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Original article

Design and synthesis of novel magnolol derivatives as potential antimicrobial and antiproliferative compounds

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ABSTRACT

A series of novel magnolol derivatives were synthesised and evaluated for *in vitro* antimicrobial and antiproliferative activities. We found that most of the compounds were effective inhibitors of *Staphylococcus aureus*, *MRSA* and *VRE* with MIC in the range of 1–64 μg/mL and MBC in the range of 1–128 μg/mL. Few derivatives also exhibited promising antifungal activity. Some magnolol analogues exhibited promising antiproliferative activity than parent magnolol when tested against three human cancer cell lines.

1. Introduction

Widespread use of antibiotics led to increased bacterial resistance that become a life threatening problem worldwide. Of particular concern are the infections caused by *Methicillin resistant Staphylococcus aureus* (*MRSA*) and *Vancomycin resistant Enterococci* (*VRE*) [1–3] which are very dangerous especially in immune compromised patients due to HIV, surgery or any other illness. In view of the limited classes of antibacterial drugs currently in use that are prone to the development of microbial drug resistance, there is an urgent unmet need for the development of new class of antibiotics to fight against multi-drug resistant bacteria. Magnolol is a naturally occurring biphenolic compound (Fig. 1) isolated from the bark of *Magnoliae officinalis* which has been used in traditional eastern medicine for the relief of flu symptoms, treatment of anxiety and stroke. Several pharmacological activities are attributed to magnolol *viz*., antimicrobial, antioxidative, antianxiety, antiinflammatory, including antiproliferative activities [4–12].

Magnolol possesses an unusual biphenolic structure with two para-allyl groups [13]. Owing to the pharmacological importance of magnolol, several groups have undertaken the structural modifications of magnolol scaffold as well as the synthesis of its structural analogues. Our literature survey revealed that antioxidant, antimicrobial and antiproliferative activities of structural analogues of magnolol which was primarily attributed to the hydroxyl group at the biphenolic moiety [14–17]. Thus, antiproliferative and proapoptotic activity of eugenol related biphenyls that are close structural analogues of magnolol on malignant melanoma cells has been reported. Similarly di-hydroxyl magnolol obtained through coupling of eugenol has been reported to exhibit mild *in vitro* cytotoxicity against cancer cells. Allylated magnolol derivatives prepared through Claisen rearrangement exhibited potent antioxidant properties [16,17]. In view of the diverse pharmacological activities of magnolol scaffold we felt the need for further structural manipulations towards development of improved structures derived from magnolol. Our continued interest in developing secondary leads based on natural product scaffolds [18–20], we report here, the synthesis and *in vitro* screening of magnolol derivatives for antimicrobial and antiproliferative activities.

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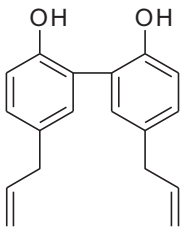


Fig. 1. The chemical structure of magnolol.

2. Results and discussion

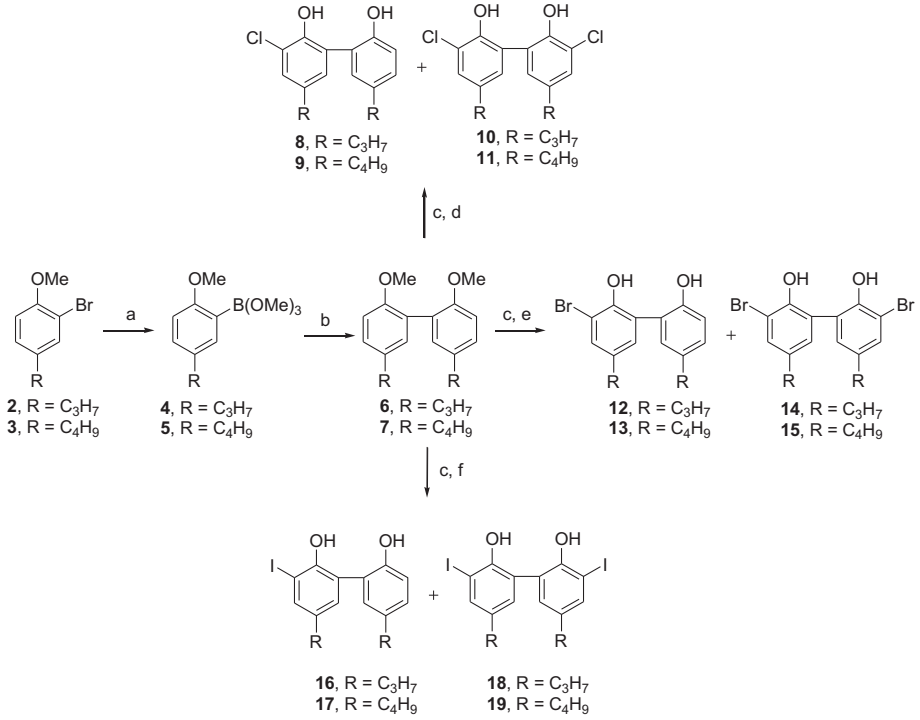
2.1. Chemistry

As illustrated in Scheme 1, magnolol derivatives were synthesised through Pd catalysed Suzuki coupling reaction. Compound **4** was synthesised by the reaction of **2** with $B(OMe)_3$ and nBuLi in dry THF at −78 °C. Compound **6** could be obtained by the reaction of **4** with $Pd(PPh_3)_4$ and Na_2CO_3 in dry DME under reflux (Suzuki coupling) in excellent yields (92%). Compound **6** on reaction with $AlCl_3$ in dimethyl sulphide, followed by the reaction with $CuCl_2$, LiCl in acetic acid gives compounds **8** and **10** (3:7) in good yields (80%). Compound **6** on reaction with $AlCl_3$ in dimethyl sulphide, followed by the reaction with Br_2 in triethylamine in toluene gives compounds **12** and **14** (4:6) in excellent yields (90%). Compound **6** on reaction with $AlCl_3$ in dimethyl sulphide, followed by the reaction with I_2 and CAN in acetonitrile gives compounds **16** and **18** (2:8) in excellent yields (93%). The synthesis of compound **18** was reported in literature with poor yields (16%), in which homo coupling of 3,6-diiodo-4-propyl phenol gives compound **18** [21]. Compound **5** was synthesised by the reaction of **3** with $B(OMe)_3$ and nBuLi in dry THF at −78 °C. Compound **7** could be obtained by the reaction of **5** with $Pd(PPh_3)_4$ and Na_2CO_3 in dry DME under reflux (Suzuki coupling) in excellent yields (90%). Compound **7** on reaction with $AlCl_3$ in dimethyl sulphide, followed by the reaction with $CuCl_2$, LiCl in acetic acid gives compounds **9** and **11** (3:7) in good yields (82%). Compound **7** on reaction with $AlCl_3$ in dimethyl sulphide, followed by the reaction with Br_2 in triethylamine and toluene afford compounds **13** and **15** (4:6) in good yields (91%). Compound **7** on reaction with $AlCl_3$ in dimethyl sulphide, followed by the reaction with I_2 and CAN in acetonitrile gives compounds **17** and **19** (2:8) in high yields (90%). Compound **21** was prepared according to literature procedure [22]. As illustrated in Scheme 2, homo peroxidative coupling of compound **20** using MTBAP gives compound **21** in moderate yields (52%). Compound **21** on reaction with $AlCl_3$ in dimethyl sulphide gives compounds **22** and **23** (2:8) in excellent yields (95%) (Scheme 2).

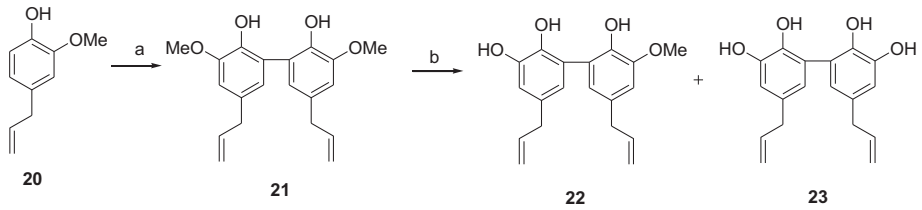
2.2. Evaluation of biological activity

2.2.1. Evaluation of antimicrobial activity

All the compounds were screened for antimicrobial activity (Table 1), and ciprofloxacin and erythromycin are taken as standard. Most of the compounds showed excellent antibacterial activity against gram positive bacteria (*S. aureus ATCC*, *MRSA 15187 and VRE*) whereas none of the compounds showed activity against gram negative bacteria. Compounds **10**–**15**, **19**, **22** and **23** showed promising antibacterial activity against *S. aureus ATCC*, *MRSA 15187*, *VRE* (MIC in the range of 1–32 μg/mL and MBC in the range of 1–128 μg/mL). Out of all the molecules synthesised compound **13** showed best activity (MIC against *S. aureus ATCC*, *MRSA 15187* and *VRE* are 2, 1 and 1 μg/mL respectively; MBC against *S. aureus ATCC*, *MRSA 15187* and *VRE* are 2, 1 and 2 μg/mL respectively). From the data (Table 1) it is clear that unsaturation at the side chain is not essential for antibacterial activity and alkyl groups like propyl, butyl, along with halogen substituents on the aromatic ring increases the antibacterial activity. The results clearly reveal the



Scheme 1. Reagents and conditions: (a) nBuLi, $B(OMe)_3$, dry THF, −78 °C, 2 h; (b) $Pd(PPh_3)_4$, Na_2CO_3, dry DME, reflux, 6 h; (c) $AlCl_3$, Me_2S, r.t. 1 h; (d) $CuCl_2$, LiCl, CH_3COOH, reflux, 12 h; (e) Br_2, triethylamine, −70 °C; (f) I_2, CAN, CH_3CN, r.t., 2 h.



Scheme 2. Reagents and conditions: (a) MTBAP, dry CH_2Cl_2, 0 °C, 15 min; (b) $AlCl_3/Me_2S$, r.t., 1 h.

pattern of activity with regard to degree and nature of halogen substitution on the aromatic rings of biaryl compounds. Even though mono-chloro and -iodo derivatives of propyl and butyl magnolol did not show appreciable activity against *MRSA*, *VRE* and *S. aureus ATCC* strains, the corresponding -monobromo derivatives showed significant inhibitory activity against these pathogens. However, the antibacterial activity of -dihalo derivatives of propyl and butyl magnolol had been comparatively significant wherein the -dibromo compounds exhibited highest activity. We have also tested all the compounds for antifungal activity against *Candida albicans ATCC 90028* and *Aspergillus fumigatus LSI-II* using Amphotericin-B as standard. Interestingly, compounds with allylic substitution i.e., **21** and **23** exhibited significant antifungal activity against *C. albicans ATCC 90028* (MIC 64 and 128 μg/mL for compounds **21** and **23** respectively) and *A. fumigatus LSI-II* (MIC 32 and 64 μg/mL for compounds **21** and **23** respectively) whereas other compounds did not show any significant antifungal activity.

2.2.2. Antiproliferative activity

In vitro antiproliferative activity of all the compounds was evaluated against a panel of three human cancer cell lines *viz.*, PC-3 (prostate cancer cells), HL-60 (human promyelocytic leukaemia cells) and MOLT-4 (human acute lymphoblastic leukaemia cell line) according to NCI guidelines and 5-FU was taken as reference compound. Among all the compounds, only alkylated-iodo and alkylated-bromo derivatives **13**, **14**, **18** and **19** exhibited better antiproliferative activity than parent magnolol (**1**) (Table 2). Compound **19**, with -diiodo and butyl substitutions, is the most active with IC50 values 2, 2 and 10 μM on HL-60, PC-3 and MOLT-4 cell lines respectively.

Table 1
MIC and MBC of magnolol derivatives in μg/mL.

Compound	*S. aureus ATCC* 29213		*MRSA* 15187		*VRE*	
	MIC	MBC	MIC	MBC	MIC	MBC
8	>256	>256	>256	>256	>256	>256
9	>256	>256	>256	>256	>256	>256
10	4	4	4	4	8	8
11	8	8	8	8	8	16
12	8	8	16	16	16	32
13	2	2	1	1	1	2
14	4	4	4	4	4	8
15	8	8	8	8	8	16
16	>256	>256	>256	>256	>256	>256
17	>256	>256	>256	>256	>256	>256
18	64	64	64	64	64	64
19	4	4	4	4	4	8
21	>256	>256	>256	>256	>256	>256
22	16	16	16	64	32	128
23	8	8	16	16	16	32
Magnolol	16	32	32	32	32	32
Erythromycin	0.5	1	64	64	64	64
Ciprofloxacin	0.25	0.5	8	8	32	64

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration.

Table 2
IC_{50} values (μM) of magnolol derivatives against a panel of cell lines.

Compound	HL-60	PC-3	MOLT-4
8	85	>100	>100
9	>100	>100	>100
10	>100	>100	>100
11	90	>100	>100
12	>100	>100	>100
13	25	74	35
14	30	95	55
15	>100	>100	>100
16	45	63	60
17	50	60	66
18	7	7	18
19	2	2	14
21	>100	>100	>100
22	>100	>100	>100
23	>100	>100	>100
Magnolol	48	62	58
5-FU	266	2.1	3.2

2.2.3. DNA fragmentation and flow cytometric analysis

DNA fragmentation, which is a typical hallmark of the apoptotic cell death was analysed in HL-60 cells. From the *in vitro* cytotoxicity

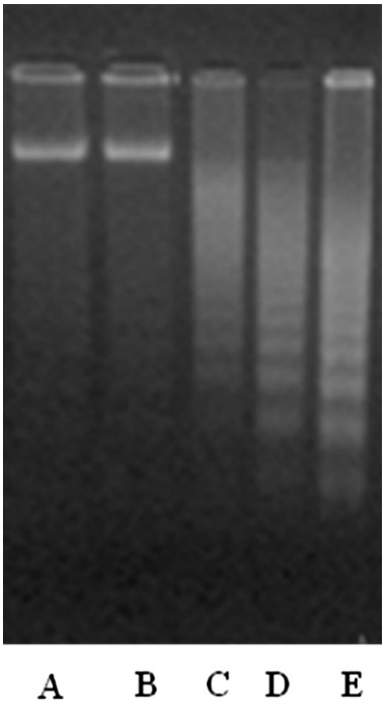


Fig. 2. Agarose gel electrophoresis of DNA extracted from HL-60 cells. The figure represents HL-60 cells treated with compound **19** for 24 h. DNA from the cells was extracted and electrophoresed in 1% agarose gel and visualized by ethidium bromide staining under UV illumination. Lanes A, B, C, D and E represent compound **19** at 0, 1, 5, 10 and 30 μM.

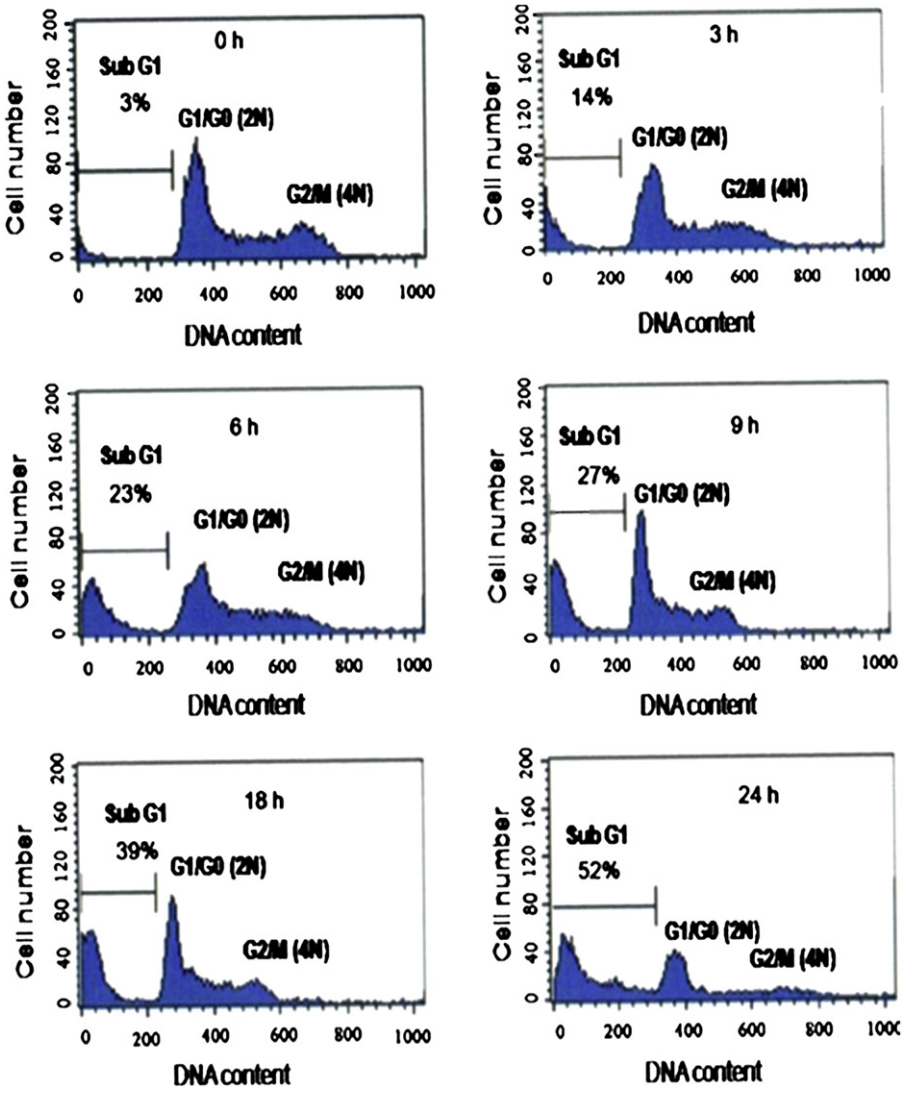


Fig. 3. Flow cytometric analysis of cells treated with compound **19** (10 μM) at different time intervals.

studies it was found that compound **19** significantly inhibits the growth of human promyelocytic leukaemia cells HL-60, and was therefore studied further to determine the mechanism of cell death in the same cell line. The DNA fragmentation analysis revealed that compound **19** induced a discrete ladder pattern in HL-60 cell line at 5, 10 and 30 μM after 24 h of incubation (Fig. 2). The flow cytometric analysis of compound **19** showed a dose dependent increase in the sub-G1 population in HL-60 cell line after 24 h. Flow cytometric analysis showed that the ratio of apoptosis in HL-60 cells for compound **19** at 1, 10, 30 and 100 μM were 12, 53, 67 and 78% respectively. In order to examine DNA profiles at different time points, flow cytometric analysis of compound **19** has been carried out at 10 μM concentration at time intervals of 0, 6, 9, 18, 24 h and ratio of apoptosis was found to be 3.7, 21, 33, 44 and 52% respectively (Fig. 3).

3. Conclusions

In conclusion, a series of novel magnolol derivatives synthesised and screened for antimicrobial and antiproliferative activity. Our study found that most of the alkylated halogenated magnolol derivatives exhibited excellent antibacterial activity against multi-drug resistant bacteria like *MRSA* and *VRE*. Alkylated-iodo and alkylated-bromo substituted derivatives showed improved anti-proliferative activity as compared to magnolol against PC-3, HL-60 and MOLT-4 human cancer cell lines. DNA laddering and flow cytometric analysis clearly revealed the mechanism involving apoptotic pathway to cell death induced by the novel biphenolic derivatives. We found that some of the compounds bearing allylic side chain exhibited significant antifungal activity against *C. albicans* and *A.* strains.

4. Experimental

Melting points were recorded on Buchi Melting point apparatus D-545 and IR spectra (KBr) on Bruker Vector 22 instrument. NMR spectra were recorded on Bruker DPX500 instrument in $CDCl_3$ with TMS as an internal standard. Chemical shift values are reported in δ (ppm) and coupling constants in hertz. Mass spectra were recorded on Q-STAR XL mass spectrometer (Applied Biosystems, USA) instrument. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F254 plates of thickness 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm and iodine.

4.1. Synthesis of magnolol derivatives

4.1.1. General procedure for Suzuki coupling

To a solution of compound **4** in dry DME were added $Pd(PPh_3)_4$ (catalytic amount) and Na_2CO_3 (3 equiv). The reaction mixture was refluxed for 6 h. After completion, the reaction mixture was diluted with 80 mL of water and extracted with ethyl acetate (3×50 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The crude product obtained was purified by flash chromatography to get pure compound (**6**) in excellent yields (92%).

4.1.2. General procedure for demethylation using $AlCl_3/Me_2S$

To a solution of $AlCl_3$ (1.2 equiv) in Me_2S was added compound (**21**) dissolved in Me_2S drop wise at room temperature under N_2 atmosphere. The reaction mixture was stirred for 1 h. Check the TLC, two new spots will appear due to partial and complete demethylation (compounds **22** and **23**). If the reaction mixture was stirred for 2 h, complete demethylation takes place and compound **23** form as sole product. After completion, the reaction mixture was quenched with ice-cold water and 2 N HCL, and then extracted with ethyl acetate. The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The crude product obtained was purified by flash chromatography to get pure compounds **22** and **23** (2:8) in good yields (95%).

4.1.3. General procedure for chlorination

To a solution of alkylated magnolol (1 equiv) in CH_3COOH were added $CuCl_2$ (1.5 equiv) and LiCl (1.5 equiv) under O_2 atmosphere. The reaction mixture was refluxed for 12 h. After completion, the reaction mixture was diluted with 100 mL of water and extracted with ethyl acetate (3×50 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The crude product obtained was purified by flash chromatography to get pure compounds **8** and **10** (3:7) in good yields (80%).

4.1.4. General procedure for bromination

To a solution of triethylamine (4 equiv) in toluene solution at −20 °C was added bromine drop wise. Then temperature was decreased to −70 °C. Then alkylated magnolol dissolved in anhydrous dichloromethane was added drop wise. The reaction mixture was stirred at −70 °C for 10 min then allows the reaction mixture to room temperature. After completion, the reaction mixture was diluted with 100 mL of water and extracted with ethyl acetate (3×50 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The crude product obtained was purified by flash chromatography to get pure compounds **12** and **14** (4:6) in excellent yields (90%).

4.1.5. General procedure for iodination

To a solution of alkylated magnolol in acetonitrile were added I_2 and CAN. The reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, solvent was evaporated in vacuum. Then the reaction mixture was diluted with 100 mL of water and extracted with ethyl acetate (3×50 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The crude product obtained was purified by flash chromatography to get pure compounds **16** and **18** (4:6) in excellent yields (90%).

4.1.6. Typical procedure for preparation of MTBAP

To a round bottom flask containing an aqueous solution (75% w/w) of methyltributylammonium chloride (1.258 g, 4 mmol) and dichloromethane (100 mL) was added a solution of potassium permanganate (632 mg, 4 mmol) in water (35 mL). The resulting mixture was stirred for 1 h, the phases were separated and the organic layer was dried over Na_2SO_4. After filtration, the dichloromethane was removed under reduced pressure and the product, a dark purple solid, was kept under high vacuum for 1 h affording 1.277 g (90%) of the oxidant salt MTBAP.

4.1.7. Typical procedure for oxidative coupling of eugenol (*21*)

To a solution of eugenol **20** (493 mg, 3 mmol) in dry dichloromethane (5 mL) under N_2 atmosphere at 0 °C, was added a solution of MTBAP (479 mg, 1.5 mmol) in dry dichloromethane (10 mL). After 15 min, water (10 mL) was added into the reaction flask followed by the bubbling of SO_2. The organic layer was separated, washed with a 0.1 M solution of HCl (3×5 mL), dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure. The product obtained was purified by flash chromatography to get pure compound **21** (52%).

4.1.8. 3-Chloro-5,5′-dipropyl-2,2′-biphenyldiol (*8*)

White colour solid; mp: 100 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.95 (t, J = 7.32, 6H), 1.57–1.53 (m, 4H), 2.5 (t, J = 7.3, 4H), 5.88 (s, −OH, 1H), 6.12 (s, −OH, 1H), 6.94 (d, J = 8.23, 1H), 7.02 (d, J = 1.86, 1H), 7.04 (d, J = 1.91, 1H), 7.11 (d, J = 8.2, 1H), 7.20 (d, J = 1.91, 1H); ^{13}C NMR: 13.76, 13.88, 24.56, 24.79, 37.03, 37.23, 117.11, 120.65, 124.35, 125.88, 128.7, 129.81, 130.59, 130.98, 135.63, 136.60, 145.92, 151.02; IR (KBr): 3518.38, 2954.5, 2872.24, 1239.23, 1078.7, 742.50 cm^{-1}; HRMS for $C_{18}H_{21}O_2Cl$ $[M + Na]^+$ Calc. 327.1127; found 327.1144; Anal. Calc. for $C_{18}H_{21}O_2Cl$: C 70.93, H 6.94; found: C 70.86, H 6.98.

4.1.9. 3-Chloro-5,5′-dibutyl-2,2′-biphenyldiol (*9*)

White colour solid; mp: 100–101 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.94 (t, J = 7.2, 6H), 1.39–1.35 (m, 4H), 1.61–1.58 (m, 4H), 2.55 (t, J = 7.24, 4H), 6.96 (d, J = 8.1, 1H), 7.03 (d, J = 1.88, 1H), 7.05 (d, J = 1.90, 1H), 7.10 (d, J = 8.12, 1H), 7.2 (d, J = 1.92, 1H); ^{13}C NMR: 13.99, 22.34, 22.44, 29.78, 33.64, 33.74, 34.68, 34.71, 115.06, 119.6, 123.6, 125.9, 126.18, 128.9, 129.33, 130.9, 136.1, 136.12, 144.5, 149.5; IR (KBr): 3356.5, 2956.6, 2928.12, 1468.17, 1236.15, 756.6 cm^{-1}; HRMS for $C_{20}H_{25}O_2Cl$ $[M + Na]^+$ Calc. 355.5217; found 355.5225; Anal. Calc. for $C_{20}H_{25}O_2Cl$: C 72.17, H 7.57; found: C 72.12, H 7.6.

4.1.10. 3,3′-Dichloro5,5′-dipropyl-2,2′-biphenyldiol (*10*)

White colour solid; mp: 103 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.95 (t, J = 7.32, 6H), 1.57–1.52 (m, 4H), 2.46 (t, J = 7.3, 4H), 5.86 (s, −OH, 2H), 6.93 (d, J = 2.01, 2H), 7.12 (d, J = 1.98, 2H); ^{13}C NMR: 12.7, 23.48, 35.94, 119.86, 124.49, 127.92, 129.29, 135.02, 145.39; IR (KBr): 3518.38, 2959.20, 2870.54, 1240.23, 1077.72, 742.5 cm^{-1}; HRMS for $C_{18}H_{20}O_2Cl_2$ $[M + Na]^+$ Calc. 362.6344; found 362.6347; Anal. Calc. for $C_{18}H_{20}O_2Cl_2$: C 63.73, H 5.94; found: C 63.66, H 5.98.

4.1.11. 5,5′-Butyl-3,3′-dichloro-2,2′-biphenyldiol (*11*)

White colour solid; mp: 105 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93 (t, J = 7.19, 6H), 1.45–1.22 (m, 4H), 1.66–1.51 (m, 4H), 2.56 (t, J = 7.19, 4H), 6.98 (d, J = 2.05, 2H), 7.2 (d, J = 2.05, 2H); ^{13}C NMR: 13.89, 22.24, 33.54, 34.59, 120.87, 125.48, 128.87, 130.08, 136.25, 146.38: IR (KBr): 3518.95, 2959.20, 2927.47, 1475.14, 1243.23, 1077.72, 795.57 cm^{-1}; HRMS for $C_{20}H_{24}O_2Cl_2$ $[M + Na]^+$ Calc. 390.6466; found 390.6470; Anal. Calc. for $C_{20}H_{24}O_2Cl_2$: C 65.40, H 6.59; found: C 65.36, H 6.63.

4.1.12. 3-Bromo-5,5′-dipropyl-2,2′-biphenyldiol (*12*)

White colour solid; mp: 95 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, J = 7.26, 6H), 1.6–1.55 (m, 4H), 2.52 (t, J = 7.21, 4H), 5.8 (s, −OH, 1H), 6.01 (s, −OH, 1H), 6.86 (d, J = 8.12, 1H), 6.97 (d, J = 1.9, 1H), 6.99 (d, J = 1.92, 1H), 7.03 (d, J = 6.41, 1H), 7.18 (d, J = 1.92, 1H); ^{13}C NMR: 13.28, 13.47, 23.89, 24.09, 36.22, 36.62, 110.37, 116.33, 123.71, 125.0, 129.1, 130.4, 131.2, 131.63, 134.92, 136.25, 146.12, 150.27; IR (KBr):

3521.3, 2962.60, 2871.54, 1472.12, 1249.23, 1080.72, 760.33, 745.60 cm^{-1}; HRMS for $C_{18}H_{21}O_2Br$ $[M + Na]^+$ Calc. 369.9727; found 369.9732; Anal. Calc. for $C_{18}H_{21}O_2Br$: C 61.9, H 6.06; found: C 61.82, H 6.09.

*4.1.13. 3-Bromo-5,5′-dibutyl-2,2′-biphenyldiol (**13**)*

Pale brown colour solid; mp: 97–98 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.93 (t, $J = 7.23$, 6H), 1.38–1.22 (m, 4H), 1.67–1.45 (m, 4H), 2.58 (t, $J = 7.29$, 4H), 5.7 (s, –OH, 1H), 5.95 (s, 1H), 6.92 (d, $J = 8.19$, 1H), 7.01 (d, $J = 1.92$, 1H), 7.05 (d, $J = 1.94$, 1H), 7.14 (d, $J = 6.41$, 1H), 7.35 (d, $J = 1.69$, 1H); ^{13}C NMR: 13.99, 22.34, 29.78, 33.74, 33.93, 34.71, 34.86, 114.70, 118.54, 121.3, 124.5, 125.9, 129.5, 130.6, 131.1, 135.9, 136.3, 144.0, 150.3; IR (KBr): 3526.02, 2958.30, 2869.8, 1481.27, 1468.93, 1240.81, 1093.81, 756.61 cm^{-1}; HRMS for $C_{20}H_{25}O_2Br$ $[M + Na]^+$ Calc. 398.0854; found 398.0859; Anal. Calc. for $C_{20}H_{25}O_2Br$: C 63.66, H 6.68; found: C 63.60, H 6.12.

*4.1.14. 5,5′-Dipropyl-3,3′-dibromo-2,2′-biphenyldiol (**14**)*

White colour solid; mp: 96 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.95 (t, $J = 7.32$, 6H), 1.66–1.59 (m, 4H), 2.53 (t, $J = 7.47$, 4H), 5.88 (s, –OH, 2H), 7.01 (d, $J = 2.03$, 2H), 7.35 (d, $J = 2.05$, 2H); ^{13}C NMR: 13.80, 24.58, 37.06, 120.98, 125.59, 129.03, 130.25, 136.13, 146.52; IR (KBr): 3455.5, 2957.36, 2928.27, 2870.6, 1375.6, 1245.75, 781.9 cm^{-1}; HRMS for $C_{18}H_{20}O_2Br_2$ $[M + Na]^+$ Calc. 448.9727; found 448.9739; $C_{18}H_{20}O_2Br_2$: C 50.49, H 4.71; found: C 50.41, H 4.75.

*4.1.15. 5,5′-Dibutyl-3,3′-dibromo-2,2′-biphenyldiol (**15**)*

Brown colour solid; mp: 98 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.93 (t, $J = 7.22$, 6H), 1.49–1.26 (m, 4H), 1.66–1.51 (m, 4H), 2.56 (t, $J = 7.33$, 4H), 5.83 (s, –OH, 2H), 7.01 (d, $J = 1.7$, 2H), 7.35 (d, $J = 1.67$, 2H); ^{13}C NMR: 13.79, 24.60, 36.81, 36.93, 111.12, 125.52, 131.02, 132.09, 136.64, 147.34; IR (KBr): 3450.83, 2958.33, 2928.21, 2869.88, 1377.99, 1241.67, 787.13 cm^{-1}; HRMS for $C_{18}H_{20}O_2Br_2$ $[M + Na]^+$ Calc. 469.0754; found 469.0769; Anal. Calc. for $C_{20}H_{24}O_2Br_2$: C 52.65, H 5.30; found: C 52.58, H 5.33.

*4.1.16. 3-Iodo-5,5′-dipropyl-2,2′-biphenyldiol (**16**)*

White colour solid; mp: 108 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.95 (t, $J = 7.27$, 6H), 1.54–1.5 (m, 4H), 2.5 (t, $J = 7.36$, 4H), 7.36–7.23 (m, 4H), 7.83 (d, $J = 1.96$, 1H), 10.84 (s, –OH, 2H); ^{13}C NMR: 13.75, 13.82, 24.72, 24.86, 37.66, 37.73, 117.154, 121.5, 124.36, 125.93, 129.1, 130.6, 131.5, 132.9, 135.6, 136.50, 145.9, 151.02; IR (KBr): 3524.3, 2958.5, 1472.4, 1070.3, 761.30 cm^{-1}; HRMS for $C_{18}H_{21}O_2I$ $[M + Na]^+$ Calc. 419.1215; found 419.1244; Anal. Calc. for $C_{18}H_{21}O_2I$: C 54.56, H 5.34; found: C 54.50, H 5.37.

*4.1.17. 3-Iodo-5,5′-dibutyl-2,2′-biphenyldiol (**17**)*

Pale brown colour solid; mp: 110 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.94 (t, $J = 7.21$, 6H), 1.39–1.23 (m, 4H), 1.69–1.47 (m, 4H), 2.56 (t, $J = 7.36$, 4H), 7.37–7.24 (m, 4H), 7.75 (d, $J = 1.73$, 1H), 10.71 (s, –OH, 2H); ^{13}C NMR: 13.86, 22.12, 30.3, 33.5, 33.66, 34.75, 34.93, 113.96, 118.42, 121.54, 124.27, 126.6, 129.75, 131.4, 131.9, 136.24, 136.57, 144.42, 150.64; IR (KBr): 3526.02, 2958.30, 2869.8, 1468.93, 1093.81, 756.61 cm^{-1}; HRMS for $C_{20}H_{25}O_2I$ $[M + Na]^+$ Calc. 447.3354; found 447.3367; Anal. Calc. for $C_{20}H_{25}O_2I$: C 56.61, H 5.94; found: C 56.56, H 5.98.

*4.1.18. 5,5′-Dipropyl-3,3′-diiodo-2,2′-biphenyldiol (**18**)*

Light yellow colour solid; mp: 111–112 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.98 (t, $J = 7.26$, 6H), 1.74–1.59 (m, 4H), 2.63 (t, $J = 7.29$, 4H), 7.35 (d, $J = 2.16$, 2H), 7.99 (d, $J = 2.23$, 2H), 10.84 (s, 2H); ^{13}C NMR: 13.84, 22.73, 35.84, 122.54, 125.91, 130.86, 132.45, 139.1, 150.5; IR (KBr): 3520.5, 2962.0, 2873.45, 1685.0, 1472.0, 1081.55, 763.3 cm^{-1}; HRMS for $C_{18}H_{20}O_2I_2$ $[M + Na]^+$ Calc. 545.3352; found 545.3358; Anal. Calc. for $C_{18}H_{20}O_2I_2$: C 41.4, H 3.86; found: C 41.33, H 3.89.

*4.1.19. 5,5′-Butyl-3,3′-diiodo-2,2′-biphenyldiol (**19**)*

Light yellow colour solid; mp: 113–114 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 0.95 (t, $J = 7.11$, 6H), 1.44–1.3 (m, 4H), 1.68–1.63 (m, 4H), 2.65 (t, $J = 7.56$, 4H), 7.45 (d, $J = 2.06$, 2H), 7.99 (d, $J = 2.03$, 2H), 10.84 (s, 2H); ^{13}C NMR: 13.94, 22.13, 32.84, 33.84, 123.5, 126.51, 131.06, 133.71, 139.19, 151.35; IR (KBr): 3518.45, 2960.0, 2870.42, 1475.14, 1077.5, 770.3 cm^{-1}; HRMS for $C_{20}H_{24}O_2I_2$ $[M + Na]^+$ Calc. 573.3456; found 573.3460; Anal. Calc. for $C_{20}H_{24}O_2I_2$: C 43.66, H 4.40; found: C 43.56, H 4.43.

*4.1.20. 3-Hydroxy-3′-methoxy-5,5′-dipropyl-2,2′-biphenyldiol (**22**)*

White colour solid; mp: 114–115 °C; ^1H NMR (500 MHz, $CDCl_3$): δ 3.32 (d, $J = 6.51$, 4H), 3.92 (s, 3H), 5.11–5.03 (m, 4H), 6.04–5.94 (m, 2H), 6.80–6.67 (m, 4H); ^{13}C NMR: 39.80, 40.00, 56.11, 110.28, 114.41, 115.63, 115.99, 120.9, 121.75, 123.36, 125.98, 133.16, 133.83, 137.38, 137.63, 138.72, 139.20, 146.35, 146.50; IR (KBr): 3525.3, 2935.0, 2870.24, 1233.23, 1125.1, 1069.6, 744.54 cm^{-1}; HRMS for $C_{19}H_{20}O_4$ $[M + Na]^+$ Calc. 335.1259; found 335.1264; Anal. Calc. for $C_{19}H_{20}O_4$: C 73.06, H 6.45; found: C 73.06, H 6.48.

4.2. Antibacterial activity

Antibacterial activity of the samples was performed using micro dilution method [23] against 3 g positive strains (*S. aureus ATCC 29213*, *Methicillin resistant S. aureus*, *Vancomycin resistant Enterococcus*). Bacterial suspensions were prepared in sterile normal saline from 24 h grown culture. The Minimum Inhibitory Concentration (MIC) was performed in Muller Hinton Broth (MHB; BD Biosciences, USA). Two-fold serial dilutions of samples were prepared in MHB in 100 μL volume in a 96 well U bottom microtitre plates (Tarson, Mumbai, India). The final concentrations of the samples ranged from 0.25 to 256 μg mL^{-1}. The turbidity of bacterial suspensions was adjusted to 0.5 McFarland ($\sim 1.5 \times 10^8$ CFU mL^{-1}), which was further diluted in MHB and, a 100 μL volume of this diluted inoculum was added to each well of the plate, resulting in a final inoculum of 5×10^6 CFU mL^{-1}. The plates were incubated at 37 °C for 24 h and were read visually. The minimum concentration of the sample showing no turbidity was recorded as MIC. The Minimum Bactericidal Concentration (MBC) was also determined from the same microtitre plates but after 24 h incubation. 20 μL of suspension from the well showing MIC value and wells containing 2×, 4×, 8× and 16× concentration of MIC value was spotted onto the Muller Hinton Agar plate. The spotted plate was incubated for 24 h and CFU count was taken simultaneously. The minimum concentration of the sample showing 3 log reductions in the inoculums size as compared to the original inoculums size was considered as the MBC.

4.3. DNA fragmentation assay

DNA fragmentation was determined by electrophoresis of extracted genomic DNA from HL-60 cells. Cells (2×10^6/mL medium) in 60 mm tissue culture plate were treated with compound **19** at 1, 5, 10 and 30 μM for 24 h. Cells were harvested, washed with PBS, pellets were dissolved in lysis buffer (10 mM EDTA, 50 mM tris pH 8.0, 0.5% w/v SDS and proteinase K (0.5 mg/mL)) and incubated at 50 °C for 1 h. Finally the DNA obtained was heated rapidly to 70 °C, supplemented with loading dye and immediately resolved on to 1.5% agarose gel at 50 V for 2–3 h.

4.4. Flow cytometric analysis

Effect of compound **19** on DNA content by cell cycle phase distribution was assessed using HL-60 cells by incubating the cells 1×10^6 mL/well with compound **19** (1, 10, 30 and 100 μL each) for

24 h. The cells were then washed twice with ice-cold PBS, harvested, fixed with ice-cold PBS in 70% ethanol and stored at −20 °C for 30 min. After fixation, these cells were incubated with RNase A (0.1 mg/mL) at 37 °C for 30 min, stained with propidium iodide (50 μg/mL) for 30 min on ice in dark, and then measured for DNA content using BDLSR flow cytometer (Becton Dickinson, USA) equipped with electronic doublet discrimination capability using blue (488 nm) excitation from argon laser. Data were collected in list mode on 10,000 events for FL2-A vs. FL2-W.

4.5. Evaluation of antiproliferative activity

The effect of magnolol derivatives on the growth of cancer cell lines was evaluated according to the procedure adopted by the National Cancer Institute for *in vitro* anticancer drug screening that uses the protein-binding dye sulforhodamine B to estimate cell growth [24]. Briefly, cells in their log phase of growth were harvested, counted and seeded (10^4 cells/well in 100 mL medium) in 96-well microtitre plates. After 24 h of incubation at 37 °C and 5% CO_2 to allow cell attachment, cultures were treated with varying concentrations (0.1–100 mM) of test samples made with 1:10 serial dilutions. Four replicate wells were set up for each experimental condition. Test samples were left in contact with the cells for 48 h under same conditions. Thereafter cells were fixed with 50% chilled TCA and kept at 4 °C for 1 h, washed and air-dried. Cells were stained with sulforhodamine B dye. The adsorbed dye was dissolved in tris-buffer and the plates were gently shaken for 10 min on a mechanical shaker. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was calculated by subtracting mean OD value of the respective blank from the mean OD value of experimental set. Percentage of growth in the presence of test material was calculated considering the growth in the absence of any test material as 100% and the results are reported in terms of IC_{50} values. 5-FU was taken as positive control.

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