Phytochem Rev (2026) 25:4829–4856
https://doi.org/10.1007/s11101-025-10200-y

Diospyrin: biosynthesis, distribution in the plant kingdom, and therapeutic potential

Lutfun Nahar · Satyajit D. Sarker

Received: 18 March 2025 / Accepted: 10 November 2025 / Published online: 20 November 2025
© The Author(s) 2025

Abstract Diospyrin, a naturally occurring bisnaphthoquinone, is chemically known as 5-hydroxy-6-(5-hydroxy-7-methyl-1,4-dioxonaphthalen-2-yl)-7-methylnaphthalene-1,4-dione. It was first isolated from the heartwood of *Diospyros montana* Roxb. (Ebenaceae) and later was found in several species of the genera *Diospyros* and *Euclea*. This bisnaphthoquinone is biosynthesized through the dimerization of 7-methyljuglone, a 1,4-naphthoquinone, via C-2 and C-6' coupling. Previous *in vitro*, *in vivo* and *in silico* studies have established diospyrin as a bioactive compound with therapeutic potentials; the most prominent therapeutically relevant bioactivities include its antibacterial (especially against *Mycobacterium* and *Staphylococcus* species), anticancer/antitumour (mainly against Ehrlich ascites carcinoma, blood, breast, cervical, colon, lung, prostate and skin cancer cell lines), and antiparasitic (particularly against *Leishmania*, *Plasmodium* and *Trypanosoma* parasites) properties. In addition to three major activities, diospyrin was found to possess antiallergic, antidiabetic, antifungal, anti-inflammatory, antinociceptive, antioxidant, antiviral, neuroprotective, and sedative properties. Some information on the plausible mechanisms of action of diospyrin for its major bioactivities is also available in the literature. Diospyrin was shown to produce a certain level of cytotoxicity in normal cells, hepatotoxicity and mutagenicity. Overall, diospyrin could be considered a structural lead for developing new therapeutics for cancers and bacterial and parasitic infections, albeit further preclinical and clinical studies with diospyrin and its structural analogues are still needed. This review presents a comprehensive and updated overview, and a critical appraisal of published information until February 2025 on diospyrin (**1**), including its occurrence in the plant kingdom, biosynthesis, therapeutic potential, mechanisms of action and toxicity.

Keywords Diospyrin · Euclein · *Diospyros* · Anticancer · Antiparasitic · *Leishmania* · *Plasmodium* · *Trypanosoma* · Antitumour · Antibacterial

Abbreviations

ARG131	Arginine 131
A375	Malignant skin melanoma
ADMET	Absorption, distribution, metabolism, excretion and toxicity
AP	Alkaline phosphate

L. Nahar (✉)
Laboratory of Growth Regulators, Palacký University and Institute of Experimental Botany, The Czech Academy of Sciences, Šlechtitelů 27, 78371 Olomouc, Czech Republic
e-mail: nahar@ueb.cas.cz; profnahar@outlook.com

S. D. Sarker (✉)
Centre for Natural Products Discovery, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK
e-mail: S.Sarker@ljmu.ac.uk

BACTEC	A blood culture system that detects bacteria, yeast, fungi, and mycobacteria in clinical samples
CNS	Central nervous system
CYS51	Cysteine 51
EAC	Ehrlich ascites carcinoma
ED_{50}	Effective dose 50% (the median effective dose)
ERK	Extracellular signal-regulated kinase
GDP	Guanosine diphosphate
GMP	Guanosine monophosphate
GOT	Glutamate oxaloacetate transaminase
GPT	Glutamate pyruvate transaminase
HeLa	Cervical cancer
Hep2	Epidermoid laryngeal carcinoma
HIV	Human immunodeficiency virus
HL-60	Human leukaemia cell line (promyeoloblast cells isolated from the peripheral blood of an Acute promyelocytic leukaemia patient)
HSP100	Heat shock protein 100 of *Leishmania donovani*
HSV	Herpes simplex virus
IC_{50}	Inhibitory concentration 50% (half maximal inhibitory concentration)
ID_{50}	The dose of a pathogen that will cause disease in 50% of exposed susceptibles
K-562	Lymphoblast cells (chronic myelogenous leukaemia)
LDH	Lactate dehydrogenase
MAPK	Mitogen-activated protein kinase
MCF-7	Breast cancer
MG-63	Human osteosarcoma
MIC	Minimum inhibitory concentration
NF-κB	Nuclear factor kappa B
PAM	PI3K/Akt/mTOR signalling pathway
PBMC	Human peripheral blood mononuclear cells
ROS	Reactive oxygen species
SER74	Serine 74
TYR32	Tyrosine 32

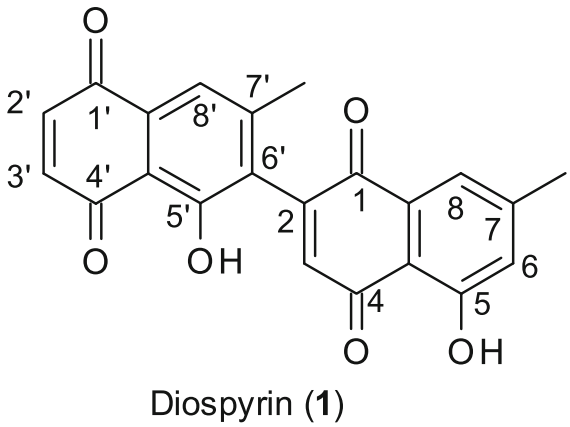
Introduction

Diospyrin (**1**, $C_{22}H_{14}O_6$, mw 374 Da) is chemically known as 5-hydroxy-6-(5-hydroxy-7-methyl-1,4-dioxonaphthalen-2-yl)-7-methylnaphthalene-1,4-dione. It is a bisnaphthoquinone, which was first isolated from the heartwood of *Diospyros montana* Roxb. (Ebenaceae) (Fig. 1) (Kapil and Dhar 1961; Yoshimoto et al. 1971; Yoshida and Mori 2000; Nematollahi et al. 2012; Hussain et al. 2015). This compound has another trivial name euclein, originated from the name of the genus *Euclea* L., which also produces this compound.

The structure of diospyrin (**1**) (Fig. 2) was initially incorrectly proposed as a C-2/C-3' linked dimer of 7-methyljuglone (Ganguly and Govindachari 1966; Yoshida and Mori 2000), but later, the structure was corrected as a C-2/C-6' linked dimer (Sidhu and Pardhasaradhi 1970; Sidhu et al. 1976). After a quarter of a century, the structure was further confirmed by X-ray crystallographic analysis, where the geometric parameters were reported as normal and the angle between the planes of the two ring systems was 59.74 (2)° (Harrison and Musgrave 2004). Furthermore, the crystal packing was found to be influenced by O—H···O hydrogen bonds and possible short C—H···O and π–π stacking interactions. The physical appearance of **1** is orange-red crystal cubes, and it has a melting point of 285–288 °C. This compound also exists as an amorphous powder at room temperature. It is quite stable at ambient temperature, but it is recommended to be stored at around 0 °C and in a dry and dark storage condition. Its water-solubility is



Fig. 1 *Diospyros montana* Roxb.—the first reported source of diospyrin (**1**)



Diospyrin (**1**)

Fig. 2 Structure of diospyrin (**1**)

only 7.9E^{-5} g/L at 25 °C (poorly soluble), but it is more soluble in acetone, chloroform and ethanol. Diospyrin (**1**) is a bioactive natural product and has been shown to possess various therapeutically relevant bioactivities, albeit with some toxicities (Nahar et al. 2025). There have been a couple of review articles covering the anticancer activity of **1** published in the literature (Sagar et al. 2010; Rauf et al. 2024). However, this present review articulates a comprehensive and updated overview, and a critical appraisal of published information (January 1961–February 2025) on diospyrin (**1**), including its occurrence in the plant kingdom, biosynthesis, therapeutic potential, mechanisms of action and toxicity.

Methodology

An extensive literature search was performed using Web of Science, PubMed and Google Scholar, and other published materials, including relevant books and PhD theses. In various combinations, with diospyrin and euclein being present in all combinations, biosynthesis, distribution, bioactivity, toxicity and therapeutic potential were used as the keywords for the literature search. The review covers publications until February 2025. Publications on diospyrin (**1**), which are not within the remit of this article, e.g., total and partial synthesis, and synthesis of diospyrin derivatives, were excluded.

Biosynthesis

Diospyrin (**1**) is formed through the dimerization of 7-methyljuglone, a 1,4-naphthoquinone, via C-2 and C-6' coupling (Fig. 3). Studies involving radio-labelled precursors or stable-isotope feeding proposed the most plausible biosynthetic route to the formation of compound **1** through the acetate-polymalonate pathway, which is also known as the polyketide pathway (Vickery and Vickery 1981; Widhalm and Rhodes 2016), as shown in Fig. 3. This pathway in plants is generally located in the cytosol of plant cells and involves CoA-linked acetate and malonate substrates that are found widely in the plant kingdom and is responsible for the production of several plant 1,4-naphthoquinones, including 7-methyljuglone and bis-1,4-naphthoquinones like **1**. The involvement of polyketide synthases in the acetate-polymalonate pathway was demonstrated previously (Bringmann et al. 2000; Bringmann and Feineis 2001; Widhalm and Rhodes 2016). Polyketide synthases belong to the type II class of enzymes and catalyse C–C bond formation in a single active site through decarboxylation, condensation, and cyclization reactions utilizing a CoA-ester substrate like acetyl CoA and a CoA-ester extender like malonyl CoA. The acetate-polymalonate pathway tends to rely on a polyketide reductase for the removal of the oxygen atom of the third acetate unit before the initial cyclization. The formation of the naphthalene intermediate likely involves multistep enzyme-mediated reactions, including cyclization. It was postulated that oxidation of the naphthalene intermediate could lead to the formation of 7-methyljuglone (Zenk et al. 1969; Culham and Gornall 1994; Widhalm and Rhodes 2016). The dimerization of 7-methyljuglone could occur through C-2 and C-6' coupling to form diospyrin (**1**).

Distribution

Since the discovery of diospyrin (**1**) from the heartwood of *D. montana* (Kapil and Dhar 1961; Yoshida and Mori 2000; Hussain et al. 2015), this compound has been reported from at least 32 other *Diospyros* species (Table 1). However, this compound was not found in all *Diospyros* species investigated to date; a phytochemical study with 17 African *Diospyros*

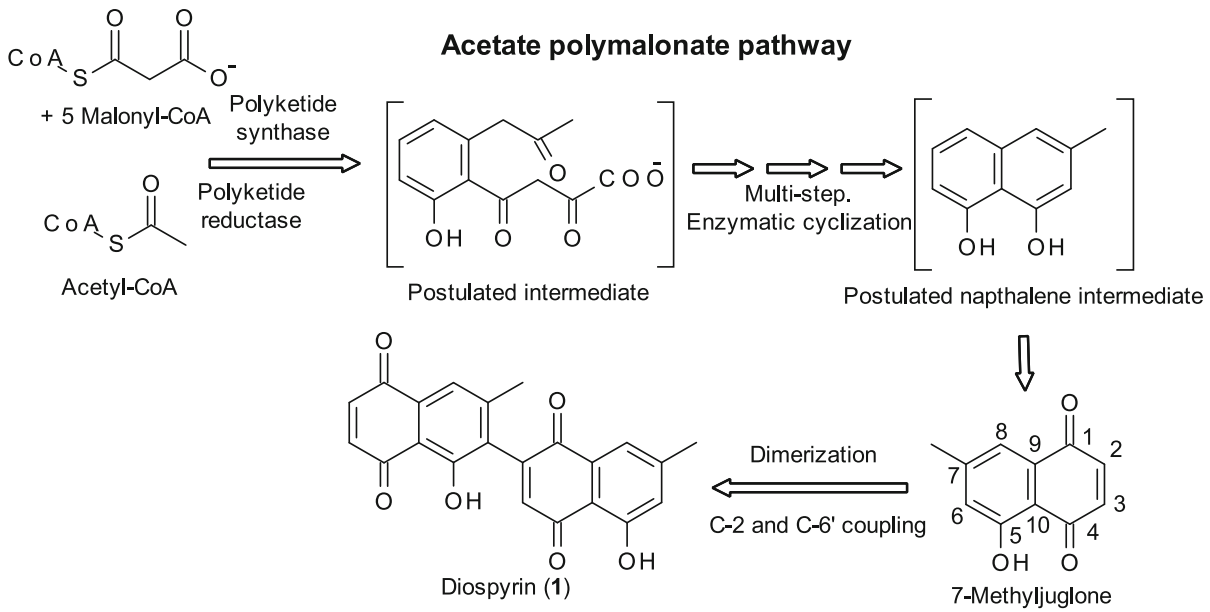


Fig. 3 Plausible biosynthetic pathway of diospyrin (**1**)

species revealed the presence of **1** only in the 11 species with no signs of **1** in the other six (Zhong et al. 1984). Of the 11 diospyrin-positive *Diospyros* species, only five species, i.e., *D. abyssinica, D. cinnabarina, D. fragrans, D. kamerunensis* and *D. longiflora*, showed the presence of any reasonable amount of this compound, while the other six species contained a negligible amount. Also, only a small portion of over 350 species of *Diospyros* (Mallavadhani et al. 1998) has ever been phytochemically investigated, and this makes it difficult to understand any distribution pattern within the genus *Diospyros*. In addition to the genus *Diospyros*, another genus *Euclea,* from the same family of Ebenaceae, also produces **1** (Table 1). At least three species from the *Euclea* genus, i.e., *E. divinoforum*, *E. natalensis* (including two varieties) and *E. pseudebenus* were found to contain this compound (Ferreira et al. 1974; Lall et al. 2005a,b; Joubert et al. 2006; Bapela et al. 2007, 2008a,b; Thaver 2010; Mahapatra et al. 2012). It is interesting to note that the genera *Diospyros* and *Euclea* are phylogenetically closely related as established from their anatomical, molecular and morphological studies (Duangjai et al. 2006; Geeraerts et al. 2009). The cooccurrence of diospyrin (**1**) in the species of these two genera further supports this phylogenetic relationship. The distribution of **1** within a plant species appears not to be restricted to one part of the plant but is in various parts of a plant, including fruit, leaf, root, and root bark, seed, seedling, stem and stem bark and wood (Table 1). However, the stem and root possess the highest amounts of this compound (Table 1).

Therapeutic potential

The therapeutic potential of a molecule is considered its ability to treat diseases or to help the healing process (Tate 1967). Several previous *in vitro, in vivo* and *in silico* studies with diospyrin (**1**) have established this compound as a bioactive compound with therapeutic potentials (Fig. 4; Table 2) as an antibacterial agent particularly against *Mycobacterium* infections (tuberculosis) as well as the infections caused by *Staphylococcus* species (Lall and Meyer 2001; Lall et al. 2003, 2005b; Van der Kooy et al. 2006; Mahapatra et al. 2007; Bapela et al. 2008a; Thaver 2010; Karkare et al. 2013a, b; Sundarrajan et al. 2015), in the treatment of parasitic diseases caused by *Leishmania* (Hazra et al. 1987, 2002, 2013; Ganapaty et al. 2006a; Mukherjee et al. 2009; Adibpour et al. 2012; Dev et al. 2012; Jha et al. 2020; Ortiz-Perez et al. 2021), *Plasmodium* (Hazra et al. 1995a; Likhitwitayawuid et al. 1999; Ganapaty et al. 2006a; Dev et al. 2012; Ortiz-Perez et al. 2021) and *Trypanosoma* parasites (Yardley et al. 1996; Cushion et al. 2000;

Table 1 Distribution of diospyrin (**1**) in the plant kingdom

Plant name	Common name	Plant part	Native source	References
Diospyros abyssinica (Hiern) F. White	Giant diospyros	Bark and wood	Sub-Saharan African countries	Zhong et al. (1984)
Diospyros assimilis Bedd. Synonym: *D. ebenum* J. Koenig Ex Retz	Malabar Ebony	Roots	India	Ganapaty et al. (2006a, b)
Diospyros batocana Hiern Synonym: *D. odorata* Hiern ex Greves	Batoka jackal-berry	Root bark	Angola, Botswana, Congo, Zambia, Zimbabwe and Zaire	Alves et al. (1983)
Diospyros canaliculate De Wild Synonym: *D. cauliflora* De Wild	Flint bark	Bark and wood	Angola, Cabinda, Cameroon, Congo, Gabon, Ghana, Ivory Coast, Liberia, Nigeria and Zaïre	Zhong et al. (1984)
Diospyros candolleana Wight Synonyms: *D. arnottiana* Miq. ex Thwaites, *D. canarica* Bedd. And *D. oligandra* Bedd	Karimaram	Roots	India and Sri Lanka	Dev et al. (2012)
Diospyros chamaethamnus Dinter ex Mildbr	Sand apple	Root bark	Namibia	Costa et al. (1998)
Disopyros dendo Welw. *ex* Hiern Synonyms: *D. atropurpurea* Gurke and *D. coccinea* Gurke ex De Wild	Billetwood	Wood	Angola, Cabinda, Cameroon, Central African Republic, Congo, Gabon, Nigeria and Zaïre	Zhong et al. (1984)
Diospyros discolor Willd Synonym: *D. blancoi* A. DC	Velvet apple	Leaves and fruits	Bangladesh and India	Nahar et al. (2025)
Disopyros cinnabarina (Gurke) F. White	Guinea ebony	Stem bark and root	Cameroon, Congo, Equatorial Guinea, Gabon, Ivory Coast and Nigeria	Waterman et al. (1979); Zhong et al. (1984)
Disopyros fragrans Gurke	Fragrant diospyros	Wood	Cabinda, Cameroon, Congo, Equatorial Guinea and Gabon	Zhong et al. (1984)
Disopyros gabunensis Gurke Synonym: *D. castaneifolia* A. Chev, and *D. deltoidei* F. White	Flint bark tree	Bark	Burundi, Cameroon, Congo, Equatorial Guinea, Gabon, Ghana, Ivory Coast, Liberia, Nigeria, Rwanda, Sierra Leone, Tanzania, Zambia and Zaïre	Zhong et al. (1984)
Diospyros hoyleana F. White	Tolbos	Bark	Angola, Cabinda, Cameroon, Congo, Gabon, North Rhodesia and Zimbabwe	Zhong et al. (1984)
Disopyros iturensis (Gurke) Let. & F. White Synonym: *D. insculpta* Hutch. & Dalzei and *Maba bequaertii* De Wild	Benin ebony	Bark	Angola, Cameroon, Central African Republic, Congo, Gabon, Nigeria and Zaïre	Zhong et al. (1984)
Diospyros japonica Sieb. &Zucc	Japanese date-plum	Bark	China, Japan and Taiwan	Kuroyana et al. (1971)

Table 1 continued

Plant name	Common name	Plant part	Native source	References
Diospyros kamerunensis Gurke Synonym: *D. pallenscens* A. Chev	African ebony or Awran or Otutu	Bark	Cameroon, Gabon, Ghana, Ivory Coast and Liberia	Zhong et al. (1984)
Diospyros longiflora Let. & F. White	Gabon date-plum	Bark	Cameroon, Congo and Gabon	Zhong et al. (1984)
Diospyros lotus L	Date-plum	Roots	Temperate Asia and southeast Europe	Rauf et al. (2017); Bawazeer et al. (2019); Bawazeer and Rauf (2021); Rauf et al. (2021)
Diospyros malabarica (Desr.) Kostel Synonym: *D. biflora* Blanco	Malabar ebony	Fruits and leaves	Indian subcontinent	Uddin et al. (2022)
Diospyros mannii Hiern Synonym: *D. aggregata* Gurke	Mann's diospyros	Stem bark	West and West-Central Africa	Jeffreys et al. (1983)
Diospyros melanoxylon Roxb	Tendu	Leaves	India and Sri Lanka	Sharma et al. 2018)
Diospyros mollis Griff	Makleua	Stem bark	Bangladesh, India, Sri Lanka and Thailand	Yoshihira et al. (1971)
Disopyros monobuttensis Gurke	Yoruba ebony or walking-stick ebony	Bark	Lama forest in Benin, Ghana, Ivory coast, Nigeria and Togo	Zhong et al. (1984)
Diospyros montana Roxb Synonym: *D. auriculata* Wight ex Hiern	Bistendu	Stem bark	Australia, China, India and Sri Lanka	Pardhasaradhi and Krishnakumaru (1979); Ravishankara et al. (2000)
Disopyros nigresence (Dalzell) C. J. Saldanha Synonym: *D. ferrea* (Willd.) Bakh	Narrow-leaved ebony	Roots	Bangladesh, India, Sri Lanka, the Democratic Republic of Congo, Thailand and Zimbabwe	Dev et al. (2012)
Diospyros obliquifolia (Hiern ex Gurke) F. White Synonym: *D. stapfiana* F. White	Guinea ebony	Stem bark	Cameroon, Congo, Equatorial Guinea, Gabon, Ivory Coast and Nigeria	Waterman and Mbi (1979)
Diospyros oocarpa Thwaites Synonym: *D. marmorata* Griff	Kalu-kadumberiya	Roots	India and Sri Lanka	Dev et al. (2012)
Diospyros piscatoria Gurke	Ongbalo	Roots	Sierra Leone to Cameroon, south to Gabon, Congo and DR Congo	Adeniyi et al. (2000)
Disopyros sanza-mintka A. Chev	Sanza	Bark	West and West-Central Africa: Sierra Leone to Ghana, also in Cameroon, Equatorial Guinea, Gabon and Congo	Zhong et al. (1984)
Diospyros sylvatica Roxb	Forest ebony	Roots	India and Sri Lanka	Adeniyi et al. (2000)
Diospyros verrucosa Hiern	Warty star apple	Roots and stem bark	Tanzania and Mozambique	Khan et al. (1987)

Table 1 continued

Plant name	Common name	Plant part	Native source	References
Diospyros virginiana L	Common persimmon	Roots	USA	Carter et al. (1978); Wang et al. (2011)
Euclea divinoforum Hiern	Diamond leaf	Roots	Eastern and Southern Africa	Costa et al. (1976)
Euclea natalensis A. DC Synonym: *E. multiflora* Hiern	Natal guarri	Roots	Ethiopia, Somalia and South Africa	Lall et al. (2005b); Bapela et al. (2008a); Thaver (2010); Mahapatra et al. (2012)
Euclea natalensis A. DC. subspecies *natalensis*	Natal guarri	Roots	Ethiopia, Somalia and South Africa	Bapela et al. (2008b)
Euclea natalensis A. DC. subspecies *natalensis*	Natal guarri	Seeds and seedlings	Ethiopia, Somalia and South Africa	Joubert et al. (2006); Bapela et al. (2007)
Euclea pseudebenus E. Mey. ex A. DC Synonym: *Euclea angustifolia* Benth	Cape ebony	Roots	Angola, Namibia and South Africa	Ferreira et al. (1974); Joubert et al. (2006)

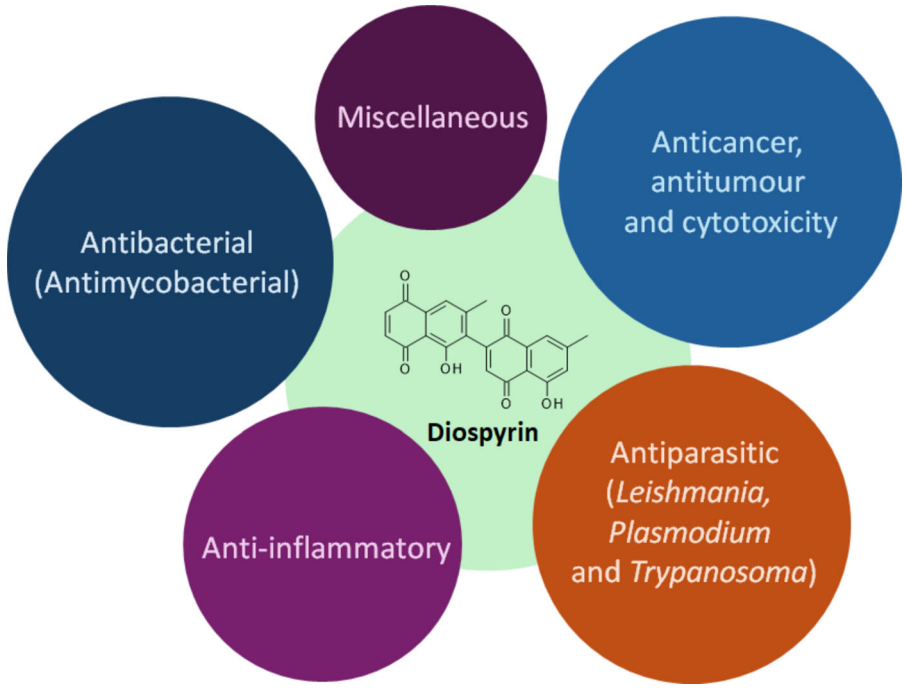


Fig. 4 Graphical representation of bioactivities of diospyrin (**1**) [The size of the circles represents the relative amount of published information; the bigger the size, the more available information]

Kaneshiro et al. 2000; Ganapaty et al. 2006a; Dev et al. 2012; Ortiz-Perez et al. 2021), and in the management of various cancers (Sagar et al. 2010; Rauf et al. 2021, 2024) and inflammatory conditions (Landa et al. 2012; Uddin et al. 2014, 2016; Kim et al. 2020; Rauf et al. 2021; Shahidullah et al. 2020; Bawazeer and

Table 2 Bioactivity and therapeutic potential of diospyrin (**1**)

Activity	Type of assays	Summary	References
Anti-allergic	*In vitro*	Diospyrin showed basophil-degranulation and allergy-related enzymes modulating properties. It inhibited soybean lipoxidase (IC_{50} = 28.9 µM). Maximal inhibition of IgE/antigen degranulation (< 15% at 1 µM), A23187 degranulation (56.8% at 10 µM), hyaluronidase (< 15% at 20 µM) and lipoxidase (65.6% at 36.7 µM) was determined	Pinho et al. (2014)
Antibacterial	*In vitro*	Diospyrin was found to be active against *Neisseria gonorrhoeae*, *Shigella* spp and *Staphylococcus aureus*	Khan et al. (1978)
	In vitro	The MICs of diospyrin against *Streptococcus pyogenes* and *Streptococcus pneumoniae* were between 1.56 and 50 µg/mL, while those against *Salmonella choleraesuis* serotype *typhi* and *Mycobacterium chelonae* ranged 25–100 µg/mL	Adeniyi et al. (2000)
	In vitro	Diospyrin, isolated from the roots of *Euclea natalensis*, showed inhibitory activity against the drug-resistant *Mycobacterium tuberculosis* H37 Rv (ATCC 27294) strain with an inhibitory concentration of 100 µg/mL. It was also active against other drug-resistant strains of *M. tuberculosis* at a concentration of 100 µg/mL	Lall and Meyer (2001); Lall et al. (2003)
	In vitro	Diospyrin, isolated from the roots of *Euclea natalensis*, showed inhibitory activity against the drug-resistant *Mycobacterium tuberculosis* H37 Rv (ATCC 27294) strain with an inhibitory concentration of 8 µg/mL	Lall et al. (2005b)
	In vitro	Diospyrin inhibited the growth of *Mycobaterium tuberculosis* (MIC = 8 µg/mL). The lowest tested concentration was 5 µg/mL	Van der Kooy et al. (2006); Mahapatra et al. (2007)
	In vitro	Diospyrin was found to be an inhibitor of the uptake of K^+ by *Mycobacterium tuberculosis* and *M. smegmatis*, with inhibition detected at submicrogram concentrations	Bapela et al. (2008a); Thaver (2010)
	In vitro and *in silico*	Diospyrin could show anti-*Staphylococcus aureus* activity through binding in the *N*-terminal domain of GyrB. It could partially inhibit the ATPase activity of GyrB in an allosteric manner. Diospyrin could bind to a novel binding site between the ATPase domain and the transducer domain. It could also inhibit both the relaxation and the DNA cleavage ability of gyrase, suggesting it inhibits gyrase with a novel mechanism	Chung (2012)
	In vitro	Showed antibacterial activity by inhibiting protein synthesis. Diospyrin was found to be an inhibitor of DNA gyrase by binding to the *N*-terminal domain of GyrB	Karkare et al. (2013a); Wang et al. (2022)
	In vitro	Antimycobacterial potential of diospyrin was demonstrated from its inhibitory activity against DNA gyrase mediated through a binding to the *N*-terminal domain of GyrB, which contains the ATPase active site, but is not a competitive inhibitor of the ATPase reaction	Karkare et al. (2013b)
	In vitro	The antimicrobial activity of diospyrin was evaluated against a broad panel of MDR and extensively drug-resistant tuberculosis strains, rapid-growing mycobacteria and other bacterial isolates. The MIC of this compound against multiple drug-resistant mycobacterial strains ranged between 4 and 256 µg/mL. Diospyrin also demonstrated antibacterial activity against several other bacterial strains, including *Bacillus, Enterococcus, Escherichia* and *Staphylococcus* species (MIC = 64–256 µg/mL)	Dey et al. (2014)
	In silico	A combination of structure-based virtual screening with physicochemical and pharmacokinetic studies against rhamnose pathway enzymes identified potential leads against *Mycobacterial tuberculosis*, and diospyrin was one of them, with good binding affinity towards the rhamnose pathway proteins	Sundarrajan et al. (2015)
	In vitro	Showed antibacterial activity against *Staphylococcus epidermis* (zone of inhibition 15 mm with 2 mg/mL; IC_{50} = 72 µg/mL)	Rauf et al. (2016)
	In vitro enzyme inhibition assay	Activity against *Helicobacter pylori*. Diospyrin showed 31.9% urease inhibition at 0.5 µM concentration	Rauf et al. (2017)

Table 2 continued

Activity	Type of assays	Summary	References
Anticancer, antitumour and cytotoxicity	*In vitro* and *in vivo*	Cytotoxic activity of diospyrin against EAC (Ehrlich ascites carcinoma) in Swiss mice was reported	Hazra et al. (1984), (1994)
	In vivo	Diospyrin showed significant inhibitory activities against murine tumours in the Swiss A mice model	Pal et al. (1996)
	In vitro	Diospyrin was assessed by an *in vitro* assay utilizing the activation of Epstein-Barr virus (EBV) early antigen expression in EBV-genome-carrying human lymphoblastoid cells	Norhanom and Hazra (1997)
	In vitro	Topoisomerase I, an extensively studied target for anticancer drugs, was selectively inhibited by diospyrin	Bailly (2000); Tazi et al. (2005)
	In vitro	Diospyrin induced apoptosis in human cancer cell lines, including the acute myeloblastic leukaemia (HL-60), chronic myelogenic leukaemia (K-562), breast adenocarcinoma (MCF-7) and cervical epithelial carcinoma (HeLa)	Chakrabarty et al. (2002)
	In vitro and *in vivo*	Diospyrin showed enhanced inhibitory activity against murine tumour *in vivo* and human cancer cell lines *in vitro* through liposomal encapsulation	Hazra et al. (2005)
	In vitro	Cytotoxicity towards rat skeletal myoblasts (L-6 cells) with the IC_{50} value of 6.38 μM	Ganapaty et al. (2006a)
	In vitro and *in vivo*	The tumour inhibitory activity of diospyrin was assessed *in vivo* against Ehrlich ascites carcinoma (EAC), and cytotoxicity was determined *in vitro* on EAC and MCF7 cancer cells by MTT assay. Diospyrin was found to display tumour inhibitory activity, cytotoxicity and ROS generation activity	Hazra et al. (2007)
	In vitro	Disopyrin showed cytotoxicity against Vero cells (IC_{50} = 17.8 μg/mL) with a selectivity index of 2.2	Mahapatra et al. (2007)
	Animal model study and *in vitro*	Inhibited Ehrlich ascites carcinoma in rodents Cytotoxicity towards tumour cells, A375 (malignant skin melanoma; IC_{50} = 0.82 μM), Hep2 (epidermoid laryngeal carcinoma; IC_{50} = 3.58 μM) and EAC (Ehrlich ascites carcinoma; IC_{50} = 0.84 μM) and PBMC (human peripheral blood mononuclear cells; IC_{50} = 78.32 μM)	Sarma et al. (2007); Rauf et al. (2024)
	In vitro and *in vivo*	Diospyrin inhibited the growth of malignant skin melanoma and epidermoid laryngeal carcinoma cells (IC_{50} = 0.82 and 3.58 μM, respectively)	Sarma et al. (2008)
	In vitro	Diospyrin was found to regulate apoptosis at the endoplasmic reticulum as well as mitochondria by modulating cytosolic calcium in human breast carcinoma cells	Kumar et al. (2009), (2012)
	In vitro	Diospyrin showed cytotoxicity against HL-60 (IC_{50} = > 100 μM) and K-562 (IC_{50} = > 100 μM) cell lines	Sagar et al. (2010)
	In vitro	The cytotoxicity of diospyrin was evaluated against the mouse fibroblast cell line Balb/c 3T3 at a concentration range of 0.04–2.5 μM using the MTT assay. Diospyrin showed cytotoxicity against this cell line with an IC_{50} value of 0.9 μM	Landa et al. (2012)
	In vitro	Cytotoxicity towards rat skeletal myoblasts (L-6 cells) with the IC_{50} value of 2.393 μg/mL	Dev et al. (2012)
	In vitro	Diospyrin was screened against thirteen human cancer cell lines, breast cancer cell lines: MDA-MB-435, MCF-7, ZR-75–1, colon cancer cell lines: HCT-15, HT-29, Colo-205, CNS cancer cell line U373, neuroblastoma cell line IMR32, oral cancer cell line KB, prostate cancer cell lines: DU-145, PC-3, lung cancer cell line A549 and melanoma cell line SK-MEL-2 and the GI_{50} (50% growth inhibitory concentration) values were 0.18, 2.54, 2.1, 2.11, 33.9, 0.13, 2.23, 0.17, 2.46, 0.20, 2.45 and 2.21 μM, respectively	Hazra et al. (2015)
	In silico	Diospyrin showed viral E6 protein (which is implicated in cervical cancer) inhibition in the energetically optimized structure-based pharmacophore modelling, ligand docking and molecular dynamics simulations studies. It exhibited H-bond interaction with TYR32, CYS51, SER74 and ARG131 amino acid residues	Kumar et al. (2019)

Table 2 continued

Activity	Type of assays	Summary	References
	In vitro	Diospyrin exhibited potent and selective cytotoxicity to the murine myeloma NS-1 (ATCC TIB-18) cell line over neonatal foreskin cells (ATCC PCS-201), with IC_{50} values of 0.8 µM and 5.8 µM, respectively	Pullella et al. (2020)
	In vitro, *Iin vivo* and *Iin silico* assays	Significant cytotoxic activity (IC_{50} = 47.40 ppm) in the Epstein–Barr-Virus (EVA) early antigen activation assay. A 60% survival rate of the lymphoblastoid Raji cells at a concentration of 1000 (mol/ratio 32 pmol TPA). In a two-stage carcinogenesis assay on mouse skin, diospyrin significantly delayed (50% effect in the 14th week and 100% effect in the 20th week) the formation of papillomas on mouse skin. Significantly attenuated thermal-induced protein denaturation (EC_{50} = 298 µg/mL. Exhibited a promising MDR reversal effect in a dose-dependent manner against the mouse T-lymphoma cell line. Docking results showed that diospyrin had favourable docking statistics as compared with the standard	Rauf et al. (2021)
Antidiabetic	*In silico* and *in vitro*	Diospyrin exhibited protein tyrosine phosphatase 1B inhibitory activity (IC_{50} = 27.59 µM). It showed molecular interactions with GLY220, TYR46, VAL49 and ASP48 inside the active site of protein tyrosine phosphatase 1B	Bawazeer et al. (2019)
Antifungal	*In vitro*	Weak antifungal activity against *Botrytis cinerea*, *Colletotrichum* spp., *Fusarium oxysporum, Phomopsis obscurans,* and *Phomopsis viticola* in the broth dilution assay	Wang et al. (2011)
Anti-inflammatory	Enzymatic *in vitro* and *in silico* assays	Diospyrin inhibited cyclooxygenase-1 and -2 (COX-1 and COX-2; IC_{50} = 1.39 µM and 0.55 µM, respectively), the key enzymes of the arachidonic acid cascade. The activity was comparable to the positive control, indomethacin	Landa et al. (2012)
	In vivo	Diospyrin (80.54%) protected the carrageenan paw edema after 3 h	Uddin et al. (2014)
	In vitro and *in silico*	Diospyrin exhibited lipoxygenase inhibitory activity (IC_{50} = 62.7 µM). Molecular docking revealed significant molecular interactions between this compound and lipoxygenase, showing promising potential for further optimization as a potential anti-inflammatory lead compound	Uddin et al. (2016)
	In vitro, *Iin vivo* and *Iin silico* assays	Diospyrin modulated inflammation in poly I:C-induced macrophages via ER stress-induced calcium-chop pathway. It reduced nitric oxide (NO) production from RAW 264.7 after 24 h (92, 94, 93% at 1, 5 and 10 µM concentrations, respectively), granulocyte–macrophage colony-stimulating factor production, and intracellular calcium release in poly I:C-induced RAW 264.7. The phosphorylation of p38 MAPK and ERK1/2 was also significantly suppressed. It inhibited mRNA levels of NO- synthase 2, C/EBP homologous protein (CHOP), calcium/calmodulin-dependent protein kinase II alpha, signal transducers and activators of transcription 1 (STAT1), STAT3, STAT4, Janus kinase 2, first apoptosis signal receptor, c-Jun, and c-Fos in poly I:C-induced RAW 264.7	Kim et al. (2020); Rauf et al. (2021)
	In vitro	Inhibitory activity on lipopolysaccharide-induced inflammation using RAW 264.7 mouse macrophages. Diospyrin moderated the production of nitric oxide (NO), monocyte chemotactic protein-1, macrophage inflammatory protein-1, interleukin (IL)-6, IL-10, granulocyte colony-stimulating factor, granulocyte–macrophage colony-stimulating factor, vascular endothelial growth factor, leukemia inhibitory factor, and RANTES/CCL5, as well as calcium release in LPS-induced RAW 264.7, at concentrations of up to 10 M significantly. It inhibited the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and mRNA expression of C/EBP homologous protein (CHOP), and tumor necrosis factor receptor superfamilymember6(Fas), in LPS-induced RAW264.7 cells at concentrations of up to 10 µM	Shahidullah et al. (2020)
	In vitro	Diospyrin showed weak antiglycation and lipoxygenase inhibitory activity, but IC_{50} could not be determined	Bawazeer and Rauf (2021)
Antinociceptive	*In vivo*	The acetic acid-induced writing was significantly protected by diospyrin (40.87%) at a dose of 10 mg/kg, offering peripheral and central analgesic effects	Uddin et al. (2014)

Table 2 continued

Activity	Type of assays	Summary	References
Antioxidant	*In vitro*	Diospyrin was found to be a potent inhibitor of heterologously expressed human glutathione transferases (GSTs) A1-1, M1-1 and P1-1. The Ki values for diospyrin with respect to both glutathione and 1-chloro-2,4-dinitrobenzene were in the range 0.08–0.6 μM	Hayeshi et al. (2004)
	In vitro	DPPH (1,1-diphenyl-2-picrylhydrazyl) antioxidant assay showed a maximum of 72.85% inhibition (IC_{50} = 139.74 μg/mL) of DPPH-free radicals at the highest concentration of 1000 μg/mL as compared to the ascorbic acid (IC_{50} = 13.72 μg/mL)	Alam et al. (2023)
Antiparasitic	*In vitro, in vivo* and *in silico*	Diospyrin (IC_{50} = 12.8 μM) exhibited antiparasitic activity against *Leishmania donovani*. It exhibited selective inhibition of intracellular amastigotes (IC_{50} = 0.18 μM). The treatment of infected BALB/c mice with diospyrin at 2 mg/kg/day reduced the hepatic parasite load by about 38%	Hazra et al. (1987); Hazra et al. (2013)
	In vitro antiparasitic assay	Antimalarial (antiplasmodial against *Plasmodium falciparum*) potential of diospyrin was reported	Hazra et al. (1995a); Likhitwitayawuid et al. (1999)
	In vitro antiparasitic assay	Diospyrin showed antiparasitic activity against *Trypanosoma cruzi* (ED_{50} = 27 μM) and *T. brucei brucei* (ED_{50} = 50 μM)	Yardley et al. (1996)
	In vitro	Diospyrin was found to inhibit the catalytic activity of DNA topoisomerase of *Leishmania donovani*	Ray et al. (1998)
	In vitro antiparasitic assay	Diospyrin depleted the cellular ATP contents of *P. carinii* f. sp. *carinii* populations *in vitro*, expressed as percent inhibition compared to ATP levels in untreated controls. It can be noted that although initially *P. carinii* f. sp. *carinii* was classified as a protozoan, it has now been classified as a fungus based on nucleic acid and biochemical analysis	Cushion et al. (2000)
	In vitro	Diospyrin inhibited ubiquinone biosynthesis in *P. carinii* f. sp. *carinii*	Kaneshiro et al. (2000)
	In vitro	Diospyrin was found to inhibit the growth of *Leishmania major* (7, 29, 22.7% inhibition at 1.2, 2.5 and 5 μg/mL, respectively), which causes cutaneous leishmaniasis	Hazra et al. (2002)
	In vitro antiparasitic assay	Diospyrin exhibited *in vitro* antiprotozoal activity against protozoan parasites of the genera *Trypanosoma*, *Leishmania* and *Plasmodium*. The strongest activity was against *T. brucei* and *L. donovani* with IC_{50} of 1.12 and 8.82 μM	Ganapaty et al. (2006a)
	In vitro antiparasitic assay	Diospyrin, isolated from *Diospyros montana*, induced apoptosis-like death in *Leishmania donovani* promastigotes	Mukherjee et al. (2009)
	Antiparasitic and *in vitro* cytotoxicity assays	Diospyrin showed antiprotozoal activity against *Trypanosoma brucei rhodesiense* (IC_{50} = 0.42 μg/mL), *Trypanosoma cruzi* (IC_{50} = 15.3 μg/mL), *Leishmania donovani* (IC_{50} = 3.3 μg/mL), and cytotoxicity against *Plasmodium falciparum* K1 (MIC = 2.194 μg/mL) and L-6 (IC_{50} = 2.393 μg/mL) cells. At the concentrations of 5 and 10 μg/mL, diospyrin displayed anthelmintic properties (paralysis and death) against *Pheritima posthuma*	Dev et al. (2012)
	In silico	Diospyrin showed a strong bonding affinity to *Leishmania donovani* pteridine reductase 1, revealing its therapeutic potential against visceral leishmaniasis	Adibpour et al. (2012)
	In silico	Diospyrin showed guanylate kinase inhibitory activity and thus showed the antiparasitic potential, as guanylate kinase is a key enzyme that catalyzes the ATP-dependent phosphorylation of GMP (guanosine monophosphate) into GDP (guanosine diphosphate) for the survival of parasites	Ansari et al. (2017)
	In silico	Antiparasitic activity against *Leishmania* spp. Diospyrin showed outstanding docking potential with *Leishmania donovani* HSP100. The docking scores of diospyrin were better than antileishmanial miltefosine and had better ADMET properties as a drug candidate	Jha et al. (2020)
	Antiparasitic assay	Diospyrin showed antiparasitic activity against *Leishmania* spp. (Leishmaniasis), *Trypanosoma cruzi* (Chagas disease), *Plasmodium falciparum* (Malaria), *Toxoplasma gondii* (Toxoplasmosis), and *Toxocara canis* (Toxocariasis), comparable to that of the conventional drugs	Ortiz-Perez et al. (2021)

Table 2 continued

Activity	Type of assays	Summary	References
Antiviral	*In vitro*	Diospyrin displayed activity against herpes simplex virus Type 1 (HSV-1) *in vitro* on primary vervet monkey kidney cells, and cytotoxicity against these cells was observed with an ID_{50} value of 0.02 mg/mL. However, there was no antiviral activity	Lall et al. (2005a)
	In vitro	A weak inhibition of HIV-1 reverse transcriptase by diospyrin (IC_{50} = > 100 µg/mL)	Mahapatra et al. (2012)
Neuroprotective	*In vitro*, *Iin vivo* and *Iin silico* assays	Acetylcholinesterase (AchE, IC_{50} = 95 µg/mL) and butyrylcholinesterase inhibitory (BchE, IC_{50} = 110 µg/mL) activities compared to that of the standard drug donepezil (IC_{50} = 95 & 85 µg/mL, respectively). Behavioural animal models test, such as the elevated plus maze, Morris water maze and paddling Y- maze test revealed that diospyrin treatment demonstrated gradual improvement in memory and enhanced motor functionality. *In silico* studies demonstrated exceptional binding affinities of diospyrin for both AChE and BChE enzymes	Alam et al. (2023)
Sedative	*In vivo*	Diospyrin displayed mild to moderate sedative activity in the open field animal model	Uddin et al. (2014)

Rauf 2021). In addition to these major bioactivities, **1** was shown to possess, anti-allergic (Pinho et al. 2014), antidiabetic (Bawazeer et al. 2019), antifungal (Wang et al. 2011), antinociceptive (Uddin et al. 2014), antioxidant (Hayeshi et al. 2004; Uddin et al. 2022; Alam et al. 2023), antiviral (Lall et al. 2005a; Mahapatra et al. 2012), neuroprotective (Alam et al. 2023), sedative (Uddin et al. 2014) and urease inhibitory activities (Rauf et al. 2017). The major therapeutic potential of diospyrin (**1**) has been critically appraised below under the following subsections, and possible mechanisms of action behind these activities have also been discussed.

Antimicrobial property

Antimicrobial property refers to the ability of a substance or drug to inhibit or kill the growth and reproduction of microorganisms, e.g., bacteria, fungi and viruses (Sanchez Armengol et al. 2021). The antimicrobial potential of any substance is usually determined *in vitro* using one of the following methods: disk, well, plug or agar contact diffusion method, thin layer chromatography bioautography method, serial dilution methods like E-test, broth macrodilution, broth microdilution and agar dilution, time-kill method, adenosine triphosphate (ATP) bioluminescence method, cross steak, flow cytometry and poison food method. The assessment of the antimicrobial activity of **1**, as reported in the literature, was mostly *in vitro* studies using disc or well diffusion and serial dilution methods (Table 2).

Antibacterial

There are about a dozen publications on the antibacterial property of diospyrin (**1**), evaluated *in vitro* or *in silico*, available in the literature (Table 2). The focus of most of those evaluations was on its anti-*Mycobacterium* potential (Adeniyi et al. 2000; Lall and Meyer 2001; Lall et al. 2003, 2005b; Van der Kooy et al. 2006; Mahapatra et al. 2007; Bapela et al. 2008a; Thaver 2010; Karkare et al. 2013b; Sundarrajan et al. 2015), looking at its prospective therapeutic applications as a novel antitubercular agent and for the treatment of other mycobacterial infections that include a heterogenous group of infectious diseases caused by *Mycobacterium* species. The earliest report on the *in vitro* antibacterial activity of **1**, isolated from the root bark of *Euclea natalensis,* was published by Khan et al. (1978), where they described its antibacterial activity against *Neisseria gonorrhoeae*, *Shigella* spp and *Staphylococcus aureus.* However, it appears that the antibacterial studies on **1** gained momentum from the year 2000 when the antibacterial activity of this compound isolated from the root of *D. piscatoria* grown in Nigeria was reported using the *in vitro* broth dilution method against *Streptococcus pyogenes* and *Streptococcus pneumoniae* (MIC range = 1.56–50 µg/mL) and also against *Salmonella choleraesuis* serotype *typhi* and *Mycobacterium chelonae* with the MIC values ranging between 25 and 100 µg/mL (Adeniyi et al. 2000). The activity against *M. chelonae* was of particular interest as it prompted further studies with another *Mycobacterium* species, *M. tuberculosis*,

the causative microorganism of tuberculosis (Lall and Meyer 2001; Lall et al. 2003, 2005b; Van der Kooy et al. 2006; Mahapatra et al. 2007; Bapela et al. 2008a; Thaver 2010). It is worth mentioning that *M. chelonae*, a nontuberculous *Mycobacterium* species, is commonly associated with skin and soft tissue infections (Jones et al. 2019; Akram et al. 2023). Diospyrin (**1**), purified from the roots of *E. natalensis*, showed inhibitory activity against the drug-resistant *M. tuberculosis* H37 Rv strain with an inhibitory concentration of 100 µg/mL; it was also active against other drug-resistant strains of *M. tuberculosis* at a concentration of 100 µg/mL (Lall and Meyer 2001, 2003). In this study, the radiometric respiratory technique with the BACTEC apparatus was used for susceptibility testing of *M. tuberculosis* (Middlebrook et al. 1977; Siddiqi et al. 1981). Later, Lall et al. (2005b) reported the MIC value of 8 µg/mL for **1** against *M. tuberculosis* H37 Rv strain but using the microplate Alamar blue Assay (Collins and Franzblau 1997). It is interesting to note that 7-methyljuglone, which is the monomer of **1**, was found to have a much more potent antibacterial activity against this microbial strain (MIC = 0.5 µg/mL) as well as against several other clinical isolates of *M. tuberculosis*, than the known anti-*Mycobacterium* antibiotic rifampicin (MIC = 0.125 µg/mL). The anti-*Mycobacterium* effect of diospyrin (**1**) was weakly bactericidal. Nonetheless, its effectiveness against the drug-resistant *Mycobacterium* strains is noteworthy, as multi-drug-resistant (MDR) strains of *M. tuberculosis* have become a major health concern worldwide, particularly in poor countries where tuberculosis remains a major cause of death in the population (Rattan et al. 1998; Schami et al. 2023). A few other studies surprisingly reported the same MIC value of 8 µg/mL for **1** against *M. tuberculosis* (Van der Kooy et al. 2006; Mahapatra et al. 2007). One of the key mechanisms of anti-*Mycobacterium* effect of **1** was found to be the inhibition of the uptake of K^+ by *M. tuberculosis* and *M. smegmatis*, with inhibition detected at submicrogram concentrations (Bapela et al. 2008a; Thaver 2010). It is known that any disruption of an *M. tuberculosis* K^+ uptake system may reduce its ability to respond to several key ionic cues and produce diminished host colonization (McGilvary et al. 2019).

The antimicrobial activity of **1** was evaluated against a broad panel of MDR and extensively drug-resistant tuberculosis strains, rapid-growing mycobacteria and other bacterial isolates (Dey et al. 2014). The MIC of this compound against MDR mycobacterial strains ranged between 4 and 256 µg/mL. Diospyrin (**1**) also demonstrated antibacterial activity against several other bacterial strains, including *Bacillus, Enterococcus, Escherichia* and *Staphylococcus* species (MIC = 64–256 µg/mL), some of which are the producers of β-lactamase, extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase, and metallo-beta-lactamase (MBL) enzymes. Later, Rauf et al. (2016) reported antibacterial activity against *Staphylococcus epidermis* (IC_{50} = 72 µg/mL).

Urease enzyme, which belongs to the urea amidohydrolase class of enzymes, has a crucial role in the survival of *Helicobacter pylori* that induces gastrointestinal diseases, in particular, gastritis, duodenal and peptic ulcers, and gastric cancer (Mahernia et al. 2015). In a bioassay-guided isolation study for discovering novel and selective urease inhibitors from the roots of *D. lotus*, compound **1** was isolated as an active compound with significant phosphodiesterase -I and carbonic anhydrase-II-inhibitory activity (IC_{50} = 15.2 and 13.4 µM, respectively), but this compound showed only 31.9% urease inhibition at the 0.5 µM concentration, and an IC_{50} value could not be established. The IC_{50} value for the positive control thiourea was 21 µM against urease. Therefore, the therapeutic prospect of **1** as an anti-*Helicobacter pylori* agent does not appear to be that great.

A few studies attempted to establish the plausible modes of action of diospyrin (**1**) for its anti-*Mycobacterium* activity (Karkare et al. 2013a, b; Wang et al. 2022) (Fig. 5). This bisnaphthoquinone exhibited antibacterial activity by inhibiting bacterial protein synthesis. It inhibited DNA gyrase, mediated through a binding to the *N*-terminal domain of GyrB, which contains the ATPase active site, but is not a competitive inhibitor of the ATPase reaction (Karkare et al. 2013a, b; Wang et al. 2022). In an *in silico* study, a combination of structure-based virtual screening with physicochemical and pharmacokinetic studies against rhamnose pathway enzymes identified potential leads against *M. tuberculosis*, and **1** was one of them with good binding affinity towards the rhamnose pathway proteins (Sundarrajan et al. 2015). A similar mode of action was also demonstrated using a combination of *in vitro* and *in silico* studies, with **1** showing anti-*Staphylococcus aureus* activity through binding in the *N*-terminal domain of GyrB (Chung et al. 2012). It

could partially inhibit the ATPase activity of GyrB in an allosteric manner. Diospyrin (**1**) could bind to a novel binding site between the ATPase and transducer domains. It could also inhibit both the relaxation and the DNA cleavage ability of gyrase, suggesting that it could inhibit gyrase with a novel mechanism. Overall, the antibacterial potential of **1**, like many plant-derived antimicrobial agents, is much less potent than conventional antibiotics. Diospyrin may not be powerful enough for its therapeutic use as an antibacterial agent, but its activity against drug-resistant bacterial strains makes this compound an ideal template for further structural modifications aiming at the discovery of new antibacterial agents, especially effective against drug-resistant microbes, particularly *Mycobacterium* species that cause tuberculosis.

Antifungal

There appears to be only one published report on the assessment of the antifungal property of diospyrin (**1**) (Wang et al. 2011). In that study, napthoquinone **1** and other compounds isolated from the roots of *D. virginiana* were assessed *in vitro* for their antifungal activities against seven fungal strains, including *Botrytis cinerea*, *Colletotrichum fragariae*, *C. gloeosporioides*, *C. acutatum*, *Fusarium oxysporum*, *Phomopsis obscurans*, and *P. viticola,* using a 96-well micro-dilution broth assay. While diospyrin (**1**) displayed a weak antifungal activity against the tested fungal strains at the tested concentrations, 7-methyljuglone, the monomer of **1**, and isodiospyrin, an isomer of **1**, at a concentration of 30 µM, displayed a significant antifungal activity against some of the tested strains, e.g., *Phomopsis* species.

Antiviral

There are at least two reports on assessing any antiviral prospects of diospyrin (**1**) published in the literature (Lall et al. 2005a; Mahapatra et al. 2012). Lall et al. (2005a) reported an antiviral activity study on this compound obtained from *E. natalensis* against herpes simplex virus Type 1 (HSV-1) *in vitro* on primary vervet monkey kidney cells and cytotoxicity against these cells was observed with an ID_{50} value of 0.02 mg/mL. Although diospyrin (**1**) was cytotoxic to vervet monkey kidney cells, it did not show any activity against the virus, and this finding was in line with a previous report of inactivity of two other naphthoquinones against the HSV-1 virus (Send et al. 1996). However, juglone, which is structurally closely related to 7-methyljuglone, was previously reported to possess inhibitory activity against this virus (Vanden Berghe and Vlietinck 1986). Later, a weak HIV-1 reverse transcriptase inhibitory property of **1**, isolated from the roots of *E. natalensis,* was reported using recombinant HIV-1 enzyme (IC_{50} = > 100 µg/mL) (Mahapatra et al. 2012). However, 7-methyljuglone showed exceptionally potent HIV-1 reverse transcriptase inhibitory activity. The dimerization of 7-methyljuglone to form diospyrin (**1**) was found to significantly reduce its antiviral activity (Mahapatra et al. 2012). From the limited antiviral activity assessments, it appears that compound **1** may not have any therapeutically relevant antiviral potential.

Anticancer, antitumour activity, and cytotoxicity

There are two review articles covering the anticancer activity of diospyrin (**1**) and its structural analogues, the first one was published in 2010 (Sagar et al. 2010) and the most recent one was published last year (Rauf et al. 2024). However, to provide a comprehensive and holistic overview of the therapeutic potential of **1** against various illnesses, for the sake of completeness, as well as to gain a deep insight, the anticancer, antitumour and cytotoxic activities of this compound have been critically and comprehensively appraised in this review article. While one of the first reports on the anticancer activity potential of **1** was published by Hazra et al. (1984, 1994), where they demonstrated cytotoxic activity of this compound against the EAC (Ehrlich ascites carcinoma) in Swiss mice, several other subsequent publications demonstrated the anticancer potential of this bisnaphthoquinone mainly against HeLa, HL-60, K-562, MCF-7 and MG-63 cell lines (Rauf et al. 2024) (Table 2).

In a study involving diospyrin (**1**) and its synthetic derivatives, **1** showed significant inhibitory activities against murine tumours in the Swiss A mice model *in vivo* (Pal et al. 1996). The growth of the EAC tumour in mice caused substantial damage to the histopathological status of the liver, with moderate changes in the spleen and kidneys. The treatment with **1** was found to restore to normality. Diospyrin (**1**) was assessed *in vitro* utilizing the activation of Epstein-Barr virus (EBV) early antigen expression in EBV-

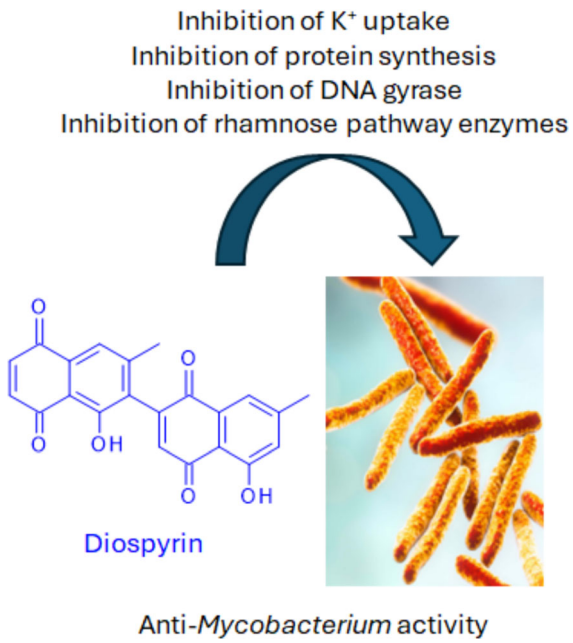


Fig. 5 Plausible mechanisms of anti-*Mycobacterium* activity of diospyrin (**1**)

genome-carrying human lymphoblastoid cells (Raji cell line) and this compound inhibited tumour promotion (Norhanom and Hazra 1997). It can be noted that lymphoblastoid cell lines are produced by Epstein–Barr virus (EBV) transformation of the B-lymphocytes within the peripheral blood lymphocyte population. The anticancer potential of **1** was demonstrated from its topoisomerase I inhibitory capacity (Bailly 2000; Tazi et al. 2005). Topoisomerase I (topoisomerases in general) is an extensively studied target for anticancer drug development (Delgado et al. 2018; Talukdar et al. 2022). Diospyrin (**1**) inhibited camptothecin-dependent topoisomerase I-mediated DNA cleavage (Tazi et al. 2005); the formation of nicked DNA, arising from single-stranded cleavage of supercoiled DNA by camptothecin-trapped topoisomerase I, was reduced (> 50%) when the reaction took place in the presence of **1**. Chakrabarty et al. (2002) demonstrated that **1** induced apoptosis (Elmore 2007) in human cancer cell lines, including HeLa (IC_{50} = 100 µM), HL-60 (IC_{50} = > 100 µM), K-562 (IC_{50} = > 100 µM) and MCF-7 (IC_{50} = 100 µM). The IC_{50} value for the positive control, doxorubicin, was 1 µM against all these cell lines, indicating that the apoptotic potency of these compounds was much less than the positive control. The cytotoxicity was maximum after 48 h of treatment of the cells. The apoptotic pathway, involved in the cytotoxic activity of **1**, was further demonstrated in a study where this compound was found to regulate apoptosis at the endoplasmic reticulum as well as mitochondria by modulating cytosolic calcium in MCF-7 cells (Kumar et al. 2009, 2012). In cells grown in relevant media, **1** initiated cytotoxicity as assessed by Trypan Blue dye exclusion, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide reduction and DNA synthesis. However, the potency of **1** was found to be much less than its derivatives. *In silico* studies have become popular in providing predictive mechanisms of action of bioactive compounds (Sarker and Nahar 2024). In such an *in silico* study, Kumar et al. (2019) observed viral E6 protein (which is implicated in cervical cancer) inhibition by **1** in energetically optimized structure-based pharmacophore modelling, ligand docking and molecular dynamics simulations studies. It exhibited H-bond interactions with TYR32, CYS51, SER74 and ARG131 amino acid residues.

Liposomal encapsulation of diospyrin (**1**) was found to enhance inhibitory activity against murine tumour *in vivo* and human cancer cell lines *in vitro* (Hazra et al. 2005). Aiming at reducing the toxicity towards normal cells and enhancing the efficacy towards tumour cells, **1** was encapsulated in a liposomal vesicle and its antitumour potential was observed in the growth of Ehrlich ascites tumour in Swiss mice. The treatment with liposome-encapsulated diospyrin (**1**) significantly enhanced the longevity of the mice bearing the tumour. The substantially altered activities of the liver function enzymes, e.g., LDH, AP, GOT and GPT in the blood serum of the tumour-bearing mice were restored to near normal by the treatment of liposome-encapsulated **1**. This study demonstrated the importance of liposomal encapsulation of **1** in enhancing (> 10%) its antitumour property *in vivo* and reducing its toxicity.

It has been shown that the anticancer activity of diospyrin (**1**) is mediated through a series of pathways including mainly apoptotic, NF-κB, MAPK/ERK, PAM (PI3K/Akt/mTOR) and Wnt/b-catenin pathways (Chakrabarty et al. 2002; Rauf et al. 2024), and through inhibition of enzymes like topoisomerase I (Bailly 2000; Tazi et al. 2005; Delgado et al. 2018) (Fig. 6). The apoptotic pathway is related to the up- or down-regulation of distinct genes such as p53 and bcl-2 (Chakrabarty et al. 2002). The mode of antitumour

action of **1** was attributed to the facile electron-transfer mechanism operating in the highly conjugated structures of **1** (Kovacic et al. 1988; Thompson et al. 1992; Norhanom and Hazra 1997). The cytotoxicity of **1** was shown to involve oxidative stress (Chakrabarty et al. 2002). It is known that naturally occurring quinones, including naphthoquinones and bisnaphthoquinones (e.g., diospyrin), can produce ROS plausibly through a redox cycling mechanism and thus offer cytotoxicity by interacting with the nucleic acids of cancer cells (Rauf et al. 2024).

Hazra et al. (2007) conducted *in vitro* and *in vivo* studies to establish the antitumour potential of diospyrin (**1**). The *in vivo* study used the EAC model, and *in vitro* cytotoxicity was determined against EAC and MCF7 cancer cells by MTT assay. Diospyrin (**1**) was found to display tumour inhibitory activity and cytotoxicity, predominantly mediated through ROS generation. Later, a similar *in vivo* study established a significant inhibitory effect of **1** against EAC in rodents, and at the same time, *in vitro* cytotoxicity against tumour cells A375 (IC_{50} = 0.82 μM), Hep2 (IC_{50} = 3.58 μM), EAC (IC_{50} = 0.84 μM), and epidermoid laryngeal carcinoma (IC_{50} = 3.58 μM) cells and noncancerous PBMC (IC_{50} = 78.32 μM) cells (Sarma et al. 2007, 2008; Rauf et al. 2024). Diospyrin (**1**) showed selective cytotoxicity towards the cancer cells, evident from the high IC_{50} value against the PBMC normal human lymphocytes. This selective cytotoxicity suggested the potential of **1**, which could be used as a structural template for developing novel antitumour agents. This compound generated substantial amounts of ROS in EAC cells, more or less commensurate with their respective IC_{50} values (Sarma et al. 2008).

Several *in vitro* cytotoxicity assays against various cancer cell lines exhibited considerable cytotoxicity of diospyrin (**1**), and the cell lines included HL-60 (IC_{50} = > 100 μM), K-562 (IC_{50} = > 100 μM), mouse fibroblast cell line (Balb/c 3T3; IC_{50} = 0.9 μM), skeletal myoblasts (L-6; IC_{50} = 2.93 μg/mL) cell lines (Table 2) (Sagar et al. 2010; Dev et al. 2012; Landa et al. 2012). The cytotoxicity of **1** was further studied against rat skeletal myoblasts (L-6) cells and the IC_{50} value was 6.38 μM, which was much less toxic than the positive control podophyllotoxin (Ganapaty et al. 2006a, b). *In vitro* cytotoxicity of **1** was assessed against thirteen human cancer cell lines, breast cancer cell lines: MDA-MB-435, MCF-7, ZR-75–1, colon cancer cell lines: HCT-15, HT-29, Colo-205, CNS cancer cell line U373, neuroblastoma cell line IMR32, oral cancer cell line KB, prostate cancer cell lines: DU-145, PC-3, lung cancer cell line A549 and melanoma cell line SK-MEL-2 and the GI_{50} (50% growth inhibitory concentration) values were 0.18, 2.54, 2.1, 2.11, 33.9, 0.13, 2.23, 0.17, 2.46, 0.20, 2.45 and 2.21 μM, respectively (Hazra et al. 2015). In a recent study, **1** exhibited more potent and selective cytotoxicity to the murine myeloma NS-1 (ATCC TIB-18) cell line than neonatal foreskin cells (ATCC PCS-201), with IC_{50} values of 0.8 μM and 5.8 μM, respectively (Pullella et al. 2020).

Rauf et al. (2021) reported a considerable cytotoxic activity (IC_{50} = 47.40 ppm) of diospyrin (**1**) in the Epstein–Barr-Virus early antigen activation assay. A 60% survival rate of the lymphoblastoid Raji cells at a concentration of 1000 (mol/ratio 32 pmol TPA). In a two-stage carcinogenesis assay on mouse skin, diospyrin significantly delayed (50% effect in the 14th week and 100% effect in the 20th week) the formation of papillomas on the mouse skin. Significantly attenuated thermal-induced protein denaturation (EC_{50} = 298 μg/mL. Exhibited a promising MDR reversal effect in a dose-dependent manner against the mouse T-lymphoma cell line. Docking results showed that diospyrin had favourable docking statistics as compared with the standard (Rauf et al. 2021).

All *in vitro*, *in vivo* and *in silico* studies performed with **1**, as discussed above and in Table 2, indicate that this compound could be used as a structural lead for developing novel anticancer agents, albeit more preclinical and clinical studies are required with diospyrin (**1**) and its analogues to develop them as clinically relevant cancer chemotherapeutic agents.

Anti-inflammatory activity

Inflammation, manifested by pain, redness, swelling, loss of function and heat, is a natural immune response of the human body to injuries and infections (Furman et al. 2019). Plants produce anti-inflammatory agents, e.g., curcumin from *Curcuma longa* L. (Nisar et al. 2023). The anti-inflammatory activity of diospyrin (**1**) was first reported by Landa et al. (2012), who assessed the cyclooxygenase-1 and -2 (COX-1 and COX-2) inhibitory activity of this compound. It can be mentioned that COX-1 and COX-2 are key enzymes of the arachidonic acid cascade. They are involved in

the production of prostaglandins that cause inflammation, pain and fever (Hawkey 2001). Therefore, COX-1 and COX-2 inhibitors offer anti-inflammatory responses and have the potential as anti-inflammatory agents, e.g., non-steroidal anti-inflammatory (NSAIDs) drugs. Diospyrin (**1**) was found to inhibit COX-1 and COX-2 with IC_{50} values of 1.39 μM and 0.55 μM, respectively. The anti-inflammatory response of **1** through COX-1 and COX-2 inhibition activity was comparable to the positive control, indomethacin. In *in silico* studies, e.g., molecular docking, the mode of inhibition action of **1** was predicted as a competitive binding mechanism comparable to conventional NSAIDs (Landa et al. 2012).

In vivo animal models, e.g., carrageenan-induced paw oedema in mice, can be useful in establishing the anti-inflammatory potential of test samples (Posadas et al. 2004). Diospyrin (**1**), isolated from the roots of *D. lotus*, was found to dose-dependently protect carrageenan-induced paw oedema in mice by 80.54% after 3 h (Uddin et al. 2014), and the finding was broadly comparable to that of the positive control diclofenac (*ca.* 90%). Diospyrin (**1**) treatment at the doses of 5 and 10 mg/kg inhibited carrageenan-induced paw oedema by 40% and 78%, respectively, at the third hour of carrageenan injection. Later, a combination of spectrometric *in vitro* and *in silico*

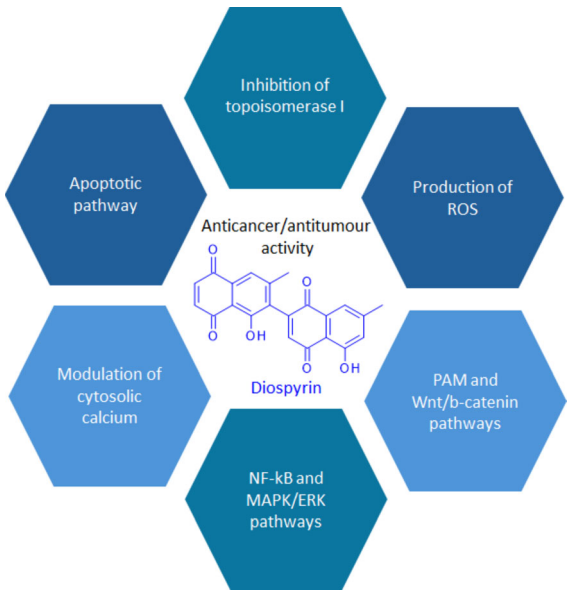


Fig. 6 Major mechanisms of anticancer/antitumour activity of diospyrin (**1**)

(molecular docking) studies revealed the lipoxygenase inhibitory potential of **1** (Uddin et al. 2016). The IC_{50} value determined from the *in vitro* assay was 62.7 μM. In the molecular docking study, this compound interacted with important subsites inside the catalytic pocket of lipoxygenase; the molecular shape and electrostatic condition favourably matched with the electrostatic environment of the active site inside this enzyme, and various molecular interactions were noted between **1** and lipoxygenase. The antiglycation and lipoxygenase inhibitory properties of **1** were also reported by other researchers (Bawazeer and Rauf 2021). Lipoxygenases, usually classified as 5-, 8-, and 15-lipoxygenases based on their selectivity to oxygenated fatty acids in a specific position, are oxidative enzymes having a non-heme iron atom in their active site, and they usually regulate inflammatory responses by generating pro-inflammatory mediators, e.g., leukotrienes (Wisastra and Dekker 2014). Thus, any compound that inhibits lipoxygenases has the potential as an anti-inflammatory agent with therapeutic values, and diospyrin (**1**), based on the *in vitro, in vivo* and *in silico* findings, can be considered one of those anti-inflammatory leads.

Inhibitory activity of **1** on lipopolysaccharide (LPS)-induced inflammation using RAW 264.7 mouse macrophages was established by a series of cell-based *in vitro* assays (Shahidullah et al. 2020). This bisnaphthoquinone moderated the production of nitric oxide (NO), monocyte chemotactic protein-1, macrophage inflammatory protein-1, interleukin (IL)-6, IL-10, granulocyte colony-stimulating factor, granulocyte–macrophage colony-stimulating factor, vascular endothelial growth factor, leukaemia inhibitory factor, and RANTES/CCL5, as well as calcium release in LPS-induced RAW 264.7, at concentrations of up to 10 μM significantly. Diospyrin (**1**) inhibited the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and mRNA expression of C/EBP homologous protein (CHOP) and tumour necrosis factor receptor superfamily member 6 (Fas), in LPS-induced RAW264.7 cells at concentrations of up to 10 μM. The percentages of NO production in LPS-induced RAW 264.7 cells incubated with **1** at the concentrations of 0.1, 1, 5, and 10 μM for 24 h were 99.15%, 97.94%, 85.76%, and 57.35%, respectively, of the control group treated with LPS only. Clearly, the inhibition of NO production by **1** was dose-dependent. Diospyrin (**1**) significantly inhibited calcium release in

LPS-induced RAW 264.7 cells in a dose-dependent manner, 75.84%, 46.06%, 39.5% and 33.63% at the concentrations of 0.1, 2, 5 and 10 mM in 18 h. This bisnaphthoquinone significantly reduced the excessive synthesis of monocyte chemotactic protein (MCP)-1 (61.22% MCP-1 production with a diospyrin concentration of 10 μM), macrophage inflammatory protein (MIP)-1β (only 67.74% MIP-1β production with a diospyrin concentration of 10 μM), granulocyte colony-stimulating factor (G-CSF) (only 43.5% G-CSF production with a diospyrin concentration of 10 μM), granulocyte macrophage colony-stimulating factor(GM-CSF) (only 1.12% GM-CSF production with a diospyrin concentration of 10 μM), vascular endothelial growth factor (VEGF) (only 21.55% VEGF production with a diospyrin concentration of 10 μM), RANTES/CCL5 (only 21.08% RANTES/CCL5 production with a diospyrin concentration of 10 μM), leukaemia inhibitory factor (LIF; IL-6 class cytokine) (only 1.13% LIF production with a diospyrin concentration of 10 μM), interleukin (IL)-6 (only 4.94% IL-6 production with a diospyrin concentration of 10 μM), and IL-10 (only 11.3% IL-10 production with a diospyrin concentration of 10 μM), in LPS-induced RAW 264.7 cells. The percent production was calculated against a 100% production in the control group. Furthermore, diospyrin (**1**) significantly inhibited mRNA expression of CHOP (C/EBP homologous protein, a transcription factor) and Fas in LPS-induced RAW 154.7 cells; the phosphorylation of p38 MAPK in LPS-induced RAW 264.7 cells incubated with **1** at concentrations of 1, 5, and 10 μM for 15 min were 94.76%, 84.67% and 77.77%, respectively, of the control group treated with LPS (0.1 μg/mL). Thus, compound **1** displayed anti-inflammatory properties mediated via inhibition of NO, and cytokines in LPS-induced mouse macrophages via the ER-stressed calcium-p38 MAPK/CHOP/Fas pathway (Shahidullah et al. 2020).

A similar work, incorporating *in vitro, in vivo* and *in silico* assays, was conducted to further demonstrate the anti-inflammatory potential of **1** (Kim et al. 2020; Rauf et al. 2021). Diospyrin (**1**) was shown to modulate inflammation in poly I:C-induced macrophages via the endoplasmic reticulum stress-induced calcium-chop pathway. It reduced nitric oxide (NO) production from RAW 264.7 after 24 h (92, 94, 93% at 1, 5 and 10 μM concentrations, respectively), granulocyte–macrophage colony-stimulating factor production, and intracellular calcium release in poly I:C-induced RAW 264.7. The phosphorylation of p38 MAPK and ERK1/2 was also significantly suppressed. It inhibited mRNA levels of NO-synthase 2, C/EBP homologous protein (CHOP), calcium/calmodulin-dependent protein kinase II alpha, signal transducers and activators of transcription 1 (STAT1), STAT3, STAT4, Janus kinase 2, first apoptosis signal receptor, c-Jun, and c-Fos in poly I:C-induced RAW 264.7.

While further work, particularly preclinical and clinical trials, is necessary to establish the true therapeutic potential of **1** as an anti-inflammatory agent, the studies reported to date (Kim et al. 2020; Shahidullah et al. 2020; Rauf et al. 2021) certainly revealed the anti-inflammatory potential as well as the mechanisms (Fig. 7) of action of this compound in *in vitro*, *in vivo* and *in silico* studies as outlined above. It is, therefore, reasonable to state that **1** could, at least, be used as a template for the generation of new non-steroidal anti-inflammatory lead compounds.

Antiparasitic property

Parasitic diseases, caused by various parasites like worms and single-celled organisms like protozoa that are living and reproducing in the host's body, may spread by contaminated food, water, or bites from infected insects like mosquitoes. While malaria, a life-threatening illness caused by *Plasmodium* species transmitted through mosquito bites, is the most talked-about and well-researched parasitic disease, several other less-studied and somewhat neglected parasitic diseases also adversely affect human life and cause fatality, e.g., Chagas disease and Leishmaniasis, caused, respectively, by *Trypanosoma* and *Leishmania* species (Ismail et al. 2020). Since the discovery of the first antimalarial drug, quinine from the bark of the *Cinchona succirubra* tree, the search for further antimalarial agents from plant sources began. Artemisinin, from *Artemisia annua*, is another success story in the fight against malaria, and this discovery earned Tu Youyou a Nobel Prize in Physiology or Medicine in 2015 (Liu and Liu 2016). The discovery of antimalarial agents from plants led to further work involving plants and plant-derived compounds for their potential against parasites like *Leishmania* and *Trypanosoma* (Ismail et al. 2020). The need for new and affordable antiparasitic drugs was further triggered by the emerging drug resistance in parasites and

the side effects of conventional drugs (Hazra et al. 2013). As a part of that continued exploration, diospyrin (**1**) was assessed for its effectiveness against *Leishmania* (Hazra et al. 1987, 2002, 2013; Ray et al. 1998; Ganapaty et al. 2006a; Mukherjee et al. 2009), *Plasmodium* (Hazra et al. 1995a; Likhitwitayawuid et al. 1999; Cushion et al. 2000; Kaneshiro et al. 2000; Ganapaty et al. 2006a) and *Trypanosoma* (Yardley et al. 1996; Ganapaty et al. 2006a; Dev et al. 2012) parasites mainly using parasitic assays (Table 2).

One of the earliest studies on diospyrin (**1**) for its antiparasitic activity was conducted against *Leishmania donovani*, which causes 'kala-azar', a fever that is still endemic in parts of Bangladesh, China and India (Hazra et al. 1987, 2013). The *in vitro* susceptibility of cultured *L. donovani* towards **1** was found to be significant; it exhibited antiparasitic activity against *L. donovani* with an IC_{50} value of 12.8 µM. Diospyrin (**1**) showed selective inhibition of intracellular amastigotes (IC_{50} = 0.18 µM). In this study, it was found that the growth of parasitic cells in a liquid culture medium was stopped by **1** at a low concentration (1 µg/mL), and **1** at a dose of 5 µg/mL caused almost total inhibition of respiration within cells. Later, in addition to an *in vitro* study, an *in vivo* animal study was conducted with **1**, isolated from *D. montana* (Hazra et al. 2013). It was observed that the treatment of intracardially infected BALB/c mice with **1** at 2 mg/kg/day reduced the hepatic parasite load by about 38%. An *in silico* molecular docking study on selected enzymes of trypanothione metabolism, viz., trypanothione reductase and ornithine decarboxylase, followed by enzyme kinetic studies, was performed with **1**. It is interesting to note that in another study conducted by Yardley et al. (1996), no antiparasitic activity was found for **1** against intracellular amastigotes of *L. donovani* at the tested concentrations. Diospyrin (**1**) was also found to induce apoptosis-like death in *L. donovani* promastigotes (Mukherjee et al. 2009).

Diospyrin (**1**), purified from the stem bark of *D. montana*, was found to inhibit the growth of *L. major* (7, 29, 22.7% inhibition at 1.2, 2.5 and 5 µg/mL, respectively), which causes cutaneous leishmaniasis (Hazra et al. 2002). This study concluded that **1** could be used as a 'lead molecule' for drug design based on the structure–activity-relationship information appearing in the literature on its structural analogues possessing antileishmanial activity *in vitro* against *L. major* parasites. It can be noted that cutaneous leishmaniasis is the most common form of leishmaniasis that causes skin lesions and ulceration on the skin.

Antimalarial (antiplasmodial against *Plasmodium falciparum*) potential of diospyrin (**1**) was first reported by Hazra et al. (1995a) and later by Likhitwitayawuid et al. (1999). Its activity against *P. carinii* f. sp. *carinii* was also reported (Kaneshiro et al. 2000).

The *in vitro* anti-trypanosomal activity of **1** was reported against extracellular *T. brucei brucei* bloodstream form trypomastigotes and *T. cruzi* in macrophages with ED_{50} values of 49.9 and 26.6 µM, respectively (Yardley et al. 1996). The ED_{50} of the positive controls, pentamidine and nifurtimox, were 0.02 and 2.7 µM, respectively.

Diospyrin (**1**) exhibited *in vitro* antiprotozoal activity against protozoan parasites of the genera *Trypanosoma*, *Leishmania* and *Plasmodium*. The strongest activity was against *L. donovani*, *Plasmodium falciparum* and *T. brucei rhodesiense* with IC_{50} values of 8.82, 5.85 and 1.12 µM (Ganapaty et al. 2006a). It was also weakly active against *T. cruzi* (IC_{50} = 40.9 µM). Dev et al. (2012) further demonstrated the anti-protozoal activity against *T. brucei rhodesiense* (IC_{50} = 0.42 µg/mL), *T. cruzi* (IC_{50} = 15.3 µg/mL), *L. donovani* (IC_{50} = 3.3 µg/mL), and cytotoxicity against *P. falciparum* K1 (MIC = 2.194 µg/mL) and L-6 (IC_{50} = 2.393 µg/mL) cells. At the concentrations of 5 and 10 µg/mL, diospyrin (**1**) displayed anthelmintic properties (paralysis and death) against *Pheritima posthuma*. Diospyrin (**1**) was reported to have significant antiparasitic activity against *Leishmania* spp., *P. falciparum*, *T. cruzi*, *Toxoplasma gondii*, and *Toxocara canis* comparable to that of conventional drugs (Ortiz-Perez et al. 2021).

In recent years, *in silico* screening of natural products to predict their bioactivity, formulation and preformulation factors, bioavailability, mechanisms of action and overall therapeutic potential has become popular (Sarker and Nahar 2024). In line with this development, studies with diospyrin (**1**) for their therapeutic efficacy and toxicity have also adopted *in silico* options (Adibpour et al. 2012; Ansari et al. 2017; Jha et al. 2020). In such an *in silico* study, compound **1** was reported to show a strong bonding affinity to *L. donovani* pteridine reductase 1 (PTR1; binding energy = − 8.37 kcal/mol; final dock energy = −

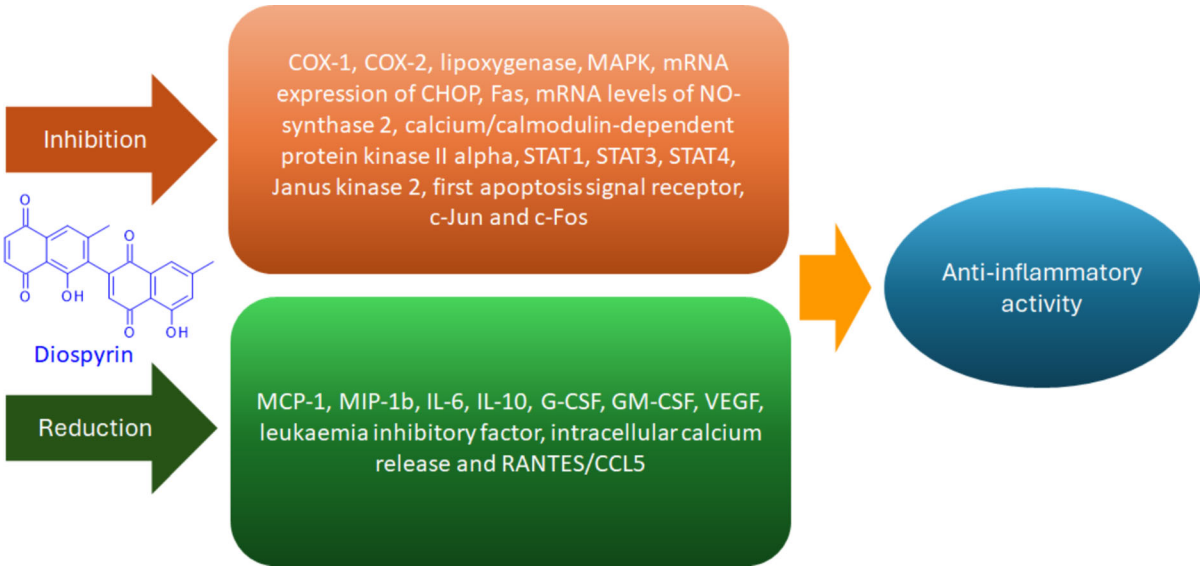


Fig. 7 Major mechanisms of anti-inflammatory activity of diospyrin (**1**)

10.48 kcal/mol), revealing its therapeutic potential against visceral leishmaniasis (Adibpour et al. 2012). PTR1 of *Leishmania* demonstrably differs structurally and functionally from the other protozoan proteins, and PTR1 inhibitors could potentially be developed as antileishmanial chemotherapeutic agents. A few years later, Ansari et al. (2017) performed *in silico* studies and demonstrated the guanylate kinase inhibitory activity of **1** and thus showed the antiparasitic potential, as guanylate kinase is a key enzyme that catalyzes the ATP-dependent phosphorylation of GMP into GDP for the survival of parasites. Diospyrin (**1**) showed higher binding activities (docking score = 49.314; fitness score = 52.69) with the amino acids ARG45, GLU102, TYR35, TYR54, SER38, TYR82, ILE103, ASP104, LYS106, LYS18, PRO13 and SER14. The sigma pi interaction between ILE103 with **1** established greater stable complex formation. In another similar *in silico* study aiming at the identification of potential inhibitors for anti-leishmanial therapeutics (Jha et al. 2020), this 1,4-naphthoquinone was shown to possess outstanding docking potential with *L. donovani* HSP100. It is known that HSP100 is released when the *Leishmania* parasite encounters heat stress in the macrophage of a mammalian host, making it an effective target for anti-leishmanial drug development. The docking scores of **1** were better than antileishmanial miltefosine and had better ADMET properties as a drug candidate. It is worth noting that all *in silico* studies need to be followed up with appropriate *in vitro* or *in vivo* studies to establish the findings beyond doubt.

To understand the mechanism of how **1** shows antiparasitic activity against *L. donovani*, it was shown that this compound could reversibly inhibit the catalytic activity of type 1 DNA topoisomerase of this parasite using an assay (Chakraborty et al. 1993) involving the relaxation of supercoiled DNA in a Mg12-dependent, ATP-independent reaction (Ray et al. 1998). Like camptothecin, diospyrin (**1**) could induce topoisomerase I-mediated DNA cleavage *in vitro*. It was suggested that this dimeric 1,4-napthoquinone could offer its inhibitory effect by binding with the enzyme and stabilizing the topoisomerase I-DNA "cleavable complex. Generally, hydroxynaphthoquinonoids like **1** are analogous to reduced coenzyme Q (ubiquinone), which has several functions in protozoan metabolism, mainly in the electron transfer system (Ellis 1994). These quinoids are expected to inhibit parasite growth by causing disruptions in their mitochondrial electron transport chain. It was also postulated that the generation of free radicals during the interaction of these naphthoquinones with the respiratory chain might be responsible for their activity against *L. donovani* (Croft et al. 1985) and *T. cruzi* (Docampo and Moreno 1984). It was shown that **1** could exert its anti-plasmodial activity by depleting the cellular ATP contents of

Plasmodium carinii f. sp. *carinii* populations *in vitro*, expressed as percent inhibition compared to ATP levels in untreated controls (Cushion et al. 2000). It was also shown that this compound could inhibit ubiquinone (a vital lipid that helps with cellular energy metabolism) biosynthesis in *Plasmodium carinii* f. sp. *carinii* (Kaneshiro et al. 2000). It can be noted that although initially *P. carinii* f. sp. *carinii* was classified as a protozoan, it has now been classified as a fungus based on nucleic acid and biochemical analysis. Diospyrin (**1**), isolated from *Diospyros montana*, induced apoptosis-like death in *L. donovani* promastigotes (Mukherjee et al. 2009), suggesting that this could be one of the main mechanisms by which this compound kills these parasitic cells. The mode of cell death in promastigotes was assessed through the externalisation of membrane-associated phosphatidylserine, mitochondrial membrane depolarisation, DNA laddering and in situ labelling of DNA fragmentation by terminal deoxyribonucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) methods (Mukherjee et al. 2009).

Based on the findings from a series of *in vitro*, *in vivo* and *in silico* studies as outlined above, it is clear that diospyrin (**1**) has therapeutic potential for treating parasitic diseases like leishmaniasis, malaria and Chagas disease. The plausible mechanisms of action of this compound have also been established scientifically. However, further studies, especially preclinical and clinical evaluations, are essential to develop **1** as an antiparasitic drug.

Miscellaneous activities

The antiallergic activity of diospyrin (**1**), isolated from the root bark of *D. chamaethamnus*, was, for the first time, demonstrated in *in vitro* cell line assay using the rat basophilic leukaemic RBL-2H3 cell line, where degranulation was induced by two stimuli, calcium ionophore and an immunologic stimulus, and evaluating inhibition of enzymes involved in allergic responses (Pinho et al. 2014) (Table 2). Quercetin, a well-known natural antioxidant, was used as a positive anti-degranulation control. Diospyrin (**1**) exhibited basophil-degranulation and allergy-related enzyme-modulating properties. It inhibited soybean lipoxidase (IC_{50} = 28.9 μM). Maximal inhibition of IgE/antigen degranulation (< 15% at 1 μM), A23187 degranulation (56.8% at 10 μM), hyaluronidase (< 15% at 20 μM) and lipoxidase (65.6% at 36.7 μM) was determined. This finding was in line with the previously reported anti-allergic properties of several other naturally occurring 1,4-naphthoquinones (Tewtrakul et al. 2009). This study highlighted the potential of **1** as a lead compound for structural modification and generating structural analogues for developing novel anti-allergic therapeutics.

Bawazeer et al. (2019) showed the antidiabetic potential of **1**, isolated from the roots of *Diospyros lotus*, using a detailed *in silico* study followed up by the PTP1B inhibition assay in 96-well plates using 3,3-dimethyl glutarate buffer. Molecular docking (Sarker and Nahar 2024), the simulation technique used to model the interaction between two molecules, was performed using Open Eye software**.** It was shown that this compound had protein tyrosine phosphatase 1B inhibitory activity (IC_{50} = 27.59 μM). Diospyrin (**1**) also displayed molecular interactions with GLY 220, TYR 46, VAL 49 and ASP 48 inside the active site of protein tyrosine phosphatase 1B (PTP1B), an intracellular non-receptor type PTP. It can be noted that PTP1B is involved in the negative control of insulin and leptin receptors and thus, implicated in diabetes (Comeau et al. 2010). Therefore, the inhibition of PTP1B offers a new way of regulating type 2 diabetes and obesity.

The acetic acid-induced writing in mice is a standard test to assess the antinociceptive activity of any test materials (Le Bars et al. 2001). The acetic acid-induced writhing in mice was used to assess the antinociceptive activity of **1**, isolated from the roots of *D. lotus*, and acetic acid-induced writhing was significantly protected by this compound (40.87%) at 10 mg/kg, offering peripheral and central analgesic effects (Uddin et al. 2014). In the hot-plate test using mice, **1** exhibited a dose-dependent central antinociceptive effect in a thermally induced pain model (Uddin et al. 2014). The sedative effect of **1** in the experimental model (open field) was also investigated (Uddin et al. 2014). The positive control diazepam, a well-known sedative agent, displayed significant sedation compared to the saline control group, while diospyrin (**1**) showed a significant sedative effect at the doses of 5 and 10 mg/kg. The sedative effect of **1** could be considered as mild to moderate, as this effect was less potent than that of diazepam.

As diospyrin (**1**) has phenolic hydroxyls in its structure, a certain level of antioxidant property in this

compound is expected. The antioxidant potential of **1** was assessed by its effects on oxidative enzymes (Hayeshi et al. 2004) as well as by its ability to scavenge 1,2-diphenyl-2-picyl-hydrazyl (DPPH) stable free radicals (Alam et al. 2023). Diospyrin (**1**), isolated from the stem bark of *D. montana*, inhibited heterologously expressed human glutathione transferases (GSTs) A1-1, M1-1 and P1-1 *in vitro*. The Ki values for diospyrin, concerning both glutathione and 1-chloro-2,4-dinitrobenzene, were in the range 0.08–0.6 μM (Hayeshi et al. 2004). This compound was found to inhibit the three tested human glutathione transferases (GST) isoforms with IC_{50} values in the range 0.1–0.5 μM. It was concluded that **1** could potentially be a GST chemomodulator.

Neuroprotection, like several other oxidative stress-related ailments, is often linked to the reduction of oxidative stress caused by ROS, and antioxidant therapies have been found effective in neuroprotection (Teleanu et al. 2019). Alam et al. (2023) investigated the neuroprotective potential of **1**, isolated from the roots of *D. lotus*, against drug-induced Alzheimer's disease by assessing its antioxidant properties and conducting the *in vitro* DPPH radical-scavenging assay. In that assay, diospyrin (**1**) showed a maximum of 72.85% inhibition (IC_{50} = 139.74 μg/mL) of DPPH-free radicals at the highest concentration of 1000 μg/mL as compared to ascorbic acid (IC_{50} = 13.72 μg/mL). This finding indicated that **1** could provide neuroprotection. The neuroprotective function of this compound was further substantiated by the *in vitro* acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory assays as well as a battery of *in vivo* behavioural animal models tests, including elevated plus maze (EPM), Morris water maze (MWM) and paddling Y-maze test. The IC_{50} values of **1** in the AchE and BchE assays were found to be 95 and 110 μg/mL, respectively, while the IC_{50} values for the positive control donepezil were 95 and 85 μg/mL, respectively. In the animal study using mice, this bisnaphthoquinone treatment exhibited gradual improvement in memory and enhanced motor functionality. Diospyrin (**1**) improved locomotor activity, memory, and learning, and reduced anxiety in experimental mice. In the MWM test, this compound significantly enhanced memory by reducing escape latency and increasing stay time in the desired quadrant by the experimental animals. In the EPM test, diospyrin-treated group showed a decrease in transfer latency time compared to the disease-control group (scopolamine-treated). In the Y-Maze test, the diospyrin-treated group showed noticeable improvement in alternation behaviour compared to the scopolamine group. Furthermore, *in silico* molecular docking studies (Alam et al. 2023) showed that **1** could favourably bind to the *AChE* and *BChE* enzymes. It was concluded that, based on the *in vitro*, *in vivo* and *in silico* findings, **1** could potentially be developed as a drug candidate for managing neurodegenerative disorders like Alzheimer's disease.

Toxicological aspects

Hazra et al. (2013) reported that diospyrin (**1**) did not show any acute toxicity in BALB/c mice and there was no mortality. The acute toxicity of **1** was assessed using a mouse model (Uddin et al. 2014). The treatment dose of 25–50 mg/kg of this compound displayed slight sedation with no mortality during 24 h of assessment (Uddin et al. 2014). The discussion presented in the earlier subsection on anticancer, antitumour and cytotoxicity shows the cytotoxic potential of **1** (Table 1). Although diospyrin (**1**) showed selective cytotoxicity against cancer cell lines, this compound can also be toxic to normal cells. Landa et al. (2012) reported the cytotoxicity of **1** against the mouse fibroblast cell line Balb/c 3T3 at a concentration range of 0.04–2.5 μM using the MTT assay, and the value was 0.9 μM. Based on that finding, it was suggested that **1** could display toxicity *in vivo*. This suggestion could be further substantiated by the fact that the monomeric 1,4-naphthoquinone, juglone, which is structurally related to **1**, was found to exert severe toxicity *in vivo* in the mouse model with an LD_{50} value of 2.5 mg/kg (Landa et al. 2012). A moderate level of cytotoxicity (IC_{50} = 17.8 μg/mL) was observed with **1** against Vero cells (Lall et al. 2005b; Mahapatra et al. 2007). The cytotoxicity of **1** was also observed against rat skeletal myoblasts (L-6) cells and the IC_{50} value was 6.38 μM, which was much less toxic than the positive control podophyllotoxin (Ganapaty et al. 2006a). An *in vitro* cytotoxicity study using the MTT assay against RAW macrophage cell line performed by Hazra et al. (2013) revealed that **1** had an IC_{50} value of 7.2 μM after 72 h incubation period.

The mutagenic property of **1** was reported by Hazra et al. (1995b). It can be mentioned that mutagenicity is the ability of a substance to cause permanent changes to DNA, leading to mutations. However, diospyrin (**1**) was found not to exert any significant liver and kidney toxicity in mice, as evident from the normal level of enzyme activity (Hazra et al. 2013). Histological examination of the liver, kidney and spleen of the mice treated with **1** revealed moderate levels of toxicities: the central veins were slightly congested, follicle showed hyperplasia, spleen sinusoid and proximal tubule were dilated, but the portal vein, cell architecture, distal tubule and glomerular tubule were normal, no fatty change was observed in the liver, but fatty change was observed in the kidneys (Pal et al. 1996).

Conclusion

Diospyrin (**1**) is a bioactive bisnaphthoquinone, which has been shown to possess therapeutic potential in treating bacterial infections, especially caused by *Mycobacterium* and *Staphylococcus* species, various forms of cancers and tumours, particularly Ehrlich ascites carcinoma, blood, breast, cervical, colon, lung, prostate and skin cancers, and antiparasitic infections, mainly caused by *Leishmania*, *Plasmodium* and *Trypanosoma* parasites. Some information on the plausible mechanisms of action of **1** for these therapeutic properties has begun to emerge in the literature. A certain level of toxicity of **1** in normal cells, hepatotoxicity and mutagenicity, has been reported, which identifies the need for further studies to establish the therapeutic window of this compound. Further preclinical and clinical studies with diospyrin (**1**) and its structural analogues are still needed to establish them as therapeutic agents for treating cancers and bacterial and parasitic infections.

Acknowledgements Lutfun Nahar gratefully acknowledges the support of the European Regional Development Fund—Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868) and the Czech Agency Grants—Project 23-05474S and Project 23-05389S.

Author contributions Both authors contributed equally to retrieving information from the published literature, designing the layout, manuscript preparation and editing.

Funding European Regional Development Fund, CZ.02.1.01/0.0/0.0/16_019/0000868, Lutfun Nahar, Czech Agency Grants, 23-05474S, Lutfun Nahar, 23-05389S, Lutfun Nahar.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

Adeniyi BA, Fong HHS, Pezzuto JM, Luyengi L, Odelola HA (2000) Antibacterial activity of diospyrin, isodiospyrin and bisisodiospyrin from the root of *Diospyros piscatorial* (Gurke) (Ebenaceae). Phytother Res 14:112–117. https://doi.org/10.1002/(sici)1099-1573(200003)14:2%3c112::aid-ptr488%3e3.0.co;2-t

Adibpour N, Rahim F, Rezaeei S, Khalaj A, Ebrahimi A (2012) In silico designing selective inhibitor of drugs, medicinal plants compounds and experimental ligands for pteridine reductase targeting visceral leishmaniasis. Afr J Microbiol Res 6:917–926. https://doi.org/10.5897/AJMR11.738

Akram SM, Rathish B, Saleh D (2023) *Mycobacterium chelonae* infection. NIH National Library of Medicine – National Center for Biotechnology Information. Available at https://www.ncbi.nlm.nih.gov/books/NBK430806/. Accessed on 13 March 2025

Alam A, Ali G, Nawaz A, Al Omar TS, Rauf A, Ayaz M, Ahmad S, Almasoud N, Al Omar AS, Khalil AA (2023) Neuroprotective evaluation of diospyrin against drug-induced Alzheimer's disease. Fitoterapia 171:105703. https://doi.org/10.1016/j.fitote.2023.105703

Alves AC, Costa MAC, Paul MI (1983) Naphthoquinones of *Diospyros batocana*. Planta Med 47:121–124

Ansari MY, Ahsan MJ, Yasmin S, Sahoo GC, Saini V, Das P (2017) *In silico* identification of novel antagonists and binding insights by structural and functional analyses of guanylate kinase of *Leishmania donovani* and interaction with inhibitors. Gene Rep 8:134–143. https://doi.org/10.1016/j.genrep.2017.07.003

Bailly C (2000) Topoisomerase I poisons and suppressors as anticancer drugs. Curr Med Chem 7:39–58. https://doi.org/10.2174/0929867003375489

Bapela MJ, Lall N, Meyer JJM (2007) Variation in the content of naphthoquinones in seeds and seedlings of *Euclea natalensis*. S Afr J Bot 73(4):606–610. https://doi.org/10.1016/j.sajb.2007.06.003

Bapela MJ, Lall N, Meyer JJM (2008a) Fertilization-induced changes on growth parameters and antimycobacterial activity of *Euclea natalensis*. South Afr J Bot 74:244–250. https://doi.org/10.1016/j.sajb.2007.11.011

Bapela MJ, Lall N, Meyer JJM (2008b) Seasonal variation of naphthoquinones in *Euclea natalensis* subspecies *natalensis*. South Afr J Bot 74:218–224. https://doi.org/10.1016/j.sajb.2007.11.007

Bawazeer S, Rauf A (2021) In vitro antiglycation and lipoxygenase inhibition on naphthoquinones isolated from *Diospyros lotus* Linn. S Afr J Bot 143:406–409. https://doi.org/10.1016/j.sajb.2021.03.014

Bawazeer S, Rauf A, Shahidullah A, Mishra AP, Faraone I, Milella L, Ullah K, Uddin G, Khan I, Patel S, Shah ZA (2019) Structural insights behind protein tyrosine phosphatase 1B inhibitory activity of diospyrin. Indian J Pharm Sci 81(3):565–568. https://doi.org/10.36468/pharmaceutical-sciences.546

Bringmann G, Feineis D (2001) Stress-related polyketide metabolism of Dioncophyllaceae and Ancistrocladaceae. J Exp Bot 52:2015–2022. https://doi.org/10.1093/jexbot/52.363.2015

Bringmann G, Rischer H, Wohlfarth M, Schlauer J, Assi LA (2000) Droserone from cell cultures *Triphyophyllum peltatum* (Dioncophyllaceae) and its biosynthetic origin. Phytochemistry 53:339–343. https://doi.org/10.1016/s0031-9422(99)00543-9

Carter FL, Garlo AM, Stanley JB (1978) Termiticidal components of wood extracts – 7-methyljuglone from *Diospyros virginiana*. J Agric Food Chem 26:869–873. https://doi.org/10.1021/jf60218a018

Chakrabarty S, Roy M, Hazra B, Bhattacharya RK (2002) Induction of apoptosis in human cancer cell lines by diospyrin, a plant-derived bisnaphthoquinonoid, and its synthetic derivatives. Cancer Lett 188(1–2):85–93. https://doi.org/10.1016/S0304-3835(02)00494-9

Chakraborty AK, Gupta A, Majumder HK (1993) A type I DNA topoisomerase from the kinetoplast hemoflagellate *Leishmania donovani*. Indian J Biochem Biophys 30:257–263

Chung T (2012) *Staphylococcus aureus* DNA gyrase: mechanism and drug targeting. PhD thesis, University of East Anglia, UK, indexed on 20 May 2023

Collins LS, Franzblau SG (1997) Microplate Alamar blue assay versus BACTEC 460 system for high throughput screening of compounds against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Antimicrob Agents Chemother 41:1004–1009. https://doi.org/10.1128/AAC.41.5.1004

Comeau AB, Critton DA, Page R, Seto CT (2010) A focused library of protein tyrosine phosphatase inhibitors. J Med Chem 53:68–72. https://doi.org/10.1021/jm100528p

Costa MAC, Lopes MH, Paul MI, Ferreira MA, Alves AC (1976) Naphthoquinones and triterpenoids of *Euclea divinorum*. Phytochemistry 15:829–829

Costa MA, Alves AC, Seabra RM, Andrade PB (1998) Naphthoquinones of *Diospyros chamaethamnus*. Planta Med 64:391

Croft SL, Evans AT, Neal RA (1985) The activity of plumbagin and other electron carriers against *Leishmania donovani* and *Leishmania mexicana amazonensis*. Ann Trop Med Parasitol 79(6):651–653. https://doi.org/10.1080/00034983.1985.11811974

Culham A, Gornall RJ (1994) The taxonomic significance of naphthoquinones in the Droseraceae. Biochem Syst Ecol 22(5):507–515. https://doi.org/10.1016/0305-1978(94)90045-0

Cushion MT, Collin M, Hazra B, Kaneshiro ES (2000) Effects of atovaquone and diospyrin-based drugs on the cellular ATP of *Pneumocyctis carinii* f. sp. *Carinii*. Antimicrob Agents Chemother 44:713–719. https://doi.org/10.1128/AAC.44.3.713-719.2000

Delgado JL, Hsein C-M, Chan N-L, Hiasa H (2018) Topoisomerases as anticancer targets. Biochem J 475:373–398. https://doi.org/10.1042/BCJ20160583

Dev MJA, Rajarajeshwari N, Ganapathy S, Parixit B, Brun R (2012) Antiprotozoal and anthelmintic naphthoquinones from three unexplored species of *Diospyros*. J Nat Remedies 12:129–134. https://doi.org/10.18311/jnr/2012/266

Dey D, Ray R, Hazra B (2014) Antitubercular and antibacterial activity of quinoid natural products against multi-drug resistant clinical isolates. Phytother Res 28:1014–1021. https://doi.org/10.1002/ptr.5090

Docampo R, Moreno SNJ (1984) Free radical metabolites in the mode of action of chemotherapeutic agents and phagocytic cells in *Trypanosoma cruzi*. Clin Infect Dis 6(2):223–238. https://doi.org/10.1093/clinids/6.2.223

Duangjai S, Wallnofer B, Samuel R, Munzinger J, Chase MW (2006) Generic delimitation and relationships in *Ebenaceae sensu lato*: evidence from six plastid DNA regions. Am J Bot 93(12):1808–1827. https://doi.org/10.3732/ajb.93.12.1808

Ellis JE (1994) Coenzyme Q homologs in parasitic protozoa as targets for chemotherapeutic attack. Parasitol Today 10:297–301. https://doi.org/10.1016/0169-4758(94)90079-5

Elmore S (2007) Apoptosis: a review of programmed cell death. Toxicol Pathol 35:495–516. https://doi.org/10.1080/01926230701320337

Ferreira MA, Aureacru M, Correiaa A, Lopes MH (1974) Naphthoquinones from *Euclea pseudebenus*. Phytochemistry 13:1587–1589. https://doi.org/10.1016/0031-9422(74)80333-X

Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, Ferruci L, Gilroy DW, Fasano A, Miller GW, Miller AH, Mantovani A, Weyand CM, Barzilai N, Goronzy JJ, Rando TA, Effros RB, Lucia A, Kelinstreuer N, Slavich GM (2019) Chronic inflammation in the etiology of disease across the life span. Nat Med 25:1822–1832. https://doi.org/10.1038/s41591-019-0675-0

Ganapaty S, Thomas PS, Karagianis G, Waterman PG (2006a) Antiprotozoal and cytotoxic naphthalene derivatives from *Diospyros assimilis*. Phytochemistry 67(17):1950–1956. https://doi.org/10.1016/j.phytochem.2006.05.039

Ganapaty S, Thomas PS, Karagianis G, Waterman PG (2006b) Mono and dimeric naphthalene derivatives from the roots of *Diospyros assimilis*. Nat Prod Res 20(9):783–787. https://doi.org/10.1080/14786410500046687

Ganguly AK, Govindachari TR (1966) Structure of diospyrin. Tet Lett 7:3373–3376. https://doi.org/10.1016/S0040-4039(00)90692-0

Geeraerts A, Raeymaekers JAM, Vinckier S, Pletsers A, Smets E, Huysmans S (2009) Systematic palynology in Ebenaceae with focus on Ebenoideae: morphological diversity and character evolution. Rev Palaeobot Palynol 153:336–353. https://doi.org/10.1016/j.revpalbo.2008.10.001

Harrison WTA, Musgrave OC (2004) Diospyrin. Acta Crystallogr Sect C Cryst Struct Commun 60(6):O399–O401. https://doi.org/10.1107/S0108270104007826

Hawkey CJ (2001) COX-1 and COX-2 inhibitors. Best Pract Res Clin Gastroenterol 15(5):801–820. https://doi.org/10.1053/bega.2001.0236

Hayeshi R, Mukanganyama S, Hazra B, Abegaz B, Hasler J (2004) The interaction of selected natural products with human recombinant glutathione transferases. Phytother Res 18:877–883. https://doi.org/10.3390/molecules26082399

Hazra B, Sur P, Sur B, Banerjee A (1984) Biological activity of diospyrin towards Ehrlich ascites carcinoma in Swiss mice. Planta Med 51:295–297. https://doi.org/10.1055/s-2007-969713

Hazra B, Saha AK, Ray R, Roy DK, Sur P, Banerjee A (1987) Antiprotozoal activity of diospyrin towards *Leishmania donovani* promastigotes in vitro. Trans R Soc Trop Med Hyg 81(5):738–741. https://doi.org/10.1016/0035-9203(87)90013-7

Hazra B, Pal S, Banerjee A (1994) New diospyrin derivatives with improved tumor inhibitory activity towards Ehrlich ascites-carcinoma. Med Sci Res 22:351–353

Hazra B, Ghosh R, Banerjee A, Kirby GC, Warhurst DC, Phillipson JD (1995a) In vitro antiplasmodial effects of diospyrin, a plant derived naphthoquinoid, and a novel series of derivatives. Phytother Res 9(1):72–74. https://doi.org/10.1002/ptr.2650090118

Hazra B, Pal S, Shetty TK, Bhattacharya RK (1995b) An investigation on the mutagenic activity of diospyrin and its semisynthetic derivatives. Med Sci Res 23:449–450

Hazra B, Golenser J, Nechemiya O, Bhattacharya S, Azzam T, Domb A, Frankenburg S (2002) Inhibitory activity of diospyrin derivatives against *Leishmania major parasites* in vitro. Indian J Pharmacol 34:422–427

Hazra B, Kumar B, Biswas S, Pandey BN, Mishra KP (2005) Enhancement of the tumour inhibitory activity, in vivo, of diospyrin, a plant-derived quinonoid, through liposomal encapsulation. Toxicol Lett 157(2):109–117. https://doi.org/10.1016/j.toxlet.2005.01.016

Hazra B, Sarma SD, Kumar B, Basu S, Das K, Pandey BN, Mishra KP (2007) Cytotoxicity of diospyrin and its derivatives in relation to the generation of reactive oxygen species in tumour cells in vitro and in vivo. Chemotherapy 53:173–176. https://doi.org/10.1159/000100514

Hazra S, Ghosh S, Das Sarma M, Sharma S, Das M, Saudagar P, Prajapati VK, Dubey VK, Sundar S, Hazra B (2013) Evaluation of a diospyrin derivative as antileishmanial agent and potential modulator of ornithine decarboxylase of *Leishmania donovani*. Exp Parasitol 135(2):407–413. https://doi.org/10.1016/j.exppara.2013.07.021

Hazra S, Ghosh S, Kumar A, Pandey BN, Hazra B (2015) Acetylamine derivative of diospyrin, a plant-derived binaphthoquinonoid, inhibits human colon cancer growth in *Nod*-Scid mice. Invest New Drugs 33:22–31

Hussain H, Al-Harrasi A, Green IR, Abbas G, Ahmed I (2015) Recent advances in the chemistry and biology of natural dimeric quinones. Stud Nat Products Chem 46:447–517

Ismail FMD, Nahar L, Zhang K, Sarker SD (2020) Medicinal natural products – A disease-focused approach. In: Sarker SD, Nahar L (eds) Antiparasitic natural products, annual reports medical chemistry. Elsevier, UK

Jeffreys JAD, Zakaria MB, Waterman PG (1983) 3'-methoxydisopyrin, a 7-methyljuglone dimer from *Diospyros mannii*. Phytochemistry 22:1832–1833

Jha A, Verma S, Kumar A (2020) Identification of potential inhibitors targeted for strengthening search of anti-leishmanial therapeutics. Biologia 75:437–445

Jones RS, Shier KL, Master RN, Bao JR, Clark RB (2019) Current significance of the *Mycobacterium chelonae-abscessus* group. Diagn Microbiol Infect Dis 94(3):248–254. https://doi.org/10.1016/j.diagmicrobio.2019.01.021

Joubert A, van der Kooy F, Meyer JJM, Lall N (2006) HPLC in the comparative study of the content of naphthoquinones (quinonoid constituents) in *Euclea* species of South Africa. Chromatographia 64(7–8):399–403. https://doi.org/10.1365/s10337-006-0055-z

Kaneshiro ES, Sul D, Hazra B (2000) Effects of atovaquone and diospyrin-based drugs on ubiquinone biosynthesis in *Pneumocystis carinii* organisms. Antimicrob Agents Chemother 44:14–18. https://doi.org/10.1128/aac.44.1.14-18.2000

Kapil RS, Dhar MM (1961) A new bisnaphthoquinone from *Diospyros montana*. J Sci Ind Res 20B:498–500

Karkare S, Chung TTH, Collin F, Mitchenall LA, McKay AR, Greive SJ, Meyer JJM, Lall N, Maxwell A (2013a) Synthesis, in vitro and in silico studies of indolequinone derivatives against clinically relevant bacterial pathogens. J Biol Chem 288:5149–5156

Karkare S, Chung TTH, Collin F, Mitchenall LA, McKay AR, Greive SJ, Meyer JJM, Lall N, Maxwell A (2013b) The naphthoquinone diospyrin is an inhibitor of DNA gyrase with a novel mechanism of action. J Biol Chem 288(7):5149–5156. https://doi.org/10.1074/jbc.M112.419069

Khan MR, Mutasa S, Naddalio G, Wevers H, Sawhney AN (1978) Antibiotic action of constituents of root bark of *Euclea natalensis*. Pak J Sci Ind Res 21:197–199

Khan MR, Kishimba MA, Locksley H (1987) Extractives from Eebenaceae – constituents of the root and stem barks of *Diospyros verrucosa*. Planta Med 5:498–498

Kim H-J, Khan I, Shahidullah A, Halimi SMA, Rauf A, Lee J-Y, Kim Y-J, Kim B-Y, Park W (2020) Diospyrin modulates inflammation in poly I:C-induced macrophages via ER stress-induced calcium-CHOP pathway. Processes 8:1050. https://doi.org/10.3390/pr8091050

Kovacic P, Ames JR, Grogan JW, Hazra B, Ryan MD (1988) Anticancer quinones and hydroquinolines: Mode of action via electron transfer and oxidative stress. In: Dryhurst G, Niki K (eds) Redox chemistry and interfacial behavior of biological molecules. Plenum Press, New York, pp 295–307

Kumar B, Kumar A, Pandey BN, Mishra KP, Hazra B (2009) Role of mitochondrial oxidative stress in the apoptosis induced by diospyrin diethylether in human breast carcinoma (MCF-7) cells. Mol Cell Biochem 320:185–195. https://doi.org/10.1007/s11010-008-9920-4

Kumar B, Kumar A, Ghosh S, Pandey BN, Mishra KP, Hazra B (2012) Diospyrin derivative, an anticancer quinonoid, regulates apoptosis at endoplasmic reticulum as well as mitochondria by modulating cytosolic calcium in human breast carcinoma cells. Biochem Biophys Res Commun 417:903–909. https://doi.org/10.1016/j.bbrc.2011.12.072

Kumar A, Rathi E, Kini SG (2019) Identification of E6 inhibitors employing energetically optimized structure-based pharmacophore modelling, ligand docking and molecular dynamics simulations studies. ChemistrySelect 4:10701–10708. https://doi.org/10.1002/slct.201902105

Kuroyana M, Yoshihir K, Natori S (1971) Naphthoquinone derivatives from Ebenaceae. 3. Shinanolone from *Diospyros japonica* Sieb. Chem Pharm Bull 19:2314–2326

Lall N, Meyer JJM (2001) Inhibition of drug-resistant strains of *Mycobacterium tuberculosis* by diospyrin, isolated from *Euclea natalensis*. J Ethnopharmacol 78:213–216. https://doi.org/10.1016/S0378-8741(01)00356-7

Lall N, Sarma SD, Hazra B, Meyer JJM (2003) Antimycobacterial activity of diospyrin derivatives and a structural analogue of diospyrin against *Mycobacterium tuberculosis* in vitro. J Antimicrob Chemother 51:435–438. https://doi.org/10.1093/jac/dkg068

Lall N, Meyer JJM, Taylor MB (2005a) Anti-HSV-1 activity of *Euclea natalensis*. S Afr J Bot 71(3–4):444–446. https://doi.org/10.1016/S0254-6299(15)30118-6

Lall N, Meyer JJM, Wang Y, Bapela NB, van Rensburg CEJ, Fourie B, Franzblau SG (2005b) Characterization of intracellular activity of antitubercular constituents from the roots of *Euclea natalensis*. Pharm Biol 43:353–357. https://doi.org/10.1080/13880200590951829

Landa P, Kutil Z, Temml V, Vuorinen A, Malik J, Dvorakova M, Marsik P, Kokoska L, Pribylova M, Schuster D, Vanek T (2012) Redox and non-redox mechanism of in vitro cyclooxygenase inhibition by natural quinones. Planta Med 78:326–333. https://doi.org/10.1055/s-0031-1280430

Le Bars D, Gozariu M, Cadden SW (2001) Animal models of nociception. Pharmacology 53:597–652

Likhitwitayawuid K, Dej-adisai S, Jongbunprasert V, Krungkrai J (1999) Antimalarials from *Stephania venosa, Prismatomeris sessiliflora, Diospyros montana* and *Murraya siamensis*. Planta Med 65:754–756. https://doi.org/10.1055/s-2006-960858

Liu W, Liu Y (2016) Youyou Tu: significance of winning the 2015 nobel prize in physiology or medicine. Cardiovasc Diagn Ther 6:1–2. https://doi.org/10.3978/j.issn.2223-3652.2015.12.11

Mahapatra A, Motovandlela SPN, Binneman B, Fourie PB, Hamilton CJ, Meyer JJM, van der Kooy F, Houghton P, Lall N (2007) Activity of 7-methyljuglone derivatives against *Mycobacterium tuberculosis* and as subversive substrates for mycothiol disulfide reductase. Bioorg Med Chem 15:7638–7646. https://doi.org/10.1016/j.bmc.2007.08.064

Mahapatra A, Tshikalange TE, Meyer JJM, Lall N (2012) Synthesis and HIV-1 reverse transcriptase inhibition activity of 1,4-naphthoquinone derivatives. Chem Nat Compd 47:883–887

Mahernia S, Bagherzadeh K, Mojab F, Amanlou M (2015) Urease inhibitory activities of some commonly consumed herbal medicines. Iran J Pharm Sci 14:943–947. https://doi.org/10.1007/s10600-012-0094-7

Mallavadhani UV, Panda AK, Rao YR (1998) Review article number 134: pharmacology and chemotaxonomy of *Diospyros*. Phytochemistry 49:901–951. https://doi.org/10.1016/S0031-9422(97)01020-0

McGilvary NJ, Kevorkian YL, Tan S (2019) Potassium response and homeostasis in *Mycobacterium tuberculosis* modulates environmental adaptation and is important for host colonization. PLoS Pathog 15:e1007591. https://doi.org/10.1371/journal.ppat.1007591

Middlebrook G, Reggiards Z, Tigertt WD (1977) Automable radiometric detection of growth of *Mycobacterium tuberculosis* in selective media. Am Rev Respir Dis 115:1067–1069. https://doi.org/10.1164/arrd.1977.115.6.1066

Mukherjee P, Majee SB, Ghosh S, Hazra B (2009) Apoptosis-like death in *Leishmania donovani* promastigotes induced by diospyrin and its ethanolamine derivative. Int J Antimicrob Agents 34(6):596–601. https://doi.org/10.1016/j.ijantimicag.2009.08.007

Nahar L, Ahmed MN, Afroze CA, Sangthong S, Guo M, Sarker SD (2025) Phytometabolites, pharmacological effects, ethnomedicinal properties, and bioeconomic potential of Velvet Apple (*Diospyros discolor* Willd.): a review. Chem Biodivers 22:e202402168. https://doi.org/10.1002/cbdv.202402168

Nematollahi A, Aminimogjadamfarouj N, Wiart C (2012) Reviews on 1,4-naphthoquinones from *Diospyros* L. J Asian Nat Prod Res 14:80–88. https://doi.org/10.1080/10286020.2011.633515

Nisar A, Jagtap S, Vyavahare S, Deshpande M, Harsulkar A, Ranjekar P, Prakash O (2023) Phytochemicals in the treatment of inflammation-associated diseases: the journey from preclinical trials to clinical practice. Front Pharmacol 14:1177050. https://doi.org/10.3389/fphar.2023.1177050

Norhanom AW, Hazra B (1997) Inhibition of tumour promoter-induced Epstein-Barr virus activation by diospyrin, a plant-derived antitumour compound, and its synthetic derivatives. Phytother Res 11:588–590. https://doi.org/10.1002/(SICI)1099-1573(199712)11:8%3c588::AID-PTR152%3e3.0.CO;2-8

Ortiz-Perez E, Rivera G, Salas CO, Zarate-Ramos JJ, Trofymchuk OS, Hernandez-Soberanis L, Perales-Flores JD, Vazquez K (2021) Natural and synthetic naphthoquinones as potential anti-infective agents. Curr Top Med Chem 21(22):2046–2069. https://doi.org/10.2174/1568026621666210915121348

Pal S, Banerjee A, Hazra B, Ray R, Bhattacharya DK (1996) Pharmacological studies on the effect of the treatment of Swiss A mice with diospyrin, a tumour-inhibitory plant product, and its synthetic derivatives. Phytother Res 10:393–397. https://doi.org/10.1002/(SICI)1099-1573(199608)10:5%3c393::AID-PTR859%3e3.0.CO;2-X

Pardhasaradhi M, Krishnakumari L (1979) Tetrahydrodiospyrin -reduced binaphthoquinone from the bark of *Diospyros montana*. Phytochemistry 18:684–685

Pinho BR, Sousa C, Valentao P, Oliveira JMA, Andrade PB (2014) Modulation of basophils' degranulation and allergy-related enzymes by monomeric and dimeric naphthoquinones. PLoS ONE 9(2):e90122. https://doi.org/10.1371/journal.pone.0090122

Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, Cirino G (2004) Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. Br J Pharmacol 142:331–338. https://doi.org/10.1038/sj.bjp.0705650

Pullella GA, Vuong D, Lacey E, Piggott MJ (2020) Total synthesis of the antitumor-antitubercular 2,6'-bijuglone natural product diospyrin and its 3,6'-isomer. J Nat Prod 83:3623–3634. https://doi.org/10.1021/acs.jnatprod.0c00800

Rattan A, Kalia A, Ahmad N (1998) Multidrug-resistant *Mycobacterium tuberculosis*: molecular perspectives. Emerg Infect Dis 4:195–209. https://doi.org/10.3201/eid0402.980207

Rauf A, Uddin G, Khan H, Arfan M, Siddiqui BS (2016) Bioassay-guided isolation of antibacterial constituents from *Diospyros lotus* roots. Nat Prod Res 30(4):426–428. https://doi.org/10.1080/14786419.2015.1013957

Rauf A, Uddin G, Siddiqui BS, Khan A, Farooq U, Khan FA, Bukhari SM, Khan SB (2017) Bioassay-guided isolation of novel and selective urease inhibitors from *Diospyros lotus*. Chin J Nat Med 15(11):865–870. https://doi.org/10.1016/S1875-5364(18)30021-9

Rauf A, Khan A, Abu-Inzeid T, Alhumaydhi FA, Bawazeer S, Raza M, Khan H, Patel S, Al-Harrasi A (2021) Novel anticancer dimeric naphthoquinones from *Diospyros lotus* having anti-tumor, anti-inflammatory and multidrug resistance reversal potential: In vitro, in vivo and in silico evidence. Anticancer Agents Med Chem 21:2089–2097

Rauf A, Akram Z, Hafeez N, Khalil AA, Khalid A, Hemeg HA, Aljohani ASM, Al Abdoulmonem W, Quradha M, Qahtan AMF (2024) Anticancer potential of diospyrin and its analogues: an updated review. Food Sci Nutr 12:6047–6054. https://doi.org/10.1002/fsn3.4237

Ravishankara MN, Shrivastava N, Jayathirtha MG, Padh H, Rajani M (2000) Sensitive high-performance thin-layer chromatographic method for the estimation of diospyrin, a tumour inhibitory agent from the stem bark of *Diospyros montana* Roxb. J Chromatogr B Biomed Sci Appl 744(2):257–262. https://doi.org/10.1016/S0378-4347(00)00277-2

Ray S, Hazra B, Mittra B, Das A, Majumder HK (1998) Diospyrin, a bisnaphthoquinone: a novel inhibitor of type 1 DNA topoisomerase of *Leishmania donovani*. Mol Pharmacol 54:994–999. https://doi.org/10.1124/mol.54.6.994

Sagar S, Kaur M, Minneman KP, Bajic VB (2010) Anticancer activities of diospyrin, its derivatives and analogues. Eur J Med Chem 45(9):3519–3530. https://doi.org/10.1016/j.ejmech.2010.06.021

Sanchez Armengol E, Harmanci M, Laffleur F (2021) Current strategies to determine antifungal and antimicrobial activity of natural compounds. Microbiol Res 252:126867. https://doi.org/10.1016/j.micres.2021.126867

Sarker SD, Nahar L (2024) Computational phytochemistry, 2nd edition. Elsevier, UK

Sarma MD, Ghosh R, Patra A, Hazra B (2007) Synthesis and antiproliferative activity of some novel derivatives of diospyrin, a plant-derived naphthoquinonoid. Bioorg Med Chem 15(11):3672–3677. https://doi.org/10.1016/j.bmc.2007.03.050

Sarma MD, Ghosh R, Patra A, Hazra B (2008) Synthesis of novel aminoquinonoid analogues of diospyrin and evaluation of their inhibitory activity against murine and human cancer cells. Eur J Med Chem 43(9):1878–1888. https://doi.org/10.1016/j.ejmech.2007.11.028

Schami A, Islam MN, Belisle JT, Torrelles JB (2023) Drug-resistant strains of *Mycobacterium tuberculosis*: cell envelope profiles and interactions with the host. Front Cell Infect Microbiol 13:1274175. https://doi.org/10.3389/fcimb.2023.1274175

Send A, Chen JL, Jolad SD, Stoddart C, Rozhon E, Kernan M (1996) Two new naphthoquinones with antiviral activity from *Rhinacanthus nasutus*. J Nat Prod 59:808–811. https://doi.org/10.1021/np9601871

Shahidullah A, Lee JY, Kim YJ, Halimi SMA, Rauf A, Kim HJ, Kim BY, Park W (2020) Anti-inflammatory effects of diospyrin on lipopolysaccharide-induced inflammation using RAW 264.7 mouse macrophases. Biomedicines 8:11. https://doi.org/10.3390/biomedicines8010011

Sharma AK, Sharma TC, Singh R, Payal P, Gupta R, Sharma MC (2018) A novel compound lup-20(29)-ene-3α,6β-diol identified in petroleum ether extract of *Diospyros melanoxylon* Roxb. leaves and to reveal its antidiabetic activity in rats. Pharmacogn Mag 14(55):S245. https://doi.org/10.4103/pm.pm_429_17

Siddiqi SH, Libonati JP, Middlebrook G (1981) Evaluation of a rapid radiometric method for drug susceptibility testing of *Mycobacterium tuberculosis*. J Clin Microbiol 13:908–913

Sidhu GS, Pardhasaradhi M (1970) Diospyrin – position of quinone benzene linkage. Ind J Chem 8:569–570

Sidhu GS, Pardhasaradhi M, Babu MH (1976) Mass spectra of diospyrin and its derivatives. Ind J Chem Section B – Org Chem 14:218–220

Sundarrajan S, Lulu S, Arumugam M (2015) Computational evaluation of phytocompounds for combating drug resistant tuberculosis by multi-targeted therapy. J Mol Model 21:247. https://doi.org/10.1007/s00894-015-2785-z

Talukdar A, Kundu B, Sarkar D, Goon S, Mondal MA (2022) Topoisomerase I inhibitors: challenges, progress and the road ahead. Eur J Med Chem 236:114304. https://doi.org/10.1016/j.ejmech.2022.114304

Tate GT (1967) Therapeutic Potential. In: Strategy of Therapy. Springer, Berlin, Heidelberg

Tazi J, Bakkour N, Soret J, Zekri L, Hazra B, Laine W, Baldeyrou B, Lansiaux A, Bailly C (2005) Selective inhibition of topoisomerase I and various steps of spliceosome assembly by diospyrin derivatives. Mol Pharmacol 67:1186–1194. https://doi.org/10.1124/mol.104.007633

Teleanu RI, Chircov C, Grumezescu AM, Volceanov A, Teleanu DM (2019) Antioxidant therapies for neuroprotection – a review. J Clin Med 8(10):1659. https://doi.org/10.3390/jcm8101659

Tewtrakul S, Tansakul P, Panichayupakaranant P (2009) Anti-allergic principles of *Rhinacanthus nasutus* leaves. Phytomedicine 16:929–934. https://doi.org/10.1016/j.phymed.2009.03.010

Thaver V (2010) Isolation and characterization of the cytotoxicity, intracellular bioactivity and mechanism of antimycobacterial action of *Euclea natalensis*-derived naphthoquinones. PhD thesis, University of Pretoria, South Africa

Thompson DC, Thompson JA, Sugumaran M, Moldeus P (1992) Biological and toxicological consequences of quinone methide formation. Chem Biol Interact 86:129–162. https://doi.org/10.1016/0009-2797(93)90117-h

Uddin G, Rauf A, Siddiqui BS, Muhammad N, Khan A, Shah SUA (2014) Anti-nociceptive, anti-inflammatory and sedative activities of the extracts and chemical constituents of *Diospyros lotus* L. Phytomedicine 21:954–959. https://doi.org/10.1016/j.phymed.2014.03.001

Uddin G, Rauf A, Arfan M, Waliullah, Rehman TU, Khan AZ, Ali G, Rehman B, Zia-ul-Haq M (2016) Molecular docking of diospyrin as a LOX inhibitory compound. J Saudi Chem Soc 20:S448–S450. https://doi.org/10.1016/j.jscs.2013.01.009

Uddin MJ, Patari P, Nag SK, Sil SK (2022) Physico-chemical analysis and antioxidant properties of tinctures of *Diospyros malabarica* (Desr.) Kostel: a preliminary report. Mater Today: Proceed 65:2723–2730. https://doi.org/10.1016/j.matpr.2022.05.574

Van der Kooy F, Meyer JJM, Lall N (2006) Antimycobacterial activity and possible mode of action of newly isolated neodiospyrin and other naphthoquinones from *Euclea natalensis*. S Afr J Bot 72(3):349–352. https://doi.org/10.1016/j.sajb.2005.09.009

Vanden Berghe DA, Vlietinck AJ (1986) Plant products as potential antiviral agents. Bull De L'institut Pasteur 84:101–105

Vickery ML, Vickery B (1981) The acetate-malonate pathway. Secondary plant metabolism. Palgrave, London, pp 56–87

Wang XN, Habib E, Leon F, Radwan MM, Tabanca N, Gao JT, Wedge DE, Cutler SJ (2011) Antifungal metabolites from the roots of *Diospyros virginiana* by overpressure layer chromatography. Chem Biodivers 8:2331–2340. https://doi.org/10.1002/cbdv.201000310

Wang W, Chang C-WT, Zhang Q (2022) 1,4-naphthoquinone analogs and their application as antibacterial agents. ChemistrySelect 7:e202203330. https://doi.org/10.1002/slct.202203330

Waterman PG, Mbi CN (1979) Sterol and dimeric naphthoquinones of the barks of 3 West Africa *Diospyros* species. Planta Med 37:241–246

Widhalm JR, Rhodes D (2016) Biosynthesis and molecular actions of specialised 1,4-naphthoquinone natural products produced by horticultural plants. Hortic Res 3(1):16046. https://doi.org/10.1038/hortres.2016.46

Wisastra R, Dekker FJ (2014) Inflammation, cancer and oxidative lipoxygenase activity are intimately linked. Cancers 6:1500–1521. https://doi.org/10.3390/cancers6031500

Yardley V, Snowdon D, Croft S, Hazra B (1996) In vitro activity of diospyrin and derivatives against *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei brucei*. Phytother Res 10:559–562. https://doi.org/10.1002/(SICI)1099-1573(199611)10:7%3c559::AID-PTR891%3e3.0.CO;2-V

Yoshida M, Mori K (2000) Synthesis of diospyrin, a potential agent against Leishmaniasis and related parasitic protozoan diseases. Eur J Org Chem 2000:1313–1317

Yoshihira K, Tezuka M, Kanchanapee P, Natori S (1971) Studies on Thai medicinal plants. 6. Naphthoquinone derivatives from Eebenaceae. 1. Diospyrol and related naphthoquinones from *Diospyros mollis* Groff. Chem Pharm Bull 19:2271–2272

Yoshimoto M, Hiraoka T, Kuwano H, Khisisda Y (1971) Four new naphthoquinone derivatives from *Diospyros* species. Chem Pharm Bull 19:851–854

Zenk MH, Fürbringer M, Steglich W (1969) Occurrence and distribution of 7-methyljuglone and plumbagin in the Droseraceae. Phytochemistry 8:2199–2200. https://doi.org/10.1016/S0031-9422(00)88181-9

Zhong SM, Waterman PG, Jeffreys JAD (1984) Naphthoquinones and triterpenes from African *Diospyros* species. Phytochemistry 23:1067–1072. https://doi.org/10.1016/S0031-9422(00)82610-2

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.