

Research Article

Synthesis, Characterization, and Pharmacological Evaluation of Selected Aromatic Amines

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Received 3 December 2014; Revised 24 January 2015; Accepted 25 January 2015

Academic Editor: Shu Taira

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Aromatic amines 1-amino-4-phenoxybenzene (A-1A), 2-(4-aminophenoxy) naphthalene (A-2A), and 1-(4-aminophenoxy) naphthalene (A-3A) were synthesized by the reduction of corresponding nitroaromatics with hydrazine monohydrate and Pd/C 5% (w/w). The newly synthesized compounds were characterized by FTIR, ¹H NMR, ¹³C NMR, UV-visible spectrophotometer, and mass spectrometry and their biological activities were investigated along with structurally similar 4-(4-aminophenoxy) biphenyl (A-A). Results of brine shrimp cytotoxicity assay showed that almost all of the compounds had LD₅₀ values <1 μg/mL. The compounds also showed significant antitumor activity with IC₅₀ values ranging from 67.45 to 12.2 μg mL⁻¹. The cytotoxicity and antitumor studies correlate the results which suggests the anticancerous nature of compounds. During the interaction study of these compounds with DNA, all of the compounds showed hyperchromic effect indicating strong interaction through binding with the grooves of DNA. Moreover, A-3A also showed decrease in λ_{max} confirming higher propensity for DNA groove binding. In DPPH free radical scavenging assay, all the compounds showed potential antioxidant capability. The compounds were highly active in protecting DNA against hydroxyl free radicals. DNA interaction and antioxidant results back up each other indicating that these compounds have potential to be used as cancer chemopreventive agents. Additionally, one compound (A-1A) showed significant antibacterial and antifungal activity as well.

1. Introduction

Aromatic amines are a class of organic compounds in which an amino (–NH₂) group is directly attached to aromatic carbon. These are used for the synthesis of many compounds like azo dyes [1], Schiff's bases [2], zeolites [3], polyimides, polyamides [4], stationary phase for HPLC [5], epoxy resins [6], and plastics [7]. These compounds also act as a catalyst for the cross-linking of polyester, a stabilizer for phenolic resins, coagulants, and antiknock additives for gasoline and diesel fuel [7]. Due to their biological activities, amines are also named as alkaloids in phytochemistry. Amine group bearing hydrazide hydrazones is a class of compounds possessing numerous biological activities like antitumor, antimycobacterial, antimicrobial, inhibitor of anthrax lethal factor, anti-inflammatory, trypanocidal, leishmanicidal, antidiabetic, and

antimalarial agents [8]. Paclitaxel is an amine containing chemotherapy drug that can be used to treat cancers [9].

It is believed that oxidative processes encourage carcinogenesis, even though the mechanisms for this are not well defined [10]. The main mechanism proposed for protective action against harmful oxidative processes is associated with the free radical scavenging activity of antioxidant compounds [11]. The antioxidants may cause the deterioration of premalignant tumors and inhibit their expansion into cancer [10]. Initial studies have reported that some antioxidants, like β-carotene, may be of advantage in the treatment of precancerous circumstances such as oral leukoplakia, probably a precursor of oral cancer [10].

It is of immense help to recognize the structural properties of DNA, the cause of some diseases, and mechanism of some antiviral and antitumor drugs, to design efficient and

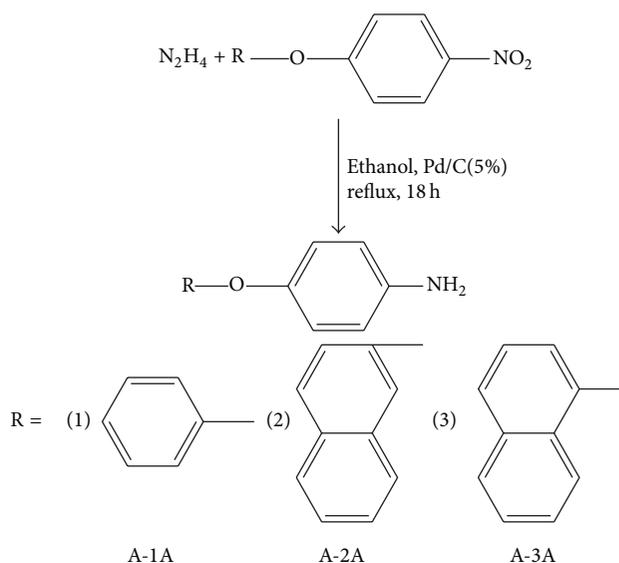
new DNA targeted drugs to deal with diseases [12]. A drug can interact with DNA by two ways, either by intercalation or as grooves binders. Grooves binders drugs interact with DNA either at minor groove or at major groove. While binding with minor groove, the drug interacts with walls of the groove and hydrogen bonding is established or electrostatic interactions occur with the bases and the phosphate backbone. On the other hand, drug establishes hydrogen bonding with major groove, forming a triple helix of DNA [13]. Both groove binders and intercalating agents are typified as antiviral, antibacterial, antifungal, and antitumor agents [14–16].

In the present study, new aromatic amines were synthesized and biological potential was evaluated along with one previously reported compound (A-A) [17] due to structural similarity. These compounds were screened by using brine shrimp cytotoxicity assay followed by potato disc antitumor assay, antibacterial assay, antifungal assay, DPPH free radical scavenging assay, and DNA damage assay. Also these compounds were investigated for the interaction with DNA to understand their possible mechanism of action in cancer chemoprevention.

2. Experimental

2.1. Materials. All the reagents used were of analytical grade. Pd/C 5% (w/w), hydrazine monohydrate ($\geq 98\%$), and ethanol ($\geq 97\%$) were used as acquired from supplier. 4-(4-Nitrophenyloxy) biphenyl, 1-nitro-4-phenoxybenzene, 2-(4-nitrophenoxy) naphthalene, and 1-(4-nitrophenoxy) naphthalene used were synthesized by our research group and were recrystallized for further use. Solvents used were dried and purified according to standard procedures [18]. The reactions were carried out in an inert atmosphere by purging dry N_2 gas. The pace of reactions and purity of products were checked by thin layer chromatography on precoated Kieselgel 60HF TLC plates. Elemental analysis was carried out on a CHNS 932 (Leco, USA) elemental analyzer. Melting points were determined on Gallen Kamp apparatus and are uncorrected. Infrared measurements ($4000\text{--}400\text{ cm}^{-1}$) were taken on ThermoScientific NICOLET 6700 FTIR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were obtained on a Bruker 300 MHz NMR spectrophotometer in deuterated chloroform using tetramethylsilane as internal reference. GC-MS spectra were recorded in methanol on a micromass platform II instrument.

2.2. General Procedure for the Synthesis of Aromatic Amines. Aromatic amines were synthesized by a reported procedure [19]. Mixture of corresponding nitroaromatic compound, hydrazine monohydrate, and Pd/C 5% (w/w) was refluxed in ethanol for 18 hours in 250 mL flask equipped with a magnetic stirrer under an inert atmosphere created by purging dry nitrogen. After the completion of reaction, Pd/C was removed by filtering the hot mixture. Concentrating the filtrate on rotary evaporator resulted in precipitation. The precipitates were separated by filtration and recrystallized from methanol [20] (Scheme 1).



SCHEME 1: General scheme for the aromatic amine synthesis.

2.2.1. Synthesis of 1-Amino-4-phenoxybenzene (A-1A). 1-Amino-4-phenoxybenzene (A-1A) was synthesized using 2.00 g (9.30 mmol) 1-nitro-4-phenoxybenzene, 5.00 mL hydrazine monohydrate, and 0.05 g Pd/C 5% (w/w).

Color: yellow, Yield 79%, melting point 83°C . FT-IR: (ν/cm^{-1}) (N-H) 3391(asym) 3315(sym), (C-O-C) 1225, (aromatic C=C) 1597. ^1H NMR (CDCl_3 , δ ppm): 3.99(2H, s, NH_2), 7.33–6.57 (m, aromatic ring protons), ^{13}C NMR (CDCl_3 , δ ppm): 159.44, 145.93, 130.12, 122.17, 121.39, 118.47, 116.85, 115.334, MS (m/z): 185(M^+) CHN found (calcd) for $\text{C}_{12}\text{H}_{11}\text{NO}$: C: 77.89 (77.84), H: 5.87 (5.95), N: 7.52 (7.57), UV-vis: λ_{max} (nm) 241.

2.2.2. Synthesis of 2-(4-Aminophenoxy) Naphthalene (A-2A). 2-(4-Aminophenoxy) naphthalene (A-2A) was prepared by mixing 2.00 g (6.94 mmol) 2-(4-nitrophenoxy) naphthalene, 5.00 mL hydrazine monohydrate, and 0.05 g Pd/C 5% (w/w), following the general procedure for synthesis of aromatic amines as outlined in Section 2.2.

Color: reddish brown, Yield 75%, melting point 116°C . FTIR: (ν/cm^{-1}) (N-H) 3393(asym) 3323(sym), (C-O-C) 1247, (aromatic C=C) 1622, ^1H NMR (CDCl_3 , δ ppm): 4.04(2H, s, NH_2), 7.89–6.23(m, aromatic ring protons), ^{13}C NMR(CDCl_3 , δ ppm): 157.49, 146.17, 145.81, 134.43, 130.20, 129.48, 128.02, 127.23, 126.98, 124.56, 121.59, 119.24, 110.85. MS (m/z): 235(M^+) CHN found (calcd) for $\text{C}_{16}\text{H}_{13}\text{NO}$: C: 81.44 (81.07), H: 5.51 (5.53), N: 5.92 (5.96), UV-vis: λ_{max} (nm), 222.

2.2.3. Synthesis of 1-(4-Aminophenoxy) Naphthalene (A-3A). 1-(4-Aminophenoxy) naphthalene (A-3A) was prepared by reaction of 2.00 g (6.94 mmol) 1-(4-nitrophenoxy) naphthalene, 5.00 mL hydrazine monohydrate, and 0.05 g Pd/C 5% (w/w) under the conditions maintained for the synthesis of 2-(4-aminophenoxy) naphthalene (A-2A).

Color: brown, Yield 74%, melting point 55°C, FT-IR: (ν/cm^{-1}) (N-H) 3400(asym), 3327(sym), (C-O-C) 1242, (aromatic C=C) 1592. ^1H NMR (CDCl_3 , δ ppm): 4.01(2H, s, NH_2), 8.25–6.61 (m, aromatic ring protons), ^{13}C NMR (CDCl_3 , δ ppm): 155.25, 146.29, 146.07, 134.83, 128.10, 127.11, 126.50, 125.77, 121.94, 121.85, 121.31, 115.40, 110.10, MS (m/z): 235(M^+) CHN found (calcd) for $\text{C}_{16}\text{H}_{13}\text{NO}$: C: 81.84 (81.07), H: 5.59 (5.53), N: 5.94 (5.96), UV-vis: λ_{max} (nm) 215.

2.3. Biological Assays

2.3.1. Brine Shrimp Cytotoxicity Assay. Brine shrimp cytotoxicity assay was used to determine the toxicity of the compounds [21]. *Artemia salina* (brine shrimp) eggs (Ocean Star Inc., USA) were hatched in seawater (34 gL^{-1}). After 24 h, ten shrimps were transferred to each vial using Pasteur pipette. The compounds with final concentrations of 10, 1, 0.5, 0.25, 0.125, and $0.0625\ \mu\text{g mL}^{-1}$ were added and the volume was raised up to 5 mL of artificial seawater. Vincristine sulphate was used as positive control and DMSO was used as negative control. The experiment was performed in triplicate and vials were incubated under illumination at 28°C. After 24 h, survivors were counted and LD_{50} (lethal dose) values were calculated by using the Finney software [22].

2.3.2. Potato Disc Antitumor Assay. Potato disc antitumor method [23] was used to test the antitumor activity of the compounds. Inoculum was prepared with three concentrations (10, 100, and $1000\ \mu\text{g mL}^{-1}$) of sample containing *Agrobacterium tumefaciens*. Potatoes were surface sterilized with 0.1% mercuric chloride and $5\text{ mm} \times 8\text{ mm}$ potatoes cylinders were placed on agar plates. Then $50\ \mu\text{L}$ of inoculum was applied to the top of each disc (10 discs per plate). Vincristine sulphate was used as positive control and DMSO was used as negative control. Experiment was performed in triplicate and plates were placed at 28°C. After 28 days, discs were stained with Lugol's solution (10% KI and 5% I_2) and the number of tumors was counted. Percentage inhibition was determined as follows:

$$\text{Percentage inhibition} = 100 - \left[\left(\frac{X}{Y} \right) \times 100 \right], \quad (1)$$

where X is average number of tumors of test sample and Y is average number of tumors of -ve control.

2.3.3. DNA-Drug Interaction Assay. Interaction of compounds with DNA was studied by using UV spectrophotometer method [24] with little modifications. InnuPREP blood DNA mini kit (Analytik Jena) was used for the extraction of genomic DNA from human blood and concentration was calculated at 260 nm by applying the extinction coefficient of $6600\ \text{M}^{-1}\text{cm}^{-1}$ [25]. The working DNA solution (100 mM) was prepared and all the compounds were dissolved in SM (solvent mixture, consisting of methanol and water 9:1). Molar concentrations of compounds were adjusted on the spectrophotometer to ensure that absorbance was between 0.1 and 1 at λ_{max} so that Beer-Lambert law could be applied. The spectrum was recorded and, to this, DNA was stepwise

added starting from the lowest concentration to higher concentrations (0.5×10^{-6} , 1×10^{-6} , 0.5×10^{-5} , and $1 \times 10^{-5}\ \text{M}$) keeping the compound concentration in the reaction mixture constant. To attain the stable interaction, reaction mixture was allowed to stay for 5 min before each measurement. SM was used as blank and spectra were recorded in the form of spectral peaks.

2.3.4. DPPH Free Radical Scavenging Assay. Radical scavenging activity of compounds was determined spectrophotometrically [26] at 200, 66.6, 22.4, 7.4, and $2.4\ \mu\text{g mL}^{-1}$ final concentration. DPPH (0.1 mM) was prepared in methanol. Ascorbic acid and DMSO were used as positive and negative control, respectively. Each concentration was assayed in triplicate and reaction mixture was incubated at 37°C in the dark. After 30 minutes, absorbance was measured at 517 nm on a UV-vis spectrophotometer (Agilent 8453, G1103A). The percent scavenging of DPPH free radical for each concentration of each test compound was calculated by the following formula:

$$\text{Percentage Scavenging} = \left[\left(\frac{X - Y}{X} \right) \times 100 \right], \quad (2)$$

where X is absorbance of control and Y is absorbance of test sample.

2.3.5. DNA Damage Assay. DNA protection activity of the compounds against oxidative DNA damage was evaluated *in vitro* by DNA damage assay [12, 27]. The reaction was carried out in a PCR tube with $15\ \mu\text{L}$ of total volume having $3\ \mu\text{L}$ pBR322 DNA ($0.5\ \mu\text{g}$), $3\ \mu\text{L}$ of 2 mM FeSO_4 , $4\ \mu\text{L}$ of 30% H_2O_2 , and $5\ \mu\text{L}$ of tested compound with 1000, 100, and $10\ \mu\text{g mL}^{-1}$ final concentration. A positive control was used which contains pBR322 DNA treated with 2 mM FeSO_4 + 30% H_2O_2 , and untreated pBR322 DNA was used as negative control (P). A control containing compound and untreated pBR322 (C + P) was also used to check the damaging or protective effect of compound on DNA. Then the mixture was incubated at 37°C for 1 h. All reaction mixtures were subjected to 1% agarose gel electrophoresis in 1X TBE buffer using 1 kb ladder (L). Gels were analyzed by scanning with Gel-Doc (Bio Rad) computer program and intensity of the bands was determined.

2.3.6. Antibacterial Assay (96-Well Plate Method). Antibacterial activity of compounds was determined by "microtiter plate method" [28] with minor modifications. Five strains of bacteria two Gram positive (*Micrococcus luteus* (ATCC 10240) and *Staphylococcus aureus* (ATCC 6538)) and three Gram negative (*Bordetella bronchiseptica* (ATCC 4617), *Escherichia coli* (ATCC 15224), and *Enterobacter aerogenes* (ATCC 13048)) cultured in nutrient broth at 37°C for 24 h were used. A sterile 96-well plate was labeled and serial dilutions were performed with 200, 100, 50, 25, 12.5, 6.25, 3.12, and $1.56\ \mu\text{g mL}^{-1}$ final concentration. Then $190\ \mu\text{L}$ of bacterial inoculum was added to each well compared with McFarland turbidity standard. A column with a broad spectrum antibiotic as positive control (kanamycin sulfate), negative control

(DMSO), and blank (nutrient broth) were used on each plate. The experiment was performed in triplicate and incubated at 37°C. After 24 h, to each well 20 μL of TTC indicator solution was added and plates were incubated at 37°C for 15–20 minutes and change in colour was determined visually. Colour change from red to pink was recorded as negative (viable bacteria) and no colour change was considered as positive (dead bacteria). The lowest concentration at which no colour change appeared was taken as MIC value.

2.3.7. Antifungal Assay (6-Well Plate Dilution Method). The compounds were screened for their antifungal activity by agar plate dilution method [29] with modifications. Two strains *Aspergillus flavus* (FCBP 0064) and *Fusarium solani* (FCBP 0291) cultured on SDA at 28°C for 24 h were used. Test compound with 200, 100, 50, 25, and 12.5 $\mu\text{g mL}^{-1}$ final concentration was poured in each well of 6-well plate. After solidification each well was inoculated with 4 mm fungal mycelia. Terbinafine and DMSO served as positive control and negative control, respectively. Plates were incubated at 28°C for 24 h. Antifungal activity was calculated by measuring the growth diameter of fungus and MIC value was taken as the lowest concentration at which no growth appeared.

3. Results and Discussion

3.1. Spectral Characterization

3.1.1. FTIR Spectroscopy. The FTIR spectra of aromatic amines (A-1A, A-2A, and A-3A) exhibited characteristic broad absorption bands at 1225–1242 cm^{-1} due to C–O–C vibration, 1587–1608 cm^{-1} due to $\nu_{\text{C}=\text{C}}$ of aromatic ring vibrations, and 3391–3468 (asymmetric) and 3315–3372 (symmetric) cm^{-1} because of N–H stretching bands [30].

3.1.2. ^1H and ^{13}C NMR Spectral Analysis. The ^1H NMR spectra showed characteristic signals in the region 4.62–3.63 ppm corresponding to primary aromatic amine protons. Phenyl protons are present in all the compounds which are verified by the appearance of multiplets at 8.25–6.23 ppm according to the substituents attached. The characteristic singlets at 3.99, 4.04, and 4.01 ppm were due to resonance of amino group protons ($-\text{NH}_2$) whereas phenyl protons appeared as multiplets in the ranges of 7.33–6.57, 7.89–6.23, and 8.25–6.61 ppm for A-1A, A-2A, and A-3A, respectively.

^{13}C NMR spectroscopic studies also confirmed the formation of all the aromatic amines. The carbon atoms of the aromatic rings attached directly to the oxygen atoms of ether linkage were strongly deshielded showing downfield signals at 157.62, 157.49, and 155.25 ppm for A-1A, A-2A, and A-3A, respectively [31].

3.1.3. Mass Spectral Studies. The mass spectral data of the aromatic amines confirms their formation as molecular ion peaks are obtained at (m/z) 185 for A-1A and 235 for A-2A and A-3A. The mass spectra of the compounds display molecular ions as base peaks.

TABLE 1: Cytotoxic activity of the amine compounds in brine shrimp cytotoxicity assay.

Sr. number	Compound code	LD ₅₀ value ($\mu\text{g mL}^{-1}$)
1	A-A	0.28
2	A-1A	0.50
3	A-2A	0.16
4	A-3A	1.0
5	Vincristine sulphate	1.0

LD₅₀: lethal dose that killed 50% of shrimps.

3.2. Biological Evaluation

3.2.1. Brine Shrimp Cytotoxicity Assay. To screen out the biologically active compounds, brine shrimp cytotoxicity assay was performed and results are summarized in Table 1. Brine shrimp cytotoxicity assay serves as prescreen test for the identification of bioactive compounds to evaluate anticancer potential of any compound. All the tested compounds are highly cytotoxic with LD₅₀ value as low as 0.16 $\mu\text{g mL}^{-1}$ (Table 1). It is important to note that these compounds are more potent than the vincristine sulphate (positive control) which means that these compounds have strong potential for the anticancer screening.

3.2.2. Potato Disc Antitumor Assay. As the compounds showed good cytotoxic activity, in the prescreen assay, they were screened for possible antitumor activity by using potato disc tumor assay and results are summarized in Table 2. The compounds A-A, A-1A, and A-2A showed significant antitumor activity with IC₅₀ values 30.47, 67.45, and 12.2 $\mu\text{g mL}^{-1}$, respectively. The results were compared with that of the positive control, vincristine sulphate (5.1 $\mu\text{g mL}^{-1}$), which predicts the anticancer nature of compounds.

3.2.3. DNA-Drug Interaction Assay. For the evaluation of the potential of an anticancerous compound, study of its binding to the DNA is the key step of drug innovation. The drug binding to DNA has been characterized through absorption titrations [32]. Generally, when drugs intercalate with DNA, blue shift or red shift is observed in the absorption spectra [33]. Additionally, increase or decrease in absorbance is also observed when drugs bind with the grooves of DNA [34]. Initially, UV-spectra of the compounds in SM (solvent mixture) consisting of methanol and water (9:1) were recorded in terms of λ_{max} . The compound A-3A showed hyperchromic effect along with blue shift of 15 nm which is indicative of higher susceptibility for intercalation with DNA. Furthermore, all of the compounds A-A, A-1A, A-2A, and A-3A with λ_{max} 206, 241, 222, and 215 nm, respectively, showed hyperchromism with increasing the concentrations of the DNA (Figure 1), suggesting their groove binding property. Groove binding compounds are of practical importance for the development of new anticancer agents because they localize in the nuclear DNA of whole cells and possess a profile of promising biological activity [35]. It has been reported previously that the interaction of copper(II) complex with DNA by increase in DNA concentration results in hyperchromic

TABLE 2: Tumor inhibition activity of the amine compounds in potato disc antitumor assay.

Sr. number	Compound code	Percentage inhibition			IC ₅₀ μgml^{-1}
		1000 μgml^{-1}	100 μgml^{-1}	10 μgml^{-1}	
1	A-A	60	57.5	12.5	30.47
2	A-1A	57.5	52.5	15	67.45
3	A-2A	100	95	13.3	12.2
4	A-3A	25	22.5	10	>1000
5	Vincristine sulphate	100	100	9.5	5.1

IC₅₀: inhibitory concentration that inhibited 50% tumors.

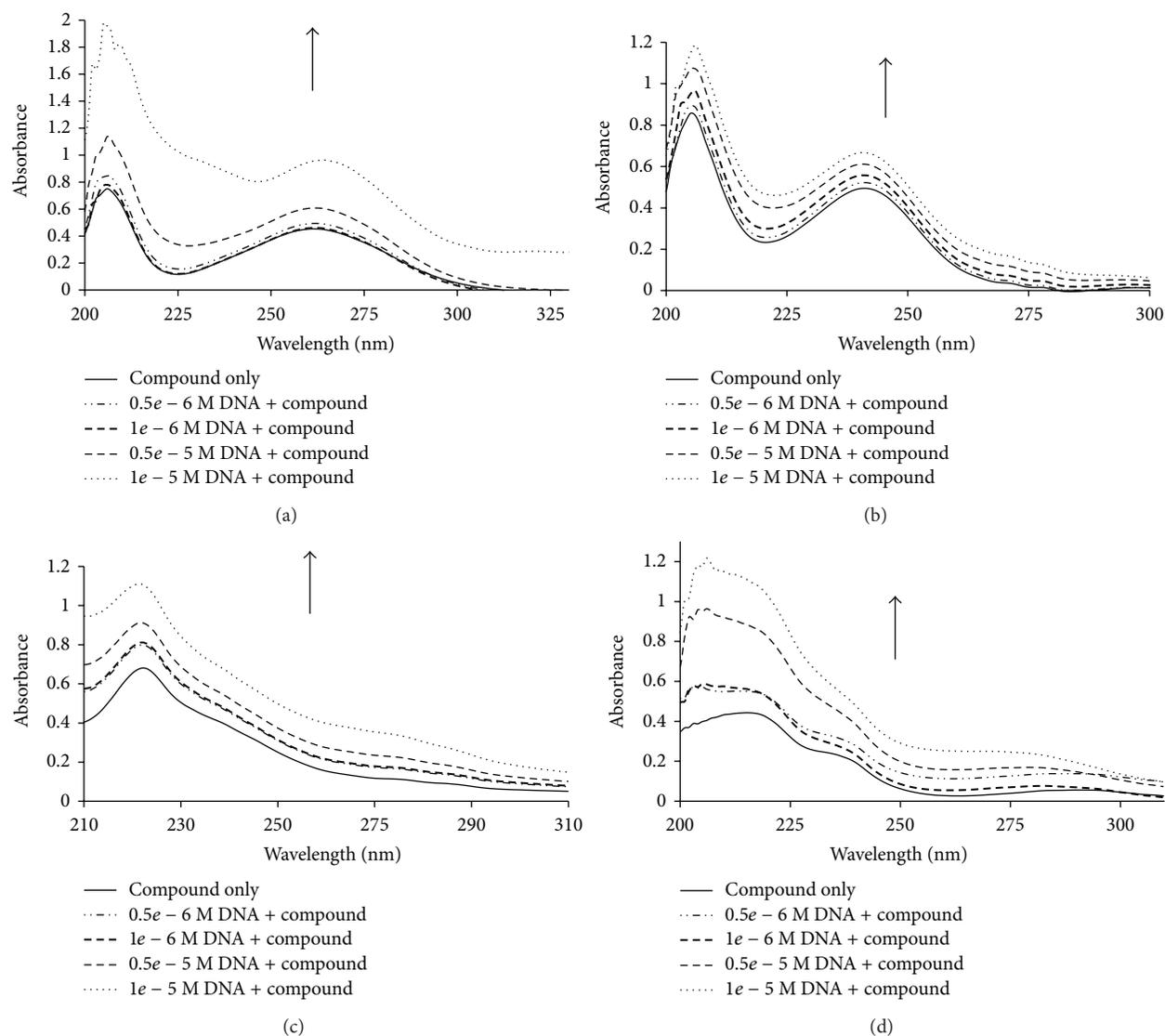


FIGURE 1: Absorption spectrum of (a) A-A, (b) A-1A, (c) A-2A, and (d) A-3A in the absence and presence of human DNA. The arrow direction shows trend in the change of absorbance.

effect. This spectral change might be indicative of groove binding as the organic ligand-copper coordination facilitates the formation of Van der Waals contacts or hydrogen bonds during interaction with DNA grooves [36]. In another study two complexes $\text{C}_{10}\text{H}_{22}\text{N}_2\text{O}_5\text{SnCl}_2$ and $\text{C}_{10}\text{H}_{22}\text{N}_2\text{O}_5\text{ZrCl}_2$

exhibited intraligand absorption bands in the UV. By the addition of increasing amounts of CT-DNA to complexes, a sharp hyperchromic effect in the absorption bands with a moderate red shift of 5 and 3 nm, respectively, was observed. Hyperchromic effect reflects the corresponding changes of

TABLE 3: DPPH free radical scavenging activity of the amine compounds.

Sr. number	Compound code	Percentage scavenging				IC ₅₀ μgml^{-1}
		200 μgml^{-1}	66.66 μgml^{-1}	22.22 μgml^{-1}	7.4 μgml^{-1}	
1	A-A	60.74	51.85	45.42	29.81	47.29
2	A-1A	67.51	59.65	42.18	28.47	32.50
3	A-2A	59.26	51.11	39.26	26.59	61.61
4	A-3A	63.70	51.34	36.80	24.59	59.66
5	Vincristine sulphate	100	95.6	59.1	25.2	9.6

IC₅₀: inhibitory concentration that scavenged 50% free radicals.

TABLE 4: DNA protection activity of the amine compounds in DNA damage assay.

Sr. number	Compound code	DNA protection activity at different concentrations		
		1000 μgml^{-1}	100 μgml^{-1}	10 μgml^{-1}
1	A-A	+++	+++	++
2	A-1A	+++	+++	++
3	A-2A	+++	+++	++
4	A-3A	+++	+++	++

+++; significant protection; ++; good protection.

DNA in its conformation and structure after the complex-DNA interaction has occurred [37].

3.2.4. DPPH Free Radical Scavenging Assay. DPPH free radical scavenging activity of the compounds was evaluated spectrophotometrically at four different concentrations (200, 66.6, 22.2, and 7.4 μgml^{-1}). All compounds showed significant antioxidant potential with IC₅₀ values of 47.29, 32.50, 61.61, and 59.66 μgml^{-1} , respectively, in comparison with ascorbic acid (9.6 μgml^{-1}). Results of this assay are summarized in Table 3.

3.2.5. DNA Damage Assay. Keeping in view the good antioxidant activity, the compounds were screened for their protection against DNA damage assay at three concentrations (1000, 100, and 10 μgml^{-1}). This assay based on the attack of $\cdot\text{OH}$ produced from the Fenton reaction and super coiled plasmid DNA is broken into open circular or linear form. By analyzing the intensity of bands formed on 1% agarose gel, results were recorded and tabulated (Table 4, Figures 2 and 3). All the compounds showed significant protection at 1000 and 100 μgml^{-1} concentrations, while showing good protection at 10 μgml^{-1} concentration. The results indicate that these compounds could be regarded as potential antioxidant and anticancerous agents after further investigation.

3.2.6. Antibacterial Assay. Antibacterial activity was determined through 96-well plate method using 2,3,5-triphenyltetrazolium chloride (TTC) as indicator which enzymatically reduced to red 1,3,5-triphenylformazan (TPF) in living tissues due to the activity of various dehydrogenases [38]. Kanamycin sulfate was used as positive control and

MIC was calculated against *B. bronchiseptica* (125 μgml^{-1}), *S. aureus* (32 μgml^{-1}), *E. aerogenes* (30 μgml^{-1}), *M. luteus* (32 μgml^{-1}), and *E. coli* (32 μgml^{-1}). The results showed that compound A-1A (Figure 3) exhibited significant antibacterial activity against all the tested strains *B. bronchiseptica*, *S. aureus*, *E. aerogenes*, *M. luteus*, and *E. coli* with MIC values of 25, 50, 50, 100, and 100 μgml^{-1} , respectively. This means that compound A-1A can act as a broad spectrum antibiotic. This is a simple and rapid method from which accurate MIC (minimum inhibitory concentration) can be generated.

3.2.7. Antifungal Assay. The compounds were tested for their antifungal activity, using 6-well agar dilution method. Here, we report the use of 6-well plate because the main advantage of the plates is that you can grow six cultures under identical conditions in the same culture plate. Moreover, smaller wells in plates are useful for application of expensive reagents in smaller volumes. Among all the compounds, A-1A showed significant antifungal activity against *F. solani* and *A. flavus* with MIC value 12.5 μgml^{-1} for both. MIC value for terbinafine was calculated against *F. solani* (16 μgml^{-1}) and *A. flavus* (1 μgml^{-1}). It is interesting to know that A-1A possesses both significant antibacterial and antifungal activity which means that this compound can be used for dual purpose as antibacterial and antifungal agents.

4. Conclusion

Three new aromatic amines 1-amino-4-phenoxybenzene (A-1A), 2-(4-aminophenoxy) naphthalene (A-2A), and 1-(4-aminophenoxy) naphthalene (A-3A) were prepared in high purity and high yield. *In vitro* investigation of the four amine compounds showed that activity ranges from being selective (active in one assay) to being broad spectrum (active in all assays). The compound A-1A was active against all the biological assays which means it can be used as a potential candidate for drug development after advanced investigation. The obtained findings showing significant activity in brine shrimp cytotoxicity assay and antitumor assay provide the evidence for a very strong positive correlation between these two assays and for prediction of some valuable anticancerous principles present in these compounds. On the other hand, the compounds showed significant antioxidant activity and DNA protective effect against oxidative damage. Interestingly, in the DNA-drug interaction study, it was observed that

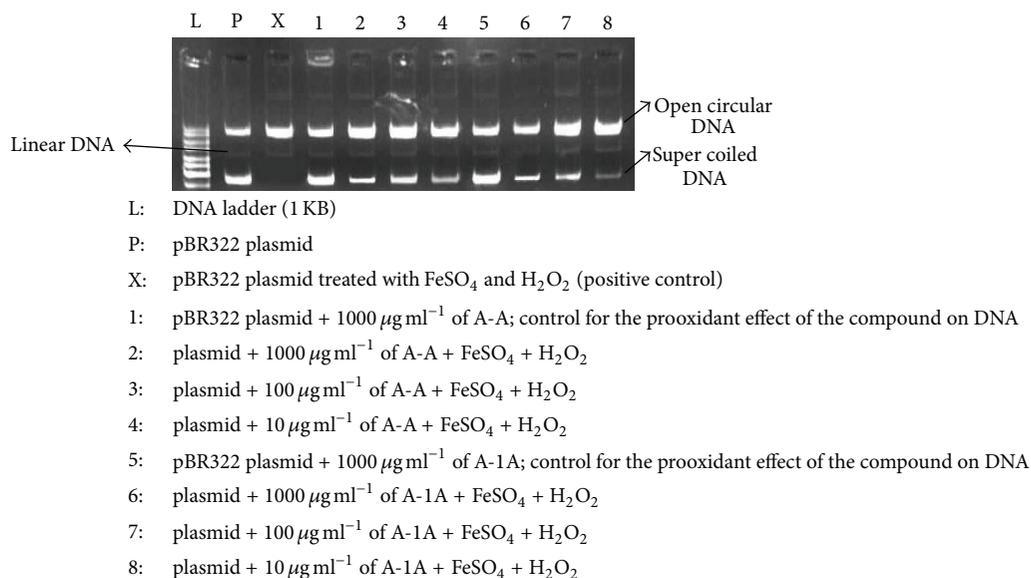


FIGURE 2: Effect of the compounds A-A and A-1A on pBR322 plasmid DNA.

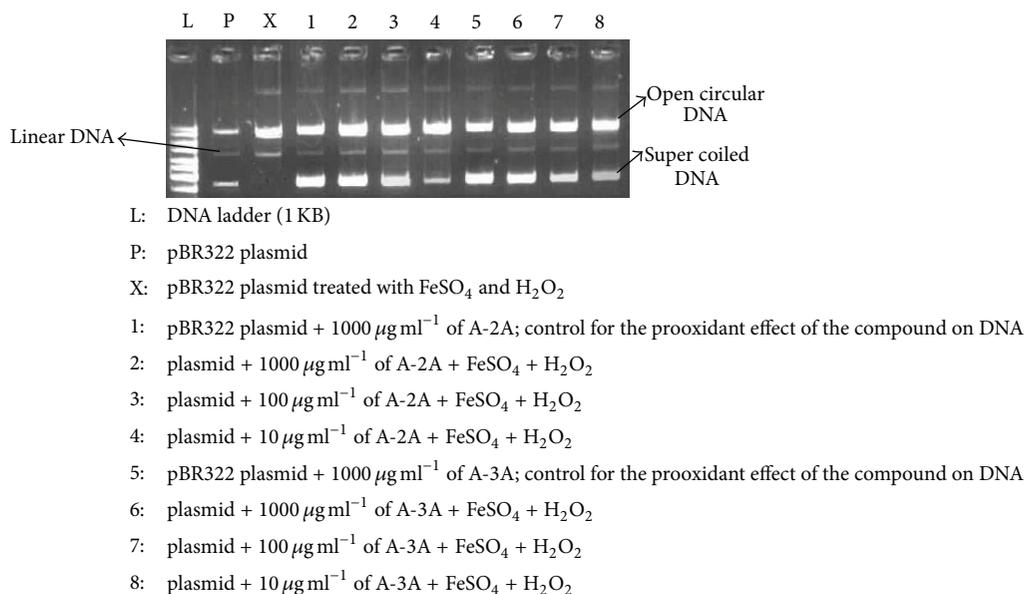


FIGURE 3: Effect of compounds A-2A and A-3A on pBR322 plasmid DNA.

these compounds bind with the grooves of DNA which can affect the binding of transcription factors with DNA and ultimately gene expression leading to the antitumor behavior. The findings of this study support the view that some of these compounds can be a promising source of potential antitumor drugs.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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