Synthesis and Characterization of 5-Substituted 1H-Tetrazoles in the Presence of Nano-TiCl\textsubscript{4}–SiO\textsubscript{2}

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1. Introduction

Tetrazoles are an increasingly popular functionality with wide ranging applications. There is considerable interest in the medicinal and biological applications of tetrazoles,\textsuperscript{1,2} due to their reported anti-allergic and anti-asthmatic,\textsuperscript{3,4} antiviral and anti-inflammatory,\textsuperscript{5,6} anti-neoplastic,\textsuperscript{7} cognition disorder activities.\textsuperscript{8} Tetrazoles are also applied as ligands in coordination chemistry\textsuperscript{9} as explosives and rocket propellants and they are used as isosteric replacements for carboxylic acids in drug design.\textsuperscript{10}

Tetrazole derivatives are used as antibiotics and optically active tetrazole-containing antifungal preparations of azole type was reported. There is always a need for new and effective antifungal and antibacterial agents with broad-spectrum activities. It was decided to develop this interest by ascertaining the molecules features essential for activity and utilizing them to develop a new class of potential drugs.

The conventional method of synthesizing tetrazoles is via the addition of azide ions to organic nitriles or cyanamides.\textsuperscript{11} Later Sharpless and co-workers reported an innovative and safe procedure for the synthesis of tetrazoles by the addition of sodium azide to nitriles using stoichiometric amounts of Zn(II) salts in water.\textsuperscript{12} Pizzo and co-workers efficiently synthesized tetrazoles by the addition of TMSN\textsubscript{3} to organic nitriles using 10 mol% TBAF as catalyst.\textsuperscript{13} Several syntheses of 5-substituted tetrazoles were reported through the [2+3] cycloaddition of nitriles to Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} or TMSN\textsubscript{3} in the presence of catalysts such as Montmorillonite K-10 Clay,\textsuperscript{14} Cu\textsubscript{2}O,\textsuperscript{15} FeCl\textsubscript{3}–SiO\textsubscript{2},\textsuperscript{16} Zeolite and sulfated zirconia,\textsuperscript{17} BaWO\textsubscript{4},\textsuperscript{18} Nano-TiO\textsubscript{2},\textsuperscript{19} MoO\textsubscript{3}–SiO\textsubscript{2}\textsuperscript{20}

However, each method has certain restrictions with regard to scope and reaction conditions; for example, costs of synthesis, unrecoverable catalysts, strong acidic conditions, long reaction times, low yields, difficult work up and harsh reaction conditions. To avoid these limitations, our studies attempted the development of more efficient methods accompanied with higher yields for the synthesis of tetrazoles in the presence of nano-TiCl\textsubscript{4}–SiO\textsubscript{2} and to evaluate their antibacterial and antifungal properties.

2. Experimental

2.1. General

The chemicals were purchased from Merck and used without any additional purification. The products were characterized by FT-IR (ATR), \textsuperscript{1}H-NMR, and a comparison of their physical properties with those reported in the literature. FT-IR (ATR) spectra were acquired on a Bruker, Eqinox 55 spectrometer. A Bruker (DRX-400 Avance) nmr was used to record the \textsuperscript{1}H NMR spectra. The X-ray diffraction (XRD) patterns of materials were obtained by employing a Philips Xpert MPD diffractometer (The instrument is a PAN alytical X'Pert Pro MPD, powered by a Philips PW3040/60 X-ray generator and fitted with an X'Celerator detector. Diffraction data is acquired by exposing powder samples to Cu-K\alpha X-ray radiation, which has a characteristic wavelength of 1.5418 Å. X-rays were generated from a Cu anode supplied with 40 kV and a current of 40 mA), equipped with a Cu Kz anode (\lambda = 1.54 Å) in the 2θ range from 10 to 80 °. The SEM of nanoparticles determined with VEGA/TESCAN scanning electron microscope (model: Mira 3-XMU) and TEM photograph was prepared by Leo 912AB OMEGA microscope. The thermal gravimetric analysis (TGA) was performed with a ‘NETZSCHE TG 209 F1 Iris’ instrument, Spectrophotometer (UV/Vis biotek model UVIKONXL), Vortex mixer (Heidolph, d-9112 schwabach), Retsch Mixer Mill MM400, 100-240 VAC, 50/60 Hz, Sonication, Bandelin SONOPULS, HD3220 homogenizer, TT13 probe, Microwave, KENWOOD K25MW11 Microwave Oven, Melting points were determined with a Thermo Scientific Electrothermal digital apparatus (Thermo Fisher Scientific Inc.).

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2.2. General Method for the Synthesis of Tetrazole Derivatives:

Nano-TiCl$_4$SiO$_2$ (0.1 g) was added to a mixture of benzonitrile (1 mmol), sodium azide (2 mmol) in DMF (5 mL) at reflux for 2 h. After completion of reaction (as monitored by TLC), the mixture was allowed to cool to room temperature, the catalyst was removed by filtration. Then by adding ice water and 4N HCl (5 mL) to the residue, a white solid was obtained. This was then washed with cold chloroform. This simple procedure yielded pure tetrazole with good yields.

All the products are known and were identified by comparison of their physical and spectral data with those of authentic samples.

2.3. Determination of Antifungal Activities

2.3.1. Microorganisms

The antifungal activities of the synthetic compounds against eighteen American Type Culture Collection (ATCC) strains of fungi, including Candida albicans (ATCC 10261, 562, 1949, 1912, 5982, 2730), C. tropicalis (ATCC 750), C. krusei (ATCC 6258), C. glabrata (ATCC 90030, 6144, 2175, 863), C. parapsilosis (ATCC 4344), C. dubliniensis (ATCC 8500, 7987, 8501), Cryptococcus neoformance (ATCC 9011), Aspergillus flavus (ATCC 64025), A. fumigatus (ATCC 14110) and A. clavatus (CBS 514.65) were determined as the lowest concentration yielding no more than 50% of the microbes in the initial inoculums.

The antibacterial activities of the EO against standard species of S. aureus (ATCC 700698), Escherichia coli (ATCC 25912), E. faecalis (ATCC11700), P. aeruginosa (ATCC 27853), and B. cereus (11778) were also determined in this study. The susceptibility of all clinical isolates of bacteria and fungi against select antibiotics were examined by microdilution and disk diffusion methods. The susceptibility of all isolates of fungi against select antibiotics was examined by microdilution and disk diffusion methods. Oxacillin and ciprofloxacin were used as reference drugs for bacteria and fluconazole as a reference drug for fungi.

2.3.2. Determination of Minimum Inhibitory Concentration

MICs were determined using broth microdilution method recommended by the CLSI with some modifications.$^{22–24}$ Briefly, for determination of antifungal activities against fungi, serial dilutions of the synthetic compounds (0.25 to 256.0 µg mL$^{-1}$) were prepared in 96-well microtitre plates using RPMI-1640 media (Sigma, St. Louis, USA) buffered with MOPS (Sigma, St. Louis, USA). To determine the antibacterial activities, serial dilutions of the synthetic compounds (0.25 to 256.0 µg mL$^{-1}$) were prepared in Muller-Hinton Broth media (Merck, Darmstadt, Germany). Test fungi or bacteria strains were suspended in the media and the cell densities were adjusted to 0.5 McFarland standards at 530 nm wavelength using a spectrophotometric method (this yields stock suspension of 1-5 × 10$^6$ cells mL$^{-1}$ for yeast and 1-1.5 × 10$^8$ cells mL$^{-1}$ for bacteria). One hundred microlitre of the working inoculums was added to the microlitre plates which were incubated in a humid atmosphere at 30 °C for 24–48 h (fungi) or at 37 °C for 24 h (bacteria). Two hundred microlitre of the uninoculated medium was included as a sterility control (blank). In addition, growth controls (medium with inoculums but without drugs) were also included. The growth in each well was compared with that of the growth control well. MICs were visually determined and defined as the lowest concentration of the synthetic compounds or drugs produced no visible growth. Each experiment was performed in triplicate. Each experiment was performed in triplicate. In addition, media from wells with fungi showing no visible growth were further cultured on Sabouraud Dextrose Agar (Merck, Darmstadt, Germany) and from wells with bacteria showing no visible growth on Muller-Hinton agar (Merck, Darmstadt, Germany) to determine the minimum fungicidal concentration (MFC) and minimum bactericidal concentration (MBC). MBCs and MFCs were determined as the lowest concentration yielding no more than four colonies, which corresponds to a mortality of 99.9 % of the microbes in the initial inoculums.

3. Results and Discussion

3.1. Optimization of the Reaction Conditions

We investigated the synthesis of 5-substituted 1H-tetrazole in the presence of nano-TiCl$_4$SiO$_2$. However our study is the first to characterize and report the IR spectra of nano-SiO$_2$, nano-TiCl$_4$. In literature, the XRD pattern of nano-SiO$_2$ has a strong peak in the 2θ value of 21.8024° with full width at half maximum (FWHM) equal to 0.1771. By contrast, our data showed that the particle size of nano-TiCl$_4$SiO$_2$ in TEM and SEM patterns are calculated as 14–20 nm and 37–41 nm, respectively (Fig. 1). Thermal gravimetric analysis (TGA) pattern of nano-TiCl$_4$SiO$_2$ was detected from 25.43 to 513.43 °C. The catalyst is stable below 173.43 °C and only 2.98 % of its weight was reduced at 173.43 °C, which is related to removal of catalyst moisture.

Initially, in an effort to develop better reaction conditions, different solvents and conditions were screened for the preparation of 5-substituted 1H-tetrazole from the reaction of benzonitrile with sodium azide in the presence of nano-TiCl$_4$SiO$_2$ and the results are summarized in Table 1. Among the different solvents screened, DMF gave the product in good yield at reflux tempera-
ture (Table 1, entry 1). Other solvents such as toluene gave the desired products in low yield (Table 1, entry 3). On the other hand, the product was formed in 60% when the reaction was performed under solvent-free conditions (Table 1, entry 5).

According to the obtained data, the best conditions were at reflux temperature in DMF using 0.2 g of TiCl₄·SiO₂ or 0.1 g of nano-TiCl₄·SiO₂ (Table 1, entries 1, 13).

Once the scope of the reaction condition was established, the reusability of the catalyst was examined. After performing the reaction, the catalyst was separated, washed with acetone, dried and re-used up to three times in reaction (Table 1, entries 15, 16). The catalyst was reusable although a gradual decline was observed in its activity.

Also, we tried to reaction of benzonitrile with sodium azide using mixer mill, ultrasonic and microwave, but these conditions didn’t give the product in good yield (Table 1, entries 6, 7 and 8).

Under the optimized reaction conditions, we chose a variety of structurally divergent benzonitriles to explore the scope and generality of the nano-TiCl₄·SiO₂ promoted [2+3] cycloaddition reaction to form 5-substituted 1H-tetrazoles and the results are presented in Table 2. It seems that the nature of the substituents on the aromatic ring of benzonitriles exert different influences. It is of interest to note that electron-withdrawing groups that increase the polarity of the cyanide group inductively (Table 2, entry 4-nitro) give higher yields of products compared to the electron-donating groups (Table 2, entry 3-hydroxy). Different halogen substituted benzonitriles, such as 4-chlorobenzonitrile and 4-bromobenzonitrile reacted smoothly and gave the desired products in decent yields (Table 2, entries 5, 6).

Heteroaromatic nitriles such as 2-pyridinecarbonitrile gave the corresponding tetrazoles in shorter reaction times with excellent yields (Table 2, entry 2). Unfortunately the present method is not amenable for aliphatic nitriles.

Although some of the previous studies reported promising antimicrobial activities for tetrazole derivatives, none of the synthetic compounds in this study exhibited antifungal activity against the examined fungi at the tested concentrations which is similar to the study of Bekhit et al. Moreover, the examined compounds failed to inhibit the growth of the tested Gram-positive and Gram-negative bacteria at the concentration up to 256 µg mL⁻¹.

Besides to antimicrobial activities, some of the tetrazole derivatives exhibit biological properties including anti-inflammatory, inhibition of cyclo-oxygenase, antidiabetic, anticonvulsant, and anticancer activities. Hence, further studies are required to investigate the other potential biological activities of these synthetic compounds.

4. Conclusion
We have demonstrated a simple method for the preparation of 5-substituted 1H-tetrazole derivatives using nano-TiCl₄·SiO₂ as eco-friendly and efficient catalyst in a one-pot procedure. Short reaction times, high yields, a clean process, easy work-up and green conditions are advantages of this protocol. Since some of the tetrazole derivatives previously showed antibacterial and antifungal activities, further studies are still required for the design and synthesis of more novel tetrazole derivatives with better antimicrobial activities by this simple green method.

Supplementary material
The IR, ¹³C and ¹H NMR spectra of the novel 5-substituted...
Table 2  Synthesis of 5-substituted 1H-tetrazole derivatives at reflux/DMF in the presence of nano-TiCl₄-SiO₂.

| Entry  | R (Ar)     | Products | Yield/%b | Time/h | MP/°C | Found | Reported {[Ref]} |
|--------|------------|----------|----------|--------|-------|-------|-----------------|
| 1      | Ph         | ![ArCN + NaN₃ → TiCl₄-SiO₂ Reflux/DMF](image) | 95       | 1.5    | 214–216 | 213–214[14] |
| 2      | 4-CH₂Ph    | ![image](image) | 84       | 2.5    | 251–152 | 250–251[15] |
| 3      | 4-OMePh    | ![image](image) | 81       | 3.5    | 232–233 | 231–232[14] |
| 4      | 3-NO₂Ph    | ![image](image) | 92       | 1.5    | 217–220 | 219[16] |
| 5      | 4-ClPh     | ![image](image) | 91       | 2      | 261–262 | 262–263[15] |
| 6      | 4-BrPh     | ![image](image) | 90       | 2      | 267–269 | 267–269[15] |
| 7      | PhCH₂      | ![image](image) | 78       | 3      | 122–123 | 123–123[14] |
| 8      | 4-OMePhCH₂ | ![image](image) | 81       | 3.5    | 232–233 | 231–232[15] |
| 9      | 4-CIPh₂    | ![image](image) | 84       | 3      | 260–261 | – |
| 10     | (Ph)CH     | ![image](image) | 88       | 2      | 164–166 | 164–165[15] |
| 11     | 3,4-diClIPh₂ | ![image](image) | 84       | 2      | 128–130 | – |
| 12     | Py         | ![image](image) | 92       | 1.2    | 218–219 | – |

a  Reaction conditions: nitrile (1 mmol), NaN₃ (2 mmol), (0.1 g), nano-TiCl₄-SiO₂-DMF (5 ml) at reflux.

b  Isolated yields.

1H-tetrazole derivatives (Table 2, compounds 9, 11 and 12) are presented in the online supplement.

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