An expeditious and environmentally benign synthesis of dispiro-3-phenylpyrrolothiazoles in ACI/EG eutectic mixture and its antioxidant and antimicrobial activities against urinary tract pathogens

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Abstract
The present study reveals the robust and facile methodology for the synthesis of massively selective dispiro-3-phenylpyrrolothiazole hybrids via one-pot 1,3-dipolar cycloaddition reaction by environmentally supported solvents and to evaluate their biological activities. The quaternary ammonium salt eutectic mixture, acetylcholine iodide-ethylene glycol (ACI/EG) medium brings an efficient environment for the synthesis of dispiropyrrrolothiazole with excellent yield in shorter reaction time than imidazolium ionic liquids. The eutectic mixture was recovered and reused without any significant drop in their catalytic activity. Among the eight synthesized compounds 4a–h, halogen derivatives are exhibiting significant antimicrobial activities against selected uropathogens pathogens. Interestingly, chloro and bromo derivatives exhibits the minimum inhibitory concentration (MIC) of 12.5 μg/ml and 6.25 μg/ml towards Escherichia coli, Klebsiella pneumonia, and Staphylococcus aureus respectively. In addition, the IC50 values of DPPH radicals with synthesized compounds are interesting, particularly compounds 4a, 4d and 4e shows lower than the control BHA indicating their potent scavenging ability of free radicals.

Keywords: Eutectic mixture, ACI/EG, Green protocol, Dispiro-3-phenylpyrrolothiazole, Uropathogens

Introduction
Adult women become frequent victims than men at a ratio of 8:1 for urinary tract infection (UTI) because of their anatomical differences. In general, Gram-positive and Gram-negative bacteria and certain fungi are the causative agents of UTI. Frequent infection is the most important factor contributing to high risk UTI which results in pyelonephritis with sepsis, renal damage, kidney damage, bladder inflammation and urethritis. By nature, urinary track is sterile but colonization of pathogens, particularly Uropathogenic Escherichia coli (UPEC) in the urinary tract will leads to highly complicated UTI [1]. The pathogens viz. Staphylococcus saprophyticus, Enterococcus faecalis, Pseudomonas aeruginosa and Candida spp. may create non-complicating infections in the initial stage. Unfortunately, followed by UPEC infection, Enterococcus spp., Klebsiella pneumoniae, Candida spp., Staphylococcus aureus, P. aeruginosa, P. mirabilis, and group B Streptococcus (GBS) are the responsible pathogens for complicated UTI. UTI is more precarious at the time of pregnancy for both parental and infant health because it can affect kidney easily [2]. Nosocomial infections [3, 4] and increasing resistance to antimicrobial agents [5] are the other unavoidable risk factors associated with UTI treatment. Though antibiotics can be used to treat UTI, long-term treatment creates antibiotic
resistance which leads to negative side-effect of UTI treatment including exhaustion of beneficial gut and mucosal microorganism, hypersensitivity, and suppressing immune development in the body [6]. Certainly, there is a need to develop a highly potent and efficient antimicrobial drug for the treatment of infectious diseases which is suitable to treat patients.

Heteroatom consisting cyclic hydrocarbons are fascinating molecules because of their treasured applications in medicinal field. Particularly, structurally rigid spiro heterocyclic analogues showed highly pronounced pharmacological properties and exist in many naturally occurring alkaloids [7]. Having medicinal values, thiazolidine ring systems play an important role in organic synthesis particularly antimicrobial substances such as penicillins, cephalosporins, narcocidins, thienamycin and other compounds that have physiological activities have been prepared from thiazolidine [8]. It is noted that spiropyrrollothiazole analogues are interesting because of their wide range of biological activities such as anti-cancer [9], anti-diabetic [10], antibiotic [11], anti-inflammatory [12], hepatoprotective [13], anti-convulsant [14], anti-leukemic agents [15], Alzheimer disease [16], and also good in anti-mycobacterial [17]. Spiro indolin-2-one nucleus was reported to treat diabetic patients, HIV-1 protease inhibitors, potent gastrin/CCK-B receptor antagonist and for growth hormone secretagogue receptor agonists [18]. Also, indane-1,3-dione displays anti-blood coagulation, anti-inflammatory, anti-biotic and anti-convulsant activities [19]. Synthesis and antimicrobial screening of a series of structurally complexed molecules with the above mentioned molecular units viz. indane-1,3-dione, indolin-2-one, and pyrrolothiazole ring with spiro junction will be a novel compound with efficient antimicrobial activities against UTI.

The design of present molecular framework stems from the promising UTI activity of thiazolidine units reported by Reese et al. [20]. In addition, the supposition that the rigidity of spiro molecular frameworks would easily binds with the biomolecules, prompted us to synthesize spiro-pyrrolothiazolidine derivatives for UTI applications. In these contexts, multicomponent 1,3-dipolar cycloaddition reactions are highly suitable and powerful methodology for the construction of pharmacologically valuable heterocyclic compounds. Particularly the chemistry of azomethine ylide 1,3-dipolar cycloaddition reaction is an interesting, less time consuming, and significant for construction of heterocyclic compounds with high stereo and chemoselectivity [21, 22]. Considering framework of environmental green chemical approach, alternative medium for organic solvents occupying predominating place in the organic synthesis. The required reaction medium must have environmentally benign criteria such as non-toxicity, biodegradability, availability, recyclability and also in economically beneficial among others. However, interesting green protocols have been reported in the literature for the synthesis of heterocyclic compounds through multicomponent reaction including microwave assisted [23], solid support and montmorillonite clay catalyzed [24, 25], ultrasonic triggered [26], and with different class of eutectic mixture solvent mediated synthesis [27, 28]. Among the various green protocols, room temperature eutectic mixtures are perspective and effective method for chemical transformations.

Environmentally green quaternary ammonium salt eutectic mixture, Acetylcholine iodide-ethylene glycol (ACI/EG) mediated protocol is an efficient reaction medium for the synthesis of heterocyclic molecules through multicomponent reaction methodology has been reported by our research group recently [29]. In continuous of our research program on the synthesis of spiro heterocyclic derivatives, for the first time, herein we wish to report that ACI/EG as an efficient eutectic mixture medium for the synthesis of biologically significant dispiro-3-phenylpyrrollothiazole analogues and their UTI activities.

**Results and discussion**

**Chemistry**

Cyclization of l-cysteine with benzaldehyde in water medium undergoes a smooth reaction yielding an analogue of proteinogenic amino acid, 2-phenyl-1,3-thiazolidine-4-carboxylic acid (1) at room temperature [30] which is used to generate in situ azomethine ylide with ninhydrin (2). The dipolarophile bearing indolin-2-one group was prepared by reacting oxindole with various p-substituted aromatic aldehde through base catalyst condensation reaction. In the present investigation in a green protocol, the cyclic amino acid, 2-phenyl-1,3-thiazolidine-4-carboxylic acid (1) was reacted with triketone, ninhydrin (2) to generate in situ azomethine ylide, which undergoes one-pot 1,3-dipolar cycloaddition with various para-alkyl/halide substituted 3-arylideneoxindoles (3a–h), as dipolarophiles under optimized reaction conditions. In the initial stage, a pilot reaction was carried out with cyclic amino acid (1), ninhydrin (2), and 3-benzylideneindolin-2-one (3a) by using imidazolium eutectic mixtures viz., [Bmim][OH], [Bmim][Br], [Bmim][BF₄], [Bmim][PF₆], [Bmim][Cl], [Emim][ClO₄], [Emim][CF₃SO₃], [Emim][PF₆], [Emim][NO₃] and also in quaternary ammonium salt eutectic mixture ACI/EG as solvent. Among the various solvent medium, our recent research [29, 31] heads-up us to utilize ACI/EG eutectic mixture as a green solvent reaction medium and it furnished dispiro-3-phenylpyrrollothiazole 4a in excellent yield of 89% in short reaction time at 50 °C.
possesses similar physio-chemical properties as ionic liquids [32], the rationale for the formation of spirocycloaduct follows the same pathway as in ionic liquids [33, 34]. Optimization of the reactions in room temperature and 50 °C were compared and presented in Table 1. The required inexpensive quaternary ammonium salt eutectic mixture, ACI/EG was prepared in good yield by mixing acetylcholine iodide and ethylene glycol at a 1: 9 molar ratio and then the mixture was heated at 70 °C [35].

Having optimized reaction conditions, all the subsequent reactions were performed through decarboxylative azomethine ylide condensation of an equimolar mixture of the reactants 1, 2 and 3a–h in one-pot treated in ACI/EG eutectic mixture at 50 °C for 1 h (Scheme 1). After completion of the reaction (monitored by TLC), the crude product was washed with water and the pure novel dispiro-3-phenylpyrrolothiazoles (4a–h) was isolated through flash column chromatography by using EtOAc:hexane (2:8).

| Table 1 Optimization of the synthesis of dispiropyrolothiazole 4a |
|---|
| **Entry** | **Solvent** | **Time (h)** | **Yield (%)** |
| | | **RT** | **50 °C** |
| 1 | [Bmim][OH] | 4 | 25 | 45 |
| 2 | [Bmim][Br] | 4 | 30 | 48 |
| 3 | [Bmim][Cl] | 4 | 30 | 46 |
| 4 | [Bmim][BF4] | 4 | 32 | 46 |
| 5 | [Bmim][PF6] | 4 | 28 | 49 |
| 6 | [Emim][ClO4] | 4 | 32 | 46 |
| 7 | [Emim][CF3SO3] | 4 | 46 | 52 |
| 8 | [Emim][PF6] | 4 | 38 | 58 |
| 9 | [Emim][NO3] | 4 | 30 | 42 |
| 10 | ACI/EG | 1 | 78 | 89 |

The structure and stereochemistry of all the cycloaducts were confirmed by their spectral data. In the IR spectrum of cycloaduct 4d, the carbonyl groups of indane-1,3-dione exhibited two absorption bands at 1740 cm⁻¹ and 1703 cm⁻¹ and the amide carbonyl carbon exhibited a band at 1695 cm⁻¹. ¹H NMR spectrum of compound 4d showed a doublet at δ 4.42 for Hₐ proton and a multiplet at δ 5.12–5.20 for H₇ proton and their correlation was also evidenced by ¹H-¹H COSY spectrum. The benzyl proton of the thiazolidine ring resonated as a singlet at δ 5.31 and the corresponding carbon signal appeared at 73.6 ppm as evidenced from its ¹H-¹³C COSY by the appearance of isolated contour plot. The two multiplets appeared at δ 3.10–3.16 and δ 3.66–3.73 were due to H₄ protons. In hetero-COSY spectrum, the carbon corresponding to these H₄ protons appeared at 38.2 ppm, which is further confirmed from DEPT-135 analysis. The H₉ proton in tertiary carbon appeared as a multiplet between δ 5.12 and 5.20. The NH proton of the oxindole ring system resonated as a singlet at δ 8.90. In the ¹³C NMR spectrum of cycloaduct 4d, two spiro quaternary carbons appeared at 68.40 and 77.85 ppm. The oxindole carbonyl carbon resonated at 172.76 ppm and indanedione carbonyl carbons appeared at 193.44 and 200.20 ppm. For further clarification, 1D and 2D NMR spectra of compound 4d is provided in the Additional file 1. Finally, the mass spectrum of cycloaduct 4d exhibited the molecular ion peak at m/z 563.07 which confirmed the formation of cycloaduct and the compound gave satisfactory elemental analysis. All the spectral data agreed well with the deduced structure of the cycloaduct. The data in Table 2 show that the ability of formation of cycloaducts in the presence of either electron releasing or electron withdrawing groups at the dipolarophile. The pictorial representation of the chemical shift values for cycloaduct 4d is presented in Fig. 1.

![Scheme 1 Synthesis of indanedione and oxindole tethered dispiropyrolothiazole hybrids](image-url)
The recovered wet eutectic mixture ACI/EG from the reaction mixture was completely purified by applying high vacuum at 60 °C. The catalytic activity of the purified eutectic mixture was further investigated and identified its efficiency for consecutive reaction. The results presented in Fig. 2, clearly show that the catalytic activity and efficiency of the reused eutectic mixture are good and effective for four consecutive runs.

Antimicrobial activity
In the biological studies, the antibacterial activity assessment of dispiropyrrolothiazole compounds 4a–h against the three Gram-negative and three Gram-positive uropathogens and two ATCC reference strains were studied using the agar well diffusion method. The data accompanying with the antibacterial prospective of the dispiropyrrolothiazole compounds are presented in Table S1 in the Additional file 1. The efficacy of antibacterial inhibitory activity diverges with respect to each compound against a panel of uropathogens. The zone of inhibition diameters was a maximum of 26.00 mm and a minimum of 9.00 mm whereas the standard antibiotic streptomycin had a higher zone of inhibition ranging from 15.00 to 30.00 mm (Fig. 3). The dispiropyrrolothiazole compounds exhibited antibacterial activity against almost all tested uropathogens except E. faecalis. The highest inhibition zone was observed on 4e against K. pneumoniae (24.00 mm) and E. coli (22.00 mm) followed by S. aureus (18.00 mm).

| Entry | Compound | Mp (°C) | Yield (%)^a |
|-------|----------|---------|-------------|
| 1.    | 4a       | 132–134 | 89          |
| 2.    | 4b       | 122–124 | 90          |
| 3.    | 4c       | 128–130 | 92          |
| 4.    | 4d       | 140–142 | 88          |
| 5.    | 4e       | 138–140 | 86          |
| 6.    | 4f       | 152–154 | 85          |
| 7.    | 4g       | 112–114 | 85          |
| 8.    | 4h       | 166–168 | 90          |

^a Reactions were carried out 50 °C and the isolated yield of the product was purified by flash column chromatography.
The moderate inhibitory activity was displayed by 4d \textit{K. pneumoniae} (15.00 mm) and \textit{E. coli} (14.00 mm) followed by \textit{S. aureus} (13.00 mm) and \textit{P. aeruginosa} (10.00 mm) and 4a \textit{E. coli} (12.50 mm), \textit{S. aureus} (9.00 mm) and \textit{P. aeruginosa} (10.5 mm)) was found to be less effective than compound 4d. On the other hand, other dispiro-3-phenylpyrrolothiazole derivatives such as 4b, 4c, 4f, 4g and 4h showed least efficacy (7.10 to 9.50 mm) against tested uropathogens. Among all tested synthetic dispiro-3-phenylpyrrolothiazole analogues, 4a, 4d and 4e were more efficient and the order of potential antibacterial activity is 4e > 4d > 4a.

The MIC test of dispiropyrrolothiazole derivatives against six uropathogens and two ATCC strains were carried out with the tube dilution technique. The MIC results are shown in Table 3. The half of tested uropathogens was showed resistant to compound 4a, later it was exempted from MIC study. The MIC values were range from 6.25 to 100.00 µg/ml. The most susceptible uropathogens were \textit{K. pneumoniae} and \textit{E. coli} (6.25 µg/ml), followed by \textit{S. aureus} (12.50 µg/ml), whereas the other uropathogens MICs range from 25.00 to 100.00 µg/ml and it was deliberately less susceptible (Table 3).

**Antioxidant activity by DPPH method**

The free radical scavenging activity of dispiropyrrolothiazole compounds 4a–h was carried out in the presence of 1,1-diphenyl-2-picrylhydrazyl (DPPH-free radical) and using tert-butyl-4-hydroxyanisole (BHA) antioxidant agents as positive control. The DPPH method is highly reliable, rapid and also one of the most appropriate methods for determines the
antioxidant activity. The inhibitory effects of different concentrations of synthesized dispiropyrrolothiazole compounds 4a–h on DPPH radical are depicted in Fig. 4. The antioxidant activity is expressed in terms of % inhibition and IC50 (effective concentration for scavenging 50% of the initial DPPH) value (μM).

Based on the results, among all the synthesized dispiropyrrololthiazoles compounds 4a–h showed scavenging activity towards DPPH. These compounds were shown an active inhibitory effect against DPPH radical at 250 μM concentration and inhibition rates were: 97.20% ± 1.05% (for 4d), 94.00% ± 1.25% (for 4a), and 92.30% ± 1.50% (for 4e) better than the positive control BHA (89.10% ± 1.30%). Whereas, the compounds 4b, 4c and 4g were exposed less inhibitory activity than the BHA. These compounds 4a, d, e inhibited the DPPH activity with an IC50 = 35.30 μM (4a), 32.50 μM (4d) and 36.80 μM (4e) which is better than the specific inhibitor of BHA (IC50 = 58.60 μM).

| Table 3 MIC of dispiropyrrlothiazole derivatives 4d and 4e against uropathogens |
|----------------------------------|----------------|----------------|
| UTI bacterial pathogens         | MIC range (µg) |
|                                 | 4d   | 4e   |
| E. coli                         | 25.00 | 6.25 |
| E. coli ATCC 25922              | 12.50 | 12.50|
| K. pneumoniae                   | 12.50 | 6.25 |
| P. aeruginosa                   | 50.00 | 100.00 |
| S. aureus                       | 25.00 | 12.25 |
| S. epidermidis                  | 100.00| 100.0 |
| S. aureus ATCC 29213            | 12.50 | 6.25 |

Fig. 3 Antibacterial activity of dispiropyrlolthiazoles 4a–h against uropathogens
Materials and methods

Bacterial strain
The uropathogens being used were *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Enterococcus faecalis* (*E. faecalis*). In addition, *Escherichia coli* (*E. coli* ATCC 25922) and *Staphylococcus aureus* (*S. aureus* ATCC 29213), the two ATCC reference cultures, are also included. The bacterial cultures were obtained from BioLine Laboratory, Coimbatore, Tamil Nadu. The organisms were periodically sub-cultured and maintained in a nutrient agar slant at 4 °C.

Inoculum preparation
The uropathogens were grown in a 5 ml brain heart infusion (BHI) broth at 37 °C antibacterial activity assessment. Eighteen hour old pure bacterial culture was used to prepare a density of 10⁸ cells/ml of 0.5 McFarland standard at the time of each experiment. Muller-Hinton agar (MHA) was prepared according to the manufacturer’s instruction, autoclaved and dispensed in a sterile plate. All the culture media were purchased from HiMedia Pvt. Ltd., Mumbai, India.

Antibacterial susceptibility tests

*Agar well diffusion*
The UTI bacterial broth culture was prepared to a density of 10⁸ cells/ml of 0.5 McFarland standards. The aliquot was spread evenly onto Muller Hinton Agar plates with a sterile cotton swab. Then, the plated medium was allowed to dry at room temperature for 30 min [36]. On each plate, equidistant wells were made with a 6 mm diameter sterilized, cork borer, 2 mm from the edge of the plate. Fifty microliter of each dispiro-3-phenylpyrrolothiazole compounds (100 µg/ml) were aseptically introduced into an agar well. Streptomycin (15 µg/ml) were used as positive controls and the DMSO was included as negative controls. This was followed by allowing the agar plate on the bench for 20 min pre-diffusion followed by incubation at 37 °C for 24 h. The formation of a clear inhibition zone of ≥ 7 mm diameters around the wells was regarded as significant susceptibility of the organisms to the dispiro-3-phenylpyrrolothiazoles 4a–h. All the experiment was performed in triplicate.

*Determination of minimum inhibitory concentration (MIC)*
The tube dilution technique [37] was used to determine the MIC of dispiro-3-phenylpyrrolothiazole derivatives that shows a maximum zone of inhibition at agar well
methods. The dispiro-3-phenylpyrrolothiazole derivatives 4d and 4e was used to determine the MIC by tube dilution technique which was shown maximum zone of inhibition at agar well method. Both compounds were serially diluted in the range from 3.125 to 100 μg/ml. The tubes were inoculated with 100 μl of UTI bacterial pathogens at a concentration of 10⁶ cells/ml. Standard antibiotics streptomycin was included in the assay for comparison. Nutrient broth with the inoculum only was used as control. All the experiments were carried out in triplicate. The tubes were incubated aerobically at 37 °C for 18 h. The growths of inoculum were decreased in the next dilution was taken as MIC values.

**Antioxidant activity by DPPH method**

The antioxidant activity of all the synthesized compounds was evaluated by DPPH method with some modifications and compared with standard BHA. The 400 μM solution of DPPH (2 ml) in ethanol was added to tested sample solutions (2 ml) of different concentrations (75, 150, and 250 μM) in aceton−ethanol 4:96 v/v. The samples were kept in the dark at room temperature. After 30 min the absorbance values were measured at 517 nm and were converted into the percentage antioxidant activity (%) using the formula [38]:

\[
\% = \left(1 - \frac{(A_{\text{sample}} - A_{\text{sample blank}})}{A_{\text{control}}}\right) \times 100
\]

where, \(A_{\text{control}}\) was the absorbance of DPPH solution without sample, \(A_{\text{sample}}\) was the absorbance of sample solution with DPPH, \(A_{\text{sample blank}}\) was the absorbance of the sample solutions without the DPPH.

All analyses were undertaken on three replicates and the results averaged. The IC₅₀ values were calculated by linear regression plots, where the absorbance represented the concentration of tested compound solution (75, 150, and 250 μM) and the ordinate represented the average percent of antioxidant activity from three separate tests. The absorbance was measured on a spectrophotometer.

**Experimental**

**General procedure for the synthesis of dispiropyrrlolothiazoles 4a−h**

To a suspension of 2-phenylthiazolidine-4-carboxylic acid (1) (209 mg, 1.0 mmol) in ACI/EG (3.0 ml) were added ninhydrin (2) (160 mg, 1.0 mmol) and 3-arylidene isatin (3a−h) (1.0 mmol) at room temperature. The reaction mixture was stirred at 50 °C for 1 h. After completion of the reaction, tested by TLC, the crude product was washed with water and purified by flash column chromatography over silica gel with a hexane−ethyl acetate mixture (8:2) to give pure dispiropyrrloisoquinoines (4a−h) in good to excellent yield.

**Spectral data of the synthesized cycloadducts**

Hexahydro-3,7-diphenylspiro[5.2′]-2H-indene-1′,3′-dione-spiro[6.3″] oxindolopyrrolo[1,2-c]thiazole, 4a. Yellow solid, mp 132–134 °C; IR (KBr) 3388, 1744, 1706, 1698 cm⁻¹; ¹H (300 MHz, CDCl₃) δ 3.13–3.17 (m, 1H, H₄); 3.65–3.71 (m, 1H, H₂); 4.37 (d, 1H, H₃, J = 9.3 Hz); 5.16–5.23 (m, 1H, H₃); 5.24 (s, 1H, H₃); 6.42–8.14 (m, 18H, ArH); 9.91 (s, 1H, NH). ¹³C (75 MHz, CDCl₃) 38.2, 55.5, 68.4, 71.5, 75.3, 78.3, 109.3, 120.9, 122.4, 122.6, 124.4, 126.5, 127.1, 127.2, 127.5, 127.6, 127.9, 128.1, 128.4, 133.2, 135.3, 135.9, 139.6, 140.6, 141.1, 172.4, 192.8, 199.8. EI-MS m/z 528.62 (M⁺). Anal. Calcd. for C₃₄H₂₆N₂O₄S: C, 74.91; H, 4.65; N, 5.22%.

Hexahydro-3-phenyl-7-[(p-chlorophenyl)spiro[5.2′]-2H-indene-1′,3′-dione-spiro[6.3″] oxindolopyrrolo[1,2-c]thiazole, 4b. Yellow solid, mp 120–122 °C; IR (KBr) 3392, 1751, 1710, 1700 cm⁻¹; ¹H (300 MHz, CDCl₃) δ 2.35 (s, 3H, Me); 3.15–3.19 (m, 1H, H₄); 3.63–3.69 (m, 1H, H₂); 4.34 (d, 1H, H₃, J = 9.3 Hz); 5.14–5.22 (m, 1H, H₃); 5.46 (s, 1H, H₃); 6.46–8.21 (m, 17H, ArH); 9.91 (s, 1H, NH). ¹³C (75 MHz, CDCl₃) 26.4, 38.2, 53.5, 64.7, 68.4, 71.5, 73.4, 77.9, 109.2, 120.9, 122.5, 122.6, 124.4, 125.8, 127.2, 127.7, 127.5, 127.6, 128.0, 128.2, 128.4, 133.2, 135.2, 135.9, 140.5, 140.7, 141.1, 172.3, 192.9, 200.1. EI-MS m/z 542.65 (M⁺). Anal. Calcd. for C₃₃H₂₄N₂O₃S: C, 75.25; H, 4.83; N, 5.16%. Found: C, 75.19; H, 4.76; N, 5.22%.

Hexahydro-3-phenyl-7-[(p-methoxyphenyl)spiro[5.2′]-2H-indene-1′,3′-dione-spiro[6.3″] oxindolopyrrolo[1,2-c]thiazole, 4c. Yellow solid, mp 128–130 °C; IR (KBr) 3392, 1751, 1710, 1691 cm⁻¹; ¹H (300 MHz, CDCl₃) δ 3.12–3.17 (m, 1H, H₄); 3.67 (s, 3H, OMe); 3.69–3.73 (m, 1H, H₂); 4.40 (d, 1H, H₃, J = 9.3 Hz); 5.11–5.19 (m, 1H, H₃); 5.32 (s, 1H, H₃); 6.42–8.27 (m, 17H, ArH); 9.96 (s, 1H, NH). ¹³C (75 MHz, CDCl₃) 38.3, 55.1, 55.8, 68.5, 71.8, 73.9, 78.1, 109.5, 113.4, 122.3, 123.1, 124.9, 125.1, 127.1, 127.9, 128.1, 128.9, 129.1, 129.7, 135.6, 136.4, 139.4, 140.2, 140.8, 159.1, 172.8, 193.6, 200.3. EI-MS m/z 558.65 (M⁺). Anal. Calcd. for C₃₂H₂₆N₂O₃S: C, 73.10; H, 4.69; N, 5.01%. Found: C, 73.17; H, 4.77; N, 4.94%.

Hexahydro-3-phenyl-7-[(p-chlorophenyl)spiro[5.2′]-2H-indene-1′,3′-dione-spiro[6.3″] oxindolopyrrolo[1,2-c]thiazole, 4d. Yellow solid, mp 140−142 °C; IR (KBr) 3383, 1740, 1703, 1695 cm⁻¹; ¹H (300 MHz, CDCl₃) δ 3.10–3.16 (m, 1H, H₄); 3.66–3.73 (m, 1H, H₂); 4.42 (d, 1H, H₃, J = 9.6 Hz); 5.12–5.20 (m, 1H, H₃); 5.31 (s, 1H, H₃); 6.45–8.24 (m, 17H, ArH); 8.90 (s, 1H, NH). ¹³C (75 MHz, CDCl₃) 38.2, 55.5, 68.4, 71.6, 73.6, 77.8, 109.8, 122.4, 123.1, 123.2, 124.5, 127.1, 128.0, 128.2, 128.2, 128.9, 129.3, 129.9, 131.7, 133.8, 135.7, 136.5, 139.4, 140.1, 140.5, 140.9, 172.8, 193.4, 200.2. EI-MS m/z 563.07 (M⁺).
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**Conclusion**

Environmentally green ACI/EG eutectic mixture mediated synthesis of novel substituted dispiropyrolothiazole analogues through azomethineylide one-pot three component 1,3-dipolar cycloaddition reactions have been developed. This eutectic mixture mediated synthesis has the advantages of good to excellent yield, mild reaction conditions and with high regio- and stereo- selectivity. Further, the reusability of recovered eutectic mixture showed their stability and efficiency for the consecutive applications in synthesis. All the synthesized compounds were responding for commonly existing uropathogens, and 4a, 4d, and 4e were effective against the uropathogens. The antioxidant activity of the synthesized compounds were assessed based on the scavenging activity of stable DPPH free radical. Interestingly, compounds 4a (IC50 = 35.30 μM), 4d (IC50 = 32.50 μM) and 4e (IC50 = 36.80 μM) showed effective free radical inhibition better than standard inhibitor BHA (IC50 = 58.60 μM). With additional optimization, we trust our compounds would be promising antimicrobial drugs for to treat against uropathogens causing urinary tract infections.

**Additional file**

**Additional file 1** Additional Figures and Tables.

**Abbreviations**

ACI: acetylcholine iodide; EG: ethylene glycol; Bmim: 1-butyl-3-methylimidazolium; DMSO: dimethyl sulfoxide; FTIR: Fourier-transform infrared spectroscopy; NMR: nuclear magnetic resonance; COSY: Correlated SpectroscopY; DEPT: distortionless enhancement by polarization transfer; UTI: urinary tract infection; UPEC: uropathogenic Escherichia coli; MIC: minimum inhibitory concentration; BHI: brain heart infusion; E. coli: Escherichia coli; S. pneumoniae: Streptococcus pneumoniae; P. aeruginosa: Pseudomonas aeruginosa; IC50: 50% inhibitory concentration; BHA: tert-butyl-4-hydroxyanisole; DPPH: 1,1-diphenyl-2-picrylhydrazyl; Bmim: 1-butyl-3-methylimidazolium; DMSO: dimethyl sulfoxide; CDCl3: chloroform-d; EI-MS: electron ionization mass spectrometry; m/z: mass-to-charge ratio.

**Authors' contributions**

GP designed, synthesized, characterized the compounds and wrote the manuscript. KP and NAA carried out the antimicrobial studies and wrote the corresponding results and discussion. NA and RSK contributed to one and/or other part of the experimental and spectroscopic studies and also in manuscript.
writing. DP and SA carried out the literature survey, synthesizing and purification the compounds and contributing to the manuscript preparation. MR and AA contributed to revise and finalizing the manuscript. All authors read and approved the final manuscript.

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Acknowledgements

Authors are express their appreciation to the Deanship of Scientific Research at King Saud University for their support to complete this research. Also, GP thank to the University of Madras for providing research facilities for the initial work.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Funding

Deanship of Scientific Research at King Saud University through research group no. RG‑1436‑005.

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Received: 3 September 2018 Accepted: 8 March 2019 Published online: 28 March 2019

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