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Original article

A natural product based DOS library of hybrid systems

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ABSTRACT

Here we described a natural product inspired modular DOS strategy for the synthesis of a library of hybrid systems that are structurally and stereochemically disparate. The main scaffold is a pyrroloisoquinoline motif, that is synthesized from tandem Pictet-Spengler lactamization. The structural diversity is generated *via* "privileged scaffolds" that are attached at the appropriate site of the motif. Screening of the library compounds for their antiplasmodial activity against chloroquine sensitive 3D7 cells indicated few compounds with moderate activity (20–50 μM). A systematic comparison of structural intricacy between the library members and a natural product dataset obtained from ZINC® revealed comparable complexity.

1. Introduction

Hybrid systems are defined as assembly of diverse molecular entities (in general two), natural or synthetic, to afford functional molecules, which intrinsically enhance or modulate the biological properties of individual components or, may exhibit new properties [1,2]. Due to their application towards discovering better drug molecules for some of the most critical segments of pathological research *viz.* cancer and malaria, in recent years they have generated substantial interest among scientists in the pharmaceutical community [3–5].

Hence hybrid systems can be veritably regarded as key target structures in diversityoriented synthesis (DOS) of natural product-based libraries for drug discovery screening. The concept of diversity oriented synthesis have revolutionized the fundamental approach of generating compound libraries [6–9]. There has been a paradigm shift from conventional one-dimensional libraries to structurally and stereochemically disparate libraries. In this regard, we have reported a diversity oriented synthesis (DOS) of a library of molecules which are hybrid of pyrroisoquinoline scaffold and few "privileged" scaffolds. DOS determined the choice of various "privileged" scaffolds used to generate the final molecules [6–9]. "Privileged scaffolds", are frequently found among variety of bioactive natural products and drug candidates capable of modulating multiple biological targets [10]. Keeping in mind their success in the past, their presence in chemical libraries increases their propensity to delivering molecules with interesting biological properties [11].

Synthesis of natural product inspired DOS libraries is not new. Interestingly there are quite a few examples of such library designs [12–19]. Very recently, Hergenrother and co-workers have worked out and illustrated an elegant ring distortion strategy of library formation employing quite a few natural products such as gibberellic acid, adrenosterone and quinine [20]. In another noteworthy instance, Aube and co-workers have come up with a library generated from templates modeled on and sustained by natural alkaloids [21].

Biologically active alkaloids such as (−)-3-demethoxyerythratidinone **1** and Crispine A **2**, blazed the trail for our natural product template (**10** and **19**) of the hybrid molecules [22,23]. The "privileged" scaffolds consisting of oxoindoles **3**, quinolones **4**, cyclopent-anes/enes (**5**/**6**) and pyrrolidinone (**7**) formed the second domain (Fig. 1).

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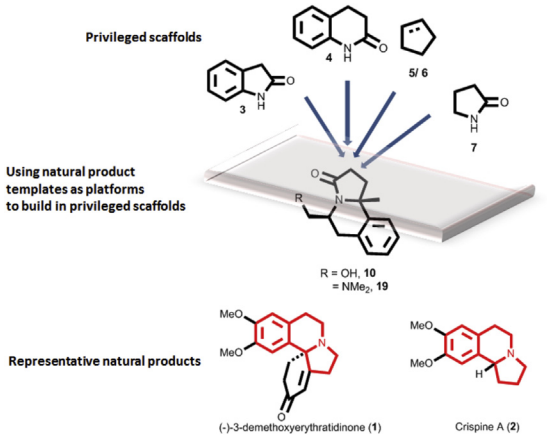


Fig. 1. DOS of hybrids *via* Platform technology.

Ours is a reagent based DOS strategy that utilized the pyrroloisoquinoline motif as a platform containing pluripotent reaction site (i.e. the methylene functionality α-to the tertiary amide) where appropriate functionalities were installed followed by facile cyclization reaction to construct the privileged scaffolds. The stereochemical and architectural diversity of the template in conjunction with the privileged scaffolds contributed to the variety and complexity of the library (Fig. 1). All the intermediates, privileged scaffolds and final hybrids were screened against *Plasmodium Falciparum*. We obtained a moderately potent series that inhibits the parasite growth. We hope this effort of ours will be a useful addition to the existing rich repertoire of DOS based synthesis of bioactive libraries.

2. Results

2.1. DOS library synthesis

To facilitate the Pictet Spengler Lactamization reaction L-phenyl alanine methyl ester was reacted with methyl levulinate. The reaction was optimized with various catalytic acids, *viz.* trifluoroacetic acid (TFA), *p*-toluenesulfonic acid (PTSA), polyphosphoric acid (PPA), acetic acid (AcOH), boron trifluoride-diethyl ether ($BF_3 \cdot OEt_2$) and titanium chloride ($TiCl_4$). As indicated in the table below (Table 1), PTSA yielded the best. The average diastereoselectivity of these reactions were poor (d.r. 70:30 → 80:20). The major diastereomer **9** was separated by flash column chromatography and was used for generation of the library.

Facile reduction of **9** with lithium aluminum hydride afforded **10**. Diallylation of **10** with allyl bromide and lithium hexamethyl disilyl amide (LHMDS) as base, at −78 °C → room temperature (rt) in teterahydrofuran (THF) generated **12**. It underwent ring closing metathesis with Grubbs I catalyst to afford **16** in 37% yield over 2 steps. Subsequent hydrogenation of **16** in presence of 10% *w/w* Pd–C and hydrogen at normal atmospheric pressure afforded **17** in 58% yield. In a different effort **10** was further acylated with ethyl chloroformate in presence of LHMDS at −78 °C followed by enolate generation with LHMDS at −78 °C and subsequent michael addition with nitrostyrene provided a mixture of diastereomeric intermediates **11**/**13** (d.r. 60:40). They were subjected to hydrogenation which in turn facilitated lactamization to the desired products **14** and **15** (Scheme 1). The relative configuration of **14** was confirmed by NOE. However poor yields encountered in this early effort prompted transformation of the hydroxymethyl appendage of **10** to less interfering dimethyl amino functionality in **20** *via* **19**. It was achieved by swern oxidation of **10** to the corresponding aldehyde followed by condensation with dimethylamine to generate **19**. The next set of functionality variant was developed by S_NAr (aromatic nucleophilic substitution) with 2,4-dinitrofluorobenzene and alkylation with *o*-nitrobenzylbromide on **20** in presence of sodium hydride (NaH), that afforded diastereomeric intermediates **21**/**22** and **23**/**24** in a diastereomeric ratio of 60:40 and 65:35 respectively. Subsequent hydrogenation of these intermediates provided the final molecules **25**–**28** (Scheme 1). All the diastereomeric intermediates and final molecules were isolated by flash column chromatography. It is noteworthy that even though the diastereoselectivity of the reactions were poor, accessing individual diastereomers other than the variation in molecular frameworks added more value to the diversity quotient of the library. The relative configuration of **21** was confirmed by single X-ray crystallography and that of **27** by NOE.

Table 1
Optimization of Pictet-Spengler lactamization reaction.

Entry	Catalyst	Yield[a]
1	PTSA	59
2	PPA	25
3	TFA	28
4	$BF_3 \cdot OEt_2$	21
5	$TiCl_4$	12
6	AcOH	44

[a] Isolated yield.

2.2. Biological evaluation

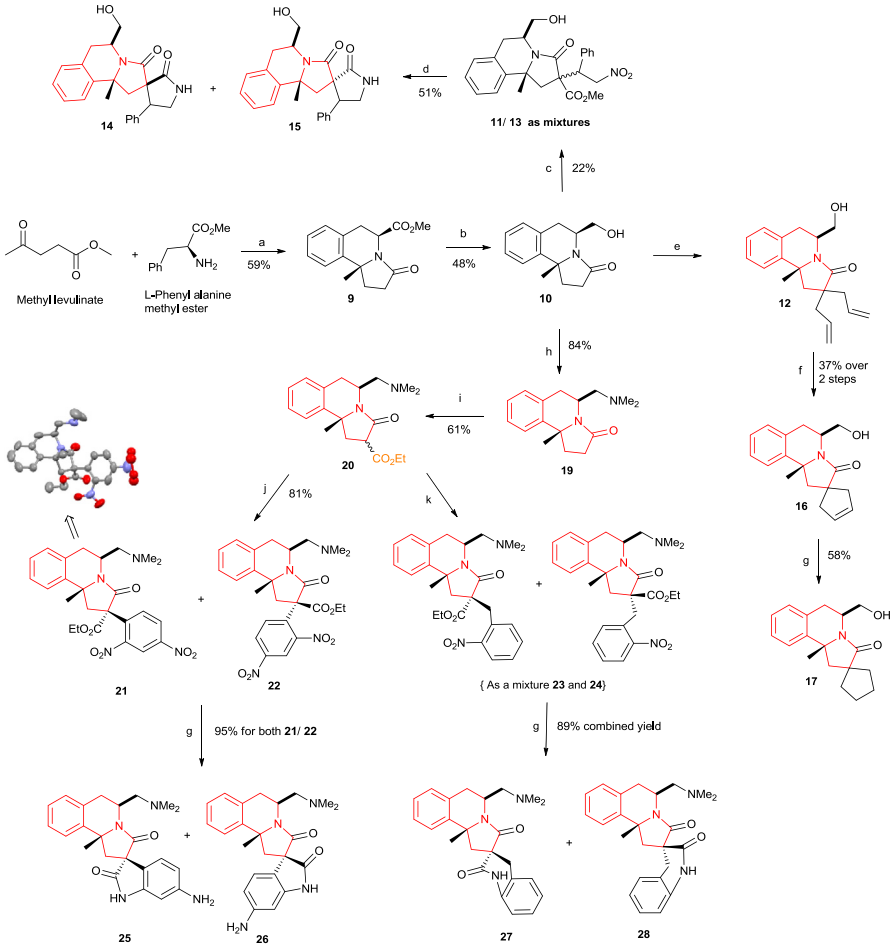
2.2.1. Antimalarial assay

DOS and phenotypic screening has been applied successfully in identifying potent antimalarial compounds [24–26]. In 2012, Schreiber and co-workers discovered an antimalarial macrocyclic lactam using diversity oriented synthesis and phenotypic screening of *P. falciparum* asexual blood-stage parasites [24]. In a similar endeavor, **NITD 609** was discovered in 2010 by a consortium led by Novartis Institute of Tropical Disease (NITD) [25,26]. Inspired from those reports we conducted screening of our library molecules, against malaria parasite (*3D7*) (refer SI-2 Table 1).

P. falciparum clones *3D7* was cultured in O+ human erythrocytes, with RPMI 1640 (Invitrogen, USA) supplemented with 24 mM sodium bicarbonate (Sigma, USA), 0.1 mM hypoxanthine (Invitrogen, USA), 25 mg mL^{-1} gentamicin (Invitrogen,USA) and 0.5% AlbuMax I (Invitrogen, USA), according to methods described earlier [27]. Parasite culture was maintained in mixed gas environment (5% O_2, 5% CO_2 and 90% N_2). Parasites were synchronized by sorbitol treatment at ring stage. The antimalarial drug CQDP was used as a reference. We were gratified to observe that 6 compounds, out of which 3 are final hybrids *viz.* **26**, **28**, **27**, 2 advanced intermediates *viz.* **21** and **22** and one early intermediate **20** showed moderate (60%) inhibition against 3D7 *P. Falciparum* at 50 μM in a growth inhibition assay (refer SI-3, Table 3). The list was further prioritized to five compounds (**21**–**22** and **26**–**28**) based on dose–response data with two concentrations against 3D7 strain. All these five scaffolds were subjected to full–dose response with 6 concentrations and the IC_{50} was determined to be 15–53 μM against *3D7* strain (Fig. 2a) (refer SI-2, Table 2) (where CQDP exhibited a potency of 45 nM).

2.2.2. Parasite progression assay

To accurately determine the sensitivity of the parasite blood stage against our active hybrids and to assess the time required for these molecules to act, *in vitro* drug sensitivity assays were performed by treating the parasites with **26**, **27** and **28** at 50 μM and then their progression through all stages over time (12 h, 24 h, 36 h and 48 h) were observed. It was observed that the trophozoites



Scheme 1. Library synthesis from scaffold **10**. (a) PTSA (10 mol%), PhMe, 110 °C; (b) $NaBH_4$, MeOH, 0 °C; (c) Methylchloroformate, LiHMDS, THF, −78 °C, then Nitrostyrene, LiHMDS, THF, 0 °C; (d) Pd–C (10% w/w), ammonium acetate, EtOAc; (e) Allyl bromide, LiHMDS, THF, −78 °C; (f) Hoveyda Grubbs-(I) catalyst (10 mol%); (g) Pd–C (10% w/w), ammonium acetate, EtOAc; (h) $(COCl)_2$, Et_3N, DMSO, 0 °C, then Me_2NH, $NaCNBH_4$, THF, rt; (i) Ethylchloroformate, LiHMDS, THF, −78 °C; (j) 2, 4-dinitroflurobenzene, NaH, THF, 0 °C to rt; (k) 2-nitrobenzylbromide, NaH, THF, 0 °C to rt.

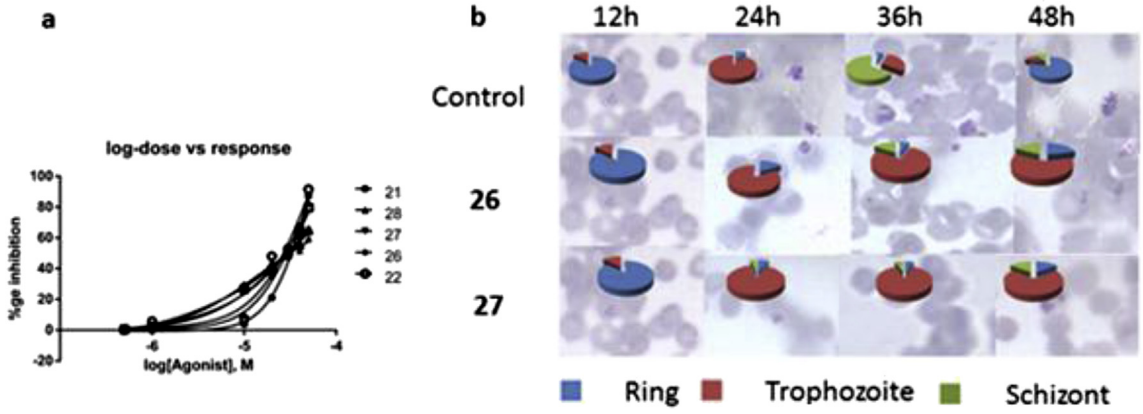


Fig. 2. (a) Log-dose *vs* Response curve of the most efficacious compounds. (b) Sensitivity of parasite against **26** and **27** in the blood stage.

were most susceptible towards all the 4 compounds belonging to 2 distinct chemotypes indicating the target's vulnerability at this stage (Fig. 2b depicts data for 2 representative molecules; also refer SI-2, Table 3).

It is noteworthy that these compounds were not cytotoxic when tested against HePG2 cells suggesting selective antiplasmodial activity (Table 5, SI).

3. Discussion

3.1. Synthetic chemistry and library design

Diversity in molecular framework is the key feature of a compound library. The DOS strategy discussed here addressed the requirement for such a library with multifarious molecular

frameworks. It used natural product scaffold as a reaction platform and systematically developed privileged scaffolds on them. In the process transformed them into more complicated structures. Readily available L-Phenyl alanine methyl ester was used as the appropriate key starting materials. The tandem PSL assembled the pyrroloisoquinoline scaffold which provided the pluripotent reaction site for aromatic nucleophilic substitution (S_NAr), Michael addition and alkylation followed by a bevy of cyclization reactions including ring closing metathesis and lactamization to afford the privileged scaffolds. The approach successfully generated hybrid molecules with higher degree of structural variety. In all, 8 hybrid systems based on a natural product template and 4 "privileged" scaffolds were prepared efficiently *via* this advanced synthetic strategy with an attractive steps/scaffold ratio of 1.39.

Unlike most small molecule library design where the starting materials with simple frameworks are transformed into more complex structures, ours by virtue of starting from natural product templates involved complexity from the initial stages. Consequently all the intermediates possessed complex framework and can be considered as an integral aspect of the library in their own right. For example to access 8 final hybrid molecules 11 complex structures were used (refer Scheme 1). In an effort to quantify the architectural complexity and diversity of the compounds key descriptors known to correlate with biological activities were analyzed.

3.2. Informatics study

Recently researchers interrogated several attributes *viz*. mol.wt., ClogP, polar surface area, rotatable bonds, aromaticity, number of chiral centers and Fsp3 of compounds synthesized by medicinal chemists till date and then compared to the commercial drugs [28–30]. They concluded that increment of aromaticity is detrimental towards compound developability, and more structural complexity of molecules (measured by Fsp3) and chiral centers correlate with the success of a compound's transition from preclinical testing to drugs [28–30]. Natural products and their analogs addressed these aspects and as a result 41% of anticancer drugs and 65% of antibacterial drugs are natural products or their derivatives [31]. Fsp3, aromaticity and number of chiral centers of the library molecules were calculated and compared to a collection of ~150,000 natural products and their derivatives from ZINC® database [32]. Analysis revealed that the average Fsp3 (Fsp3 is number of sp3 hybridized carbon divided by the total number of carbons in a molecule) count of our library (0.39) is much better than the natural product collection (0.33) (Fig. 3). Average stereocenters among the library molecules (2.24) is nearly the same as the natural products (2.5) and average aromatic carbons in our molecules are lower (9) compared to the natural product collection (15). This demonstrated that not only the complexity quotient of the library is equal to the natural products but the detrimental attribute of aromatic carbon count is also less compared to it. Furthermore the library molecules possessed orthogonal functional groups ready for forward structure activity relationship studies through chemoselective reactions (Scheme 1). The preliminary mechanistic interrogation rationalized the biological activity of the active compounds against malaria and provided opportunities for a thorough drug discovery investigation.

4. Conclusion

Herein we have described a modular DOS strategy of hybrid systems between pyrroloisoquinoline and "privileged scaffolds" in an efficient "steps/scaffold" ratio of 1.4. Among the molecules synthesized few of them showed moderate potency of 30–50 μM. Interestingly compound **20** an early intermediate exhibited similar potency as the most active final compounds. This definitely provides an opportunity for further diversification on a different direction. And could be the foundation of the next series of compounds. Even though it is unrealistic to expect highly potent molecules through unbiased campaigns like DOS, yet the molecules with low micromolar potency (as in the library above) would act as suitable hit compounds which could be developed into potent leads.

5. Experimental

5.1. General experiments

Air and moisture-sensitive reactions were carried out in oven-dried glassware that was sealed with rubber septa under positive pressure of dry argon. Sensitive liquids and solutions were transferred by syringe. Reactions were stirred with Teflon-coated magnetic stirrer bars. Elevated temperatures were maintained by using Thermostat-controlled silicon oil baths. Organic solutions were concentrated on a rotary evaporator with a desktop vacuum pump. THF, Et_2O, dioxane, benzene, and toluene were distilled from sodium and benzophenone prior to use. CH_2Cl_2 was distilled from calcium hydride prior to use. Compound **9** was synthesized from literature protocol [33]. Analytical TLC was performed on 0.25 mm silica-gel G plates with a 254 nm fluorescent indicator. The TLC plates were visualized by using UV light and treated with a phosphomolybdic acid stain, followed by gentle heating. Purification of the products was performed by flash chromatography on silicagel and the purified compounds showed a single spot by analytical TLC. The diastereomeric ratio and the regioisomeric ratio were determined by ^1H NMR spectroscopy of the crude reaction mixtures. Data for ^1H NMR spectra are reported as follows: chemical shift (ppm, referenced to TMS; s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, ddd = doublet of doublet of doublets, m = multiplet), coupling constant (Hz), and integration. Data for ^{13}C NMR spectra are reported in terms of chemical shift (ppm) relative to residual solvent peaks ($CDCl_3$:77.0 ppm).

***P. falciparum* parasite and human erythrocytes.** *P. falciparum* clones used in this study was 3D7 [34–36]. Parasites were cultured were cultured in O^+ human erythrocytes, with RPMI 1640 (Invitrogen, USA) supplemented with 24 mM sodium bicarbonate (Sigma, USA), 0.1 mM hypoxanthine (Invitrogen, USA), 25 mg mL^{-1} gentamicin (Invitrogen,USA) and 0.5% AlbuMax I (Invitrogen, USA), according to methods described earlier [27]. Parasite culture was maintained in mixed gas environment (5% O_2, 5% CO_2 and 90% N_2). Parasites were synchronized by sorbitol treatment at ring stage.

5.2. Growth inhibition assay

Growth inhibition assays were done by flowcytometer with slight modification as described previously [37]. Briefly, the parasites were first synchronized by the purification of schizont-stage parasites on a Percoll gradient, followed by 2–3 rounds of treatment of the ring-stage parasites with sorbitol. Schizont-stage parasites at an initial parasitemia level of 0.3% at 2% hematocrit were incubated with compounds for one cycle of parasite growth (40 h post invasion). The parasite-infected erythrocytes were stained with ethidium bromide dye and measured by a fluorescence activated cell sorter (FACS)-based assay, as described previously (Singh et al., 2010). The whole sample was collected and washed twice with PBS and subjected to staining with ethidium bromide (10 μM) for 15 min at room temperature in dark. The cells were washed with PBS, and analyzed by flow cytometry on FACSCalibur (Becton

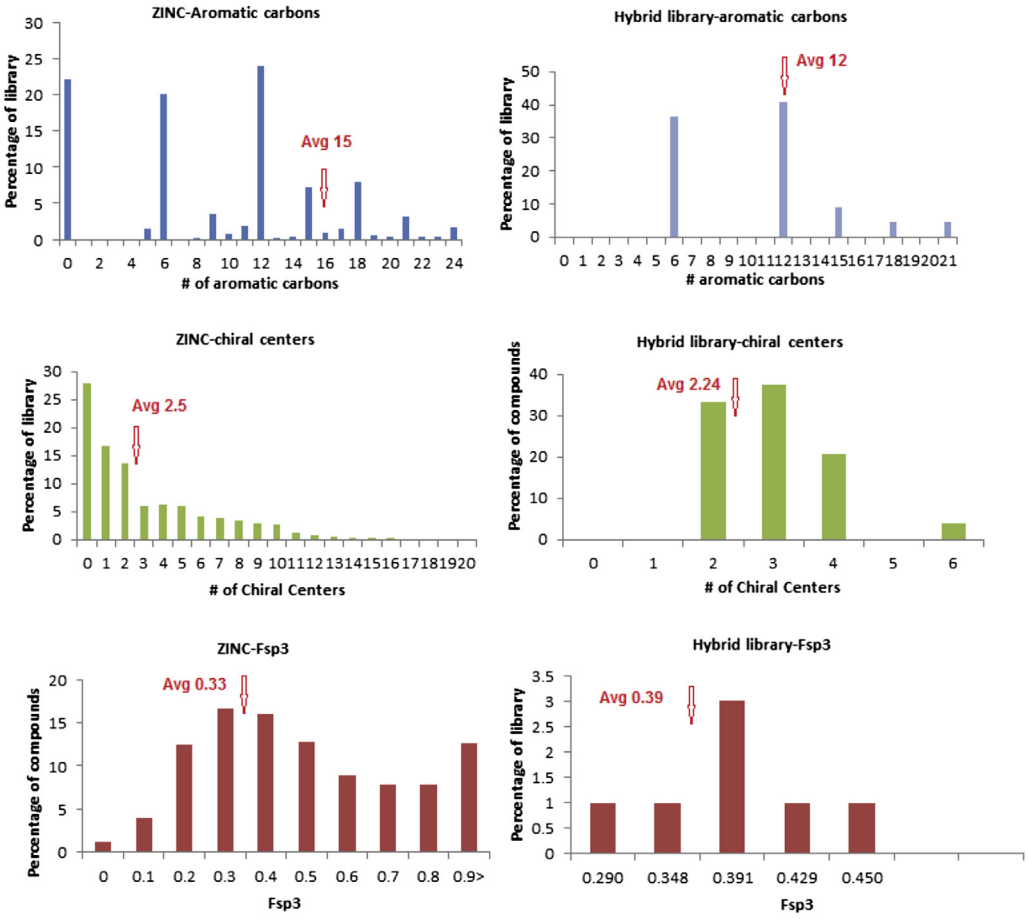


Fig. 3. Comparison of compounds created through the platform technology with natural product collection.

Dickinson) using CellQuest software. Fluorescence signal (FL-2) was detected with the 590 nm band pass filter using an excitation laser of 488 nm collecting 100,000 cells per sample. Following acquisition, data was analyzed for % parasitemia of each sample by determining the proportion of FL-2-positive cells using Cell Quest. Growth inhibition (% Inhibition) was calculated with respect untreated control infected erythrocytes (iRBCs) to the drug treated iRBCs at different concentration by applying the formula:

$$\%\ \text{Inhibition} = [1 - \text{parasitemia test (treated iRBCs)}/\text{parasitemiacontrol (untreated iRBCs)}*100]$$

Three independent experiments were done in duplicates.

5.3. Statistical analysis

The data for the IC_{50} value and % growth inhibition activity of compounds are expressed as the mean ± standard deviation (SD) of three independent experiments done in duplicates.

Half maximal inhibitory concentrations for each compound were calculated by using Graph-Pad Prism software.

To see the effect of drugs on the growth of parasite we monitored the progression at different stages (Rings, Trophozoites, Schizonts) of the parasite in erythrocytic cycle. Breifly, ring-stage parasite culture was diluted to 1% parasitemia and 2% hematocrit in a complete RPMI medium and seeded in triplicate in 96 well plate and further incubated at 37 °C for 12, 24 and 48 h erythrocytic cycle to monitor progression at each stage in presence of compounds at 50 μM concentration.

Morphological analysis was done by visualizing the parasitized RBCs under the microscope (NIKON Eclipse Ti, 100x) and counting (~2000 cells/Giemsa-stained slides in duplicates) revealed healthy rings 12 h post-invasion (h.p.i.) and trophozoites 24 h.p.i., either in presence and untreated as control.

5.4. Synthesis

5.4.1. Synthesis of ***10***

Compound **9** (1 g, 5.71 mmol) were dissolved in 30 mL of THF and was cooled to 0 °C. $NaBH_4$ (216 mg, 5.71 mmol) was then added to it in portions. The reaction mixture was slowly warmed to rt. After 12 h LCMS indicated the formation of **10** and completion of the reaction was confirmed by TLC. The reaction mixture was cooled to room temperature and was evaporated in a rotavapor. The crude was taken up in ethyl acetate, washed with water followed by

brine solution, dried over anhydrous Na_2SO_4, concentrated under reduced pressure and was purified by column chromatography using silicagel (50–100% EtOAc in Hexane) to afford **10** (644 mg, 48%) as off white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.09–7.26 (m, 4H), 4.89–4.85 (t, J = 14.8 Hz, 1H), 4.05–3.99 (m, 1H), 3.7–3.6(bs, 1H), 3.14–3.08 (m, 1H), 2.74–2.69 (m, 2H), 2.47–2.38 (m, 2H), 2.18–2.15 (m, 1H), 1.63 (s, 3H); **^{13}C NMR** (300 MHz, DMSO-d_6) δ172.90, 143.26, 132.65, 128.97, 126.77, 123.26, 62.33, 59.71, 51.32, 38.67, 33.88, 30.30, 28.84, 27.39 ppm; **LCMS** $[M+H]^+$: calcd. 232.12, found 232.05.

*5.4.2. Synthesis of **19***

To a stirred solution of **10** (0.3 g, 1.3 mmol) in DCM (25 mL) was added Dimethoxyperiodinane (1.1 g, 2.6 mmol) in portions for 5 min and stirred for 15 h at ambient temperature. After confirming the completion of the reaction by TLC, the reaction mixture was quenched with cold water, separated the organic layer, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford the crude **18**, it was taken to the next step as such (**LCMS** $[M+H]^+$: calcd. 230.11, found 230.10).

To a stirred solution of **18**, N, N-Dimethylamine hydrochloride (0.127 g, 1.56 mmol) and Triethylamine (0.435 mL, 3.1 mol) in DCM (5 mL) was added sodium cyanoborohydride (0.26 g, 0.0042 mol) in portion for 5 min and stirring was continued at ambient temperature for 15 h. After confirming the completion of the reaction by TLC, the reaction mixture was quenched with cold water, separated the organic layer, dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford the crude. The crude product thus obtained was purified by column chromatography using silicagel (Hexane/EtOAc, 1:1) to afford (0.18 g, 84%Yield) as pale yellow liquid. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.35–7.0 (m, 4H), 4.5–4.0 (m, 1H), 3.0–2.9 (m, 1H), 2.8–2.7 (m, 1H), 2.7–2.5 (m, 2H), 2.5–1.8 (m, 10H), 1.60 (s, 3H); **^{13}C NMR** (400 MHz, DMSO-d_6) δ172.69, 141.87, 130.78, 129.21, 126.89, 124.64, 61.55, 60.29, 51.32, 38.87, 33.88, 30.92, 28.64, ppm; **LCMS** $[M+H]^+$: calcd 259.17, found 259.21.

*5.4.3. Synthesis of **20***

To a solution of compound **19** (2 g, 0.00864 mol) in THF (80 mL) was added LiHMDS in THF (20% w/v in THF, 18 mL 3.6 g, 0.0216 mol) at −78 °C. The mixture was stirred at −78 °C for 1 h. Ethylchloromate (2.06 g, 1.82 mL, 0.019 mol) diluted with THF (5 mL) was added dropwise to the above mixture at −78 °C under a N_2 atmosphere. The mixture was stirred at −78 °C for 0.5 h under N_2 atmosphere. Completion of the reaction was confirmed by TLC. The mixture was quenched with saturated aq.NH_4Cl solution, diluted with EtOAc, washed the organic layer with water followed by brine solution. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford crude product. The crude residue thus obtained was purified by column chromatography on silicagel (n-hexane/EtOAc, 80:20) to afford (1.55 g, 61% yield) mixture of diastereomers as off white solid. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.24–7.13 (m, 4H), 4.7–4.55 (m, 1H), 4.3–4.15 (m, 2H), 3.85–3.7 (m, 1H), 3.2–3.1 (m, 1H), 2.85–2.75 (m, 1H), 2.6–2.5 (m, 3H), 2.35–2.24 (m, 1H), 2.25 (s, 6H), 1.7–1.6 (m, 3H), 1.35–1.2 (m, 3H); **LCMS** $[M+H]^+$: calcd. 331.19, found 331.28.

*5.4.4. Synthesis of **21** and **22***

A solution of compound **20** (0.5 g, 0.0015 mol) in THF (5 mL) was added to a suspension of NaH (60% suspension, 0.086 g, 0.0021 mol) at 0 °C under a N_2 atmosphere. The mixture was stirred at 0 °C for 1.5 h. 2,4-dinitro-1-fluoro benzene (0.42 g, 0.0022 mol) dissolved in THF(3 mL) was added dropwise at 0 °C under a N_2 atmosphere and continued stirring for 0.5 h at the same temperature. Completion of the reaction was confirmed by TLC. The reaction mixture was quenched with saturated aq.NH_4Cl solution at 0 °C. The reaction mixture was diluted with EtOAc, washed the organic layer with water followed by brine solution. The organic layer separated was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude residue. The resulting residue was purified by column chromatography on silicagel (EtOAc in Hexane, 80–100% gradient elution) to afford (0.608 g, 81% combined yield, d.r: 70:30) diastereomers **21** and **22** as off white solid. **^{1}H NMR** (**21**, 400 MHz, $CDCl_3$) δ 9.04–9.03 (d, J = 2.4 Hz, 1H), 8.49–8.11 (m, 1H), 8.13–8.11 (d, J = 8 Hz, 1H), 7.35–7.1 (m, 4H), 4.95–4.8 (m, 1H), 4.2–4.1 (m, 2H), 3.8–3.7 (m, 1H), 3.3–3.2 (m, 1H), 2.8–2.6 (m, 3H), 2.4 (s, 6H), 2.3–2.2 (m, 1H), 1.35 (s, 3H), 1.12–1.08 (t, J = 14 Hz, 3H); **^{13}C NMR** (300 MHz, DMSO-d_6) δ 181.70, 164.56, 164.44, 162.07, 161.95, 149.42, 142.60, 142.52, 142.44, 140.23, 133.17, 128.82, 128.72, 127.41, 125.31, 125.27, 125.14, 124.07, 120.83, 110.96, 110.72, 110.19, 104.15, 103.90, 103.65, 97.26, 72.08, 70.15, 62.86, 58.49, 42.30, 29.66, 22.98 ppm; **LCMS** $[M+H]^+$: calcd. 497.19, found 497.31.

^{1}H NMR (**22**, 400 MHz, $CDCl_3$) δ 9.04–9.03 (d, J = 2.4 Hz, 1H), 8.8 (s, 1H), 8.2–8.1 (m, 1H), 8.0–7.9 (m, 1H), 7.3–6.9 (m, 4H), 4.6–4.4 (m, 1H), 4.2–4.0 (m, 2H), 3.6–3.4 (m, 1H), 3.3–3.2 (m, 1H), 3.0–2.8 (m, 2H), 2.7–2.4 (m, 2H), 2.4(bs, 6H), 1.7 (s, 3H), 1.2–1.0 (m, 3H); **^{13}C NMR** (300 MHz, DMSO-d_6) δ 177.24, 164.26, 164.14, 161.78, 150.89, 142.71, 140.21, 129.84, 129.18, 128.90, 128.43, 127.15, 125.19, 124.95, 123.92, 120.21, 111.19, 111.07, 110.99, 109.88, 104.11, 103.87, 103.62, 96.49, 74.74, 71.96, 63.26, 53.12, 46.84, 29.67, 24.07 ppm; **LCMS** $[M+H]^+$: calcd. 497.19, found 497.25.

*5.4.5. Synthesis of **25***

To solution of **21** (0.065 g, 0.00013 mol) in EtOAc (10 mL) added Pd/C (50% moist, 0.015 g) and stirred under H_2 atmosphere for 12 h. Reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered over celite, washed the celite with EtOAc (2 × 10 mL). The filtrate collected was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude. The crude product thus obtained was column chromatography on silicagel (MeOH in EtOAc, 5–15% gradient elution) to afford **25** (0.048 g, 95% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.4–7.1 (m, 4H), 6.8–6.9 (m, 1H), 6.3 (s, 1H), 6.1–6.0 (m, 1H), 5.4(bs, 1H), 5.1 (s, 2H), 4.4–4.2 (m, 1H), 3.2–3.0 (m, 2H), 3.0–2.7 (m, 2H), 2.6–2.3 (m, 2H), 2.2 (s, 6H), 1.60 (s, 3H); **^{13}C NMR** (300 MHz, DMSO-d_6) δ170.51, 169.79, 149.37, 143.60, 131.31, 128.83, 126.76, 124.43, 122.39, 105.82, 94.85, 62.30, 59.04, 55.73, 45.96, 45.48, 44.98, 32.52, 30.99 ppm; **LCMS** $[M+H]^+$: calcd. 391.20, found 391.27.

*5.4.6. Synthesis of **26***

To solution of **22** (0.065 g, 0.0001 mol) in EtOAc (10 mL) added Pd/C (50% moist, 0.015 g) and stirred under H_2 atmosphere for 12 h. Reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered over celite, washed the celite with EtOAc (2 × 10 mL). The filtrate collected was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude. The crude product thus obtained was column chromatography on silicagel (MeOH in EtOAc, 5–15% gradient elution) to afford **26** (0.048 g, 95% yield). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.4–7.2 (m, 4H), 6.19 (s, 1H), 6.0–5.8 (m, 2H), 5.43 (s, 1H), 5.0 (s, 1H), 4.35–4.2 (m, 1H), 3.2–3.1 (s, 2H), 2.95–2.85 (m, 1H), 2.7–2.6 (m, 1H), 2.5–2.4 (m, 3H), 2.2 (s, 6H), 1.8 (s, 3H); **LCMS** $[M+H]^+$: calcd. 391.20, found 391.29.

*5.4.7. Synthesis of **27** and **28***

A solution of compound **20** (0.8 g, 1.77 mmol) in THF (15 mL) was added to a suspension of NaH (60% suspension, 0.086 g, 0.0021 mol) at 0 °C under a N_2 atmosphere. The mixture was stirred at 0 °C for 1.5 h. *o*-nitrobenzylbromide (0.42 g, 2.2 mmol) dissolved

in THF (5 mL) was added dropwise at 0 °C under a N_2 atmosphere and continued stirring for 0.5 h at the same temperature. Completion of the reaction was confirmed by TLC. The reaction mixture was quenched with saturated aq.NH_4Cl solution at 0 °C. The reaction mixture was diluted with EtOAc, washed the organic layer with water followed by brine solution. The organic layer separated was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude residue. It was dissolved in EtOAc (10 mL) added Pd/C (50% moist, 0.2 g) and stirred under H_2 atmosphere for 8 h. Reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered over celite, washed the celite with EtOAc (2 × 25 mL). The filtrate collected was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude. The crude product thus obtained was purified by Preparative HPLC to afford (0.7 g, 89% combined yield) **27** and **28** as off white solid. **^{1}H NMR** (**27**, 400 MHz, $CDCl_3$) δ 8.4 (bs, 1H), 7.3–6.9 (m, 8H), 4.8–4.7 (m, 1H), 3.6–3.4 (m, 1H), 3.2–3.1 (m, 1H), 3.0–2.9 (m, 1H), 2.9–2.8 (m, 1H), 2.8–2.7 (m, 1H), 2.7–2.4 (m, 3H), 2.6 (s, 6H), 1.3 (s, 3H); **^{13}C NMR** (300 MHz, $CDCl_3$) δ171.04, 143.17, 136.35, 131.66, 129.31, 128.43, 127.76, 127.14, 126.80, 123.75, 123.40, 121.99, 115.04, 63.17, 59.94, 51.06, 46.50, 45.93, 45.72, 36.45, 31.40, 30.56 ppm; **LCMS** $[M+H]^+$: calcd. 390.21, found 390.22. **^{1}H NMR** (**28**, 400 MHz, $CDCl_3$) δ 8.1 (s, 1H), 7.3–6.9 (m, 7H), 6.7 (s, 1H), 4.6(bs, 1H), 3.7–3.5 (m, 1H), 3.3–3.3 (m, 2H), 3.0–2.9 (m, 2H), 2.7–2.5 (m, 1H), 2.4–2.1 (m, 7H), 2.0–1.9(bs, 1H), 1.3 (s, 3H); **^{13}C NMR** (300 MHz, $CDCl_3$) δ171.58, 170.20, 142.18, 136.58, 131.32, 129.40, 128.30, 127.87, 126.99, 126.79, 124.09, 123.23, 121.42, 114.91, 62.70, 59.52, 51.69, 46.29, 45.81, 44.39, 36.38, 33.07, 31.4 ppm; **LCMS** $[M+H]^+$: calcd. 390.21, found 390.23.

5.4.8. Synthesis of *11/13*

Step1

To a solution of compound **10** (200 mg, 0.86 mmol) in THF (20 mL) was added LiHMDS (20% solution in THF (1.8 mL, 2.15 mol) at −78 °C. The mixture was stirred at −78 °C for 1 h. Methylchloromate (100 mg, 0.86 mol) diluted with THF (3 mL) was added dropwise to the above mixture at −78 °C under N_2 atmosphere. The mixture was stirred at −78 °C for 0.5 h under N_2 atmosphere. Completion of the reaction was confirmed by TLC. The mixture was quenched with saturated aq.NH_4Cl solution, diluted with EtOAc, washed the organic layer with water followed by brine solution. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford crude product.

Step2

A solution of crude product from above was added to a suspension of NaH (60% suspension(34 mg, 0.89 mmol) at 0 °C under N_2 atmosphere. The mixture was stirred at 0 °C for 1.5 h. Nitrostyrene (110 mg, 0.86 mmol) dissolved in THF (2 mL) was added drop-wise at 0 °C under N_2 atmosphere and continued stirring for 0.5 h at the same temperature. Completion of the reaction was confirmed by TLC. The reaction mixture was cooled to 0 °C and then quenched with saturated aq.NH_4Cl solution. The reaction mixture was diluted with EtOAc, washed the organic layer with water followed by brine solution. The organic layer separated was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude residue. The resulting residue was purified by column chromatography on silicagel (EtOAc in n-Hexane, 2:8 as eluent) to afford **11** and **13** (70 mg, 22% yield) as off white solid. **^{1}H NMR**, 400 MHz, DMSO-d_6) δ 7.35–7.14 (m, 10H), 5.4–5.0 (m, 1H), 4.75–4.6 (m, 1H), 4.3–4.1 (m, 2H), 4.1–3.95 (m, 2H), 3.85–3.65 (m, 1H), 2.9–2.8 (m, 2H), 2.7–2.6 (m, 1H), 2.45–2.3 (m, 2H), 1.3 (s, 3H), 1.12–1.08 (t, J = 16 Hz, 3H); **^{13}C NMR** (100 MHz, CDCl3) δ 167.43, 160.08, 136.66, 133.46, 130.39, 129.65, 128.58, 127.89, 126.93, 119.55, 113.79, 90.89, 89.42, 58.70, 55.22, 46.76, 29.70, 28.64; **LCMS** $[M+H]^+$: calcd. 453.19, found 453.21.

5.4.9. Synthesis of *14* and *15*

Step A

To solution of **11**/**13** (0.4 g, 0.8 mmol) in EtOAc (20 mL) was added Pd/C (50% moist, 0.28 g) followed by ammonium formate (0.83 g, 0.0131 mol) and stirring was continued at ambient temperature 15 h. Reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered over celite, washed the celite with EtOAc (2 × 20 mL). The filtrate collected was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford (0.4 g) the crude which was purified by preparative HPLC to afford (0.12 g of **14** and 0.11 g of **15** as solid compounds.

Compound 14: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.2–6.9 (m, 6H), 6.9–6.8 (m, 2H), 6.8–6.7 (m, 1H), 5.0–4.9 (m, 1H), 4.3–4.1 (m, 1H), 4.1–4.0 (m, 1H), 4.0–3.9 (m, 1H), 3.8–3.7 (m, 1H), 3.7–3.6 (m, 1H), 3.5–3.4 (m, 1H), 3.4–3.2 (m, 1H), 2.7–2.65 (m, 1H), 2.56–2.52 (d, J = 16 Hz, 1H), 1.97–1.94 (d, J = 12 Hz, 1H), 1.73 (s, 3H); **^{13}C NMR** (100 MHz, CDCl3) δ174.99, 174.26, 171.582, 144.09, 143.27, 141.13, 138.00, 131.82, 131.68, 129.49, 128.88, 128.55, 128.43, 128.33, 127.85, 127.36, 126.75, 126.62, 123.72, 123.55, 62.97, 61.72, 59.32, 59.17, 59.09, 58.41, 52.19, 50.25, 49.64, 48.40, 46.31, 41.67, 40.34, 40.05, 39.78, 39.50, 39.23, 38.94, 38.66, 38.45, 30.87, 30.57, 29.27, 28.90 ppm; **LCMS** $[M+H]^+$: calcd 377.17, found 377.25.

Compound 15: **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.5–7.30 (m, 3H), 7.3–7.00 (m, 5H), 6.10–6.00 (m, 1H), 4.4–4.2 (m, 2H), 4.20–4.15 (m, 1H), 4.0–3.68 (m, 3H), 3.66–3.50 (m, 1H), 3.15–3.00 (dd, 1H), 3.0–2.95 (d, J = 16 Hz, 1H), 2.80–2.70 (m, 1H), 2.10–2.00 (d, J = 16 Hz, 1H), 1.30 (s, 3H); **^{13}C NMR** (100 MHz, CDCl3) δ175.12, 171.99, 143.47, 138.41, 132.37, 128.88, 128.48, 128.35, 127.64, 127.13, 126.62, 126.48, 123.60, 61.28, 59.89, 58.25, 52.56, 47.74, 30.57, 29.68 ppm; **LCMS** $[M+H]^+$: calcd 377.17, found 377.36.

5.4.10. Synthesis of *12*

To a solution of compound **10** (150 mg, 0.65 mmol) in THF (10 mL) was added LiHMDS 1 M solution in THF (1.4 mL, 1.44 mmol) at −78 °C. The mixture was stirred at −78 °C for 1 h. Allyl bromide (1.2 mL, 1.44 mmol) diluted with THF (2 mL) was added dropwise to the above mixture at −78 °C under N_2 atmosphere and stirring was continued at the same temperature for 0.5 h. Completion of the reaction was confirmed by TLC. The mixture was quenched with saturated aq.NH_4Cl solution, diluted with EtOAc, washed the organic layer with water followed by brine solution. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford **12** as a crude liquid which was taken to the next step as such (**LCMS** $[M+H]^+$: calcd 312.18, found 312.20).

Compound **12** was dissolved in (10 mL) of dry DCM and the solution was degassed with argon. To this was added a DCM solution of Grubb's catalyst (1st generation, 0.2 g, 0.24 mmol). The reaction mixture was refluxed for 6 h. The reaction mixture was evaporated and purified by column chromatography using silicagel (50% EtOAc in Hexane as eluent) to afford **16** (24 mg, 37% Yield) as off white solid. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.25–7.17 (m, 4H), 5.61 (s, 2H), 3.86–3.76 (m, 2H), 3.31–3.23 (m, 1H), 3.06–2.93 (m, 3H), 2.75–2.56 (m, 4H), 2.25–2.21 (d, J = 12 Hz, 1H), 2.05–1.99 (d, J = 18 Hz, 1H), 1.51 (s, 3H); **^{13}C NMR** (400 MHz, DMSO-d_6) δ178.27, 143.40, 132.67, 129.10, 128.62, 128.10, 126.62, 126.59, 124.03, 60.74, 60.23, 52.05, 50.16, 48.87, 45.65, 45.27, 29.80, 29.59 ppm; **LCMS** $[M+H]^+$: calcd. 284.15, found 284.16.

*5.4.11. Synthesis of **17***

To solution of **16** (0.15 g, 5.3 mmol) in EtOAc (10 mL) added Pd/C (50% moist, 15 mg) followed by ammonium formate (67 mg, 1.06 mmol) and stirred under H_2 atmosphere for 15 h. Reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered over celite and washed the celite layer with plenty of EtOAc. The filtrate collected was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the crude. The crude product was purified by column chromatography using silicagel (EtOAc in Hexane, 50–70% as eluent) to afford **17** (90 mg) as pale pink solid. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.23–7.16 (m, 4H), 4.93–4.9 (t, J = 12 Hz, 1H), 3.3–3.28 (m, 1H), 2.99–2.94 (m, 2H), 2.44–2.41 (d, J = 12.8 Hz, 1H), 2.16–2.13 (t, J = 12.4 Hz, 1H), 1.95–1.8 (m, 1H), 1.8–1.6 (m, 4H), 1.6–1.5 (m, 4H), 1.3–1.2 (m, 1H); **^{13}C NMR** (400 MHz, DMSO-d_6) δ169.85, 168.2, 141.37, 130.51, 129.21, 126.85, 126.81, 124.66, 61.28, 60.71, 58.94, 47.81, 45.31, 44.66, 30.84, 28.87, 13.99 ppm; **LCMS** $[M+H]^+$: calcd 286.17, found 286.18.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.03.023.

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