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ORIGINAL PAPER

Photochemical and DNA degradation studies on tenoxicam, lornoxicam, and their photolysis products

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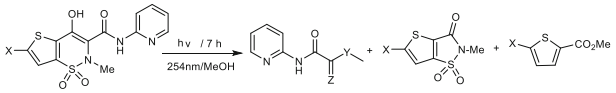
Abstract Tenoxicam and lornoxicam are nonsteroidal anti-inflammatory drugs, were subjected to photoirradiation at 254 nm led to the photodegradation of the pharmaceutical agents. Both, the isolated photodegradation products and the pharmaceutical agents were examined toward DNA binding and degradation. The photodegradation products degrade calf thymus in concentration dependent manner.

Graphical abstract



Keywords Oxicams · Photostability · Photodegradation · Zwitterionic effect · DNA degradation

Introduction

Oxicams are nonsteroidal anti-inflammatory drugs (NSAIDs) which are used for the treatment of rheumatic arthritis, osteoarthritis, and post-operative inflammations [1, 2]. Several other functions of this group of drugs have been reported which include UV-protection and sensitization, chemoprevention and chemosuppression of cancer, and finally they function as effective anti-oxidants [3–7]. In the year 1980, piroxicam (PRX, 4-hydroxy-2-methyl-*N*-(pyridinyl-2-yl)-2*H*-1,2-benzothiazine-3-carboxamide 1,1-dioxide; Scheme 1, $R^2 = Me$, $R^1 = CONH$-2-pyridinyl) was launched into the European market for clinical use as a lead compound of the oxicams [8]. Since then, new members of these pharmaceutical agents have been synthesized based on structure–activity relationships (SARs) and the isosteric substitution concept, which starts by substituting the lead compound with moieties of similar stereoelectronic features which might improve the pharmacological efficacy and other pharmacokinetic properties [9].

Shortly after the pharmaceutical agent became widely available for the therapeutic use, it was associated with the development of adverse light-induced draw-back which was explained in terms of phototoxicity of either the photoproducts or the metabolite end products of the drug [10–14]. Therefore, studying the photochemical and photophysical properties and of course the photostability of the oxicams were the main concern of many literature reports [15–17]. Also, contradictory data concerning the photostability of the oxicams can be found in the literature. While piroxicam was proved to be stable to photolysis even after prolonged exposure to light [13, 14, 18], tenoxicam showed 50 % loss of the drug when exposed to sunlight for 3 h [19]. On the other hand, it was reported that direct

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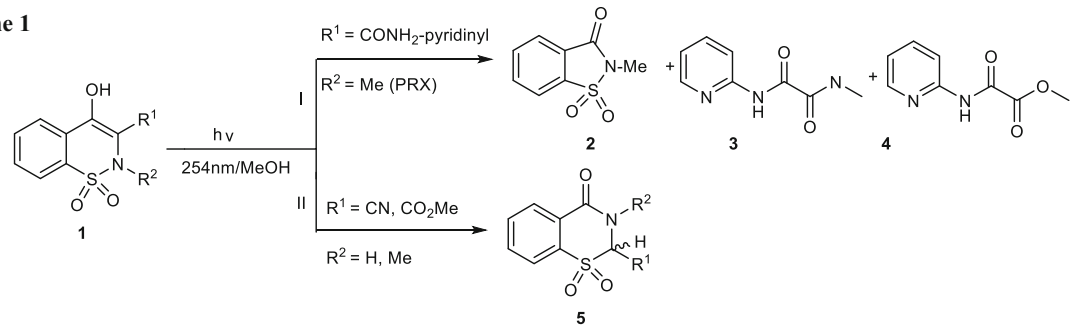
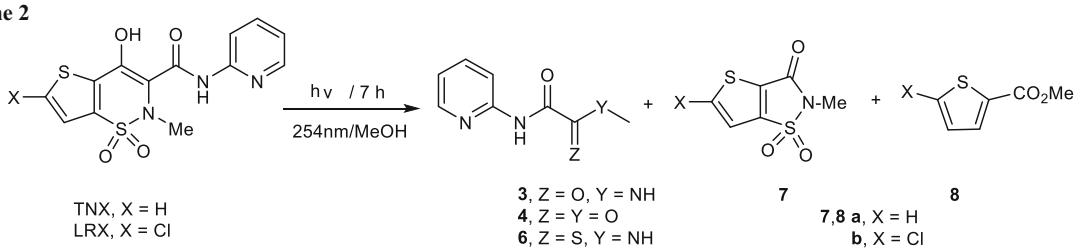
Scheme 1



Scheme 2



irradiation of piroxicam in dichloromethane afforded two main photodegradation products namely, *N*-methylsaccharin (**2**) (Scheme 1, pathway I) and *N*-(2-pyridinyl)oxamic acid [20]. This was explained in terms of a plausible mechanism via formation of dioxetane intermediate [20, 21]. Furthermore, in another literature report, when a methanolic solution of piroxicam was subjected to photolysis, two more products beside *N*-methylsaccharin were isolated and identified as *N*-methyl-*N*′-(2-pyridinyl)oxalamide (**3**) and methyl (2-pyridinylcarbamoyl)formate (**4**) (Scheme 1, pathway I) [22].

In a previous study, we reported a different photochemical behavior for this ring system. When 3-substituted 4-hydroxy-1,2-benzothiazine-1,1-dioxides were subjected to 254 nm aerobic/anaerobic irradiation in methanol for 7 h, a single photoisomerization product was obtained in moderate yield 75 % (Scheme 1) which was demonstrated to be the 2-substituted 4-oxo-1,3-2*H*-benzothiazine-1,1-dioxides **5** [23] (Scheme 1, pathway II). This was explained in terms of two-step free radical mechanism via homolytic cleavage at S–N bond in the thiazine ring.

The obvious question that arises, if they both have a common benzothiazine ring system and photolyzed under the same aerobic conditions with the same solvent and wavelength, why do we have two different photochemical behaviors? Such question has no straight forward answer. But from the chemical structure point of view, the only difference between the piroxicam and the analogue compounds is the substitution pattern in the thiazine ring. The 2-pyridinylcarboxamide group in the piroxicam is replaced by cyano or ester groups in the analogues. Furthermore, the piroxicam (PRX) is benzothiazine oxicam, while tenoxicam (TNX) and lornoxicam (LRX) are thienothiazine oxicams (Scheme 2). All of these oxicams have the thiazine ring with the same substitution pattern as 2-pyridinylcarboxamide at position three in the thiazine ring. This prompted us to study the photochemical behavior of tenoxicam and lornoxicam under the same conditions which could reveal some answers and explanation for what seems a contradicted photochemical behavior of this group of compounds.

Results and discussion

When the methanolic solution of tenoxicam (TNX) and lornoxicam (LRX) is irradiated at 254 nm for 7 h, five main photodegradation products were identified namely *N*-methyl-*N*′-(pyridin-2-yl)oxalamide (**3**), methyl(pyridin-2-ylcarbamoyl)formate (**4**), *N*-methyl-*N*′-(pyridin-2-yl carbamoyl)methanethioamide (**6**), *N*-methyl-3-oxothieno[3,2-*d*]-1,2-thiazole-1,1-dioxides **7a**, **7b** and methyl thiophene-2-carboxylates **8a**, **8b** (Scheme 2).

The structures of the photodegradation products were assigned by ^{1}H NMR, ^{13}C NMR, 2D-NMR, IR, and GC–MS. Moreover, UV–Vis spectra for both tenoxicam (TNX), lornoxicam (LRX), and the isolated photodegradation products were measured (Fig. 1). A plausible mechanism

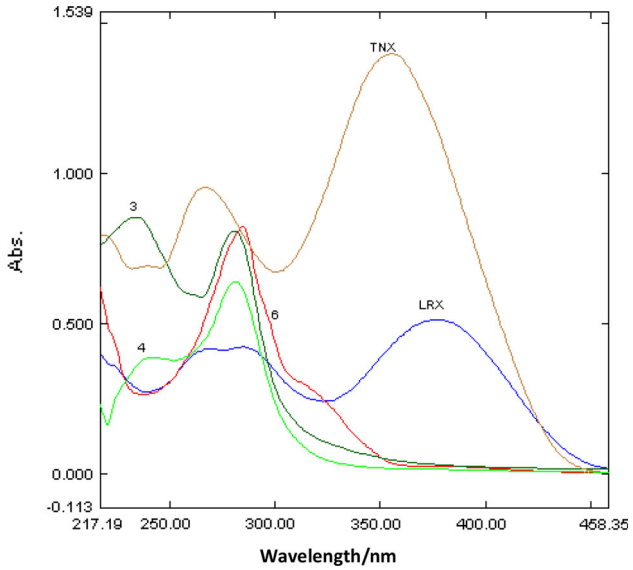


Fig. 1 UV–Vis spectra of TNX, LRX, and photodegradation products **3**, **4**, **6** in methanol

explaining the formation of these products is depicted in Scheme 3.

Photoexcited oxicam reacts with singlet oxygen at C_3=C_4 in the thiazine ring to form dioxetane intermediate **9**. Subsequent dioxetane ring opening leads to intermediate **10**. The later intermediate undergoes either nucleophilic attack by the solvent lone pair to form methyl (pyridin-2-ylcarbamoyl)formate (**4**), and *N*-methyl-3-oxothieno[3,2-*d*]-1,2-thiazole-1,1-dioxides **7a**, **7b** or extrusion of sulfur dioxide and methylation to yield *N*-methyl-*N*′-(pyridin-2-yl)oxalamide (**3**) and methyl thiophene-2-carboxylates **8a**, **8b** [24].

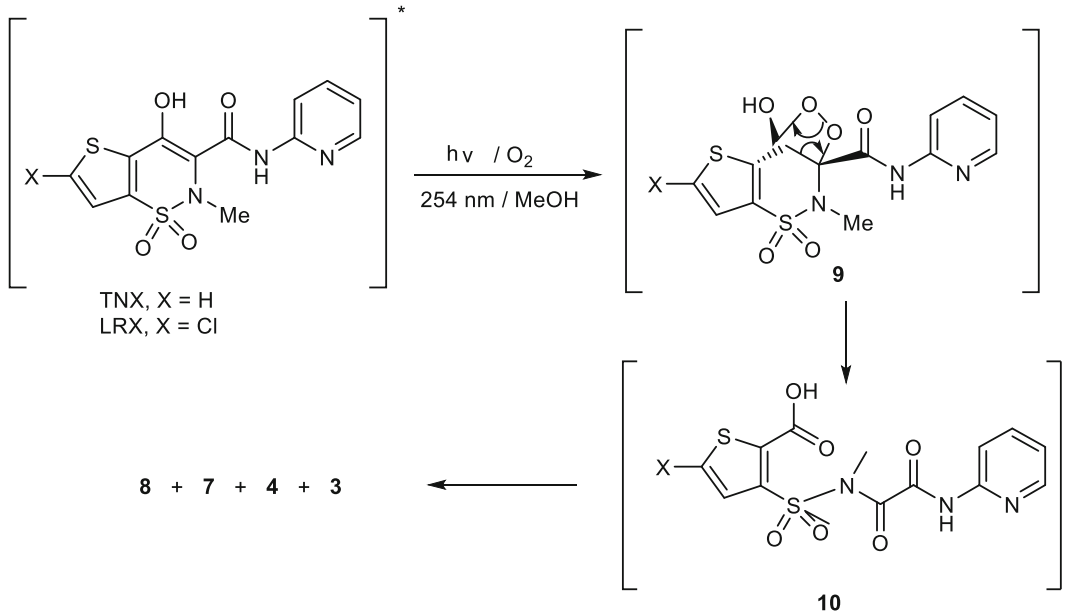
However, formation of photoproduct **6** as one of the aerobic photolysis products of tenoxicam and lornoxicam (Scheme 2) is quite abnormal and unexpected for such photodegradation, and was not detected or reported as one of the photolysis, oxidation or metabolites of such oxicams [25, 26]. The chemical structure of compound **6** was assigned and confirmed by ^{1}H NMR, ^{13}C NMR, 2D-NMR, IR, and GC–MS.

First, the other isomeric structures for compound **6** were excluded by closer look into the MS fragmentation pattern (Fig. 2) which revealed fragment at $m/z = 121$ attributed to [PyrNHCO^{+}], as a base peak followed by a peak at $m/z = 166$ attributed to [PyrNHCOCS^{+}].

Second, the only difference between structure **3** and **6** is that, one of the carbonyl oxygen atoms in **3** is replaced by one sulfur atom. The mechanism of formation of this product is not clear but its formation only on photolysis of tenoxicam and lornoxicam reveals that, the thiophene ring has a role in its formation as a source of sulfur. On the other hand, the participation of the other sulfur atom in the thiazine ring system in the formation of this product is excluded based on compound **6** was not detected or isolated on the photolysis of piroxicam [20–22].

The formation of the other photodegradation products (Scheme 3) is compatible with the previously reported photolysis pattern of piroxicam in term of chemical structure similarity between the three pharmaceutical agents [20–22]. Piroxicam, tenoxicam, and lornoxicam have benzothiazine ring with 2-pyridinyl carboxamide and hydroxyl groups at position 3 and 4, respectively, show

Scheme 3



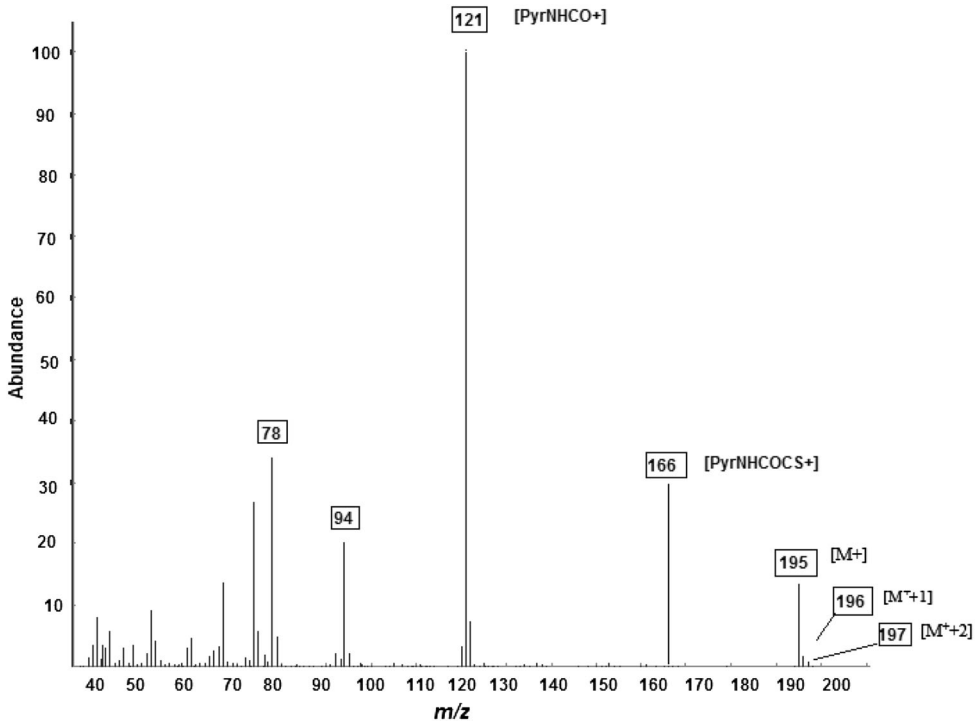


Fig. 2 Mass spectrum of compound **6**

different tautomeric forms due to fast internal proton transfers between the hydroxyl group and the 2-pyridinylcarboxamide group in the thiazine ring. This might cause unusual sensitive behavior to any changes in, chemical substitution, solvent and temperature [27, 28].

Thus, piroxicam, tenoxicam, and lornoxicam show a zwitterionic nature in polar organic solvents as demonstrated by ^{13}C NMR spectroscopy and obtained results from molecular orbital calculations [21, 29]. Therefore, by increasing the charge density on C_3=C_4 in the thiazine ring, the electrophilic attack of singlet oxygen is enhanced leading to the formation of the dioxetane intermediate **9** as a key step in the photodegradation process (Scheme 1, pathway I). On the other hand, benzothiazines with other substitution pattern at position 3 like CN or CO_2Me reacted differently and gave the photoisomerization product **5** via homolytic cleavage at S–N bond in the thiazine ring (Scheme 1, pathway II). To summarize these results, in the presence of oxygen, photodegradation (photo-oxidation) mechanism is preferred over the photoisomerization mechanism for oxicams with the 2-pyridinylcarboxamide group at position three in the thiazine ring.

To support these interpretations tenoxicam and lornoxicam were subjected to anaerobic photolysis under the same conditions from solvent and wavelength. Therefore, the methanolic solutions of the pharmaceutical agents were purged with argon for 1 h prior to photolysis at wavelength 254 nm, and the photolysis process was monitored by GC–MS. Neither the above photodegradation nor the photoisomerization products were detected in the photolysis solution during the course of photolysis.

Pharmacology

The competency of the isolated photolysis products **3**, **4**, and **6** to have aptitude to interact and cleave calf thymus DNA was assessed and interrelated to lornoxicam (LRX) and tenoxicam (TNX) as controls. Nonsignificant degradation on the calf thymus DNA was recorded at 2 μM concentration of both the photolysis products and the controls as shown in Fig. 3a. At 4 μM concentration the tenoxicam exhibited a considerable degradation effect on the tested calf thymus DNA, Fig. 3b while, the photolysis products **3**, **4**, **6** and the lornoxicam displayed a feeble DNA degradation at the same concentration. The tenoxicam completely degrade the calf thymus DNA at 6 μM concentration, while, lornoxicam and the photolysis products **3**, **4**, **6** at 6 μM concentration displayed a considerable degradation activities on the tested DNA (Fig. 3c).

The tenoxicam has higher degradation activity on the calf thymus DNA than the lornoxicam at 6 μM concentration. The lower degradation effect of lornoxicam on the DNA may be due to electronic effect of the chlorine atom as an electron withdrawing moiety in the thiophene ring of lornoxicam. It was reported that the presence of an electron withdrawing groups in the chemical structure decrease the DNA degradation activities [30].

The photolysis products **3**, **4**, **6** and the tested controls have a powerful degradation effect on calf thymus DNA at 8 μM concentration (Fig. 3d). It is obvious from the results that photolysis products **3**, **4**, **6** and tested controls bind and interact with the DNA in a concentration dependent

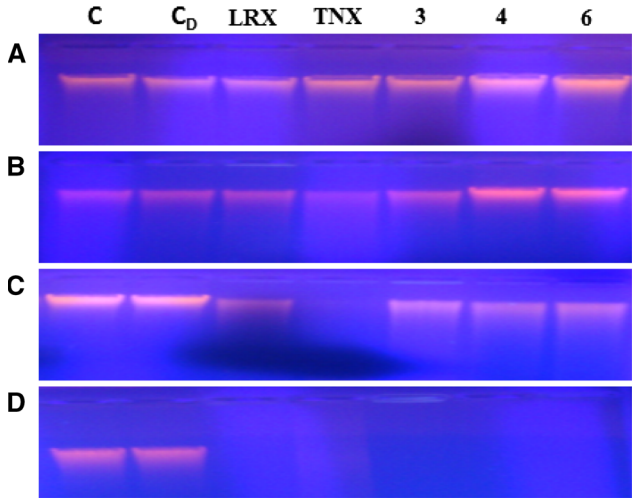


Fig. 3 A figure presenting the degradation effect of 2 μM (**a**), 4 μM (**b**), 6 μM (**c**), and 8 μM (**d**) of photolysis products **3**, **4**, and **6** on the calf thymus DNA. *Lane 1* DNA; *lane 2* DNA DMSO; *lane 3* lornoxicam (LRX); *lane 4* tenoxicam (TNX); *lanes 5, 6*, and *7* photolysis products **3**, **4**, and **6**, respectively

manner. Binding and interacting activities of the photolysis products with calf thymus DNA have encouraged competency to performance as a nuclease like activities and degrade calf thymus DNA. The existing study verifies that both the photolysis products and tenoxicam have significant degradation activities on calf thymus DNA without any addition. The calf thymus DNA-binding assessments are significant for the rational approach and get-together of novel and additional-effective medicines directing the heritable ingredients in eukaryotes. The DNA degradation competence in the absence of any chemical is an appreciated feature for TNX, **3**, **4**, and **6** which delivers a possible demand of these compounds as chemotherapeutic application in antitumor treatments.

Conclusion

The photochemical behavior of oxicams and analogues is very sensitive and depends on: (1) the type of the substituent on position three in the thiazine ring; (2) there is a correlation between the 2-pyridinylcarboxamide group at this position and the photo-oxidation mechanism. The behavior of lornoxicam and tenoxicam is nearly compatible with that of piroxicam, except formation of product **6** which is a novel photolysis product of oxicams. The photolysis products **3**, **4**, **6**, lornoxicam, and tenoxicam bind and interact with the DNA in a concentration dependent manner and a powerful degradation effect on calf thymus DNA at 8 μM concentration was noticed.

Experimental

The NMRs were recorded on a 500 MHz NMR spectrometer (Bruker, DRX500). EI GC/MS spectra were recorded using a Shimadzu GC17A/GCMS-QP 5050A mass spectrometer equipped with a standard EI source. EI mass spectra were recorded using an Autospec X magnetic sector mass spectrometer with EBE geometry (Vacuum Generators, Manchester, UK) equipped with a standard EI source. Samples were introduced by push rod in Aluminum crucibles. Ions were accelerated by 8 kV. The spectra shown here were recorded and processed with the opus software (V3.6 Micromass 1998) by the accumulation and averaging of several single spectra. UV/Vis spectra were recorded on a Perkin Elmer Lambda 40 UV/Vis spectrometer. Melting points were measured on a Buchi B-540. Photodegradation was performed with a RPR-100 Rayonet Photochemical Chamber Reactor (Southern New England Ultraviolet Company, Branford, USA) at 254 nm using quartz tubes. Tenoxicam and lornoxicam were purchased from Kemprotec Limited United Kingdom and used directly without any further purification. All the chromatographic separations were performed on 20 × 20 cm or 5 × 20 cm Merck glass plates covered with silica gel 60 F254 layer thicknesses 0.25 mm.

General photolysis procedure

Tenoxicam (TNX) and lornoxicam (LRX) 100 mg were dissolved in 100 mm^3 methanol and irradiated at 254 nm in a RPR-100 Rayonet Photochemical Chamber Reactor for 7 h. After concentration, the residue was subjected to chromatography on silica gel plates with 1 % methanol in chloroform. The R_f values of the appropriate zones are given below.

N-Methyl-N′-(pyridin-2-yl)oxalamide (**3**)

It was obtained in 18–22 % conversion as colorless powder after sublimation at 60–65 °C under 60 mbar vacuum, and crystallization from diethyl ether and *n*-pentane (1:1). $R_f = 0.33$; m.p.: 120–121 °C ([24, 25] 121 °C).

Methyl (pyridin-2-ylcarbamoyl)formate (**4**)

It was obtained in 12–15 % conversion as colorless powder after sublimation at 60–65 °C under 60 mbar vacuum, and crystallization from diethyl ether and *n*-pentane (1:1). $R_f = 0.63$; m.p.: 102–103 °C ([24, 25] 102 °C).

N-Methyl-N′-(pyridin-2-ylcarbamoyl)methanethioamide (**6**, $C_8H_9N_3OS$)

It was obtained in 12–14 % conversion as pale yellow powder after sublimation at 60–65 °C under 60 mbar vacuum, and crystallization from diethyl ether and *n*-pentane (1:1). $R_f = 0.78$; m.p.: 133–134 °C; ^1H NMR ($CDCl_3$): $\delta = 3.32$ (d, $^3J = 5$ Hz, 3H, CH_3), 7.14 (dd,

$^3J = 5$ Hz, $^3J = 5$ Hz, 1H), 7.78 (dd, $^3J = 8$ Hz, $^3J = 8$ Hz, 1H), 8.22 (d, $^3J = 8$ Hz, 1H), 8.41 (d, $^3J = 5$ Hz, 1H), 9.56 (s, 1H, N*H*CH_3), 10.59 (s, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$): $\delta = 33.1$ ($NHCH_3$), 113.7 (CH), 120.8 (CH), 138.4 (CH), 148.5 (CH), 150.0 (CNHCO), 156.2 (CNH*C*O), 186.4 (*C*SNHCH_3) ppm; IR (KBr): $\overline{V} = 3265$ (NH), 1683 (C=O), 1059 (C=S) cm^{-1}; MS: *m/z* (%) = 197 ($[M + 2]^+$, 1), 196 ($[M + 1]^+$, 2), 195 ($[M]^+$, 14), 166 ($[PyrNHCOCS]^+$, 35), 121 ($[PyrNHCO]^+$, 100), 94 ($[PyrNH_2]^+$, 17), 78 ($[Pyr]^+$, 30), 74 ($[CH_3NHCS]^+$, 23), 67 (14).

N-Methyl-3-oxothieno[2,3-d]-1,2-thiazole-1,1-dioxide (**7a**)
It was obtained in 8–10 % conversion as white crystals from methanol/H_2O (1:1). M.p.: 141 °C ([32] 142–143 °C).

5-Chloro-N-methyl-3-oxothieno[2,3-d]-1,2-thiazole-1,1-dioxide (**7b**)
It was obtained in 7–10 % conversion as off white crystals from ethanol. M.p.: 161 °C ([32, 33] 159 °C).

Methyl thiophene-2-carboxylate (**8a**)
It was obtained in 8–10 % conversion as viscous oil. B.p.: 80–82 °C/10 mbar ([34] 120–121 °C/13 mbar, [35] 40–44 °C/0.7 mbar).

Methyl 5-chlorothiophene-2-carboxylate (**8b**)
It was obtained in 9–10 % conversion as viscous oil. B.p.: 95–96 °C/9 mbar ([34] 87–88 °C/7 mbar, [36] 95–97 °C/9 mbar).

DNA degradation and agarose gels electrophoresis
The photolysis products **3**, **4**, and **6** in DMSO were added independently to 1 μg of calf thymus DNA (life technologies) and incubated at 37 °C for 60 min. The lornoxicam (LRX) and the tenoxicam (TNX) were used as parental and controls for this assay. The DNA was examined by utilizing agarose gels electrophoresis [31]. The electrophoresis performance was achieved through 1 % (w/v) agarose gels in Tris–acetate-EDTA buffer (0.04 M Tris–HCl, 5 mM sodium acetate, and 1 mM EDTA (pH 8). The ethidium bromide (0.5 μg/cm^3) was added to agarose gels for staining. The DNA in agarose gels was visualized with UV transilluminator and photographed with digital camera.

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