



## MAOS protocols for the general synthesis and lead optimization of 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazines

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### ABSTRACT

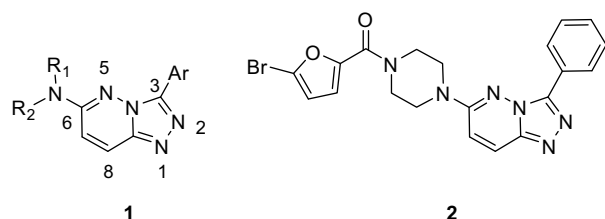
General, high-yielding MAOS protocols for the expedient synthesis of functionalized 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazines are described amenable to an iterative analog library synthesis strategy for the lead optimization of an M1 antagonist screening hit. Optimized compounds proved to be highly selective M1 antagonists.

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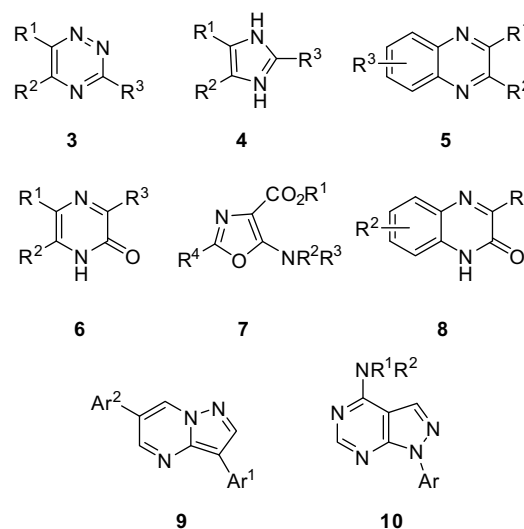
In the course of our program in small molecule probe development for the Molecular Library Screening Center Network (MLSCN),<sup>1</sup> a high-throughput screen identified the 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazine scaffold **1** as an attractive hit for a CNS target (Fig. 1). While numerous reports describe syntheses of **1**, yields are typically moderate (<50%) with prolonged reaction times at high temperatures (steps requiring 18- to >60 h at reflux).<sup>2–5</sup> In order to employ an iterative analog library synthesis approach for the lead optimization of **2**, a weak, but selective muscarinic acetylcholine receptor antagonist (M1 IC<sub>50</sub> = 22 μM, M4 IC<sub>50</sub> > 150 μM), significant refinements were required in the synthetic protocols for delivering analogs **1**, with diversity at both C3 and C6.<sup>2–5</sup>

As many of the leads identified from HTS campaigns are small heterocyclic compounds, our laboratory has devoted significant effort to develop efficient protocols for the preparation of diverse

heterocyclic templates employing microwave-assisted organic synthesis (MAOS).<sup>6–12</sup> In recent reports, we have described *general*, high-yielding MAOS protocols for the expedient synthesis of 1,2,4-triazines **3**,<sup>6</sup> imidazoles **4**,<sup>7</sup> quinoxalines **5**,<sup>8</sup> pyrazinone **6**,<sup>9</sup> 5-aminoxazoles **7**,<sup>10</sup> quinoxalinones **8**,<sup>11</sup> pyrazolo[1,5-*a*]pyrimidines **9**,<sup>12</sup> and pyrazolo[3,4-*d*]pyrimidines **10**<sup>12</sup> from simple starting materials (Fig. 2). Therefore, application of MAOS to develop a general, high-yielding, and expedient synthesis of the 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazine scaffold **1** seems warranted (see Scheme 1).



**Figure 1.** Generic structure of 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazine **1** and our M1 antagonist screening lead **2**.

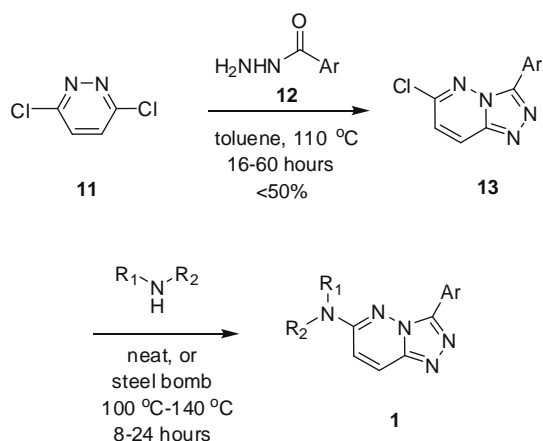


**Figure 2.** Heterocyclic templates accessed through MAOS in our laboratory.

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**Scheme 1.** Classical synthesis of 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazines **1**.

Classical conditions for the synthesis of 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazines **1** involve refluxing 3,6-dichloropyridazine **11** with an acylhydrazide **12** in toluene for 16 h, or more typically for 60 h, to provide the 3-aryl-6-chloro-[1,2,4]triazolo[4,3-*b*]pyridazine **13** in yields less than 50%.<sup>3–5</sup> Introduction of the amino moiety in the 6-position was accomplished through an  $S_NAr$  reaction employing either neat or steel bomb conditions at 100–140 °C for 8–30 h to deliver analogs **1** in yields ranging from 40% to 70%.<sup>3–6</sup> Moreover, previous efforts were focused on traditional medicinal chemistry approaches and the development of structure–activity relationships (SARs), with little concern for achieving high chemical yields or reaction generality for either the heterocycle synthesis or the  $S_NAr$  reaction. Indeed, the 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazine scaffold **1** has been an important pharmacophore for the development GABAA receptor agonists at the  $\alpha 2/\alpha 3$ -subunit.<sup>3–5</sup> Interestingly, microwave-assisted organic synthesis has never before been applied to this heterocyclic system, and even more surprising when one considers a 1–6 day reaction time to deliver a single derivative of **1**.

**Table 1**  
Optimization of MAOS conditions to produce **15**

Entry	T (°C)	Solvent	Time (min)	15:16 <sup>a</sup>
1	140	HOAc	10	42:58
2	160	HOAc	10	28:72
3	180	HOAc	10	24:76
4	200	HOAc	10	13:87
5	150	50% HOAc/EtOH	10	78:22
6	170	50% HOAc/EtOH	10	64:36
7	150	10% HOAc/EtOH	10	79:21
8	170	10% HOAc/EtOH	10	74:26
9	150	5% HOAc/EtOH	10	85:15
10	170	5% HOAc/EtOH	10	77:23
11	135	5% HOAc/EtOH	20	80:20
12	135	EtOH <sup>b</sup>	10	—
13	150	5% HCl/EtOH <sup>c</sup>	10	100:0

<sup>a</sup> Ratio determined by analytical LC–MS and <sup>1</sup>H NMR; conversion >95%.

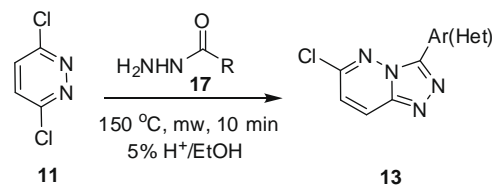
<sup>b</sup> No product formed without acid catalysis.

<sup>c</sup> 5% 4 N HCl/dioxane.

By varying solvent and temperature parameters, microwave conditions were rapidly developed to accelerate and generalize the synthesis of the 3-phenyl-6-chloro-[1,2,4]triazolo[4,3-*b*]pyridazine **15** core employing 3,6-dichloropyridazine **11** and acylhydrazide **14** (Table 1). When HOAc was employed as a solvent or catalyst, a corresponding acetylated phenyl acylhydrazide **16** was obtained in varying quantities. Optimal HOAc conditions employed 5% HOAc/EtOH at 150 °C for 10 min to afford the desired **15**, along with **16** in an 85:15 ratio (Table 1, entry 9). Despite the side product, the conversion to **15/16** was quantitative and isolated yields of **15** exceeded 82%. Application of the same MAOS conditions, but replacement of HOAc with 5% 4 N HCl in dioxane, afforded 100% conversion to **15** in 95% isolated yield without producing **16** (Table 1, entry 13). Thus, a reaction that previously required up to 60 h of conventional heating to provide <50% yield,<sup>3–5</sup> now afforded 95% yield of the desired product **15** in 10 min by virtue of MAOS—a 360-fold reduction in reaction time.<sup>13</sup>

Attention was now directed at the application of these new MAOS conditions to a diverse array of acylhydrazides to ensure that this new protocol would indeed be general. As shown in Table 2, the MAOS protocol, employing either catalytic HOAc (Method A) or HCl (Method B), proved to be general with respect to a wide range of electron-rich (entry **13g**), electron-deficient (entry **13f**), and hindered acylhydrazides (entry **13a**) **17** as well as heterocyclic

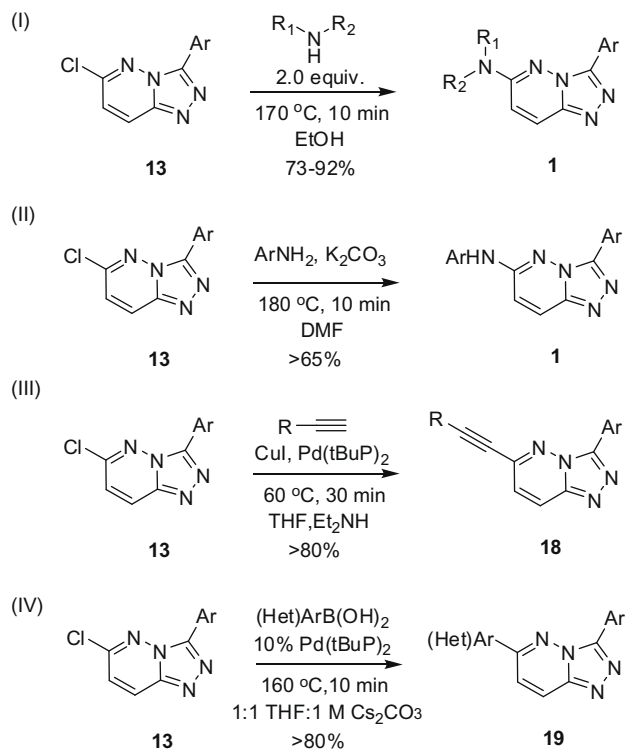
**Table 2**  
Generality of the MAOS protocol to deliver analogs **13**



Compound	R	Yield <sup>a</sup> (%)	Yield <sup>b</sup> (%)
<b>13a</b>		79	92
<b>13b</b>		77	93
<b>13c</b>		81	91
<b>13d</b>		87	96
<b>13e</b>		80	95
<b>13f</b>		75	97
<b>13g</b>		74	96
<b>13h</b>		70	88
<b>13i</b>		72	86

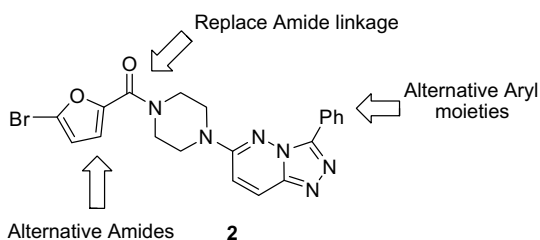
<sup>a</sup> 5% HOAc/EtOH, remaining mass balance congeners or **16**.

<sup>b</sup> 5% 4 N HCl/dioxane. All yields for isolated, analytically pure materials.

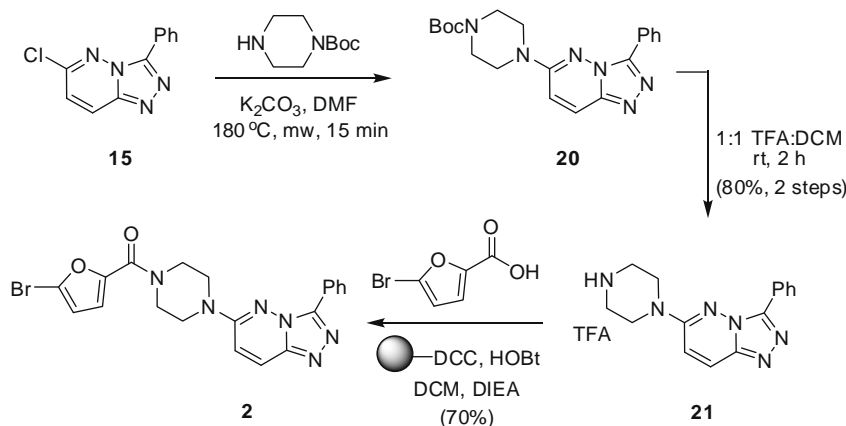


**Scheme 2.** MAOS protocols to functionalize the 3-aryl-6-chloro-[1,2,4]triazolo[4,3-*b*]pyridazine **13**.

congeners (entries **13h**, **13i**) affording the desired 3-aryl/heteroaryl-6-chloro-[1,2,4]triazolo[4,3-*b*]pyridazines **13** in isolated yields ranging from 74% to 97% in 10 min at 150 °C.



**Figure 3.** Synthetic plan to optimize **2** for M1 antagonist potency, while maintaining selectivity versus M2–M5.



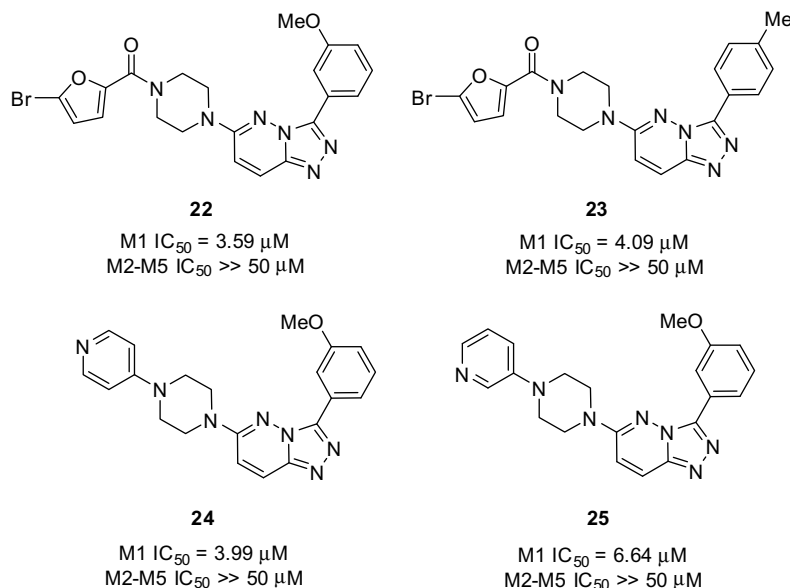
**Scheme 3.** Application of MAOS protocols for the resynthesis of the M1 antagonist HTS hit **2**.

Developing a general MAOS-mediated  $S_NAr$  protocol for the reaction of diverse amines with analogs **13** to deliver 3-aryl, 6-amino-[1,2,4]triazolo[4,3-*b*]pyridazines **1** proved more difficult. Nucleophilic amines (benzyl, aliphatic, piperidines, and piperazines) reacted smoothly in EtOH at 170 °C for 10 min to produce analogs **1** in yields ranging from 73% to 92% (Scheme 2). Less nucleophilic amines, such as anilines, required  $K_2CO_3$  in DMF with microwave irradiation for 15 min at 180 °C to produce analogs **1** in yields exceeding 65%.<sup>14</sup> Furthermore, analogs **13** readily participated in general microwave-assisted Sonogashira and Suzuki couplings to afford analogs **18** and **19** in yields exceeding 80% in every case examined (Scheme 2).

Utilizing these new MAOS protocols, we resynthesized the M1 versus M4 selective antagonist HTS hit **2** (Scheme 3). Beginning with **15**, delivered in 95% yield (Table 1), an  $S_NAr$  reaction with Boc-piperazine provided **20**, which was then deprotected using 1:1 TFA:DCM to afford **21** in 80% yield for the two steps. **21** was then acylated employing standard polymer-supported reagents and scavengers to generate the original HTS hit **2** in 70% yield.<sup>15</sup> Evaluation of **2** against M1–M5 indicated that **2** was indeed a selective M1 antagonist (M1  $IC_{50}$  = 23  $\mu$ M, M2–M5  $IC_{50}$  >> 50  $\mu$ M). Prior to this discovery there was only one other M1 selective small molecule antagonist,<sup>16</sup> and prior to its discovery, the only M1 selective antagonist was MT7, a 71 amino acid peptide toxin from the green mamba snake.<sup>17</sup> Encouraged by this result, we employed an iterative parallel synthesis approach, employing our new MAOS protocols, to rapidly develop structure–activity relationships in an attempt to improve the M1 antagonist potency while maintaining selectivity for M2–M5.

As shown in Figure 3, we simultaneously varied the substituents at the C-3 and C-6 positions, synthesizing small 12- to 24-member libraries employing the synthetic routes depicted in Schemes 2 and 3. Analogs of **2** were triaged in a single point 10  $\mu$ M screen for the compound's ability to decrease an  $EC_{80}$  concentration of acetylcholine.

SAR for this series was rather 'flat', with subtle changes leading to a complete loss of M1 inhibitory activity. Out of ~60 analogs, only four demonstrated significant M1 antagonism; however, we managed to improve upon HTS hit **2**. As shown in Figure 4, exploration of the C3 position identified both the 3-OMe phenyl derivative **22** and the 4-Me phenyl congener **23** as engendering more potency (M1  $IC_{50}$  = 3.59  $\mu$ M and 4.09  $\mu$ M, respectively), while maintaining selectivity (M2–M5  $IC_{50}$  >> 50  $\mu$ M). When holding the 3-OMe phenyl moiety constant at C3 and exploring alternatively functionalized piperazines for the bromofuranic amide at C6, we identified two piperazinyl piperazine analogs, **24** and **25**, which maintained M1 antagonism (M1  $IC_{50}$  = 3.99  $\mu$ M and 6.64  $\mu$ M, respectively) and selectivity (M2–M5  $IC_{50}$  >> 50  $\mu$ M).



**Figure 4.** Optimized analogs of **2** as highly selective M1 antagonists with improved M1 inhibitory activity as compared to HTS hit **2**.

Moreover, these latter analogs, with basic amines, afforded improved solubility and physicochemical characteristics.

In summary, we have applied MAOS to the preparation of 3,6-disubstituted-[1,2,4]triazolo[4,3-*b*]pyridazines **1**, and developed general and high-yielding protocols with over a 360-fold acceleration in reaction rate. For both the heterocyclic synthesis and the subsequent S<sub>N</sub>Ar steps, reaction time, yield, and overall reaction generality were dramatically improved under these MAOS protocols; more importantly, these new protocols allow for an iterative analog library synthesis approach for lead optimization to be employed for the rapid synthesis of large numbers of analogs of **1**. Employing these new MAOS protocols, a lead optimization campaign centered on the selective, but weak M1 antagonist hit **2** (M1 IC<sub>50</sub> = 23 μM) delivered two analogs, **22** and **24**, with over a 6-fold increase in M1 inhibitory activity (M1 IC<sub>50</sub> = 3.99 μM and 6.64 μM, respectively) while maintaining selectivity versus M2–M5 (IC<sub>50</sub> >> 50 μM). These compounds represent a novel chemotype of selective, small molecule M1 antagonists, and hold promise as leads for potential new therapeutic agents for Parkinson's Disease and dystonia.

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- Typical MAOS experimental for 6-chloro-3-*p*-tolyl-[1,2,4]triazolo[4,3-*b*]pyridazine (**13e**). (Method A) To a 5 mL microwave reaction vessel were added 3,6-dichloropyridazine (100 mg, 0.671 mmol) and *p*-toluic hydrazide (111 mg, 0.738 mmol) in a 3 mL solution of 5% AcOH/EtOH. The vial was irradiated in a microwave synthesizer at 150 °C for 10 min. LC–MS (single peak, 2.91 min, *m/e*, 245.1 (M+1)) indicated that all starting material had been consumed affording 131 mg (80%) of 6-chloro-3-*p*-tolyl-[1,2,4]triazolo[4,3-*b*]pyridazine as a white solid following column purification. (Method B) To a 5 mL microwave reaction vessel were added 3,6-dichloropyridazine (100 mg, 0.671 mmol) and *p*-toluic hydrazide (111 mg, 0.738 mmol) in a 3 mL solution of 5% 4 N HCl/EtOH. The vial was irradiated in a microwave synthesizer at 150 °C for 10 min. LC–MS (single peak, 2.91 min, *m/e*, 245.1 (M+1)) indicated that all starting material had been consumed affording 156 mg (95%) of 6-chloro-3-*p*-tolyl-[1,2,4]triazolo[4,3-*b*]pyridazine as a white solid following a silica plug and concentration in vacuo. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ (ppm): 2.41 (s, 3H), 7.43 (d, *J* = 8 Hz, 2H), 7.53 (d, *J* = 9.7 Hz, 1H), 8.19 (d, *J* = 8.2 Hz, 2H), 8.52 (d, *J* = 9.7 Hz, 1H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz) δ (ppm): 21.1, 122.5, 122.8, 127.1, 127.3, 129.5, 140.3, 143.8, 146.8, 149.1; LC–MS: single peak, 2.91 min, *m/e*, 245.1 (M+1).
- Typical MAOS experimental for *N*-(4-methoxybenzyl)-3-*p*-tolyl-[1,2,4]triazolo[4,3-*b*]pyridazin-6-amine. To a 5 mL microwave reaction vessel were added 6-chloro-3-*p*-tolyl-[1,2,4]triazolo[4,3-*b*]pyridazine (50 mg, 0.205 mmol) and 4-methoxybenzyl amine (35 μL, 6 mmol) in 3 mL of ethanol. The vial was initially heated in a microwave synthesizer to 170 °C for 25 min. Preparative LC–MS afforded 51.6 mg (73%) of *N*-(4-methoxybenzyl)-3-*p*-tolyl-[1,2,4]triazolo[4,3-*b*]pyridazin-6-amine as a white solid. <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>, 600 MHz) δ (ppm): 2.38 (s, 3H), 3.71 (s, 3H), 4.41 (d, *J* = 5.5 Hz, 2H), 6.88 (d, *J* = 9.9 Hz, 1H), 6.92 (d, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 6.7 Hz, 2H), 7.36 (d, *J* = 8.5 Hz, 2H), 7.97 (d, *J* = 9.8 Hz, 1H), 8.21 (d, *J* = 8.2 Hz, 2H). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>, 150 MHz) δ (ppm): 21.5, 44.7, 55.5, 114.2, 117.0, 124.4, 124.6, 127.0, 129.3, 129.6, 130.9, 139.4, 143.8, 146.3, 154.1, 158.8; LC–MS: single peak, 3.00 min, *m/e*, 346.2 (M+1).
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