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Anticancer Activity of Novel Indenopyridine Derivatives

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Eighteen new 4-[2-amino-3-cyano-5-oxo-4-substitutedaryl-4*H*-indeno[1,2-b]pyridin-1-(5*H*)-yl]benzenesulfonamide derivatives **6a-q** were synthesized via a reaction of aromatic aldehydes, enaminone **3** and malononitrile in one-pot reaction. Also, compounds **6a-q** were obtained, via another route by reaction of enaminone **3** with arylidenemalononitriles **4a-q**. The structure of the synthesized compounds was characterized by microanalysis, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. All the target compounds were subjected to *in vitro* anticancer activity against breast cancer cell line (MCF7). Compound **6d** showed a higher potency with IC_{50} value (4.34 μM) than that of the Doxorubicin (5.40 μM), as the reference drug, while compound **6n** with IC_{50} value (6.84 μM) is nearly as active as Doxorubicin. Also, compounds **6a-c**, **6e**, **6f**, **6h** and **6p** exhibited a moderate activity, while compounds **3**, **6g**, **6i-m**, **6o** and **6q** showed weak activity.

Key words: Indenopyridines, Sulfonamides, Anti-breast cancer activity

INTRODUCTION

Cancer is the leading cause of mortality to human beings. Thus, there exists a great urgency to develop highly efficacious and minimally toxic treatments for cancer. Although tremendous progress has been achieved in the development of novel cancer treatments, most of the current cancer drugs usually exhibit high toxicity and are severely resisted by tumor cells, in clinical practice. This dilemma is particularly true for DNA-damaging agents, the mainstay of cancer treatment (Hurley, 2002). Indenopyridines were found to possess several pharmacological properties, including anticancer activity (Israel et al., 1972; Utsugi et al., 1997; Manpadi et al., 2007; Vigante et al., 2007; Ghahremanzadeh et al., 2010). Also, the sulfonamides constitute an important class of drugs, with several types of pharmacological agents, which possesses anticancer activity (Abbate et al., 2004; Ghorab et al., 2006; Ismail et al., 2006; Rostom, 2006) among others. A large number of structurally novel sulfonamides have ultimately been reported to show substantial anticancer activity *in vitro* and *in vivo* (Supuran et al., 2004). Several mechanisms have been reported for anticancer activity of the sulfonamide compounds and the most prominent of these mechanisms were through the inhibition of the carbonic anhydrase (Maren, 1976; Supuran and Scozzafava, 2000; Kivelä et al., 2005; Turkmen et al., 2011). The mechanism of tumor inhibition by sulfonamide carbonic anhydrase (CA) inhibitor was suggested by Chegwidden et al. (2000). These compounds may reduce the provision of bicarbonate for the synthesis of nucleotides and other cell components, such as membrane lipids. In continuation of our work (Ghorab et al., 2010) it seemed of interest to design and synthesize a novel series of indeno[1,2-*b*]pyridines, carrying a biologically active sulfonamide moiety to be evaluated as potential anticancer agents.

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MATERIALS AND METHODS

Materials

Melting points (°C, uncorrected) were determined in open capillaries on a Gallenkemp melting point apparatus (Sanyo Gallenkemp). Precoated silica gel plates (silica gel 0.25 mm, 60 G F 254; Merck) were used for thin layer chromatography, dichloromethane-methanol (9.5:0.5 mL) mixture was used as a developing solvent

system and the spots were visualized by ultraviolet light and/or iodine. Infrared spectra were recorded, in KBr discs, using IR-470 Shimadzu spectrometer (Shimadzu). ^{1}H-NMR spectra (in DMSO-d_6) were recorded on Bruker Ac-300 ultra shield NMR spectrometer (Bruker, δ ppm), at 300 MHz, using TMS as internal standard. Electron impact Mass Spectra were recorded on a, Shimadzu Gc-Ms-Qp 5000 instrument (Shimadzu). Elemental analyses were performed on Carlo Erba 1108 Elemental Analyzer (Heraeus). All compounds were within ± 0.4% of the theoretical values.

Synthesis

4-(3-Oxo-3*H*-inden-1-yl-amino)benzenesulfonamide (3)

A mixture of indane-1,3-dione **1** (1.49 g, 0.01 mol) and sulfanilamide **2** (1.72 g, 0.01 mol) in ethanol (30 mL) was refluxed for 4 h. The reaction mixture was cooled and poured onto ice-water. The obtained solid was crystallized from dioxane to give **3**.

Yield, 74%; m.p. 194-196ºC; IR (KBr, cm^{-1}): 3345, 3330, 3300 (NH, NH_2), 1715 (C=O), 1372, 1158 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 7.3-7.9 [m, 10H, Ar-H + SO_2NH_2], 9.0 [s, 1H, CH], 10.5 [s, 1H, NH]. ^{13}C-NMR (DMSO-d_6): 98.0, 116.9, 125.1, 127.2, 128.4, 130.0, 130.5, 134.2, 135.6, 137.0, 145.8, 158.3, 200.4. MS m/z (%): 300 [M$^+$] (15.6), 64 (100). Anal. Calcd. for $C_{15}H_{12}N_2O_3S$: C, 59.99; H, 4.03; N, 9.33. Found: C, 59.60; H, 4.40; N, 9.70.

4-[2-Amino-3-cyano-5-oxo-4-substitutedaryl-4*H*-indeno[1,2b]-pyridine-1(5*H*)-yl]benzenesulfonamide (6a-q)

Method (A): A mixture of **3** (3 g, 0.01 mol) and arylidenemalononitriles **4a-q** (0.01 mol) in ethanol (30 mL), containing 3 drops of triethylamine, was refluxed for 4 h. The reaction mixture was filtered while hot and the obtained solid was crystallized from ethanol, to give **6a-q**.

Method (B): A mixture of **3** (3 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol) and aldehyde (0.01 mol) in ethanol (30 mL), containing 3 drops of triethylamine, was refluxed for 4 h. The reaction mixture was filtered while hot and the obtained solid was crystallized from ethanol, to give **6a-q** (m.p and mixed m.p and the same R_f).

4-[2-Amino-3-cyano-5-oxo-4-phenyl-4*H*-indeno[1,2-*b*] pyridin-1-(5*H*)-yl]-benzenesulfonamide (6a)

Yield, 79%; m.p. 179-181ºC; IR (KBr, cm^{-1}): 3436, 3344, 3280 (NH_2), 3067 (CH arom.), 2190 (C≡N), 1705 (C=O), 1373, 1152 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 4.3 [s, 1H, CH], 6.6 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.9 [m, 15H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 39.0, 57.9, 102.9, 118.3, 119.6, 125.1, 126.5, 127.8, 128.2, 128.8, 129.1, 129.9, 130.2, 135.7, 138.6, 139.2, 140.5, 147.3, 153.0, 169.4, 194.6. MS m/z (%) 454 [M$^+$] (1.92), 233 (100). Anal. Calcd for $C_{25}H_{18}N_4O_3S$: C, 66.07; H, 3.99; N, 12.33. Found: C, 66.30; H, 3.70; N, 12.70.

4-[2-Amino-3-cyano-5-oxo-4-tolyl-4*H*-indeno[1,2-*b*] pyridin-1-(5*H*)-yl]benze-nesulfonamide (6b)

Yield, 81%; m.p. 104-106ºC; IR (KBr, cm^{-1}): 3432, 3327, 3290 (NH_2), 2986, 2840 (CH aliph.), 2191 (C≡N), 1720 (C=O), 1368, 1188 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 2.3 [s, 3H, CH_3], 4.4 [s, 1H, CH], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.1-7.8 [m, 14H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 24.3, 40.0, 61.8, 96.0, 118.5, 119.8, 123.7, 127.0, 127.9, 128.8, 129.5, 130.7, 135.7, 137.3, 138.2, 139.1, 140.4, 147.3, 153.0, 168.6, 196.2. MS m/z (%) 468 [M$^+$] (3.58), 105 (100). Anal. Calcd for $C_{26}H_{20}N_4O_3S$: C, 66.65; H, 4.30; N, 11.96. Found: C, 66.40; H, 4.60; N, 11.70.

4-[2-Amino-3-cyano-5-oxo-4-(2-hydroxyphenyl)-4*H*-indeno[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6c)

Yield, 69%; m.p. 170-172ºC; IR (KBr, cm^{-1}): 3390 (OH), 3343, 3300, 3270 (NH_2), 2184 (C≡N), 1684 (C=O), 1333, 1152 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 5.1 [s, 1H, CH], 6.8 [s, 2H, NH_2 exchangeable with D_2O], 9.1 [s, 1H, OH]. ^{13}C-NMR (DMSO-d_6): 30.8, 56.0, 102.0, 116.8, 118.5, 119.8, 121.9, 124.3, 126.4, 127.8, 128.4, 128.6, 129.0, 129.8, 130.2, 131.1, 134.8, 137.2, 140.7, 149.8, 160.8, 165.9, 190.4. MS m/z (%) 470 [M$^+$] (1.5), 63 (100). Anal. Calcd for $C_{25}H_{18}N_4O_4S$: C, 63.82; H, 3.86; N, 11.91. Found: C, 63.60; H, 3.50; N, 11.60.

4-[2-Amino-3-cyano-5-oxo-4-(4-hydroxyphenyl)-4*H*-indeno[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6d)

Yield, 63%; m.p. 95-97ºC; IR (KBr, cm^{-1}): 3410 (OH), 3367, 3260, 3210 (NH_2), 2164 (C≡N), 1685 (C=O), 1396, 1168 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 4.4 [s, 1H, CH], 6.5 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.8 [m, 14H, Ar-H + SO_2NH_2], 8.3 [s, 1H, OH]. ^{13}C-NMR (DMSO-d_6): 39.2, 56.0, 105.8, 114.5, 116.1, 119.6, 123.0, 126.9, 127.5, 128.9, 130.5, 131.7, 134.1, 135.1, 136.4, 137.5, 140.8, 154.5, 159.6, 162.3, 186.4. Anal. Calcd for $C_{25}H_{18}N_4O_4S$: C, 63.82; H, 3.86; N, 11.91. Found: C, 63.60; H, 3.50; N, 11.60.

E-4-[2-amino-3-cyano-5-oxo-4-styryl-4*H*-indeno[1,2-*b*] pyridin-1-(5*H*)-yl]ben-zenesulfonamide (6e)

Yield, 71%; m.p. 124-126ºC; IR (KBr, cm^{-1}): 3390, 3343, 3310 (NH_2), 3060 (CH arom.), 2940, 2860 (CH aliph.),

2190 (C≡N), 1701 (C=O), 1349, 1153 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 3.8 [d, 1H, CH-4, *J* = 7.2 Hz], 6.6-6.7 [m, 2H, CH=CH], 6.8 [s, 2H, NH_2 exchangeable with D_2O], 7.3-7.5 [m, 15H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 27.6, 58.1, 104.0, 117.8(2), 119.2, 123.4, 125.7(2), 127.8, 129.0, 129.5(2), 129.7, 129.9(2), 130.6, 130.8, 131.3, 135.1, 136.5, 136.9, 138.0, 146.1, 153.4, 168.8, 194.7. MS m/z (%) 480 [M^+] (4.3), 219 (100). Anal. Calcd for $C_{27}H_{20}N_4O_3S$: C, 67.48; H, 4.20; N, 11.66. Found: C, 67.10; H, 4.50; N, 11.40.

4-[2-Amino-3-cyano-5-oxo-4-(2-methoxyphenyl)-4*H*-indeno-[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6f)

Yield, 88%; m.p. 208-210ºC; IR (KBr, cm^{-1}): 3407, 3345, 3243 (NH_2), 3066 (CH arom.), 2940, 2853 (CH aliph.), 2212 (C≡N), 1710 (C=O), 1368, 1191 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 3.7 [s, 3H, OCH_3], 4.4 [s, 1H, CH], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.9 [m, 14H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 32.7, 56.0, 61.8, 98.1, 116.9, 118.3, 119.3, 122.2, 123.7, 127.2, 128.4, 129.2, 130.0, 130.6, 131.3, 135.7, 137.6, 140.1, 145.7, 152.4, 158.4, 168.6, 195.2. MS m/z (%) 484 [M^+] (9.1), 63 (100). Anal. Calcd for $C_{26}H_{20}N_4O_4S$: C, 64.54; H, 4.16; N, 11.56. Found: C, 64.70; H, 4.40; N, 11.20.

4-[2-Amino-3-cyano-5-oxo-4-(4-methoxyphenyl)-4*H*-indeno-[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6g)

Yield, 78%; m.p. 230-232ºC; IR (KBr, cm^{-1}): 3472, 3344, 3300 (NH_2), 3100 (CH arom.), 2920, 2840 (CH aliph.), 2208 (C≡N), 1706 (C=O), 1349, 1188 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 3.8 [s, 3H, OCH_3], 4.3 [s, 1H, CH], 6.9 [s, 2H, NH_2 exchangeable with D_2O], 7.0-8.0 [m, 14H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 39.3, 56.0, 61.8, 98.1, 116.1, 117.0, 118.5, 127.2, 128.4, 129.1, 130.0, 130.7, 131.3, 135.7, 137.0, 139.1, 147.1, 153.0, 158.4, 168.6, 191.8. MS m/z (%) 484 [M^+] (2.4), 44 (100). Anal. Calcd for $C_{26}H_{20}N_4O_4S$: C, 64.45; H, 4.16; N, 11.56. Found: C, 64.10; H, 4.50; N, 11.30.

4-[2-Amino-4-(benzo[d][1,3]dioxol-5-yl)-3-cyano-5-oxo-4*H*-indeno[1,2-*b*]-pyridin-1-(5*H*)-yl]benzenesulfonamide (6h)

Yield, 68%; m.p. 85.87ºC; IR (KBr, cm^{-1}): 3448, 3363, 3232 (NH_2), 2985, 2897 (CH aliph.), 2191 (C≡N), 1705 (C=O), 1380, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 4.3 [s, 1H, CH], 6.1 [s, 2H, O-CH_2-O], 6.3 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.7 [m, 13H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 38.9, 61.8, 101.3, 102.3, 115.9, 116.0, 118.2, 118.6, 122.9, 124.8, 127.9, 128.5, 129.2, 130.7, 133.7, 134.1, 135.8, 137.5, 146.5, 146.9, 148.9, 152.9, 163.8, 195.1. Anal. Calcd for $C_{26}H_{18}N_4O_5S$: C, 62.64; H, 3.64; N, 11.24. Found: C, 62.40; H, 3.90; N, 11.60.

4-[2-Amino-3-cyano-5-oxo-4-(4-N-dimethylphenyl)-4*H*-indeno[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6i)

Yield, 66%; m.p. 167-169ºC; IR (KBr, cm^{-1}): 3421, 3367, 3280 (NH_2), 2920, 2816 (CH aliph.), 2210 (C≡N), 1701 (C=O), 1375, 1188 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 3.0 [s, 6H, $2CH_3$], 4.9 [s, 1H, CH], 6.8 [s, 2H, NH_2 exchangeable with D_2O], 7.7-8.0 [m, 14H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 38.9, 39.9, 62.9, 100.2, 115.4, 116.2, 119.7, 123.9, 128.6, 129.1, 130.2, 131.5, 133.5, 134.8, 136.9, 137.7, 141.4, 146.3, 151.7, 154.2, 162.1, 190.4. Anal. Calcd for $C_{27}H_{23}N_5O_3S$: C, 65.17; H, 4.66; N, 14.08. Found: C, 65.50; H, 4.30; N, 14.40.

4-[2-Amino-3-cyano-5-oxo-4-(3-nitrophenyl)-4*H*-indeno-[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6j)

Yield, 72%; m.p. 160-161ºC; IR (KBr, cm^{-1}): 3370, 3352, 3225 (NH_2), 3046 (CH arom.), 2213 (C≡N), 1702 (C=O), 1349, 1192 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 4.4 [s, 1H, CH], 7.1 [s, 2H, NH_2 exchangeable with D_2O], 7.4-8.4 [m, 14H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 39.1, 56.0, 95.5, 118.7, 119.7, 122.6, 124.1, 126.4, 128.6, 129.2, 129.6, 130.1, 130.9, 135.4, 136.0, 136.9, 139.2, 145.7, 147.2, 147.7, 153.1, 168.7, 194.8. MS m/z (%) 499 [M^+] (3.3), 63 (100). Anal. Calcd for $C_{25}H_{17}N_5O_5S$: C, 60.11; H, 3.43; N, 14.02. Found: C, 60.40; H, 3.10; N, 14.30.

4-[2-Amino-3-cyano-5-oxo-4-(4-nitrophenyl)-4*H*-indeno-[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6k)

Yield, 83%; m.p. 123-125ºC; IR (KBr, cm^{-1}): 3410, 3380, 3344 (NH_2), 3088 (CH arom.), 2212 (C≡N), 1709 (C=O), 1347, 1190 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 4.3 [s, 1H, CH], 7.1 [s, 2H, NH_2 exchangeable with D_2O], 7.4-8.3 [m, 14H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 38.9, 57.8, 95.1, 115.5, 118.5, 123.4, 126.9, 128.3, 129.2, 130.1, 131.2, 131.6, 134.3, 135.6, 139.2, 145.7, 146.6, 147.7, 153.0, 169.1, 194.8. MS m/z (%) 499 [M^+] (0.49), 283 (100). Anal. Calcd for $C_{25}H_{17}N_5O_5S$: C, 60.11; H, 3.43; N, 14.00. Found: C, 59.80; H, 3.70; N, 13.70.

4-[2-Amino-3-cyano-5-oxo-4-(2-chlorophenyl)-4*H*-indeno-[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6l)

Yield, 69%; m.p. 146-148ºC; IR (KBr, cm^{-1}): 3344, 3310, 3260 (NH_2), 3064 (CH arom.), 2214 (C≡N), 1711 (C=O), 1368, 1189 (SO_2), 736 (C-Cl). ^{1}H-NMR (DMSO-d_6): δ 4.3 [s, 1H, CH], 7.1 [s, 2H, NH_2 exchangeable with D_2O], 7.4-8.0 [m, 14H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 31.9, 61.8, 98.2, 118.9, 119.0, 123.8, 127.1, 127.2,

127.5, 128.4, 129.1, 129.6, 130.7, 131.5, 134.2, 135.5, 137.1, 139.3, 143.6, 145.4, 153.0, 168.6, 194.8. MS m/z (%) 488 [M^+] (2.4), 44 (100). Anal. Calcd for $C_{25}H_{17}ClN_4O_3S$: C, 61.41; H, 3.50; N, 11.47. Found: C, 61.10; H, 3.80; N, 11.70.

4-[2-Amino-3-cyano-5-oxo-4-(2,4-dichlorophenyl)-4*H*-indeno-[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6m)

Yield, 88%; m.p. 142-144°C; IR (KBr, cm^{-1}): 3380, 3344, 3290 (NH_2), 3056 (CH arom.), 2193 (C≡N), 1717 (C=O), 1370, 1153 (SO_2), 734 (C-Cl). ^{1}H-NMR (DMSO-d_6): δ 4.3 [s, 1H, CH], 6.3 [s, 2H, NH_2 exchangeable with D_2O], 7.2-7.9 [m, 13H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 31.3, 60.3, 95.6, 115.1, 119.7, 125.1, 127.7, 128.8, 129.1, 129.8, 130.4, 131.7, 132.8, 133.8, 134.3, 135.5, 137.2, 138.4, 142.4, 145.4, 153.1, 168.6, 195.7. MS m/z (%) 523 [M^+] (2.9), 159 (100). Anal. Calcd for $C_{25}H_{16}Cl_2N_4O_3S$: C, 57.37; H, 3.08; N, 10.70. Found: C, 57.70; H, 3.40; N, 10.40.

4-[2-Amino-3-cyano-4-(2-hydroxynaphthalen-l-yl)-5-oxo-4*H*-indeno[1,2-*b*]-pyridin-1-(5*H*)-yl]benzenesulfonamide (6n)

Yield, 76%; m.p. 177-179°C; IR (KBr, cm^{-1}): 3410 (OH), 3360, 3344, 3320 (NH_2), 3048 (CH arom.), 2192 (C≡N), 1708 (C=O), 1348, 1153 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 4.1 [s, 1H, CH], 6.8 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.9 [m, 16H, Ar-H + SO_2NH_2], 10.3 [s, 1H, OH]. ^{13}C-NMR (DMSO-d_6): 29.4, 56.7, 104.3, 116.6, 117.7, 118.7, 119.0, 122.0, 123.9, 125.0, 125.4, 126.3, 127.7, 128.0, 128.3, 129.2, 130.2, 134.0, 135.7, 137.0, 137.1, 147.9, 152.1, 153.4, 162.2, 195.3. MS m/z (%) 520 [M^+] (3.3), 63 (100). Anal. Calcd for $C_{29}H_{20}N_4O_4S$: C, 66.91; H, 3.87; N, 10.76. Found: C, 66.60; H, 3.50; N, 10.40.

4-[2-Amino-3-cyano-4-(2-methoxynaphthalen-1-yl)-5-oxo-4*H*-indeno[1,2-*b*]-pyridin-1-(5*H*)-yl]benzenesulfonamide (6o)

Yield, 73%; m.p. 160-162°C; IR (KBr, cm^{-1}): 3478, 3377, 3342 (NH_2), 3071 (CH arom.), 2960, 2840 (CH aliph.), 2189 (C≡N), 1701 (C=O), 1348, 1150 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 3.8 [s, 3H, OCH_3], 4.0 [s, 1H, CH], 7.0 [s, 2H, NH_2 exchangeable with D_2O], 7.3-7.9 [m, 16H, Ar-H + SO_2NH_2]. MS m/z (%) 534 [M^+] (6.2), 47 (100). Anal. Calcd for $C_{30}H_{22}N_4O_4S$: C, 67.40; H, 4.15; N, 10.48. Found: C, 67.70; H, 4.50; N, 10.10.

4-[2-Amino-3-cyano-4-(4-methoxynaphthalen-1-yl)-5-oxo-4*H*-indeno[1,2-b]-pyridin-1-(5*H*)-yl]benzenesulfonamide (6p)

Yield, 78%; m.p. 101-103°C; IR (KBr, cm^{-1}): 3441, 3356, 3251 (NH_2), 3066 (CH arom.), 2981, 2839 (CH aliph.), 2186 (C≡N), 1701 (C=O), 1392, 1157 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 3.7 [s, 3H, OCH_3], 4.0 [s, 1H, CH], 6.8 [s, 2H, NH_2 exchangeable with D_2O], 7.0-8.3 [m, 16H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 38.9, 55.5, 56.4, 103.6, 104.7, 115.4, 116.1, 122.0, 123.7, 124.3, 124.9, 125.3, 125.6, 126.5, 127.6, 128.0, 128.7, 130.4, 132.2, 133.7, 135.3, 137.8, 140.7, 155.4, 158.5, 162.8, 193.8. Anal. Calcd for $C_{30}H_{22}N_4O_4S$: C, 67.40; H, 4.15; N, 10.48. Found: C, 67.70; H, 4.50; N, 10.10.

4-[2-Amino-3-cyano-4-(2-thienyl-1-yl)-5-oxo-4*H*-indeno-[1,2-*b*]pyridin-1-(5*H*)-yl]benzenesulfonamide (6q)

Yield, 81%; m.p. 47-49°C; IR (KBr, cm^{-1}): 3434, 3380, 3290 (NH_2), 2190 (C≡N), 1707 (C=O), 1390, 1158 (SO_2). ^{1}H-NMR (DMSO-d_6): δ 4.1 [s, 1H, CH], 6.8 [s, 2H, NH_2 exchangeable with D_2O], 7.0-7.9 [m, 13H, Ar-H + SO_2NH_2]. ^{13}C-NMR (DMSO-d_6): 27.8, 56.8, 103.6, 116.9, 117.4, 123.1, 123.3, 123.4, 124.1, 126.7, 128.3, 129.0, 132.5, 135.5, 137.0, 138.4, 143.5, 151.3, 169.1, 196.4. Anal. Calcd for $C_{23}H_{16}N_4O_3S_2$: C, 59.98; H, 3.50; N, 12.17. Found: C, 59.60; H, 3.20; N, 12.50.

In vitro cytotoxic screening

The cytotoxic activity of the newly synthesized compounds was evaluated *in vitro,* using the Sulfo-Rhodamine-B stain (SRB) assay by the method of Skehan et al. (1990). The *in vitro* anticancer screening was done by the pharmacology unit, at the National Cancer Institute, Cairo University.

Cells were plated in 96-multiwell microtiter plate (10^4 cells/well) for 24 h, before treatment with the compound (s) to allow the attachment of cells to the wall of the plate. The tested compounds dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compounds under test (5, 12.5, 25, and 50 μg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 h, at 37°C, and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained for 30 min with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Excess unbound dye was removed, by four washes, with 1% acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for breast tumor cell line, after the specified time (Skehan et al., 1990). The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table I.

Table I. *In vitro* cytotoxic activity of the newly synthesized compounds against human breast cancer cell line (MCF-7)

Compd. No.	Compound concentration (μg/mL)				IC_{50}[a] (μg/mL)	IC_{50}[b] (μM)
	5	12.5	25	50		
	Surviving fraction (Means ± S.E.)[a]					
Doxorubicin	0.1943 ± 0.11	0.1717 ± 0.02	0.1855 ± 0.01	0.2013 ± 0.03	2.97	5.40
3	0.5251 ± 0.11	0.3857 ± 0.02	0.3556 ± 0.04	0.1778 ± 0.01	5.72	19.06
6a	0.3823 ± 0.08	0.1714 ± 0.03	0.1566 ± 0.07	0.1362 ± 0.03	4.40	9.68
6b	0.5005 ± 0.09	0.4878 ± 0.08	0.3661 ± 0.07	0.1588 ± 0.02	4.60	9.82
6c	0.2815 ± 0.04	0.1411 ± 0.08	0.1407 ± 0.01	0.1431 ± 0.01	3.30	7.02
6d	0.2263 ± 0.02	0.1346 ± 0.04	0.1212 ± 0.02	0.1153 ± 0.02	2.04	4.34
6e	0.8502 ± 0.10	0.6243 ± 0.03	0.3514 ± 0.03	0.2735 ± 0.01	4.26	8.86
6f	0.3539 ± 0.03	0.2782 ± 0.06	0.2443 ± 0.03	0.2779 ± 0.05	3.98	8.22
6g	0.6704 ± 0.10	0.1404 ± 0.03	0.1828 ± 0.04	0.1577 ± 0.03	10.90	22.52
6h	0.3172 ± 0.06	0.1250 ± 0.07	0.0804 ± 0.09	0.0591 ± 0.03	3.64	7.08
6i	0.5989 ± 0.09	0.2995 ± 0.08	0.2239 ± 0.07	0.2220 ± 0.02	12.00	24.14
6j	0.6809 ± 0.05	0.2977 ± 0.06	0.2616 ± 0.04	0.1029 ± 0.02	8.90	17.82
6k	0.6993 ± 0.03	0.2720 ± 0.01	0.2296 ± 0.02	0.2164 ± 0.04	14.40	28.86
6l	0.5770 ± 0.04	0.4562 ± 0.08	0.3451 ± 0.03	0.1580 ± 0.01	9.52	19.50
6m	0.6036 ± 0.10	0.3978 ± 0.03	0.3268 ± 0.04	0.3101 ± 0.03	15.10	28.87
6n	0.2827 ± 0.07	0.2987 ± 0.07	0.3405 ± 0.02	0.3576 ± 0.06	3.56	6.84
6o	0.6339 ± 0.05	0.3730 ± 0.02	0.3564 ± 0.06	0.3525 ± 0.03	15.40	28.84
6p	0.3846 ± 0.10	0.2198 ± 0.03	0.1358 ± 0.04	0.0990 ± 0.03	3.92	7.34
6q	0.6814 ± 0.06	0.1574 ± 0.04	0.1946 ± 0.02	0.1351 ± 0.03	11.20	24.45

[a]Each value is the mean of three values ± S.E.; [b]IC_{50} value: concentration causing 50% inhibition of cell viability.

RESULTS AND DISCUSSION

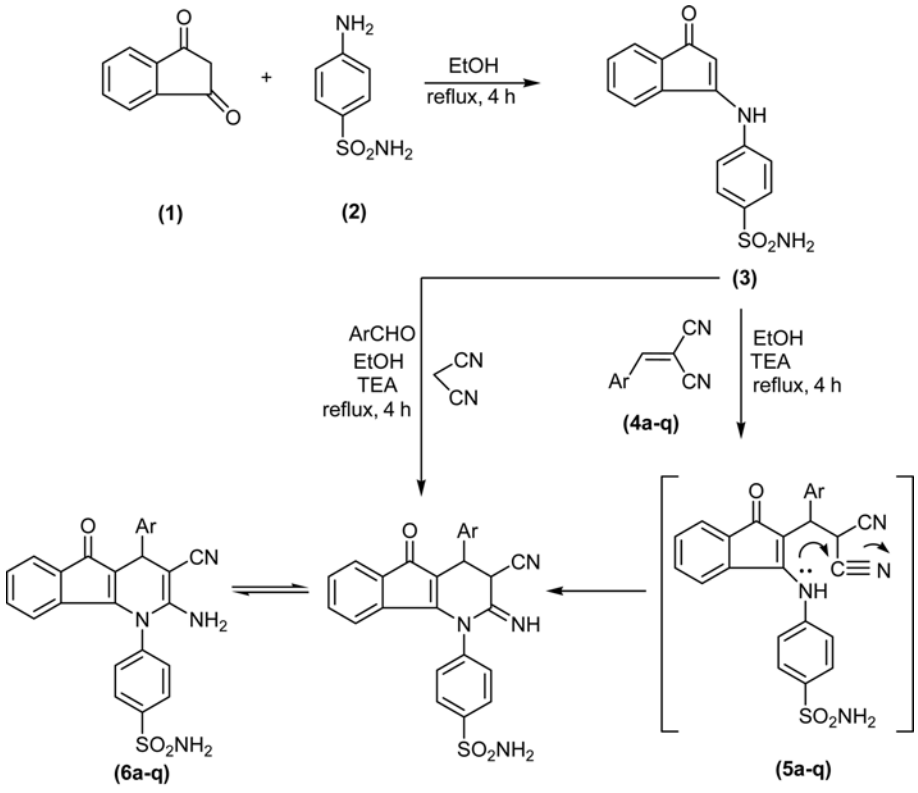
Chemistry

The aim of this work was the design and synthesis of some new series of indeno[1,2-b]pyridine **6a-q**, which carries a biologically active benzenesulfonamide moiety at position-1, free amino group at position-2 and cyano group at position-3 (Scheme 1), to evaluate their anticancer activity. Enaminone derivatives are highly reactive intermediates extensively used for synthesis of heterocyclic compounds. Thus, condensation of indan-1,3-dione **1** with sulfanilamide **2** yielded the corresponding 4-(3-oxo-3*H*-inden-1-yl-amino]benzenesulfonamide **3**. The structure of compound **3** was confirmed by elemental analysis and spectral data. The IR spectrum revealed bands at 3345, 3330, 3300 cm^{-1} (NH, NH_2), 1715 cm^{-1} (C = O), 1372, 1158 cm^{-1} (SO_2). Also, ^{1}H-NMR spectrum indicated the presence of signal at 10.5 ppm, which could be assigned to NH of the enaminone **3**. Treatment of the enaminone **3**, with arylidenemalononitriles, **4a-q** in the presence of catalytic amount of triethylamine, as a base catalyst, yielded the corresponding 4-[2-amino-3-cyano-5-oxo-4-substituted-aryl-4*H*-indeno[1,2-*b*]pyridine-1-(*5H*)-yl]benzenesulfonamide **6a-q**, via the formation of the intermediate Michael type products, followed by intramolecular cyclization (Scheme 1).

The *N*-Aryl-substituted benzenesulfonamide decreases the nucleophilicity of the enaminone **3** towards arylidenemalononitriles **4a-q**. The base catalyst triethylamine was required to generate the anion of the enaminone **3**, thus, facilitating the addition to the unsaturated nitriles **4a-q** (Scheme 2). On the other hand, compounds **6a-q** were unambiguously synthesized by another route, which involves one-pot condensation of the aldehyde, malononitrile and enaminone **3**, in a molar ratio (1:1:1) in refluxing ethanol that contains triethylamine, as catalyst. In this case, formation of compounds **6a-q** is illustrated in terms of initial condensation of the appropriate aldehyde with malononitrile affording the activated arylidenemalononitriles **4a-q**, followed by addition of the enaminone **3** to arylidenemalononitriles **4a-q**. The IR spectra of compounds **6a-q** exhibited bands at 3472-3225 cm^{-1} corresponding to (NH_2), 2214-2164 cm^{-1} (C≡N), 1720-1684 cm^{-1} (C = O), 1392-1150 cm^{-1} (SO_2).

In vitro cytotoxic screening

All the newly synthesized compounds were evaluated for their *in vitro* cytotoxic activity against breast cancer cell line (MCF 7), compared with that of doxorubicin (CAS 23214-92-8), as the positive control.



6a; Ar = C_6H_5
6b; Ar = $C_6H_4CH_3$-4
6c; Ar = C_6H_4OH-2
6d; Ar = C_6H_4OH-4
6e; Ar = C_6H_5CH=CH
6f; Ar = $C_6H_4OCH_3$-2
6g; Ar = $C_6H_4OCH_3$-4
6h; Ar = pipronyl
6i; Ar = $C_6H_4N(CH_3)_2$-4
6j; Ar = $C_6H_4NO_2$-3
6k; Ar = $C_6H_4NO_2$-4
6l; Ar = C_6H_4Cl-2
6m; Ar = $C_6H_3Cl_2$-2,4
6n; Ar = $C_{10}H_6OH$-2
6o; Ar = $C_{10}H_6OCH_3$-2
6p; Ar = $C_{10}H_6OCH_3$-4
6q; Ar = 2-thienyl

Scheme 1. The formation of indenopyridine derivatives (**6a**-**q**)

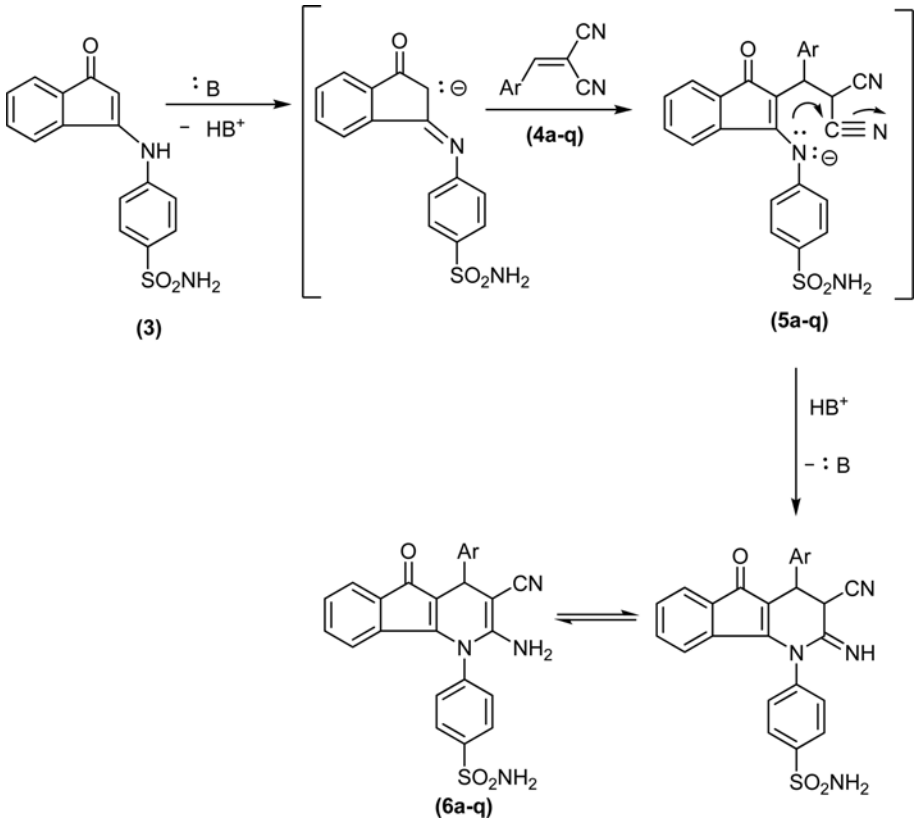
Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain survival curve of breast cancer cell line (MCF 7). The response parameter calculated was IC_{50} value, which corresponds to the concentration required for 50% inhibition of cell viability.

From these results (Table I), it can be seen that despite the variation in biological activity between the compounds, it was not very high-yet, as the following points can still be concluded.

It is clear from the results that the comparison of cytotoxic of the indenopyridine derivatives has shown the cells killing potency, which follows the order of indenopyridine **6d**, having 4-hydroxyphenyl at 4-position with (IC_{50} value = 4.34 μM) > 2-hydroxyl-1-naphthalene **6n** with (IC_{50} value = 6.84 μM) > 2-hydroxyphenyl **6c** with (IC_{50} value = 7.02 μM) > benzo[*d*][1,3] dioxol **6h** with (IC_{50} value = 7.08 μM) > 4-methoxy-1-naphthalene **6p** with (IC_{50} value = 7.34 μM) > 2-methoxyphenyl **6f** with (IC_{50} value = 8.22 μM) > styryl **6e** with (IC_{50} value = 8.86 μM) > unsubstituted phenyl **6a** with (IC_{50} value = 9.68 μM) > 4-tolyl **6b** with (IC_{50} value = 9.82 μM). The presence of 4-hydroxyphenyl at 4-position of indenopyridine ring **6d**, with cyano group, at 3-position increased the cytotoxic activity, which showed a higher potency than the tested compounds and more potency than the reference drug doxorubicin.

These results exhibited the nature of substituent at C-4 of the indenopyridine ring, by 4-hydroxyphenyl, has an important role on the cytotoxic activity and potency.

On the other hand the indenopyridine derivative **6n**, which bears 2-hydroxy-1-naphthalene moiety at 4-position with (IC_{50} value = 6.84 μM) is nearly as active



Scheme 2. Postulated mechanism for the formation of compounds (**6a**-**q**)

as Doxorubicin, and exhibited a higher potency than the indenopyridine derivative **6p** that carries 4-methoxy-1-naphthalene moiety at the same position with (IC_{50} value = 7.34 μM).

In addition, it was found that the indenopyridine **6a**, carrying unsubstitutedphenyl at 4-position with (IC_{50} value = 9.68 μM), revealed a higher potency than that of 4-tolyl **6b** with (IC_{50} value = 9.82 μM). According to these results, it was found that the tested compounds **6a-c**, **6e**, **6f**, **6h**, and **6p** exhibited a moderate activity and was found to be less active than the reference drug, while compounds **3**, **6g**, **6i-m**, **6o**, and **6q** showed weak activity.

In conclusion, the present data showed that, some compounds combining both indenopyridine and benzenesulfonamide moieties, exhibited a promising *in vitro* cytotoxic activity, against breast cancer cell line (MCF7), specially those containing substituted phenyl with either 4-hydroxyphenyl **6d**, 2-hydroxy-1-naphthalene **6n**, 2-hydroxyphenyl **6c**, and pipronyl **6h**, at 4-position with (IC_{50} value = 4.34, 6.84, 7.02, and 7.08 μM). Compound **6d** showed the highest *in vitro* cytotoxic activity, when compared with that of the other tested compounds and Doxorubicin, as the reference drug.

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REFERENCES

Abbate, F., Casini, A., Owa, T., Scozzafava, A., and Supuran, C. T., Carbonic anhydrase inhibitors: E7070, a sulfonamide anticancer agent, potently inhibits cytosolic isozymes I and II, and transmembrane, tumor-associated isozyme IX. *Bioorg. Med. Chem. Lett.*, 14, 217-223 (2004).

Chegwidden, W. R., Dodgson, S. J., and Spencer, I. M., The roles of carbonic anhydrase in metabolism, cell growth and cancer in animals. In The carbonic anhydrases new horizons. Birkhauser Verlog, Basel, Boston, Berlin, pp. 343-363, (2000).

Ghahremanzadeh, R., Shakibaei, G. I., Ahadi, S., and Bazgir, A., One-pot, pseudo four-component synthesis of a spiro [diindeno[1,2-b:2',1'-e]pyridine-11,3'-indoline]-trione library. *J. Comb. Chem.*, 12, 191-194 (2010).

Ghorab, M. M., Ismail, Z. H., and Abdalla, M., Synthesis and biological activities of some novel triazoloquinazolines and triazinoquinazolines containing benzenesulfonamide moieties. *Arzneimittelforschung*, 60, 87-95 (2010).

Ghorab, M. M., Noaman, E., Ismail, M. M., Heiba, H. I., Ammar, Y. A., and Sayed, M. Y., Novel antitumor and radioprotective sulfonamides containing pyrrolo [2,3-d]pyrimidines. *Arzneimittelforschung*, 56, 405-413 (2006).

Hurley, L. H., DNA and its associated processes as targets for cancer therapy. *Nat. Rev. Cancer*, 2, 188-200 (2002).

Ismail, M. M., Ghorab, M. M., Noaman, E., Ammar, Y. A., Heiba, H. I., and Sayed, M. Y., Novel synthesis of pyrrolo [2,3-d] pyrimidines bearing sulfonamide moieties as potential antitumor and radioprotective agents. *Arzneimittelforschung*, 56, 301-308 (2006).

Israel, M., Jones, L. C., and Modest, E. J., 6H-indeno[1,2-b] pyrido[3,2-e]pyrazines. A new heterocyclic ring system. *J. Heterocycl. Chem*., 9, 255-262 (1972).

Kivelä, A. J., Kivelä, J., Saarnio, J., and Parkkila, S., Carbonic anhydrases in normal gastrointestinal tract and gastrointestinal tumours. *World J. Gastroenterol.*, 11, 155-163 (2005).

Manpadi, M., Uglinskii, P. Y., Rastogi, S. K., Cotter, K. M., Wong, Y. S., Anderson, L. A., Ortega, A. J., Van Slambrouck, S., Steelant, W. F., Rogelj, S., Tongwa, P., Antipin, M. Y., Magedov, I. V., and Kornienko, A., Three-component synthesis and anticancer evaluation of polycyclic indenopyridines lead to the discovery of a novel indenoheterocycle with potent apoptosis inducing properties. *Org. Biomol. Chem*., 5, 3865-3872 (2007).

Maren, T. H., Relatons between structure and biological activity of sulfonamides. *Annu. Rev. Pharmacol. Toxicol*., 16, 309-327 (1976).

Rostom, S.A., Synthesis and *in vitro* antitumor evaluation of some indeno[1,2-c]pyrazol(in)es substituted with sulfonamide, sulfonylurea(-thiourea) pharmacophores, and some derived thiazole ring systems. *Bioorg. Med. Chem*., 14, 6475-6485 (2006).

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R., New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst*., 82, 1107-1112 (1990).

Supuran, C. T., Casini, A., Mastrolorenzo, A., and Scozzafava, A., COX-2 selective inhibitors, carbonic anhydrase inhibition and anticancer properties of sulfonamides belonging to this class of pharmacological agents. *Mini Rev. Med. Chem*., 4, 625-632 (2004).

Supuran, C. T. and Scozzafava, A., Carbonic anhydrase inhibitors. *Exp. Opin. Ther. Patents*, 10, 575-600 (2000).

Turkmen, H., Zengin, G., and Buyukkircali, B., Synthesis of sulfanilamide derivatives and investigation of *in vitro* inhibitory activities and antimicrobial and physical properties. *Bioorg. Chem*., 39, 114-119 (2011).

Utsugi, T., Aoyagi, K., Asao, T., Okazaki, S., Aoyagi, Y., Sano, M., Wierzba, K., and Yamada, Y., Antitumor activity of a novel quinoline derivative, TAS-103, with inhibitory effects on topoisomerases I and II. *Jpn. J. Cancer Res*., 88, 992-1002 (1997).

Vigante, B., Trizitis, G., Tirzite, D., Chekavichus, B., Uldrikis, J., Sobolev, A., and Duburs, G., 4-(10-Methyl-10H-phenothiazin-3-yl)-1,4-dihydropyridines, 4,5-dihydroindeno[1,2-b] and 5,5-dioxo-4,5-dihydrobenzothieno[3,2-b]pyridines. *Chem. Heterocyclic Compounds*, 43, 225-232 (2007).