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1,3-DIPOLAR CYCLOADDITION OF DIAZOALKANES WITH MONOSUBSTITUTED ALKENES AND A,B-UNSATURATED ENONES AND EVALUATION OF THEIR ANTITUBERCULAR ACTIVITY

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Abstract
The regiospecific synthesis of pyrazolines has been accomplished through the 1,3-dipolar cycloaddition of diazoalkanes. The reaction of 2-diazopropane **1** with monosubstituted alkenes **2** has been studied. It led to pyrazolines derivatives **3**. In addition the reactivity of *p*-toluldiazomethane **4** towards α,β-unsaturated enones **5** is reported, affording Δ^2-pyrazolines. Products were screened for their antimycobacterial activity against *Mycobacterium tuberculosis H37Rv* strain .

Keywords: 1,3-Dipolar cycloaddition; Diazoalkanes; Pyrazolines; Regioselectivity, antimycobacterial.

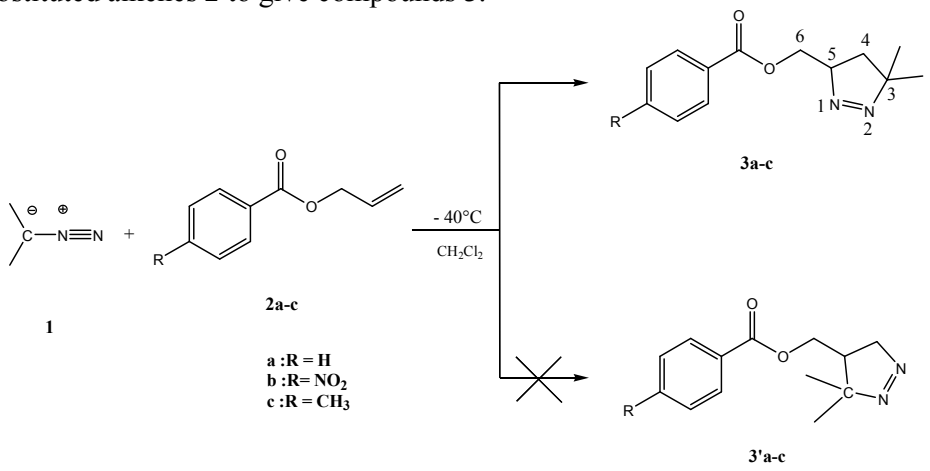
1. Introduction

The 1,3-dipolar cycloaddition also known as the Huisgen cycloaddition[I-IV] have been recognized as one of the most efficient reactions for the construction of five-membred heterocyclic compounds[V-VII]. 1,3-Dipolar reactions of alkenes with diazoalkanes have been used to prepare pyrazolines[VIII]. The chemistry of these compounds has generated intensive scientific interest due to their biological activities such as antibacterial, antifungal, ansecticidal, anti-inflammatory, etc[IX-XIII].
Our group has a current interest in the synthesis of pyrazolines derivatives based on 1,3-dipolar cycloaddition of 2-diazopropane (DAP) to alkenes. In continuation of this theme, we synthesized new pyrazolines. Furthermore, an impressive effort has been devoted to the synthetic application of the cycloaddition of aryldiazomethanes to α,β-unsaturated carbonyls to give other pyrazolines.

2. Results and discussion

At low temperature (-40°C), Δ^1-pyrazolines **3** have been previously prepared by cycloaddition of 2-diazopropane **1** to alkenes **2**[XIV-XV]. It is conceivable that the cycloaddition

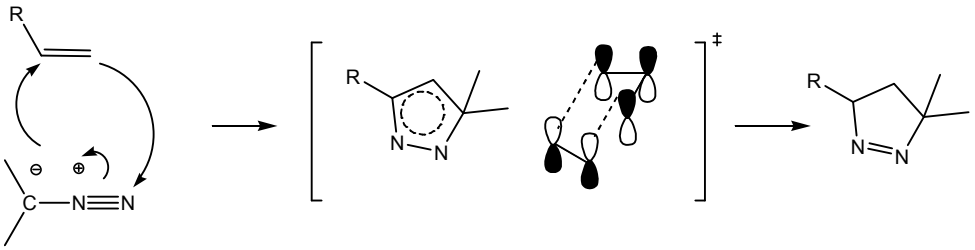
starts by the attack of the carbanion of DAP **1** on the C=C double bond of the monosubstituted alkenes **2** to give compounds **3**.



Scheme 1

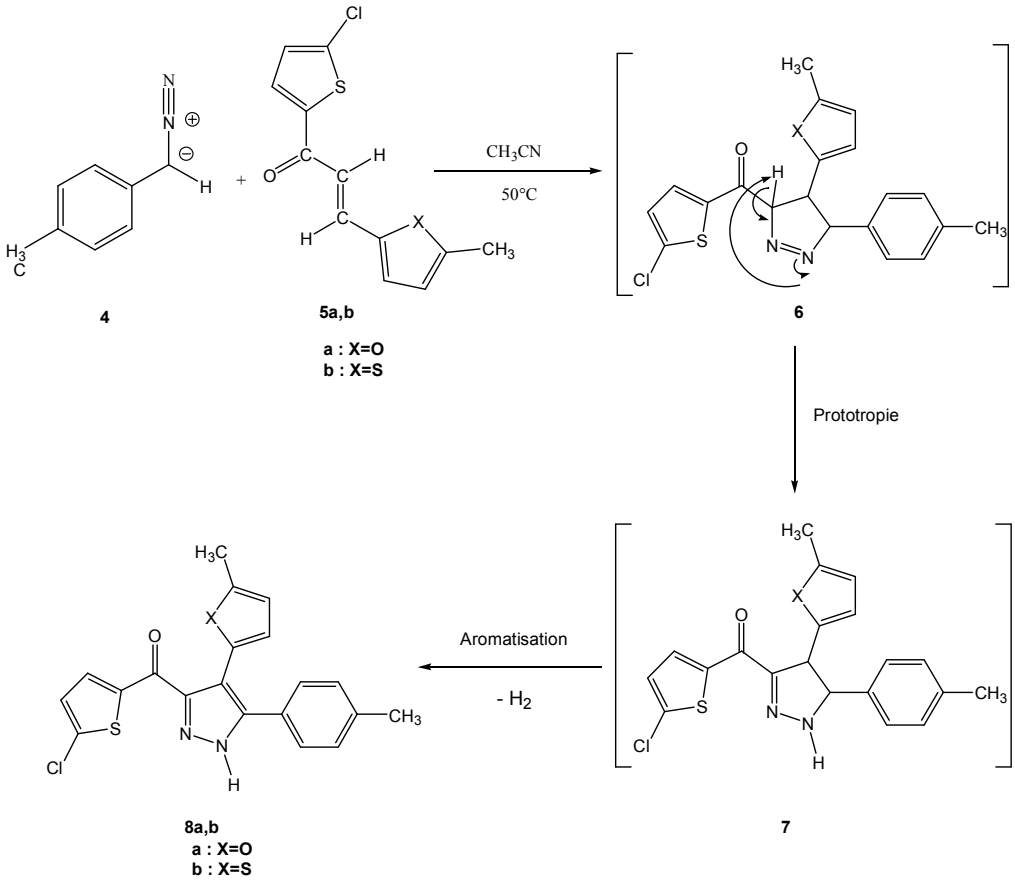
During this reaction, it can theoretically form two regioisomer **3** and **3'**. In our case we obtained only compounds **3** in good yields (70-85 %) without any secondary product (**Scheme 1**). The multiplet near 4.8 ppm observed in all ^{1}H-NMR spectra of cycloadducts corresponds to the proton H_5 of **3** or H_4 of **3'**, the H_4 is less deshielded than H_5 [XVI-XVII]. Indeed, the chemical shift of proton H_5 indicates that it is deshielded because of its proximity to a nitrogen atom (**Scheme 1**).

The 2-diazopropane react with the dipolarophile in a concerted, often asynchronous, and symmetry-allowed $_\pi4_s + _\pi2_s$ fashion through a thermal six-electron Huckel aromatic transition state (**Scheme 2**).



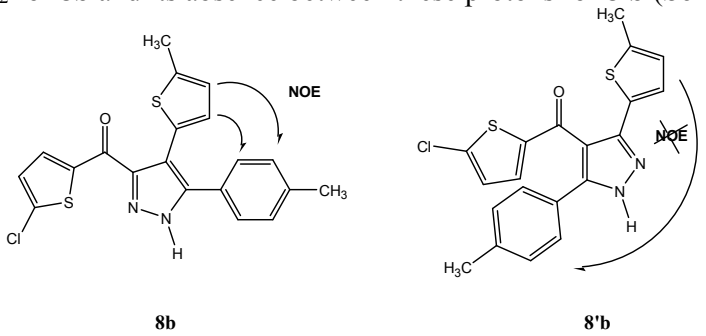
Scheme 2

The 1,3-dipolar cycloaddition reactions between *p*-toluldiazomethane **4** and α,β-unsaturated enones such as **5a-b** at 50 °C in acetonitrile form relatively unstable Δ^1-pyrazolines as the initial cycloadducts that undergo a 1,3-proton migration and aromatization[XVIII-XIX] to give the thermodynamically more stable Δ^2-pyrazoline derivatives (**Scheme 3**).



Scheme 3

The 1,3-dipolar cycloaddition of *p*-toluldiazomethane **4** is, in each case, regiospecific. The structure elucidation for regioisomers **8a** and **8b** was achieved with the aid of 2D-NMR techniques (NOESY), wherein an interaction was observed among the aryl group with protons H_1 and H_2 for **8b** and its absence between these protons for **8'b** (**Scheme 4**).

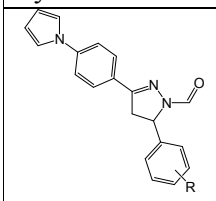


Scheme 4

All the target molecules (**3a-c**) were screened against *M. tuberculosis H37RV* (ATCC27294) using Agar dilution method. Their antimycobacterial activity was evaluated in terms of minimum inhibitory concentration (MIC) values. The MIC values in lg/mL of **3a-c** along with those of standard drugs for comparison. The MIC values of the compounds are in the range 3.125–25 lg/mL. It is evident that among two compounds, **3a** and **3c** show potent anti-tubercular activity with MIC of 3.125±0.056 lg/mL. The MIC of these two compounds is

comparable with that of the standard drug, ciprofloxacin and pyrrolyl pyrazoline **4**[XX]. Compound **3b** showed moderate inhibition activity with MIC of 12.5 lg/mL.
There was no statistically significant difference between the synthesized substances and the reference drug.

Table 1. MICs results for ciprofloxacin, pyrrolyl pyrazoline **4** and pyrazolines **3a-c** using the Alamar Blue (MABA) technique.

Compounds	**MIC (g/mL)**
ciprofloxacin	3.96 ± 0.067
Pyrazoline 3a	3.125 ± 0.056
Pyrazoline 3b	12.5 ± 0.24
Pyrazoline 3c	3.125 ± 0.056
 R = p-Cl, 3-OCH_3, 3-Br, 4-$CH(CH_3)_2$ **4**	3.125-25

Data are expressed as mean ± standard deviation (n = 3) and different letters show significant difference at the 5% level in Duncan's test ($p < 0.05$).

3. Conclusion

In conclusion, our studies have revealed that the 1,3-dipolar cycloaddition of diazoalcanes with various electron deficient olefins gives pyrazoline derivatives with complete regioselectivity. Also, none of the active molecules is toxic to a normal cell line. Hence, these compounds with significant *anti-TB* activity could serve as antitubercular agents.

4. Experimental procedure

Caution: Diazoalkanes are toxic and should be used in an efficient fume hood.

4.1. General

Flash chromatography was performed using silica gel Merck 60 (particle size 0.040–0.063 mm). All anhydrous reactions were performed under nitrogen using anhydrous solvents. NMR spectra were obtained on a Bruker AC 300 spectrometer operating at 300 MHz for ^1H and at 75.64 MHz for ^{13}C. Melting points were determined on a Buchi-510 capillary melting point apparatus. Chemical shifts are given in parts per million relative to tetramethylsilane (TMS) and the coupling constants *J* are given in Hertz. The spectra were recorded in $CDCl_3$ as solvent at room temperature. Elemental analysis was recorded on a PERKIN–ELMER 240B microanalyzer. Mass spectra were recorded on a Finnigan LCQ DECA XP plus.

4.2. 1,3-Dipolar cycloaddition of 2-diazopropane 1 with monosubstituted alkenes 2

To a solution of dipolarophiles **2a-c** (300 mg) in 100 mL of CH_2Cl_2, cooled at 40°C, was added portionwise 2.6 M of freshly prepared 2-diazopropane. The reaction was kept at the same temperature during 2 h. The solvent was removed and chromatography (SiO_2; ethyl acetate/petroleum ether, 8:2) to afford compounds **3a-c**.

(4,5-dihydro-5,5-dimethyl-3H-pyrazol-3-yl)methyl benzoate 3a

Yield = 72%. M.p = 131-132 °C [ethanol] (white crystals). IR (KBr) υmax/cm^{-1}: 1630 (N=N); 1730 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.24 (dd, $J_{H4a-H4b}$ = 12.6 Hz, J_{H4a-H5} = 8.7 Hz, 1H, H_{4a}), 1.27 (s, 3H, CH_3) 1.47 (s, 3H, CH_3), , 1.71 (dd, $J_{H4b-H4a}$ = 12.6 Hz, J_{H4b-H5} = 8.7 Hz, 1H, H_{4b}), 4.77 (dd, $J_{H6a-H4b}$ = 11.4 Hz, J_{H6a-H5} = 5.1 Hz, 1H, H_{6a}), 5.69 (dd, $J_{H6a-H6b}$ = 11.4 Hz, J_{H6b-H5} = 3.9 Hz, 1H, H_{6b}) 4.91 (m, 1H, H_5), 7.14, 7.42 (m, 5H, H_{arom}). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.4 (CH_3), 22.5 (CH_3), 30.3 (C_4), 60.4 (C_6), 81.2 (C_3), 91.0 (C_5), 131.4-126.6 (C_{arom}), 164.3 (C=O). Elemental analysis: $C_{13}H_{16}N_2O_2$ requires C, 67.22; H, 6.94; N, 12.06; found C 67.23, H 6.97, N 12.01%.

(4,5-dihydro-5,5-dimethyl-3H-pyrazol-3-yl)methyl 4-nitrobenzoate 3b

Yield = 68%. M.p = 128-129 °C [ethanol] (white crystals). IR (KBr) υmax/cm^{-1}: 1635 (N=N); 1740 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25 (dd, $J_{H4a-H4b}$ = 12.6 Hz, J_{H4a-H5} = 8.7 Hz, 1H, H_{4a}), 1.28 (s, 3H, CH_3) 1.55 (s, 3H, CH_3), 1.76 (dd, $J_{H4b-H4a}$ = 12.6 Hz, J_{H4b-H5} = 8.7 Hz, 1H, H_{4b}), 4.76 (dd, $J_{H6a-H4b}$ = 11.4 Hz, J_{H6a-H5} = 5.1 Hz, 1H, H_{6a}), 4.82 (m, 1H, H_5), 4.95 (dd, $J_{H6a-H6b}$ = 11.4 Hz, J_{H6b-H5} = 3.9 Hz, 1H, H_{6b}) 8.15- 8.35 (AA'BB', $J_{AA'BB'}$ = 9 Hz, 4H, H_{arom}). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 24.4 (CH_3), 26.4 (CH_3), 33.2 (C_4), 64.6 (C_6), 85.7 (C_3), 89.7 (C_5), 123.1-150.2 (C_{arom}), 163.9 (C=O). Elemental analysis: $C_{13}H_{15}N_3O_4$ requires C, 56.31; H, 5.45; N, 15.15; found C 56.33, H 5.40, N 15.12%.

(4,5-dihydro-5,5-dimethyl-3H-pyrazol-3-yl)methyl 4-methylbenzoate 3c

Yield = 85%. M.p = 153-154 °C [ethanol] (white crystals). IR (KBr) υmax/cm^{-1}: 1630 (N=N); 1730 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.22 (s, 3H, CH_3), 1.25 (dd, $J_{H4a-H4b}$ = 12.6 Hz, J_{H4a-H5} = 8.7 Hz, 1H, H_{4a}), 1.55 (s, 3H, CH_3), 1.79 (dd, $J_{H4b-H4a}$ = 12.6 Hz, J_{H4b-H5} = 8.7 Hz, 1H, H_{4b}), 2.10 (s, 3H, CH_3), 4.75 (dd, $J_{H6a-H4b}$ = 11.4 Hz, J_{H6a-H5} = 5.1 Hz, 1H, H_{6a}), 4.92 (m, 1H, H_5), 5.51 (dd, $J_{H6a-H6b}$ = 11.4 Hz, J_{H6b-H5} = 3.9 Hz, 1H, H_{6b}) 6.59-7.67 (AA'BB', $J_{AA'BB'}$ = 7.8 Hz, 4H, H_{arom}). ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 20.2 (CH_3), 23.1 (CH_3), 26.4 (CH_3), 39.9 (C_4), 61.9 (C_6), 85.1 (C_3), 92.3 (C_5), 123.2-138.7 (C_{arom}), 164.6 (C=O). Elemental analysis: $C_{14}H_{18}N_2O_2$ requires C, 68.27; H, 7.37; N, 11.37; found C 68.30, H 7.40, N 11.36%.

4.2. 1,3-dipolar cycloaddition of p-toluldiazomethane 4 with α,β-unsaturated enones 5a-b

A mixture of 1 eq. of *p*-tolyl tosylhydrazone, quatalitique amount of NaOH (5M) in 20 mL of acetonitrile was stirred at 50°C. We add at once 200 mg of the enone **5**. A TLC control being performed on the mixture to regular time intervals during the reaction. At the end of this reaction, the acetonitrile is removed on a rotary evaporator and the product is purified by column chromatography (elution: 8 hexane / 2 ethyl acetate).

(5-chlorothiophen-2-yl)(4-(5-methylfuran-2-yl)-5-p-tolyl-1Hpyrazol-3-yl) methanone 8a

Yield = 40%. M.p = 154-155 ° C (yellow crystals). IR (KBr) υmax/cm^{-1}: 1640 (C=N); 1730 (C=O); 3300 (N–H). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.39 (s, 3H, CH_3) 2.47 (s, 3H, CH_3), 5.32 (s, 1H, NH), 6.65 (d, J_{H2-H1} = 3.3 Hz, 1H, H_2), 6.84 (d, J_{H1-H2} = 3.3 Hz, 1H, H_1), 6.94 (d, J_{H4-H3} = 4.2 Hz, 1H, H_4), 7.19, 7.33 (AA'BB ', $J_{AA'BB'}$ = 8.1 Hz, 4H, H_{arom}), 7.93 (d, J_{H4-H3} = 4.2 Hz, 1H, H_3).^{13}C NMR (75.5MHz, $CDCl_3$) δ 15.3 (CH_3), 21.3 (CH_3), 140.9-125.2 (C_{arom}), 178.2 (C=O). Elemental analysis: $C_{20}H_{15}ClN_2O_2S$ requires C, 62.74; H, 3.95; N, 7.32; found C 62.71, H 3.98, N 7.35%.

(5-chlorothiophen-2-yl)(4-(5-methylthiophen-2-yl)-5-p-tolyl-1Hpyrazol-3-yl) methanone 8b

Yield = 45%. M.p = 186-187 ° C (yellow crystals).). IR (KBr) υmax/cm^{-1}: 1645 (C=N); 1740 (C=O); 3300 (N–H). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.27 (s, 3H, CH_3) 2.38 (s, 3H, CH_3), 4.45 (s, 1H, NH), 6.56 (d, J_{H2-H1} = 3.3 Hz, 1H, H_2), 6.75 (d, J_{H1-H2} = 3.3 Hz, 1H, H_1), 6.86 (d, J_{H4-H3} = 3.9 Hz, 1H, H_4), 7.10-7.23 (AA'BB ', $J_{AA'BB'}$ = 8.1 Hz, 4H, H_{arom}), 7.86 (d, J_{H4-H3} = 3.9 Hz, 1H, H_3).^{13}C NMR (75.5MHz, $CDCl_3$) δ 15.3 (CH_3), 21.4 (CH_3), 125.2-140.9

(C_{arom}), 178.3 (C= O). Elemental analysis: $C_{20}H_{15}ClN_2OS_2$ requires C, 60.21; H, 3.79; N, 7.02; found C 60.30, H 3.81, N 6.98%.

4.3. Determination of antimycobacterial activity

The microplate Alamar blue assay (MABA) was used to measure the minimal inhibitory concentration (MIC) for the tested compounds (minimum concentration necessary to inhibit 90% growth of *M. tuberculosis* $H_{37}Rv$ ATCC 27294)[XXI]. In a sterile 96-well microplate was added 200 µL of distilled water in each well of the outer-perimeter, to avoid water evaporation during incubation. The test compounds (pyrazolines **3a-c** and ciprofloxacin) were diluted in DMSO to obtain solutions, and thereafter, were diluted in Middlebrook 7H9 to obtain variable concentrations of the compounds, with starting concentration of 250 g/mL. The *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) strain was cultivated in 7H9 broth at 37 °C until reaching the turbidity equivalent to McFarland 1 scale. The culture was diluted 25 times, and then 100 µL of bacterial suspension was inoculated in each well containing the compound solutions. The microplates were sealed with parafilm and incubated at 37 °C for 6 days, when Alamar Blue solution was added to the control wells containing the mycobacterial strain. The plates were reincubated for 24 h, when the reading was performed. The blue color in the wells was defined as negative bacterial growth, while the pink color development was defined as positive growth. The microplates showing wells with violet color were reincubated for 24 h, and if color change to pink was detected, the growth was considered positive. If the color was maintained, the growth is negative.

4.4. Statistical analysis

Statistical analysis was performed based on one-way analysis of variance (ANOVA) and Duncan's mean comparison tests at 5% significant level using IBM SPSS Statistics 22 (IBM Corporation, Armonk, NY, USA)

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