RSC Advances

PAPER

View Article Online
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Cite this: *RSC Adv.*, 2022, **12**, 22054

A green synthesis and antibacterial activity of ferrocene-based thiazole derivatives in choline chloride/glycerol eutectic solvent†

Di Zhao,[a] Yanying Liu,[b] Yang Li *[a] and Yu Chen*[c]

Received 23rd July 2022
Accepted 2nd August 2022

DOI: 10.1039/d2ra04587g

rsc.li/rsc-advances

In this work, a green Hantzsch synthesis of 4-ferrocenylthiazole derivatives has been accomplished successfully. The Hantzsch reaction between bromoacetylferrocene and various aryl thioureas, 1-alkylindole-3- or 9-alkylcarbazole-3-carbothioamides proceeded efficiently in a deep eutectic solvent (DES) that is, choline chloride/glycerol (ChCl/Gly) (1 : 2 molar ratio) at 80 °C, avoiding the use of common volatile organic solvents. Moreover, the DES media could be reused up to three times without any appreciable decrease in the yield. The synthetic strategy has the attractive features such as mild and environmentally benign reaction conditions, experimental simplicity, easy work-up procedure and good yields. Subsequently, a preliminary screening for *in vitro* antibacterial activities of all these newly-synthesized compounds revealed that the halo-substituted (F, Cl, Br) compounds **3f–h** showed significant antibacterial activities against Gram (+) bacterial *B. subtilis* and Gram (–) *E. coli*, among which the fluoro-substituted **3f** possessed the best activity with the MIC value of 7.8125 μg mL^{-1}, being higher than the reference drug ciprofoxacin (15.625 μg mL^{-1}).

Introduction

Thiazole is a valuable heterocyclic unit present in numerous pharmaceuticals.[1,2] Derivatives of this family have exhibited a broad spectrum of biological activities such as antimicrobial,[3] anticancer,[4] antitubercular[5] and anti-inflammatory properties,[6] and have been extensively studied for current drug discovery. Consequently, considerable synthetic efforts have been invested surrounding the thiazole ring for further modification and functionalization to enhance the potency of this class of heterocycles.[7] On the other hand, it has been well established that the introduction of ferrocene nucleus into organic heterocyclic compounds might often lead to a hybrid product with enhanced or unexpected activity compared to that of the parent compound.[8] This could be rationalized as being due to the unique properties of the ferrocene nucleus such as membrane permeation, aqueous stability, anomalous metabolism, and redox behaviour.[9] In light of these findings, the incorporation of ferrocene moiety and thiazole ring into a molecular framework would be recognized as an attractive way to endow important candidates for medicinal applications, though the related reports concerning the synthesis of such ferrocene–thiazole hybrids are very few so far. In this context, Ma *et al.*[10] reported the synthesis of ferrocene-based thiazole Schiff bases with potential antibacterial activity, involving the Hantzsch reaction of chloroacetylferrocene with thiourea followed by the condensation with aromatic aldehydes (eqn (a), Scheme 1). Wrona *et al.*[11] described the synthesis of 2-ferrocenyl-4-hydroxythiazoles by cyclization of ferrocenyl thioimidates in the presence of sodium ethoxide (eqn (b), Scheme 1). Tarraga *et al.*[12] reported the synthesis of ferrocenylthiazoles and 2,5-bis(ferrocenyl)thiazoles *via* acylation of α-aminoacetylferrocene with benzoyl chloride or chlorocarbonyl ferrocene followed by the action of the Lawesson's reagent (LR) (eqn (c), Scheme 1).

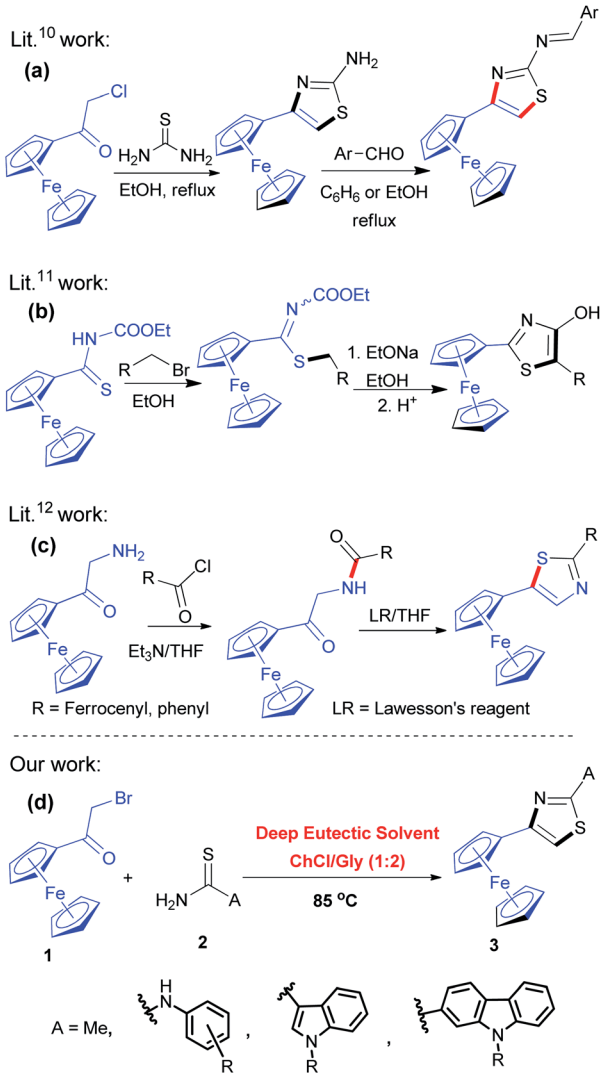
In light of the current stringent environmental requirements and safety consideration in the chemical production, increasing research effort has been focused on the development of sustainable and environmentally benign reaction procedures to replace those efficient but somewhat outdated methods, particularly, to replace hazardous volatile organic solvents.[13] During the past decade, deep eutectic solvents (DESs), as an emerging class of unconventional solvents derived from the combination of two or three safe and cheap components of Lewis or Brønsted acids and bases through hydrogen bond formation, have attracted enormous attention due to their unique properties including wide liquid range, negligible vapor pressure, low toxicity, non-flammability, high biodegradability, low cost of components and convenient preparation,[14] thus rendering them widely acknowledged as an excellent alternative to volatile organic solvents in the development of

[a]*College of Chemistry and Chemical Engineering, Bohai University, Jinzhou 121013, China. E-mail: bhuzh@163.com*

[b]*Research Institute of Jinzhou Petrochemical Company, Jinzhou 121013, China*

[c]*School of Life Science and Biopharmaceutics, Shenyang Pharmaceutical University, Shenyang, 110016, China. E-mail: 569180398@qq.com*

† Electronic supplementary information (ESI) available. See https://doi.org/10.1039/d2ra04587g



Scheme 1 Some synthetic routes for ferrocene-based thiazole hybrids.

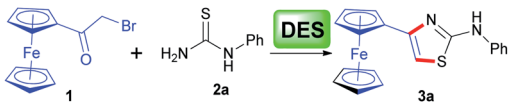
environmentally friendly organic reactions.[15] Against this background and in continuation of our interest toward the development of new efficient synthetic methodologies for the construction of heterocycles,[16,17] we would like to report, herein, a new and green Hantzsch thiazole synthesis of various structurally intriguing ferrocene-based derivatives (**3**) by using the DES choline chloride/glycerol (ChCl/Gly) as a safe, eco-friendly and unconventional solvent as outlined in eqn (d) of Scheme 1. As far as we know, no related reports are available concerning the application of the DES for the green synthesis of ferrocene-based thiazole derivatives.

Results and discussion

As well known, the classical and well-established Hantzsch reaction protocol is a preferred approach for the construction of thiazole derivatives with the use of α-haloketones and thiourea as the reactants in refluxing organic solvents such as methanol or ethanol.[18,19] Currently, DESs have been increasingly applied as possible alternative 'green' and bio-renewable solvents for organic transformations, avoiding many disadvantages of common hazardous volatile organic solvents.[20] Thus, following the line of green chemistry we envisioned that the application of deep eutectic solvents in the Hantzsch thiazole synthesis of ferrocene-based derivatives would attract significant interest.

Accordingly, we initially conducted the model reaction of bromoacetylferrocene (**1**) with 1-phenylthiourea (**2a**) by using the widely used DES, namely ChCl–urea (1 : 2) as the media at 80 °C, which was reported to yield good results in the heterocyclodehydration reaction between α-chloroketones and guanidine derivatives.[21] However, we observed that in our case a mixture of products was produced as shown by TLC analysis after completion of the reaction, from which the desired product was isolated only in a low yield of 19% (entry 1, Table 1), while the unexpected by-product, identified as 4-ferrocenyloxazol-2-amine, was formed as a major product in 63% yield. The reason for this attributed presumably to the fact that α-bromoketone **1** might be more inclined to react with the urea component of the ChCl/urea to give the corresponding 2-aminooxazole. It was worthy to mention that in consistent with our observations, Singh *et al.*[22] also described a similar reaction, wherein phenacyl bromide reacted with the urea component of ChCl–urea to deliver the 4-phenyloxazol-2-amine as a final product. Switching ChCl–urea to ChCl/thiourea was also to no avail (entry 2, Table 1). Recently, Xiao *et al.*[23] reported the use of ChCl/$ZnCl_2$ as solvent for the efficient Hantzsch dihydropyridine synthesis. However, our attempt to follow the purported approach was frustrated as well by a poor yield with a number of side-products being evident (entry 3, Table 1). In order to pick a suitable DES for the model reaction, a series of ChCl-based DESs were screened. Upon using ChCl/glucose (ChCl/Glu),[24] no formation of the desired product was observed and the starting materials were recovered unchanged (entry 4, Table 1). We also attempted to use ChCl/oxalic acid (ChCl/OA)[25] or ChCl/polyethylene glycol (ChCl/PEG)[26] as solvent, but the results were still not satisfactory as we expected (entries 5 and 6, Table 1). After these fruitless attempts, we were delighted to find that ChCl/Gly[27] could be used as an efficient solvent, in which the reaction proceeded smoothly, giving the desired compound **3a** in a remarkably high yield of 73% (entry 7, Table 1). The reason for the effectiveness of ChCl/Gly solvent might be due to its relatively low viscosity, good solubility, high stability and positive synergic effect on the reaction through extensive hydrogen bonding in comparison with other DESs. Further, in order to determine the optimum reaction conditions, the effect of different reaction temperature and molar ratio of the ChCl/Gly was examined. We observed that the best result could be achieved when the reaction was carried out at 85 °C (entry 10, Table 1), but altering the molar ratio resulted in no further improvement in the product yield (entries 13 and 14, Table 1). In addition, in order to highlight the advantages of the DES solvent, we also carried out the model reaction only in glycerol media at 85 °C or in organic solvent such as ethanol at refluxing temperature as described in literature.[10] We found that the yield of the product obtained from the reaction in glycerol was only

Table 1 Hantzsch reaction of bromoacetylferrocene (**1**) with 1-phenylthiourea (**2a**) in different DES media[a]



Entry	Solvent	Temp./°C	Time/h	Yield[b]/%
1	ChCl/urea (1 : 2)	80	10	19
2	ChCl/thiourea (1 : 2)	80	10	13
3	ChCl/$ZnCl_2$ (1 : 2)	80	10	27
4	ChCl/Glu (1 : 1)	80	10	NR[c]
5	ChCl/OA (1 : 1)	80	10	21
6	ChCl/PEG (1 : 2)	80	10	44
7	ChCl/Gly (1 : 2)	80	6	73
8	ChCl/Gly (1 : 2)	70	6	61
9	ChCl/Gly (1 : 2)	75	6	66
10	**ChCl/Gly (1 : 2)**	**85**	**6**	**82**
11	ChCl/Gly (1 : 2)	90	6	78
12	ChCl/Gly (1 : 2)	95	6	76
13	ChCl/Gly (1 : 1)	85	6	38
14	ChCl/Gly (1 : 3)	85	6	53
15	Glycerol	85	12	38
16	EtOH	Reflux	12	65
17	ChCl/Gly (1 : 2)[d]	85	6	82
18	ChCl/Gly (1 : 2)[e]	85	6	81
19	ChCl/Gly (1 : 2)[f]	85	6	79
20	ChCl/Gly (1 : 2)[g]	85	6	67

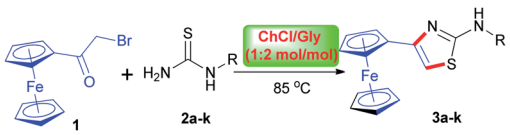
[a] Reaction conditions: bromoacetylferrocene **1** (0.5 mmol) and 1-phenylthiourea (**2a**) (0.55 mmol). [b] Isolated yield. [c] NR means no reaction. [d] Reaction in the 1st recovered DES solvent. [e] Reaction in the 2nd recovered DES solvent. [f] Reaction in the 3rd recovered DES solvent. [g] Reaction in the 4th recovered DES solvent.

38% after 12 hours (entry 15, Table 1), which suggested that the reaction gave a good yield due to ChCl/Gly as solvent and not due to its individual glycerol component. Likewise, the model reaction in ethanol medium delivered the product **3a** in a moderate yield of 65% after 12 hours (entry 16, Table 1). Thus, we could conclude that the application of ChCl/Gly is superior over the conventional solvent procedure.

Finally, the recyclability of the ChCl/Gly for the model reaction was investigated under the optimized conditions. After completion of the reaction, an equal volume of water was added to the reaction mixture to completely precipitate out the resulting product, which was then extracted using dichloromethane. And the DES ChCl/Gly could be recovered by removing water from the aqueous layer under vacuum and reused for the next run. Thus, the fresh substrates **1** and **2a** were added to the recovered ChCl/Gly to repeat the model reaction. We found that the recovered ChCl/Gly could be re-used up to three consecutive runs without significant decrease in **3a** yield (entries 17–19, Table 1), though a slight darkening of the eutectic mixture was observed after recycling. However, starting from the fourth cycle, a significant drop in the product yield was noticed (entry 20, Table 1).

To demonstrate the synthetic potential by using ChCl/Gly as the privileged reaction medium in the Hantzsch thiazole

Table 2 Yields and physical properties of the targeted compounds **3a–k**



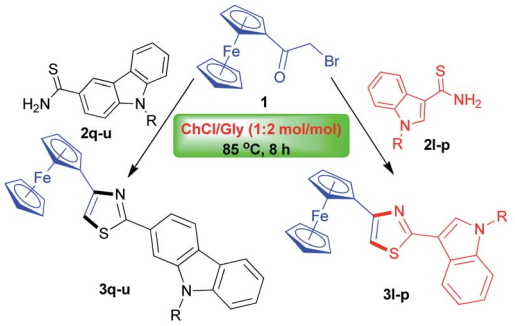
Entry	Compd	R	Yield[a]/%	Mp/°C
1	**3a**	Phenyl	82	161–162
2	**3b**	*o*-Tolyl	79	167–168
3	**3c**	*p*-Tolyl	87	159–160
4	**3d**	4-Methoxyphenyl	91	168–169
5	**3e**	4-Ethylphenyl	85	139–141
6	**3f**	4-Fluorophenyl	76	145–146
7	**3g**	4-Chlorophenyl	80	177–178
8	**3h**	4-Bromophenyl	83	163–164
9	**3i**	*o*-Hydroxyphenyl	74	189–190
10	**3j**	Methyl	76	144–145
11	**3k**	Piperonyl	81	175–176

[a] Isolated yield.

synthesis, we extended the reaction to other substituted 1-phenylthioureas in a similar fashion. Satisfactorily, these substances were equally amenable to the reaction process without any experimental difficulties, successfully furnishing the corresponding **3b–k** in satisfactory yields of 76–91% as listed in Table 2. Moreover, in all cases, the recyclability of the DES system could be successfully achieved. It appeared that the nature of the substituent present in the benzene ring did not exert an obvious influence on the product yields. For example, products **3b–e** with electron-donating methyl, methoxyl or ethyl substituent (entries 2–5, Table 2) on the benzene ring were obtained in comparable yields with products **3f–h** bearing electron-withdrawing groups such as fluoro, chloro, and bromo substituent (entries 6–8, Table 2). Further, we attempted the reaction with 1-(2-hydroxyphenyl)thiourea, 1-methylthiourea and 1-(benzo[*d*][1,3]dioxol-5-yl)thiourea under the same reaction conditions. As expected, these species were also viable substrate for this transformation, invariably giving the expected products **3i**, **3j** and **3k** in satisfactory yields of 74%, 76% and 81%, respectively (entries 9–11, Table 2).

Recently, the synthesis of biologically interesting indole- or carbazolo-based thiazole hybrids have been frequently reported through molecular hybridization approach,[28–30] and these hybrids have showed potent antimicrobial, antioxidant and anticancer activities.[31–33] Prompted by these reports and with the aim of further diversifying our work to give rise to a new dimension of structural diversity as potential candidates for biological evaluation, we became very interested in the synthesis of the novel ferrocene-based indole/carbazolo-thiazole hybrids. To this purpose, an analogous series of reactions were performed using the readily synthesized 1-alkylindole-3- (**2l–p**) or 9-alkylcarbazole-3-substituted carbothioamides (**2q–u**)[34] as the reactants in place of aryl thioureas under the same reaction conditions. To our delight, their

Table 3 Yields and physical properties of ferrocene-based indole/carbazolo-thiazole hybrids



Entry	Compd	R	Yield[a]/%	Mp/°C
1	**3l**	Me	74	122–123
2	**3m**	Et	71	116–118
3	**3n**	*n*-Bu	68	95–96
4	**3o**	Bn	76	167–168
5	**3p**	*P*-ClBn	73	178–180
6	**3q**	Me	72	163–164
7	**3r**	Et	68	149–150
8	**3s**	*n*-Bu	65	108–109
9	**3t**	Bn	70	161–162
10	**3u**	*P*-ClBn	66	178–180

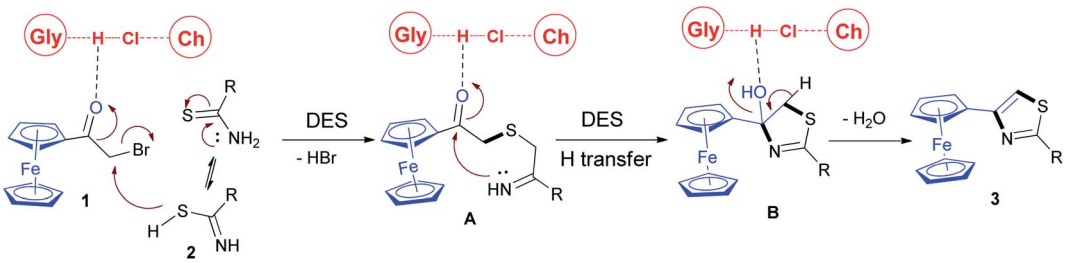
[a] Isolated yield.

Hantzsch reaction proceeded smoothly as well, furnishing the expected 2-(1-alkyl-1*H*-indol-3-yl)-4-ferrocenylthiazole (**3l–p**) and 2-(9-alkyl-9*H*-carbazol-2-yl)-4-ferrocenylthiazoles (**3q–u**) as listed in Table 3. From the experiments of Table 3, we found that in the Hantzsch reaction with bromoacetylferrocene the reactivity of 1-alkylindole-3-carbothioamides and 9-alkylcarbazole-3-carbothioamides was slightly lower in comparison with those of aryl thioureas, thus requiring slightly longer reaction time of 8 hours with somewhat lower product yields of 65–74%.

On the basis of a recent report concerning the reactivity of α-chloro oximes in DESs,[27] a proposed reaction mechanism for the synthesis of the title compounds in ChCl/Gly was outlined in Scheme 2. The reaction might first involved in the nucleophilic attack of the thiourea or thioamide sulfur atom on the α-carbon of the bromoacetyl group to form the intermediate **A** with the elimination of an equivalent of HBr. In this reaction process, the interaction of ChCl/Gly with the oxygen atom of the α-bromoketone could enhance the reactivity of the bromoacetyl moiety and facilitate the nucleophilic attack by the sulfur atom. Subsequently, this conversion was followed by the *in situ* intramolecular nucleophilic cyclization reaction involving the participation of the imine N-atom and the carbonyl C=O group to form the intermediate **B**.[35] In the cyclisation reaction step the activation of intermediate **A** by extensive hydrogen bonding with DES played a key role, increasing the electrophilicity of the carbonyl group and enhanced the rate of the cyclisation.[36] Subsequently, the generated hydroxyl group formed hydrogen bonded with the DES, thus facilitating a loss of water to produce the final products **3**.

Currently, due to the serious concern related to the resistance of pathogenic bacteria towards the clinically used antibacterial drugs, screening new class of compounds for development of new antibacterial drugs is an urgent priority to overcome the increasing danger of drug-resistant problems. Thus, considering thiazole ring being a characteristic component of numerous antibacterial reagents,[37] a preliminary evaluation for their *in vitro* antibacterial activities against *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) as Gram (+), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) as Gram (−) was assayed by measuring minimum inhibitory concentrations (MICs), and the test results were recorded in Table 4.

As shown in Table 4, the series of *N*-substituted-4-ferrocenylthiazol-2-amines (**3a–k**) exhibited moderate to good antibacterial activity against the Gram (+) bacterial *B. subtilis* and Gram (−) *E. coli.*, whereas 2-(indol-3-yl)- (**3l–p**) and 2-(carbazol-2-yl)-4-ferrocenylthiazoles (**3q–u**) showed poor antibacterial activities against all the tested pathogenic bacteria. In the series of **3a–k**, compound *N*-phenyl-4-ferrocenylthiazol-2-amine (**3a**) showed moderate activities against *B. subtilis* and *E. coli* (entry 1, Table 4). The introduction of electron-donating substituents such as methyl, methoxyl and ethyl group into phenyl moiety as in compounds **3b–e**, resulted in much lower activities against the two bacterial strains (entries 2–5, Table 4), which demonstrated that the introduction of the electron-donating substituents was not conducive to their inhibitory activities. Interestingly, we observed that the presence of halo group such as F, Cl and Br gave an significant improvement of the inhibitory activities, increasing the antibacterial potential with the order of F > Br > Cl (entries 6–8, Table 4). Especially, the most active fluoro-substituted **3f** with the MIC value of 7.8215



Scheme 2 The proposed mechanistic pathway for synthesis of the title compounds **3**.

Table 4 Antibacterial activity of the compounds **3a–u** [MIC/(μg mL^{-1})]

Entry	Compd	*B. subtilis*	*S. aureus*	*E. coli*	*P. aeruginosa*
1	**3a**	31.25	62.5	31.25	125
2	**3b**	125	62.5	125	125
3	**3c**	125	250	125	250
4	**3d**	31.25	62.5	62.5	125
5	**3e**	125	125	62.5	250
6	**3f**	7.8215	62.5	7.8125	125
7	**3g**	15.625	31.25	15.625	62.5
8	**3h**	7.8215	31.25	15.625	125
9	**3i**	31.25	15.625	31.25	125
10	**3j**	31.25	125	31.25	62.5
11	**3k**	62.5	31.25	31.25	62.5
12	**3l**	62.5	125	31.25	125
13	**3m**	250	250	125	125
14	**3n**	250	125	125	125
15	**3o**	62.5	125	125	125
16	**3p**	62.5	125	31.25	125
17	**3q**	250	250	250	250
18	**3r**	62.5	125	125	125
19	**3s**	62.5	125	31.25	125
20	**3t**	250	250	125	125
21	**3u**	250	125	125	125
Ref.	Ciprofoxacin	15.625	15.625	15.625	15.625

μg mL^{-1} against *B. subtilis* and *E. coli* was much superior to the reference drug ciprofoxacin (entry 6, Table 4), and thus might be interesting and promising candidates for further biological research. In addition, it has been found that the *o*-hydroxyphenyl-substituted product **3i** exhibited a good antibacterial effect against *S. aureus* with the MIC value of 15.625 μg mL^{-1}, being comparable with the reference drug (entry 9, Table 4). These insights from the *in vitro* antibacterial activity might provide valuable information for further optimization of the series of derivatives, and hopefully have the potential to further exploitation in new antibacterial drug discovery. Our next efforts will mainly focus on the structural activity relationship study by structural optimization towards the ultimate goal of providing intriguing lead compounds for the development of novel and effective antibacterial agents.

Conclusions

In conclusion, a green and facile synthesis of structurally intriguing ferrocene-based thiazoles using the readily available, environmentally benign and non-toxic DES ChCl/Gly as reaction medium has been accomplished. Our synthetic strategy have the attractive advantages of mild reaction conditions, simple experimental operation, wide range of applicable substrates, easy purification procedure, and good product yields. The recyclability and biodegradability of the ChCl/Gly make this methodology highly sustainable and reliable. These synthesized compounds belong to a new class of ferrocene–thiazole hybrids and a preliminary evaluation for their *in vitro* antibacterial bioassay revealed that compounds bearing halogen substitution showed potent and promising activity against Gram (+) bacterial *B. subtilis* and Gram (−) *E. coli* with the MIC value being equipotent or even better than the reference drug ciprofoxacin. These results might give an important insight to future optimization of the series of ferrocene–thiazole hybrids. Currently, work is ongoing, mainly focusing on the further elaboration and application of these compounds, which represent an intriguing goal that we are contemplating, and these results will be a part of future reports.

Author contributions

Y. Li and Y. Chen conceptualization, D. Zhao and Y. Liu methodology, investigation and synthesis, Y. Chen antibacterial activity assay, Y. Li writing.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank the Doctoral Start-up Foundation of Liaoning Province (grant no. 2019-BS-004) and the Scientific Research Foundation of the Education Department of Liaoning Province (grant no. LQ2019006).

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