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The discovery of a series of N-substituted 3-(4-piperidinyl)-1,3-benzoxazolinones and oxindoles as highly brain penetrant, selective muscarinic M_1 agonists

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ABSTRACT

A series of N-substituted 3-(4-piperidinyl)-1,3-benzoxazolinones and oxindoles are reported which were found to be potent and selective muscarinic M_1 agonists. By control of the physicochemical characteristics of the series, particularly the lipophilicity, compounds with good metabolic stability and excellent brain penetration were identified. An exemplar of the series was shown to be pro-cognitive in the novel object recognition rat model of temporal induced memory deficit.

The acetylcholine (ACh) activated muscarinic family of G-protein coupled receptors (GPCRs) are widely distributed throughout both the central and peripheral nervous systems. There are five known receptor sub-types, M_{1-5}.[1] The M_1 receptor is highly expressed in the hippocampus and cerebral cortex, areas of the brain linked with memory and learning function.[2] This makes it an attractive target for pharmaceutical intervention in diseases associated cognitive deficits, such as schizophrenia and Alzheimer's disease.[3]

The first generation of muscarinic agonists mimicked ACh and utilised the ACh binding site. Due to the high sequence homology of this orthosteric binding site, these ligands were non-selective for the different muscarinic sub-types.[4] Molecules such as xanomeline **1**[5] and sabcomeline **2**[6] (Fig. 1) did progress into the clinic; however, studies were discontinued, despite some promising signs of pharmacological action, due to intolerable dose limiting side effects.[7] These side effects are thought to be mediated primarily by the M_2 and M_3 receptor sub-types, highlighting the need for a selective ligand.

A second generation of selective M_1 ligands was heralded by the disclosure of AC-42 **3** by Acadia, which was shown to bind at an allosteric site.[8] The evolutionary pressure on the receptor away from the binding site of the endogenous ligand is much reduced, leading to a much greater heterogeneity across M_{1-5}. This offers the opportunity to achieve selectivity over the M_{2-5} sub-types. Compound **3** was soon followed by further ligands such as TBPB **4**.[9]

We have previously reported the identification of a series of 2′-biaryl amides, exemplified by compound **5**, which were identified from a high throughput screen (HTS) of the GlaxoSmithKline corporate compound collection.[10,11] A further hit identification initiative utilised virtual screening of the compound collection against a pharmacophore of AC-42 **3**. This led to the discovery of a series of benzimidazolinones which, although closely related in structure to TBPB **4**, showed diverse structure activity relationships (SAR), especially for substitution of the aromatic ring. This programme of work led to the identification of compound **6**, which demonstrated a pro-cognitive effect in the rat passive avoidance model and other models of cognition.[12] The benzimidazolinones offered excellent pharmacological and pharmacokinetic profiles, but the modest brain penetration for **6** in rat suggested the potential involvement of active efflux out of the brain (e.g., via P-glycoprotein (P-gp) transporter). Herein we report the identification of a series of oxindoles **7** and benzoxazolinones **8**, describing strategies to prepare compounds, which are selective M_1 agonists, with excellent brain penetration and without P-gp liability.

The common right hand intermediates **9a–d** were synthesised starting from either the protected cyclohexanol **10** or cyclohexa-

Abbreviations: Ach, acetylcholine; GPCRs, G-protein coupled receptors; HTS, high throughput screen; SAR, structure activity relationship; P-gp, P-glycoprotein.

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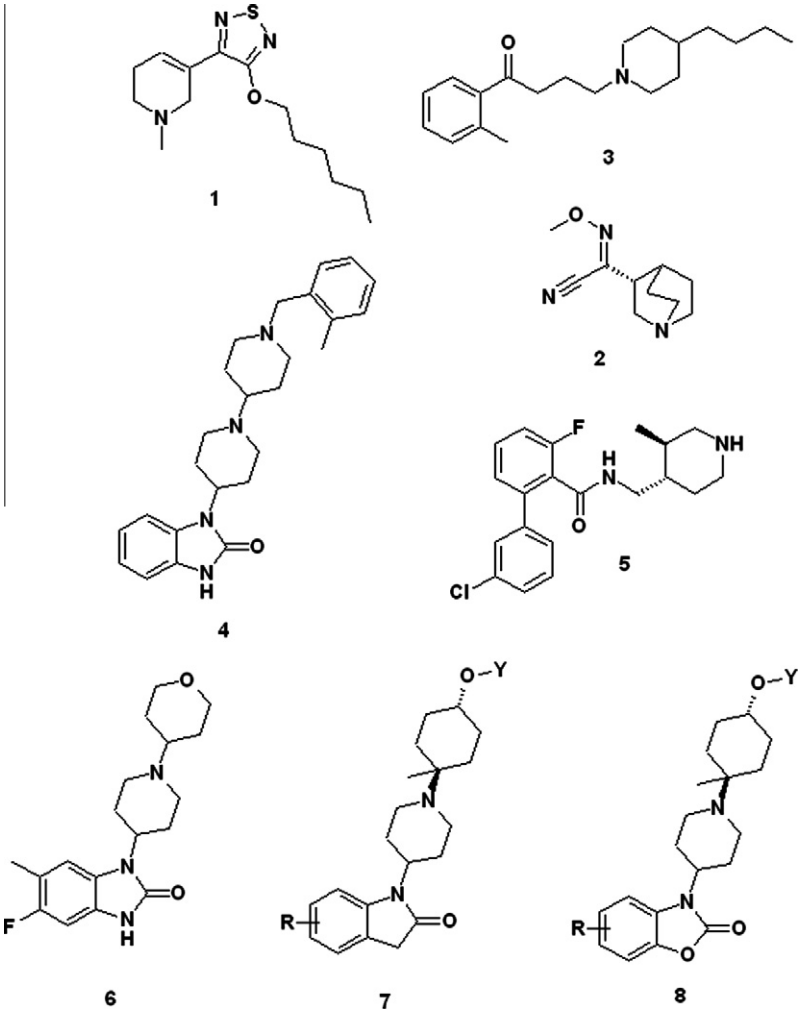


Figure 1. Muscarinic agonists.

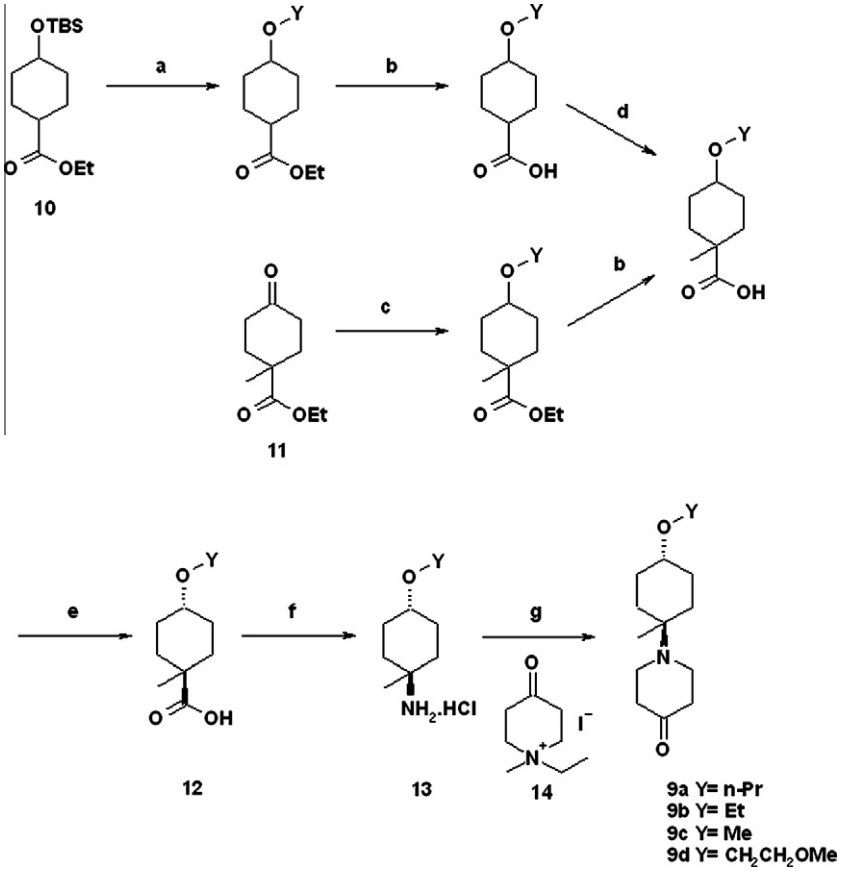
none **11** (Scheme 1). Reductive etherification, catalysed by either bismuth tribromide or iron trichloride, installed the desired capping ether, with saponification using aqueous sodium hydroxide giving the carboxylic acid. For intermediates starting from cyclohexanol **10** the quaternary methyl group was installed using lithium diisopropylamide (LDA) and methyl iodide. Separation of the *cis* and *trans* isomers was achieved using thionyl chloride.[13] The isomerically pure acid **12** was converted to amine **13** by a Curtius rearrangement using diphenyl phosphoryl azide (DPPA), followed by hydrolysis of the isocyanate using aqueous hydrochloric acid. The final piperidinone ring was formed by reaction of amine **13** with piperidinone salt **14** to give the right hand ketone **9**.

The oxindoles **7a–c** were prepared using the method described by Forbes, (Scheme 2).[14] S_NAr displacement using di-*t*-butyl malonate gave the nitro aromatic compound **15**. Reduction to the aniline **16** was followed by reductive amination with the right hand component **9** to give the substituted aniline **17**. Treatment with *para*-toluenesulfonic acid (*p*-tsa), in toluene at reflux, produced the final cyclisation and decarboxylation to give the oxindole **7**.[15]

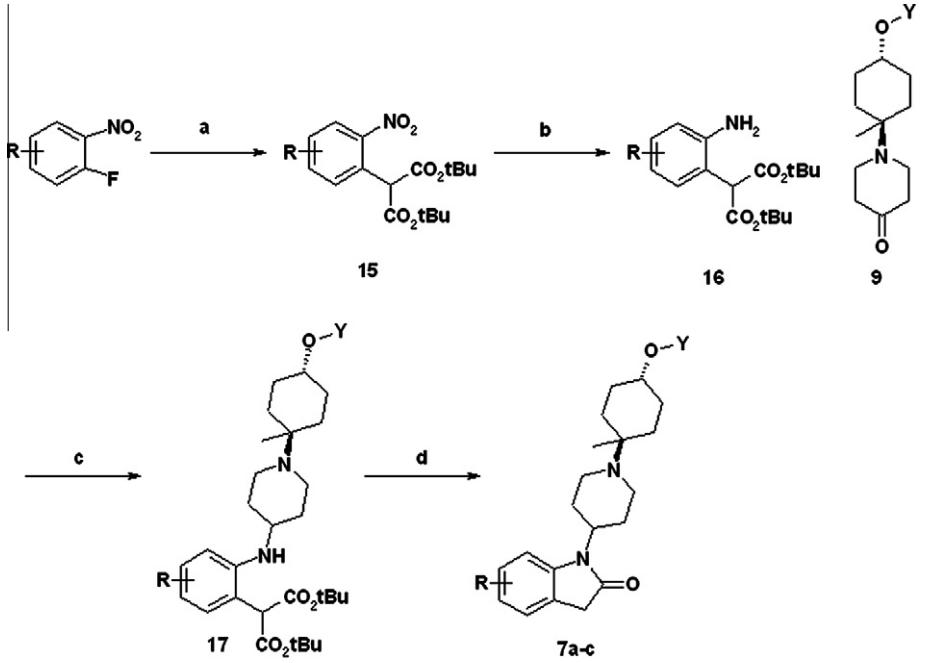
The benzoxazolinones **8a–k** were prepared by a two step procedure, starting from the substituted aminophenol **18** (Scheme 3). Reductive amination with the ketone **9**, using polymer supported cyanoborohydride, was followed by cyclisation with either triphosgene or carbodiimidazole (CDI) to give the target benzoxazolinone **8**.

We started from a series of benzimidazolinones related to **6**, with replacement of the tetrahydropyranyl (THP) capping group by alkoxy substituted cyclohexyl groups giving an improved M_1 potency (cf. **19** and **6**, Table 1).[16,17] The strategy focused on replacing the benzimidazolinone, as the presence of the hydrogen bond donating N-H group has been implicated in P-gp interaction.[18] Oxindoles **7a–c** were prepared and **7a** and **7b** maintained an excellent potency for the M_1 receptor, with a pEC_{50}s of 9.3 and 9.4 (Table 1). However, the use of the methoxy group (**7c**) led to a reduction in potency of just under a log unit. Pharmacokinetic profiling of **7a** in rat revealed excellent brain penetration (Br:Bl ratio of 5.7). Furthermore, the free concentrations in the brain and blood (2.5 nM and 2.6 nM, respectively) suggested passive diffusion across the brain blood barrier with no transporter efflux (Table 2).[19] However **7a** had a high estimated blood clearance in rat of 85 mL/min/kg. It was presumed this was due to metabolic instability driven by the increased lipophilicity of **7a** compared to **6**.

Benzoxazolinone analogues **8** were investigated as an alternative to the oxindole **7**. With a simpler synthetic scheme and only a moderate reduction in potency (cf. **8a** with **7c**), the benzoxazolinone core was selected to probe the effects of aromatic substitution on potency and physicochemical properties. No substitution led to a marked reduction in M_1 potency (**8b**), while small substituents were tolerated at R^4 and R^5 (see **8c** and **8d**). The electronic nature of R^6 had little impact on muscarinic potency, with the

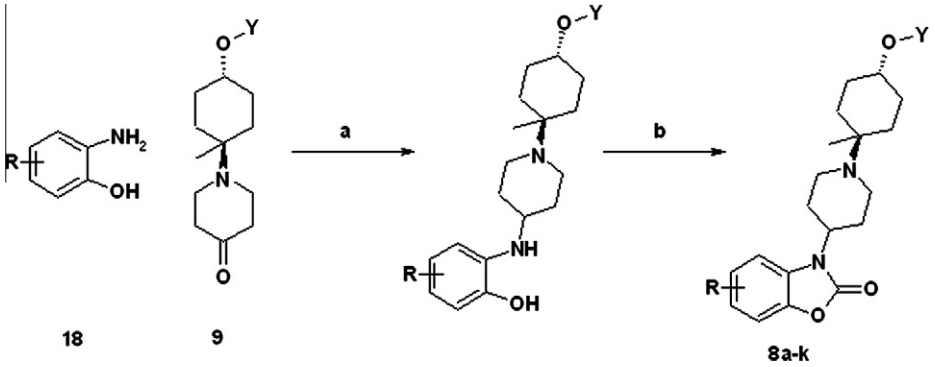


Scheme 1. Synthesis of right hand capping group **9**. Reagents and conditions: (a) Y-CHO, $BiBr_3$ or $FeCl_3$, Et_3SiH, MeCN, 87%; (b) concd NaOH, MeOH/THF, 96%; (c) Y-OTBS, $BiBr_3$ or $FeCl_3$, Et_3SiH, MeCN, 83%; (d) (i) 2 equiv LDA; (ii) MeI, 87%; (e) $SOCl_2$, PhMe, 85 °C, 34–40%; (f) (i) DPPA, Et3 N, PhMe 90 °C, 94–100%; (ii) 5 M HCl, THF, 53–100%; (g) K_2CO_3, H_2O, EtOH, 90 °C, 45–63%.



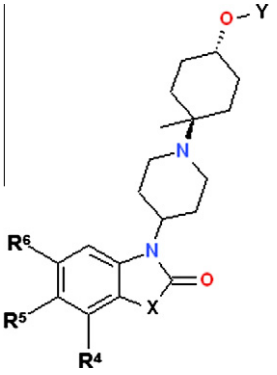
Scheme 2. Synthesis of oxindole analogues. Reagents and conditions: (a) di-*t*-butyl malonate, NaH, DMF, 29–56%; (b) H_2, 10% Pd/C, EtOH, 96–100%; (c) PS-$B(CN)H_3$, AcOH, DCM, 100 °C microwave; (d) *p*-tsa, PhMe, reflux, 62–89% over two steps.

electron donating methoxy analogue **8e** and electron withdrawing nitrile analogue **8f** having very similar M_1 potencies and selectivities. Introducing the highly electron withdrawing methyl sulfone substituent (**8g**) did lead to a reduction in M_1 potency, and the



Scheme 3. Reagents and conditions: (a) PS-B(CN)H_3, AcOH, DCM, 100 °C microwave, 29–92%; (b) triphosgene or CDI, DIPEA, DCM, 0 °C, 6–80%.

Table 1
Muscarinic agonist data



Compd	X	Y	R^6	R^5	R^4	*c* log *P*	pEC_{50}[a]				
							M_1[b]	M_2[b]	M_3[b]	M_4[b]	M_5[b]
6	NH	N/A[c]	Me	F	H	1.90	8.3	6.1	5.6	6.6	6.1
19	NH	*n*-Pr	Me	F	H	4.18	9.4	7.7	7.1	7.9	7.9
7a	CH_2	*n*-Pr	Me	H	H	3.48	9.3	7.2	7.2	7.4	7.7
7b	CH_2	Et	Me	H	H	2.95	9.4	7.0	6.9	7.5	7.7
7c	CH_2	Me	Me	H	H	2.56	8.5	6.2	6.5	6.8	6.9
8a	O	Me	Me	H	H	2.73	8.1	5.9	5.7	6.3	6.4
8b	O	Et	H	H	H	2.62	7.6	<4.8	<4.8	6.9	6.0
8c	O	Et	Me	F	H	3.26	9.0	6.3	6.1	7.0	7.3
8d	O	Et	Me	H	F	3.26	8.9	6.1	5.7	6.7	6.8
8e	O	Et	MeO	H	H	2.54	8.6	6.4	5.8	6.8	6.8
8f	O	Et	CN	H	H	2.05	8.5	5.6	5.6	6.1	6.4
8g	O	Et	$MeSO_2$	H	H	0.98	7.9	5.6	5.9	5.8	5.9
8h	O	Et	$EtSO_2$	H	H	1.51	6.7	<4.8	6.4	5.8	5.3
8i	O	*n*-Pr	$MeSO_2$	H	H	1.51	8.6	6.1	5.5	6.3	6.5
8j	O	$MeOCH_2CH_2$	Me	H	H	2.59	8.6	6.2	6.2	6.7	6.8
8k	O	$MeOCH_2CH_2$	CN	H	H	1.53	8.0	5.6	5.4	6.1	6.0

[a] Mean value of at least five test occasions.
[b] Ref. 16.
[c] THP cap.

Table 2
Pharmacokinetic data

Compd	Est. Cl_b[a] (mL/min/kg)	Br:Bl	BTB[b] (%)	WBB[b] (%)	$C_{free,\ brain}$[a] (nM)	$C_{free,\ blood}$[a] (nM)
7a	85	5.7[c]	94	80	2.5	2.6
8i	11	0.8[a]	74	60	168	378

[a] Estimated from 3 mg/kg po dose.
[b] Brain tissue binding (BTB); Whole blood binding (WBB) Ref. 20.
[c] Determined from 1 mg/kg iv dose.

use of the more lipophilic ethyl sulfone (**8h**) caused a further loss in activity at M_1, suggesting a steric limit to the binding pocket. Combining the methyl sulfone with the *n*-propoxy capping group gave compound **8i** with a *c* log *P* of 1.51 and an increased M_1 potency of 8.6. Pharmacokinetic analysis of **8i** gave a low estimated rat blood clearance of 11 mL/min/kg, demonstrating that good metabolic stability could be achieved in the series (Table 2). This encouraging result was tempered by a 2.25-fold difference between free brain concentration of **8i** compared to the free blood concentration, suggesting **8i** was a substrate for P-gp.[19]

This result led us to target further analogues with a *c* log *P* of ~1.5, replacing the sulfone with alternative substituents at R^6 and varying alkoxide Y. It was anticipated that the reduced hydrogen bond acceptor character of the molecule would prevent P-gp recognition. The combination of a nitrile substituent with the insertion of a second oxygen atom in the capping alkoxy group

Table 3
Pharmacokinetic data for **8k**

Compd	Est. Cl_b[a] (mL/min/kg)	Br:Bl[a]	BTB[b] (%)	WBB[b] (%)	$C_{free,\ brain}$[a] (nM)	$C_{free,\ blood}$[a] (nM)
8k	23	1.7	61	42	261	265

[a] Estimated from 3 mg/kg po dose.
[b] Ref. 20.

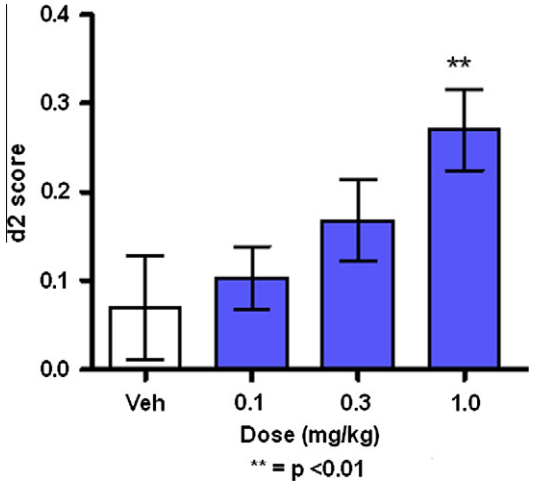


Figure 2. NOR results for **8k**.

(**8k**) showed a slightly reduced potency at M_1 of 8.0 but a *c* log *P* of 1.53. The pharmacokinetic profile in rat of **8k** gave a low estimated blood clearance (23 mL/min/kg) and the free concentrations in the brain (261 nM) and blood (265 nM) were almost exactly the same (Table 3). This gave evidence that **8k** was not a substrate for transporters in the blood brain barrier.

Modulation of global physicochemical properties of the molecule had furnished a compound with the desired potency, metabolic stability and brain penetration. Compound **8k** was therefore selected for further profiling in the CEREP high-throughput panel, only showing significant activity at the sigma-receptor (75% inhibition of control specific binding at 10 μM).[21] With its selective pharmacology demonstrated **8k** was advanced into an in vivo cognition model. The novel objection recognition model of temporal induced memory deficit in rat was selected and **8k** was dosed from 0.1–1 mg/kg (Fig. 2).[22] It demonstrated a robust improvement in the memory of rats in the 1 mg/kg dose group compared to vehicle, as well producing a dose related increase across the dosing groups. This showed **8k** to have a pro-cognitive effect in pre-clinical species.

In conclusion, we have identified a series of N-substituted 3-(4-piperidinyl)-benzoxazolinones and oxindoles as highly brain penetrant, selective muscarinic M_1 agonists. By modulation of the physicochemical properties, particularly lipophilicity, we were able to achieve good metabolic stability without compromising potency or brain penetration. This led to the discovery of compound **8k**, which was further profiled in an in vivo model for cognition, demonstrating a robust pro-cognitive effect with a minimum efficacious dose of 1 mg/kg.

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22. The novel object recognition (NOR) model was used to assess the effects on recognition memory in rats. Ennaceur and Delacour (*Behavioural Brain. Res.*, **1988**, *31*, 47) and is a non-rewarded test of spontaneous behaviour based on the differential exploration of novel and familiar objects. The NOR test comprised of two sessions, T1 and T2, each lasting 3 min. On the first test day (T1), following a 3 min habituation to the empty test box, the rat was placed into the annex and 2 identical objects were placed into the test arena. The rat was then returned to the test area and allowed to freely explore the objects for 3 min. On the second day (T2), a similar protocol was adopted, except that one of the 'familiar' objects was substituted for a novel one of the same colour, material and similar size but different shape. In order to detect a potential cognition-enhancing effect an inter-trial interval (ITI) of 24 h between T1 and T2 was used to induce a performance deficit. The objects comprised of black hardened plastic geometric shapes, such as towers and pyramids, that were approximately 6.5 cm high × 6 cm diameter. Exploration was scored as time spent sniffing or licking the objects. Sitting on or climbing over the objects was not considered to be exploratory behaviour. Between trials, objects were cleaned with 70% ethanol to remove olfactory cues. All trials were recorded via camera. Exploration was scored retrospectively by an experimenter blind to the novel object. The presentation of objects was balanced across days, trials and box position (left/right) to minimise bias. Data were expressed as a d1 index (time spent exploring novel object —time spent exp exploring familiar object) and d2 index ([time exploring novel object—time exploring familiar object]/total exploration time). T1 exploration times were also considered since hyperactivity or sedation may confound the results. The d1 and d2 index scores were analyzed using a two-way analysis of variance (ANOVA, object × treatment) followed by planned comparisons for between groups analysis using Statistica version 6 (Statsoft Inc., Tulsa). Data are expressed as mean ± SEM and were considered significant when $p < 0.05$.