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Short Communication

A Fast and Efficient Track to Allosteric Modulators of Muscarinic Receptors: Microwave-Assisted Syntheses

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Summary. By using microwave irradiation bisammonium- and bispyridinium-type allosteric modulators of muscarinic receptors can be obtained fast and efficiently.

Keywords. Medicinal chemistry; Microwave irradiation; Muscarinic receptor; Alkylation; Imide formation.

Introduction

Allosteric modulators of the muscarinic receptor are established active agents capable of selectively binding to one of the subtypes of the acetylcholine receptors M_1–M_5. They bind to a topographically different side than classical orthosteric ligands and are able to influence both the dissociation and association of orthosteric ligands such as *N*-methylscopolamine (*NMS*) [1–3]. Equilibrium binding can be either increased (positive cooperativity) or decreased (negative cooperativity) by allosteric modulators. Muscarinic allosteric modulators described so far are structurally divergent and many of them are characterized by being positively charged, *e.g.* alcuronium-like compounds [4], gallamine [5], alkylbisammonium-type derivatives such as W84 or naphmethonium [6–11] and bispyridinium-type derivatives, *e.g.* DUO 3 [12, 13] (structural formulae, see Figs. 1 and 2). Moreover, by application of *Schwyzer*'s message-address-concept the allosteric modulators can guide the way to subtype-selective agonists of the muscarinic receptors (unpublished results). Thus, for drug development a fast and efficient synthesis pathway is important.

Conventional syntheses of W84-type compounds and DUO 3 were described in literature [6–13]. The synthesis pathway to W84 derivatives (see Fig. 1) is characterized mainly by the formation of the imide by conversion of a primary amine **8** and phthalic acid anhydride **7** and the alkylation of the obtained amine with dibromoalkane and can take several days up to weeks. The same is true for the DUO-synthesis pathway consisting of the formation of the oxime ether and subsequent connection of two pyridine moieties with dibromoalkanes (see Fig. 2). Especially the alkylation step is time consuming. This stands in the way of efficient drug development demanding series of compounds of huge variety of the substitution pattern.

In the last years a growing interest in the use of microwave-assisted reactions in organic synthesis and medicinal chemistry could be observed [14]. Effects noticed with microwave dielectric heating are different from heating, *e.g.*, with an oil bath [15]: The energy is directly introduced in a reaction mixture, resulting in a different temperature profile of the reaction in comparison to conventional methods of heating and a more efficient exploitation of the irradiated energy. This often results in a shortening of

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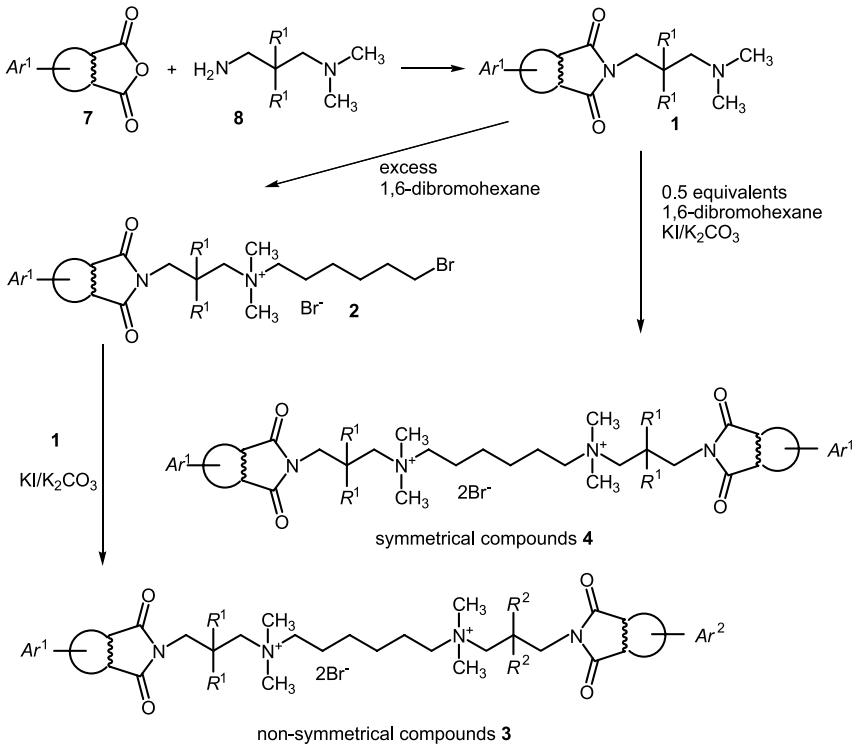

	Ar^1	Ar^2	R^1	R^2
1a	*phth*		H	
1b	*phth*		CH_3	
1c	*naphth*		CH_3	
1d	*naphth*		H	
2a	*phth*		CH_3	
2b	*naphth*		CH_3	
3a	*naphth*	*phth*	CH_3	H
3b	*phth*	*phth*	CH_3	H
4a	*phth*		H	
4b	*naphth*		H	

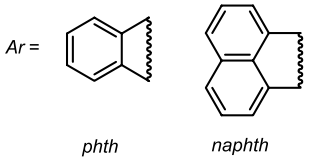

Fig. 1. Syntheses pathway of alkylbisammonium-like compounds

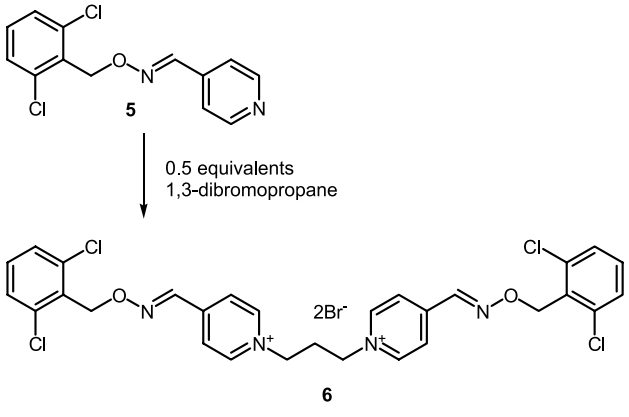

Fig. 2. Synthesis pathway of DUO 3

the reaction time, rate enhancement, better selectivity, and reduction of thermally degradative products when compared to conventional syntheses.

Recently, microwave irradiation has been utilized for forming phthalimides and bisphthalimides [16–18, commercial microwave ovens were used], for N-alkylation of phthalimides [19, 20] and for N-alkylation of halopyridines [21]. Furthermore *Lidström et al.* described in a comprehensive review [22] the advantages of microwave-assisted syntheses in organic chemistry by means of different kind of reactions, *e.g.* N-alkylation or condensation.

The aim of this investigation was to synthesize allosteric modulators of the alkylbisammonium-type and bispyridinium-type, to reduce the reaction time

substantially and additionally to increase the yield by using microwave irradiation instead of conventional energy sources.

Results and Discussion

Conventional Synthesis

In order to compare the conventional and the microwave syntheses corresponding reaction conditions were tried. The results of the conventional synthesis have been published already and could be verified [6, 8, 9, 11, 13].

Microwave-Assisted Synthesis

The microwave synthesis, *i.e.* the alkylation step, was executed in acetonitrile at 85°C (ramp: 20°C/min, 800 W). The reaction was monitored by TLC (silica gel, CH_3OH:0.2 M $NH_4NO_3 = 3{:}2$, $R_f = 0.3$–0.5) in order to find the earliest time point of quantitative conversion.

The comparison between conventional alkylation reactions and microwave-assisted syntheses in the synthesis pathway of hexamethonium-type and bispyridinium-type compounds is reported in Table 1. The identity of each compound was established by NMR and IR spectroscopy. The most remarkable finding is the tremendous reduction of reaction time from days to hours. Comparing the conversion times revealed the highest reduction for the reactions which took the longest under conventional reflux conditions: *e.g.* the formation of W84 precursor **1a** is four times faster in the microwave and the formation of the naphmethonium precursor **2b** which took 11 days under conventional heating was speed up by a factor of about 30. A similar observation was made for the bispyridinium compound **6**. Additionally, the yield could be increased by means of microwave-assisted synthesis, especially for **2b**.

Conclusion

Taken together, using microwave-assisted heating the synthesis of allosteric modulators **3**–**6** can be executed within a day instead of weeks, which substantially speeds up the drug development. Additionally, the application of the Milestone MLS rotaPREP allows the preparative parallel synthesis of compounds further enhancing the development of new allosteric modulators and subtype-selective agonists of muscarinic receptors.

Experimental

^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.61 MHz) spectra were recorded on a Bruker Advance 400 MHz spectrometer, equipped with XWIN-NMR software running on a Microsoft Windows PC. $CDCl_3$ was applied as solvent and the centre of the signal of $CDCl_3$ was used as an internal reference. IR spectra were obtained using a Biorad PharmalyzIR FT-IR spectrometer. Thin layer chromatography was performed using silica gel F_{254} plastic sheets. All chemicals were purchased from Lancaster (Mühlheim, Germany) and Aldrich (Steinheim, Germany) and were used without further purification.

All products are known compounds and were identified by comparing their physical and spectroscopical data with those of authentic samples [6, 8, 9, 11, 13].

Microwave reactions were carried out in a Milestone MLS-Ethos 1600 using a double tube system (*PTFE* inner tube, *PEEK* outer tube) equipped with a 20 bar excess pressure valve

Table 1. Comparison of reaction time and yield using the conventional route and microwave irradiation, respectively

Compd	$\frac{\text{time}_{con}}{\text{h}}$	$\frac{\text{time}_{micro}}{\text{h}}$	$\frac{\text{yield}_{con}}{\%}$	$\frac{\text{yield}_{micro}}{\%}$	$\frac{\text{temp}_{micro}}{°\text{C}}$	Ref.$_{con}$
1a	2	0.5	85	91	115	[11]
1b	3	1.5	63	64	115	[11]
1c	24	3	82	86	115	[6]
1d	3	2.5	88	90	115	[9]
2a	120	7	35	65	80	[11]
2b	264	8	55	70	80	[6]
3a	48	5	67	70	90	[9]
3b	48–96	7	60	70	90	[11]
4a	15	2	–[a]	97	100	[8]
4b	48	4	29	46	100	[11]
6	144	3	53	54	85	[13]

con Conventional route; *micro* microwave irradiation; [a] yield is not exactly reported [8]

procured by Mikrowellen-Laborsysteme (MWS, Leutkirch/Germany). Weflon-tablets (*PTFE* with 10% graphite) were used in apolar solvents, *e.g.* toluene.

Preparation via the Conventional Route

The compounds **1c** and **2b** were synthesized conventionally according to Ref. [6], **4a** according to Ref. [8], **1d** and **3a** according to Ref. [9], **1a**, **1b**, **2a**, **3b**, and **4b** according to Ref. [11] and compounds **5** and **6** according to Ref. [13].

Preparation via Microwave Heating

*Synthesis of the Imides **1a**, **1b**, **1c**, and **1d***

A mixture of the appropriate anhydride (50 mmol), the corresponding primary amine (50 mmol), and a catalytic amount of *p*-toluenesulfonic acid and two weflon-tablets (*PTFE* with 10% graphite) were heated at 115°C in toluene by using a water separator (ramp: 30°C/min, 800 W). When the reaction was completed the solvent was evaporated and the obtained crystals were washed several times with petroleum ether and recrystallized from methanol.

*Synthesis of the Intermediate Compounds **2a** and **2b***

The corresponding imides **1b** and **1c** (10 mmol) were dissolved in a fifteen fold excess of 1,6-dibromohexane (150 mmol) and stirred at 80°C (ramp: 20°C/min, 800 W). After cooling to room temperature the obtained precipitate was filtered off and washed with hot diethyl ether.

*Synthesis of the Non-symmetrical Compounds **3a** and **3b***

The precursors **2a** and **2b** (10 mmol), **1a** and a catalytic amount of KI/K_2CO_3 (1:1) were dissolved in 150 cm^3 acetonitrile and heated to 90°C (ramp: 20°C/min, 800 W). After cooling to room temperature the resulting precipitate was filtered off and washed with acetonitrile and *n*-pentane.

*Synthesis of the Symmetrical Compounds **4a** and **4b***

Two equivalents of the corresponding imides **1a** and **1d** (2 mmol), respectively, and a catalytic amount of KI/K_2CO_3 (1:1) were dissolved in 1 equivalent of 1,6-dibromohexane (1 mmol) and heated at 100°C (ramp: 20°C/min, 800 W). After cooling to room temperature the obtained precipitate was filtered off and washed with acetonitrile and *n*-pentane.

(E,E)-1-1′-(1,3-Propanediyl)bis[4-[[(2,6-dichlorobenzyloxy)imino] methyl]pyridinium] dibromide (**6**, $C_{29}H_{26}Br_2Cl_4N_4O_2$)

Pyridine-4-carboxaldehyde (*E*)-O-(2,6-dichlorobenzyl)-oxime **5** (4.0 g, 14.2 mmol) and 1,3-dibromopropane (1.4 g, 7.1 mmol) were dissolved in acetonitrile (70 cm^3) and heated at 85°C (ramp: 20°C/min, 800 W) for 3 h. After cooling to room temperature the crystalline solid was filtered off and recrystallized from ethanol to give 2.9 g (54%) of fine yellowish needles.

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