystallographic data for **4Aa** (CIF)
X-ray crystallographic data for **4Ea** (CIF)
X-ray crystallographic data for **4Fb** (CIF)
X-ray crystallographic data for **4Gb** (CIF)
X-ray crystallographic data for **4Bc** (CIF)
X-ray crystallographic data for **4Gc** (CIF)
X-ray crystallographic data for **6a** (CIF)

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Notes

The authors declare no competing financial interest.

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(19) The crystallographic data of **4Aa**, **4Ea**, **4Fb**, **4Gb**, **4Bc**, **4Gc**, and **6a** (CCDC 1412502−1412508) can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: + 44 1223 336033; or deposit@ccdc.cam.ac.uk).

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