SYNTHESIS AND $S_{RN}1$ REACTION OF THE FIRST QUINONE-OXAZOLE BIOREDUCTIVE ALKYLATING AGENT

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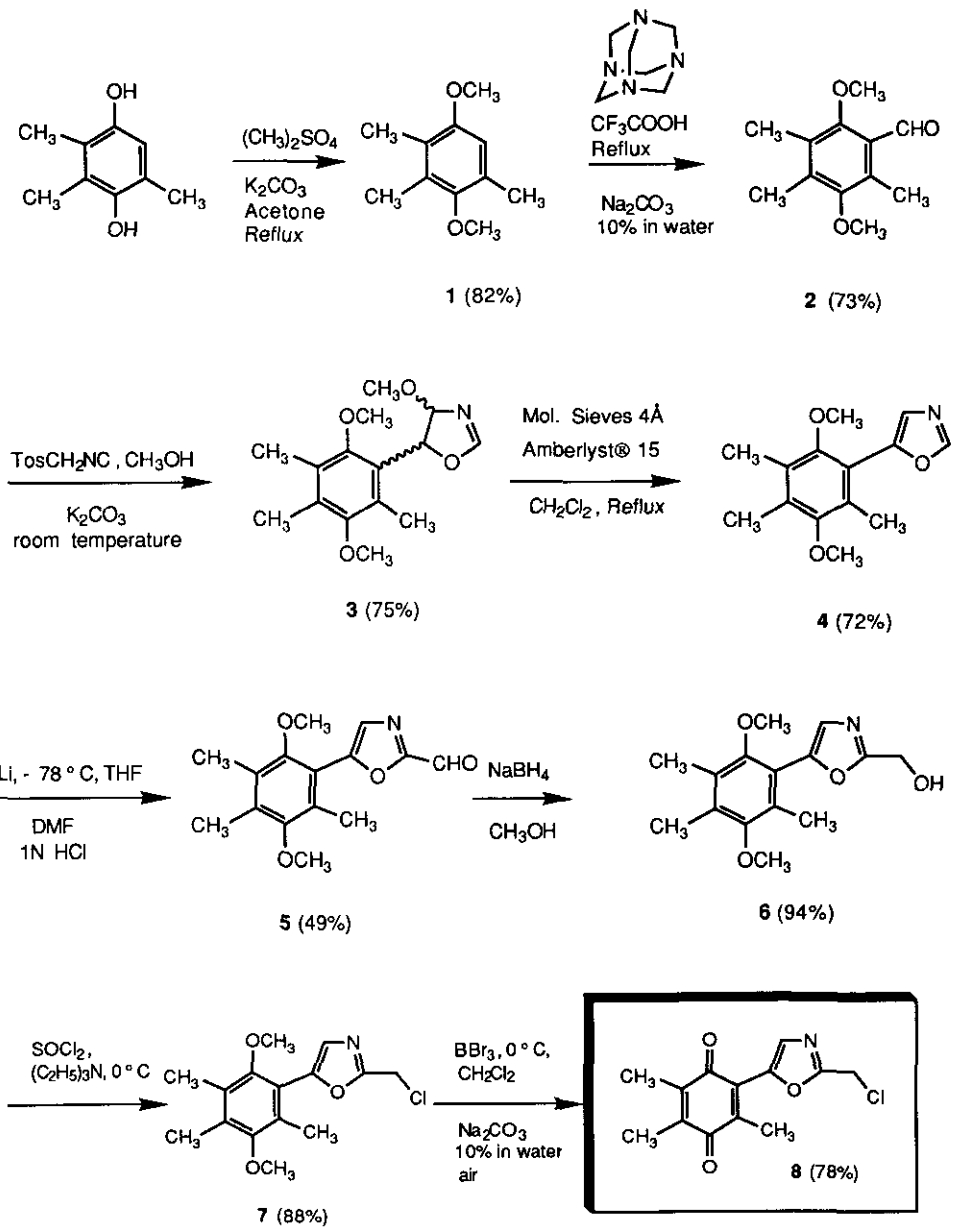
Abstract - 2-(2-Chloromethyloxazol-5-yl)-3,5,6-trimethyl-1,4-benzoquinone, the first compound of a new quinone-heterocyclic class of bioreductive alkylating agent, was prepared from trimethylhydroquinone in 8 steps and reacted by $S_{RN}1$ reaction with lithium salt of 2-nitropropane. The C-alkylation derivative showing its ability to react as one electron bioreductive alkylating agent was obtained in 88% yield .

The importance of quinone group is well-established for several classes of bioreductive alkylating agents which exhibit antitumor activities and are used in treatment of solid tumors and leukemia.[1-3] In addition, various trimethylbenzoquinone derivatives inhibit selectively enzymes by disturbing the arachidonate cascade system and are potent pharmacological agents in inflammatory and allergic diseases.[4] As a part of our program directed toward the study of $S_{RN}1$ reactions in nitroheterocycles[5-8] and quinones,[9-10] we wish to report the synthesis of 2-(2-chloromethyloxazol-5-yl)-3,5,6-trimethyl-1,4-benzoquinone (**8**) and the first results concerning its reactivity in electron transfer reactions (Scheme 1). This compound, prepared to study the influence of the planarity of quinone-heterocycle system on one electron transfer reactivity, is the first heterocyclic example of a potential bioreductive alkylating agent bearing a non-fused tetrasubstituted *p*-benzoquinone moiety .

Scheme 1



After methylation of trimethylhydroquinone,[11] derivative (**1**)[12] was converted into the aldehyde (**2**)[12] *via* a straightforward formylation process employing hexamethylenetetramine and trifluoroacetic acid.[13] Whereas it is known that oxazole can be obtained by reaction of tosylmethyl isocyanide (TosMIC) and aldehydes in equimolar

quantities,[14] in these conditions, we could only observed the formation of the unexpected 4-methoxy-2-oxazoline (**3**) as the major product contamined by a small amount of the oxazole derivative (**4**).The oxazoline (**3**) could not be obtained pure neither by Kugelrohr distillation nor by silica gel column chromatography and its structure was tentatively assigned from Nmr spectrum and this mixture used for the preparation of oxazole (**3**).

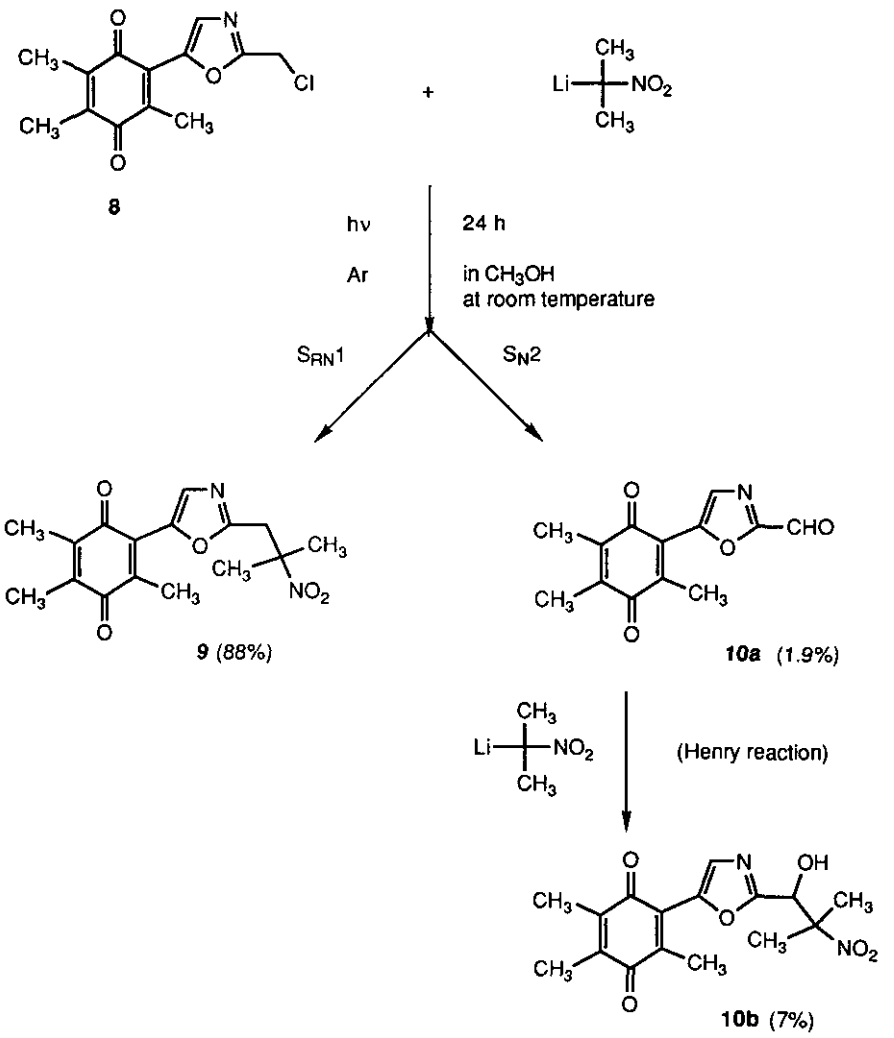
TosMIC has been already used for the synthesis of 4-ethoxy-2-oxazoline, but in the presence of thallium ethoxide ion in a ethanol / DME mixture (4 : 1) at room temperature.[15] The formation of **3** may be attributed to steric hindrance which affects the elimination of toluenesulfinic acid and favours the substitution by methanol or to the anchimeric assistance of the aromatic ring in the β–position of tosyl group.[16-17]

Acid catalyzed reaction of the mixture of oxazoline (**3**) and oxazole (**4**) using Amberlyst® 15 ion-exchange resin under anhydrous conditions led to the formation of the oxazole derivative (**4**). Metalation of **4** with *n*-butyllithium followed by addition of DMF produced **5**.[18] The aldehyde (**5**) was reduced into the corresponding alcohol (**6**) by $NaBH_4$, and latter was converted into the chloride (**7**) after chlorination with $SOCl_2$.

Oxidative demethylation with cerium ammonium nitrate[19] (CAN) was not applicable to the preparation of **8** because of the high reactivity of the oxazole ring towards oxidizing agents.[20] The quinone chloride (**8**) was obtained after demethylation of **7** with boron tribromide[21] followed by air oxidation of the hydroquinone intermediate in basic conditions.

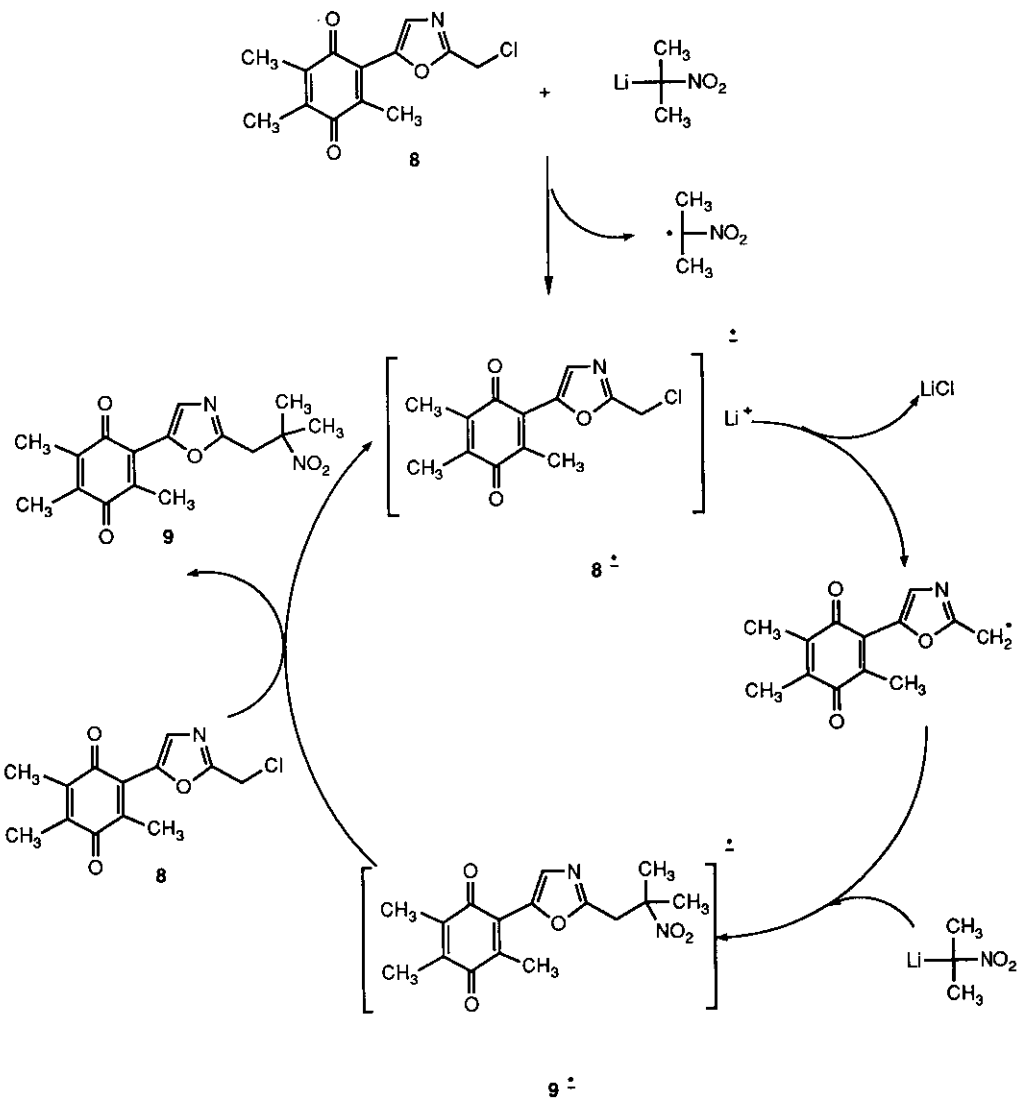
The chloride (**8**) was treated under conditions conducive to $S_{RN}1$ reactions (inert atmosphere, light) to afford selectively the C-alkylation product 2-[2-(2-methyl-2-nitropropyl)oxazol-5-yl]-3,5,6-trimethyl-1,4-benzoquinone (**9**), with small amounts of 2-(2-formyloxazol-5-yl)-3,5,6-trimethyl-1,4-benzoquinone (**10a**) (1.9%) and 2-[2-(1-hydroxy-2-methyl-2-nitropropyl)oxazol-5-yl]-3,5,6-trimethyl-1,4-benzoquinone (**10b**) (7%) as side products. This product (**10b**) can be explained as the result of the Henry reaction of 2-nitropropane salt with the aldehyde (**10a**). It is generally assumed that aldehyde arises from the instable nitronic ester formed in the reaction of nitroparaffin salt with benzylic halide, which may occur as an S_N2 oxygen-alkylation (Scheme 2).

Scheme 2



$S_{RN}1$ mechanism was accessed when comparing the yield of the reaction in the presence (or not) of two different inhibitors[22] (cupric chloride and di-*tert*-butyl nitroxide). When catalytical amounts of these inhibitors were added the yield of **9** dropped from 88% to 59% and 51% respectively. These data provided good evidence for assigning the $S_{RN}1$ mechanism to the reaction between the oxazole chloride derivative of 1,4-benzoquinone (**8**) and 2-nitropropane salt, as fully illustrated by Scheme 3.

Scheme 3



In conclusion, we have developed an access to a new quinone-oxazole bioreductive alkylating agent and shown that conjugation of the quinone and the oxazole systems is enough expanded to permit the $S_{RN}1$ C-alkylation to be the major process. We also have described the first example of an $S_{RN}1$ reaction involving oxazole system.

EXPERIMENTAL SECTION

GENERAL METHODS : The ^{1}H and ^{3}C-Nmr spectra were recorded on a Bruker AC 200 MHz spectrometer. Chemical shifts are reported in δ units (ppm) relative to internal TMS. Melting points were determined with a

Büchi micromelting points apparatus and are uncorrected. Products were purified by either Kugelrohr distillation or silica gel column chromatography. Elemental analyses were performed by the Elemental Analysis Center, Faculty of Science of St Jérôme, Aix-Marseille University, France.

1,4-Dimethoxy-2,3,5-trimethylbenzene (**1**).

A mixture of trimethylhydroquinone (100 g, 0.658 mol), dimethyl sulphate (249 g, 1.98 mol), K_2CO_3 (363 g, 2.63 mol) and acetone (1 l) was refluxed for 24 h. The solvent was distilled off and water (1 l) was added to the residue. The aqueous solution was extracted with ether twice. The organic layer was washed with a solution of 33% ammonia, dried over $MgSO_4$ and removed under reduced pressure. Vacuum distillation afforded 98 g (82%) of **1**. bp 64 °C (0.004 Torr) [lit.,[12] bp 144 °C (30 Torr)]. ^{1}H-Nmr ($CDCl_3$) δ 2.11 (s, 3H); 2.20 (s, 3H); 2.30 (s, 3H); 3.65 (s, 3H); 3.77 (s, 3H); 6.53 (s, 1H).

2,5-Dimethoxy-3,4,6-trimethylbenzaldehyde (**2**).

Trifluoroacetic acid (150 ml, 1.95 mol) was slowly added to a mixture of **1** (26 g, 144 mmol) and hexamethylenetetramine (20 g, 144 mmol). After 24 h refluxing, the mixture was poured into cold water, neutralized with 10% aqueous Na_2CO_3 and extracted with chloroform. The organic layer was dried over $MgSO_4$ and the solvent was evaporated under reduced pressure. The residue was triturated in a small amount of petroleum ether, the solution was separated and petroleum ether was evaporated under reduced pressure. The crude solid was recrystallized from water-ethanol (1:1) to afford 22 g (73%) of yellow solid **2**. mp 80 °C (lit.,[12] mp 83.5-84.5 °C from ethanol). ^{1}H-Nmr ($CDCl_3$) δ 2.18 (s, 3H); 2.27 (s, 3H); 2.50 (s, 3H); 3.65 (s, 3H); 3.77 (s, 3H); 10.50 (s, 1H).

1-(4-Methoxyoxazolin-5-yl)-2,5-dimethoxy-3,4,6-trimethylbenzene (**3**)

Solid K_2CO_3 (66 g, 480 mmol) was added under N_2 to a solution of **2** (50 g, 240 mmol) and TosMIC (70 g, 360 mmol) in 500 ml of dry methanol. The reaction mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the solid residue was washed with a saturated NaCl solution then dichloromethane. The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was distilled under reduced pressure to give **3** (50 g, 75%). bp 155 °C (0.006 Torr) contaminated with **4**. ^{1}H-Nmr ($CDCl_3$) δ 2.18 (s, 3H); 2.22 (s, 6H); 3.52 (s, 3H); 3.64 (s, 6H); 5.36 (d, J = 6.21 Hz, 1H); 5.51(d, J = 6.21 Hz, 1H); 7.13 (s, 1H).

1-(Oxazol-5-yl)-2,5-dimethoxy-3,4,6-trimethylbenzene (**4**).

A mixture of **3** (6.44 g, 23.1 mmol) contaminated with **4**, Amberlyst® 15 ion-exchange resin (6 g) and molecular sieves 4 Å (10 g) in dichloromethane (150 ml) was refluxed for 5 days. After cooling down to room temperature and filtration, the solvent was evaporated. The residue was purified by chromatography on a silica gel column, eluted with chloroform. Distillation led to 4.1 g (72%) of **4**. bp 100 °C (0.006 Torr). White solid. mp 50 °C (sublimation). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.00; H, 6.93; N, 5.66. Found : C, 67.93; H, 7.01; N, 5.52. ^{1}H-Nmr ($CDCl_3$) δ 2.18 (s, 3H); 2.21 (s, 3H); 2.25 (s, 3H); 3.47 (s, 3H); 3.69 (s, 3H); 7.21 (s, 1H); 8.03 (s, 1H). ^{13}C-Nmr ($CDCl_3$) δ 12.60; 13.02; 13.51; 60.06; 60.89; 119.74; 126.02; 128.82; 129.25; 133.05; 147.63; 150.65; 153.33; 153.34.

1-(2-Formyloxazol-5-yl)-2,5-dimethoxy-3,4,6-trimethylbenzene (**5**).

A solution of *n*-BuLi (6.5 ml, 10.56 mmol, 1.6 M in *n*-hexane) was added dropwise to a cooled (- 78 °C) and stirred solution of **4** (2.33 g, 9.43 mmol) and LiBr (0.82 g, 9.43 mmol) in anhydrous THF (20 ml). After 30 min stirring, the solution was quenched with DMF (0.8 ml, 10.33 mmol). When addition was completed, the reaction mixture was allowed to warm up to room temperature and ether was added. The reaction mixture was neutralized with 1 N HCl. After washing, the solvent was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on a silica gel column, eluted with dichloromethane-acetone (93:7) to give **5** (1.27 g; 49%) .Yellow solid. mp 62 °C (cyclohexane). Anal. Calcd for $C_{14}H_{17}NO_4$: C, 65.44; H, 6.22; N, 5.09. Found : C, 65.45; H, 6.29; N, 5.04. ^{1}H-Nmr ($CDCl_3$) δ 2.22 (s, 3H), 2.66 (s, 6H); 3.52 (s, 3H); 3.68 (s, 6H); 7.55 (s, 1H); 9.78 (s, 1H). ^{13}C-Nmr ($CDCl_3$) δ 12.72; 13.24; 13.79; 14.10; 60.23; 61.24; 118.42; 129.20; 130.06; 134.40; 151.92; 153.35; 153.40; 153.44; 157.75; 177.16.

1-(2-Hydroxymethyloxazol-5-yl)-2,5-dimethoxy-3,4,6-trimethylbenzene (**6**).

Sodium borohydride (0.19 g, 5 mmol) was added to **5** (1.38 g, 5 mmol) in solution in dry methanol (10 ml). After 30 min, the reaction mixture was poured into water (50 ml). After extraction with dichloromethane and purification by chromatography on silica gel (chloroform-acetone, 8:2), **6** (1.3 g) was obtained in 94% yield, as a white solid. mp 105 °C (petroleum ether). Anal. Calcd for $C_{15}H_{19}NO_4$: C, 64.97; H, 6.91; N, 5.05. Found : C, 64.60; H, 6.87; N, 5.01. ^{1}H-Nmr ($CDCl_3$) δ 2.19 (s, 3H); 2.20 (s, 3H); 2.24 (s, 3H); 3.49 (s, 3H); 3.67 (s, 3H); 4.03 (br s, 1H); 4.82 (s, 2H); 7.13 (s, 1H). ^{13}C-Nmr ($CDCl_3$) δ 12.69; 13.11; 13.68; 57.22; 60.16;

61.04; 119.89; 126.13; 128.84; 129.11; 133.08; 147.59; 153.16; 163.40.

1-(2-Chloromethyloxazol-5-yl)-2,5-dimethoxy-3,4,6-trimethylbenzene (7).

Thionyl chloride (2.17 ml, 29.2 mmol) was added dropwise at 0 °C to a solution of **6** (2.13 g, 7.69 mmol) and triethylamine (1.07 ml, 7.69 mmol) in 50 ml of dichloromethane. The reaction mixture was stirred for 1 h at room temperature and the solvent was removed under reduced pressure. The residue was diluted with dichloromethane, washed with a 10% aqueous solution of Na_2CO_3. The solvent was dried over $MgSO_4$ and removed under reduced pressure to give a white solid. Purification by chromatography on silica gel column eluting with dichloromethane gave **7** (2 g; 88%) as a white solid. mp 75 °C (sublimation). Anal. Calcd for $C_{15}H_{18}NO_3Cl$: C, 60.91; H, 6.13; N, 4.74; Cl, 11.99. Found : C, 60.92; H, 6.14; N, 4.76; Cl, 12.00. ^{1}H-Nmr ($CDCl_3$) δ 2.21 (s, 6H); 2.25 (s, 3H); 3.51 (s, 3H); 3.68 (s, 3H); 4.69 (s, 2H); 7.17 (s, 1H). ^{13}C-Nmr ($CDCl_3$) δ 12.70; 13.14; 17.67; 36.19; 60.18; 61.06; 119.46; 127.30; 128.93; 129.18; 133.28; 148.94; 153.30; 158.53.

2-(2-Chloromethyloxazol-5-yl)-3,5,6-trimethyl-1,4-benzoquinone (8).

To a stirred solution of chloride (**7**) (1 g, 3.38 mmol) in dichloromethane (100 ml) was added over 5 min 13.5 ml (13.5 mmol) of boron tribromide 1 M solution in dichloromethane at 0 °C. After 4 h stirring at room temperature, the reaction mixture was poured into a 10% aqueous solution of Na_2CO_3. The mixture was then stirred vigorously for 15 min and the aqueous solution was extracted with dichloromethane. The organic layer was dried over $MgSO_4$ and the solvent was removed in vacuo. The oily residue obtained was purified by chromatography on a silica gel column eluting with chloroform to yield 0.7 g (78%) of **8** as orange oil which gradually solidified in a red semisolid. This product cannot be purified by recrystallization or sublimation. These two methods gave poor elemental analyses. Anal. Calcd for $C_{13}H_{12}NO_3Cl$: C, 58.77; H, 4.45; N, 5.27; Cl, 13.34. Found : C, 57.66, 56.67; H, 4.87, 4.92; N, 4.20, 3.84; Cl, 12.60, 12.50. ^{1}H-Nmr ($CDCl_3$) δ 2.08 (s, 6H); 2.34 (s, 3H); 4.68 (s, 2H); 7.75 (s, 1H). ^{13}C-Nmr ($CDCl_3$) δ 12.49; 12.64; 13.75; 35.89; 128.96; 133.02; 140.80; 141.18; 146.13; 159.68; 184.29; 186.70.

General Procedure for $S_{RN}1$ Reactions

The chloride (**8**) (0.34 g, 1.28 mmol) and lithium salt of 2-nitropropane (0.122 g, 1.28 mmol) in dry methanol (20 ml) were stirred at room temperature for 24 h under argon and irradiated with two 60W fluorescent lamps. The reaction mixture was poured into water and extracted with dichloromethane. The organic layers were dried

over $MgSO_4$ and evaporated under reduced pressure. Purification by chromatography on a silica gel column eluting with dichloromethane gave 3 products. 0.36 g (88%) of 2-[2-(2-Methyl-2-nitropropyl)oxazol-5-yl]-3,5,6-trimethyl-1,4-benzoquinone (**9**). Orange solid. mp 87 °C (ether-pentane, 1:1). Anal. Calcd for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70; N, 8.80. Found : C, 60.30; H, 5.74; N, 8.85. ^{1}H-Nmr ($CDCl_3$) δ 1.58 (s, 6H); 1.94 (s, 6H); 2.13 (s, 3H); 3.39 (s, 2H); 7.62 (s, 1H). ^{13}C-Nmr ($CDCl_3$) δ 12.51; 12.66; 13.55; 26.00; 26.61; 38.80; 86.33; 129.15; 132.73; 140.05; 140.76; 141.15; 145.47; 159.99; 184.52; 186.87. 0.006 g (1.9%) of 2-[2-Formyloxazol-5-yl]-3,5,6-trimethyl-1,4-benzoquinone (**10a**). An authentic sample of **10a** was prepared following the procedure used for **8** from **5** in 40% yield. Orange solid. mp 83 °C (cyclohexane). Anal. Calcd for $C_{13}H_{11}NO_4$: C, 63.67; H, 4.52; N, 5.71. Found : C, 63.45; H, 4.49; N, 5.70. ^{1}H-Nmr ($CDCl_3$) δ 2.10 (s, 6H); 2.39 (s, 3H); 8.04 (s, 1H); 9.82 (s, 1H). ^{13}C-Nmr ($CDCl_3$) δ 12.02; 12.52; 13.99; 128.45; 134.57; 141.01; 141.59; 143.18; 157.66; 177.15; 183.97; 186.33. 0.03 g (7%) of 2-[2-(1-Hydroxy-2-methyl-2-nitropropyl)oxazol-5-yl]-3,5,6-trimethyl-1,4-benzoquinone (**10b**). Orange solid. mp 121 °C (cyclohexane). Anal. Calcd for $C_{16}H_{18}N_2O_6$: C, 57.48; H, 5.43; N, 8.38. Found : C, 57.40; H, 5.51; N, 8.45. ^{1}H-Nmr ($CDCl_3$) δ 1.63 (s, 3H); 1.73 (s, 3H); 2.07 (s, 6H); 2.26 (s, 3H); 4.00 (br s, 1H); 5.41 (s, 1H); 7.73 (s, 1H). Inhibition studies with $CuCl_2$ or di-*tert*-butyl nitroxide were carried out by adding the required amount of $CuCl_2$, $2H_2O$ (0.02 g, 0.13 mmol) or di-*tert*-butyl nitroxide (0.019 g, 0.13 mmol) to the reaction mixture. The yields of **9** from these studies were respectively 59% and 51%.

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