ELSEVIER

European Journal of Medicinal Chemistry 37 (2002) 255–260

EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY

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Short communication

Synthesis and anticancer and anti-HIV testing of some pyrazino[1,2-*a*]benzimidazole derivatives

Şeref Demirayak *, Usama Abu Mohsen, Ahmet Çağri Karaburun

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Anadolu, 26470 Eskişehir, Turkey

Received 12 April 2001; received in revised form 15 November 2001; accepted 15 November 2001

Abstract

In this study, some 1-methylene-2,3-diaryl-1,2-dihydropyrazino[1,2-*a*]benzimidazole and some 1-(2-arylvinyl)-3-arylpyrazino[1,2-*a*]benzimidazole derivatives were synthesised. The structure elucidation of the compounds was performed by IR, ^{1}H-NMR and MASS spectroscopic data and elemental analyses results. Anticancer and anti-HIV activities of the compounds were examined, however no anti-HIV activity was seen; highly notable anticancer activity was obtained. It was also observed that the compounds were more potent against leukaemia cell lines. © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.

Keywords: Pyrazino[1,2-*a*]benzimidazoles; Anticancer activity; Anti-HIV activity

1. Introduction

It has been known that α-methylene-γ-lactone derivatives, which are sesquiterpenic natural compounds [1–7] (i.e. Helenalin **I**) and their structural analogues, quinone methide derivatives [8–13] possess anticancer activity. For these compounds, it can be said that the most important structural requirement for cytotoxicity is an O=C–C=CH_2 residue, which is a part of an ester, lactone or ketone [5]. The natural compounds, mitomycines **II** [14–20] are also thought to be acting as a precursor of quinone methide; however, mitomycines have limited use because of their toxicity. Efforts to obtain less toxic compounds led to the new anti-tumour agents of indole **III** [21–24], benzimidazole **IV** [25–32] and quinazoline **V** [33–36] derivatives. It was reported that these compounds interact with biological nucleophiles such as L-systein and sulfhydryl bearing enzymes in a Michael-type addition reaction and possess cytotoxic and antitumour activity as an alkylating agent [37,38]. Considering α-methylene-γ-lactone residue as the pharmacophoric group, we should lead to a much simpler structure; α,β-unsaturated ketones [39–42] as a bioisostere of this structure. In this study, the synthesis of some 1-methylene-2,3-diaryl-1,2-dihydropyrazino-[1,2-*a*]benzimidazole derivatives (**5a**–**s**) and some 1-(2-arylvinyl)-3-arylpyrazino[1,2-*a*]benzimidazole derivatives (**10a**–**h**) was attempted. On the other hand, our compounds should also be assumed as the structural analogues of nonsteroidal antiestrogenic triarylethylene

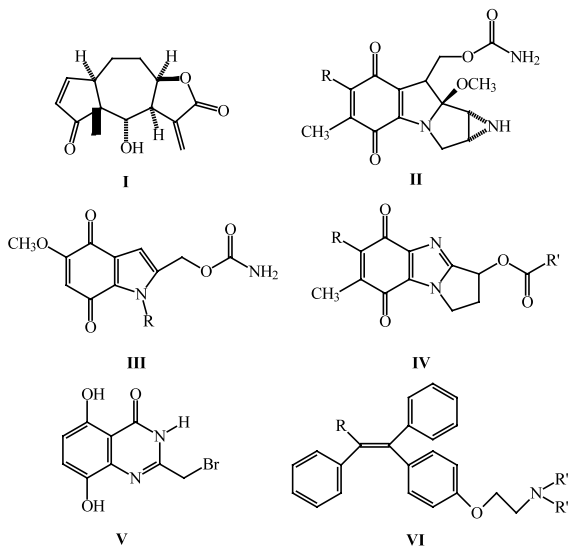
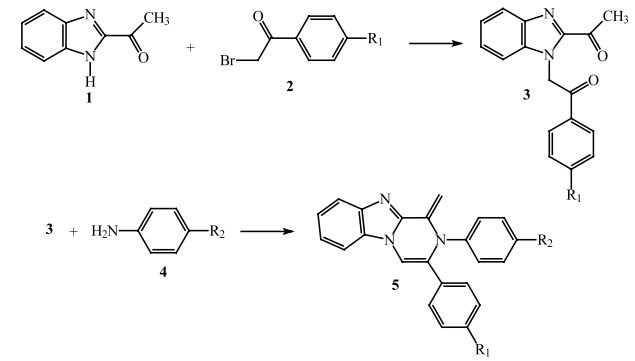
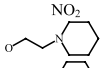
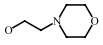


Fig. 1.

* Correspondence and reprints.
E-mail address: sdemiray@anadolu.edu.tr (Ş. Demirayak).

0223-5234/02/$ - see front matter © 2002 Published by Éditions scientifiques et médicales Elsevier SAS.
PII: S0223-5234(01)01313-7



	R_2
4a	H
4b	CH_3
4c	OCH_3
4d	Cl
4e	NO_2
4f	
4g	

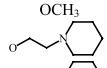
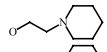
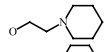
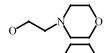
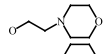
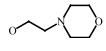
	R_1	R_2
5a	H	H
5b	H	CH_3
5c	H	OCH_3
5d	H	Cl
5e	H	NO_2
5f	CH_3	H
5g	CH_3	CH_3
5h	CH_3	OCH_3
5i	OCH_3	H
5j	OCH_3	OCH_3
5k	Cl	H
5l	Cl	OCH_3
5m	H	
5n	OCH_3	
5o	Cl	
5p	H	
5r	OCH_3	
5s	Cl	

Fig. 2.

derivatives **VI** including Tamoxifen and Clomiphene which were known for their anticancer activity [37] (Fig. 1).

2. Chemistry

For the synthesis of the aimed heterocycles, the reaction sequences outlined in Figs. 2 and 3 were followed. To obtain 1-(2-aryl-2-oxoethyl)-2-acetylbenzimidazoles **3**, which are starting materials of the compounds **5**, 2-acetylbenzimidazole **1** and a suitable α-bromoacetophenone **2** derivative was reacted in the presence of potassium carbonate in acetone. 1-Methylene-2,3-diaryl-1,2-dihydropyrazino[1,2-*a*]benzimidazoles **5a**–**s** were obtained by reacting **3** and **4a**–**g** in acetic acid. Although the compounds **4a**–**e** were commercially available, 4-[(2-(piperidin-1-yl)ethoxy]aniline **4f** and 4-[2-(morpholin-4-yl)ethoxy]aniline **4g** derivatives, the starting materials of compounds **5m**–**s** were obtained by the hydrolyses of the ethers formed by reacting 4-hydroxyacetanilide with 2-(piperidin-1-yl)ethylchloride and 2-(morpholine-4-yl)ethylchloride, respectively. These compounds were synthesised and used without any further purification or structure elucidation.

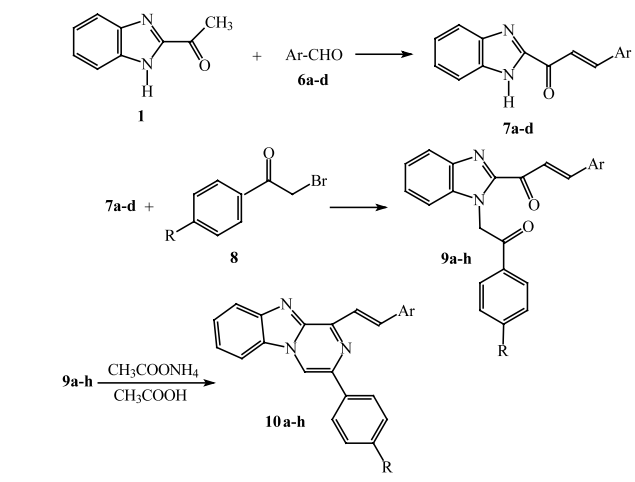
The other group of compounds whose syntheses was attempted was pyrazinobenzimidazole derivatives **10a**–**h**.

Reaction of 2-acetylbenzimidazole **1** with substituted benzaldehydes **6a**–**d** afforded 1-(benzimidazol-2-yl)-3-aryl-2-propenone derivatives **7a**–**d**, as described in the literature [43]. 1-[1-(2-Aryl-2-oxoethyl)benzimidazol-2-yl]-3-arylpropenones **9** were then synthesised by reacting **7a**–**d** with α-bromoacetophenones **8** in acetone in the presence of potassium carbonate. Finally, heating **9** with ammonium acetate in acetic acid gave **10**.

3. Results and discussion

3.1. Anticancer activity

According to the test method, the compounds having $\log_{10} GI_{50}$ (GI_{50}: growth inhibition of 50%) values greater than -4 are considered as inactive. It can be seen that for all of our compounds, $\log_{10} GI_{50}$ values are smaller than -4. Therefore, we may conclude that all of our compounds provide a notable activity level. Melphalan and *cis*-diaminodichloroplatinum, two of



6 and 7	Ar
a	
b	
c	
d	

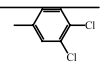
9 and 10	R	Ar
a	H	
b	Cl	
c	CH_3	
d	OCH_3	
e	Cl	
f	CH_3	
g	OCH_3	
h	Cl	

Fig. 3.

the commonly used chemotherapeutic agents, were used as standard compounds. When the meangraph midpoint (MG-MID) values of the compounds melphalan and *cis*-diaminodichloroplatinum, i.e. −5.09 and −6.20 respectively, are considered, it is observed that our compounds provide high activity levels. It is especially noteworthy that MG-MID values of compounds **5e**,**f** and **i** are between the values obtained from the standard compounds. However, when the MG-MID values, whose average values against various cancer types were given, are taken into consideration four of the compounds with MG-MID values lower than −5 attract attention, i.e. **5e**,**f**,**i**,**l**. Each of these four compounds is carrying a 1-methylene residue and may be considered as a structural analogue of O=C–C=CH_2 residue. Also, we should say that their structure resembles Tamoxifen group drugs of oestrogen antagonist activity. Nevertheless, the compounds **5n**,**o**,**r**,**s**, carrying a 2-substituted aminoethyl residue and especially prepared to emphasise this similarity, were failed to sustain the expected increase in activity. It is observed that the activity levels of the α,β-unsaturated ketone analogue compounds **9a**,**b**,**d**,**e**,**f**,**h** and their cycled forms, compounds **10a**,**b**,**c**,**e**,**g**,**h**; could not reach the levels obtained from the 1-methylene residue carrying compounds **5**. Nonetheless, α,β-unsaturated ketone analogues **9**, among which compounds **9b**,**e**,**h** have activity values almost approximate to −5, appear to be more active than pyrazinone derivatives **10**. With regard to all these data, in the three groups of newly synthesised compounds, no significant difference was observed among substituents. When these data obtained are examined according to their activity against various cancer types, it is observed that both the standard and the tested compounds are effective against leukaemia in lower concentrations; for 12 of the compounds this value is lower than −5. The most noteworthy compound is **5o**, i.e. it is even more active than melphalan against leukaemia.

3.2. Anti-HIV activity

None of our compounds showed any anti-HIV activity.

4. Experimental protocols

4.1. Chemistry

Melting points were determined using an Electrothermal 9100 digital melting point apparatus and were uncorrected. Spectroscopic data were recorded on the following instruments. IR: Shimadzu 435 IR spectrophotometer; ^{1}H-NMR: Bruker DPX 400 NMR spectrometer, in DMSO-d_6, TMS as internal standard; MS: VG Platform Mass spectrometer. Analyses for C, H, N was within ±0.4% of the theoretical values. 2-Acetylbenzimidazole [44] and α-bromoacetophenone [45] derivatives were prepared according to the methods in the literature. Some characteristics of the compounds were given in Table 1.

4.1.1. General method

*4.1.1.1. 1-Methylene-2,3-diaryl-1,2-dihydropyrazino-[1,2-a]benzimidazoles (**5a–s**).* A mixture of the suitable **1** (2 mmol) and an arylamine **4** (2 mmol) was refluxed for 10 h in acetic acid (50 mmol). In the case of the compounds **5b–e**,**g**,**j**, the cooled solution was poured into ice water and neutralised with sodium carbonate. The precipitate was crystallised from ethanol. In the case of the compounds **5a**,**f**,**h**,**i**,**k**,**l**, the solution was allowed to crystallise in a refrigerator. The crystallised compound was recrystallised in acetic acid to give the acetic acid salt of the compound. In the case of the compounds **5m–s**, the cooled solution was poured into ice water and neutralised with sodium carbonate. The precipitate formed was filtered and dissolved in methanol. 1 mL concentrated hydrochloric acid was added into the solution. The solvent was evaporated under vacuum. The precipitate was crystallised from methanol to give the hydrochloric acid salt of the compound.

*4.1.1.2. 1-[1-(2-Aryl-2-oxoethyl)benzimidazol-2-yl]-3-arylpropenones (**9a–h**).* A mixture of **7a–d** (5 mmol), an appropriate **8** (5 mmol) and potassium carbonate (6 mmol) in acetone (100 mL) was stirred at room temperature for 6 h. The solvent was evaporated at low temperature. The residue was washed with water and then ethanol. The raw product was recrystallised from ethanol.

*4.1.1.3. 1-(2-Arylvinyl)-3-arylpyrazino[1,2-a]benzimidazole derivatives (**10a–h**).* A solution of suitable **9** (3 mmol) and ammonium acetate (30 mmol) in 50 mL of acetic acid was refluxed for 1 h. The solution was cooled, poured into ice water and neutralised with sodium carbonate. The precipitate formed was filtered and crystallised in ethanol.

5a IR (KBr) ν_{max} (cm^{-1}): 3433–3252 (O–H), 2600–2350 (N^+–H), 1708 (C=O), 1594–1479 (C=N, C=C), 1270 (C–O). ^{1}H-NMR δ (ppm): 1.89 (s, 3H), 7.00 (t, 1H), 7.33–7.51 (m, 10H), 7.73 (d, $J = 7.63$ Hz, 2H), 7.85 (d, $J = 8.16$ Hz, 1H), 8.39 (d, $J = 8.11$ Hz, 1H), 8.58 (s, 1H), 8.83 (s, 1H), 12.00 (s, 1H). EIMS: m/z: 336.5 [M + 1], 335.5 [M +], 334.5 [M − 1, %100], 258, 167, 142. ESMS: m/z: 336.2 (%100).

5c IR (KBr)ν_{max} (cm^{-1}): 3443 (O–H), 1598–1490 (C=N, C=C), 1240 (C–O). ^{1}H-NMR δ (ppm): 3.76 (s, 3H), 7.00 (d, $J = 8.78$ Hz, 2H), 7.37–7.68 (m, 8H), 7.72

Table 1
Some characteristics of the compounds

Compound	m.p. (°C)	Yield (%)	Formulae	Molecular weight
5a	126–7	60	$C_{23}H_{17}N_3 \cdot C_2H_4O_2 \cdot 1/2H_2O$	404.47
5b	202–3	62	$C_{24}H_{19}N_3 \cdot 2H_2O$	385.47
5c	197–8	54	$C_{24}H_{19}N_3O \cdot H_2O$	383.45
5d	148–9	60	$C_{23}H_{16}ClN_3 \cdot 1/2H_2O$	378.86
5e	231–2	53	$C_{23}H_{16}N_4O_3$	396.41
5f	132–3	58	$C_{24}H_{19}N_3 \cdot 2C_2H_4O_2$	427.51
5g	130–2	65	$C_{25}H_{21}N_3 \cdot H_2O$	381.48
5h	107–8	55	$C_{25}H_{21}N_3O \cdot C_2H_4O_2$	406.49
5i	141–2	62	$C_{24}H_{19}N_3O \cdot C_2H_4O_2$	443.51
5j	181–2	60	$C_{25}H_{21}N_3O_2 \cdot 5/2H_2O$	440.51
5k	138–9	68	$C_{23}H_{16}ClN_3 \cdot 2C_2H_4O_2 \cdot 1/4H_2O$	456.94
5l	128–9	67	$C_{24}H_{18}ClN_3O \cdot C_2H_4O_2$	417.90
5m	261–2	58	$C_{30}H_{30}N_4O \cdot 2HCl \cdot H_2O$	553.53
5n	332–3	57	$C_{31}H_{32}N_4O_2 \cdot 2HCl \cdot H_2O$	583.56
5o	288–9	63	$C_{30}H_{29}ClN_4O \cdot 2HCl \cdot H_2O$	587.98
5p	227–8	68	$C_{29}H_{28}N_4O_2 \cdot 2HCl \cdot H_2O$	555.50
5r	275–6	61	$C_{30}H_{30}N_4O_3 \cdot 2HCl \cdot H_2O$	585.53
5s	203–4	64	$C_{29}H_{27}ClN_4O_2 \cdot 2HCl \cdot H_2O$	589.95
9b	211–2	82	$C_{24}H_{16}Cl_2N_3O_2 \cdot H_2O$	467.32
9c	185–7	78	$C_{23}H_{18}N_2O_3 \cdot H_2O$	388.41
9d	151–2	80	$C_{23}H_{18}N_2O_4 \cdot H_2O$	404.41
9e	160–1	80	$C_{22}H_{15}ClN_2O_3 \cdot H_2O$	408.83
9f	194–5	81	$C_{23}H_{18}N_2O_2S \cdot H_2O$	404.47
9g	192–3	76	$C_{23}H_{18}N_2O_3S \cdot H_2O$	420.47
9h	196–7	80	$C_{22}H_{15}ClN_2O_2S \cdot H_2O$	424.89
10b	183–5	77	$C_{24}H_{15}Cl_2N_3$	581.32
10c	214–5	75	$C_{23}H_{17}N_3O$	351.39
10d	180–2	82	$C_{23}H_{17}N_3O_2$	367.39
10e	225–7	80	$C_{22}H_{14}ClN_3O$	371.80
10f	206–7	78	$C_{23}H_{17}N_3S$	367.45
10g	152–4	82	$C_{23}H_{17}N_3OS$	383.45
10h	178–9	76	$C_{23}H_{14}ClN_3S$	399.87

(d, $J = 7.63$ Hz, 2H), 7.89 (d, $J = 8.16$ Hz, 1H), 8.57 (d, $J = 8.11$ Hz, 1H), 8.91 (s, 1H), 9.02 (s, 1H). EIMS: m/z: 366 [M + 1, %100], 365 [M +], 351, 334, 258, 244, 167.

5h IR (KBr)ν_{max} (cm^{-1}): 2600–2300 (N^+–H), 1705 (C=O), 1592–1480 (C=N, C=C), 1236 (C–O). ^{1}H-NMR δ (ppm): 1.91 (s, 3H), 2.35 (s, 3H), 3.77 (s, 3H), 6.98 (d, $J = 8.92$ Hz, 2H), 7.29 (d, $J = 8.00$ Hz, 2H), 7.43 (d, $J = 8.89$ Hz, 2H), 7.59 (d, $J = 8.10$ Hz, 2H), 7.36–7.55m, 2H, 7.08 (s, 1H), 7.86 (d, $J = 8.16$ Hz, 1H), 8.39 (d, $J = 8.11$ Hz, 1H), 8.36 (s, 1H), 8.73 (s, 1H), 12.00 (s, 1H).

5k IR (KBr)ν_{max} (cm^{-1}): 2660–2240 (N^+–H), 1704 (C=O), 1597–1464 (C=N, C=C), 1255 (C–O). ^{1}H-NMR δ (ppm): 1.92 (s, 3H), 7.03 (t, 1H), 7.35 (d, $J = 8.68$ Hz, 2H), 7.33–7.56 (m, 7H), 7.78 (d, $J = 8.39$ Hz, 2H), 7.87 (d, $J = 8.16$ Hz, 1H), 8.40 (d, $J = 8.11$ Hz, 1H), 8.62 (s, 1H), 8.88 (s, 1H), 12.00 (s, 1H). EIMS: m/z: 369 [M +], 334, 292, 258, 167, 166 (%100), 142.

5m IR (KBr) ν_{max} (cm^{-1}): 2650–2450 (N^+–H), 1602–1500 (C=N, C=C), 1250 (C–O). ^{1}H-NMR δ (ppm): 3.15–3.4 (m, 4H), 3.60 (t, 2H), 3.90–4.10 (m, 4H), 4.56 (t, 2H), 7.06 (d, $J = 8.86$ Hz, 2H), 7.41 (d, $J = 8.86$ Hz, 2H), 7.43–7.52 (m, 2H), 7.59–7.70 (m, 4H), 7.75–7.81 (m, 1H), 7.94 (d, $J = 8.32$ Hz, 1H), 8.67 (d, $J = 8.39$ Hz, 1H), 9.12 (s, 1H), 9.51 (s, 1H), 12.03 (bs, 1H).

5s IR (KBr) ν_{max} (cm^{-1}): 2660–2400 (N^+–H), 1598–1500 (C=N, C=C), 1262 (C–O). ^{1}H-NMR δ (ppm): 3.30–3.45 (m, 4H), 3.71 (t, 2H), 3.75–3.85 (m, 4H), 4.46 (t, 2H), 6.95 (d, $J = 8.92$ Hz, 2H), 7.29 (d, $J = 8.90$ Hz, 2H), 7.37 (s, 1H), 7.45 (d, $J = 7.45$ Hz, 2H), 7.49–7.51 (1H), 7.55–7.62 (1H), 7.66 (d, $J = 8.53$ Hz, 2H), 7.99 (d, $J = 8.33$ Hz, 1H), 8.51 (d, $J = 8.31$ Hz, 1H), 9.05 (s, 1H), 9.13 (s, 1H), 11.19 (s, 1H).

9c IR (KBr) ν_{max} (cm^{-1}): 3490 (O–H), 1702–1660 (C=O), 1598–1450 (C=N, C=C). ^{1}H-NMR δ (ppm): 2.38 (s, 3H), 6.25 (s, 2H), 6.52 (bs, 1H), 7.02 (bs, 1H), 7.12 (d, $J = 8.50$ Hz, 2H), 7.42–7.58 (m, 3H), 7.95–8.05 (m, 3H), 8.28 (d, $J = 17.05$ Hz, 2H), 8.52 (m, 1H).

9e IR (KBr) ν_{max} (cm^{-1}): 3450 (O–H), 1696–1662 (C=O), 1610–1500 (C=N, C=C). ^{1}H-NMR δ (ppm): 6.22 (s, 2H), 6.56 (bs, 1H), 7.05 (bs, 1H), 7.40–7.65 (m, 3H), 7.71 (d, $J = 8.12$ Hz, 2H), 7.80–7.90 (m, 2H), 8.11 (d, $J = 8.22$ Hz, 2H), 8.32 (d, $J = 16.72$ Hz, 2H), 8.45–8.50 (m, 1H).

9g IR(KBr)ν_{max} (cm^{-1}): 3470 (O–H), 1695–1655 (C=O), 1605–1450 (C=N, C=C). ^{1}H-NMR δ (ppm): 3.68 (s, 3H), 6.25 (s, 2H), 7.05 (t, 1H), 7.34–7.52 (m, 7H), 7.68 (d, $J = 8.61$ Hz, 2H), 7.92 (d, $J = 16.21$ Hz, 1H), 8.02 (d, $J = 7.59$ Hz, 1H), 8.32 (d, $J = 16.18$ Hz, 1H).

10b IR (KBr) ν_{max} (cm^{-1}): 1617–1450 (C=N, C=C). ^{1}H-NMR δ (ppm): 7.47–7.65 (m, 5H), 7.73 (d, $J = 8.32$ Hz, 2H), 7.82 (d, $J = 8.12$ Hz, 2H), 7.98 (d, $J = 16.20$ Hz, 2H), 8.08 (d, $J = 8.32$ Hz, 2H), 8.22 (d, $J = 8.16$ Hz, 2H), 8.65 (s, 1H).

10c IR (KBr) ν_{max} (cm^{-1}): 1630–1446 (C=N, C=C). ^{1}H-NMR δ (ppm): 3.81 (s, 3H), 6.61 (bs, 1H), 6.98 (bs, 1H), 7.12–7.60 (m, 6H), 7.88 (d, $J = 16.02$ Hz, 1H), 8.02–8.10 (m, 3H), 8.28 (d, $J = 16.07$ Hz, 1H), 8.71 (s, 1H).

10h IR (KBr) ν_{max} (cm^{-1}): 1627–1450 (C=N, C=C). ^{1}H-NMR δ (ppm): 7.04–7.56 (m, 8H), 7.90–8.12 (m, 4H), 8.26 (d, $J = 16.20$ Hz, 1H), 8.81 (s, 1H).

4.2. Pharmacology

4.2.1. Anticancer activity

The cytotoxic and/or growth inhibitory effects of the compounds were evaluated in vitro against approximately 60 human tumour cell lines derived from nine neoplastic diseases, namely: leukaemia (L), non-small cell lung cancer (NSCLC), colon cancer (CC), central nervous system cancer (CNSC), melanoma (M), ovarian cancer (OC), renal cancer (RC), prostate cancer (PC), and breast cancer (BC). The evaluation of anti-

Table 2
Antiproliferative activities of the compounds (log GI_{50})

Compound	L	NSCLC	CC	CNSC	M	OC	RC	PC	BC	MG-MID
5a	−4.50	−4.36	−4.65	−4.29	−4.27	−4.27	−4.27	−4.23	−4.45	−4.37
5b	−4.62	−4.18	−4.47	−4.05	−4.19	−4.09	−4.27	−4.15	−4.33	−4.27
5c	−4.64	−4.86	−4.75	−4.91	−4.68	−4.62	−4.69	−4.65	−4.66	−4.72
5d	−4.00	−4.59	−4.48	−4.57	−4.48	−4.62	−4.70	−4.37	−4.53	−4.50
5e	−5.39	−5.23	−5.19	−5.00	−4.87	−5.14	−5.38	−4.81	−5.08	−5.14
5f	−5.52	−5.43	−5.63	−5.49	−5.55	−5.06	−4.85	−5.51	−5.83	−5.42
5g	−4.54	−4.82	−4.73	−4.38	−4.45	−4.39	−4.22	−4.41	−4.79	−4.54
5h	−5.02	−4.43	−5.05	−4.18	−4.44	−4.00	−4.03	−4.47	−4.67	−4.47
5I	−5.76	−4.96	−5.50	−4.89	−5.24	−4.91	−4.81	−4.63	−5.54	−5.17
5j	−4.23	−4.74	−4.56	−5.14	−4.65	−4.88	−5.03	−4.64	−4.81	−4.75
5k	−4.42	−5.22	−4.78	−5.03	−4.75	−4.87	−4.74	−4.53	−4.78	−4.82
5l	−4.43	−5.07	−4.96	−5.31	−5.02	−5.07	−5.28	−4.81	−5.17	−5.02
5n	−5.51	−4.76	−5.04	−4.80	−4.85	−4.66	−4.70	−4.89	−4.97	−4.90
5o	−6.40	−4.40	−4.00	−4.92	−4.47	−4.00	−4.00	−4.00	−4.63	−4.62
5r	−5.44	−4.54	−4.82	−4.50	−4.87	−4.41	−4.44	−4.68	−4.73	−4.72
5s	−5.14	−4.45	−4.73	−4.25	−4.74	−4.44	−4.35	−4.64	−4.64	−4.59
9a	−4.70	−4.65	−4.70	−4.53	−4.60	−4.67	−4.64	−4.50	−4.62	−4.64
9b	−5.33	−4.69	−4.89	−4.75	−4.73	−4.77	−4.67	−4.66	−4.72	−4.81
9d	−4.74	−4.38	−4.51	−4.42	−4.54	−4.36	−4.42	−4.42	−4.53	−4.50
9e	−5.14	−4.59	−4.71	−4.61	−4.84	−4.50	−4.68	−4.62	−4.78	−4.75
9f	−4.80	−4.32	−4.49	−4.22	−4.46	−4.32	−4.29	−4.31	−4.46	−4.42
9h	−5.11	−4.63	−4.75	−4.69	−4.80	−4.58	−4.65	−4.58	−4.75	−4.73
10a	−4.49	−4.25	−4.38	−4.21	−4.13	−4.26	−4.28	−4.10	−4.28	−4.28
10b	−4.06	−4.00	−4.00	−4.00	−4.00	−4.05	−4.00	−4.00	−4.04	−4.02
10c	−4.61	−4.09	−4.12	−4.10	−4.22	−4.15	−4.05	−4.06	−4.26	−4.18
10e	−4.74	−4.37	−4.40	−4.47	−4.37	−4.32	−4.45	−4.12	−4.57	−4.45
10g	−5.02	−4.32	−4.50	−4.17	−4.47	−4.27	−4.02	−4.00	−4.54	−4.41
10h	−4.48	−4.06	−4.07	−4.03	−4.08	−4.17	−4.01	−4.17	−4.34	−4.14
A	−5.48	−5.17	−5.11	−5.12	−5.08	−5.18	−4.99	−4.49	−4.79	−5.09
B	−6.39	−6.20	−6.14	−6.18	−6.08	−6.45	−6.17	−6.41	−6.05	−6.20

Cells were exposed for 48 h to serial dilutions of compounds tested. Growth inhibition was evaluated by SRB assay. The optical density was read with titertek microplate reader at 540 nm. Subsequent data analyses were performed with STATISTICA Microsoft software. Growth inhibition dose (GI_{50}; M) represents the concentrations which the percentage growth ii + 50 as compared with control untreated cells (+100). The compounds, whose $\log_{10} GI_{50}$ was higher than −4 were considered as not active. Leukaemia (L), non-small cell lung cancer (NSCLC), colon cancer (CC), central nervous system cancer (CNSC), melanoma (M), ovarian cancer (OC), renal cancer (RC), prostate cancer (PC), breast cancer (BC). Standard agents: **A**, melphalan; **B**, *cis*-diaminedichloroplatinum.

cancer activity was performed at the National Cancer Institute of Bethesda, USA, following the in vitro screening program, which is based upon the use of multiple panels of 60 human tumour cell lines against which our compounds were tested at 10-fold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. A 48 h continuous drug exposure protocol was followed and a sulforhodamine B (SRB) protein assay was used to estimate cell viability of growth [46,47]. The results of the anticancer activity tests were given in Table 2.

4.2.2. Anti-HIV Activity

The compound was dissolved in dimethylsulfoxide, and then diluted 1:100 in culture medium before preparing serial half $\log_{10}$ dilutions. T4 lymphocytes (CEM cell line) were added and after a brief interval HIV-1 was added resulting in a 1:200 final dilution of the compound. Uninfected cells without the compound served as basic control; the cultures were incubated at 37 °C in 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt XTT was added to all the wells and the cultures were incubated to allow the development of formazan colour by viable cells. Each well was analysed spectrophotometrically to qualitate formazan production and it was also viewed microscopically for detection of viable cells and confirmation of protective activity. Drug treated virus-infected cells were compared with drug treated non-infected cells.

Acknowledgements

Authors are grateful to Anadolu University Research Fund for the support of this work and to V.L. Narayanan and J.P. Bader of the National Institute of Health, Maryland, Bethesda, USA, for anticancer and anti-HIV screening.

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