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ARTICLE

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Introducing new method for the synthesis of polycyclic compounds containing [1,3]dithiine derivatives, with anticancer and antibacterial activities against common bacterial strains between aquatic and human

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Abstract

In this study, glycerol:potassium carbonate used as a green deep eutectic solvent, for synthesis of polycyclic compounds containing [1,3]dithiine derivatives. The antimicrobial properties of the derivatives against *Lactococcus garvieae* and *Edwardsiella tarda* were tested as bacterial strains between aquatic and human based on the minimum inhibitory concentration (MIC), the minimum lethal concentration (MBC) and inhibition zone diameter (IZD). In addition to antimicrobial properties, cytotoxicity testing was performed against MCF-7 breast cancer cells via MTT cell viability assay.

1 | INTRODUCTION

Deep eutectic solvent as green and environmentally friendly solvents, cheap and non-toxic have many applications in the synthesis of heterocyclic compounds. These solvents can be used as Lewis acid and base in the synthesis of heterocyclic compounds [1–3]. Glycerol:potassium carbonate can be used as a deep eutectic solvent in the synthesis of heterocyclic compounds. According to reports published by Beyzaei et al., glycerol:potassium has been used as a green and deep eutectic solvent in the synthesis of isoxazole and pyrazole derivatives [2,4].

[1,3]Dithiine heterocyclic compounds contain two sulfur atoms in their structure. The use of carbon disulfide, daimedone, and activated methylene in the presence of triethylamine as the base catalyst [5] and the use of 1 and 3 propane thiols and compounds containing the carbonyl group in the presence of various catalysts [6,7] were among the methods that presented in the synthesis of [1,3]dithiine derivatives. Recently, heterocyclic compounds containing [1,3]dithiine derivatives with antibacterial properties have been reported. In addition to antibacterial properties, antioxidant properties of heterocyclic compounds containing [1,3]dithiine derivatives have also been reported [8–10]. Other biological properties such as histone deacetylase and DNA topoisomerase II-targeted inhibitors [11] and glycineamide ribonucleotide formyltransferase [12] have been reported from compounds containing [1,3]dithiine derivatives.

In the field of aquaculture, bacterial infections are the most important problem [13]. *Lactococcus garvieae* is a gram-positive bacterium found in cattle, dogs, cats, buffalo, and animal products, including cow's milk. It also causes *Lactococcosis* in fish, especially rainbow trout [14–18].

Edwardsiella tarda is a gram-negative bacterium of the Hafniaceae family. This bacterium causes *Edwardsiella* septicemia in fish [19].

In this study, to continue our research, to provide new methods in the synthesis of polycyclic compounds containing [1,3]dithiine derivatives with biological activity [9], we

synthesized [1,3]dithiine derivatives by glycerol:potassium carbonate as a green deep eutectic solvent and biological activity such as antibacterial properties against common bacterial strains between aquatic and human and anticancer activity against MCF-7 breast cancer cells were evaluated.

2 | RESULTS AND DISCUSSION

2.1 | Synthesis of [1,3]dithiine derivatives

In this study, according to Scheme 1, by using carbonyl group, malononitrile, carbon disulfide and active methylene groups (barbituric acid, thiobarbituric acid, and dimedone), and glycerol:potassium carbonate as deep eutectic solvent, polycyclic compounds containing [1,3] dithiine derivatives were synthesized.

2.2 | Reaction optimization

In order to obtain the optimal ratios of solvent constituents in the synthesis of polycyclic compounds containing [1,3]dithiine derivatives, first the solvent was made with different ratios of glycerol and potassium carbonate and the synthesis of the compounds was examined and tested. The results are given in Table 1.

The results show that the reaction is not performed under solvent-free conditions and using glycerol or potassium carbonate alone.

According to previously reported studies, in the ratio of less than 4:1 (glycerol:potassium carbonate), the mixture was not completely dissolved and homogeneous and does not create a suitable and transparent solvent [20].

The highest yield was observed using a ratio of 4:1 (glycerol:potassium carbonate).

Using optimal conditions seven polycyclic compounds containing [1,3]dithiine derivatives were synthesized (Table 2).

The proposed mechanism was given in Scheme 2.

2.3 | Method comparison

According to previous reports, the present reaction has already been performed by piperidine as a catalyst.

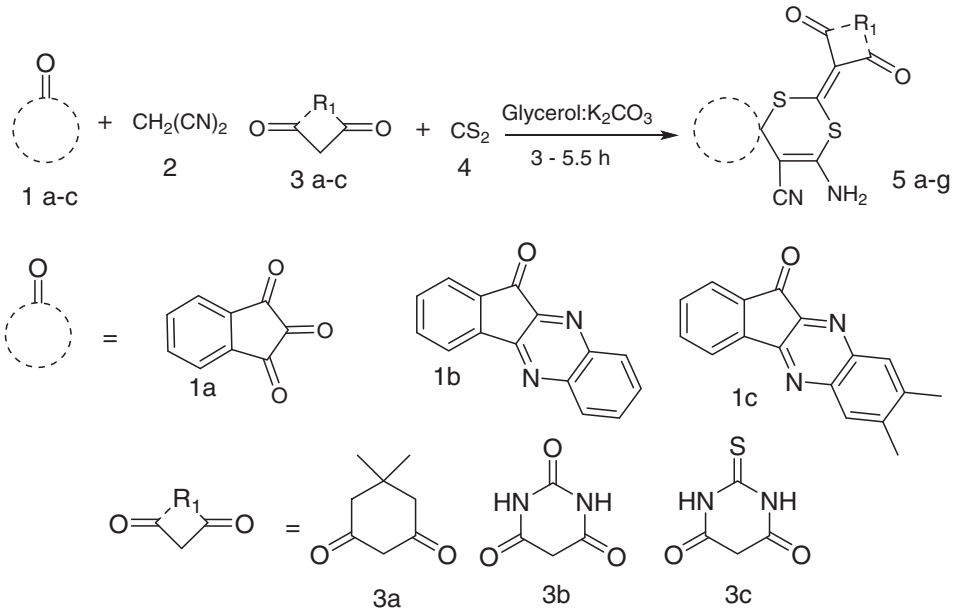
The results of the study in Table 3 show that the use of deep eutectic solvent reduces the reaction time and also increases the reaction efficiency.

2.4 | Antibacterial activity

The antibacterial effects of derivatives against *L. garvieae* and *E. tarda* as common bacterial strains between aquatic and human were evaluated and the results were shown in Table 4.

The order of antibacterial activity of derivatives based on the minimum inhibitory concentration (MIC) and the minimum lethal concentration (MBC) were **5g** > **5c** > **5f** > **5b** > **5e** > **5d** > **5a**. Examination of the results shows that there is a clear relationship between the structure of compounds and their antibacterial properties.

In general, compounds containing active methylene with sulfur have the highest effect. Other factors were also influential, including the presence of pyrazine rings in the final structure of the compounds.

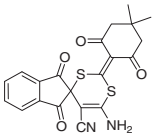
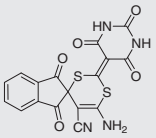
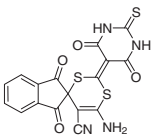
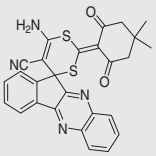
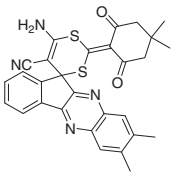
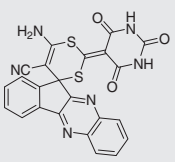
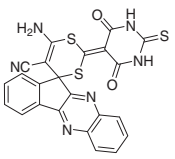


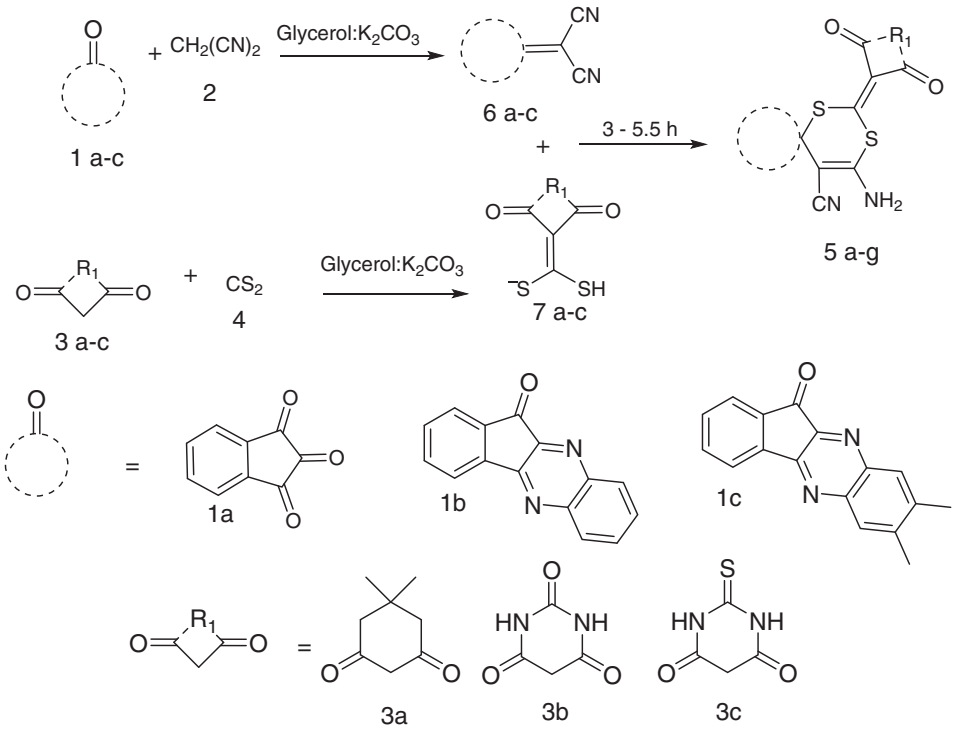
SCHEME 1 Synthesis of 6′-amino-2′-(arylidene)spiro[indeno[1,2-*b*]quinoxaline[1,3]dithiine]-5′-carbonitrile derivatives

TABLE 1 Optimizing the ratio of solvent ingredients

Entry	Compound	Ratio Glycerol:potassium carbonate	Yield (%)
1	**5a**	Solvent free	0
2	**5a**	Glycerol	0
3	**5a**	Potassium carbonate	0
4	**5a**	4:1	75
5	**5a**	5:1	61
6	**5a**	6:1	46
7	**5a**	10:1	38

TABLE 2 Synthesis of polycyclic compounds containing [1,3]dithiine derivatives

Entry	Structure	Time (h)	Yield (%)	MP (°C) Found	MP (°C) Reported [9]
5a		5	75	295–297	297–298
5b		5.5	83	274–276	273–274
5c		5.5	80	279–283	279–281
5d		3	85	286–289	285–288
5e		4	84	262–265	264–266
5f		4.5	92	275–278	273–275
5g		5	91	255–256	254–256



SCHEME 2 Proposed mechanism for synthesis of 6′-amino-2′-(arylidene)spiro[indeno[1,2-*b*]quinoxaline[1,3]dithiine]-5′-carbonitrile derivatives

TABLE 3 Comparison of derivative synthesis under the use of piperidine and deep eutectic solvent conditions

	Use of piperidine [9]		This work	
Derivative	**Time (h)**	**Yield (%)**	**Time (h)**	**Yield (%)**
5a	10.5	70	5	75
5b	11	81	5.5	83
5c	12	77	5.5	80
5d	9.5	85	3	85
5e	10	81	4	84
5f	10	90	4.5	92
5g	10.5	88	5	91

TABLE 4 Antimicrobial activities of derivatives against *Lactococcus garvieae* and *Edwardsiella tarda*

	Bacteria					
	Lactococcus garvieae			*Edwardsiella tarda*		
Product/antibiotics	**IZD**	**MIC**	**MBC**	**IZD**	**MIC**	**MBC**
5a	10.98	1024	2048	12.46	2048	2048
5b	13.87	256	512	14.57	512	512
5c	15.75	128	64	14.17	128	64
5d	12.82	1024	2048	13.58	512	1024
5e	14.01	512	1024	13.79	512	1024
5f	14.85	256	256	15.37	256	128
5g	16.27	64	32	15.90	16	16
Gentamicin	16.51	16	32	16.47	4	4
Penicillin	17.12	2	4	15.24	4	16

Note: IZD values reported as mm; MIC and MFC values reported as μg/mL.

TABLE 5 IC_{50} value of derivatives against MCF-7 breast cancer cells

Entry	Compound	Molecular weight	IC_{50} (μM/mL)
1	**5a**	424.49	187
2	**5b**	412.39	154
3	**5c**	428.46	168
4	**5d**	496.60	133
5	**5e**	526.67	127
6	**5f**	486.52	85
7	**5g**	502.59	104

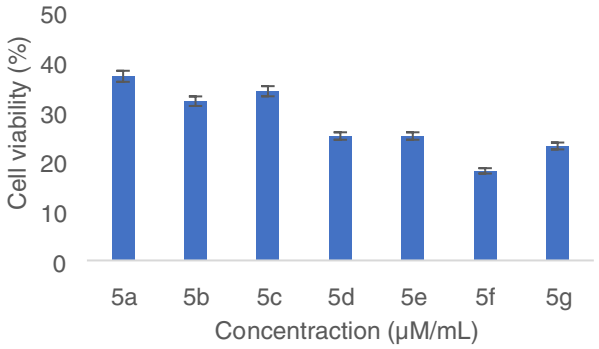


FIGURE 1 Cell proliferation and viability of derivatives against MCF-7 breast cancer cells. Data represents mean ($n = 3$) ± SD

3 | ANTICANCER ACTIVITY

The cytotoxic effect of derivatives based on IC_{50} value for exposure on MCF-7 breast cancer cells was shown in Table 5 and Figure 1.

The results of Table 5 show that the effect of derivatives based on IC_{50} values is **5f** > **5g** > **5e** > **5d** > **5b** > **5c** > **5a** with values of 85, 104, 127, 133, 154, 168, and 178 μM/mL.

In general, it can be concluded that the presence of pyrazine ring has increased the effectiveness.

Cell proliferation and viability of derivatives than control during the test with the high concentrations of derivatives were shown in Figure 1.

Maximum cell proliferation and viability were observed about 18% than control for **5f** with the concentrations of 300 μM/mL.

4 | CONCLUSIONS

As a conclusion, glycerol:potassium carbonate, has been used as green, non-toxic, cheap, and environmentally friendly deep eutectic solvent for synthesis of polycyclic compounds containing [1,3]dithiine derivatives. The synthesized compounds had higher efficiencies and less time than the previously reported method. Antibacterial properties of derivatives against *L. garvieae*, *E. tarda* as common bacterial strains between aquatic and human based on the MIC and the MBC were evaluated and acceptable results related to the structure of the derivatives were obtained. In addition, anti-cancer properties of the derivatives against MCF-7 breast cancer cells were evaluated using the MTT method and IC_{50} value of derivatives were reported. In addition, cell proliferation and viability of **5f** were observed about 18% than control.

5 | EXPERIMENTAL SECTION

5.1 | Material and method

All chemicals and solvents were purchased from Merck and Sigma-Aldrich. Kruss type KSP1 N melting point meter was used to determine melting points of compounds. Absorption given in cm^{-1} of compounds by a Bruker Tensor 27 Fourier-transform infrared spectrometer was taken. By using a Bruker Ultra Shield-250 spectrometer (250 and 75 MHz, respectively), ^{1}H and ^{13}C NMR spectra of compounds in Dimethyl sulfoxide (DMSO-d6) were measured. Performed elemental analyses C, H, N, and S by Thermo Finnigan Flash EA microanalyzer were done. By using Jenway 6405 UV–V spectrophotometer, the concentration of bacterial suspensions was determined.

5.2 | Synthesis of glycerol:potassium carbonate deep eutectic solvent

The amount of 1.40 g (0.01 mol) of potassium carbonate and 3.60 g (0.04 mol) of glycerol was stirred for 2 h at 80°C. The colorless homogeneous solution prepared as deep eutectic solvent was used in synthesis of derivatives.

5.3 | General procedure for the synthesis of polycyclic compounds containing [1,3] dithiine derivatives

First, 1 mmol of malononitrile (0.066 g), 1 mmol of multiring compounds containing a carbonyl group (**4**, **8a**,**b**), and 1 g prepared deep eutectic solvent were stirred for 0.5 h at 50°C. In another container, 1 mmol of active methylene compound (**1a**–**c**), 3 mmol of carbon disulfide (0.2284 g), and 1 g prepared deep eutectic solvent was stirred for 0.5 h at 50°C. Then, the two mixtures were mixed and stirred at 50°C. After completing the reaction (monitored using thin-layer chromatography, hexane/ethyl acetate), 10 mL of

ethanol:water (1:1) was added to the mixture and stirred for 0.5 h at room temperature then sediments were separated and recrystallized in acetonitrile.

5.4 | Selected spectral data [9]

5.4.1 | 6′-Amino-7,8-dimethyl-2′-(2,4,6-trioxotetrahydropyrimidin-5[2*H*]-ylidene) spiro[indeno[1,2-*b*]quinoxaline-11,4′-[1,3] dithiine]-5′-carbonitrile (**7a**)

IR (KBr, cm^{-1}): 3352, 3275 (NH_2), 2164 (CN), 1718, 1673 (CO); ^1H NMR (250 MHz, DMSO-d_6) δ 0.99 (3H, s, Me), 1.05 (3H, s, Me), 2.01–2.25 (4H, m, $2CH_2$), 6.65 (1H, d, $J = 7.5$ Hz, H-Ar), 6.80–7.01 (3H, m, H-Ar), 7.24 (2H, br s, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.8, 27.9, 33.7, 47.6, 51.3, 58.1, 110.4, 111.5, 117.1, 122.7, 123.6, 129.1, 135.3, 143.4, 160.1, 173.9, 177.9, 193.4, 194.6. Anal. Calcd for $C_{21}H_{16}N_2O_4S_2$: C, 59.42; H, 3.79; N, 6.59; S, 15.11. Found: C, 59.37; H, 3.83; N, 6.62; S, 15.15.

5.4.2 | 6′-Amino-2′-(4,4-dimethyl-2,6-dioxocyclohexylidene) spiro[indeno[1,2-*b*] quinoxaline-11,4′-[1,3]dithiine]-5′-carbonitrile (**10a**)

IR (KBr, cm^{-1}): 3222 and 3218 (NH_2), 2162 (CN), 1672 (CO); ^1H NMR (DMSO-d_6) δ 0.99 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 1.99–2.05 (m, 2H, CH_2), 2.57–2.75 (m, 2H, CH_2), 7.30 (br s, 2H, NH_2), 7.49–7.59 (m, 3H, H-Ar), 7.78 (t, $J = 7.75$ Hz, 2H, H-Ar), 8.01–8.16 (m, 3H, H-Ar). ^{13}C NMR (DMSO-d_6) δ 27.4, 28.0, 32.4, 47.5, 50.5, 59.0, 112.3, 117.9, 121.9, 124.9, 129.3, 129.5, 129.8, 130.1, 132.8, 136.6, 141.4, 142.1, 152.3, 154.6, 159.3, 165.3, 159.9.185.4. Anal. Calcd for $C_{27}H_{20}N_4O_2S_2$: C, 65.30; H, 4.06; N, 11.28; S, 12.91. Found: C, 65.34; H, 4.08; N, 11.31; S, 12.95.

5.5 | Antibacterial activity

According to the clinical and laboratory standards institute (CLSI) guidelines M07-A9, M26-A, M02-A11, M44-A, and M27-A2, broth microdilution, and time-kill susceptibility based on the inhibition zone diameter, MIC, and MBC values against *L. garvieae* (IBRC-M 10900) and *E. tarda* (IBRC-M 10718) were measured [8–10,21,22].

5.6 | Anticancer activity

MTT method and previous reports [23,24] were used to evaluate the anticancer properties of derivatives against MCF-7 breast cancer cells. Concentrations of 6.25, 12.5, 25, 75, 150, and 300 μM/mL of derivatives for 48 h were tested to evaluate the anticancer activity.

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DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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