Compounds Structurally Related to Tamoxifen as Openers of Large-Conductance Calcium-Activated K^+ Channel

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We found that a variety of compounds containing partial structures of tamoxifen showed activity as chemical modulators of large-conductance calcium-activated K^+ channels (BK channels).

Key words large-conductance calcium-activated K^+ channel; tamoxifen; channel opener; (xeno)estrogen; electrophysiology

Large-conductance calcium-activated K^+ channels (BK channels) characteristically respond to two distinct physiological stimuli, *i.e.*, changes in membrane voltage and in cytosolic Ca^{2+} concentration.[1] The BK channel opens in response to an increase in cytosolic Ca^{2+} concentration and membrane depolarization, resulting in an increase of K^+ efflux, which leads to rapid hyperpolarization of the excitatory membrane and thus reduces Ca^{2+} influx through voltage-dependent Ca^{2+} channels. The BK channel is formed by a tetramer of the pore-forming α-subunit and up to four β-subunits that function to modulate the BK channel.[2,3] Recent cloning studies also revealed the presence of multiple splice variants of α-subunits[4—6] and multiple subtypes of β-subunits (β_1, β_2/β_3 and β_4).[7—9] Thus, there is a large diversity of BK channels, which may be specific to tissues and organs.[4—6] Except for cardiac myocytes, the BK channels are expressed in a number of organ systems, such as smooth muscle cells, skeletal muscle cells, neuronal cells, and secretary epithelial cells,[10] and they have important physiological roles in modulating muscle contraction and neuronal activities, such as synaptic transmission.[11]

These features and the widespread distribution of the channel throughout the central nervous system and in peripheral tissues offer rich opportunities for discovering novel therapeutic agents based on BK channel modulators, particularly openers.[12,13] Chemical channel openers are expected to quench excitatory events that pathologically elevate the cytosolic Ca^{2+} and induce depolarization of the cell membranes, and potentially have specificity for tissues and organs of interest. Well-characterized BK channel openers could be used to treat acute stroke, epilepsy, and bladder overactivity.[14] There is some evidence for the utility of BK channel openers in the treatment of asthma, hypertension, gastric hypermotility and psychoses.[1] Recent studies have shown that the BK channel is one of the targets for the non-genomic effects of (xeno)estrogens, such as tamoxifen and estradiol.[15—19] The stimulatory action of tamoxifen and 17β-estradiol on the BK channel activity requires the presence of the β_1 subunit.[15—19] Herein, we show that compounds containing partial structures of tamoxifen can activate the human BK channels, possibly through action on the β_1 subunit.

Compounds **3a**—**o** were synthesized by means of McMurry condensation reaction of a substituted benzophenone derivative and 3-pentanone in the presence of $TiCl_4$ and zinc powder in dry THF with heating at reflux for 4—20 h.[20] Preliminary assay by using the fluorescent dye method with $DiBAC_4(3)$ was applied with rat rSlo α and β_1 stably expressing human embryonic kidney (HEK 293) cell lines.[21] The results are shown in Fig. 1. The magnitude of release of the dye from the inside of the cells upon opening of the BK channels is shown in terms of the normalized decrease of fluorescence of the dye, relative to that in the case of tamoxifen, defined as 100 (%). The observed relative magnitudes were based on a large set of data (n=11—19). The larger the value, the stronger the opening activity of a compound. As shown in Fig. 1, the *N*,*N*-dimethylaminoethyloxy group of tamoxifen is not necessary for activity (see **3a**). The acidic phenol functionality is also not essential, but rather hydrophobicity, such as a methoxy (**3c**) or trifluoromethyl group (**3h**), is crucial, particularly at the *para* position. Compounds in which the two benzenes are substituted are less potent (**3l**—**o**). While diethylstilbestrol (DES) is more potent than tamoxifen in the dye assay, 4,4′-dimethoxystilbene, **4a**, is more potent than the present series of the compounds **3a**—**o**. In DES and **4a**, the arrangement of the two benzenes with

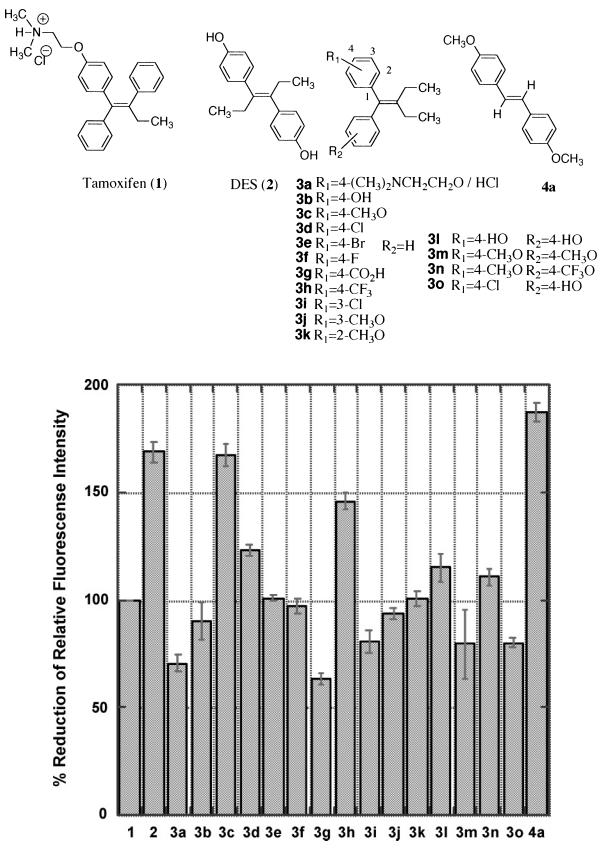

Fig. 1. Fluorescent Dye Assay Result
n=11—19.

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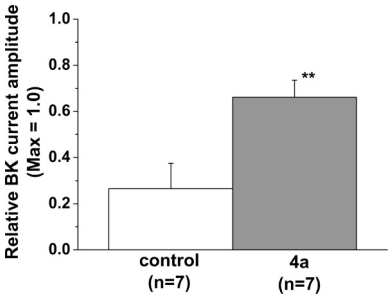

Fig. 2. Electrophysiological Effect of **4a** on the BK Channel Current

Vehicle containing DMSO at 0.05—0.1% was used as control. Statistical analysis was performed with Student's *t*-test. $p<0.05$ was accepted as statistically significant (**).

respect to the central double bond is different from that in **3a**—**o**.

We further investigated the channel opening activity of **4a** electrophysiologically (Fig. 2).

The BK channel currents were recorded by the inside-out patch-clamp technique from HEK-293 cells expressing human hSlo α and β_1 subunits.[22] We also repeated the same experiment with rSlo α and β_1 subunits. Compound **4a** apparently increased the relative current amplitude, activated by the test potential to 120 mV from the holding potential of -60 mV, as compared with the control. **4a** did not show significant effects when hSlo α was expressed without β_1 subunit (data not shown). The half activation potential was shifted from 136.5 ± 4.9 mV ($n=7$) to 120 ± 6.1 mV ($n=7$) by **4a**, indicating that the BK channel activity was facilitated by **4a**. Clearly, a hydroxyl group on the aromatic ring is not essential for the BK channel opening activity of the tamoxifen derivatives. Thus, the present work suggests a simple but important criterion for the design of tamoxifen derivatives as BK channel openers.

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