Research Article

In Vitro Antimicrobial Activity of Some New Azo Compounds Synthesized from 2-Aminoethyl Piperazine

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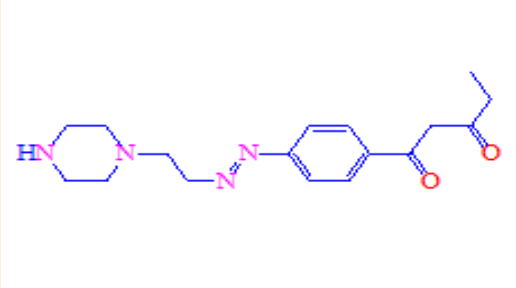
Abstract

Six new azo compounds were synthesized by the reaction of 2-aminoethyl piperazine with different coupling agents. New compounds were characterized by UV-visible, FT-IR and ^{1}H NMR spectroscopy. Compounds were tested *in vitro* for their antimicrobial activity against clinically isolated strains. Variable and modest activities were observed against the investigated strains of bacteria and fungi. Compounds **3b**, **3d** and **3f** demonstrated good antimicrobial activity against all the tested microbial strains.



Keywords: 2-Aminoethyl piperazine, Azo coupling agents, Antimicrobial activity.

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Introduction

Nowadays synthetic azo compounds are widely used as medicines, cosmetics, food, paints, plastics, shipbuilding, automobile industry, cable manufacture and in analytical chemistry [1-8]. Pharmaceutical importance of azo compounds is well known for their use as antineoplastics, antidiabetics, antiseptics, anti-inflammatory and other useful chemotherapeutic agents [9]. The existence of an azo moiety in different types of compounds have made them to involve in a number of biological reactions such as protein synthesis, inhibition of DNA, RNA, carcinogenesis and biological activity against bacteria and fungi [10]. Majority of these compounds are derived from the coupling of diazotized hetereocyclic amines with aromatic amino and hydroxyl compounds. The medicinal properties of azo compounds particularly synthesized from acetyl salicylic acid, thymol, aldimine and β-napthol etc have been frequently reported. Developing antimicrobial drugs and maintaining their potency, in opposite on to resistance by different classes of microorganisms as well as a broad spectrum of antibacterial activity are some of the major concern of research in this area. Synthesis and antimicrobial activity of azo compound using imatinib intermediate and naphthalene-2-ol has been reported [11]. Piperazine moiety has already been used in the clinical treatment of depression. Aminoethyl piperazine is a derivative of piperazine. Keeping in view of these, the present paper reporting the synthesis of some new azo compounds and evaluate them for antimicrobial activity. This strategy was extended in the present research for preparing of new group of coloring azo compounds.

Experimental

Materials and Reagents

All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd. Melting range was determined by Veego Melting Point VMP III apparatus. The FT-IR spectra were recorded using KBr discs on FT-IR Jasco 4100 infrared spectrophotometer and were quoted in cm^{-1}.^{1}H NMR spectra was recorded on Bruker DMX 300 spectrometer

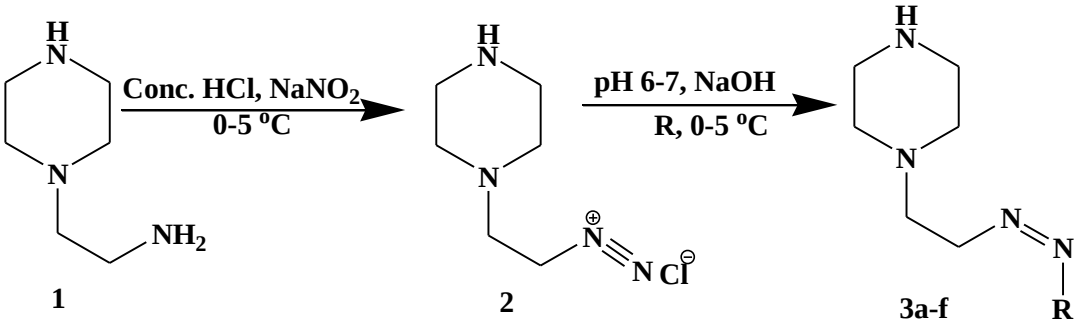
using DMSO-d_6 as solvent and TMS as an internal standard. The purity of compounds was checked by TLC. The crude products were recrystallized from chloroform.

Diazotization of 2-aminoethyl piperazine (1)

The amine **(1)** (0.721 mmol) was dissolved in 6 N HCl (25-30 mmol). The mixture was cooled by means of an ice-water bath till it attain 0-5 °C and an aqueous solution of $NaNO_2$ (0.901 mmol, 10 ml) was added drop wise within 15 min with continuous stirring. Finally the excess of HNO_2 was destroyed by adding solid urea (0.5 g). The intermediate diazonium compound (**2**) was obtained.

General procedure for the synthesis of diazotized derivatives of 2-aminoethyl piperazine compounds (3a-f)

Compounds **3a-f** was synthesized by the reaction of diazonium compound (**2**) and different coupling agents. During the procedure, the pH value was maintained within 6-7 by 6N NaOH and the temperature at 0-5 °C. The mixture was stirred for 6-8 hrs. The precipitated crude compound was collected by filtration at vacuum and washed with water. The obtained compounds (**Scheme 1**) were recrystallized from the chloroform.



Scheme 1

4-(2-2-(Piperazin-1-yl)ethyldiazenyl)naphthalene-1-ol (3a)

Off white solid. Yield: 65%. FT-IR (KBr, cm^{-1}): 3500 (O-H), 3350 (N-H), 3010 (Ar-H), 1600 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.41 (t, 2H, CH_2), 2.08 (s, 1H, NH pip), 2.40 (t, 2H, CH_2), 2.52 (m, 4H, $2CH_2$), 2.70 (m, 4H, $2CH_2$), 5.24 (d, 1H, OH), 6.54 (d, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 7.65 - 8.07 (m, 4H, Ar-H).

1-(Naphthalene-5-yl)-2-(2-piperazin-1-yl)ethyldiazene (3b)

Off brown solid. Yield: 72%. FT-IR (KBr, cm^{-1}): 3450 (O-H), 3400 (N-H), 3010 (Ar-H), 1590 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.40 (t, 2H, CH_2), 2.11 (s, 1H, NH pip), 2.40 (t, 2H, CH_2), 2.53 (m, 4H, $2CH_2$), 2.74 (m, 4H, $2CH_2$), 5.25 (d, 1H, OH), 6.53 (d, 1H, Ar-H), 7.16 (d, 1H, Ar-H), 7.63 - 8.04 (m, 4H, Ar-H).

2-(2-(2-Piperazine)-1-yl)ethyldiazonyl)-1H-indole-3-carbaxyaldehyde (3c)

Off yellow solid.Yield: 85 %. FT-IR (KBr, cm^{-1}): 3450 (N-H), 3300 (N-H indole), 3000 (Ar-H), 1590 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.40 (t, 2H, CH_2), 2.13 (s, 1H, NH pip), 2.45 (t, 2H, CH_2), 2.48 (m, 4H, $2CH_2$), 2.64 (m, 4H, $2CH_2$), 7.15 (s, 1H, Ar-H), 7.45 (m, 4H, Ar-H), 10.05 (s, 1H, indole-NH).

1-(4-(2-(Piperazin-1-yl)ethyl)diazenyl)phenyl)butane-1,3-dione) (3d)

Off white solid. Yield: 88 %. FT-IR (KBr, cm^{-1}): 3450 (N-H), 3025 (Ar-H), 1750 (C=O), 1590 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.41 (t, 2H, CH_2), 1.50-1.55 (m, 4H, $2CH_2$), 2.08 (s, 3H, CH_3), 2.39 (m, 4H, $2CH_2$), 2.50 (t, 2H, CH_2), 3.33 (s, 2H, CH_2), 5.73 (s, 1H, NH pip), 7.42 (d, 2H, Ar-H), 7.82 (d, 2H, Ar-H).

5-((2-Piperazin-1-yl)ethyl)diazenyl)nicotinic acid (3e)

Off brown solid. Yield: 85 %. FT-IR (KBr, cm^{-1}): 3400 (N-H), 3020 (Ar-H), 1700 (COOH), 1600 (N=N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.42 (t, 2H, CH_2), 2.14 (s, 1H, NH pip), 2.40 (t, 2H, CH_2), 2.45 (m, 4H, $2CH_2$), 2.68 (m, 4H, $2CH_2$), 8.23 (s, 1H, Ar-H), 8.80 (s, 1H, Ar-H), 8.98 (s, 1H, Ar-H), 10.85 (s, 1H, COOH).)

1-(4-((2-Piperazin-1-yl)ethyl)diazeneyl)phenyl)pentane-1,3-dione (3f)

Off white solid. Yield: 85 %. FT-IR (KBr, cm^{-1}): 1600 (N=N), 1725 (C=O), 3005 (Ar-H), 3400 (NH). ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.15 (t, 3H, CH_3), 1.45 (t, 2H, CH_2), 2.13 (s, 1H, NH pip), 2.40 (t, 2H, CH_2), 2.48 (q, 2H, CH_2), 2.43 (m, 4H, $2CH_2$), 2.64 (m, 4H, $2CH_2$), 3.65 (s, 2H, CH_2), 7.25 (d, 2H, Ar-H), 7.85 (d, 2H, Ar-H).

Antibacterial activity

Antibacterial activity of the synthesized compounds was determined against Gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative bacteria (*Salmonella typhi* and *Escherichia coli*) in DMF by disc diffusion method on nutrient agar medium [12]. The sterile medium (Nutrient Agar Medium, 15 ml) in each petriplates was uniformly smeared with cultures of Gram positive and Gram negative bacteria. Sterile discs of 10 mm diameter (Hi-Media) was made in each of the petriplate, to which 50 µl (1 mg/ml i.e., 50 µg/disc) of the synthesized compound was added. The treatments also included 50 µl of DMF as negative, streptomycin as positive control for comparison. For each treatment, three replicates were maintained. The plates were at 25 ± 2 ºC for 24 h and the size of the resulting zone of inhibition, if any, was determined.

Antifungal activity

The synthesized compoounds were screened for their antifungal activity against *Cladosporium oxysporum* and *Aspergillus niger* in DMF by poisoned food technique [13]. Potato Dextrose Agar (PDA) media was prepared and about 15 ml of PDA was poured into each petriplate and allowed to solidify. 5 mm disc of seven days old culture of the test fungi was placed at the center of the petriplates and incubated at 26 °C for 7 days. After incubation the percentage inhibition was measured and three replicates were maintained for each treatment. Nystatin was used as standard. The synthesized compound was tested (at the dosage of 500 µl of the novel compound/petriplate, where the concentration was 0.1 mg/ml) by poisoned food technique.

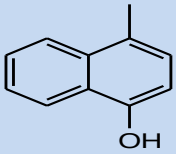
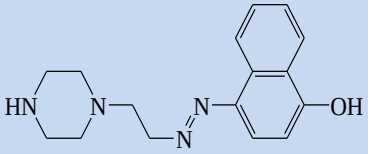
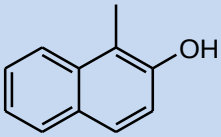
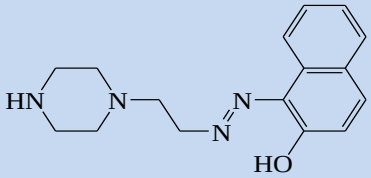
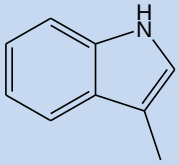
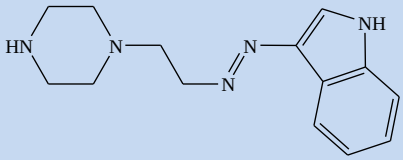
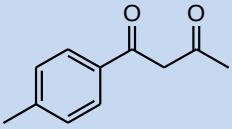
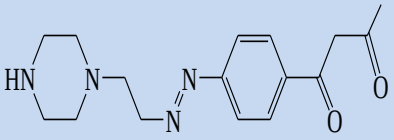
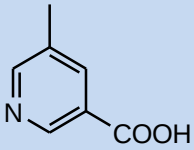
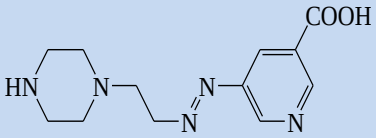
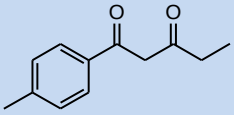
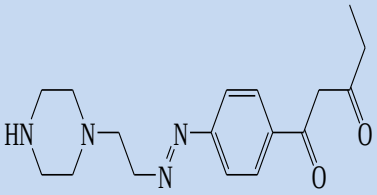
Results and Discussion

Chemistry

The diazotized derivatives of 2-aminoethyl piperazine (**3a-f**) were synthesized according to Scheme 1. Formation of diazotized derivatives of 2-aminoethyl piperazine was confirmed by recording their ^{1}H NMR, FT-IR and UV-visible spectra. The synthesis employs readily available starting materials and simple procedures making this method very attractive and convenient for the synthesis of various azo compounds. The chemical structures and physical data of all the compounds were tabulated in **Table 1**.

The absorptions around 3000 cm^{-1} in synthesized compounds confirm the aromatic stretching vibrations, and the appearance of a medium to strong absorption bands above 1600 cm^{-1} due to a stretching vibration of the N=N bond formation in synthesized compounds. The characterization of new compounds was based upon a careful comparison of ^{1}H NMR spectra. An important characteristic feature in the ^{1}H NMR spectra of **1** showed NH_2 proton in 5.42 ppm, which was absent in the spectra of **3a-f**. The ^{1}H NMR spectra of new compounds showed multiplet (piperazine ring) in the region of δ, 2.40 - 2.74. Similarly a doublet appeared at δ, 6.53 - 8.98 are due to the protons of the aromatic group. The ^{1}H NMR spectra of **3a-f** showed NH group in the region of δ, 2.08-2.14.

Table 1 The chemical structure and physical data 3a-f

Compound	R	Structure	M.F.	M.W.	M.P °C
3a			$C_{16}H_{20}N_4O$	284.36	102-104
3b			$C_{16}H_{20}N_4O$	284.16	105-107
3c			$C_{14}H_{19}N_5$	257.33	168-170
3d			$C_{16}H_{22}N_4O_2$	302.17	63-65
3e			$C_{12}H_{17}N_5O_2$	263.30	120-122
3f			$C_{17}H_{24}N_4O_2$	316.40	98-99

In vitro antimicrobial activity

The investigation of antibacterial screening data revealed that all the tested compounds showed antibacterial activity against four pathogenic bacterial strains. Among the series **3a-f**, compound **3f** exhibited an elevated antibacterial activity against Gram positive (zone of inhibition 18 - 19 mm) and Gram negative (zone of inhibition 13 - 16 mm) bacteria. Compounds **3b** and **3d** showed good antibacterial activity against all the tested organisms. Compounds **3a** and **3e** also showed moderate inhibitory activity. The results were compared with standard drugs as depicted in **Table 2**. The *in vitro* antifungal activity of the synthesized compounds **3a-f** was studied against *Cladosporium oxysporum* and *Aspergillus niger*. The results were compared with the standard drug nystatin. Compounds **3b** and **3f** showed good antifungal activity. Compounds **3a, 3c, 3d** and **3e** showed weak antifungal activity against tested fungal strains.

Table 2 *In vitro* antibacterial and antifungal activities of 3a-f

Compound	Zone of inhibition in diameter (mm)					
	Bs	**Sa**	**St**	**Ec**	**Co**	**An**
3a	11	---	---	10	08	---
3b	20	15	14	09	11	12
3c	14	12	---	09	08	07
3d	18	14	12	---	09	09
3e	14	11	10	---	---	08
3f	19	18	16	13	12	13
Streptomycin	21	18	14	15	---	----
Nystatin	---	---	---	---	15	16

Abbreviations: Bs-Bacillus subtilis, Sa-Staphylococcus aureus, St-Salmonella typhi, Ec-Escherichia coli, Co-Cladosporium oxysporum, An- Aspergillus niger.

Conclusions

In conclusion, diazotized derivatives of 2-aminoethyl piperazine compounds (**3a-f**) were synthesized in good yield, characterized by different spectral studies and their antimicrobial activities have been evaluated. Compounds **3b**, **3d** and **3f** demonstrated good antimicrobial activity against all the tested microbial strains. Readily available starting materials and simple synthesizing procedures make this method very attractive and convenient for the synthesis of various diazotized compounds.

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