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Design, synthesis and cytotoxic activities of novel hybrid compounds between 2-phenylbenzofuran and imidazole

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ABSTRACT

A series of novel hybrid compounds between 2-phenylbenzofuran and imidazole have been prepared and evaluated in vitro against a panel of human tumor cell lines. The results suggest that substitution of the imidazolyl-3-position with a naphthylacyl or bromophenacyl group, were vital for modulating cytotoxic activity. In particular, hybrid compound **15** was found to be the most potent compound against 4 strains human tumor cell lines and more active than cisplatin (DDP), and exhibited cytotoxic activity selectively against liver carcinoma (SMMC-7721).

Cancer remains one of the most difficult diseases worldwide to treat and is the leading cause of human mortality exceeded only by cardiovascular diseases.[1] Therefore, developing new anticancer drugs and more effective treatment strategies for cancer is of importance.[2] Natural products represent a significant source of inspiration for the design of structural analogues with improved pharmacological profile in medicinal chemistry.[3] Naturally occurring substituted-benzofurans are an important class of biologically active oxygen-containing heterocycles. Natural products possessing the 2-phenylbenzofuran moiety exhibit a broad range of biological and pharmacological activities.[4] Recently, naturally occurring benzofurans have been identified to possess antitumor activity. As exemplified in Scheme 1, ebenfuran III[5] and moracins O[6] are also 2-phenylbenzofuran derived compounds exhibiting potent cytotoxic activities against human breast cancer cells and hepatocellular cancer cells.[5,6]

Imidazole and its derivatives have attracted considerable interests in recent years for their versatile properties in chemistry and pharmacology. Biological activities of imidazolium salts have been reported,[7] especially antitumor activity.[8] For example, two new imidazolium halides (Scheme 1), Lepidiline A and Lepidiline B, isolated from the roots of *Lepidium meyenii*, showed potent cytotoxic activity against the human cancer cell lines.[9] Recently, we have reported the synthesis of a series of novel hybrid compounds of dihydrobenzofurans and imidazols moieties such as NMIB (Scheme 1) and their potential antitumor activity.[10]

Molecular hybridization as a drug discovery strategy involves the rational design of new chemical entities by the fusion of two drugs, both active compounds and/or pharmacophoric units recognized and derived from known bioactive molecules.[11] Considering the anticancer activities of naturally occurring 2-phenylbenzofurans as well as the potent cytotoxic activities of natural and synthetic imidazole derivatives, we were interested in synthesizing a number of new hybrid compounds bearing 2-phenylbenzofuran and imidazole moieties (Scheme 1).

Although benzofuran-triazoles hybrid molecules through a heptyloxybenzene chain were synthesized and found to exhibit CYP26A1 inhibitory activity by Simons,[12] to the best of our knowledge, no reports concerning antitumor activity for hybrid compounds between 2-phenylbenzofuran and imidazole have been reported.

In the present research, we have designed and synthesized a series of novel hybrid compounds of imidazole scaffold-based 2-phenylbenzofurans. The purpose of this study was to investigate the antitumor activity of 2-phenylbenzofuran-imidazole hybrids, with the ultimate aim of developing novel potent antitumor agents.

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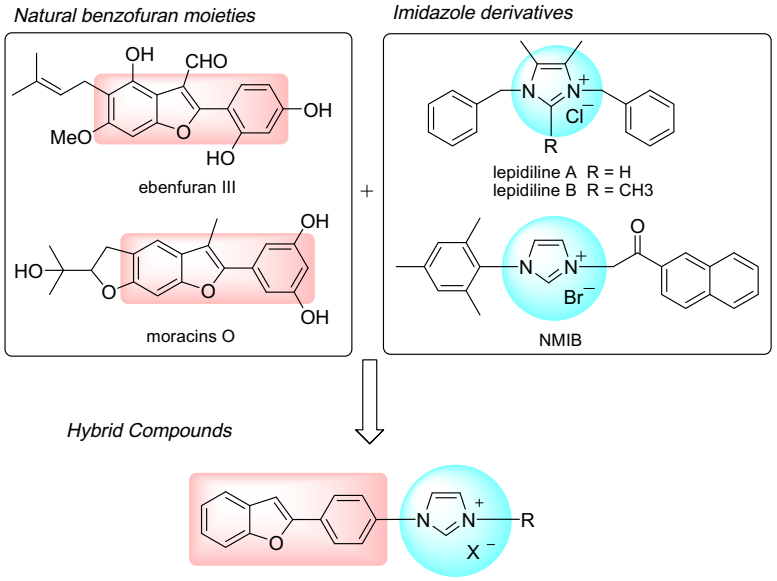
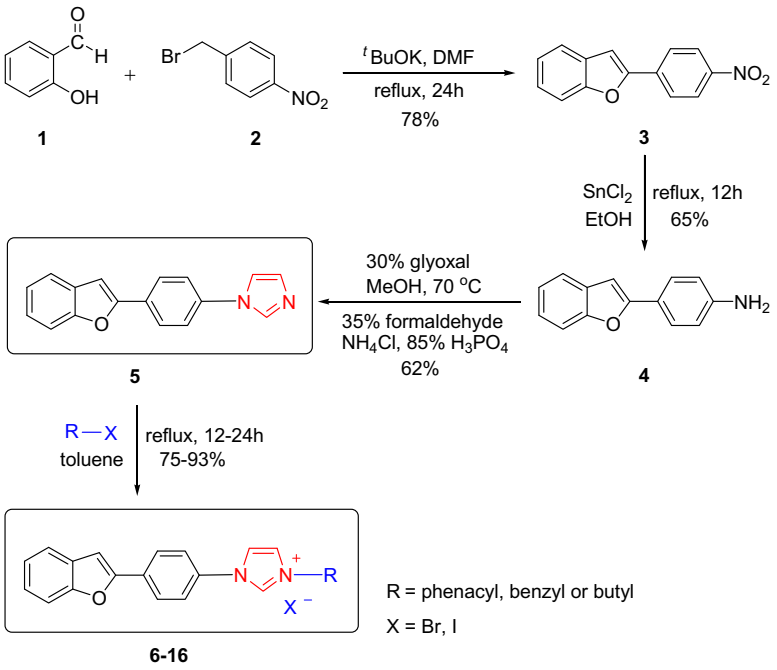


Scheme 1. Design of novel hybrid compounds.

As shown in Scheme 2, the key step in the formation of the phenylbenzofuran backbone was readily achieved by reacting salicylaldehydes (**1**) with 4-nitrobenzyl bromide (**2**) to produce 2-(4-nitrophenyl)benzofuran (**3**, 78% yield) in refluxing DMF.[13] The corresponding amino derivative **4** was prepared from compound **3** by reduction with $SnCl_2$ in refluxing toluene in 65% yield. Base on our previous synthesis[14], 1-(4-(benzofuran-2-yl)phenyl)-substituted imidazole **5** were prepared by treatment of 4-(benzofuran-2-yl)benzenamine **4** with ammonia, formaldehyde and glyoxal.[15] Finally, eleven phenylbenzofuran-based imidazolium salts (**6**–**16**) were prepared with excellent yields by reaction of 1-phenylbenzofuran-substituted imidazoles **5** with the corresponding phenacyl and alkyl halides in refluxing toluene.[16] The structures and yields of hybrid compounds are shown in Tables 1.

The potential cytotoxicity of all newly synthesized hybrid compounds was evaluated in vitro against a panel of human tumor cell lines according to procedures described in the literature.[17] The panel consisted of myeloid liver carcinoma (SMMC-7721), colon carcinoma (SW480), breast carcinoma (MCF-7), lung carcinoma (A549) and leukaemia (HL-60).[18] Cisplatin (DDP) was used as the reference drug. The results are summarized in Table 2 (IC_{50} value, defined as the concentrations corresponding to 50% growth inhibition).

As shown in Table 2, the structures of the hybrid compounds have an obvious influence on the cytotoxic activities. 2-Phenylbenzofuran-imidazole hybrid **5** lacked activity against all tumor cell lines investigated at the concentration of 40 μM. Meanwhile, its imidazolium salt **11** with a 4-hydroxyphenacyl substituent at position-3 of imidazole ring was inactive.



Scheme 2. Synthesis of hybrid compounds **5**–**16**.

Table 1
Structures and yields of hybrid compounds **5–16**

Compound	R	X	Molecular formula	Mp (°C)	Yields (%)
5	–	–	$C_{25}H_{19}BrN_2O_2$	Oil	62
6	Benzyl	Br	$C_{24}H_{19}BrN_2O$	159–161	93
7	2-Bromobenzyl	Br	$C_{24}H_{18}Br_2N_2O$	220–222	86
8	Allyl	Br	$C_{20}H_{17}BrN_2O$	Oil	89
9	Butyl	I	$C_{21}H_{21}IN_2O$	Oil	85
10	Phenacyl	Br	$C_{25}H_{19}BrN_2O_2$	155–157	85
11	4-Hydroxyphenacyl	Br	$C_{25}H_{19}BrN_2O_3$	255–257	90
12	4-Methoxyphenacyl	Br	$C_{26}H_{21}BrN_2O_3$	215–217	92
13	4-Fluorophenacyl	Br	$C_{25}H_{18}BrFN_2O_2$	177–179	84
14	4-Bromophenacyl	Br	$C_{25}H_{18}Br_2N_2O_2$	252–254	88
15	Naphthylacyl	Br	$C_{29}H_{21}BrN_2O_2$	221–223	82
16	2′-Phenyl-phenacyl	Br	$C_{31}H_{23}BrN_2O_2$	194–196	75

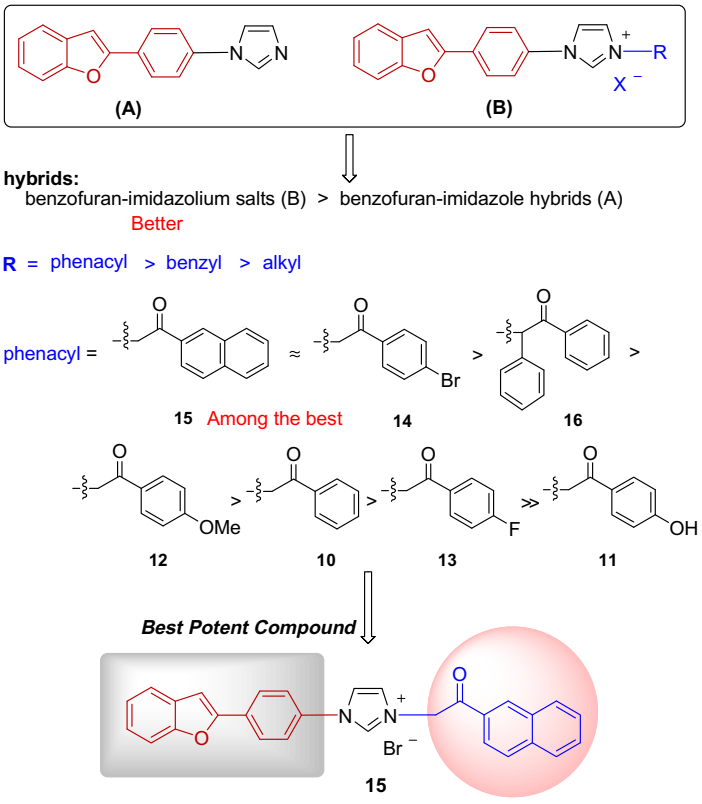
Table 2
Cytotoxic activities of hybrid compounds **5–16** in vitro[b] (IC_{50}, μM[a])

Compound	SMMC-7721	SW480	MCF-7	A549	HL-60
5	>40	>40	>40	>40	>40
6	15.10	19.92	26.94	25.35	5.37
7	4.38	12.71	14.29	9.77	1.97
8	27.04	>40	13.65	33.49	14.49
9	12.73	>40	31.39	21.71	11.76
10	3.71	10.34	11.90	12.94	2.61
11	>40	>40	>40	>40	>40
12	3.71	6.93	11.28	9.79	2.26
13	13.54	16.77	16.69	19.65	12.33
14	3.39	2.85	2.84	8.46	3.15
15	1.65	3.38	5.87	10.93	2.49
16	3.31	6.93	6.90	6.79	2.70
DDP	8.86	15.92	16.65	11.68	1.81

[a] Cytotoxicity as IC_{50} for each cell line, is the concentration of compound which reduced by 50% the optical density of treated cells with respect to untreated cells using the MTT assay.
[b] Data represent the mean values of three independent determinations.

However, imidazolium salt hybrids **6–9** with alkyl substituent at position-3 of imidazole ring exhibited some degree of cytotoxic activities. Among them, compound **7**, bearing a 2-bromobenzyl substituent at position-3 of imidazole, was the most active compound, which displayed similar cytotoxic activity in vitro compared with DDP.

Compared with above alkyl substituent imidazolium salt derivatives **6–9**, hybrid compounds **10–16** with phenacyl substituent at position-3 of imidazole ring exhibited higher cytotoxic activity. Most of this kind of derivatives showed moderate or potent activity (except compound **11**. The loss of potency in imidazolium salt **11** is possibly due to the presence of a phenolic hydroxyl group in phenacyl substituent.). Especially, compounds **14**, **15** and **16**, bearing a naphthylacyl, bromophenacyl or phenylphenacyl substituent at position-3 of the imidazole ring, displayed similar cytotoxic activity in vitro compared with DDP. Interestingly, hybrid compound **15**, a naphthylacyl substituent at position-3 of imidazole, was found to be the most potent derivative against all of human



Scheme 3. Structure–activity relationship of hybrid compounds.

tumor cell lines investigated and more active than DDP (except against HL-60). Notably, this compound exhibited cytotoxic activity selectively against liver carcinoma (SMMC-7721) with IC_{50} value 5.4-fold more sensitive to DDP.

The results suggest that substitution of the imidazolyl-3-position with a naphthylacyl or bromophenacyl group were vital for modulating cytotoxic activity. The structure-activity relationship (SAR) results were summarized in Scheme 3.

In conclusion, a series of novel 2-phenylbenzofuran-imidazole hybrid compounds prepared in this research proved to be potent antitumor agents. The hybrids **14** and **15**, bearing a bromophenacyl or naphthylacyl substituent at position-3 of the imidazole ring, were found to be the most potent compounds. Compound **15** was found to be the most potent derivative against 4 strains human tumor cell lines investigated and more active than DDP, and exhibited cytotoxic activity selectively against liver carcinoma (SMMC-7721). The phenylbenzofuran-based imidazolium salts **15**, **14** and **16** can be considered promising leads for further structural modifications guided by the valuable information derivable from our detailed SARs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.02.094.

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15. *General procedure for the preparation of 2-phenylbenzofuran-imidazole hybrid* **5**. To a magnetically stirred solution of the 30% glyoxal (1.2 mmol) and 35% formaldehyde (1.2 mmol) in methanol (30 ml) at 70 °C, 4-(benzofuran-2-yl) benzenamine **6** (1.0 mmol) and 25% ammonia (1.2 mmol) was added and reaction mixture was stirred for 8 h at the same temperature. Reaction progress was monitored by TLC. After removed the solvent, the dark residue was poured into ice water (20 ml) and extracted by ethylacetate; organic layer was washed by water and brine, dried (anhyd Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel (petroleum ether 60–90 °C: ethyl acetate = 3:1) to afford **5** in 62% yield. Compound **5**: yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91–7.88 (3H, m), 7.62 (2H, dd, J = 7.2, 6.6 Hz), 7.40 (2H, d, J = 8.4 Hz), 7.28–7.22 (4H, m), 7.01 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.98, 154.44, 136.98, 135.42, 130.54, 129.65, 129.01, 128.78, 126.27, 124.73, 123.20, 121.48, 121.09, 117.97, 111.22, 102.08. HR-ESI-MS m/z Calcd for $C_{17}H_{12}N_2O$ 260.0950, found 260.0944.
16. *General procedure for the preparation of 2-phenylbenzofuran imidazolium bromides* **6**–**16**. A mixture of 2-phenylbenzofuran-imidazole hybrids **5** (1 mmol) and alkyl bromides (1.2 mmol) was stirred in toluene (10 ml) at reflux for 12–24 h. A white solid was formed. After completion of the reaction as indicated by TLC, the precipitate was filtered through a small pad of Celite, and washed with toluene (3 × 10 ml), then dried to afford **6**–**16** in 75–93% yields. Pure samples were obtained after recrystallization from appropriate solvent (acetone or methanol). Compound **15**: white powder, yield 82%, mp 221–223 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.03 (1H, s), 8.92 (1H, s), 8.53 (1H, s), 8.25–8.07 (9H, m), 7.84 (1H, d, J = 7.5), 7.78–7.66 (4H, m), 7.44–7.30 (2H, m), 6.36 (2H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 190.81, 153.95, 150.81, 136.68, 135.58, 134.54, 132.03, 130.86, 130.68, 129.71, 128.84, 128.41, 127.87, 127.42, 126.21, 125.52, 125.15, 123.38, 123.16, 122.47, 120.82, 120.37, 114.46, 111.23, 63.44. HRMS (ESI-TOF) m/z Calcd for $C_{29}H_{21}N_2O_2$ $[M\text{-}Br]^+$ 429.1598, found 429.1596. Compound **14**: white powder, yield 88%, mp 252–254 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.62 (1H, s), 8.20 (1H, s), 8.10 (2H, s), 8.09 (2H, s), 7.77–7.71 (3H, m), 7.60 (1H, d, J = 7.2), 7.58 (2H, d, J = 7.8), 7.50 (1H, d, J = 7.8), 7.30–7.19 (3H, m), 6.07 (2H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 191.33, 155.75, 153.98, 136.56, 134.34, 133.26, 131.88, 130.15, 129.04, 128.04, 127.82, 126.55, 124.32, 123.51, 122.95, 121.87, 115.32, 112.53, 103.45, 56.98. HRMS (ESI-TOF) m/z Calcd for $C_{25}H_{18}BrN_2O_2$ $[M-Br]^+$ 457.0546, found 457.0543.
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18. The assay was in five kinds of cell lines (SMMC-7721, SW480, MCF-7, A549 and HL-60). Cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal serum and dispersed in replicate 96-well plates. Compounds were then added. After 48 h exposure to the compounds, cells viability were determined by the [3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide] (MTT) cytotoxicity assay by measuring the absorbance at 570 nm with a microplate spectrophotometer. Each test was performed in triplicate.