


 Cite this: *RSC Adv.*, 2021, **11**, 3216

 Received 30th October 2020  
 Accepted 24th December 2020

DOI: 10.1039/d0ra09247a

[rsc.li/rsc-advances](http://rsc.li/rsc-advances)

## Synthesis of 2-ethoxycarbonylthieno[2,3-*b*]quinolines in biomass-derived solvent $\gamma$ -valerolactone and their biological evaluation against protein tyrosine phosphatase 1B<sup>†</sup>

 Xu-Yang Mu,<sup>‡,a</sup> Zhi-Jia Wang,<sup>‡,a</sup> Bo Feng,<sup>‡,a</sup> Lei Xu,<sup>b</sup> Li-Xin Gao,<sup>b</sup> Rajendran Satheeshkumar,<sup>ID,ac</sup> Jia Li,<sup>b</sup> Yu-Bo Zhou<sup>\*b</sup> and Wen-Long Wang<sup>ID,\*a</sup>

A series of 2-ethoxycarbonylthieno[2,3-*b*]quinolines were synthesized in the bio-derived "green" solvent  $\gamma$ -valerolactone (GVL) and evaluated for their inhibitory activities against PTP1B, the representative compound **6a** displayed an  $IC_{50}$  value of  $8.04 \pm 0.71 \mu\text{M}$  with 4.34-fold preference over TCPTP. These results provided novel lead compounds for the design of inhibitors of PTP1B as well as other PTPs.

### Introduction

Protein tyrosine phosphatase 1B (PTP1B) is a prototypic member of the protein tyrosine phosphatase (PTP) family that appears to be involved in the regulation of several cellular functions, including insulin cascade,<sup>1</sup> and other important pathways related to human breast and ovarian cancers.<sup>2</sup> Consequently, the inhibition of PTP1B is considered to be a potential therapeutic for the treatment of type 2 diabetes and cancers. Various PTP1B inhibitors have been developed over the past decade.<sup>3</sup> However, only two candidates have entered clinical trials while no commercial drugs have been approved to date due mainly to limited bioavailability.<sup>3</sup> Therefore, the rapid development of a potent and bioavailable PTP1B specific inhibitor remains necessary.

Thienoquinolines are an important subset of the quinoline family and consequential structural units in the domain of medicinal chemistry due to their myriad bioactivities.<sup>4-6</sup> This class of compounds are well documented with urea transporter inhibitory (compound **1**),<sup>7</sup> anti-inflammatory (compound **2**),<sup>8</sup> anti-oxidant (compound **3**)<sup>8</sup> activities (Fig. 1).

Our efforts to develop modulators of protein tyrosine phosphatases started from *1H*-2,3-dihydroperimidines.<sup>9-14</sup> Using the scaffold hopping strategy,<sup>15,16</sup> we developed 3-aryl-1-oxa-2,8-diazaspiro[4.5]dec-2-enes<sup>11</sup> as PTP1B inhibitors, bis-aryl amides<sup>9</sup> and benzo[c][1,2,5]thiadiazoles as SH2-Containing Protein Tyrosine Phosphatase-2 (SHP2) inhibitors.<sup>13</sup> We noticed that the scaffold of

thieno[2,3-*b*]quinolines showed high similarity with that of *1H*-2,3-dihydroperimidines and might provide novel scaffold to develop novel PTP1B inhibitors (Fig. 2).

In the pharmaceutical industry, there is a strong desire to embed sustainable principles earlier in the drug discovery process,<sup>17,18</sup> in which the development of more environmentally friendly methods for the isolation and purification of active molecules continues to represent a major challenge.<sup>17</sup> There are many examples of *Green Chemistry* principles successfully applied in medicinal chemistry,<sup>17-21</sup> such as using microwave chemistry<sup>22,23</sup> and high-throughput screening<sup>18,24</sup> to provide drug candidates consuming less material, with less waste, and in less time. In recent years there has been a trend towards the use of bio-derived, green solvents as replacements for hazardous and/or environmentally-damaging solvents for oxidation, nitration, rearrangement, phosphorylation, amide condensation, urea formation, and metal catalyzed cross-coupling.<sup>21,25-37</sup> Increasing attention is directed to  $\gamma$ -valerolactone (GVL),<sup>38-44</sup> which is a naturally occurring chemical found in fruits.<sup>38</sup> Due to the low toxicity and the outstanding physicochemical properties of biodegradability and low vapor pressure,<sup>38</sup> GVL has been recognized as sustainable dipolar aprotic solvents and has been developed as a greener alternative to classical solvents used in metal-catalyzed cross-coupling reactions,<sup>44</sup> transformation of  $\text{CO}_2$  to formamides,<sup>38</sup> and membrane preparation.<sup>41</sup> With our interest in the area of sustainable synthesis in discovery-phase medicinal chemistry,<sup>45</sup> we developed an environmentally benign and sustainable protocol

<sup>a</sup>School of Pharmaceutical Sciences, Jiangnan University, Wuxi, 214122, China.  
 E-mail: wwenlong2011@163.com

<sup>b</sup>State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, 201203, China. E-mail: ybzhou@simm.ac.cn

<sup>c</sup>Departamento de Química Orgánica, Facultad de Química y de Farmacia, Pontificia Universidad Católica de Chile, Santiago de Chile, 702843, Chile

<sup>†</sup> Electronic supplementary information (ESI) available. See DOI: 10.1039/d0ra09247a

<sup>‡</sup> These authors contributed equally to this work.

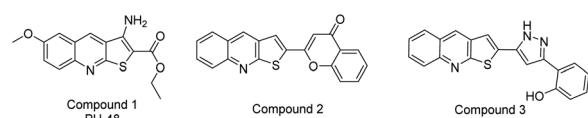


Fig. 1 Biologically active thienoquinolines.



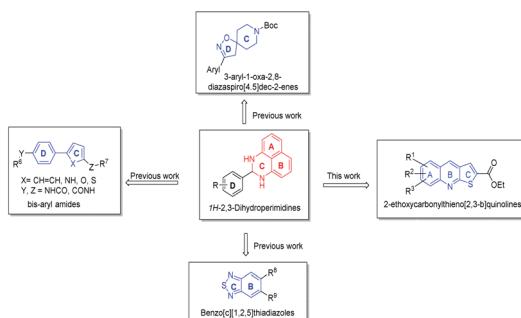


Fig. 2 The scaffolds of modulators of protein tyrosine phosphatases derived from 1*H*-2,3-dihydroperimidines.

for the synthesis of 2-ethoxycarbonylthieno[2,3-*b*]quinolines by condensation of 2-chloro-3-formylquinolines with ethyl mercaptoacetate at 90 °C in  $\gamma$ -valerolactone as a solvent, in which the simple addition of water allowed for complete removal of GVL without the need for extensive isolation and purification.

The obtained 2-ethoxycarbonylthieno[2,3-*b*]quinolines were evaluated for their inhibitory activities against PTP1B, several derivatives were identified as PTP1B inhibitors, the representative compound **6a** was also subjected to selectivity analyses to determine whether its biological properties made it suitable for further development.

## Results and discussion

To optimize the experimental parameters (Table 1), 2-chloroquinoline-3-carbaldehyde (**4a**), which was prepared as ref. 7, was chosen as model substrate using precipitate

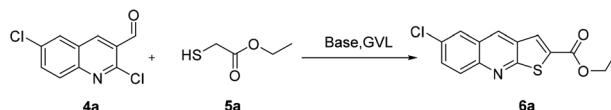
procedure by simple addition of water (Table 1). A series of bases, such as  $\text{Na}_2\text{CO}_3$ , DIPEA, DBU, and  $\text{Et}_3\text{N}$ , were investigated at six equivalents compared to 2-chloroquinoline-3-carbaldehyde. Among them,  $\text{Et}_3\text{N}$  showed best result with 66% yield (entry 4). Increase or decrease of the equivalent of  $\text{Et}_3\text{N}$  showed no positive effects on the yield (entries 5 and 6). After investigation of the equivalent of ethyl mercaptoacetate (entries 7 and 8), the results demonstrated that 1.2 equivalent was the optimal (entry 4). The reaction temperature also was investigated (entries 9–11), and the results displayed that the temperature obviously affected the yield and the suitable temperature was 90 °C (entry 4). After an increase in concentration (entries 12–14), the yield was improved remarkably and the suitable concentration was 0.5 mol L<sup>−1</sup> (entry 13). Prolonged the reaction time from one hour (entry 13) to two hours (entry 15) did not benefit the reaction yield.

Based on the optimized reaction conditions, a series of 2-ethoxycarbonylthieno[2,3-*b*]quinolines were synthesized from compounds **4a–4n**, which were prepared as ref. 7. The results showed that this protocol could be applied to various of 2-chloroquinoline-3-carbaldehydes with electro-withdrawing and electro-donating group on phenyl ring, and had good substrate compatibility in moderate to excellent yields from 51% to 92% (Table 2).

### Protein tyrosine phosphatase 1B inhibitory activities and structure–activity relationships

The inhibitory activities of all synthesized compounds against PTP1B were measured using 6,8-difluoro-4-methylumbelliferyl phosphate (DiFMUP) as the substrate and NSC-87877 as

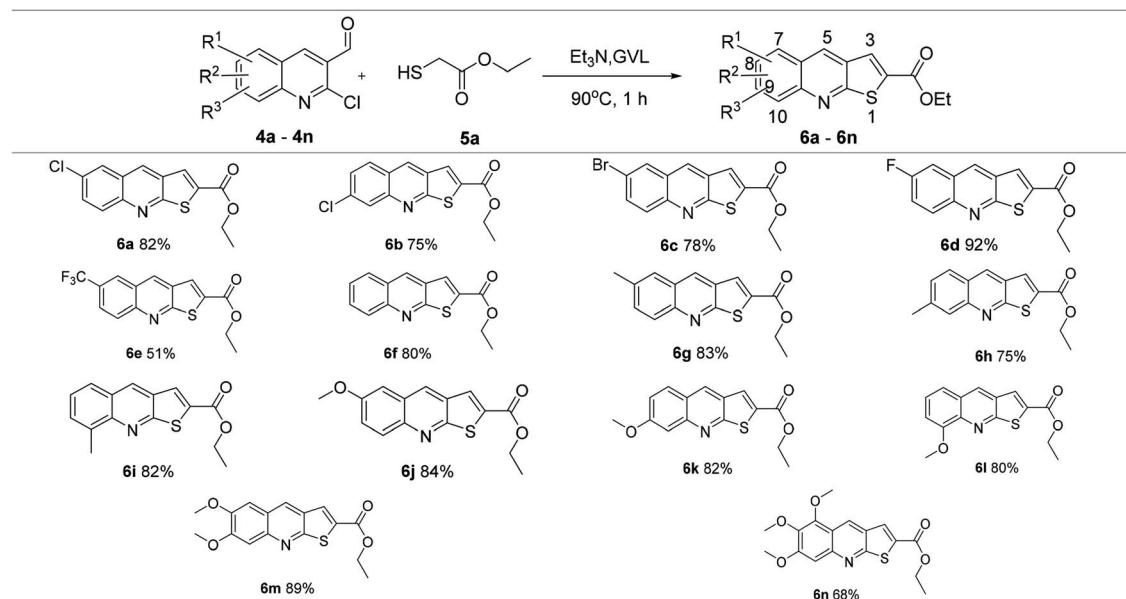
Table 1 Optimization of synthesis of 2-ethoxycarbonylthieno[2,3-*b*]quinoline (**6a**) in GVL



Entry	Base (equiv.)	5a (equiv.)	Concentration 4a (mol L <sup>−1</sup> )	Temp. (°C)	Time (h)	Yield (%)
1	$\text{Na}_2\text{CO}_3$ (6)	1.2	0.05	90	1	49 <sup>a</sup>
2	DIPEA (6)	1.2	0.05	90	1	21 <sup>a</sup>
3	DBU (6)	1.2	0.05	90	1	56 <sup>a</sup>
4	$\text{Et}_3\text{N}$ (6)	1.2	0.05	90	1	66 <sup>a</sup>
5	$\text{Et}_3\text{N}$ (4)	1.2	0.05	90	1	59 <sup>a</sup>
6	$\text{Et}_3\text{N}$ (8)	1.2	0.05	90	1	59 <sup>a</sup>
7	$\text{Et}_3\text{N}$ (6)	1	0.05	90	1	62 <sup>a</sup>
8	$\text{Et}_3\text{N}$ (6)	1.4	0.05	90	1	65 <sup>a</sup>
9	$\text{Et}_3\text{N}$ (6)	1.2	0.05	30	1	Trace
10	$\text{Et}_3\text{N}$ (6)	1.2	0.05	60	1	53 <sup>a</sup>
11	$\text{Et}_3\text{N}$ (6)	1.2	0.05	120	1	63 <sup>a</sup>
12	$\text{Et}_3\text{N}$ (6)	1.2	0.1	90	1	73 <sup>a</sup>
13	$\text{Et}_3\text{N}$ (6)	1.2	0.5	90	1	82 <sup>a</sup>
14	$\text{Et}_3\text{N}$ (6)	1.2	0.8	90	1	76 <sup>a</sup>
15	$\text{Et}_3\text{N}$ (6)	1.2	0.5	90	2	82 <sup>a</sup>

<sup>a</sup> Precipitation and washing.



Table 2 Synthesis of 2-ethoxycarbonylthieno[2,3-*b*]quinolines under optimal conditions

positive control,<sup>46–48</sup> and the results were detailed in Table 3. As for compounds **6a**–**6b**, compound **6a** with chloride at 8 position showed  $98.8 \pm 0.1\%$  inhibition at the concentration of  $50 \mu\text{M}$ , exhibited obviously better inhibitory activity against PTP1B compared to the compound **6b** with chloride at the 9 position. This result indicated that the position of chloride on the phenyl ring significantly affected the inhibitory activity. As for compounds **6a**, **6c**–**6f** with substituents on the 8 position, compounds **6a** with chloride and compound **6c** with bromide showed excellent inhibitory activities against PTP1B at the concentration of  $50 \mu\text{M}$ , compound **6e** with trifluoromethyl group exhibited moderate inhibitory activity, while compound **6d** with fluoride and compound **6f** with H atom obviously lose inhibitory activity. These results indicated that the electronic property and substitute size played important role on the inhibitory activity. Among compounds (**6g**–**6i**) with methyl group on the phenyl ring, compound **6g** showed better inhibitory activity than the compound **6h** and **6i**. These results were similar with that from compounds with chloride (compounds **6a** and **6b**) and further demonstrated that the position of

substrates on phenyl ring affected the inhibitory activities. Among compounds with methoxyl group (**6j**–**6n**), all of them did not show obviously inhibitory activities. These results implied that electro-donating group on the phenyl ring was detrimental to the inhibitory activity. In general, the biological activities of 2-ethoxycarbonylthieno[2,3-*b*]quinolines were affected by three issues of the substitute on phenyl ring A, including position, electronic property and substitute size.

### Selectivity against other PTPs

PTP1B shares the close homology with other PTPs, for example, T-cell protein tyrosine phosphatase (TCPTP) shares a structurally very similar active site with PTP1B and about 80% homologous in the catalytic domain, making it difficult to design inhibitors that are specific for PTP1B.<sup>46</sup> In addition to the potency exploration, we investigated the selectivity of the representative compounds **6a**, **6c** against other PTPs (TCPTP, SHP-2) (Table 4). Homogeneous T-cell protein tyrosine phosphatase (TCPTP) inhibitory activities were investigated simultaneously by the same method.<sup>47–49</sup> Compounds **6a** and **6c**

Table 3 Protein tyrosine phosphatase 1B inhibitory activities of compounds **6a**–**6n**

Comp.	Inhibition (%) at $50 \mu\text{M}$	$\text{IC}_{50}^a$	Comp.	Inhibition (%) at $50 \mu\text{M}$	$\text{IC}_{50}^a$
<b>6a</b>	$98.8 \pm 0.1$	$8.04 \pm 0.71$	<b>6h</b>	$12.7 \pm 6.5$	NT <sup>b</sup>
<b>6b</b>	$45.7 \pm 9.5$	NT <sup>b</sup>	<b>6i</b>	$22.5 \pm 1.4$	NT <sup>b</sup>
<b>6c</b>	$98.2 \pm 0.1$	$8.96 \pm 1.22$	<b>6j</b>	$20.8 \pm 1.9$	NT <sup>b</sup>
<b>6d</b>	$26.1 \pm 6.5$	NT <sup>b</sup>	<b>6k</b>	$21.6 \pm 3.2$	NT <sup>b</sup>
<b>6e</b>	$71.6 \pm 3.0$	$21.21 \pm 1.50$	<b>6l</b>	$23.0 \pm 0.4$	NT <sup>b</sup>
<b>6f</b>	$4.5 \pm 2.6$	NT <sup>b</sup>	<b>6m</b>	$35.5 \pm 2.1$	NT <sup>b</sup>
<b>6g</b>	$52.1 \pm 4.8$	NT <sup>b</sup>	<b>6n</b>	$25.2 \pm 0.5$	NT <sup>b</sup>
NSC-87877	—	$26.18 \pm 8.58$	—	—	—

<sup>a</sup>  $\text{IC}_{50}$  values were determined by regression analyses and expressed as means  $\pm$  SD of three replications. <sup>b</sup> NT means not tested.



Table 4 The IC<sub>50</sub> values of compounds **6a** and **6c** against PTPs<sup>a</sup>

Comp.	IC <sub>50</sub> (μM)			
	PTP1B	TCPTP	SHP2	TCPTP/PTP1B
<b>6a</b>	8.04 ± 0.71	34.93 ± 3.21	12.17 ± 3.13	4.34
<b>6c</b>	8.96 ± 1.22	30.75 ± 2.97	7.84 ± 0.98	3.43
NSC-87877	26.18 ± 8.58	71.87 ± 3.87	5.09 ± 2.03	2.74

<sup>a</sup> TCPTP, T-cell protein tyrosine phosphatase; SHP-2, SH2-Containing Protein Tyrosine Phosphatase-2; IC<sub>50</sub> values were determined by regression analyses and expressed as means ± SD of three replications.

exhibited 4.34-fold and 3.43-fold greater selectivity for PTP1B than for TCPTP respectively. Besides TCPTP, we tested the inhibitory activity of **6a** and **6c** against SHP2, both of them showed similar activities against PTP1B. These results indicated that the scaffold of thieno[2,3-*b*]quinolines preferred PTP1B compared to the homogeneous scaffolds, such as bis-aryl amides and benzo[*c*][1,2,5]thiadiazoles fit for SHP2, and the scaffold hopping strategy provided an efficient way to obtain selective modulator for specific PTPs.

## Conclusions

In summary, we have developed a sustainable protocol for synthesis of 2-ethoxycarbonylthieno[2,3-*b*]quinolines with a simple precipitate procedure by addition of water for the removal of the high boiling solvent GVL, providing an important alternative approach to the current industrial use of toxic solvent DMF and THF. In addition, Biological evaluation demonstrated that some of the synthesized 2-ethoxycarbonylthieno[2,3-*b*]quinolines showed inhibitory activity against PTP1B, and the representative compound **6a** displayed an IC<sub>50</sub> value of 8.04 ± 0.71 μM with 4.34-fold preference over TCPTP. These preliminary results provided a possible opportunity for the development of novel PTP1B inhibitors.

## Conflicts of interest

The authors have declared no conflict of interest.

## Acknowledgements

This work was supported by National Natural Science Foundation of China (21772068), National Science & Technology Major Project “Key New Drug Creation and Manufacturing Program”, China (Number: 2018ZX09711002), Natural Science Foundation of Jiangsu Province (BK20190608).

## Notes and references

- 1 D. E. Moller, *Nature*, 2001, **414**, 821–827.
- 2 R. A. Bartolome, Á. Martín-Regalado, M. Jaén, M. Zannikou, P. Zhang, V. Ríos, I. V. Balyasnikova and J. I. Casal, *Cancers*, 2020, **12**, 500.

- 3 H. Hussain, I. R. Green, G. Abbas, S. M. Adekenov, W. Hussain and I. Ali, *Expert Opin. Ther. Pat.*, 2019, **29**, 689–702.
- 4 L. Wu, Y. Wang, H. Song, L. Tang, Z. Zhou and C. Tang, *Adv. Synth. Catal.*, 2013, **355**, 1053–1057.
- 5 B. M. Kiran, B. P. Nandeshwarappa, V. P. Vaidya and K. M. Mahadevan, *Phosphorus, Sulfur Silicon Relat. Elem.*, 2007, **182**, 969–980.
- 6 A. A. Geies, E. A. Bakhite and H. S. El-Kashef, *Pharmazie*, 1998, **53**, 686.
- 7 Y. Zhao, M. Li, B. Li, S. Zhang, A. Su, Y. Xing, Z. Ge, R. Li and B. Yang, *Eur. J. Med. Chem.*, 2019, **172**, 131–142.
- 8 P. Mahajan, M. Nikam, A. Asrondkar, A. Bobade and C. Gill, *J. Heterocycl. Chem.*, 2016, **54**, 1415–1422.
- 9 R. Satheeshkumar, R. Zhu, B. Feng, C. Huang, Y. Gao, L. Gao, C. Shen, T. Hou, J. Li, Y. Zhou and W. Wang, *Bioorg. Med. Chem. Lett.*, 2020, **30**, 127170.
- 10 W. Wang, C. Huang, L. Gao, C. Tang, J. Wang, M. Wu, L. Sheng, H. Chen, F. Nan, J. Li, J. Li and B. Feng, *Bioorg. Med. Chem. Lett.*, 2014, **24**, 1889–1894.
- 11 W. Wang, X. Chen, L. Gao, L. Sheng, J. Li, J. Li and B. Feng, *Chem. Biol. Drug Des.*, 2015, **86**, 1161–1167.
- 12 W. Wang, H. Luo, Y. Gao, L. Gao, L. Sheng, Y. Zhou, J. Li, J. Li and B. Feng, *Chin. J. Org. Chem.*, 2016, **36**, 2142–2149.
- 13 W. Wang, X. Chen, Y. Gao, L. Gao, L. Sheng, J. Zhu, L. Xu, Z. Ding, C. Zhang, J. Li, J. Li and Y. Zhou, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 5154–5157.
- 14 W. Wang, D. Yang, L. Gao, C. Tang, W. Ma, H. Ye, S. Zhang, Y. Zhao, H. Xu, Z. Hu, X. Chen, W. Fan, H. Chen, J. Li, F. Nan, J. Li and B. Feng, *Molecules*, 2013, **19**, 102–121.
- 15 G. Schneider, W. Neidhart, T. Giller and G. Schmid, *Angew. Chem.*, 1999, **38**, 2894–2896.
- 16 G. Hessler and K. Baringhaus, *Drug Discovery Today: Technol.*, 2010, **7**, e263–e269.
- 17 M. C. Bryan, B. Dillon, L. G. Hamann, G. J. Hughes, M. E. Kopach, E. A. Peterson, M. Pourashraf, I. Raheem, P. Richardson, D. Richter and H. F. Sneddon, *J. Med. Chem.*, 2013, **56**, 6007–6021.
- 18 I. Aliagas, R. Berger, K. Goldberg, R. T. Nishimura, J. Reilly, P. Richardson, D. Richter, E. C. Sherer, B. A. Sparling and M. C. Bryan, *J. Med. Chem.*, 2017, **60**, 5955–5968.
- 19 J. B. Zimmerman, P. T. Anastas, H. C. Erythropel and W. Leitner, *Science*, 2020, **367**, 397–400.
- 20 J. L. Tucker, *Org. Process Res. Dev.*, 2006, **10**, 315–319.
- 21 R. A. Sheldon, *Curr. Opin. Green Sustainable Chem.*, 2018, **18**, 13–19.
- 22 B. P. Nandeshwarappa, D. B. Aruna Kumar, H. S. Bhojya Naik and K. M. Mahadevan, *J. Sulfur Chem.*, 2005, **26**, 373–379.
- 23 A. Kumar, Y. E. Jad, J. M. Collins, F. Albericio and B. G. D. L. Torre, *ACS Sustainable Chem. Eng.*, 2018, **6**, 8034–8039.
- 24 K. D. Collins, T. Gensch and F. Glorius, *Nat. Chem.*, 2014, **6**, 859–871.
- 25 A. Widener, *C&EN Global Enterprise*, 2019, **97**, 14.
- 26 F. Gao, R. Bai, F. Ferlin, L. Vaccaro, M. Li and Y. Gu, *Green Chem.*, 2020, **22**, 6240–6257.



27 R. K. Henderson, C. Jiménez-González, D. J. C. Constable, S. R. Alston, G. G. A. Inglis, G. Fisher, J. Sherwood, S. P. Binks and A. D. Curzons, *Green Chem.*, 2011, **13**, 854–862.

28 C. J. Clarke, W. Tu, O. Levers, A. Bröhl and J. P. Hallett, *Chem. Rev.*, 2018, **118**, 747–800.

29 B. H. Lipshutz, F. Gallou and S. Handa, *ACS Sustainable Chem. Eng.*, 2016, **4**, 5838–5849.

30 Z. Li, K. H. Smith and G. W. Stevens, *Chin. J. Chem. Eng.*, 2016, **24**, 215–220.

31 H. J. Salavagione, J. Sherwood, M. D. Bruyn, V. L. Budarin, G. J. Ellis, J. H. Clark and P. S. Shuttleworth, *Green Chem.*, 2017, **19**, 2550–2560.

32 S. Lawrenson, M. North, F. Peigneguy and A. Routledge, *Green Chem.*, 2017, **19**, 952–962.

33 F. G. Calvo-Flores, M. J. Monteagudo-Arrebola, J. A. Dobado and J. Isac-García, *Top. Curr. Chem.*, 2018, **376**, 18.

34 U. Tilstam, *Org. Process Res. Dev.*, 2012, **16**, 1273–1278.

35 L. Mistry, K. Mapesa, T. W. Bousfield and J. E. Camp, *Green Chem.*, 2017, **19**, 2123–2128.

36 T. W. Bousfield, K. P. R. Pearce, S. B. Nyamini, A. Angelis-Dimakis and J. E. Camp, *Green Chem.*, 2019, **21**, 3675–3681.

37 K. L. Wilson, J. Murray, C. Jamieson and A. J. B. Watson, *Org. Biomol. Chem.*, 2018, **16**, 2851–2854.

38 J. Song, B. Zhou, H. Liu, C. Xie, Q. Meng, Z. Zhang and B. Han, *Green Chem.*, 2016, **18**, 3956–3961.

39 O. A. Musaimi, A. El-Faham, A. Basso, B. G. D. I. Torre and F. Albericio, *Tetrahedron Lett.*, 2019, **60**, 151058.

40 H. Q. Lê, J. Pokki, M. Borrega, P. Uusi-Kyyny, V. Alopaeus and H. Sixta, *Ind. Eng. Chem. Res.*, 2018, **57**, 15147–15158.

41 M. A. Rasool and I. F. J. Vankelecom, *Green Chem.*, 2019, **21**, 1054–1064.

42 J. Winters, W. Dehaen and K. Binnemans, *Green Chem.*, 2020, **22**, 6127–6136.

43 F. Ferlin, L. Luciani, S. Santoro, A. Marrocchi, D. Lanari, A. Bechtoldt, L. Ackermann and L. Vaccaro, *Green Chem.*, 2018, **20**, 2888–2893.

44 G. Strappaveccia, E. Ismalaj, C. Petrucci, D. Lanari, A. Marrocchi, M. Drees, A. Facchetti and L. Vaccaro, *Green Chem.*, 2015, **17**, 365–372.

45 C. Zhang, R. Zhu, Z. Wang, B. Ma, A. Zajac, M. Smiglak, C. Xia, S. L. Castle and W. Wang, *RSC Adv.*, 2019, **9**, 2199–2204.

46 L. F. Iversen, K. B. Moller, A. K. Pedersen, G. H. Peters, A. S. Petersen, H. S. Andersen, S. Branner, S. B. Mortensen and N. P. Moller, *J. Biol. Chem.*, 2002, **277**, 19982.

47 L. K. Debarba, F. M. V. Vechiato, H. Veida-Silva, B. C. Borges, M. C. Jamur, J. Antunes-Rodrigues and L. L. K. Elias, *Mol. Cell. Endocrinol.*, 2019, **482**, 62–69.

48 S. Welte, K. Baringhaus, W. Schmider, G. Müller, S. Petry and N. Tennagels, *Anal. Biochem.*, 2005, **338**, 32–38.

49 N. Krishnan, D. Koveal, D. H. Miller, B. Xue, S. D. Akshinthala, J. Kragelj, M. R. Jensen, C. Gauss, R. Page, M. Blackledge, S. K. Muthuswamy, W. Peti and N. K. Tonks, *Nat. Chem. Biol.*, 2014, **10**, 558–566.

