ACS Combinatorial Science

Research Article

pubs.acs.org/acscombsci

Regio- and Stereoselective Synthesis of a Library of Bioactive Dispiro-Oxindolo/Acenaphthoquino Andrographolides via 1,3-Dipolar Cycloaddition Reaction Under Microwave Irradiation

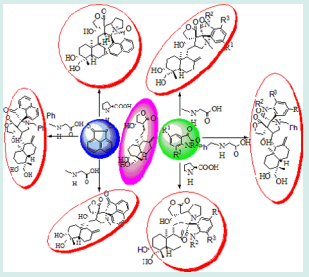
Abhijit Hazra,[†] Yogesh P. Bharitkar,[†] Debanjana Chakraborty,[†] Susanta Kumar Mondal,[‡] Nupur Singal,[‡] Shyamal Mondal,[†] Arindam Maity,[†] Rupankar Paira,[†] Sukdeb Banerjee,[†] and Nirup B. Mondal*[,†]

[†]Department of Chemistry, Indian Institute of Chemical Biology, Council of Scientific and Industrial Research, 4, Raja S. C. Mullick Road, Jadavpur, Kolkata 700 032, India

[‡]Chembiotek, TCG Lifesciences Ltd, BIPL, Block-EP & GP, Sec-V, Kolkata 700091, India

Supporting Information

ABSTRACT: Dispiro-pyrrolidino/pyrrolizidino fused oxindoles/acenaphthoquinones have been derived from andrographolide via azomethine ylide cycloaddition to the conjugated double-bond under microwave (MW) irradiation. The reactions are chemo-, stereo-, and regioselective in nature. Change in amino acid from sarcosine/*N*-benzyl glycine to L-proline changes the regiochemistry. A representative library of 40 compounds along with in vitro anticancer evaluation is reported.



KEYWORDS: *dispiroheterocycles, pyrrolidino/pyrrolizidino, andrographolide, dipolar cycloaddition, azomethine ylide*

INTRODUCTION

Exploring novel pharmacological agents with minimum number of synthetic steps and in less time is a major challenge to the chemists.[1] Besides, chemists are facing another challenge for the past two decades, namely, that of developing new transformations that are not only efficient, selective, and high yielding but also environmentally benign. In general, the conventional approaches involve the use of multistep reaction sequences. These are typically associated with low yields and high cost, entail tedious isolation of the resulting products, and seldom become commercially viable. The use of multicomponent reactions (MCRs) has become a modern approach to synthesize such novel compounds[2] of biological significance. The MCR strategy, where three or more simple and flexible molecules are brought together to rapidly introduce structural complexity and diversity, offers significant advantages over conventional linear-type syntheses.[3−5] Thus, the choice of an appropriate reaction condition meeting the aforesaid challenges is of crucial importance for successful syntheses now-a-days.

Highly substituted pyrrolidines have gained much prominence because they make the central skeleton of many natural products.[6] Some spiropyrrolidines are potential antileukemic and anticonvulsant agents,[7] and possess antiviral and local anesthetic activities.[8] Furthermore, the 3′-spirooxindoles have been of interest to organic chemists because many such derivatives are characterized by interesting biological properties.[9−11] Spirooxindoles find many biological applications as antimicrobial, antitumoral, and inhibitors of human NK-1 receptor, and so forth,[12] and constitute the central skeleton for numerous alkaloids and pharmacologically important compounds. Spiropyrrolidinyloxindole ring systems are also found in a number of alkaloids like horsifiline, spirotryprostatine A and B, elacomine, and so forth.[13] Construction of this type of substituted pyrrolidine or pyrrolizidine rings with spirooxindole moiety was achieved via cycloaddition reaction of 1,3-dipoles with olefinic or acetylenic dipolarophiles. Discovered by Huisgen[14a] and developed by Woodward and Hoffman[14b] and also by Houk et al., this regio- and stereoselective reaction has since become an important as well as general method for the construction of five-membered heterocyclic rings.[15−17]

Reports on the synthesis of derivatives of andrographolide, the major diterpene constituent of *Andrographis paniculata*, for modification of the biological activities are available in the literature. Bioevaluation of the derivatives has shown anticancer, α-glucosidase inhibitory[18] as also TNF-α and IL-6 expression inhibitory activity.[19] Encouraged by these reports, recently we have synthesized and reported a few dispiro analogues of andrographolide via azomethine ylide cycloaddition in refluxing methanol.[20] The results of the biological evaluation of these compounds encouraged us further to build a library of dispiro analogues of andrographolides for further biological evaluation. In this endeavor to access the library of compounds containing both spiro-oxindole and pyrrolidine/pyrrolizidine rings attached with the well-known bioactive

Received: September 19, 2012
Revised: November 14, 2012
Published: November 20, 2012

   dx.doi.org/10.1021/co3001154 | *ACS Comb. Sci.* 2013, 15, 41−48

natural core andrographolide, we decided to explore the applicability of microwave (MW) irradiation technique to develop an efficient, convenient, high yielding, and less time-consuming green protocol. This technique is being employed in many reactions in recent years for achieving energy efficiency and enhancing the rate of reaction as well as product yields.

RESULTS AND DISCUSSION

Initially, the three-component azomethine ylide cycloaddition reaction of isatin, sarcosine/proline/*N*-benzyl glycine and andrographolide was investigated to optimize the green reaction conditions. Various green reaction protocols, for example, *β*-cyclodextrin-water (*β*-CD), micelle (cetyltrimethylammonium bromide/CTAB-water), water immiscible ionic liquid 1-butyl-3-methylimidazolium hexafluorophosphate ([bmim] PF_6), and MW irradiation were screened for the optimization of the reaction condition (Table 1). The best result was obtained under MW irradiation with excellent yield (80%) in shorter reaction time (15−20 min).

Table 1. Synthesis of 3a/4a/5a under Different Reactions Conditions

entry	condition	temp. (°C)	time (h)	yield[a] (%)
1	methanol	reflux	22	62
2	water-*β*CD	reflux	20	35
3	water-CTAB	reflux	22	30
4	ionic liquid	80	12	60
5	MW-MeOH	100	0.25	82

[a]Isolated yield.

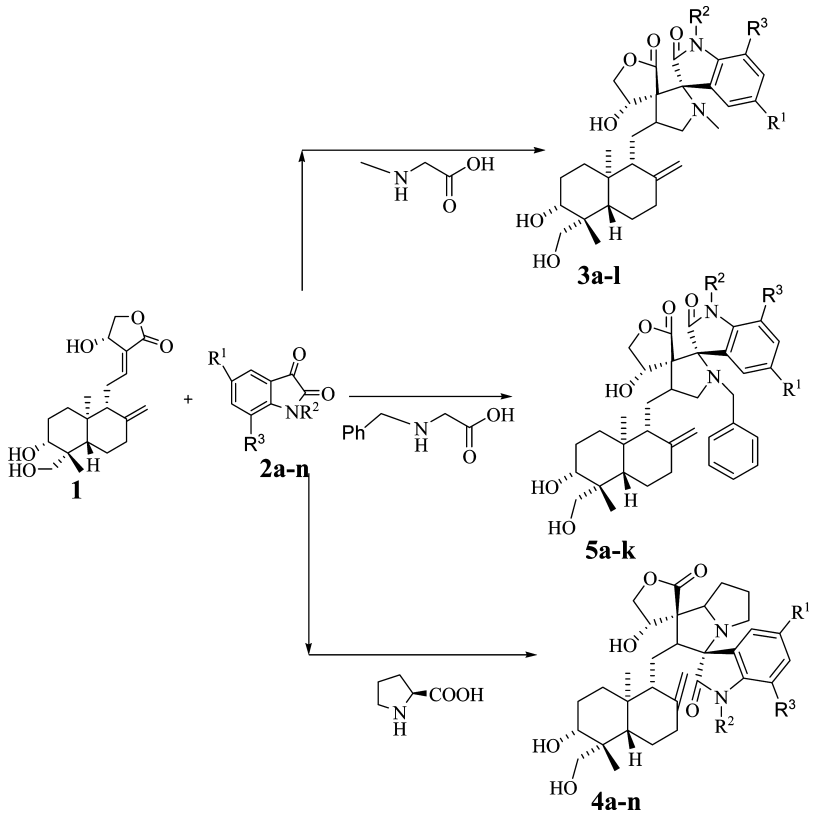
The reaction has been carried out by mixing the three-components using 2−3 mL of methanol and irradiating under MW at 100 °C until completion of the reaction as evidenced by TLC. The reaction proceeds through decarboxylative condensation of isatin with amino acid to generate azomethine ylide, which subsequently undergoes 1,3-dipolar cycloaddition with andrographolide to afford novel dispiro cycloadduct (Scheme 1). Control of the relative stereochemistry at the spiro center was observed. *Anti*-ylides are involved in the reactions which add to dipolarophiles to form the cycloadducts. Formation of *syn*-ylide is not observed because of the unfavorable steric repulsion between the carbonyl group of oxindole and methyl group of sarcosine or benzyl group of *N*-benzyl glycine.[21]

Characterization of the products was performed by detailed spectral analysis. In all the cases mass spectral analysis of the products provided evidence for the success of the cycloaddition reaction which was further supported by the NMR spectral data. In the spectra of the products, the signals for the fused six-membered ring fragments of andrographolide appeared almost unchanged. From 2D NMR spectroscopic analysis it was revealed that the reverse regioisomer is predominant in case of proline and the normal one in case of sarcosine as was reported in our previous communication.[20]

For the generalization of the method, we extended this reaction of andrographolide and sarcosine/proline/*N*-benzyl glycine with variously substituted isatins and the corresponding dispiropyrrolidino/pyrrolizidino andrographolide derivatives were produced in high yields (Tables 2−4).

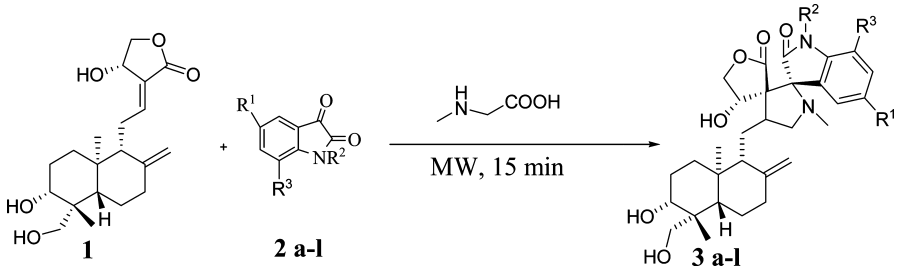
In case of *N*-benzyl glycine also the addition follows the pattern of sarcosine, with the isatin part added from the C13 end and the *N*-benzyl part from the C12 end. The crucial

Scheme 1. Synthesis of Novel Di-spiro Pyrrolidino/Pyrrolizidino Andrographolides



dx.doi.org/10.1021/co3001154 | ACS Comb. Sci. 2013, 15, 41−48

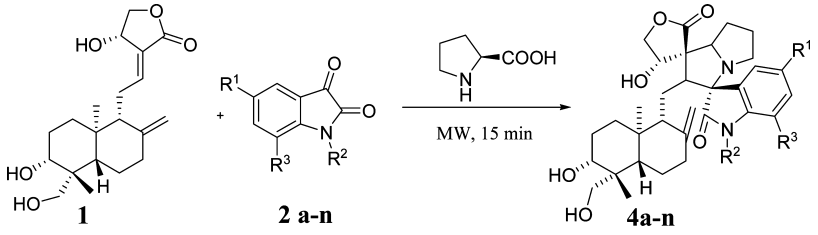
Table 2. Yields of 3a–l under Microwave Irradiation



entry	R^1	R^2	R^3	product[a] (3a–l)	yield (%)[b]
1	H	H	H	**3a**	82
2	Me	H	H	**3b**	82
3	Cl	H	H	**3c**	81
4	I	H	H	**3d**	80
5	F	H	H	**3e**	84
6	OMe	H	H	**3f**	82
7	Br	H	H	**3g**	79
8	H	Me	H	**3h**	80
9	Me	H	Me	**3i**	82
10	H	H	F	**3j**	76
11	H	H	Br	**3k**	74
12	H	Ph	H	**3l**	70

[a]Reaction was performed at 100 °C for 15 min. [b]Isolated yield.

Table 3. Yields of 4a–n under Microwave Irradiation



entry	R^1	R^2	R^3	product[a] (4a–n)	yield (%)[b]
1	H	H	H	**4a**	85
2	Me	H	H	**4b**	82
3	Cl	H	H	**4c**	85
4	I	H	H	**4d**	84
5	F	H	H	**4e**	88
6	OMe	H	H	**4f**	81
7	Br	H	H	**4g**	78
8	H	Me	H	**4h**	80
9	H	Ph	H	**4i**	70
10	H	CH_2Ph	H	**4j**	84
11	Me	H	Me	**4k**	83
12	H	H	F	**4l**	77
13	H	H	Br	**4m**	75
14	H	H	I	**4n**	73

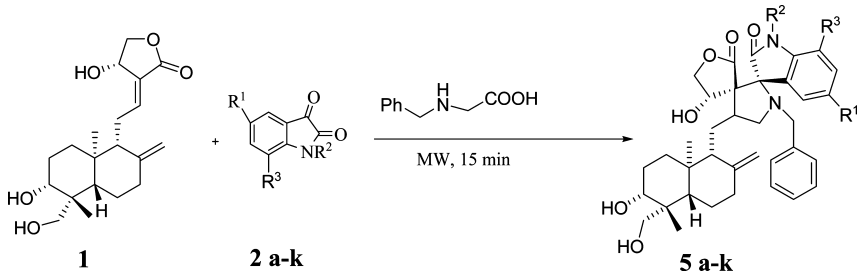
[a]Reaction was performed at 100 °C for 15 min. [b]Isolated yield.

evidence in support of this addition came from the observed HMBC correlation in the spectrum of **5c** between $-CH_2N$ (δ 56.2) and H-11 (δ 2.24) and of C-11 (δ 24.1) with $-CH_2N$ protons (δ 3.18, 2.73) (Figure 1). This was further supported by the COSY relationship in between $-CH_2N$ proton (δ 3.18) and H-12 (δ 2.53) coupled with the NOESY relationships between H-9 (δ 1.33) and $-CH_2N$ protons (δ 3.18, 2.73) and between H-11 (δ 2.24) and $-CH_2N$ proton (δ 2.73). The NOESY correlation of β oriented H-9 (δ 1.33) with H-12 (δ 2.53) suggests the latter to be β oriented.

In case of 7-substituted and *N*-phenyl isatins the reaction rate was to some extent slow (20 min in MW) with marginally lower yield of the products as shown in Tables 2–4. The reaction took place also in water in presence of β-CD or CTAB, but the rate of the reaction was very slow, and yields were about 30–35% (Table 1). Formation of some insoluble byproduct may be the reason for the low yield. But under MW irradiation the reaction occurred within 20 min in a clean manner. The yields were quite high (up to 80%) with no significant byproducts. The reaction was also performed in ionic liquid

dx.doi.org/10.1021/co3001154 | *ACS Comb. Sci.* 2013, 15, 41–48

Table 4. Yields of 5a–k under Microwave Irradiation



entry	R^1	R^2	R^3	product[a] (5a–k)	yield (%)[b]
1	H	H	H	**5a**	82
2	Me	H	H	**5b**	84
3	Cl	H	H	**5c**	82
4	I	H	H	**5d**	80
5	F	H	H	**5e**	81
6	OMe	H	H	**5f**	78
7	Br	H	H	**5g**	79
8	Me	H	Me	**5h**	79[c]
9	H	H	F	**5i**	75[c]
10	H	H	Br	**5j**	72[c]
11	H	Ph	H	**5k**	70[c]

[a]Reaction was performed at 100 °C for 15 min. [b]Isolated yield. [c]After 20 min reaction.

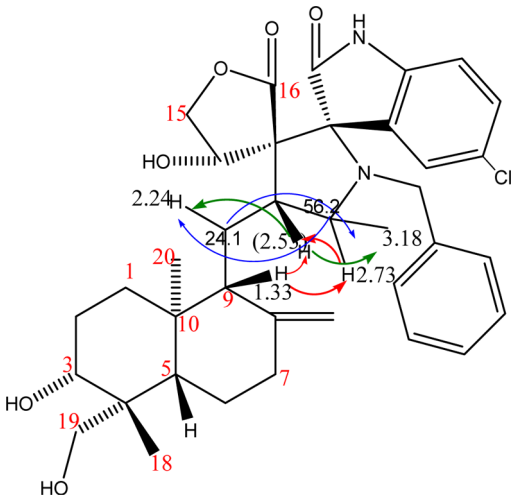


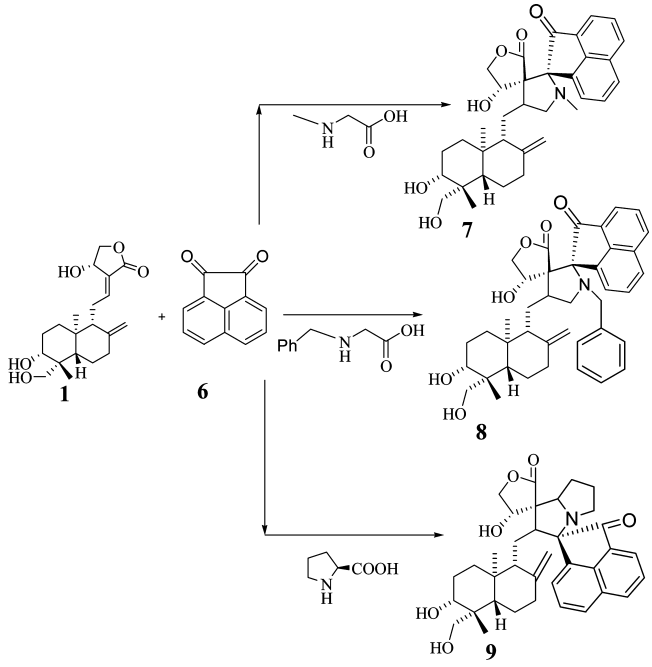
Figure 1. Important correlations of **5c** [HMBC (C →blue arrow H), COSY (green arrow), NOESY (red arrow)].

(IL). Though clean and high yielding, it was comparatively slower with respect to MW irradiation. However it was faster than in methanol. The real hurdle faced was the separation of IL from the product for its recycling, because of the polar nature of both the product and IL. Considering all the factors, reaction in methanol assisted by microwave irradiation works the best.

Using the optimized protocol (for MW) we extended the synthetic methodology toward another 1,2-diketone acenaphthoquinone (**6**) in place of isatins to synthesize some more novel dispiro derivatives (**7**, **8**, and **9**) with high yield (~80%) (Scheme 2).

In case of sarcosine and *N*-benzyl glycine the acenaphthoquinone part got added from the C13 end and the methyl/*N*-benzyl part from the C12 end mimicking the case of isatin. The crucial evidence in support of this addition came from the observed HMBC correlation in the spectrum of **7** between

Scheme 2. Synthesis of Novel Di-spiro Andrographolide Derivatives Using Acenaphthoquinone in Place of Isatin



signals of $-CH_2N$ (δ 60.3) and H-11 (δ 2.38, 2.26) as also C-11 (δ 24.9) and $-CH_2N$ protons (δ 3.56, 3.15). Further, COSY relationship between peaks for $-CH_2N$ proton (δ 3.56) and H-12 (δ 3.11) together with the NOESY relationships among peaks for H-9 (δ 1.61) and $-CH_2N$ protons (δ 3.15) as also between exomethylene protons (δ 4.73) and a $-CH_2N$ proton (δ 3.11) strongly supported the proposed structure (Figure 2). Similar relationship was also observed in case of **8** [HMBC between $-CH_2N$ (δ 57.1) and H-11 (δ 2.49, 2.18), C-11 (δ 24.5) and $-CH_2N$ protons (δ 3.57, 2.97); COSY between one $-CH_2N$ proton (δ 3.57) and H-12 (δ 2.97) as also H-11 (δ

dx.doi.org/10.1021/co3001154 | *ACS Comb. Sci.* 2013, 15, 41–48

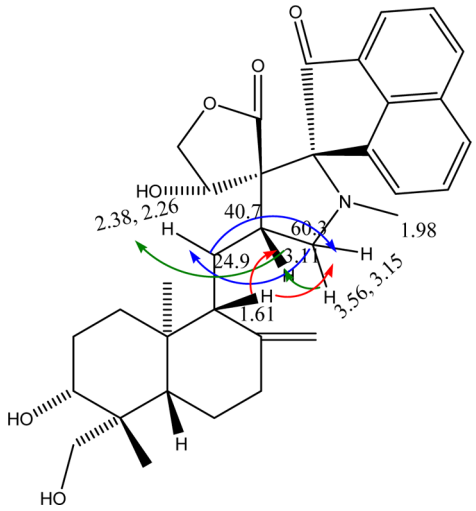


Figure 2. Important correlations of **7** [HMBC (C →blue arrow H), COSY (green arrow), NOESY (red arrow)].

2.49); NOESY relationships between H-9 (δ 1.51) and $-CH_2N$ protons (δ 3.57, 2.97) and also between exomethylene protons (δ 4.80) and $-CH_2N$ proton (δ 2.97)], strongly supporting the proposed structure (Figure 3). The NOESY relationship of the

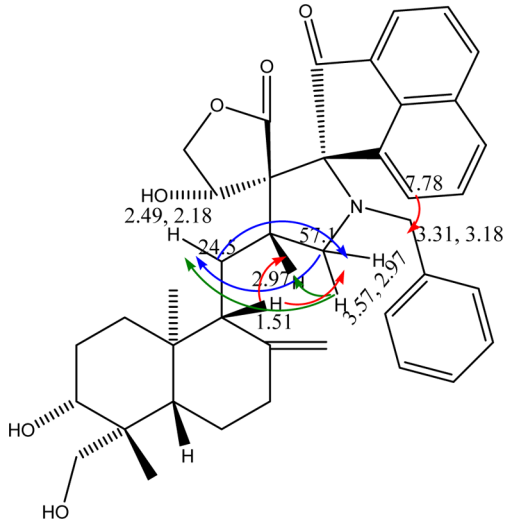


Figure 3. Important correlations of **8** [HMBC (C →blue arrow H), COSY (green arrow), NOESY (red arrow)].

acenaphthoquinone aromatic proton (δ 7.78) (2′) with the N-benzylic proton (δ 3.31) also helped in the confirmation of structure **8** (Figure 3). The NOESY correlation of β oriented H-9 (δ 1.61 or 1.51) with H-12 (δ 3.11 or 2.97) confirms the latter also to be β oriented.

This was ultimately confirmed by single crystal X-ray crystallography of **8** (Figure 4) where the β-orientation of C7 and C9 protons was clearly visible, and the chirality deduced at the C7, C16, and C20 center are respectively R, R, and R (according to X-ray crystallographic numbering). This is applied to all the figures presented.

As noted in reactions with isatin, the acenaphthoquinone part added from the C12 end when proline was introduced in the reaction (in case of **9**) and the proline part from the C13 end (Figure 5) as evidenced from detailed NMR spectra.

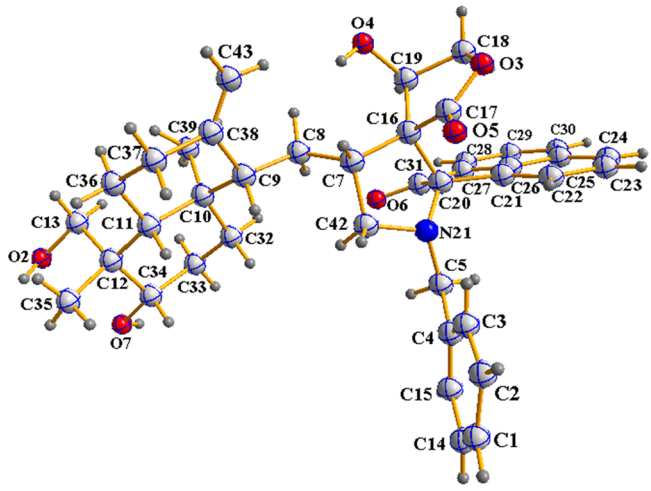


Figure 4. ORTEP representation of **8**.

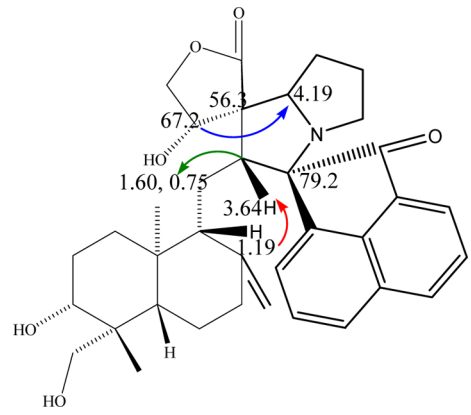


Figure 5. Important correlations of **9** [HMBC (C →blue arrow H), COSY (green arrow), NOESY (red arrow)].

Inspired by the positive results from the biological screening of the previous 15 compounds against some cancer cell lines [unpublished data], anticancer activity of these 40 compounds compared to andrographolide was evaluated against seven cancer cell lines (Table 5).

The enhancement of activity in the *N*-benzyl glycine series seems to be very promising, as indicated from their IC_{50} values, and demand further evaluation for SAR analysis, toxicity profiles, and mechanistic pathways.

CONCLUSION

In conclusion, a facile, atom-economic synthesis of novel dispiro compounds of andrographolide has been achieved via 1,3-dipolar cycloaddition of azomethine ylides (derived from isatins or acenaphthoquinone and few aminoacid derivatives) under MW irradiation to Δ^{12} of andrographolide. When the amino acid was changed from sarcosine or *N*-benzyl glycine to proline, the opposite regioisomer was produced, as determined by 2D NMR and confirmed by X-ray crystallographic analysis. All the reactions are chemoselective in nature as only one of the two double bonds takes part in the reaction. Biological evaluation indicates the promise held out by *N*-benzyl glycine derivatives that need to be further explored.

dx.doi.org/10.1021/co3001154 | *ACS Comb. Sci.* 2013, 15, 41−48

Table 5. IC_{50} Values of Compounds 3a−3l, 4a−4n, 5a−5k, and 7−9

	IC_{50} (μM)						
Comp ID	CHO	HepG-2	HeLa	A431	MCF-7	Caco-2	MDCK
3a	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	ND[a]
3b	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	ND
3c	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	ND
3d	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	ND
3e	>50.0	>50.0	>50.0	>50.0	>50.0	40.9	ND
3f	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	ND
3g	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	ND
3h	>50.0	>50.0	>50.0	>50.0	>50.0	ND	>50.0
3i	>50.0	>50.0	>50.0	>50.0	>50.0	ND	>50.0
3j	>50.0	>50.0	>50.0	>50.0	>50.0	ND	>50.0
3k	>50.0	>50.0	>50.0	>50.0	>50.0	ND	>50.0
3l	>50.0	>50.0	49.0	49.2	>50.0	ND	>50.0
4a	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	ND
4b	31.2	>50.0	>50.0	>50.0	33.4	28.9	ND
4c	9.8	18.4	31.8	36.2	14.3	>50.0	ND
4d	12.9	18.5	18.8	21.5	31.6	>50.0	16.4
4e	32.8	46.0	>50.0	>50.0	>50.0	>50.0	ND
4f	>50.0	>50.0	>50.0	>50.0	>50.0	>50.0	ND
4g	13.1	22.2	28.8	36.5	26.4	30.6	ND
4h	27.0	>50.0	35.5	>50.0	>50.0	>50.0	ND
4i	32.4	25.0	24.8	26.7	36.4	ND	17.7
4j	26.9	22.0	23.7	>50.0	38.9	39.0	>50.0
4k	20.0	26.2	28.5	25.4	23.1	ND	27.1
4l	>50.0	>50.0	>50.0	>50.0	>50.0	ND	>50.0
4m	24.9	39.9	36.3	37.1	33.7	ND	>50.0
4n	24.2	26.8	26.1	26.2	30.9	ND	26.4
5a	22.0	20.9	14.0	9.5	18.9	18.8	ND
5b	19.4	13.9	13.7	12.1	10.9	12.4	ND
5c	10.2	9.7	9.8	9.0	12.7	12.5	10.0
5d	48.7	43.9	40.4	33.1	43.2	43.5	37.8
5e	16.2	16.5	14.6	9.0	14.4	12.0	ND
5f	14.4	12.3	12.7	16.6	15.7	13.3	12.7
5g	13.1	10.0	8.9	10.0	12.8	ND	10.6
5h	8.1	7.5	7.5	8.1	15.4	ND	7.3
5i	10.8	12.9	12.2	13.9	13.7	ND	13.8
5j	11.5	10.6	10.4	12.5	13.9	ND	13.0
5k	13.8	9.3	8.9	8.2	16.0	ND	13.4
7	>50.0	>50.0	>50.0	>50.0	>50.0	ND	>50.0
8	9.6	8.1	7.4	10.5	8.9	ND	10.8
9	30.9	30.3	29.6	39.2	>50.0	>50.0	39.3
1	46.0	33.4	27.1	15.0	29.4	14.1	20.9

[a]ND: Not done.

EXPERIMENTAL PROCEDURES

General Information. *Chemistry.* All the compounds evaluated in this work were synthesized in one-pot sequences using a mono mode Discover microwave reactor (CEM Corp., Matthews, NC, U.S.A.). Melting points were determined in capillaries and are uncorrected. IR spectra were recorded as KBr pellets using a JASCO 410 FTIR spectrometer. The NMR spectra were recorded using a Bruker 600 DPX spectrometer operating at 600 MHz for ^{1}H and 150 MHz for ^{13}C respectively in DMSO-d_6/$CDCl_3$ with TMS as internal standard. Mass spectra (positive mode) were obtained on a Q-TOF micro TM mass spectrometer in the electrospray ionization mode. Andrographolide was isolated from *A. paniculata* in the usual way. Isatins, acenaphthoquinone and α-amino acids were purchased from Alfa-Aesar Company. All other solvents and chromatographic absorbents were procured from E. Merck (Germany) and SRL (India) Ltd. unless otherwise indicated.

Biology. Cell lines (CHO, HepG2, HeLa, A-431, MCF-7, MDCK and Caco-2) were obtained from National Centre for Cell Sciences, Pune, India. MEM-alpha, FBS (fetal bovine serum), penicillin−streptomycin were purchased from Gibco, India. Cell culture flasks, plates, and tips were obtained from Tarsons, Kolkata, India. DMEM, other components of media, and all other chemicals were purchased from Sigma, St. Louis, U.S.A.

Typical Experimental Procedure for Synthesis of Dispiro Products. A mixture of **1** (0.58 mmol, 200 mg), isatin (0.6 mmol, 90 mg) or acenapthoquinone (0.6 mmol, 110 mg), and proline (0.61 mmol, 70 mg) or sarcosine (0.61 mmol, 54 mg) or *N*-benzyl glycine (0.61 mmol, 101 mg) was taken in a conical flask. The contents of the flask were mixed thoroughly

dx.doi.org/10.1021/co3001154 | *ACS Comb. Sci.* 2013, 15, 41−48

with 2−3 mL methanol. Then the crude mixture was irradiated under MW at 100 °C. The progress of the reaction was monitored by TLC in chloroform−methanol (9:1) and visualization was accomplished in UV or by LB spray. After completion of the reaction, the contents were directly dissolved in chloroform−methanol (1−2%) and subjected to column chromatography using chloroform−methanol (0.5% methanol in chloroform) as eluant. The product was crystallized from an acetonitrile-benzene mixture.

Cell Culture and MTT Assay. MDCK cells were maintained in growth media containing MEM-alpha (Gibco) supplemented with 10% FBS (Gibco) and penicillin−streptomycin (100U/mL for each). Other cell lines were maintained in DMEM media containing 10% FBS and 40 μg/mL gentamycin.

HeLa cells were plated (in 96 well plates) at 6000 cells/well/180 μL media. For other cell lines it was 4000 cells/well/180 μL media. Cell seeding density was optimized so that the wells without any inhibitor can make up to 90% confluency at the end of the incubation period/experiment. The plates containing cells were placed in 37 °C incubator with 5% CO_2 and 95% relative humidity for 24 h. Media was aspirated off and replaced with 180 μL of fresh media. Test compounds were dissolved at a concentration of 5 mM (DMSO), and further serial half dilutions were made in DMSO to reach to a concentration of 0.078 mM. These substocks were further 10-fold diluted in respective growth media. Then 20 μL of growth media containing test compounds was added (n = 2) in 96-well test plate to produce final working conc of 50 to 0.78 μM (DMSO: 1%). Each plate had cell control, vehicle control, media control, and standard inhibitor Doxorubicin at 10 μM.

The plates were placed back into the incubator for 72 h. 20 μL of MTT (5 mg/mL of PBS pH7.2) was added in each well, and the plates further incubated for 4 h. The plates were centrifuged (2500 rpm, 10 min), media flicked off and formazan crystals dissolved in 150 μL of DMSO. Absorbance was measured at 510 nm using Spectramax M5 (Molecular Devices, U.S.A.). Cell death at each conc was determined based on OD difference of the test well from that of wells of vehicle control. If the highest test concentration (50 μM) shows less than 50% cell death then IC_{50} (concentration that causes 50% cell death) is reported as >50 μM or else it was calculated using GraphPad Prism 5.0 software. The MTT assay experiment was performed in duplicate wells each day and in three separate days.[22,23]

■ ASSOCIATED CONTENT

Supporting Information

^1H, ^{13}C NMR, and HRMS data along with spectral copy of all compounds associated with this Article. This material is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data in CIF format are available free of charge via the Internet from the Cambridge Crystallographic Data Centre under deposition no. CCDC 893411. These data 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, U.K.; fax: +44 1223 336033; or deposit@ccdc.cam.ac.uk).

■ AUTHOR INFORMATION

Corresponding Author

*Fax: +91-33-2473-5197. E-mail: nirup@iicb.res.in.

Funding

This work received financial assistance from the Council of Scientific and Industrial Research (CSIR, Net Work Project-0009), Government of India and Department of Science and Technology (West Bengal-India). A.H., B.Y., A.M., S.M., and R.P. are recipients of Research Fellowships from CSIR.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Our thanks are due to Dr. B. Achari (Emeritus Scientist, CSIR) for helpful suggestions and Mr. K. Sarkar, E. Padmanabhan, and S. Samaddar for recording HRMS, NMR, and IR spectra, respectively. Special thanks go to Prof. B.C. Ranu of Indian Association for the Cultivation of Science for giving permission to use the microwave reactor.

■ REFERENCES

(1) (a) Schreiber, S. L. Target-Oriented and Diversity-Oriented Organic Synthesis in Drug Discovery. *Science* **2000**, *287*, 1964−1969. (b) Dömling, A. Recent advances in isocyanide-based multicomponent chemistry. *Curr. Opin. Chem. Biol.* **2002**, *6*, 303−313.

(2) (a) Orru, R. V. A.; de Greef, M. Recent Advances in Solution-Phase Multicomponent Methodology for the Synthesis of Heterocyclic Compounds. *Synthesis* **2003**, 1471−1499. (b) Balme, G.; Bossharth, E.; Monteiro, N. Pd-Assisted Multicomponent Synthesis of Heterocycles. *Eur. J. Org. Chem.* **2003**, 4101−4111. (c) Bräse, S.; Gil, C.; Knepper, K. The recent impact of solid-phase synthesis on medicinally relevant benzoannelated nitrogen heterocycles. *Bioorg. Med. Chem.* **2002**, *10*, 2415−2437. (d) Dömling, A.; Ugi, I. Multicomponent Reactions with Isocyanides. *Angew. Chem., Int. Ed.* **2000**, *39*, 3168−3210.

(3) (a) Ohno, H.; Ohta, Y.; Oishi, S.; Fujii, N. Direct Synthesis of 2-(Aminomethyl)indoles through Copper(I)-Catalyzed Domino Three-Component Coupling and Cyclization Reactions. *Angew. Chem., Int. Ed.* **2007**, *46*, 2295−2298. (b) Bonne, D.; Dekhane, M.; Zhu, J. Modulating the Reactivity of α-Isocyanoacetates: Multicomponent Synthesis of 5-Methoxyoxazoles and Furopyrrolones. *Angew. Chem., Int. Ed.* **2007**, *46*, 2485−2488. (c) Pinto, A.; Neuville, L.; Zhu, J. Palladium-Catalyzed Three-Component Synthesis of 3-(Diarylmethylene)oxindoles through a Domino Sonagashira/Carbopalladation/C−H Activation/C−C Bond-Forming Sequence. *Angew. Chem., Int. Ed.* **2007**, *46*, 3291−3295. (d) Komagawa, S.; Saito, S. Nickel-Catalyzed Three-Component [3 + 2+2] Cocyclization of Ethyl Cyclopropylideneacetate and Alkynes—Selective Synthesis of Multisubstituted Cycloheptadienes. *Angew. Chem., Int. Ed.* **2006**, *45*, 2446−2449. (e) Yoshida, H.; Fukushima, H.; Ohshita, J.; Kunai, A. CO_2 Incorporation Reaction Using Arynes: Straightforward Access to Benzoxazinone. *J. Am. Chem. Soc.* **2006**, *128*, 11040−11041. (f) Dondas, H. A.; Fishwick, C. W. G.; Gai, X.; Grigg, R.; Kilner, C.; Dumrongchai, N.; Kongkathip, B.; Kongkathip, N.; Polysuk, C.; Sridharan, V. Stereoselective Palladium-Catalyzed Four-Component Cascade Synthesis of Pyrrolidinyl-, Pyrazolidinyl-, and Isoxazolidinyl Isoquinolines. *Angew. Chem., Int. Ed.* **2005**, *44*, 7570−7574. (g) Pache, S.; Lautens, M. Palladium-Catalyzed Sequential Alkylation−Alkenylation Reactions: New Three-Component Coupling Leading to Oxacycles. *Org. Lett.* **2003**, *5*, 4827−4830.

(4) (a) Siamaki, A. R.; Arndtsen, B. A. A Direct, One Step Synthesis of Imidazoles from Imines and Acid Chlorides: A Palladium Catalyzed Multicomponent Coupling Approach. *J. Am. Chem. Soc.* **2006**, *128*, 6050−6051. (b) Duan, X. H.; Liu, X. Y.; Guo, L. N.; Liao, M. C.; Liu, W. M.; Liang, Y. M. Palladium-Catalyzed One-Pot Synthesis of Highly Substituted Furans by a Three-Component Annulation Reaction. *J. Org. Chem.* **2005**, *70*, 6980−6983.

(5) (a) Tietze, L. F.; Brasche, G.; Gericke, K. *Domino Reactions in Organic Synthesis*; Wiley-VCH: Weinheim, Germany, 2006, pp 1−617.

(b) Zhu, J.; Bienaymé, H. *Multicomponent Reactions*; Wiley-VCH: Weinheim, Germany, 2005; pp 33−75.
(6) (a) Daly, J. W.; Spande, T. F.; Whittaker, N.; Highet, R. J.; Feigl, D.; Nishimori, N.; Tokuyama, T.; Meyers, C. W. Alkaloids from Dendrobatid Frogs: Structures of Two ω-Hydroxy Congeners of 3-Butyl-5-Propylindolizidine and Occurrence of 2,5-Disubstituted Pyrrolidines and a 2,6-Disubstituted Piperidine. *J. Nat. Prod.* **1986**, *49*, 265−280. (b) Waldmann, H. Amino Acid Esters: Versatile Chiral Auxiliary Groups for the Asymmetric Synthesis of Nitrogen Heterocycles. *Synlett* **1995**, 133−141.
(7) Abou-Gharbia, M. A.; Doukas, P. H. Synthesis of Tricyclic Arylspiro Compounds as Potential Antileukemic and Anticonvulsant Agents. *Heterocycles* **1979**, *12*, 637−640.
(8) Kornet, M. J.; Thio, A. P. Oxindole-3-spiropyrrolidines and -piperidines. Synthesis and local anesthetic activity. *J. Med. Chem.* **1976**, *19*, 892−898.
(9) (a) Joshi, K. C.; Chand, P. Biologically active indole derivatives. *Pharmazie* **1982**, *37*, 1−12. (b) Da-Silva, J. F. M.; Garden, S. J.; Pinto, A. C. The Chemistry of Isatins: a Review from 1975 to 1999. *J. Braz. Chem. Soc.* **2001**, *12*, 273−324. (c) Abdel-Rahman, A. H.; Keshk, E. M.; Hanna, M. A.; El-Bady, Sh. M. Synthesis and evaluation of some new spiro indoline-based heterocycles as potentially active antimicrobial agents. *Bioorg. Med. Chem.* **2004**, *12*, 2483−2488.
(10) Kobayashi, J.; Tsuda, M.; Agemi, K.; Shigemori, H.; Ishibashi, M.; Sasaki, T.; Mikami, Y. Purealidins B and C, new bromotyrosine alkaloids from the okinawan marine sponge psammaplysilla purea. *Tetrahedron* **1991**, *47*, 6617−6622.
(11) James, D. M.; Kunze, H. B.; Faulkner, D. J. Two New Brominated Tyrosine Derivatives from the Sponge Druinella (=Psammaplysilla) purpurea. *J. Nat. Prod.* **1991**, *54*, 1137−1140.
(12) (a) Okita, T.; Isobe, M. Synthesis of the pentacyclic intermediate for dynemicin a and unusual formation of spiro-oxindole ring. *Tetrahedron* **1994**, *50*, 11143−11152. (b) Rosenmond, P.; Hosseini-Merescht, M.; Bub, C. Ein einfacher Zugang zu den tetracyclischen Vorläufern der Hetero- und Secoyohimbane, Strychnos- und Oxindolalkaloide. *Liebigs Ann. Chem.* **1994**, *2*, 151−154.
(13) Hilton, S. T.; Ho, T. C. T.; Pljevaljcic, G.; Jones, K. A New Route to Spirooxindoles. *Org. Lett.* **2000**, *2*, 2639−2641.
(14) (a) Huisgen, R. 1,3-Dipolar Cycloadditions. Past and Future. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 565−598. (b) Woodward, R. B.; Hoffmann, R. The Conservation of Orbital Symmetry. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 781−853.
(15) (a) Houk, K. N.; Sims, J.; Duke, R. E.; Strozier, R. W.; George, J. K. Frontier molecular orbitals of 1,3 dipoles and dipolarophiles. *J. Am. Chem. Soc.* **1973**, *95*, 7287−7301. (b) Houk, K. N.; Sims, J.; Watts, C. R.; Luskus, L. J. Origin of reactivity, regioselectivity, and periselectivity in 1,3-dipolar cycloadditions. *J. Am. Chem. Soc.* **1973**, *95*, 7301−7315.
(16) (a) Pellissier, H. Asymmetric 1,3-dipolar cycloadditions. *Tetrahedron* **2007**, *63*, 3235−3285. (b) Pinho e Melo, T. M. V. D. Conjugated Azomethine Ylides. *Eur. J. Org. Chem.* **2006**, 2873−2888. (c) Pandey, G.; Banerjee, P.; Gadre, S. R. Construction of Enantiopure Pyrrolidine Ring System via Asymmetric [3 + 2]-Cycloaddition of Azomethine Ylides. *Chem. Rev.* **2006**, *106*, 4484−4517. (d) Coldham, I.; Hufton, R. Intramolecular Dipolar Cycloaddition Reactions of Azomethine Ylides. *Chem. Rev.* **2005**, *105*, 2765−2810. (e) Rück-Braun, K.; Freysoldt, T. H. E.; Wierschem, F. 1,3-Dipolar cycloaddition on solid supports: nitrone approach towards isoxazolidines and isoxazolines and subsequent transformations. *Chem. Soc. Rev.* **2005**, *34*, 507−516. (f) Kanemasa, S. Metal-Assisted Stereocontrol of 1,3-Dipolar Cycloaddition Reactions. *Synlett* **2002**, 1371−1387.
(17) (a) Najera, C.; Sansano, J. M. Azomethine Ylides in Organic Synthesis. *Curr. Org. Chem.* **2003**, *7*, 1105−1150. (b) Harwood, L. M.; Vickers, R. J. *Synthetic Application of 1,3-Dipolar Cycloaddition Chemistry Toward Heterocycles and Natural Products*; Padwa, A., Pearson, W. H., Eds.; John Wiley & Sons: New York, 2002; p 169. (c) Gothelf, K. V. *Cycloaddition Reactions in Organic Synthesis*; Kobaya-Shi, S., Jorgensen, K. A., Eds.; Wiley-VCH: Weinheim, Germany, 2002; p 211. (d) Karlsson, S.; Högberg, H. E. Asymmetric 1,3-Dipolar Cycloadditions for the Construction of Enantiomerically Pure Heterocycles. *Org. Prep. Proced. Int.* **2001**, *33*, 103−172. (e) Gothelf, K. V.; Jørgensen, K. A. Asymmetric 1,3-Dipolar Cycloaddition Reactions. *Chem. Rev.* **1998**, *98*, 863−910. (f) Sustmann, R.; Sicking, W.; Huisgen, R. Regiochemistry in cycloadditions of diazomethane to thioformaldehyde and thioketones. *J. Org. Chem.* **1993**, *58*, 82−89. (g) Padwa, A. *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; John Wiley & Sons: New York, 1984; p 277. (h) Huisgen, R.; Graf, H. Stereochemistry of tetracyanoethylene cycloadditions to thioenol ethers. *J. Org. Chem.* **1979**, *44*, 2595−2596.
(18) Dai, G. F.; Xu, H. W.; Wang, J. F.; Liu, F. W.; Liu, H. M. Studies on the novel α-glucosidase inhibitory activity and structure−activity relationships for andrographolide analogues. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2710−2713.
(19) Li, Z.; Huang, W.; Zhang, H.; Wang, X.; Zhou, H. Synthesis of andrographolide derivatives and their TNF-α and IL-6 expression inhibitory activities. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6891−6894.
(20) Hazra, A.; Paira, P.; Sahu, K. B.; Naskar, S.; Saha, P.; Paira, R.; Mondal, S.; Maity, A.; Luger, P.; Weber, M.; Mondal, N. B.; Banerjee, S. Chemistry of Andrographolide: formation of novel di-spiropyrrolidino and di-spiropyrrolizidino oxindole adducts via one-pot three-component [3 + 2] azomethine ylide cycloaddition. *Tetrahedron Lett.* **2010**, *51*, 1585−1588.
(21) (a) Amornraksa, K.; Grigg, R.; Gunaratna, H. Q. N.; Kemp, J.; Sridharan, V. X=Y−ZH Systems as potential 1,3-dipoles. Part 8. Pyrrolidines and Δ^5-pyrrolines (3,7-diazabicyclo[3.3.0]octenes) from the reaction of imines of α-amino acids and their esters with cyclic dipolarophiles. Mechanism of racemisation of α-amino acids and their esters in the presence of aldehydes. *J. Chem.Soc., Perkin Trans. 1* **1987**, 2285−2296. (b) Rehn, S.; Bergman, J.; Stensland, B. The Three-Component Reaction between Isatin, α-Amino Acids, and Dipolarophiles. *Eur. J. Org. Chem.* **2004**, 413−418. (c) Cardoso, A. L.; Kaczor, A.; Silva, A. M. S.; Fausto, R.; Melo, T.M.V.D.P.; Gonsalves, A. M. R. Intermolecular cycloaddition of nonstabilized azomethine ylides generated from 1,3-thiazolidine-4-carboxylic acids: synthesis of 5,7a-dihydro-1*H*,3*H*-pyrrolo[1,2-*c*]thiazoles. *Tetrahedron* **2006**, *62*, 9861−9871. (d) Jayashankaran, J.; Manian, R. D. R. S.; Raghunathan, R. A facile entry into a novel class of dispiroheterocycles through 1,3-dipolar cycloaddition. *Arkivoc* **2005**, No. xi, 32−39.
(22) Talupula, B. K. Cytotoxicity of PBN spin trap on A204 cells. *J. Adv. Pharm. Res.* **2011**, *2*, 9−17.
(23) Al-Qubaisi, M.; Rozita, R.; Yeap, S. K.; Omar, A. R.; Ali, A. M.; Alitheen, N. B. Selective Cytotoxicity of Goniothalamin against hepatoblastoma HepG2 cells. *Molecules* **2011**, *16*, 2944−2959.