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# Design, Synthesis and *In Vitro* Evaluation of Novel Benzo[*b*]thiophene Derivatives as Serotonin *N*-acetyltransferase (AANAT) Inhibitors

C. MESANGEAU[a], S. YOUS[a,*], P. CHAVATTE[a], G. FERRY[b], V. AUDINOT[b], J.A. BOUTIN[b], P. DELAGRANGE[b], C. BENNEJEAN[b], P. RENARD[b] and D. LESIEUR[a]

[a]*Laboratoire de Chimie Thérapeutique, Faculté des Sciences Pharmaceutiques et Biologiques, BP 83, 59006 Lille Cedex, France;* [b]*Institut de Recherches Internationales Servier, 6 place des pléiades, 92415 Courbevoie Cedex, France*

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**Serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AANAT) is the penultimate enzyme in melatonin (5-methoxy-*N*-acetyltryptamine) biosynthesis. It is the key-enzyme responsible of the nocturnal rhythm of melatonin production in the pineal gland. Specific AANAT inhibitors could be useful for treatment of different physiopathological disorders encountered in diseases such as seasonal affective disorders or obesity. On the basis of previous works and 3D-QSAR studies carried out in our laboratory, we have synthesized and evaluated four novel benzo[b]thiophene derivatives designed as AANAT inhibitors. Compound 13 exhibited high inhibitory activity ($IC_{50} = 1.4\,\mu M$) and low affinities for both $MT_1$ (1100 nM) and $MT_2$ (1400 nM) receptors.**

*Keywords*: Benzothiophene; Arylalkylamine *N*-acetyltransferase; Serotonin *N*-acetyltransferase; Melatonin; Inhibition; QSAR

## INTRODUCTION

Melatonin (*N*-acetyl-5-methoxytryptamine) is a neurohormone principally synthesized and secreted by the pineal gland during the daily dark period. It is primarily known for its role in the control of circadian rhythms but it is also involved in a wide range of physiological or pathophysiological processes. Its effects are mediated through high-affinity receptors called $MT_1$ and $MT_2$ and through another putative binding site, termed $MT_3$. Melatonin, as a chronobiotic, might provide valuable therapy for resynchronisation of disturbed biological rhythms, such as jet-lag or sleep disorders. However, its action seems harmful for some diseases such as seasonal affective disorders or obesity.[1–3] For that reason, beside the design of melatoninergic agonists and antagonists, our laboratory has launched a program that was aimed at the inhibition of the hormone biosynthesis. Serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AANAT) is the penultimate enzyme in melatonin biosynthesis. It catalyses the reaction of serotonin with acetylcoenzyme A to produce *N*-acetylserotonin. Since AANAT is the rate-limiting step in melatonin synthesis, it is considered as a valuable target for the control of circulating melatonin levels. In previous works, Cole and coworkers[4,5] have reported the discovery of a bisubstrate analogue inhibitor that mimics the structure of the intermediate formed during the catalysis and they have concluded that *N*-bromoacetyltryptamine might react with coenzyme A and would be a precursor.[6] For that reason, we synthesised compounds that would be able to react with coenzyme A to form such an inhibitor. *N*-[(5-hydroxy-benzo-[b]thiophen-3-yl)ethyl]bromoacetamide S27481 (Figure 1) was shown to be the most potent AANAT inhibitor ($IC_{50} = 0.18\,\mu M$), but exhibited too high affinities for both $MT_1$ ($Ki = 43.5$ nM) and $MT_2$ ($Ki = 3.6$ nM) receptors for its development as a valuable drug.[7] So, our investigations turned to obtaining compounds showing an important decrease in affinity for melatonin receptors while maintaining totally or partially their AANAT inhibitory potential. On the basis of previous studies carried out in our laboratory and supported by three-dimensional

*Corresponding author. Tel./Fax: +33 320 96 43 75. E-mail: syous@phare.univ-lille2.fr

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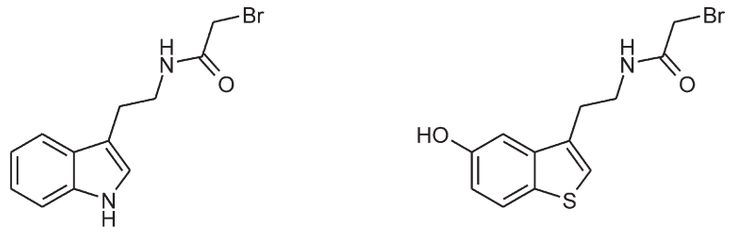
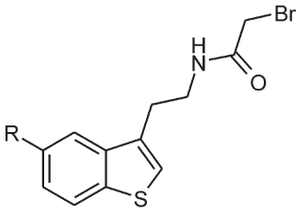



***N*-Bromoacetyltryptamine**




**S27481**




**R = $COOCH_3$, COOH, $NO_2$, $NHSO_2CH_3$**

FIGURE 1 Structure of *N*-bromoacetyltryptamine, S27481 and new benzo[*b*]thiophene derivatives.

quantitative structure-activity relationship (3D-QSAR) studies,[8] we have designed, synthesized and evaluated four novel benzo[*b*]thiophene derivatives as AANAT inhibitors.

## MATERIALS AND METHODS

### Instrumentation

Melting points were determined on a Büchi SMP-535 apparatus and are uncorrected. Column chromatography was carried out using silica gel (silica gel 60, 70–230 Mesh, ASTM, Merck) with an appropriate solvent. IR spectra were recorded on a Vector 22 Bruker spectrophotometer. $^1$H NMR spectra were recorded on a Bruker AC 300 P spectrometer. Chemical shifts are reported in δ units (ppm) relative to $(Me)_4Si$. An asterisk denotes signals of protons exchangeable in $D_2O$. Elemental analyses (C,H,N) for final compounds were performed by CNRS Laboratories (Vernaison, France) and the values were within 0.4% of the theoretical values.

### Synthesis

#### *(3-Methyl-benzo[**b**]thiophen-5-yl)carbonitrile (1)*

A mixture of 5-chloro-3-methyl-benzo[*b*]thiophene (8 g, 44 mmol), and copper cyanide (29.5 g, 330 mmol) in dimethylformamide (90 mL) was refluxed for 24 h. The reaction mixture was then poured into water (250 mL) and extracted with ethyl acetate (250 mL). After filtration through Celite, the organic layer was washed with water, dried over $MgSO_4$ and evaporated under vacuum. The residue was recrystallized from methanol to afford **1** as brown crystals (60% yield). Mp 44–45°C. $^1$H NMR (DMSO-$d_6$) δ 2.45 (s, 3H, $CH_3$), 7.63 (s, 1H, H-2), 7.74 (d, 1H, J = 8.3 Hz, H-6), 8.22 (d, 1H, J = 8.3 Hz, H-7), 8.34 (s, 1H, H-4). IR (KBr) $\nu$ 2227 $cm^{-1}$ (C≡N).

#### *(3-Methyl-benzo[**b**]thiophen-5-yl)carboxylic Acid (2)*

Compound **1** (2.6 g, 15 mmol) was dissolved in methanol (60 mL) and a solution of NaOH (6 g, 150 mmol) in water (60 mL) was added. The reaction mixture was heated under reflux for 15 h and then the methanol was evaporated. The aqueous phase was washed twice with diethyl ether and acidified to pH 1 with 3M HCl. The resulting precipitate was filtered, dried and recrystallized from toluene/cyclohexane (9/1) to afford **2** as white crystals (79% yield). Mp 215–219°C. $^1$H NMR (DMSO-$d_6$) δ 2.46 (s, 3H, $CH_3$), 7.52 (s, 1H, H-2), 7.92 (d, 1H, J = 8.3 Hz, H-6), 8.09 (d, 1H, J = 8.3 Hz, H-7), 8.34 (s, 1H, H-4), 13.03 (br s, 1H, OH). IR (KBr) $\nu$ 1676 $cm^{-1}$ (C=O).

#### *(3-Methyl-benzo[**b**]thiophen-5-yl)carboxylic Acid Methyl Ester (3)*

A mixture of **2** (7 g, 36 mmol) and potassium carbonate (15.1 g, 109 mmol) in dry acetone (100 mL) was heated at reflux for 1 h. Methyl iodide (3.2 mL, 51 mmol) was then added dropwise and the reaction mixture refluxed for 5 h. After cooling to room temperature, the solid was filtered and washed with acetone. The filtrate was evaporated to leave

a residue which was recrystallized from methanol/water (8/2) to afford **3** as brown crystals (75% yield). mp 38–39°C. $^1$H NMR (DMSO-$d_6$) δ 2.46 (s, 3H, $CH_3$), 3.90 (s, 1H, $CH_3$), 7.54 (s, 1H, H-2), 7.93 (d, 1H, J = 8.3 Hz, H-6), 8.11 (d, 1H, J = 8.3 Hz, H-7), 8.34 (s, 1H, H-4). IR (KBr) $\nu$ 1715 cm$^{-1}$ (C=O).

### *(3-Bromomethyl-benzo[**b**]thiophen-5-yl)carboxylic Acid Methyl Ester (4)*

To a solution of **3** (3.6 g, 17.5 mmol) in dry carbon tetrachloride (150 mL) was added *N*-bromosuccinimide (3.2 g, 18 mmol), and dibenzoyl peroxide (0.24 g, 1 mmol). The reaction mixture was heated under reflux for 4 h, and then immediately filtered. After evaporation *in vacuo*, the residue was triturated with petroleum ether to afford the crude product which was recrystallized from acetonitrile to afford **4** as brown crystals (55% yield). mp 99–100°C. $^1$H NMR (DMSO-$d_6$) δ 3.94 (s, 3H, $CH_3$), 5.11 (s, 2H, $CH_2$), 7.55 (s, 1H, H-2), 7.91 (d, 1H, J = 8.3 Hz, H-6), 8.10 (d, 1H, J = 8.3 Hz, H-7), 8.34 (s, 1H, H-4). IR (KBr) $\nu$ 1709 cm$^{-1}$ (C=O).

### *(3-Cyanomethyl-benzo[**b**]thiophen-5-yl)carboxylic Acid Methyl Ester (5)*

To a stirred suspension of potassium cyanide (3.1 g, 34.4 mmol) in dimethylsulfoxyde (50 mL) was added compound **4** (4.9 g, 17.2 mmol). The mixture was stirred at room temperature for 1 h and then poured into cold water. The aqueous layer was extracted twice with ethyl acetate. The organic layer was washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized from acetonitrile to afford **5** as red crystals (63% yield). mp 128–131°C. $^1$H NMR (DMSO-$d_6$) δ 3.92 (s, 3H, $CH_3$), 4.40 (s, 2H, $CH_2$), 7.89 (s, 1H, H-2), 7.99 (d, 1H, J = 8.5 Hz, H-6), 8.19 (d, 1H, J = 8.5 Hz, H-7), 8.48 (s, 1H, H-4). IR (KBr) $\nu$ 2248 (C≡N), 1707 cm$^{-1}$ (C=O).

### *[3-(2-Aminoethyl)-benzo[**b**]thiophen-5-yl] carboxylic Acid Methyl Ester Hydrochloride (6)*

Under $N_2$, a solution of **5** (0.9 g, 3.8 mmol) in anhydrous THF (30 mL) was added dropwise to 5.3 mL (5.3 mmol) of a borane-tetrahydrofuran complex (1 M solution in THF). After 2.5 h at 50°C, methanol (50 mL) was added and the contents were evaporated to dryness. The residue was taken up in 50 mL of saturated methanolic hydrochloric acid solution, and heated at 50°C for 2 h. After removal of the solvent, the crude product was dissolved in water. The aqueous layer was washed with ethyl acetate, basified to pH 8 with potassium carbonate and extracted with methylene chloride. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford an oil which was dissolved in dry ether and treated with gaseous HCl. The precipitate was filtered and recrystallized from ethyl acetate/cyclohexane (8/2) to afford **6** as white crystals (59% yield). mp > 200°C. $^1$H NMR (DMSO-$d_6$) δ 3.16–3.33 (m, 4H, $CH_2CH_2$), 3.91 (s, 3H, $CH_3$), 7.76 (s, 1H, H-2), 7.96 (d, 1H, J = 8.4 Hz, H-6), 8.17 (d, 1H, J = 8.4 Hz, H-7), 8.23 (br s, 3H, $NH_3$), 8.45 (s, 1H, H-4). IR (KBr) $\nu$ 3300–2500 ($NH_3^+$), 1713 cm$^{-1}$ (C=O).

### *[3-(2-(Bromoacetylamino)ethyl)-benzo[**b**]thiophen-5-yl]carboxylic Acid Methyl Ester (7)*

Potassium carbonate (0.66 g, 4.8 mmol) was added to a solution of **6** (0.55 g, 1.4 mmol) in water (10 mL) and ethyl acetate (30 mL). The mixture was cooled to 0°C and bromoacetyl bromide (0.2 mL, 2 mmol) was added dropwise. The reaction mixture was then stirred at room temperature for 2 h. The organic layer was separated, washed with water, dried over $MgSO_4$ and evaporated. The crude product was purified by silica gel column chromatography, with ethyl acetate/cyclohexane (5/5) as eluent and recrystallized from toluene/cyclohexane (3/1) to afford **7** as white crystals (55% yield). mp 155–158°C. $^1$H NMR (DMSO-$d_6$) δ 3.05 (m, 2H, C$H_2$CH$_2$), 3.44 (m, 2H, CH$_2$C$H_2$), 3.85 (s, 2H, $CH_2Br$), 3.91 (s, 3H, $CH_3$), 7.62 (s, 1H, H-2), 7.93 (d, 1H, J = 8.0 Hz, H-6), 8.15 (d, 1H, J = 8.6 Hz, H-7), 8.43 (s, 1H, H-4), 8.49 (br t, 1H, NH). IR (KBr) $\nu$ 1710, and 1644 cm$^{-1}$ (C=O). Found: C, 47.47; H, 4.10; N, 3.87. ($C_{14}H_{14}BrNO_3S$) requires: C, 47.20; H, 3.96; N, 3.93%.

### *[3-(2-(Bromoacetylamino)ethyl]-benzo[**b**]thiophen-5-yl]carboxylic Acid (8)*

To a solution of **7** (0.3 g, 0.84 mmol) in methylene chloride (20 mL) was added portionwise boron tribromide (0.2 mL, 2.2 mmol) at 0°C. The mixture was stirred for 1 h at room temperature and poured dropwise into a stirred solution of 5% potassium carbonate in water (80 mL). The aqueous layer was washed with ethyl acetate (50 mL) and acidified with 1M HBr. The resulting precipitate was collected by filtration and dried *in vacuo* to afford **8** as white crystals (70% yield). mp 226–230°C. $^1$H NMR (DMSO-$d_6$) δ 3.04 (t, 2H, C$H_2$CH$_2$), 3.44 (m, 2H, CH$_2$C$H_2$), 3.85 (s, 2H, $CH_2Br$), 7.60 (s, 1H, H-2), 7.92 (d, 1H, J = 8.4 Hz, H-6), 8.11 (d, 1H, J = 8.4 Hz, H-7), 8.42 (s, 1H, H-4), 8.49 (br t, 1H, NH), 13.0 (br s, 1H, OH). IR (KBr) $\nu$ 3270 (NH), 1684, and 1644 cm$^{-1}$ (C=O). Found: C, 45.70; H, 3.58; N, 4.06. ($C_{13}H_{12}BrNO_3S$) requires: C, 45.63; H, 3.53; N, 4.09%.

### *(5-Amino-benzo[**b**]thiophen-3-yl)acetonitrile Hydrochloride (9)*

A solution of (5-nitro-benzo[*b*]thiophen-3-yl)acetonitrile (1 g, 4.6 mmol) in dioxane (40 mL) containing

10% palladium on charcoal (200 mg) was hydrogenated at 1 atm for 8 h at room temperature. The mixture was then filtered through Celite and the filtrate was evaporated to dryness. The residue was dissolved in methylene chloride and hydrochloric acid gas was bubbled through it. The resulting precipitate was collected by filtration, dried and recrystallized from methanol to afford **9** as white crystals (88% yield). $^1$H NMR (DMSO-$d_6$) δ 4.32 (s, 2H, $CH_2$), 7.44 (d, 1H, J = 8.3 Hz, H-6), 7.79 (d, 1H, J = 1.7 Hz, H-4), 7.91 (s, 1H, H-2), 8.16 (d, 1H, J = 8.3 Hz, H-7), 10.48 (br s, 3H, $NH_3$). IR (KBr) $\nu$ 3000–2700 ($NH_3^+$), 2251 cm$^{-1}$ (C≡N).

### *N-(3-Cyanomethyl-benzo[**b**]thiophen-5-yl) methanesulfonamide (10)*

Methanesulfonyl chloride (0.7 mL, 9.5 mmol) was slowly added to a solution of **9** (1.3 g, 5.9 mmol) in pyridine (10 mL) at room temperature. The reaction mixture was stirred for 1 h at room temperature, poured into 6M HCl and extracted with ethyl acetate. The organic layer was separated, washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was recrystallized from acetonitrile to afford **10** as pink crystals (66% yield). mp 200–204°C. $^1$H NMR (DMSO-$d_6$) δ 3.02 (s, 3H, $CH_3SO_2$), 4.26 (s, 2H, $CH_2$), 7.31 (d, 1H, J = 8.3 Hz, H-6), 7.67 (s, 1H, H-4), 7.78 (s, 1H, H-2), 8.00 (d, 1H, J = 8.3 Hz, H-7), 9.90 (s, 1H, NH). IR (KBr) $\nu$ 3297 (NH), 2254 cm$^{-1}$ (C≡N).

### *N-[3-(2-aminoethyl)-benzo[**b**]thiophen-5-yl] methanesulfonamide Hydrochloride (11)*

A 1M solution of borane in tetrahydrofuran (12.8 mL, 12.8 mmol) was added portionwise to a solution of **10** (1.14 g, 4.3 mmol) in anhydrous tetrahydrofuran (25 mL). The mixture was refluxed for 4 h under nitrogen and then 6M HCl (15 mL) was added dropwise. Refluxing was continued for 1 h and the solvent was evaporated *in vacuo*. The crude product was recrystallized from ethyl acetate/methanol (6/4) to afford **11** as white crystals (67% yield). mp 238–240°C. $^1$H NMR (DMSO-$d_6$) δ 2.99 (s, 3H, $CH_3SO_2$), 3.10 (m, 2H, $CH_2CH_2$), 3.16 (m, 2H, $CH_2CH_2$), 7.28 (d, 1H, J = 8.7 Hz, H-6), 7.63 (s, 1H, H-4), 7.66 (s, 1H, H-2), 7.98 (d, 1H, J = 8.7 Hz, H-7), 8.08 (br s, 3H, $NH_3$), 8.23 (br s, 3H, $NH_3$), 8.45 (s, 1H, NH). IR (KBr) $\nu$ 3277 (NH), 3000–2500 cm$^{-1}$ ($NH_3^+$).

### *2-(5-Nitro-benzo[**b**]thiophen-5-yl)ethylamine Hydrochloride (12)*

The reaction was carried out from (5-nitro-benzo[*b*]thiophen-3-yl)acetonitrile as described for compound **11**. Recrystallisation from ethyl acetate/methanol (7/3) afforded **12** as yellow crystals (47% yield). mp > 240°C. $^1$H NMR (DMSO-$d_6$) δ 3.15 (m, 2H, $CH_2CH_2$), 3.27 (m, 2H, $CH_2CH_2$), 7.91 (s, 1H, H-2), 8.14 (br s, 3H, $NH_3$), 8.22 (d, 1H, J = 8.9 Hz, H-6), 8.31 (d, 1H, J = 8.9 Hz, H-7), 8.76 (d, 1H, J = 2.1 Hz, H-4). IR (KBr) $\nu$ 3200–2500 cm$^{-1}$ ($NH_3^+$).

### *N-[2-(5-Methanesulfonylamino-benzo[**b**]thiophen-3-yl]ethyl]bromoacetamide (13)*

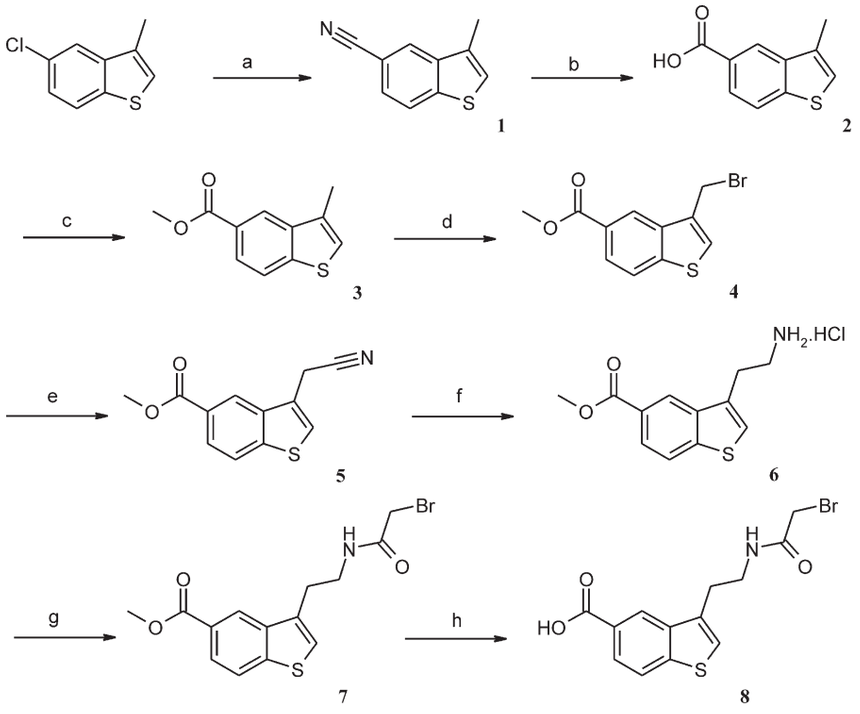
The reaction was carried out as described for compound **7**. Recrystallisation from toluene/cyclohexane (8/2) to afford **13** as white crystals (40% yield). mp 155–157°C. $^1$H NMR (DMSO-$d_6$) δ 2.92 (m, 2H, $CH_2CH_2$), 2.99 (s, 3H, $CH_3SO_2$), 3.45 (m, 2H, $CH_2CH_2$), 3.85 (s, 2H, $CH_2$Br), 7.26 (d, 1H, J = 8.6 Hz, H-6), 7.51 (s, 1H, H-2), 7.63 (d, 1H, J = 2.0 Hz, H-4), 7.94 (d, 1H, J = 8.6 Hz, H-7), 8.47 (br t, 1H, NH), 9.77 (s, 1H, NH). IR (KBr) $\nu$ 3362, and 3244 (NH), 1664 cm$^{-1}$ (C=O). Found: C, 39.78; H, 3.82; N, 7.10. ($C_{13}H_{15}BrN_2O_3S_2$) requires: C, 39.90; H, 3.86; N, 7.16%.

### *N-[2-(5-Nitro-benzo[**b**]thiophen-3-yl)ethyl] bromoacetamide (14)*

The reaction was carried out as described for compound **7**. Recrystallisation from toluene afforded **14** as yellow crystals (55% yield). mp 204–206°C. $^1$H NMR (DMSO-$d_6$) δ 3.09 (m, 2H, $CH_2CH_2$), 3.45 (m, 2H, $CH_2CH_2$), 3.84 (s, 2H, $CH_2$Br), 7.77 (s, 1H, H-2), 8.19 (d, 1H, J = 9.0 Hz, H-6), 8.28 (d, 1H, J = 9.0 Hz, H-7), 8.49 (br t, 1H, NH), 8.73 (d, 1H, J = 2.1 Hz, H-4), 8.47 (br t, 1H, NH). IR (KBr) $\nu$ 3366 (NH), 1660 cm$^{-1}$ (C=O). Found: C, 42.27; H, 3.40; N, 8.14. ($C_{12}H_{11}BrN_2O_3S$) requires: C, 42.00; H, 3.23; N, 8.16%.

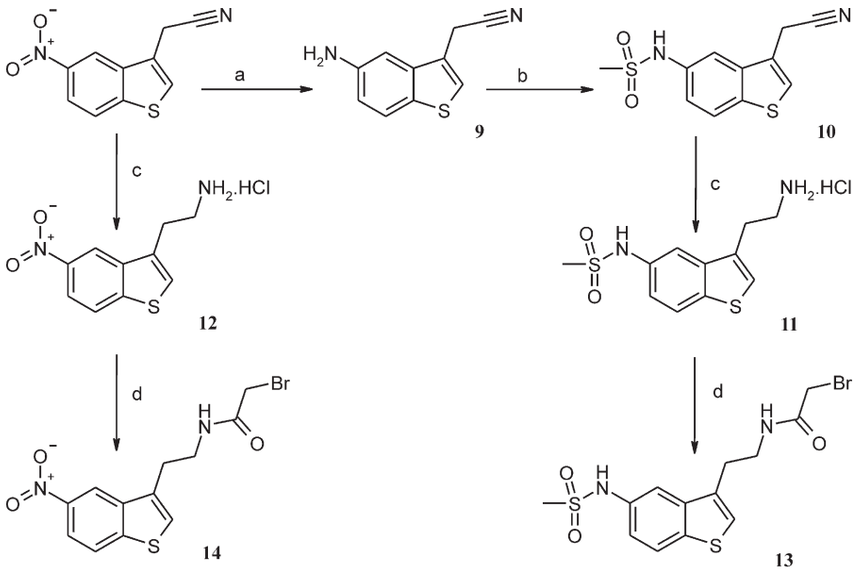
## Pharmacology

The studied compounds were evaluated in a human serotonin *N*-acetyltransferase assay as described by Ferry *et al.*[9] In brief, the reaction mixture contained 10 µl enzyme (1 µg for the human partially purified enzyme) in a phosphate buffer (50 mM sodium phosphate, pH 6.8, containing 500 mM NaCl and 2 mM EDTA), 10 µl [$^3$H]acetyl-CoA (129 GBq/mmol), 1 mM acetyl-CoA, 4 mM serotonin, in a final volume of 100 µl. After 30 min incubation at 37°C, the reaction was stopped by the addition of 50 µl of a 10% trichloroacetic acid solution. Thirty µl of this solution were analysed by reversed-phase HPLC using a Platinum EPS C8 (53 × 7 mm, Alltech, France), column on a Hewlett Packard 1100 system. The column was developed with a linear gradient of 5–35% acetonitrile in $H_2O$/0.1% TFA at a flow rate of 2 ml/min for 5 min. Radioactivity was followed on-line after addition of the scintillation cocktail (2 ml/min) using a Berthold detector and a slaved pump (EGG, Bad Wildbad, Germany).



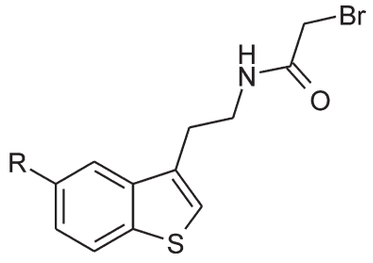
SCHEME 1 Preparation of compounds **7** and **8**. Reagents: (a) CuCN, DMF; (b) NaOH, $H_2O$, $CH_3OH$; (c) $CH_3I$, $K_2CO_3$, acetone; (d) *N*-bromosuccinimide, dibenzoyl peroxyde, $CCl_4$; (e) KCN, DMSO; (f) 1M $BH_3$-THF, anhydrous THF; (g) $BrCOCH_2Br$, $K_2CO_3$; (h) $BBr_3$, $CH_2Cl_2$.

The compounds were also tested in a binding assay using the cloned human receptors $MT_1$ and $MT_2$ as described by Nosjean *et al.*[10] In brief, membrane samples containing 40 μg of proteins diluted in binding buffer (20 mM Tris-HCl, pH 7.4 containing 5 mM $MgCl_2$) were incubated for 2 h at 37°C with 25 pM ($MT_1$) or 200 pM ($MT_2$) 2-[$^{125}$I]-melatonin in the presence (non-specific binding) or in the absence (total binding) of 1 μM melatonin and with varying concentrations of the test compounds. Incubations were carried out in triplicate in 96-well microplates and were terminated by filtration



SCHEME 2 Preparation of compounds **13** and **14**. Reagents: (a) 10% Pd/C, $H_2$, dioxane; (b) $CH_3SO_2Cl$, pyridine; (c) 1M $BH_3$-THF, anhydrous THF; (d) $BrCOCH_2Br$, $K_2CO_3$.

TABLE I Inhibition values of human arylalkylamine *N*-acetyltransferase and $MT_1$ and $MT_2$ receptor binding affinities of compounds **7**, **8**, **13**, **14**




| Compound | R | Predicted $IC_{50}$ (μM) | NAT inhibition $IC_{50}$ ± SEM (μM) | Affinities Ki ± SEM (nM) | |
|---|---|---|---|---|---|
| | | | | $MT_1$ | $MT_2$ |
| **S27481** | OH | – | 0.18 ± 0.03 | 43.5 ± 19 | 3.63 ± 0.21 |
| **7** | $COOCH_3$ | 1.9 | 2.2 ± 0.1 | 0.9 ± 0.1 | 0.6 ± 0.09 |
| **8** | COOH | 0.8 | 15%[a] | 7700 ± 420 | 1200 ± 110 |
| **13** | $NHSO_2CH_3$ | 2.4 | 1.4 ± 0.3 | 1100 ± 90 | 1400 ± 250 |
| **14** | $NO_2$ | 1.0 | 35%[a] | 34 ± 2 | 5.4 ± 0.3 |

[a] Percent activity at 10 μM.

through 96-well format glass-fiber plates (GF/B Unifilter, Packard) using a Filtermate (Packard) apparatus. Membranes were then washed three times with 2 mL of 50 mM Tris-HCl (pH 7.5) buffer before the addition of 30 μL per well of scintillation liquid (Microscint 20, Packard) and counting in a scintillation counter (TopCount NXT, Packard). Binding data were analyzed by non-linear regression using the program PRISM (Graphpad Software Inc., San Diego, CA).

## RESULTS AND DISCUSSION

### Chemistry

5-Chloro-3-methylbenzo[b]thiophene was involved in an aromatic nucleophilic substitution reaction in DMF using copper cyanide as reactant to give compound **1**. Hydrolysis of the nitrile function with sodium hydroxide afforded the corresponding acid **2** which was then converted to the corresponding ester **3** using methyl iodide and potassium carbonate as the base. Compound **3** was brominated by *N*-bromosuccinimide in the presence of benzoyl peroxyde to afford **4**. Then, **4** was reacted with potassium cyanide to give the cyanomethyl derivative **5**. The amine **6** was prepared from **5** by action of a borane-tetrahydrofuran complex in anhydrous THF (Scheme 1).

The nitro group of 5-nitro-3-cyanomethyl-benzothiophene[11] was selectively hydrogenated over 10% Pd/C to give compound **9**. Treatment of the amine **9** with methylsulfonylchloride afforded the corresponding methanesulfonylamino compound **10**. Amines **11** and **12** were prepared by reduction of compound **10** and of (5-nitro-benzo[*b*]thiophen-3-yl)acetonitrile respectively with a borane-tetrahydrofuran complex in anhydrous THF (Scheme 2).

The *N*-acetylated derivatives (**7**, **13** and **14**) were prepared from the appropriate amine hydrochlorides (**6**, **11** and **12**) by treatment with bromoacetylbromide in the presence of potassium carbonate as base, in a biphasic medium according to a variant of the Schotten-Bauman procedure (Schemes 1 & 2).

The acidic derivative **8** was obtained by hydrolysis of the corresponding ester using boron tribromide in methylene chloride (Scheme 1).

### Pharmacology

Four benzothiophenic amidic compounds **7**, **8**, **13** and **14** were evaluated as AANAT inhibitors (Table I). In a previous paper, we described a potent AANAT inhibitor ((S27481; $IC_{50} = 0.18$ μM) that exhibited nanomolar affinity values for both $MT_1$ (Ki = 43.5 nM) and $MT_2$ (Ki = 3.6 nM) receptors. Our goal was then to find a new lead compound with low affinities for $MT_1$ and $MT_2$ melatonin receptors. A three-dimensional quantitative structure-activity relationship (3D-QSAR) approach using comparative molecular field analysis (CoMFA) was recently applied to a series of 40 compounds synthesized in our laboratory and evaluated as AANAT inhibitors. The best model was used to predict inhibitory activities for a wide range of compounds. Four of them were synthesized on the basis of their potential low affinities for melatonin receptors according to

our knowledge of classical structure-affinity relationships for melatonin ligands. So, the phenolic group of *N*-[(5-hydroxy-benzo[*b*]thiophen-3-yl)ethyl] bromoacetamide was replaced by carboxylic acid methyl ester, carboxylic acid, methanesulfonylamino and nitro functions. As shown in Table I, the inhibition potency of ester **7** ($IC_{50} = 2.2\,\mu M$) is close to the predicted one ($IC_{50} = 1.9\,\mu M$) but affinity values, contrary to all expectations, were comparable to that for melatonin ($10^{-10}$ M). As expected, affinity values for compound **8** are very low (micromolar) but, unfortunately, the inhibitory potency is poor (15% at $10^{-5}$ M). Replacement of the hydroxy group by a nitro function, as for compound **14**, led to a dramatic loss in inhibitory activity while the same degree of affinity was retained. The methanesulfonylamino compound **13** appeared to be the best compromise between inhibitory activity and affinity values: it exhibited an $IC_{50}$ value of 1.4 μM, slightly lower than the predicted value and only eight times higher than the value of the reference compound; however, the affinity values for both $MT_1$ and $MT_2$ were high, reaching 1100 and 1400 nM respectively, but 25 and 385 times lower than those of S27481. Consequently, compound **13** can be considered as a promising lead for the search for valuable AANAT inhibitors.

## *References*


[1] Delagrange, P. and Guardiola-Lemaitre, B. (1997), *Clin. Neuropharmacol.* **20**, 482–510.

[2] Le Gouic, S., Delagrange, P., Atgie, C., Nibbelink, M., Hanoun, N., Casteilla, L., Renard, P., Lesieur, D., Guardiola-Lemaitre, B. and Ambid, L. (1996) *Int. J. Obes. Relat. Metab. Disord.* **20**, 661–667.

[3] Dawson, D. and Encel, N. (1993) *J. Pineal Res.* **15**, 1–12.

[4] De Angelis, J., Gastel, J., Klein, D.C. and Cole, P.A. (1998) *J. Biol. Chem.* **273**, 3045–3050.

[5] Khalil, E.M. and Cole, P.A. (1998) *J. Am. Chem. Soc.* **120**, 6195–6196.

[6] Khalil, E.M., De Angelis, J., Ishii, M. and Cole, P.A. (1999) *Proc. Natl Acad. Sci. USA* **96**, 12418–12423.

[7] Beaurain, N., Mesangeau, C., Chavatte, P., Ferry, G., Audinot, V., Boutin, J.A., Delagrange, P., Bennejean, C. and Yous, S. (2002) *J. Enz. Inhib. Med. Chem.* **17**, 409–414.

[8] Chavatte, P., Yous, S., Beaurain, N., Mésangeau, C., Ferry, G. and Lesieur, D. (2002) *Quant. Struct. Act. Relat.* **20**, 414–421.

[9] Ferry, G., Loynel, A., Kucharczyk, N., Bertin, S., Rodriguez, M., Delagrange, P., Galizzi, J.P., Jacoby, E., Volland, J.P., Lesieur, D., Renard, P., Canet, E., Fauchère, J.L. and Boutin, J.A. (2000) *J. Biol. Chem.* **275**, 8794–8805.

[10] Nosjean, O., Nicolas, J.P., Klupsch, F., Delagrange, P., Canet, E. and Boutin, J.A. (2001) *Biochem. Pharmacol.* **61**, 1369–1379.

[11] Chapman, N.B., Scrowston, R.M. and Westwood, R. (1969) *J. Chem. Soc., Perkin Trans.* **14**(1), 1855–1858.