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BIOACTIVITY AND CONFORMATIONAL STUDY OF NOVEL GLYCOL AZO DERIVATIVES

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ABSTRACT

Four novel derivatives of azo glycol derivatives **(9-12)** have been synthesized through multi-step synthetic scheme starting from glycerol. Compounds were also found to be active against gram-positive bacteria (*Streptococcus pneumoniae, Staphylococcus epidermidis* and *Bacillus pumilus*) and gram-negative bacteria (*Pseudomonus aeruginosa and E.coli*) as well as yeast (Candida albicans). In addition, urease inhibition activity of all compounds (**9-12**) is also reported. Most stable conformation and optimal dihedral angle of glycol moiety is also evaluated by computational method.

Keywords: Glycerol, Dye. Antimicrobial activity, Urease, Conformational analysis, Dihedral angle.

INTRODUCTION

Our work here included exploring the bioactive capabilities of the four new derivatives **(9-12).** The most stable conformation of the molecule and dihedral angle of diol groups with respect to each other and binding potential of these synthetic molecules with synthetic polar substrates are also calculated. Forcefield opimized molecular structure of **(9)** is shown in Fig. **1**. The most stable conformation of the molecule and dihedral angle of diol groups with respect to each other and binding potential of these synthetic molecules with synthetic polar substrates are also calculated.

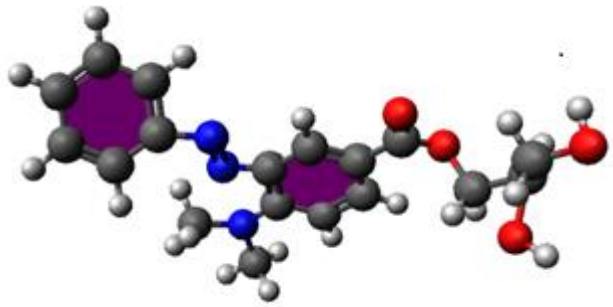
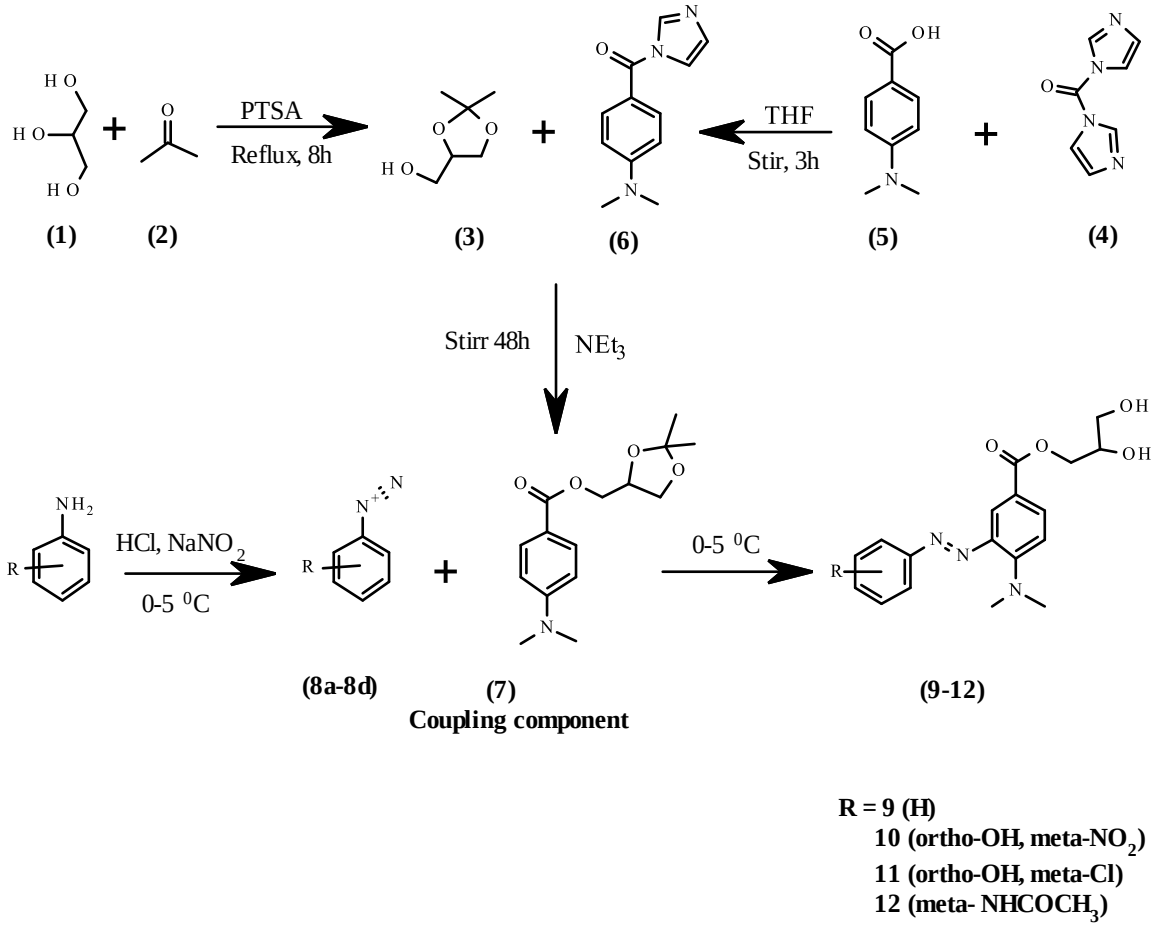


Fig. 1. Force field optimized molecular structure of Azo glycol derivative **(9).**

Synthesis route (Scheme-1) involved esterification of the isopropylidene glycerol **(3)** with 4 (dimethylamino)benzoic acid to form **(7)**. Compound **(7)** served as a coupling component for diazonium compound **(8a-8d)** to synthesize azo chromophores **(9-12)**.

Synthesized molecules **(9-12)** were found to be bioactive against bacteria and yeast as well. Many glycerol derivatives such as fatty acid glycerides have been reported as bioactive against gram-positive, gram-negative, yeast and protozoa. Examples include monolaurin and monocaprin against *Helicobacter pylori* (Petschow *et al*., 1996), *E. coli* (Kabara, 1978) *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* beta hemolytic and alpha hemolytic, *Corynebacterium*, *Giardia lamblia* (Zeinab *et al*., 2014) and *Candida* (Kabara *et al*., 1972). Azo chromophore itself shows antimicrobial activity against gram-positive bacteria (*staphylococcus aureus, streptococcus pyogenes*), gram-negative bacteria (*Pseudomonas aeruginosa*, *Proteus vulgaris* and *E. coli*) and yeast specie *Candida albicans* (Moanta and Radu, 2008; Moanta and Radu, 2009). Antibacterial and antifungal activity

are exhibited by azo schiff bases against *Bacillus subtilis* and some fungi including *Candida albicans*, *Cryptococcus neoformans* and *S Tricophyton mentagrophytes* (Jarrahpour *et al*., 2004).



Scheme 1. Synthetic route for compounds (**9-12**)

E.C.3.5.1.5) give end products CO_2 and NH_3 with approximately, 10^{14} times faster rate than non catalytic reaction (Mobley *et al*., 1995). High activity of urease enzyme in agriculture causes ammonia toxicity in environment and also takes away essential nutrients from plants (Ciurli *et al*., 2002). While in human body, Urease play primary role for many human diseases like ulcer, urolithiasis, pyelonephritis, ammonia and hepatic encephalopathy, hepatic coma, and urinary catheter encrustation (Amtul *et al*., 2002). Owing to these reasons, in recent year's urease inhibition studies have gained the attention of researchers. Related to the designed molecules **(9-10),** mono easters of glycerol have been reported as urease inhibitors and effectively used for inhibit the growth of *Helicobacter pylori* (Kajiwara, 2003; Loughlin, 2003).

MATERIAL AND METHODS

Preparation of Solution

Extract was dissolved in 6% dimethylsulfoxide (DMSO) to afford concentration of 100 mg/ml. Gentamycin was used as reference standard (positive control). 6% dimethylsulfoxide (DMSO) served as negative control.

Antibacterial Assay

The agar well diffusion method was used to determine antibacterial activity by plant extract and its fractions (Ahmed *et al*., 1998). In this procedure100 µL of inoculums (diluted to 10^6 CFU/ mL) of test culture was mixed with 20 mL of molten sterile tryptic soya agar. This mixture was poured in to pre-sterilized petri dishes under sterile condition. Plates were allowed to set at 4ºC for 30-40 minutes. Holes (6 mm diameter) were made in center of each seeded plates. 0.1 mL of test solution i.e. compound **(9-12)** were then filled in holes with two concentrations (0.1%

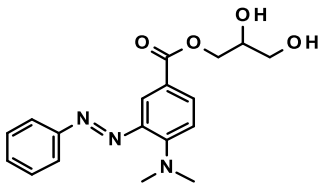
and 0.05%). Standard disc of antibiotic gentamycin (10μg) served as positive antibacterial control. DMSO was used as negative control. All plates were then incubated at 37ºC ±1ºC for 24 hours. Zone of inhibition around the wall was observed for evaluation of the antibacterial activity. Vernier Caliper was used to measure the diameter (in mm) of inhibition zone. Readings were made repeatedly to minimize test error.

Urease assay and inhibition

Reaction mixtures comprising of 25 μL of enzyme (Jack bean Urease) solution and 55 μL of buffers containing 100 mM urea were incubated with 5 μL of test compounds (1 mM concentration) at 30^{o}C for 15 min in 96-well plates. Urease activity was determined by measuring ammonia production using the indophenol method as described by Weatherburn (1967). Briefly, 45 μL each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 μL of alkali reagent (0.5% w/v NaOH and 0.1 % active chloride NaOCl) were added to each well. The increasing absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 μL. The results (change in absorbance per min) were processed by using SoftMax Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M $K_2HPO_4.3H_2O$, 1 mM EDTA and 0.01 M $LiCl_2$). Percentage inhibitions were calculated from the formula 100–($OD_{testwell}/OD_{control}$) x100. Thiourea was used as the standard inhibitor of urease.

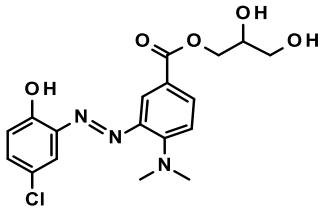
RESULTS AND DISCUSSION

Structure of all synthetic compounds (**9-12**) were same, the only difference is the presence of different substitutions on benzene ring as shown in Fig. **2**. Compound (**9**) has no substitution, while compound 12 has a substitution on *meta* position. Compounds (**10**) and (**11**) both ware di-substituted, they have -OH group on *para* position and electron withdrawing groups (Cl, NO_2) on *meta* position as shown in Fig. **2**.



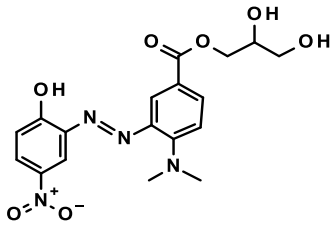
2,3-dihydroxypropyl 4-(dimethylamino)-3-(phenyldiazenyl)benzoate

(9)



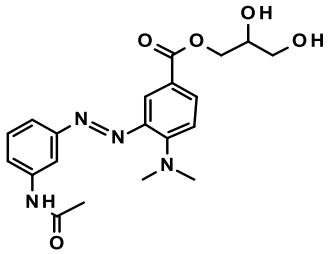
2,3-dihydroxypropyl3-((5-chloro-2-hydroxyphenyl)diazenyl)4 (dimethylamino)benzoate

(10)



2,3-dihydroxypropyl4-(dimethylamino)-3-((2-hydroxy-5-nitrophenyl)diazenyl) benzoate

(11)



2,3-dihydroxypropyl 3-((3-acetamidophenyl)diazenyl)-4-(dimethylamino)benzoate

(12)

Fig. 2. Molecular structure of compounds **(9-12).**

All synthesized compounds **(9-12)** were subjected to antimicrobial activity against gram-positive (*Streptococcus pneumoniae*, *Staphylococcus epidermidis* and *Bacillus pumilus*), gram-negative (*Pseudomonus aeruginosa and*

E.coli) and *yeast* (*Candida albican*). Many glycerol derivatives such as fatty acid glycerides have been reported as bioactive against gram-positive, gram-negative, yeast and protozoa. Examples include monolaurin and monocaprin against *Helicobacter pylori* (Petschow *et al*., 1996), *E. coli* (Kabara, 1978) *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus* beta hemolytic and alpha hemolytic, *Corynebacterium, Giardia lamblia* (Zeinab *et al*., 2014) and *Candida* (Kabara *et al*., 1972). Azo chromophore itself shows antimicrobial activity against gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes*), gram-negative bacteria (*Pseudomonas aeruginosa, Proteus vulgaris* and *E. coli*). There are also few examples of azo moiety activity against yeast species *Candida albicans* (Moanta and Radu, 2008; 2009). Antibacterial and antifungal activity is exhibited by azo schiff bases against *Bacillus subtilis* and some fungi including *Candida albicans*, *Cryptococcus neoformans* and *S Tricophyton mentagrophytes* (Jarrahpour *et al*., 2004).

In this research work, gentamycin (10 µg) served the role of standard while 0.1% and 0.05% concentrations of synthetic compounds **(9-12)** were used. According to the zone inhibition study, it was found that all compounds exhibited good inhibition activity towards gram-negative bacteria at 0.05% and 0.1% and compounds **(10)** and **(11)** were found to be the most active molecules. Both compounds (**10**) and (**11**) were di-substituted with OH group on *ortho* position and electron withdrawing groups on *meta* position, these molecules were found to be active against gram-negative bacteria. At 0.05% concentration, compound **(12)** did not show any activity against *E.coli* and compounds **(9)** and **(12)** were also found to be inactive against *Pseudomonus aeruginosa*. All four compounds (**9-12**) were also found to be active against *Candida albican* at both concentrations except compound **(12)** which is inactive at 0.05% concentration. Compound **(9)** and **(12)** were inactive at both concentrating against *Streptococcus pneumoniae,* while compound **(10)** showed high activity. Compound **(11)** was found highly active against other gram positive bacteria. Details of bioactivity of the synthesized molecules are given in Table **1**.

Urease enzyme carries Ni^{+2} in its organometallic structure. It promotes the hydrolysis of urea into constituents NH_3 and CO_2. There are many health related hazards that can directly be attributed to NH_3 and urease gives CO_2 and NH_3 with approximately 10^{14} times faster rate than non catalytic reaction (Mobley *et al*., 1995). High activity of urease enzyme in agriculture causes ammonia toxicity in environment and also takes away essential nutrients from plants (Ciurli *et al*., 2002). In human body, urease play primary role for many human diseases like ulcer, stoney concentrations (urolithiasis), pyelonephritis, hepatic encephalopathy, hepatic coma, and urinary catheter encrustation (Amtul *et al*., 2002). Owing to these reasons, in recent years urease inhibition studies have gained the attention of researchers. Desire for urease inhibition has led to synthesis of molecules known as urease enzyme inhibitors. Related to the designed molecules in this research work **(9-10)**, mono esters of glycerol have been reported as urease inhibitors and effectively used for inhibit the growth of *Helicobacter pylori* (Kajiwara, 2003; Loughlin, 2003).

All synthesized compounds **(9-10)** were also tested for inhibition activity against urease with thiourea serving as the standard inhibitor with LC_{50} value was (21.6 ± 0.12 mM). It was found that compounds **(9-10)** were weak to moderately active. The LC_{50} value range was (48.3 ± 0.44 mM to > 200 mM). Compound **(10)** was found to be the most active urease inhibitor. The LC_{50} values are listed in Table **2**.

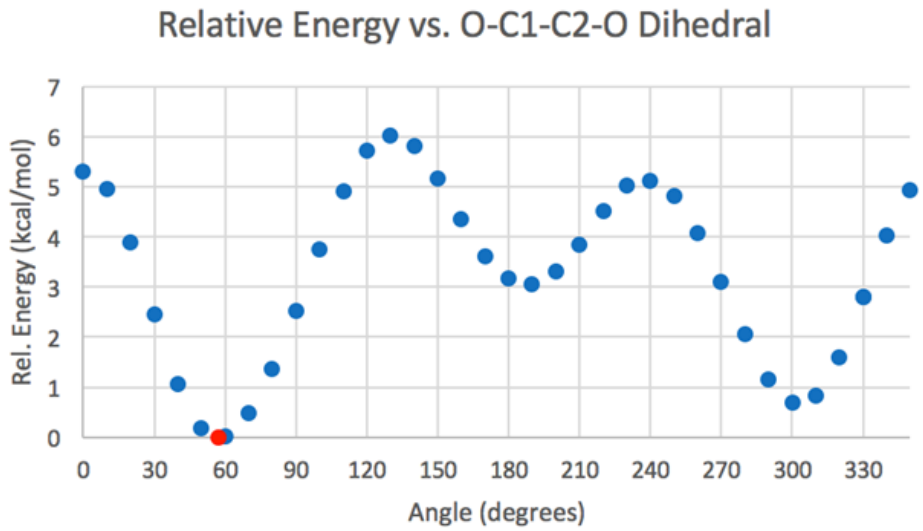


Fig. 3. Relaxed dihedral angle scan (shown in blue) and optimized structure (shown in red).

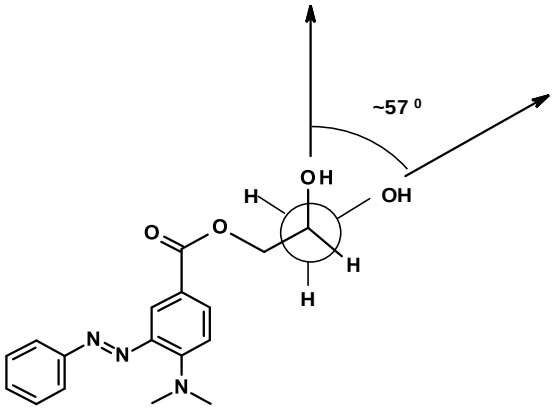


Fig. 4. Dihedral angle and Newman projection across C_1 and C_2

Table 1. Bioactivity of compounds **(9-12)**.

Zone of inhibition (mm)																		
	Yeast			***Gram-positive***									***Gram-negative***					
	Candida Albican			***Streptococcus pneumoniae***			***Staphylococcus epidermidis***			***Bacillus pumilus***			***Escherichia Coli***			***Pseudomonas aeruginosa***		
	mm			**mm**			**mm**			**mm**			**mm**			**mm**		
Conc. %	0.1	0.05	Std.	0.1	0.05	Std.	0.1	0.05	Std.	0.1	0.05	Std.	0.1	0.05	Std.	0.1	0.05	Std.
9	17.6	14.6	20	-	-	20	16	-	20	15.6	-	20	16	13.6	22	12.3	-	20
10	19	14.3	20	18.3	16.6	21	15	-	20	18.3	13.6	22	17.6	14.7	23.5	18	12.6	19.3
11	19	17.6	21	-	-	20	17.6	14.6	21	17.6	14.6	20	16	14.3	24	17.6	14.6	20
12	16	-	20	14	-	20	-	-	21	14	-	20	16.3	-	22	14	-	20

Table 2. Urease inhibition.

Sr. #	**Compound**	**Urease Inhibition (LC_{50} ± SEM μM)** [a]	**Percent Inhibition at 100 μM**
1	9	> 200 μM	15.5
2	10	48.3 ± 0.44	66.3
3	11	50.1 ± 0.19	64.7
4	12	89.3 ± 0.22	38.8
5	Thiourea [std.*]	21.6 ± 0.12	96.3

a = (Mean ± Standard error of mean), Std. = Standard deviation.

From the limited structure activity relationship, it was established that increase in no. of aryl substitutions increased the urease inhibition activity. Compound (**9**) has no substitution so it was the least active compound among others and have IC_{50} > 200 μM. In compound (**12**) acetanilide -$NHCOCH_3$ group is situated at *meta* position

and it showed almost half activity compared to compound (**10**). Compounds (**10** and **11**) were most active compounds and their IC_{50} values were (48.3 ± 0.44 µM) and (50.1 ± 0.19 µM) respectively. Compounds (**10** and **11**) were di-substituted which caused increase in the activity, both having -OH at *ortho* position. In compound (**10**) there is a single -Cl while in compound (**11**) a $–NO_2$ group was present at *meta* position with respect to the -OH group.

Computational and Conformational analysis

Computational analysis of compound **(9)** was performed in order to determine how its structural features are related to its binding to fabric and absorbance spectrum.

Conformational analysis was carried out, so that further computations could be performed on the most highly populated conformer. Using DFT at the B3LYP/6-31+G(d) level of theory, a relaxed scan was performed on the O-C1-C2-O dihedral angle in 10° increments. This showed that the total energy was lowest at an angle of 60° (Fig. **3**). This conformation was then fully optimized, leading to the finding that an angle of 57.2° gave the minimum energy.

Relative to this energy, the rotational barrier was slightly over 6 kcal/mol. Both glycerol hydroxyl groups were available for hydrogen bonding with acceptor groups on fiber chains. Dihedral angle of the vicinal diols is shown in Newman projection across C_1 and C_2 of the alkyl part of the bonded glycerol in Fig **4**.

Force field optimized molecule **(9)** is shown in Fig **5.** Diols of **(9)** are shown with dihedral angle of 57^o forming interchain H-bonds with polar protic site (R_2N-H) of polymeric substrate such as polyamide chains.

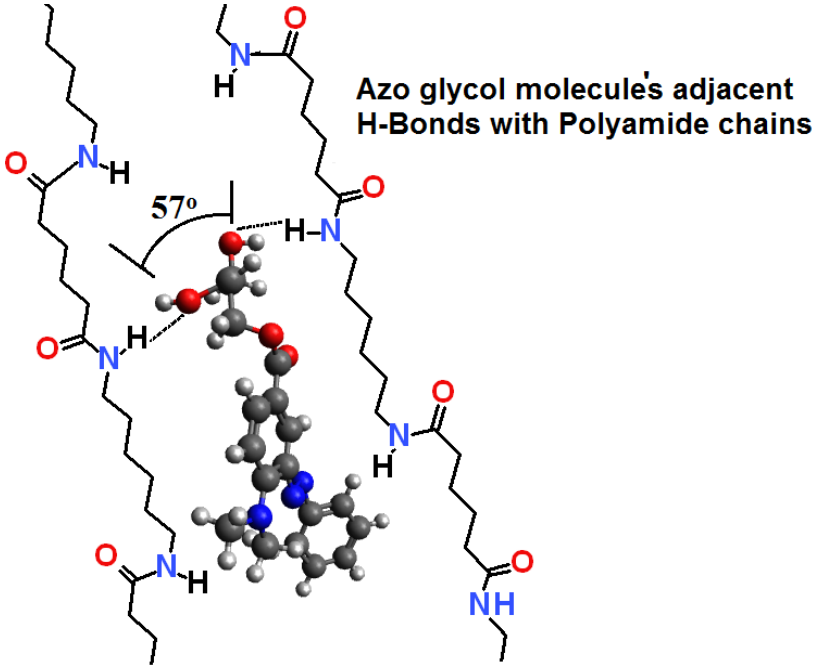


Fig. 5. Interchain H-bonds formed by diols.

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