Screening of natural products for new leads as inhibitors of IκBα kinase: 16-Oxo-cleroda-3, 13E-dien-15-oic acid from Polyalthia longifolia of Annonaceae family

N V S Ramakrishna*, E K S Vijaya Kumar, A K Jain, B S Kalakoti, R D Gupte, R B Panicker & R V S V Vadlamudi
Research Centre, Hoechst Marion Roussel Limited, LBS Marg, Mulund (W), Mumbai 400 080

Received 22 August 1999; accepted 3 April 2000

16-Oxo-cleroda-3, 13E-dien-15-oic acid\(^1\) has been isolated from pet. ether extract of twigs of Polyalthia longifolia by bioactivity monitored purification. \(^1\) shows good IκBα kinase inhibitory activity with an IC\(_{50}\) of 14.9 \(\mu M\).

IκBα kinase is responsible for phosphorylation of IκB, which is an important step in the activation of the transcription factor NFκB. Phosphorylated IκB is then degraded through the ubiquitin-proteosome pathway. It therefore, appears possible that inhibition of IκBα kinase would finally inhibit activation of NFκB, through inhibition of phosphorylation and subsequent degradation of IκB.

A critical role for NFκB activation in arthritis is evident from the following facts: (i) The therapies that are used for the treatment of arthritis, such as prednisone and gold compounds are known to block activation of NFκB\(^2\), (ii) Glucocorticoids reportedly inhibit NFκB by two mechanisms. First several groups have reported that activated glucocorticoid receptors directly interact with and inhibit activated NFκB subunits. A second mechanism involves the transcriptional activation of the IκBα gene in response to treatment with glucocorticoids. Glucocorticoids, by upregulating IκBα protein levels, function to block nuclear translocation of NFκB and DNA binding\(^3,8\) and (iii) Salicylates which are non-steroidal antiinflammatory drugs inhibit the activation of NFκB at concentrations used to treat arthritis\(^7\).

IκBα kinase inhibitory activity is assayed by carrying out enzyme-substrate reaction in a microtitre plate, in the presence of test sample at a specific concentration. The amount of phosphopeptide formed during the reaction, is then assayed by transferring it to an ELISA plate coated with a specific antibody against the phosphopeptide. Final colour development is carried out by horse radish peroxidase reaction using tetramethyl benzidine as a substrate. The details of biological assay will be reported elsewhere.

Polyalthia longifolia belonging to the family Annonaceae and is widely distributed in India and popularly known as Asoka. The morphological details and distribution of the plant species of these genera are described in literature\(^9\). The phytochemical studies on this plant have been carried out by several groups, which resulted in the isolation and characterization of several diterpenes, alkaloids and proanthocyanidines\(^11,12\). 16-Oxo-cleroda-3, 13E-dien-15-oic acid \(^1\) is one of the diterpenes isolated from stem bark\(^1\) and also from the leaves\(^1\). \(^1\) was reported to have antifeedant activity against castorlooper\(^1\). This communication describes the bioactivity guided isolation, characterization and IκBα kinase inhibitory activity of 16-oxo-cleroda-3, 13E-dien-15-oic acid \(^1\) isolated from twigs of Polyalthia longifolia.

The pet. ether extract of the twigs of Polyalthia longifolia was chromatographed over silica gel column followed by preparative TLC as described in the experimental section. The isolation of compound \(^1\) was monitored both by TLC and IκBα kinase inhibitory activity. The compound \(^1\) was identified as 16-oxo-cleroda-3, 13E-dien-15-oic acid by comparison of its spectral data with the data reported in literature. The isolated compound inhibited IκBα kinase with an IC\(_{50}\) of about 14.9 \(\mu M\).

\(^*\)Current address: Zydus Cadila Research Centre, Zydus Tower, Satellite Cross Road, Sarkhej-Gandhinagar Highway, Ahmedabad 380 015
Experimental Section

The melting point was determined on a Bristoline apparatus and uncorrected. IR spectrum was recorded on a Perkin-Elmer 782 spectrophotometer. 1H NMR spectra were recorded on a Bruker ACP 300 spectrometer and mass spectrum on a VG Quattro II mass spectrometer. The TLC was carried out using precoated silica plate (Article No. 5554, E. Merck) using 5% methanol in chloroform as developing solvent and spots were visualized under UV.

Extraction

The twigs of Polyalthia longifolia were collected in our Factory premises, Mulund, Mumbai. Coarse pulvrisered and dried Polyalthia longifolia twigs (1 kg) were extracted at 45°C with pet. ether (5 litres, 18 hr). The extract was filtered and the residual plant material was then extracted with CH2Cl2 (2x5 litres, 18 hr). The combined CH2Cl2 extracts were concentrated to dryness in vacuo at 45°C to get crude plant extract (40 g).

Isolation

Crude plant extract (3 g) was passed through a silica gel flash column (5 cm × 25 cm). The column was eluted with 10:90 (2 litres), 30:70 (1 litre) and 50:50 (1 litre) ethyl acetate-pet. ether respectively. Eluates were collected in 30 mL volumes each. All the fractions were monitored by HPLC on a 4 mm × 250 mm, Merck LiChrocart RP-Select B (5 μ) column using a gradient of 0.1% aqueous orthophosphoric acid (pH 2.5) to CH3CN in 20 min at a flow rate of 1 mL/min and UV detection at 240 nm at 40°C. The fractions containing the compound I were combined and concentrated in vacuo at 45°C to obtain enriched material (0.5 g).

The above material was subjected to a second silica gel column (3 cm × 25 cm) and eluted with 10:90 (3 litres) and 30:70 (2 litres) ethyl acetate-pet. ether respectively. The fractions containing the compound I were mixed, concentrated and dried in vacuo at 45°C to get semi-pure material (0.2 g).

The semi-pure material (0.15 g) was finally purified by preparative TLC on silica gel (Article No. 13895, E. Merck) using 50:50 ethyl acetate-pet. ether for developing and ethyl acetate for elution. The spot corresponding to the compound I was detected under UV light at 254 nm. The ethyl acetate eluates on concentration in vacuo at 45°C gave pure compound I (0.070 g).

16-Oxo-cleroda-3, 13E-dien-15-oic acid: Semi-solid; soluble in CH2Cl2, CHCl3, EtOAc, MeOH, CH3CN and DMSO; [α]20° -22° (c 0.02, MeOH); Anal: Found: C, 75.35; H, 9.56. Caled for C20H30O5: C, 75.47; H, 9.43%; ESIMS: 317 (M-H); UV: 218 nm; IR: 3000-2950, 1710, 1650, 1480, 1400, 1280, 1240 and 900 cm⁻¹; 1H NMR (300 MHz, CDCl3): δ 9.56 (s, CH), 6.48 (s, CH), 5.22 (bs, CH), 2.59 (m, CH2), 2.11 (m, CH2), 1.74 (m, CH2), 1.61 (m, CH), 1.59 (bs, CH3), 1.42 (m, CH2), 1.24 (m, CH2), 1.00 (s, CH3), 0.85 (d, CH3) and 0.69 (s, CH3); 13C NMR (75 MHz, CDCl3): δ 194.95 (d), 171.35 (s), 158.61 (s), 145.07 (s), 134.35 (d), 121.50 (d), 47.36 (d), 40.13 (s), 39.01 (s), 37.82 (t), 37.62 (t), 37.10 (d), 28.35 (t), 27.57 (t), 20.69 (q), 20.03 (t), 18.93 (t), 18.82 (q) and 16.65 (q). The compound is identical in all respects to 16-oxo-cleroda-3, 13E-dien-15-oic acid.

Acknowledgement

Authors wish to acknowledge the support received from Dr A Batzer, Dr A Ehnsen and Dr J Wink of HMR, Frankfurt.

References

1. Indian Patent application No. 100/BOM/98. European Patent application No. 98107396.8.