Novel Anthracene-9-Sulfonyl Derivatives As Anticancer Agents: 
Synthesis And In Vitro Biological Evaluation

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Abstract

In this study, a new series of anthracene-9-sulfonyl derivatives incorporated with different heterocyclic moieties were synthesized and screened for their in vitro anticancer activity against colon carcinoma cell lines (HCT-116), hepatic carcinoma cell lines (HepG 2) and breast carcinoma cell lines (MCF-7). Among them, compound 4-acetylphenyl anthracene-9-sulfonate (8) showed selective high cytotoxic activity over colon carcinoma cell lines (HCT-116), also compounds 2-(anthracen-9-ylsulfonyl)malononitrile (7), N-(4-fluorophenyl)anthracene-9-sulfonamide (5b) and N-((1H-benzo[d]imidazol-2-yl)methyl)anthracene-9-sulfonamide (10) showed the significant selective cytotoxic effect over breast carcinoma cell lines (MCF-7). All the compounds are subjected to explore their safety on normal human skin cell lines (BJ-1), the results revealed that all the compounds are safe and have insignificant weak cytotoxicity over normal human cells.

Keywords: Anthracene-9-sulfonyl. Anticancer. HCT-116 cells. HepG 2 cells. MCF-7 cells.

1. Introduction

Anthracene is the simplest tricyclic aromatic compound, consisting of three fused benzene rings. Anthracene exhibit promising biological activities [1-4]. Anthracene derivatives have a broad range of biological activities for example, anti-inflammatory [5], antibacterial [6-9], antifungal [9] and anticancer activity [10-20]. Also anthracene was reported to be active against specific skin ailments [21]. Three-ring system of the anthracene has potential for overlapping with the DNA base pairs [22]. Many-sided chemistry of anthracene provides a suitable route to synthesis a number of closely related derivatives [23]. In the present study, we studied the anticancer activity of synthesized compounds (1-13) against colon carcinoma cell lines (HCT-116), hepatic carcinoma cell lines (HepG 2) and breast carcinoma cell lines (MCF-7).

Experimental Section

Chemistry

All melting points are uncorrected and were taken in open capillary tubes using Electrothermal apparatus 9100. Elemental microanalyses were carried out at Microanalytical Unit, Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt, using VarioElementar and were found within ±0.4% of the theoretical values. Infrared spectra were recorded on
a FT/IR- 4100 Jasco-Japan, Fourier transform, Infrared spectrometer at cm⁻¹ scale using KBr disc technique at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. ¹H NMR and ¹³C NMR spectra were determined by using a JEOL AS-500 NMR spectrometer at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. Varian Gemini200-Oxford 300 MHz and Merury Plus-Oxford 400 MHz at Ministry of defense, Chemical Warfare Department, The Main Chemical Warfare Laboratories, Cairo, Egypt. Chemical shifts were expressed in δ (ppm) downfield from tetramethylsilane as an internal standard. The mass spectra were measured with a GC MSQp1000EX Shimadzu, Cairo University, Cairo, Egypt, and with a Finnigan MAT SSQ-7000 mass spectrometer at Central Services Laboratory, National Research Centre, Dokki, Cairo, Egypt. Follow up of the reactions and checking the purity of the compounds were made by thin layer chromatography on silica gel-precoated aluminium sheets (Type 60, F 254, Merck, Darmstadt, Germany) using chloroform/methanol (20:2, v/v), and the spots were detected by exposure to UV lamp at λ254 nanometer for few seconds and by iodine vapor.

(Anthracen-9-ylsulfonyl)glycinoyl chloride (4)
To a mixture of 1 (2.76 g, 0.01 mol) and glycine (0.75 g, 0.01 mol) in dioxane (10 mL), aqueous saturated sodium carbonate was added drop wise until pH=7.5-8. After stirring for 0.5 h, the mixture was acidified with 1N hydrochloric acid and the formed precipitate was collected, washed several times with dry benzene and recrystallized from acetone to give 4.

Yield: 60%; M.p. > 300°C. IR spectrum (KBr, v, cm⁻¹): 3222 (NH), 1689 (C=O). ¹H NMR (DMSO-d₆): δ: 4.3 (s, 2H, CH₂), 7.5-8.6 (m, 9H, Ar-H), 9.3 (s, 1H, 1NH, D₂O exchangeable). ¹³C NMR (DMSO-d₆): δ 21.1, 21.3, 45.6, 123.7, 123.6, 125.9, 125.8, 125.6, 126.5, 126.8, 129.1, 129.3, 131.3, 131.4, 133.0, 162.5. MS: m/z = 333 (3%) (M⁺), m/z = 335 (6%) (M⁺+2); Anal. Calcd. For C₁₇H₁₄N₂O₃S (333.79): C, 57.57; H, 3.62; N, 4.20%; found: C, 57.45; H, 3.55; N, 5.13%.

N-(substituted)anthracene-9-sulfonamide 5(a, b)
A mixture of 1 (2.76 g, 0.01 mol) and derivatives of amines namely: 4-aminoantipyrine and 4-fluoroaniline (0.01 mol) in absolute ethanol (20 mL) containing triethylamine (1 mL) was stirred for 3 h at room temperature. The formed precipitate was filtered off, washed with water, dried and recrystallized from ethanol-dioxane mixture to give 5(a, b).

N-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)anthracene-9-sulfonamide (5a).
Yield: 80%; M.p. 159-161°C. IR spectrum (KBr, v, cm⁻¹): 3185 (NH), 1692 (C=O). ¹H NMR (DMSO-d₆): δ: 2.2 (s, 3H, CH₃), 3.2 (s, 3H, CH₃ of N-CH₂), 7.1-8.5 (m, 14H, Ar-H), 9.1 (s, 1H, 1NH, D₂O exchangeable). ¹³C NMR (DMSO-d₆): δ 13.3, 33.9, 114.5, 122.4, 123.4, 123.9, 124.2, 124.5, 124.6, 124.8, 127.3, 127.5, 127.6, 128.7, 128.2, 128.6, 129.1, 129.2, 129.7, 130.9, 132.1, 132.6, 133.3, 136.5, 161.6. MS: m/z = 443 (20%) (M⁺); Anal. Calcd. For C₂₁H₁₇N₂O₃S (443.52): C, 67.70; H, 4.77; N, 9.47%; found: C, 67.52; H, 4.26; N, 9.24%.

N-(4-fluorophenyl)anthracene-9-sulfonamide (5b).
Yield: 70%; M.p. 244-246°C. IR spectrum (KBr, ν, cm⁻¹): 3222 (NH). ¹H NMR (DMSO-d₆): δ 6.8-7.9 (m, 13H, Ar-H), 10.2 (s, 1H, NH, D₂O exchangeable).¹³C NMR (DMSO-d₆): δ 114.3, 114.9, 117.2, 117.6, 123.2, 123.4, 123.8, 123.9, 126.4, 126.5, 126.6, 126.8, 127.3, 127.8, 132.0, 132.3, 133.9, 136.8, 156.5. MS: m/z = 376 (18%) (M⁺); Anal. Calcd. For C₂₅H₁₉O₅S (376.43): C, 69.89; H, 4.14%.

N'-isonicotinoylanthracene-9-sulfonohydrazide (9)
A mixture of 1 (2.76 g, 0.01 mol) and isonicotinic hydrazide (1.37 g, 0.01 mol) in absolute ethanol (20 mL) containing triethylamine (1 mL) was stirred for 3 h at room temperature. The formed precipitate was filtered, washed with water, dried and recrystallized from DMF to give 9. Yield: 85%; M.p. 164-166°C. IR spectrum (KBr, ν, cm⁻¹): 3100, 3045, 3020, 3010 (4NH), 1687, 1675 (C=O). M.p. 245°C. Anal. Calcd. For C₂₉H₂₃NO₅S (4219): C, 68.20; H, 4.42; N, 10.85%; found: C, 68.30; H, 4.42; N, 10.74%.

NN'-[(1H-benzo[d]imidazol-2-yl)methyl]anthracene-9-sulfonamide (10)
Solution of 4 (0.33 g, 0.001 mol) in dry benzene (20 mL) and o-phenylenediamine (0.10 g, 0.001 mol) were refluxed for 30 min. The solid obtained was filtered and washed with cold water and then recrystallized from ethanol-dioxane mixture to give 10. Yield: 85%; M.p. 198-200°C. IR spectrum (KBr, ν, cm⁻¹): 3288, 3200 (2NH). ¹H NMR (DMSO-d₆): δ 4.1 (s, 2H, CH₂), 6.9-8.1 (m, 13H, Ar-H), 10.5, 12.1 (2s, 2H, NH, D₂O exchangeable).¹³C NMR (DMSO-d₆): δ 43.2, 116.3, 116.5, 122.2, 124.2, 124.3, 124.5, 124.6, 127.3, 127.4, 127.6, 127.9, 128.0, 129.3, 131.6, 131.7, 136.5, 137.4, 137.8, 142.5. MS: m/z = 387 (8%) (M⁺); Anal. Calcd. For C₂₉H₂₃NO₅S (387.46): C, 68.19; H, 4.42; N, 10.65%; found: C, 68.23; H, 4.30; N, 10.74%.

123.6, 123.7, 123.8, 123.9, 124.1, 126.1, 126.2, 126.3, 126.6, 126.5, 126.5, 126.7, 128.1, 128.4, 128.4, 128.6, 129.7, 129.8, 132.3, 133.6, 132.7, 132.7, 134.2, 134.2, 136.8, 136.9, 167.5, 167.5. MS: m/z = 702 (29%); (M+)1+; Anal. Calcd. For C24H22N6O4S (702.80): C, 60.18; H, 4.00; N, 19.76% found: C, 60.08; H, 3.99; N, 19.76%. Also 135.6, 137.2, 141.5, 169.3.

NN′-[[(1,1′-biphenyl)-4,4′-diyl]bis(2-(anthracene-9-sulfonamido)acetamide)] (12)

Solution of 4 (0.66 g, 0.002 mol) in dry benzene (20 mL) and benzidine (0.18 g, 0.001 mol) were refluxed for 30 min. The solid obtained was filtered and washed with cold water and then recrystallized from DMF to give 12.

Yield: 65%; M.p. 209-211°C. IR spectrum (KBr, ν, cm⁻¹): 3016, 3053, 3000, 2920 (NH), 1691, 1687 (C=O). 1H NMR (DMSO-d6): δ 4.3 (s, 4H, 2CH2), 6.9-8.4 (m, 26H, Ar-H), 8.9, 11.0 (2s, 2H, 2NH, D-O exchangeable). 13C NMR (DMSO-d6): δ 44.4, 44.5, 118.2, 118.3, 118.8, 118.9, 124.2, 124.3, 124.5, 124.6, 124.6, 124.7, 124.9, 127.1, 127.1, 127.3, 127.4, 127.4, 127.5, 127.6, 127.7, 127.8, 127.8, 127.9, 127.9, 128.1, 128.1, 129.4, 129.6, 131.5, 131.6, 131.7, 131.7, 135.6, 135.7, 135.8, 135.8, 138.2, 139.2, 169.1, 169.2. MS: m/z = 779 (35%) (M+1); Anal. Calcd. For C44H30N6O4S2 (778.90): C, 67.85; H, 4.40; N, 7.19%; found: C, 67.74; H, 4.28; N, 7.09%.

2-(Anthracene-9-sulfonamido)-N-(4-sulfamoylphenyl)acetamide (13)

Solution of 4 (0.33 g, 0.001 mol) in dry benzene (20 mL) and 4-aminobenzenesulfonamide (0.17 g, 0.001 mol) were refluxed for 30 min. The solid obtained was filtered and washed with cold water then recrystallized from dioxane to give 13.

Yield: 70%; M.p. 210-212°C. IR spectrum (KBr, ν, cm⁻¹): 3405, 3286 (NH2), 3185, 3100, 1691 (C=O). 1H NMR (DMSO-d6): δ 4.1 (s, 2H, CH2), 6.8 (s, 2H, NH, D-O exchangeable), 6.9-7.8 (m, 13H, Ar-H), 9.2, 10.1 (2s, 2H, 2NH, D-O exchangeable). 13C NMR (DMSO-d6): δ 45.2, 118.2, 118.6, 124.2, 124.3, 124.6, 124.7, 127.2, 127.4, 127.6, 127.8, 128.1, 128.8, 129.3, 129.9, 133.2, 133.5, 135.6, 137.2, 141.5, 169.3. MS: m/z = 469 (26%); (M+)1+; Anal. Calcd. For C24H19N3O4S2 (469.53): C, 66.28; H, 4.08; N, 8.95%; found: C, 65.08; H, 3.99; N, 8.65%.

Pharmacology (Cell lines):

Colon carcinoma cell lines (HCT-116), hepatic carcinoma cell lines (HepG 2), breast carcinoma cell lines (MCF-7) and BJ-1 °A telomerase-immortalized normal foreskin fibroblast cell line were obtained from Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden.

Cell viability assay:

Procedure was done according to [24], the cells were seeded at concentration of 10x10⁵ cells per well in case of HepG 2 and MCF-7, 20x10⁵ cells/well in case of HCT-116 cell lines and 40 x10⁵ cells/well in a fresh complete growth medium in case of BJ-1 using 96-well microtiter plastic plates at 37 °C for 24 hours under 5% CO₂ in a water jacketed carbon dioxide incubator. DMSO was used as negative control, Doxorubicin as positive control. After 48 hours’ incubation, the medium was aspirated and then MTX salt were added to each well and incubated for further four hours at 37 °C under 5% CO₂. To stop the reaction and dissolve the formed crystals, 10% sodium dodecyl sulphate (SDS) was added to each well and incubated overnight at 37 °C. The absorbance was measured using a microplate multiwell reader at 595 nm and a reference wavelength of 690 nm. Cell viability was assessed according to the mitochondrial- dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyl tetrazolium bromide) to purple formazan.

Results and discussion

Chemistry

Anthracene-9-sulfonyl chloride 1 synthesized as the reported method [25]. Acylation of glycine with compound 1 in the presence of aqueous saturated sodium carbonate gave Compound 2 according to the method [26]. (Scheme 1). 1H NMR for compound 2 confirmed the proposed structure due to the appearance of a singlet at 9.3 and 13.0 due to NH and OH in compound 2, respectively. Treatment of the latter compound with ammonium thiocyanate and acetic anhydride in the presence of anhydrous pyridine led to the formation of compound 3 according to the method [26]. Also compound 2 reacted with acetic anhydride and thionyl chloride to give compound 4 as the reported method [27]. (Scheme 1). Characteristic IR bands provide significant indication for the formation of compounds 3 and 4, for example the disappearance of (OH).
indicates the formation of compounds 3 and 4. Compounds (5a, b), 6, 7 and 9 prepared by the reaction of compound 1 with 4-aminoantipyrine, 4-fluoroaniline, hydrazene hydrate, malononitrile and isonicotinic hydrazide, respectively, according to the method [28]. (Scheme 2).

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\[ \text{Cl} \quad \text{SO}_2 \quad \text{OH} \quad \text{Cl} \]
\[ \text{H} \quad \text{O} \quad \text{C} \quad \text{O} \]

\[ \text{HN} \quad \text{NHCH}_2\text{COOH} \quad \text{H}_2\text{N} \quad \text{POH} \]
\[ \text{aq} \text{Na}_2\text{CO}_3 \]

\[ \text{HN} \quad \text{S} \quad \text{O} \quad \text{SO}_2 \]
\[ \text{HN} \quad \text{S} \quad \text{O} \quad \text{SO}_2 \]

\[ \text{SO}_2\text{NHCH}_2\text{COCl} \]

Scheme 1. Synthesis of anthracene-9-sulfonyl derivatives 1-4

Confirmation of the synthesized compounds 11 and 12 was done using \(^1\)H NMR spectroscopic data. For example appearance of a multiplet at 7.1-8.9 and 6.9-8.4 due to 22H and 26H of Ar-H in compounds 11 and 12, respectively. When acid chloride 4 was treated with sulfanilamide, it gave the corresponding
Table 1: In vitro cytotoxicity percent of 14 compounds, at concentration 100 µg/ml. the result is an average of 3 replicate.

<table>
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<tr>
<th>Compounds</th>
<th>HepG2</th>
<th>HCT-116</th>
<th>MCF-7</th>
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<tr>
<td>1</td>
<td>3.8%</td>
<td>34.2%</td>
<td>64.1%</td>
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<tr>
<td>2</td>
<td>3.5%</td>
<td>31.8%</td>
<td>45.9%</td>
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<td>3</td>
<td>12.9%</td>
<td>53.3%</td>
<td>68.8%</td>
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<td>4</td>
<td>9.6%</td>
<td>8.3%</td>
<td>59.7%</td>
</tr>
<tr>
<td>5a</td>
<td>4.8%</td>
<td>23.9%</td>
<td>63.4%</td>
</tr>
<tr>
<td>5b</td>
<td>29%</td>
<td>25%</td>
<td>89.8%</td>
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<tr>
<td>6</td>
<td>10.7%</td>
<td>43.2%</td>
<td>37.5%</td>
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<td>34.3%</td>
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<td>72%</td>
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<td>9</td>
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<td>46.2%</td>
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<td>40.8%</td>
<td>85.6%</td>
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<tr>
<td>Doxorubicin</td>
<td>99.5%</td>
<td>98%</td>
<td>99%</td>
</tr>
</tbody>
</table>

sulfanilamide 13 as reported method [27]. (Scheme 3). Characteristic IR bands provide significant indication for the formation of compound, the appearance of the characteristic band at 3405, 3286 due to the presence of (NH₂) in compound 13.

Pharmacology

According to our results, all the compounds gave weak cytotoxic effects on the hepatic cell lines (HepG 2) ranged from 3.5-40.7%. While compound 8 showed selective high cytotoxic activity (72%) over colon carcinoma cell lines (HCT-116), while the rest of the compounds had weak to moderate activity ranged from 2.6- 53.3%. For breast carcinoma cell lines (MCF-7), compounds 7, 5b, and 10 showed the significant selective cytotoxic effect with 93.4, 89.8 and 85.6% respectively as shown in Table 1 & Figure 1. All the compounds are subjected to explore their safety on normal human skin cell lines (BJ-1), the results revealed that all the compounds (1-13) are safe and have insignificant weak cytotoxicity over normal human cells. Compounds 7, 5b, 10, and 8 which possessed high cytotoxic activity over 70% were further screened at different concentrations at their corresponding cell line ranged from (100-12.5 ppm) to calculate their LC₅₀ values as shown in Figure 2 & 3.

From Figures 2 & 3, compounds 7, 5b, and 10 showed significant potentiality in a dose-dependent manner with LC₅₀ 34.7±0.4, 49.6±0.7 and 20±0.6, respectively. Where, Doxorubicin LC₅₀ on MCF-7 = 26.1±1.3. While compound 8 showed LC₅₀ 68±1.2 on colon HCT-116 cell lines Figure 3. While, Doxorubicin LC₅₀ = 37.6(±1.5).

Fig 3: Compound 8 cytotoxic activity over colon carcinoma cell lines (HCT-116) at different concentrations, the result is an average of 3 replicate.

Conclusion

The purpose of the present work is the synthesis of new anthracene-9-sulfonyl derivatives, which are projected to show potent anticancer activity. Thus, the parent anthracene-9-sulfonyl chloride 1 was subjected to a series of various reactions to get the target compounds. In vitro cytotoxic evaluation of all the novel anthracene-9-sulfonyl derivatives against colon carcinoma cell lines (HCT-116), hepatic carcinoma cell lines (HepG 2) and breast carcinoma cell lines (MCF-7). The activity profile revealed that the compounds containing p-acetyl phenyl-sulfonate, cyano group, p-fluorophenyl and benzimidazole ring have exerted a highly potent activity.

Conflict of interests: The authors declare that they have no conflict of interest

References


