From the Selective Serotonin Transporter Inhibitor Citalopram to the Selective Norepinephrine Transporter Inhibitor Talopram: Synthesis and Structure−Activity Relationship Studies

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Citalopram and talopram are structurally closely related, but they have very distinct pharmacological profiles as selective inhibitors of the serotonin and norepinephrine transporters, respectively. A systematic structure−activity relationship study was performed, in which each of the four positions distinguishing the two compounds were varied. The inhibitory potencies of the resulting 16 compounds were tested at both serotonin and norepinephrine transporters. This showed that particularly two of the four positions are determinants for the biological activity.

Introduction

The serotonin transporter (SERT)[a] and the norepinephrine transporter (NET) are integral membrane proteins that facilitate the reuptake of the neurotransmitters 5-hydroxytryptamine (5-HT, serotonin) and norepinephrine (NE), respectively, from the extracellular space into neurons.[1] SERT and NET are important drug targets for treatment of psychiatric diseases such as depression and anxiety.[2] In particular, the development of selective serotonin reuptake inhibitors (SSRIs) and combined serotonin/norepinephrine reuptake inhibitors (SNRIs) has resulted in important drugs used in the treatment of depression such as the SSRIs sertraline, paroxetine, and fluoxetine in addition to the SNRIs venlafaxine and duloxetine (Figure 1).[3–5]

In the search for novel antidepressants, the ring structure from tricyclic antidepressants (TCAs) was replaced by bicyclic ring structures (e.g., indanes, indenes, and phtalanes) at H. Lundbeck A/S (Valby, Denmark) in the 1960s.[6,7] The phenylsubstituted phtalanes were found to be the most potent compounds, and a series of approximately 60 phenylsubstituted phtalanes were studied as inhibitors of SERT.[8] Among these, citalopram (**1**, Figure 1) was shown to be a highly selective and potent inhibitor of SERT. Further studies demonstrated that the inhibitory activity of citalopram almost exclusively resided in the (*S*)-enantiomer (escitalopram).[9] Following this discovery, it was found that escitalopram has an improved clinical effect as compared to SSRIs including citalopram.[10–12] Talopram (**2**, Figure 1)[7] was synthesized in the same series of compounds, and although the two compounds are structurally closely related, they have very distinct pharmacological profiles. Citalopram (**1**) is a potent, selective inhibitor of SERT,[13] whereas talopram (**2**) is a potent, selective inhibitor of NET.[6,14] The two compounds have the same phenylsubstituted phthalane skeleton, as well as a propylamine moiety, and they differ in four positions only (Figure 1). Citalopram (**1**) has two aromatic substituents, a fluorine and a cyano group, whereas talopram (**2**) has no aromatic substituents. On the other hand, talopram (**2**) has two methyl substituents on the dihydro-isobenzenzofurane moiety, while citalopram has no substituents in that position. Finally, citalopram (**1**) contains an *N*,*N*-dimethyl propylamine group, while talopram (**2**) has an *N*-methyl propylamine.

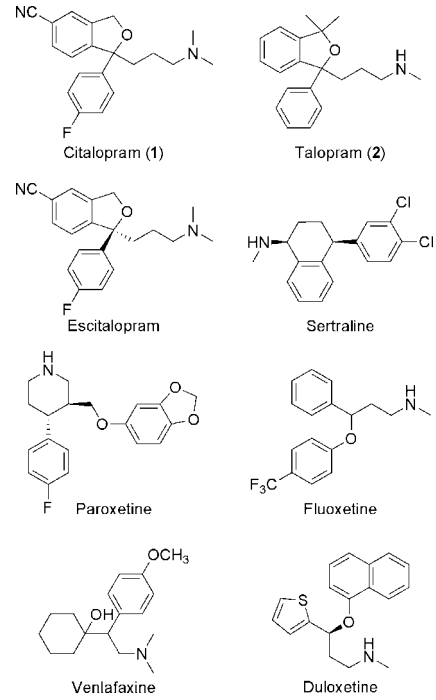

Figure 1. Structures of citalopram (**1**), talopram (**2**), escitalopram sertraline, paroxetine, fluoxetine, venlafaxine, and duloxetine.

In a previous SAR study of citalopram analogues it was demonstrated that the aromatic substituents were important for inhibitory activity at SERT.[8] However, a systematic SAR study of citalopram and talopram analogues that could explain the distinct differences in potency toward SERT and NET has so far not been performed. Therefore, a SAR study was carried out, where each of the four positions distinguishing citalopram (**1**) and talopram (**2**) were modified independently leading to 16 analogues (Table 1). These compounds were synthesized, and their inhibitory potencies at SERT and NET were determined. This enabled an analysis of the structural elements

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[a] Abbreviations: NET, norepinephrine transporter; SERT, serotonin transporter; SSRI, selective serotonin reuptake inhibitor.

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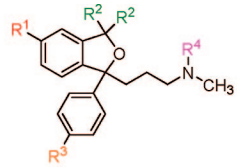
Table 1. Structures of Compounds **1**−**16**



Compound	R^1	R^2	R^3	R^4
1 (citalopram)	CN	H	F	CH_3
3	CN	H	F	H
4	CN	CH_3	F	CH_3
5	CN	CH_3	F	H
6	CN	H	H	CH_3
7	CN	H	H	H
8	CN	CH_3	H	CH_3
9	CN	CH_3	H	H
10	H	H	F	CH_3
11	H	H	F	H
12	H	CH_3	F	CH_3
13	H	CH_3	F	H
14	H	H	H	CH_3
15	H	H	H	H
16	H	CH_3	H	CH_3
2 (talopram)	H	CH_3	H	H

required for selective inhibition of SERT and NET, respectively, by the phenylsubstituted phthalanes.

Results and Discussion

Chemistry. Of the 16 analogues included in this study (Table 1), citalopram (**1**), talopram (**2**), and compounds **3**, and **10**−**16** had previously been synthesized[7,8] and were available in our laboratories. Compounds **4**−**9** have not previously been described. Thus, these compounds were prepared as described below; this method was also used for a revised synthesis of compound **12**.[7]

Phenylsubstituted phthalanes have generally been synthesized by two different routes (Figure 2): routes A (the anion strategy) and B (the double Grignard strategy), respectively.[7,8] In both cases, the starting point is a phthalide (**I**), which is reacted with an aryl magnesium halide giving a 2-hydroxymethyl benzophenone (**II**). In the anion strategy, **II** is ring closed by use of acid

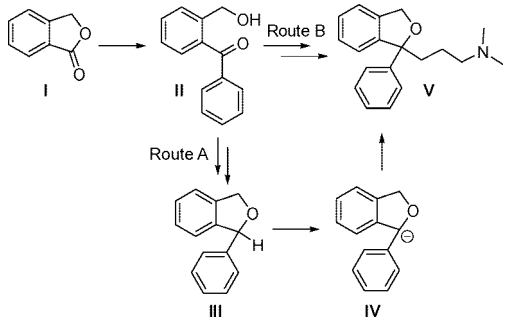
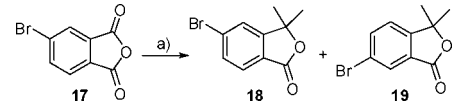


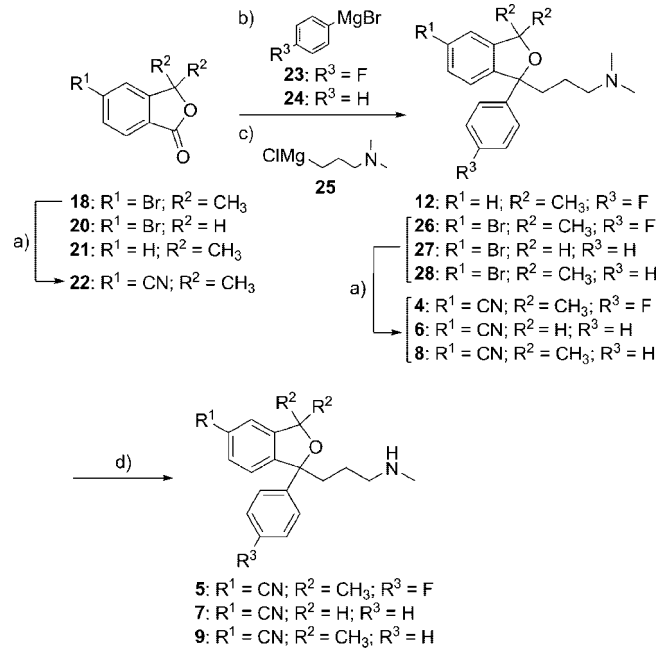
Figure 2. Two synthetic routes generally used for the synthesis of phenylsubstituted phthalanes: routes A (the anion strategy) and B (the double Grignard strategy).

Scheme 1[a]



[a] Reagents and conditions: (a) MeMgCl, THF, 0 °C → rt, 1 h.

Scheme 2[a]



[a] Reagents and conditions: (a) $NiBr_2$, NaCN, NMP, microwave, 200 °C, 0.5 h. (b) **23** or **24**, THF, 0 °C, 0.5 h. (c) (i) **25**, THF, reflux, 1 h; (ii) EtOH/HCl (1:1). (d) Chloroethyl chloroformate, dichloroethane, microwave, 180 °C, 0.5 h.

and subsequently reduced providing **III**. Treatment of **III** with strong base affords anion **IV**, which is alkylated with a *N*,*N*-dimethylaminopropyl halide to give the desired compound **V**. Alternatively, in route B **V** is obtained directly from **II** by treatment with the Grignard reagent derived from (3-chloropropyl)-dimethyl-amine followed by acid promoted ring-closure.

For the synthesis of compounds **4**−**9** and **12** both strategies were initially attempted, but it was quickly realized that the double Grignard strategy (route B) was superior in this case. Initially, 5-bromoisobenzofuran-1,3-dione (**17**) was treated with methyl magnesium chloride to provide bromo-phtalide **18**. The reaction gave a mixture of two isomers **18** and **19**[15] (Scheme 1) in a 2:1 ratio. Gratifyingly, compound **18** was readily isolated by crystallization from ethanol in an overall yield of 33%. Bromo-phtalide **18** was reacted with aryl magnesium bromide (**23** or **24**) generating a 2-(hydroxymethyl)-benzophenone in situ, which was treated with Grignard reagent **25**, and the desired intermediates, compounds **26** and **28**, were obtained after acid promoted ring-closure (Scheme 2). In a similar manner, phtalides **20** and **21**[16] were reacted with aryl magnesium bromides **23** or **24**, followed by reaction with **25** providing intermediate **27** and final compound **12**.

Inspired by the commercial production of citalopram (**1**), where a double Grignard strategy is applied, we evaluated compound **22** as a starting material for cyanated analogues **4**−**9**. Despite the fact that this route works very well for the preparation of **1** it was found that the acid-promoted ring-closure after the double Grignard reaction could not be performed with compound **22**. The only structural difference compared to the

corresponding starting material for **1** is the two methyl groups (R^2, Scheme 2) in compound **22**.

Next, conditions for cyanation of compounds **26**−**28** were investigated using bromo-phtalide (**18**) as a model substrate. Initially, cyanation was attempted by treatment of **18** with either CuCN and K_2CO_3 in *N*-methylpyrrolidone (NMP)[17] or using Buchwald's aromatic Finkelstein cyanation procedure[18] under microwave irradiation. However, in both cases only very low degree of conversion was observed (as determined by LC-MS). Fortunately, applying Leadbeater's protocol using NaCN and $NiBr_2$ in NMP under microwave irradiation[19] provided the cyanated analogue **22** in high yield (Scheme 2). These conditions were then applied in the cyanation of **26**−**28** to provide the desired compounds **4**, **6**, and **8**, respectively.

The *N*-monomethylated compounds **5**, **7**, and **9** were obtained by demethylation of **4**, **6**, and **8**, respectively, by treatment with chloroethyl chloroformate under microwave irradiation and subsequent treatment with warm (70 °C) ethanol.[20]

Biology. The potency of citalopram (**1**), talopram (**2**), and their analogues for inhibition of SERT and NET function has over the course of time been determined in widely different model systems such as human blood platelets,[8] rat brain synaptosomes,[14] or mammalian cell lines transiently expressing recombinant SERT or NET.[13,21] The inherent biological differences of these model systems complicate direct comparison of pharmacological profiles of many of these compounds. In the present study, the % inhibition at 100 nM of compounds **1**−**16** was determined at human SERT (hSERT) and NET (hNET) expressed in the COS-7 kidney cell line through stable transfection of hSERT and hNET cDNA, respectively. A reuptake inhibition assay was used,[22] where COS-7 cells stably expressing either hSERT or hNET were incubated with [^{3}H]5-HT (hSERT assay) or [^{3}H] dopamine (hNET assay) and 100 nM inhibitor or no inhibitor (control). Values for % inhibition are given as mean ± SEM determined in six independent experiments each performed in triplicate.

The results of the biological testing at hSERT and hNET show a distinct relationship between the substitution pattern of the phenylsubstituted phthalanes and their biological activity (Figure 3 and Table 1 in Supporting Information). Citalopram (**1**) together with compounds **3** and **7** are the most potent analogues at hSERT with 52−58% inhibition when tested at 100 nM, whereas compounds **6**, **10**, and **11** were relatively potent with 20−27% inhibition. Compound **3** is a metabolite of citalopram (**1**), and it has previously been demonstrated to be equipotent to the parent compound.[8] All other analogues were much less potent and had <10% inhibition when tested at 100 nM. Characteristic for the six most potent compounds is that none of them have methyl substituents in the R^2-position (Table 1), which thus can be identified as the primary factor in reducing hSERT inhibitory activity. The aromatic CN substitutent (R^1, Table 1), which is present in compounds **1**, **3**, **6**, and **7**, is important for enhancing SERT inhibitory activity. Conversely, the aromatic fluorine atom (R^3, Table 1) and particularly the amino substitution (R^4, Table 1) are less important factors for hSERT inhibition.

For hNET activity, talopram (**2**) is the most potent compound with a 63% inhibition when tested at 100 nM, while compounds **13** and **15** are relatively potent with 37% and 36% inhibition, respectively. Interestingly, compound **11** is among the more potent hNET inhibitors with a 19% inhibition, and this compound also showed significant potency (20% inhibition) at hSERT, thus being a SNRI. The remaining analogues generally produced less than 10% inhibition at 100 nM. The common

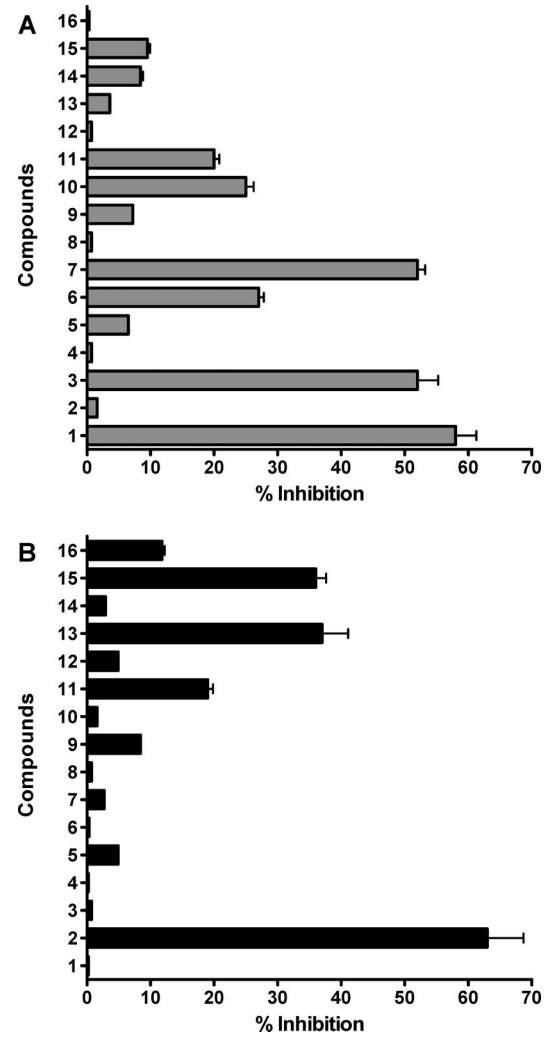


Figure 3. Biological activity of compounds **1**−**16** at SERT and NET, respectively. % inhibition of [^{3}H]5-HT (SERT, **A**) and [^{3}H]DA (NET, **B**) uptake by 100 nM inhibitor. Bars represent mean ± SEM obtained from six independent experiments each performed in triplicate.

structural feature of the most potent compounds is the absence of a CN substitutent (R^1, Table 1), while the presence of methyl substituents in the R^2-position (Table 1) increases potency. Similarly to hSERT activity, the aromatic fluorine atom (R^3, Table 1) and the amino substitution (R^4, Table 1) are less important for hNET activity.

Conclusions

Citalopram (**1**) and talopram (**2**) are structurally related but show very different pharmacological profiles, being highly selective and potent inhibitors of SERT and NET, respectively. The two compounds share the phenyl-substituted phthalane scaffold, and the differences in chemical structure are limited to substitutions in four positions. A systematic variation of these substitutions leads to 16 analogues, which have been investigated in the present study. Nine of these compounds had been prepared before and were available in-house, one compound was synthesized according to a revised procedure, and six compounds were successfully synthesized according to a double Grignard strategy. Pharmacological characterization of the series of compounds at cloned hSERT and hNET show that two of the four positions, the cyano and dimethyl substituents, are the primary determinants for potency at either hSERT or hNET. The data demonstrates that the parent compounds citalopram

(**1**) and talopram (**2**) are the most potent and most selective for hSERT and hNET, respectively, within this series compounds. Studies aiming at understanding the molecular basis for this selectivity are under way in our laboratories.

Experimental Section

General Procedure A. Synthesis of Compounds 4, 6, and 8. Compound **26**, **27**, or **28** (1.0 equiv, approximately 0.50 g) was loaded into a MW vial and dissolved in NMP (15 mL). $NiBr_2$ (1.2 equiv) and NaCN (2.4 equiv) were added, and the reaction was conducted under MW irradiation for 0.5 h at 200 °C. EtOAc (100 mL) was added, and the mixture was washed with a mixture of brine and concentrated NaOH (5:1) (2 × 100 mL). The organic phase was dried and purified by flash chromatography (heptane to heptane/EtOAc/Et_3N 65:35:5) giving a brown crude oil.

General Procedure B. Synthesis of Compounds 5, 7, and 9. Compound **4**, **6**, or **8** (1 equiv, 0.10−2.50 g) was dissolved in 1,2-dichloroethane (10 mL) and loaded into a MW vial. Chloroethyl chloroformate (5 mL, 46 mmol) was added, and the reaction was conducted under MW irradiation for 0.5 h at 180 °C. The reaction mixture was purified by flash chromatography, flushing the column once with EtOAc giving crude yellow oil. The oil was dissolved in 99% EtOH (50 mL) and stirred for 12 h at 70 °C. The mixture was concentrated in vacuo, and EtOAc and 15% aqueous NaOH (1:1) were added. The product was extracted with EtOAc (2 × 100 mL), and the combined organic layers were concentrated in vacuo giving the crude product as an oil. The product was purified by flash chromatography (heptane/EtOAc/Et_3N 65:35:5 to EtOH/EtOAc/Et_3N 50:50:10) giving an orange oil.

{3-[1-(4-Fluoro-phenyl)-3,3-dimethyl-1,3-dihydro-isobenzofuran-1-yl]-propyl}-dimethyl-amine (12). Phthalide **21** (5 g, 31 mmol) was dissolved in dry THF (40 mL), and the mixture was cooled to 0 °C on an ice bath. Grignard reagent **23** (37 mL, 37 mmol) was added dropwise over 0.5 h subsequently allowing the reaction mixture to stir at rt for 0.5 h. Grignard reagent **25** (69 mL, 62 mmol; see Supporting Information for the preparation of this solution) was added dropwise over 0.5 h and the mixture refluxed for 1 h. The reaction mixture was quenched with 10% aqueous HCl (250 mL). EtOAc (200 mL) was added. The product was extracted using 10% aqueous HCl (2 × 100 mL). The combined aqueous layers were basified with 15% aqueous NaOH (400 mL; pH = 11), and the product was extracted with EtOAc (2 × 250 mL). The organic phases were concentrated in vacuo giving a brown oil. This material was dissolved in a mixture of 99% EtOH and 37% aqueous HCl (1:1) and then concentrated in vacuo giving the crude product as a brown oil. The product was dissolved in EtOAc, basified with 15% aqueous NaOH (pH = 11), and extracted with EtOAc (2 × 250 mL). The combined organic phases were dried and concentrated in vacuo giving a brown oil, which was dried for 12 h. The free base (2.79 g, 9 mmol) was dissolved in acetone and treated with oxalic acid (1 equiv) in acetone to precipitate the oxalate of **12** as a white solid (3.04 g, 48%). Anal. ($C_{21}H_{26}FNO \cdot C_2H_2O_4 \cdot 0.59H_2O$) C, H, N.

Compounds **1**−**3**, **10**, **11**, and **13**−**16** were available in our laboratories and have been previously described.[6–8,14,21,23,24]

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Note Added after ASAP Publication. This paper was published on April 23, 2008, with errors in Figure 1 and Table 1. The correct version was published on May 1, 2008.

Supporting Information Available: Experimental details for the synthesis of compounds **4**−**9**, **12**, **18**, and **25**−**28**, spectroscopic data, and elemental analysis results, as well as experimental procedures for the biological testing. This material is available free of charge via the Internet at http://pubs.acs.org.

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