# Synthesis and Antitumor Activity of Some New 2-Chloroethylnitrosoureas

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The synthesis of a series of *N*-(2-chloroethyl)-*N'*-(9*H*-xanthen-9-yl)-*N*-nitrosoureas and *N*-(2-chloroethyl)-*N'*-(9*H*-thioxanthen-9-yl)-*N*-nitrosoureas is described. The title compounds were evaluated against NSCLCN6 L16 bronchial epidermoid carcinoma *in vitro* and some of them were found to be active. *N*-(2-chloroethyl)-*N'*-(2-methoxy-9*H*-xanthen-9-yl)-*N*-nitrosourea (**8e**) was active against leukemia P388 tumor system in mice.

**Synthese und pharmakologische Prüfung von neuen 2-Chlorethylnitrosoharnstoffen**

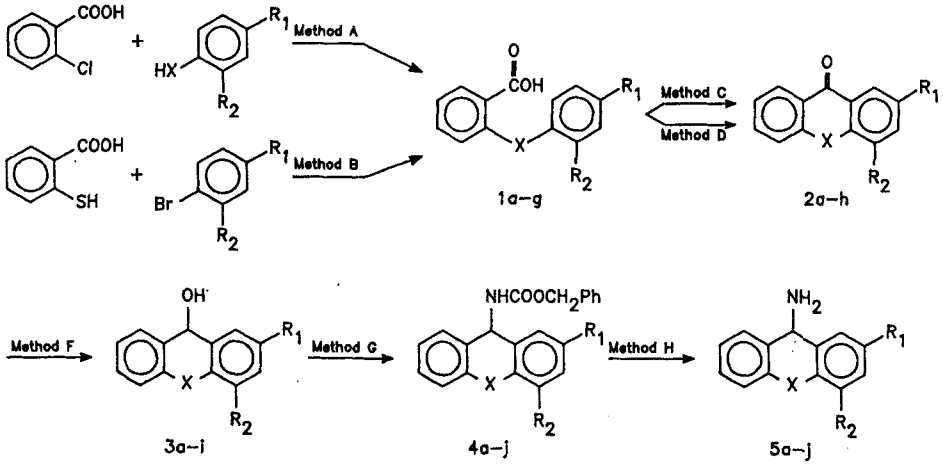
Eine Reihe von neuen *N*-(2-chlorethyl)-*N'*-(9*H*-xanthen-9-yl)-*N*-nitrosoharnstoffen und *N*-(2-chlorethyl)-*N'*-(9*H*-thioxanthen-9-yl)-*N*-nitrosoharnstoffen wurde dargestellt. Die neuen Verbindungen wurden auf Aktivität gegen NSCLCN6 L16 (Epidermoides Bronchialkarzinom) *in vitro* geprüft: einige erwiesen sich als wirksam. Verbindung **8e** ist auch gegen das Leukämie P388-Tumorsystem *in vivo* wirksam.

2-Chloroethylnitrosoureas are highly effective cancer chemotherapeutic agents that are in common clinical use (reviews: ref.[1,2]). The most known members of this class are *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU), *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU), and *N*-(2-chloroethyl)-*N'*-(4-methylcyclohexyl)-*N*-nitrosourea (MeCCNU).

Synthesis and testing of nitrosourea analogues have led to the conclusion that the 1-(2-chloroethyl)-1-nitrosourea moiety is the basis for antitumor activity and that modification of the *N*-3 substituent can effectively alter the *in vivo* antitumor activity[1,2].

Despite the large number of nitrosoureas that have been synthesized, the effect of tricyclic systems to their activity has not yet been studied.

The fact that some derivatives of 9*H*-xanthene and 9*H*-thioxanthene have exhibited anticancer activity[3] provided us with a good reason to use these tricyclic systems as *N*-3 substituents.




reagents
A: MeONa, $Cu_2O$ / MeOH
B: $K_2CO_3$, Cu, KI / DMF
C: $H_2SO_4$ 98%
D: $Ac_2O$, $H_2SO_4$ 98%
F: Na-Hg / EtOH
G: benzyl carbamate / $CH_3COOH$
H: KOH / EtOH

**Scheme 1**

  0365-6233/93/0808-0451 $ 5.00 + .25/0

*Chemistry*

Nitrosoureas **8a-m** (tables 1;2) were prepared according to *Martinez* et al.[4] from the appropriate amines **5a-j**, **6a-b**, **7** (table 3) and *N*-[*N*-(2-chloroethyl)-*N*-nitrosocarbamoyl-oxy]succinimide in anhydrous DMF at 0°C (*method K*).

The 9*H*-xanthen-9-amines and 9*H*-thioxanthen-9-amines **5a-j** tables 3;4) were obtained by following the route shown in Scheme 1.

The *o*-phenoxybenzoic acids and *o*-phenylthiobenzoic acids **1a-g** were prepared from a routine *Ullmann* ether synthesis either from the *o*-chlorobenzoic acid and the appropriate phenol (*method A*)[5] or from the *o*-thiosalicylic acid and bromotoluene (*method B*)[6]. - The xanthones and thioxanthones **2a-h** were prepared by cyclization of these acids either with conc. $H_2SO_4$ (*method C*)[7] or with $Ac_2O$-conc. $H_2SO_4$ (*method D*)[8].

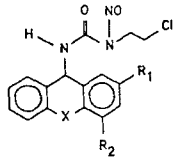
Compound **2e** (2-ethoxyxanthone) was prepared by direct ethylation of 2-hydroxyxanthone[9] (*method E*).

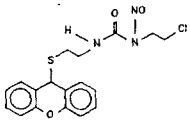
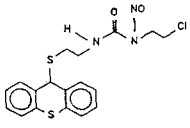
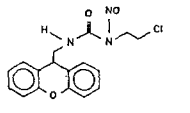
The xanthydrols and thioxanthydrols **3a-i** (Tables 5; 6) were synthesized by Na/Hg reduction[10,11] of the pertinent ketones (*method F*). Finally the amines **5a-j** (Table 3) were obtained by hydrolysis of their benzyl carbamates **4a-j** (Tables 7; 8, *method H*) in refluxing EtOH with 15% KOH[12]. The carbamates were obtained by condensation of a xanthydrol or thioxanthydrol with benzyl carbamate in glacial AcOH (*method G*)[12].

Compounds **6a** and **6b** (Table 3) were prepared from xanthydrol or thioxanthydrol and cysteamine hydrochloride in refluxing $CH_3CN$ (*method I*)[13].

Compound **7** (Table 3) was obtained from 9*H*-xanthene-9-carboxylic acid by $LiAlH_4$ reduction of 9*H*-xanthene-9-carboxamide (*method* **J**)[14].

**Table 1**:. 2-Chloroethylnitrosoureas **8a-m**



| Compd | $R_1$ | $R_2$ | X | mp[a] (°C) | Yield (%) | Formula[b] |
|---|---|---|---|---|---|---|
| 8a | H | H | O | 103-105 | 72 | $C_{16}H_{14}ClN_3O_3$ |
| 8b | $CH_3$ | H | O | 101-103 | 79 | $C_{17}H_{16}ClN_3O_3$ |
| 8c | H | $CH_3$ | O | 130-132 | 77 | $C_{17}H_{16}ClN_3O_3$ |
| 8d | $CH_3$ | $CH_3$ | O | 107-110 | 79 | $C_{18}H_{18}ClN_3O_3$ |
| 8e | $OCH_3$ | H | O | 112-113 | 82 | $C_{17}H_{16}ClN_3O_4$ |
| 8f | $OC_2H_5$ | H | O | 120-122 | 80 | $C_{18}H_{18}ClN_3O_4$ |
| 8g | H | $OCH_3$ | O | 125-127 | 73 | $C_{17}H_{16}ClN_3O_4$ |
| 8h | H | H | S | 80-82 | 71 | $C_{16}H_{14}ClN_3O_2S$ |
| 8i | $CH_3$ | H | S | 93-96 | 82 | $C_{17}H_{16}ClN_3O_2S$ |
| 8j | H | $CH_3$ | S | 121-123 | 76 | $C_{17}H_{16}ClN_3O_2S$ |
| 8k |  | | | 88-90 | 73 | $C_{18}H_{18}ClN_3O_3S$ |
| 8l |  | | | 116-118 | 69 | $C_{18}H_{18}ClN_3O_2S_2$ |
| 8m |  | | | 105-108 | 78 | $C_{17}H_{16}ClN_3O_3$ |

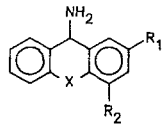
[a] From ether-pentane. [b] Anal (C, H, N).

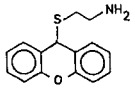
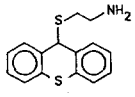
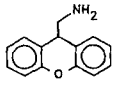
**Table 2:** $^1$H-NMR spectral data of nitrosoureas

| No | $^1$H NMR[a] |
|---|---|
| 8a[b] | 3.55 (t, 2H, $NCH_2CH_2$, J=5.7), 4.22 (t, 2H, $NCH_2CH_2$, J=5.7), 6.62 (d, 1H, H-9, J=8.5), 7.10-7.22 (m, 3H, ArH), 7.22-7.42 (m, 3H, ArH), 7.42-7.61 (m, 2H, ArH), 9.85 (d, 1H, NH, J=8.5). |
| 8b | 2.28 (s, 3H, $CH_3$), 3.67 (t, 2H, $NCH_2CH_2$, J=6), 4.16 (t, 2H, $NCH_2CH_2$, J=6), 6.37 (d, 1H, H-9, J=8.4), 6.97-7.22 (m, 4H, ArH), 7.22-7.40 (m, 2H, ArH), 7.40-7.52 (m, 1H, ArH), 9.81 (d, 1H, NH, J=8.4). |
| 8c | 2.40 (s, 3H, $CH_3$), 3.66 (t, 2H, $NCH_2CH_2$, J=6), 4.14 (t, 2H, $NCH_2CH_2$, J=6), 6.38 (d, 1H, H-9, J=9), 6.97-7.26 (m, 4H, ArH), 7.26-7.43 (m, 2H, ArH), 7.43-7.53 (d, 1H, ArH), 9.80 (d, 1H, NH, J=9). |
| 8d | 2.20 (s, 3H, $CH_3$), 2.29 (s, 3H, $CH_3$), 3.62 (t, 2H, $NCH_2CH_2$, J=6), 4.10 (t, 2H, $NCH_2CH_2$, J=6), 6.32 (d, 1H, H-9, J=9), 6.84-7.20 (m, 4H, ArH), 7.20-7.48 (m, 2H, ArH), 9.76 (d, 1H, NH, J=9). |
| 8e | 3.63 (t, 2H, $NCH_2CH_2$, J=6), 3.70 (s, 3H, $OCH_3$), 4.12 (t, 2H, $NCH_2CH_2$, J=6), 6.38 (d, 1H, H-9, J=9), 6.82-7.02 (m, 2H, ArH), 7.02-7.22 (m, 3H, ArH), 7.22-7.41 (m, 1H, ArH), 7.41-7.51 (m, 1H, ArH), 9.85 (d, 1H, NH, J=9). |
| 8f | 1.30 (t, 3H, $OCH_2CH_3$, J=7), 3.66 (t, 2H, $NCH_2CH_2$ , J=6), 3.97 (q, 2H, $OCH_2CH_3$, J=7), 4.14 (t, 2H, $NCH_2CH_2$ , J=6), 6.37 (d, 1H, H-9, J=9), 6.90-7.00 (m, 2H, ArH), 7.06-7.20 (m, 3H, ArH), 7.30-7.38 (m, 1H, ArH), 7.42-7.49 (m, 1H, ArH), 9.81 (d, 1H, NH, J=9). |
| 8g | 3.67 (t, 2H, $NCH_2CH_2$ , J=6), 3.91 (s, 3H, $OCH_3$), 4.14 (t, 2H, $NCH_2CH_2$, J=6), 6.41 (d, 1H, H-9, J=9), 7.00-7.30 (m, 5H, ArH), 7.30-7.54 (m, 2H, ArH), 9.84 (d, 1H, NH, J=9). |
| 8h | 3.72 (t, 2H, $NCH_2CH_2$, J=6), 4.18 (t, 2H, $NCH_2CH_2$, J=6), 5.88 (d, 1H, H-9, J=9), 7.25-7.45 (m, 4H, ArH ), 7.47-7.70 (m, 4H, ArH), 9.89 (d, 1H, NH, J=9). |
| 8i | 2.33 (s, 3H, $CH_3$), 3.73 (t, 2H, $NCH_2CH_2$, J=6), 4.22 (t, 2H, $NCH_2CH_2$, J=6), 5.87 (d, 1H, H-9, J=9), 7.11-7.62 (m, 7H, ArH), 9.91 (d, 1H, NH, J=9). |
| 8j | 2.48 (s, 3H, $CH_3$), 3.42 (t, 2H, $NCH_2CH_2$, J=6), 4.11 (t, 2H, $NCH_2CH_2$, J=6), 6.33 (d, 1H, H-9, J=9), 7.09-7.72 (m, 7H, ArH). |
| 8k[b] | 2.57 (t, 2H, $SCH_2CH_2$, J=6), 3.30 (q, 2H, $SCH_2CH_2$, J=6), 3.45 (t, 2H, $NCH_2CH_2$, J=6), 4.12 (t, 2H, $NCH_2CH_2$, J=6), 5.34 (s, 1H, H-9), 6.90 (br t, 1H, NH), 7.02-7.52 (m, 8H, ArH). |
| 8l[b] | 2.61 (t, 2H, $SCH_2CH_2$, J=6), 3.12 (q, 2H, $SCH_2CH_2$, J=6), 3.45 (t, 2H, $NCH_2CH_2$, J=6), 4.15 (t, 2H, $NCH_2CH_2$, J=6), 5.38 (s, 1H, H-9), 7.11 (br t, 1H, NH), 7.22-7.60 (m, 8H, ArH). |
| 8m[b] | 3.45 (t, 2H, $NCH_2CH_2$, J=6), 3.83 (m, 2H, $CH_2NHCO$), 4.27 (t, 2H, $NCH_2CH_2$, J=6), 4.43 (t, 1H, H-9, J=5.7), 7.08 (br t, 1H, NH), 7.17-7.50 (m, 8H, ArH). |

[a] In DMSO (270 MHz). [b] in $CDCl_3$ (200 MHz)

**Table 3.** Amines **5a-j**[a], **6a-b**[b], **7**[b].



| Compd | $R_1$ | $R_2$ | X | Method | mp (°C) | Yield (%) | Formula[c] |
|---|---|---|---|---|---|---|---|
| 5a | H | H | O | H | 153-154[d] | 70 | $C_{15}H_{15}NO_3$ |
| 5b | $CH_3$ | H | O | H | 145-147 | 62 | $C_{16}H_{17}NO_3$ |
| 5c | H | $CH_3$ | O | H | 133-135 | 60 | $C_{16}H_{17}NO_3$ |
| 5d | $CH_3$ | $CH_3$ | O | H | 123-124 | 57 | $C_{17}H_{19}NO_3$ |
| 5e | $OCH_3$ | H | O | H | 134-136 | 61 | $C_{16}H_{17}NO_4$ |
| 5f | $OC_2H_5$ | H | O | H | 128-130 | 60 | $C_{17}H_{19}NO_4$ |
| 5g | H | $OCH_3$ | O | H | 137-139 | 57 | $C_{16}H_{17}NO_4$ |
| 5h | H | H | S | H | 152-155[d] | 71 | $C_{15}H_{15}NO_2S$ |
| 5i | $CH_3$ | H | S | H | 123-125 | 74 | $C_{16}H_{17}NO_2S$ |
| 5j | H | $CH_3$ | S | H | 152-155 | 61 | $C_{16}H_{17}NO_2S$ |
| 6a |  | | | I | 189-191[e] | 55 | $C_{15}H_{16}ClNO$ |
| 6b |  | | | I | 222-224 | 65 | $C_{15}H_{16}ClNS$ |
| 7 |  | | | J | 235-236[f] | 45 | $C_{14}H_{14}ClNO$ |

[a] $CH_3COOH$ salts (from hexane). [b] HCl salts (from ether). [c] Anal.: (C, H). [d] ref 12. [e] ref. 13. [f] ref. 14.

The $^1$H-NMR spectra of nitrosoureas **8a-m** (Table 2) showed the $CH_2CH_2Cl$ group signals as triplets. H-9 of 9*H*-xanthene and 9*H*-thioxanthene couples with the NH proton (compds. **8a-j**). The -NH signal disappeared and the H-9 signal became a singlet upon addition of $D_2O$. In the $^1$H-NMR spectra of **8k-m**, NH (exchangeable with $D_2O$) couples with the $CH_2N$ hydrogens.

## *Pharmacological results and discussion*

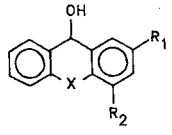
Compounds **8a-m** were tested against NSCLCN6 L16 (bronchial epidermoid carcinoma cell line of human origin)[18,19]; results: Table 9. Cytostatic activity is considered to be very interesting when $IC_{50} \leq 10$ µg/ml. Although the nitrosoureas (*e.g.* CCNU) which are in clinical use were inactive in this model[20], eight of the tested compounds were active. These are mainly derivatives of 9*H*-xanthene either substituted with $CH_3$ (**8b**, **8c**) or $OCH_3$ (**8e**, **8g**) or without any substituents (**8a**,**8m**). Derivatives with $OC_2H_5$ (**8f**) or two $CH_3$ (**8d**) groups were inactive. The 9*H*-thioxanthene derivatives **8h-k** were also inactive with the exception of derivative **8l**.

**Table 4:** Spectral data of amines

| No | IR[a] (cm$^{-1}$) ($\nu NH_3^+$) | $^1$H NMR[b] |
|---|---|---|
| 5b | 2170 | 1.97 (s, 3H, $CH_3COO^-$), 2.40 (s, 3H, $CH_3$), 5.02 (s, 1H, H-9), 6.80-8.02 (m, 7H, ArH). |
| 5c | 3400, 2180 | 1.88 (s, 3H, $CH_3COO^-$), 2.36 (s, 3H, $CH_3$), 5.00 (s, 1H, H-9), 6.98-7.66 (m, 7H, ArH). |
| 5d | 2185 | 2.02 (s, 3H, $CH_3COO^-$), 2.47 (s, 6H, 2x$CH_3$), 5.00 (s, 1H, H-9), 7.00-8.50 (m, 6H, ArH). |
| 5e | 3380, 2180 | 2.00 (s, 3H, $CH_3COO^-$), 3.98 (s, 3H, $OCH_3$), 5.00 (s, 1H, H-9), 6.90-8.20 (m, 7H, ArH). |
| 5f | 3300, 2150 | 1.40 (t, 3H, $OCH_2CH_3$, J=7), 1.84 (s, 3H, $CH_3COO^-$), 4.00 (q, 2H, $OCH_2CH_3$, J=7), 4.95 (s, 1H, H-9), 6.75-7.70 (m, 7H, ArH) |
| 5g | 3410, 2150 | 1.90 (s, 3H, $CH_3COO^-$), 3.85 (s, 3H, $OCH_3$), 5.00 (s, 1H, H-9), 6.95-7.70 (m, 7H, ArH). |
| 5i | 3410, 2163 | 2.02 (s, 3H, $CH_3COO^-$), 2.48 (s, 3H, $CH_3$), 4.98 (s, 1H, H-9), 7.20-8.25 (m, 7H, ArH). |
| 5j | 3410, 2200 | 1.95 (s, 3H, $CH_3COO^-$), 2.50 (s, 3H, $CH_3$), 4.75 (s, 1H, H-9), 6.95-8.20 (m, 7H, ArH). |
| 6b | 3420 | 2.52 (m, 2H, $SCH_2CH_2$), 2.92 (m, 2H, $SCH_2CH_2$), 5.68 (s, 1H, H-9), 7.30-7.60 (m, 8H, ArH). |

[a] KBr.- [b] DMSO-$d_6$ (200 MHz).

**Table 5:** 9*H*-xanthydrols, 9*H*-thioxanthydrols **3a-i**



| Compd | $R_1$ | $R_2$ | X | mp[a] (°C) | Yield (%) | Formula[b] |
|---|---|---|---|---|---|---|
| 3a | $CH_3$ | H | O | 79-82 | 96 | $C_{14}H_{12}O_2$ |
| 3b | H | $CH_3$ | O | 89-92 | 94 | $C_{14}H_{12}O_2$ |
| 3c | $CH_3$ | $CH_3$ | O | 92-94 | 96 | $C_{15}H_{14}O_2$ |
| 3d | $OCH_3$ | H | O | 104-105 | 94 | $C_{14}H_{12}O_3$ |
| 3e | $OC_2H_5$ | H | O | 98-100 | 95 | $C_{15}H_{14}O_3$ |
| 3f | H | $OCH_3$ | O | 125-128 | 83 | $C_{14}H_{12}O_3$ |
| 3g | H | H | S | 96-97[c] | 94 | $C_{13}H_{10}OS$ |
| 3h | $CH_3$ | H | S | 89-90[c] | 91 | $C_{14}H_{12}OS$ |
| 3i | H | $CH_3$ | S | 102-104 | 92 | $C_{14}H_{12}OS$ |

[a] From benzene-petroleum ether. [b] Anal.: (C, H). [c] ref 17.

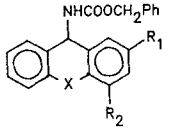
One of the most active compounds (**8e**) was tested *in vivo* versus P388 lymphocytic leukemia in mice. Antitumor activity was expressed as T/C ratio, where T is the median survival time of treated mice, and C is the median survival time of control mice. A drug is considered to be active when T/C(%) > 130%[21]. Compound **8e** increased the median survival time of mice with a T/C% maximum of 163 between 100 and 150 mg/kg. The median survival time of the test mice was compared to that of the control mice and a confirmed increased life span (ILS) of 63 was found for doses between 100 and 150 mg/ml.

**Table 6:** Spectral data of xanthydrols - thioxanthydrols

| No | IR[a] ($cm^{-1}$) (νOH) | $^1$H NMR[b] |
|---|---|---|
| 3a | 3320-3230 | 2.28 (d, 1H, OH, J=9), 2.41 (s, 3H, $CH_3$), 5.85 (d, 1H, H-9, J=9), 7.03-7.88 (m, 7H, ArH). |
| 3b | 3360-3260 | 2.48 (s, 3H, $CH_3$), 2.57 (d, 1H, OH, J=8.8), 5.93 (d, 1H, H-9, J=8.8), 6.95-7.98 (m, 7H, ArH). |
| 3c | 3320-3220 | 2.08 (d, 1H, OH, J=9), 2.37 (s, 3H, $CH_3$), 2.47 (s, 3H, $CH_3$), 5.92 (d, 1H, H-9, J=9), 7.15-7.97 (m, 6H, ArH). |
| 3d | 3320-3220 | 2.40 (d, 1H, OH, J=9), 3.89 (s, 3H, $OCH_3$), 5.88 (d, 1H, H-9, J=9), 6.92-7.91 (m, 7H, ArH). |
| 3e | 3300-3200 | 1.45 (t, 3H, $OCH_2CH_3$, J=7), 2.20 (d, 1H, OH, J=9), 4.16 (q, 2H, $OCH_2CH_3$, J=7), 5.94 (d, 1H, H-9, J=9), 6.94-8.00 (m, 7H, ArH). |
| 3f | 3490-3280 | 2.42 (d, 1H, OH, J=9), 3.90 (s, 3H, $OCH_3$), 5.78 (d, 1H, H-9, J=9), 6.80-6.90 (m, 1H, ArH), 7.00-7.39 (m, 5H, ArH), 7.50-7.62 (m, 1H, ArH). |
| 3i | 3300-3200 | 2.50 (s, 3H, $CH_3$), 2.62 (d, 1H, OH, J=9), 5.65 (d, 1H, H-9, J=9), 7.05-7.95 (m, 7H, ArH). |

[a] Nujol mulls. [b] $CDCl_3$ (200 MHz).

**Table 7:** Benzyl carbamates **4a-j**




| Compd | $R_1$ | $R_2$ | X | mp[a] (°C) | Yield (%) | Formula[b] |
|---|---|---|---|---|---|---|
| 4a | H | H | O | 165-166[c] | 85 | $C_{21}H_{17}NO_3$ |
| 4b | $CH_3$ | H | O | 158-159 | 88 | $C_{22}H_{19}NO_3$ |
| 4c | H | $CH_3$ | O | 153-155 | 65 | $C_{22}H_{19}NO_3$ |
| 4d | $CH_3$ | $CH_3$ | O | 174-176 | 65 | $C_{23}H_{21}NO_3$ |
| 4e | $OCH_3$ | H | O | 127-129 | 85 | $C_{22}H_{19}NO_4$ |
| 4f | $OC_2H_5$ | H | O | 146-148 | 86 | $C_{23}H_{21}NO_4$ |
| 4g | H | $OCH_3$ | O | 132-134 | 65 | $C_{22}H_{19}NO_4$ |
| 4h | H | H | S | 134-135[c] | 68 | $C_{21}H_{17}NO_2S$ |
| 4i | $CH_3$ | H | S | 101-103 | 75 | $C_{22}H_{19}NO_2S$ |
| 4j | H | $CH_3$ | S | 117-120 | 79 | $C_{22}H_{19}NO_2S$ |

[a] From ethanol. [b] Anal.: (C, H). [c] ref. 12.

The appearance of this therapeutic plateau between 100 and 150 mg/kg for compound **8e** and its weak toxicity in *in vivo* experiments, make this product very interesting in comparison with the nitrosoureas which are in clinical use and are characterised by an extremely high toxicity and very often by quick degradation (ref.[1;2]).

We express our appreciation to Dr. *L. Skaltsounis* and Dr. *A. Tsotinis* for mass spectral and $^1$H-NMR measurements.

## Experimental Part

*Chemistry*

Melting points: Büchi apparatus, uncorrected.- $^1$H-NMR spectra: $CDCl_3$ or DMSO-$d_6$, Bruker AC-200 (200 MHz) or Bruker HX-270 (270 MHz) spectrometer, ppm (δ units) downfield of internal $Me_4Si$. J in Hz.- IR-spectra: Perkin Elmer 388 spectrometer.- UV-spectra: Perkin Elmer Lambda-7 spectrometer.- Mass spectra: Nermag R 1010 C instrument.- Elemental analyses: Service Central d'Analyse (CNRS France), Analyses indicated by the symbols of the elements were within ± 0.4% of theoretical values.

**Table 8:** Spectral data of benzyl carbamates

| No | IR[a] ($cm^{-1}$) (νNH), (νCO) | $^1$H NMR[b] |
|---|---|---|
| 4b | 3316, 1684 | 2.43 (s, 3H, $CH_3$), 5.41 (s, 2H, $CH_2Ph$), 5.47 (d, 1H, NH, J=10), 6.39 (d, 1H, H-9, J=10), 7.10-8.00 (m, 12H, ArH). |
| 4c | 3290, 1680 | 2.39 (s, 3H, $CH_3$), 5.18 (s, 2H, $CH_2Ph$), 5.22 (d, 1H, NH, J=9.8), 6.21 (d, 1H, H-9, J=9.8), 6.97-7.21 (m, 4H, ArH), 7.26-7.40 (m, 7H, ArH), 7.54 (d, 1H, ArH, J=7). |

**Table 9**: In vitro activity of **8a-m** against NSCLCN6 L16.

| Compd. | $IC_{50}$[a] (μg/ml) |
|---|---|
| CCNU | >10[a] |
| thiotepa | 9.4±1.5 |
| 8a | 3.5±1.6 |
| 8b | 7.0±1.5 |
| 8c | 3.4±1.5 |
| 8d | >10[b] |
| 8e | 3.5±0.8 |
| 8f | >10[b] |
| 8g | 2.6±1.8 |
| 8h | >10[b] |
| 8i | >10[b] |
| 8j | >10[b] |
| 8k | >10[b] |
| 8l | 7.6±1.4 |
| 8m | 6.7±1.3 |

[a] $IC_{50}$: Concentration required to reduce cellular growth by 50%
[b] inactive

*2-(4-Methylphenoxy)benzoic acid* (**1a**) *(Method A)*

Sodium (2.10 g, 0.09 mol) was dissolved in 35 mL of anhydrous MeOH and 7 g (0.045 mol) of *o*-chlorobenzoic acid were added followed by 9.72 g (0.090 mol) of *p*-cresol and catalytical amount of $Cu_2O$. MeOH was removed by distillation and the residue was heated at 180°C for 1 h, cooled to 100°C, and poured into ice. The solution was extracted several times with $Et_2O$ and the aqueous layer was acidified with 10% HCl, cooled and filtered. The solid was washed with $H_2O$, dried, and recrystallized from benzene/petroleum ether. Yield 95%, mp. 117-118°C (lit.[15]).
The following compounds were prepared similarly.

*2-(2-Methylphenoxy)benzoic acid* (**1b**)

Yield 82%, mp. 131-134°C (lit.[15]).

*2-(2,4-Dimethylphenoxy)benzoic acid* (**1c**)

Yield 69%, mp. 150-152°C (lit.[15]).

*2-(4-Methoxyphenoxy)benzoic acid* (**1d**)

Yield 50%, mp. 140-142°C (lit.[15]).

*2-(2-Methoxyphenoxy)benzoic acid* (**1e**)

Yield 42%, mp. 110-112°C (lit.[15]).

*2-(4-Methylphenylthio)benzoic acid* (**1f**) *(Method B)*

*o*-Thiosalicylic acid (5 g, 0.032 mol), anhydrous $K_2CO_3$ (6.91 g, 0.050 mol), KI (0.30 g), copper bronze (0.33 g) and DMF (90 mL) were refluxed for 18 h. The mixture was poured into ice-water and filtered. The solution was extracted several times with $Et_2O$ and treated as described in method *A*. Yield 87%, mp. 212-215°C (lit.[16]).

*2-(2-Methylphenylthio)benzoic acid* (**1g**) Yield 88%, mp. 171-174°C.- $^1$H-NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.45 (s; 3H, $CH_3$), 6.87-7.00 (m; 1H, ArH), 7.25-8.00 (m; 6H, ArH), 8.25-8.60 (m; 1H, ArH), 10.55 (br s; 1H, COOH).- Anal.: (C,H).

*2-Methylxanthone* (**2a**) *(Method C)*

A mixture of 2-(4-methylphenoxy)benzoic acid and 10 times its weight of conc. $H_2SO_4$ was heated with stirring in a water bath for 1 h and poured into ice-water. The precipitate was collected, treated with N-$Na_2CO_3$, washed with water and recrystallized from ethanol. Yield 75%, mp. 119-120°C (lit.[15]).

The following compounds were prepared similarly:

*4-Methylxanthone* (**2b**)

Yield 82%, mp. 122-125°C (lit.[15]).

*2,4-Dimethylxanthone* (**2c**)

Yield 76%, mp. 150-152°C (lit.[15]).

*2-Methylthioxanthone* (*2g*)

Yield 69%, mp. 120-123°C (lit.[16]).

*4-Methylthioxanthone* (**2h**)

Yield 85%, mp. 145-147°C.- $^1$H-NMR (200 MHz, $CDCl_3$): δ (ppm) = 2.65 (s; 3H, $CH_3$), 7.53-8.10 (m; 5H, ArH), 8.80-9.17 (m; 2H, ArH). Anal.: (C,H).

*2-Methoxyxanthone* (**2d**) *(Method D)*

A mixture of 10 g of 2-(4-methoxyphenoxy)benzoic acid (0.059 mol), $Ac_2O$ (100 mL) and conc. $H_2SO_4$ (1 mL) was heated as described in method *C*. Yield 75%, mp. 130-131°C (lit.[15]).

*2-Ethoxyxanthone* (**2e**) *(Method E)*

A mixture of 2-hydroxyxanthone (4.2 g, 0.020 mol), EtBr (2.59 g, 1.77 mL, 0.024 mol), anhydrous $K_2CO_3$ (13.8 g, 0.1 mol) and dry acetone (140 mL) was stirred and heated at reflux for 6 h. The mixture was filtered and the inorganic material was washed with hot acetone. The solvent was removed under reduced pressure and the residue was crystallized from ethanol to give 2-ethoxyxanthone. Yield 67%, mp. 116-118°C.- $^1$H-NMR (200 MHz, $CDCl_3$) δ (ppm) = 1.45 (t; 3H, $OCH_2\underline{CH_3}$, J = 7), 4.13 (q; 2H, $OC\underline{H_2}CH_3$, J = 7), 7.24-7.50 (m; 4H, ArH), 7.62-7.78 (m; 2H, ArH), 8.28-8.35 (m; 1H, ArH). Anal.: (C,H).

*2-Methylxanthydrol* (**3a**) *(Method F)*

A mixture of 375 g (1.87 mol) of Hg, 13.8 g (0.065 mol) of 2-methylxanthone and 80 mL of 95% EtOH was placed in a pressure bottle. Small pieces of Na (4.6 g, 0.2 mol) were added during a period of 15 min while the mixture was being shaken. After the mixture had been shaken mechanically for an additional 30 min the alcohol layer was decanted. The mercury amalgam was washed with two 15 mL portions of hot EtOH and the combined alcoholic solutions were filtered and poured into ice-water. The precipitate was collected, dried and recrystallized.

*N-(9H-Xanthen-9-yl) benzyl carbamate* (**4a**) *(Method G)*

A solution of 2.18 g (0.011 mol) of xanthydrol and 2.0 g (0.013 mol) of benzyl carbamate in 25 mL of glacial AcOH was stirred at room temp. overnight and poured into ice-water (150 ml). The precipitate was dried and recrystallized.

*9H-Xanthene-9-amine ($CH_3COOH$ salt)* (**5a**) *(Method H)*

The solution of KOH (5.0 g, 0.09 mol) in EtOH (50 mL) and 0.005 mol of the carbamate **4a** was heated for 12 h, cooled and concentrated *i.vac.* to a volume of ca. 15 mL. The concentrate was shaken with 25 mL of $H_2O$ and extracted with $Et_2O$. The org. layer was washed with $H_2O$, dried ($Na_2SO_4$) and concentrated *i.vac.*. The residual oil was dissolved in 200 mL of hexane and 1 mL of AcOH was added slowly with constant stirring. The precipitate was collected, washed with hexane and dried.

*9-[2-(Aminoethyl)thio]-9H-xanthene hydrochloride* (**6a**) *(Method I)*

A mixture of xanthydrol (10.9 g, 0.055 mol), cysteamine hydrochloride (6.25 g, 0.055 mol) and $CH_3CN$ (250 mL) was heated at reflux for 2 h. The solution was concentrated *i.vac.* and the residue was crystallized from EtOH/$Et_2O$.

*9H-Xanthene-9-methanamine* (**7**) *(Method J)*

9*H*-xanthene-9-carboxamide (2.25 g, 0.01 mol) [from 9*H*-xanthene-9-carboxylic acid (2.8 g, 0.012 mol)] was reduced with $LiAlH_4$ (1.90 g, 0.05 mol) in 200 mL of $C_6H_6$/$Et_2O$ (1/1). The mixture was refluxed for 24 h and treated as usual to give the 9*H*-xanthene-9-methanamine.

*N-(2-Chloroethyl)-N'-(9H-xanthen-9-yl)-N-nitrosourea* (**8a**) *(Method K)*

To a cooled (0°C) solution of DMF (5 mL) containing *N*-[*N*-(2-chloroethyl)-*N*-nitrosocarbamoyloxy]-succinimide (2.74 g, 0.011 mol) was added, under stirring, 9*H*-xanthene-9-amine (2.16 g, 0.011 mol). The mixture was stirred at 0°C overnight and poured into ice-water. The water was decanted carefully and the residue was dissolved in $Et_2O$, washed several times with $H_2O$, dried ($Na_2SO_4$) and concentrated *i.vac.*. The residue was crystallized slowly from ether/pentane.

*Pharmacology*

*In vitro* study: Cell line NSCLCN6 L16 bronchial epidermoid carcinoma.

This cell line was established from the tumor of a 67 year old man with a 45 pack/year history of smoking as described by *Roussakis* et al.[18,19].

Pharmacological tests were done in 96 hole microplates Falcon 3072 (flat bottom microtest III plate with lid). Cells 0.07 x $10^5$ were dropped in each hole containing 50 µl of RPMI (Gibco) medium supplement with 5% fetal calf serum and 2 mM glutamin. 50 µl of the solution to be tested were added in decreasing concentrations at the ratio of two holes for each dose. The microtest plates were incubated for 72 h at 37°C in 5% $CO_2$-air. Cell proliferation was estimated by a colorimetric method[22]. 10 µl of M.T.T.([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl] tetrazolium bromide) were added; after 4 h the dark blue crystals which formed during the reduction of the M.T.T. with dehydrogenase in the mitochondria of the living cells, were made soluble with 100 µl of isopropanol.

Microplates were read by ELISA using a multiskanner (Titertek multiskan MK II) with 570 nm filter. The optical density of each hole led to a dose/effect curve and the $IC_{50}$ (inhibition concentration for 50% of cell growth) to be determined for each product relative to controls (8 holes with 0.07 x $10^5$ cells in 100 µl of medium).

*In vivo* study: Lymphocytic leucemia P 388.

The P 388 leucemia model is used as the prescreen because it is sensitive to many different types of chemical structures. For testing, $10^6$ lymphocytic leucemia mouse cells are implanted intraperitoneally into $DBA_2F_1$ mice (20 to 22 g) on day 0, and compound **8e** is administered intraperitoneally on day 1, day 5 and day 9 treatment schedule.

## References


1 S.K. Carter, F.M. Schabel Jr., L.E. Broder, and T.P. Johnston, Adv. Cancer Res. *16*, 273 (1972).
2 R.J. Weinkam and H.S. Lin, Adv. Pharmacol. Chemother. *19*, 1 (1982).
3 E. Hirschberg, A. Gellhorn, M.R. Murray, and E.F. Elslager, J. Natl. Cancer Inst. *22*, 567 (1959).
4 J. Martinez, J. Oiry, J. Imbach, and F. Winternitz, J. Med. Chem. *25*, 178 (1982).
5 J.R. Pfister, R.W. Ferraseri, I.T. Harisson, W.H. Rooks, A.P. Roszkowski, A. Van Horn, and J.H. Fried, J. Med. Chem. *15*, 1032 (1972).
6 S. Archer, K.J. Miller, R. Rej, C. Periana, and L. Fricker, J. Med. Chem. *25*, 220 (1982).
7 F.L. Allen, P. Koch, and H. Suschitzky, Tetrahedron *6*, 315 (1959).
8 A.A. Goldberg and A.H. Wragg, J. Chem. Soc. *1958*, 4227.
9 J.S.H. Davies, F. Lamb and H. Suschitzky, J. Chem. Soc. *1958*, 1790.
10 A.F. Hollemann, Org. Synth., Coll. Vol. I, pp. 554-555, Sec. Edition, Ed. by A.H. Blatt, John Willey & Sons, Inc., New York, 1947.
11 A.A. Goldberg and A.H. Wragg, J. Chem. Soc. *1957*, 5120.
12 J.E. Ollmann and D.T. Witiak, J. Org. Chem. *39*, 1589 (1974).
13 J.A. Bristol, E.H. Gold, I. Gross, and R.G. Lovey, J. Med. Chem. *24*, 1010 (1981).
14 V. Evdokimoff and A. Calo, Ann. Chim. (Rome) *49*, 1321 (1959); C.A. *54*, 8803g (1960).
15 F. Ullmann and M. Zlokasoff, Ber. Dtsch. Chem. Ges. *38*, 2111 (1905).
16 F. Ullmann and V. Glenck, Ber. Dtsch. Chem. Ges. *49*, 2491 (1916).
17 C. Angelini, G. Grandolini, and L. Mignini, Ann. Chim. (Rome) *46*, 235 (1956); C.A. *51*, 369d (1975).
18 C. Roussakis, G. Dadouis, C. Gratas et al., 5th NCI-EORTC symposium on new drugs in cancer therapy, Abstract n° 248 (1986); C.A. 105, 218477u (1986).
19 C. Gratas, N. Robillard and C. Roussakis, Biol. Cell *68*, 133 (1990).
20 R.T. Eagan, D.T. Carr, D.T. Coles, D.E. Dines and R.E. Ritts, Cancer Chemother. Rep. *58*, Part 1, 913, NCI Editions, Bethesda, Maryland, (1974).
21 G.I. Geran, N.H. Greenberg, M.M. Macdonald, A.M. Schumaker, and B.J. Abbott, Cancer Chemotherap. Rep. 2, 7 (1972).
22 T. Mosmann, J. Immunol. Methods *65*, 55 (1983).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