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# Triazene Derivatives. I. Sulfaguanidine as a Carrier for Substituted Triazeno Group*

## Triazen Türevleri I. Sülfaguanidin Molekülünde Substitüe Triazen Yapılarının Oluşturulması

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Most compounds which contain a di-2-chloroethyl amino group possessing a certain level of chemical reactivity and a number of substituted triazeno derivatives are cytotoxic to proliferating tissues [5, 6, 7, 8, 12, 14, 15, 17, 18, 13]

However a number of such compounds have limited use as chemotherapeutic agents because of the lack of specific action on malignant growths, also many normal proliferating tissues are equally affected by most of the derivatives.

It was shown by DANIELLI (Nature **170,** 863, 1952) that the cells may be highly selective in the types of molecule which they concantrate within their plasma membranes. Following this many authors tried to incorporate the di-2-choloroethyl amino group into molecules of different physical and chemical properties varying in their anionic, cationic, liphophilic and hydrophilic character as well as trying to have different structures permitting the chlorine atom to hydrolize in different rates[6]- Manv others have tried to incorporate triazeno moiety into suitable carrier molecules and some others have tried to combine triazeno structure with di-2-chloro ethyl moietv to increase the activity [13, 15, 19, 20]

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*** This work is the first of a series of research with triazene derivatives.**

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Besides the compounds such as Nitrogen Mustard (NSC- 762), Cyclophosphamide (NSC-26271), Melphalan (NSC-8806), Chlorambucil (NSC- 3088) Imidazole Mustard (NSC- 82196), BCNU (NSC-409962), CCNU (NSC-79037), many others have been prepared and tried for antitumor activity among which only the ones carrying either sulfamide structure or triazenc structure concern the present research.

Triazeno derivatives of various ring systems such as substituted imidazoles[13,] and pyrazoles[18] both carrying either carboxamide or carboxilic acid groups have been synthesized and a number of these have displayed antineoplastic activity among which Imidazole Mustard (NSC - 82196) has been clinically accepted. ELKS and HEY[5] have prepared a number of l-aryl-3,3-dimethyl triazenes from aniline, m-and p-nitro aniline, anthranilic acid, methyl anthranilate 2-naphtylamine,4-amino phtalimide, methyl-4-amino phtalate and 5-amino quinoline. LECOCQ, has synthesized some compounds of the type sulfanilic acid and thioanisidine, carrying di-2-choloroethyl amino function at the para position and also p-di-N, N-2-chloroethyl or hydroxy ethyl sulfamoil aniline. The antibacterial activity of these compounds were found inferior than that of sulfanilamide. The analogues of the latter compound have also been been prepared by Ross and Wilson[11] replacing the p-amino function with various groups. Again by replacing p- amino function by several others Boucherle et al[2] and Di Modica and Angeletti[4] have prepared several sulfonic and sulfanilic acid derivatives. Benn et al.[1] have prepared derivatives of p-di-2-chloroethyl amino substituted sulfanilic acid esters and sulfonamides. Pearison, Holland and Midget[10] using the method of Elks and Hey, have synthesized some triazeno derivatives of sulphanilamide carrying 3,3-di(2— hydroxyethyl), diethyl, cyclopentyl, piperidine, ethoxyethyl, morpholino groups and also triazeno derivatives of sulfadiazine carrying diethyl and cyclopentyl groups. Then assayed the antibacterial activities of these compounds coming to the conclusion that the antibacterial affect is slight in comparison with sulfanilamide.

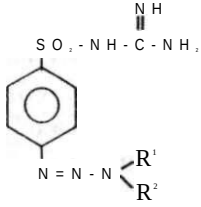
Considering that the antineoplastic activity- is born by the substituted triazeno groups but heterocyclic moiety provides the molecules a high selectivity to be concentrated in the plasma membranes[18] along

with the fact that either amide or ester moiety increases the antileukemic activity when tested in standard mouse L-1210 leukemia assay, it has been though to incorporate triazeno group into some suitable molecule which has already been proved to involve in metabolic processes. Since sulfonamides posses the amide moiety and have been proved to involve in metabolic processes with little toxic side effects they meet the requirements mentioned above. Additionally Ross et al.[3] have shown that sulfonamides deposit selectively in the neoplastic tissues because the pH of such tissues is lower then most of the normal tissues due to the high rate production of lactic acid from glucose under under aerobic conditions[3] et ref. loc. cit.

After all the considerations mentioned above, one of the sulfonamides, sulfaguanidine was selected as a carrier for substituted triazeno group.

The compounds synthesized are tabulated in Table. I along with their melting points.

**Table I. — The triazeno derivatives of Sulfaguanidine**



| Compound | R | $R^2$ | m.p. C° |
|---|---|---|---|
| I | 2-hydroxy ethyl | 2—hydroxy ethyl | 152 |
| II | ethyl | ethyl | 236 |
| III | — H | 2-hydroxy ethyl | 193 |
| IV | — H | morpholino | 198 |
| V | — H | N-piperidino | 183 |
| VI | — H | cyclohexyl | 222 |
| VII | — H | benzyl | 218 |
| VIII | — H | propyl | 229 |
| IX | cyclohexyl | cyclohexyl | chars at 300 |
| X | — H | phenyl | 210 |
| XI | - H | 2-butyl | 195 |
| XII | — H | t-butyl | 220 |
| XIII | n-butyl | n-butyl | 215 |
| XIV | methyl | methyl | 202 |

Generally the triazeno derivatives of sulfaguanidine are highly insoluble products both in polar and nonpolar solvents but sparingly soluble in ethanol. They are insoluble in aqueous sodium carbonate but but soluble in aqueous sodium hydroxide developing a brown color. They are also soluble in acid medium giving a dark color, but nmr spectrum taken in trifluoroacetic acid has shown decomposition of the structure and loss of alkyl groups situated at the 3 position of triazeno moiety. In the ir spectra of triazeno sulfaguanidines there are two sets of bands. A set of them are steady in all spectra but characteristic of sulfamide structure and the other set of bands are characteristic of triazene structure. These bands are listed in Table.II.

**Table II. The ir bands of the derivatives of triazeno sulfaguanidine**

| | |
|---|---|
| **Steady bands in all derivatives:** | **(in $cm^{-1}$)** |
| **3200 — 3500** | **Generally a trident** |
| **1600 — 1640** | **a fork** |
| **1550 s** | |
| **1250 s** | |
| **1140 — 1150 s** | |
| **1080 — 1100 ms** | |
| **820 — 850 m** | |
| **550 m** | |
| **Characteristic bands of triazenes:** | |
| **1450 — 1470** | **a spike** |
| **1400 — 1410** | **a spike** |
| **1050 — a small spike or a dent** | |
| **730 — 740 a small spike or a dent** | |
| **650 — a spike, a fork or a triple band** | |

The UV spectra of the compounds synthesized are very similar. Generally the bands shift very slightly. The values of the bands are: 208-210 nm the bands shift very slightly. The values of the bands are: 208- 210 max., 226-228 nm min., 236-250 nm max., 268-278 nm min., 350-366 nm max. The tlc of all the derivatives on Silicagel plates with n-Bu OH: AcOH: Wa (5: 2: 3) allways gave one spot having an Rf value of about 0.50 - 0.66. Many of these compounds are being biologically tested. The results are to be published later.

## EXPERIMENTAL

All the solvents used were technical grade except those used for recystallization and spectrometry for which only analytical grade and spectral grade solvents were used. Melting points have been taken with a Mettler FP - 5 instrument. All melting points are uncorrected. UV spectra were taken with a Pye-Unicam SP - 1700 instrument and ır spectra were taken with a Pye - Unicam SP-1100 instrument, nmr spectra were taken with a Perkin Elmer R 32, 90 MHz instrument. For the synthesis of all the derivatives basicly the same ractions have been applied.

### General Method:

Reaction A- In a 1 It flask 40.00 g (0.187 Mol) Sulfaguanidine was dissolved in 170 ml (conc.) HCl , to this solution 400 ml water was added. The flask was kept in an ice-bath. When the inner temperature lowered to 0-5°C sodium nitrite solution (13 g / 50 ml in water and cooled to 0°C) was added dropwise. This addition was carried on till it gave reaction with the KI-starch paper.

Reaction B- When the diazotization reaction was over, 0.3 Mol of the amine derivative which was dissolved in the mininum quantity of water and cooled to 0°C was added to this mixture for the coupling reaction. The product which precipitated was filtered and washed with cold water. During the entire reaction the temperature was kept below 5°C. When possible, the product was recrystallized from ethanol/water and dried in vacuum over sulphuric acid.

## DISCUSSION

One of the major problems in cancer chemotherapy is the difficulty of finding a compound that acts differentially on the cancer tissues but not on normal proliferating tissues. In order to realize such a discrimination it was thought to benefit from the differences between normal and neoplastic tissues considering their biochemical properties, particularly the pH. The fundamental metabolic property of all cancer cells is the production of lactic acid from glucose under aerobic conditions. Consequently the pH of neoplastic tissues has a lower value than most of normal tissues. Therefore one of the ways

in which this lower pH of tumour tissue can be exploited is to utilize this property to accumulate an effective carcinostatic chemical gruop via a carrier of suitable PKa value.

In this research,expecting a favorable carcinostatic activity, sulfaguanidine molecule has been chosen as carrier and substituted triazeno group has been chosen as carcinostatic moiety and they were incorporated.

## SUMMARY

One of the major biochemical property of neoplastic tissues is their low pH value. This property has been taken into consideration to incorporate a well known carcinostatic group, substituted triazeno moiety onto a suitable carrier, sulfaguanidine.

By substitution of triazeno moiety fourteen different molecules have been synthetized. All of the compounds have been characterized by their melting points, Rf value, uv, ir and nmr spectra.

These compounds are being tested for antineoplastic activity, the data are to be published later.

## ÖZET

Kanserli hücrelerin temel biyokimyasal özelliklerinden birisi de düşük pH değerleridir. Bu özellik dikkate alınarak iyi bilinen bir karsinostatik grup, sübstitüe trizeno grubu uygun bir taşıyıcıya, sülfaquanidine takılmıştır.

Triazen yapısının sübstitüsyonu ile 14 değişik molekül sentez edilmiştir. Tüm bileşikler E.n. ları, Rf değerleri, UV, İR ve NMR spektrumları ile tanımlanmışlardır.

Bu bileşikler henüz antineoplastik etki yönünden deneylenmektedir. Elde edilen bilgiler daha sonra yayınlanacaktır.

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Fakülte Profesörler Kurulunun 10.3.1970 tarih ve 358 sayılı Kararı ile Fakülte Mecmuasında yayınlanacak yazılar için tebit edilen esaslar

1) Dergide, başka bir mecmuada aynı isimle ve aynı tarzda neşredilmemiş orijinal çalışmalar yayınlanır.

2) Yazılar Komisyona verildiği tarih sırasıyla yayınlanır.

3) Metin 15 daktilo sayfasını geçmemek üzere Türkçe veya yabancı dilde yazılabilir. Metin başlığı ve özeti Türkçe ve yabancı dilde yazılacaktır.

Yabancı dilde yazılmış başlık, metin ve özetlerin dil kurallarına uygun olmasının temini, yazara aittir.

4) Yazılar, kâğıdın bir yüzüne, daktilo ile ve normal aralıkla yazılmalı, italik yazılacak kelimelerin altı çizilmeli, klişesi yapılacak grafik, şema, formül gibi şekiller, çini mürekkep ile, aydinger kâğıdına çizilmeli; fotoğraflar parlak kâğıda ve kontraslı olarak çekilmelidir. Şekillerin her biri ayrı kâğıtlarda olmalı ve kâğıdın üzerinde yazarın adı, kaçıncı şekil olduğu, resim altı yazılması istenen ibare kaydedilmelidir.

5) Yazı plânı aşağıdaki şekilde olmalıdır: Konunun takdimi, bulgular, denel kısım, münakaşa, Türkçe özet, yabancı dilde özet, literatür.

Konunun takdimi 2 daktilo sahifesini geçmemeli; materyal, metot ve yapılan ameliyeler "denel kısım" da yer almalı, "münakaşa" kısmı, gerekli ise konmalıdır.

Literatür, metinde parentez içindeki numaralarla belirtilmesi ve metin sonunda bu numaralara uygun olarak sıralanmalıdır. Sırasıyla yazarın soaydı, adının ilk harfi, mecmuanın milletlerarası kullanılan kısaltılmış ismi, cilt numarası (italik), sayfa ve parentez içinde tarih yazılmalıdır.

6) Tashihler yazar tarafından yapılacaktır.

7) Yazara 50 ayrı baskı verilir.