

SYNTHESIS AND ANTINEOPLASTIC ACTIVITY OF CERTAIN TRIAZENE AND TRIAZENO-ACRIDINE COMBILIXIN DERIVATIVES

Samir M. El-Moghazy Aly¹, Hanan H. Georgey¹, Mohammed A. Mohammed¹, Nagwa M. Abdel Gawad¹ and Noha H. Amin²

Department of Pharmaceutical Chemistry, Faculty of Pharmacy,
¹Cairo University and ²Bani-Sueif University, Egypt

تمتاز مشتقات الأكردين بفاعليتها كمضادات للسرطان وقد عرفت بتأثيرها الإرتباطي على الحمض النووي وقد ذكر التراث العلمي العديد من تلك المشتقات و خاصة التي تحتوي على مدمج الأكردينو: تريازين و التي أثبتت فاعليته كمضادات للسرطان. لذلك تم في هذا البحث تحضير أربعة سلاسل من ثنائي الفاعلية مدمج الأكردينو: تريازين تم أيضا تحضير ثلاثة سلاسل جديدة من التريازينو: بنزين سلفوناميد كوسيط لتحضير تلك المدمجات.

تم تحضير هذه السلاسل عن طريق تفاعل - كلوروبنزويك أسيد مع انثرانيلك أسيد ليعطي مركب الذي تمت حلقتة إلى أكريدون - كربوكسيلك أسيد بواسطة حمض الكبريتيك. مع ثيونايل كلوريد أدى إلى تكون الذي تم تفاعلة مع - بييرازين - - يل دايزينايل) بنزين سلفوناميد أو مع (مستبدل - (- هيدروكسي إيثيل) بييرازين - (دايزينايل) بنزين سلفوناميد السابق تحضيرهما للحصول على المستبدلات و على التوالي التي استخدمت كوسيط لتشبيد و بموجب تحليلهما المائي أو تشبيد و عن طريق تفاعلها مع السلفوناميدات المختلفة. تم عمل مسح بيولوجي لأربعة عشر مركب ضد السرطان على خلايا الثدي في المعهد القومي للأورام.

Four series of bifunctional ligands have been synthesized as DNA-binding combilexins. These novel agents contain a triazeno-benzene sulfonamide linker moiety that is attached to an intercalating acridine or acridone chromophore by a functionalized amide or ester residue. In order to obtain these combilexins three series of the anticipated antitumor triazeno-benzene sulfonamide were synthesized. The synthesis and bioscreening of the new antineoplastic compounds are depending on the structural correlation with several reported antineoplastic acridines.

2-Chlorobenzoic acid was reacted with anthranilic acid to give N-(2-carboxyphenyl) anthranilic acid which upon cyclodehydration

with sulfuric acid afforded 9-oxo-9,10-dihydroacridine-4-carboxylic acid, (acridone-4-carboxylic acid) **8**. This latter intermediate has been converted to 9-chloroacridine carbonyl chloride **9** using thionyl chloride. Selective substitution of **9** with derivatives of 4-(piperazine-1-yl)diazanyl benzenesulfonamides **4a-e** or derivatives of 4-(2-hydroxyethyl)piperazine-1-yl)diazanyl benzenesulfonamides **5a-e** to yield their 9-chloroacridine-4-carboxamides **10a-e** and 9-chloroacridine-4-carboxylic acid esters **13a-e** respectively. Those intermediates have been reacted either with different sulfonamides to give derivatives of 4-[4-[4-(4-sulfamoylphenyldiazanyl)piperazine-1-carbonyl]-9-ylamino]benzenesulfonamides **11a-h** and derivatives of 2-[(4-(4-sulfamoylphenyl)diazanyl)piperazine-1-yl]ethyl 9-(4-sulfamoylphenylamino)-9,10-dihydroacridine-4-carboxylates **14a-i** respectively or subjected to mild acid hydrolysis to yield derivatives of 4-[4-(9-oxo-9,10-dihydroacridine-4-carbonyl)piperazine-1-yl]diazanyl-benzenesulfonamide **12a-e** and derivatives of 2-[4-(4-sulfamoylphenyl)diazanyl]piperazine-1-yl]ethyl-9-oxo-9,10-dihydroacridine-4-carboxylate **15a-e** respectively.

Besides, the synthesis of derivatives of 4-(piperazine-1-yl)diazanyl benzenesulfonamides **4a-e** and derivatives of 4-(2-hydroxyethyl)piperazine-1-yl)diazanyl benzenesulfonamides **5a-e** has been achieved via diazotization of various substituted benzene sulfonamides with sodium nitrite and hydrochloric acid followed by various amines coupling to yield the target triazeno-benzene sulfonamides.

Fourteen new compounds were selected for screening their antitumor activity against breast cell line in National Cancer Institute. Six of them were found to be active as antitumor agents, while two were found to be mild active.

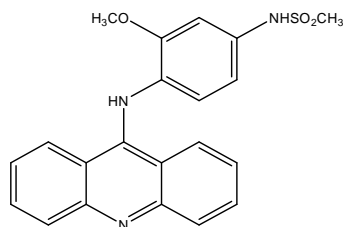
INTRODUCTION

Many publications describe the biological importance of acridine derivatives as antitumor agents^{1&2}, which are related to their ability to intercalate DNA³. The binding energy is obtained by insertion a flat aromatic portion of the nucleus between the base pairs of DNA double helix^{4&5}, that causes DNA

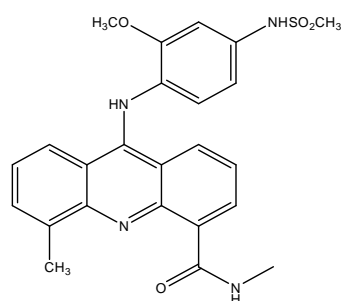
break and chromosome damage⁶. The DNA binding propensities and topoisomerase II-inhibitory activities of acridines have been exploited in the development of clinically-active antitumor agents⁷.

9-Anilinoacridine derivatives have been extensively studied and one congener, namely m-AMAS **I** has become useful in clinical application for treatment of leukemia and

lymphoma^{8&9}. Furthermore, the introduction of 4-carboxamide group such as compound **II** have led to increase in the activity^{10&11}.



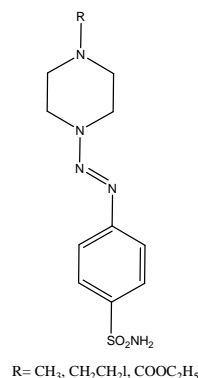
I



II

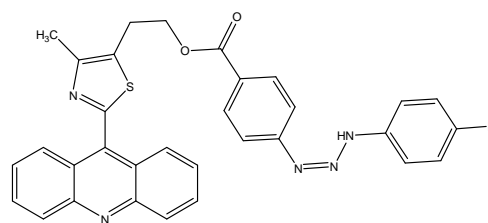
On the other hand, a class of alkylating agents represented by triazene compounds¹²⁻¹⁴, are able to alkylate DNA molecule, acting mainly on the N-7 or O-6 atoms of guanine^{15&16} as diazenopiperazine derivatives **III**^{17&18}. Additionally, the formation of acridine-triazene combilexins **IV** and **V**^{19&20} showed potential antitumor activity.

Based on the aforementioned facts, it was intended to investigate the synthesis of the new triazene derivatives **3-5** and the acridine-triazene combilexin derivatives **11** and **14** have been synthesized



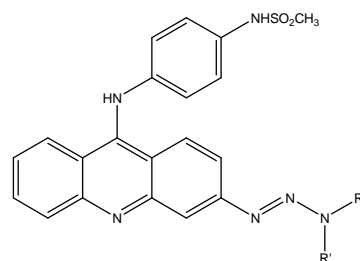
R = CH₃, CH₂CH₃, COOC₂H₅

III



R = CONH₂, CO(CH₂)₂N(1-piperidinyl)

IV



R=R' = methyl, ethyl, propyl

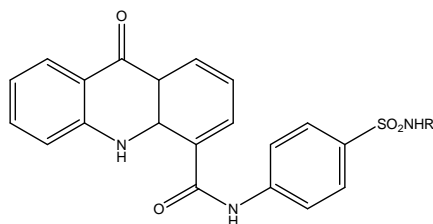
V

containing a triazene moiety, which itself possesses a cytotoxic activity, linked by an amide or ester linkage in the 4-position to the active 9-substituted anilinoacridine analog. Such hybridization between the self active moieties was expected to

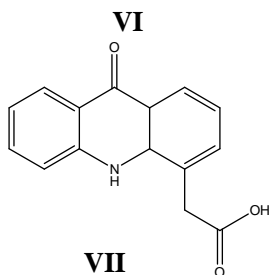
enhance the observed overall antineoplastic profile.

Nevertheless, the presence of a carboxamide side chain, which attached to a terminal ring peri to an electron withdrawing atom in a linear tricyclic chromophore, represents an interesting class of DNA intercalator²¹. Among this, amide derivative **VI** which has shown significant antitumor activity and a subpanel disease selectivity against prostate cancer²². As a result, compounds **12** have been prepared in which the 4-carboxamido moiety of **VI** is replaced by the piperazinocarbonyl function.

Finally, acridone-4-acetic acid **VII** showed significant antitumor activity²³. Consequently, the effect of methyl removal together with the formation of prodrug containing the anticipated antitumor triazenopiperazine side chain have been studied through the synthesis of compound **15**.



R= different heterocycles



CHEMISTRY

The target compounds **3**, **4**, **5**, **11**, **12**, **14**, and **15** were synthesized as outlined in schemes 1, 2 and 3. The synthesis of compounds **3** and **5** has been achieved via diazotization of various substituted benzene sulfonamides followed by coupling with ethoxycarbonyl piperazine or with hydroxyethylpiperazine in a neutral medium adjusted with sodium acetate¹⁸. The structure of the **3** and **5** was established through spectroscopic (IR, ¹H-NMR, Mass) as well as elemental analyses data. IR spectra of **3a-e** and **5a-e** revealed the presence of NH band (=3279-3448), SO₂ groups (= 1133-1159 and 1308-1356) in addition to the triazene band (=1422-1439). ¹H-NMR revealed the presence of aromatic protons (=7.29-7.93), the characteristic two triplet piperazine protons at (=2.49-3.73 and 3.72-3.39) beside the presence of the triplet and quartet ethyl protons of **3** (= 1.23 and 4.07-4.09) or the presence of the triplet ethylene protons of **5** (= 2.57-2.72 and 2.88-3.35). Mass spectra of compounds **3d** and **5a** showed the molecular ion peaks of the compounds (=383(M⁺) and 313 (M⁺) respectively).

Furthermore, alkaline hydrolysis of compound **3** followed by decarboxylation led to the mono-substituted piperazine derivatives **4**. IR spectra of compounds **4a-e** revealed the disappearance of the ester carbonyl band (=1683-1652). ¹H-NMR revealed the disappearance of the characteristic ethyl protons.

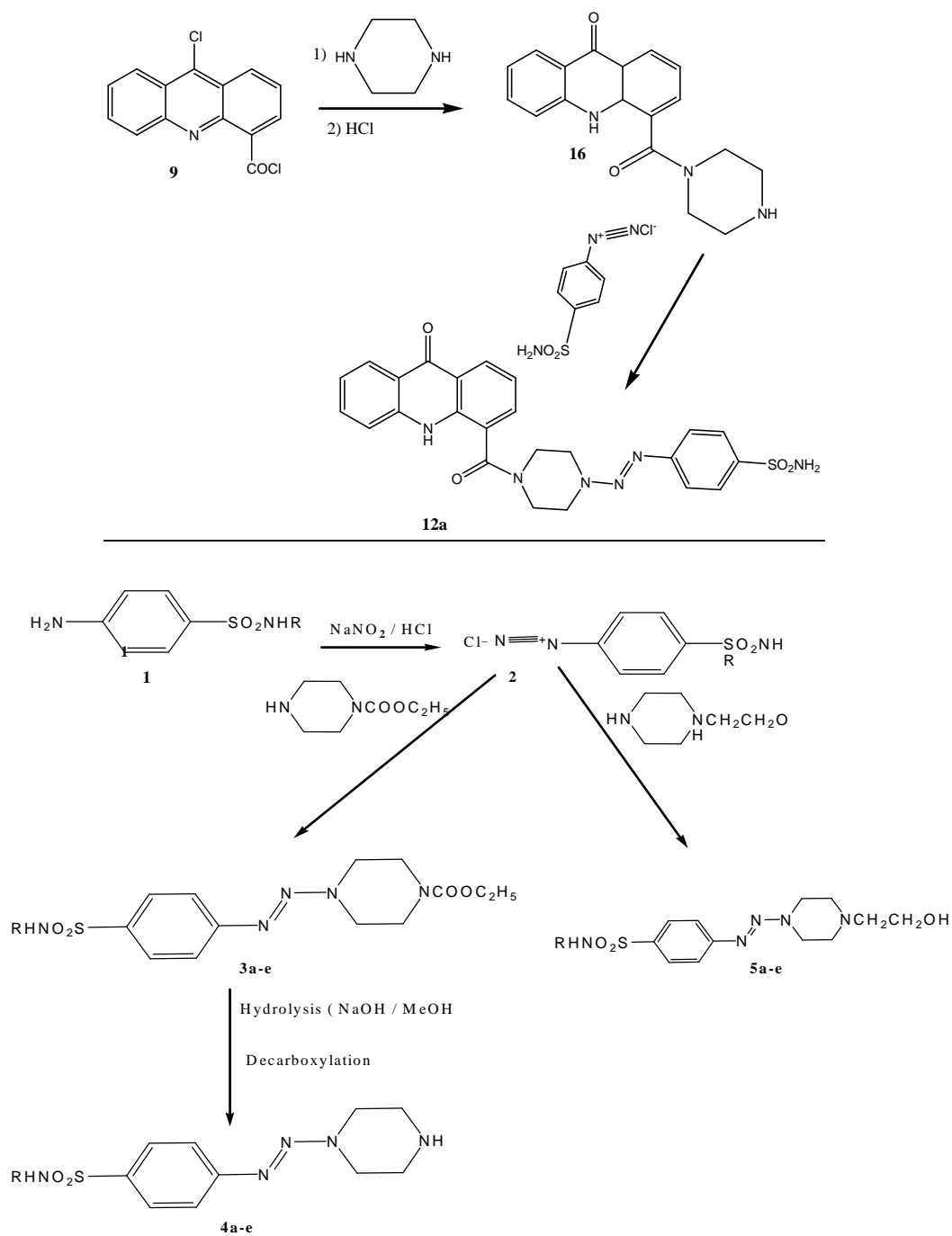
Mass spectra of compounds **4a** and **4d** showed the molecular ion peaks of the compounds (= 269 (M^+) and 313 ($M+2$) respectively).

9-Chloroacridine-4-carbonyl chloride **9** was reacted with the trizenosulfonamide derivatives **4a-e** or **5a-e** in N,N-dimethylformamide in presence of triethylamine to yield **10a-e** or **13a-e** that was directly added to acidified solution of the appropriate sulfonamides in N,N-dimethylformamide to obtain **11a-h** or **14a-j** respectively. IR spectra showed the appearance of NH band (=3427-3100), carbonyl band (= 1690-1650) in addition to the characteristic band of triazene (= 1444-1432) and SO₂ group (= 1334-1314 and 1178-1138). ¹H-NMR exhibited the presence of the piperazine protons (=2.43-2.50 and 3.15-4.00) together with the amino protons that disappeared on deuterium exchange together with the aromatic protons that was integrated for the corresponding compound (= 6.80-8.32). Mass spectra of compounds **11a** and **14a** revealed the molecular ion peaks of the compounds (= 726 ($M-2$) and 772 (M^+)) respectively).

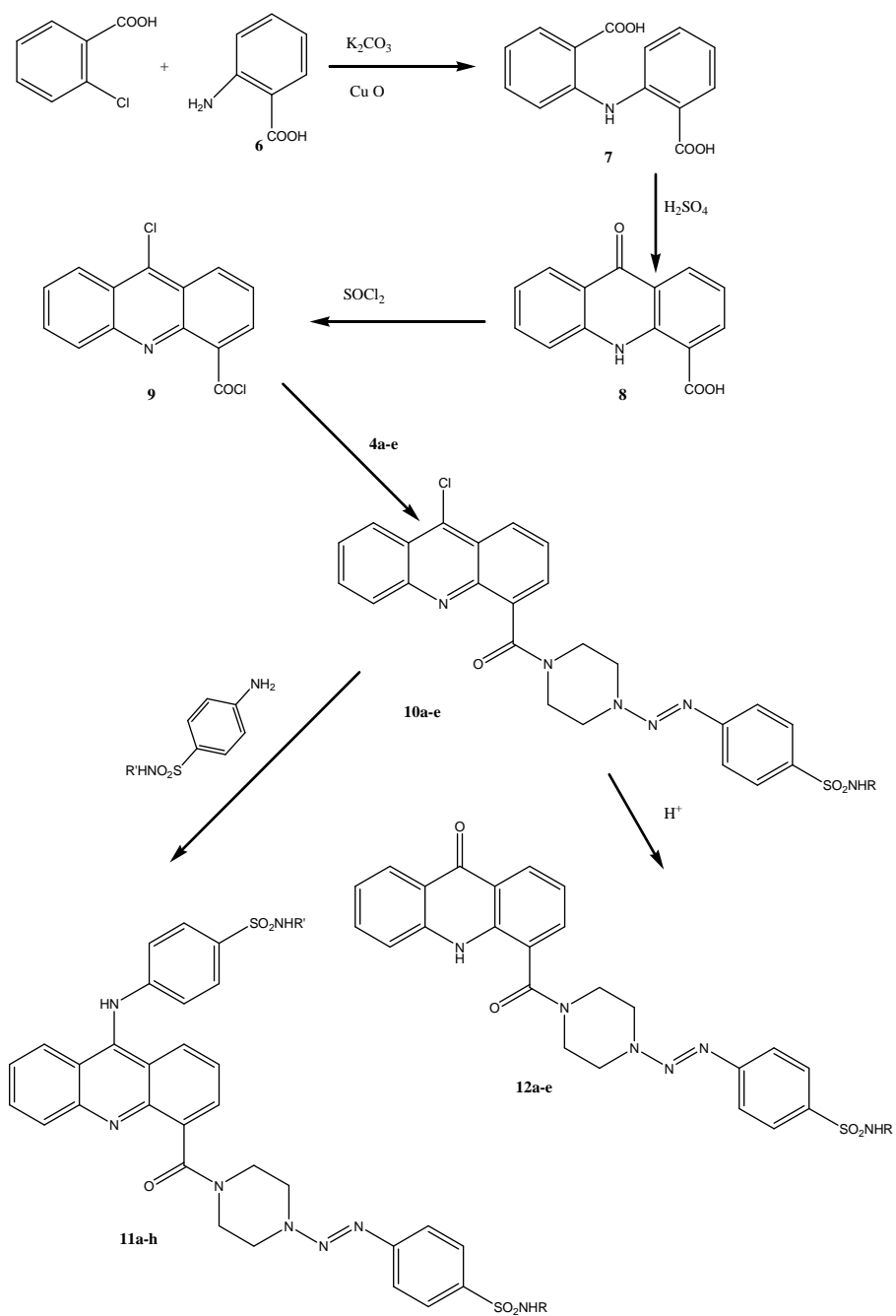
Moreover, mild acid hydrolysis of **10a-e** and **13a-e** afforded compounds **12a-e** and **15a-e** respectively. IR spectra showed the appearance of NH band (=3427-3194), carbonyl band (= 1696-1654), in addition to the

Characteristic band of triazene (= 1444-1438) and SO₂ group (= 1369-1320 and 1157-1142). ¹H-NMR exhibited the presence of the piperazine protons (=2.47-2.88 and 3.31-3.40) together with the amino protons that disappeared on deuterium exchange and the aromatic protons that was integrated for the corresponding compound (= 6.81-8.54). Mass spectra of compounds **12b** and **15a** exhibited the molecular ion peaks of the compounds (= 566 ($M-2$) and 538 ($M+4$)) respectively).

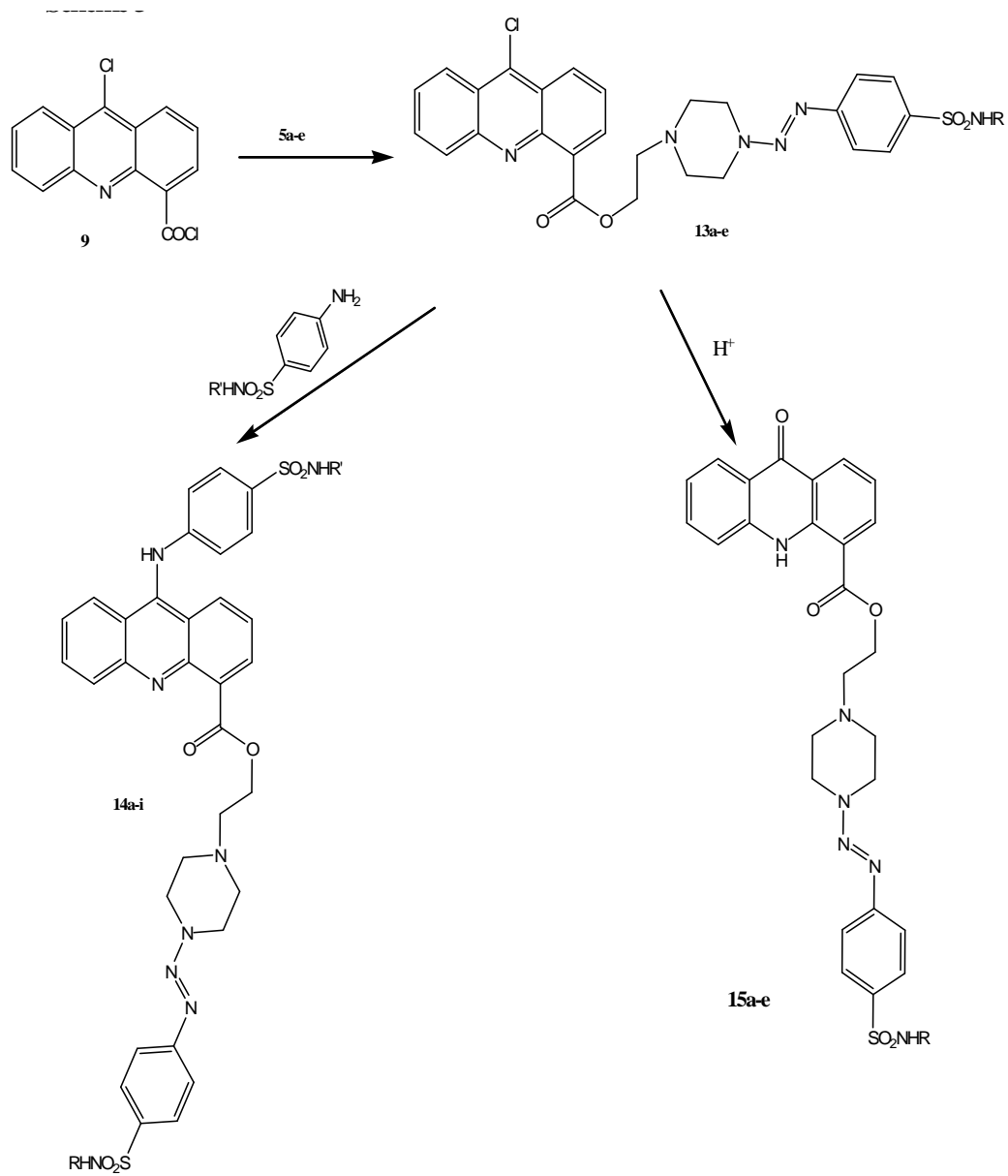
Another method has been adopted for the synthesis of **12a** via coupling of 9-chloroacridine-4-carbonyl chloride with anhydrous piperazine using the biphasic technique to yield **16** which was reacted with diazotized sulfanilamide to produce **12a**. Unfortunately, the yield of **16** was very low (13%), which limited the usage of this pathway. IR of **16** showed the appearance of NH bands (3433, 3300), carbonyl band (= 1639), triazene band (= 1415) and SO₂ bands (= 1338 and 1152). ¹H-NMR exhibited two triplets peaks for piperazine protons (= 2.81-2.93 and 3.17-3.25), aromatic protons (= 7.24-8.64) in addition to a singlet for the amino proton which disappeared on deuterium exchange (= 10.02). Finally, mass spectrum showed the molecular ion peaks of the compound (= 307 (M^+)).



Scheme 1



Scheme 2



Scheme 3

EXPERIMENTAL

Chemistry

All melting points were determined by the open capillary tube method using IA 9100MK-digital melting point apparatus and are uncorrected. IR spectra were recorded on Bruker Vector 22 (Germany) or Jasco FTIR plus 460 (Japan). ¹H-NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ¹H-NMR. Spectra were run at 300 MHz in deuterated chloroform (CDCl₃), dimethylsulfoxide (DMSO-d₆). Chemical shifts were quoted in δ units and were related to that of the solvents. Mass spectra were recorded on Finnigan MAT, SSQ 7000, mass spectrometer at 70 eV. Elemental Microanalysis were carried out using Heraeus and Vario EL III (elementar), CHNS analyzer (Germany) at the Microanalytical Center, Cairo University. Compounds **2**¹⁹, **3a**¹⁹, **7**²⁴, **8**⁴ and **9**⁴ were prepared according to the reported methods.

Ethyl 4-((4-substituted sulfamoylphenyl)diazanyl)piperazine-1-carboxylates **3b-e**

The freshly prepared diazonium salt of 4-substituted sulfamoyl benzenediazonium chloride **2** (10 mmol) was neutralized with saturated aqueous sodium acetate solution (pH= 6-7) in an ice bath (0°C). Excess ethoxycarbonyl piperazine (2 mL, 12.6 mmol) was added to this stirred reaction mixture and stirring was continued for further 30 minutes while keeping the temperature at 0°C,

then neutralized with sodium acetate once more and left to stir for further an hour. The product was filtered, washed with water, dried and crystallized from hexane. ¹H-NMR (DMSO-d₆) of **3d**: δ = 1.23 (t, 3H, CH₂CH₃), 3.56 (t, 4H, piperazine C-3, 5), 3.81 (t, 4H, piperazine C-2, 6), 4.07-4.09 (q, 2H, CH₂CH₃), 6.81 (s, 4H, NH₂, 2NHs (D₂O exchange)), 7.41 (d, 2H, aromatic H C-2, 6), 7.70 (d, 2H, aromatic H C-3, 5). Mass spectrum of **3d**: m/z (%): M+2= 385 (4.21%).

4-(Piperazine-1-yl)diazanylsubstituted benzenesulfonamides **4a-e**

Ethyl 4-((4-substituted sulfamoylphenyl)diazanyl)piperazine-1-carboxylate **3a-e** (10 mmol) was refluxed with sodium hydroxide (1.2 g, 30 mmol) dissolved in methanol (70 mL), then the alcoholic layer was distilled off slowly during a two hour period. Heating was continued until the temperature of the residue was 130-135°C and left for 10 minutes at the same temperature in a sand bath. The residue was poured onto crushed ice and pH was adjusted to 7-8 using glacial acetic acid. The product was filtered, washed with water, dried and crystallized from N,N-dimethylformamide / water. ¹H-NMR (DMSO-d₆) of **4a**: δ = 2.49 (t, 4H, piperazine C-3, 5), 3.32 (t, 4H, piperazine C-2, 6), 7.28 (s, 3H, NH and NH₂ (D₂O exchange)), 7.48 (d, 2H, aromatic H C-2, 6), 7.78 (d, 2H, aromatic H C-3, 5). ¹H-NMR (DMSO-d₆) of **4d**: δ = 2.48 (t, 4H, piperazine C-3, 5), 3.89 (t, 4H, piperazine C-2, 6), 7.03 (s, 5H,

3NHs and NH₂ (D₂O exchange)), 7.39-7.72 (m, 4H, aromatic H). Mass spectrum of **4a**: m/z (%): M⁺ 269 (2.73 %). Mass spectrum of **4d**: m/z (%): M+2= 313 (29.57 %).

4-((4-(2-Hydroxyethyl)piperazine-1-yl)diazenyl)substituted benzenesulfonamides 5a-e

The freshly prepared diazonium salt of 4-substituted sulfamoyl-benzenediazonium chloride **2** (10 mmol) was neutralized with saturated aqueous sodium acetate solution (pH= 6-7) in an ice bath (0°C). Excess hydroxyethyl piperazine (1.6 mL, 12.6 mmol) was added to this stirred reaction mixture and stirring was continued for further 30 minutes in an ice bath, then neutralized with sodium acetate once more and left to stir for further an hour. The product was filtered, washed with water, dried and crystallized from N,N-dimethylformamide / water. ¹H-NMR (DMSO-d₆) of **5a**: = 2.49 (t, 4H, piperazine C-3, 5), 2.72 (s, 2H, OCH₂CH₂), 2.88 (s, 2H, OCH₂CH₂), 3.26 (t, 4H, piperazine C-2, 6), 3.7 (s, 1H, OH (D₂O exchange)), 7.29-7.92 (m, 4H, aromatic H), 7.94 (s, 2H, NH₂ (D₂O exchange)). ¹H-NMR(DMSO-d₆) of **5d**: = 2.43 (t, 4H, piperazine C-3, 5), 2.57 (t, 2H, OCH₂CH₂), 3.35 (t, 2H, OCH₂CH₂), 3.73 (t, 4H, piperazine C-2, 6), 4.01 (s, 1H, OH (D₂O exchange)), 6.84 (s, 4H, 2NHs and NH₂ (D₂O exchange)), 7.37 (d, 2H, aromatic H C-3, 5), 7.67 (d, 2H, aromatic H C-2, 6). Mass spectrum of **5a**: m/z (%): M⁺ 313 (11.93 %).

4-((4-(9-Chloroacridine-4-carbonyl)piperazin-1-yl)diazenyl)-N-substituted benzenesulfonamides 10a-e

Freshly prepared 9-chloroacridine-4-carbonyl chloride **9** obtained from acridone-4-carboxylic acid **8** (1 g, 4.18 mmol) was dissolved in dry N,N-dimethylformamide (5 mL) and then added to a solution of **4a-e** (4.18 mmol) in dry N,N-dimethylformamide (5 mL) catalyzed with triethylamine (0.5 mL). The mixture was heated in a water bath for an hour. The resulting solution was used directly in the next step due to its instability.

N-(4-(4-((4-Substituted sulfamoyl)-phenyl)diazenyl)piperazine-1-carbonyl) acridin-9-ylamino)-substituted benzene sulfonamides 11a-h

The appropriate sulfonamide (4.18 mmol) was dissolved in dry N,N-dimethylformamide (5 mL), acidified with dilute hydrochloric acid (3 drops), added to the stirred mixture of the corresponding **10a-e**, and left to stir in a water bath for another one hour. The reaction mixture was diluted with ice-water and dilute ammonium hydroxide solution was added till complete precipitation (pH=7). The product was filtered, washed with water, dried and crystallized from N,N-dimethylformamide / water. ¹H-NMR (DMSO-d₆) of **11a**: = 2.43-2.49 (m, 8H, piperazine H), 2.88 (s, 3H, COCH₃), 6.76 (s, 2H, 2NHs (D₂O exchange)), 6.99 (s, 2H, NH₂(D₂O exchange)), 7.31-8.32 (m, 15H, aromatic H), 8.43

(s, 1H, NH (D₂O exchange)), 10.87 (s, 1H, NH (D₂O exchange)). ¹H-NMR (DMSO-d₆) of **11b**: = 2.24 (s, 3H, CH₃), 2.57 (s, 3H, CH₃), 2.73 (s, 4H, piperazine C-2, 6), 2.88 (s, 4H, piperazine C-3, 5), 6.60-8.55 (m, 17H, aromatic H and NH), 10.95 (s, 2H, SO₂NH₂), 11.60 (s, 1H, SO₂NH). ¹H-NMR (DMSO-d₆) of **11h**: = 2.72-3.15 (m, 8H, piperazine H), 7.36 (s, 4H, 2NHs, NH₂ (D₂O exchange)), 7.56-8.17 (m, 18H, aromatic H). Mass spectrum **11a**: m/z (%): M-2= 726 (12.75 %).

4-((4-(9-Oxo-9,10-dihydroacridine-4-carbonyl)piperazine-1-yl)-diazanyl)-N-substituted benzene-sulfonamides 12a-e

The stirred reaction mixture of **10a-e** was acidified with dilute hydrochloric acid (5 mL), and left to stir in a water bath for another hour. The reaction mixture was diluted with ice-water and dilute ammonium hydroxide solution was added till complete precipitation (pH = 7). The product was filtered, washed with water, dried and crystallized from N,N-dimethylformamide / H₂O. ¹H-NMR (DMSO-d₆) of **12a**: = 2.72 (t, 4H, piperazine C-2, 6), 2.88 (t, 4H, piperazine C-3, 5), 6.81-8.41 (m, 14H, aromatic H), 8.50 (s, 1H, NH (D₂O exchange)), 8.54 (s, 1H, NH (D₂O exchange)). ¹H-NMR (TFAA) of **12d**: 3.15 (t, 4H, piperazine C-2, 6), 3.23 (t, 4H, piperazine C-3, 5), 7.82-9.12 (m, 16H, 11aromatic H, 3NHs and NH₂). ¹H-NMR (DMSO-d₆) of **12e**: = 2.70 (t, 4H, piperazine C-2, 6), 2.92 (s, 3H, COCH₃), 3.01 (t,

4H, piperazine C-3, 5), 7.20-8.30 (m, 11H, aromatic H), 10.70 (s, 1H, NH (D₂O exchange)), 10.80 (s, 1H, NH (D₂O exchange)). Mass spectrum of **12b**: m/z (%): M-2= 566 (16.36 %).

2-(4-((4-N-substituted sulfamoyl-phenyl)diazanyl)piperazin-1-yl)-ethyl-9-chloroacridin-4-carboxylates 13a-e

Freshly prepared 9-chloroacridine-4-carbonyl chloride **9** obtained from acridone-4-carboxylic acid **8** (1 g, 4.18 mmol) was dissolved in dry N,N-dimethylformamide (5 mL) and then added to a solution of the appropriate **5a-e** (4.18 mmol) in dry N,N-dimethylformamide (5 mL) catalyzed with triethylamine (0.5 mL). The mixture was heated in a water bath for an hour. The resulting solution was used directly in the next step due to its instability.

2-(4-((4-N-substituted sulfamoyl-phenyl)diazanyl)piperazin-1-yl)-ethyl-9-(4-N-substituted sulfamoyl-phenylamino)-acridine-4-carboxylate 14a-j

The appropriate sulfonamide (4.18 mmol) was dissolved in dry N,N-dimethylformamide (5 mL), acidified with dilute hydrochloric acid (3 drops), added to the stirred mixture of **13a-e** and stirring was continued in a water bath for another hour. The reaction mixture was diluted with ice-water and dilute ammonium hydroxide solution was added till complete precipitation (pH = 7). The product was filtered, washed with water, dried and crystallized from N,N-dimethylformamide / water. ¹H-

NMR (DMSO- d_6) of **14a**: = 2.49 (t, 4H, piperazine C-2, 6), 2.65 (t, 2H, OCH_2CH_2), 2.85 (t, 2H, OCH_2CH_2), 3.59 (t, 4H, piperazine C-3, 5), 6.77(s, 7H, 3NHs and 2NH₂s (D₂O exchange)), 7.28-8.47 (m, 15H, aromatic H), 10.90 (s, 2H, 2NHs (D₂O exchange)). ¹H-NMR (DMSO- d_6) of **14d**: = 2.49 (t, 4H, piperazine C-2, 6), 2.71 (t, 2H, OCH_2CH_2), 2.87 (t, 2H, OCH_2CH_2), 4.00 (t, 4H, piperazine C-3, 5), 6.80-8.53 (m, 20H, 18 aromatic H and 2NHs), 9.50 (s, 2H, NH₂). ¹H-NMR (DMSO- d_6) of **14f**: = 2.48 (t, 4H, piperazine C-2, 6), 2.72 (t, 2H, OCH_2CH_2), 2.88 (t, 2H, OCH_2CH_2), 3.45 (t, 4H, piperazine C-3, 5), 7.34-8.50 (m, 22H, 18 aromatic H, 2NHs and NH₂ (D₂O exchange)). Mass spectrum of **14a**: m/z (%): M⁺ 772 (0.13 %).

2-(4-((4-N-substituted sulfamoyl-phenyl) diazenyl) piperazin-1-yl) ethyl-9-oxo-9,10-dihydro-acridine-4-carboxylate 15a-e

The stirred reaction mixture of the appropriate **13a-e** was acidified with dilute hydrochloric acid (5 mL), and left to stir in a water bath for another hour. The reaction mixture was diluted with ice-water and dilute ammonium hydroxide solution was added till complete precipitation (pH = 7). The product was filtered, washed with water, dried and crystallized from N,N-dimethyl-formamide / water. ¹H-NMR (DMSO- d_6) of **15a**: = 2.49 (t, 4H, piperazine C-2, 6), 2.70 (t, 2H, OCH_2CH_2), 2.90 (t, 2H, OCH_2CH_2), 3.41 (t, 4H,

piperazine C-3, 5), 7.30-8.52 (m, 14H, 11 aromatic H, NH and NH₂ (D₂O exchange)). ¹H-NMR (TFAA) of **15d**: = 2.19 (t, 4H, piperazine C-2, 6), 2.30 (t, 2H, OCH_2CH_2), 3.10 (t, 2H, OCH_2CH_2), 3.30 (t, 4H, piperazine C-3, 5), 7.61-9.08 (m, 20H, 15 aromatic H, 3NHs and NH₂ (D₂O exchange)). Mass spectrum of **15a**: m/z (%): M⁺+4= 538 (0.9 %).

Biological screening

Fourteen representative compounds have been chosen and tested for their cytotoxic activity against human mammary carcinoma cell line in the National Cancer Institute, Cairo University. The screening involves calculation of the percentage growth of surviving fraction of the drug treated cell lines compared by untreated control using Sulforhodamine B (SRB) colorimetric assay. Sulforhodamine B is a bright pink aminoxanthene anionic dye with two sulfonic acid groups that bind electrostatically to protein basic amino acid residues of trichloroacetic acid fixed cells under mild acidic conditions²⁵. Cultures fixed with trichloroacetic acid were stained for 30 minutes with 0.4% w/v Sulforhodamine B dissolved in 1% acetic acid, and protein-bound dye was extracted with 10 mM tris base for determination of optical density in a computer-interfaced, 96-well microtiter plate reader.

The optical density measured is linear to the cell number of the surviving fraction. Therefore, the

Table 1: Physical, microanalytical and I.R. data of compounds **3b-e**, **4a-e**, **5a-e**, **12a-e** and **15a-e**.


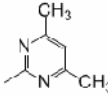
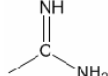
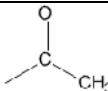

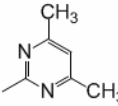
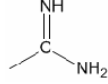
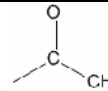
Cpd. No.	R	Molecular Formula M. Wt.	Yield (%) M.P.(°C)	Microanalysis (%)		IR (cm ⁻¹)
				Calc.	Found	
3b		C ₁₇ H ₂₁ N ₇ O ₄ S 419.46	59 230-2	C 48.67 H 5.04 N 23.37	48.90 5.01 23.38	3396 (NHs), 1655 (CO), 1436 (N=N-N), 1340, 1159 (SO ₂).
3c		C ₁₉ H ₂₅ N ₇ O ₄ S 447.51	56 200-2	C 50.99 H 5.63 N 21.90	51.19 5.31 22.21	3447 (NH), 1652 (CO), 1431 (N=N-N), 1350, 1150 (SO ₂).
3d		C ₁₄ H ₂₁ N ₇ O ₄ S 383.14	65 235-7	C 43.85 H 5.52 N 25.57	44.06 5.33 25.66	3445, 3339, 3203 (NH ₂ , NHs), 1683 (CO), 1427 (N=N-N), 1350, 1133 (SO ₂).
3e		C ₁₅ H ₂₁ N ₅ O ₅ S 383.42	53 215-17	C 46.98 H 5.52 N 18.26	46.30 5.12 18.30	3311 (NH), 1688 (CO), 1439 (N=N-N), 1338, 1149 (SO ₂).
4a	H	C ₁₀ H ₁₅ N ₅ O ₂ S 269.32	58 255-7	C 44.59 H 5.61 N 26.00	44.55 5.50 25.90	3080, 3060 (NH ₂ , NH), 1420 (N=N-N); 1320, 1150 (SO ₂).
4b		C ₁₄ H ₁₇ N ₇ O ₂ S 347.39	66 155-7	C 48.40 H 4.93 N 28.22	48.30 5.20 28.12	3400, 3298 (NHs), 1447 (N=N-N), 1355, 1154 (SO ₂).
4c		C ₁₆ H ₂₁ N ₇ O ₂ S 375.45	64 109-11	C 51.18 H 5.63 N 26.11	C 51.10 H 5.50 N 25.56	3100 (NH), 1440 (N=N- N), 1320, 1140 (SO ₂).
4d		C ₁₁ H ₁₇ N ₇ O ₂ S 311.11	70 182-4	C 42.43 H 5.50 N 31.48	C 42.26 H 5.80 N 31.78	3446, 3339, 3200 (NH ₂ , NHs), 1427 (N=N-N), 1349, 1133 (SO ₂).
4e		C ₁₂ H ₁₇ N ₅ O ₃ S 311.36	62 154-6	C 46.29 H 5.50 N 20.39	46.03 5.93 20.15	3313, 3243 (NHs), 1687 (CO), 1436 (N=N-N), 1338, 1152 (SO ₂).
5a	H	C ₁₂ H ₁₉ N ₅ O ₃ S 313.37	56 290-1	C 45.99 H 6.11 N 22.34	45.80 6.10 22.32	3419 (OH), 3375 (NH ₂ , NHs), 1438 (N=N-N), 1334, 1156 (SO ₂).

Table 1: Continued.


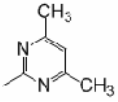
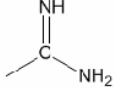
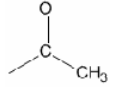

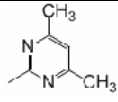
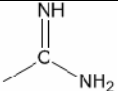
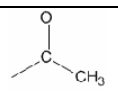

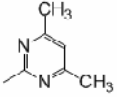
Cpd. No.	R	Molecular Formula M. Wt.	Yield (%) M.P.(°C)	Microanalysis (%)		IR (cm ⁻¹)
				Calc.	Found	
5b		C ₁₆ H ₂₁ N ₇ O ₃ S 391.45	68 252-4	C 49.09 H 5.40 N 25.04	48.58 5.73 25.04	3446 (OH), 3279 (NH), 1612, 1422 (N=N-N), 1311, 1153 (SO ₂).
5c		C ₁₈ H ₂₅ N ₇ O ₃ S 419.50	65 200-2	C 51.53 H 6.00 N 23.37	51.29 6.23 23.37	3444 (OH, NH), 1428 (N=N-N), 1308, 1147 (SO ₂).
5d		C ₁₃ H ₂₁ N ₇ O ₃ S 355.41	72 216-8	C 43.93 H 5.95 N 27.58	43.81 5.73 27.68	3448, 3371, 3207 (OH, NH ₂ , NHs), 1433 (N=N- N), 1363, 1132 (SO ₂).
5e		C ₁₄ H ₂₁ N ₅ O ₄ S 355.41	59 155-7	C 47.31 H 5.95 N 19.70	47.10 5.90 19.60	3368 (OH), 3291 (NH), 1436 (N=N-N), 1330, 1156 (SO ₂).
12a	H	C ₂₄ H ₂₂ N ₆ O ₄ S 490.14	60 290-2	C 58.76 H 4.52 N 17.13	C 58.85 H 4.31 N 17.08	3359, 3258 (NH ₂ , NHs), 1686 (CO), 1440 (N=N- N), 1369, 1142 (SO ₂).
12b		C ₂₈ H ₂₄ N ₈ O ₄ S 568.60	72 278-9	C 59.14 H 4.25 N 19.70	C 59.01 H 4.54 N 19.60	3261, 3194 (NHs), 1690 (CO), 1444 (N=N-N), 1320, 1150 (SO ₂).
12c		C ₃₀ H ₂₈ N ₈ O ₄ S 596.66	70 222-4	C 60.39 H 4.73 N 18.78	60.50 4.90 18.85	3418, 3294 (NHs), 1690 (CO), 1438 (N=N-N), 1330, 1152 (SO ₂).
12d		C ₂₅ H ₂₄ N ₈ O ₄ S 532.58	75 285-7	C 56.38 H 4.54 N 21.03	56.19 4.30 20.99	3423, 3225 (NH ₂ , NHs), 1696 (CO), 1440 (N=N- N), 1332, 1143 (SO ₂).
12e		C ₂₆ H ₂₄ N ₆ O ₅ S 532.57	65 237-9	C 58.63 H 4.54 N 15.78	58.51 5.10 15.33	3422 (NHs), 1690 (CO), 1439 (N=N-N), 1328, 1157 (SO ₂).
15a	H	C ₂₆ H ₂₆ N ₆ O ₅ S 534.58	58 280-2	C 58.41 H 4.90 N 15.72	C 58.69 H 5.05 N 15.62	3500, 3250 (NH ₂ , NHs), 1688 (CO), 1439 (N=N- N), 1326, 1146 (SO ₂).
15b		C ₃₀ H ₂₈ N ₈ O ₅ S 612.66	71 215-6	C 58.81 H 4.60 N 18.28	58.58 4.91 18.48	3400, 3224 (NHs), 1668 (CO), 1444 (N=N-N), 1330, 1142 (SO ₂).
15c		C ₃₂ H ₃₂ N ₈ O ₅ S 640.71	66 175-6	C 59.98 H 5.03 N 17.48	59.66 4.79 17.51	3422 (NHs), 1654 (CO), 1436 (N=N-N), 1326, 1150 (SO ₂).

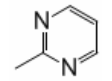
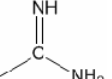
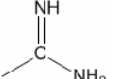
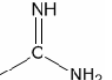
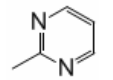
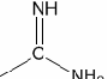
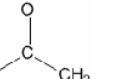
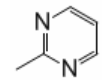
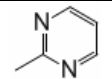
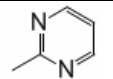
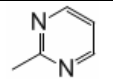
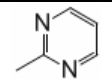
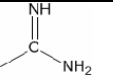
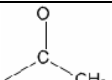
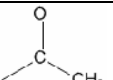
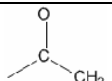
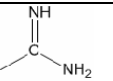
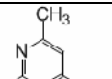
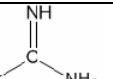
Table 1: Continued.

Cpd. No.	R	Molecular Formula M. Wt.	Yield (%) M.P.(°C)	Microanalysis (%)		IR (cm ⁻¹)
				Calc.	Found	
15d		C ₂₇ H ₂₈ N ₈ O ₅ S 576.63	77 255-6	C 56.24 H 4.89 N 19.43	56.20 4.50 19.42	3424 (NH ₂ , NHs), 1670 (CO), 1440 (N=N-N), 1329, 1141 (SO ₂).
15e		C ₂₈ H ₂₈ N ₆ O ₆ S 576.17	62 195-7	C 58.32 H 4.89 N 14.57	C 58.59 H 4.73 N 14.57	3421 (NHs), 1688 (CO), 1440 (N=N-N), 1327, 1153 (SO ₂).

Table 2. Physical, microanalytical and I.R. data of compounds **11a-h** and **14a-j**.

Cpd. No.	R	R'	Molecular Formula M. Wt.	Yield (%) M.P. (°C)	Microanalysis (%)		IR (cm ⁻¹)
					Calc.	Found	
11a			C ₃₃ H ₃₂ N ₁₀ O ₆ S ₂ 728.80	235-6 70	C 54.38 H 4.42 N 19.21	54.90 4.25 19.11	3427, 3335, 3219 NH ₂ , NHs), 1690 (CO), 1438 (N=N-N), 1328, 1138 (SO ₂).
11b	H		C ₃₆ H ₃₄ N ₁₀ O ₅ S ₂ 750.85	65 200-2	C 57.58 H 4.56 N 18.65	57.81 4.45 18.49	3221, 3074 (NH ₂ , NHs), 1658 (CO), 1432 (N=N-N), 1314, 1151 (SO ₂).
11c			C ₃₅ H ₃₂ N ₁₂ O ₅ S ₂ 764.84	72 215-7	C 54.96 H 4.21 N 21.97	54.81 4.20 21.90	3216 (NH ₂ , NHs), 1658 (CO), 1440 (N=N-N), 1328, 1151 (SO ₂).
11d			C ₃₇ H ₃₆ N ₁₂ O ₅ S ₂ 792.89	71 195-7	C 56.04 H 4.57 N 21.19	55.79 4.59 21.27	3200 (NH ₂ , NHs), 1678 (CO), 1440 (N=N-N), 1326, 1146 (SO ₂).
11e		H	C ₃₁ H ₃₀ N ₁₀ O ₅ S ₂ 686.76	75 255-7	C 54.21 H 4.40 N 20.39	54.60 4.70 20.15	3325, 3218 (NH ₂ , NHs), 1658 (CO), 1439 (N=N-N), 1316, 1137 (SO ₂).
11f			C ₃₇ H ₃₆ N ₁₂ O ₅ S ₂ 792.89	74 270-2	C 56.04 H 4.57 N 21.19	56.30 4.88 21.42	3417, 3327 (NH ₂ , NHs), 1658 (CO), 1440 (N=N-N), 1326, 1138 (SO ₂).
11g			C ₃₄ H ₃₂ N ₈ O ₇ S ₂ 728.80	69 190-2	C 56.03 H 4.42 N 15.37	56.10 4.62 15.21	3572, 3276(NH ₂ , NHs), 1658 (CO), 1441 (N=N-N), 1329, 1147 (SO ₂).

Table 2: Continued.

Cpd. No.	R	R'	Molecular Formula M. Wt.	Yield (%) M.P. (°C)	Microanalysis (%)		IR (cm ⁻¹)
					Calc.	Found	
11h		H	C ₃₄ H ₃₀ N ₁₀ O ₅ S ₂ 722.80	72 200-2	C 56.49 H 4.18 N 19.37	56.50 4.00 19.62	3423, 3280 (NH ₂ , NHs), 1650 (CO), 1444 (N=N-N), 1334, 1152 (SO ₂).
14a			C ₃₄ H ₃₆ N ₁₂ O ₆ S ₂ 772.86	66 230-2	C 52.83 H 4.69 N 21.74	52.90 4.70 21.24	3427, 3337, 3220 (NH ₂ , NHs), 1690 (CO), 1440 (N=N-N), 1329, 1138 (SO ₂).
14b			C ₃₇ H ₃₆ N ₁₂ O ₆ S ₂ 808.89	67 245-7	C 54.94 H 4.48 N 20.77	54.83 4.49 20.47	3428, 3333, 3219 (NH ₂ , NHs), 1658 (CO), 1433 (N=N-N), 1332, 1136 (SO ₂).
14c			C ₃₅ H ₃₆ N ₁₀ O ₇ S ₂ 772.85	66 290-2	C 54.39 H 4.69 N 18.12	54.60 4.50 18.43	3222(NH ₂ , NHs), 1678 (CO), 1440 (N=N-N), 1330, 1141 (SO ₂).
14d		H	C ₃₆ H ₃₄ N ₁₀ O ₆ S ₂ 766.21	72 160-2	C 56.38 H 4.46 N 18.26	56.75 4.55 18.26	3190(NH ₂ , NHs), 1659 (CO), 1444 (N=N-N), 1324, 1178 (SO ₂).
14e			C ₄₀ H ₃₆ N ₁₂ O ₆ S ₂ 844.92	75 255-7	C 56.86 H 4.29 N 19.89	56.65 4.55 20.09	3197(NH ₂ , NHs), 1678 (CO), 1441 (N=N-N), 1326, 1147 (SO ₂).
14f	H		C ₃₆ H ₃₄ N ₁₀ O ₆ S ₂ 766.21	66 200-2	C 56.38 H 4.46 N 18.26	56.13 4.55 18.26	3383, 3255 (NH ₂ , NHs), 1653 (CO), 1435 (N=N-N), 1333, 1156 (SO ₂).
14g			C ₃₇ H ₃₆ N ₁₂ O ₆ S ₂ 808.89	74 230-2	C 54.94 H 4.48 N 20.77	54.60 4.70 20.87	3250, 3100 (NH ₂ , NHs), 1688 (CO), 1444 (N=N-N), 1329, 1151 (SO ₂).
14h			C ₃₆ H ₃₆ N ₈ O ₈ S ₂ 772.85	75 255-7	C 55.94 H 4.69 N 14.49	55.60 4.50 14.12	3263 (NH ₂ , NHs), 1658 (CO), 1442 (N=N-N), 1326, 1154 (SO ₂).
14i			C ₃₅ H ₃₆ N ₁₀ O ₇ S ₂ 772.85	73 267-9	C 54.39 H 4.69 N 18.12	54.58 4.75 18.21	3329 (NH ₂ , NHs), 1658 (CO), 1442 (N=N-N), 1329, 1154 (SO ₂).
14j			C ₃₉ H ₄₀ N ₁₂ O ₆ S ₂ 836.94	73 270-2	C 55.96 H 4.81 N 20.08	56.03 5.05 19.64	3300, 3200 (NH ₂ , NHs), 1658 (CO), 1439 (N=N-N), 1327, 1147 (SO ₂).

assay is a sensitive measure of drug induced cytotoxicity with the best signal to noise ratio. The assay also, provides a colorimetric end point that is nondestructive, indefinitely stable and visible to naked eye²⁵.

Method

Cells were plated in 97-multiwell plate (10^4 cells/well) for 24 hours before treatment with the compounds to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (0, 1, 2.5, 5 and 10 $\mu\text{g/mL}$) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were then incubated with the compound for 48 hours at 37°C and in atmosphere of 5% CO_2 . After this time, cells were fixed, washed and stained with Sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. The color intensity was measured in an LISA reader. Finally, the relation between surviving fraction and drug concentration was plotted to get the survival curve the tumor cell line after the specific compound.

Analysis of data

Data were collected, revised and analyzed by SPSS statistical package version 11. Excel computer program was used to tabulate the results (table 3), and represent it graphically (Figure 1-14). Probit regression analysis procedure has been introduced to select the best model

that describes the relationship between the probit (percentages of protection) as a dependant variable in order to be used for prediction of the concentration of the drug that causes inhibition of 50% (IC_{50}) or 90% (IC_{90}) of cancer cells. The *in vitro* growth inhibition properties of each drug were described by (IC_{50}) or (IC_{90}) and the degree of inhibition of cancer cell line was described by the equation:

The probit (P) = intercept + (regression coefficient x conc).

RESULTS AND DISCUSSION

Data are presented in Table 3 to reveal the drug concentration and probit for the mammary cell line and illustrated by a series of curves (Fig. 1-Fig.14) as a plot of the probit against drug concentration of the tested compounds.

The data of the survival curves of the chosen compounds revealed that The IC_{50} compounds **3a**, **3d**, **4a**, **4d**, **5a** and **5d** were **13.38**, **14.41**, **11.49**, **12.73**, **13.34** and **15.12** respectively. These results show that, all the tested triazeno-benzene sulfonamides are inactive except the unsubstituted sulfamoyl derivative **4a** that possesses mild cytotoxic activity. In addition to, the monosubstituted piperazine derivative **4a** has cytotoxic activity than its ethylcarboxylate analogue **3a**.

The 4-substituted carboxamide derivatives containing unsubstituted sulfamoyl derivatives **11a** and **12a** provide higher cytotoxic activity than those of the carbamimidoylsulfamoyl

Table 3: Antitumor activity of the newly synthesized derivatives.

Compound No	IC ₅₀	IC ₉₀
3a	13.38	27.18
3d	14.41	28.81
4a	11.49	21.13
4d	12.73	22.75
5a	13.34	25.57
5d	15.12	30.71
11a	8.02	16.89
11b	8.61	20.32
12a	7.26	18.42
12d	9.89	20.46
14a	6.97	20.08
14f	15.42	29.69
15a	5.65	19.29
15d	7.91	20.02

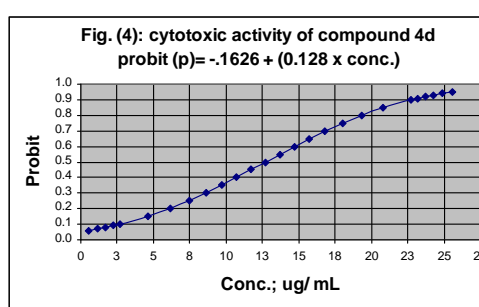
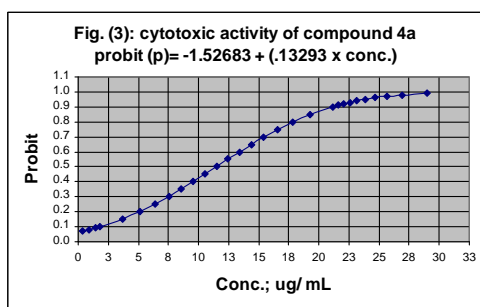
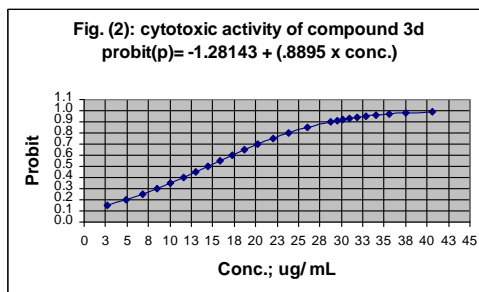
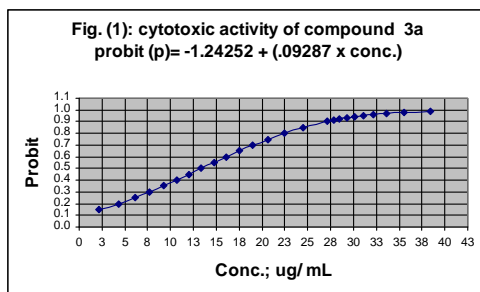
Fig.1-Fig.14. Cytotoxic activity of the newly synthesized derivatives.

Fig. (5): cytotoxic activity of compound 5a
probit (p)= -1.39868 + .10481 x conc.)

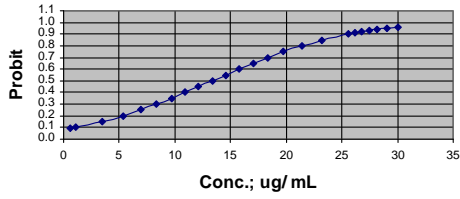


Fig. (6): cytotoxic activity of compound 5d
probit (p)= -1.24189 + (.08216 x conc.)

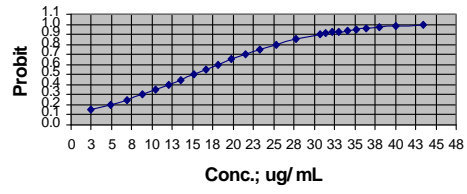


Fig. (7): cytotoxic activity of compound 11a
probit (p)= -1.15872 + (.14444 x conc.)

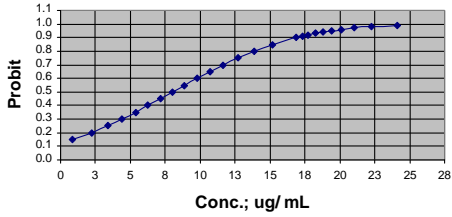


Fig. (8): cytotoxic activity of compound 11b
probit (p)= -.94190 + (.10943x conc.)

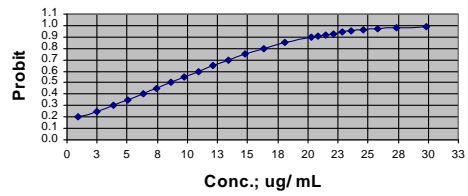


Fig. (9): cytotoxic activity of compound 12a
probit (p)= -.83417 + (.11484 x conc.)

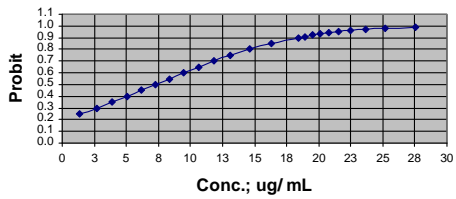


Fig. (10): cytotoxic activity of compound 12d
probit (p)= -1.19823 + (.12118 x conc.)

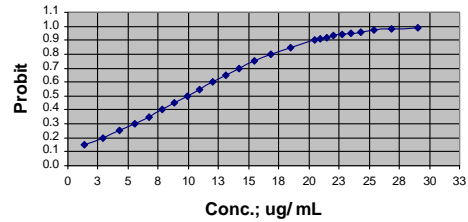


Fig. (11): cytotoxic activity of compound 14a
probit (p)= -.068205 + (.09781 x conc.)

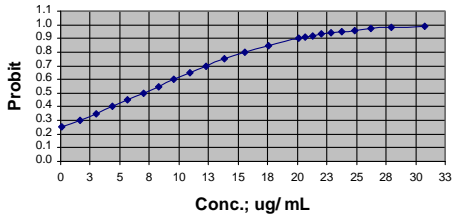
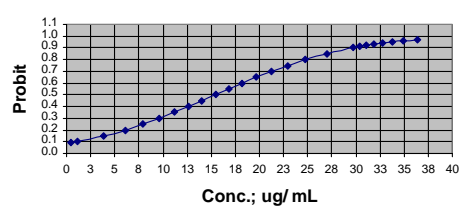
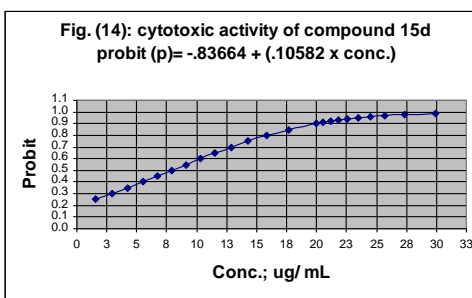
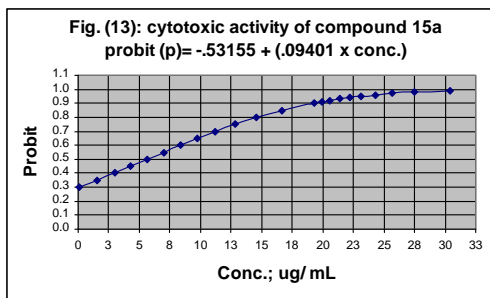


Fig. (12): cytotoxic activity of compound 14f
probit (p)= -1.38561 + (.08984 x conc.)





analogues **11d** and **12d** and IC_{50} for compounds **11a**, **11b**, **12a** and **12d** were 8.02, 8.61, 7.26 and 9.89 respectively. These results indicate that, increasing the polarity of the compound decrease the cytotoxic activity. On the other hand, **11a** shows a slight decrease in the cytotoxic activity than **11b** attributed to the presence of 4,6-dimethyl-2-pyrimidinyl group. Furthermore, IC_{50} for compounds **14a**, **14f**, **15a** and **15d** were 6.97, 15.42, 5.65 and 7.91 respectively. These results show that similar to the 4-substituted carboxamide derivatives, the 4-substituted ester derivatives containing unsubstituted sulfamoyl derivatives **15a** shows higher cytotoxic activity than the carbamimidoylsulfamoyl analogue **15d**. On the other hand, the 4-substituted ester derivatives containing unsubstituted sulfamoyl derivatives **14f** shows no cytotoxic activity compared to its carbamimidoylsulfamoyl analogue **14a** that may be attributed to the presence of 2-pyrimidinyl moiety.

Finally, the 4-substituted ester derivative containing unsubstituted sulfamoyl derivatives **15a** shows higher cytotoxic activity than its carboxamide analogue **14a** which indicate that the lipophilicity increase the activity.

Conclusion

- The hybrid structure between an acridine nucleus and a triazene moiety results in an active cytotoxic combilexin, the case which complies with the aim of this work.
- Unsubstituted sulfamoyl derivatives show higher cytotoxic activity than the carbamimidoylsulfamoyl analogues.
- Substitution in the 4 position of acridone by sulfamoyl(phenyl)diazenyl)piperazin-1-yl)ethyl group to form ester derivatives are more active than the 4-substituted sulfamoyl(phenyl)diazenyl)piperazin-1-yl)carboxamide derivatives.
- Acridone derivatives are more potent than the substituted acridine derivatives.
- Lipophilicity increases the activity.

- Finally it can be concluded that the cytotoxic activity decreases in the following order: **15a** > **14a** > **12a** > **15d** > **11b** > **11a** while two compounds show mild activity that are **12d** > **4a**.

REFERENCES

- 1- S. M. E. Moghazy Aly and H. M. Safwat, *Egypt J. Pharm. Sci.*, 31, 505 (1990).
- 2- I. Antonini, P. Polucci, L.R. Kelland, S. Spinelli, N. Pescalli and S. Martelli, *J. Med. Chem.*, 43, 4801 (2000).
- 3- J. B. Hansen, E. Langvad, F. Frandsen and O. Buchardt, *ibid.*, 26, 1510 (1983).
- 4- G. J. Atwell, B. F. Cain, B. C. Baguley, G. J. Finlay and W. A. Denny, *ibid.*, 27, 1481 (1984).
- 5- W. A. Denny, I. A. G. Roos and L. P. G. Wakelin; *Anti-cancer-Drug Res.*, 1, 141 (1986).
- 6- W. A. Denny, G. J. Atwell and B. C. Baguley, *J. Med. Chem.*, 26, 1619 (1983).
- 7- G. J. Finlay, J. F. Rion, and B. C. Baguley, *Eur. J. Cancer, Part A*, 32, 708 (1996).
- 8- W.A. Denny, B. C. Baguley, B. F. Cain and R. J. Waring, "Molecular Aspects of Anticancer Drug Action", Ed. Neidle and Waring, 1983, p. 1.
- 9- B. F. Cain, R. N. Seelye and G. J. Atwell, *J. Med. Chem.*, 17, 922 (1974).
- 10- W. A. Denny, B. F. Cain, G. J. Atwell, C. Hansch, A. Panthanickal and A. Leo, *ibid.*, 25, 276, (1982).
- 11- B. C. Baguley, W. A. Denny, G. J. Atwell, G. J. Finlay, G. W. Rewcastle, S. J. Twigden and W. R. Wilson, *Cancer Res.*, 44, 3245 (1984).
- 12- E. Carvalho, A. P. Francisco, J. Iley, E. Rosa, *Bioorg. Med. Chem.*, 8, 1719 (2000).
- 13- V. Gadjeva and G. Vesselina, *Int. J. Pharm.*, 247, 39 (2002).
- 14- P. Diana, P. Barraja, A. Lauria, A. M Almerico, G. Cirrincione, A. Loi, C. Musiu, A. Pani, P. Lacolla and M. Marongiu, *Eur. J. Med. Chem.*, 34, 353 (1999).
- 15- C. V. Catapano, M. Broggin, E. Erba, M. Ponti, L. Mariani, L. Citti and M. D'Incalci, *Cancer Res.*, 47, 4884 (1987).
- 16- J. H. M. Van Delft, A. Luiten-Schuite, V. L. Souliotis, S. A. Kyrtopoulos, J. Ouwerkerk, H. J. Keizer and R. A. Baan, *Biomarkers*, 1, 94, (1996)
- 17- V. R. Little and K. Vaughan, *Can. J. Chem.*, 82, 1294 (2004).
- 18- I. Lalezari and F. Afghahi, *J. Pharm. Sci.*, 64, 698 (1975).
- 19- A. W. McConnaughie and T. C. Jenkins, *J. Med. Chem.*, 38, 3488 (1995).
- 20- G. J. Atwell, B. F. Cain and W. A. Denny, *ibid.*, 20, 520, (1977).
- 21- K. Hano, A. Akashi, I. Yamamoto, S. Narumi, H. Iwata, *Gann*, 59, (3), 207 (1968), through *Chem. Abst.*, 69, 425279 (1968).

- 22- M. Y. Ebeid, S. M. El-Moghazy Aly, A. N. Mikhael and A. A. Eissa, *Egypt. J. Pharm. Sci.*, 33, 293 (1992).
- 23- S. A. Gamage, G. W. Reweastle, G. J. Atwell, B. C. Baguley and W. A. Denny, *Anticancer Drug Des.*, 7, 403, (1992).
- 24- N. S. Drozdov and A. F. Bekhli; *J. Gen. Chem. USSR*, 8, 1505, (1938), through *Chem. Abstr.*, 33, 45966 (1939).
- 25- H. Kalant and W. H. E. Roschlau, "Principles of Medical Pharmacology" 6th edition, New York, Oxford, Oxford University Press, 1998, p. 774.