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Synthesis, tautomerism, and antimicrobial, anti-HCV, anti-SSPE, antioxidant, and antitumor activities of arylazobenzosuberones

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ABSTRACT

2-Dimethylaminomethylene-1-benzosuberone 1 was coupled with diazotized aniline derivatives to afford a series of the hitherto unreported 2-arylazo-1-benzosuberones **3a–i**. The tautomeric structure and the effect of substituents on the tautomeric form (s) of the products **3a–i** were discussed. Similar coupling of the enaminone **1** with diazonium salts of heterocyclic amines gave the respective fused azolotriazino-benzosuberones. Some of the newly synthesized compounds showed potent antimicrobial, anti-HCV, antioxidant, antitumor (as topoisomerase I inhibitors), and antimicrobial activities.

1. Introduction

In our previous papers,[1–10] we were interested in the azo-hydrazone tautomerism of various arylazo heterocycles as many of them are useful in the field of material sciences and theoretical chemistry.[11,12] In addition to these applications, azo compounds are used as photosensitive species in photographic or electrophotographic systems and are the dominant organic photoconductive materials in commercial copiers.[13] Also, hydrazones are known as one of the most important classes of organic compounds, some of which show significant biological activities such as analgesic, antitumor, anti-inflammatory, antibacterial, and antifungal, and they also play the role of anticancer, and immunomodulating agents.[14–19] On the other hand, benzosuberone ring system and its fused systems present interesting pharmacological and biological activities as potent antitumor agents[20,21] against L1210 murine leukemia and HT29 cell lines, as potent inhibitors of tubulin polymerization,[22] and as blood platelet aggregation inhibitors.[23] Based on these findings, we report herein the results of coupling of 2-dimethylaminomethylene-1-benzosuberone **1** with diazonium salts of aniline derivatives and heterocyclic amines and elucidation of the tautomeric structure(s) of the resulting products. Also, we were interested in investigating the biological activities of the synthesized benzosuberone derivatives in inhibiting Hepatitis C Virus (HCV) and Subacute Sclerosing Panencephalitis (SSPE), besides their proved potent antioxidant activities.

2. Results and discussion

2.1. Chemistry

The required starting 2-dimethylaminomethylene-1-benzosuberone **1** was prepared by reacting 1-benzosuberone with dimethylformamide–dimethylacetal as previously described.[24] Coupling reaction of the enaminone **1** with diazotized anilines gave the respective arylazo derivatives **3** and/or **4** (Scheme 1). On the basis of their elemental and spectral (IR and mass) analyses (see Section 4), the isolated products were assigned structure **3** rather than structure **4**. For example, the mass spectra revealed, in each case, that the molecular ion peak was compatible with structure **3** rather than with structure **4**. The formation of the products (**3a–i**) is assumed to proceed via Japp–Klingemann-type cleavage of dimethyl aminomethylene moiety[25] as depicted in Scheme 1.

The isolated products **3** can have one or more of the three tautomeric structures, namely, the keto-hydrazone **3A**, hydroxy-azo **3B,** and CH-azo structure **3C** (Scheme 1). To elucidate the actual tautomeric form(s) of these compounds, their electronic absorption spectra were first studied. The spectra of the compounds in dioxane showed in each case three absorption bands in the regions

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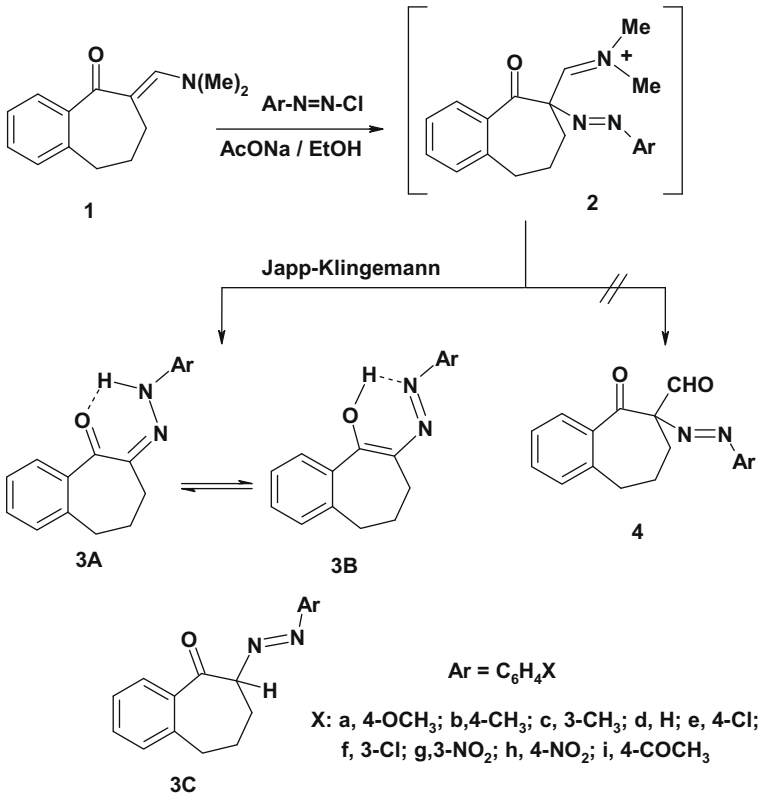


Scheme 1.

423–404, 288–284, and 276–231 nm (Table 1). This absorption pattern is analogous to that reported for the azo chromophore.[26] Furthermore, the spectrum of **3d**, taken as a typical example of the series studied, in solvents of different polarities, showed little, if any, shift (Table 1). The small shift in λ_{max} of **3d** in different solvents is due to solute–solvent interaction. This finding is in agreement with the observation that *o*- and *p*-arylazo derivatives of phenols and naphthols, unlike those of arylhydrazones derived from the reaction of quinones with *N*-alkyl-*N*-phenylhydrazine, are largely independent of the solvent polarity.[27] This finding excludes the hydrazone tautomeric form **3A** (Scheme 1).

Table 1
UV Spectral data in dioxane and K_T of compounds **3a–i** in (DMSO-d_6)

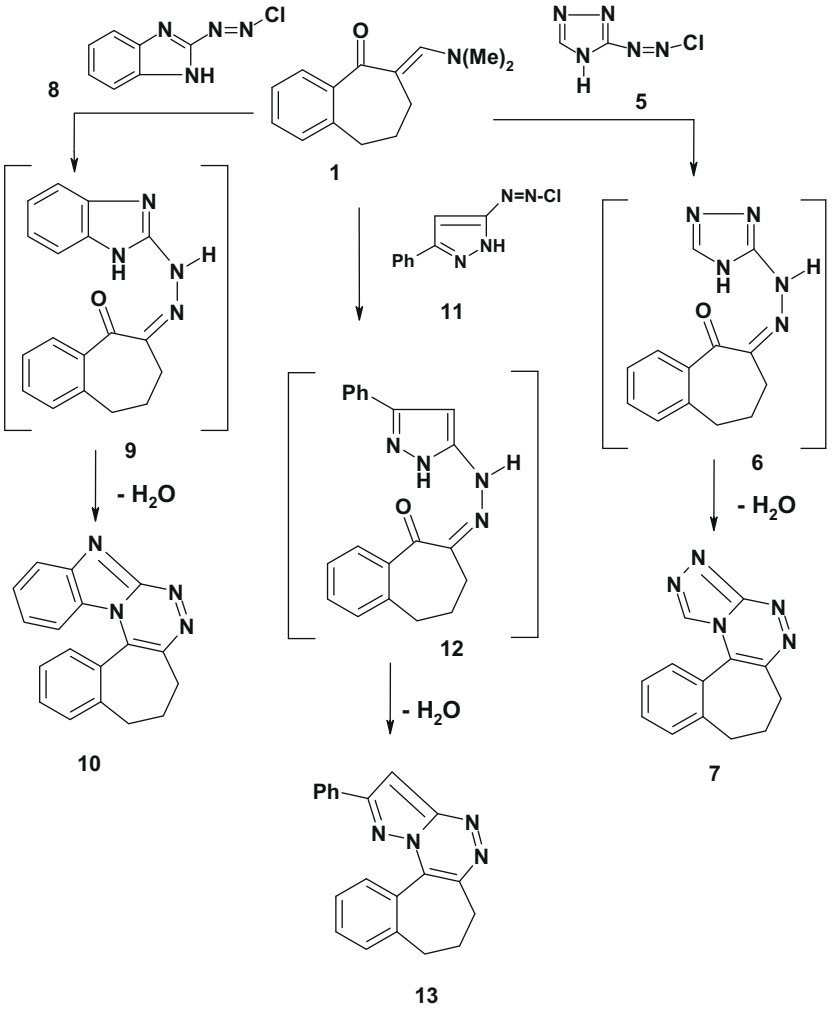
Compound no.	λ_{max} (log ε)	Ratio of **3A** in ^{1}H NMR	Ratio of **3B** in ^{1}H NMR	K_T
3a	404 (5.32), 288 (4.75), 233 (4.27)	0.2	0.8	0.25
3b	423 (4.65), 284 (4.14), 234 (4.15)	0.39	0.61	0.64
3c	410 (4.72), 286 (4.19), 233 (4.17)	0.4	0.6	0.67
3d[a]	415 (4.27), 285 (3.98), 231 (3.91)	0.44	0.56	0.79
3e	415 (4.96), 287 (4.42), 232 (4.27)	0.53	0.47	1.13
3f	406 (4.55), 286 (4.13), 233 (4.08)	0.54	0.46	1.17
3g	412 (4.62), 287 (4.35), 233 (4.18)	0.55	0.45	1.78
3h	409 (4.70), 276 (3.93), 234 (4.02)	0.67	0.33	2.03
3i	415 (4.10), 285 (3.91), 232 (3.80)	0.70	0.30	2.33

[a] Solvent λ_{max} (log ε): acetic acid 415 (4.43), 274 (3.85), 232 (3.94); acetone 411 (4.40), 298 (4.00), 231 (3.91); chloroform 418 (4.55), 282 (4.07), 236 (4.04); DMF 410 (4.36), 277 (3.81), 238 (3.89); ethanol 413 (4.51), 279 (3.96), 233 (4.01).

Furthermore, the ^{1}H NMR spectra of compounds **3** in deuterated chloroform are characterized, in each case, by a singlet signal assignable to the OH of the hydroxy-azo form near δ 14.0 ppm[28] and revealed absence the singlet signal near δ 5.27[29] for the CH proton of the CH-azo form **3C**. This finding indicates that the products **3a–i** prepared exist in $CDCl_3$ as a single tautomeric form, namely hydroxy-azo form **3B** (Scheme 1).

However, the ^{1}H NMR spectra of compounds **3** in deuterated dimethylsulfoxide indicated that such compounds exist as an equilibrium of two tautomeric forms namely, the keto-hydrazone form **3A** and the hydroxy-azo form **3B**. This is because their ^{1}H NMR spectra revealed, in each case, two characteristic singlet signals near δ 10.0 and 14.0 ppm assignable to NH and OH protons, respectively. The data in Table 1 indicate that the ratio of these two signals which is a function of the equilibrium constants (K_T = **[3A]**/**[3B]**) was found to depend on the substituent on the aryl moiety. This ratio revealed that the ratio of the keto-hydrazone form **3A** increases and that of the hydroxy-azo form **3B** decreases as the electron-donating property of the substituent increases.

Next, we investigated the behavior of the enaminone **1** toward some diazotized heterocyclic amines as potential precursors for fused heterocyclic systems.[25] Thus, coupling the reaction of enaminone **1** with the diazonium salt of 3-amino-1,2,4-triazole **5** in pyridine afforded the corresponding hydrazone **6** which undergoes in situ intramolecular cyclization to give 7,8-Dihydro-6H-benzo [6′,7′] cyclohepta [1′,2′-*e*] [1,2,4] triazolo [3,4,-*c*] [1,2,4] triazine **7** (Scheme 2). The structure of the isolated product **7** was established by its spectral (IR, ^{1}H NMR and MS) data and elemental analyses (see Section 4). Its mass spectrum revealed molecular ion peak at *m/z* 237(M^+) and its ^{1}H NMR spectrum showed a characteristic signal at δ 8.95 of the triazole CH proton.[30]



Scheme 2.

In a similar manner, enaminone **1** coupled readily with diazonium salt of 2-aminobenzimidazole **8** and 5-amino-3-phenylpyrazole **11** under the same experimental conditions, to afford a single product in each case according to TLC (Scheme 2). The structure assigned for the isolated products was based on the elemental analyses and spectral data. For example, ^{1}H NMR spectrum of compound **13** revealed a singlet signal at δ 8.54 which is characteristic of the proton of pyrazole ring.[31]

2.2. Biological screening

2.2.1. Antimicrobial activity

In vitro antimicrobial screening of seven of the synthesized hydrazones **3** was evaluated against four antibacterial species, namely, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Escherichia coli,* and four fungal strains, namely, *Aspergillus fumigatus*, *Penicillium italicum, Syncephalastrum racemosum,* and *Candida albicans.* Activities of the compounds and the minimum inhibitory concentration (MIC) of the tested compounds were evaluated by agar diffusion method. Compounds **3b**, **3c, 3g,** and **3i** exhibited highly significant activity against bacterial species as compared with the reference drugs and also, the tested compounds had moderate activity against the fungi as compared with the reference (Terbinafin) as shown in Table 2. In addition, the MIC was considered to be the lowest concentration of the tested substance exhibiting no visible growth of the bacteria or fungi on the plate as shown in Table 2I.

2.2.2. Pharmacology

Benzosuberone derivatives have some cytotoxic, antioxidant, and anti-angiogenic activities.[32] Also, in our previous work,[24] we demonstrated the ability of nine newly synthesized benzosuberone derivatives to inhibit Hepatitis C Virus (HCV) and Subacute Sclerosing Panencephalitis (SSPE), besides their proved potent antioxidant activities. Based on these strong biological activities, we aimed to test the antioxidant, anti-HCV, and antitumor activities.

2.2.2.1. Hepatitis C virus (HCV) NS3-4A protease inhibitory activities in both HCV replicon cells and hamster brains. In order to monitor the potential of eight newly synthesized compounds **3** to inhibit HCV, we used two methods for the determination of minimum inhibitory concentration (MIC) of these compounds. Firstly, their antiviral activities in HCV replicon cells were measured by their Hepatitis C Virus (HCV) NS3-4A Protease Inhibitory activities. Secondly, their antiviral chemotherapy for Subacute Sclerosing Panencephalitis (SSPE) was determined in hamster brains. Ribaverin was used in both methods as a positive control.

Table 3 shows the MIC values for the tested compounds **3** and ribaverin against HCV and SSPE. The results showed that all the nine tested compounds are highly effective at very low concentrations compared to ribaverin in the following descending order: **3f**, **3a**, **3c**, **3h**, **3g**, **3d**, **3i,** and **3b**. It is worth to mention that all the tested compounds are more potent than ribaverin.

Table 2
Preliminary antimicrobial activity for tested compounds

Microorganism	**3b** (μg/mL)			**3c** (μg/mL)			**3f** (μg/mL)		
	5.0	2.5	1.25	5.0	2.5	1.25	5.0	2.5	1.25
Aspergillus fumigatus	22	13	9	22	14	9	14	8	6
Penicillium italicum	0	0	0	14	6	3	0	0	0
Syncephalastrum racemosum	18	12	7	19	12	8	16	9	6
Candida albicans	14	6	2	9	6	2	15	11	7
Staphylococcus aureus	12	8	5	12	8	5	12	9	5
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0
Bacillus subtilis	16	6	4	15	10	8	17	10	7
Escherichia coli	7	0	0	12	6	0	9	0	0
	3g			**3h**			**3i**		
Aspergillus fumigatus	20	11	8	18	8	5	20	11	6
Penicillium italicum	0	0	0	0	0	0	17	6	4
Syncephalastrum racemosum	19	12	9	16	9	4	16	9	6
Candida albicans	15	8	5	12	6	0	10	7	3
Staphylococcus aureus	10	6	0	9	3	0	15	8	5
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0
Bacillus subtilis	19	13	8	14	8	3	16	10	7
Escherichia coli	12	5	0	8	0	0	12	0	0

Microorganism	**7** (μg/mL)			St. (μg/mL)		
	5.0	2.5	1.25	5.0	2.5	1.25
Aspergillus fumigatus	10	0	0	24	18	11
Penicillium italicum	8	0	0	19	9	4
Syncephalastrum racemosum	18	9	0	21	13	9
Candida albicans	9	4	0	19	10	6
Staphylococcus aureus	14	8	5	15	6	4
Pseudomonas aeruginosa	0	0	0	11	5	0
Bacillus subtilis	16	7	4	22	18	11
Escherichia coli	9	0	0	27	20	13

St. = reference standard *Terbinafin* was used as a standard antifungal agent while *chloramaphenicol* was used as a standard antibacterial agent.

Table 2l
The minimum inhibitory concentration (MIC)

Tested microorganisms	**3b**	**3c**	**3i**
Aspergillus fumigatus	0.313	0.313	0.625
Penicillium italicum	—	1.25	1.25
Syncephalastrum racemosum	0.625	0.313	0.313
Candida albicans	1.25	1.25	1.25
Staphylococcus aureus	0.625	0.625	0.625
Bacillis subtilis	0.625	0.313	0.313
Escherichia coli	—	2.50	—

2.2.2.2. DPPH radical scavenging activity. Radical scavenging activities of eight newly synthesized compounds **3** were measured using a model colorimetric test: DPPH radical scavenging test. The results are summarized in Table 4. The stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds, plant and fruit extracts, and food materials. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance drop at 515 nm. Among the 10 compounds tested for antioxidant activity using DPPH method, six exhibited good scavenging activity against the DPPH radical in the following descending order: **3f, 3a, 3c, 3h, 3g, 3d, 3i, and 3b.** It is worth to mention that all the tested compounds are more potent than ascorbic acid.

Table 3
MIC of ribaverin and the eight tested compounds against HCV and SSPE

Tested compound	MIC (μg/ml)	
	HCV	Subacute sclerosing panencephalitis (SSPE)
Ribaverin	16.15	77.89
3a	1.011	6.010
3b	1.141	7.06
3c	1.021	6.011
3d	1.071	6.122
3f	1.000	6.001
3g	1.043	6.111
3h	1.034	6.079
3i	1.092	6.123

2.2.2.3. Inhibition of peroxynitrite-induced tyrosine nitration. The reactive nitrogen species peroxynitrite ($ONOO^-$) has been implicated in human pathology and its role is usually inferred from the measurement of 3-nitrotyrosine (3-NT). Peroxynitrite and species derived from it can oxidize and nitrate lipids,[33] proteins,[34] DNA[35,36], and carbohydrates[37] leading to tissue damage in a number of pathological conditions in humans and in experimental animals. Due to the cytotoxicity of $ONOO^-$ and its apparent formation at sites of tissue injury,[38,39] there has been considerable interest in the ability of natural and synthetic antioxidants to diminish $ONOO^-$ damage. In the present work, the ability of ten synthetic compounds to prevent $ONOO^-$-mediated damage was examined using the model system: $ONOO^-$-mediated tyrosine nitration. When the amino acid tyrosine is exposed to $ONOO^-$ at pH 7.4, 3-NT is formed.[40]

The relative inhibitory activity of these compounds is summarized in Table 5 as IC_{50} values. Under our experimental conditions the relative potencies are in descending order: **3f**, **3a**, **3c**, **3h**, **3g**, **3d**, **3i,** and **3b**.

It is worth to mention that all the tested compounds are more potent than trolox (Table 5).

2.2.2.4. In vivo efficacy study. The in vivo activity of the tested compounds and etoposide against murine Colon 38 cancer was tested (Table 6). Treatment of Colon 38 implanted into mice with ER-37328 induced a clear dose-dependent inhibition of tumor

Table 4
Decrease of DPPH absorbance (%) by tested compounds

Compound no.	Decrease of DPPH absorbance% mean ± SD (n = 3)
Ascorbic acid (standard)	79.042 ± 2.928
3a	83.89 ± 0.099
3b	80.12 ± 0.088
3c	83.11 ± 0.091
3d	80.98 ± 0.064
3f	84.13 ± 0.092
3g	81.14 ± 0.082
3h	82.00 ± 0.081
3i	80.98 ± 0.089

Table 5
IC_{50} values of the compounds tested for the inhibition of $ONOO^-$-mediated 3-nitrotyrosine formation

Compound no.	IC_{50} (μM) mean ± SD (n = 3)
Trolox	58.430 ± 5.9
3a	54.190 ± 0.0576
3b	56.345 ± 0.0511
3c	55.088 ± 0.0555
3d	56.007 ± 0.0533
3f	54.099 ± 0.0587
3g	56.00 ± 0.0543
3h	5.130 ± ± 0.0555
3i	56.123 ± 0.0522

Table 6
Antitumor activities

Compound no	Average GI50	Relative potency to etoposide
3a	0.61	4.918032787
3b	0.7	4.285714286
3c	0.64	4.6875
3d	0.68	4.411764706
3f	0.6	5
3g	0.66	4.545454545
3h	0.65	4.615384615
3i	0.69	4.347826087

growth. Moreover, marked tumor regression was observed at 3 mg/kg. No body weight was observed at 3 mg/kg. All the tested compounds showed potent antitumor activities and the order of activity is in descending order: **3f**, **3a**, **3c**, **3h**, **3g**, **3d**, **3i,** and **3b**.

In a progress to determine the mechanism of action of the antitumor activities. The correlation coefficients with etoposide, doxorubicin, SN-38 (topoisomerase I inhibitor), and cisplatin were calculated according to the NCI COMPARE analysis procedure using the tested compound as the benchmark. These calculations culminated on the newly synthesized compounds that act as topoisomerase inhibitors (Table 7).

To confirm this mechanistic pathway of action the following assays are done.

2.2.2.5. Inhibition of topoisomerase II activity. The effects of the tested compounds and etoposide on topoisomerase II activity were analyzed by means of a relaxation assay. The tested compounds and etoposide inhibited topoisomerase II activity, and the relative potency of each compound was determined and confirmed in the following descending order: **3f**, **3a**, **3c**, **3h**, **3g**, **3d**, **3i,** and **3b** (see Table 8a).

All the previously mentioned measurements revealed that all the tested compounds are more active than etoposide and act similar to its mechanism of action as topoisomerase I inhibitors (see Table 8b).

Table 7
The correlation coefficients of the newly synthesized compounds with topoisomerase I inhibitors

Compound no.	The correlation coefficients with etoposide, doxorubicin, SN-38 (topoisomerase I inhibitor), and cisplatin, calculated according to the NCI COMPARE analysis procedure using the tested compound as the benchmark			
	Etoposide	Doxorubicin	SN-38	Cisplatin
3a	0.38	0.36	0.35	0.33
3b	0.32	0.29	0.27	0.25
3c	0.37	0.35	0.34	0.32
3d	0.34	0.32	0.3	0.25
3f	0.40	0.37	0.36	0.34
3g	0.35	0.33	0.33	0.2
3h	0.36	0.34	0.32	0.31
3i	0.33	0.31	0.29	0.26

Table 8a
The relative potencies of the newly synthesized compounds to etoposide

Compound no.	Relative potency to etoposide
3a	4.90
3b	4.27
3c	4.66
3d	4.40
3f	5
3g	4.53
3h	4.60
3i	4.33

Table 8b
The relative potencies of the newly synthesized compounds to etoposide

Compound no.	Relative potency to etoposide
3a	4.80
3b	4.20
3c	4.46
3d	4.30
3f	4.90
3g	4.50
3h	4.40
3i	4.30

3. Conclusion

In conclusion, we synthesized new hydrazones through the coupling of enaminone **1** with diazotized aniline derivatives and the tautomeric structures of the product were studied. Also, coupling of enaminone **1** with the diazonium salt of heterocyclic amine afforded tetra and penta heterocyclic ring systems. The newly synthesized compounds seem to be interesting for biological activity studies. These compounds were investigated for antimicrobial, anti-HCV, antioxidant, and antitumor (as topoisomerase I inhibitors) activities. All the tested compounds are highly effective at very low concentrations compared to the reference in all biological activity studies.

4. Experimental

4.1. Chemistry

Melting points were uncorrected. IR spectra were recorded (KBr) on a Pye Unicam SP-1000 Spectrophotometer. ^{1}H NMR spectra were obtained on a Varian Gemini 300 MHz spectrometer using TMS as an internal reference. Chemical shifts were expressed as δ (ppm). Mass spectra were recorded on a GCMS-QP 1000 EX mass

spectrometer operating at 70 eV. The elemental analyses were performed at the Microanalytical Center at Cairo University, Giza, Egypt. 2-Dimethylaminomethylene-1-benzosuberone (**1**) was prepared by following the literature method.[24]

4.1.1. Preparation of 2-(arylhydrazono)-1-benzosuberone (3a–i)

To a stirred solution of the enaminone **1** (1.10 g, 5 mmol) in ethanol (20 mL) was added sodium acetate trihydrate (0.7 g, 5 mmol) and the mixture was cooled in an ice bath to 0–5 °C. To the resulting solution, while being stirred, was added dropwise over a period of 20 min a solution of the appropriate arenediazonium chloride, prepared as usual by diazotizing the respective aniline (5 mmol) in hydrochloric acid (6 M, 3 mL) with sodium nitrite solution (5 mmol) in (5 mL) water. The whole mixture was then left in a refrigerator overnight. The precipitated solid was collected, washed with water, and finally crystallized from the appropriate solvent to give the respective hydrazones (**3a–i**).

4.1.1.1. 2-[4-Methoxyphenylhydrazono]-1-benzosuberone (3a).

Red solid, yield (0.91 g, 62%), mp 214–216 °C (Ethanol/Dioxane); ^{1}H NMR (DMSO-d_6) 2.02–2.04 (m, 2H, CH_2), 2.51–2.82 (m, 4H, $2CH_2$), 3.84 (s, 3H, OCH_3), 7.33–7.61 (m, 4H, ArH), 7.64 (d, J = 9 Hz, 2H, ArH), 8.09 (d, J = 9 Hz, 2H, ArH), 12.20 (s, 0.2H, NH), 14.87 (s, 0.8H, OH); ^{1}H NMR ($CDCl_3$) 2.01–2.06 (m, 2H, CH_2), 2.50–2.82 (m, 4H, $2CH_2$), 3.82 (s, 3H, OCH_3), 7.33–7.61 (m, 4H, ArH), 7.64 (d, J = 9 Hz, 2H, ArH), 8.09 (d, J = 9 Hz, 2H, ArH), 14.87 (s, 1H, OH); IR ν (cm^{-1}): 3421 cm^{-1}; Ms m/z (%) 294 (M^+, 61), 133(6), 132(67), 117(50), 107(89), 91(33), 88(39), 77(39), 74(28), 63 (100). Anal. Calcd for $C_{18}H_{18}N_2O_2$ (294.36); C, 73.45; H, 6.16; N, 9.52. Found: C, 73.33; H, 6.09; N, 9.41.

4.1.1.2. 2-[4-Methylphenylhydrazono]-1-benzosuberone (3b).

Yellow needles, yield (1.14 g, 82%), mp 146–148 °C (Ethanol); ^{1}H NMR (DMSO-d_6) 1.97–2.01 (m, 2H, CH_2), 2.27 (s, 3H, CH_3), 2.27–2.80 (m, 4H, $2CH_2$), 7.24 (d, J = 9 Hz, 2H, ArH), 7.31 (d, J = 9 Hz, 2H, ArH), 7.36–7.63 (m, 4H, ArH), 10.18 (s, 0.39H, NH), 13.86 (s, 0.61H, OH); ^{1}H NMR ($CDCl_3$) 1.97–2.04 (m, 2H, $1CH_2$), 2.25 (s, 3H, CH_3), 2.41–2.75 (m, 4H, $2CH_2$), 7.24 (d, J = 9 Hz, 2H, ArH), 7.31 (d, J = 9 Hz, 2H, ArH), 7.36–7.63 (m, 4H, ArH), 14.04 (s, 1H, OH); IR ν (cm^{-1}): 3436 cm^{-1}; Ms m/z (%) 278 (M^+, 83), 172(27), 119(13), 116(50), 115(30), 107(53), 106(20), 91(30), 89(30), 79(87), 77(87), 65(100). Anal. Calcd for $C_{18}H_{18}N_2O$ (278.36): C, 77.67; H, 6.52; N, 10.06. Found: C, 77.45; H, 6.41; N, 10.23.

4.1.1.3. 2-[3-Methylphenylhydrazono]-1-benzosuberone (3c).

Yellow solid, yield (0.71 g, 51%), mp 110–112 °C (ethanol); ^{1}H NMR (DMSO-d_6) 1.99–2.04 (m, 2H, CH_2), 2.51 (s, 3H, CH_3), 2.51–2.84 (m, 4H, $2CH_2$), 7.04–7.79 (m, 8H, ArH), 10.18 (s, 0.4H, NH), 14.02 (s, 0.6H, OH); ^{1}H NMR ($CDCl_3$); 1.98–2.04 (m, 2H, CH_2), 2.50 (s, 3H, CH_3), 2.51–2.84 (m, 4H, $2CH_2$), 7.04–7.79 (m, 8H, ArH), 14.02 (s, 1H, OH); IR ν (cm^{-1}): 3420 cm^{-1}; Ms m/z (%) 278 (M^+, 18), 172(60), 131(23), 118(26), 116(74), 104(21), 91(100), 77(58), 65(51). Anal. Calcd for (278.36): C,77.67; H, 6.52; N, 10.06. Found: C,77.39; H, 6.46; N, 9.88.

4.1.1.4. 2-Phenylhydrazono-1-benzosuberone (3d).

Orange needles, yield (0.95 g, 72%), mp 90 °C. (Ethanol/Dioxane); ^{1}H NMR (DMSO-d_6) 1.97–2.01 (m, 2H, CH_2), 2.51–2.81 (m, 4H, $2CH_2$), 6.90–7.65 (m, 9H, ArH), 10.23 (s, 0.44H, NH), 13.77 (s, 0.56H, OH); ^{1}H NMR ($CDCl_3$); 1.95–2.76 (m, 6H, $3CH_2$), 6.94–7.55 (m, 9H, ArH), 14.00 (s, 1H, OH) IR ν (cm^{-1}): 3420 cm^{-1}; Ms m/z (%) 265 (M^++1, 9), 264 (M^+, 36), 172(39), 145(11), 118(18), 116(38), 91(80), 77(85), 65(100). Anal. Calcd for $C_{17}H_{16}N_2O$ (264.33): C, 77.25; H, 6.10; N, 10.60. Found: C, 77.02; H, 6.34; N, 10.52.

4.1.1.5. 2-[4-Chlorophenylhydrazono]-1-benzosuberone (3e).

Yellow needles, yield (1.27 g, 85%), mp 146–148 °C (Ethanol); ^{1}H NMR (DMSO-d_6) 1.97–2.01 (m, 2H, CH_2), 2.27–2.80 (m, 4H, $2CH_2$), 7.24 (d, J = 9 Hz, 2H, ArH), 7.31 (d, J = 9 Hz, 2H, ArH), 7.36–7.63 (m, 4H, ArH), 10.32 (s, 0.53H, NH), 13.86 (s, 0.47H, OH); ^{1}H NMR ($CDCl_3$); 1.97–2.76 (m, 6H, $3CH_2$), 7.12–7.36 (m, 4H, ArH),7.38 (d, J = 9 Hz, 2H, ArH), 7.51 (d, J = 9 Hz, 2H, ArH), 13.93 (s, 1H, OH); IR ν (cm^{-1}): 3406 cm^{-1}; Ms m/z (%) 298 (M^+, 83), 172(27), 119(13), 116(50), 115(30), 107(53), 106(20), 91(30), 89(30), 79(87), 77(87), 65(100). Anal. Calcd for $C_{17}H_{15}ClN_2O$ (298.77): C, 68.34; H, 5.06; N, 9.38. Found: C, 68.51; H, 4.99; N, 9.24.

4.1.1.6. 2-(3-Chlorophenylhydrazono)-1-benzosuberone (3f).

Orange needles, yield (0.91 g, 61%), mp 158–160 °C (Ethanol); ^{1}H NMR (DMSO-d_6) 1.97–2.46 (m, 2H, CH_2), 2.62–2.82 (m, 4H, $2CH_2$), 6.92–7.66 (m, 8H, ArH), 10.34 (s, 0.54H, NH), 13.52 (s, 0.46H, OH); ^{1}H NMR ($CDCl_3$); 1.97–2.46 (m, 2H, CH_2), 2.62–2.82 (m, 4H, $2CH_2$), 6.92–7.66 (m, 8H, ArH), 13.62 (s, 1H, OH); IR ν (cm^{-1}): 3448 cm^{-1}; Ms m/z (%) 300 (M^++2, 5), 299 (M^++1, 10), 298 (M^+, 19), 172(49), 127(27), 117(39), 116(38), 111(31), 91(100), 77(58), 63(98). Anal. Calcd for $C_{17}H_{15}ClN_2O$ (298.77): C, 68.34; H, 5.06; N, 9.38. Found: C, 68.19; H, 5.21; N, 9.16.

4.1.1.7. 2-(3-Nitrophenylhydrazono)-1-benzosuberone (3g).

Yellow needles, yield (1.30 g, 84%), mp 160–162 °C (Ethanol/Dioxane); ^{1}H NMR (DMSO-d_6) 1.99–2.04 (m, 2H, CH_2), 2.51–2.81 (m, 4H, $2CH_2$), 7.33–8.26 (m, 8H, ArH), 10.60 (s, 0.55H, NH), 13.51 (s, 0.45H, OH); ^{1}H NMR ($CDCl_3$); 1.99–2.04 (m, 2H, CH_2), 2.51–2.81 (m, 4H, $2CH_2$), 7.33–8.26 (m, 8H, ArH), 13.50 (s, 1H, OH); IR ν (cm^{-1}): 3433 cm^{-1}; Ms m/z (%) 310 (M^++1, 14), 309 (M^+, 77), 172(76), 144(19), 117(44), 115(47), 91(100), 89(31), 77(36), 64(68). Anal. Calcd for $C_{17}H_{15}N_3O_3$ (309.33): C, 66.01; H, 4.89; N, 13.58. Found: C, 65.95; H, 4.75; N, 13.49.

4.1.1.8. 2-(4-Nitrophenylhydrazono)-1-benzosuberone (3h).

Yellow needles, yield (1.36 g, 88%), mp 210–212 °C (Ethanol/Dioxane); ^{1}H NMR (DMSO-d_6) 1.97–2.06 (m, 2H, $1CH_2$), 2.68–2.86 (m, 4H, $2CH_2$), 7.31–7.68 (m, 4H, ArH), 7.70 (d, J = 9 Hz, 2H, ArH), 8.19 (d, J = 9 Hz, 2H, ArH), 10.82 (s, 0.67H, NH), 13.43 (s, 0.33H, OH); ^{1}H NMR ($CDCl_3$); 1.97–2.79 (m, 6H, $3CH_2$), 7.14(d, J = 9 Hz, 2H, ArH), 7.18–7.56 (m, 4H, ArH), 8.14 (d, J = 9 Hz, 2H, ArH), 13.85 (s, 1H, OH); IR ν (cm^{-1}): 3413, 3278, 1658 cm^{-1}; Ms m/z (%) 310 (M^++1, 10), 309 (M^+, 65), 172(66), 138(17), 122(9), 116(97), 115(69), 103(51), 91(100), 77(59), 64(90). Anal. Calcd for $C_{17}H_{15}N_3O_3$ (309.33): C, 66.01; H, 4.89; N, 13.58. Found: C, 66.32; H, 4.77; N, 13.74.

4.1.1.9. 2-(4-Acetylphenylhydrazono)-1-benzosuberone (3i).

Yellow solid, yield (1.19 g, 78%), mp 200–202 °C (Dioxane); ^{1}H NMR (DMSO-d_6) 1.96–2.05 (m, 2H, CH_2), 2.52 (s, 3H, $COCH_3$), 2.66–2.84 (m, 4H, $2CH_2$), 7.30–7.92 (m, 4H, ArH), 7.93 (d, J = 8 Hz, 2H, ArH), 7.95 (d, J = 8 Hz, 2H, ArH), 10.55 (s, 0.7H, NH), 13.59 (s, 0.3H, OH); ^{1}H NMR ($CDCl_3$); 1.96–2.05 (m, 2H, CH_2), 2.52 (s, 3H, $COCH_3$), 2.66–2.84 (m, 4H, $2CH_2$), 7.30–7.92 (m, 4H, ArH), 7.93 (d, J = 8 Hz, 2H, ArH), 7.95 (d, J = 8 Hz, 2H, ArH), 13.59 (s, 1H, OH); IR ν (cm^{-1}): 3395, 1702 cm^{-1}; Ms m/z (%) 306 (M^+, 63), 172(75), 115(36), 107(80), 106(31), 91(30), 89(43), 77(97), 65(100). Anal. Calcd for $C_{19}H_{18}N_2O_2$ (306.37): C, 74.49; H, 5.92; N, 9.14. Found: C, 74.31; H, 5,87; N, 9.00.

4.1.2. Coupling of enaminone 1 with diazonium salts of heterocyclic amines

A solution of enaminone **1** (1.10 g, 5 mmol) in pyridine (20 ml) was cooled in an ice bath at 0–5 °C while being stirred. To the resulting cold solution was added portionwise a cold solution of

the appropriate diazonium salt of 3-amino-1,2,4-triazole **5,** 2-aminobenzimidazole **8**, or 5-amino-3-phenylpyrazole **11,** prepared as usual by diazotized heterocyclic amine (5 mmol) in nitric acid (6 M, 3 ml) with sodium nitrite (0.35 g, 5 mmol) in water (5 ml). After all the diazonium salt was added, the mixture was stirred for further 30 min while cooling in an ice bath. The reaction mixture was then left in a refrigerator for three days. The solid that precipitated was filtered off, washed with water, dried, and finally crystallized from the appropriate solvent to give product **7**, **10,** or **13**, respectively. The physical constants and the spectral data of the isolated products **7, 10,** and **13** are listed below.

4.1.2.1. 7,8-Dihydro-6*H*-benzo[6′,7′]cyclohepta[1′,2′-*e*] [1,2,4]triazolo[3,4-*c*] [1,2,4]triazine (7). Pale yellow solid, yield (1.01 g, 85%), mp196–198 °C (Dioxane); ^{1}H NMR (DMSO-d_6) 2.39–2.71 (m, 6H, 3CH$_2$), 7.51–8.20 (m, 4H, ArH), 8.95 (s, 1H, triazole-H); Ms *m/z* (%) 238 (M$^+$+1, 12), 237 (M$^+$, 12), 127(19), 101(21), 89(27), 87 (27), 74(15), 63(23), 53(100). Anal. Calcd for $C_{13}H_{11}N_5$ (237.27): C, 65.81; H, 4.67; N, 29.52. Found: C, 65.98; H, 4.80; N, 29.31.

4.1.2.2. 9,10-Dihydro-8*H*-benzo[6′,7′]cyclohepta[1′,2′-*e*]benzimidazo[2,1-*c*] [1,2,4]triazine (10). Dark yellow solid, yield (1.29 g, 90%), mp 206 °C. (Dioxane); ^{1}H NMR (DMSO-d_6) 2.05–2.81 (m, 6H, 3CH$_2$), 7.40–8.16 (m, 8H, ArH); Ms *m/z* (%) 287 (M$^+$+1, 24), 286 (M$^+$, 100), 257(39), 129 (29), 128(40), 127(17), 115(39), 102 (28), 91(12), 89(32), 77(40), 63 (58). Anal. Calcd for $C_{18}H_{14}N_4$ (286.34): C, 75.51; H, 4.93; N, 19.57. Found: C, 75.24; H, 4.77; N, 19.29.

4.1.2.3. 2-Phenyl-7,8-Dihydro-6*H*-benzo[6′,7′]cyclohepta[1′,2′-*e*]pyrazolo[5,1-*c*] [1,2,4]triazine (13). Yellow solid, yield (1.25 g, 80%), mp 172 °C. (Ethanol/Dioxane); ^{1}H NMR ($CDCl_3$) 2.06–2.90 (m, 6H, 3CH$_2$), 6.32–7.53 and 7.56–7.80 (m, 9H, ArH), 8.54 (s, 1H, pyrazole-H); Ms *m/z* (%) 313 (M$^+$+1, 3), 312 (M$^+$, 22), 160(43), 144(46), 131(100), 118 (59), 115(489, 91(54), 89(60), 77(85). Anal. Calcd for $C_{20}H_{16}N_4$ (312.38): C, 76.90; H, 5.16; N, 17.94. Found: C, 76.72; H, 5.02; N, 18.14.

4.1.3. Determination of minimum inhibitory concentration (MIC) of ribavirin and different tested compounds in HCV replicon cells

Briefly, 1×10^4 replicon cells per well were plated in 96-well plates. On the following day, replicon cells were incubated at 37 °C for the indicated period of time with antiviral agents were serially diluted in DMEM plus 2% FBS and 0.5% dimethyl sulfoxide (DMSO). Total cellular RNA was extracted using an RNeasy-96 kit (QIAGEN, Valencia, CA), and the copy number of HCV-RNA was determined using a quantitative RT-PCR (QRT-PCR) assay. Each datum point represents the average of five replicates in cell culture. The cytotoxicity of tested compound was measured under the same experimental settings using a tetrazolium (MTS)-based cell viability assay (Promega, Madison, WI). For the cytotoxicity assay with human hepatocyte cell lines, 1×10^4 parental Huh-7 cells per well or 4×10^4 HepG2 cells per well were used.

4.1.4. MIC of the tested compounds in hamster brains for antiviral chemotherapy for subacute sclerosing panencephalitis (SSPE)

Under ether anesthesia, 50 ml of ribavirin or tested compound solutions at dosages of 5, 10, and 20 mg/kg/day was injected for 10 days intracranially to a depth of 2 mm by using a 27-gauge needle and was placed within the subarachnoid space. On 1, 2, 3, 5, 7, 10, 12, 15, and 20 days after the initial injection, four hamsters from each group were sacrificed. The brains were aseptically removed, washed twice with phosphate-buffered saline (PBS), homogenized, and suspended in PBS. The suspension was centrifuged at 1600 for 10 min. The supernatant was collected, ethanol was added to remove proteins, and the mixture was heated at 90 °C to evaporate the ethanol. The protein-free samples were used to evaluate the MIC in brain tissue by HPLC and bioassay.

4.1.5. Radical scavenging assays

DPPH radical scavenging assay: The antioxidant activities of the tested compounds and the standard were assessed on the basis of the radical scavenging effect of the stable DPPH· free radical.[41] Weighed quantities of the ten compounds were dissolved in distilled DMSO and used. Solution of ascorbic acid used as a standard for this study was prepared in distilled H_2O. All these solutions were serially diluted with respective solvents to get lower dilutions.

Each compound (10 μl) or standard (from 0.0 μM/ml to 100 μM/ml) was added to 90 μl of DPPH· in methanol solution (100 μM) in a 96-well microtitre plate. After incubation in the dark at 37 °C for 30 min, the decrease in absorbance of each solution was measured at 515 nm using ELISA microplate reader (Bio Rad Laboratories Inc., California, USA, Model 550). Absorbance of blank sample containing the same amount of DMSO and DPPH· solution was done and measured as well. The experiment was carried out in triplicate. The scavenging potential was compared with a solvent control (0% radical scavenging) and ascorbic acid. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Reduction of absorbance} = [(A_B - A_A)/AB] \times 100,$$

where A_B indicates absorbance of blank sample; and A_A indicates absorbance of tested extract solution (t = 30 min).

4.1.6. Reaction with reactive nitrogen species

4.1.6.1. Synthesis of peroxynitrite. Peroxynitrite ($ONOO^-$) was synthesized as previously described.[40] Briefly, an acidic solution (0.6 M HCl) of H_2O_2 (0.7 M) was mixed with KNO_2 (0.6 M) on ice for one second and the reaction was quenched with ice-cold NaOH (1.2 M). Residual H_2O_2 was removed by mixing with granular MnO_2 prewashed with NaOH (1.2 M). The stock solution was filtered and then frozen overnight (−20 °C) and the top layer of the solution was collected for the experiment. Concentrations of stock $ONOO^-$ were redetermined before each experiment at 302 nm using a molar absorption coefficient of 1670 cm^{-1} M^{-1}. Concentrations of 200–250 mM were usually obtained. Once thawed, $ONOO^-$ solutions were kept in ice for no longer than 30 min before use.

4.1.7. Reaction of compounds with peroxynitrite

The ability of tested compounds to inhibit peroxynitrite-induced tyrosine nitration was investigated via reaction of equimolar concentrations (100 μM) of tyrosine and peroxynitrite in the presence of increasing concentrations of each tested compound (0–300 μM) in 100 mM phosphate buffer, pH 7.4, at 37 °C for 15 min. Snap-freezing reaction mixtures prior to HPLC analysis successfully terminated the reactions. The formation of 3-nitrotyrosine (3-NT) was monitored by HPLC analyzed with photodiode array detection (see below). 3-NT formed was characterized and quantified by use of an authentic standard (elution time and unique spectral characteristics).

4.1.8. HPLC analysis

Reaction mixtures were analyzed using reverse-phase HPLC analysis performed on an Agilent 1100 system with a Zorbax ODS C18 column (150 × 4.6 mm i.d., 4 μm) and a guard column (15 × 4.6 mm i.d., 4 μm). Mobile phase A consisted of methanol/water/ 5 N HCl (5:94.9:0.1 v/v/v) and mobile phase B consisted of acetonitrile/water/5 N HCl (50:49.9:0.1 v/v/ v). The following gradient system was used (min/% acetonitrile): 0:0, 5:0, 40:50, 60:100, 65:100, and 65.1/0 with a flow rate of 0.7 ml/min. The eluent was

monitored by photodiode array detection at 280 nm for 3-NT measurements with spectra of products obtained over the 220–600 nm range.

4.1.9. Antitumor activity of novel topoisomerase II inhibitors

DNA topoisomerase II has been shown to be an important therapeutic target in cancer chemotherapy. Herein, we describe studies on the antitumor activity of novel topoisomerase II inhibitors.

The tested compounds inhibited topoisomerase II activity at 10 times lower concentration than etoposide in relaxation assay and cells. Murine Colon 38 cancer, murine leukemia P388, and human lung cancer LX-1 were obtained from the Cancer Chemotherapy Center, Japan Foundation for Cancer Research (Tokyo, Japan). Human lung cancer PC-1 and human gastric cancer MKN-1, MKN-7, MKN-28, and MKN-74 were obtained from Immuno Biology Laboratory (Gunma, Japan). Human lung cancer A549 and human colon cancer WiDr were obtained from Dainippon Pharmaceutical Co., Ltd (Osaka, Japan). Human gastric cancer HGC-27 and GT3TKB were obtained from Riken Cell Bank (Ibaraki, Japan).The other cell lines were purchased from American Type Culture Collection (Rockville, MD).

4.1.10. Growth-inhibitory effect

Exponentially growing solid tumor cells in 0.1 ml of medium were seeded in 96-well plates on day 0. On day 1, 0.1 ml aliquots of medium containing graded concentrations of test drugs were added to the cell plates. After incubation at 37 °C for 72 h, the cell number was estimated by sulforhodamine B (SRB) assay, as described by Skehan et al. The cell number on day 1 was also measured separately. GI50 values are the drug concentrations causing a 50% reduction in the net protein increase in control cells. The antitumor spectrum of ER-37328 was compared with those of other drugs by means of the NCI COMPARE analysis procedure. Exponentially growing P388 cells were seeded in 96-well plates on day 0. On day 1, 0.1 ml aliquots of medium containing graded concentrations of test drugs were added to the wells. After incubation at 37 °C for 72 h, the cell number was determined by MTT assay. IC_{50} values were calculated as the drug concentrations that reduced the number of cells to 50% of the control number.

4.1.11. In vivo efficacy study

Female C57BL/6 mice (6 weeks of age; Charles River, Atsugi, Japan) were housed in barrier facilities on a 12 h light/dark cycle, with food and water ad libitum. About 30 mg of Colon 38 tumor tissue was inoculated s.c. into the flank of animals. Mice were randomized and separated into control and treatment ($n = 5$ each) groups when the tumor volume reached approximately 300 mm^3 on day 1. The tested compound was dissolved in 5% glucose. Etoposide was diluted in saline and doxorubicin was dissolved in saline. The tested compound was administered on day 1 (single dose) by iv injection into the tail vein, and etoposide and doxorubicin were given at the maximum tolerated dose on the reported administration schedule. Control animals received 5% glucose. Tumor volume and body weight were measured on the days indicated in the figures. Tumor volume was calculated according to the following equation: tumor volume (mm^3) = (length − $width^2$)/2.

4.1.12. Relaxation assay

Topoisomerase II was purified from P388 cells. One unit of the enzyme was defined as the activity to relax completely 0.125 μg of supercoiled pBR-322 DNA at 30 °C for 1 h. For the assay, 0.125 μg of supercoiled pBR-322 DNA (Takara Shuzo Co., Ltd, Tokyo, Japan) was relaxed with one unit of topoisomerase II in 20 μl of the assay buffer [50 mM Tris–HCl (pH8.0), 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.5 μM EDTA, 1 mM ATP, and 30 μg/ml BSA] in the presence or absence of an inhibitor at 30 °C for 1 h. Samples were subjected to electrophoresis in 0.7% agarose gels with TBE buffer [89 mM Tris, 89 mM boric acid, and 2 mM EDTA (pH 8.0)]. The DNA was stained with ethidium bromide and photographed under UV light.

4.1.13. Growth-inhibitory effect

The growth-inhibitory effects of the tested compounds 28 on 21 human solid tumor cell lines (8 lung, 7 colon, and 6 gastric cancer cell lines) were determined. The tested compounds inhibited the growth of these cell lines with an average GI50 given in Table 1 and relative potency to etoposide were determined. The correlation coefficients with etoposide, doxorubicin, SN-38 (topoisomerase I inhibitor), and cisplatin, calculated according to the NCI COMPARE analysis procedure using the tested compound as the benchmark, are given in Table 2, respectively. That is, the pattern of the growth-inhibitory effect of the tested compounds was similar to that of etoposide, but less similar to that of doxorubicin.

References and notes

1. Shawali, A. S.; Farghaly, T. A. *Tetrahedron* **2009**, *65*, 644.
2. Farghaly, T. A.; Abdallah, Z. A. *Arkivoc* **2008**, *xvii*, 295.
3. Shawali, A. S.; Mosselhi, M. A. N.; Farghaly, T. A.; Shehata, M. R.; Tawfik, N. M. *J. Chem. Res.* **2008**, 452.
4. Shawali, A. S.; Mosselhi, M. A. N.; Altablawy, F. M. A.; Farghaly, T. A.; Tawfik, N. M. *Tetrahedron* **2008**, *64*, 5524.
5. Shawali, A. S.; Sherif, S. M.; Farghaly, T. A.; Darwish, M. A. A. *Afinidad* **2008**, *65*, 314.
6. Shawali, A. S.; Mosselhi, M. A. N.; Farghaly, T. A. *J. Chem. Res.* **2007**, 479.
7. Shawali, A. S.; Sherif, S. M.; Farghaly, T. A.; Shehata, M. R.; Darwish, M. A. A. *J. Chem. Res.* **2007**, 44.
8. Shawali, A. S.; Farghaly, T. A.; Edrees, M. M. *Int. J. Pure Appl. Chem.* **2006**, *1*, 531.
9. Shawali, A. S.; Zayed, M. M.; Farghaly, T. A. *J. Heterocycl. Chem.* **2005**, *42*, 185.
10. Shawali, A. S.; Farghaly, T. A. *Tetrahedron* **2004**, *60*, 3051.
11. Bhaskar, M.; Gnanamani, A.; Ganeshjeevan, R. J.; Chandraskar, R.; Sadulla, S.; Radhakrishnan, G. *J. Chromatogr., A* **2003**, *1018*, 117.
12. Navarro, A.; Sanz, F. *Dyes Pigments* **1999**, *40*, 131.
13. Carpicz, R.; Gulbinas, V.; Undzenas, A. *J. Chin. Chem. Soc.* **2000**, *47*, 589.
14. El-Gazzar, A. B. A.; El-Enany, M. M.; Mahmoud, M. N. *Bioorg. Med. Chem.* **2008**, *16*, 3261.
15. Alagarsamy, V.; Soloma, V. R.; Dhanabal, K. *Bioorg. Med. Chem.* **2007**, *15*, 235.
16. Ganguly, A. K.; Doll, A. K.; Girijavallabhan, V. M. *Curr. Med. Chem.* **2001**, *8*, 1419.
17. Kandile, N. G.; Mohamed, M. I.; Zaky, H.; Mohamed, H. M. *Eur. J. Med. Chem.* **2009**, *44*, 1989.
18. Vicini, P.; Incerti, M.; La Colla, P.; Loddo, R. *Eur. J. Med. Chem.* **2009**, *44*, 1801.
19. Banachiewicz, B. M.; Kaminska, T. *Eur. J. Med. Chem.* **2001**, *36*, 93.
20. Afonso, A.; Kelly, J. M.; Weinstein, J.; Wolin, R. L.; Rosenblum, S. B. US Patent 6 218 401, 2001; *Chem. Abstr.* **2001**, *134*, 295746.
21. Joseph, B.; Algille, D.; Mérour, J. Y.; Léonce, S. *Chem. Pharm. Bull.* **2000**, *48*, 1872.
22. Sriram, M.; Hall, J. J.; Grohmann, N. C.; Strecker, T. E.; Wootton, T.; Franken, A.; Trawick, M. L.; Pinney, K. G. *Bioorg. Med. Chem.* **2008**, *16*, 8161.
23. Nakayama, T.; Takashi, H. Jpn Kokai. Tokkyo Koho JP 03167178 A2, 1991; *Chem. Abstr.* **1991**, *115*, 256204.
24. Farghaly, T. A.; Abdel Hafez, N. A.; Ragab, E. A.; Awad H. M.; Abdalla, M. M. *Eur. J. Med. Chem.*, in press.
25. Al-Mousawi, S.; Abdelkhalik, M. M.; John, E.; Elnagdi, M. H. *J. Heterocycl. Chem.* **2003**, *40*, 689.
26. Jones, R.; Ryan, A. J.; Sternhell, S.; Wright, S. E. *Tetrahedron* **1963**, *19*, 1497.
27. Daunis, J.; Follet, M.; Marzin, C. *Org. Magn. Reson.* **1980**, *13*, 330.
28. Nawrot-Modranka, J.; Nawrot, E.; Graczyk, J. *Eur. J. Med. Chem.* **2006**, *41*, 1301.
29. Hegarty, A. F.; Cashman, M. P.; Scott, F. L. *Chem. Commun.* **1971**, *13*, 884.
30. Wagle, S.; Adhikari, A. V.; Kumari, N. S. *Eur. J. Med. Chem.* **2009**, *44*, 1135.
31. Konova, E. S.; Makarov, V. A.; Alekeeva, L. M.; Avramenko, G. V.; Granik, V. G. *Russ. Chem. Bull. Int. Ed.* **2006**, *55*, 735.
32. Grubbs, C. J.; Lubet, R. A.; Atigadda, V. R.; Christov, K.; Deshpande, A. M.; Tirmal, V.; Xia, G.; Bland, K. I.; Eto, I.; Brouillette, W. J.; Muccio, D. D. *Carcinogenesis* **2006**, *27*, 1232.
33. Radi, R.; Beckman, J. S.; Bush, K. M.; Freeman, B. A. *Arch. Biochem. Biophys.* **1991**, *288*, 481.
34. Ischiropoulos, H.; Al-Mehdi, A. B. *FEBS Lett.* **1995**, *364*, 279.
35. Yermilov, V.; Rubo, J.; Oshima, H. *FEBS Lett.* **1996**, *376*, 207.
36. Spencer, J. P. E.; Wong, J.; Jenner, A.; Aruoma, O. I.; Cross, C. E.; Halliwell, B. *Chem. Res. Toxicol.* **1996**, *9*, 1152.
37. Beckman, J. S.; Beckman, T. W.; Chen, J.; Marshall, P. A.; Freeman, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1620.
38. Halliwell, B.; Zhao, K.; Whiteman, M. *Free Radicals Res.* **1999**, *31*, 651.
39. Ischiropoulos, H. *Arch. Biochem. Biophys.* **1998**, *356*, 1.
40. Beckman, J. S.; Chen, J.; Ischiropoulos, H.; Crow, J. P. *Methods Enzymol.* **1994**, *233*, 229.
41. Gamez, E. J. C.; Luyengi, L.; Lee, S. K.; Zhu, L. F.; Zhou, B. N.; Fong, H. H. S.; Pezzuto, J. M.; Kinghorn, A. D. *J. Nat. Prod.* **1998**, *61*, 706.