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Synthesis, Antioxidant and Antimicrobial Activity of 4-Aminophenol and 2-Aminobenzoic Acid Based Novel Azo Compounds

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In present work, 4-aminophenol and 2-aminobenzoic acid were diazotized at low temperature and novel azo compounds from 2-aminobenzoic acid, benzamide, 4-aminophenazone, 2-bromonitrobenzene and 4-dichlorobenzene were synthesized. The resulting compounds were characterized by m.p., UV-visible and FTIR spectroscopic technique. The synthesized compounds were screened for their antibacterial activity by using disc diffusion method. The bacterial strains *Escherichia coli, Shigella sonnei, Streptococcus pyrogenes, Staphylococcus aureus* and *Neisseria gonorrhoea* were used. The antioxidant activity was also performed by using DPPH method. Results revealed that azo compounds of 4-aminophenol with 2-aminobenzoic acid and 4-aminophenazone showed much better antibacterial activity against all strains while same azo compounds had good antioxidant activity.

Keywords: 4-Aminophenol, 2-Aminobenzoic acid, Azo compounds, Antibacterial and antioxidant activities.

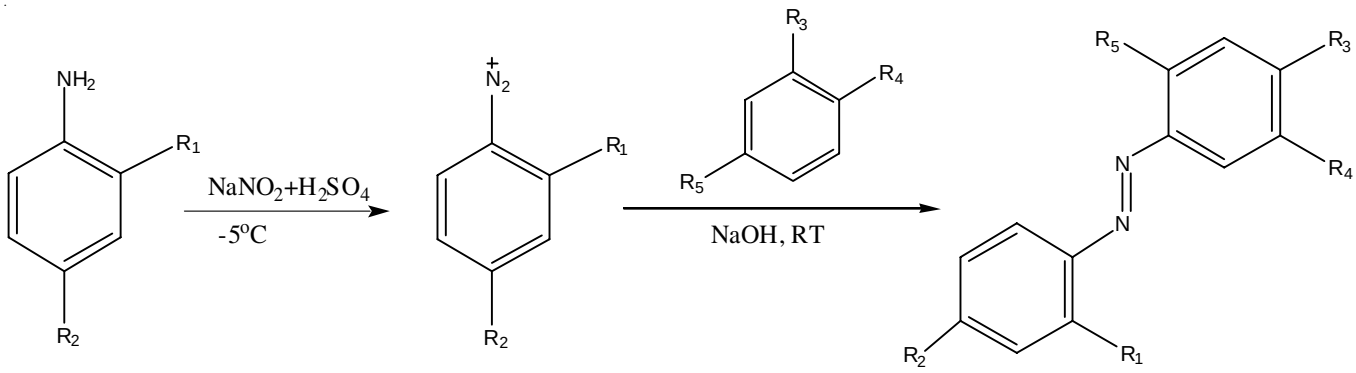
INTRODUCTION

Azo compounds are an important and versatile class of synthetic organic compounds with variety of applications. Azo compounds are also found among the largest classes of industrially synthesized organic compounds.These compounds generally have high molar extinction coefficient and the medium to high light and wet fastness properties[1]. Due to these properties such compounds have been widely used in the areas like dyeing of textile fibers, plastics, leather, paper and bio-medical studies[2]. Optical storage capacity, optical switching, holography and nonlinear optical properties, polymers with azo units represent promising candidates for photoactive materials[3]. The physico-chemical properties of these compounds have strong relation to their tautomeric forms since they potentially have enol-azo, keto-azo and hydro-azo tautomeric forms in solution and in solid state[4-6]. The azo dyes possess antiseptic and antiprotozoal properties and also promote wound healing. Such compounds have also been useful for antibacterial activity against different organism[7-13] and antioxidant activity[14]. The cationic dyes are more active in acidic medium and preferably attack on gram positive bacteria as compared to anionic dyes. Azo compounds are also known for their medicinal importance and are well recognized for their use as antineoplastics[15], antidiabetics[16], antiseptics[17], antibacterial[18-22], pesticidal activities[23], antitumor [24] and antifungal[25].

EXPERIMENTAL

All the chemicals were purchased from Merck and were used without further purification. The melting points were measured by using standard melting point apparatus from Stuart and were uncorrected. The UV-visible (ORI Germany UV4000 spectrophotometer) spectra were recorded in methanol with at concentration rate of 10^{-4} M. FTIR spectra were recorded in the region of 4000 to 400 cm^{-1} on a FTIR-ALPHA BRUKER IR spectrometer in KBr pellets.

General procedure: The aniline derivatives (4-aminophenol and 2-aminobenzoic acid) were added in distilled water (15 mL) and conc. H_2SO_4 (2 mL). The mixture were warmed (T ≈ 50 °C) to get clear solutions. Sodium nitrite was dissolved in distilled water (10 mL). Both the solutions were cooled to temperature below 5 °C by ice water bath. Added cooled sodium nitrite solution drop wise into the solution of aniline derivatives with vigorous stirring. To these solutions, separately prepared solutions of different active aromatic compounds (equimolar)were added slowly with parallel addition of sodium hydroxide (2 M) (**Scheme-I**). The precipitates of different colours were formed by adjusting the pH at 7. Precipitates were filtered by suction filtration and washed several times with distilled water to remove the extra acid and salt from the product. The yield of these synthesized compounds was 40-80 %.



1.	R_1 = H,	R_2 = OH,	R_3 = NH_2,	R_4 = COOH,	R_5 = H
2.	R_1 = H,	R_2 = OH,	R_3 = H,	R_4 = $CONH_2$,	R_5 = H
3.	R_1 = H,	R_2 = OH,	R_3 = $C_5H_8N_3O$,	R_4 = H	R_5 = H
4.	R_1 = H,	R_2 = OH,	R_3 = NH_2,	R_4 = NO_2,	R_5 = H
5.	R_1 = COOH,	R_2 = H,	R_3 = Br,	R_4 = NO_2,	R_5 = H
6.	R_1 = COOH,	R_2 = H,	R_3 = H,	R_4 = Cl,	R_5 = Cl

Scheme-I: Synthetic scheme for the synthesis of azo compounds

Bacterial strains: The following strains were used for antibacterial activity: (1) *Escherichia coli* (ATCC 35318); (2) *Escherichia coli* (ATCC 25922); (3) *Shigella sonnei* (ATCC 25931); (4) *Staphylococcus aureus* (ATCC 38541); (5) *Streptococcus pyrogenes* (ATCC 19615); (6) *Staphylococcus aureus* (ATCC 25923); (7) *Neisseria gonorrhea* (ATCC 49226). All bacterial strains were maintained on nutrient agar medium at ± 37 °C.

Antibacterial activity: Antibacterial activity was evaluated by disc diffusion method[26,27]. Disc diffusion method is a popular method for determining the antibacterial activity. Nutrient agar media was prepared and sterilized and was poured into sterile petri dishes under sterile environment. The plates were inoculated by 15 µL suspension of bacterial growth culture. Stock solutions of the compounds were prepared *i.e.*, 10 µg/1.0 µL for all the compounds.Filter discs soaked in drug solutions were placed on these inoculated plates with the help of a sterilized forceps. These plates were then incubated overnight at 37 °C. The results were collected after 24 h by determining the inhibition zone diameter values of each compound.

Antioxidant activity by DPPH method: The antioxidant activity of compounds **1-6** was determined by DPPH method[28,29]. Solutions of all synthesized compounds were prepared in 1 mg/1.0 mL. 50 µL solution of each compound was added into the 2 mL of freshly prepared 0.2 mM DPPH solution in methanol. All the solutions were incubated at 37 °C for 20 min. The absorbance were taken at 517 nm with the help of double beam spectrophotometer (NIL). Ascorbic acid (1 mM) was used as a positive control. Percentage scavenging activity of the synthesized compounds was calculated by using the following formula:

$$\text{Scavenging } (\%) = \left(\frac{A_o - A_T}{A_o}\right) \times 100$$

where, A_o = Absorbance of DPPH solution; A_T = Absorbance of sample solution.

RESULTS AND DISCUSSION

Azo compounds **1-6** were prepared by diazotization of 4-aminophenol and 2-aminobenzoic acid and reacted with active aromatic compounds at temperature below 5 °C[30]. The aromatic compounds used were 2-aminobenzoic acid, benzamide, 4-aminophenazone, 2-bromonitrobenzene and 1,4-dichlorobenzene. The structural formulae of the synthesized are given in Fig. 1.

2-Amino-3-[(*Z*)-(4-hydroxyphenyl)diazenyl]benzoic acid (1): Yield: 40 %, λ_{max} = 352 nm, m.p. 133-134 °C, FTIR (KBr, ν_{max}, cm^{-1}): 3642 (OH stretching), 3410 (NH_2), 1688 (C=O of COOH), 1514.4 (N=N), 1461 (C=C of aromatic ring), 750 (C-H of aromatic ring).

3-[(*E*)-(4-Hydroxyphenyl)diazenyl]benzamide (2): Yield: 47 %, λ_{max} = 355 nm, m.p. 123-125 °C, FTIR (KBr, ν_{max}, cm^{-1}): 3650 (O-H stretching), 3165.6 (NH_2 of amide), 1655.1 (C=O of amide), 1622 (C=C of aromatic), 1577.0 (N=N), 769 (C-H of aromatic ring).

4-Amino-2-{4-[(*E*)-(4-hydroxyphenyl)diazenyl]phenyl}-1,5-dimethyl-1,2-dihydro-3*H*-pyrazol-3-one (3): Yield: 79 %, λ_{max} = 359 nm, m.p. 190-191 °C, FTIR (KBr, ν_{max}, cm^{-1}): 3642 (O-H stretching), 3415 (NH_2), 2973.1 (C-H of CH_3), 1601 (C=C of aromatic ring), 1592.2 (N=N), 757 (C-H of aromatic ring),

4-[(*Z*)-(4-Amino-3-nitrophenyl)diazenyl]phenol (4): Yield: 44 %, λ_{max} = 352 nm, m.p. 85-86 °C, FTIR (KBr, ν_{max}, cm^{-1}): 3570 (OH), 3420 (NH_2), 1580 (C=C of aromatic ring), 1521.1 (N=N), 1510 (NO_2), 740 (C-H of aromatic ring).

2- [(*E*)-(4-Bromo-3-nitrophenyl)diazenyl]benzoic acid (5): Yield: 58 %, λ_{max} = 358 nm, m.p. 52-54 °C, FTIR (KBr, ν_{max}, cm^{-1}): 1652.3 (C=O of COOH), 1577.8 (N=N), 1560 (NO_2), 1455.6 (C=C of aromatic ring), 726 (C-H of aromatic ring), 641 (C-Br)

2-[(*E*)-(2,5-Dichlorophenyl)diazenyl]benzoic acid (6): Yield: 32 %, λ_{max} = 365 nm, m.p. 240-242 °C, FTIR (KBr, ν_{max}, cm^{-1}): 2961.4 (O-H stretching), 1680.4 (C=O of COOH), 1590.8 (N=N), 1454.9 (C=C of aromatic ring), 761 (C-H of aromatic ring), 540 (C-Cl).

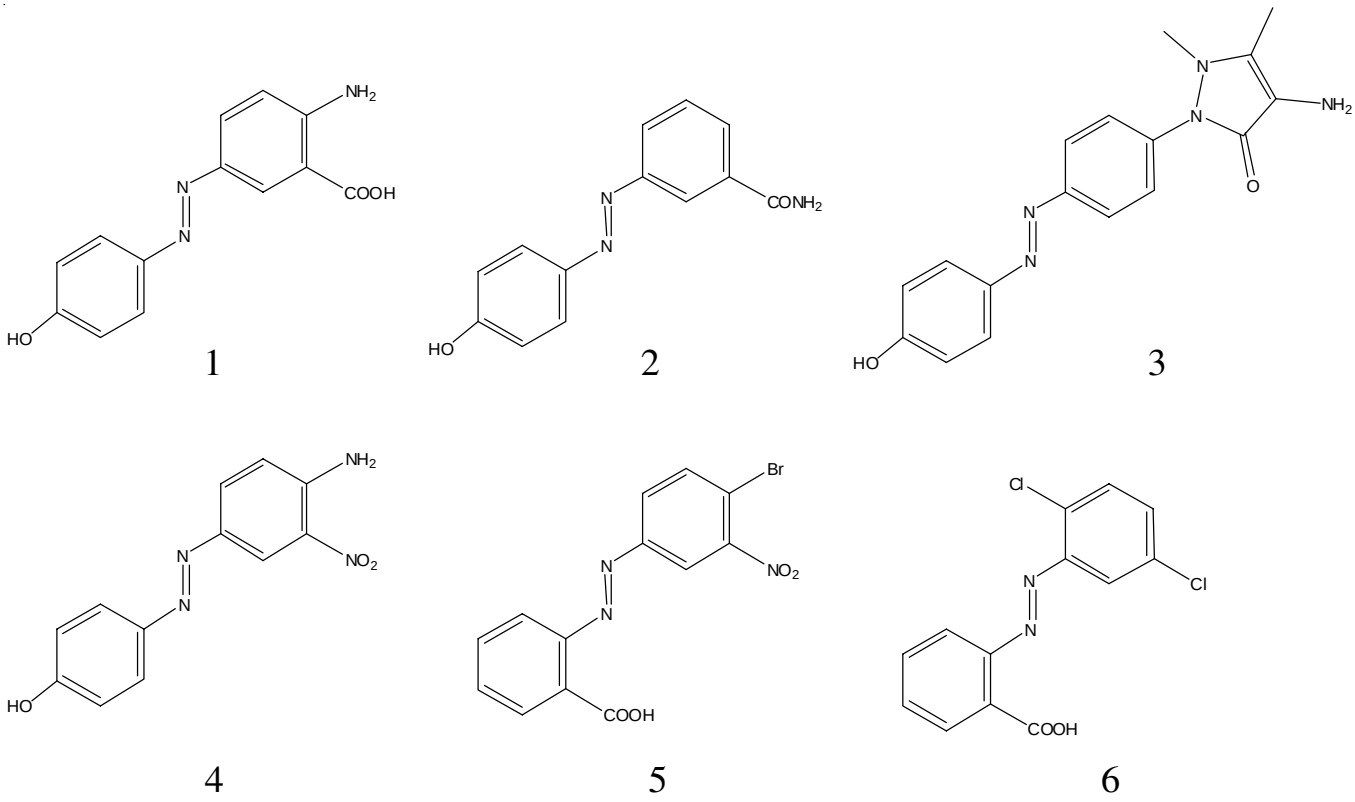


Fig. 1. Structural formulae of the synthesised azo compounds

Antibacterial activity: Azo compounds (**1**-**6**) were evaluated for their antibacterial activity against seven microbes mentioned above. Compounds (**1**-**6**) showed medium to good antibacterial activity against all strains (Table-1). Compound **2** and **5** showed good results against *E. coli* (ATCC 35318) while **3** and **5** exhibited comparable activity against *S. aureus* (ATCC 38541) to that of standard drug used as reference. All compounds were remarkably active against *S. sonnei* (ATCC 25931). Compounds **4** and **5** also give good results against *E. coli* (ATCC 25922). Medium activity of test compounds has been observed against *S.pyrogenes* (ATCC 19615). Test compounds were found less active against *S. aureus* (ATCC 25923) and *N. gonorrhea* (ATCC 49226) (Fig. 2).

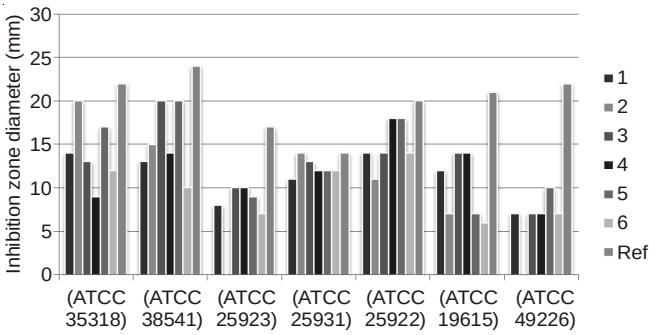


Fig. 2. Antibacterial activity of synthesized compounds against seven different ATCC bacterial strains. Cefpodoxime is used ae reference drug

DPPH scavenging activity: Compounds (**1**-**6**) were also analyzed for their antioxidant potential. DPPH method was used to determine the free radical scavenging activities of the compounds[31]. Solution of test compounds (50 µL each) was added to 2 mL of 0.2 mM ethanolic solution of DPPH. After incubation for 20 min at temperature = 37 °C, absorbance of the mixtures was noted at λ = 517 nm. Ascorbic acid (1 mM) was used as positive control. Free radical scavenging (% age) of the samples was calculated by the formula as given before.

The decrease in absorbance by the test samples is due to the pairing up the free electron of DPPH which correspond to its ability as antioxidant[32-37]. Compounds **1** and **3** showed better antioxidant activity as compared to other compounds. Compound **6** was inactive. The results of antioxidant activity are summarized in Table-2.

TABLE-1
ANTIBACTERIAL ACTIVITY OF SYNTHESIZED COMPOUNDS (**1-6**) (IZD ± MEAN)
CEFPODOXIME IS USED ARE REFERENCE DRUG

Bacterial strains	Compounds						Reference
	1	**2**	**3**	**4**	**5**	**6**	
E. coli (ATCC 35318)	14 ± 0.2	20 ± 0.3	13 ± 0.4	9 ± 0.7	17 ± 0.4	12 ± 0.4	22 ± 0.3
S. aureus (ATCC 38541)	13 ± 0.6	15 ± 0.2	20 ± 0.2	14 ± 0.8	20 ± 0.6	10 ± 0.6	24 ± 0.4
S. aureus (ATCC 25923)	8 ± 0.3	Nil	10 ± 0.5	10 ± 0.3	9 ± 0.7	7 ± 0.2	17 ± 0.6
S. sonnei (ATCC 25931)	11 ± 0.1	14 ± 0.3	13 ± 0.2	12 ± 0.1	12 ± 0.1	12 ± 0.4	14 ± 0.2
E. coli (ATCC 25922)	14 ± 0.6	11 ± 0.2	14 ± 0.3	18 ± 0.6	18 ± 0.3	14 ± 0.5	20 ± 0.2
S. pyrogenes (ATCC 19615)	12 ± 0.4	7 ± 0.7	14 ± 0.5	14 ± 0.7	7 ± 0.6	6 ± 0.5	21 ± 0.1
N. gonorrhoea (ATCC 49226)	7 ± 0.1	Nil	7 ± 0.8	7 ± 0.1	10 ± 0.2	7 ± 0.5	22 ± 0.4

TABLE-2
PERCENTAGE SCAVENGING OF SYNTHESIZED COMPOUNDS (**1-6**) AT CONCENTRATIONS (1 µg/µL)

Compd.	Observation	Activity	Difference	Scavenging (%)
1	1.014	Moderate	0.377	27.10
2	1.356	Weak	0.035	2.5
3	0.772	Good	0.619	44.50
4	1.280	Weak	0.111	7.97
5	1.334	Weak	0.057	4.1
6	1.850	Inactive	–	–
Ascorbic acid	0.201	+ve Control	1.19	85.55

Conclusion

In this research, a series of azo compounds were prepared from 4-aminophenol and 2-aminobenzoic acid. Their characterization was checked along with biological activities including antibacterial and antioxidant activity. Results reveal that these compounds have moderate to excellent antibacterial activity against all seven strains in comparison to reference drug (cefpodoxime). Thus these synthesized azo compounds can be used in potent drug for bacterial infections.

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REFERENCES

1. O.O. Ajani, O.E. Akinremi, A.O. Ajani, A. Edobor-Osoh and W.U. Anake, *Phys. Rev. Res. Int.*, **3**, 28 (2013).
2. E. Aktan, B. Babur, Z. Seferoglu, T. Hokelek and E. Sahin, *J. Mol. Struct.*, **1002**, 113 (2011).
3. J. He, S. Bian, L. Li, J. Kumar, S. Tripathy and L. Samuelson, *J. Phys. Chem. B*, **104**, 10513 (2000).
4. N. Ertan, *Dyes Pigments*, **44**, 41 (1999).
5. F. Karci and N. Ertan, *Dyes Pigments*, **55**, 99 (2002).
6. I. Sener, F. Karci, N. Ertan and E. Kilic, *Dyes. Pigments*, **70**, 143 (2006).
7. K.M. Rathod and N.S. Thakre, *Chem. Sci. Trans.*, **2**, 25 (2012).
8. A.A. Jarrahpour, M. Motamedifar, K. Pakshir, N. Hadi and M. Zarei, *Molecules*, **9**, 815 (2004).
9. A.Z. Sayed, M.S. Aboul-Fetouh and H.S. Nassar, *J. Mol. Struct.*, **1010**, 146 (2012).
10. M.N. Mohammed, *J. Chem. Chem. EN*, **6**, 885 (2012).
11. Swati, Ginni, R. Karnawat, I.K. Sharma and P.S. Verma, *Int. J. Appl. Biol. Pharm. Technol.*, **2**, 332 (2012).
12. A.H. Shridhar, J. Keshavayya, H.J. Hoskeri and R.A.S. Ali, *Int. Res. J. Pure Appl. Chem.*, **1**, 119 (2011).
13. I.H.R. Tomi, A.H.R. Al-Daraji, R.R.T. Al-Qaysi, M.M. Hasson and K.H.D. Al-Dulaimy, **7**, 687 (2014).
14. C.T. KeerthiKumar, J. Keshavayya, T. Rajesh and S.K. Peethambar, *Int. J. Pharm. Pharm. Sci*., **5(Suppl. 1)**, 296 (2013).
15. R.G. Child, R.G. Wilkinson and A. Tomcu-Fucik, *Chem. Abstr.*, **87**, 6031 (1997).
16. H.G. Garg and C. Prakash, *J. Med. Chem.*, **15**, 435 (1972).
17. C.H. Browing, J.B. Cohen, S. Ellingworth and R. Gulbransen, *Br. Med. J*., **2(3269)**, 326 (1923).
18. A. Khalid, M. Arshad and D.E. Crowley, *Appl. Microbiol. Biotechnol.*, **78**, 361 (2008).
19. U. Pagga and D. Brown, *Chemosphere*, **15**, 479 (1986).
20. I.M.A. Awad, A.A.M. Aly, A.M. Abdel-Alim, R.A. Abdel-Aal and S.H. Ahmed, *J. Inorg. Biochem.*, **33**, 77 (1988).
21. A.G. Makhsumov, M.A. Ergashev and F.A. Normatov, *Pharm. Chem. J.*, **25**, 798 (1991).
22. S.A. Ibrahim, M.A. El-Gahami, Z.A. Khafagi and S.A. El-Gyar, *J. Inorg. Biochem.*, **43**, 1 (1991).
23. S. Samadhiya and H. Halve, *Oriental J. Chem*., **17**, 119 (2001).
24. T.A. Farghaly and Z.A. Abdallah, *ARKIVOC*, 295 (2008).
25. H. Xu and X. Zeng, *Bioorg. Med. Chem. Lett.*, **20**, 4193 (2010).
26. L. Barros, R.C. Calhelha, J.A. Vaz, I.C.F.R. Ferreira, P. Baptista and L.M. Estevinho, *Eur. Food Res. Technol.*, **225**, 151 (2007).
27. K. Uthayarasa, K. Pathmanathan, J.P. Jeyadevan and E.C. Jeyaseelan, *Int. J. Integ. Biol*., **10**, 76 (2010).
28. M. Nagai, M. Tani, Y. Kishimoto, M. Iizuka, E. Saita, M. Toyozaki, T. Kamiya, M. Ikeguchi and K. Kondo, *J. Clin. Biochem.*, **48**, 203 (2011).
29. S. Shamim, S. Murtaza and M.F. Nazar, *J. Chem. Soc Pak.*, (Submitted).
30. H. Karabiyik, N.O. Iskeleli, C. Albayrak and E. Agar, *Struct. Chem.*, **18**, 87 (2007).
31. D.N. Nicolaides, D.R. Gautam, K.E. Litinas, D.J. Hadjipavlou-Litina and K.C. Fylaktakidou, *Eur. J. Med. Chem.*, **39**, 323 (2004).
32. R.S. Upadhayaya, J.K. Vandavasi, R.A. Kardile, S.V. Lahore, S.S. Dixit, H.S. Deokar, P.D. Shinde, M.P. Sarmah and J. Chattopadhyaya, *Eur. J. Med. Chem.*, **45**, 1854 (2010).
33. T. Chandra, N. Garg, S. Lata, K.K. Saxena and A. Kumar, *Eur. J. Med. Chem.*, **45**, 1772 (2010).
34. R. Kalla and J. Zablocki, *Annu. Rep. Med. Chem.*, **44**, 265 (2009).
35. M.W. Sabaa, N.A. Mohamed, R.R. Mohamed, N.M. Khalil and S.M. Abd El Latif, *Carbohydr. Polym.*, **79**, 998 (2010).
36. S.A.F. Rostom, *Bioorg. Med. Chem.*, **18**, 2767 (2010).
37. S.A.F. Rostom, H.M.A. Ashour, H.A.A. El Razik, A. El Fattah H.A. El Fattah and N.N. El-Din, *Bioorg. Med. Chem.*, **17**, 2410 (2009).