Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2803–2806

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Solid-Phase Synthesis and Bioevaluation of Lupeol-Based Libraries as Antimalarial Agents†

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Received 7 June 2002; accepted 29 July 2002

Abstract—The use of the triterpenoid lupeol as a scaffold for the synthesis of lupeol-based libraries is described. Lupeol was anchored to a solid support (Rink amide/Sieber Amide) through aliphatic dicarboxylic acid moieties, which also served as a site for introducing diversity. The resulting polymer linked 3β-*O* (resin-alkanoyl)-lup-20(29)-ene **3** was used to generate key intermediates 3β-*O* (resin-alkanoyl)-30-bromo-lup-20(29)-ene **4** and 3β-*O* (resin-alkanoyl)-30-amino-lup-20(29)-ene **6** for the generation of libraries based on disubstituted lupeol derivatives. A 96-member library was screened for its in-vitro antimalarial activity against *Plasmodium falciparum*.

Natural products play an important role in drug discovery and many approved therapeutics as well as drug candidates have been derived from natural sources. They also represent promising scaffolds for diversification by using combinatorial techniques, as they have been selected by nature for their ability to undergo transformations in a three-dimensional space. Library construction around such scaffolds thus has the potential for both lead discovery and lead optimization. Nevertheless any such diversification is usually functional group based and the original structure of the template remains unchanged. In recent years, libraries based on natural products have received wide attention since they give an easy access to analogues having superior therapeutic potential than the parent scaffold.[1]

Out of the variety of structurally diverse compounds obtained from tropical plants, terpenoids are often found in significant quantities, and a wide array isolated and characterized. The lupane type of triterpenoids and their derivatives represent a unique and one of the more important classes of biologically active natural products. Among this class of compounds, the pentacyclic triterpene lupeol [lup-20(29)-en-3β-ol] obtained in excellent yield from the stem bark of *Crataeva nurvula*, is of particular interest because of it's wide spectrum of biological activity exhibited by it. The activities include urolithic, anticalciuric[2] and antimalarial activity[3] against the chloroquine-resistant *Plasmodium falciparum*. Thus, lupeol provides an interesting template for diversification, which may result in the identification of more potent analogues. In continuation of our interest in the design and synthesis of combinatorial libraries based on natural products with antimalarial activity,[4] we have now targeted the template-directed synthesis of libraries based on lupeol with the view to enhance its antimalarial activity. We envisioned developing synthetic intermediates that have the potential for the synthesis and amplification of triterpenoid based derivatives with at least two-point diversity. Combinatorial approach has been successfully used by us[5] and others[6] for enhancing the biological activity of lead molecules with weak activity profile. In this paper we describe solid phase synthesis and antimalarial screening of novel libraries based on 3- and 30-substituted lup-20(29)-ene derivatives.

Chemistry

In the first step of our approach, we developed a linking strategy to produce polymer-linked lupeol to allow maximum introduction of diversity while maintaining a

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†CDRI Communication No. 6271.

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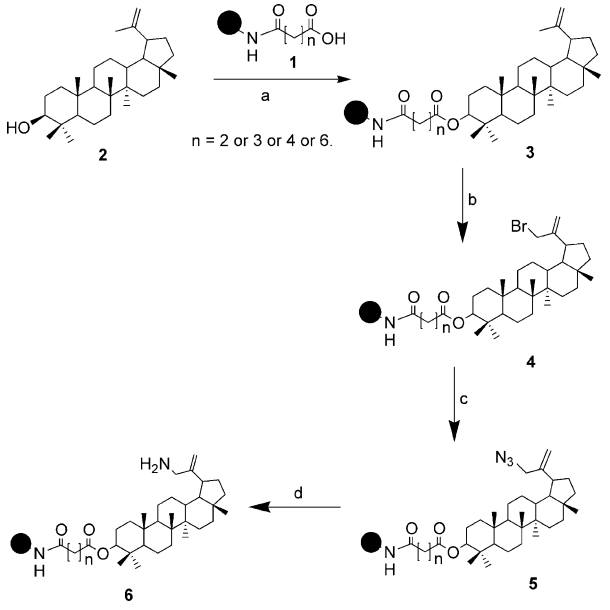
PII: S0960-894X(02)00623-6

reliable and efficient cleavage procedure. We proposed to incorporate bifunctional linkers, which on one side will remain linked to the resin whereas on the other side can be linked with the hydroxyl group of lupeol at C-3 position. Among the variety of commercially available bifunctional moieties, we selected aliphatic dicarboxylic acid anhydrides, as it can be conveniently used to derivatize the amide-based resin. The aliphatic chain present in these linkers may also enhance the liphophilicity of lupeol; thereby enhancing its biological activity. For our solid-phase synthesis we preferred to use amide-based resin, as hydroxy based resin such as wang resin when acylated with aliphatic dicarboxylic acid anhydrides resulted in poor loading. The synthesis was initiated by loading various anhydrides onto the Rink/Sieber amide resin (0.50 mmol/gm) using pyridine in DMF at room temperature for 12 h to obtain resin **1**. The completion of the reaction was monitored by a negative Kaiser test. This was followed by coupling of lupeol to the carboxyl function on the resin using the standard DIC/DMAP procedure to afford polymer linked 3β-O(resin-alkanoyl)-lup-20(29)-ene **3** as outlined in Scheme 1. The completion of the reaction was monitored by the carboxyl group test.[7] This linking strategy to produce the polymer bound tri-terpenoid scaffold also provides an additional site for the introduction of diversity at C-30, the resin bound **3** was treated with *N*-bromosuccinimide to give 3β-*O* (resin-alkanoyl)-30-bromo-lup-20(29)-ene (Scheme 1). The intermediate **4** has been further derivatized to produce a library using a variety of robust solid–phase substitution reactions, for example reaction with a variety of primary and secondary amines and reaction with aromatic alcohols to give compounds **7** and **8** (Scheme 2), respectively. In the first instance a library of 48 compounds has been generated in parallel format using automation with 36 compounds represented by **7** and 12 compounds represented by **8** (Scheme 2), respectively.



Scheme 1. Reagents and conditions: (a) DIC, DMAP, DMF/THF (1:1); (b) NBS, DCM: CCl_4 (1:1), 6 h; (c) NaN_3, DMSO, 80 °C, 24 h; (d) $SnCl_2$, PhSH, Et_3N, THF, 2 h.

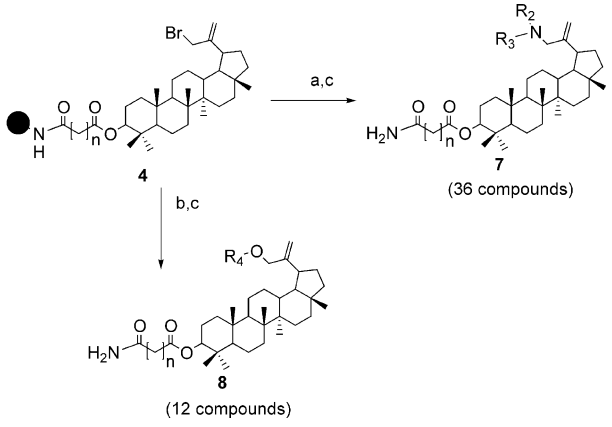
The monomers used for introducing diversity have been summarized in Scheme 2. The product purities ranged from 60 to 90% based on analytical HPLC (Table 1).

The second library, based on lupeol, involves generation of yet another key intermediate 3β-*O* (resin-alkanoyl)-30-amino-lup-20(29)-ene **6** derived from the bromo derivative **4**. The method involves treatment of compound **4** with sodium azide in DMSO for 24 h at 80 °C to obtain intermediate 3β-*O* (resin-alkanoyl)-30-azido-lup-20(29)-ene **5**. The azide was then reduced to amine **6** by treatment with stannous chloride and thiophenol in the presence of triethylamine for 2 h at room temperature (Scheme 1). Derivatisation of intermediate **6** with a variety of isocyanate, amino acids/aromatic acids and aromatic aldehydes led to the synthesis of structurally diverse derivatives **9**, **10** and **11** (Scheme 3). Thus, a second library of 48 compounds was generated in parallel format using automation with eight compounds represented by **9**, 32 compounds represented by **10** and eight compounds represented by **11**.

The monomers used for introducing diversity have been depicted in Scheme 3. Resin bound intermediates and products were cleaved with 1–2% TFA in DCM and characterized using HPLC, NMR and FAB-MS.[8]

Antimalarial Activity Against *Plasmodium falciparum* In Vitro

The antiparasitic activity of compound was assessed by evaluating the minimum inhibitory concentration (MIC) against *P. falciparum* in vitro.[9] 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.[10] Parasite suspension medium RPMI-1640 at 1–2% parasitimia and 3% hematocrit was dispersed into sterile

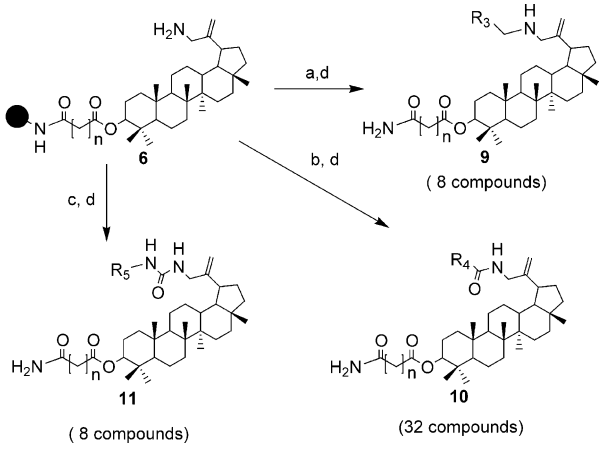


Scheme 2. Reagents and conditions: (a) primary or secondary amine, DBU, 16 h; (b) Benzyl alcohol derivatives, DBU, 16 h; (c) 1% TFA-DCM, 10 min, $n=2$ (**X1**), 3 (**X2**), 4 (**X3**), 6 (**X4**), R_2 = cyclohexyl-, R_3 = H (**Y1**), R_2 = benzyl-, R_3 = H (**Y2**), R_2 = furfuryl-, R_3 = H (**Y3**), R_2 = 2-pyridyl ethyl-, R_3 = H (**Y4**), R_2R_3 = piperidenyl- (**Y5**), R_2R_3 = morpholinyl- (**Y6**), R_2R_3 = *N*-methyl piperazinyl (**Y7**), R_2 = *N*, *N*-diethyl amino ethyl- R_3 = H (**Y8**), R_2 = *N*-cyclohexyl-, R_3 = *N*′-cyclohexyl-, (**Y9**) R_4 = 4-bromo benzyl- (**Y10**), benzyl- (**Y11**), 4-methoxy benzyl- (**Y12**).

Table 1. Structure, yields and purities of representative compounds from library based on structure **7**, **8**, **9**, **10** and **11**

Entry	X	Y	Z	Yield[a]	Purity[b]	FABMS[c]
1	2 (X1)	Cyclohexyl amino- (Y1)	—	75	90	623
2	2 (X1)	2-Pyridyl ethyl amino- (Y4)	—	75	60	646
3	3 (X2)	Morpholinyl- (Y6)	—	73	90	625
4	3 (X2)	*N,N*-Dicyclohexyl amino- (Y9)	—	80	75	719
5	4 (X3)	Piperidineyl- (Y5)	—	85	80	637
6	4 (X3)	4-Bromo benzyl- (Y10)	—	75	85	738
7	6 (X4)	*N*-Methyl piperazinyl- (Y7)	—	78	88	680
8	6 (X4)	4-Methoxy benzyl- (Y12)	—	80	80	718
9	2 (X1)	—	4-Hydroxy phenyl- (Z1)	82	75	648
10	2 (X1)	—	4-Chloro benzoyl- (Z3)	85	80	680
11	3 (X2)	—	Pyridyl-(3-acetyl)- (Z4)	80	75	675
12	3 (X2)	—	2-Amino-3-phenyl propionyl- (Z9)	85	70	653
13	4 (X3)	—	Acetyl- (Z7)	75	72	612
14	4 (X3)	—	Benzoyl- (Z8)	80	75	671
15	6 (X4)	—	Benzyl- (Z11)	85	80	731
16	6 (X4)	—	P-Tolyl- (Z12)	78	85	731

[a]Crude yields.
[b]As determined by analytical HPLC.
[c]All compounds in the library gave the corresponding $M^+ + 1$ in the FABMS(+).



Scheme 3. Reagents and conditions: (a) R_3CHO, TMOF/DMF (2:1), 2 h then $NaCNBH_3$, 1% AcOH in TMOF, 1 h; (b) R_4COOH, HOBt, DIC, DMF, 6 h; (c) R_5NCO, DCM, 24 h; (d) 1% TFA-DCM, 10 min, $n=2$ (**X1**), 3 (**X2**), 4 (**X3**), 6 (**X4**) R_3 = 4-hydroxy phenyl- (**Z1**), 3-pyidyl- (**Z2**), R_4CO = 4-chloro benzoyl- (**Z3**), pyridyl-3-acetyl- (**Z4**), 4-hydroxy cinnamoyl- (**Z5**), 2-prolyl- (**Z6**), acetyl- (**Z7**), benzoyl- (**Z8**), 2-amino-3-phenyl propionyl- (**Z9**), 7-aminoheptanoyl- (**Z10**), R_5 = benzyl (**Z11**), P-tolyl- (**Z12**).

96-well plates; and test compounds were serially diluted in duplicate wells to obtain final concentration of 50, 10 and 2 μg/mL. The culture plates were incubated in a candle jar at 37 °C for 30–40 h. Thin blood smears from each well were microscopically examined and the concentration, which fully inhibited the maturation of the ring parasites into schizont stage, was recorded as MIC. The in vitro results of some of the most active compounds have been summarized in Table 2. Out of the 96 compounds screened for their biological activity, 15 compounds were found to be more active than lupeol, others were either inactive or equipotent to lupeol (data not shown). Out of the 15 compounds a majority of the compounds were derived from 3β-*O* (resin-alkanoyl)-30-bromo-lup-20(29)-ene **4** interme-diate and the most active compound was found to be X4Y10 (Table 2) with MIC value of 13.07 μM in comparison to the MIC value of 117 μM for lupeol. The standard drug chloroquine exhibited MIC value of 0.24 μM. The compound X4Y10 with suberic acid and 4-bromobenzyl alcohol as monomers was found to be at least 9 times more potent than lupeol.

Table 2. Antimalarial activity of Lupeol derivatives against *P. falciparum*

Compd	MIC (μM)	Compd	MIC (μM)
X1Y1	16.00	X4Y10	13.07
X1Y3	16.10	X4Y11	13.92
X1Y4	15.40	X1Z11	14.83
X1Y6	16.36	X1Z12	14.83
X2Y8	15.31	X2Z3	14.43
X2Y11	15.82	X2Z10	14.66
X2Y12	15.50		
X3Y1	15.36	Lupeol	117
X3Y9	13.66	Chloroquine	0.24

Antimalarial Activity Against *Plasmodium berghei* In Vivo

In order to study the in vivo efficacy of the congeners identified using combinatorial approach, one of the compounds X1Z11 was evaluated for in vivo activity against *P. berghei*. Swiss mice (25 ± 1 gm) of either sex were inoculated with 1×10^6 *P. berghei* parasitized cells on day zero. A group of six mice was administered aqueous suspension of the compound X1Z11 at 100 mg/kg dose from day zero to three via intraperitoneal route; while another six mice were administered the vehicle alone. Thin blood smears from the treated mice were observed daily to record the degree of parasitaemia till the animals died.

The results summarized in Table 3 show that the mean percent parasitaemia in treated group on day 4 was 1.7 ± 0.6% as against 4.4 ± 0.5% in the control group. Thus compound X1Z11 exhibited nearly 60% suppression after 4-day treatment, however, the subsequent progression of the parasitaemia was not significantly altered.

Table 3. In vivo antimalarial activity of compound X1Z11 against *P. berghei* in Swiss mice

Group	Dose (mg/kg/day)	% Parasitaemia on day (mean ± SE)		Survival time in days (mean ± SE)
		Day 4	Day 6	
Compd X1Z11	100	1.7 ± 0.6	6.8 ± 1.6	16.5 ± 3.3
Vehicle (control)	—	4.4 ± 0.5	9.4 ± 1.5	13.3 ± 1.3

In summary, we have demonstrated that lupeol-based libraries can be generated employing robust synthetic methodologies on solid supports. Naturally available lupeol can be successfully transformed into various key scaffolds followed by generation of libraries using automated synthesizer. Screening of the library for antimalarial activity against *P. falciparum* in vitro led to the identification of compounds with seven to nine fold increase in the biological activity in comparison to lupeol. Thus, for the first time using combinatorial approach we have demonstrated an appreciable increase in the antimalarial activity of lupeol thereby confirming the validity of combinatorial approach for lead optimization. Though in the present investigation, we have used a limited set of monomers, these fifteen compounds lay foundation for the introduction of more structural diversity using a variety of carefully selected monomers. Further modifications are nevertheless necessary to optimise our lead structure in order to enhance its antimalarial activity.

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8. (a) **General experimental procedure for 7** or **8:** To the suspension of Sieber amide resin (100 mg, 0.05 mmol) linked to succinic acid in DMF/THF (2:1) mixture was added 4-DMAP (0.1 equiv, 0.05 mmol), lupeol (3 equiv, 0.15 mmol) and DIC (3 equiv, 0.15mmol). After shaking the mixture for 16 h at rt, the solvent was drained and the resin was washed sequentially with DMF, MeOH, DCM (5×3 mL each) and dried in vacuo to afford **3.** In the next step, the 3β-*O*-(resin-alkanoyl) lupeol **3** (100 mg, 0.05 mmol) was suspended in DCM/CCl_4 (1:1) and to it was added NBS (4 equiv, 0.20 mmol) and the reaction was shaken at 600 rpm for 6 h. Thereafter the resin was sequentially washed with DMF, DCM and ether (5×3 mL each) and dried to yield 3-*O* (resin-alkanoyl)-30-bromo-lup-20(29)-ene **4**. This was suspended in DMSO and to it was added DBU (2 equiv, 0.1 mmol) and primary or secondary amine or substituted benzyl alcohol (10 equiv, 0.5 mmol). The reaction was shaken for 16 h at rt and then the resin was washed with DMF, MeOH, DCM, ether (5×3 mL each) and then dried in vacuo. The desired compound was cleaved from the resin using 1% TFA in DCM to obtain prototypes **7** or **8**. 3β-*O*-(4-amido succinyl)-30-*N* [benzyl amino]-lup-20(29)-ene [X1Y1; 7]: FAB MS 631 (M + H); ^{1}H NMR (300 MHz, $CDCl_3$): 5.45 (brs, 1H, H-29-α), 5.66 (brs, 1H, H-29-β), 4.50 (m, 1H, H-3), 3.45 (s, 2H, H-30), 1.25 (s, 3H, H-26), 1.15 (s, 3H, H-27), 1.13 (s, 3H, H-25), 0.92 (s, 3H, H-23), 0.88 (s, 3H, H-24), 0.78 (s, 3H, H-28) Benzyl amino moiety, 4.03 (s, 2H, CH_2), 7.31(m, 5H, C6H5) Succinyl moiety, 2.55 (t, 2H, $J = 12$ Hz, CH2), 2.67 (t, 2H, $J = 12$, Hz CH_2). (b) **General procedure for 5**, **6 and 11:** To the 3β-*O*-(resin-alkanoyl)-30-bromo-lup-20(29)-ene **4** (100 mg, 0.05 mmol) on Sieber amide resin suspended in DMSO was added Sodium azide (10 equiv, 0.5 mmol) and the reaction mixture shaken at 80 °C for 16 h. The solvent was drained and the resin was washed with DMF (5×3 mL), H_2O (5×3 mL), MeOH (5×3 mL), DCM (5×3 mL), and dried in vacuo to get intermediate **5**. Thereupon 3β-O (resin-alkanoyl)-30-azido-lup-20(29)-ene was suspended in THF and treated sequentially with triethylamine (25 equiv, 1.25 mmol), thiophenol (20 equiv, 1.0 mmol) and stannous chloride (5 equiv, 0.25 mmol). After shaking at rt for 2 h, the solvent was drained and the resin was washed with MeOH (5×3 mL), DCM (5×3 mL), MeOH (5×3 mL), DCM (5×3 mL), and then dried in vacuo to get 3β-*O* (resin-alkanoyl)-30-amino-lup-20(29)-ene **6.** This was suspended in DMF: DCM (1:1) and then treated with 4-methyl phenyl isocyanate (10 equiv, 0.5 mmol) at rt for 16 h. The solvent was drained and the resin was washed sequentially with DMF (5×3 mL), MeOH (5×3 mL), DCM (5×3 mL), and then dried in vacuo. The desired compound was cleaved from the resin using 1% TFA in DCM to obtain prototype **11**. 3β-*O*-(4-amido succinyl)-30-*N* [*N*′-(4-methyl phenyl) urea]-Lup-20(29)-ene [X1Z12]: FAB MS 674 (M + H); ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.48 (brs, 1H, H29-α), 5.70 (brs, 1H, H29-β), 4.45 (m, 1H, H-3), 3.78 (s, 2H, H-30), 1.34 (s, 3H, H-26), 1.32 (s, 3H, H-27), 1.27 (s, 3H, H-25), 1.25 (s, 3H, H-23), 1.22 (s, 3H, H-24), 1.20 (s, 3H, H-28), *N*′-[4-methylphenyl] urea moiety, 2.32 (s, 3H, CH_3), 6.95 (d, 2H, $J = 6$ Hz), 7.11(d, 2H, $J = 6$ Hz), Succinyl moiety, 2.55 (t, 2H, CH_2, $J = 12$ Hz), 2.71 (t, 2H, CH_2, $J = 12$ Hz).
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