Synthesis of Combinatorial Libraries Based On Terpenoid Scaffolds

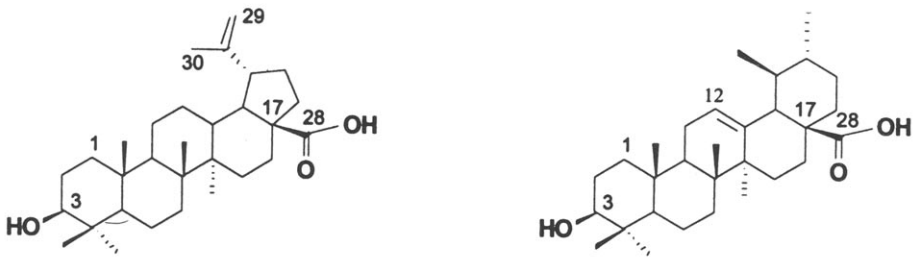
A. Pathak, S.K. Singh, M.A. Farooq Biabani, D.K. Kulshreshtha, S.K. Puri[#], S. Srivastava and B. Kundu*

Medicinal Chemistry Division, [#]Parasitology Division, Central Drug Research Institute, Lucknow 226 001, India

Abstract: Triterpenoid-based scaffolds betulinic acid (**1a**) and ursolic acid (**1b**), have been used for the generation of combinatorial libraries in parallel format using solid phase organic synthesis method. These templates have the potential for the synthesis and amplification of triterpenoid-based compounds with one and two-point diversity. This has been demonstrated by the synthesis of two small libraries comprising 18 derivatives each of betulinic acid and ursolic acid with structural diversity at C-3 and C-28 positions. The primary screening of antimalarial activity of these libraries against *P. falciparum in vitro* led to the identification of four compounds with 5 fold increase in the activity compared to betulinic and ursolic acids.

INTRODUCTION

Solid Phase Organic Synthesis (SPOS) and synthesis of combinatorial libraries have been used predominantly for the preparation of libraries of peptides, heterocyclic compounds, sugars and peptide nucleic acids etc. [1]. Recently however several organic chemistry laboratories have initiated work on anti-HIV activities. Some of the most prominent lead compounds identified are: an anti-inflammatory agent pyracrenic acid [3β-(3,4-dihydroxycinnamoyl) oxylup-20-(29)-en-28-oic]acid [5], RPR 103611, which blocks HIV infection with an IC_{50} of about 10nM [6] and betulinic acid as an anti-malarial agent [7]. From the structure activity relationship studies carried out on a series of ω-alkanoic acid



Betulinic acid (1a) **Ursolic acid (1b)**

Fig. (1). Representative examples of lupane and ursane class of triterpenoids.

methods for the synthesis of all kinds of complex natural product [2] and natural product like structures using combinatorial methods [3]. This is in view of the fact that even today natural products remain the most prolific source of lead compounds and novel scaffolds [4]. Among natural products, the terpenoid class of compounds represents a unique and most important class of bioactive compounds. The tropical plants often contain significant quantities of wide array of structurally different triterpenoids in their tissue. Among these lupane and ursane type of compounds such as betulinic acid (**1a**) and ursolic acid (**1b**) and their derivatives have been focus of numerous biological investigations such as anti-inflammatory, anti-malarial, and [8] and ω-undecanoic amide [9] derivatives of betulinic acid, it appears that derivatization of C-3 hydroxyl and C-28 carboxyl can be carried out without loss in the biological activities. Thus, these triterpenoid scaffolds provides an interesting pharmacophore for synthesizing libraries with the view to get more potent antiinfectious agents.

In continuation of our interest in the design and synthesis of combinatorial libraries based on small organic molecules and peptide libraries [10], we have now targeted the synthesis of triterpenoid based libraries. The automated parallel synthesis of the two novel series: betulinic and ursolic acid derivatives have been carried out using solid phase methodology. These scaffolds (Fig. **1**) have the potential for the synthesis and amplification of triterpenoid-based derivatives with two-point diversity and set the stage for further advances in the field of synthesis of combinatorial libraries which can then be screened to provide lead structure. In the present paper antimalarial activity against *P. falciparum* by novel betulinic and ursolic acid derivatives are reported.

*Address correspondence to this author at the Medicinal Chemistry Division, Central Drug Research Institute, Lucknow 226 001, India; Tel: 0091-522-212411-18, Ext: 4383; Fax: 0091-522-223045; E-mail: Bijoy_Kundu@yahoo.com

CDRI communication No. **6139**

1386-2073/02 $35.00+.00

EXPERIMENTAL SECTION

2-chlorotrityl chloride resin (loading capacity 1.3 mmol/gram, Sieber amide resin (loading capacity 0.60 mmol/gram) and Rink amide resin (loading capacity 0.63 mmol/gram) were purchased from Advanced ChemTech (Louisville, KY). Fmoc and amino acids were purchased from Novabiochem. All other reagents and solvents were of standard quality and used without further purification. The following abbreviations were used: DCM = dichloromethane, DMF = dimethylformamide, DMSO = dimethylsulphoxide, PDC = pyridenium dichromate, TFA = trifluoroacetic acid, $NH_2OH.HCl$ = hydroxylamine hydrochloride DMAP = dimethylaminopyridine, DIPEA = diisopropylethylamine, DIC = diisopropylcarbodiimide, HOBt = 1-hydroxybenzotriazole, Fmoc = 9-fluorenylmethoxycarbonyl, TBTu = 2-(1H-benzitriazol-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate. NMR spectra were recorded on a Bruker 220 and 300 MHz instrument and MS (FAB) data were generated on JEOL (JAPAN) / SX-102, DA-6000. Analytical HPLC was carried out using a Shimadzu LC10AS using a reverse phase column (4.6 × 250 mm) with acetonitrile-water as mobile phase. FT-IR sample prepared on KBr disk spectrum was recorded on a Perkin-Elmer 16 PC Fourier transform infrared spectrometer. The libraries were synthesized in parallel format using an Advanced Chemtech multiple organic synthesizers 496 Ω.

LINKING STRATEGY

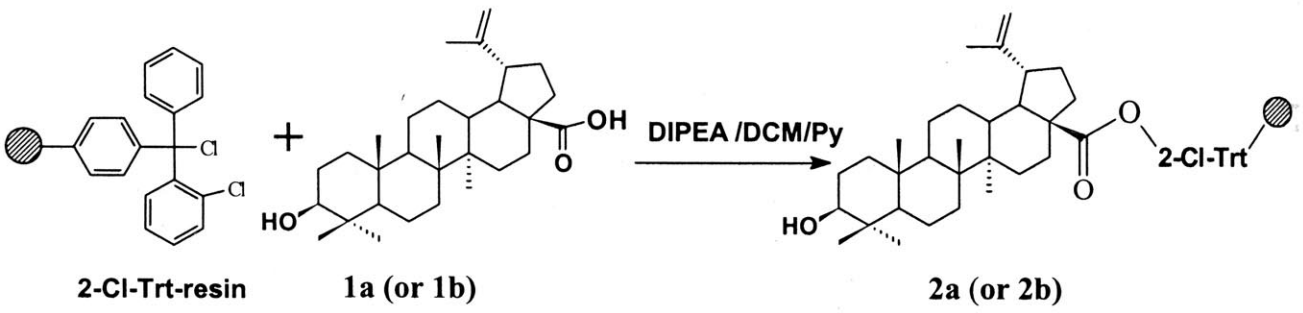
Initially an attempt was made to directly attach betulinic and ursolic acids to the resins via ester and amide bond formation at C-28 position (Scheme 1), as it will also serve as a means for providing protection to the carboxyl functionality. Our objective was to develop a linking strategy in a manner so as to get polymer linked triterpenoid based scaffolds which can be readily cleaved under mild acidic conditions. The triterpenoid scaffolds used in the present study were found to be highly unstable under harsh acidic conditions involving 90% trifluoroacetic acid. We therefore investigated loading of triterpenoids using several commercially available resins such as 2-chlorotrityl chloride, Sieber amide, and Rink amide. These resins are well known for their super sensitivity to acids thus facilitating cleavage of compounds under mild conditions. Among the resins mentioned above, 2-chlorotrityl chloride resin and Sieber amide resin yielded desired products in high purity though coupling yields were found to be less than 80%. On the contrary with Rink amide resin, though coupling went to completion, cleavage with 10-20% TFA-DCM resulted in compounds with undesired byproducts.

2-chlorotrityl chloride and Sieber amide resins were therefore selected for our further studies. Next, an attempt was made to improve the yield of loading of terpenoids by introducing amino acids as a spacer between triterpenoids and the resin (Scheme 2). The use of resin bound amino acids is also desirable as it would allow insertion of structurally diverse amino acids at C-28 carboxyl position as a means of diversity. Initially we proceeded with the loading of betulinic and ursolic acids on to the Sieber amide resin pre-derivatised with amino acids. Evidence that the loading step went to completion included a negative Kaiser test and the recovery of products in high purities after cleavage from the resin. The cleaved products were also characterized using ^{1}H NMR, FAB-MS and FT-IR. Similar results were obtained when 2-chlorotrityl chloride resin was used as polymeric support except that the compounds were obtained as acids instead of amides.

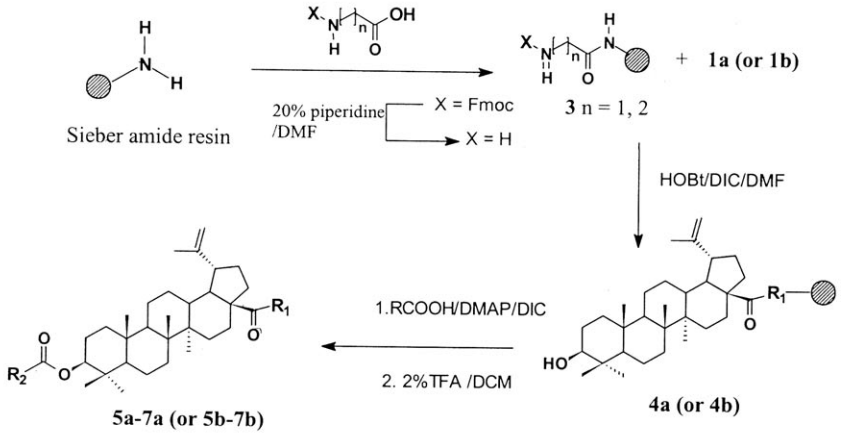
APPROACHES TO INCORPORATE DIVERSITY INTO TRITERPENOID SCAFFOLDS AT POSITION C-3 AND C-28 POSITIONS

A series of betulinic acid and ursolic acid derivatives were obtained by introducing diversity at the C-3 and C-28 positions. Initially diversity was introduced at C-28 position by loading two amino acids: Fmoc-glycine and Fmoc-β-alanine on Sieber amide resin. The pre-derivatized amino acid resins were then coupled with triterpenoid scaffolds **1a** and**1b** via C-28 position to provide immobilized scaffolds **4a** and **4b**. This was followed by introduction of diversity at C-3 position by treating immobilized triterpenoids scaffolds with a variety of acids such as: aliphatic, aromatic and amino acids (Fig. **2**). The products obtained after cleavage from the resin were characterized using HPLC, ^{1}H NMR and FAB-MS, FT-IR. The purities of these compounds ranged from 60-90% based on analytical HPLC. Interestingly treatment of intermediates **2a** and **2b** with a variety of acids such as: aliphatic, aromatic and amino acids also resulted in derivatives with one point diversity at the C-3 position of betulinic and ursolic acids (data not shown).

In yet another series of diversity, immobilized triterpenoid scaffolds **4a** and **4b** were subjected to oxidation to provide 3-oxo triterpenoid scaffolds **8a** and **8b**. These



Scheme 1. Triterpenoid coupling with 2-chlorotrityl chloride resin.

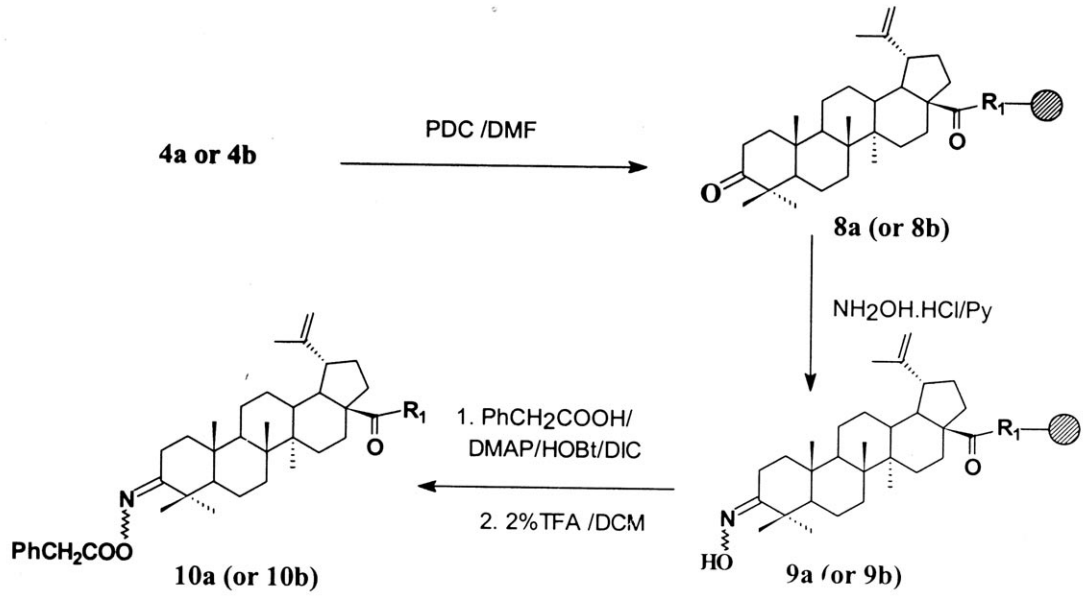


Scheme 2. General scheme for the incorporation of diversity at C-3 and C-28.

keto scaffolds upon treatment with hydroxylamine furnished 3-oxime triterpenoid scaffolds **9a** and **9b**. The oxime scaffolds were further subjected to esterification using phenylacetic acid to furnish oxime ester **10a** and **10b** (Scheme 3). The products obtained after cleavage from the resin were characterized using HPLC, ^{1}H NMR and FAB-MS. The purities of these compounds were in the range of 60-80% based on analytical HPLC.

Table 1. Representative Examples of R_1 and R_2 Diversity on Betulinic and Ursolic Acid

	R_1	R_2	MS-FAB(M+1)	yield (%)
Betulinic acid				
5a	-OH	$C_6H_5CH_2$-	577	65
6a	-$NHCH_2CONH_2$	3-C_5H_4N-	618	91
7a	-$NHCH_2CH_2CONH_2$	$C_6H_5CH_2$-	645	86
Ursolic acid				
5b	-OH	$C_6H_5CH_2$-	439 [MH-$C_6H_5CH_2CO_2H$]$^+$	68
6b	-$NHCH_2CONH_2$	$C_6H_5CH_2CH(NH_2)$-	660	78
7b	-$NHCH_2CH_2CONH_2$	3,5-$(CH_3O)_2C_6H_3CH_2$-	705	80



Scheme 3. Preparation of solid-phase oxime esters.

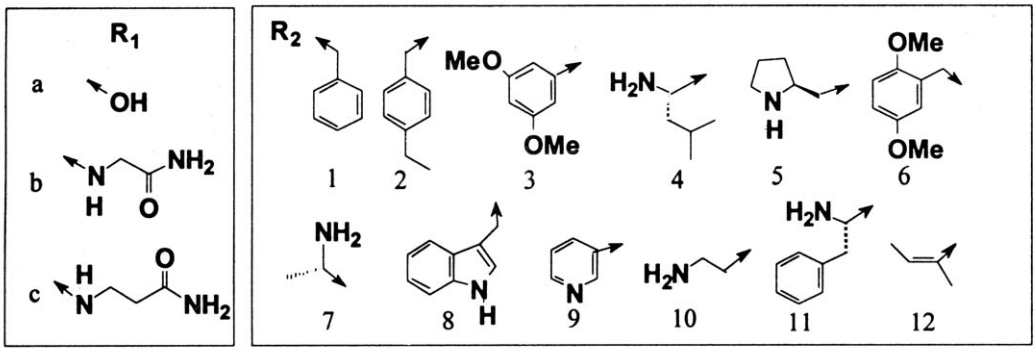


Fig. (2). R_1 and R_2 diversity on betulinic and ursolic derivatives.

The scope and limitation of our synthetic strategy for introducing diversity at C-28 and C-3 was explored by generating small libraries of betulinic and ursolic acids. Thus, a model library of 18 compounds each of betulinic and ursolic acids was made using automated multiple organic synthesizer. A set of 12 different carboxylic acids and two amino acids has been used for generating the libraries in parallel format using both the scaffolds.

GENERAL PROCEDURE TO ATTACH TRITERPENOIDS ON 2-CHLOROTRITYL CHLORIDE RESIN

To a suspension of resin 2-chlorotrityl chloride (100 mg, 0.130 mmol) in DCM (1mL) was added solution of betulinic acid (177.84 mg, 0.39 mmol) in DCM (500 µL), Pyridine (500 µL) and DIPEA (166.73 µL, 0.97 mmol) drop-wise. Reaction mixture was stirred at room temperature for 12 h. The reaction mixture was treated with 100 µL methanol to cap unloaded resin. The resin was filtered and washed sequentially with DCM (3 × 5 ml), DMF (3 × 5 ml), DCM (3 × 5 ml), diethylether (3 × 5 ml) and dried in vacuo.

GENERAL PROCEDURE TO ATTACH AMINO ACIDS ON SIEBER AMIDE RESIN

A premixed solution of Fmoc-β-alanine (55.9 mg, 0.180 mmol), HOBt (27.5 mg, 0.180 mmol), and DIPEA was added to a suspension of Sieber amide resin (100 mg, 0.06 mmol) in DMF followed by addition of TBTu (57.7 mg, 0.180 mmol). The reaction mixture was stirred at room temperature for 18 h and the resin was filtered, washed sequentially with DMF (6 × 5 ml), methanol (3 × 5 ml), DCM (3 × 5 ml), diethylether (3 × 5 ml) and dried in vacuo.

GENERAL PROCEDURE TO COUPLE TRITERPENOID ON AMINO ACID LOADED SIEBER AMIDE RESIN

To a suspension of amino acid loaded Sieber amide resin (100 mg, 0.06 mmol) in DMF (500 µL) and pyridine (500 µL) was added a premixed solution of betulinic acid (109.4 mg, 0.48 mmol), HOBt (73.4 mg, 0.24 mmol) and DIC (37.1 µL, 0.24 mmol). The reaction mixture was stirred at room temperature for 48 h. The resin was filtered, washed sequentially with DMF (6 × 5 ml), methanol (3 × 5 ml), DCM (3 × 5 ml), diethylether (3 × 5 ml) and dried in vacuo.

GENERAL PROCEDURE FOR SOLID-PHASE ESTERIFICATION

A premixed solution of carboxylic acid (0.49 mmol), DMAP (4 mg, 0.049 mmol) and DIC (76.3 µL, 0.496 mmol) in DMF (1mL) was added to a suspension of triterpenoid scaffold **4a** or **4b** (100mg, 0.06 mmol) in DMF (1mL). The reaction mixture was stirred at room temperature for 24 h and the resin was filtered, washed sequentially with DMF (3 × 5 ml), methanol (3 × 5 ml), DCM (3 × 5 ml), diethylether (3 × 5 ml) and dried in vacuo.

SOLID-PHASE OXIDATION

Freshly prepared PDC (225.6 mg, 0.60 mmol) was added slowly at 0°C with stirring to a polymer supported C-3 hydroxy-triterpenoid scaffold (100 mg, 0.062 mmol) suspension in DMF (1 mL). The reaction mixture was stirred at 0°C for 6 h and the resin was filtered, washed sequentially with DMF (6 × 5 ml), methanol (3 × 5 ml), water (3 × 5 ml), methanol (3 × 5 ml), DCM (3 × 5 ml), and diethylether (3 × 5 ml) and finally dried in vacuo.

SOLID-PHASE PREPARATION OF OXIME

$NH_2OH.HCl$ (33.12 mg, 0.48 mmol) was added to a suspension of polymer bound 3-oxo-triterpenoid scaffold (100 mg, 0.06 mmol) suspension in pyridine (1mL). The reaction mixture was stirred for 12 h at room temperature, and the resin was filtered and washed sequentially with DMF (6 × 5 ml), methanol (3 × 5 ml), water (3 × 5 ml), methanol (3 × 5 ml), DCM (3 × 5 ml), and diethylether (3 × 5 ml) and then dried in vacuo.

Table 2. Antimalarial Activity of Betulinic Acid Derivatives Against *P. falciparum*

Entry No.	R_1	R_2	MIC μg/ml
1.	Glycyl amide (b)	3,5 dimethoxybenzoyl (3)	>50
2.	Glycyl amide (b)	2,5 dimethoxybenzoyl (6)	50
3.	Glycyl amide (b)	Nicotinic acid (9)	>50
4.	Glycyl amide (b)	Indole-3-acetyl (8)	50
5.	Glycyl amide (b)	Trans 2,3 dimethylacryloyl (12)	50
6.	**Glycyl amide (b)**	**Prolyl (5)**	**10**
7.	Glycyl amide (b)	Phenylalanyl (11)	50
8.	Glycyl amide (b)	Leucyl (4)	50
9.	Glycyl amide (b)	Alanyl (7)	50
10.	β-alanylamide (c)	Phenylacetyl (1)	**10**
11.	β-alanylamide (c)	p-ethyl phenylacetyl (2)	>50
12.	β-alanylamide (c)	3,5 dimethoxybenzoyl (3)	>50
13.	β-alanylamide (c)	2,5 dimethoxybenzoyl (6)	>50
14.	β-alanylamide (c)	Nicotinic acid (9)	>50
15.	β-alanylamide (c)	Indole-3-acetyl (8)	50
16.	β-alanylamide (c)	Alanyl (7)	50
17.	β-alanylamide (c)	Leucyl (4)	50
18.	β-alanylamide (c)	Prolyl (5)	>50

Betulinic acid = 50 μg/ml and Chloroquine = 0.04 μg/ml

Table 3. Antimalarial Activity of Ursolic Acid Derivatives Against *P. falciparum*

Entry No.	R_1	R_2	MIC μg/ml
21	Glycyl amide (b)	2,5 dimethoxybenzoyl (6)	50
22	Glycyl amide (b)	Nicotinic acid (9)	50
23	Glycyl amide (b)	Indole-3-acetyl (8)	50
24	Glycyl amide (b)	Trans 2,3 dimethylacryloyl (12)	50
25	Glycyl amide (b)	Alanyl (7)	**10**
26	Glycyl amide (b)	Phenylalanyl (11)	50
27	Glycyl amide (b)	Leucyl (4)	50
28	**Glycyl amide (b)**	**Prolyl (5)**	**10**
29	β-alanylamide (c)	3,5 dimethoxybenzoyl (3)	>50
30	β-alanylamide (c)	2,5 dimethoxybenzoyl (6)	>50

Ursolic acid = 50 μg/ml and Chloroquine = 0.04 μg/ml

SOLID-PHASE OXIME ESTERIFICATION

To a suspension of polymer bound triterpenoid oxime scaffold **9a** or **9b** (100 mg, 0.06 mmol) in DMF (1mL) was added a premixed solution of phenyl acetic acid (48.96 mg, 0.36 mmol) and DIC (55.74 μl, 0.36 mmol) in DMF (1mL). The reaction mixture was stirred at room temperature for 24 h and then the resin was filtered, washed sequentially with DMF (3 × 5 ml), methanol (3 × 5 ml), DCM (3 × 5 ml), and diethylether (3 × 5 ml) and dried in vacuo.

REMOVAL OF FMOC GROUP

The Fmoc amino acid loaded Sieber amide resin (100 mg, 0.06 mmol) was swollen in DMF (2 × 3mL) and treated with 30% piperidine at room temperature for 30 min. The resin was filtered and washed with DMF (6 × 5 ml) and then dried in vacuo.

GENERAL PROCEDURE FOR THE CLEAVAGE

For the cleavage of final compounds, the resin was treated with 1-2% TFA for 30 min at room temperature. The resin was then filtered, washed thoroughly with methanol (3 × 0.5 ml), and the filtrate was evaporated to dryness *in vacuo.*

SYNTHESIS OF LIBRARIES ON MULTIPLE ORGANIC SYNTHESIZER

After optimizing the reaction conditions in syringes, the libraries of the betulinic acid and ursolic acid were synthesized in parallel format in the 96 well reaction block using the 496 automated synthesizer. The immobilized scaffolds were prepared separately in bulk quantities in four reaction vessels, followed by distribution in 36 well of reaction block. Monomers were added to the desired well using dispense sequence file. The structure of monomers R1 and R2 used for generating libraries are shown in Fig. **2**. Finally the compounds were cleaved from the resin using 1-2% TFA in DCM and evaporated to dryness. Before screening the compounds were then lyophilized by dissolving them in t-butanol/water. Purities of the compounds were assessed by RP HPLC and were in the range of 60-90%, which was sufficient for *in vitro* evaluation.

CHARACTERIZATION DATA FOR REPRESENTATIVE COMPOUNDS

3β-phenylacetoxylup-20(29)-en-28-oic Acid (5a)

MS(FAB) m/z 577 $[M+H]^+$, 441 $[MH\text{-}C_6H_5CH_2CO_2H]^+$, FT-IR 1688, 1734 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.72 (s, 3H), 0.75 (s, 3H), 0.82 (s, 3H), 0.92 (s, 3H), 1.25 (s, 3H), 1.68 (s, 3H), 4.44 (m, 1H), 4.73 (s, 1H), phenylacetyl moiety 3.60 (m, 2H), 7.29 (m, 5H).

3β-phenylacetoxyurs-12-en-28-oic Acid (5b)

MS(FAB) m/z 439 $[MH\text{-}C_6H_5CH_2CO_2H]^+$, FT-IR 1674, 1728 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.78 (s, 3H), 0.80 (s, 3H), 0.88 (s, 3H), 0.90 (brs, 6H), 1.01 (s, 3H), 1.25 (s, 3H), 5.25 (m, 1H), phenylacetyl moiety 7.30 (m, 5H), 4.01 (m, 2H).

N-[3β-nicotinyloxylup-20(29)-en-28-oyl] acetamide (6a)

MS(FAB) m/z 618 $[M+H]^+$, 495 $[MH\text{-}C_5H_4NCO_2H]^+$, FT-IR 1685, 1720 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.83 (s, 3H), 0.89 (s, 3H), 0.91 (s, 3H), 0.93 (s, 3H), 0.99 (s, 3H), 1.69 (s, 3H), 4.61 (s, H), 4.74 (s, 1H), nicotin moiety 7.56 (m, 1H), 8.47 (m, 1H), 8.85 (m, 1H), 9.33 (m, 1H), glycine moiety 3.80 (m, 2H).

N-[3β-phenylalanyloxyurs-12-en-28-oyl] acetamide (6b)

MS(FAB) m/z 660 $[M+H]^+$, 495 $[MH\text{-}C_6H_5CH_2CH(NH_2)CO_2H]^+$, FT-IR 1683, 1720$cm^{-1}$, ^1H NMR ($CDCl_3$) δ 0.70 (s, 3H), 0.76 (s, 3H), 0.83 (s, 3H), 0.91 (brs, 6H), 1.04 (s, 3H), 1.09 (s, 3H), 5.41 (brs, 1H), 4.56 (m, 1H), phenylalanyl moiety 7.36 (m, 5H), 3.95 (m, 1H), 3.60 (m, 2H), glycine moiety 3.95 (m, 2H).

N-[3β-phenylacetoxylup-20(29)-en-28-oyl] propanamide (7a)

MS(FAB) m/z 645 $[M+1]^+$, FT-IR 1680, 1718 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.72 (s, 3H), 0.75 (s, 3H), 0.81 (s, 3H), 0.89 (s, 3H), 0.94 (s, 3H), 1.63 (s, 3H), 4.44 (m, 1H), 4.59 (s, 1H), 4.72 (s, 1H), phenylacetyl moiety 7.30 (m, 5H), 3.60 (brs, 2H), β-ala moiety 2.50 (brs, 2H), 3.59 (m, 2H).

N-[3β-(2,5-dimethoxy)phenylacetoxyurs-12-en-28-oyl] propanamide (7b)

MS(FAB) m/z 705 $[M+1]^+$, 509 (M-(2,5-$(CH_3O)_2$C6H3CH_2CO2), FT-IR 1671, 1722 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.74 (d, 3H, J=6Hz), 0.81 (s, 3H), 0.86 (d, 3H, J=6Hz), 0.91 (s, 3H), 0.94 (s, 3H), 1.08 (brs, 6H), 4.48 (m, 1H), 5.42 (brs, 1H), 2,5-dimethoxy benzyl moiety 6.80 (m, 1H), 6.77 (m, 2H), 3.75 (brs, 5H), 3.58.(s, 3H) β-ala-moiety 2.94 (m, 2H), 2.94 (m, 2H).

N-[3-phenylacetoxyoximelup-20(29)-en-28-oyl] propanamide (10a)

MS(FAB) m/z 658 $[M+H]^+$, 522 $[MH\text{-}C_6H_5CH_2CO_2H]^+$, FT-IR 1669, 1703 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.82 (s, 3H), 0.85 (s, 3H), 0.88 (s, 3H), 0.91 (s, 3H), 0.95 (s, 3H), 1.67 (s, 3H), 4.59 (s, 1H), 4.87 (s, 1H), phenylacetyl moiety 3.65 (brs, 2H), 7.33 (m, 5H), β-ala moiety 2.52 (m, 2H), 3.54 (m, 2H).

N-[3-phenylacetoxyoximeurs-12-en-28-oyl] acetamide (10b)

MS(FAB) m/z 508 $[MH\text{-}C_6H_5CH_2CO_2H]^+$, FT-IR 1660, 1709 cm^{-1}, ^1H NMR ($CDCl_3$) δ 0.76 (d, 3H, J=6Hz), 0.83 (s, 3H), 0.86 (d, 3H, J=6Hz), 0.96 (brs, 6H), 1.04 (s, 3H), 1.08 (s, 3H), 5.54 (brs, 1H), phenylacetyl moiety 3.66 (brs, 2H), 7.30 (m, 5H).

ANTIMALARIAL ACTIVITY AGAINST *P. FALCIPARUM*

The antiparasitic activity of each compound was assessed by evaluating the minimum inhibitory concentration (MIC) against *P. falciparum in vitro* [11]. Asynchronous parasites obtained from the cultures of *P. falciparum* (strain NF-54)

were synchronized after 5% sorbital treatment so as to obtain only ring stage parasites [12]. Parasite suspension medium RPM 1-1640 at 1-2% parasitimia and 3% hematocrit was dispersed into sterile 96 well plates; and test compounds were serially diluted in duplicate wells to obtain final concentration of 50 μg/ml, 10 μg/ml and 2 μg/ml. The culture plates were incubated in a candle jar at 37^0C for 30-40 h. Thin blood smears from each well were microscopically examined and the concentration, which fully inhibited the maturation of ring parasites into schizont stage, was recorded as MIC.

RESULTS AND DISCUSSION

The structures of betulinic acid and ursolic acid correlate closely in the A-through D-rings, but differ significantly in the E-ring, betulinic acid has five-membered E-ring, an isopropenyl substituent, while ursolic acid contain a six-membered E-ring, and two methyl substituents. Thus, introduction of chemical diversity in betulinic acid and ursolic acid have been carried out using a common synthetic strategy for both and under identical reaction conditions.

In the first instance, the modifications have been introduced at C-3 hydroxyl and C-28 carboxyl group of betulinic and ursolic acid by using a two-point diversity approach. In this approach first a series of amino acids is introduced as diversity between the resin and C-28 carboxyl group of the triterpenoids to furnish immobilized betulinic acid and ursolic acid scaffolds. Subsequently modifications were introduced at C-3 hydroxyl group by esterification using variety of carboxylic acids. In another series of chemical modifications diversity was introduced by the oxidation of 3-hydroxyl group to provide 3-oxo as an intermediate followed by treatment with hydroxylamine to yield 3-oxime triterpenoid scaffolds. These oxime intermediates can be then further diversified via esterification using series of carboxylic acids to furnish a novel series of oxime esters.

The libraries were screened for their antimalarial activity with the view to study the affect of modifications on biological activity. The antimalarial activity of each libraries was evaluated against *P. falciparum in vitro*. Out of the eighteen derivatives of betulinic acid screened, two congeners (**6** and **10**) exhibited five-fold increase in the antimalarial activity in comparison to betulinic acid. The MIC values for both the congeners **6** and **10** were found to be 10 μg/ml whereas for betulinic acid it was 50 μg/ml**.** The standard drug chloroquine exhibited MIC at 0.04 μg/ml. Similarly out of the 10 congeners of ursolic acid screened, two congeners 2**5** and 2**8** exhibited MIC at 10 μg/ml in comparison to a MIC of 50 μg/ml for ursolic acid whereas other congeners were either less active or equipotent to ursolic acid. It is interesting to note that out of the four active compounds with MIC 10 μg/ml, **6** and **28** derived from betulinic and ursolic acid respectively have the same set of monomers, glycyl residue at R_1 and prolyl residue at R_2. Thus among the monomers used in the present investigation, glycine and proline appears to be the most favored at R_1 and R_2 respectively for both the terpenoids.

CONCLUSIONS

In summary, we have identified triterpenoids as a novel natural product scaffold for the generation of combinatorial libraries. These scaffolds are indeed good substrates for automated synthesis of combinatorial libraries. This has been demonstrated by successfully carrying out synthesis of two small libraries comprising 18 derivatives each of betulinic and ursolic acid. Screening of libraries for their antimalarial activity against *P. falciparum in vitro* led to the identification of compounds **6, 10, 25** and **28** with a five-fold increase in the biological activity in comparison to betulinc and ursolic acids. Though in the present investigation we have used a limited set of monomers, **6** and **28** lay foundation for the introduction of more structural diversity using a variety of carefully selected monomers at both positions of betulinic and ursolic acids.

REFERENCES

[1] For reviews, see (a) Dolle, R.E. *J. Comb. Chem*., **2000**, *2*, 383. (b) Kundu, B.; Khare, S.K. Rastogi, S.K. *Progress in Drug Research,* **1999**, *53*, 91.

[2] Hall, D.G.; Manku, S.; Wang.F. *J. Comb. Chem*., **2001**, *3*, 125.

[3] Nicolaou, K.C.; Pfefferkorn, J.A.; Roecker, J.A.; Cao, G.-Q.; Barluenga, S.; Mitchell, H.J. *J. Am. Chem. Soc.*, **2000**, *122*, 9939.

[4] Shu, Y.-Z. *J. Nat. Prod*., **1998**, *61*, 1053.

[5] Otsuka, H.; Fujioka, S.; Komiya, T.; Goto, M.; Hirmatsu, Y.; Fujimura, H. *Chem. Pharm. Bull*., **1981**, *29*, 3099.

[6] Mayaux, J-F.; Bousseau, A.; Pauwels, R.; Huet, T.; Henin, Y.; Dereu, N.; Evers, M.; Soler, F.; Poujade, C.; Clercq, E.D.; Le Pecq, J-B. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 3564.

[7] Bringmann, G.; Saeb, W.; AkeAssi, L.; Francois, G.; Narayanan, A.S.S.; Peters, K.; Peters, E-M. *Planta Medica,* **1997**, *63*, 255.

[8] Soler, F.; Paoujade, C.; Evers, M.; Carry, J.C.; Henin, Y.; Bousseau, A.; Pauwels, R.; De Clercq, E.; Mayaux, J.F.; Le Pecq, J.B.; Dereu, N. *J. Med. Chem.,* **1996**, *39*, 1069.

[9] Evers, M.; Paoujade, C.; Soler, F.; Ribeil, Y.; James, C.; Lelievre, Y.; Gueguen, J.-C.; Reisdorf, D.; Morize, I.; Pauwels, R.; De Clercq, E.; Henin, Y.; Bousseau, A.; Mayaux, J.F.; Le Pecq, J.B.; Dereu, N. *J. Med. Chem.*, **1996**, *39*, 1056.

[10] (a) Batra, S.; Rastogi, S.K.; Kundu, B.; Patra, A.; Bhaduri, A.P. *Tetrahedron Lett.* **2000**, *41*, 5971. (b) Kundu, B.; Rastogi, S.K.; Batra, S.; Raghuvanshi, S.K.; Shukla, P.K. *Bioorg. Med. Chem. Lett.*, **2000**, *10*, 1779. (c) Srinivasan, T.; Gupta, P.; Kundu, B. *Tetrahedron Lett*., **2001**, *42*, 5993.

[11] Bruce-Chwatt, L-T.; Black, R.H.; Canfield, C.J.; Clyde, D.F.; Peters, W.; and Wesusdorfer, W.H.; Chemotherapy of malaria, **1986**, 2nd Ed World Health Organizaion, Geneva.

[12] Lambros, C.; Vanderberg, J.P. *J. Parasitol.*, **1979**, *65*, 418.