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Research Article

Novel 5-Hydroxy, 5-Substituted Benzenesulfonamide Pyrimidine-2,4,6-Triones Attenuate Lipopolysaccharide-Induced Acute Lung Injury via Inhibition of the Gelatinases, MMP-2 and MMP-9

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT A novel series of ten 5-hydroxy, 5-substituted benzene sulfonamide pyrimidine-2,4,6-triones were synthesized and their structures ascertained using ^{1}H-NMR, ^{13}C-NMR, mass and elemental analysis. These compounds were subsequently tested for inhibition of MMP-2 and MMP-9 where most exhibited activity with compound **5i** being the most potent against MMP-2 and MMP-9 with IC_{50} values of 2.35 nM and 8.24 nM, respectively. Compound **5i** was further analyzed in a mouse LPS-induced acute lung injury model where it had protective activity. Histochemical studies indicated that **5i** improved the vascular integrity of the lung.

Key words: synthesis; sulfonamide pyrimidine-2,4,6-triones; MMP-2; MMP-9

INTRODUCTION

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are two major life threatening sickness associated with acute and severe inflammation of lungs which leads to respiratory failure [Malemud, 2006]. Extensive studies on the pathogenesis of ALI have allowed elucidation of alterations in lung endothelial and epithelial barriers as causal to the disease. The characteristic of ALI include disruption of alveolar capillary membranes which results in excessive neutrophil infiltration, pulmonary edema, release of pro-inflammatory, and cytotoxic mediators [Grommes and Soehnlein, 2011]. Due to compromised lung compliance in ALI, it is associated with significant mortality (34–58%; MacCallum and Evans, 2005] and morbidity with no effective treatment.

Matrix metalloproteinases (MMP) are a diverse family of zinc containing extracellular proteinases metal enzymes that are responsible for degrading all types of extracellular matrix proteins [Nelson et al., 2000] overactivation and/or dysregulation of which may be involved in various inflammatory, malignant, and degenerative disease states. Aberrant expression of MMP has been associated with numerous chronic lung diseases [Vignola et al., 1988]. The bronchoalveolar lavage (BAL) fluid of patients with ARDS

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secondary to severe trauma or septic shock has elevated levels of MMP-9 in comparison with controls [Ricou et al., 1996]. A similar pattern was observed in the lungs of newborns with ARDS, along with elevated concentrations of MMP-2 [Cederqvist et al., 2001]. Patients with pneumonia also have increased MMP-9 expression [Hartog et al., 2003]. Thus, selective inhibition of MMP-2 and MMP-9 may represent a treatment option for ALI patients.

The present manuscript describes the synthesis and inhibitory activity of a series of novel 5-hydroxy, 5-substituted benzene sulfonamide pyrimidine-2,4,6-trione derivatives against MMP-2 and MMP-9. Further, to assess the beneficial effect on ALI, the most active inhibitor, **5i** was also tested in Lipopolysaccharide (LPS)-stimulated lung injury in mice.

METHODS AND MATERIALS

Chemistry

Analytical grade chemical were used directly. Melting points were determined in open capillary tube melting point apparatus and are uncorrected. Silica gel-G coated Al-plates (0.5 mm thickness; Merck) were used to check the completion of reaction and the plates were illuminated under UV (254 nm). FTIR spectra were recorded on Perkin Elmer-Spectrum RX-I spectrometer, while elemental analysis was carried out on a Vario EL III CHNOS elemental analyzer. ^{1}H-NMR spectra were recorded on a Bruker Avance II 400 NMR Spectrometer and ^{13}C-NMR spectra on a Bruker Avance II 100 NMR spectrometer in DMSO-d_6 using TMS as internal standard. Mass spectra were obtained using a VG-AUTOSPEC spectrometer. For induction of ALI, LPS (*Escherichia coli*, serotype 0111:B4) was used along with dimethyl sulfoxide (DMSO), and other reagents, were procured from Sigma–Aldrich (USA). The final volume of DMSO in the reaction mixture was less than 0.5%.

Synthesis of compound ***3*** was performed as previously reported [Karimi Zarchi and Aslani, 2012; Xiang et al., 2012].

Synthesis of compound ***5(a–j)***: The relevant methyl ketone (**3**) and 0.5 mmol of alloxan monohydrate (0.08 g) were suspended in 5 mL of glacial acetic acid and refluxed at 115°C for 3 h. The resulting mixture was precipitated, cooled, and re-crystallized from ethanol to afford pure products.

N-*(4-(2-(5-Hydroxy-2,4,6-trioxohexahydropyrimidin-5-yl)acetyl)phenyl)benzenesulfonamide (5a)*

Yield: 67%; m.p: 204–205°C; MW: 417.39; R_f: 0.52; FTIR (ν_{max}; cm^{-1} KBr): 3272 (N—H), 3052 (C—H$_{Aromatic}$), 2935 (C—H$_{aliphatic}$), 1672 (C=O), 1402, 1132 (SO_2), 887, 658; ^{1}H-NMR (400MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.62 (s, 2H, 2 × NH), 7.86–7.72 (m, 4H,4 × CH, Ar-H), 7.70–7.06 (m, 5H, 5 × CH, Ar—H), 3.87 (br,s, 1H, NH), 3.61 (s, 1H, OH), 3.18 (s, 2H,CH_2); ^{13}C-NMR (100MHz, $CDCl_3$) δ ppm:201.1, 170.2, 150.4, 142.2, 139.7, 131.9, 129.6, 129.1, 127.3, 126.7, 116.2, 91.5, 41.2; Mass: 418.41 $(M + H)^+$; Elemental analysis for $C_{18}H_{15}N_3O_7S$: Calculated: C, 51.80; H, 3.62; N, 10.07. Found: C, 51.82; H, 3.61; N, 10.07.

*4-Fluoro-*N*-(4-(2-(5-hydroxy-2,4,6-trioxohexahydropyrimidin-5 yl)acetyl)phenyl)benzenesulfonamide (5b)*

Yield: 72%; m.p: 193–195°C; MW: 435.38; R_f: 0.62; FTIR (ν_{max}; cm^{-1} KBr): 3275 (N—H), 3059 (C—H$_{Aromatic}$), 2937, 2932 (C—H$_{aliphatic}$), 1664 (C=O), 1408, 1135 (SO_2), 883 (F), 652; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.58 (s, 2H, 2 × NH), 7.98–7.72 (m, 4H, 4 × CH, Ar—H), 7.38–7.05 (m, 4H, 4 × CH, Ar—H), 3.89 (br,s, 1H, NH), 3.63 (s, 1H, OH), 3.21 (s, 2H,CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 201.1, 170.2, 166.1, 150.5, 142.2, 135.3, 130.8, 129.6, 126.7, 116.3, 115.9, 91.6, 41.2; Mass: 436.37 $(M + H)^+$; Elemental analysis for $C_{18}H_{14}FN_3O_7S$: Calculated: C, 49.66; H, 3.24; N, 9.65. Found: C, 49.68; H, 3.23; N, 9.64.

*3-Fluoro-*N*-(4-(2-(5-hydroxy-2,4,6-trioxohexahydropyrimidin-5-yl)acetyl)phenyl)benzenesulfonamide (5c)*

Yield: 63%; m.p: 208–209°C; MW: 435.38; R_f: 0.54; FTIR (ν_{max}; cm^{-1} KBr): 3279 (N—H), 3064 (C—H$_{Aromatic}$), 2942, 2939 (C—H$_{aliphatic}$), 1667 (C=O), 1412, 1139 (SO_2), 889 (F), 659; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.62 (s, 2H, 2 × NH), 7.89–7.72 (m, 3H,3 × CH, Ar—H), 7.70–7.08 (m, 5H, 5 × CH, Ar—H), 3.85 (br, s, 1H, NH), 3.67 (s, 1H, OH), 3.24 (s, 2H,CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 201.2, 170.2, 161.7, 150.4, 142.1, 141.3, 130.6, 129.6, 126.7, 122.9, 118.7, 116.2, 113.9, 91.4, 41.2; Mass: 436.42 $(M + H)^+$; Elemental analysis for $C_{18}H_{14}FN_3O_7S$: Calculated: C, 49.66; H, 3.24; N, 9.65. Found: C, 49.65; H, 3.25; N, 9.65.

N-*(4-(2-(5-Hydroxy-2,4,6-trioxohexahydropyrimidin-5-yl)acetyl)phenyl)− 3-methylbenzenesulfonamide (5d)*

Yield: 68%; m.p: 212–213°C; MW: 431.42; R_f: 0.49; FTIR (ν_{max}; cm^{-1} KBr): 3282 (N—H), 3068 (C—H$_{Aromatic}$), 2946, 2935 (C—H$_{aliphatic}$), 1673 (C=O), 1417, 1142 (SO_2), 881, 664; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.64 (s, 2H, 2 ×

NH), 7.77–7.72 (m, 3H,3 × CH, Ar—H), 7.67–7.06 (m, 5H, 5 × CH, Ar—H), 3.87 (br, s, 1H, NH), 3.69 (s, 1H, OH), 3.23 (s, 2H,CH_2), 2.28 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 201.3, 170.2, 150.3, 142.2, 139.7, 138.8, 132.2, 129.7, 128.9, 126.7, 124.3, 116.2, 91.5, 41.2, 21.3; Mass: 432.48 $(M + H)^+$; Elemental analysis for $C_{19}H_{17}N_3O_7S$: Calculated: C, 52.90; H, 3.97; N, 9.74. Found: C, 52.92; H, 3.98; N, 9.74.

N-*(4-(2-(5-Hydroxy-2,4,6-trioxohexahydropyrimidin-5-yl)acetyl)phenyl)−4-methylbenzenesulfonamide (5e)*

Yield: 71%; m.p: 221–223°C; MW: 431.42; R_f: 0.58; FTIR (ν_{max}; cm^{-1} KBr): 3284 (N—H), 3066 (C—$H_{Aromatic}$), 2942, 2939 (C—$H_{aliphatic}$), 1678 (C=O), 1415, 1149 (SO_2), 881, 669; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.62 (s, 2H, 2 × NH), 7.74–7.72 (m, 4H,4 × CH, Ar—H), 7.40–7.06 (m, 4H, 4 × CH, Ar—H), 3.89 (br,s, 1H, NH), 3.64 (s, 1H, OH), 3.21 (s, 2H,CH_2), 2.31 (s, 3H, CH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 201.2, 170.4, 150.4, 142.3, 137.8, 136.9, 129.7, 129.4, 128.3, 126.8, 116.3,91.4, 41.2, 21.3; Mass: 432.45 $(M + H)^+$; Elemental analysis for $C_{19}H_{17}N_3O_7S$: Calculated: C, 52.90; H, 3.97; N, 9.74. Found: C, 52.91; H, 3.96; N, 9.72.

N-*(4-(2-(5-Hydroxy-2,4,6-trioxohexahydropyrimidin-5-yl)acetyl)phenyl)−3-nitrobenzenesulfonamide (5f)*

Yield: 74%; m.p: 218–219°C; MW: 462.39; R_f: 0.64; FTIR (ν_{max}; cm^{-1} KBr): 3283 (N—H), 3068 (C—$H_{Aromatic}$), 2949, 2941 (C—$H_{aliphatic}$), 1681 (C=O), 1423, 1152 (SO_2), 878 (NO_2), 671; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.65 (s, 2H, 2 × NH), 8.54–8.02 (m, 4H,4 × CH, Ar—H), 7.72–7.06 (m, 4H, 4 × CH, Ar—H), 3.87 (br, s, 1H, NH), 3.62 (s, 1H, OH), 3.24 (s, 2H,CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 201.2, 170.3, 150.4, 148.2, 142.2, 140.3, 133.5, 129.8, 129.6, 127.3, 126.8, 123.1, 116.2, 91.2, 41.2; Mass: 463.42 $(M + H)^+$; Elemental analysis for $C_{18}H_{14}N_4O_9S$: Calculated: C, 46.76; H, 3.05; N, 12.12. Found: C, 46.77; H, 3.06; N, 12.11.

N-*(4-(2-(5-Hydroxy-2,4,6-trioxohexahydropyrimidin-5-yl)acetyl)phenyl)−4-nitrobenzenesulfonamide (5g)*

Yield: 67%; m.p: 226–228°C; MW: 462.39; R_f: 0.69; FTIR (ν_{max}; cm^{-1} KBr): 3287 (N—H), 3072 (C—$H_{Aromatic}$), 2952, 2947 (C—$H_{aliphatic}$), 1685 (C=O), 1427, 1157 (SO_2), 875 (NO_2), 678; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.61 (s, 2H, 2 × NH), 8.39–8.12 (m, 4H,4 × CH, Ar—H), 7.72–7.05 (m, 4H, 4 × CH, Ar—H), 3.86 (br,s, 1H, NH), 3.61 (s, 1H, OH), 3.22 (s, 2H,CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 201.3, 170.3, 150.5, 145.9, 142.2, 129.7, 128.2, 126.7, 124.2, 116.2, 91.5, 41.2; Mass: 463.40 $(M + H)^+$; Elemental analysis for $C_{18}H_{14}N_4O_9S$: Calculated: C, 46.76; H, 3.05; N, 12.12. Found: C, 46.78; H, 3.04; N, 12.13.

N-*(4-(2-(5-Hydroxy-2,4,6-trioxohexahydropyrimidin-5-yl)acetyl)phenyl)−2-(trifluoromethyl)* benzenesulfonamide *(5h)*

Yield: 62%; m.p: 241–242°C; MW: 485.39; R_f: 0.79; FTIR (ν_{max}; cm^{-1} KBr): 3293 (N—H), 3069 (C—$H_{Aromatic}$), 2958, 2952 (C—$H_{aliphatic}$), 1691 (C=O), 1429, 1155 (SO_2), 782 (CF_3), 682; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.59 (s, 2H, 2 × NH), 7.90–7.72 (m, 4H,4 × CH, Ar—H), 7.62–7.06 (m, 4H, 4 × CH, Ar—H), 3.85 (br, s, 1H, NH), 3.63 (s, 1H, OH), 3.24 (s, 2H,CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 201.2, 170.3, 150.4, 142.2, 135.2, 132.2, 130.1, 129.6, 128.2, 127.1, 126.7, 125.4, 116.2, 120.2, 91.4, 41.2; Mass: 486.42 $(M + H)^+$; Elemental analysis for $C_{19}H_{14}F_3N_3O_7S$: Calculated: C, 47.01; H, 2.91; N, 8.66. Found: C, 47.03; H, 2.90; N, 8.68.

N-*(4-(2-(5-Hydroxy-2,4,6-trioxohexahydropyrimidin-5-yl)acetyl)phenyl)−4-(trifluoromethy) benzenesulfonamide (5i)*

Yield: 73%; m.p: 245–246°C; MW: 485.39; R_f: 0.72; FTIR (ν_{max}; cm^{-1} KBr): 3295 (N—H), 3064 (C—$H_{Aromatic}$), 2962, 2958 (C—$H_{aliphatic}$), 1682 (C=O), 1431, 1152 (SO_2), 786 (CF_3), 689; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.63 (s, 2H, 2 × NH), 7.90–7.79 (m, 4H,4 × CH, Ar—H), 7.72–7.05 (m, 4H, 4 × CH, Ar—H), 3.87 (br,s, 1H, NH), 3.61 (s, 1H, OH), 3.21 (s, 2H,CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 202.1, 170.2, 150.4, 143.1, 142.2, 134.3, 129.7, 128.8, 127.2, 126.8, 124.1, 116.2, 91.4, 41.2; Mass: 486.38 $(M + H)^+$; Elemental analysis for $C_{19}H_{14}F_3N_3O_7S$: Calculated: C, 47.01; H, 2.91; N, 8.66. Found: C, 47.02; H, 2.92; N, 8.66.

N-*(4-(2-(5-Hydroxy-2,4,6-trioxohexahydropyrimidin-5-yl)acetyl)phenyl)−4-methoxybenzenesulfonamide (5j)*

Yield: 76%; m.p: 194–195°C; MW: 447.42; R_f: 0.63; FTIR (ν_{max}; cm^{-1} KBr): 3291 (N—H), 3062 (C—$H_{Aromatic}$), 2959,2952 (C—$H_{aliphatic}$), 1676 (C=O), 1435, 1158 (SO_2), 794, 678; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ ppm: 10.61 (s, 2H, 2 × NH), 7.72–7.64 (m, 4H,4 × CH, Ar—H), 7.12–7.06 (m, 4H, 4 × CH, Ar—H), 3.89 (br, s, 1H, NH), 3.82 (s, 3H, OCH_3), 3.64 (s, 1H, OH), 3.24 (s, 2H,CH_2);

^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 201.2, 170.2, 150.3, 142.2, 132.3, 129.6, 126.8, 126.2, 116.2, 114.2, 91.4, 55.9, 41.3; Mass: 448.44 $(M + H)^+$; Elemental analysis for $C_{19}H_{17}N_3O_8S$: Calculated: C, 51.00; H, 3.83; N, 9.39. Found: C, 51.02; H, 3.83; N, 9.40.

Animals

The present study was performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals and approved by the Institutional Committee on the Ethics of Animal Experiments of the Medical School of Southeast University. Sodium phenobarbital was used as anesthetic prior to all surgeries. For the study, specific pathogen-free male BALB/c mice (16–20 g; 6–8 weeks) were maintained under specific pathogen-free conditions in the animal center facilities of the Institute. Mice were kept in a temperature-controlled room (12-h dark and light cycles) and provided *ad libitum* access to food and water. They were acclimated for at least for 7 days to the surrounding environment prior to experimentation.

For induction of ALI, LPS (*Escherichia coli*, serotype 0111:B4) was used along with DMSO, and other reagents were procured from Sigma–Aldrich (USA). The final volume of DMSO in the reaction mixture was less than 0.5%.

Murine model of LPS-induced lung inflammation

LPS was used to induce ALI in mice as described in previous studies [Kuo et al. 2011; Li et al. 2012]. Forty mice were taken and randomly divided into five groups, including a sham operation group and four treatment groups. The sham operation group received vehicle ip for 30 min followed by intratracheal (i.t.) instillation of 50 μL saline. The four treatment groups were injected with vehicle, 10, 100, 1000 μg/kg of compound **5i** i.p. for 30 min respectively, followed by 100 μg/50 μL of LPS i.t. After 6 h, the mice were sacrificed and samples collected.

Bronchoalveolar lavage fluid collection

BAL was performed as described previously [Kuo et al. 2011; Li et al. 2012]. Briefly, after euthanasia, the trachea was exposed and intubated with a tracheal cannula. Collection was performed after repeated washing of airways and lungs with 1 mL of cold saline three times. Then respective BALFs were pooled and collected on ice. These were then centrifuged at 500 × *g* for 5 min at 4°C. The resulting cell-free supernatant was stored at −20°C for measurement of protein concentration using the Bio-Rad protein assay. Gemsa stain was used for the determination of total leukocyte content by counting the cells in the pellet.

RESULTS AND DISCUSSION

Chemistry

The synthesis of the target compounds was achieved in two-step reaction as shown in Figure 1. The reaction was started with synthesis of *N*-(4-acetyl-*N*-(*P*-substituted) phenyl benzenesulfonamide, **3** reacting compound **1** with relevant aromatic sulphonamides (**2**) in the presence of pyridine under vigorous reflux conditions. Compound **3** was also used as starting substrate to react with compound **4** to furnish compounds **5**(**a–j**). The structure of the

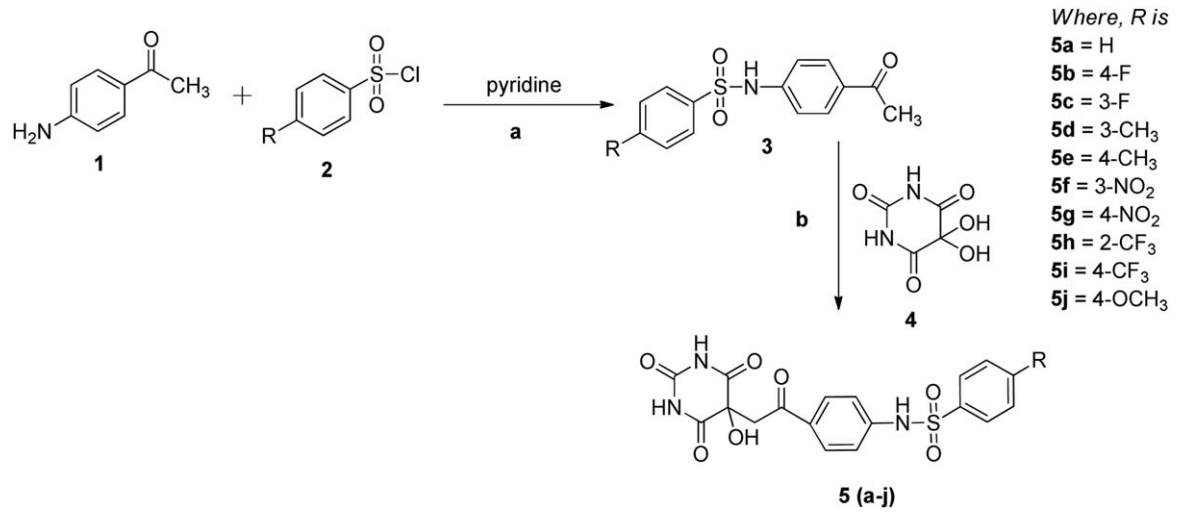

Fig. 1. Reagents and condition: (**a**) Reflux for 4 h; (**b**) Reflux, 3 h.

TABLE 1. MMP Inhibition Study of Compound 5(a–j)

	IC_{50}* (in nM)	
Compound	MMP-2	MMP-9
5a	980 ± 0.13	887.03 ± 0.63
5b	14.81 ± 0.66	29.11 ± 0.41
5c	21.01 ± 0.73	28.32 ± 0.53
5d	49.43 ± 0.36	63.26 ± 0.11
5e	61.23 ± 0.34	79.46 ± 0.68
5f	39.21 ± 0.83	41.16 ± 0.60
5g	28.34 ± 0.71	36.04 ± 0.39
5h	5.31 ± 0.63	15.76 ± 0.15
5i	2.35 ± 0.21	8.24 ± 0.29
5j	643.62 ± 0.15	443.45 ± 0.24
CGS27023A	26.5 ± 0.2	7.3 ± 0.62

*The mean of three experiments ± standard deviation.

synthesized compounds were confirmed on their physicochemical characteristics and spectroscopic (IR, ^{1}H-NMR,^{13}C-NMR, and mass spectral data) investigation. The strong intensity bands at 3430, 3139 and 2543 cm^{-1} were attributable to 2NH and C═N, respectively confirming the formation of a cyanoacetylhydrazono derivative. An upward field singlet in ^{1}H-NMR spectrum at 3.1 ppm was observed for CH_2 group and downfield singlet appearing at 9.2 ppm was due to addition of NH group. Moreover,^{13}C-NMR displayed a signal at 31.5 ppm for CH_2 and another signal at 125.7 ppm assigned to C═N.

Pharmacological Activity

MMP inhibition studies

As a result of the attractive drug like properties of barbiturates several 5,5-disubstituted pyrimidine-2,4,6-triones, exhibiting high inhibition over several MMP subtypes have been discovered [Foley et al., 2001; Blagg et al., 2005; Reiter et al., 2006;Wang et al., 2011]. The presence of a sulfonamide group also contributes to MMP inhibition. To search for novel MMP inhibitors, these two pharmacophores was linked together to generate novel hybrid conjugates. Their design was based on the assumption that, the appropriate balance between hydrophilic/lipophilic properties was efficiently achieved on substituting a 5-OH group because of its hydrogen bonding properties. As shown in Table 1, most of the compounds exhibit potent inhibitory activity against both the target MMPs as compared to CGS27023A, a reference standard [Kasaoka et al., 2008]. Among the newly synthesized derivatives, compound **5i** was the most potent analog against both MMP-2 and MMP-9, with IC_{50} values of 2.35 ± 0.21 and 8.24 ± 0.29 nM, respectively. Changing the substitution pattern of the trifluoromethyl as in compound **5h** leads to a slight loss of activity. The least activity was associated by compound possessing no phenyl substituent **5a**. More surprisingly, introduction of a fluorine substituent, compound **5b**, led to marked improvement of potency against both MMP-2 and MMP-9. Changing the pattern of substitution from *ortho-* to *para-* (compound **5c**), resulted in a marginal loss in activity. Activity further declined on introducing electron donating methyl substituents, compound **5d** and **5e** against both MMPs. Replacing methyl with an electron withdrawing group, e.g., nitro in compound **5f**, increased activity against MMP-2 and MMP-9, i.e., 39.21 ± 0.83 and 41.16 ± 0.60 nM, respectively, while a modest increase in activity was observed in the isomer, *para*-NO_2, compound **5g**.

From the structure–activity relationship studies (SAR), the inhibitory potency of the compounds described is greatly influenced by minor structural variations. Compounds containing electron withdrawing groups (**5b**, **5c**, **5f**, **5g**, **5h,** and **5i**) exhibit greater inhibition than their analogs with electron donating substituent (**5d**, **5e**, and **5j**) although no substitution render any compound inactive. Apart from this, the

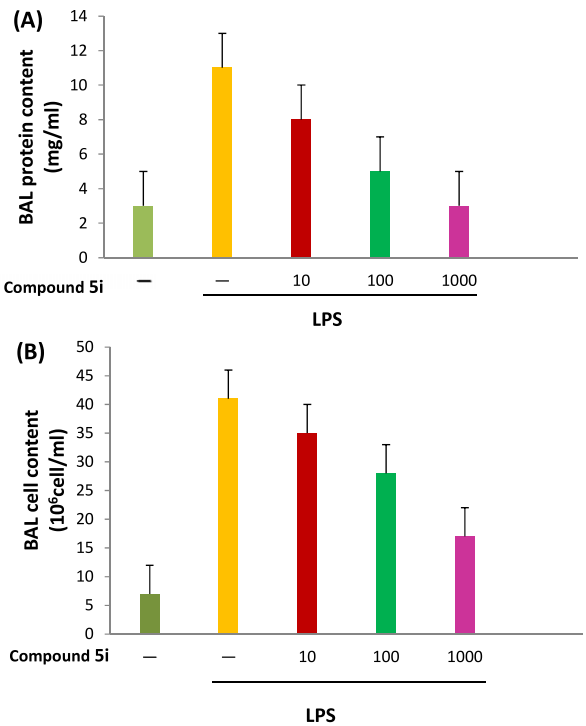

Fig. 2. Effect of compound **5i** on LPS-induced protein accumulation and leukocytes migration in BALF. **A:** Protein contents quantification in cell-free BALF exemplified pulmonary permeability. **B:** Leukocyte count in BALF. Values are expressed as mean ± S.D.

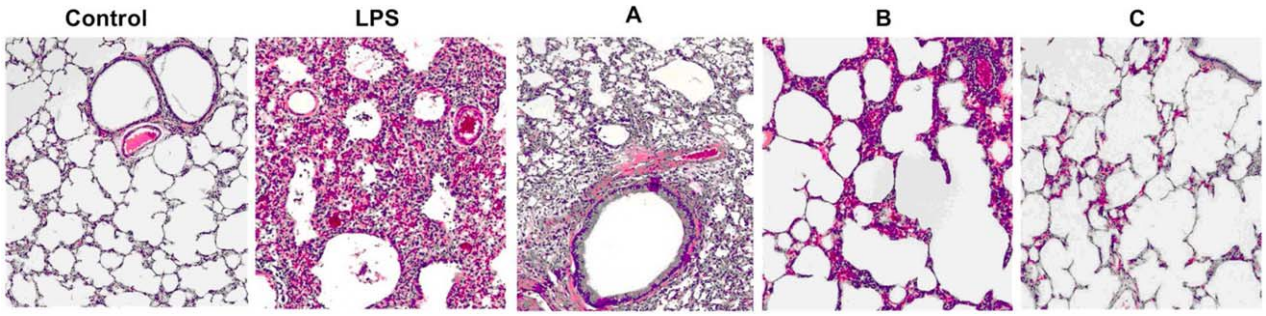

Fig. 3. Compound **5i** improves LPS induced lung injury (original magnification ×100). Healthy lung (control) and LPS induced lung injury; Treatment with compound **5i** at concentrations of (**A**) 25, (**B**) 50, and (**C**) 100 mg/kg. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

position of substitution has much impact on activity with *para*-substituted compounds being more active than their *meta* analogs.

*Effect of compound **5i** on LPS-induced ALI in mice*

Infection caused by Gram-negative pathogens is responsible for ALI progression with LPS, an endotoxin constituting major portion of outer membrane of Gram-negative bacteria serving as a determinant pathological factor in ALI [Chen et al., 2010]. In the present study, administration of LPS i.t. leads to acute lung inflammation in mice via the recruitment of macrophages and neutrophils leading to a loss of vascular integrity resulting in protein leakage and leukocyte infiltration.

The effect of compound **5g** was evaluated for effects on vascular permeability and leukocyte migration in ALI mice measuring the total BALF protein concentration to assess the integrity of the vascular membrane barrier. IB i.t. administration of LPS, protein concentration of BALF was increased nearly fourfold in comparison to control mice. In mice pretreated with compound **5i** at 100 and 1000 μg/kg for 30 min the BALF protein concentration was reduced (Fig. 2A). LPS administration for 6 h led to an approximate fivefold increase in lung leukocyte infiltration (Fig. 2B), With 30 min of **5i** pretreatment, LPS-induced leukocyte migration was inhibited in a dose-dependent manner with an approximate IC_{50} value of 1000 μg/kg (Fig. 1b).

Histochemical changes of lungs following administration of LPS included infiltration of inflammatory cells, tissue damage, and marked alveolar wall edema (Fig. 3) was observed. These changes were dose-dependently reversed by treatment with compound **5i** (25, 50, and 100 mg/kg).

On the basis of these results, compound **5i** exerted protective effect in LPS-induced ALI mice.

CONCLUSIONS

In the present paper a novel series of potent MMP-2 and MMP-9 inhibitors were synthesized among which compound **5i** was the most potent analog against MMP-2 and MMP-9, with IC_{50} values of 2.35 ± 0.21 and 8.24 ± 0.29 nM. Compound **5i** was also active in vivo in reducing LPS-induced ALI.

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