PAPER

Sulphonated graphene oxide catalyzed continuous flow synthesis of pyrazolo pyrimidinones, sildenafil and other PDE-5 inhibitors†

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Sulphonated graphene oxide was used for cascade condensation and cyclization reactions towards accessing substituted pyrazolo pyrimidinones. Further, sulphonation and amination reactions were integrated through continuous flow chemistry to access PDE-5 inhibitors. Herein, we report a simple continuous synthetic platform that reduce tedious manual operations and accelerate the synthesis of several potent inhibitors of phosphodiesterase type-5. The developed platform enabled us to perform one-flow multi-step, multi-operational process to synthesize the PDE-5 inhibitors such as sildenafil and its analogues in 32.3 min of the reaction time, with minimal human intervention and single solvent.

Pyrazolo pyrimidinone is a fundamental structural analogue of nucleobase purines (Fig. 1) and is one of the most versatile adenosine receptor antagonists. This scaffold has attained global importance because of a wide range of pharmacological properties[1–4] such as, anti-bacterial, anti-cancer, antifungal activity, anti-microbial, fungicidal and pesticide, *etc.*, making it an interesting synthetic target for organic chemists. In recent years, there are numerous synthetic methods and tools developed for the manufacturing of pyrazolo pyrimidinone based drugs like Viagra or Sildenafil (PDE-5 inhibitor).[5,6] The first batch protocol for the formation of pyrazolo pyrimidinone is dated on 1996,[7] wherein aromatic acid[8] or acid chlorides[9] were used for the cyclization reaction which was attempted in a two-step sequence amidation followed by oxidative cyclization and was promoted by excess amounts of $SOCl_2$ or bases (TEA, NaOH, pyridine & *t*-BuOk) at reflux temperature. The recent developments over a decade in the oxidative cyclization have demonstrated the use of simple and commercially available aromatic aldehydes as substrates in the presence of oxidants like $K_2S_2O_8$,[10] H_2O_2, $K_2S_2O_5$, *etc.* in stoichiometric amounts. On the other hand, to minimize the usage of oxidants, several novel transition metal (Pd, Cu, In, Sn) catalytic reactions under homogeneous and heterogeneous conditions were employed.[11]

Later on, a series of methods have been developed for coupling and cyclization reactions.[12–15] The other reagents used

for the transformation include H_2O_2/NaOH, $HPOCl_3$, PCl_5, $K_2S_2O_5$, $NaHSO_3$, Al_2O_3, *t*-BuOK/*t*-BuOH, PPA, AcOH *etc.* in stoichiometric amounts.[16–19] Though the batch protocols are assisted by using various reagents, the reaction systems in
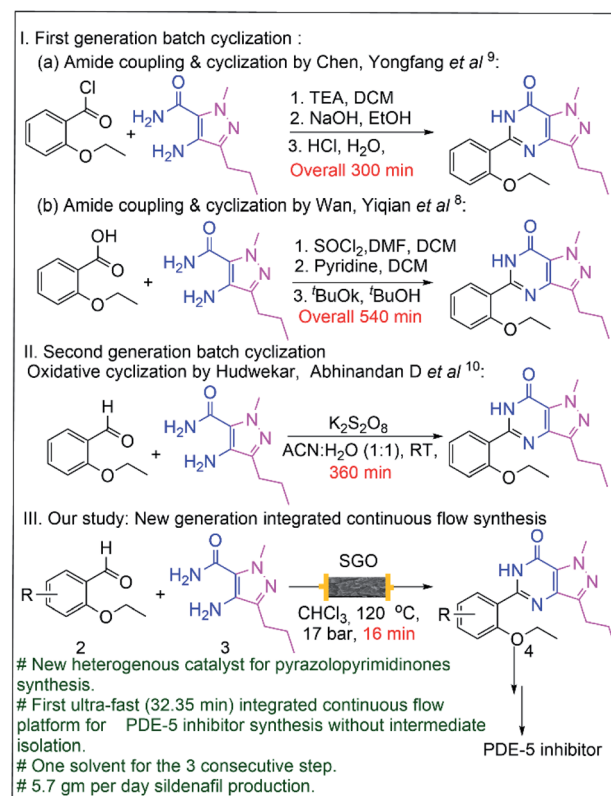

Fig. 1 Synthetic transformations of pyrazolo pyrimidinones.

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stoichiometric amounts results in generation of huge side products and waste. Batch process have a low surface to volume ratio and inefficient mixing, results in longer reaction times (4–24 h) to complete the reaction with off-quality product and are hence inappropriate for automation.[20–22]

The existing batch process involves labour and cost-consuming multiple operational processes that need to be absolutely avoided by the chemical industry. In recent years, to minimize the wastage in the chemical reaction, heterogeneous catalysis with packed bed systems is well explored. Therefore, fabrication of suitable heterogenous catalyst, continuous-flow, oxidant free, one-flow, one solvent, with minimal human intervention, towards accessing broad range of substrates in fast manner in reactors is highly desired for PDE-5 synthesis.[23–27]

As proof of the concept for the PDE-5 inhibitor synthesis under continuous flow process system, a model reaction between **2a** and **3** was attempted in flow manner. To optimize the reaction conditions for the continuous flow process system, the feasibility reaction was carried out by infusing the solution containing reactants with the solvent(s) under a stoichiometric molar ratio. The stock solution was passed through Amberlyst® 15 (1.0 g) filled in SS-cartridge (id = 7 mm, length 100 mm, vol = 4 mL) at 120 °C with 17 bar pressure with a flow rate of 250 μL min⁻¹ (retention time 16 min) which typically led to generation of the yellowish solution with 20% yield of the desired product **4a** (Table 1, entry 1). The result was also similar when GO was used affording 23% yield of the desired product along with major amount of unreacted starting materials (Table 1, entry 2).
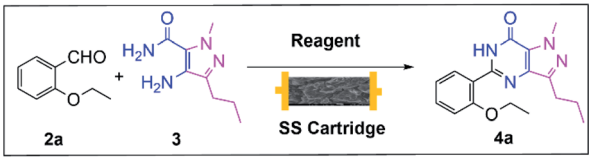
We anticipated that incorporating a hard acid –SO₃H functional group into the graphene oxide backbone, the catalytic efficiency could be further improved. In this context, we have prepared the sulphonated graphene oxide (SGO)[28] (see details in ESI†) and examined its reactivity. To further elucidate the

optimized reaction parameters, we have investigated several experiments at different conditions such as various flow rates (10 μL min⁻¹, 50 μL min⁻¹, 100 μL min⁻¹, 250 μL min⁻¹, 500 μL min⁻¹, 1 mL min⁻¹ & 2 mL min⁻¹), at different temperatures (60 °C, 80 °C, 100 °C, 120 °C & 140 °C), at different pressures (5 bar, 10 bar, 17 bar and 32 bar) and using different solvents (CHCl₃, 1,2-DCE, DCM & ᵗBuOH) respectively. We noticed that under lower temperature & pressures, no reaction or low yields was observed (see the ESI, Table S1,† entries 5–9), which may be attributed due to the insufficient energy and shorter duration of time for the cyclization reaction to occur. Finally, we have achieved optimized continuous flow conditions to yield 85% of compound **4a** (Table 1, entry 3) in CHCl₃ as a solvent at 120 °C temperature and 17 bar pressure with a flow rate of 250 μL min⁻¹. Further, to check the long-term durability of SGO, we run the system continuously for 30 h and obtained **4a** with *ca* 0.67 mmol h⁻¹ productivity. It is worth to mention that the SGO works efficiently for the one-pot synthesis *i.e.*, the condensation and cyclization reaction without an external oxidant (see the ESI Comparative Table S2,† entries 1–6). The detailed mechanistic study to check the oxidative pathway (involvement of aerial oxidation) is discussed (see ESI Mechanistic study, Fig. S7†).

After optimization of reaction conditions for the synthesis of pyrazolo pyrimidinones, we further explored the substrate scope and limitations of this strategy. Thus, several substituted aryl aldehydes with F, Cl, Br and –OMe (**2c–g**) and un-substituted aldehyde **2b** were investigated under optimized conditions to afford the corresponding pyrazolo pyrimidinones with 80–85% yield (**4a–g**, Fig. 2).[29]

After completion of the step-1 cyclization reaction and validation of SGO activity, further two step protocols, the sulphonation followed by amine coupling reaction to obtain the PDE-5 inhibitors were needed to be performed. In conventional batch process for the sulphonation reaction, chloro sulphonic acid, which is highly hygroscopic, corrosive and powerful

Table 1 Condensation and cyclization reaction of the 2-ethoxy benzaldehyde (**2a**) and 4-amino-1-methyl-3-propyl-1*H*-pyrazole-5-carboxamide (**3**) under continuous flow process[a]

Entry	Flow rate (μL min⁻¹)	Reagent	Time (min)	Yield (%)
1	250	Amberlyst[b]	16	20
2	250	GO	16	23
3	**250**	**SGO**	**16**	**87**
4	500	SGO	08	70
5	100	SGO	40	85

[a] Reaction conditions: feed solution molar ratio [**2a** : **3** : CHCl₃ (1 : 1.1 : 236)]; reagent (1 g) filled SS-cartridge (id = 7 mm, length 100 mm, vol = 4 mL); temperature 120 °C; under 17 bar pressure; yields are based on the isolated yields. [b] Amberlyst® 15 hydrogen form.
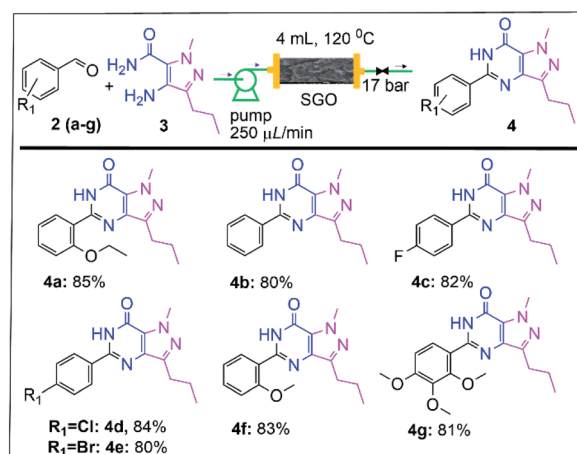

Fig. 2 General synthetic scheme for the synthesis of pyrazolo pyrimidinone; reaction condition: feed solution molar ratio [**2a** : **3** : CHCl₃ (1 : 1.1 : 236)]; [0.053 M in CHCl₃ each stock solution]; catalyst (1.0 g) filled SS-cartridge (id = 7 mm, length 100 mm, vol = 4 mL); time 0.26 h; at temperature 120 °C; yields are based on the isolated yields.
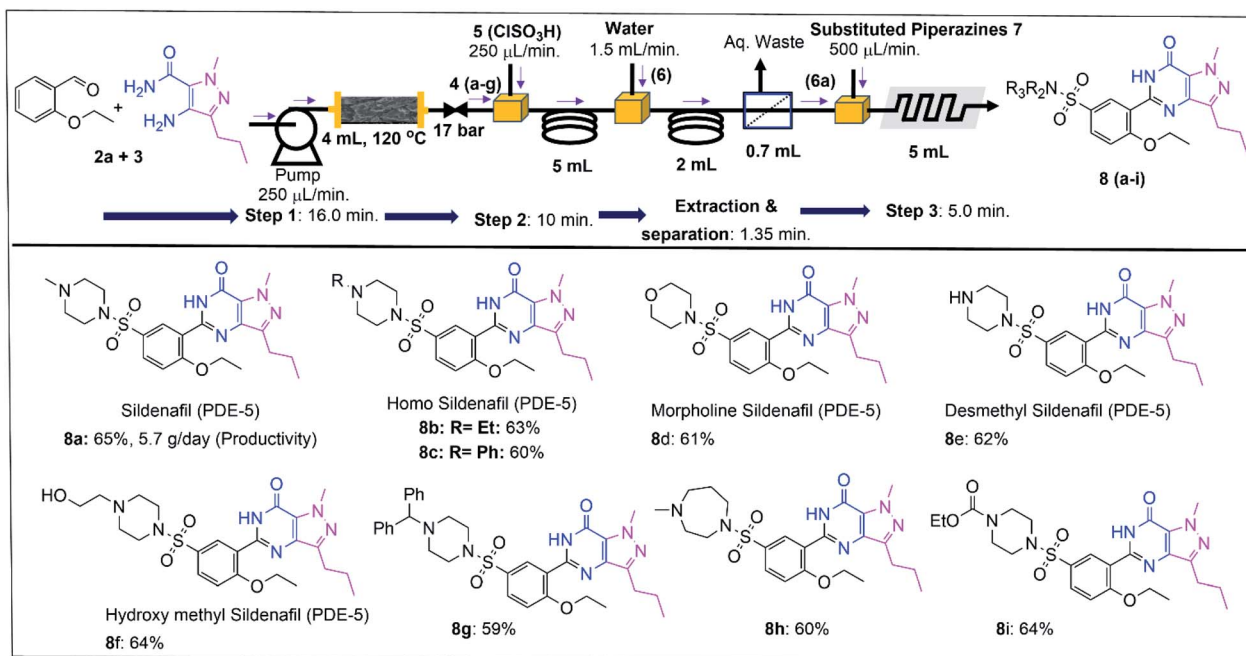
Fig. 3 New generation continuous micro-flow ultra-fast PDE-5 API synthesis route, reaction condition: stock solution molar ratio of **2a** : **3** : CHCl$_3$ (1 : 1.1 : 236); 0.053 M in CHCl$_3$]; stock solution of 5 (0.032 M in CHCl$_3$); room temperature 25 ± 5 °C; stock solution of **6a** (0.021 M in CHCl$_3$); stock solution of 7 (0.021 M in CHCl$_3$); room temperature 25 ± 5 °C; yields are based on the isolated.

lachrymatory agent is employed.[30] Apart from this, the reaction also requires additional energy for maintaining the reactor temperature from 0 °C to −10 °C. To minimize the toxic exposure of chloro sulphonic acid and to maintain the ambient reaction temperature, flow technique was employed.[31] In this regard, individual sulphonation reaction was optimized under the continuous flow for the synthesis of compound (**6a**) (see the ESI, Table S3,† entry 4). Herein, we observed the sulphonated product **6** was obtained in 10 min, at ambient temperature with 88% yield (see the ESI, Table S3,† entry 4). As shown in (ESI Table S4†), further to remove the excess amount of the chloro sulfonic acid which is present in the reaction medium and also to minimize the post-downstream purification process, we further appended continuous aqueous quenching, and droplet-based extraction strategy[32–37] (see the ESI; Fig. S8 and Table S4†). The clean separation was achieved after regulating the back pressure and allowing the transmission of the integrated process for the sulphonation (10.0 min), and extraction/separation (1.3 min) of pyrazolo pyrimidinone with excellent yields (~88% yields) resulting in *ca.* 0.32 mmol h^{-1} productivity. It was observed that the conventional batch process for similar reaction recorded almost same yield in longer reaction time (4–6 h) and at low temperature (0 °C to −10 °C) to furnish the desired product.

Next, we focused for developing the continuous flow amination process to synthesize the sulphonamide API[38] compounds such as sildenafil, and other PDE-5 inhibitors. The sulphonated product **6a** was used as a model compound and treated with *N*-methyl piperazine to produce **8a**. After investigating several reaction conditions, we were gratified to observe

that the results are feasible and 94% yield of **8a** was obtained in just 5 min of retention time at ambient temperature. Under this optimized condition, **8a** was obtained with a *ca.* 0.6 mmol h^{-1} productivity (see the ESI, Fig. S9 and Table S5,† entry 6). After the successful completion of the individual optimization reactions in continuous flow manner, we have planned to build a fully integrated continuous automated platform for API sildenafil free base and its analogues synthesis. Fig. 3 illustrates a basic set-up to the integrated continuous flow synthesis reactor. Using this platform, the reactants substituted aldehyde **2a** and amine **3** were introduced to a micro-reactor[39] to get sildenafil (**8a**) in overall moderate yield (~65%) (Fig. 3) in 32.35 min (condensation and cyclization reaction (16.0 min), *in situ* sulphonation (10 min), quenching, extraction (1.0 min) and separation (0.35 min), and followed by amination (5 min) resulting in *ca.* 0.5 mmol h^{-1} productivity. Thus, we were able to produce 5.7 g of sildenafil after running the reaction for 24 h. Finally, after the development of a fully integrated process for the synthesis of sildenafil, we were interested in testing the scope and limitation of our newly developed platform. As shown in Fig. 3, we have successfully developed the micro-flow conditions that have emerged as a superior alternative for the cascade PDE-5 inhibitor synthesis obtained by batch protocol (see the ESI Comparative Table S6,† entries 1–8). A range of amino substrates (substituted piperazines and morpholine) were found to be well tolerated furnishing the corresponding substituted PDE-5 inhibitors in 59–65% overall yield. In contrast, a series of PDE-5 inhibitors such as sildenafil, homo-sildenafil, hydroxy-methyl sildenafil, and morpholine sildenafil, des-methyl sildenafil *etc.*, are accessible quickly in a continuous

flow process on small scale as well as large scale in one-solvent system. The present protocol avoids multi-solvents/multi-solvent system and highly laborious post-synthetic work-ups in comparison with the traditional batch methods including many unusual unit operations that takes several hours/days to produce them.

In conclusion, we have developed a new and highly efficient integrated continuous flow platform for the synthesis of biologically active pyrazolo pyrimidinones, sildenafil and other PDE-5 inhibitors. The system is multi-operational, time-effective, scalable, real-time in fast manner in 32.35 min reaction time in one solvent providing an improved 65% overall yield with 5.7 g day^{-1} productivity of sildenafil. The integrated machine has all the desired attributes required in continuous manufacturing process development. To our best of knowledge, this integrated continuous flow process will be useful in small scale as well as large scale industrial synthesis of sildenafil and other analogue PDE-5 inhibitors.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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