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A Machine-Assisted Approach for the Preparation of Follow-on Pharmaceutical Compound Libraries

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Christiane Schotten, Lisa G. T. Leist, A. Lisa Semrau, Duncan. L. Browne*

Follow-on drugs represent the majority of new drug introductions and show improved properties to the pioneer drug. In order to fast-follow, R&D needs to be able to quickly generate a library of analogues that can then be screened for desired properties. Here we have designed a hybrid machine-assisted approach which makes use of both multi-step continuous flow processing and robotic microwave reactors to generate a library of indoles over two technology steps (four reaction steps; diazotization, reduction, unmasking of the hydrazine and Fischer indole reaction). This 'machine' is then used to prepare a small library of hitherto unreported analogues of the 5-HT agonist Zolmitriptan in good yields, thus demonstrating that the integration and use of machines is a powerful tool to expedite drug discovery.

Introduction

The discovery and development of new pharmaceutical compounds is crucial to the continued improvement of human health. The majority of new drug compounds introduced to the market consist of so called "follow-on" or "me-too" drugs. The term "me-too" refers to new compounds developed in parallel to the pioneer drug which do not necessarily show improved properties, whereas, the term "follow-on" usually refers to those compounds that are introduced after the pioneer drug. Follow-on compounds often exhibit improvements over the already approved pioneer drug, for example, through lowered dosing rates or weakened side effects. Follow-on compounds are either developed by the pharmaceutical company who introduced the original pioneering molecule or by a competing company aiming for a market share. Even though follow-on drugs have been criticised for not driving forward innovation in the same manner as pioneering drugs, they can increase availability of important pharmaceutical agents through lowering prices and raising the standard of medication through their enhanced properties.[1]

Recently, the use of a machine-assisted approaches for the synthesis of compounds or compound collections with useful properties has been discussed and explored.[2] Such approaches take advantage of automation, computer control and robotics to perform manual and repetitive operations and thus free-up human operators and minimize errors derived from repetitive tasks. Furthermore, integration of several reaction steps and/or workup processes can lead to decreased handling operations and streamline the synthesis process.

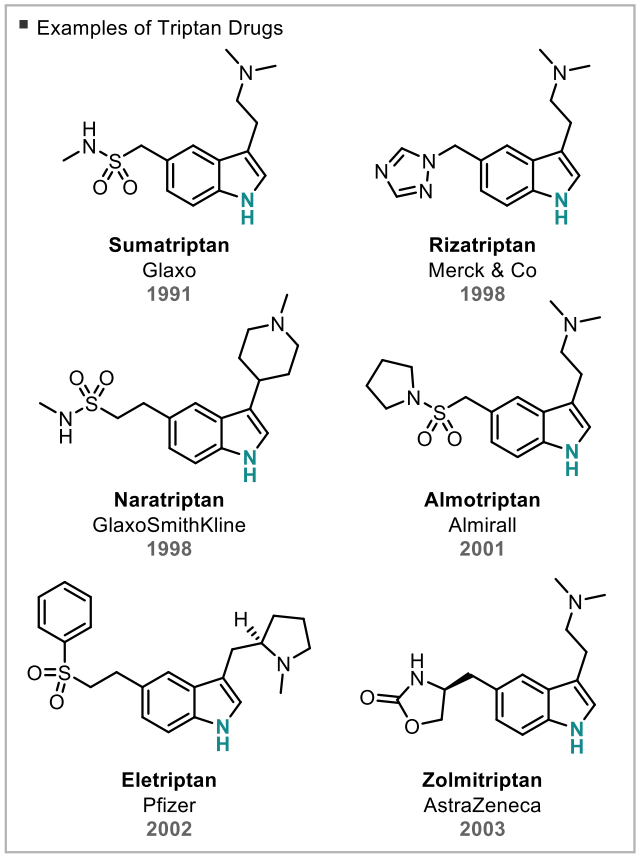


Figure 1. Examples of Triptan Drugs with marketing company and year of FDA approval

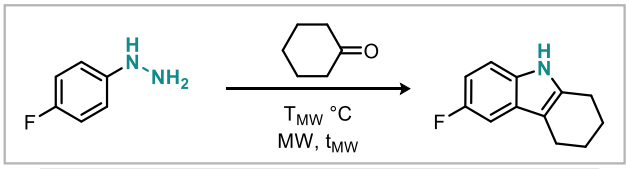
Within the context of follow-on pharmaceutical compounds, we considered a continuous flow + robotic microwave hybrid machine. Such a hybrid would be capable of conducting multistep synthesis, including the generation and consumption

[a] School of Chemistry, Cardiff University Main Building, Park Place, Cardiff, CF10 3AT, UK. E-mail: dlbrowne@cardiff.ac.uk

of hazardous materials on the front-end of the 'machine' followed by rapid and automated robotic microwave processing as a point to diversify compound collections in the second-part of the 'machine'. Herein we report the establishment of a proof-of-principle towards delivering such a machine for the preparation of a series of follow-on compounds. The Triptan drugs, an example sequence of follow-on compounds, used for the treatment of migraines, are a series of indole based 5-HT agonists. Sumatriptan was the first Triptan to market, delivered by Glaxo in 1991. Following this, further Triptan drugs were marketed for the treatment of different migraine types (Figure 1).

The synthesis of indoles has been of interest for many years due to their useful biological applications in the agrochemical and pharmaceutical industry.[3] A broad number of indole syntheses are known, with the Fischer indole reaction remaining one of the most commonly used due its versatility. Indeed, the Fischer indole reaction is still employed in recent total syntheses and is currently the key heterocycle forming reaction for the commercial manufacture of the Triptan drugs. However, one of the main drawbacks, and perhaps one of the reasons for the plethora of alternative syntheses that have been developed, is the limited commercial availability of hydrazines. Owing to this drawback, other ways to access the key Fischer indole intermediate hydrazones and enhydrazines have been established, such approaches are often referred to as 'interrupted-Fischer' syntheses. For example, the intermediate enhydrazines can be formed by a transition-metal catalyzed hydroamination of alkynes in a Larock-type synthesis with a variety of aromatic precursors.[4] The derivatives of the intermediate hydrazones can be formed by a transition-metal catalyzed cross coupling of activated aromatics and a hydrazine starting material.[5] Indoles can also be prepared in a modified Japp-Klingemann reaction.[6] Alternatively the hydrazine can be formed prior to the Fischer Indole reaction either *via* an S_NAr process between hydrazine and an appropriately activated aromatic or by the reduction of the corresponding diazonium salts.[7]

In line with the latter approach, this project aims to use the continuous flow part of the hybrid machine to first prepare and then consume a diazonium salt. This will deliver an array of hydrazines on demand from their corresponding aniline input feeds (Scheme 1, Step 1). The emerging masked hydrazines will then be directly fed into the second part of the machine to undergo expedited Fischer indole reactions with a range of ketones and aldehydes using a robotic microwave reactor (Scheme 1, Step 2). The approach is modular in setup, where a common stream of hydrazine from Step 1 can be distributed to a range of microwave vials, prefilled with the required ketones or aldehydes, prior to microwave irradiation in Step 2. Such an approach takes advantage of the safe handling of unknown yet potentially hazardous materials under flow conditions before then being manipulated by automated equipment for the final part of the reaction.



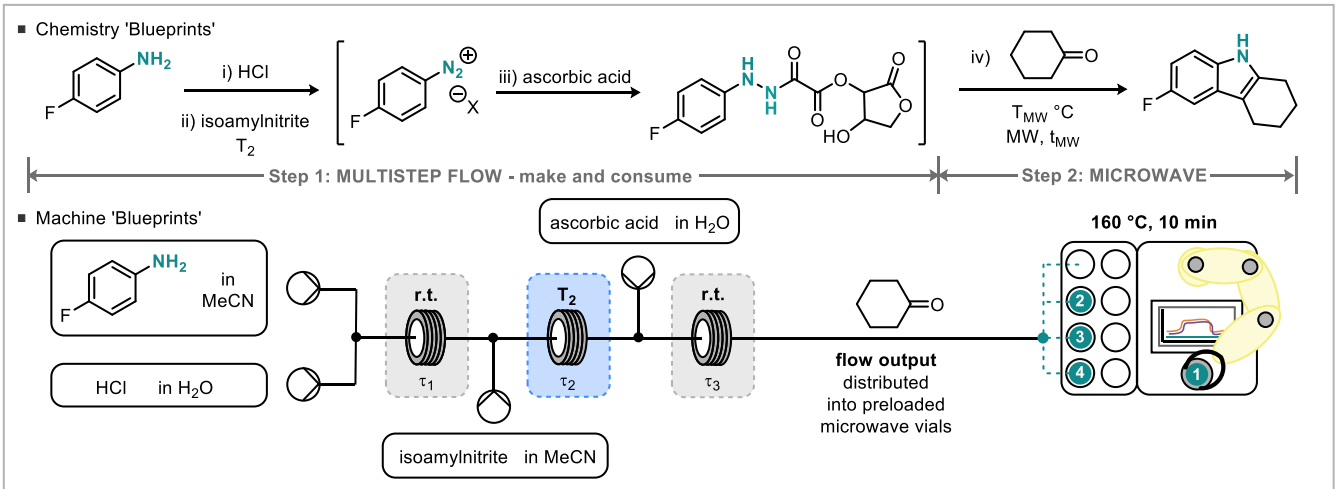
entry	hydrazine source	T_{MW} [°C]	t_{MW} [min]	^{19}F yield [%]
1	commercial	120	10	98 (97)
2	in situ prepared	120	10	15
3	in situ prepared	140	10	30
4	in situ prepared	160	10	52 (47)
5	in situ prepared	180	10	46
6	in situ prepared	160	30	48
7[a]	in situ prepared	160	10	67
8[a,b]	**in situ prepared**	**160**	**10**	**73**

Table 1. Optimisation of Microwave Parameters, Step 2; isolated yields in parenthesis; batch hydrazine formation: 1 mmol *p*-fluoroaniline, 1 mmol HCl, 1 mmol isoamylnitrite, 1 mmol Vit C; a) 5 mmol of HCl; b) 1.2 mmol isoamylnitrite

Results and Discussion

In order to establish machine parameters for both the flow and microwave components, we started by exploring the microwave conditions for the Fischer indole reaction with the commercially available *p*-fluorophenyl hydrazine and cyclohexanone (Table 1). It was found that running this reaction for 10 minutes at 120 °C in acetonitrile solvent resulted in 97 % isolated yield (Table 1, entry 1). Next, the complex reaction mixture that would result from the *in situ* vitamin C reduction of a diazonium salt was tested for its performance in the indole formation under microwave conditions. When applying microwave irradiation for 10 minutes at 120 °C the yield was significantly lower than with pure hydrazine (15%, Table 1, entry 2), highlighting the difficulty of merging one multistep process directly into another. After screening of reaction temperature, equivalents of acid and equivalents of isoamylnitrite used to deliver the masked hydrazine, an improvement to 73% yield was acquired (Table 1, entry 8).

With optimum conditions for merging the hydrazine generation with the microwave step in hand, the flow setup was designed to fit with this (Scheme 1). The flow part of the machine (Step 1) consisted of merging of the aniline with hydrochloric acid in a T-piece and passage through a mixing coil of residence time τ_1. This stream was then merged with a solution of isoamylnitrite and passed through a second reaction coil with the residence time τ_2, where the diazonium salt was formed at 0 °C. The resultant stream was merged with an ascorbic acid solution to induce reduction to the masked hydrazine, in the form of an oxalyl hydrazide, which could then be hydrolysed to the hydrazine in the presence of acid in the microwave step. To permit analysis and a measurement of effectiveness of the process, an aliquot of the exit stream was then transferred to a microwave vial and the Fisher reaction carried out to provide the indole.



Optimization of Step 1 machine parameters

entry	c (HCl) [mol/L]	c (isoamylnitrite) [mol/L]	c (Vit C) [mol/L]	T_2 [°C]	τ_1 [min]	τ_2 [min]	τ_3 [min]	^{19}F yield %
1	0.2	0.24	0.2	0	0.6	3.3	6.3	41
2	1	0.2	0.2	0	0.6	3.3	6.3	72
3	1	0.24	0.24	0	0.6	3.3	6.3	62
4	1	0.24	0.2	rt	0.6	3.3	6.3	53
5	1	0.24	0.2	0	5	3.3	6.3	60
6	1	0.24	0.2	0	0.6	1.7	6.3	64
7	1	0.24	0.2	0	0.6	8.3	6.3	65
8	1	0.24	0.2	0	0.6	3.3	2.5	64
9	1	0.24	0.2	0	0.6	3.3	12.5	64
10	**1**	**0.24**	**0.2**	**0**	**0.6**	**3.3**	**6.3**	**74 (68)**

Scheme 1. Outline of synthesis, machinery and optimization

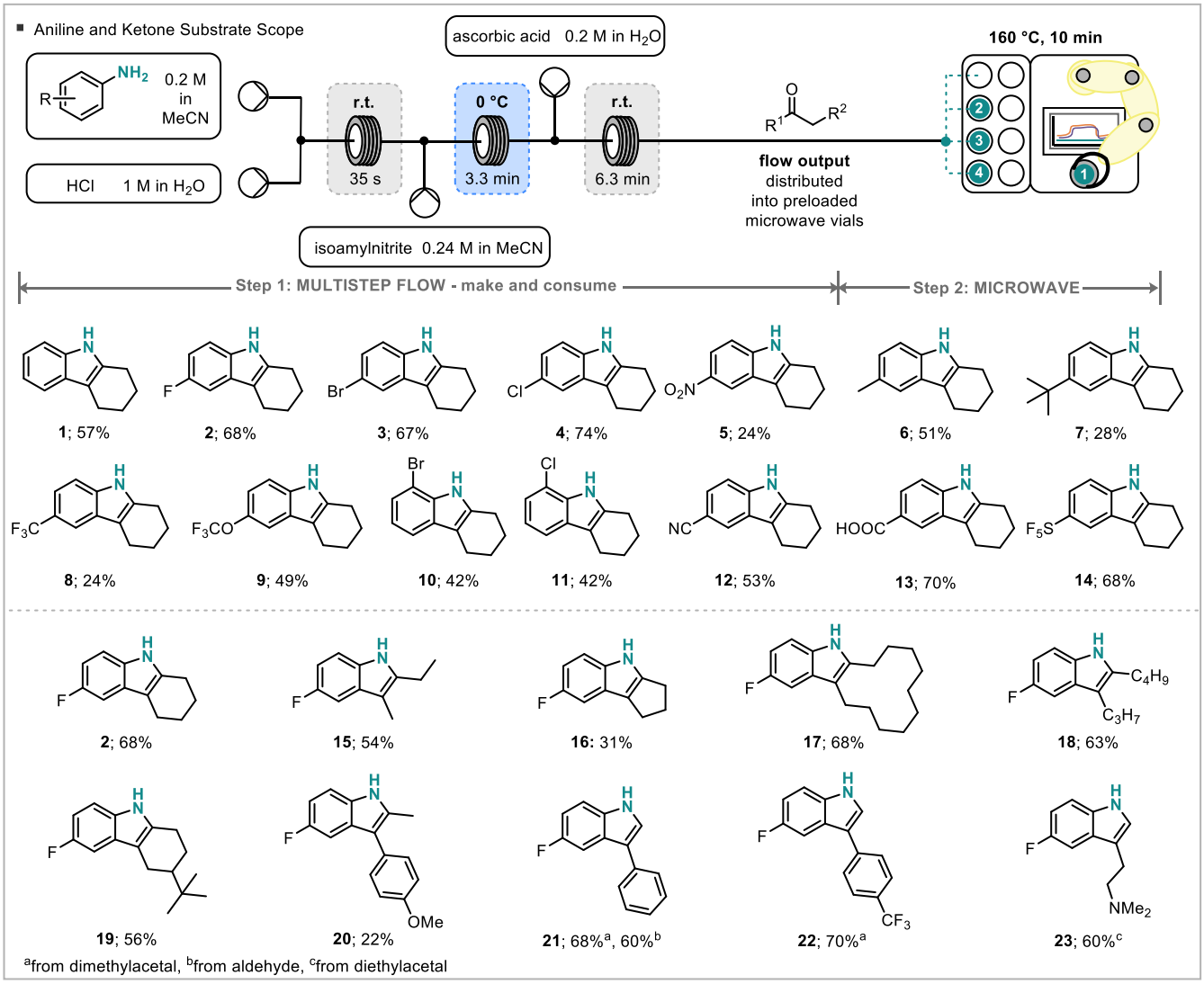
Analysis of the ^{19}F NMR with an internal standard served as a yield measure to guide the parameter optimization.

During parameter screening the concentration of hydrochloric acid, isoamylnitrite and ascorbic acid, the temperature of the second reaction coil (diazonium salt formation) and the residence times in all reaction coils were screened. A selection of these results is shown in Scheme 1. Best conditions for the first step proved to be 1 M hydrochloric acid in H_2O (5 equiv), 0.24 M isoamylnitrite in MeCN (1.2 equiv), 0.2 M ascorbic acid in H_2O (1 equiv), 0 °C in the second reaction coil, residence times of 0.6 min (τ_1, 0.23 mL reaction coil), 3.3 min (τ_2, 2 mL reaction coil) and 6.3 min (τ_3, 5 mL reaction coil) at a flowrate of 0.2 mL/min for each pump (Scheme 1, optimization table, entry 10).

Following reaction optimization, a small substrate scope of anilines was performed with cyclohexanone as the ketone partner. This reaction scoping also served to assess the robustness of the processing conditions against the propensity for blockage formation. Using the two-step machine-assisted process, 14 indoles were isolated in moderate to excellent yields over the four chemical reaction steps (Scheme 2). Notably, at the concentrations studied for the flow step, no blockages occurred across the screened substrates. The indole derived from *p*-chloroaniline (**4**) gave the highest yield of 74%.

Strongly electron-withdrawing groups such as *p*-nitro or *p*-CF_3 groups (**5, 8**) produced lower yields (24%). This could be attributable to the competing radical formation with ascorbic acid, which is reportedly best with electron-poor aromatics.[7i, j, m] *Ortho* substituted anilines provided lower yields of the indoles than their corresponding para substituted anilines.

A ketone/aldehyde substrate scope was then performed using *p*-fluoroaniline as partner to prepare a range of indoles (Scheme 2). To achieve this, a common flow stream with the *in situ* prepared oxalyl hydrazide (Step 1) was transferred into a series of microwave vials preloaded with the desired carbonyl compound (or carbonyl precursor). Cyclic and acyclic ketones with varying chain lengths, benzylic ketones, aldehydes and acetals all participated in the reaction, affording the corresponding indoles in good to excellent yields over the four-step process (31-68%, **15**-**22**). Pleasingly, 4-dimethyl aminobutyraldehyde diethyl acetal, including a tertiary amine could be incorporated to give the desired indole in 60% yield (**23**). This is particularly notable in the context of the Triptan series, where this exact side-chain is present in several examples, Sumatriptan, Rizatriptan, Almotriptan and Zolmitriptan.



Scheme 2. Aniline and Ketone Substrate Scope

After demonstrating the feasibility of the method over a range of both aniline and carbonyl substrates the machine was then used to prepare Zolmitriptan itself. Machine input feeds for this consisted of the appropriate oxazolidinone substituted aniline, Hydrocholoric acid, isoamyl nitrite, ascorbic acid and 4-dimethyl aminobutyraldehyde diethyl acetal and afforded the Triptan in 56% yield. Following this demonstration a range of nine *hitherto* unreported analgoues of Zolmitriptan were synthetized in good to excellent yields (Scheme 3, **24**-**32**).

Conclusion

In conclusion, we have designed a hybrid machine-assisted approach that incorporates a multistep continuous flow processes and robotic microwave reactors to expedite the rapid preparation of a library of indoles. This type of approach could be important for the screening and discovery of follow-on drugs. Key to realizing this ability was the optimization of the reaction conditions as the crude reaction mixture passed from one machine step to the next. In this manner 33 indoles, including nine analogues of the anti-migraine drug Zolmitriptan and Zolmitriptan itself, were synthesized. Notably, only ten of the examples presented here had been characterized in the literature prior to this report. The complexity of the equipment described here could be further advanced depending on desired capability and budget (see the Supporting Information for some proposed alterations).

Conflicts of interest

There are no conflicts to declare

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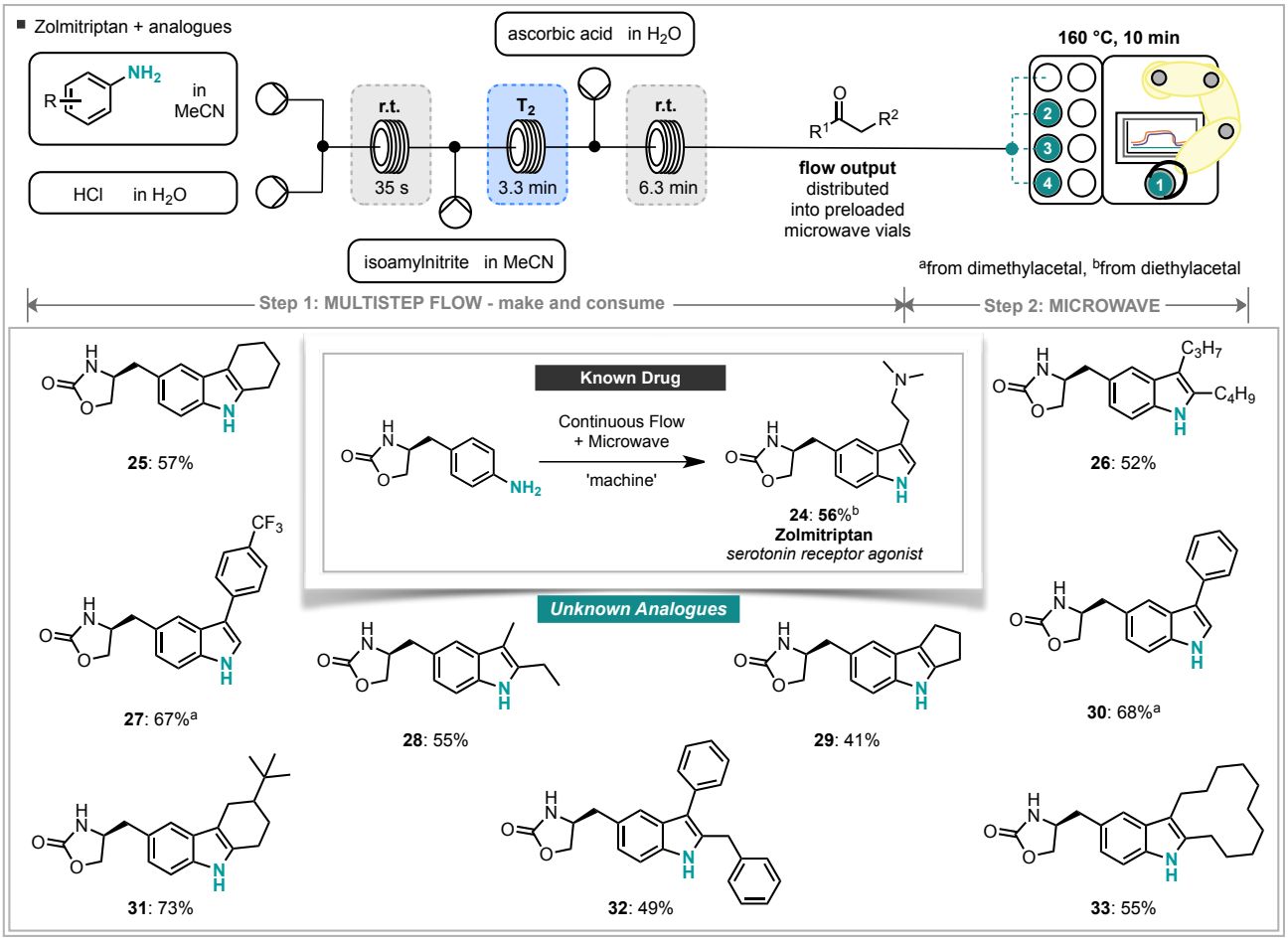

Scheme 3. Zolmitriptan Analogue Scope

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