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Design, Synthesis and *In Vitro* Evaluation of Novel Derivatives as Serotonin *N*-Acetyltransferase Inhibitors

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Serotonin *N*-acetyltransferase (arylalkylamine *N*-acetyltransferase, AANAT) is an enzyme that catalyses the first rate limiting step in the biosynthesis of melatonin (5-methoxy-*N*-acetyltryptamine). Different physiopathological disorders in human may be due to abnormal secretion of melatonin leading to an inappropriate exposure of melatonin receptors to melatonin. For that reason, we have designed, synthesized and evaluated as inhibitors of human serotonin *N*-acetyltransferase, a series of compounds that were able to react with coenzyme A to give a bisubstrate analog inhibitor. Compound 12d was found to be a potent AANAT inhibitor ($IC_{50} = 0.18\,\mu M$).

Keywords: Arylalkylamine *N*-Acetyltransferase; Melatonin; Inhibition

INTRODUCTION

Melatonin (*N*-acetyl-5-methoxytryptamine) is a neurohormone synthesized by the pineal gland during the dark period and this hormonal signal is a highly reliable measure of night time whatever the species considered. Different physiopathological disorders in humans may be due to abnormal secretion of melatonin leading to an inappropriate exposure of melatonin receptors to endogenous melatonin. This is the case for seasonal affective disorders or some cases of insomnia.[1,2] For that reasons, it seems interesting to inhibit its biosynthesis by inhibiting serotonin *N*-acetyltransferase (Arylalkylamine *N*-acetyltransferase, AANAT, EC 2.3.1.87) that is the penultimate rate-limiting step in its production in the pineal gland following a nocturnal rhythm.[3,4] This enzyme catalyzes the transfer of the acetyl group from acetylcoenzyme A (AcCoA) to serotonin, with the involvement of an intermediate ternary complex[5,6] to produce *N*-acetylserotonin. On the basis of this suggested mechanism, Khalil and Cole have first described and characterized a bisubstrate analog inhibitor that mimics the structure of the intermediate formed during catalytic reaction.[7] This discovery led to the hypothesis that *N*-bromoacetyltryptamine (**1**, Table I) might react with coenzyme A (CoASH) and the resulting bisubstrate analog would be responsible for the enzyme inhibition.[8,9] Starting from these results, we searched our laboratory compounds library for synthetic compounds including structural features that were able to react with CoASH and we screened them as potential inhibitors of AANAT. Compound **8a** was found to be an inhibitor of this enzyme ($IC_{50} = 1.5\ 10^{-6}\,M$) but exhibited high affinity (picomolar) for melatonin receptors ($K_i = 1.8\ 10^{-12}\,M$). On the basis of previous work carried out in our laboratory,[10] we designed new compounds in order to obtain a decrease in melatonin receptor affinity and an increase in the inhibitory potency. Here we describe the synthesis and pharmacological evaluation of these novel *N*-acetyltransferase inhibitors (Table I).

MATERIALS AND METHODS

Instrumentation

Melting points were determined on a Büchi SMP-535 apparatus and are uncorrected. Column

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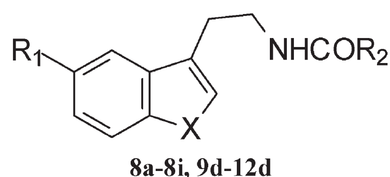
TABLE I Inhibition values of human arylalkylamine *N*-acetyltransferase (AANAT) and MT_1 and MT_2 receptor binding affinities for compounds **8a–8i, 9d–12d**



8a-8i, 9d-12d

No	R_1	X	R_2	Mp (°C)	AANAT inhibition $IC_{50} \pm$ SEM (μM)	Affinities Ki ± SEM (nM)	
						MT_1	MT_2
1	H	NH	CH_2Br	–	1.43 ± 0.70	ND	ND
8a	OCH_3	CH = CH	CH_2Br	100–101	1.5 ± 0.50	0.0018[a]	
8b	OCH_3	NH	CH_2Br	131–133	56 ± 2%[b]	0.056 ± 0.015	0.3 ± 0.05
8c	OCH_3	O	CH_2Br	87–88	40 ± 6	0.15 ± 0.02	0.34 ± 0.02
8d	OCH_3	S	CH_2Br	116–119	0.61 ± 0.08	0.27 ± 0.10	0.05 ± 0.01
9d	OCH_3	S	$(CH_2)_3Cl$	75–76	21 ± 2%[b]	0.94 ± 0.01	0.59 ± 0.01
10d	OCH_3	S	$CH = CH_2$	94–95	25 ± 2%[b]	1.6 ± 0.2	0.19 ± 0.02
11d	OCH_3	S	$CH_2CH = CH_2$	82–84	53 ± 5%[b]	0.13 ± 0.02	0.04 ± 0.01
8e	H	S	CH_2Br	75–77	0.68 ± 0.08	6.63 ± 1.35	2.54 ± 0.03
8f	Cl	S	CH_2Br	179–180	58 ± 2%[b]	0.51 ± 0.02	0.15 ± 0.04
8g	Br	S	CH_2Br	138–140	3.78 ± 0.00	1.22 ± 0.32	0.16 ± 0.01
8h	F	S	CH_2Br	98–100	0.39 ± 0.16	20.6 ± 9.0	0.36 ± 0.04
8i	C_2H_5	S	CH_2Br	77–79	0.70 ± 0.10	2.13 ± 0.37	1.04 ± 0.11
12d	OH	S	CH_2Br	127–129	0.18 ± 0.02	43.5 ± 19	3.63 ± 0.21

[a]The affinity was determined in the ovine *pars tuberalis*. [b]% Inhibition at 10^{-5} M.

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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 Brüker spectrophotometer. ^{1}H NMR spectra were recorded on a Brüker 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). The results obtained were within 0.4% of the theoretical values.

Synthesis

4-(4-Ethyl-phenysulfanyl)-3-oxo-butyric Acid Ethyl Ester (2)

4-Chloro-3-oxo-butyric acid ethyl ester (4.20 g, 0.025 mole) was slowly added to a cold stirred solution of 4-ethylthiophenol (3.20 g, 0.025 mole) and pyridine (8.0 mL, 0.1 mole) in ether (50 mL). The mixture was stirred for 2 h, then poured into ice-water and extracted with ether. The organic layer was separated, washed with 1 M HCl, then with water, dried over magnesium sulfate and evaporated under reduced pressure. The resulting oil was purified by silica gel column chromatography with diethylether/hexane/light petroleum: (2/2/1) as eluent, affording **2** as an oil (80% yield). ^{1}H-NMR (DMSO-d_6) δ 1.23 (t, 3H, J = 7.9 Hz, $CH_2C\boldsymbol{H_3}$), 1.32 (t, 3H, J = 7.3 Hz, $CO_2CH_2C\boldsymbol{H_3}$), 2.66 (q, 2H, J = 7.9 Hz, $C\boldsymbol{H_2}CH_3$), 3.71 (s, 2H, $SC\boldsymbol{H_2}$), 3.78 (s, 2H, $COC\boldsymbol{H_2}$), 4.25 (q, 2H, J = 7.3 Hz, $C\boldsymbol{H_2}CH_3$), 7.23–7.37 (m, 4H, H-2, H-3, H-5, H-6). IR (KBr) ν 1740, 1710 cm^{-1}.

(5-Ethyl-benzo[b]thiophen-3-yl)acetic Acid Ethyl Ester (3)

To a mixture of polyphosphoric acid (30 g) and celite (20 g) were added phosphorus pentoxide (0.7 g) and toluene (100 mL). The reaction mixture was stirred at 110°C for 1 h and then **2** (3.0 g, 0.012 mole) was added. Stirring was continued at this temperature for 5 h and after cooling the reaction mixture was filtered and the organic layer was washed with an aqueous solution of potassium carbonate, dried over magnesium sulfate and evaporated. The resulting oil was purified by silica gel column chromatography with diethylether/hexane/light petroleum (2/2/1) as eluent, affording **3** as an oil (60% yield). ^{1}H-NMR (DMSO-d_6) δ 1.15 (t, 3H, J = 7.6 Hz, $CH_2C\boldsymbol{H_3}$), 1.23 (t, 3H, J = 6.9 Hz, $CO_2CH_2C\boldsymbol{H_3}$), 2.74 (q, 2H, J = 7.6 Hz, $C\boldsymbol{H_2}CH_3$), 3.92 (s, 2H, $C\boldsymbol{H_2}CO$), 4.17 (q, 2H, J = 6.9 Hz, $CO_2C\boldsymbol{H_2}CH_3$), 7.23 (dd, 1H, J = 8.3 and 1.4 Hz, H-6), 7.67–7.70 (m, 2H, H-2, H-4), 7.92 (d, 1H, J = 8.3 Hz, H-7). IR (KBr) ν 1740 cm^{-1}.

(5-Ethyl-benzo[b]thiophen-3-yl)acetic Acid (4)

To a solution of **3** (3 g, 0.012 mole) in methanol (20 mL) was added a solution of sodium hydroxide (4.8 g, 0.12 mole) in water (20 mL). The reaction mixture was refluxed for 5 h and then the methanol was evaporated. The aqueous layer was extracted

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twice with ether and acidified with 6 M HCl. The resulting solid was filtered, washed with water and recrystallized from ethanol/water (1/7) affording **4** (70% yield). mp 125–127°C. ^{1}H-NMR (DMSO-d$_6$) δ 1.18 (t, 3H, J = 7.6 Hz, CH$_2$C***H***$_3$), 2.78 (q, 2H, J = 7.6 Hz, C***H***$_2$CH$_3$), 3.89 (s, 2H, C***H***$_2$COOH), 7.22 (d, 1H, J = 8.3 Hz, H-6), 7.36 (s, 1H, H-4), 7.56 (s, 1H, H-2), 7.76 (d, 1H, J = 8.3 Hz, H-7), 10.0 (br s*, 1H, OH). IR (KBr) ν 1700 cm^{-1}.

2-(5-Ethyl-benzo[b]thiophen-3-yl)acetamide (5)

To a solution of **4** (1.40 g, 0.006 mole) in chloroform (50 mL) was added SOCl$_2$ (2.8 mL, 0.024 mole). The mixture was refluxed for 2 h and then evaporated. After cooling, the resulting oil was dissolved in anhydrous ether (30 mL) and a solution of 28% ammonium hydroxide (6 mL, 0.09 mole) was added. After stirring for 30 min, the white precipitate was filtered, washed with water and recrystallized from ethanol affording **5** (65% yield). mp 201-203°C. ^{1}H-NMR (DMSO-d$_6$) 1.22 (t, 3H, J = 7.4 Hz, CH$_2$C***H***$_3$), 2.77 (q, 2H, J = 7.4 Hz, C***H***$_2$CH$_3$), 3.63 (s, 2H, C***H***$_2$CONH$_2$), 7.00 (m, 2H, N***H***$_2$), 7.19 (dd, 1H, J = 8.0 and 1.4 Hz, H-6), 7.52 (s, 1H, H-2), 7.66 (d, 1H, J = 1.4 Hz, H-4), 7.84 (d, 1H, J = 8.3 Hz, H-7). IR (KBr) ν 3340, 3160, 1650 cm^{-1}.

(5-Ethyl-benzo[b]thiophen-3-yl)acetonitrile (6)

To a solution of **5** (2.50 g, 0.011 mole) in anhydrous tetrahydrofuran (40 mL) was added triethylamine (3.50 mL, 0.025 mole). The mixture was cooled at 0°C and trifluoroacetic anhydride (1.70 mL, 0.012 mole) was added dropwise. After stirring for 1 h at room temperature, the solvent was evaporated under *vacuum* and the residue was taken up with water. The obtained solid was filtered, dried and recrystallized from ethanol–water (4/1) affording **6** (62% yield). mp 59–60°C. ^{1}H-NMR (DMSO-d$_6$) δ 1.22 (t, 3H, J = 7.5 Hz, CH$_2$C***H***$_3$), 2.81 (q, 2H, J = 7.5 Hz, C***H***$_2$CH$_3$), 4.27 (s, 2H, C***H***$_2$CN), 7.32 (m, 1H, J = 8.3and1.3 Hz, H-6), 7.72–7.79 (m, 2H, H-2, H-4), 7.98 (d, 1H, J = 8.3 Hz, H-7). IR (KBr) ν 2230 cm^{-1}.

2-(5-Ethyl-benzo[b]thiophen-3-yl)ethylamine Hydrochloride (7i)

A solution of boron-tetrahydrofuran complex (1 M in THF; 15 mL, 0.015 mol) was added portionwise to a solution of 6 (1.0 g, 0.005 mole) in anhydrous tetrahydrofuran (20 mL). The mixture was refluxed for 2 h under nitrogen and then HCl (6 M, 12 mL) was added dropwise. Refluxing was continued for 1 h and the solvent was evaporated under *vacuum*. The resulting solid was recrystallized from ethanol affording **7i** (60% yield). mp 159–161°C. ^{1}H-NMR (DMSO-d$_6$) δ 1.32 (t, 3H, J = 7.5 Hz, CH$_2$C***H***$_3$), 2.75 (q, 2H, J = 7.5 Hz, C***H***$_2$CH$_3$), 3.10–3.14 (m, 4H, C***H***$_2$C***H***$_2$), 7.22 (d, 1H, J = 8.3 Hz, H-6), 7.53 (s, 1H, H-2), 7.71 (s, 1H, H-4), 7.96 (d, 1H, J = 8.3 Hz, H-7), 8.24 (br s*, 3H, NH$_3$). IR (KBr) ν 3240–2600 cm^{-1}.

General Procedures for Preparation of N-acylated Derivatives (8a–8i and 9d–11d)

The method adopted for the synthesis *N-[2-(7-methoxy-napht-1-yl)ethyl] bromoacetamide* (**8a**) is described. Potassium carbonate (2.62 g, 0.019 mole) was added to a solution of *2-(7-methoxy-napht-1-yl) ethylamine hydrochloride* (**7a**; 1.5 g, 0.006 mole) in water (40 mL) and methylene chloride (80 mL). The mixture was cooled to 0°C and bromoacetyl bromide (0.83 mL, 0.009 mole) 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 magnesium sulfate and evaporated under *vacuum* yielding a residue that was recrystallized from toluene–cyclohexane (2/1) affording **8a** (93% yield). mp 100–101°C. ^{1}H-NMR (DMSO-d$_6$) δ 3.15 (t, 2H, C***H***$_2$CH$_2$), 3.41 (m, 2H, CH$_2$C***H***$_2$), 3.88 (s, 2H, CH$_2$Br), 3.95 (s, 3H, OCH$_3$), 7.18 (d, 1H, J = 8.8 Hz, H-6), 7.35 (m, 2H, H-2, H-3), 7.57 (s, 1H, H-8), 7.74 (d, 1H, J = 7.8 Hz, H-7), 7.83 (d, 1H, J$_{5-6}$ = 8.8 Hz, H-5), 8.54 (br t, 1H, NH). IR (KBr) ν 3303, 1645 cm^{-1}. Found: C, 55.91; H, 5.03; N, 4.35. (C$_{15}$H$_{16}$BrNO$_2$) requires: C, 55.87; H, 5.04; N, 4.37%.

N-[2-(5-Methoxy-benzo[b]thiophen-3-yl)-ethyl]vinylacetamide (11d)

Vinylacetic acid (1.05 mL, 0.0123 mole) and 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride (2.36 g, 0.0123 mole) were dissolved in anhydrous methylene chloride (40 mL). After 30 min at −10°C, a solution of 2-(5-methoxy-benzo[b]thiophen-3-yl)ethylamine (**7d**) (1.7 g, 0.082 mole) in anhydrous methylene chloride (20 mL) was added dropwise and the reaction mixture was stirred for a further 2 h at −10°C and for 1 h, at room temperature. Methylene chloride was evaporated and the residue taken up in ethyl acetate and washed with a 10% aqueous solution of potassium carbonate and water. The organic layer was dried over magnesium sulfate and evaporated under *vacuum*. The residue was recrystallized from diisopropyl ether affording **11d** (47% yield). mp 82–84°C. ^{1}H-NMR (DMSO-d$_6$) δ 2.93 (m, 4H, C***H***$_2$CH$_2$ and COC***H***$_2$), 3.39 (m, 2H, CH$_2$C***H***$_2$), 3.85 (s, 3H, OCH$_3$), 5.08 (m, 2H, C*H*), 5.90 (m, 1H, C***H***), 7.0 (d, 1H, J = 8.8 and 2.1 Hz, H-6), 7.39 (d, 1H, J = 2.1 Hz, H-4), 7.44 (s, 1H, H-2), 7.83 (d, 1H, J = 8.8 Hz, H-7), 8.05 (br t, 1H, NH). IR (KBr) ν 3270, 1633 cm^{-1}. Found: C, 65.11; H, 6.08; N, 5.18. (C$_{15}$H$_{17}$NO$_2$S) requires: C, 65.43; H, 6.22; N, 5.09%.

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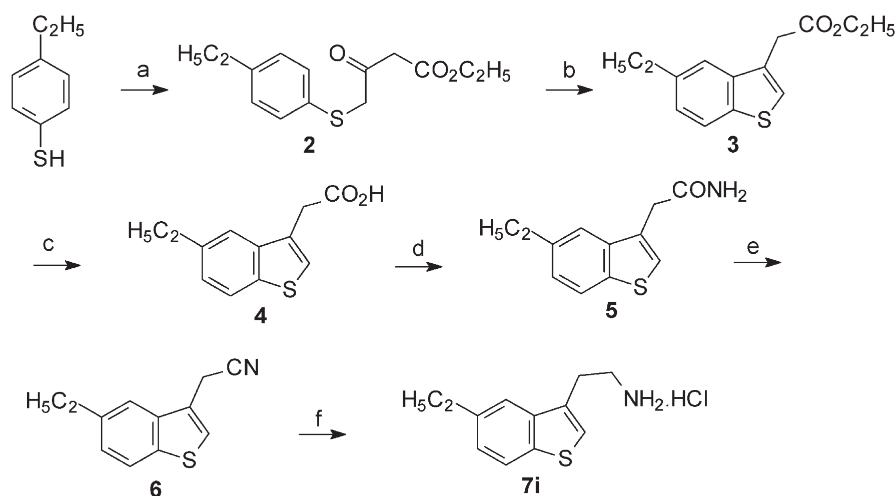

SCHEME 1 Preparation of 2-(5-ethyl-benzo[b]thiophen-3-yl)ethylamine hydrochloride **(7i)**. Reagents: (a) $ClCH_2COCH_2CO_2C_2H_5$, py; (b) polyphosphoric acid, P_2O_5; (c) NaOH, H_2O, CH_3OH; (d) i) $SOCl_2$, $CHCl_3$, ii) NH_4OH, diethyl ether; (e) $(CF_3CO)_2O$, $N(C_2H_5)_3$, anhydrous THF; (f) BH_3-THF, 1M, anhydrous THF.

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N-[2-(5-Hydroxy-benzo[b]thiophen-3-yl)ethyl] Bromoacetamide (12d)

To a solution of **8d** (1.06 g, 0.0032 mole) in methylene chloride (30 mL) was added portionwise boron tribromide (0.92 mL, 0.0097 mole) at 0°C and under a nitrogen atmosphere. The mixture was stirred for 2 h at room temperature, poured into water and extracted with methylene chloride. The organic layer was washed with water, dried over magnesium sulfate and evaporated under *vacuum*. The residue was recrystallized from toluene affording **12d** in 84% yield. mp 127–129°C. ^{1}H-NMR (DMSO-d_6) δ 3.18 (m, 2H, C$\boldsymbol{H}_2$CH$_2$), 3.73 (s, 2H, CH$_2$C$\boldsymbol{H}_2$), 4.0 (s, 2H, C$\boldsymbol{H}_2$Br), 7.06 (dd, 1H, J = 8.6and2.3 Hz, H-6), 7.39 (d, 1H, J = 2.3 Hz, H-4), 7.45 (s, 1H, H-2), 7.83 (d, 1H, J = 8.6 Hz, H-7), 9.6 (br s*, 1H, OH). IR (KBr) ν 3304, 1648 cm^{-1}. Found: C, 45.23; H, 3.86; N, 4.41. ($C_{12}H_{12}BrNO_2S$) requires: C, 45.27; H, 3.85; N, 4.46%.

Pharmacology

The compounds were evaluated in a human serotonin *N*-acetyltransferase assay as described by Ferry *et al.*[4] In brief, the reaction mixture contained 10 µl enzyme (1 µg partially purified human enzyme), in a phosphate buffer (50 mM sodium phosphate, pH6.8, containing 500 mM NaCl and 2 mM EDTA), 10 µl [^{3}H]acetyl-CoA (129GBq/mmol), 1 mM acetyl-CoA, 4 mM serotonin, in a final volume of 100 µl. After incubation of 30 min 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 analyzed by reverse-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).

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.*[11] In brief, membrane samples containing 40 µg of proteins diluted in binding buffer (20 mM TRIS–HCl, pH7.4 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 absence (total binding) of 1 µM melatonin and with varying concentrations of test compounds. Incubations were carried out in triplicate in 96-well microplates and were terminated by filtration 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 counted 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

Key intermediates in the synthesis of the target compounds described here are the corresponding amine hydrochlorides. Some of them were commercially available (**7b**, **7e**) or previously described (**7a**, **7c–d**, **7f–h**).[12–15] To obtain the amine hydrochloride

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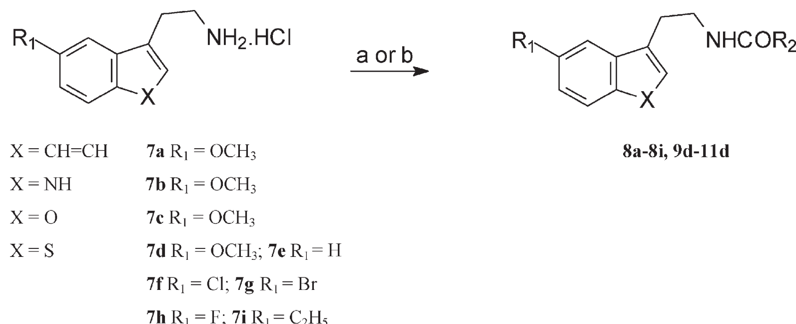


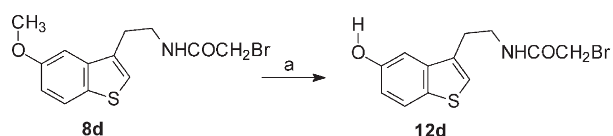
SCHEME 2 Preparation of *N*-acetylated derivatives (**8a–8i**, **9d–11d**). Reagents: (a) R_2COCl or R_2COBr, K_2CO_3, CH_2Cl_2, H_2O; (b) $CH_2 = CHCH_2CO_2H$, EDCI, CH_2Cl_2.

7i, we used the general synthetic approach described by Campaigne (Scheme 1).[14]

Condensation of 4-ethylthiophenol with ethyl-4-chloroacetoacetate in pyridine gave the ketoester **2**, which was cyclized with polyphosphoric acid to give compound **3**. Hydrolysis of the ester function with sodium hydroxide afforded the corresponding acid **4**. Treatment of this acid with thionyl chloride and further with ammonia gave the amide **5**, which was then converted to the nitrile **6** by reaction with trifluoroacetic anhydride in anhydrous THF. The amine **7i** was finally prepared by action of the boron–tetrahydrofuran complex in anhydrous THF.

The *N*-acetylated derivatives (**8a–i**, **9d–10d**) were prepared from the appropriate amine hydrochlorides (**7a–i**) by treatment with the required acid chloride or bromide in the presence of potassium carbonate as base and in a biphasic medium according to a variant of the Schotten–Bauman procedure (Scheme 2).[16]

The allyl amide **11d** was prepared using a method involving a coupling step between amine **7d** and vinylacetic acid in the presence of 1-[3-(dimethylamino)propyl]-3-ethyl carbodiimide hydrochloride. Finally, the phenolic derivative **12d** was obtained by *O*-demethylation of the amide **8d** by action of boron tribromide in dichloromethane at 0°C (Scheme 3).



SCHEME 3 Preparation of phenolic compound **12d**. Reagents: (a) BBr_3, CH_2Cl_2.

Pharmacology

A series of heterocyclic amidic compounds was synthesized and evaluated as AANAT inhibitors (Table I). A preliminary screening of our laboratory compounds library has provided compound **8a** that was found as potent as a AANAT inhibitor as compound **1** previously described by Khalil *et al*.[8] However, **8a** exhibited a very high (picomolar) affinity for melatonin receptors. We have synthesized analogues in which the naphthalene ring of **8a** was replaced by bioisosteric ones (indole, benzofurane or benzothiophene). The benzothiophenic compound **8d** appeared to be two folds more potent than **1** and **8a** but retained the same degree of MT_1 and MT_2 binding affinities. We therefore decided to choose **8d** as a new lead compound. The suggested mechanism of AANAT inhibition by compound **1** involved a reaction between the bromomethyl group and the reduced CoA, resulting in the production of a bisubstrate analog inhibitor.[8] For that reason, we have also investigated the possibility of replacing the reactive bromomethyl group of the acetamido side chain by chloropropyl (**9d**), vinyl (**10d**) or allyl (**11d**) groups that might react with CoASH. Unfortunately, these modifications led to a loss of inhibitory activity. In other respects, the methoxy group was found to be an important structural feature for the melatonin receptors affinity.[10] Consequently, in order to decrease the affinity for both MT_1 and MT_2 receptors, or to obtain MT_1 and MT_2 antagonist activity, we have replaced the methoxy group of **8d** by various substituents such as hydrogen (**8e**), chloride (**8f**), bromide (**8g**), fluoride (**8h**), ethyl (**8i**) or hydroxyl (**12d**). This pharmacomodulation resulted in an increase in the inhibitory activity for compounds (**8h**, **12d**). Compound **12d** emerged as the most favorable one since it exhibited a IC_{50} value of 0.18 μM, whereas its affinity values (Ki) are 43.5 and 3.6 nanomolar for MT_1 and MT_2, respectively.

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