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ORIGINAL ARTICLE

Eco-sustainable synthesis and biological evaluation of 2-phenyl 1,3-benzodioxole derivatives as anticancer, DNA binding and antibacterial agents

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Benzodioxole;
Anticancer;
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DNA binding;
Green chemistry

Abstract The current research and development scenario in medicinal chemistry demands small molecules synthesized in a simple, fast and effective way with enhanced activity and fewer side effects than the existing ones. Therefore, one-pot, microwave assisted green and efficient synthesis of a series of derivatives belonging to 2-phenyl 1,3-benzodioxole (**1a**–**14a**) and 2-phenyl 1,3-benzodioxole-4-ol (**1b**–**14b**) class were carried out and subsequently investigated for their anticancer, antibacterial and DNA binding potential. Compound **3c** proved to be the most active one among the screened derivatives possessing anticancer and antibacterial potency greater than the standard reference compound (cisplatin and cinoxacin for anticancer and antibacterial activity, respectively). The most active compound in terms of DNA binding capacity was found to be **5b**. A rewarding feature of the work is a facile, convenient, eco friendly one step synthesis of compounds demonstrating attenuated activity against cancer and bacterial cell with an inherent potential of binding

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to DNA. Subsequently, a hit molecule for further anticancer, antibacterial (compound **3c**) and DNA binding studies (compound **5b**) was also identified.

1. Introduction

The design of small molecules for better treatment of diseases has become an important therapeutic objective, given the wide-ranging side effects of existing molecules and rapid resistance developed to them (Leaf, 2004; Allerberger and Mittermayer, 2008). The benzothiazole nucleus is found in many such promising small molecule anticancer and antibacterial agents which were evaluated up to advanced preclinical stage (Racane et al., 2013). Among the small molecules with a benzothiazole nucleus, the study of 2-phenyl substituted benzothiazole (Fig. 1) derivatives is of considerable current interest owing to their diverse biophysical and biological properties. They are reported to possess antitumor (Kadri et al., 2008), antibacterial (Bandyopadhyay et al., 2011), antifungal (Dutta Gupta et al., 2010), antiparasitic (Kuvaev et al., 2005) and antioxidant activity (Sharma et al., 2013). Further they have strong activity profile as imaging agents for β-amyloid protein which aids in non-invasive diagnosis of Alzheimer's disease (Weekes and Westwell, 2009).

The benzodioxole ring which is an isostere of benzothiazole nucleus is also studied as antitumor (Wei et al., 2012), antibacterial (Leite et al., 2004), antifungal (Bakhite and Radwan 1999), antiparasitic (Kamau et al., 2011), antimalarial (Nelson and Hoosseintehrani 1982) and antioxidant agents (Zhao et al., 1997). Additionally some benzodioxole derivatives are also used as pesticides or pesticide intermediates and herbicides (Ugolini et al., 2005). The 1,3-benzodioxole system is also an integral part of many natural products like sesamol (Shenoy et al., 2011) and piperine (Srinivasan 2007). The extensive review of the literature revealed that the 2-phenyl substituted benzodioxole ring system is yet to be explored for various biological activities.

Discovery and development of a new drug molecule is a lengthy and costly affair which may lead to stifling of innovation (DiMasi et al., 2003). Moreover, environment contamination involved in the discovery process of new chemical entities is a global concern. Therefore, current medicinal chemistry research strives for rapid synthesis of small molecules with increased efficacy and lesser side effects than the existing ones in a simple, effective and environment friendly fashion.

From the above mentioned facts and based on the principle of bioisosterism, fourteen compounds belonging to 2-phenyl 1,3-benzodioxole (Fig. 2a) and 2-phenyl 1,3-benzodioxol-4-ol series (Fig. 2b) were synthesized using green chemistry approach and subsequently evaluated for anticancer and antibacterial activity. Subsequently, DNA binding studies were also carried out to ascertain the mechanism of action of the compounds.

2. Experimental

2.1. Chemistry

The chemicals, reagents and solvents employed for synthesis were procured from Hi-media Laboratories (Mumbai, India) and SD fine-chem limited (Mumbai, India). The progress of the reaction and purity were monitored by using TLC Silica gel 60 F_{254} aluminium sheets (Merck F_{254}, Darmstadt, Germany) developed in mobile phase containing ethyl acetate and petroleum ether (1:1). The melting point of the synthesized compounds was determined by DRK Digital melting point apparatus. IR spectra were recorded on Shimadzu IR-Affinity spectrometer using KBr pellets. The ^1H spectra of the compounds synthesized were acquired in deuterated DMSO on a Bruker ARX 400 MHz (Bruker AG, Fallanden, Switzerland) instrument. Tetramethylsilane was used as the internal standard and all chemical shift values were expressed in parts per million (δ, ppm). The mass spectra were obtained from 6120 Quadrupole LC/MS mass spectrometer using electron spray ionization method. (Agilent Technologies, California, USA).

Compounds **1(a)** to **14(a)** were synthesized according to the literature (Dutta Gupta et al., 2012).

2.1.1. General Procedure for the synthesis of *1(b)* to *14(b)*

Pyrogallol (1 mol equivalent) and benzoic acid derivatives (1.05 mole equivalent) were heated in the microwave (Biotage Initiator 2.5 at 350 W, 100 °C for 30–120 s) in the presence of polyphosphoric acid (0.1 mol equivalent, Tables 1 and 2). TLC was observed to monitor the completion of the reaction by using ethyl acetate: petroleum ether in the ratio of 1:1 as the mobile phase. The reaction mass was neutralized with 10% NaOH solution and then filtered. The crude product was recrystallized using 70% alcohol (Fig. 3).

2.2. In vitro cytotoxicity studies

The *in-vitro* cytotoxicity potential of the test compounds was evaluated on A549 human lung carcinoma cells using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] based cell proliferation assay (Van Meerloo et al., 2011; Ferrari et al., 1990). The carcinoma cell lines were obtained from National Centre for Cell Science (NCCS), Pune (India) and cultivated in Dulbecco's modified Eagle's red medium (DMEM) (Sigma Life Science, USA) containing 10% foetal bovine serum (FBS). An equal number of cells were incubated at 37 °C with 5% CO_2 in a 96 well micro plates. Thereafter, the cells were treated with test compounds and standard at concentration of 100 μg/ml. Control cells were supplemented with 0.5% DMSO. After 72 h treatment, 5 μl of MTT reagent along with 45 ml of phenol red and FBS free DMEM was added to each well and plates were incubated at 37 °C with 5% CO_2 for 4 h. Subsequently, 50 ml of solubilization buffer was added to each well to solubilize the coloured

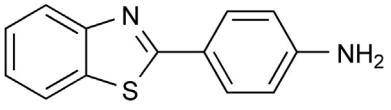


Figure 1 2-aminophenyl benzothiazole.

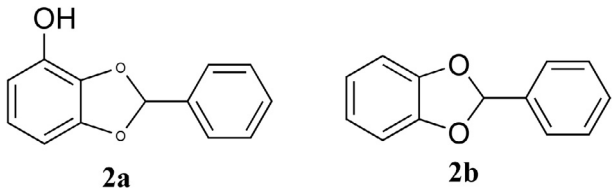


Figure 2 Chemical structures of benzodioxole nucleus synthesized.

formazan crystals produced by the reduction of MTT. After 24 hrs, the optical density was measured at 550 nm using a spectrophotometer in a microplate reader (Bio-Rad, USA). Cisplatin was used as standard reference compound.

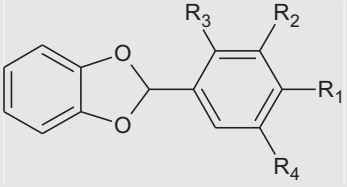
2.3. DNA binding studies

The interaction of the compounds with calf thymus DNA was studied in phosphate buffer of pH 7 using UV visible spectrophotometer (Shimadzu, UV 1800) (Mansuri-Torshizi et al., 2001). The hypochromic effect observed in the absorption spectra of the molecules with increasing concentrations of DNA (0–100 μM) is shown in Fig 4. The change in absorbance was measured at 210–320 nm for synthetic compounds–DNA complex. Scatchard equation was utilized to build the binding isotherm. The half reciprocal UV plot obtained on the basis of absorbance data for synthetic compounds-DNA complex is depicted in Fig. 5. The linear plots obtained indicate the involvement of one binding process with independent binding sites on DNA. The parameters, λ_{max}, hypochromicity, isobestic point and binding constant were found from the absorption spectra (Mansouri-Torshizi et al., 2008). The intrinsic binding constant (K_i) for a given complex with DNA was obtained from a plot of $D/\Delta\varepsilon_{app}$ versus D according to equation, $D/\Delta\varepsilon_{app} = D/\Delta\varepsilon + 1/\Delta\varepsilon \times K$, where D = concentration of DNA in base molarities, $\Delta\varepsilon_{app} = |\varepsilon_a - \varepsilon_f|$ and $\Delta\varepsilon = |\varepsilon_b - \varepsilon_f|$, where ε_a and ε_f are respective extinction coefficients of the complex in the presence and absence of DNA. The apparent extinction coefficient ε_a is obtained by calculating Aobs/[Acridones]. The data were fitted to the equation with a slope equal to $1/\Delta\varepsilon$ and Y-intercept equal to $1/(\Delta\varepsilon \times K)$. Thereafter the intrinsic binding constant (K_i) was determined from the slope of Y-intercept.

2.4. Antibacterial studies

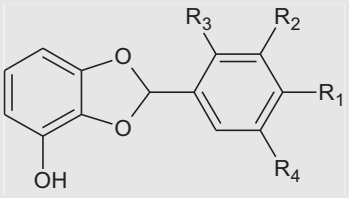
The microorganisms were procured from MTCC, Mumbai, India. The in vitro antibacterial studies were carried out by disc diffusion method in nutrient agar medium (Dutta Gupta et al., 2010, Ednie et al., 2000) The screening of the compounds was performed against *Escherichia coli* (MTCC 40, Gram negative), *Pseudomonas aeruginosa* (MTCC 424, Gram negative) *Bacillus subtilis* (MTCC 441, Gram positive) and *Staphylococcus aureus* (MTCC 3160, Gram positive) at concentration of 60, 80, 100, 120 and 140 μg/ml. Cinoxacin was used as standard reference drug. The required concentrations of the compounds were prepared in sterile DMSO. The sterile growth medium was poured into the petri plate and allowed to solidify. Subsequently, the microbial suspension was swabbed on agar bed using a sterile cotton swab. This was followed by placing a sterile paper disc uniformly on the agar bed. The synthesized compounds and standard drug contained in sterile paper disc were allowed to diffuse for 10 min. Thereafter, the petri plates were incubated for 24 h at 37 °C. The zone of inhibition was measured in mm to determine the antimicrobial potency of the compounds.

Table 1 Details of 2-phenyl benzodioxole derivatives synthesized.



S. No.	R_1	R_2	R_3	R_4	Reactant II	Reaction time (s)	Physical description	Melting point °C	Rf value	% yield
1a	H	H	H	H	Benzoic acid	30	White crystals	50–51	0.703	80.2
2a	Cl	H	H	H	4-Chloro benzoic acid	45	Light grey powder	102–105	0.393	75.8
3a	Me	H	H	H	4-Methyl benzoic acid	60	Brownish powder	57–58	0.721	78.3
4a	Me	OMe	H	H	3-methoxy-4-methyl benzoic acid	45	Greyish powder	99–101.	0.750	82.5
5a	H	H	OH	H	2-hydroxy benzoic acid	90	White crystals	115–118	0.786	60.6
6a	NH_2	H	H	H	4-amino benzoic acid	120	Brownish crystals	120–125	0.667	65.5
7a	H	NH_2	H	H	3-amino benzoic acid	120	Creamish powder	130–134	0.634	68.9
8a	H	H	NH_2	H	2-amino benzoic acid	105	Light brown crystals	188–190	0.626	62.8
9a	H	H	N-CH_3	H	N-methyl-2-amino benzoic acid	120	Light brown powder	174–175	0.661	68.4
10a	OH	OCH_3	H	H	Vanillic acid	45	White crystals	54–59	0.421	60.6
11a	Cl	H	Cl	H	2,4–dichloro benzoic acid	90	White powder	171–174	0.722	78.1.
12a	OH	OH	H	OH	Gallic acid	120	Greyish crystals	178–180	0.328	61.3
13a	H	H	OAc	H	Aspirin	105	White crystals	78–80	0.698	81.3
14a	H	H	COOH	H	Phthalic acid	60	Greyish crystals	102–105	0.746	70.5

Table 2 Details of 2-phenyl benzodioxole-4-ol derivatives synthesized.



S. No.	R_1	R_2	R_3	R_4	Reactant II	Reaction time (s)	Physical description	Melting point °C	Rf value	% yield
1b	H	H	H	H	Benzoic acid	30	White crystals	113–114	0.323	72.6
2b	Cl	H	H	H	4-Chloro benzoic acid	30	Pale white crystals	99–103	0.256	78.2
3b	Me	H	H	H	4-Methyl benzoic acid	90	Greyish powder	122–125	0.465	82.4
4b	Me	OMe	H	H	3-Methoxy-4-methyl benzoic acid	60	Dark brown powder	87–89	0.333	68.6
5b	H	H	OH	H	2-Hydroxy benzoic acid	60	White crystals	110–112	0.286	70.8
6b	NH_2	H	H	H	4-Amino benzoic acid	90	Light brown crystals	165–168	0.376	65.1
7b	H	NH_2	H	H	3-Amino benzoic acid	90	Creamish powder	154–156	0.365	60.6
8b	H	H	NH_2	H	2-Amino benzoic acid	120	Brownish crystals	180–182	0.343	61.4
9b	H	H	N-CH_3	H	N-Methyl-2-amino benzoic acid	120	Brown powder	164–168	0.423	68.8
10b	OH	OCH_3	H	H	Vanillic acid	30	Cream coloured crystals	72-73	0.289	62.6
11b	Cl	H	Cl	H	2,4-Dichloro benzoic acid	90	White powder	184–185	0.523	80.5
12b	OH	OH	H	OH	Gallic acid	120	Cream coloured powder	180–183	0.275	60.2
13b	H	H	OAc	H	Aspirin	90	White crystals	87–90	0.462	75.5
14b	H	H	COOH	H	Phthalic acid	45	Greyish powder	112–113	0.491	76.8

3. Results and discussion

3.1. Chemistry

A series of 2 phenyl substituted 1,4-benzodioxole derivatives were synthesized (Fig. 3) by green chemistry approach and characterized as per literature (Dutta Gupta et al., 2012). In a similar environment friendly fashion, one-pot synthesis of a series of 2 phenyl substituted 1,4-benzdioxole-4-ol derivatives were carried out by using polyphosphoric acid as solvent/catalyst under microwave irradiation (Fig. 3). The physical properties of the synthesized compounds are summarized in Tables 1 and 2. The reaction is believed to proceed by the attack of the hydroxyl group of benzoic acid by 'H' of polyphosphoric acid to form the protonated benzoic acid which is attacked by catechol/pyrogallol to form a tetrahedral intermediate that further cyclizes to form the 1,3-benzodioxole ring. The formation of the synthesized compounds was confirmed by IR, ^{1}H NMR and mass spectral analysis. In the IR spectra of compounds (**1b**–**14b**), the stretching bands due to one OH group were detected in the range of 3498–3317 cm^{-1}. The compounds **6b**–**9b** showed a characteristic NH stretching band between 3325 and 3381 cm^{-1}. In case of compound **2b** and **11b**, corresponding C–Cl stretching was observed at 732 cm^{-1} and 761 cm^{-1}, respectively. In ^{1}H NMR spectra of the compounds (**1b**–**14b**), a single peak corresponding to the CH proton of the dioxole ring system was observed between 7.0 and 7.5. The physical properties and percentage yield of the synthesized compounds along with their IR, ^{1}H NMR and mass spectral data are given below.

3.1.1. 2-Phenylbenzo[d][1,3]dioxole (*1a*) (Cole et al., 1980)

It was obtained as a light grey solid, 80.2% yield, mp 50–51 °C.

3.1.2. 2-(4-Chlorophenyl)benzo[d][1,3]dioxole (*2a*)

It was obtained as a light grey solid, 75.8% yield, mp 102–105 °C. IR (KBr cm^{-1}): 1089 (C—O stretching), 3115 (aromatic CH stretching), 758 (C—Cl stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.8(d, 1H, ArH, J = 6.4), 6.9 (d, 1H, ArH, J = 6.5), 7.1 (d, 2H, ArH, J = 7.1), 7.4 (s, 1H, dioxole CH), 7.6 (t, 2H, ArH), 8.1 (d, 2H, ArH, J = 7.6). Mass (m/z): 199 (M + 1).

3.1.3. 2-(4-Methylphenyl) benzo[d][1,3]dioxole (*3a*)

It was obtained as a brown solid, 78.3% yield, mp 57–58 °C. IR (KBr cm^{-1}): 1174 (C—O stretching), 3032 (aromatic CH stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.8 (d, 2H, ArH, J = 6.4), 6.9 (d, 2H, ArH, J = 6.5), 7.1 (t, 2H, ArH), 7.4 (s, 1H, dioxole CH), 8.0 (d, 2H, ArH, J = 7.6), 2.4 (s, 1H, CH_3). Mass (m/z): 232.6 (M + 1).

3.1.4. 2-(3-Methoxy-4-methylphenyl benzo[d][1,3]dioxole (*4a*)

It was obtained as a grey solid, 82.5% yield, mp 99-101 °C. IR (KBr cm^{-1}): 1149 (C—O stretching), 3066 (aromatic CH stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.9 (d, 1H, ArH, J = 6.5), 7.0 (d, 1H, ArH, J = 6.5), 7.1 (s, 1H, ArH), 7.3 (s, 1H, dioxole CH), 7.5 (d, 1H, ArH), 7.6 (d, 1H, ArH, J = 7.3), 7.7 (t, 2H, ArH, J = 7.1), 2.3 (s, 1H, CH_3), 3.9 (s, 1H, OCH_3). Mass (m/z): 242.2 (M + 1).

3.1.5. 2-(2-Hydroxyphenyl benzo[d][1,3]dioxole (*5a*)

It was obtained as a white solid, 60.6% yield, mp 115–118 °C. IR (KBr cm^{-1}): 1155 (C—O stretching), 3007 (aromatic CH stretching), 3238 (OH stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.9 (d, 3H, ArH, J = 6.4), 7.5 (d, 1H, ArH, J = 7.5), 7.5 (t, 1H, ArH), 7.5 (s, 1H, dioxole CH), 7.7 (t,

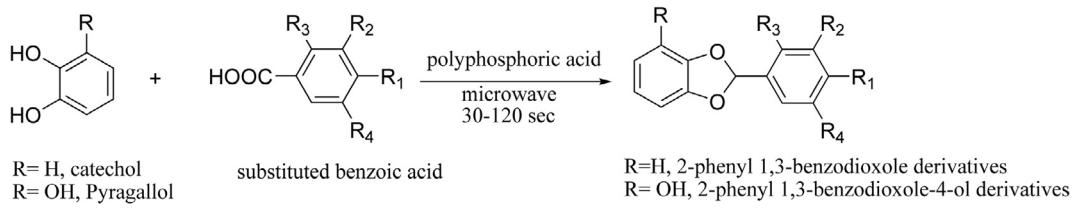


Figure 3 Scheme of synthesis.

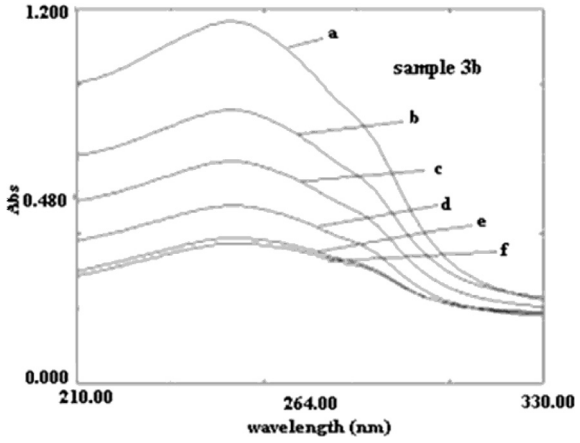


Figure 4 Effect of CT DNA on synthesized compound 3b.

1H, ArH), 7.7 (t, 2H, ArH, J = 7.1), 5.2 (s, 1H, OH). Mass (m/z): 214.0 (M + 1).

*3.1.6. 2-(4-Aminophenyl) benzo[d][1,3]dioxole (**6a**)*

It was obtained as a brown solid, 65.5% yield, mp 120–125 °C. IR (KBr cm^{-1}): 1070 (C—O stretching), 3008 (aromatic CH stretching), 3361 (NH stretching), 1332 (C≡N stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.6 (d, 3H, ArH, J = 6.3), 7.2 (d, 1H, ArH, J = 7.0), 7.6 (t, 1H, ArH), 7.2 (s, 1H, dioxole CH), 7.8 (t, 1H, ArH), 7.8 (d, 2H, ArH, J = 7.4), 4.0 (s, 1H, NH stretching). Mass (m/z): 213.0 (M + 1).

*3.1.7. 2-(3-Aminophenyl) benzo[d][1,3]dioxole (**7a**)*

It was obtained as a cream solid, 68.9% yield, mp 130–134 °C. IR (KBr cm^{-1}): 1153 (C—O stretching), 2985 (aromatic CH stretching), 3404 (NH stretching), 1274 (C≡N stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.7 (d, 3H, ArH, J = 6.3), 7.0 (d, 2H, ArH, J = 6.5), 7.1 (t, 2H, ArH), 7.6 (s, 1H, dioxole CH), 7.1 (d, 1H, ArH, J = 6.5), 4.9 (s, 1H, NH stretching). Mass (m/z): 213.0 (M + 1).

*3.1.8. 2-(2-Aminophenyl) benzo[d][1,3]dioxole (**8a**)*

It was obtained as a light brown solid, 62.8% yield, mp 188–190 °C. IR (KBr cm^{-1}): 1091 (C—O stretching), 2950 (aromatic CH stretching), 3300 (NH stretching), 1313 (C≡N stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.5(d, 2H, ArH, J = 6.3), 6.7 (d, 2H, ArH, J = 6.5), 7.6 (t, 1H, ArH), 7.2 (s, 1H, dioxole CH), 7.6 (d, 1H, ArH, J = 7.2), 7.7(t, 1H, ArH), 4.9 (s, 1H, NH stretching). Mass (m/z): 213.0 (M + 1).

*3.1.9. 2-(N-methyl-2-aminophenyl) benzo[d][1,3]dioxole (**9a**)*

It was obtained as a light brown solid, 68.4% yield, mp 174–175 °C. IR (KBr cm^{-1}): 1100 (C—O stretching), 2941 (aromatic CH stretching), 3387 (NH stretching), 1253 (C≡N stretching).. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.5 (d, 2H, ArH, J = 6.3), 6.6 (d, 2H, ArH, J = 6.3), 7.3 (t, 2H, ArH), 7.3 (s, 1H, dioxole CH), 7.7 (t, 1H, ArH), 2.8 (s, 1H, CH_3), 4.8 (s, 1H, NH stretching). Mass (m/z): 227.1 (M + 1).

*3.1.10. 2-(3-Methoxy-4-hydroxyphenyl) benzo[d][1,3]dioxole (**10a**)*

It was obtained as a white solid, 60.6% yield, mp 54–59 °C. IR (KBr cm^{-1}): 1100 (C—O stretching), 3242 (aromatic CH stretching), 3435 (OH stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.2 (d, 2H, ArH, J = 6.0), 6.6 (s, 2H, ArH), 6.9 (t, 1H, ArH), 7.3 (s, 1H, dioxole CH), 7.3 (t, 1H, ArH), 3.8 (s, 3H, OCH_3), 5.1 (s, 1H, OH stretching). Mass (m/z): 243.1 (M + 1).

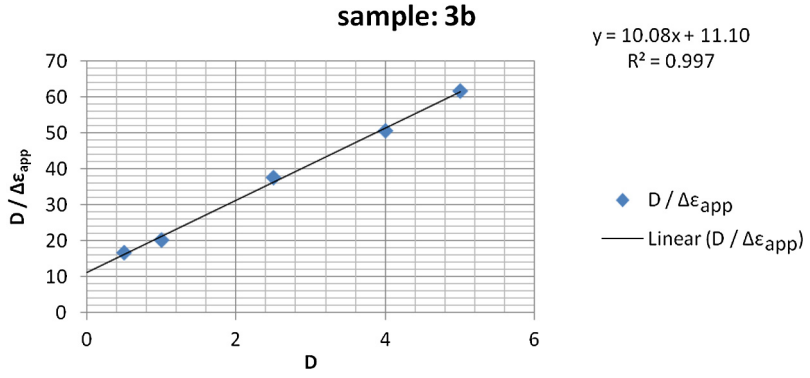


Figure 5 Half reciprocal UV plots for the binding of synthesized compound 3b to DNA.

*3.1.11. 2-(2,4-Dichlorophenyl) benzo[d][1,3]dioxole (**11a**)*

It was obtained as a white solid, 78.1% yield, mp 171–174 °C. IR (KBr cm^{-1}): 1116 (C—O stretching), 2993 (aromatic CH stretching), 696 (C—Cl stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.2 (d, 2H, ArH, J = 6.8), 7.5 (s, 2H, ArH), 7.7 (d, 1H, ArH, J = 7.3), 7.6 (s, 1H, dioxole CH), 7.8 (t, 2H, ArH). Mass (m/z): 267.1 (M + 1).

*3.1.12. 2-(3,4,5-Trihydroxyphenyl) benzo[d][1,3]dioxole (**12a**)*

It was obtained as a grey solid, 61.3% yield, mp 178–180 °C. IR (KBr cm^{-1}): 1072 (C—O stretching), 3051 (aromatic CH stretching), 3460 (OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.7 (s, 2H, ArH), 6.7 (d, 1H, ArH, J = 6.3), 6.9 (d, 1H, ArH, J = 6.4), 7.4 (s, 1H, dioxole CH), 6.9 (t, 2H, ArH), 5.1 (s, 1H,OH stretching). Mass (m/z): 246.9 (M + 1).

*3.1.13. 2-(2-Acetyloxyphenyl) benzo[d][1,3]dioxole (**13a**)*

It was obtained as a white solid, 81.3% yield, mp 78–80 °C. IR (KBr cm^{-1}): 1105(C—O stretching), 3028(aromatic CH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.9 (d, 1H, ArH, J = 6.4), 7.1 (d, 1H, ArH, J = 6.5), 7.3 (d, 1H, ArH, J = 6.8), 7.4 (s, 1H, dioxole CH), 7.7 (t, 1H, ArH), 7.4 (d, 1H, ArH), 7.6 (d, 1H, ArH, J = 7.3) 7.7 (d, 1H, ArH, J = 7.2), 7.9 (t, 1H, ArH), 2.2 (s, 1H, OCH_3). Mass (m/z): 256.1 (M + 1).

*3.1.14. 2-(2-Carboxyphenyl) benzo[d][1,3]dioxole (**14a**)*

It was obtained as a grey solid, 70.5% yield, mp 102–105 °C. IR (KBr cm^{-1}): 1111 (C—O stretching), 2980 (aromatic CH stretching), 3429 (OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.1 (d, 1H, ArH, J = 6.5), 7.5 (d, 3H, ArH, J = 7.2), 7.5 (s, 1H, dioxole CH), 7.6 (d, 2H, ArH, J = 7.3), 7.6 (t, 2H, ArH), 13 (s, 1H, COOH). Mass (m/z): 242.2 (M + 1).

*3.1.15. 2-Phenylbenzo[d][1,3]dioxol-4-ol (**1b**)*

It was obtained as a white solid, 72.6% yield, mp 113–114 °C. IR (KBr cm^{-1}): 1141 (C—O stretching), 3182 (aromatic CH stretching), 3498 (OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 7.4 (d, 2H, ArH J = 7.3), 7.6 (d, 2H, ArH J = 7.4), 7.3 (s, 1H, dioxole CH), 8.1(t, 4H, ArH), 5.2 (s, 1H, OH). Mass (m/z): 213.9 (M−1).

*3.1.16. 2-(4-Chlorophenyl) benzo[d][1,3]dioxol-4-ol (**2b**)*

It was obtained as a pale white solid, 78.2% yield, mp 99–103 °C. IR (KBr cm^{-1}): 1107 (C—O stretching), 3050 (aromatic CH stretching), 732 (C—Cl stretching). 3412 (OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.4 (d, 2H, ArH, J = 6.3), 6.6 (d, 1H, ArH, J = 6.4), 6.8(d, 1H, ArH), 7.3 (s, 1H, dioxole CH), 7.4 (d, 1H, ArH, J = 7.2), 7.9 (d, 1H, ArH, J = 7.4), 7.9 (t, 1H, ArH), 5.2 (s, 1H, OH). Mass (m/z): 248.7(M + 1).

*3.1.17. 2-(4-Methylphenyl) benzo[d][1,3]dioxol-4-ol (**3b**)*

It was obtained as a grey solid, 82.4% yield, mp 122–125 °C. IR (KBr cm^{-1}): 1107 (C—O stretching), 3050 (aromatic CH stretching), 3414(OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.2 (d, 2H, ArH, J = 6.1), 6.4 (d, 1H, ArH, J = 6.2), 6.8 (d, 1H, ArH, J = 6.7), 7.3 (s, 1H, dioxole CH), 7.4 (d, 1H, ArH, J = 7.3), 7.8 (d, 1H, ArH, J = 7.4), 7.8 (t, 1H, ArH), 2.3(s, 3H, CH_3). Mass (m/z): 22.1 (M + 1).

*3.1.18. 2-(3-Methoxy-4-methyl phenyl) benzo[d][1,3]dioxol-4-ol (**4b**)*

It was obtained as a dark brown solid, 68.6% yield, mp 87–89 °C. IR (KBr cm^{-1}): 1103 (C—O stretching), 2937(aromatic CH stretching), 3417 (OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.6 (d, 2H, ArH, J = 6.3), 7.2 (s, 1H, dioxole CH), 7.5(s, 1H, ArH), 7.5 (d, 2H, ArH, J = 7.3), 7.6 (d, 1H, ArH, J = 7.3), 7.6(t, 1H, ArH), 2.3 (s, 3H, –OCH_3), 2.3 (s, 3H, CH_3). Mass (m/z): 258.8 (M + 1).

*3.1.19. 2-(2-Hydroxyphenyl) benzo[d][1,3]dioxol-4-ol (**5b**)*

It was obtained as a white solid, 70.8% yield, mp 110–112 °C. IR (KBr cm^{-1}): 1128 (C—O stretching), 2974 (aromatic CH stretching), 3381 (OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.9 (d, 3H, ArH, J = 6.5), 7.3(s, 1H, dioxole CH), 7.5 (d, 1H, ArH, J = 7.3), 7.5 (t, 1H, ArH), 7.7 (d, 2H, ArH, J = 7.4), 5.0 (s, 2H, OH). Mass (m/z): 230.1 (M + 1).

*3.1.20. 2-(4-Aminophenyl) benzo[d][1,3]dioxol-4-ol (**6b**)*

It was obtained as a light brown solid, 65.1% yield, mp 165–168 °C. IR (KBr cm^{-1}): 1128(C—O stretching), 3051(aromatic CH stretching), 1325(C≡N stretching), 3381 (N—H stretching), 3460 (OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.2 (d, 3H, ArH, J = 6.0), 6.5 (d, 1H, ArH, J = 6.3), 6.5 (t, 1H, ArH), 7.2 (s, 1H, dioxole CH), 7.6 (t, 2H, ArH), 4.2 (s, 1H, –NH), 5.0 (s, 1H, OH). Mass (m/z): 229.1 (M + 1).

*3.1.21. 2-(3-Aminophenyl) benzo[d][1,3]dioxol-4-ol (**7b**)*

It was obtained as a cream solid, 60.6% yield, mp 154–156 °C. IR (KBr cm^{-1}): 1153 (C—O stretching), 2985 (aromatic CH stretching), 1332 (C≡N stretching), 3300 (N—H stretching), 3406(OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.2 (d, 1H, ArH, J = 6.0), 6.4 (d, 1H, ArH, J = 6.3), 6.7(d, 2H, ArH, J = 6.3), 6.7 (t, 1H, ArH), 7.0 (t, 2H, ArH), 7.1 (s, 1H, dioxole CH), 4.0(s, 1H, –NH), 5.0 (s, 1H, OH). Mass (m/z): 228.1 (M−1).

*3.1.22. 2-(2-Aminophenyl) benzo[d][1,3]dioxol-4-ol (**8b**)*

It was obtained as a brown solid, 61.4% yield, mp 180–182 °C. IR (KBr cm^{-1}): 1249 (C—O stretching), 3028 (aromatic CH stretching), 1319 (C≡N stretching), 3373 (N—H stretching), 3473 (OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.4(d, 1H, ArH, J = 6.3), 6.6 (d, 1H, ArH, J = 6.4), 7.1 (s, 1H, dioxole CH), 7.3(d, 2H, ArH, J = 7.2), 7.6(t, 1H, ArH), 7.7 (t, 2H, ArH), 5.6(s, 1H, OH), 4.0(s, 1H, -NH). Mass (m/z): 229.1 (M + 1).

*3.1.23. 2-(N-methyl-2-aminophenyl) benzo[d][1,3]dioxol-4-ol (**9b**)*

It was obtained as a brown solid, 68.8% yield, mp 164–168 °C. IR (KBr cm^{-1}): 1095 (C—O stretching), 3050 (aromatic CH stretching), 1280 (C≡N stretching), 3325 (N—H stretching), 3450 (OH stretching). ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.5(d, 1H, ArH, J = 6.3), 6.6 (d, 1H, ArH, J = 6.4),7.0 (d, 1H, ArH, J = 6.6), 7.3(d, 1H, ArH, J = 7.1), 7.3(t, 1H, ArH),

7.4 (s, 1H, dioxole CH), 7.7 (t, 2H, ArH), 5.6 (s, 1H, OH), 4.8 (s, 1H, –NH), 2.8(s, 3H, CH_3). Mass (*m*/*z*): 243.1 (M + 1).

*3.1.24. 2-(3-Methoxy-4-hydroxyphenyl) benzo[d][1,3]dioxol-4-ol (**10b**)*

It was obtained as a cream coloured solid, 62.6% yield, mp 72–73 °C. IR (KBr cm^{-1}): 1100 (C—O stretching), 3174 (aromatic CH stretching), 3431 (OH stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.5 (d, 2H, ArH, J = 6.3), 6.9 (d, 1H, ArH, J = 6.4), 7.3 (s, 1H, dioxole CH), 7.7 (s, 1H, ArH), 7.8 (t, 1H, ArH), 5.2 (s, 1H, OH), 3.8 (s, 3H, OCH_3). Mass (*m*/*z*): 260.0 (M + 1).

*3.1.25. 2-(2,4-Dichlorophenyl) benzo[d][1,3]dioxol-4-ol (**11b**)*

It was obtained as a white solid, 80.5% yield, mp 184–185 °C. IR (KBr cm^{-1}): 1091 (C—O stretching), 2981 (aromatic CH stretching), 761 (C—Cl stretching). 3431 (OH stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.2 (d, 2H, ArH, J = 6.1), 6.4(d, 1H, ArH, J = 6.2), 7.5 (s, 1H, dioxole CH), 7.7 (d, 1H, ArH, J = 6.3), 7.8 (s, 1H, ArH), 7.9 (t, 1H, ArH), 5.2(s, 1H, OH). Mass (*m*/*z*): 283.0 (M + 1).

*3.1.26. 2-(3,4,5-Trihydroxyphenyl) benzo[d][1,3]dioxol-4-ol (**12b**)*

It was obtained as a cream coloured solid, 60.2% yield, mp 180–183 °C. IR (KBr cm^{-1}): 1095 (C—O stretching), 3050 (aromatic CH stretching), 3456 (OH stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.2(d, 1H, ArH, J = 6.1), 6.4 (d, 1H, ArH, J = 6.3), 6.7(s, 1H, ArH), 6.9 (s, 1H, ArH), 6.9 (t, 1H, ArH), 6.9 (t, 1H, ArH), 7.0 (s, 1H, dioxole CH), 5.1 (s, 2H, OH), 5.2 (s, 2H, OH). Mass (*m*/*z*): 262.1 (M + 1).

*3.1.27. 2-(2-Acetyloxyphenyl) benzo[d][1,3]dioxol-4-ol (**13b**)*

It was obtained as a white solid, 75.5% yield, mp 87–90 °C. IR (KBr cm^{-1}): 1149(C—O stretching), 3018(aromatic CH stretching), 3452(OH stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.8(d, 2H, ArH, J = 6.5), 7.1 (d, 1H, ArH, J = 6.9), 7.4 (d, 1H, ArH, J = 7.1), 7.3(s, 1H, dioxole CH), 7.6 (d, 1H, ArH, J = 7.2), 7.7 (d, 1H, ArH, J = 7.3), 7.8(t, 1H, ArH), 5.0 (s, 1H, OH), 2.2 (s, 3H, OCH_3). Mass (*m*/*z*): 272.2 (M + 1).

*3.1.28. 2-(2-Carboxyphenyl) benzo[d][1,3]dioxol-4-ol (**14b**)*

It was obtained as a grey solid, 76.8% yield, mp 112–113 °C. IR (KBr cm^{-1}): 1095 (C—O stretching), 3051 (aromatic CH stretching), 3452(OH stretching). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.2 (s, 1H, dioxole CH), 7.5 (d, 2H, ArH, J = 7.1), 7.5 (d, 3H, ArH, J = 7.2), 7.6 (d, 1H, ArH, J = 7.4), 7.6 (t, 1H, ArH), 5.1 (s, 1H, OH), 12.8 (s, 1H, COOH). Mass (*m*/*z*): 258.8 (M + 1).

3.2. In vitro cytotoxicity studies

All the compounds exhibited considerable amount of anticancer activity against A 549 human lung carcinoma cells in terms of percentage cell proliferation inhibition (% CPI) at a concentration of 100 µgm/ml. Compound **3a** (% CPI = 78.80) and **9b** (% CPI = 52.90) were found to be more potent than the standard (% CPI = 49.50). It was revealed that mostly compounds belonging to benzodioxole-4-ol category (**2b**, **6b**–**14b**) showed better anticancer activity than the compounds without a hydroxy group at fourth position of the benzodioxole ring (**2a**, **6a**–**14a**). However this phenomenon was reversed for compounds with unsubstituted phenyl ring (**1a**, **1b**), methyl (**3a**, **3b**) or hydroxy (**4a**, **5a**, **4b**, **5b**) substituted phenyl ring. Subsequently it was observed that among 2-phenyl benzodioxole derivatives, mono substitution at para position of the phenyl ring with a methyl group (**3a**) was the most potent. In case of 2-phenyl benzodioxole-4-ol derivatives, mono substitution with a methyl amino group at second position of the phenyl ring (**9b**) was found to be the most active compound. The percentage cell proliferation inhibition of the synthesized compounds is depicted in Table 3.

3.3. DNA binding studies

The ability of the synthesized compounds to bind with DNA as determined by binding constant value was found to be moderate to mild. This can be attributed to a different mechanism of action via which the compounds exhibit their cytotoxic effects. The 2-phenyl benzodioxole-4-ol derivatives (**1b**–**14b**) were found to be more effective than the 2-phenyl benzodioxole class of compounds (**1a**–**14a**). Compound with a hydroxyl group at second position of the phenyl ring (**5a** and **5b**) was found to be the most active in their respective series. The binding constant (K_i), λ_{max}, % hypochromicity and isobestic point is highlighted in Table 4.

3.4. Antibacterial studies

Antibacterial studies on the compounds bearing 2-phenyl benzodioxole ring system (**1a**–**14a**) revealed that compound **3a** is more potent than the standard against all the strains of bacteria (Table 5). Compound **4a** exhibited better activity than the standard in terms of inhibiting S. *aureus*. It was further disclosed that the activity of the compounds either

Table 3 *In-vitro* cytotoxicity studies of synthesized benzodioxole derivatives against A549 human lung carcinoma cells.

Compound code	% Proliferation inhibition	Compound code	% Proliferation inhibition
1a	32.10	**1b**	12.30
2a	24.00	**2b**	34.80
3a	78.80	**3b**	31.40
4a	30.90	**4b**	10.64
5a	24.90	**5b**	19.80
6a	24.60	**6b**	36.00
7a	9.74	**7b**	20.30
8a	32.70	**8b**	40.30
9a	33.90	**9b**	52.90
10a	28.10	**10b**	43.10
11a	30.90	**11b**	36.10
12a	21.10	**12b**	28.30
13a	28.10	**13b**	38.50
14a	26.90	**14b**	32.70
Cisplatin	49.5		

Table 4 Binding constant and photometric properties of 2-phenyl 1,2-benzodioxole derivatives in complex with CT DNA.

Compound Code	Binding constant (K_i)	λ_{max} (nm)	% Hypochromicity	Isobestic point	Compound Code	Binding constant (K_i)	λ_{max} (nm)	% Hypochromicity	Isobestic point
1a	1.6100	255	53.2	210	**1b**	1.7215	275	76.9	225
2a	0.6739	270	50.6	Unclear	**2b**	0.7346	295	43.2	Unclear
3a	0.6763	255	42.0	220	**3b**	0.9081	267	39.0	210
4a	0.9134	260	40.3	220	**4b**	1.3237	270	48.5	210
5a	2.5118	289	60.7	Unclear	**5b**	5.8739	310	83.5	218
6a	1.1641	296	41.0	245	**6b**	0.4257	315	36.6	220
7a	0.0442	290	22.3	250	**7b**	0.5481	309	30.8	Unclear
8a	1.4361	260	44.8	Unclear	**8b**	1.6860	273	52.5	215
9a	1.6878	260	65.6	210	**9b**	0.3419	271	45.5	Unclear
10a	0.3444	258	76.2	205	**10b**	0.6581	270	82.0	230
11a	0.6978	261	47.3	215	**11b**	0.7346	275	43.2	Unclear
12a	1.5067	260	58.2	218	**12b**	2.6540	275	64.4	235
13a	0.6154	259	60.5	Unclear	**13b**	1.1289	269	50.2	210
14a	1.5186	255	55.8	209	**14b**	0.5602	268	72.6	Unclear

Table 5 Antibacterial activity of the compounds.

Compound Code	Zone of inhibition (mm) in 100 μg/ml				Compound Code	Zone of inhibition (mm) in 100μg/ml			
	Strain I	Strain II	Strain III	Strain IV		Strain I	Strain II	Strain III	Strain IV
1a	28	16	24	20	**1b**	18	14	16	12
2a	20	14	18	12	**2b**	12	12	16	12
3a	32	18	30	24	**3b**	20	15	22	16
4a	22	17	18	21	**4b**	12	9	18	11
5a	25	15	22	19	**5b**	15	14	20	14
6a	26	16	24	20	**6b**	16	16	22	18
7a	22	16	20	18	**7b**	12	14	16	13
8a	20	14	15	16	**8b**	10	10	13	12
9a	20	14	14	16	**9b**	12	11	12	11
10a	16	15	14	15	**10b**	11	12	10	13
11a	18	14	15	15	**11b**	16	14	15	14
12a	18	15	16	16	**12b**	15	11	16	16
13a	24	16	20	22	**13b**	17	14	19	19
14a	18	13	13	15	**14b**	11	11	9	10
Cinoxacin	31	16	28	20					

Note: Strain I: Bacillus subtilis MTCC 441; Strain II: Staphyllococcous aureus MTCC 3160.
Strain III: Escherichia coli MTCC 40; Strain IV: *Pseudomonas aeruginosa* MTCC 424.

remained same or decreased when the phenyl ring is substituted with any group other than methyl at para position. Substitution at other position of the phenyl ring did not result in any significant increase in potency. The activity data of compounds belonging to 2-phenyl benzodioxole-4-ol category (**1b**–**14b**) showed that all have considerable antibacterial activity but none are potent than the reference standard. Subsequently, a closer look at the activity profile of this series of compounds demonstrated that there is no marked improvement in antibacterial property of the compounds with a substitution in the phenyl ring at any position as compared to unsubstituted phenyl ring (**1b**). However, in this series also, compound **3b** (methyl group at para position of the phenyl ring, i.e. analogue of **3a**) was the exception which exhibited enhanced activity among all the derivatives of the series. The result of antibacterial studies of the synthesized compounds against various strains of bacteria is depicted in Table 5.

4. Conclusion

The present study identified some 2-phenyl 1,3-benzodioxole derivatives with significant anticancer and antibacterial property, which may be associated with their DNA binding capacity. For 2-phenyl 1,3-benzodioxole series, it was concluded that substitution of 4 position of the phenyl ring with an electron donating group having a atom with no unshared pair of electron (CH_3) markedly increases anticancer and antibacterial potential. In case of 2-phenyl 1,3-benzodioxole-4-ol series, it was revealed that there is a drastic increase in anticancer and antibacterial property when the second position of the phenyl ring was substituted with a mono substituted atom bearing a lone pair of electron (NCH_3). Subsequently, the research work identifies 2-(4-methyl phenyl) 1,3-benzodioxole (**3a**) as a hit molecule for further improvement of anticancer and antibacterial potency and 2-(2-hydroxy phenyl) 1,3-benzodioxole-4-ol (**5b**) as a hit molecule for the future development of DNA

binding agents. This study also provides a protocol for simple, rapid, eco-sustainable and effective synthesis of 2-phenyl 1,3-benzodioxole derivatives which may be explored further for better activity profile against cancer and bacterial cells.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2014.08.004.

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