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ANTICANCER ACTIVITY OF NEW (TETRAZOL-5-YL)METHYLINDOLE DERIVATIVES AND THEIR ACYCLIC *C*-NUCLEOSIDE ANALOGS

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Abstract: New (tetrazol-5-yl)methylindole derivatives were synthesized from 2-phenylindole. Furthermore, the sugar acetyl hydrazones of the tetrazole derivatives as well as their derived acyclic *C*-nucleoside analogs were prepared. The synthesized compounds were studied for their anticancer activity against human liver carcinoma cell line (HepG2) and the results showed that arylidine substituted tetrazole derivatives **7c** and **7d** were the most active.

Keywords: indole, tetrazoles, sugar hydrazones, acyclic nucleosides, anticancer activity

The chemistry of indole containing compounds has been increasingly interesting because of their biological activities (1–4). They have been shown to possess anti-inflammatory (5), antibacterial (6), antiviral (7), anti-TB (8), antifungal (9), analgesic (10) and anti-tumor activities (11, 12). Structure–activity relationship correlations in many indole compounds indicated that *N*-alkylation (13, 14) was effective in causing a marked rise in activity against various bacteria, fungi, and viruses. On the other hand, tetrazole derivatives found wide applications as carboxylic surrogates, bioisosteres of carboxylic acids (15, 16) and lipophilic spacers in pharmaceuticals, resulting in compounds with antihypertensive, anti-allergic and antibiotic activities (17). Furthermore, *N*-substitution of many tetrazoles derivatives resulted in interesting antinociceptive activity (18). The nucleosides as well as their acyclic and *C*-nucleoside analogs possess a wide range of medicinal properties, including antibiotic, antiviral, and antitumor activities (19–23). Due to these interesting biological significances of indole, tetrazole and nucleoside analogs, we assumed that newly synthesized compounds incorporating the three structural constituents, with *N*-substitution of the indole and tetrazole, could be of interest as new active anticancer leads. Our interest (24–27) in search for novel biologically active compounds through attachment of carbohydrate moieties to newly synthesized heterocycles, also encouraged us to report the synthesis and anticancer activity of new substituted indolyltetrazole derivatives and their acyclic nucleoside analogs.

EXPERIMENTAL

Chemistry

Melting points were determined with a Kofler block apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer model 1720 FTIR spectrometer for KBr discs. NMR spectra were recorded on a Varian Gemini 200 NMR spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Bruker Ac-250 FT spectrometer at 250 MHz for ^{1}H and at 62.9 MHz for ^{13}C with TMS as a standard. The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F_{245}. Elemental analyses were performed at the Microanalytical Data Centre at Faculty of Science, Cairo University, Egypt.

1-Acetonitril-2-phenylindole (2)

2-Phenylindole **1** (1.93 g, 0.01 mole) and sodium hydride (0.5 g, 0.02 mole) were dissolved in DMF (20 mL) and the solution was stirred for 1 h. Chloroacetonitrile (3 mL) was added and the reac-

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tion mixture was stirred for 24 h, then diluted with cold water (20 mL). The obtained precipitate was filtered off, dried and recrystallized from ethanol to give compound **2** as yellow solid.

1-[(2*H*-Tetrazol-5-yl)methyl]-2-phenyl-1*H*-indole (3)

A mixture of compound **2** (2.32 g, 0.01 mole), sodium azide (0.65 g, 12 mmole) and ammonium chloride (0.53 g, 0.01 mole) in DMF (10 mL) was heated under reflex for 24 h, then diluted with cold water (20 mL). The obtained precipitate was filtered off, washed with water, dried and recrystallized from ethanol to afford compound **3**.

Ethyl 2-{5-[(2-phenyl-1*H*-indol-1-yl)methyl]-2*H*-tetrazol-2-yl}acetate (4)

To a stirred solution of compound **3** (2.75 g, 0.01 mole) and sodium hydride (0.5 g, 0.02 mole) in DMF (15 mL), ethyl chloroacetate (0.3 mL) was added dropwise at room temperature. The reaction mixture was stirred for 48 h, and then diluted with cold water (20 mL). The obtained precipitate was filtered off, washed with water, dried and recrystallized from ethanol to afford compound **4** as white solid.

2-{5-[(2-Phenyl-1*H*-indol-1-yl)methyl]-2*H*-tetrazol-2-yl}acetohydrazide (5)

A mixture of ester the **4** (3.61 g, 0.01 mole) and hydrazine hydrate (1 mL) in absolute ethanol (20 mL) was refluxed for 72 h, the solvent was evaporated under vacuum and the obtained precipitate was filtered off and recrystallized from ethanol to afford the hydrazide **5** as white solid.

General procedure for the synthesis of 6–8

To a solution of hydrazide **5** (3.47 g, 0.01 mole) in ethanol (10 mL), the aldehydes, namely *p*-fluorobenzaldehyde, *p*-bromobenzaldehyde and/or *N*,*N*-dimethylaminobenzaldehyde (0.01 mole) and glacial acetic acid (0.5 mL) were added. The mixture was refluxed for 5 h and the solvent was concentrated under reduced pressure. After cooling, the obtained solid was filtered off, dried and recrystallized from the proper solvent to afford the corresponding hydrazone derivatives **6–8,** respectively.

Sugar 2-{5-[(2-phenyl-1*H*-indol-1-yl)methyl]-2*H*-tetrazol-2-yl}acetylhydrazone (9–12)

To solution of the hydrazide **5** (3.47 g, 0.01 mole) in ethanol (30 mL) containing few drops of glacial acetic acid, the respective sugar (0.01 mole) in water (1 mL) was added. The reaction mixture was heated under reflux for 30 h and the solvent volume was reduced under vacuum. After cooling, the precipitated solid was filtered, dried and recrystallized from ethanol-DMF mixture to give the corresponding sugar hydrazone **9–12** as yellowish powder.

***O*-Acetylsugar-2-{5-[(2-phenyl-1H-indol-1-yl)methyl]-2*H*-tetrazol-2-yl}acetylhydrazone (13–16)**

To a solution of the sugar hydrazone **9–12** (0.005 mole) in dry pyridine (3 mL), acetic anhydride (0.02 mole) was added and the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was poured into ice-cold water and the obtained precipitate was filtered off, dried and crystallized from ethanol to afford the corresponding protected sugar hydrazone derivative **13–16**.

2-(*O*-Acetylsugar)-5-{[5-[(2-phenyl-1*H*-indol-1-yl)methyl]-2*H*-tetrazol-2-yl]methyl}-2,3-dihydro-1,3,4-oxadiazole (17–20)

A solution of the sugar hydrazones **9–12** (0.005 mole) in acetic anhydride (3 mL) was heated at 100°C for 1.5 h. The reaction mixture was poured onto crushed ice and the precipitated solid was filtered off, washed with potassium hydrogen carbonate solution and water, dried and recrystiallized from ethanol to give corresponding oxadiazoline derivatives **17–20**.

Anticancer screening

The synthesized compounds were evaluated and screened against human liver carcinoma cell line (HepG2). Four concentrations were taken of each compound: 0, 5, 12, 25 and 50 μg/mL.

The human tumor cell line (HEPG2) was available at the National Cancer Institute, Cairo, Egypt. The antitumor activity of the newly synthesized compounds was measured using the sulforhodamine-B stain (SRB) assay by the method of Skehan et al., (28). Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Tested compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the tested compounds (0, 5, 12.5, 25 and 50 μM) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds 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% (w/v) with sulforho-

damine-B stain (SRB) dissolved in 1% acetic acid. Unbounded dye was removed by four washes with 1% acetic acid, and attached stain was recovered with Tris–EDTA buffer. Cell survival and drug activity were determined by measuring color intensity using an ELISA reader. Data are representative of three individual experiments, performed in three replicates for each individual dose and measured by SRB assay. Control values did not exhibit significant changes compared to the DMSO vehicle. The IC_{50} was determined by using a program Graph-Pad PRISM version 3. Statistical significance was determined by using one-way analysis of variance (ANOVA) test. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and compared with the reference drug, doxorubicin and the results are given in Table 1.

RESULTS AND DISCUSSION

The starting compound 2-phenylindole (**1**) was prepared from acetophenone, phenylhydrazine and polyphosphoric acid according to previously reported procedure (29). Reaction of 2-phenylindole with chloroacetonitrile in the presence of sodium hydride afforded 1-acetonitrile-2-phenylindole (**2**). When the nitrile derivative **2** was allowed to react with sodium azide in the presence of ammonium chloride, the tetrazole derivative **3** was obtained in 77% yield. Its IR spectrum revealed the absence of the CN absorption band and instead absorption band at 3286 cm^{-1} corresponding to the NH group appeared. Reaction of the tetrazole **3** with ethyl chloroacetate gave the corresponding ethyl ester derivative **4** which on treatment with hydrazine in ethanol afforded the corresponding hydrazide derivative **5** in 78% yield. The IR spectrum of the ester **4** revealed the presence of the ester carbonyl absorption band at 1738 cm^{-1} which disappeared in the IR spectrum of the hydrazide **5** which showed absorption band at 3320–3290 cm^{-1} for the NH and NH_2 groups. The ^1H NMR spectrum of the ester **4** showed the ethyl group signals as triplet and quartet which disappeared in the ^1H NMR spectrum of the hydrazide **5** and instead signals corresponding to NH and NH_2 groups appeared. Reaction of the hydrazide **5** with *p*-fluorobenzaldehyde, *p*-bromobenzaldehyde and *N*,*N*-dimethylaminobenzaldehyde resulted in the formation of the arylidene derivatives **6**–**8**, respectively.

When the hydrazide **5** was allowed to react with D-galactose, D-mannose, D-xylose or D-ribose in an aqueous ethanolic solution and a catalytic amount of acetic acid, the corresponding sugar hydrazones **9**–**12** were obtained, respectively. Their ^1H NMR spectra showed the signals of the sugar chain protons at δ 3.30–5.80 ppm and the C-1 methine proton as doublet in the range δ 7.51–7.54 ppm in addition to the aromatic protons in the region of δ 7.27–7.67 ppm.

Acetylation of the sugar hydrazones **9**–**12** with acetic anhydride in pyridine at room temperature afforded the corresponding per-*O*-acetyl derivatives **13**–**16**, respectively. It is well known that reaction of sugar aroylhydrazones with acetic anhydride gives the respective per-*O*-acetyl derivatives. However, it has been reported (30–34) that when the reaction was carried out at high temperature in boiling acetic anhydride, cyclization usually takes place in addition to per-*O*-acetylation to afford *C*-nucleoside analogs. We reported previously (30, 35) the synthesis of 1,2,4-triazolo[1,3,4]oxadiazole and *N*-acetyl-1,3,4-oxadiazoline acyclic nucleoside analogs by the reaction of hydrazinyl sugars with boiling acetic anhydride. Thus, when the hydrazones **9**–**12** were heated in acetic anhydride at 100°C, they gave the 1,3,4-oxadiazoline acyclic nucleoside analogs **17**–**20**, respectively. The IR

Table 1. Effect of the selected compounds on liver carcinoma cell line (HepG2)

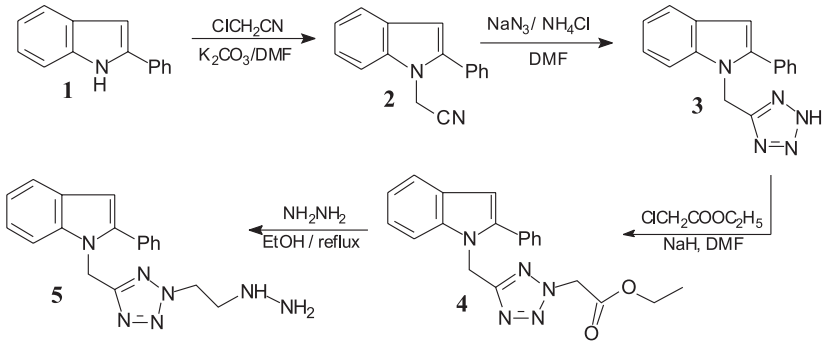
Compd.	IC_{50}* [μg/mL]
2	–
3	22
4	–
5	–
6	21
7	–
8	–
9	22.5
10	4.2
11	15
12	12
13	–
16	27
20	22
Doxorubicin	4.0

*IC_{50}: Dose of the compound that reduces the surviving cells by 50%. Data were represented in three individual experiments, performed in three replicates. Cell survival and drug activity were determined by measuring color intensity using an ELISA reader Data were expressed as the mean absorbance value ± S.E. Significant difference of absorbance of treated cells compared to control value at $p < 0.05$.

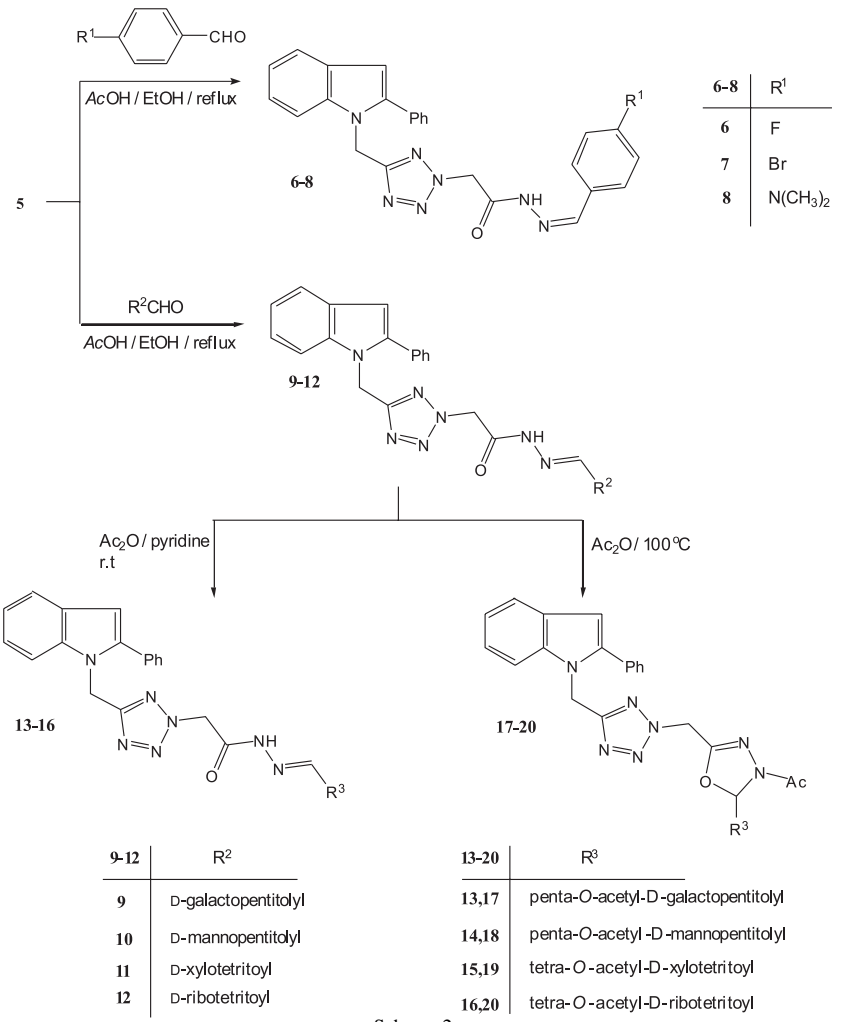
Table 2. Physical and analytical data of the synthesized compounds.

Compd.	M.p. (°C)	Yield (%)	Mol. formula (Mol. wt.)	Analysis (%) calc. /found		
				C	H	N
2	255–256	80	$C_{16}H_{12}N_2$ 232	82.73 82.60	5.21 5.17	12.06 11.86
3	147–149	76.5	$C_{16}H_{13}N_5$ 275	69.80 69.63	4.76 4.69	25.44 25.30
4	174–176	78	$C_{20}H_{19}N_5O_2$ 361	66.47 66.23	5.30 5.19	19.38 19.18
5	174–176	78	$C_{18}H_{17}N_7O$ 347	62.24 62.09	4.93 4.80	28.23 28.16
6	215–216	77	$C_{25}H_{20}FN_7O$ 453	66.22 66.07	4.45 4.50	21.62 21.39
7	219–220	78	$C_{25}H_{20}BrN_7O$ 514	58.33 58.26	3.92 3.82	19.06 18.89
8	226–227	74	$C_{27}H_{26}N_8O$ 478	67.77 67.61	5.48 5.35	23.42 23.27
9	195–196	78	$C_{24}H_{27}N_7O_6$ 509	56.58 56.31	5.34 5.22	19.24 19.15
10	202–203	79	$C_{24}H_{27}N_7O_6$ 509	56.57 56.29	5.34 5.24	19.24 19.18
11	205–206	72	$C_{23}H_{25}N_7O_5$ 479	57.61 57.49	5.26 5.20	20.45 20.29
12	98–99	79	$C_{23}H_{25}N_7O_5$ 479	57.61 57.44	5.26 5.22	20.45 20.31
13	102–103	80	$C_{34}H_{37}N_7O_{11}$ 719	56.74 56.58	5.18 5.28	13.62 13.52
14	103–104	76	$C_{34}H_{37}N_7O_{11}$ 719	56.74 56.49	5.18 4.98	13.62 13.50
15	106–107	71	$C_{31}H_{33}N_7O_9$ 647	57.49 57.11	5.14 5.11	15.14 15.02
16	104–103	72	$C_{31}H_{33}N_7O_9$ 647	57.49 57.22	5.14 5.05	15.14 14.91
17	111–112	66	$C_{36}H_{39}N_7O_{12}$ 761	56.76 56.52	5.16 5.11	12.87 12.69
18	102–103	69	$C_{36}H_{39}N_7O_{12}$ 761	56.76 56.57	5.16 5.12	12.87 12.71
19	107–108	61	$C_{33}H_{35}N_7O_{10}$ 689	57.47 57.29	5.12 5.09	14.22 14.05
20	114–115	64	$C_{33}H_{35}N_7O_{10}$ 689	57.47 57.52	5.12 5.05	14.22 14.19

spectra showed absorption bands in the carbonyl frequency region at 1668–1672 cm^{-1} and 1732–1738 cm^{-1} corresponding to the carbonyl amide and the carbonyl ester groups, respectively, indicating the presence of *N*-acetyl group in addition to the *O*-acetyl groups. The ^1H NMR spectra showed signals corresponding to the *O*-acetyl-methyl protons in addition to the *N*-acetyl-methyl protons each as singlet and signals corresponding to the rest of the alditolyl chain protons. The ^{13}C NMR spectra of **17** showed the resonances of the acetyl-methyl carbons at δ 19.24–24.05 ppm. The value of the chemical shift of the *C*-N-Ac (C-1 in the original sugar chain moiety and C-2 in the oxadiazoline ring) appeared at δ 89.10 ppm, which indicated its *N,N*-acetal nature rather than being from C=N group. The signals at δ 169.64–172.44 ppm correspond to the carbonyl groups (Scheme 2).



Scheme 1.



6-8	R^1
6	F
7	Br
8	$N(CH_3)_2$

9-12	R^2
9	D-galactopentitolyl
10	D-mannopentitolyl
11	D-xylotetritoyl
12	D-ribotetritoyl

13-20	R^3
13,17	penta-*O*-acetyl-D-galactopentitolyl
14,18	penta-*O*-acetyl-D-mannopentitolyl
15,19	tetra-*O*-acetyl-D-xylotetritoyl
16,20	tetra-*O*-acetyl-D-ribotetritoyl

Scheme 2.

Anticancer activity

Fourteen synthesized compounds were selected for testing at the Department of Tumor Pathology, National, Cancer Institute, Cairo, Egypt. Human liver carcinoma cell lines were used for the evaluation according to the method described by Skehan et al. (32).

The results are expressed in the form of the concentration of compound that causes 50% inhibition of cells growth. The *in vitro* evaluation revealed

Table 3. Spectral data of prepared compounds

Compd.	^{1}H NMR [DMSO, δ, ppm], IR [KBr, cm^{-1}]
2	^{1}H NMR: 4.82 (s, 2H, CH_2), 7.22 (m, 2H, Ar-H), 7.32 (m, 3H, Ar-H), 7.41 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.67 (m, 2H, Ar-H), 7.85 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.62 (s, 1H, Ar-H). IR: 3061 (CH), 2205 (CN).
3	^{1}H NMR: 4.94 (s, 2H, CH_2), 7.24 (m, 2H, Ar-H), 7.35 (m, 3H, Ar-H), 7.42 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.69 (m, 2H, Ar-H), 7.88 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.65 (s, 1H, Ar-H), 10.05 (s, 1H, NH). IR: 3042 (CH), 1610 (C=N).
4	^{1}H NMR: 1.72 (t, *J* = 6.2 Hz, 3H, CH_3), 4.15 (q, *J* = 6.2 Hz, 2H, CH_2), 4.49 (s, 2H, CH_2), 4.93 (s, 2H, CH_2), 7.23 (m, 2H, Ar-H), 7.35 (m, 3H, Ar-H), 7.44 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.67 (m, 2H, Ar-H), 7.89 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.66 (s, 1H, Ar-H). IR: 3042 (CH), 1738 (C=O), 1612 (C=N).
5	^{1}H NMR: 4.52 (s, 2H, CH_2), 4.94 (s, 2H, CH_2), 5.88 (bs, 2H, NH_2), 7.25 (m, 2H, Ar-H), 7.36 (m, 3H, Ar-H), 7.42 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.68 (m, 2H, Ar-H), 7.89 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.65 (s, 1H, Ar-H), 10.12 (s, 1H, NH). IR: 3040 (CH), 1675 (C=O), 1614 (C=N).
6	^{1}H NMR: 4.52 (s, 2H, CH_2), 4.94 (s, 2H, CH_2), 6.88 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.25 (m, 2H, Ar-H), 7.30 (s, 1H, C*H*=N), 7.36 (m, 3H, Ar-H), 7.42 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.58 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.68 (m, 2H, Ar-H), 7.89 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.65 (s, 1H, Ar-H), 10.12 (s, 1H, NH). IR: 3048 (CH), 1672 (C=O), 1612 (C=N).
7	^{1}H NMR: 4.53 (s, 2H, CH_2), 4.97 (s, 2H, CH_2), 6.88 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.25 (m, 2H, Ar-H), 7.31 (s, 1H, C*H*=N), 7.37 (m, 3H, Ar-H), 7.42 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.58 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.68 (m, 2H, Ar-H), 7.82 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.60 (s, 1H, Ar-H), 10.10 (s, 1H, NH). IR: 3044 (CH), 1671 (C=O), 1614 (C=N).
8	^{1}H NMR: 3.22 (s, 6H, $2CH_3$), 4.53 (s, 2H, CH_2), 4.97 (s, 2H, CH_2), 6.88 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.25 (m, 2H, Ar-H), 7.31 (s, 1H, C*H*=N), 7.37 (m, 3H, Ar-H), 7.42 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.58 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.68 (m, 2H, Ar-H), 7.82 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.60 (s, 1H, Ar-H), 10.10 (s, 1H, NH) IR: 3052 (CH), 1670 (C=O), 1609 (C=N).
9	^{1}H NMR: 3.32–3.39 (m, 2H, H-6',6''), 3.72 (m, 1H, H-5'), 4.14 (m, 1H, H-4'), 4.27 (t, *J* = 7.4 Hz, 1H, H-3'), 4.37 (dd, *J* = 7.4 Hz, *J* = 7.8 Hz, 1H, H-2'), 4.46 (m, 1H, OH), 4.49 (d, *J* = 6.4 Hz, 1H, OH), 4.55 (s, 2H, CH_2), 4.96 (s, 2H, CH_2), 5.19 (m, 1H, OH), 5.63 (t, *J* = 4.6 Hz, 1H, OH), 5.79 (t, *J* = 4.6 Hz, 1H, OH), 7.27 (m, 2H, Ar-H), 7.39 (m, 3H, Ar-H), 7.44 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.52 (t, *J* = 7.6 Hz, 1H, H-1'), 7.69 (m, 2H, Ar-H), 7.88 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.67 (s, 1H, Ar-H), 10.11 (s, 1H, NH). IR: 3510-3480 (OH), 1678 (C=O), 1612 (C=N).
10	^{1}H NMR: 3.30–3.39 (m, 2H, H-6',6''), 3.70 (m, 1H, H-5'), 4.12 (m, 1H, H-4'), 4.27 (t, *J* = 7.4 Hz, 1H, H-3'), 4.39 (dd, *J* = 7.4 Hz, *J* = 7.8 Hz, 1H, H-2'), 4.47 (m, 1H, OH), 4.50 (d, *J* = 6.4 Hz, 1H, OH), 4.58 (s, 2H, CH_2), 4.97 (s, 2H, CH_2), 5.21 (m, 1H, OH), 5.65 (t, *J* = 4.6 Hz, 1H, OH), 5.80 (t, *J* = 4.6 Hz, 1H, OH), 7.28 (m, 2H, Ar-H), 7.38 (m, 3H, Ar-H), 7.45 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.51 (t, *J* = 7.8 Hz, 1H, H-1'), 7.67 (m, 2H, Ar-H), 7.89 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.67 (s, 1H, Ar-H), 10.14 (s, 1H, NH). IR: 3497–3477 (OH), 1672 (C=O), 1610 (C=N).
11	IR: 3495-3485 (OH), 1670 (C=O), 1611 (C=N).
12	^{1}H NMR: 3.33–3.40 (m, 2H, H-5',5''), 3.62 (m, 1H, H-4'), 4.28 (t, *J* = 7.4 Hz, 1H, H-3'), 4.79 (dd, *J* = 7.4 Hz, *J* = 7.8 Hz, 1H, H-2'), 4.51 (d, *J* = 6.4 Hz, 1H, OH), 4.56 (m, 1H, OH), 4.60 (s, 2H, CH_2), 5.02 (s, 2H, CH_2), 5.25 (t, *J* = 4.6 Hz, 1H, OH), 5.62 (t, *J* = 4.6 Hz, 1H, OH), 7.29 (m, 2H, Ar-H), 7.42 (m, 3H, Ar-H), 7.45 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.54 (t, *J* = 7.8 Hz, 1H, H-1'), 7.70 (m, 2H, Ar-H), 7.88 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.66 (s, 1H, Ar-H), 10.12 (s, 1H, NH). IR: 3040 (CH), 1735 (C=O), 1612 (C=N).
13	^{1}H NMR: 1.85, 1.97, 2.03, 2.12, 2.16 (5s, 15H, $5CH_3$), 4.08 (dd, *J* = 11.4 Hz, *J* = 2.8 Hz, 1H, H-6'), 4.20 (dd, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H, H-6''), 4.48 (s, 2H, CH_2), 4.82 (m, 1H, H-5'), 4.91 (s, 2H, CH_2), 5.12 (m, 1H, H-4'), 5.26 (dd, *J* = 6.5 Hz, *J* = 7.4 Hz, 1H, H-3'), 5.54 (dd, *J* = 7.4 Hz, *J* = 7.2 Hz, 1H, H-2'), 7.29 (m, 2H, Ar-H), 7.35 (m, 3H, Ar-H), 7.47 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.54 (d, *J* = 7.2 Hz, 1H, H-1'), 7.66 (m, 2H, Ar-H), 7.97 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.67 (s, 1H, Ar-H), 10.14 (s, 1H, NH). IR: 3052 (CH), 1737 (C=O), 1615 (C=N).

Table 3. cont.

Compd.	^{1}H NMR [DMSO, δ, ppm], IR [KBr, cm^{-1}]
15	IR: 3069 (CH), 1732 (C=O), 1615 (C=N). **14** ^{1}H NMR: 1.86, 1.97, 2.04, 2.12, 2.18, 2.28 (6s, 18H, 6CH_3), 3.97 (dd, *J* = 11.4 Hz, *J* = 2.8 Hz, 1H, H-5'), 4.12 (dd, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H, H-5''), 4.48 (s, 2H, CH_2), 4.66 (m, 1H, H-4'), 4.91 (s, 2H, CH_2), 5.12 (m, 1H, H-3'), 5.29 (dd, *J* = 6.5 Hz, *J* = 7.4 Hz, 1H, H-2'), 5.50 (dd, *J* = 7.4 Hz, *J* = 7.6 Hz, 1H, H-1'), 7.29 (m, 2H, Ar-H), 7.39 (m, 3H, Ar-H), 7.52 (d, *J* = 7.2 Hz, 1H, H-1'), 7.46 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.65 (m, 2H, Ar-H), 7.92 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.69 (s, 1H, Ar-H), 10.17 (s, 1H, NH). IR: 3069 (CH), 1732 (C=O), 1615 (C=N).
16	IR: 3079 (CH), 1739 (C=O), 1612 (C=N).
17	^{1}H NMR: 1.87, 1.99, 2.03, 2.11, 2.18, 2.29 (6s, 18H, 6CH_3), 3.98 (dd, *J* = 11.4 Hz, *J* = 2.8 Hz, 1H, H-5'), 4.14 (dd, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H, H-5''), 4.48 (s, 2H, CH_2), 4.66 (m, 1H, H-4'), 4.91 (s, 2H, CH_2), 5.10 (m, 1H, H-3'), 5.30 (dd, *J* = 6.5 Hz, *J* = 7.4 Hz, 1H, H-2'), 5.49 (dd, *J* = 7.4 Hz, *J* = 7.6 Hz, 1H, H-1'), 5.72 (d, *J* = 7.6 Hz, 1H, oxadiazoline-H), 7.28 (m, 2H, Ar-H), 7.39 (m, 3H, Ar-H), 7.47 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.68 (m, 2H, Ar-H), 7.92 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.68 (s, 1H, Ar-H). ^{13}C NMR ($CDCl_3$) 19.24, 19.45, 20.15, 20.21, 21.28, 24.05 (6*C*H_3CO), 50.30, 53.25 (2CH_2), 61.79 (C-5), 64.28 (C-4), 68.82 (C-3), 71.28 (C-2), 71.90 (C-1), 89.10 (oxadiazoline C-2), 118.12-150.12 (Ar-14C), 156.42 (tetrazole C-4), 157.14 (oxadiazoline C-5), 169.60, 170.29, 170.86, 171.27, 171.49, 172.18 (6C=O). IR: 3058 (CH), 1738 (C=O), 1610 (C=N)
18	^{1}H NMR: 1.89, 1.98, 2.03, 2.10, 2.17, 2.27 (6s, 18H, 6CH_3), 3.95 (dd, *J* = 11.4 Hz, *J* = 2.8 Hz, 1H, H-5'), 4.12 (dd, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H, H-5''), 4.47 (s, 2H, CH_2), 4.69 (m, 1H, H-4'), 4.91 (s, 2H, CH_2), 5.12 (m, 1H, H-3'), 5.28 (dd, *J* = 6.5 Hz, *J* = 7.4 Hz, 1H, H-2'), 5.50 (dd, *J* = 7.4 Hz, *J* = 7.6 Hz, 1H, H-1'), 5.74 (d, *J* = 7.6 Hz, 1H, oxadiazoline-H), 7.28 (m, 2H, Ar-H), 7.39 (m, 3H, Ar-H), 7.48 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.68 (m, 2H, Ar-H), 7.93 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.69 (s, 1H, Ar-H). IR: 3079 (CH), 1739 (C=O), 1612 (C=N).
19	^{1}H NMR: 1.86, 2.05, 2.12, 2.16, 2.27 (5s, 15H, 5CH_3), 3.98 (dd, *J* = 11.4 Hz, *J* = 2.8 Hz, 1H, H-4'), 4.15 (dd, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H, H-4''), 4.48 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 5.10 (m, 1H, H-3'), 5.27 (dd, *J* = 6.5 Hz, *J* = 7.4 Hz, 1H, H-2'), 5.52 (dd, *J* = 7.4 Hz, *J* = 7.6 Hz, 1H, H-1'), 5.71 (d, *J* = 7.6 Hz, 1H, oxadiazoline-H), 7.27 (m, 2H, Ar-H), 7.39 (m, 3H, Ar-H), 7.50 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.67 (m, 2H, Ar-H), 7.92 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.62 (s, 1H, Ar-H). IR: 3069 (CH), 1740 (C=O), 1611 (C=N).
20	^{1}H NMR: 1.88, 2.02, 2.10, 2.14, 2.24 (5s, 15H, 5CH_3), 3.95 (dd, *J* = 11.4 Hz, *J* = 2.8 Hz, 1H, H-4'), 4.14 (dd, *J* = 11.4 Hz, *J* = 3.2 Hz, 1H, H-4''), 4.52 (s, 2H, CH_2), 4.85 (s, 2H, CH_2), 5.12 (m, 1H, H-3'), 5.27 (dd, *J* = 6.5 Hz, *J* = 7.4 Hz, 1H, H-2'), 5.54 (dd, *J* = 7.4 Hz, *J* = 7.6 Hz, 1H, H-1'), 5.73 (d, *J* = 7.6 Hz, 1H, oxadiazoline-H), 7.28 (m, 2H, Ar-H), 7.39 (m, 3H, Ar-H), 7.54 (d, 1H, *J* = 8.0 Hz, Ar-H), 7.68 (m, 2H, Ar-H), 7.91 (d, 1H, *J* = 8.2 Hz, Ar-H), 8.66 (s, 1H, Ar-H), IR: 3082 (CH), 1742 (C=O), 1614 (C=N).

that some of the tested compounds revealed high inhibition activity while other compounds displayed moderate or little activity. According to the obtained results outlined in Table 1, compound **10** was the highly active among the series of tested compounds and affected the cell viability in a dose dependent manner with IC_{50} values of 4.2 μg/mL followed by compounds **12** and **11** with IC_{50} values of 12 and 15 μg/mL, respectively..

During the past decades, the killing of tumors through the induction of apoptosis has been recognized as a novel strategy for the identification of anticancer drugs (36–38). The apoptosis itself also plays an important role in the development of various diseases including cancer (39, 40). In previous studies (45), compounds incorporating indolyl moiety as the basic structural constituent exhibited a caspase-3-dependent HepG2 cells apoptosis since the protein abundance of caspase-3 and caspase-3-like protease activity in HepG2 cells were up-regulated by these compounds. Indole containing compounds were shown to cause HepG2 cells cycle arrest at G0/G1 phase, thus preventing cells from entering S or G2/M phase and finally apoptosis occurred. It has been reported (41) that compounds having indole ring system in its basic skeleton were

also shown to reduce the expression of cyclooxygenase-2 in HepG2 cells. Cyclooxygenase is a rate-limiting enzyme in prostanoids (PGs) biosynthesis. The cyclooxygenase-2 gene has been characterized as an immediate early gene and associated with inflammation, pain and cellular proliferation as well as apoptosis. Lines of evidences suggest that the over-expression of cyclooxygenase-2 might be one of the leading factors in hepatic carcinogenesis (42). Non-steroid anti-inflammatory drugs (NSAIDs) are commonly used drugs to specially inhibit the cyclooxygenase-2 activity. NSAIDs can inhibit the tumor cell proliferation, induce apoptosis through down-regulation of bcl-2 expression and levels of prostanoids (43–46). Hoechst staining has been applied, in a previous study (41), on a number of indole containing compounds to indicate whether HepG2 underwent cell death *via* apoptosis or necrosis. It has been found that HepG2 exposed to indole containing compounds (with higher inhibition activities) revealed marked nuclear condensation, membrane blebbing, nuclear fragmentation and apoptotic bodies all of which are the characteristics of apoptotic programmed cell death. These facts indicate that these HepG2 underwent cell death *via* apoptosis and not necrosis.

All the above facts can lead us to assume that the synthesized indole compounds (with high activity) resulted in HepG2 cell death *via* apoptosis. We, also, are willing to find a more effective anti-tumor drug by properly modifying the structure of tested compounds, since many indole containing derivatives such as harringtonine, vincaleukoblastine and vincristine, which have strong anti-tumor effects, share the indole ring in their chemical structures.

Structure activity relationship

From the anti-tumor activity results, obtained for the selected tetrazolylindole derivatives, the activity of the tested compounds could be correlated with structure variation and modification. In case of tetrazolylsugar hydrazones, structure activity correlation evidenced that the attachment of acyclic mannopentitolyl sugar moiety to the acetyl hydrazinyl substituted in the tetrazole ring system displayed higher activity than the corresponding analogs carrying galactopentitolyl, ribotetritolyl and xylotetritolyl sugar moieties. This is obviously clear as the activity was decreased when the mannopentitolyl was replaced, especially, with the galactopentitolyl analogue. This variation of activity spectrum between these derivatives indicates the importance of the hydroxyl configuration in this series of acylic sugar moieties.

The sugar hydrazones of {(indol-1-yl)methyl]-2*H*-tetrazol-2-yl} derivatives with the free unprotected hydroxyl groups revealed higher inhibition activities than the corresponding protected acetylated analogs or the corresponding oxadiazoline derivatives substituted with acyclic acetylated sugar moieties.

The results also revealed the effect of halogen substitution, in the *para* position, on the phenyl ring of the arylidine derivatives **6a–c**. Substitution with fluorine atom at the *para* position revealed relatively higher activity than the corresponding chlorine atom or the dimethylamino group at the same position.

Correlation of the results indicated also that the tetrazole **3** with free NH group showed relatively higher activity than its *N*-substituted derivatives as the activity was reduced for compounds **4** and **5** in which the free nitrogen atom was substituted by alkyl ester or hydrazide functionalities.

REFERENCES

1. Nakashima Y., Kawashima Y., Amanuma F., Sota K., Tanaka A., Kameyama T.: Chem. Pharm. Bull. 32, 4271 (1984).
2. Chen I., McDougal A., Wang F., Safe S.: Carcinogenesis 19, 1631 (1998).
3. Hong C., Firestone G.L., Bjeldanes L.F.: Biochem. Pharmacol. 63, 1085 (2002).
4. Chang X., Tou J.C., Hong C.: Carcinogenesis 26, 771 (2005).
5. Misra U., Hitkari A., Saxena A.K., Gurtu S., Shanker K.: Eur. J. Med. Chem. 31, 629 (1996).
6. Samosorn S., Bremner J. B., Ball Lewis A.K.: Bioorg. Med. Chem. 14, 857 (2006).
7. Bal T.R., Anand B., Yogeeswari P., Sriram D.: Bioorg. Med. Chem. Lett. 15, 4451 (2005).
8. Sriram D., Yogeeswari P., Gopal G.: Eur. J. Med. Chem. 40, 1373 (2005).
9. Lundt B.F.: Ger. Offen. 2,024,966 (1970), Chem. Abstr. 74, 53520 (1971).
10. Dekker W.H., Selling H.A., Overeem J.C.: J. Agric. Food Chem. 23, 785 (1975).
11. Blum C.A., Xu M., Orner G.A., Fong A.T., Bailey G.S., Stoner G. D., Horio D.T., Dashwood R.H.: Carcinogenesis 22, 315 (2001).
12. Zhang H.Z.: J. Med. Chem. 48, 5215 (2005).
13. Webber S.E., Tikhe J., Worland S.T., Fuhrman S.A., Hendrickson T.F., Matthews D.A. et al.: J. Med. Chem. 39, 5072 (1996).
14. Velezheva V.S., Brennan P.J., Marshakov V.Y., Gusev D.V., Lisichkina I.N., Peregudov A.S., Tchernousova L.N. et al.: J. Med. Chem. 47, 3455 (2004).

15. Master H.E., Khan S.I., Poojari K.A.: Bioorg. Med. Chem. 13, 4891 (2005).
16. Herr R.J.: Bioorg. Med. Chem. 10, 3379 (2002).
17. Bond A.D., Fleming A., Kelleher F., McGinkley J., Prajapati V.: Tetrahedron 62, 9577 (2006).
18. Rajasekaran A., Thampi P.P.: Eur. J. Med. Chem. 40, 1359 (2005).
19. Holy A.: Nucleosides Nucleotides 6, 147 (1987).
20. Remy R.J., Secrist J.A.: Nucleosides Nucleotides 4, 411 (1985).
21. El Ashry E.S.H., El Kilany Y.: Adv. Heterocycl. Chem. 69, 129 (1998).
22. Markar G.M., Keseru G.M.: J. Med. Chem. 40, 4154 (1997).
23. Franchetti P., Cappellacci L., AbuSheikha G., Jayaram H.N.: J. Med. Chem. 40, 1731 (1997).
24. El-Sayed W.A., Ramiz M.M.M., Abdel-Rahman A.A.-H.: Monatsh. Chem. 139, 1499 (2008).
25. El-Sayed W.A., Fathi N.M., Gad W.A., El-Ashry E.S.H.: J. Carbohydr. Chem. 27, 357 (2008).
26. El-Sayed W.A., Abdel-Rahman A.A.-H., Ramiz M.M.M.: Zeitschrift für Naturforschung 64c, 323 (2009).
27. El-Sayed W.A., Rashad A.E., Awad S.M., Ali M.M.: Nucleosides Nucleotides Nucleic Acids, 28, 261 (2009).
28. Skehan P., Storeng R., Scudiero D.: J. Natl. Cancer Inst. 82, 1107 (1990).
29. Furniss B.S., Hannaford A.J., Smith P.W.G., Tatchell A.R.: Vogel's Text Book of Practical Organic Chemistry, 5th edn., Longman Scientific & Technical and John Wiley & Sons, Inc., New York 1991.
30. Abdel-Aal M.T., El-Sayed W.A., El-Kosy S.M., El-Ashry E.S.H.: Arch. Pharm. Chem. Life Sci. 341, 307 (2008).
31. Abdel Aal M.T., El-Sayed W.A., El-Ashry E.S.H.: Arch. Pharm. Chem. Life Sci. 339, 656 (2006).
32. Ali O.M., Amer H.H., Abdel Rahman A.A.-H.: Synthesis 2823 (2007).
33. El-Ashry E.S.H., Nassr M., Abdel Rahman M.A., Rashed N., Makkawy K.: Carbohydr. Res. 82, 149 (1980).
34. El-Ashry E.S.H., Rashed N., Mousaad A.: Carbohydr. Res. 100, C39 (1982).
35. Abdel Aal M.T., El-Sayed W.A., El-Ashry E.S.H.: Arch. Pharm. Chem. Life Sci. 339, 656 (2006).
36. Panchal R.G.: Biochem. Pharmacol. 55, 247 (1998).
37. Smets L.A.: Anticancer Drugs 5, 3 (1994).
38. Watson A.J.: Alimen. Pharmacol. Ther. 9, 215 (1995).
39. Fisher D.E.: Cell 78, 539 (1994)
40. McConkey D.J., Zhivotovsky B., Orrenius S.: Mol. Aspects Med. 17, 1 (1996).
41. Deng X.U., Yin W., Li W. D., Yin F. Z., Lu X. Y., Zhang X. C., Hua Z. C., Cai B. C.: J. Ethnopharmacol. 106, 179 (2006).
42. Koga H., Sakisaka S., Ohishi M.: Hepatology 29, 688 (1999).
43. Li H.L., Zhang H.W., Chen D.D., Zhong L., Ren X.D., Tu R.: Acta Pharmacol. Sin. 23, 631 (2002).
44. Li X.H., Li J.J., Zhang H.W.: Acta Pharmacol. Sin. 24, 1045 (2003).
45. Sheng H., Shao J., Kirkland S.: J. Clin. Invest. 99, 2254 (1997).
46. Tsujii M., DuBois R.N.: Cell 83, 493 (1995).

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