Arabian Journal of Chemistry (2021) **14**, 103408

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Synthesis and antimicrobial, antiproliferative and anti-inflammatory activities of novel 1,3,5-substituted pyrazoline sulphonamides

Thi-Dan Thach [a,b], T. My-Thao Nguyen [a], T. Anh-Thu Nguyen [a], Chi-Hien Dang [b,c,*], Thanh-Binh Luong [b,c], Van-Su Dang [d,*], Kien-Sam Banh [c], Van-Sieu Luc [c], Thanh-Danh Nguyen [b,c,*]

[a] *Tra Vinh University, Tra Vinh City, Tra Vinh Province, Viet Nam*
[b] *Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam*
[c] *Institute of Chemical Technology, Vietnam Academy of Science and Technology, 1A, TL29 Street, Thanh Loc Ward, District 12, Ho Chi Minh City, Viet Nam*
[d] *Department of Chemical Technology, Ho Chi Minh City University of Food Industry, Viet Nam*

Received 9 July 2021; accepted 25 August 2021
Available online 31 August 2021

KEYWORDS

Synthesis;
Sulphonamide;
Pyrazoline;
Antimicrobial;
Antiproliferative;
Anti-inflammatory

Abstract The design of novel molecules is imperative for the discovery of potent drugs in the medicinal chemistry field. In this work, new 1,3,5-substituted pyrazoline sulphonamides were synthesised using a two-step process with microwave assistance and evaluated biologically for their antimicrobial, antiproliferative, and anti-inflammatory properties. Most of the sulphonamides bearing 3-OH or 4-Cl groups exhibited significant inhibition of two Gram-positive bacteria, *Bacillus subtillis* and *Staphylococcus aureus*, and the yeast *Candida albicans*. Six compounds showed good activity against the cancer cell lines cervix carcinoma (Hep-2C) and human lung carcinoma (A549) with IC_{50} in the range 16.03 ± 1.63 to 22.75 ± 0.19 μM and 18.64 ± 1.02 to 20.66 ± 2.09 μM, respectively, and exhibited low toxicity against mammalian Vero cells. In evaluating *in vitro* anti-inflammatory behaviour, five compounds showed high inhibition of NO production over the standard reference, with low toxicity against murine macrophage cell line RAW 264.7.

* Corresponding authors at: Institute of Chemical Technology, Vietnam Academy of Science and Technology, 1A, TL29 Street, Thanh Loc Ward, District 12, Ho Chi Minh City, Vietnam (C.-H. Dang and T.-D. Nguyen).
E-mail addresses: dangchihien@gmail.com (C.-H. Dang), sudv@hufi.edu.vn (V.-S. Dang), ntdanh@ict.vast.vn (T.-D. Nguyen).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.arabjc.2021.103408

Further investigation found that two compounds, **1b** and **18b,** exhibited the highest activity when testing mouse ear oedema. The findings are promising for the discovery of potent new drugs.

1. Introduction

Sulphonamides are an emerging class of therapeutic agents with a wide range of biological properties, including antimicrobial (Abdul Qadir et al., 2015; Genç et al., 2008), antidepressant (De Oliveira et al., 2011; Zajdel et al., 2013), anti-inflammatory (Bonardi et al., 2020; Naim et al., 2018; Supuran, Briganti et al., 2001), anticancer (Alafeefy et al., 2013; Mettu et al., 2020; Scozzafava et al., 2002), and antidiabetic (Gao et al., 2016; Markowicz-Piasecka et al., 2019) properties. Thus, sulphonamides are particularly interesting for the development of new medicines, and numerous drugs containing sulphonamide moieties have been discovered in recent decades (Fig. 1). In addition, pyrazoline derivatives are versatile heterocyclic compounds with a wide range of biological properties (Ali et al., 2007; Amir et al., 2008; Kaplancıklı et al., 2010; Ozmen Ozgun et al., 2019). Thus, pyrazoline sulphonamide compounds promise to be an important class in the search for new antibacterial, anticancer and anti-inflammatory agents (Mete et al., 2016; Ozmen Ozgun et al., 2019; Sadashiva et al., 2017).

Sulphonamides with antimicrobial effects represent a large group of antimicrobial drugs. Sulphonamides are well known as competitive antagonists of *p*-aminobenzoic acid due to the similarity between structures (Fernández-Villa et al., 2019; Michelini et al., 2013); *p*-aminobenzoic acid is an essential compound in the synthesis of folic acid, which induces bacterial growth. Thus, sulphonamides inhibit the synthesis of folic acid, leading to a bacteriostatic effect. Furthermore, 1,3,5-substituted pyrazoline derivatives exhibit good microbial activity against bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Manna and Agrawal, 2009; Samshuddin et al., 2012), as well as fungi such as *Aspergillus* niger and *Candida* Albicans (Mishra et al., 2017; Turan-Zitouni et al., 2005).

Anticancer agents based on sulphonamide structures are in advanced clinical trials and are being evaluated on lung, colon, gastric and pancreatic cancers (Bano et al., 2011). For instance, E7070, a sulphonamide antitumour agent, inhibits human non-small lung tumour cell (A549) growth (Fig. 1). This compound has been shown to inhibit the phosphorylation of pRb, leading to decreasing expression of cyclin A, B1, CDK2, and CDC2 proteins and suppressing catalytic activity of CDK2 with the induction of p53 and p21 proteins in A549 cells (Fukuoka et al., 2001). Moreover, pyrazoline derivatives are receiving significant attention as potent anticancer drugs because of their efficacy in the inhibition of cancer cell lines, including HepG-2, Hela and A549 cells (Akhtar et al., 2021; Shamsuzzaman et al., 2016). Wang et al. showed inhibition of HepG-2 cell growth via the mechanism of upregulated expression of cleaved PARP, cleaved caspase-3, Bax and p53 proteins, and downregulated expression of Bcl-2 protein in HepG-2 cells (Wang et al., 2017).

In recent years, pyrazoline and sulphonamide derivatives have attracted attention for their anti-inflammatory activity, which stems from nitric oxide (NO) inhibitory effects (Hamada and Abdo, 2015; Shaaban et al., 2012). NO, a signalling molecule, plays an important role in inflammation pathogenesis and is considered a pro-inflammatory mediator. NO is synthesised and released into endothelial cells with the help of NO synthases that convert arginine into citrulline, producing NO in the process (Sharma et al., 2007; Tuteja et al., 2004). Thus, inhibitors of NO production represent an important therapeutic advance in the management of inflammatory diseases. However, the evaluation on anti-inflammatory activity of compounds which structurally composed of a sulphomanide and a pyrazoline is rarely reported so far. Therefore, the synsthesis of new pyrazoline derivatives bearing sulphonamide moiety are an important approach to find potent drugs in the pharmaceutical field.

In our search for new bioactive agents, 1,3,5-substituted pyrazoline derivatives bearing a benzenesulphonamide moiety were synthesised. All the synthesised compounds were evaluated for antimicrobial activity against bacteria, yeasts and fungi, for *in vitro* cytotoxic activity against cancer cell lines (Hep-2C and A549), and for *in vitro* anti-inflammatory activity through inhibiting NO production. With anti-inflammatory behaviour, the most active compounds were further evaluated for their *in vivo* activity using tests on mouse ear oedema.

2. Experimental

2.1. Chemistry

2.1.1. Microwave-assisted synthesis of the dihydro-1H-pyrazol-1-ylbenzenesulfonamides (*1b-26b*)

A solution of chalcones (**1a-26a**) (3 mmol) and 4-hydrazinylbenzenesulfonamide hydrochloride (699 mg; 3 mmol) in anhydrous methanol (30 mL) was irradiated by the microwave for 1 h. The reaction mixture was cooled overnight at 0 °C. The separated solids were filtered and washed with hexane (10 mL × 3). The pure compounds (**1b-26b**) was obtained by recrystallization from ethanol and dried under the vacuum for 8 h.

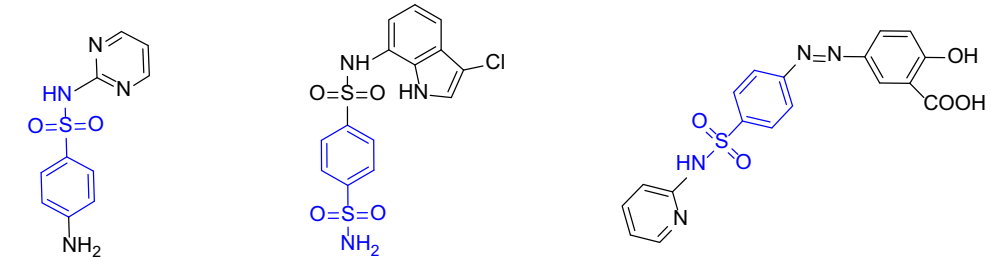


Fig. 1 Structure of example sulphonamide drugs.

2.1.1.1. 4-(5-(3-hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(1b)***. Yellow powder. m.p. 125–127 °C. Yield 63 %. IR (KBr, ν_{max} cm^{-1}): 3481 (–OH), 3452 (—NH_2), 3324 (—NH_2), 1594 (C═C, benzene), 1510, 1458 (C═C, benzene), 1403, 1332 (—SO_2), 1311, 1277, 1192 (—SO_2), 1099, 861, 759, 697. ^{1}H NMR (500 MHz, DMSO) δ 9.42 (s, 1H), 7.82–7.76 (m, 2H), 7.63–7.57 (m, 2H), 7.47–7.39 (m, 3H), 7.43–7.37 (m, 1H), 7.14 (t, *J* 7.5 Hz, 1H), 7.11–7.05 (m, 2H), 7.00 (s, 2H), 6.72 (dt, *J* 8.0, 1.5 Hz, 1H), 6.64 (ddd, *J* 8.0, 1.5 Hz, 1H), 6.61 (t, *J* 2.0 Hz, 1H), 5.54 (dd, *J* 12.0, 5.0 Hz, 1H), 3.94 (dd, *J* 17.5, 12.0 Hz, 1H), 3.17 (dd, *J* 17.5, 5.0 Hz, 1H). ^{13}C NMR (125 MHz, DMSO): δ 157.8, 149.6, 145.9, 143.1, 133.0, 131.8, 130.2, 129.3, 128.7, 127.1, 126.0, 116.3, 114.6, 112.0, 111.9, 62.3, 56.0, 42.9, 18.5. ESI-MS (*m*/*z*): 394.1221 $[M+H]^+$ calcd for $C_{21}H_{19}N_3O_3S$ ($[M+H]^+$ = 394.1225).

2.1.1.2. 4-(5-(2-methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(2b)***. Yellow powder. m.p. 236–238 °C. Yield 80 %. ^{1}H NMR (500 MHz, DMSO) IR (KBr, ν_{max} cm^{-1}): 3391 (—NH_2), 3292 (—NH_2), 3062 (>C—H, benzene), 2836, 1594 (C═C, benzene), 1557, 1491, 1467 (C═C, benzene), 1408, 1341 (—SO_2), 1285, 1150 (—SO_2), 1097, 1005, 871, 688. δ 7.78 (dd, *J* 8.5, 1.5 Hz, 3H), 7.60 (d, *J* 9.0 Hz, 2H), 7.47 –7.42 (m, 2H), 7.42–7.36 (m, 1H), 7.26 (td, *J* 9.0, 8.0, 2.0 Hz, 1H), 7.11 (d, *J* 8.0 Hz, 1H),7.03–6.98 (m, 4H), 6.87 (dd, *J* 7.5, 1.5 Hz, 1H), 6.83 (t, *J* 7.0 Hz, 1H), 5.73 (dd, *J* 12.0, 5.0 Hz, 1H), 3.96 (dd, *J* 17.5, 12.5 Hz, 1H), 3.91 (s, 3H), 3.10 (dd, *J* 17.5, 5.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 156.1, 150.1, 145.8, 132.9, 131.8, 129.2, 128.9, 128.6, 128.2, 127.2, 126.0, 125.7, 120.6, 111.6, 57.2, 55.7, 41.8. ESI-MS (*m*/*z*): 408.1377 $[M+H]^+$ calcd for $C_{22}H_{21}N_3O_3S$ ($[M+H]^+$ = 408.1382).

2.1.1.3. 4-(5-(2-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(3b)***. Yield 79 %. (Fioravanti et al., 2017)

2.1.1.4. 4-(5-(3-nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(4b)***. Yellow powder. m.p. 220–222 °C. Yield 62 %. IR (KBr, ν_{max} cm^{-1}): 3557 (—NH_2), 3289 (—NH_2), 3298 (>C—H, benzene), 2309, 1595 (C═C, benzene), 1534, 1494, 1444 (C═C, benzene), 1399, 1348 (—SO_2), 1156, 1137 (—SO_2), 1098, 1081, 871, 689. ^{1}H NMR (500 MHz, DMSO) δ 8.18 (t, *J* 1.5 Hz, 1H), 8.14 (dt, *J* 7.5, 1.5 Hz, 1H), 7.83–7.77 (m, 2H),7.70–7.63 (m, 2H), 7.61 (d, *J* 9.0 Hz, 2H), 7.50–7.39 (m, 3H), 7.12 (d, *J* 9.0 Hz, 2H), 7.03 (s, 2H), 5.87 (dd, *J* 12.0, 5.0 Hz, 1H), 4.04 (dd, *J* 17.5, 12.0 Hz, 1H), 3.30 (dd, *J* 17.5, 5.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 149.8, 148.2, 145.6, 143.7, 133.5, 132.4, 131.5, 130.8, 129.4, 128.7, 127.2, 126.2, 122.6, 120.9, 112.1, 61.5, 42.7. ESI-MS (*m*/*z*): 445.0929 $[M+H]^+$ calcd for $C_{21}H_{18}N_4O_4S$ ($[M+H]^+$ = 445.0946).

2.1.1.5. 4-(5-phenyl-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl) benzenesulfonamide ***(5b)***. Yield 75 %. (Gul et al., 2017a)

2.1.1.6. 4-(5-(3-hydroxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(6b)***. Yellow powder. m.p. 219–220 °C. Yield 80 %. IR (KBr, ν_{max} cm^{-1}): 3394 (—NH_2), 3297 (—NH_2), 3061 (>C—H, benzene), 2834, 1592 (C═C, benzene), 1556, 1494, 1461 (C═C, benzene), 1418, 1351 (—SO_2), 1282, 1160 (—SO_2), 1087, 1015, 872, 689. ^{1}H NMR (500 MHz, DMSO) δ 9.38 (s, 1H), 7.71–7.65 (m, 2H), 7.62–7.56 (m, 2H), 7.26 (d, *J* 8.5 Hz, 2H), 7.14 (t, *J* 7.5 Hz, 1H), 7.09–7.02 (m, 2H), 6.96 (s, 2H), 6.71 (dt, *J* 7.5, 1.5 Hz,1H), 6.64 (ddd, *J* 8.0, 2.0, 1.5 Hz, 1H), 6.61 (t, *J* 2.0 Hz, 1H), 5.50 (dd, *J* 12.0, 5.0 Hz, 1H), 3.92 (dd, *J* 17.5, 12.0 Hz, 1H), 3.14 (dd, *J* 18.0, 5.5 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 157.8, 149.7, 145.9, 143.1, 138.9, 132.7, 130.1, 129.2, 129.0, 127.0, 126.0, 116.1, 114.6, 112.0, 111.8, 62.2, 43.0, 20.9. ESI-MS (*m*/*z*): 408.1375 $[M+H]^+$ calcd for $C_{22}H_{21}N_3O_3S$ ($[M+H]^+$ = 408.1382).

2.1.1.7. 4-(5-(2-methoxyphenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(7b)***. Yellow powder. m.p. 244–246 °C. Yield 78 %. IR (KBr, ν_{max} cm^{-1}): 3388 (—NH_2), 3267 (—NH_2), 1597 (C═C, benzene), 1506, 1440 (C═C, benzene), 1398, 1326 (—SO_2), 1288, 1244, 1155 (—SO_2), 1098, 992, 897, 747. ^{1}H NMR (500 MHz, DMSO) δ 7.67 (d, *J* 8.0 Hz, 2H), 7.59 (d, *J* 9.0 Hz, 2H), 7.25 (t, *J* 8.0 Hz, 3H), 7.10 (d, *J* 8.0 Hz, 1H), 7.01–6.96 (m, 4H), 6.86 (dd, *J* 8.0, 2.0 Hz, 1H), 6.83 (t, *J* 7.0 Hz, 1H), 5.70 (dd, *J* 12.5, 5.0 Hz, 1H), 3.98–3.90 (m, 1H), 3.91 (s, 3H), 3.07 (dd, *J* 17.5, 5.0 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 156.1, 150.2, 145.8, 138.9, 132.7, 129.2, 129.1, 128.9, 128.3, 127.2, 126.0, 120.6, 111.6, 111.5, 57.1, 55.7, 41.9, 20.9. ESI-MS (*m*/*z*): 422.1538 $[M+H]^+$ calcd for $C_{23}H_{23}N_3O_3S$ ($[M+H]^+$ = 422.1538).

2.1.1.8. 4-(5-(4-chlorophenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(8b)***. Yellow powder. m.p. 197–198 °C. Yield 64 %. IR (KBr, ν_{max} cm^{-1}): 3320 (—NH_2), 3248 (—NH_2), 3069 (>C—H, benzene), 2846, 1597 (C═C, benzene), 1560, 1496, 1478 (C═C, benzene), 1411, 1346 (—SO_2), 1273, 1192 (—SO_2), 1098, 1014, 877, 698. ^{1}H NMR (500 MHz, DMSO) δ 7.71–7.65 (m, 2H), 7.63–7.57 (m, 2H), 7.44–7.37 (m, 2H),7.31–7.22 (m, 4H), 7.10–7.03 (m, 2H), 7.01 (s, 2H), 5.64 (dd, *J* 12.0, 5.0 Hz, 1H), 3.94 (dd, *J* 17.5, 12.0 Hz, 1H), 3.16 (dd, *J* 17.5, 5.0 Hz, 1H), 2.34 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 149.8, 145.8, 140.6, 139.1, 133.1, 132.1, 129.3, 129.0, 128.9, 127.7, 127.1, 126.1, 111.9, 61.6, 42.8, 20.9. ESI-MS (*m*/*z*): 426.1051 $[M+H]^+$ calcd for $C_{22}H_{20}N_3O_2SCl$ ($[M+H]^+$ = 426.1043).

2.1.1.9. 4-(5-(3-nitrophenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(9b)***. Yellow powder. m.p. 169–170 °C. Yield 65 %. IR (KBr, ν_{max} cm^{-1}): 3399 (—NH_2), 3284 (—NH_2), 3108 (>C—H, benzene), 2625, 1594 (C═C, benzene), 1556, 1454, 1443 (C═C, benzene), 1398, 1351 (—SO_2), 1153, 1134 (—SO_2), 1097, 1006, 871, 700. ^{1}H NMR (500 MHz, DMSO) δ 8.16 (t, *J* 2.0 Hz, 2H), 8.13 (dt, *J* 7.5, 2.2 Hz, 1H), 7.69 (d, *J* 8.0 Hz, 2H), 7.67–7.62 (m, 2H), 7.60 (d, *J* 8.5 Hz, 2H), 7.27 (d, *J* 8.0 Hz, 2H), 7.09 (d, *J* 8.0 Hz, 2H), 7.02 (s, 2H), 5.84 (dd, *J* 12.0, 5.0 Hz, 1H), 4.01 (dd, *J* 17.5, 12.0 Hz, 1H), 3.27 (dd, *J* 18.0, 5.0 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (126 MHz, DMSO) δ 149.9, 148.2, 145.7, 143.8, 139.2, 133.4, 132.4, 130.8, 129.3, 128.6, 127.2, 126.2, 122.6, 120.9, 112.0, 61.4, 42.7, 30.6, 20.9. ESI-MS (*m*/*z*): 437.1283 $[M+H]^+$ calcd for $C_{22}H_{20}N_4O_4S$ ($[M+H]^+$ = 437.1283).

2.1.1.10. 4-(3-(4-methoxyphenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(10b)***. Yield 81 % (Thach et al., 2020)

2.1.1.11. 4-(3,5-bis(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(11b)***. Yield 80 %. (Thach et al., 2020)

2.1.1.12. 4-(3-(2,3-dimethoxyphenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(12b)***. Yellow powder. m.p. 205–206 °C. Yield 56 %. IR (KBr, vmax cm-1): 3356 (—NH_2), 3268 (—NH_2), 1596 (C═C, benzene), 1509, 1424 (C═C, benzene), 1396, 1333 (—SO_2), 1310, 1250, 1151 (—SO_2), 1099, 1019, 876, 831, 754. ^{1}H NMR (500 MHz, DMSO) δ 7.77–7.69 (m, 2H), 7.62–7.54 (m, 2H), 7.04–6.96 (m, 6H), 7.00–6.91 (m, 2H), 6.59–6.53 (m, 1H), 5.68 (dd, *J* 12.0, 5.5 Hz, 1H), 3.95 (dd, *J* 17.5, 12.0 Hz, 1H), 3.85 (s, 3H), 3.81 (s, 6H), 3.12 (dd, *J* 17.5, 5.5 Hz, 1H). ^{13}C NMR (125 MHz, DMSO) δ 160.2, 152.7, 149.9, 146.0, 145.6, 134.6, 132.4, 127.6, 127.2, 124.4, 124.4, 117.7, 114.1, 112.3, 111.4, 60.2, 57.5, 55.6, 55.3, 42.4. ESI-MS (*m*/*z*): 468.1554 $[M+H]^+$ calcd for $C_{24}H_{25}N_3O_5S$ ($[M+H]^+$ = 468.1593).

2.1.1.13. 4-(5-(2-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(13b)***. Yellow powder. m.p. 199–200 °C. Yield 69 %. IR (KBr, ν_{max} cm^{-1}): 3373 (—NH_2), 3272 (—NH_2), 1596 (C═C, benzene), 1509, 1424 (C═C, benzene), 1396, 1336 (—SO_2), 1310, 1259, 1156 (—SO_2), 1097, 839, 815, 742. ^{1}H NMR (500 MHz, DMSO) δ 7.78–7.70 (m, 2H), 7.64–7.59 (m,2H), 7.56 (dd, *J* 8.0, 1.5 Hz, 1H), 7.32 (td, *J* 8.0, 2.0 Hz, 1H), 7.26 (td, *J* 8.0, 1.5 Hz, 1H), 7.04–6.96 (m, 5H), 6.99–6.92 (m, 2H), 5.77 (dd, *J* 12.0, 5.0 Hz, 1H), 4.05 (dd, *J* 7.5, 12.0 Hz, 1H), 3.80 (s, 3H), 3.15 (dd, *J* 17.5, 5.5 Hz, 1H). ^{13}C NMR (125 MHz, DMSO) δ 160.3, 149.9, 145.7, 138.0, 133.0, 131.2, 130.1, 129.4, 127.9, 127.8, 127.3, 124.1, 114.2, 111.5, 59.8, 56.0, 55.3, 41.8, 18.5. ESI-MS (*m*/*z*): 442.0943 $[M+H]^+$ calcd for $C_{22}H_{20}N_3O_3SCl$ ($[M+H]^+$ = 442.0992).

2.1.1.14. 4-(3-(4-methoxyphenyl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(14b)***. Yellow powder. m.p. 150–151 °C. Yield 57 %. IR (KBr, ν_{max} cm^{-1}): 3373 (—NH_2), 3272 (—NH_2), 1596 (C═C, benzene), 1509, 1424 (C═C, benzene), 1396, 1336 (—SO_2), 1310, 1259, 1156 (—SO_2), 1097, 839, 815, 742. ^{1}H NMR (500 MHz, DMSO) δ 8.16 (t, *J* 1.5 Hz, 1H), 8.13 (dt, *J* 7.0, 2.0 Hz, 1H), 7.77–7.71 (m, 2H), 7.69–7.62 (m, 2H), 7.60 (d, *J* 9.0 Hz, 2H), 7.08 (d, *J* 9.0 Hz, 2H), 7.02 (dd, *J* 6.5, 2.5 Hz, 4H), 5.81 (dd, *J* 12.0, 5.5 Hz, 1H), 4.00 (dd, *J* 17.5, 12.0 Hz, 1H), 3.80 (s, 3H), 3.26 (dd, *J* 17.5, 5.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 160.4, 149.8, 148.2, 145.8, 143.9, 133.1, 132.4, 130.8, 127.8, 127.2, 124.1, 122.6, 120.9, 114.2, 111.8, 61.4, 55.9, 42.9. ESI-MS (*m*/*z*): 475.1064 $[M+H]^+$ calcd for $C_{22}H_{20}N_4O_5S$ ($[M+H]^+$ = 475.1052).

2.1.1.15. 4-(3-(4-chlorophenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(15b)***. Yield 67 %. (Gul et al., 2017a)

2.1.1.16. 4-(3-(4-chlorophenyl)-5-(3-hydroxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(16b)***. Yellow powder. m.p. 218–220 °C. Yield 67 %. IR (KBr, ν_{max} cm^{-1}): 3296 (—NH_2), 3196 (—NH_2), 1594 (C═C, benzene), 1508, 1492 (C═C, benzene), 1392, 1330 (—SO_2), 1309, 1248, 1154 (—SO_2), 1092, 917, 825, 737, 694. ^{1}H NMR (500 MHz, DMSO) δ 9.43 (s, 1H), 7.83–7.77 (m, 2H), 7.63–7.57 (m, 2H), 7.54–7.47 (m, 2H), 7.14 (t, *J* 8.0 Hz, 1H), 7.11–7.05 (m, 2H), 7.01 (s, 2H), 6.71 (dt, *J* 7.5, 1.5 Hz, 1H), 6.64 (ddd, *J* 8.0, 2.5, 1.0 Hz, 1H), 6.60 (t, *J* 2.0 Hz, 1H), 5.56 (dd, *J* 12.5, 5.5 Hz, 1H), 3.93 (dd, *J* 18.0, 12.5 Hz, 1H), 3.17 (dd, *J* 17.5, 5.0 Hz, 1H). ^{13}C NMR (125 MHz, DMSO) δ 157.9, 148.6, 145.7, 143.0, 133.7, 133.2, 130.7, 130.1, 128.7, 127.7, 127.1, 116.3, 114.7, 112.0, 62.5, 42.7. ESI-MS (*m*/*z*): 428.0853 $[M+H]^+$ calcd for $C_{21}H_{18}N_3O_3SCl$ ($[M+H]^+$ = 428.0836).

2.1.1.17. 4-(3-(4-chlorophenyl)-5-(2-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(17b)***. Yellow powder. m.p. 213–215 °C. Yield 78 %. IR (KBr, ν_{max} cm^{-1}): 3377 (—NH_2), 3265 (—NH_2), 1596 (C═C, benzene), 1509, 1492 (C═C, benzene), 1393, 1330 (—SO_2), 1315, 1244, 1154 (–SO_2), 1093, 992, 826, 745. ^{1}H NMR (500 MHz, DMSO) δ 7.82–7.76 (m, 2H), 7.60 (d, *J* 9.0 Hz, 2H), 7.52–7.46 (m, 2H), 7.30–7.22 (m, 1H), 7.10 (d, *J* 8.0 Hz, 1H), 7.04 – 6.98 (m, 4H), 6.88 (dd, *J* 7.5, 1.5 Hz, 1H), 6.83 (t, *J* 7.5 Hz, 1H), 5.74 (dd, *J* 12.0, 5.0 Hz, 1H), 3.94 (dd, *J* 17.5, 12.0 Hz, 1H), 3.90 (s, 3H), 3.10 (dd, *J* 17.5, 5.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 156.1, 149.1, 145.6, 133.6, 133.1, 130.8, 129.0, 128.7, 128.1, 127.7, 127.2, 120.6, 111.7, 111.6, 57.5, 55.7, 41.6. ESI-MS (*m*/*z*): 442.0996 $[M+H]^+$ calcd for $C_{22}H_{20}N_3O_3SCl$ ($[M+H]^+$ = 442.0992).

2.1.1.18. 4-(3-(4-chlorophenyl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(18b)***. Yellow powder. m.p. 231–232 °C. Yield 56 %. IR (KBr, ν_{max} cm^{-1}): 3363 (—NH_2), 3259 (—NH_2), 1592 (C═C, benzene), 1508, 1409 (C═C, benzene), 1391, 1336 (—SO_2), 1313, 1151 (—SO_2), 1093, 871, 828, 739. ^{1}H NMR (500 MHz, DMSO) δ 8.18 (t, *J* 2.0 Hz, 2H), 8.14 (dt, *J* 7.5, 1.5 Hz, 1H), 7.80 (d, *J* 8.5 Hz, 2H), 7.71–7.64 (m, 2H), 7.62 (d, *J* 9.0 Hz, 2H), 7.52 (d, *J* 8.5 Hz, 2H), 7.12 (d, *J* 9.0 Hz, 2H), 7.04 (s, 2H), 5.88 (dd, *J* 12.0, 5.0 Hz, 1H), 4.02 (dd, *J* 18.0, 12.0 Hz, 1H), 3.30 (dd, *J* 17.0, 5.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 148.8, 148.2, 145.4, 143.6, 133.9, 133.8, 132.4, 130.8, 130.4, 128.7, 127.8, 127.2, 122.7, 120.9, 112.2, 61.7, 42.5. ESI-MS (*m*/*z*): 479.0559 $[M+H]^+$ calcd for $C_{21}H_{17}N_4O_4SCl$ ($[M+H]^+$ = 479.0558).

2.1.1.19. 4-(3-(4-fluorophenyl)-5-(3-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(19b)***. Yellow powder. m.p. 208–209 °C. Yield 54 %. IR (KBr, ν_{max} cm^{-1}): 3404 (—NH_2), 3294 (—NH_2), 1596 (C═C, benzene), 1512, 1417 (C═C, benzene), 1351, 1308 (—SO_2), 1228, 1123 (—SO_2), 1098, 876, 738, 703. ^{1}H NMR (500 MHz, DMSO) δ 8.18 (t, *J* 1.5 Hz, 1H), 8.14 (dt, *J* 7.0, 2.0 Hz, 1H), 7.88–7.82(m, 2H), 7.70–7.62 (m, 2H), 7.61 (d, *J* 9.0 Hz, 2H), 7.30 (t, *J* 9.0 Hz, 2H), 7.11 (d, *J* 9.0 Hz, 2H), 7.03 (s, 2H), 5.86 (dd, *J* 2.0, 5.0 Hz, 1H), 4.03 (dd, *J* 18.0, 12.5 Hz, 1H), 3.30 (dd, *J* 18.0, 5.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 162.7 (d, *J* 247.5 Hz), 149.0, 148.2, 145.6, 143.7, 133.6, 132.4, 130.8, 128.4 (d, *J* 8.5 Hz), 128.1 (d, *J* 3.4 Hz), 127.2, 122.7, 120.9, 115.7 (d, *J* 21.9 Hz), 112.1, 61.7, 42.7. ESI-MS (*m*/*z*): 463.0850 $[M+H]^+$ calcd for $C_{21}H_{17}N_4O_4SF$ ($[M+H]^+$ = 463.0852).

2.1.1.20. (E)-4-(3-phenyl-5-styryl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(20b)***. Yellow powder. m.p. 224–226 °C. Yield 78 %. IR (KBr, vmax cm-1): 3304 (—NH2), 3239 (—NH2), 1593 (C═C, benzene), 1509, 1447 (C═C, benzene), 1401, 1306 (—SO2), 1267, 1144 (—SO2), 1098, 968, 799, 703. ^{1}H NMR (500 MHz, DMSO) δ 7.83–7.76 (m, 2H), 7.70–7.62 (m, 2H), 7.51–7.43 (m, 2H), 7.46–7.38 (m, 3H), 7.34–7.19 (m, 5H), 7.02 (s, 2H), 6.72 (d, *J* 16.0 Hz, 1H), 6.32 (dd, *J* 16.0, 8.0 Hz, 1H), 5.25 (ddd, *J* 12.5, 7.5, 5.5 Hz, 1H), 3.76 (dd, *J* 17.5, 12.0 Hz, 1H), 3.27 (dd, *J* 17.5, 5.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 150.4, 146.4, 135.8, 133.1, 131.9, 131.4, 129.2, 128.7, 128.6, 127.9, 127.8, 127.1, 126.5, 126.0, 112.2, 61.2, 39.8. ESI-MS (*m*/*z*): 404.1393 $[M+H]^+$ calcd for $C_{23}H_{21}N_3O_2S$ ($[M+H]^+$ = 404.1433).

2.1.1.21. (E)-4-(5-styryl-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide SP107 ***(21b)***. Yellow powder. m.p. 250–252 °C. Yield 73 %. IR (KBr, ν_{max} cm^{-1}): 3306 (—NH_2), 3238 (—NH_2), 1592 (C═C, benzene), 1503, 1448 (C═C, benzene), 1399, 1305 (—SO_2), 1250, 1155, 1141 (—SO_2), 1095, 968, 874, 702. ^{1}H NMR (500 MHz, DMSO) δ 7.72–7.61 (m, 4H), 7.45–7.39 (m, 2H), 7.33 – 7.19 (m, 7H), 7.01 (s, 2H), 6.70 (d, *J* 16.0 Hz, 1H), 6.30 (dd, *J* 16.0, 7.5 Hz, 1H), 5.22 (ddd, *J* 12.0, 8.0, 5.0 Hz, 1H), 3.73 (dd, *J* 17.5, 12.0 Hz, 1H), 3.24 (dd, *J* 17.5, 5.0 Hz, 1H), 2.35 (s, 3H). ^{13}C NMR (125 MHz, DMSO) δ 150.4, 146.5, 138.9, 135.9, 132.9, 131.4, 129.2, 129.2, 128.6, 128.0, 127.8, 127.1, 126.5, 126.0, 112.1, 61.0, 40.0, 21.0. ESI-MS (*m*/*z*): 418.1556 $[M+H]^+$ calcd for $C_{24}H_{23}N_3O_2S$ ($[M+H]^+$ = 418.1589).

2.1.1.22. (E)-4-(3-(4-methoxyphenyl)-5-styryl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(22b)***. Yellow powder. m.p. 242–243 °C. Yield 62 %. IR (KBr, ν_{max} cm^{-1}): 3311 (—NH_2), 3243 (—NH_2), 1596 (C═C, benzene), 1508, 1425 (C═C, benzene), 1399, 1306 (—SO_2), 1251, 1177, 1145 (—SO_2), 1098, 971, 796, 704. ^{1}H NMR (500 MHz, DMSO) δ 7.78–7.70 (m, 2H), 7.70–7.61 (m, 2H), 7.45–7.39 (m, 2H), 7.34–7.26 (m, 2H), 7.29–7.18 (m, 3H), 7.06–6.98 (m, 4H), 6.70 (d, *J* 16.0 Hz, 1H), 6.30 (dd, *J* 16.0, 8.0 Hz, 1H), 5.19 (ddd, *J* 12.0, 7.5, 5.5 Hz, 1H), 3.81 (s, 3H), 3.72 (dd, *J* 17.5, 11.5 Hz, 1H), 3.23 (dd, *J* 17.5, 5.0 Hz, 1H). ^{13}C NMR (125 MHz, DMSO) δ 160.2, 150.3, 146.6, 135.9, 132.6, 131.3, 128.6, 128.0, 127.8, 127.6, 127.1, 124.5, 114.2, 112.0, 61.0, 55.3, 40.0. ESI-MS (*m*/*z*): 434.1506 $[M+H]^+$ calcd for $C_{24}H_{23}N_3O_3S$ ($[M+H]^+$ = 434.1538).

2.1.1.23. (E)-4-(3-(2,5-dimethoxyphenyl)-5-styryl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(23b)***. Yellow powder. m.p. 267–269 °C. Yield 54 %. IR (KBr, ν_{max} cm^{-1}): 3355 (—NH_2), 3248 (—NH_2), 1593 (C═C, benzene), 1508, 1431 (C═C, benzene), 1392, 1334 (—SO_2), 1271, 1227, 1152 (—SO_2), 1096, 974, 872, 818, 745. ^{1}H NMR (500 MHz, DMSO) δ 7.68–7.62 (m, 2H), 7.45–7.39 (m, 3H), 7.30 (dd, *J* 15.0, 7.0 Hz, 2H), 7.26–7.21 (m, 3H), 7.05 (d, *J* 9.0 Hz, 1H), 7.01 (s, 2H), 6.99 (dd, *J* 9.0, 3.0 Hz, 1H), 6.68 (d, *J* 16.0 Hz, 1H), 6.31 (dd, *J* 16.0, 7.5 Hz, 1H), 5.16 (ddd, *J* 12.0, 7.5, 5.5 Hz, 1H), 3.84–3.79 (m, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 3.76 (d, *J* 1.8 Hz, 1H), 3.31 (dd, *J* 18.0, 5.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 153.1, 151.9, 149.4, 146.5, 135.9, 133.0, 131.3, 128.6, 128.1, 127.8, 127.1, 126.5, 121.5, 116.2, 114.0, 112.8, 12.3, 61.2, 56.4, 55.6, 43.0. ESI-MS (*m*/*z*): 464.1638 $[M+H]^+$ calcd for $C_{25}H_{25}N_3O_4S$ ($[M+H]^+$ = 464.1644).

2.1.1.24. (E)-4-(3-(4-chlorophenyl)-5-styryl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(24b)***. Yellow powder. m.p. 274–275 °C. Yield 73 %. IR (KBr, ν_{max} cm^{-1}): 3304 (—NH_2), 3238 (—NH_2), 1595 (C═C, benzene), 1509, 1413 (C═C, benzene), 1394, 1306 (—SO_2), 1233, 1156 (—SO_2), 1094, 996, 808, 613. ^{1}H NMR (500 MHz, DMSO) δ 7.86–7.77 (m, 2H), 7.72–7.62 (m, 2H), 7.57– 7.49 (m, 2H), 7.46–7.39 (m, 2H), 7.34–7.18 (m, 5H), 7.03 (s, 2H), 6.72 (d, *J* 16.0 Hz, 1H), 6.31 (dd, *J* 16.0, 7.5 Hz, 1H), 5.27 (ddd, *J* 12.5, 8.0, 5.5 Hz, 1H), 3.75 (dd, *J* 17.5, 11.5 Hz, 1H), 3.27 (dd, *J* 17.5, 5.0 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 149.3, 146.2, 135.8, 133.6, 133.3, 131.6, 130.8, 128.7, 128.6, 127.9, 127.8, 127.7, 127.1, 126.5, 112.4, 61.4, 39.6. ESI-MS (*m*/*z*): 438.1016 $[M+H]^+$ calcd for $C_{23}H_{20}N_3O_2SCl$ ($[M+H]^+$ = 438.1043).

2.1.1.25. (E)-4-(3-(4-fluorophenyl)-5-styryl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(25b)***. Yellow powder. m.p. 252–253 °C. Yield 81 %. IR (KBr, ν_{max} cm^{-1}): 3303 (—NH_2), 3238 (—NH_2), 1593.99 (C═C, benzene), 1502, 1417 (C═C, benzene), 1398, 1290 (—SO_2), 1229, 1156, 1144 (—SO_2), 1096, 823, 807, 735. ^{1}H NMR (500 MHz, DMSO) δ 7.87–7.83 (m, 2H), 7.72–7.63 (m, 2H), 7.45–7.39 (m, 2H), 7.33 – 7.28 (m, 4H), 7.28–7.22 (m, 3H), 7.03 (s, 2H), 6.71 (d, *J* 15.5 Hz, 1H), 6.31 (dd, *J* 16.0, 8.0 Hz, 1H), 5.24 (ddd, *J* 12.0, 7.5, 5.0 Hz, 1H), 3.75 (dd, *J* 17.5, 11.5 Hz, 1H), 3.27 (dd, *J* 17.5, 5.5 Hz, 1H). ^{13}C NMR (126 MHz, DMSO) δ 162.6 (d, *J* 247.0 Hz), 149.5, 146.4, 135.9, 133.1, 131.5, 128.6, 128.6, 128.2 (d, *J* 8.2 Hz), 127.9, 127.9, 127.1, 126.5, 115.7 (d, *J* 21.8 Hz), 112.3, 61.3, 39.9. ESI-MS (*m*/*z*): 422.1348 $[M+H]^+$ calcd for $C_{23}H_{20}N_3O_2SF$ ($[M+H]^+$ = 422.1339).

2.1.1.26. (E)-4-(3-(4-nitrophenyl)-5-styryl-4,5-dihydro-1H-pyrazol-1-yl)benzenesulfonamide ***(26b)***. Yellow powder. m.p. 265–266 °C. Yield 52 %. IR (KBr, ν_{max} cm^{-1}): 3310 (—NH_2), 3240 (—NH_2), 1589 (C═C, benzene), 1513, 1458 (C═C, benzene), 1399, 1304 (—SO_2), 1253, 1156, 1143 (—SO_2), 1098, 972, 880, 705. ^{1}H NMR (500 MHz, DMSO) δ 8.52 (t, *J* 2.0 Hz, 1H), 8.24 (ddd, *J* 8.0, 2.5, 1.5 Hz, 1H), 8.19 (dt, *J* 8.0, 1.5 Hz, 1H), 7.76 (t, *J* 8.0 Hz, 1H), 7.73–7.66 (m, 2H), 7.45–7.40 (m, 2H), 7.34–7.28 (m, 4H), 7.27–7.20 (m, 1H), 7.06 (s, 2H), 6.74 (d, *J* 15.5 Hz, 1H), 6.33 (dd, *J* 16.0, 8.0 Hz, 1H), 5.35 (ddd, *J* 12.0, 7.5, 5.0 Hz, 1H), 3.83 (dd, *J* 17.5, 12.0 Hz, 1H), 3.41–3.36 (m, 1H). ^{13}C NMR (125 MHz, DMSO) δ 148.5, 148.2, 145.9, 135.8, 133.8, 133.7, 132.1, 131.7, 130.4, 128.6, 127.9, 127.5, 127.1, 126.5, 123.3, 119.9, 112.7, 61.6, 40.00. ESI-MS (*m*/*z*): 449.1304 $[M+H]^+$ calcd for $C_{23}H_{20}N_4O_4S$ ($[M+H]^+$ = 449.1284).

2.2. Antimicrobial assay

The antimicrobial activity of the derivatives (**1b-26b**) was tested using a broth dilution method in 96-well microtiter microplates (Thach et al., 2020). Briefly, stock solutions (2 mg mL^{-1}) of the synthetic compounds in dimethyl sulphoxide (DMSO) were used to prepare the various concentrations in series of 25–100 µg mL^{-1} and inoculated with a suspension (100 µL) of the corresponding microorganism. Two Gram-negative bacteria (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 10145) and two Gram-positive bacteria (*B. subtillis* ATCC 11774 and *S. aureus* subsp. *aureus* ATCC 11632) were used for the evaluation of antibacterial properties, while two

filamentous fungal strains (*A. niger* (ATCC 6275) and *Fusarium oxysporum* (ATCC 7601)) and two yeast strains (*C. albicans* ATCC 7754 and *S. cerevisiae* (VTCC–Y–62)) were used for the evaluation of antifungal and yeast activities. DMSO was used as a negative control. Streptomycin and tetracycline (Sigma) were used as positive controls of the tests with the Gram-positive and Gram-negative bacteria, respectively, and nystatin (Sigma) was used as a positive control for the tests involving filamentous fungi and yeast. The minimum inhibitory concentration (*MIC*) value was expressed as the lowest concentration of the test sample.

2.3. Cytotoxicity assay

Human lung carcinoma (A549), human cervix carcinoma (HEP-2C) and Vero cells were used for the toxicity assays of the synthesised compounds. Cells were cultured in DMEM (Dulbecco's modified Eagle's medium) or MEME (minimum essential medium with Earle's salts) supplemented with *L*-glutamine, sodium pyruvate, $NaHCO_3$, PSF (penicillin–streptomycin sulphate-Fungizone), non-essential amino acids, and bovine calf serum (10%) at 37 °C in a 5% (v/v) CO_2 incubator.

A sulforhodamine B (SRB) assay was used to determine cell viability (Lin et al., 1993). Briefly, cells were plated in a 96-well plate. The cells were stained by the addition of SRB (0.4%, w/v) dissolved in acetic acid (1%). The plates were placed on a shaker for 5 min and the absorption intensity was determined at 515 nm using an ELISA plate reader. The test samples were initially dissolved in DMSO and added to the wells. Ellipticine was used as a positive control for all cell lines. Negative control groups using DMSO (10%) were added to the wells. A zero-day control was carried out by adding an equivalent number of cells to some wells of the 96-well plates and then incubating at 37 °C for 10 min. To screen the toxicity of the synthesised compounds, cell survival (CS) values were determined at a concentration of 10 μg mL^{-1} and calculated via Eq. (1).

$$CS\% = \frac{\text{Absorbance(sample)} - \text{Absorbance(zero day)}}{\text{Absorbance(control)} - \text{Absorbance(zero day)}} \times 100 \quad (1)$$

The samples with CS values below 50% were selected to test against Vero cells and determine the IC_{50} value, which is the concentration inducing 50% inhibition of cell growth. The IC_{50} value was determined graphically using a curve-fitting algorithm. All values were expressed as means ± SD at least three independent experiments.

2.4. In vitro anti-inflammatory activity

The anti-inflammatory activity of the synthesised compounds was evaluated using a modified NO inhibitory method with a murine macrophage cell line, RAW 264.7, as previously reported (Cheenpracha et al., 2010). Briefly, RAW 264.7 cells were cultured in DMEM supplemented with *L*-glutamine (2 mM), HEPES (10 mM), sodium pyruvate (1,0 mM), and 10% foetal bovine serum (FBS). The cells were seeded into the 96-well culture plates and incubated at 37 °C in a humidified atmosphere containing 5% CO_2 for 24 h. The culture was replaced by FBS-free DMEM for 3 h. The tested compounds were dissoluted in DMSO. The cells were treated with samples dissolved in DMEM at various concentrations for 2 h, followed by NO production via treatment with lipopolysaccharide (LPS) (1.0 μg mL^{-1}) for 24 h. FBS-free DMEM was used as a negative control. N^G-methyl-L-arginine acetate salt (*L*-NMMA) (Sigma) was used as a positive control. The amount of NO produced in the cultured medium was measured with a Griess Reagent System (Promega Cooperation, WI, USA). The mixture was incubated at room temperature for 10 min and the absorbance was measured at 540 nm by a microplate reader. The standard curves were created by using known concentrations of sodium nitrite. NO Inhibition (%) was calculated via Eq. (2).

$$\text{Inhibition}\% = \frac{\text{NO(LPS)} - \text{NO(sample)}}{\text{NO(LPS)}} \text{x}100 \quad (2)$$

The IC_{50} value was identified as the concentration inducing a 50% inhibition of NO production and was determined graphically using a curve-fitting algorithm with TableCurve 2Dv4 software. All values were expressed as means ± SD from three independent experiments. To evaluate the cytotoxic effect of the synthesised compounds on the RAW 264.7 cells in the assay condition, an SRB assay was used at concentrations of 20 μg mL^{-1}.

2.5. In vivo anti-inflammatory effect

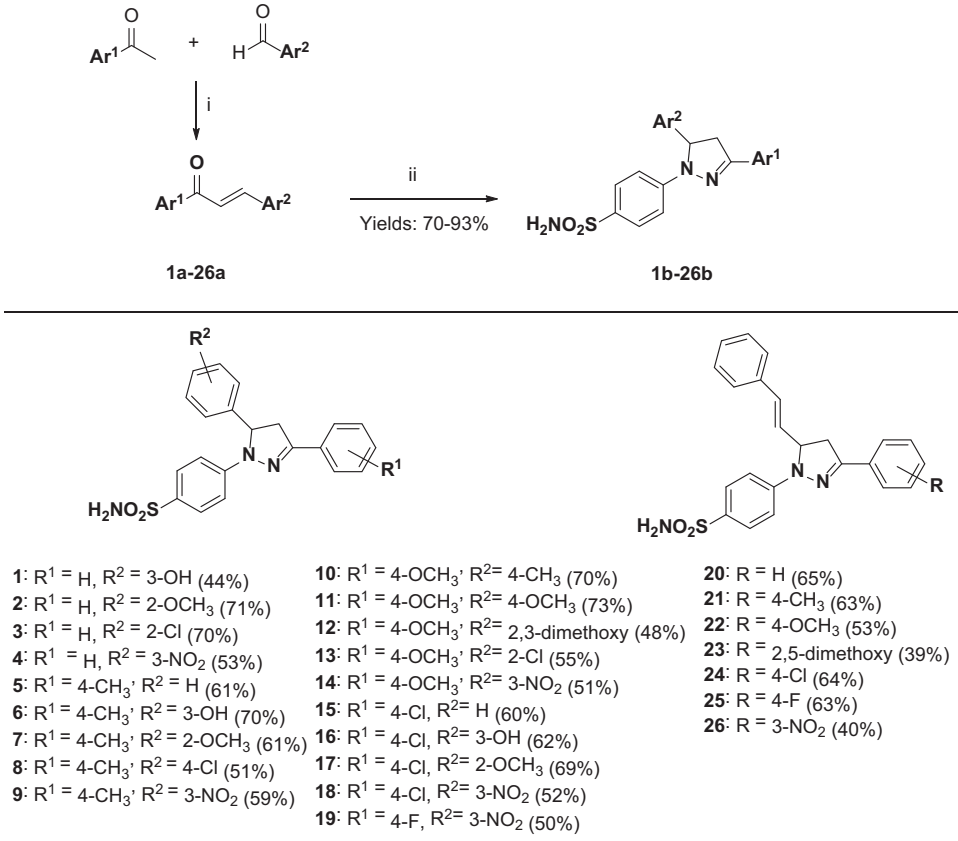
The synthetic compounds with IC_{50} values lower than the values of *L*-NMMA and CS values against RAW 264.7 cells at 20 μg mL^{-1} of more than 30% were selected for evaluation of *in vivo* anti-inflammatory activity, which was tested on mouse ear oedema induced by ethyl phenylpropionate (EPP), as previously reported (Pacheco et al., 2014). Briefly, EPP dissolved in acetone (1 mg/20 μL/ear) was applied topically to the right ear of the mice (inner and outer surfaces) to induce oedema. The same ear received the test compounds at 2 mg in 20 μL of acetone. The control group used 20 μL of acetone. The ear oedema was measured at 15 min, 30 min and 60 min after the application of the compounds. The percentage of oedema inhibition was calculated using Eq. (3).

$$\text{Inhibition}\% = \frac{\text{Oedema(control)} - \text{Oedama(sample)}}{\text{Oedema(control)}} \text{x}100 \quad (3)$$

3. Results and discussion

3.1. Chemistry

The synthesis of new triarylpyrazoline sulphonamides was performed via the two-step protocol illustrated in Scheme 1. Chalcones (**1a-26a**) were prepared by the reaction between aldehydes and ketones in the presence of bases for 4 h at room temperature with yields of 70–93%. Microwave-assisted cyclocondensation of 4-hydrazinylbenzenesulphonamide and the corresponding chalcones with some modifications (Gul et al., 2017b) afforded the sulphonamides (**1b-26b**) in total yields of 39–73% for two steps. The result showed that the synthetic performance under microwave irriation was significantly improved in comparison with common reflux (Gul et al., 2017a; Thach et al., 2020) while reaction time was shortened down from 12 h to 1 h. Twenty novel compounds (**1b**, **4b**, **6b-9b**, **12b-14b** and **16b-26b**) are reported for the first time in



Scheme 1 Synthesis of 1,3,5-substituted pyrazoline sulphonamides. Reagents: (i) EtOH, KOH aq. (2 %), rt, 4 h; (ii) 4–hydrazinylbenzenesulphonamide, MeOH, microwave, 1 h.

this study. The structures of these compounds were determined using FTIR, NMR and HR-MS spectroscopies. Of particular note, the NMR spectrum data of the triarylpyrazoline sulphonamides bearing styryl groups (**20b-26b**) showed the presence of two protons at 6.71 ppm (d, J 16.0 Hz) and 6.31 ppm (dd, J 16.0, 7.7 Hz), indicating a double bond of the styryl groups in the *trans* configuration and singlet signals around 7.0 ppm for two protons of the $-SO_2NH_2$ moiety in the structures. The signals belonging to the aliphatic protons of the pyrazoline ring (H_a,H_b and H_x) appeared at about 3.3, 3.8 acfnd 5.3 ppm, respectively. All other signals are in complete agreement with the assigned structures. Moreover, the peaks of FTIR spectra confirmed the presence of the mainly functional groups such as N—H, $-SO_2$ and benzene rings in their structure. The series of synthesised triarylpyrazoline sulphonamides was evaluated for antimicrobial, antiproliferative, and anti-inflammatory activities.

3.2. Antimicrobial activity

The 1,3,5-substituted pyrazoline sulphonamides (**1b-26b**) were tested for their antimicrobial activity against two Gram-negative bacteria (*E. coli* and *P. aeruginosa*), two Gram-positive bacteria (*B. subtillis* and *S aureus*), two fungi (*A. Niger* and *F. Oxysporum*), and two yeasts (*C. albicans* and *S. cerevisiae*). The tests were performed using a broth dilution method in the 96-well microtiter microplates. The *MIC* values were determined from three tested concentrations of each compound (25, 50 and 100 μg mL^{-1}). The data are depicted in Table 1. None of the tested compounds showed inhibition of the two Gram-negative bacteria at test concentrations. Most of the synthesised compounds did not exhibit antifungal activity, except **16b** which had *MIC* values of 100 and 50 μg mL^{-1} against *A. Niger* and *F. Oxysporum*, respectively. Additionally, none of the triarylpyrazoline sulphonamides bearing a styryl ring (**20b-26b**) inhibited any microbial strains.

Among the synthesised sulphonamides, compounds **1b**, **6b** and **16b**, containing a 3-OH group, exhibited a high level of antimicrobial activity against two Gram-positive bacteria and the yeast *C. albicans*. A previous report confirmed that the 2-OH substituent has a high level of activity against these microbial strains (Thach et al., 2020). Two other compounds, **8b** and **15b**, bearing a 4-Cl substituent on the benzene rings, also exhibited a high level of activity against these strains. In general, hydroxybenzene and 4-chlorobenzene substituents attached to the pyrazoline ring are important for the enhancement of antibacterial activity, whereas the presence of styryl substituent in the triarylpyrazoline sulphonamides does not inhibit the test microbial strains.

3.3. Antiproliferative activity

The *in vitro* antiproliferative activity of all the synthesised triarylpyrazoline sulphonamides was tested against two cancer cell lines, human lung carcinoma (A549) and human cervix carcinoma (HEP-2C), using the SRB assay method (Farooq et al., 2020; Lee et al., 2011; Yang et al., 2014). Before determining the IC_{50} value and toxicity to mammalian Vero cells,

Table 1 Antimicrobial activity of triarylpyrazoline sulphonamide derivatives.

Comp.	MIC (μg mL^{-1})							
	Bacterial Gram (−)		Bacterial Gram (+)		Fungi		Yeast	
	E. coli	*P. aeruginosa*	*B. subtillis*	*S. aureus*	*A. niger*	*F. oxysporum*	*S. cerevisiae*	*C. albicans*
1b	> 100	> 100	**50**	**25**	> 100	> 100	> 100	**25**
2b	> 100	> 100	> 100	> 100	> 100	> 100	**100**	> 100
3b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
4b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
5b	> 100	> 100	> 100	100	> 100	> 100	> 100	> 100
6b	> 100	> 100	**50**	**50**	> 100	> 100	> 100	**25**
7b	> 100	> 100	**100**	> 100	> 100	> 100	> 100	> 100
8b	> 100	> 100	**25**	**50**	> 100	> 100	> 100	**50**
9b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
10b	> 100	> 100	**25**	> 100	> 100	> 100	> 100	> 100
11b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
12b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
13b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
14b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
15b	> 100	> 100	**50**	**50**	> 100	> 100	> 100	**50**
16b	> 100	> 100	**100**	**50**	**100**	**50**	> 100	**50**
17b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
18b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
19b	> 100	> 100	**100**	> 100	> 100	> 100	> 100	> 100
20b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
21b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
22b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
23b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
24b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
25b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
26b	> 100	> 100	> 100	> 100	> 100	> 100	> 100	> 100
Tetracycline	5.5	11.0	–	–	–	–	–	–
Streptomycin	–	–	7.2	11.4	–	–	–	–
Nystatin	–	–	–	–	23.1	11.6	5.8	11.6

it was essential to perform screening studies for cell viability inhibition against the cancer cell lines (Hoang et al., 2018; Hoang et al., 2015). The pharmacological results are summarised in Table 2. Ten compounds, **2b**, **6b**, **8b-10b**, **12b**, **13b**, **15b-17b,** were found to be associated with CS values lower than 50% for both the cancer cell lines, while **14b** inhibited only Hep-2C with a CS value of 46.90 ± 2.21%. Many of the compounds, e.g. **2b**, **8b**, **15b-17b,** showed complete inhibition (CS = 0%) against both the cell lines at the test concentration of 10 μg mL^{-1}. IC_{50} values (μM), which represent the concentration required to achieve 50% inhibition of the cells by each compound, were determined and compared with ellipticine as a cytotoxic drug. The compounds displayed antiproliferative activity with IC_{50} values against Hep-2C in the range of 16.03 ± 1.63 to 22.75 ± 0.19 μM and against A549 in the range of 16.19 ± 0.99 to 20.66 ± 2.09 μM. The triarylpyrazoline sulphonamides bearing a styryl ring (**20b-26b**) did not exhibit antiproliferative activity against the cancer cells which can relate to the interaction between the compounds and the relative receptors. It indicated that the structure of the starting aryl aldehydes is particularly important in enhancing the toxicity of triarylpyrazoline sulphonamides. In general, most of the triarylpyrazoline sulphonamides bearing substituents on both the benzene rings, e.g. Me, OMe, OH and Cl groups, exhibited higher toxicity against the two cancer cell lines than the non-substituted compounds.

Interestingly, a further study on these potent compounds showed that many of them exhibited low toxic effects on mammalian cells. Six compounds, **6b**, **10b**, **12b-14b**, **16b,** had CS values for Vero cells of more than 70% at the test concentration of 10 μg mL^{-1}, whereas ellipticine induces higher toxicity at the test concentration of 5 μg mL^{-1}. Two compounds, **8b** and **15b,** had IC_{50} values (19.22 ± 0.25 μM and 23.96 ± 1.0 2 μM, respectively) against mammalian Vero cells higher than those of the reference drug (14.34 μM). The six compounds showed good cytotoxic profiles against Hep-2C with IC_{50} values of 22.75 ± 0.19, 17.10 ± 1.42, 17.23 ± 2.38, 16.03 ± 1.63, 20.69 ± 0.56, 16.62 ± 1.38 μM, respectively. Five compounds, **6b**, **10b**, **12b, 13b**, **16b**, which contained methyl, hydroxyl, and methoxyl groups on the benzene rings, showed good cytotoxicity profiles against cell line A549 with IC_{50} values of 20.32 ± 2.31, 20.66 ± 2.09, 19.82 ± 1.75, 18.64 ± 1.02, and 20. 02 ± 2.71 μM, respectively. As a result, these compounds are suggested as potential anticancer drugs, given that they have no toxicity for the mammalian cells.

3.4. Anti-inflammatory properties

Anti-inflammatory activity was investigated via NO assay, as NO produced by macrophages, endothelial cells and neurons is an important indicator of inflammation with the regulation of many different physiological processes (García-Aranda

Table 2 Antiproliferative activity of triarylpyrazoline sulphonamide derivatives.

No.	Comp.	CS (%)[a]			IC_{50} (μM)		
		Hep-2C	A549	Vero	Hep-2C	A549	Vero
1	**1b**	79.08 ± 2.16	83.54 ± 3.07	–	–	–	–
2	**2b**	0	0	6.99 ± 0.97	17.42 ± 2.95	18.16 ± 0.83	5.99 ± 0.17
3	**3b**	99.86 ± 0.09	99.57 ± 0.41	–	–	–	–
4	**4b**	99.08 ± 0.68	99.89 ± 0.07	–	–	–	–
5	**5b**	96.78 ± 1.51	97.19 ± 0.22	–	–	–	–
6	**6b**	23.62 ± 2.80	11.54 ± 3.23	99.78 ± 0.68	**22.75 ± 0.19**	**20.32 ± 2.31**	–
7	**7b**	98.17 ± 0.48	98.74 ± 1.60	–	–	–	–
8	**8b**	0	0	2.44 ± 1.20	16.56 ± 0.89	18.47 ± 2.31	19.22 ± 0.25
9	**9b**	0	3.25 ± 2.75	20.14 ± 0.86	16.26 ± 1.49	20.87 ± 2.38	12.59 ± 0.46
10	**10b**	3.23 ± 0.43	26.31 ± 2.26	70.24 ± 0.89	**17.10 ± 1.42**	**20.66 ± 2.09**	–
11	**11b**	98.23 ± 1,67	96.64 ± 1.26	–	–	–	–
12	**12b**	**19.30 ± 2.16**	**41.85 ± 1.97**	**78.57 ± 0.66**	**17.23 ± 2.38**	**19.82 ± 1.75**	–
13	**13b**	0	0	94.28 ± 0.32	**16.03 ± 1.63**	**18.64 ± 1.02**	–
14	**14b**	46.90 ± 2.21	66.65 ± 2.33	87.62 ± 0.73	**20.69 ± 0.56**	–	–
15	**15b**	0	0	43.88 ± 0.86	17.20 ± 2.11	18.44 ± 0.92	23.96 ± 1.02
16	**16b**	0	0	71.19 ± 1.63	**16.62 ± 1.38**	**20.02 ± 2.71**	–
17	**17b**	0	0	0	16.26 ± 2.17	16.19 ± 0.99	4.42 ± 0.06
18	**18b**	94.93 ± 0.84	96.38 ± 0.90	–	–	–	–
19	**19b**	99.07 ± 0.53	99.36 ± 0.44	–	–	–	–
20	**20b**	99.07 ± 1.45	97.32 ± 0.77	–	–	–	–
21	**21b**	98,70 ± 0,25	97,66 ± 1,02	–	–	–	–
22	**22b**	98.53 ± 0.77	98.91 ± 1.24	–	–	–	–
23	**23b**	59.75 ± 3.25	60.69 ± 1.83	–	–	–	–
24	**24b**	96.99 ± 1.03	95.25 ± 1.73	–	–	–	–
25	**25b**	89.91 ± 2.57	94.18 ± 2.05	–	–	–	–
26	**26b**	98.52 ± 0.24	96.31 ± 2.17	–	–	–	–
27	DMSO	100	100	100	–	–	–
28	Ellipticine[b]	1.34 ± 0.52	2.82 ± 1.69	20.66 ± 1.54	0.89 ± 0.28	1.34 ± 0.61	14.34 ± 0.16

[a] The compounds were tested at a concentration of 10 μg mL^{-1}.
[b] reference drug was tested at a concentration of 5 μg mL^{-1}.

et al., 2020; Sharma et al., 2007). As excessive NO is possibly associated with inflammation, inhibition of NO production is a principal factor for screening potential anti-inflammatory drugs. In the present work, the murine macrophage cell line RAW 264.7 was treated with LPS and used as the conditioned medium for generating NO. IC_{50} values were calculated from tests at various concentrations, and the toxicity of the synthetic compounds against RAW 264.7 cells was measured according to the SRB assay at 20 μg mL^{-1} of each compound.

The results are summarised in Table 3. Seven synthetic compounds, **1b**, **2b**, **4b**, **16b-19b,** exhibited much higher activity than did the reference. Their IC_{50} values were in the range 14.78 ± 2.44 to 28.36 ± 3.55 μM. The results indicate that the effect of substituents on the benzene ring of the starting

Table 3 In vitro anti-inflammatory activity of triarylpyrazoline sulphonamide derivatives.

No.	Comp.	IC_{50} (μM)	CS (%)	No.	Comp.	IC_{50} (μM)	CS (%)
1	**1b**	**14.78 ± 2.44**	**31.55**	15	**15b**	32.04 ± 4.06	30.12
2	**2b**	**23.76 ± 2.18**	**80.14**	16	**16b**	30.09 ± 3.09	11.95
3	**3b**	61.36 ± 8.54	42.09	**17**	**17b**	**15.5 ± 0.52**	**54.23**
4	**4b**	**19.39 ± 0.99**	**53.71**	**18**	**18b**	**28.36 ± 3.55**	**50.06**
5	**5b**	> 250	3.79	19	**19b**	16.9 ± 0.69	7.59
6	**6b**	157.44 ± 7.91	68.57	20	**20b**	> 250	57.38
7	**7b**	85.39 ± 7.05	78.54	21	**21b**	> 250	74.48
8	**8b**	34.61 ± 4.49	3.98	22	**22b**	> 250	70.38
9	**9b**	51.28 ± 5.98	26.17	23	**23b**	> 250	63.39
10	**10b**	> 250	40.95	24	**24b**	> 250	78.39
11	**11b**	139.45 ± 3.43	44.55	25	**25b**	> 250	76.28
12	**12b**	> 250	58.01	26	**26b**	> 250	85.51
13	**13b**	128.55 ± 7.5	59.18	27	*L*-NMMA	31.37 ± 1.33	91.55
14	**14b**	> 250	72.72				

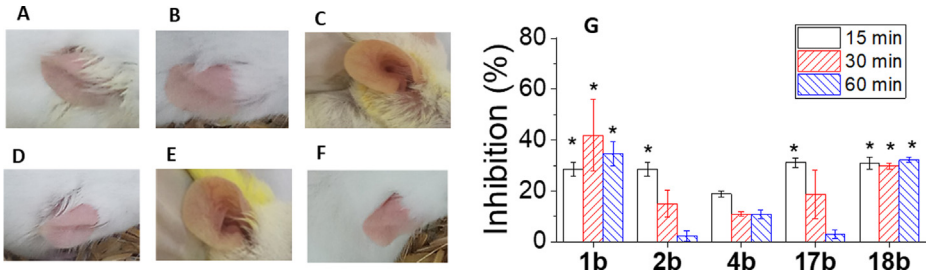


Fig. 2 Images for the treatment of mouse ear oedema using **1b** (A), **2b** (B), **4b** (C), **17b** (D), **18b** (E) and control (F) at 15 min after application and *in vivo* inhibition of mouse ear oedema of five triarylpyrazoline sulphonamides at treatment times of 15, 30 and 60 min (G). The asterisk indicates significant differences at $p < 0.05$.

acetophenones is associated with the anti-inflammatory activity of the triarylpyrazoline sulphonamides. Compounds without substituents or bearing a 4-Cl or 4-F group (**16b-19b**) incorporated into the basic acetophenone ring may suppress NO production by inhibiting iNOS enzyme activity, whereas the benzene ring containing methyl or methoxyl groups did not exhibit anti-inflammatory activity. It is noteworthy that the triarylpyrazoline sulphonamides bearing a styryl ring show no anti-inflammatory activity which can relate to weak interaction of these molecules with the relative enzymes. Five of these compounds, **1b**, **2b**, **4b**, **17b** and **18b,** exhibited low toxicity against the macrophage cells, with a CS value of more than 30% at a concentration of 20 μg mL^{-1}. These compounds could have beneficial therapeutic effects in inflammation management; thus, based on the results of the *in vitro* NO assay, these compounds were selected for investigation of *in vivo* anti-inflammatory activity.

The inhibitory potential of **1b**, **2b**, **4b**, **17b** and **18b** was assessed by measuring ear thickness, and the percentage of inhibition was calculated by comparing mouse ear thickness when treated with the compounds and the controls, at 15, 30 and 60 min after treatment (Banez et al., 2020; Sharma et al., 2007; Yun et al., 2008). Four of the test compounds, **1b**, **2b**, **17b** and **18b**, showed a significant decrease in ear oedema at 15 min, and inflammatory inhibition was found to be about 30% compared with the control (Fig. 2). Two compounds, **1b** and **18b**, showed the highest inflammatory inhibition over the treatment time. This result suggests that these compounds could be potent drugs for the treatment of inflammation.

4. Conclusion

A series of 1,3,5-substituted pyrazoline sulphonamide compounds was synthesised under microwave assisstance and the biological properties were investigated for antimicrobial potential, cytotoxicity, and anti-inflammatory activity. The compounds bearing 3-OH or 4-Cl groups exhibited significant antimicrobial activity against the bacteria *B. subtillis* and *S. aureus* and the yeast *C. albicans*. The synthetic compounds were successfully evaluated for antiproliferative activity using the cervix carcinoma cell (Hep-2C), human lung carcinoma (A549), and Vero cell. Six compounds, **6b**, **10b**, **12b-14b**, **16b**, showed good activity against the cancer cells with low toxicity against mammalian Vero cells. The results for the cytotoxicity of the novel compounds suggest that they have the potential to be chemotherapeutic agents in cancer treatment and warrant further investigation. Their anti-inflammatory behaviour was evaluated by testing *in vitro* NO production and *in vivo* mouse ear oedema. Five compounds, **1b**, **2b**, **4b**, **17b** and **18b**, showed high inhibition of NO production over the standard reference, and low toxicity for RAW 264.7 cells. Among them, compounds **1b** and **18b** showed the highest activity in inhibiting mouse ear oedema. These findings are promising for the discovery of novel potent drugs. Of particular importance, the pyrazoline-based sulphonamides bearing the styryl group did not exhibit any bioactivity in all tests. Thus, we do not recommend designing such molecules for pharmaceutical studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This study was fully supported by Tra Vinh University under grant contract number 304/2020/HĐ.HĐKH&ĐT-ĐHTV.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103408.

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