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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL TOLUENESULFONYL DERIVATIVES OF PYRAZOLES ANNELATED WITH A POLYFUNCTIONAL CYCLOHEXANE RING

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Abstract. We report a new method for tosylation by the interaction of 6-hydroxy-3,6-dimethyl-4-R-5-acetyl-4,5,6,7-tetrahydroindazoles with toluene sulfochloride in boiling acetone in the presence of triethylamine for synthesis of two new toluenesulfonyl derivatives of pyrazoles annelated with polyfunctional cyclohexane ring. Structure of newly synthesized compounds was confirmed by elemental analysis and spectral data. Agar well diffusion assay was used to screen newly synthesized compounds against Gram-positive bacteria, Gram-negative bacteria and yeast. Test compounds showed moderate antibacterial activity and no antifungal activity. Gram-negative bacteria were found to be more sensitive as compared to Gram-positive bacteria.

Keywords: *Agar well diffusion, antimicrobial activity, cyclohexane, Pyrazoles, tosylation, toluene sulfochloride.*

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1. Introduction

Functionally substituted organic compounds are being extensively investigated as potential antimicrobial agents due to ever increasing antimicrobial resistance and shortage of new antimicrobial drugs. In this regard, functionally substituted cyclohexane derivatives are best available option (Shoaib & Ganbarov, 2019). Diacetyl (diethoxycarbonyl) substituted hydroxy cyclohexanones are very valuable building blocks for synthesis of organic compounds due to presence of dioxo compounds (acetylacetone, acetoacetate), aliphatic compounds and aromatic aldehydes (Ismiev *et al.*, 2016). As polycarbonyl compounds, these compounds are able to interact with nucleophilic reagents. Thus, these compounds have ability to enter into the different kinds of systems like condensation, heterocyclization etc (Hote & Lokhande, 2014; Dyachenko *et al.*, 2015; Sukach *et al.*, 2015; Maharramov *et al.*, 2016; Semenova *et al.*, 2019).

Reactions of diacetyl (dialkoxycarbonyl) substituted hydroxy cyclohexanones with hydrazine leading to the corresponding pyrazoles annelated with cyclohexane ring have been studied in detail in the literature (Maharramov *et al.*, 2011a; Maharramov *et al.*, 2011b; Semenova *et al.*, 2019). However, the transformations of the latter by introducing functional groups on the heterocycles have not been carried out. As a part of our ongoing research, we report fictionalization of 6-hydroxy-3,6-dimethyl-4-R-5-

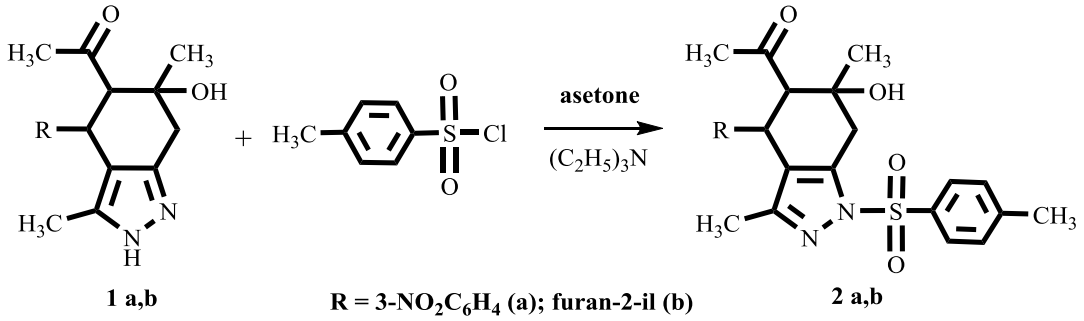
acetyl-4,5,6,7-tetrahydroindazoles (compounds **2a** and **2b**), and their interaction with toluene sulfochloride in boiling acetone in the presence of an equimolecular amount of triethylamine.

2. Materials and Methods

Synthesis of 5-acetyl-6-hydroxy-3,6-dimethyl-4-R-1-tosyl 4,5,6,7- tetrahydro-1H-indazoles (2a-2b)

Tosylation of the starting tetraindazole 5-acetyl-6-hydroxy-3,6-dimethyl-4-R-1-tosyl-4,5,6,7-tetrahydro-1H-indazoles led to synthesis of two new compounds; 5-acetyl -6-hydroxy-3,6-dimethyl-4-(3-nitrophenyl)-1-tosyl-4,5,6,7- tetra-hydro-1H-indazol (**2a**) and 5-acetyl -6-hydroxy-3,6-dimethyl-4-(furan-2-il)-1-tosyl-4,5,6,7-tetrahydro-1H-indazol (**2b**).

A solution of 5 mmol of reagent 1a-1b(for corresponding compounds **2a-2b**), 5 mmol (0.95 g) of toluenesulfonyl chloride and 5 mmol (0.50 g) of triethylamine in 20 ml of acetone was boiled for 8 hours. Resulting solution was cooled and 50 ml of cold water was added. After 24 hours, the precipitated powder was filtered and recrystallized from ethanol. Scheme 1 shows the synthesis of compounds **2a-2b**.



Scheme 1. Synthesis of compounds **2a-2b**

^1H and ^{13}C NMR spectra were recorded on a Bruker AC-300 instrument (300 MHz on 1H and 75 MHz nuclei at 13C cores) in a $(CD_3)_2SO$ and $CDCl_3$ solution, residual signals of the solvent were used as the standard. The melting points were determined on a Kofler's table. TLC monitored the purity of the resulting compounds on *Silufol UV-254* plates, eluent acetone-hexane 1:1, developer-iodine vapor, UV detector. Carlo Erba 1106 analyzer was used to perform elemental analysis for C, H, and N.

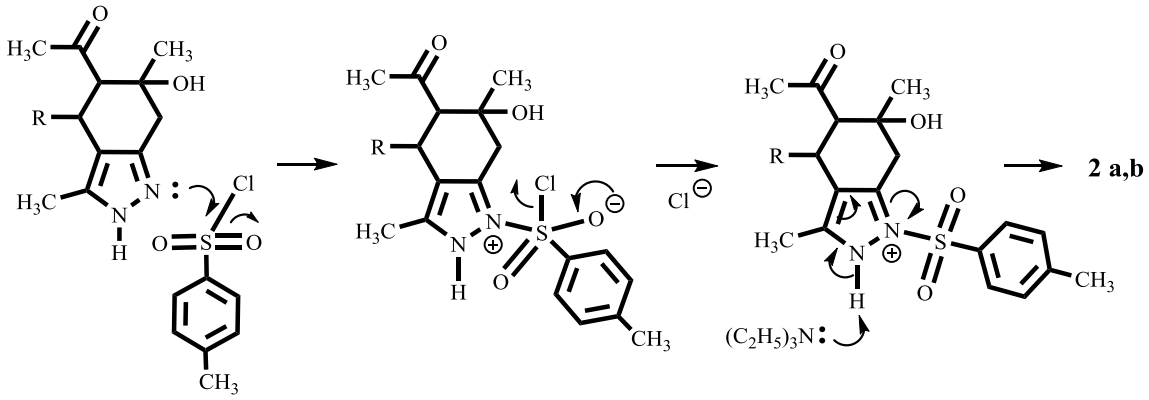
Determination of antimicrobial activity

Newly synthesized compounds were screened for antimicrobial activity using standard agar well diffusion assay (Balouiri *et al*., 2016). Mueller-Hinton agar was used to determine *in vitro* antibacterial properties were evaluated against *Escherichia coli BDU-12, Klebsiella pneumoniae BDU-44, Acinetobacter baumannii BDU-32, Pseudomonas aeruginosa BDU-49, Staphylococcus aureus BDU-23, Bacillus Subtilis BDU-50, Bacillus mesentericus BDU-51* and *Bacillus megaterium BDU-N20*. *In vitro* antifungal properties were determined against *Candida tropicalis BDU LK30, Candida pelliculosa BDU KT55* and *Candida pseudotropicalis BDU MA88* using sabouraud dextrose agar. All the test cultures were obtained from our own collection at Department of Microbiology, Baku State University. Test compounds were dissolved in Dimethyl

sulphoxide (DMSO) and three different concentrations (0.3%, 0.1% and 0.05%) were evaluated. All the experiments were performed three tomes and DMSO was used as control.

3. Results and Discussions

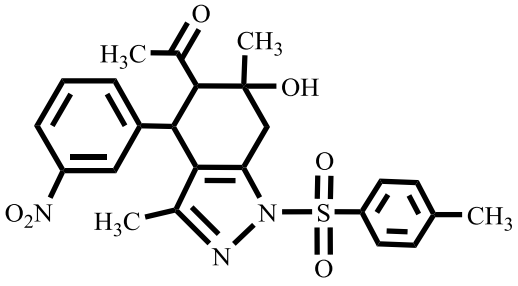
The products of tosylation of the starting tetraindazole; 5-acetyl-6-hydroxy-3,6-dimethyl-4-R-1-tosyl-4,5,6,7-tetrahydro-1H-indazoles (2a-2b) were isolated in the yields of 64-69 %.The scheme 2 shows the proposed mechanism for the formation of compounds (**2a-2b**). The pyridine nitrogen of the heterocycle is involved in the substitution reaction. In contrast to pyrrole nitrogen, a pair of pyridine nitrogen electrons is free from conjugation; for this reason, pyridine nitrogen is more nucleophilic and it carries out the nucleophilic substitution of the chlorine atom.



Scheme 2. Proposed mechanism for formation of compounds **2a-2b**

5-acetyl-6-hydroxy-3,6-dimethyl-4-(3-nitrophenyl)-1-tosyl-4,5,6,7-tetra-hydro-1H-indazol (2a):

The synthesized compound is colorless solid in the yield of 69%. Melting point was found to be 146^0C. **^{1}H NMR spectrum (300 MHz, $(CD_3)_2SO$), δ, ppm**:1.40 (s, 3H, CH_3); 1.66 (s, 3H, CH_3); 1.87 (s, 3H, CH_3); 2,40 (s,3H, CH_3); 2,98 (d, 1H, CH); 3.21 (dd,2H,CH_2); 4.48 (d,1H, CH); 5.04 (s, 1H, OH); 7.45-8.11(m, 9H, CH_{arom}). **^{13}C NMR spectrum (75 MHz, $(CD_3)_2SO$), δC, ppm:**7.90; 17.21; 23.75; 29.08; 30.43; 33.28; 54.69; 66.78; 104.03; 106.28; 125.88; 130.54; 135.94; 138.12; 157.80; 210.05. **Found, %:** C-59.71; H-5.30; N-8.78; **$C_{24}H_{25}N_3O_6$ S, Calculated, %:** C-59.62; H-5.21; N-8.69.

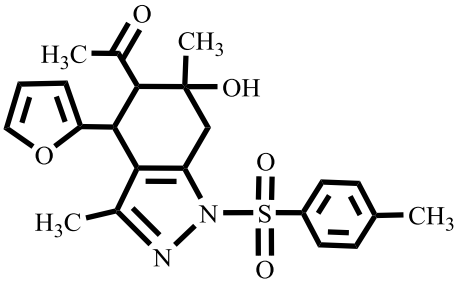


2 a

Figure 1. Structure of compound **2a**

5-acetyl -6-hydroxy-3,6-dimethyl-4-(furan-2-il)-1-tosyl-4,5,6,7-tetrahydro-1H-indazol (2b):

The synthesized compound is colorless solid in the yield of 64%. Melting point was found to be 142^0C.**^{1}H NMR spectrum (300 MHz, $CDCl_3$), δ, ppm**:1.40 (s, 3H, CH_3); 1.66 (s, 3H, CH_3); 1.87 (s, 3H, CH_3); 2,40 (s,3H, CH_3); 2,96 (d, 1H, CH); 3.21 (dd,2H,CH_2); 3.63 (s, 1H, OH); 4.21 (d,1H, CH); 6.37(s,1H, CH_{furil}); 6.66 (d,1H, CH_{furil});7.31(d, 2H, CH_{arom});7.41 (s, 1H, CH_{furil}); 7.85(d, 2H, CH_{arom}). **^{13}C NMR spectrum (75 MHz, $(CD_3)_2SO$), δC, ppm:**7.90; 17.21; 23.75; 29.08; 30.43; 33.28; 54.69; 66.78; 104.03; 106.28; 112.74; 123.18; 125.88; 130.54; 135.94; 138.12; 140.82; 147.51; 157.80; 211.12.**Found, %:** C-61.72; H-5.75; N-6.62; **$C_{22}H_{24}N_2O_5S$, Calculated, %:** C-61.67; H-5.65; N-6.54.



2 b

Figure 2. Structure of compound **2b**

Table 1. Average diameter of inhibition zone (mm) at 0.3% concentration

Test Culture	**2a**	**2b**	**DMSO**
Escherichia coli	24.7±0.3	19±0.3	-
Klebsiella pneumoniae	-	-	-
Acinetobacter baumannii	13±0.6	13±0	-
Pseudomonas aeruginosa	-	17.3±0.3	-
Staphylococcus aureus	17.3±1	14.3±0.3	-
Bacillus Subtilis	-	-	-
Bacillus megaterium	13±0.6	13±0	-
Bacillus mesentericus	14.3±0.3	-	-
Candida tropicalis	-	-	-
Candida pelliculosa	-	-	-
Candida pseudotropicalis	-	-	-

(-): Inactivity

Antimicrobial activity

The antimicrobial activity of test compounds (**2a-2b**) was evaluated against four Gram-negative bacteria, four Gram-positive bacteria and three yeast species. DMSO was used as control and showed no activity against any of tested cultures. Overall results of antimicrobial activity are listed in table 1. Tested compounds showed variable antimicrobial activity at concentration of 0.3%. All the compounds were inactive at concentration of 0.1% and 0.05%. Tested compounds showed better antibacterial activity against Gram-negative bacteria as compared to Gram-positive bacteria. Compound **2b** was stronger antibacterial agent as compared to compound **2a**. This is

due to fact that presence of furan-2-il imparts stronger antibacterial properties as compared to 3-nitrophenyl. For compound **2b**, *Acinetobacter baumannii* and *Escherichia coli* were found to be most susceptible (zone of inhibition 24.7mm and 19mm, respectively) among Gram-negative bacteria. *Klebsiella pneumoniae* was completely resistant to both the test compounds. Among the Gram-positive bacteria, *Staphylococcus aureus* was most sensitive test culture with zone of inhibition 17.3 and 14.3 mm for compounds **2a** and **2b** respectively. It is interesting to note that test compounds were inactive against all the tested species of fungi. Inactivity against fungi is attributed to complex cell wall structure of the fungi.

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