Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Synthesis and evaluation of 2-phenylamino-1,4-naphthoquinones derivatives as potential hypoglycaemic agents

Síntese e avaliação de derivados de 2-fenilamino-1,4-naftoquinonas como potenciais agentes hipoglicemiantes

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Abstract

Due to the severe side effects revealed by most of the currently used antidiabetic medicines, search for finding new and safe drugs to manage diabetes is continued. Naphthoquinones possessing strong antioxidant properties have been employed as candidates for diabetes therapy. Present study is aimed at finding the antioxidant and hypoglycaemic potential of some novel derivatives of 2-phenylamino-1,4-naphthoquinones (PAN) including chloro, nitro, methyl and bromo (5a-d) derivatives synthesized by single pot experiment. Product crystals were purified by TLC and characterized by FT-IR. The antioxidant potential of the compounds was assayed through DPPH radical scavenging and reducing power activities noted as UV-vis. absorbance. The DPPH assay has showed the powerful antioxidant activity of nitro and bromo derivatives, while the nitro derivative showed the significant reduction potential towards FRAP assay. Hypoglycaemic potential of the compounds was studied in rat animal model. All synthesized compounds revealed better hypoglycaemic activity; however, the chloro-derivative exhibited the more potent hypoglycaemic activity showing about 43% reduction in the mean blood glucose levels of the treated animals. As the bioreduction of naphthoquinones may be influenced by changing its redox properties, it has been noticed that the e-donating resonance effect (+R) of 'chloro' group has shown the significant effects on biological activity through stabalization of its imine form which limits the potential of generation of free radicals during bioreduction of quinones and thus has been proposed as the reason of its hypoglycaemic activity. Future studies employing the properties of e-donating groups of PAN may optimize the drug-receptor interaction for better drug designing and drug development strategies against diabetes and also for the clinical trials.

Keywords: diabetes, naphthoquinone derivatives, 2-phenylamino-1,4-naphthoquinones, hypoglycaemic agents, antioxidants.

Resumo

Em razão dos graves efeitos colaterais causados pela maioria dos medicamentos antidiabéticos atualmente utilizados, continua a busca por novos medicamentos seguros para o controle do diabetes. As naftoquinonas, que possuem fortes propriedades antioxidantes, têm sido empregadas como candidatas à terapia do diabetes. O presente estudo visa encontrar o potencial antioxidante e hipoglicemiante de alguns novos derivados de 2-fenilamino-1,4-naftoquinonas (PAN), incluindo derivados de cloro, nitro, metil e bromo (5a-d) sintetizados por experimento em pote único. Os cristais do produto foram purificados por TLC e caracterizados por FT-IR. O potencial antioxidante dos compostos foi testado por meio de atividades de sequestro de radicais DPPH e redução de energia observada como absorção no UV-vis. O ensaio DPPH mostrou a poderosa atividade antioxidante dos derivados nitro e bromo, enquanto o derivado nitro mostrou o potencial de redução significativo para o ensaio FRAP. O potencial hipoglicêmico dos compostos foi estudado em modelo animal de rato. Todos os compostos sintetizados revelaram melhor atividade hipoglicemiante; no entanto, o derivado cloro apresentou atividade hipoglicêmica mais potente, com redução de

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Received: July 13, 2021 - Accepted: December 23, 2021.

43% nos níveis médios de glicose no sangue dos animais tratados. Como a biorredução de naftoquinonas pode ser influenciada pela alteração de suas propriedades redox, notou-se que o efeito da doação eletrônica por ressonância (+R) do grupo "cloro" tem sido significativo na atividade biológica por meio da estabilização de sua forma imina, que limita o potencial de geração de radicais livres durante a biorredução de quinonas, e, portanto, tem sido proposto como a razão de sua atividade hipoglicemiante. Estudos futuros empregando as propriedades de grupos de doação eletrônica de PAN podem otimizar a interação droga-receptor para melhor planejamento de medicamentos e estratégias de desenvolvimento de medicamentos contra o diabetes e também para os ensaios clínicos.

Palavras-chave: diabetes, derivados da naftoquinona, 2-fenilamino-1,4-naftoquinonas, hipoglicemiantes, antioxidantes.

1. Introduction

Diabetes and its complications increase alarmingly in the world population. Most of the complications arise generally due to the uncontrolled hyperglycaemia and disturbances in rapid catabolism of lipids and protein (Pandhare et al., 2010). Oxidative stress has been considered the main cause of elevated levels of mitochondrial reactive oxygen species (ROS); mostly the superoxide anions aggravate diabetes and its complications. The natural naphthoquinones and their synthetic derivatives have been of interest to treat diseases with key redox properties of the quinone moiety present. The naphthoquinones have shown strong anticancer (Wang et al., 2015; Tabrizi and Chiniforoshan, 2017), antibacterial and antifungal activities (Sanchez-Calvo et al., 2016), and recently evaluated as metabolic enzyme inhibitors (Riaz et al., 2021) as prototypes of new antineoplastic drugs (Guimaraes et al., 2021), and also as emerging agents in combating multidrug-resistant (MDR) pathogens (Mone et al., 2021).

The naphthoquinone scaffolds have been studied as potent anti-diabetic agentsand the inhibitors of protein tyrosine phosphatases such as CDC25B (Brun et al., 2005). The amino derivatives of naphthoquinone (amino-naphthoquinones) are produced naturally in some plants for example, the plant family *Caryophyllales* (Lopez et al., 2011). These compounds have exhibited various cytotoxic activities and it has been shown that incorporation of amino groups into 1,4-naphthoquinone structures often results in an increase in their antibacterial (Andrade-Guel et al., 2011), antiparasitic (Sharma et al., 2013; Ibis et al., 2014), and antiproliferative activities (Suja et al., 2016). Further, the transition metal complexes of amino-1,4-naphthoquinone ligands have shown the promising biological activity comparable to that of parent free ligand against some bacterial and fungal species (Hassan et al., 2020).

The earlier studies have demonstrated the promising hypoglycaemic activity of thymoquinone (Al-Sa'aidi et al., 2014; Sangi et al., 2015; Bashandy et al., 2015; Saheb et al., 2016) and the rear of the present study was to synthesize some amino-naphthoquinone derivatives with structural similarity to thymoquinone, a well characterized component of essential oil of *Nigella sativa* with promising activity against diabetes (Gray et al., 2016), and to test for their comparable hypoglycaemic potential (structure-activity relationship study). The synthesized amino-naphthoquinone derivatives also showed some structural similarities to commonly used hypoglycaemic agents for example, sulfonylureas (e.g., tolbutamide **1a**) and thiazolindiones (e.g., troglitazone). However, such drugs also have revealed severe side effects and often prescribed only for specific diabetic condition. We assume that our synthesized compounds if trialled clinically, shall show better therapeutic efficiency with very limited side-effects due to structure resemblance with the natural antidiabetic compound, the thymoquinone **2**.

It would be of interest to evaluate the synthetic derivatives of amino-naphthoquinone with various substitutions for their hypoglycaemic activity as it has been established that different substituents accounts for different antidiabetic behaviour. Considering the hydrophobicity and electronic factor, the addition of amino functionality (a good nucleophilic agent for chelation or stronger hydrophilic interactions) and/ or halogen group to lead compound is quite easy and is according to the Topliss scheme (Patrick, 2002). However, the halogenated drugs have also shown the undesirable response of drug accumulation in lipid tissues. Thus, the nature of the halogen and the point of attachment to the lead, both matter for the chemical reactivity. For example, the replacement of the methyl (R^1-group) substituent by chlorine transform the antidiabetic drug 'tolbutamide' **1a** into another one 'chlorpropamide' **1b** with the significant longer half-life (Thomas, 2007).

Keeping in view the importance of halogen groups at ring structure and the limitations of antidiabetic drugs, we synthesized the two of the four amino-naphthoquinone derivatives by incorporating the halogen atoms (Cl and Br) to amino-naphthoquinone skeleton. The experiment shall lead the bases for designing the substituent(s) for amino-naphthoquinones to develop more efficient antidiabetic drugs.

2. Materials and Methods

The chemicals utilized during study were purchased from sigma Aldrich/ Merck. The solvents used in reaction were of analytical grade and used as such.

2.1. Chemical part

*Synthesis of 2-(phenylamino)-1,4-naphthoquinones (PAN) 5a-d:*The substrate 1,4-naphthoqinone **3** (0.5 g, 3.2 mmol, 2 eq) and *p*-substituted anilines **4a**-**d** (1.6 mmol, 1 eq) were dissolved in MeOH (50 ml) and stirred at 200 rpm for variable reaction duration followed by reflux at 70 °C. The resultant mixture was cooled to ambient, filtered and the title compounds were crystallized upon standing overnight (Table 1).

The thin-layer chromatography (TLC) was carried out on pre-coated silica gel (0.25 mm, 70-230 mesh) on Al-back

Table 1. The experimental and physical parameters involved in the synthesis and characterization of targeted compounds (**5a-d**).

5	m of 4 (mg)	Stirring (h)	Reflux (h)	m of 5 (mg)	Yield (%)	IR ύ (cm^{-1})
a	204	43	6	210	74	1599, 1705 (C=O), 3197 (N–H)
b	221	48	10	203	69	1504 (N=O), 1598, 1707 (C=O), 3202 (N–H)
c	171	48	6	208	79	1602, 1700 (C=O), 3199 (N–H)
d	275	45	12	249	76	1597, 1705 (C=O), 3198 (N–H)

with fluorescent indicator $60F_{254}$ (Merck, Germany) and the spots on TLC sheets were visualized using UV lamp (of 256 and 365 nm λ) or 2,4-DNP dip upon heating. The FT-IR spectra were recorded as anhydrous KBr pellets on an IR prestige-21 (200 V) FT-IR spectrometer (Schimadzu, Japan), at High-Tech Laboratory, University of Sargodha (Pakistan).

2.2. Biological part

The antioxidant potential of the compounds was noted as absorbance on UV-visible spectrophotometer (Pharma Spec-1700, Shimadzu-Japan), while the blood glucose levels were noted using glucometer (On Call EZ II, ACON® Laboratories, Inc.).

2.3. Radical scavenging activity (DPPH assay)

The assay was performed following a reported method (Martins et al., 2008). The stock solutions (10 ml of 1 mM) of four synthesized compounds were prepared in MeOH. Different dissolutions ranging from (1–200 µM) concentrations were subsequently prepared for each compound. The MeOH served as blank and ascorbic acid was used as standard. The reaction mixtures were incubated for 25-30 min at room temperature and then absorbance was recorded at 520 nm. The radical scavenging activity was calculated using following Equation 1.

$$\%Inhibition = \left[\frac{(blankabsorbance - sampleabsorbance)}{(blankabsorbance)}\right] \times 100 \qquad (1)$$

The mean of two IC50 values (concentration causing 50% inhibition) of each compound was determined graphically. Scavenging activity (%) was plotted against concentration of each of the test sample. Then IC50 was calculated from the graph. The lower IC50 value gives the highest % RSA indicating a higher antioxidant activity.

2.4. Reducing power assay

The reducing power of the synthesized PAN derivatives **5a-d** was determined following a reported method (Benzie and Strain, 1996). The stock solutions of **5a-d** were prepared in DMSO and then different dissolutions were prepared (1-200 µM). To each millilitre of different dilutions, 2.5 ml of phosphate buffer and $K_3[Fe(CN)_6]$ was added. The reaction mixture was incubated for 20 min at 50 °C. The Cl_3CCOOH (2.5 ml) was then added to the mixture and centrifuged at 3000 rpm (10 min). The upper layer was separated and mixed with 2.5 ml of distilled H_2O and a freshly prepared solution (0.5 ml) of $FeCl_3$. The absorbance was noted at 700 nm using spectrophotometer. The ascorbic acid was used as standard antioxidant.

2.5. Hypoglycaemic potential of the synthesized PAN derivatives (5a-d)

The ability of synthesized PAN compounds **5a-d** to lower the blood glucose levels was analyzed in ♂ albino rats (average weight of 200 g) purchased from University of Veterinary and Animal Sciences, Lahore (UVAS, Pakistan). These were maintained on standard laboratory condition of room temperature, humidity and under 12 h dark–light cycle at the Department of Pharmacy, University of Sargodha, Pakistan. The experiment was conducted following the instructions of animal handling procedures under the principles of laboratory animal care and use guidelines (WHO, 1985). The blood glucose levels were determined using glucometer. The animals were divided into six groups, each group contains five rats. The animals were fasted for 14 h and diabetes was induced in rats by a single intra-peritoneal injection of 150 mg/ kg of 10% alloxan **16** dissolved in isotonic NaCl. The control group was injected with the same volume of isotonic NaCl as the diabetic groups received. The rats were administered with treatments (thymoquinone **2** as standard (250 mg/ kg), and the **5a-d** as drugs (1 mg/kg) daily as oral dose for period of 14 days. The groups are discussed in Table 2.

2.6. Statistical analysis

The results are presented as means ± standard error of means. For establishing significant differences between groups, data were analyzed by One-way ANOVA. The values were considered statistically significant at $p<0.05$ using SPSS (Statistical Package for Social Sciences) software, version 21.0.

3. Results and Discussion

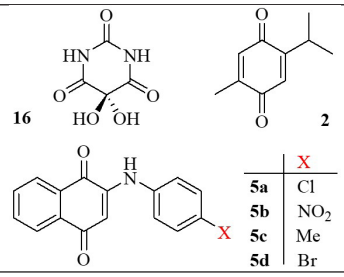
3.1. Chemistry: synthesis of 2-phenylamino-1,4-naphthoquinones (PAN) derivatives

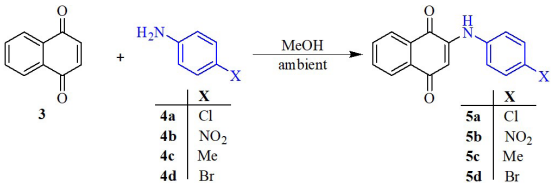
The four PAN derivatives **5a-d** were synthesized by single pot synthesis protocol. The 1,4-naphthoquinone **3** was stirred with substituted anilines **4a-d** in MeOH at ambient for a variable reaction duration. The resulting products (**5a-d**) were isolated and purified by crystallization from MeOH (Scheme 1).

Contrary to the many other cases, instead of altering the electron density of the phenyl ring of naphthoquinone, to

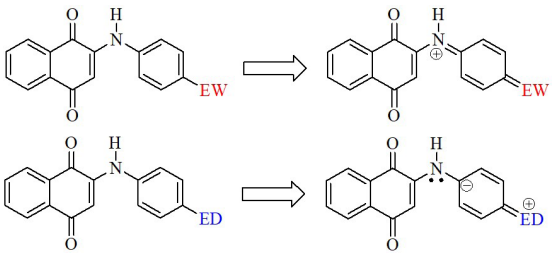
Table 2. The treatment groups and their composition.

Group Composition
1. Normal saline treated
2. Alloxan**16** + normal saline treated
3. **16** induced diabetic + **2** thymoquinone
4. **16** induced diabetic + **5a**
5. **16** induced diabetic + **5b**
6. **16** induced diabetic + **5c**
7. **16** induced diabetic + **5d**





Scheme 1. Synthesis of 2-phenylamino-1,4-naphthoquinones derivatives.



Scheme 2. Electronic effects of addition of e-withdrawing and donating groups on PAN compound (adopted [20] and redrawn).

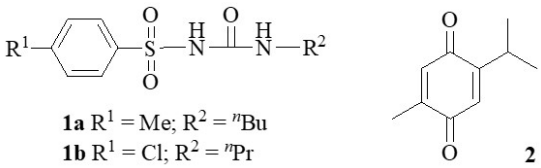


Figure 1. General structure of compounds; 1a *N*-Butyl-*N*-(4-methylbenzenesulphonyl) carbamide; 1b *N*-Propyl-*N*-(4-methylbenzenesulphonyl) carbamide; 2 2-Methyl-5-propylbenzoquinone

make it more reactive (Satheshkumar et al., 2014), we have altered the electron densities on $PhNH_2$ by incorporating the substituents with electron withdrawing or donating effects to facilitate the addition reaction in the presence of MeOH, which is indicated by the high percent yields (Table 1). The synthesized compound would show hybridization of PAN according to the electronic properties of the substituent. It has been reported that the resonance effects dominates the inductive effects when the electron donating substituents are incorporated in the quinone ring. However, in the case of electron withdrawing groups the reverse may be true (Macias-Ruvalcaba et al., 2002). Following scheme shows the effects of incorporation of e-withdrawing and donating groups to the PAN structure (Scheme 2 adopted from Satheshkumar et al., 2014, and redrawn). The e-withdrawing group causes the delocalization of electron pair of N in phenyl ring, which decreases its basicity. While an e-donating group inhibits the delocalization (due to lone pair-π electron pair repulsions) of electron pair of N in phenyl ring, which increases its basicity. The availability/ localization of electron pair on nitrogen is the key parameter in biological action. The general structure of the compound which is to be derivatized showed in Figure 1 whose nomenclature are 1a *N*-Butyl-*N*-(4-methylbenzenesulphonyl) carbamide, 1b *N*-Propyl-*N*-(4-methylbenzenesulphonyl) carbamide and 2 2-Methyl-5-propylbenzoquinone respectively

The characterization of synthesized compounds **5a-d** by IR revealed enough information to authenticate the formation of targeted compounds. The IR spectrum of naphthoquinone**3** (the substrate) shows only one broad absorbance at 1610 cm^{-1} for both carbonyls since both C=O are symmetrical but the position of signal in spectrum is quite unexpected (normal range 1690-1720 cm^{-1}). However, the IR spectra of synthesized PAN derivatives **5a-d** showed two signals around 1690 and 1600 cm^{-1} signifying a change due to the induction of PhNH moiety. The larger $\bar{\upsilon}$ value (1690 cm^{-1}) is exhibited by the C=O not in conjugation with phenylamino functionality; whereas, the smaller $\bar{\upsilon}$ value (1600 cm^{-1}) belongs to the C=O in conjugation with phenylamino moiety (Figure 2). The high energy IR region (3100–4000 cm^{-1}) also confirmed the formation of targeted compounds **5a-d**. The substrate **3** showed an intense absorbance of aromatic sp^2C–H stretching at 3053 cm^{-1}. The spectra of synthesized compounds **5a-d** exhibit an intense absorbance of hydrogen bonded N–H stretching 3200±5 cm^{-1} and a poor absorbance of aromatic sp^2C–H cm^{-1} at 3000±3 cm^{-1} (Figure 3). The appearance of intense absorbance has provided the clear indication of an amino functionality, which may exist in other tautomeric (imine) form as well (Scheme 3).

The proposed mechanism is displayed in Scheme 4. Two equivalents of 1,4-naphthoquinone **3** and one equivalent of *p*-substituted amines **5a-d** were used since **3** acts as not only substrate but also as an oxidizing agent. The conjugate addition of amines **4a-d** to

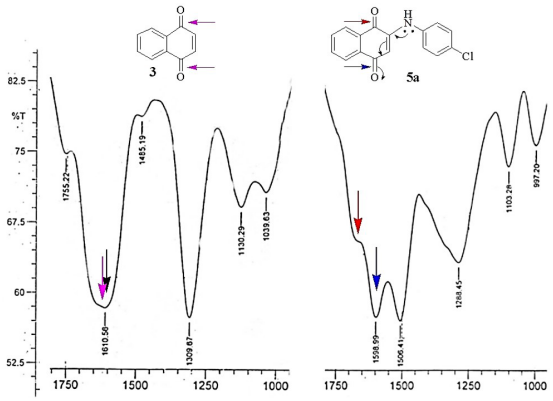


Figure 2. Portions of IR spectra (1750-1000 cm^{-1}) of substrate **3** and2-(4´-chlorophenyl amino)-1,4-naphthoquinones **5a.**

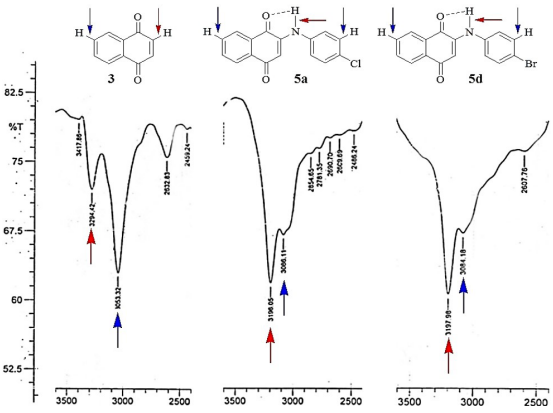
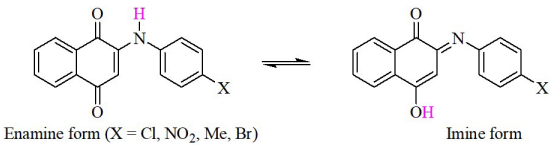


Figure 3. Portions of IR spectra (2500-3500 cm^{-1}) of substrate **3,** 2-(4´-chlorophenylamino) -1,4-naphthoquinones **5a** and 2-(4´-bromophenylamino)-1,4-naphthoquinones **5d.**

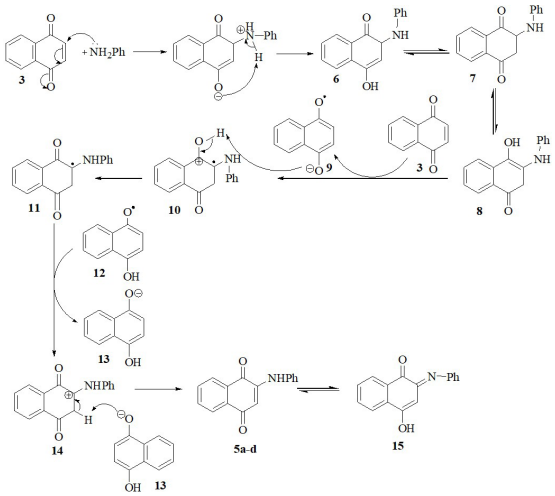


Scheme 3. Tautomeric form of 2-phenylamino-1,4-naphthoquinones (PAN)derivatives.

1,4-naphthoquinone **3** followed by deprotonation results in the formation of quinone **6** that may tautomerize to **7** or **8**. The oxidation of **8** by **3** following single electron transfer (SET) furnishes the formation of a radical cation **10** that converts to resonance stabilized free radical **11**. Another course of SET to **11** results in the formation of targeted compounds **5a-d**, which may also exist in its other tautomeric form **15** (Scheme 4).

3.2. Biology: antioxidant and hypoglycaemic potential

The knowledge about the chemical structure of a natural antioxidant (phytochemical) provides bases for synthesis of more specific chemical compounds with enhanced bioactivity. Therefore, many researchers have synthesized



Scheme 4. Proposed mechanism of formation of 2-phenylamino-1,4-naphthoquinones.

a variety of chemical compounds and evaluated their antioxidant potential. The nitrogen containing heterocyclic compounds as oxindoles were synthesized and studied for their antioxidant potential with respect to ascorbic acid. It was inferred that the structural variations bring about change in bioactivity e.g., if halogens (Cl or Br) were substituted in the aromatic ring, it sharply enhanced the antioxidant potency of the compound (Hossain et al., 2009). The radical scavenging activity of naphthoquinones has also been studied and it was found that the antioxidant potential of 2-aminothiophene compounds has increased by introducing the naphthoquinones moiety to the compounds (Gouda et al., 2013). In the present study, among four PAN derivatives tested, the nitro and bromo derivatives have shown the better radical scavenging activity as compared to the chloro derivative, however, the methyl derivative has shown no activity at all. The bromo derivative was found with the maximum antioxidant activity as exhibited by the higher %RSA (Figure 4). Fourteen different concentrations (1-200 µM) of compounds **(5a-d)** were prepared and the highest scavenging activity was noted for **5b** at 135 µM concentration and also a significant activity was noticed for **5d** at 120 µM concentration. Since the maximum %RSA was exhibited by the Br-derivative, hence, the current results further support the earlier findings (Hossain et al., 2009), and also explain the importance of electron donating groups on the PAN structure for their radical scavenging activity.

The Fe^{3+} reducing power (FRAP) assay was also conducted to evaluate the reduction potential of synthesized compounds (**5a-d)**. The assay results have been presented in Table 3.

We find that **5c** has shown more activity towards Fe^{3+} reduction (except at 30 µM) indicating the higher antioxidant potential after ascorbic acid. Here, the nitro group with greater electron withdrawing effect showed the more significant reduction potential among PAN derivatives. While evaluating **5a-d** for their potential against diabetes induced hyperglycemia, all compounds showed significant reduction in mean blood glucose levels of the

Table 3. Antioxidant potential of 5a-d towards Fe^{3+} reducing power assay (FRAP). Data are presented as mean ± standard deviation (n=5).

Conc. of compounds (μM)	A (700 nm λ)				
	Ascorbic acid	5a	5b	5c	5d
30	0.025	0.011	0.010	0.022	0.025
60	0.054	0.020	0.014	0.025	0.019
90	0.054	0.013	0.013	0.026	0.014
120	0.020	0.010	0.030	0.034	0.011
150	0.038	0.014	0.013	0.036	0.013

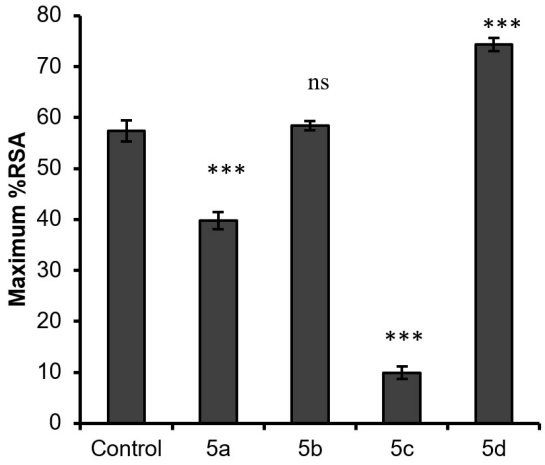


Figure 4. DPPH radical scavenging activity: Values are expressed as mean + SE (n = 3). Treatments were compared with control at p values<0.001***; nsnon-significant comparison. (Control= ascorbic acid; 5a=chloro derivative; 5b=methyl derivative; 5c=nitro derivative; 5d=bromo derivative). The data were analyzed using Oneway-ANOVA analysis and Tukey's post hoc test.

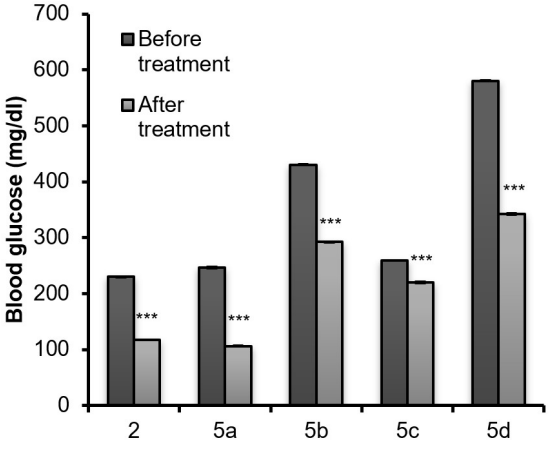


Figure 5. Hypoglycaemic potential of PAN derivatives: Blood glucose levels for treatment rats were compared before and after the test treatments at p values<0.001*** (2= thymoquinone as standard compound; 5a=chloro derivative; 5b=methyl derivative; 5c= nitro derivative; 5d= bromo derivative). Values are expressed as mean + SE (n = 5). The data were analyzed using Oneway-ANOVA.

experimental rats. However, the **5a** (chloro-derivative of PAN) showed the most significant ($p<0.05$) activity against hyperglycemia in rats bringing about 43% reduction in the mean blood glucose levels; near the control value (Figure 5).

The search of finding the most potent and safe antidiabetic agent provoked different researchers to tune their efforts for synthesis of compounds structurally related to some natural antidiabetics. Konatham et al. (2010) synthesized the curcumin derivatives and reported four derivatives (H, Cl, Me_2N, OH) with promising antidiabetic activities even more than the curcumin. Moreover, similar to our findings, they reported the Me-derivative with inferior antidiabetic activity as compared to curcumin (Konatham et al., 2010). Similarly, Kini and Ghate, 2011) reported the synthesis of 3-[5'-methyl-2'-aryl-3'-(thiazol-2"-yl amino) thiazolidin-4'-one] coumarins and found some of the compounds with Cl and Br-substituents with significant hypoglycaemic activity (Kini and Ghate, 2011). We also have found the Cl-derivative of phenylamino-naphthoquinone (**5a)** as the most potent antidiabetic agent. Both **5a** and **5d** shows similar electronic effects (–I, +R effects). However, we found more hypoglycaemic potential of **5a** as compared to **5d**; the reason would be the greater size of the Br-group, which might not fill the binding pocket of the receptor. The **5b** exhibits more electron withdrawing potential, but having high polarity (π =0.86) than **5a** (π =0.71) and large dipole moments. It has been considered as hydroneutral and thus supposed not so hydrophobic to interact with target receptor on cell membrane (Sagawa and Shikata, 2014). The incorporation of Me-groups usually reduces the water solubility and consequently enhances the ability of analogue to absorb into biological membrane but reduces its ability to be released from the biological membranes into aqueous media. It has also been noted that Me-group may develop more suitable interactions with the binding site of the receptor and best fit the pocket on the target site (Patrick, 2002).

3.3. Proposed mechanism of action for 5a

It has been reported earlier that in the biological system, the quinone may be reduced to semiquinone (a free radical) and also to hydroquinone by the enzyme cytochrome P450 reductase and other flavoprotein enzymes. These intermediary species are re-oxidized by molecular oxygen (O_2) and generate ROS, such as superoxide, $^{\cdot}OH$ and H_2O_2 (Kumagai et al., 2012). Bio-reduction of

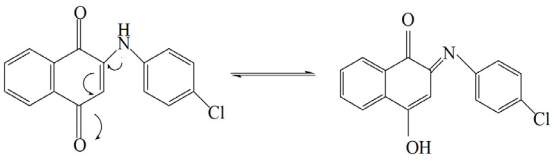


Figure 6. Proposed mechanism of action for chloro derivative of PAN.

naphthoquinones may be influenced by changing the redox properties of naphthoquinones. It has been found that presence of electron-rich substituents at the quinone moiety lowers its reduction potential, thus they become more reactive. However, for the mechanism of action of **5a**, we propose that the incorporation of amine moiety to the quinone structure limit the free radical generating ability of the quinones and also further addition of any chemical group having electron donating resonance effect may stabilize the imine form of the compound (Figure 6). By utilizing the oxidation-reduction properties of its quinone moiety, naphthoquinones may act as generators of free radicals as their semiquinone radicals may lead to the formation of reactive oxygen species. This induces the cytotoxicity. Mainly the cytotoxic properties of naphthoquinones have been reported to be highly dependent on their chemical properties (Monks et al., 1992). However, the incorporation of e^--donating group at *para* position of 2-phenylamino-1,4-naphthoquinone (PAN) has induced the favourable change in biological properties of PAN conferring more cyto-protective properties to it.

4. Conclusions

The compounds 2-phenylamino-1,4-naphthoquinones(PAN) have been the subject of interest for their diverse use in medical and biological applications as naphthoquinone amino derivatives exhibit interesting redox properties which make these potential targets of medicine. Mostly the synthetic naphthoquinone(s) have shown antimicrobial activities however; very less data is available regarding its potential against diabetes.

In present study the four synthesized PAN derivatives were tested for its antioxidant potential and hypoglycaemic activity *in vivo* to stimulate research utilizing the medicinal benefits of PAN derivatives in drug discovery and development. All the synthesized compounds showed better antioxidant and hypoglycaemic activity, however, the chloro derivative exhibited the more potent hypoglycaemic activity may be due to the presence of e-donating resonance effect (+R) of chloro group. It is inferred that the addition of chloro group to the PAN structure provide stability to its imine form which limits the potential of generation of free radicals of quinones during bio-reduction, which may be proposed as reason for the potential hypoglycaemic activity exhibited by PAN chloro derivative. The present study findings need additional efforts to optimize drug-receptor interactions for better drug designing strategies and to develop more potent antidiabetic derivatives containing the 2-phenylamino-1,4-naphthoquinones moieties. The study will stimulate the need for further clinical trials of the synthesized compounds as drug(s) based on naphthoquinone derivatives for therapy against diabetes.

Acknowledgements

The work was carried out at Department of Chemistry in collaboration with Department of Pharmacy at University of Sargodha, Pakistan. We are thankful to all the concerned persons.

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