# SYNTHESIS AND SPASMOLYTIC ACTIVITY OF MESOIONIC 1,4-DIPHENYL-5-(5-NITRO-2-FURANYL)-1,3,4-TRIAZOLIUM-2-THIOLATE HYDROCHLORIDE.

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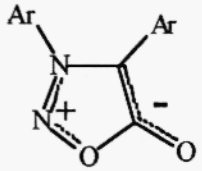
**Abstract:** Mesoionic 1,4-diphenyl-5-(5-nitro-2-furanyl)-1,3,4-triazolium-2-thiolate was prepared as its hydrochloride by the reaction of 1,4-diphenyl-thiosemicarbazide with 5-nitro-2-furoyl chloride. The structure was confirmed by elemental analysis, IR, mass, $^{1}H$ and $^{13}C$ NMR Spectra. Pharmacological tests in vitro showed that the compound posseses spasmolytic action in the guinea-pig trachea and the rat uterus, but not in the guinea-pig ileum.

**Introduction**

Compounds now classified as mesoionic were prepared late in the last century by Fischer and Besthorn and by Busch et col (1 - 8) although the currently accepted structures were not then known.

The concept and definition of mesoionic compounds were proposed by Baker and Ollis(9,10), though anticipated by Schönberg (11), on the basis of studies of Sydnones 1 (1,2,3,-oxadiazoliun-5-olates), which were first described by Earl and Mackney (12 ) (Figure -1).

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**Ar = $C_6H_5$**

**Figure 1**

Their definition evolved somewhat over the years (e.g 13,14) while we have recently proposed two definitions (15,16). These are: (a) Mesoionic Compounds are poly-heteroatomic 5-membered ring betaines, stabilized by electron-delocalization and with dipole moments not less then 5D (1D = $3.33564 \times 10^{-30}$ cm), in which electrons and a positive charge are delocalised over part of the ring and attached groups and in which electrons and a negative charge, formally on an α-atom (normally a hetero-atom) are delocalized over the remaining part of the ring; and (b) Mesoionic Compounds are those characterized by:

(a) a planar 5-menbered ring with at least one side-chain whose α-atom is also in this plane.

(b) a high dipole moment value and a high quadrupole moment value.

We have since refined and unified these into a single definition (17) viz, "Mesoionic compounds are planar 5-membered heterocyclic betaines with at least one side-chain whose α-atom is also in the ring plane and with dipole moments of the order of 5D. Electrons are delocalized over two regions separated by what are essentially single bonds. One region which includes the α-atom of the side-chain is associated with the HOMO and negative π-charge whereas the other region is associated with the LUMO and positive π-charge".

These characteristics lead to strong interactions with many biomolecules, so that a wide range of biological activity has been reported for mesoionic compounds. These reports refer inter alia to analgesic, anticonvulsant, antidepressive/psycho-stimulant, anti-inflammatory, anti-malaria, anti-neoplasic, anti-pyretic activity; also ascaricidal, bactericidal, CNS-stimulant, coccidiostatic, diuretic, fungicidal, hypoglycemic, hypotensive, insecticidal, sympathomimetic and tuberculostatic activity as well as non-competitive inhibition of monoamineoxidase (13,18-47). However, hardly any pharmacological studies on smooth muscles have been carried out (48). The present paper reports spasmolytic activity of the title compound, the hydrochloride of a member of the 1,3,4-triazolium-2-thiolate class of mesoionic compounds 3-a.

Mesoionic compounds of this class were in fact among those prepared by Busch et col. (2-8) for which they used two different methods. The first involved the reactions of mesoionic 1,3,4-thiadiazólium-2-thiolates 3 with primary amines; while the second involved the reactions of 1,4-diphenyl-thiosemicarbazide 4 with carboxylic acid chlorides 5 (see Figure 2).

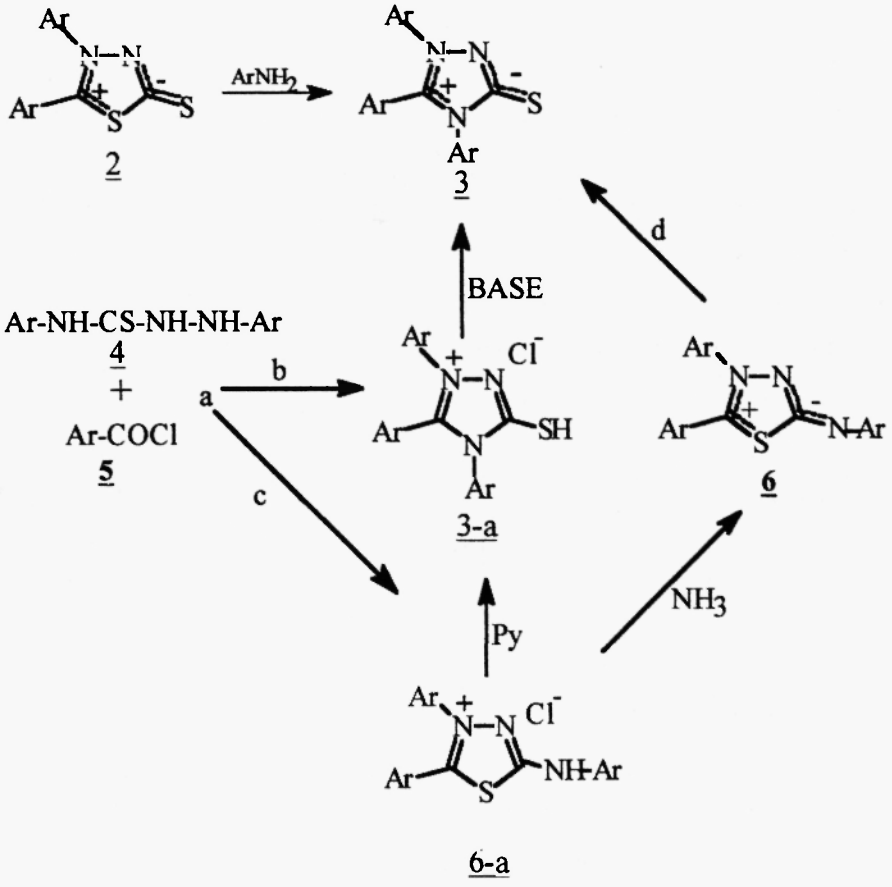
It is noteworthy that Schönberg (11), much later, suggested that the second method led to the formation of inter-convertible isomers (Figure 2); whereas it was subsequently suggested that the products are the corresponding 1,3,4-triazolium-2-thiol chlorides 3-a (41-44). However we have recently (45) restudied this reaction sequence using 1,4-diphenyl-thiosemicarbazide 4 and aroyl chlorides 5, and showed that the hydrochlorides of the mesoionic 1,3,4-thiadiazolium-2-aminides 6-a are the kinetic products; whereas the hydrochlorides of the mesoionic 1,3,4-triazolium-2-thiolates 3-a are the thermodynamic products. We also demonstrated the conditions for obtaining the products of either system ( Figure 2).

In the present work, we have prepared and studied mesoionic 1,4-diphenyl-5-(5-nitro-2-furanyl)-1,3,4-triazolium-2-thiolate 8, obtained as the hydrochloride by the reaction of 1,4-diphenyl-thiosemicarbazide 4 with 5-nitro-2-furoyl chloride 7 as in the general method shown in Figure 3. It has not previously been reported in the literature.

## Experimental

### 1 - Chemistry

Melting points were determined on a Kofler hot-plate apparatus combined with a Carl Zeiss microscope and are uncorrected, $^1$H NMR Spectra were obtained on a Varian EM 360 Spectrometer - the sample being




(a) humidity, open system
(b) anhydrous, closed system
(c) humidity, basic conditions
(d) alkaline isomerization

**Figure - 2**

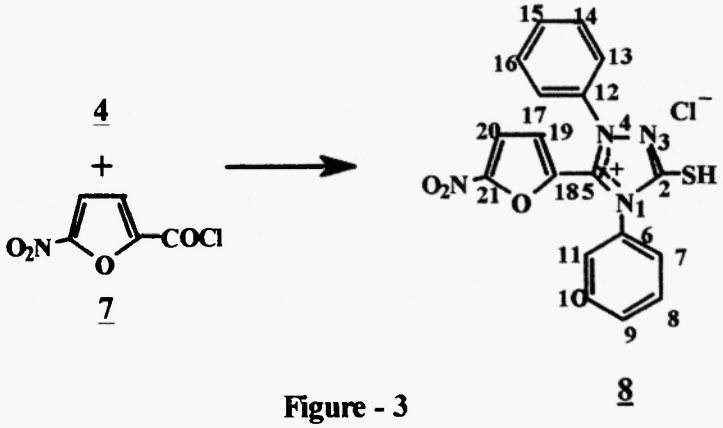



**Figure - 3**

dissolved in $CF_3CO_2H$ with TMS as reference, $^{13}C$ NMR spectra were obtained on a Varian FT 80 spectrometer - the sample being dissolved in DMSO-$d^6$ with TMS as reference, Mass Spectra were obtained on a Hewlett Packard 5890-5888a spectrometer in CG/MS mode, IR spectra were obtained on a Bomem Micheson Spectroscope with the sample in a KBr disc, Elemental Analysis was carried out on a Perkin Elmer 240 Elemental Microanalyser.

1,4-Diphenyl-5-(5-nitro-2-furanyl)-1,3,4-triazolium-2-thiol chloride. 5-nitro-2-furoyl chloride (20 mmoles) dissolved in 10ml of dioxan was added slowly with stirring to a suspension of 1,4-diphenyl-thiosemicarbazide (20 mmoles) in 10 ml of humid dioxan, while cooling in an ice-bath. After addition was complete, the reaction mixture was allowed to attain ambient temperature and stirring continued for 24 hours. The desired mesoionic hydrochloride was obtained in 32% yield in the form of orange-colored crystals with m.p. 263°C .

Elemental Analysis: Calculated; C, 54.0; H, 3,25; N, 14.0 %. Found: C,53,5; H,3,20; N, 14.3%. IR Spectrum, $\nu_{max}$ ($cm^{-1}$) 3150 and 3040 ($C_{Ar}$ - H); 2714 (S-H); 1572 and 1361 (asymmetric and symmetric vibrations of the $NO_2$ group). Mass Spectrum m/z = 364 ($M^{+}$free base)(6.14%); 215 (2.81%); 135 (9.01%); 112 (6.02%); 82 (17.55%); 77 (100%); 51 (52,23%). The peak at m/z 135 is especially characteristic of 1-phenyl-1,3,4-triazolium-2-thiolate derivatives and is attributed to $[Ph\text{-}N{=}C{=}S]^{+\cdot}$. $^1H$ NMR Spectrum δ (ppm) 7.4-8.1 (m, 11 H), protons of the two $C_6H_5$ grups and the C4 proton of the furan ring; 7.2 (s, 1H); C3 proton of the furan ring, 4.0 (s ,1H) sulphydryl proton. $^{13}C$ NMR Spectrum, δ/ppm. The chemical shifts are shown in Table I.

TABLE I - $^{13}C$ NMR Chemical Shifts: Mesoionic - 1,4 diphenyl-5-(5-nitro-2-furanyl)-1,3,4-triazolium-2-thiolate hydrochloride (see figure 3).

| C | 2 | 5 | 6 | 7, 11 | 8, 10 | 9 | 12 | 13, 17 | 14, 16 | 15 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| δ (ppm) | 167.8 | 146.5 | 136.6 | 132.3 | 130.6 | 127.7 | 133.8 | 125.7 | 130.6 | 127.7 | 141.8 | 108.7 | 114.5 | 152.7 |

**2 - Pharmacology**

**Spasmolytic Activity**

The isolated tissues were prepared according to well-established pharmacological methods (48). Briefly, the rat uterine horns, guinea-pig ileum and trachea were obtained from rats and guinea-pigs killed by an excess of pentobarbital given intraperitoneally. The tissues were suspended in isolated organ baths filled with appropriate physiological salt solutions kept at 31 - 37°C (depending on the tissue) and bubbled with a 95% $O_2$ + 5% $CO_2$ gas mixture. The responses of the tissue were registered by the use of physiographs (Beckmam 511-A model) coupled to force displacement transducers.

Initially the concentration of each stimulant (oxytocin, bradykinin, carbachol or histamine), which produced approximately 60 - 80% maximal contraction (submaximal) was obtained. This concentration was then repeated in the presence of the compound which was added 5 min before to the organ bath and the contraction was recorded as before. The percent inhibition of the contraction was calculated by comparing the responses in the absence (100%) and in the presence of different concentrations of the compound. The concentration of the compound which inhibited the contraction induced by the stimulant by 50% (IC 50) was then obtained from concentration-response curves.

The ability of the compound to reduce the spontaneous tone of the guinea-pig trachea was studied as follows. Initially a concentration of aminophylline which produced a maximal relaxation of the tissue was obtained. After washing the trachea the compound was added in a cumulative fashion until a maximal relaxation was achieved. The percent inhibition of tone obtained with each concentration of the compound was calculated by comparing the responses to that of aminophylline which was considered as 100% and IC 50 values were calculated as described above.

All the data were analysed statistically using Student's *t* test and the results were considered significant when the probability (p) was < 0.05 (see Table II).

TABLE II - The effect of mesoionic 1,4-diphenyl-5-(5-nitro-2-furanyl)-1,3,4-triazolium-2-thiolate hydrochloride on contractions induced by oxytocin (A) and bradykinin (B) in the rat uterus, carbachol (C) and histamine (D) in the guinea-pig ileum, and by carbachol (E) in the guinea-pig trachea. The compound also reduced the spontaneous tone of the guinea-pig trachea (F). The results are mean ± SEM of 5 tests *P<0.05 and **P<0.01 by using Student's *t* test.

| Experiment | Tissue | Agonist | Concentration (μM) | % inhibition Mean ± SEM | IC50 (μM) |
|---|---|---|---|---|---|
| A | Uterus | Oxytocin | 1.0 | 7.3 ± 1.6 | |
| | | | 5.0 | 22.5 ± 2.3* | 45.0 |
| | | | 10.0 | 39.7 ± 1.3** | |
| | | | 50.0 | 68.7 ± 4.9** | |
| B | Uterus | Bradykinin | 1.0 | 3.1 ± 1.6 | |
| | | | 5.0 | 34.0 ± 3.2* | 8.0 |
| | | | 10.0 | 69.0 ± 2.2** | |
| | | | 50.0 | 88.7 ± 1.6** | |
| C | Ileum | Carbachol | 100.0 | 0 | - |
| D | Ileum | Histamine | 100.0 | 0 | - |
| E | Trachea | Carbachol | 1.0 | 30.7 ± 1.6* | |
| | | | 3.0 | 48.0 ± 3.4** | 3.2 |
| | | | 10.0 | 98.7 ± 0.2** | |
| F | Trachea | - | 0.3 | 2.4 ± 1.0 | |
| | | | 1.0 | 20.7 ± 3.2* | |
| | | | 3.0 | 64.3 ± 3.0** | 2.0 |
| | | | 10.0 | 96.8 ± 0.4** | |

## Results and Discussion

### 1 - Chemistry

The conditions used for the synthesis of the title conpound were these appropriate for the isolation of the thermodynamic product of the reaction between 1,4-diphenyl-thiosemicarbazide and 5-nitro-2-furoyl-2 chloride viz., the title compound, belonging to the 1,3,4-triazolium-2-thiolate system (as the hydrochloride) 3-a (Figure 2).

The spectral data are fully consistent with those expected of the title compound. Among the most noteworthy data, as well as the elemental analysis, are (a) The I.R. peak at 2714 $cm^{-1}$ characteristic of mesoionic 2-thiol derivatives and the $^{1}H$ NMR shift at 4.0 ppm also attributed to the SH group. In the Mass spectrum, the occurrence of the molecular ion of the mesoionic free base and the peak at m/z 135 for $PhN{=}C{=}S]^{+}$ are characteristic. The $^{13}C$ NMR spectrum includes a peak at $\delta = 167.8$ (C-2 characteristic of the 2-thiol function of the hydrochloride of the parent mesoionic system).

### 2 - Pharmacology

The nonspecific spasmolytic activity of the compound measured as the inhibition of contractions induced by various smooth muscle stimulants is summarized in Table II. In the rat uterus, the compound antagonized in a concentration-dependent manner, the responses to oxytocin and bradykinin and the IC50 values obtained againist the two stimulants were 45.0 and 8.0 μM respectively. While the compound was inactive up to a concentration of 100 μM in the guinea-pig ileum against histamine and carbachol, it was found to be a potent relaxant of the guinea-pig trachea. Thus it antagonized not only the carbachol induced contractions but also reduced the spontaneous tone of the tissue and the corresponding IC50 values were 3.2 and 2.0 μM respectively. In all the tissues studied the effect of the compound was reversible after repeated washings.

The results indicate that the compound has potent tracheal muscle relaxant activity especially when compared with the therapeutically used bronchodilator theophylline, the IC50 value of which was 30.2 μM (result not shown). To our knowledge, this is the first report of tracheal muscle relaxant activity attributed to a mesoionic compound. However, mesoionic xanthine analogues have been reported (49), as inhibitors of phosphodiesterase enzymes and thus may cause smooth muscle relaxation through an increase in 3,5-cyclic adenosine monophosphate levels. Further studies on mesoionic componds may lead to new bronchodilator drugs for the treatment of asthma.

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