Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 929–933
Bioorganic & Medicinal Chemistry Letters

Regioselective oxidation of 2-amino-3-aroyl-4,5-dialkylthiophenes by DMSO

Elizabeth Joshi,[a] Mahendra D. Chordia,[a,*] Timothy L. Macdonald,[a]
Joel Linden[b] and Ray Olsson[a]

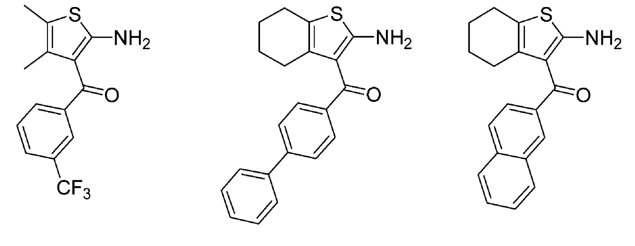
[a] *Department of Chemistry, University of Virginia, Charlottesville, VA 22901, USA*
[b] *Department of Medicine (Cardiology), University of Virginia, Charlottesville, VA 22901, USA*

Received 16 September 2003; revised 26 November 2003; accepted 2 December 2003

Abstract—Solutions of 2-amino-3-aroyl-4,5-dialkylthiophenes in DMSO (dimethyl sulfoxide) undergo regioselective oxidation of benzylic carbon under mild conditions. We describe three examples and propose a mechanism for oxidation.

Some 2-amino-3-aroyl-4,5-dialkylthiophenes are allosteric enhancers of agonist activity at the A1 adenosine receptor.[1–7] Thus, this class of compounds are potentially of use in the treatment of a variety of disorders by increasing activation of the A1 adenosine receptor, for example, the reduction of free fatty acid levels in blood[8] and the protection of tissue from ischemic injury.[9]

Owing to the limited water solubility of the 2-aminothiophenes, DMSO is frequently used as the solvent in stock solutions for pharmacological studies. In the course of such studies we noticed that over a period of weeks these compounds undergo conversion to complex mixtures of products. Here we describe evidence from ^{1}H NMR, ^{13}C NMR, and LCMS that the oxidation of benzylic carbons by DMSO is the initial step in this transformation and that these compounds are susceptible to biological oxidation by peroxidases. DMSO is a popular solvent for many organic reactions due to its polarity and is a reactant in the Swern[10] and Pfitzner–Moffatt[11] and other oxidation reagents. To our knowledge there are no reports of DMSO as an oxidant of benzylic carbons.



PD, 81 723 ATL-525 ATL-527

Figure 1. Structures of 2-amino-3-aroyl-4,5-dialkylthiophenes.

Figure 1 shows the structures of the three 2-aminothiophenes used in these studies. Solutions of each (10 mM) in DMSO-d_6 stored at 4 °C underwent analysis by LCMS and ^{1}H NMR periodically over a period of 4 weeks. Figure 2 shows the serial changes in the ^{1}H NMR spectrum[12] of one representative compound, ATL-527, that underwent a transformation to at least one new compound characterized by two triplets at 2.29 and 2.85 ppm, with concomitant disappearance of resonances of the starting material. At the end of 28 days, only a small amount of starting material remained. Simultaneously, the amount of newly formed product diminished, suggesting it underwent further transformation(s).

Figure 3 shows the changes in the ^{13}C NMR spectrum of a solution of ATL-525 stored in DMSO-d_6 for 25 days. Of the new resonances, that at 193.2 ppm is consistent with a carbonyl group, suggesting that ATL-525 had undergone oxidation.

The LCMS analysis[13] of solution of compound ATL-527 in DMSO-d_6 prior to its storage revealed a single peak having a retention time of 19.5 min with molecular

---

* Corresponding author. Tel.: +1-434-924-0595; fax: +1-434-982-2302; e-mail: mdc3x@virginia.edu

0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.12.010

ion $(M+H)^+$ of *m*/*z* 308 and fragment ions at *m*/*z* 212 (very weak), 180 (loss of naphthyl group) and 155 (complete loss of the 3-aroyl substituent). LCMS analysis of that solution after 28 days of storage revealed formation of a number of new compounds; the major component had a retention time of 6.4 min, a molecular ion $(M+H)^+$ of *m*/*z* 322 and fragment ions of *m*/*z* 226, 194 and 155 (Fig. 4). The 14 unit increase in mass of the product and the fragments is consistent with an oxidation product. Other identifiable components in the chromatogram had retention times of 8.7, 8.9, 10.2, 19.4, and 19.5 min. The mass spectra of those compounds contained molecular ions (M + H) of *m*/*z* 324, 340, 322, 324, and 340, respectively (Fig. 4). The amounts of those components were not sufficient for NMR analysis. However, their presence suggests the formation of several intermediates, as shown in Scheme 2. Similar observations were made for compound ATL-525.

Table 1 summarizes measurements by ^{1}H NMR of the rates of degradation of solutions of PD, 81723, ATL-525 or ATL-527 in DMSO. PD, 81723 was the most labile.

A similar regioselective oxidation of benzylic methylene group of 2-amido-3-carboethoxy-thiophene by $K_2Cr_2O_7$ in acetic acid[14] (Scheme 1) is a precedent for the oxidation reported here. A number of attempts were made to prepare the proposed oxidation product either by protecting 2-amino group as an acetyl/^{t}Boc group followed

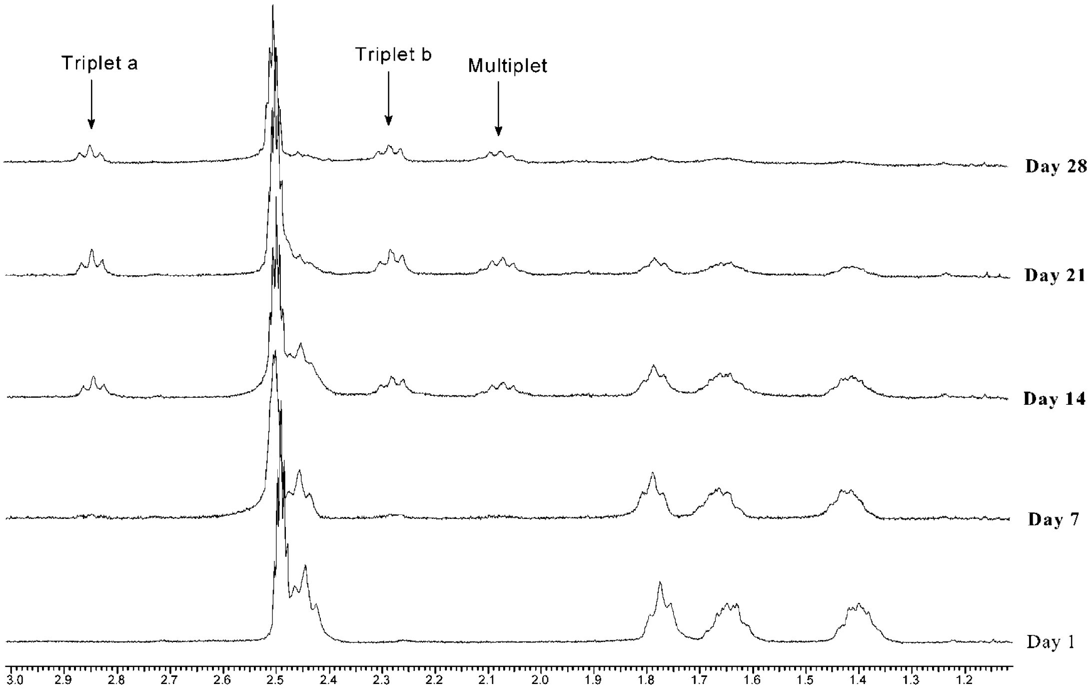


Figure 2. Sequential changes in the ^{1}H NMR spectral data of ATL-527 stored in DMSO-d_6.

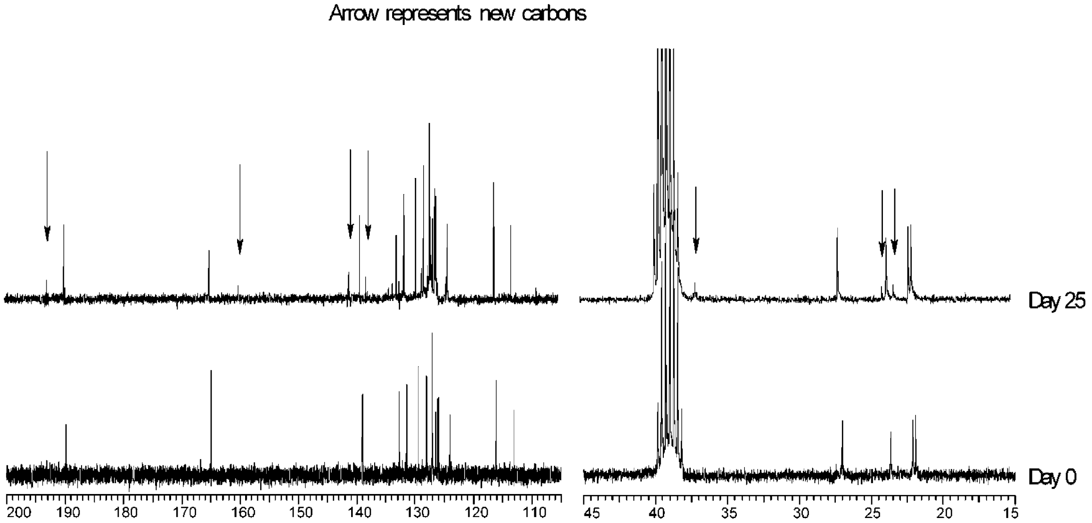
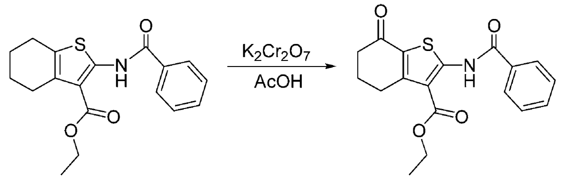


Figure 3. Changes in ^{13}C NMR spectrum of compound ATL-525 during 25 days of storage in DMSO. Arrows mark new resonances that appeared during storage.



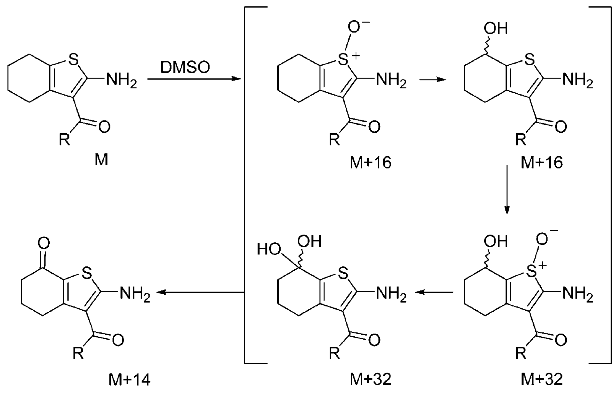
Scheme 1. Reported oxidation of 2-amido-3-carboethoxythiophene by $K_2Cr_2O_7$.

by oxidation with $K_2Cr_2O_7$ or through direct synthesis from 1,3-cyclohexanedione. In all the cases the starting material completely consumed to complex mixture with no signs of desired product formation. In addition, attempts to isolate pure oxidation product from both ATL-525 and ATL-527 after extraction with ethyl acetate followed by concentration and purification by silica gel column chromatography or by preparative TLC were unsuccessful due to the complexity of the mixture of compounds.

The direct purification of DMSO solution of ATL-527 by preparative HPLC,[15] led to isolation of partially purified oxidized ATL-527. However, upon concentration of the fractions complex mixture of products resulted.

The detailed mechanism of the oxidation is still unclear. It might occur by a radical, that is, single electron transfer pathway, but we believe an ionic pathway is more consistent with the slow reaction rate. Scheme 2 presents a possible mechanism for the oxidation.

Additional experiments examined the roles of dissolved oxygen and temperature. Working in a glove box under nitrogen, a stock solution of ATL-527 was made from a



Scheme 2. Proposed mechanism of the oxidation by DMSO.

fresh bottle of DMSO-d_6 saturated with nitrogen. The solution was transferred into a pair of NMR tubes and the tubes were sealed. In a parallel experiment the ATL-527 was prepared in DMSO-d_6 saturated with oxygen. One NMR tube of each pair was stored at 4 °C and the other at ambient temperature. Analysis by ^1H NMR at room temperature over the next 4 weeks showed:

Table 1. Rates of degradation in DMSO

Compd	% Oxidation to keto compound			
	7	14	21	28 (days)[a]
PD 81, 723	10	50	>90	—
ATL-525	10	30	40	50
ATL-527	15	40	60	>90

[a] Reactions were carried out in NMR-tube using DMSO-d_6 as a solvent, stored at 4 °C and thawed prior to ^1H NMR analysis; product formation was calculated by integrating the aliphatic protons with the residual DMSO protons as an internal standard.

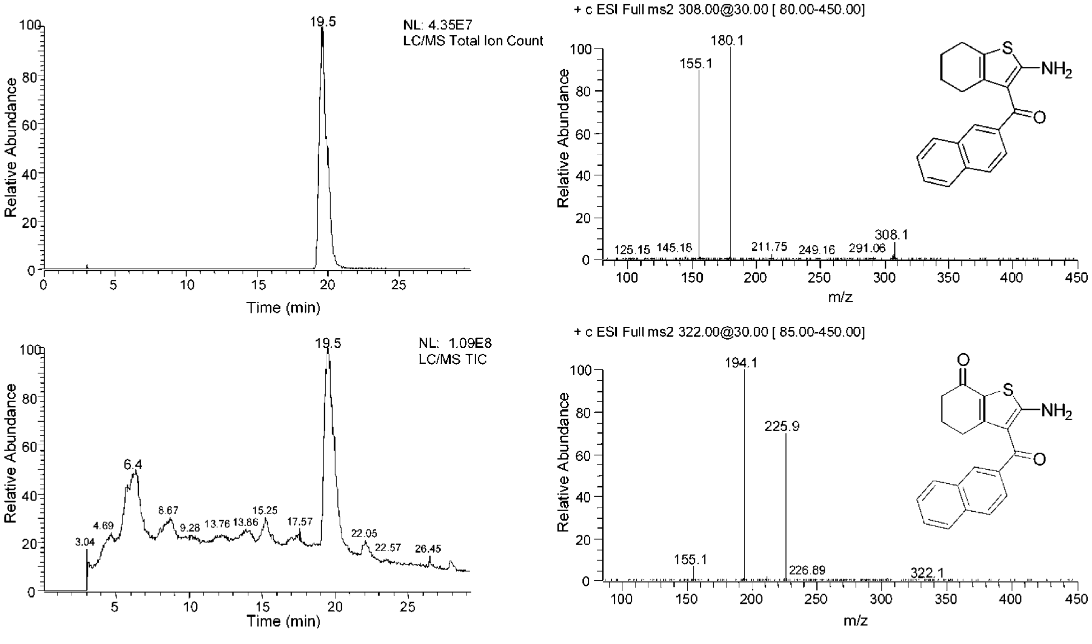


Figure 4. A. Top left: LC/MS Total Ion Count (TIC) showing peak associated with ATL-527 (R_t = 19.5 min). Top right: MS/MS spectrum of parent m/z = 308 and daughters m/z = 212, 180 (loss of napthyl) and 155 (loss of naphthoyl). B. Bottom left: LC/MS TIC of the sample at day 35 showing peaks associated with ATL-527 (Rt = 19.5 min) and major product (Rt = 6.4 min). Bottom right: MS/MS spectrum of oxidized product parent m/z = 322 and daughters m/z = 226, 194 (loss of napthyl) and 155 (loss of naphthoyl).

Table 2. Biological oxidation data

Compd	Biological oxidation (half life in seconds)[a]	
	HRP[b]	HLM[c]
PD 81, 723	<60	<60
ATL-525	<60	<60
ATL-527	~80	<60

[a] The half life data was obtained by monitoring the disappearance of substrate by LCMS analysis in relation to an internal standard.
[b] HRP = horse radish peroxidase.
[c] HLM = human liver microsomes.

(1) Degradation occurred in all four tubes, evidence that dissolved oxygen does not play a critical role; (2) The rate of degradation was about twice as great in the samples stored at 4 °C and thawed to room temperature for ^{1}H NMR analysis compared to samples maintained at room temperature suggesting the effect of freeze-thawing in DMSO on oxidation, and (3) no degradation occurred when methanol-d_4 was the solvent. Such results identify DMSO as the oxidant. Scheme 2 presents a possible mechanism for the oxidation. These results indicate that DMSO is not a suitable solvent for stock solutions of 2-amino-3-aroyl-4,5-alkylsubstituted thiophenes.

Since the chemical oxidation of 2-amino-3-aroyl-thiophenes was facile under mild conditions, susceptibility of these compounds towards biological oxidation systems was studied. All three compounds were incubated for oxidation with horse radish peroxidases (HRP) and human liver microsomes (HLM).[16] Table 2 demonstrates half lives for these compounds as analyzed by LCMS. All three compounds underwent decomposition in about 60 s.[17] At this time neither the mechanism nor the product(s) of those degradations are known.

The partially oxidized mixtures of ATL-525 and ATL-527 in DMSO were shown to possess slightly reduced allosteric enhancer activity of A1 adenosine receptor.

The oxidative lability of 2-amino-3-aroyl thiophenes could hamper their therapeutic utility. The synthesis of compounds in this class with structures resistant to oxidation is the subject of ongoing investigation.

Acknowledgements

NIH (RO1-HL56111) supported this work. We thank Chuck Arrington for semi-preparative HPLC analysis.

References and notes

1. Bruns, R. F.; Fergus, J. H.; Coughenour, L. L.; Courtland, G. G.; Pugsley, T. A.; Dodd, J. H.; Tinney, F. *J. Mol. Pharmacol.* **1990**, *38*, 950.
2. van der Klein, P. A.; Kourounakis, A. P.; IJzerman, A. P. *J. Med. Chem.* **1999**, *42*, 3629.
3. Baraldi, P. G.; Zaid, A. N.; Lampronti, I.; Fruttarolo, F.; Pavani, M. G.; Tabrizi, M. A.; Shryock, J. C.; Leung, E.; Romagnoli, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1953.
4. Tranberg, C. E.; Zickgraf, A.; Giunta, B. N.; Luetjens, H; Figler, H.; Murphree, L. J.; Falke, R.; Fleischer, H.; Linden, J.; Scammells, P. J.; Olsson, R.A. *J. Med. Chem.* **2002**, *45*, 382.
5. Baraldi, P. G.; Romagnoli, R.; Pavani, M. G.; Nunez, M. del C; Tabrizi, A.M.; Shryock, J.C.; Leung, E.; Moorman, A.R.; Uluoglu, C.; Iannotta, V.; Merighi, S.; Borea, P.A., *J. Med. Chem.* **2003**, *46*, 794.
6. Dhalla, A. K.; Shryock, J. C.; Shreeniwas, R.; Belardinelli, L. *Curr. Top. Med. Chem.* **2003**, *3*, 369.
7. Lütjens, H.; Zickgraf, A.; Figler, H.; Linden, J.; Olsson, R. A.; Scammells, P. J. *J. Med. Chem.* **2003**, *46*, 1870.
8. Merkel, L. A.; Hawkins, E. D.; Colussi, D. J.; Greenland, B. D.; Smits, G. J.; Perrone, M. H.; Cox, B. F. *Pharmcol.* **1995**, *51*, 224.
9. Mizumura, T.; Auchampach, J. A.; Linden, J.; Bruns, R. F.; Gross, G. J. *Circ. Res.* **1996**, *79*, 415.
10. Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165.
11. Pfitzner, K. E.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 5661.
12. ^{1}H and ^{13}C NMR analysis were performed on a Varian Unity Inova 300 spectrophotometer (Palo-Alto, CA) using Solaris software. The ^{1}H NMR data were obtained at 300 MHz using residual solvent (DMSO) as a reference. The ^{13}C NMR obtained at 75.4 MHz.
13. LCMS analysis was performed on a Waters 2690HPLC (Milford, MA) equipped with Thermo-Finnigan LCQ Classic Mass ion trap instrument controlled by Excalibur 1.1 software (San Jose, CA). For LCMS a Waters Symmetry C_8 reversed phase column (5 μm, 2.1 × 150 mm) eluted at 200 μL/min separated the components of 15-μL samples. Mass analysis employed ESI ionization, capillary temperature 200 °C; spray voltage, 5.0 kV; capillary voltage, 35.0 V, and sheath gas (nitrogen) flow rate, 40. Data collection was in the positive ion mode. For MS/MS experiments the *m/z* range was 150–800, and product ion spectra were generated by collision-induced dissociation (CID) of the MH+ ions of interest. Separation used a linear gradient composed of 0.02% TFA (solvent A) and methanol (solvent B). The initial composition consisted of 40:60 A:B, changing to 5:95 A:B over a 30 min period. On such gradients ATL-527 eluted at 19.5 min and the major oxidation product at 6.4 min.
14. Kharizomenova, I. A.; Kapustina, M. V.; Grinev, A. N.; Sheinker, Y. N.; Alekseeva, L. M.; Kuleshova, E. F. *Khim. Geterotsikl. Soedin. (Russ.)* **1984**, *12*, 1626.
15. Semi-preparative HPLC purification was performed on Water's 600 instrument attached to 996 PDA detector using Advantage C18 reverse phase column (250 × 10 mm) from Thompson Instrument Co (Clear Brook, VA). The data was processed with Millennium[32] software. Initially a linear gradient elution conditions were utilized to start with 10% acetonitrile (solvent A) and water (solvent B) till 10 min at which the composition was 100% acetonitrile. The column was further eluted with 100% acetonitrile for 10 more min and then equilibrated. The peak at 11.95 min, monitored at $\lambda = 254$ nm was collected as oxidized product. Two additional major fractions at 13.5 min. and 17.2 min were collected. The later fractions at 17.2 min were of the starting material.
^{1}H NMR of partially purified compound ($CDCl_3$): δ 1.95 (m, 2H, CH_2), 2.44 (t, 2H, CH_2), 2.82 (t, 2H, CH_2), 7.37 (dd, $J = 1.2$, 6.4 Hz, 1H, ArH), 7.75 (d, $J = 8.1$, 1H), 8.08 (brs, 1H), other aromatic protons could not be assigned. This patially purified material when analyzed again by LCMS revealed a small amount of oxidized material along with the formation of three more major components with R_t 12.2, 13.2, 14.0.

16. BD Genset Corp. (Woburn, MA). supplied human liver microsomes, Cat. No. 452161. Horseradish peroxidase (HRP) was from Sigma (St. Louis, MO).
17. Fresh 10 mM stock solutions of ATL-527, ATL-525, and PD-81,723 in DMSO were prepared daily.
    Half-Life Studies with Horseradish Peroxidase.
    Horseradish peroxidase (HRP) incubations were carried out at 37 °C in a 0.1 M potassium phosphate buffer (pH 8.0). HRP (2.5 units/mL) and 250 μM substrate was preincubated with buffer, and the reaction was initiated by addition of 1.1 mM H_20_2. At various time points reactions were quenched with 5% (v/v) catalase (5 mg/mL) and tetraphenylethylene (TPE) as an internal standard was added. After a few seconds, the proteins were separated by the addition of 5% (v/v) of a 10% trichloroacetic acid (TCA) solution and centrifugation. The supernatant served for all succeeding analyses. The concentration of substrate remaining at each time point was determined by HPLC. The calibration curve of the internal standard and drug was found to be linear in the range tested. The drug half-lives ($t_{1/2}$) under these conditions were determined using a first-order approximation by plotting the natural logarithm of the drug concentration versus time to give a slope equal to-k. The $t_{1/2}$ is equal to $0.693/k$.
    Half-Life Studies with Human Liver Microsomes.
    Human liver microsome (HLM)-mediated oxidations were carried out using 0.1 M potassium phosphate buffer (pH 7.4). The incubations contained 100 μM substrate, 2 mM NADPH, and 12 mM $MgCl_2 \cdot 6H_2O$. Quenching the reaction and preparation of a protein-free solution for analysis was as for the HRP-mediated oxidations.