Arch Pharm Res Vol 29, No 10, 826-833, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Synthesis of Novel Benzofuran and Related Benzimidazole Derivatives for Evaluation of *In Vitro* Anti-HIV-1, Anticancer and Antimicrobial Activities*

Samia M. Rida, Soad A. M. El-Hawash, Hesham T. Y. Fahmy[1], Aly A. Hazzaa, and Mostafa M. M. El-Meligy

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Egypt and [1]Department of Pharmaceutical Sciences, College of Pharmacy, South Dakota State University Brookings, SD 57007, U.S.A.

(Received May 10, 2006)

Previously, we synthesized and evaluated several benzofuran derivatives containing heterocyclic ring substituents linked to the benzofuran nucleus at C-2 by a two- to four-atom spacer as potential anti-HIV-1, anticancer and antimicrobial agents. Among these derivatives, **NSC 725612** and **NSC 725716** exhibited interesting anti-HIV-1 activity. To further investigate the structure-activity relationship, we synthesized several new benzofuran derivatives derived from 2-acetylbenzofuran (**2**, **3a-c**) and 2-bromoacetylbenzofuran (**6**; **7a,b**; **8a,b**). The compounds were designed to comprise the heterocyclic substituents directly linked to the benzofuran nucleus at C-2. Moreover, various related benzimidazoles derived from 2-acetylbenzimidazole and from 2-cyanomethylbenzimidazole (**12a,b**; **13a,b**; **15**; **16a,b**) were also prepared as isosteres. The synthesized compounds were preliminarily evaluated for their *in vitro* anti-HIV-1, anticancer and antimicrobial activity. Compounds **2**, **3a**, **3b**, and **12b** showed weak anti-HIV-1 activity. Compound **6** exhibited mild activity against *S. aureus*, while compound **15** had mild activity towards *S. aureus* and *C. albicans*. However, no significant anticancer activity was observed with any of the tested compounds. From these results, we conclude that the presence of the spacer between the heterocyclic substituent and the benzofuran nucleus may be essential for the biological activity.

Key words: 2-Acetylbenzofuran, Thiazolo[2,3-*c*]-1,2,4-triazoles, 2-Acetyl-1*H*-benzimidazole, Benzo[4,5]imidazo[1,2-*a*]pyridines, Anticancer, Anti-HIV-1, Antibacterial, Antifungal activities

INTRODUCTION

As a part of our research program regarding the chemistry and biological properties of benzofuran derivatives, we recently reported the synthesis and evaluation of *in vitro* anti-HIV-1, anticancer and antimicrobial activities of a number of new benzofurans (Rida *et al.*, 2006). The compounds were designed to comprise the benzoxazole nucleus linked at C-2 with various heterocyclic ring systems by a two- to four-atom spacer. Of these derivatives, the 2-[(1-benzofuran-2-yl-ethylidene)hydrazono]-3-phenylthiazolidin-4-one (NSC 725612) and 4-amino-3-butyl-2-thioxo-2,3-dihydrothiazole-5-carboxylic acid-(1-benzofuran-2-yl-ethylidene)hydrazide (NSC 725716, Fig. 1) exhibited promising anti-HIV-1 activity. In addition, a survey of the literature revealed that other 2-(substituted-phenyl)benzofurans exhibit interesting anticancer activity (Hutchinson *et al.*, 1997; Pinney *et al.*, 1998; Luc Pieters *et al.*, 1999).

The above-mentioned findings prompted us to continue our investigation of benzofuran derivatives in an attempt to generate new lead compounds for future development as anti-HIV-1, antitumor and/or antimicrobial agents. In this work, a new series of benzofurans that comprise the benzofuran nucleus directly linked at C-2 to various substituted heterocyclic ring systems was synthesized in order to investigate the effects of such structural modifications on the anticipated biological activity.

On the other hand, the literature indicated that the benzimidazole nucleus, the isoster of benzofuran, is an essential part of many clinically useful chemotherapeutic agents. For example, the benzimidazole derivatives, thiabendazol,

*This work was presented in part at The Second International Conference of Pharmaceutical and Drug Industries Division Poster P. 162, 7-9 March 2005.

Correspondence to: Soad A. M. El-Hawash, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria, AR Egypt
Tel: 002-034873273
E-mail: soadhawash@yahoo.com

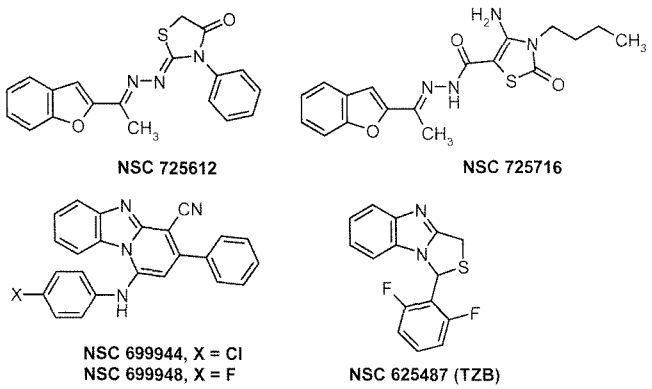


Fig. 1. Structures of biologically active benzfuran and benzimidazole derivatives

triclabendazole and mebendazole, are effective anthelmentic agents (Reynold, 1993, a). Chlormidazole is used in the treatment of fungal infections of the skin (Reynold, 1993b). Enviroxime is an effective drug against rhinovirus (Reynold, 1993a). Methyl 2-benzimidazolecarbamate (carbendazim, FB642) is an anticancer agent that induces the apoptosis of cancer cells (Hao *et al.*, 2002).

Enlightened by these findings and as a continuation of our interest in this area, we extended our investigation to the synthesis of other related benzimidazole derivatives. The target compounds were also designed to comprise the benzimidazole nucleus linked directly at C-2 with the selected heterocyclic substituent in order to explore further their *in vitro* anti-HIV-1, anticancer and antimicrobial activities.

Moreover, several pyrido[1,2-*a*]benzimidazoles (Badawey and Kappe, 1999; El-Hawash *et al.*, 1999) and pyrazino [1,2-*a*]benzimidazoles (Demirayak *et al.*, 2002) have been reported as potential anticancer agents. Among them, NSC 699944 and NSC 699948 (Fig. 1) exhibited significant antitumor activity. They were selected by NCI for further *in vivo* anticancer hollow fiber assays. These results, in addition to the selective HIV-1 reverse transcriptase inhibitory activity recorded for the non nucleoside 1-(2,6-difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole (TZB, NSC 625487 Fig. 1) (Buckheit *et al.*, 1993), motivated us to prepare some substituted pyrido[1,2-*a*]benzimidazole derivatives (**16a**,**b**) in an attempt to produce new effective chemotherapeutic agents.

MATERIAL AND METHODS

Chemistry

All melting points were determined in open-glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded using KBr discs on a Perkin-Elmer 1430 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR were recorded in $CDCl_3$ on Varian Gemini or Jeol spectrometers using TMS as an internal standard (chemical shift in δ ppm). MS were run on a Finnigan mass spectrometer model SSQ/7000 (70 ev). The microanalyses were performed at the Microanalytical Laboratory, National Research Center, Cairo, and the data were within ±0.4% of the theoretical values. Reactions were monitored by thin-layer chromatography on silica gel-protected aluminum sheets (Type 60 F254, Merck), and the spots were detected by exposure to a UV-lamp at l 254 nm for few seconds.

3-Cyano-4-(3,4-dimethoxyphenyl)-6-(1-benzofuran-2-yl)-1*H*-pyrid-2-one (2)

A mixture of 2-acetylbenzofuran (**1**) (0.32 g, 2 mmol), ethylcyanoacetate (0.23 g, 0.21 mL, 2 mmol), 3,4-dimethoxybenzaldehyde (0.31 g, 2 mmol) and ammonium acetate (0.5 g, 6 mmol) in dry ethanol (20 ml) was refluxed for 3-5 h. After cooling, the separated crystals were filtered, washed with water, air dried and recrystallized from aqueous dimethylformamide (Table I).

IR (KBr, cm^{-1}): 3112 (N-H); 2220 (CN); 1630 (C=O); 1616, 1517 (C=C). ^{1}H-NMR ($CDCl_3$, Jeol, 500 MHz, δ ppm): 4.0 (s, 6H, 2 OC<u>H</u>$_3$); 7.0 (s, 1H, benzofuran-C$_3$-<u>H</u>); 7.25-7.45 (m, 5H, 3,4-dimethoxyphenyl and benzofuran-C$_5$-<u>H</u>, C$_6$-<u>H</u>); 7.59 (d, J = 7 Hz, 1H, benzofuran-C$_4$-<u>H</u>); 7.66 (s, 1H, pyridone-C$_5$-<u>H</u>); 7.69 (d, J = 7 Hz, 1H, benzofuran-C$_7$-<u>H</u>); 8.05 (s, 1H, N-<u>H</u>).

2-Amino-4-(substituted phenyl)-6-(1-benzofuran-2-yl) pyridines-3-carbonitriles (3a-c)

A mixture of **1** (0.32 g, 2 mmol), malononitrile (0.13 g, 2 mmol), the proper aromatic aldehyde (2 mmol) and ammonium acetate (0.5 g, 6 mmol) in dry ethanol (20 mL) was refluxed for 3-4 h. After cooling, the separated crystals were filtered, washed with ethanol, air dried and recrystallized from aqueous dimethylformamide (Table I).

IR for **3a-c** (KBr, cm^{-1}): 3465-3443, 3359-3351 (N-H); 2215-2212 (CN); 1646-1633, 1617-1609 (C=N); 1583-1579, 1551-1549, 1514-1500 (C=C, δN-H). ^{1}H-NMR for **3b** ($CDCl_3$, Jeol, 400 MHz, δ ppm): 5.4 (br s, 2H, N<u>H</u>$_2$); 7.26 (s, 1H, benzofuran-C$_3$-<u>H</u>); 7.29-7.56 (m, 6H, p-chlorophenyl and benzofuran-C$_5$-<u>H</u>, C$_6$-<u>H</u>); 7.59 (d, J = 7 Hz, 1H, benzofuran-C$_4$-<u>H</u>); 7.69 (d, J = 7 Hz, 1H, benzofuran-C$_7$-<u>H</u>); 7.85 (s, 1H, pyridine-C$_5$-<u>H</u>). ^{1}H-NMR for **3c** ($CDCl_3$, 400 MHz, δ ppm, Jeol): 3.9 (s, 3H, OC<u>H</u>$_3$); 5.4 (br s, 2H, N<u>H</u>$_2$); 7.0 (s, 1H, benzofuran-C$_3$-<u>H</u>); 7.26-7.61 (m, 6H, p-methoxyphenyl and benzofuran-C$_5$-H, C$_6$-H); 7.65 (d, J = 7 Hz, 1H, benzofuran-C$_4$-H); 7.68 (d, J = 7 Hz, 1H, benzofuran-C$_7$-H); 7.8 (s, 1H, pyridine-C$_5$-H). ^{13}C-NMR for **3c** ($CDCl_3$, 100 MHz, δ ppm): 55.8 (CH_3); 105.8 (benzofuran-<u>C</u>$_3$); 111.8 (C$_{3a}$); 114.9 (phenyl-C$_{3/5}$); 116.5 (CN); 122.1, 123.5, 125, 128.7, 129.3, 130.5, 130 (C$_{4\text{-}7}$ benzofuran; C$_{3\text{-}5}$ pyridine; C$_1$, C$_{2/6}$ phenyl); 149.0, 150.0

(C_2, C_6 pyridine); 155.6, 155.7, 161.0 (C_2, C_{7a} benzofuran; C_4 phenyl).

4-(1-Benzofuran-2-yl)-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)thiazole (6)

A mixture of 4-(benzofuran-2-yl)-2-hydrazinothiazole hydrobromide (**5**) (0.31 g, 1 mmol), acetylacetone (0.1 g, 0.1 mL, 1 mmol) and anhydrous sodium acetate (0.1 g, 1.5 mmol) in absolute ethanol (20 mL) was refluxed for 3 h. The mixture was then cooled and diluted with an equal volume of water. The separated product was filtered, air dried and crystallized from aqueous ethanol (Table I). IR (KBr, cm^{-1}): 1661, 1643 (C=N); 1612, 1571, 1484 (C=C). ^{1}H-NMR ($CDCl_3$, Jeol, 500 MHz, δ ppm): 2.33 (s, 3H, pyrazole-C_5-CH_3); 2.75 (s, 3H, pyrazole-C_3-CH_3); 6.01 (s, 1H, pyrazole-C_4-H); 7.1 (s, 1H, thiazole-C_5-H); 7.22 (s, 1H, benzofuran-C_3-H); 7.27-7.37 (m, 2H, benzofuran-C_5-H, C_6-H); 7.49 (d, J = 7 Hz, 1H, benzofuran-C_4-H); 7.59 (d, J = 7 Hz, 1H, benzofuran-C_7-H).

4-(1-Benzofuran-2-yl)-2-[(4-substituted-benzylidene)hydrazino]thiazole hydrobromides (7a,b)

A mixture of 2-bromoacetyl benzofuran (**4**) (0.48 g, 2 mmol) and the appropriate thiosemicarbazone (2 mmol) in dry dioxane (10 mL) was stirred at room temperature for 2-3 h. The separated crystals were filtered, washed with ethanol, air dried and recrystallized from aqueous dimethylformamide (Table I).

IR for **7a,b** (KBr, cm^{-1}): 3437-3412 (N-H); 1662-1660, 1624-1623 (C=N); 1569-1568, 1500, 1491-1490 (C=C). ^{1}H-NMR for **7b** ($CDCl_3$, Jeol, 500 MHz, δ ppm,): 3.51 (br.s, 2H, D_2O exchangeable, N^+H_2); 7.02 (s, 1H, thiazole-C_5-H); 7.19 (s, 1H, benzofuran-C_3-H); 7.2-7.30 (m, 2H, benzofuran-C_5-H, C_6-H); 7.33 (d, J = 7 Hz, 1H, benzofuran-C_4-H); 7.45 (d, J = 7 Hz, 1H, benzofuran-C_7-H); 7.51-7.55 (2d, 4H, p-chlorophenyl); 8.08 (s, 1H, N=C-H).

5-(1-Benzofuran-2-yl)-6-bromo-3-(4-substituted-phenyl)thiazolo[2,3-*c*]1,2,4-triazoles (8a,b)

A mixture of 7a or 7b (2 mmol) and anhydrous sodium carbonate (0.3 gm) in dry chloroform (20 mL) was stirred at room temperature for half an hour; bromine (0.93 g, 0.3 mL, 6 mmol) was then added dropwise with stirring for 2 h. The chloroform was evaporated under vacuum, and the remaining residue was triturated with 10 mL of water. The formed product was filtered, washed with water, air dried and crystallized from ethanol/water (Table I).

IR for **8a,b** (KBr, cm^{-1}): 1662, 1645 (C=N); 1599, 1569, 1518 (C=C). ^{1}H-NMR for **8b** ($CDCl_3$, Jeol, 500 MHz, δ ppm): 6.92 (s, 1H, benzofuran-C_3-H); 7.28-7.39 (m, 6H, benzofuran-C_5-H, C_6-H and p-chlorophenyl); 7.41 (d, J = 7 Hz, 1H, benzofuran-C_4-H); 7.45 (d, J = 7 Hz, 1H, benzofuran-C_7-H).

Table I. Physical and analytical data of the prepared benzofuran derivatives (**2-8**)

Comp. No.	R	M.P. [°C]	Yield [%]	Mol.Formula[a] (Mol.Wt.)
2	-	265-267	30	$C_{22}H_{16}N_2O_4$ (372.38)
3a	H	242-244	25	$C_{20}H_{13}N_3O$ (311.34)
3b	Cl	298-300	30	$C_{20}H_{12}ClN_3O$ (345.79)
3c	OCH_3	198-200	25	$C_{21}H_{15}N_3O_2$ (341.37)
6	-	100-102	34	$C_{16}H_{13}N_3OS$ (395.37)
7a	H	233-235	75	$C_{18}H_{13}N_3OS.HBr$ (400.30)
7b	Cl	220-222	93	$C_{18}H_{12}ClN_3OS.HBr$ (434.75)
8a	H	88-90	95	$C_{18}H_{10}BrN_3OS$ (396.27)
8b	Cl	138-140	95	$C_{18}H_9BrClN_3OS$. (430.71)

[a] Analyzed For C, H, N and the results were within ± 0.4% of the theoretical values.

MS for 8b m/z (% relative abundance): 430 [M^+ (18)]; 432 [M^{+2} (28)]; 434 [M^{+4} (8)]; 253 (87); 144 (88); 55 (100).

2-Amino-6-(1*H*-benzimidazol-2-yl)-4-(substituted-phenyl) pyridine-3-carbonitriles (12a,b)

A mixture of 2-acetyl-1H-benzimidazole (**11**) (0.48 g, 3 mmol), the appropriate aromatic aldehyde (3 mmol), malononitrile (0.2 g, 3 mmol) and ammonium acetate (0.75 g, 9 mmol) in absolute ethanol (20 ml) was refluxed for 2-3 h, during which precipitation of the product occurred. The mixture was then cooled, and the precipitate was filtered, washed with ethanol and crystallized from aqueous dimethylformamide (Table II).

IR for **12a,b** (KBr, cm^{-1}): 3467, 3348-3347, 3237-3216 (N-H); 2217-2189 (CN); 1643, 1602-1585 (C=N); 1553-1552, 1519-1515 (δ N-H, C=C). ^{1}H-NMR for 12a ($DMSOd_6$, Jeol 500 MHz, δ ppm): 3.87 (br. s, 2H, D_2O exchangeable, NH_2); 7.26-7.69 (m, 8H, Ar-H); 7.91 (s, 1H, pyridine-C_5-H). ^{1}H-NMR for **12b** ($CDCl_3$, Jeol 500 MHz, δ ppm): 1.83 (br.s, 2H, D_2O exchangeable, NH_2); 3.81, 3.84 (2s, 6H, $2OCH_3$); 6.95-7.34 (m, 8H, Ar-H); 7.82 (s, 1H, pyridine-C_5-H).

Table II. Physical and analytical data of the prepared benzimidazole derivatives (**12-16**)

Comp. No.	R	R^1	M.P. [°C]	Yield [%]	Mol.Formula[a] (Mol.Wt.)
12a	H	Cl	>300	45	$C_{19}H_{12}ClN_5$ (345.79)
12b	OCH_3	OCH_3	180-182	80	$C_{21}H_{17}N_5O_2 \cdot H_2O$ (389.42)
13a	H	Cl	89-90	65	$C_{19}H_{11}ClN_4O$ (346.78)
13b	OCH_3	OCH_3	220-222	95	$C_{21}H_{16}N_4O_3$ (372.39)
15	---	---	215-217	50	$C_{17}H_{13}N_3S$ (291.38)
16a	C_6H_5	---	255-257	61	$C_{24}H_{14}ClN_3$ (379.85)
16b	Benzofuran-2-yl	---	198-200	95	$C_{26}H_{14}ClN_3O$ (419.87)

[a] Analyzed for C, H, N, and the results were within ± 0.4% of the theoretical values.

6-(1*H*-Benzimidazol-2-yl)-3-cyano-4-(substituted-phenyl)-1*H*-pyrid-2-ones (13a,b)

The title compounds were prepared, as described under **12a**,**b**, from **11** (0.48 g, 3 mmol), the appropriate aromatic aldehyde (3 mmol), ethyl cyanoacetate (0.34 g, 0.32 mL, 3 mmol) and ammonium acetate (0.75 gm, 9 mmol) (Table II). IR for 13a,b (KBr, cm^{-1}): 3038-3005, 2990-2976 (N-H); 2222-2219 (CN); 1726-1713 (C=O); 1660-1659, 1615-1610 (C=N); 1588, 1519-1513 (C=C). ^{1}H-NMR for **13b** ($CDCl_3$, Jeol 500 MHz, δ ppm): 3.94, 3.95 (2s, 6H, 2OC$\underline{H}_3$); 6.92, 6.94 (2s, 2H, 2N-$\underline{H}$); 7.25-7.79 (m, 7H, Ar-$\underline{H}$); 8.1 (s, 1H, pyridine-C_5-$\underline{H}$).

2-[2-Amino-4-phenylthien-3-yl]-1*H*-benzimidazole (15)

A mixture of 2-cyanomethyl-1*H*-benzimidazole (**14**) (0.31 g, 2 mmol), acetophenone (0.24 g, 0.23 mL, 2 mmol), sulfur (0.06 g, 2 mmol) and triethylamine (0.24 mL) in absolute ethanol (20 mL) was refluxed for 5 h. The mixture was then cooled, and the crystals that formed were filtered, washed with ether, air-dried and recrystallized from ethanol (Table II).

IR (KBr, cm^{-1}): 3412, 3312, 3288 (N-H); 1643, 1617 (C=N) 1586, 1553, 1517, 1500 (δN-H, C=C). ^{1}H-NMR ($CDCl_3$, Jeol 500 MHz, ä ppm): 2.0 (br. s, 2H, D_2O exchangeable, $N\underline{H}_2$); 7.24-7.61 (m, 10H, Ar-$\underline{H}$).

1-Aryl-3-(4-chlorophenyl)pyrido[1,2-a]benzimidazole-4-carbonitriles (16a,b)

A mixture of **14** (0.31 g, 2 mmol), p-chlorobenzaldehyde (0.28 g, 2 mmol), proper aromatic ketone (2 mmol) and ammonium acetate (0.5 g, 6 mmol) in absolute ethanol (20 mL) was refluxed for 2-3 h. The mixture was then cooled and diluted with an equal volume of water. The obtained precipitate was filtered, washed with ether, dried and crystallized from aqueous ethanol (Table II).

IR for **16a**,**b** (KBr, cm^{-1}): 2235-2223 (CN); 1642-1625, 1617-1608 (C=N); 1588-1587, 1519-1516 (C=C). ^{1}H-NMR for **16a** ($CDCl_3$, Jeol 500 MHz, δ ppm): 7.3-7.9 (m, 13H, Ar-$\underline{H}$); 8.4 (s, 1H, C_2-$\underline{H}$ of the fused system). ^{1}H-NMR for **16b** ($CDCl_3$, Jeol 500 MHz, δ ppm): 7.4-7.9 (m, 13H, Ar-$\underline{H}$); 8.4 (s, 1H, C_2-$\underline{H}$ of the fused system). MS for **16b** m/z (% relative abundance): 420 [M^+ (8.7)]; 421 [MH^+ (23.5)]; 422 [M^++2 (11.5)]; 423 [MH^++2 (14)]; 280 (100).

Biological evaluation *in vitro* anti-HIV-1 activity

The *in vitro* anti-HIV-1 drug testing was performed by the National Cancer Institute's Developmental Therapeutics Program, AIDS antiviral screening program, according to the reported procedure (Weislow *et al.*, 1989). The assay involved the killing of T_4 lymphocytes by HIV. The T_4 lymphocytes (CEM cell line) were exposed to HIV at a virus-to-cell ratio of approximately 0.05 and treated with the test compounds, dissolved in dimethylformamide, at doses ranging from 10^{-8} to 10^{-4}. A complete cycle of virus reproduction is necessary to obtain the required cell killing (incubation at 37°C in a 5% carbon dioxide atmosphere for 6 days). Uninfected cells treated with the compound served as a toxicity control, whereas the infected and uninfected cells without the compound served as basic controls. After incubation, the tetrazolium salt XTT was added to all wells, and cultures were incubated to allow formazan color development by viable cells. Formazan production was measured spectrophotometrically, and possible protective activity was confirmed by microscopic detection of viable cells. The effect of each compound on cell growth of HIV-infected and uninfected cells was compared to that of untreated uninfected cells. All tests were compared to AZT as a positive control carried out at the same time under identical conditions.

Antitumor screening

The antitumor screening was performed at the National Cancer Institute (NCI), Bethesda, Maryland, U.S.A.. The compounds were evaluated in three cell lines in a one-dose primary anticancer assay subsequent to the NCI preclinical antitumor drug discovery screen (Grever *et al.*, 1992). The three cell lines used were lung (NCI-H460), breast (MCF-7) and central nervous system (SF-268). In the current protocol, each cell is inoculated and preincubated on microtiter plates. Test agents are then added at a single concentration (100 mM), and the culture is incubated for 48 h. Endpoint determinations are made with alamar blue. The results for each agent are presented as the percent of growth of the treated cells compared to the untreated control cells.

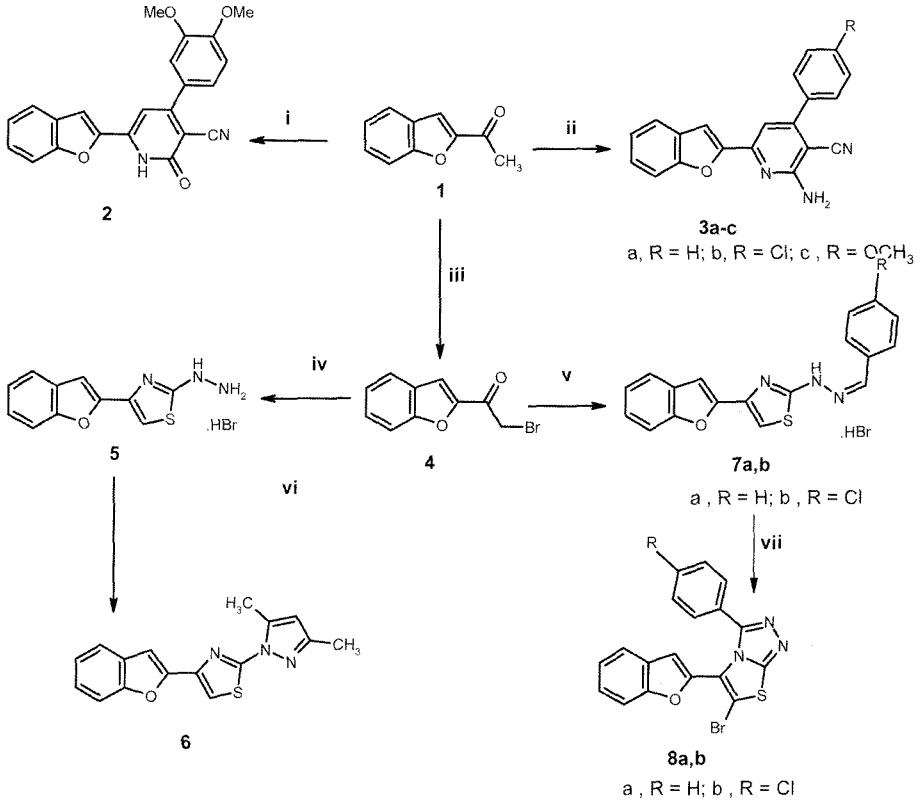
Antimicrobial activity

The test compounds were evaluated by the agar cup diffusion technique (Conte and Barriere, 1988) using a 2 mg/mL solution in DMF. The test organisms were *Staphylococcus aureus* (ATCC 6538) and *bacillus subtilis* (DB 100), Gram-positive bacteria, *Pseudomonas aeruginosa* (ATTC 27853) and *Escherichia coli* (DH5a), Gram-negative bacteria, and *Candida albicans* (0443P), a representative fungus. A control using DMF without any test compound was included for each organism. The minimal inhibitory concentration (MIC) of the most active compounds was measured using the two-fold serial broth dilution method (Scott, 1989). Ampicillin and clotrimazol in DMF were used as reference drugs.

RESULTS AND DISCUSSION

Chemistry

The target benzofuran derivatives listed in Table I were prepared according to the sequence of reactions depicted



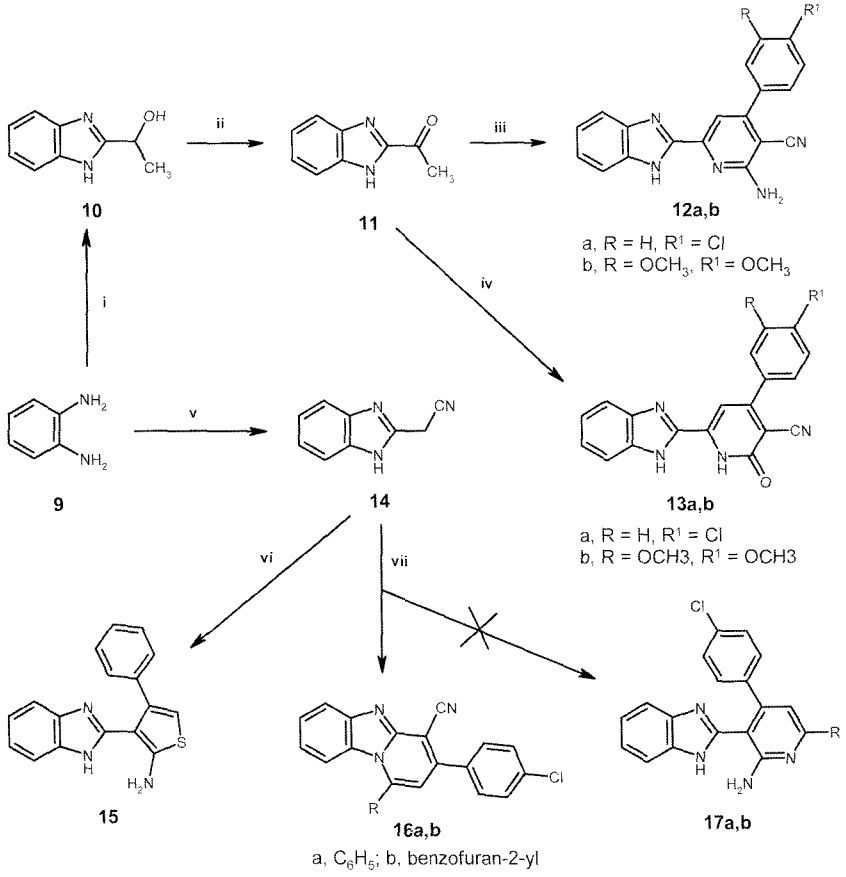
Reagents:(i) 3,4-$(CH_3O)_2C_6H_3CHO$ / $NC\text{-}CH_2CO_2C_2H_5$+ NH_4OAC, (ii) 4-RC_6H_4CHO / $CH_2(CN)_2$+NH_4OAC (iii) Br_2 / ACOH, (iv) $H_2N\text{-}NH\text{-}CS\text{-}NH_2$, (v) 4-R-$C_6H_4CH$=NNH-CS-$NH_2$, (vi) $(CH_3CO)_2CH_2$, (vii) Br_2 / Na_2CO_3

Scheme 1. Synthetic pathway of benzofuran derivatives **2-8**

in Scheme 1. The starting 2-acetylbenzofuran (**1**) was produced as previously described (Elliott, 1951). Reacting **1** with the appropriate aromatic aldehyde and ethyl cyanoacetate in the presence of ammonium acetate afforded 3-cyano-4-(substituted-phenyl)-6-(1-benzofuran-2-yl)-1*H*-pyrid-2-one (**2**). Similarly, 2-amino-4-(substituted-phenyl)-6-(1-benzofuran-2-yl)pyridine-3-carbonitriles (**3a-c**) were prepared by reacting **1** with malononitrile, a substituted aromatic aldehyde and ammonium acetate. The intermediate 2-bromoacetyl benzofuran (**4**) was prepared according to a previously described method (Ghabrial *et al.*, 1996). Treatment of **4** with thiosemicarbazide afforded 4-(1-Benzofuran-2-yl)-2-hydrazinothiazole hydrobromide (**5**) (Kempter *et al.*, 1969). Subsequent cyclocondensation of **5** with acetylacetone yielded 4-(1-Benzofuran-2-yl)-2-(3,5-dimethyl-1*H*-pyrazol-1-yl)thiazole (**6**). Likewise, 4-(1-Benzofuran-2-yl)-2-[2-(4-substituted-benzylidene)hydrazino] thiazole hydrobromides (**7a,b**) were prepared by the treatment of **4** with various thiosemicarbazones of the appropriate aromatic aldehydes. Oxidative cyclization of **7a,b** using bromine in presence of sodium carbonate resulted in the formation of 5-(1-Benzofuran-2-yl)-6-bromo-3-(4-substituted-phenyl)thiazolo [2,3-*c*]-1,2,4-triazoles (**8a,b**). It has been found that bromination at C-5 of the thiazole ring occurs during the course of this oxidative cyclization. The IR spectra of **8a,b** lacked absorption bands corresponding to NH. The ^{1}H-NMR spectra also revealed the disappearance of the broad singlet of NH_2, a singlet of thiazole C-5 and a singlet of N=CH that confirmed cyclization and bromination.

On the other hand, the desired benzimidazole derivatives (Table II) were prepared following the reaction sequence outlined in Scheme 2. The starting 2-acetyl-1*H*-benzimidazole (**11**) was prepared according to a previously reported procedure (Ozegowski and Krebs, 1965). Reacting **11** with the appropriate aromatic aldehyde and malononitrile in the presence of ammonium acetate gave 2-amino-6-(1*H*-benzimidazol-2-yl)-4-(substituted phenyl) pyridine-3-carbonitriles (**12a,b**). Similarly, 6-(1*H*-Benzimidazol-2-yl)-3-cyano-4-(substituted-phenyl)-1*H*-pyrid-2-ones (**13a,b**) were prepared by treating **11** with the appropriate aromatic aldehyde, ethyl cyanoacetate and ammonium acetate. Moreover, reacting 2-cyanomethyl-1*H*-benzimidazole (**14**), which was prepared as reported (Copeland and Day, 1943), with sulfur and acetophenone in the presence of a catalytic amount of triethylamine yielded 2-[2-amino-4-phenyl thien-3-yl]-1*H*-benzimidazole (**15**).

Refluxing a mixture of **14**, acetophenone (or 2-acetyl-



Reagents:(i) $CH_3CH(OH)CO_2H$; (ii) CrO_3/HOAc; (iii) 3-R,4-$R^1C_6H_3CHO/CH_2(CN)_2/NH_4OAc$; (iv) 3-R,4-$R^1C_6H_3CHO$/ $NCCH_2CO_2C_2H_5/NH_4OAc$; (v) $NCCH_2CO_2C_2H_5$; (vi) $C_6H_5COCH_3/S/(Et)_3N$; (vii) 4-$ClC_6H_4CHO/ArCOCH_3/NH_4OAc$.

Scheme 2. Synthetic pathway of benzimidazole derivatives **12-16**

benzofuran), *p*-chlorobenzaldehyde and ammonium acetate in ethanol, as reported for the synthesis of related substituted 2-aminopyridine derivatives (Kovalenko *et al.,* 1999), unexpectedly resulted in the formation of 1-aryl-3-(4-chlorophenyl)pyrido[1,2-*a*]benzimidazole-4-carbonitriles (**16a,b**) rather than 2-amino-3-(benzimidazol-2-yl)-4-(4-chlorophenyl)-6-aryl-3,4-dihydropyridines (**17a,b**). The proposed mechanism for the reaction sequence is illustrated in Fig. 2. The structures of **16a,b** were confirmed by microanalyses, IR, ^{1}H-NMR and MS spectra. The IR spectra were characterized by absorption bands at 2235-2223 cm^{-1}, characteristic of the CN, and the absence of NH bands, corresponding to NH_2 and the benzmidazole NH. The ^{1}H-NMR spectra revealed the absence of the D_2O-exchangeable signals corresponding to NH_2 and HH of benzmidazole. The MS for **16b** showed peaks at m/z 420 and 422 corresponding to $M^{+\cdot}$ + 1 and $M^{+\cdot}$ + 3, respectively, and a base peak at m/z 280.

Biological study *in vitro* Anti-HIV-1 activity

Six compounds (**2**, **3a-c**, **12a,b**) were selected by NCI and evaluated for their effects on HIV-1-induced cytopathogenicity in a human T_4 lymphocyte cell line (CEM). Activity is expressed as percent of protection, representing the percent of surviving HIV-infected cells that were treated with the test compound (at the indicated concentration) relative to uninfected, untreated controls. The results indicated that the test compounds failed to counteract the cytopathic effects of HIV, since the cell growth of HIV-infected cells remained between 4-17% (Table III). Owing to their weak anti-HIV activity, it is difficult to clarify the relationship between molecular structure and biological activity for these compounds.

Anticancer screening

Three compounds (**7a,b**, **8b**) were selected by NCI and evaluated for their *in vitro* antineoplastic activity against the three-cell-line panel consisting of the Breast-MCF7 cell line, the Lung-NCI-H460 cell line and the CNS-SF-268 cell line. Compounds **7a** and **7b** showed weak activity against the lung cell line. However, the test compounds were considered inactive because the percent growth

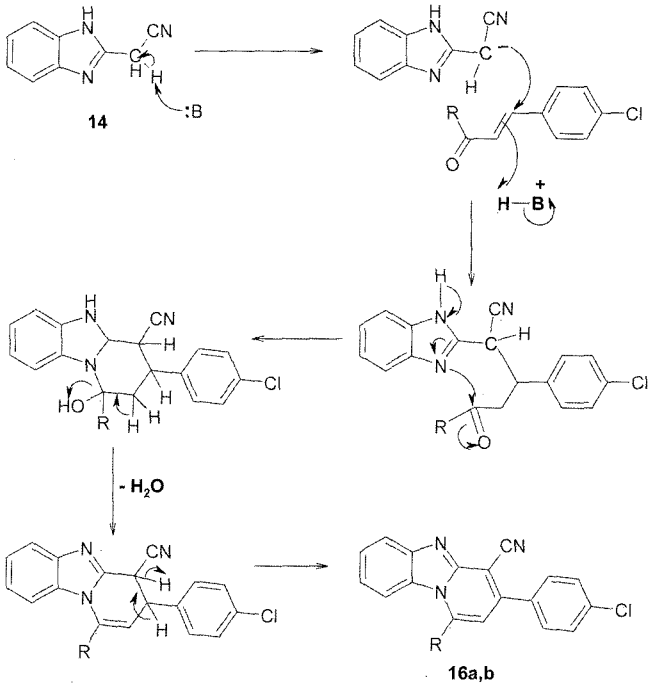


Fig. 2. Proposed mechanism of the reaction leading to compounds **16a,b**

Table III. *In vitro* Anti-HIV activity of compounds **2**, **3a-c**, **12a,b**

Comp. No.	NSC No.	Maximum % Protection	Dose (Molar)	IC_{50} (Molar)
2	725725-P	9.94	6.36×10^{-8}	8.48×10^{-5}
3a	725720-K	16.62	6.36×10^{-8}	1.53×10^{-4}
3b	725721-L	6.74	2.01×10^{-7}	1.10×10^{-4}
3c	725722-M	11.08	2.00×10^{-5}	$>2.00\times10^{-4}$
12a	725723-N	3.56	2.01×10^{-7}	6.05×10^{-5}
12b	725724-O	17.19	2.00×10^{-5}	4.04×10^{-5}

Table IV. Growth percentages of 3-cell-line panel in primary anticancer screen of selected compounds

Comp. No	NSC No	Sample Concentration	Percentage growth		
			Breast-MCF7	Lung NCI-H460	CNS SF-268
7a	S-725702-P	10^{-4} M	85	66	103
7b	S-725703-Q	10^{-4} M	72	53	87
8a	S-725704-R	10^{-4} M	87	84	132

inhibition was higher than 32% (Table IV).

Antimicrobial testing

All of the newly synthesized compounds were preliminarily evaluated for their *in vitro* activity against *S. aureus*, a Gram-positive bacteria, *E. coli*, a Gram-negative bacteria, and *C. albicans*, a fungus. Ampicillin and clotrimazole were used as reference drugs.

The results indicated that compound **6** exhibited weak activity against *S. aureus* (MIC 500 μg/mL), while compound **15** demonstrated weak activities against both *S. aureus* and *C. albicans* (MIC 500 μg/mL).

CONCLUSION

As a part of our research project regarding 2-substituted benzofurans and related bioisosteric heterocyclic ring systems and our attempts to identify novel lead compounds for future development as antitumor, anti-HIV-1 and/or antimicrobial agents, we previously reported the synthesis and biological evaluation of a series of 2-substituted benzofuran derivatives that have various heterocyclic ring systems linked to benzofuran nucleus at position 2 by two- to four-atom spacers (Rida *et al.*, 2006). The results of our earlier study revealed that two compounds exhibited promising anti-HIV-1 activity (**NSC 725612** and **NSC 725716,** Fig. 1). These compounds were selected as leads for further structural modifications in an effort to obtain more potent compounds. In the present investigation, we report the design and synthesis of a new series of 2-substituted benzofurans, which include in their structures various heterocyclic moieties directly attached to the benzofuran nucleus at position 2. The goal in making these structural changes was to explore the significance of the spacer on the respective biological activity.

Compounds **7a,b** were designed to change the position of the spacer in the lead compounds through its insertion between the thiazole ring and phenyl group in the side chain. The anticancer screening data indicated that the compounds exhibited mild activity against lung cancer NCI-460 (Table IV). However, inclusion of such spacer in a ring structure (compounds **6** and **8a**) decreased the antimicrobial and anticancer activity, respectively.

Our study was extended to the synthesis of additional derivatives containing a substituted pyridine moiety directly attached to benzofuran nucleus (**2** and **3a-c**). The anti-HIV-1 results indicated that the test compounds weakly reduced the viral cytopathic effect. (Table II). Compound **3a** (R = H) was the most active in this regard.

On the other hand, some related bioisosteric benzimidazole derivatives (**12**, **13**, and **15**) were also synthesized to investigate their biological effects. The anti-HIV-1 assays revealed that compound **12a** (R – H, R^1 = Cl) had a weak activity, while when R and R^1 = OCH_3 (**12b**), the activity increased slightly (Table III). The antimicrobial test results indicated that compound **15** exhibited mild activity against *S. aureus*.

From the aforementioned results, we concluded that the presence of a spacer linking the various heterocyclic moieties to the benzofuran ring is an essential feature for biological activity.

Moreover, substituted pyrido[1,2-a]benzimidazoles (**16a,b**)

were designed as relatives to the biologically active compounds known in the literature (**NSC 699944** and **699948**, Fig. 1). However, the antimicrobial results indicated that such compounds are devoid of activity.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. Dr. Hoda El-Shamy, Department of Microbiology, High Institute of Public Health, Alexandria, Egypt for performing the antibacterial and antifungal testing. The authors also are grateful to the staff of the Department of Health and Human Services, National Cancer Institute, Bethesda, Maryland, U.S.A. for conducting the anticancer screening of the newly synthesized compounds.

REFERENCES

Badawey, E.A. and Kappe, T., Benzimidazole condensed ring systems. XI. Synthesis of some substituted cycloalkyl pyrido[1,2-*a*]benzimidazoles with anticipated antineoplastic activity. *Eur. J. Med. Chem.*, 34, 663-667 (1999).

Buckheit, R. W. Jr, Hollingshead, M. G., Germany-Decker, J, White, E. L., McMahon, J. B., Allen, L. B., Ross, L. J., Decker, W. D., Westbrook, L., and Shannon, W. M., Thiazolobenzimidazole: Biological and biochemical anti-retroviral activity of a new non-nucleoside reverse transcriptase inhibitor. *Antiviral Res.*, 21, 247-265 (1993).

Conte, J. E. and Barriere, S. L., *Manual of Antibiotics and Infectious Diseases*, 1st Ed. Lea and Febiger, U.S.A., 135 (1988).

Copeland, R. A. and Day, A. R., The preparation and reactions of 2-benzimidazolylecarboxylic acid and 2-benzimidazolacetic acid, *J. Am. Chem. Soc.*, 65, 1072-1075 (1943).

Demirayak, S., Abu Mohsen, U., and Cagri Karaburun, A., Synthesis and anticancer and anti-HIV testing of some pyrazino[1,2-*a*]benzimidazole derivatives. *Eur. J. Med. Chem.*, 37, 255-260 (2002).

El-Hawash, S. A., Badawey, E. A., and Kappe, T., Benzimidazole condensed ring system. XII. Synthesis and anticancer evaluation of certain pyrido[1,2-*a*]benzimidazole derivatives. *Pharmazie*, 54, 341-346 (1999).

Elliott, E. D., The preparation and properties of 2-vinylbenzofuran, *J. Am. Chem. Soc.*, 73, 754 (1951).

Ghabrial, S. S., Zaki, M. Y., and Eldin, S. M., Hydrazidoylhalides in heterocyclic synthesis of several new pyrazole[5,1-c]-1,2,4-triazine,pyramidine and thiazole derivatives, *Egypt J. Pharm. Sci.*, 37(1-6) 329-339 (1996).

Grever, M., Schepartz, S., and Chabner, B., *The National Cancer Institute Cancer Drug Discovery and Development Program Semin. Oncol.*, 19, 622-638 (1992).

Hao, D., Rizzo, J. D., Stringer, S., Moore, R. V., Marty, J., Dexter, D. L., Mangold, G. L., Camden, J. B.,Von Hoff, D. D., and Weitman, S. D., Preclinical antitumor activity and pharmacokinetics of methyl-2-benzimidazolecarbamate (FB642). *Invest. New Drugs*, 20, 261-270 (2002).

Hutchinson, S., Luetjens, H., and Scammells, P., A new synthesis of the benzofuran adenosine antagonist XH-14, *Bioorg. Med. Chem. Lett.* 7, 3081-3084 (1997).

Kempter, G., Sarodnick, G., Schaefer, H., and Fiebig, H. J., Spindler, Heterocycles from 2-bromoacetyl heterocycles J., *Z. Chem.*, 8(9) 339-340 (1968), Through *C. A.*, 70, 11630b (1969).

Kovalenko, S. N., Vasil`ev, M. V., Sorokina, I. V., ChernyKh, V. P., Turov, A. V., and Rundnev, S. A., Recyclization of 2-imino-2H-1-benzopyrans using nucleophilic reagents3. Reactions of 2-iminocoumarin-3-carboxamides with o-phenylenediamines and o-amino(thio)phenols, *Chem. Heterocycl. Comp.*, 34, 1412-1415 (1999), Through *C. A.*, 131, 257452p (1999).

Ozegowski, W. and Krebs, D., Amino acid antagonists IV. Attempted synthesis of [1-methyl-5-bis(b-chloroethyl)amino-benzimidazol-2-yl]acetic acid, *J. Prakt. Chem.*, 29(1-2), 18-25, Through *C. A.*, 63, 8341g (1965).

Pieters, L, Van Dyck, S, Gao, M, Bai, R, Hamel, E., and Vlietinck A., Lemi inverted question markere G. Synthesis and biological evaluation of dihydrobenzofuran lignans and related compounds as potential antitumor agents that inhibit tubulin polymerization. *J. Med. Chem.*, 42, 5475-5481 (1999).

Pinney, K., Pettit, G., Mocharla, V., Del, P., Majia, M., and Shirali, A., Benzo[b]thiophen derivatives, useful as anti-mitotic agents which inhibit tubulin polymerization and as antitumor agents, US Appl., 813, 018, 6 Mar. (1997) 85 pp (Eng.), *C. A.*, 129, 245037c (1998).

Reynold, J. E. F., *Martindale The Extra Pharmacopeia*, 30thed., Royal Pharmaceutical Society of Great Britain, Pharmaceutical Press, London (a) P 46, 54-55 (b) P 319, (c) P 544 (1993).

Rida, S. M., El-Hawash, S. A., Fahmy, H. T., Hazza, A. A., El- and Meligy, M. M., Synthesis and in vitro Evaluation of some Novel Benzofuran Derivatives as Potential Anti-HIV-1, Anticancer and Antimicrobial Agents. *Arch. Pharm. Res.*, 29, 16-25 (2006).

Scott, A. C., Laboratory Control of Antimicrobial Therapy. In: Colle, J. G., Duguid, J. P., Fraser, A. G., Marmion, B. P., Mackie and MacCartney Practical Medical Microbiology, Churchill Livingstone, 13th edition, 2, 161-181 (1989).

Weislow, O. W., Kiser, R., Fine, D., Bader, J., Shoemaker, R. H., Boyd, M. R., New soluble-formazan assay for HIV-I cytopathic effects: application to high-flux screening of synthetic and natural products for AIDS-antiviral activity, *J. Natl. Cancer Inst.*, 81, 577-586 (1989).