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1,3-Dipolar Cycloaddition Reactions of *N*-Acetylazido-2-chlorophenothiazine

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Abstract: In the present paper, we reported the synthesis of *N*-acetylazido-2-chlorophenothiazine and its 1,3-dipolar cycloaddition reactions with alkynes and alkenes as dipolarophiles. Thus, we obtained a new series of 1,2,3-triazole and Δ^2-1,2,3-triazoline derivatives linked at the side functionalised chain of the phenothiazine ring heterocycle.

Keywords: Phenothiazine; Azide; 1,2,3-triazole; Δ^2-1,2,3-triazoline; cycloaddition.

Introduction

The phenothiazine derivatives were the first agents to be successfully used for the treatment of psychosis. At present, they are widely known for their clinical use as antipsychotic, antiemetic, antimicrobial, anti-inflammatory, antitubercular, antitumour antihypertensive, and antihistaminic agents, among other therapeutic uses.[1-8] In the recent years, phenothiazines proved to act as human cholinesterase inhibitors, and on many occasions, they have been characterized as multidrug resistance (MDR) reversal agents.[9-11] Also it has been reported that some

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phenothiazines inhibit intracellular replication of viruses including human immunodeficiency viruses (HIV).[12] Furthermore, some of these derivatives have been reported to exhibit significant anticancer activities, which has triggered a great interest in designing and synthesising new phenothiazines in order to explore their anticancer activities.[13,14]

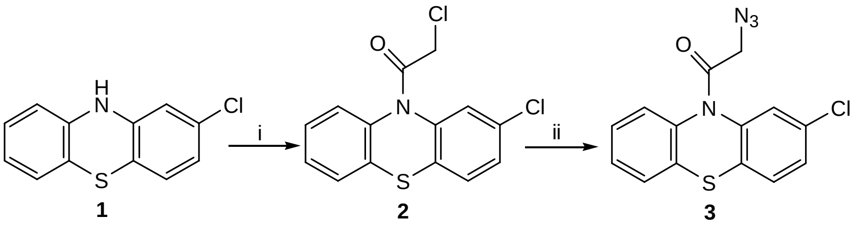
Azides represent a class of extremely important compounds, which are characterised by significant biological and industrial applications.[15,16] One of the most valuable synthetic applications of azides is the preparation of 1,2,3-triaza compounds, via 1,3- dipolar cycloaddition.[17] Consequently, azides react with olefinic dipolarophiles to create Δ^2-1,2,3- triazolines and with acetilenic dipolarophiles to originate 1,2,3-triazoles. In contrast to the latter, which are easily isolated, the triazolines might have different stabilities depending on their substituents, and thus more difficult to isolate. Most of triazolines are unstable and decompose by loosing nitrogen, affording the formation of products such as aziridines, diazo compounds, piperidines and pirrolidines.[18]

The chemistry of 1,2,3-triazoles has drawn much attention, mainly because of their wide range of potential applications. For example, these compounds have been used as fungicides, herbicides, light stabilizers, fluorescent whiteners, optical brightening agents as well as corrosion retardants.[15] Derivatives thereof have also been found to possess a large spectrum of biological activities such as antitumor, anti-inflammation, antimicrobial, antifungal, antithrombotic, antiplatelet and antiviral.[16,19] Moreover, some of the 1,2,3-triazole derivatives linked to carbohydrate moieties have shown antimicrobial and cytostatic activities, among other important pharmacological applications.[15,20]

Results and discussions

In the present paper, the side chain commonly found in phenothiazine drugs[21] has been modified by reducing its length from three to two carbon atoms. Also, the exocyclic tertiary nitrogen from the newly grafted 1,2,3-triazoles was modified.

For this purpose, we have introduced and functionalized a two carbon chain by acylating the 2-chlorophenothiazine **1** with chloroacetyl chloride, yielding the *N*-(2-chloroacetyl)-2-chlorophenothiazine[22] **2**. Treatment of this reactive halogenated derivative with sodium azide, in the presence of tetrabutylammonium bromide as phase-transfer catalyst, resulted in the formation of the *N*-azidoacetyl-2-chlorophenothiazine **3** (Scheme 1):



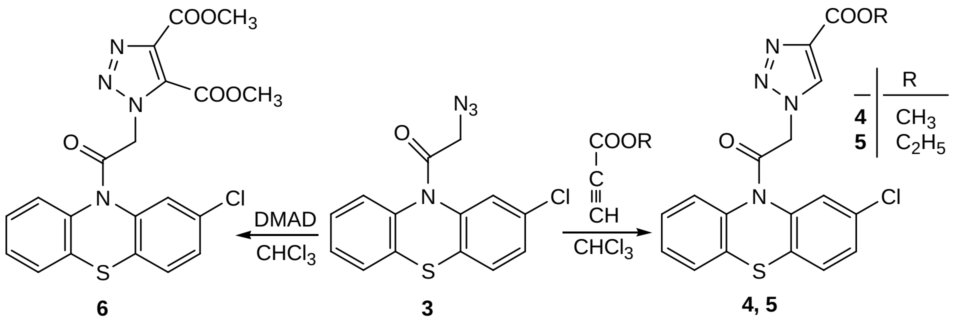
Scheme 1

Reagents and conditions: (i) $ClCH_2COCl$, toluene, reflux;
(ii) NaN_3, TBAB, CH_3Cl, H_2O, r.t.

The structure of newly organic azide has been proven by the spectroscopic analysis: IR, ^1H NMR, ^{13}C NMR and MS. More exactly, the IR spectrum shows the presence of the azide peak at 2105 cm^{-1}. In the ^1H and ^{13}C NMR spectra, the methylene appeared at δ 3.97 (2H) ppm and respectively at δ 50.9 ppm. The expected molecular weight is 316.6, value confirmed by the appearance of ion peaks at *m/z* 316 (M^+) in the ESI mass spectrum.

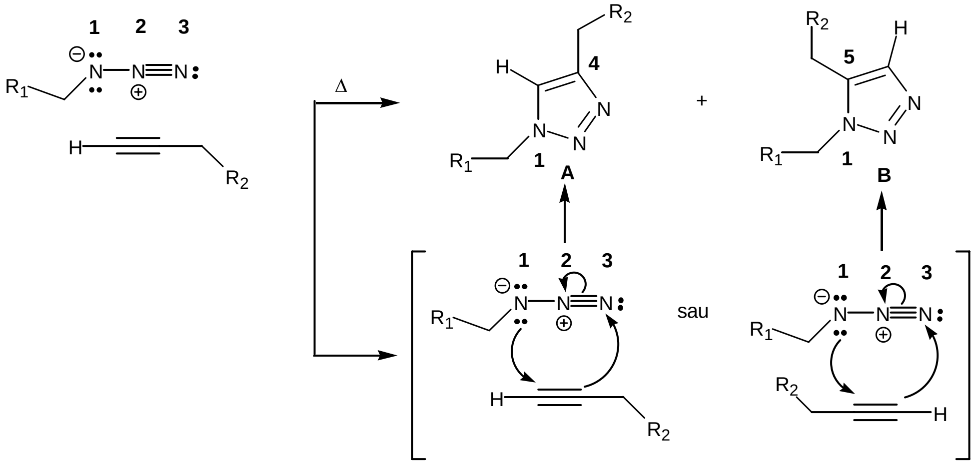
Based on the excellent pharmacological activity associated with 1,2,3-triazole derivatives, we sought to synthesize 1,2,3-triazolyphenothiazines by extending 1,3-cycloaddition reaction to the azide intermediate **3**.

For example, reflux of the *N*-azidoacetyl-2-chlorophenothiazine **3** with DMAD in chloroform for 48 h yielded a novel heterocyclic compound named dimethyl 1-[2-oxo-2-(10H-2-chlorophenothiazin-10-yl)ethyl]-1H-1,2,3-triazole-4,5-dicarboxylate in 80 per cent yield (Scheme 2):



Scheme 2

The thermal 1,3-dipolar cycloaddition of the organic azide **3** with terminal alkynes affords the formation of both 1,4- and 1,5-regioisomers **A** and **B**. It is believed that the close activation energies for the concerted process are responsible for the formation of both isomers (Scheme 3).[23]

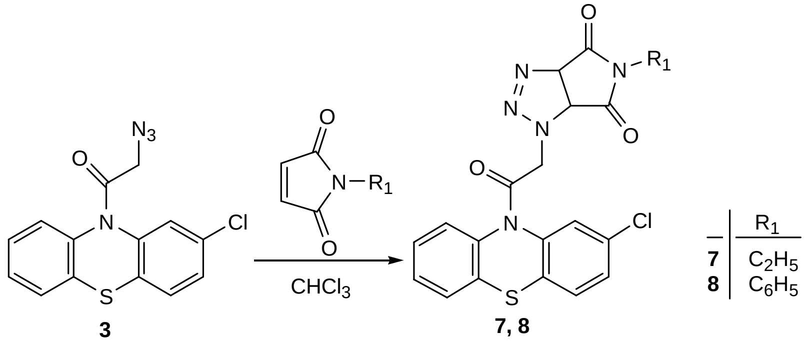


Scheme 3

In our case, the thermal reaction of 1,3-dipolar cycloaddition between the *N*-azidoacetyl-2-chlorophenothiazine **3** and methyl as well as ethyl propiolate appears to be completely regiospecific, as the only compound obtained was the 1,4-isomer in a high yield (Scheme 2).

The formation of the 1*H*-1,2,3-triazole system in compounds **4** and **5** is supported by the significant chemical shifts of 5-H observed in the ^{1}H-NMR spectra. Consequently, the signal for 5-H in compound **4** appears at 8.26 ppm, while for the compounds **5** appears at 8.30 ppm. Also, the recorded bi-dimensional spectra (^{1}H–^{1}HCOSY, ^{13}C–^{1}H HMQC and ^{13}C–^{1}H HMBC) confirm the proposed structures.

Azide **3** reacted with *N*-maleinimide in refluxing chloroform for 72 hours to give exclusively triazoline **7**, **8** (Scheme 4).



Scheme 4

The structures of the Δ^2-1,2,3- triazolines resulting from the above reaction were confirmed by both, the chemical shifts and coupling constants of the protons (experimental section).

Experimental

Melting points were obtained on a Mel-Temp II apparatus. IR spectra were recorded on a Bruker Tensor 27 instrument. NMR spectra were recorded on a Bruker DPX-300 spectrometer. Chemical shifts are reported in ppm downfield from TMS. Mass spectra were recorded on a Finnigan MAT 90X spectrometer.

N-(chloroacetyl)-2-Chlorophenothizine (**2**)

To a solution of 2-chlorophenothizine (2.3 g, 10 mmol) in toluene (40 mL), chloroacetyl chloride (1.7 g, 15 mmol) was added in portions. The mixture was refluxed for five hours. On cooling, the reaction product was separated and recrystallised from ethanol (25 mL) giving colourless crystals; yield 2 g (65%).

Mp 118-119 °C. IR (ATR): 1678, 1575, 1402, 1331, 1236, 1129, 1094, 803, 737 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 4.18$ (d, 2H, CH_2), 7.19-7.64

(m, 7 H, 7 x CH_{ar}). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 41.6$ (CH_2), 126.2, 127.0, 127.4, 127.6, 127.7, 128.2, 128.5, 132.9, 137.3, 138.8 (CH and C), 165.3 (NCO). MS (ESI): $m/z = 310$ (M^+).

N-azidoacetyl-2-Chlorophenothiazine (**3**)

To a solution of *N*-(chloroacetyl)-2-chlorophenothiazine (3.1 g, 10 mmol) in chloroform (30 mL) were added sodium azide (0.72 g, 11 mmol), water (15 mL), and tetrabutylammonium bromide (0.03 g, 0.10 mmol). The reaction mixture was vigorously stirred at room temperature for 36 h and then separated. The organic layer was washed with water (3 x 20 mL), dried off (Na_2SO_4), and concentrated in vacuum. The residue was triturated with ethanol and the resulting solid recrystallized from ethanol (30 mL) to give **3** as colourless crystals; yield 2.7 g (85%).

Mp 101-102 °C. IR (ATR): 2105, 1681, 1458, 1366, 1274, 1178, 818, 746 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): $\delta = 3.97$ (d, 2H, CH_2), 7.20-7.59 (m, 7 H, 7 x CH_{ar}). ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 50.9$ (CH_2), 126.4, 127.1, 127.5, 127.6, 127.8, 128.4, 128.6, 133.0, 136.9, 138.5 (CH and C), 166.6 (NCO). MS (ESI): $m/z = 316$ (M^+).

Methyl 1-[2-oxo-2-(10H-2-chlorophenothiazin-10-yl)ethyl]-1H-1,2,3-triazole-4-carboxylate (**4**)*; General Procedure*

A solution of *N*-azidoacetyl-2-chlorophenothiazine (3.2 g, 10 mmol) and methyl propiolate (1 g, 12 mmol) in chloroform (40 mL) was heated under reflux for 48 h. The reaction was monitored by TLC for completion. Evaporation of the solvent yielded solids that were recrystallised from ethanol. Colourless crystals; yield 3.2 g (80%).

Mp 174-176 °C. IR (ATR): 3108, 1726, 1694, 1453, 1227, 1049, 811, 761. ^{1}H NMR ($CDCl_3$): δ = 3.43 (s, 3H, OCH_3), 5.14 (bs, 1H, CH_2), 5.48 (bs, 1H, CH_2), 7.3-7.72 (m, 7H, 7 x CH_{ar}), 8.26 (s, 1H, $CH_{triazole}$). ^{13}C NMR ($CDCl_3$): δ = 51.0 (OCH_3), 51.6 (CH_2), 126.9, 127.1, 127.3, 127.7, 128.2, 133.6, 138.5 (CH and C), 163.6 (NCO), 164.7 ($COOCH_3$). MS (ESI): *m/z* = 400 (M^+).

Ethyl 1-[2-oxo-2-(10H-2-chlorophenothiazin-10-yl)ethyl]-1H-1,2,3-triazole-4-carboxylate (**5**):

Yield 70%. Mp 182-184 °C. IR (ATR): 3113, 1711, 1687, 1457, 1386, 1223, 1053, 808, 759. ^{1}H NMR ($CDCl_3$): δ = 1.38 (t, ^{3}J = 7.1 Hz, 3H, CH_3), 4.40 (q, ^{3}J = 7.1 Hz, 2H, OCH_2), 5.24 (bs, 1H, CH_2), 5.53 (bs, 1H, CH_2), 7.25-7.63 (m, 7H, 7 x CH_{ar}), 8.30 (s, 1H, $CH_{triazole}$). ^{13}C NMR ($CDCl_3$): δ = 14.2 (CH_3), 51.6 (CH_2), 61.2 (OCH_2), 126.5, 127.1, 127.8, 128.2, 128.6, 129.5, 133.1, 138.1, 140.3 (CH and C), 160.4 (NCO), 163.7 ($COOCH_2CH_3$). MS (ESI): *m/z* = 414 (M^+).

Dimethyl 1-[2-oxo-2-(10H-2-chlorophenothiazin-10-yl)ethyl]-1H-1,2,3-triazole-4,5-dicarboxylate (**6**)

Yield 75%. Mp 165-166 °C. IR (ATR): 1719, 1691, 1557, 1459, 1308, 1277, 1058, 842, 758. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.96 (s, 6H, 2 x CH_3), 5.48 (bs, 1H, CH_2), 5.77 (bs, 1H, CH_2), 7.24-7.62 (m, 7H, 7 x CH_{ar}). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 52.6 (CH_2), 52.7 (OCH_3), 53.4 (OCH_3), 127.8, 128.2, 128.7, 128.1, 128.4, 130.0, 133.5, 137.0, 138.3 (CH and C), 159. 1 ($COOCH_3$), 160.2 ($COOCH_3$), 166.6 (NCO). MS (ESI): *m/z* = 458 (M^+).

1-[2-(10H-2-Chlorophenothiazin-10-yl)-2-oxoethyl]-5-ethyl-3a,6a-cis-1H-pyrrolo[3,4-d]-1,2,3-triazole-4,6-dione (**7**)*; General Procedure*

A solution of *N*-azidoacetyl-2-chlorophenothiazine (3.2 g, 10 mmol) and *N*-ethylmaleinimide (1.25 g, 10 mmol) in chloroform (30 mL) was heated under reflux for 72 h. The solvent was evaporated and the residue purified by column chromatography on silica gel with ethyl acetate: hexane 1:1. Colourless crystals; yield 3.5 g (80%).
Mp 172-175 °C. IR (ATR): 1703, 1691, 1456, 1398, 1222, 1142, 1093, 996, 758. ^{1}H NMR ($CDCl_3$): δ = 1.1 (t, ^3J = 7.2 Hz, 3H, CH_3), 3.51 (q, ^3J = 7.2 Hz, 2H, NCH_2), 4.48 (d, ^3J = 10.8 Hz, 1H, CH), 4.96 (bs, 1H, CH_2), 5.18 (bs, 1H, CH_2), 5.62 (d, ^3J = 10.8 Hz, 1H, CH), 7.22-7.59 (m, 7H, 7 x CH_{ar}). ^{13}C NMR ($CDCl_3$): δ = 12.5 (CH_3), 34.3 (NCH_2), 50.0 (CH_2), 57.4 (CH), 83.0 (CH), 126.6, 127.2, 127.6, 127.8, 128.4, 128.8, 132.9, 136.7, 138.4 (CH and C), 166.6 (NCO), 170.0 ($COOCH_3$), 172.2 ($COOCH_3$). MS (ESI): *m/z* = 441 (M^+).

1-[2-(10H-2-Chlorophenothiazin-10-yl)-2-oxoethyl]-5-phenyl-3a,6a-cis-1H-pyrrolo[3,4-d]-1,2,3-triazole-4,6-dione (**8**)

Yield 85%. Mp 183-184 °C. IR (ATR): 1717, 1697, 1459, 1386, 1241, 1199, 1094, 812, 758, 690. ^{1}H NMR (DMSO-d_6): δ = 4.54 (d, ^3J = 10.8 Hz, 1H, CH), 5.08 (bs, 2H, CH_2), 5.80 (d, ^3J = 10.8 Hz, 1H, CH), 7.13-7.77 (m, 7H, 7 x CH_{ar}). ^{13}C NMR (DMSO-d_6): δ = 57.0 (CH_2), 57.8 (CH), 82.7 (CH), 126.5, 127.7, 127.9, 128.0, 128.7, 131.4, 132.3, 137.3 (CH and C), 167.2 (NCO), 170.3 ($COOCH_3$), 171.6 ($COOCH_3$). MS (ESI): *m/z* = 489 (M^+).

Conclusions

Using *N*-azidoacetyl-2-chlorophenothiazine, a new series of three phenothiazin-1,2,3-triazoles and two phenothiazin-Δ^2-1,2,3- triazolines was obtained and their structures were confirmed by both, analytical and spectral methods (IR, ^{1}H-NMR, ^{13}C-NMR and MS).

We expect that all of the newly obtained compounds possess the well known biological activities of phenothiazine derivatives as well as some potentially new pharmacological effects.

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