

ORIGINAL RESEARCH

Synthesis and characterization of triazenes derived from sulfonamides

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ABSTRACT: A series of novel triazene derivatives 1-3, 1a-3a were synthesized by the coupling of diazonium salts of amines (sulfaguanidine, sulfapyridine, sulfamethoxazole) with N-methylaniline / p-nitroaniline in acidic media. The structures of the synthesized compounds were confirmed by the spectral data (UV, IR, ¹H-NMR, APCI-MS) and elemental analysis. The effects of all the compounds on A 549 and L 929 cell lines growth were investigated. The cytotoxic and antitumor activities of these compounds have not been in vitro against A 549 and L 929 cell lines.

KEY WORDS: Triazene, sulfonamide, prontosil, azo reduction.

INTRODUCTION

Prontosil 4-[(2,4-diaminophenyl)azo]benzenesulfonamide (Figure 1), the first commercially available antibacterial antibiotic, was developed by a research team at the Bayer Laboratories of the IG Farben conglomerate in Germany.

The conversion of prontosil to sulfonamide is the first known examples on prodrug activation. This reaction occurs with the aid of azo reductase enzymes by the large intestinal microbiota. The reaction takes place in two steps, the formation of the hydrazo compound, followed by the reductive cleavage of the nitrogen bond (Figure 2).

The sulfonamide derivatives have attracted considerable pharmaceutical interest due to antibac-

terial, antiepileptic, antihypertensive, diuretic, hypoglycemic, antithyroid and protease inhibitory activity (1-6) In recent years, the novel sulfonamide derivatives have been reported to show potent inhibition of growth against several leukemia, non-small cell lung, ovarian, melanoma, colon, renal, prostate and breast cancer cell line (7)

A lot of antitumor drugs possess a limited bioavailability due to low chemical stability limited oral absorption or rapid metabolism (8). Because of these disadvantages, several prodrug models that can be activated into antitumor drugs have been designed. An important aspect of prodrug design is the need for converting rapidly to the active therapeutic agent *in vivo*. Some triazene derivatives provide a potential prodrug system for anticancer activity (9). On the other hand, some researchers synthesize a range of triazene derivatives and investigate as a prodrug candidates for melanocyte-directed enzyme prodrug therapy (10).

One of the most prevalent usage of the triazenes is in the development of antitumor molecules. Chemotherapeutic agents of the triazene class have been used in the clinical management of many tumors including brain, leukemias, melanomas (11,12); metastatic malignant melanoma, cancer of colon and Hodgkin's disease such as dacarbazine (12,13) Dacarbazine; [5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide] which is used in the treatment of malignant melanoma

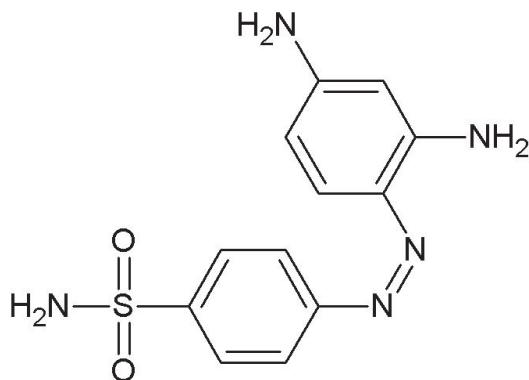


FIGURE 1. Prontosil

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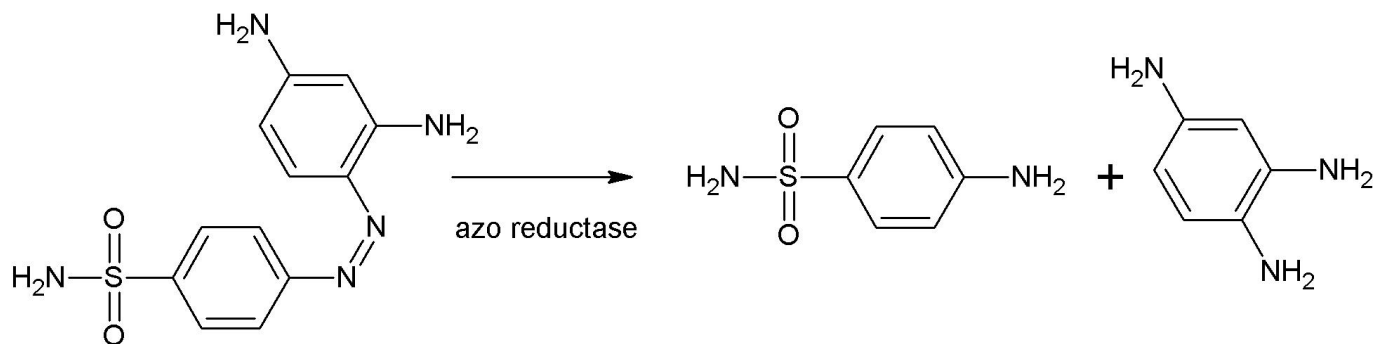


FIGURE 2. Azo reduction of prontosil

is the first generation of arylalkyltriazenes. A number of cyclic arylmonoalkyltriazenes, 8-carbamoyl-3-alkylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one, have been designed as potential therapeutic alternatives to dacarbazine (14). This antitumor activity observed in several murine tumor models has been reported to be comparable with or superior to that of dacarbazine (13, 15-17). Temozolomide, which is a triazene prodrug, was shown to be effective for the treatment of certain central nervous system neoplasms and *in vitro* against lung cancer as well as against brain metastasis from non-small lung cancer (18). The researches for more effective anticancer agents has focused to a large extent on the design of molecules capable of recognizing and binding to target DNA base sequences. Structural and biophysical studies of the antitrypanosomal agent, Berenil [bis(4-amidinophenyl)-1,3-triazene], have shown that the molecule binds in a DNA duplex minor groove with a preference for Adenine/Thymine-rich base tracts (19-21).

In our department, a series of triazenes derived from 5-(4-aminophenyl)-2,4-dihydro-4-substituted-3*H*-1,2,4-triazole-3-thiones have been synthesized for *in vitro* anticancer properties against three cell lines (22); 1-[(4-Carboxy)phenyl]-3-[4(4-allyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thio-5-yl)phenyl]-3*H*-triazene has been showed only the marked effects on breast cancer cell lines (Figure 3). Some compounds that pass the criteria for activity in this assay have been scheduled automatically for evaluation against the full panel of 60 human tumour cell lines and showed variable antitumor activity

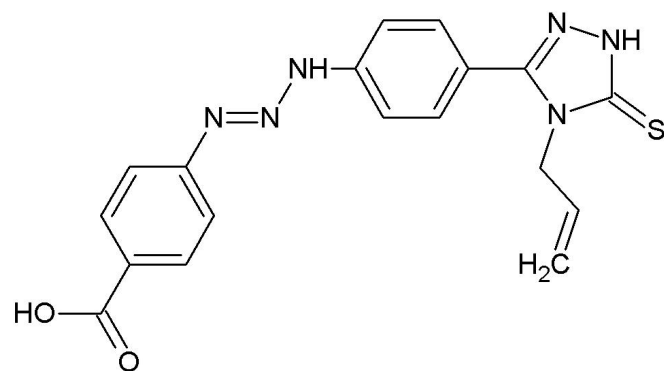


FIGURE 3. 1-[(4-Carboxy)phenyl]-3-[4(4-allyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thio-5-yl)phenyl]-3*H*-triazene

against most of the tested sub-panel tumor cell lines. In our other previous study, we reported that the synthesis of some novel triazenes and diazenes derived from *N*-methylaniline and to evaluate preliminary antitumor activity of these compounds *in vitro* against Huh-7-liver cancer cell (23).

Mathews et al. have been shown that 1,3-triazene functional group such as 1,3-diphenyl-1-triazene (DPT)) is metabolized by the pathway proposed in Figure 4. In this scheme, DPT is reduced by P450 reductase to form phenyl diazenyl radical and aniline that decomposes to form benzene and nitrogen gas (24). Antitumor activity of DPT attributed both to the aryldiazonium cations, formed by hydrolysis of an aryldimethyltriazene and to the methylation of bionucleophiles by the carbocations generated by solvolysis of arylmonomethyltriazenes.

Overexpression of epidermal growth factor receptor (EGFR) is observed in many human breast cancers. Recently, "combi-targeting concept" is termed as a novel tumor-targeting strategy by researchers in antitumor drug development. SMA41 (25); a 3-methyltriazene termed "combi-molecules" have been reported to possess EGFR/DNA targeting properties induced potent antiproliferative activity. Due to its poor hydrosolubility novel triazene compounds designed by Brahimi et al (26).

These observations led us to directed "combi-targeting concept" is termed as a novel tumor-targeting strategy. In this study, we designed novel triazene drugs due to conversation of methylating agent and sulfanilamide, is defined antitumor activity, recently.

RESULTS AND DISCUSSION

Chemistry

A synthetic route for the target triazenes **1-3**, **1a-3a** is outlined in the Figure 6. The diazonium salts derived from containing an aromatic primary amine group (sulfaguanidine, sulfapyridine, sulfamethoxazole) were coupled with *N*-methylaniline and 4-nitroaniline resulting in the formation of triazenes. Aromatic primary amines have been treated with nitrite ion under hydrochloric acid (22,23,27) and hydrochloric acid: gl.acetic acid (1/1, h/h) to form a diazonium salts, which are used to provide the desired triazene (28). In this study, we used hydrochloric acid: gl.acetic acid (1/1, h/h) for acidic media to form triazene compounds.

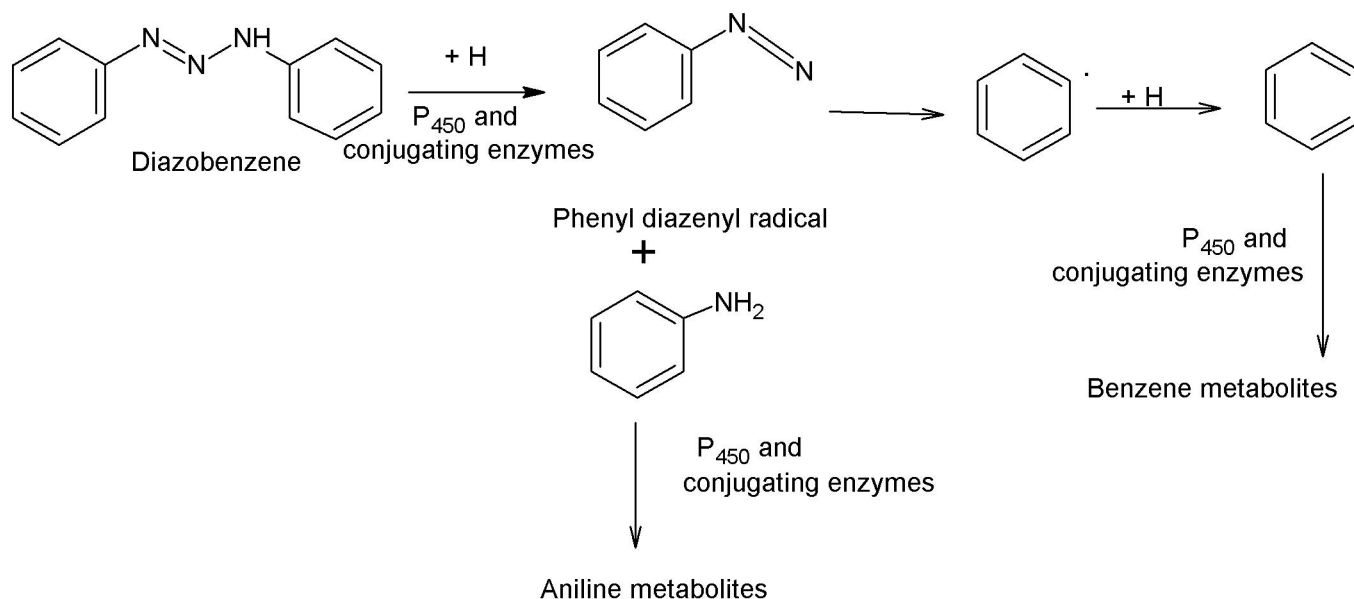


FIGURE 4. Proposed pathway for DPT metabolism

The target compounds were prepared by using the reaction sequence in Figure 6. We synthesized a series of novel triazene derivatives, in which diazonium salts of aromatic primary amines were treated with *N*-methyl aniline and *p*-nitroaniline according to literature (28). The structures of six new synthe-

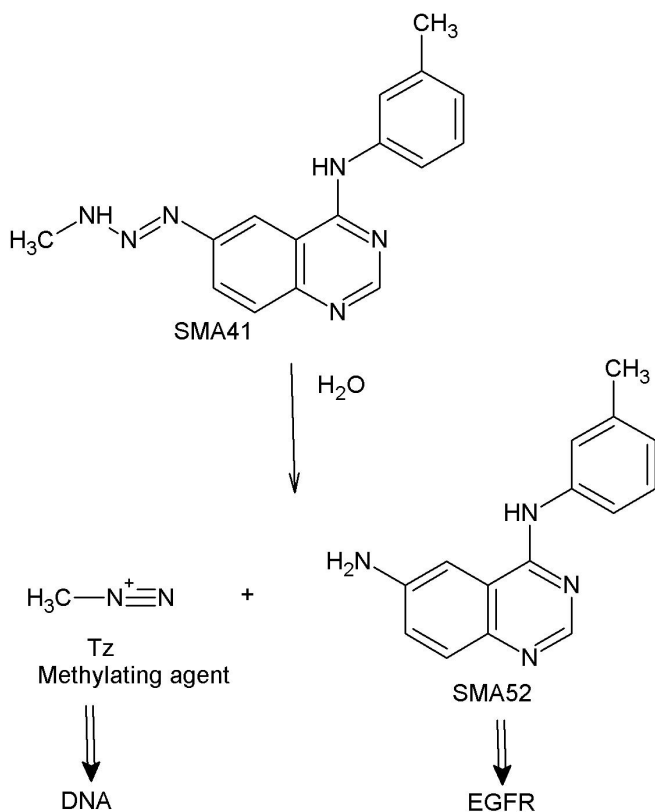


FIGURE 5. SMA41

sized compounds were confirmed by elemental analysis (C, H, N, S), UV, IR, $^1\text{H-NMR}$ and APCI-MS spectra. Analysis data of all the synthesized compounds were in full agreement with the suggested molecular structures. The chemical shifts of N-CH_3 protons of **1-3** were observed 2.09 ppm in $^1\text{H-NMR}$ spectra (29-33). The signals of triazene compounds (**1a-3a**) arising from $-\text{NH-}$ at 13.27-13.39 ppm were observed. This finding also supported the idea that the structures of these compounds should be given in triazene form. The signals of triazene compound, **2a** arising from N-H was not observed. This proton was exchangeable with $\text{DMSO-}d_6$. The remaining protons were also observed at the expected regions. The signals of *N*-methylaniline/*p*-nitroaniline and aromatic primary amines (Ar-NH_2) arising from the asym. and sym. stretching bands were not observed. Instead of these bands, The IR spectra of triazene compounds showed typical bands corresponding to the triazene group ($-\text{N}=\text{N}-\text{N}$) at $1334\text{-}1392\text{ cm}^{-1}$ (32, 34-36). In addition, triazene compounds were also characterised by their UV absorption. Maximum absorption bands of triazenes were detected at $352\text{-}389\text{ nm}$ (20,37-40). The APCI-MS spectra of **1-3** and **1a-3a** showed molecular ion $[\text{M}^++1]$ which confirmed its molecular weight. These findings also supported the idea that the structures of these compounds should be given in triazene form.

BIOLOGICAL ACTIVITY

Antitumor and Cytotoxic Activity

Cytotoxic and anticancer effects of synthesized compounds at four different concentrations tested. The CellTiter 96 Aqueous ONE Solution (Promega, Madison, WI) was used to evaluate cellular viability utilizing reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) cell culture and viability assay. A 549 and L 929 cell line were used to test both anticancer effects and cytotoxicity. Cell viability was analyzed using the MTS assay. The cytotoxic and antitumor activity of triazenes from sulfonamides on A549 lung cell growth showed not inhibition. Only

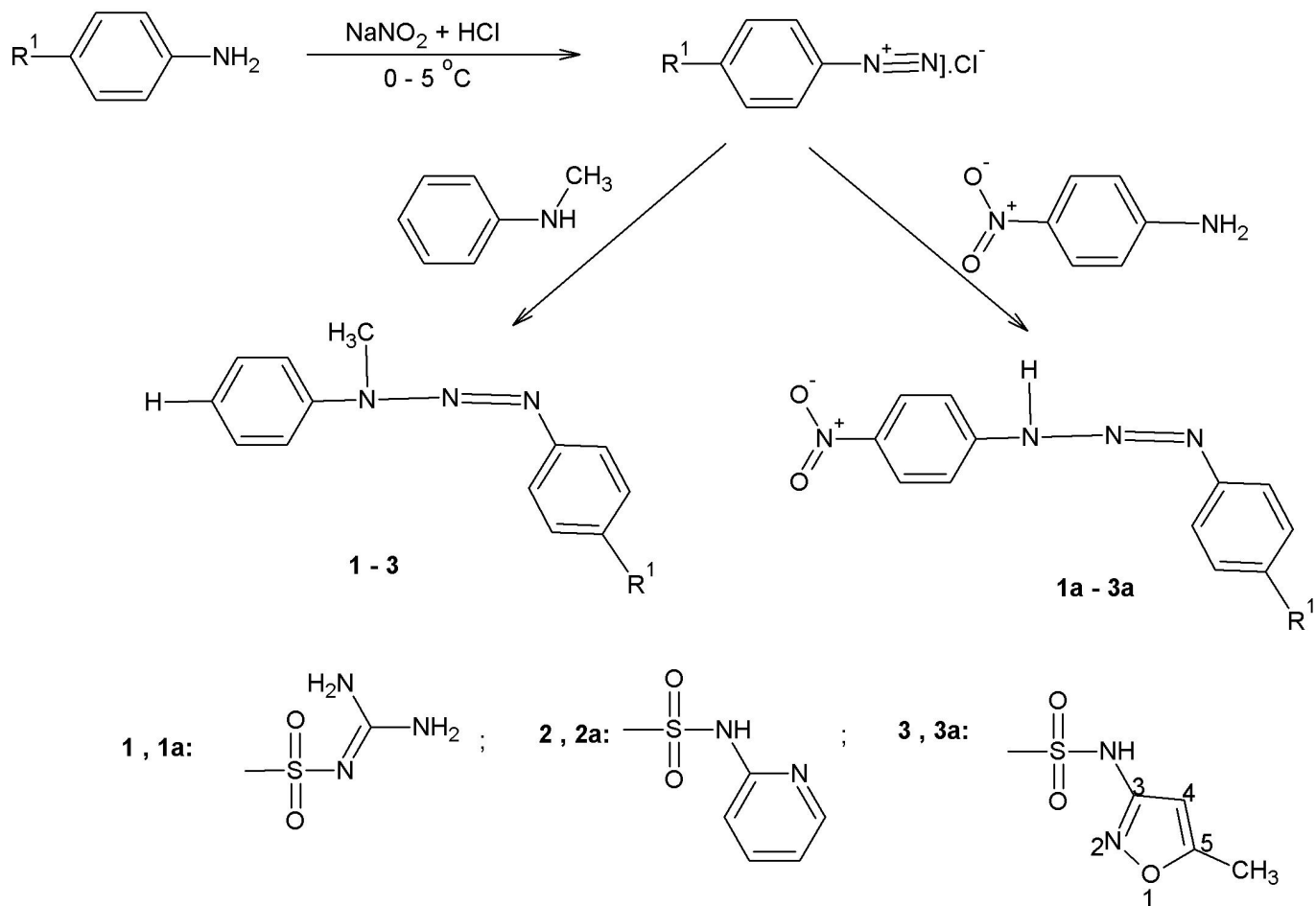


FIGURE 6. The synthesis pathway of the studied compounds

compound 3a had a cytotoxic effect on cancer cells when used at a concentration of 10 μM at the first day. After the first day the effect did not continue probably because the cancer cells metabolized the chemical.

The lipophilicity of the compounds is well known to play an important role in the penetration of these compounds into cells. The compounds of lipophilicity results were calculated using ALOGPS 2.1 software. The calculated values demonstrated that increasing/decreasing the lipophilic character of compounds the activity. Because of the lack of antitumor activity compounds through arildiazonium salts (Figure 4) can be considered.

EXPERIMENTAL

Chemistry

Melting points were determined on a Schmelzpunktbestimmer SMP II and are uncorrected. The UV spectra was measured with a Shimadzu UV-2100S. The IR spectra were recorded on Shimadzu FTIR-8400 S. $^1\text{H-NMR}$ spectra in dimethylsulfoxide- d_6 (DMSO- d_6) were obtained on a Bruker Avance-DPX 400 spectrometer. Tetramethylsilane (TMS) was used as an internal standard and all chemical shift values were recorded as δ (ppm) values. Mass spectra of synthesized compounds

were performed using Agilent 1100 MSD LC-MS. The elemental analysis for C, H, N, S was obtained on a Leco CHNS-932 instrument. ($^1\text{H-NMR}$, APCI-MS and Elemental analysis were provided by the Scientific and Technical Research Council of Turkey Instrumental Analysis Laboratories, Ankara-Turkey). All chemicals used in this study were supplied from Aldrich Chemical Co., Merck and Sigma.

General procedure for the preparation of triazenes (1-3, 1a-3a)

To a magnetically stirred cold solution (ice-bath $0-5^\circ\text{C}$) of these aromatic primary amines (3mmol) in 6 N aqueous hydrochloric acid/acetic acid (1:1, h/h), acetone (1 ml) and a ice-cold solution of sodium nitrite (0.21 g, 3 mmol) in water (1 ml) was added dropwise. Stirring was continued at $0-5^\circ\text{C}$ and then the reaction mixture was treated at the same temperature with a 40% aqueous *N*-methylaniline solution (6mmol) for **1-3** and *p*-nitroaniline (3mmol) for **1a-3a**. The reaction mixture was waited overnight at room temperature in dark. The precipitated solid that separated was washed with cold water, filtered off and then crystallized from ethanol.

N-(Diaminomethylene)-4-(3-methyl-3-phenyltriazene-1-ene-1-yl) benzenesulfonamide (1). Brown (ethanol), yield: 60%, mp 229°C ; UV λ_{max} (EtOH) (nm): 352, 238, 207. IR $\bar{\nu}_{\text{max}}$ (cm^{-1}): 3444,

3325, 3143, 2939, 1639, 1593, 1539, 1500, 1458, 1334, 1164, 825, 752. ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 2.09 (s, 3H, N-CH₃), 6.41-7.03 (b.s., 4H, N=C(-NH₂)₂), 7.19-8.37 (m, 9H, Ar-H). APCI-MS (*m/z*, %): 333 (100) [M+1]⁺, 305 (31.4), 215 (4.8), 199 (24.5), 182 (17.9); Anal. C₁₄H₁₆N₆O₂S calc. for: C, 50.59; H, 4.85; N, 25.28; S, 9.65. Found: C, 51.20; H, 4.73; N, 24.98; S, 9.53%. Log P : 2.18 ± 0.50.

N-(Diaminomethylene)-4-[3-(4-nitrophenyl)triaz-1-ene-1-yl]benzenesulfonamide (1a)

Yellow (ethanol), yield: 85%, mp 238 °C: UV λ_{max} (EtOH) (nm): 380, 248, 199. IR ν_{max} (cm⁻¹): 3479, 3367, 3290, 1627, 1601, 1530, 1508, 1481, 1334, 1172, 829. ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 6.60 (d, 2H, m-nitro, J= 9 Hz), 6.73 (s, 4H, N=C(NH₂)₂), 7.79 (d, 2H, o-triazene, J=9Hz), 7.95 (d, 2H, o-nitro, J= 9 Hz), 8.28 (d, 2H, m-triazene, J= 9 Hz), 13.27 (s, 1H, triazene NH). APCI-MS (*m/z*, %): 364 [M+1]⁺ (28.1), 336 (31.2), 215 (100), 200 (59.9). Anal. C₁₃H₁₃N₇O₄S calc. for: C, 42.97; H, 3.61; N, 26.98; S, 8.82. Found: C, 43.36; H, 3.92; N, 27.42; S, 9.18%. Log P : 2.21 ± 0.75.

4-(3-Methyl-3-phenyltriaz-1-ene-1-yl)-N-pyridine-2-ylbenzenesulfonamide (2)

Orange (ethanol), yield: 56%, mp 204-206 °C: UV λ_{max} (EtOH) (nm): 355, 239, 206. IR ν_{max} (cm⁻¹): 3217, 3028, 2931, 2812, 1631, 1596, 1531, 1500, 1461, 1361, 1155, 844, 767. ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 2.09 (s, 3H, N-CH₃), 6.86-8.02 (m, 9H, Ar-H), 11.74-12.41 (b.s., 1H, SO₂NH). APCI-MS (*m/z*, %): 368 [M+1]⁺ (100), 340 (47). Anal. C₁₈H₁₇N₅O₂S calc. for: C, 58.84; H, 4.66; N, 19.06; S, 8.73. Found: C, 59.61; H, 4.51; N, 18.96; S, 8.56%. Log P : 3.56 ± 0.50.

4-[(3-(4-Nitrophenyl)triaz-1-ene-1-yl)-N-pyridine-2-yl]benzenesulfonamide (2a)

Brown (ethanol), yield: 59%; mp 213 °C: UV λ_{max} (EtOH) (nm): 377, 243, 202. IR ν_{max} (cm⁻¹): 3217, 3078, 1631, 1596, 1504, 1473, 1446, 1392, 840. ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 6.58-6.62 (d, 4H, ArH), 6.73 (s, 4H, CH pyridine), 7.93-7.96 (d, 4H, Ar-H). APCI-MS (*m/z*, %): 399 [M+1]⁺ (4.8), 371 (23.3), 250 (100), 235 (84.6). Anal. C₁₇H₁₄N₆O₄S calc. for: C, 51.25; H, 3.54; N, 21.09; S, 8.05. Found: C, 50.94; H, 3.45; N, 20.76; S, 7.92%. Log P : 3.52 ± 0.75.

N-(5-Methylisoxazole-3-yl)-4-(3-methyl-3-phenyltriaz-1-ene-1-yl)benzenesulfonamide (3). Orange (ethanol), yield: 64%, mp 183-184 °C: UV λ_{max} (EtOH) (nm): 355, 239, 208. IR ν_{max} (cm⁻¹): 3310, 3147, 2940, 2831, 1620, 1593, 1500, 1469, 1431, 1338, 1157, 844, 756. ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 2.09 (s, 3H, C-CH₃), 2.30 (s, 3H, N-CH₃), 6.17 (s, 1H, isoxazole, C₄-H), 7.21-7.90 (m, 9H, Ar-H), 11.45 (s, 1H, SO₂NH). APCI-MS (*m/z*, %):

372 [M+1]⁺ (100), 344 (33.1). Anal. C₁₇H₁₇N₅O₃S calc. for: C, 54.97; H, 4.61; N, 18.86; S, 8.63. Found: C, 55.90; H, 4.69; N, 18.80; S, 8.63%. Log P : 3.58 ± 0.46.

N-(5-Methylisoxazole-3-yl)-4-[3-(4-nitrophenyl)triaz-1-ene-1-yl]benzenesulfonamide (3a)

Yellow (ethanol-water), yield: 63%; mp: 188 °C: UV λ_{max} (EtOH) (nm): 389, 206; IR ν_{max} (cm⁻¹): 3259, 3050, 2993, 1596, 1512, 1465, 1446, 1338, 1161, 845. ¹H-NMR (400 MHz, DMSO-d₆) δ (ppm): 2.31 (s, 3H, C₅-CH₃), 6.17 (s, 1H, isoxazole, C₄-H), 7.90-8.33 (m, 8H, Ar-H), 11.46 (s, 1H, SO₂NH), 13.39 (s, 1H, triazene NH). APCI-MS (*m/z*, %): 403 [M+1]⁺ (62.3), 375 (100), 254 (91.6). Anal. C₁₆H₁₄N₆O₅S calc. for: C, 47.76; H, 3.51; N, 20.89; S, 7.97. Found: C, 48.52; H, 3.34; N, 20.45; S, 7.94%. Log P : 3.54 ± 0.40.

BIOLOGICAL METHODS

Antitumor and cytotoxic activity

The synthesized compounds were tested for their anticancer activities and cytotoxicity properties. The CellTiter 96 Aqueous ONE Solution (Promega, Madison, WI) was used to evaluate cellular viability utilizing reduction of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS). A 549 and L 929 cell line were used to test both anticancer effects and cytotoxicity. Cells were routinely grown in a 75-mm flask in an environment containing 5% CO₂ and passed every 3 days. Cell viability was analyzed using the MTS assay. 5.000 Cells were plated in each well of a 96-well tissue culture plate. After 24 hours of growth the medium was replaced with fresh medium containing different concentration (10nM, 100nM, 1μM and 10μM) of chemicals, and the cells were grown for 4 days (41).

The MTS assay was performed according to the protocol provided by the Manufacturer. In short, 20 μL of MTS solution was added to each well, and cells were incubated at 37° C for 1 to 3 hours. The absorbance (at 490 nm) of each well was determined. Data are presented as a percentage of the values obtained from cells cultured under the same conditions in the absence of chemicals. For the time course study of chemicals' cytotoxicity, L 929 cells were treated with chemicals with the same dose used to detect anticancer activity. Cell viability was analyzed 1 to 4 days after the initiation of treatment, using the MTS assay.

Although all test compounds were dissolved in DMSO and the final concentration of DMSO was 0.1%, the solvent showed no activity in these assays at the level that was used for screening. For comparison of the anticancer activity and cytotoxicity ob-

Sülfonamidlerden türeyen triazenlerin sentezi ve karakterizasyonu

ÖZET: Bir seri yeni triazen türevleri olan 1-3, 1a-3a bileşikleri, asidik ortamda aminlerin diazonyum tuzlarının (sülfoguanidin, sülfopiridin, sülfometoksazol) N-metil anilin/p-nitroanilin ile kenetlenmesi sonucunda sentezlenmiştir. Sentezlenen bu bileşiklerin yapıları, spektral bulgular (UV, IR, ¹H-NMR) ve elemental analiz ile aydınlatılmıştır. Tüm bileşiklerin, in vitro olarak A 549 ve L 929 hücre dizilerinin büyümesine etkileri araştırılmış, sitotoksik ve antitumor aktiviteleri bulunmamıştır.

ANAHTAR KELİMELER: Triazen, sülfonamid, prontosil, azo redüksiyon

served with the test compounds, doxorubicin and taxol were selected as standard drugs.

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