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# Synthesis and biological activity of some known and putative duloxetine metabolites

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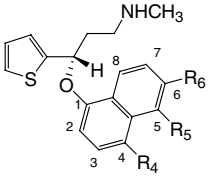
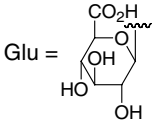
**Abstract**—Several putative phase I duloxetine metabolites, 4-hydroxy-, 5-hydroxy-, 6-hydroxy-, 5-hydroxy-6-methoxy-, 6-hydroxy-5-methoxy-, 5,6-dihydroxy-, and 4,6-dihydroxyduloxetine were synthesized, and their phase II metabolite as glucuronide or sulfate conjugates were also synthesized. Their in vitro binding activities were compared to that of parent compound duloxetine.


## 1. Introduction

Duloxetine HCl (**1**, (*S*)-*N*-methyl-3-(1-naphthalenyloxy)-2-thiophenepropanamine hydrochloride) is a potent and balanced dual reuptake inhibitor of norepinephrine and serotonin[1] that has been shown to be effective for the treatment of depression[2] and stress urinary incontinence.[3] Duloxetine undergoes substantial oxidative metabolism followed by conjugation. During the development of duloxetine, **1**, several of the metabolites (both primary and secondary) have been tentatively identified (Fig. 1) by LC–MS.[4] The major biotransformation pathways involved oxidation of the naphthyl ring at either 4-, 5-, or 6-positions followed by further oxidation, methylation, and/or conjugation. The proposed major metabolites found in plasma were 4-hydroxyduloxetine (**2**), 6-hydroxy-5-methoxyduloxetine (**7**), 4,6-dihydroxyduloxetine (**9**) and the sulfate conjugate of 5-hydroxy-6-methoxyduloxetine (**12**). In order to confirm the structures of these and other putative metabolites, it was necessary to prepare authentic samples of each of them for use as comparators. In the course of these studies, the metabolites were also evaluated in vitro for their ability to inhibit radioligand binding to the transporters of serotonin (5HT), norepinephrine (NE), and dopamine (DA).




**1**. $R_4 = R_5 = R_6 = H$
**2**. $R_4 = OH$; $R_5 = R_6 = H$
**3**. $R_4 = R_5 = H$; $R_6 = OH$
**4**. $R_4 = R_6 = H$; $R_5 = OH$
**5**. $R_4 = H$; $R_5 = R_6 = OH$
**6**. $R_4 = H$; $R_5 = OH$, $R_6 = OCH_3$
**7**. $R_4 = H$; $R_5 = OCH_3$; $R_6 = OH$
**8**. $C_5$, $C_6$ dihydro analog of **5**
**9**. $R_4 = R_6 = OH$; $R_5 = H$

**10**. $R_4 = OGlu$; $R_5 = R_6 = H$
**11**. $R_4 = R_5 = H$; $R_6 = OGlu$
**12**. $R_4 = H$; $R_5 = OSO_3^-$, $R_6 = OCH_3$
**13**. $R_4 = H$; $R_5 = OGlu$; $R_6 = OCH_3$
**14**. $R_4 = H$; $R_5 = OCH_3$; $R_6 = OGlu$
**15**. $R_4 = H$; $R_5 = OH$; $R_6 = OGlu$ or $R_5 = OGlu$; $R_6 = OH$
**16**. $R_4 = OGlu$; $R_5 = H$; $R_6 = OH$ or $R_4 = OH$; $R_5 = H$; $R_6 = OGlu$

**Figure 1.**

## 2. Discussion

### 2.1. Chemistry

**2.1.1. Synthesis of 1-fluoronaphthols, key intermediates for the synthesis of phase I metabolites.** Carbon-14 labeled duloxetine ($R_1 = R_2 = R_3 = H$) was synthesized previously in this laboratory.[5] The product was obtained by condensing a carbon-14 labeled thiophene side chain **17** with 1-fluoronaphthalene. It was postulated that the metabolites could be synthesized with the same manner by reaction of the substituted 1-fluoronaphthols,

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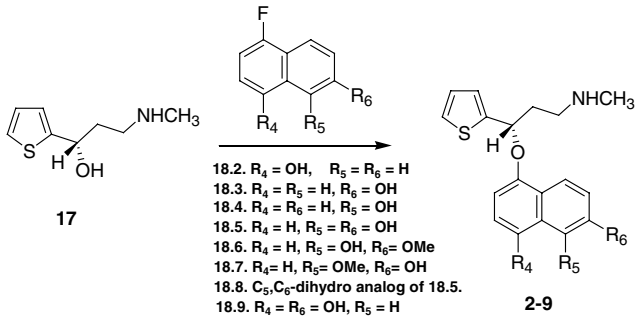
**18.2**–**18.9**, with the thiophene side chain. The initial object was to prepare these 1-fluoronapthols.

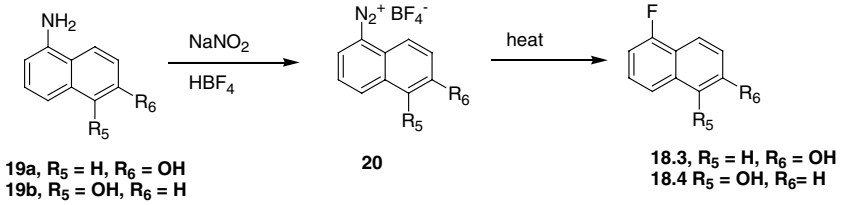



The first two compounds, 1-fluoronaphth-6-ol, **18.3**, and 1-fluoronaphth-5-ol, **18.4**, were synthesized from commercially available 1-aminonaphth-6-ol, **19a**, and 1-aminonaphth-5-ol, **19b**, using a modified Schiemann reaction.[6] The diazonium tetrafluoroborate intermediate, **20**, was isolated and dried. The dried powder was then heated in decahydronaphthalene at 160 °C to give the desired fluoronaphthols, **18.3** and **18.4**.

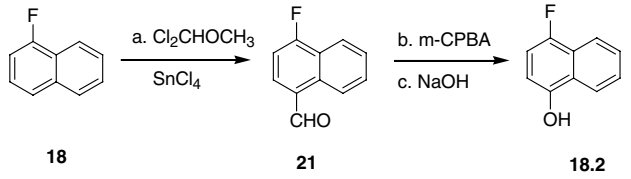
The hydroxy group on 1-fluoronaphth-6-ol, **18.3**, was protected as a methyl ether. The resulting 1-fluoro-6-methoxynaphthalene **22** was then formylated as described above. The formylation occurred exclusively at the C-5 position.[8] This aldehyde was then converted to the corresponding 1-fluoro-6-methoxynaphth-5-ol, **18.6**, by following the reactions shown in the previous reaction scheme.

Alternatively, the hydroxy group on 1-fluoronaphth-6-ol was protected as an isopropyl ether, **23**. Repeating the steps shown above in the preparation of **18.2**, yielded 1-fluoro-6-isopropyloxynaphth-5-ol (**24**). The 5-hydroxyl group was then protected as a methyl ether, and the isopropyl ether was cleaved selectively with $BCl_3$ at 0 °C to give 1-fluoro-5-methoxynaphth-6-ol, **18.7**, in good overall yield.

Treatment of 1-fluoronaphth-6-ol **18.3** with benzeneseleninic anhydride[9] in THF gave 5-fluoro-1,2-naphthoquinone **25** as a red-orange solid in 65% yield. The same naphthoquinone was also obtained by reacting with Fremy's salt, $(KSO_3)_2NO$, but in a lower yield. The

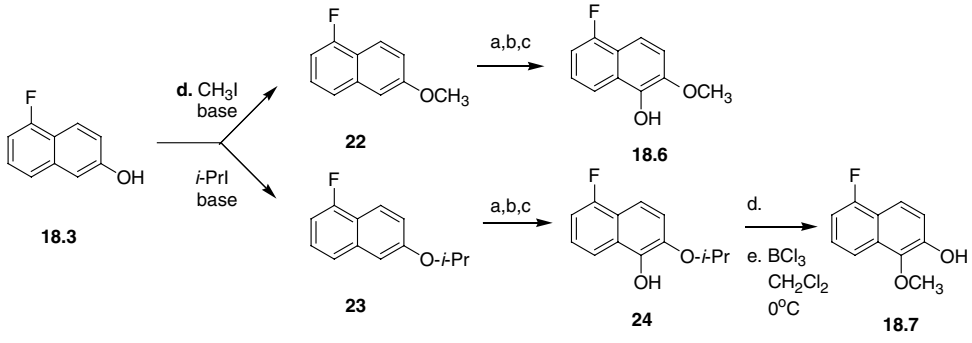


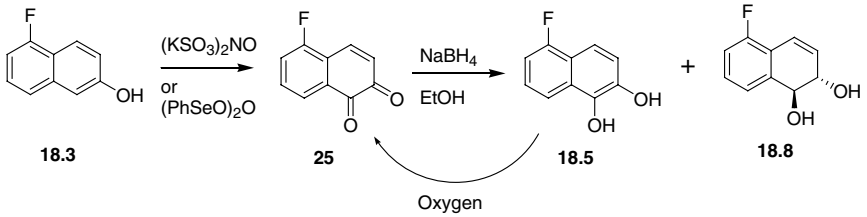

The 1-fluoronaphth-4-ol, **18.2**, was synthesized from the commercially available 1-fluoro-naphthalene, **18**. Compound **18** was formylated by stirring with 1,1-dichloromethyl methyl ether and $SnCl_4$ at 0 °C in methylene chloride to give 4-fluoro-1-naphthaldehyde, **21**.[7] Baeyer Villiger rearrangement followed by saponification of the corresponding formate gave the desired product **18.2** in good overall yield.




naphthoquinone **25** was dissolved in ethanol and stirred with sodium borohydride under an oxygen atmosphere at room temperature to give the desired product, **18.8**, in 35% isolated yield. 5,6-Dihydroxynaphthalene (**18.5**), was a by-product of the reaction, but oxidized back to naphthoquinone, which was subsequently reduced by sodium borohydride. This cycle was repeated until the naphthoquinone color was no longer detected. Demethylation of 1-fluoro-6-methoxynaphth-5-ol (**18.6**), with $BCl_3$ at room temperature yielded 1-fluoro-5,6-dihydroxynaphthalene (**18.5**).

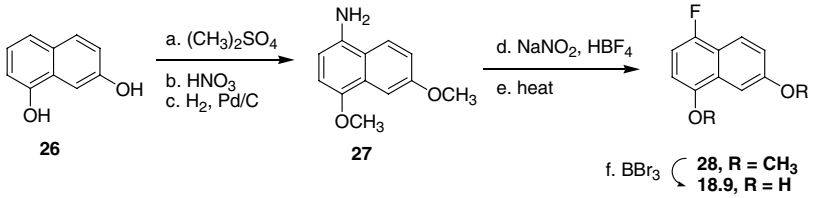
The compound 1-fluoro-4-6-dihydroxynaphthalene (**18.9**) was synthesized from the commercially available 1,7-dihydroxynaphthalene (**26**).[10] The dihydroxy groups were protected as methyl ethers by treatment with methyl sulfate in aqueous KOH. Direct nitration with






20% aqueous $HNO_3$ in benzene at room temperature gave C4 and C8-nitro isomers in 3:2 ratio as determined by proton NMR. Recrystallization from hot methanol gave pure 4,6-dimethoxy-1-nitronaphthalene. Alternatively, chromatography ($SiO_2$, 9:1 to 4:1 hexanes/methylene chloride) separated two isomers. The nitro group was reduced by hydrogenation (5% Pd/C, EtOH/THF at 60 °C), and the resulting 1-amino-4,6-dimethoxy-naphthalene (**27**) was converted to 1-fluoro-4,6-dimethoxynaphthalene (**28**) by a modified Schiemann reaction in low yield (17%). Presumably, as observed in the earlier examples, the oxygen-containing electron-rich groups on the naphthalene ring stabilized the cationic reaction center, and made it less likely to accept an incoming nucleophilic fluoride anion. Demethylation of **28** by $BBr_3$ at room temperature gave the desired 1-fluoro-4,6-dihydroxynaphthalene (**18.9**) in good yield.

Without isolation, the reaction mixture was concentrated, and the crude naphthalene ketal condensed directly with the thiophene side chain **33**. The crude reaction solution was then treated with an excess of 20% acetic acid at room temperature to cleave the ketal protective group. Chromatography gave the desired product **4**. The same process was then applied to the synthesis of other metabolites. Compounds **2**, **3**, **4**, **7**, and **9** were isolated in a reasonable yield. Low yield was obtained when **18.6** was used, possibly due to steric hindrance. When a less sterically demanding protecting group (the acetal from ethyl vinyl ether) was employed, the overall yield of **6** was greatly improved. Attempts to prepare **8** from ketal protected **18.8** gave a mixture of products in low yield. Fluoronaphthol **18.5** could not be protected as a vicinal-ketal or a vicinal-acetal due to steric hindrance. Treatment of **18.5** with trimethyl *ortho*-

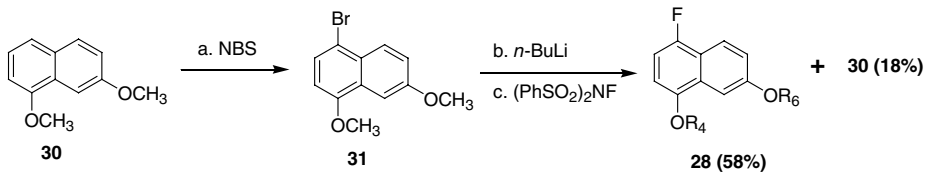



To improve the yield for the synthesis of 1-fluoro-4,6-dimethoxy-naphthalene (**28**), a new approach using an electrophilic fluorinating agent was designed. Bromination of 1,7-dimethoxynaphthalene (**30**) with NBS gave mainly 4-bromo-dimethoxynaphthalene (**31**), with a small amount of 8-bromo isomer. Bromo-lithium exchange of the crude bromide followed by electrophilic fluorination with *N*-fluorobenzenesulfonimide gave the desired product **28** in 58% yield, together with formation of **30** in 18% yield, presumably from an electron transfer mechanism.[11] Failure to degas the reaction system led to formation of a reddish orange solid, presumably the *para*-naphthoquinone.

formate gave a cyclic *ortho*-formate **35**. After condensation with **33**, the desired product was isolated as a cyclic *ortho*-formate and hydrolyzed to yield **5**, which was analyzed and identified by LC–MS.

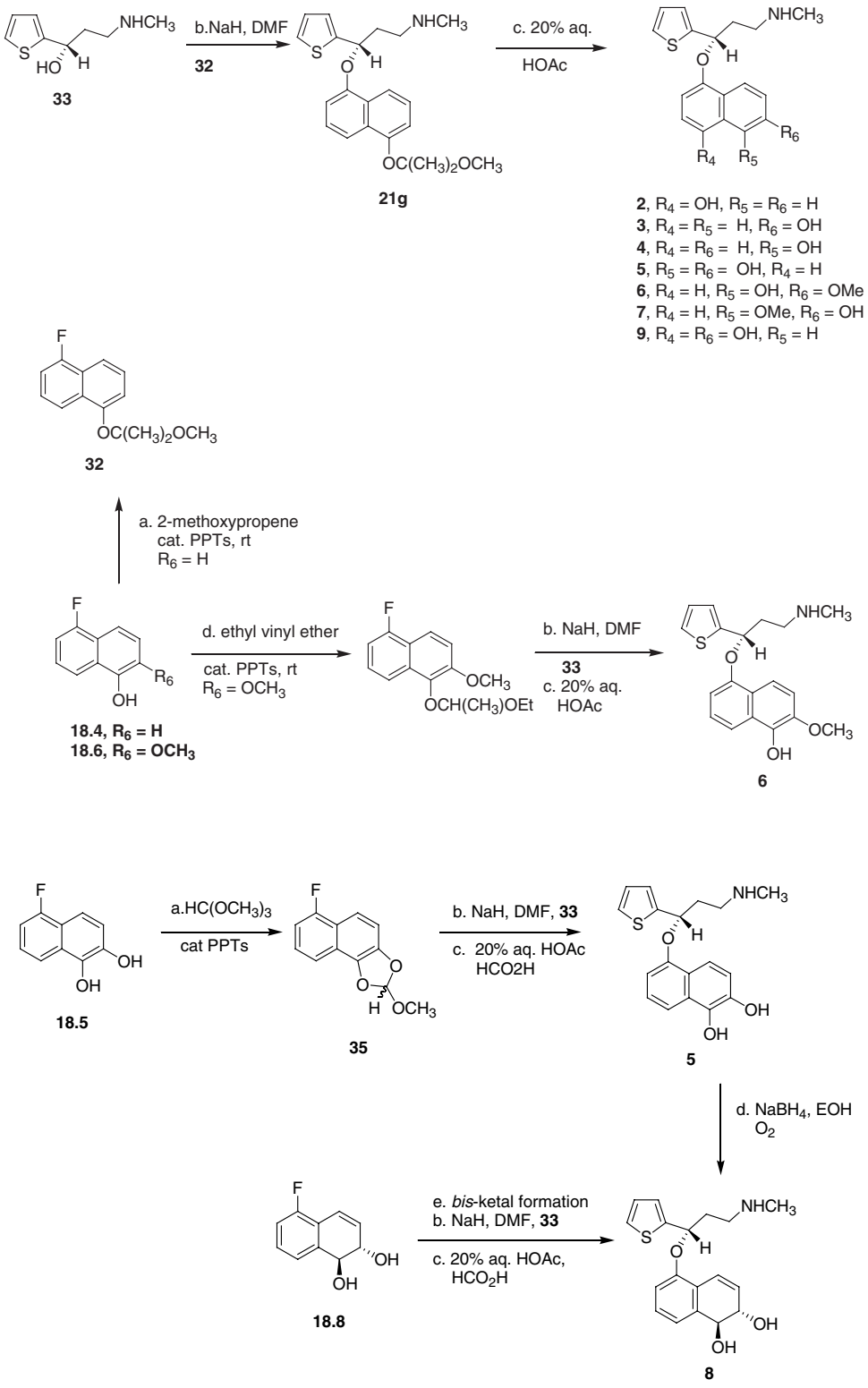
Compound **5** was then stirred under oxygen atmosphere in the presence of large excess of sodium borohydride in ethanol to yield two diastereomers of compound **8**. Attempted synthesis of **5** from **3** or **4** failed.

**2.1.3. Synthesis of phase II metabolites: glucuronide conjugates.** Treatment of **2** (4-hydroxy-duloxetine) with



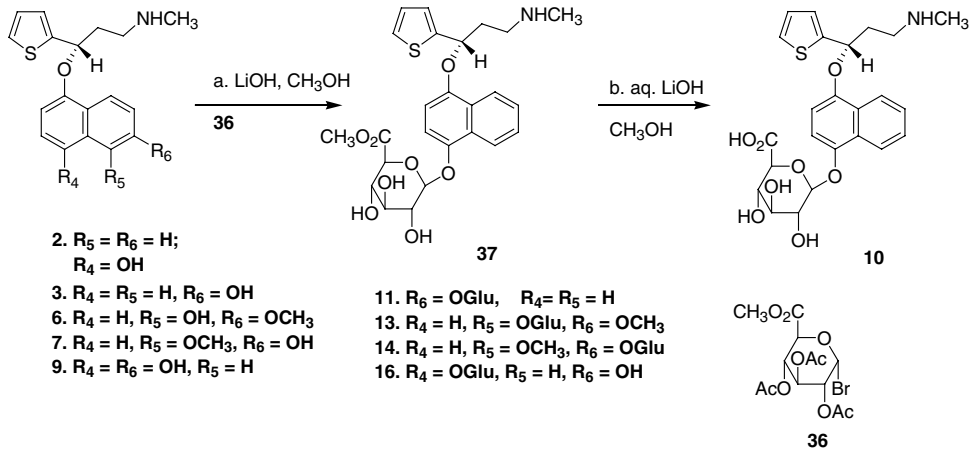

**2.1.2. Synthesis of phase I metabolites.** To synthesize the phase I metabolites by following the same manner as for duloxetine, compound **18.4** was protected as a ketal (**32**), which was partially hydrolyzed on the TLC plate.[12]

**36** and LiOH in methanol at room temperature[13] gave the conjugate **37**. Saponification with excess of LiOH in aqueous methanol gave **10**, which was identical to the metabolite as confirmed by LC–MS. Conjugates **11**, **13**,

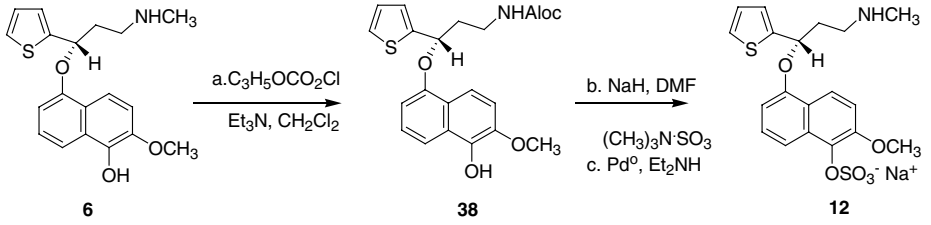



and **14** were also synthesized by this method. Glucuronide conjugate **15** was prepared from compound **5** ($R_4 = H$, $R_5 = R_6 = OH$) as above as a mixture, but neither compound matched the LC–MS profiles of the metabolite. Further evaluation of data suggested that it may be a phase II glucuronide conjugate of 4,6-dihydroxyduloxetine. 1-Fluoro-4,6-dihydroxynaphthalene **18.9** was prepared as described in Section 2.1 of the paper, and the phase I metabolite was synthesized as above using a bis-acetal protecting group. As observed for 5,6-dihydroxyduloxetine, **5**, the free base 4,6-dihydroxyduloxetine, **9**, was not very stable. To minimize problems, bioconversion of **9** to the phase II glucuronide conjugate **16** was conducted using *Actinoplanes missouriensis*. Two major products, presumably C4-OGlu and C6-OGlu, were detected by LC–MS. One of the reaction

products matched with the metabolite. The material was isolated by HPLC and its structure was confirmed by proton NMR to be the 4-OH-glucuronide conjugate **16**.




**2.1.4. Synthesis of phase II metabolites: sulfate conjugates.**[14,15] The sulfate conjugate of **12** was prepared in good yield with trimethylamine (or pyridine) sulfur trioxide complex. The synthesis was accomplished by: (a) protection of the amino group with allyl chloro formate to yield **38**, (b) conjugate formation using trimethylamine sulfur trioxide complex, (c) de-protection by hydrogenolysis with palladium reagent.




## 2.2. In vitro pharmacology

Duloxetine and its putative metabolites were evaluated for their ability to inhibit radioligand binding to the transporters of serotonin (5HT), norepinephrine (NE), and dopamine (DA) using $^3$H-paroxetine, $^3$H-nisoxetine, and $^3$H-mazindol (for duloxetine, $^3$H-WIN35428 was used), respectively, in membranes expressing the human monoamine transporters. Membranes expressing human 5HT transporters (HST), human NE transporters (HNET), and human DA transporters (HDAT) were obtained from Receptor Biology, Inc. (Beltsville, MD). Using these membranes, the following radioligand assays were performed: $^3$H-paroxetine (0.2 nM) for the HST, $^3$H-nisoxetine (1.0 nM) for the HNET, and $^3$H-mazindol (or $^3$H-WIN35,428) (1.0 nM) for the HDAT. Assays for duloxetine (for the active metabolites, duplicate assays were performed and for all others, a single assay was performed) were performed in triplicate in a final volume of 0.8 mL containing the following components: 0.2 mL drug (0.1–10,000 nM), 0.2 mL radioligand, 0.2 mL membrane suspension (10 mg/mL, HST; 17 mg/mL, HNET; 7 mg/mL HDAT), and 0.2 mL reaction media (50 mM TrisCl pH 7.4, 150 mM NaCl, 5 mM KCl for HST and HNET; 50 mM TrisCl pH 7.4,

**Table 1.** [$^3$H]-Radioligand binding to membranes expressing cloned human transporters

| Compound | $^3$H-Paroxetine 5HT, $K_i$, nM | $^3$H-Nisoxetine NE, $K_i$, nM | $^3$H-Mazindol DA, $K_i$, nM |
|---|---|---|---|
| **1** (Duloxetine) | 0.79 ± 0.039 | 7.45 ± 0.32 | 240 ± 23.1[a] |
| **2** (4-Hydroxyduloxetine) | 63.9 (57.3, 70.6) | 97.1 (88.1, 106) | 130 (150, 110) |
| **3** (6-Hydroxyduloxetine) | 1.06 (0.86, 1.25) | 4.72 (4.67, 4.77) | 163.5 (124, 203) |
| **4** (5-Hydroxyduloxetine) | 9.6 (10.1, 9.1) | 18.4 (17.9, 18.9) | 240.5 (240, 241) |
| **6** (5-Hydroxy-6-methoxyduloxetine) | 266 | 920 | 2814 |
| **7** (6-Hydroxy-5-methoxyduloxetine) | 3.66 (3.48, 3.84) | 235.5 (315, 156) | 353 (503, 203) |
| **8a** (Duloxetine-5,6-diol I) | 120 | 695 | 7275 |
| **8b** (Duloxetine-5,6-diol II) | 31.7 | 554 | >10,000 |
| **10** (4-Hydroxyduloxetine glucuronide) | >10,000 | >10,000 | 3509 |
| **11** (6-Hydroxyduloxetine glucuronide) | 1459 | 5454 | >10,000 |
| **12** (5-Hydroxy-6-methoxyduloxetine sulfate) | 3118 | >10,000 | >10,000 |
| **13** (5-Hydroxy-6-methoxyduloxetine glucuronide) | >10,000 | >10,000 | >10,000 |
| **15** (5,6-Dihydroxyduloxetine glucuronide) | >10,000 | >10,000 | >10,000 |

[a] $K_i$ value obtained with another dopamine transporter (ligand WIN35428).

100 mM NaCl for HDAT). Incubations (40 min at 37 °C for HST; 30 min at 25 °C for HNET and HDAT) were terminated by rapid vacuum filtration over Whatman GF/B filters (presoaked in 0.5% polyethylenimine) and washed four times with cold Tris–HCl pH 7.4. Non-specific binding was determined by including separate samples of 1 μM duloxetine (HST), 10 μM desipramine (HNET), and 10 μM nomifensin (HDAT).

Inhibition curves were analyzed by nonlinear least squares curve fitting to obtain $IC_{50}$ values. The $K_i$ values were calculated from $IC_{50}$ and $K_d$ values according to the method of Cheng and Prusoff.[16]

The in vitro results are tabulated in Table 1. In HST and HNET membranes, duloxetine potently inhibited the binding of $^3$H-paroxetine and $^3$H-nisoxetine with $K_i$ values of 0.79 nM and 7.45 nM, respectively. In HDAT membranes, duloxetine was a relatively weak inhibitor of $^3$H-WIN 35,428 binding, yielding a $K_i$ value of 240 nM. Compounds **3** and **4** were also potent inhibitors at HST and HNET and had low affinity for HDAT. Compound **7** inhibited $^3$H-paroxetine binding to HST with a $K_i$ value of 3.66 nM but showed low affinities for HNET and HDAT. Compound **2** had moderate to weak activity in all of the membranes. The secondary metabolites tested (**10**, **11**, **12**, **13**, **15**) were devoid of any significant binding to any of the three transporters.

## Acknowledgements

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