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Neuroprotective effect of novel celecoxib derivatives against spinal cord injury *via* attenuation of COX-2, oxidative stress, apoptosis and inflammation

Yan An, Jianing Li, Yajun Liu*, Mingxing Fan

Department of Spine Surgery, Beijing Jishuitan Hospital, Beijing 100035,China

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ABSTRACT

A novel series of celecoxib derivatives were synthesized and evaluated for cyclooxygenase (COX-1/COX-2) inhibitory activities for benefit in spinal cord injury (SCI). The title compounds were synthesized by conventional methods in good yields and subsequently tested for inhibitory activity against COX-1/COX-2. The most potent COX-2 inhibitor among the tested derivatives was further assayed for protective effect against experimental SCI of Sprague-Dawley rats. The designed compounds showed considerable inhibition of COX-2 as compared to COX-1 revealing compound **7m** as most potent inhibitor of COX-2 isoenzyme (IC_{50} = 0.04 μM). The expression of mitochondrial apoptotic genes (Bcl-2 and Bax) together with COX-2 and iNOS was restored near to normal as evidenced by western blot analysis in SCI rats. Taken altogether, compound **7m** was identified as most potent inhibitor of COX-2. It also showed protective action against SCI *via* attenuation of COX-2, oxidative stress and apoptosis and inflammation in Male Sprague-Dawley rats.

1. Introduction

The loss of motor and sensory function in a person due to spinal cord injury (SCI) is major cause of disability and mortality across the world. The physiological, biochemical and structural alterations cause irreversible damage to the spinal cord followed by SCI [1]. According to estimates, around more than 2 million people live with SCI and more than million new cases reported each year in the worldwide. This large community affected by SCI have huge, life-changing impacts and financial implication on the associated families. The studies have confirmed that, the etiology of SCI is multi-factorial in nature involving multiple mechanisms [2,3]. Moreover, the damage caused by SCI to the spine categorised specifically in two distinct forms, which are interrelated to each other, such as, primary and secondary. The primary SCI describes immediate damage by mechanical trauma causing alteration of cellular membrane, micro-vasculature system which enhances cellular permeability and loss of integrity of myelin and axons. This milieu initiates secondary injury concomitantly which are multi-faceted and can last for years. The secondary injuries consist of numerous subphases which causing loss of neuronal activity due oxidative stress, inflammation, apoptosis including other physiological changes [4–7]. Therefore, many of the therapeutic modalities against SCI are targeting complication arises due to secondary injuries, However, none of the agent/drugs proved effective till now against SCI which has put selective pressure to discover novel agents/molecules which can mitigate the consequences of SCI *via* multiple mechanisms (see Scheme 1).

Inflammation is the characteristic hallmark of SCI. It induces secretion of cytokines released by CNS or inflammatory cells [tumour necrosis factor (TNF)-α, Interleukin (IL)-1β and IL-6] and play key role in the regulation of the various steps of inflammatory reactions, i.e., recruitment and activation, but also in the downregulation of inflammatory cells [8,9]. This increase in inflammation in SCI is frequently coupled with oxidative stress due to loss of antioxidant capacity. Oxidative stress is defined as imbalance between oxidants and antioxidants, ultimately resulting in necrosis and lipid peroxidation. Malondialdehyde (MDA), glutathione (GSH) and superoxide dismutase (SOD) are important biomarkers of oxidative stress after SCI [10–12]. Apoptosis is another prominent clinical feature in the spinal cord post SCI after inflammation. It is known as programmed cell death and mainly controlled by the genes of Bcl-2 family (Bcl2 and Bax). The proportion of Bcl-2/Bax determines whether cells undergoing apoptosis or not. Besides, caspase associated cascade reaction is the important pathway for apoptosis, and caspase-3 is the key protease in the process of apoptosis [13]. The cyclooxygenase-2 (COX-2) is an inducible enzyme which produces leukotrienes and thromboxanes and reported to induce inflammatory cascade. Experimental evidences suggests that

* Corresponding author.
E-mail address: ay_jstspine@vip.163.com (Y. Liu).

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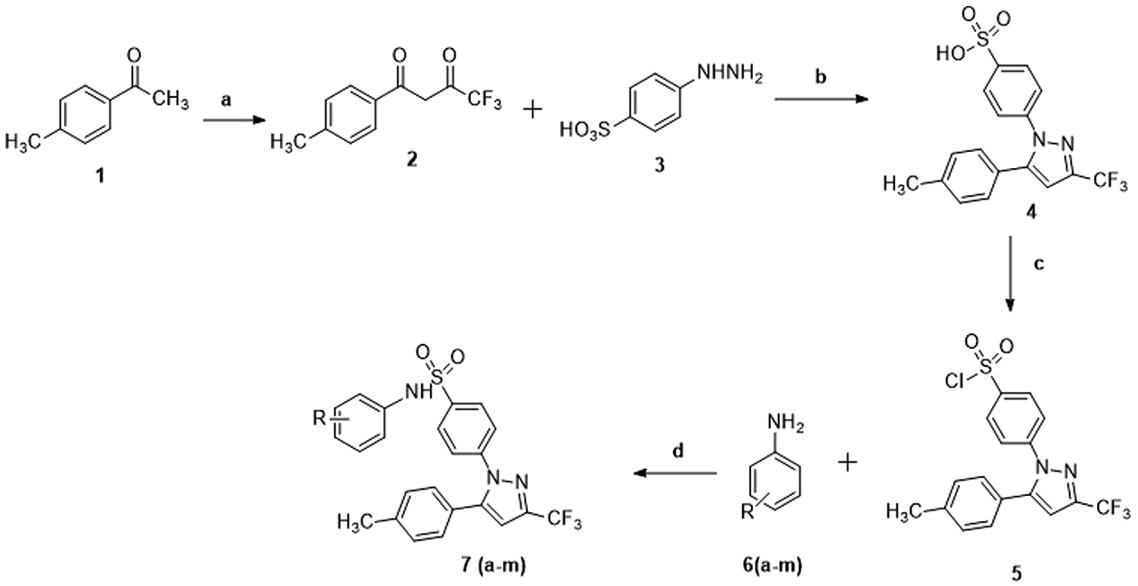

Scheme 1. . Synthesis of title analogues **7 (a-m)**. Where, (a) CF_3COOEt, NaOMe/MeOH, reflux, 95%; (b) EtOH/HCl, reflux, 82%; (c) PCl_5, CH_2Cl_2, reflux, 74% (d) Various aniline **6(a-m)**, Na2CO3, 4–6 h, room temperature.

following to SCI, the level of COX-2 was found overexpressed primarily in brain and activated macrophages in response to inflammatory stimuli or lipopolysaccharides [14]. It has been found that improving anti-oxidative capacity and inhibiting inflammation and apoptosis could ameliorate neurological impairment in SCI. Therefore, present study discusses the development of novel COX-2 inhibitors against SCI.

Pyrazole is well know heterocyclic molecule endowed with limitless therapeutic roles such, as anti-cancer [15–17], anti-viral [18–21], anti-bacterial [22–25], anti-fungal [26,27], anti-diabetic [28,29] and many more [30]. Celecoxib, a well known pyrazole based NSAID (Non-steroidal anti-inflammatory drug) and selective COX-2 inhibitor used to treat arthritis, acute pain, menstrual cramps and in familial adenomatous polyposis [31,32]. Moreover, lot of NSAIDS are derived from pyrazole nucleus as shown in Fig. 1. The potent activity of Celecoxib led to the discovery of various derivatives and pro-drugs in search of novel anti-inflammatory agents [33]. Moreover, Celecoxib has shown to ameliorate after effects of SCI alone or in combination of agents [34]. Thus, prompted by the above, the present study deals with the discovery and development of novel derivatives of Celecoxib as potent COX-2 inhibitor and their protective effect against experimentally induced spinal cord injury in rats.

2. Results and discussion

2.1. Chemistry

The compounds were synthesized by claisen condensation of the commercially available *p*-methyl-acetophenone (**1**) and trifluoroacetic acid ethyl ester gave diketone (**2**) in good yield. Compound **2** was then reacted with the commercially available *p*-hydrazino-benzenesulfonic acid (**3**) in refluxing ethanol to afford sulfonic acid derivative **4** (yield over 90%). This reaction is a regioselective transformation and the 1,5-diarylpyrazole could be generated almost exclusively by carrying out the condensation in the presence of the hydrochloride salt of the phenyl-hydrazine. The intermediate **4** was converted into its sulfonyl chloride **5** with phosphorus pentachloride in dry dichloromethane. It was later allowed to react with substituted anilines (**6a-n**) to afford target compounds (**7a-m**) in excellent yields after recrytallisation. The structures of compounds were ascertained by FT-IR, NMR, mass and elemental analysis.

2.2. In-vitro COX inhibitory activity

The newly developed Celecoxib derivatives were tested for *in-vitro* COX-1/COX-2 isozyme inhibition studies and the results are presented in Table 1. The compounds were analysed in comparison with template molecule of synthesis Celecoxib as standard drug. On closely inspecting the inhibitory activity chart, it was found that, entire set of compounds showed mild to significant inhibitory activity against COX-2 than COX-1. For instance, compound **7a**, with no-substitution on the flanked phenyl ring showed least inhibitory activity against both the tested isoenzyme. The activity was significantly increased upon insertion of *para*-methyl group at the phenyl against both COX-1 and COX-2 with enhanced activity against COX-2 isoenzyme (**7b**). On changing position of methyl group from *para* to *ortho* (**7c**), the activity was significantly decreased. The inhibitory activity was further dropped in the case of compound **7d** and **7e** having *para* and *ortho* methoxy group, respectively. To our surprise, the inhibitory activity against COX-2 as compared COX-1 was significantly increased on insertion of *para*-nitro group with drastic improvement in SI, **7f**. The changing of substitution pattern of *para*-nitro group to *ortho* showed marked reduction in inhibitory activity against both the tested isoenzyme together with drop in SI, **7g**. The activity was further dropped in the case of compound **7h** and **7i** having *para* and *ortho* substituted chloro atom, respectively. The replacement of chloro with fluoro atom (**7k** and **7l**) showed drastic improvement in inhibitory activity and SI. In the next instance, the activity was considerably improved upon introduction of tri-fluro methyl group (**7l** and **7m**). Out of which, compound **7m** containing *para*-trifluoro methyl group showed most potent inhibitory (IC_{50} = 0.04 μM) activity against COX-2 than COX-1 among all the tested derivatives with highest selectivity against COX-2.

The structure-activity relationship (SAR) studies suggest that minor changing of substitution pattern have marked influence on the activity. It has been found that compounds containing electron withdrawing substituent showed significant inhibition of COX-2 as compared to COX-1. Moreover, compounds containing electron donating group showed least activity against COX-1. Among the pattern of substituent, *para* is more favourable for inhibitory activity than *ortho*-substituted counterparts. This has also strong influence on the SI.

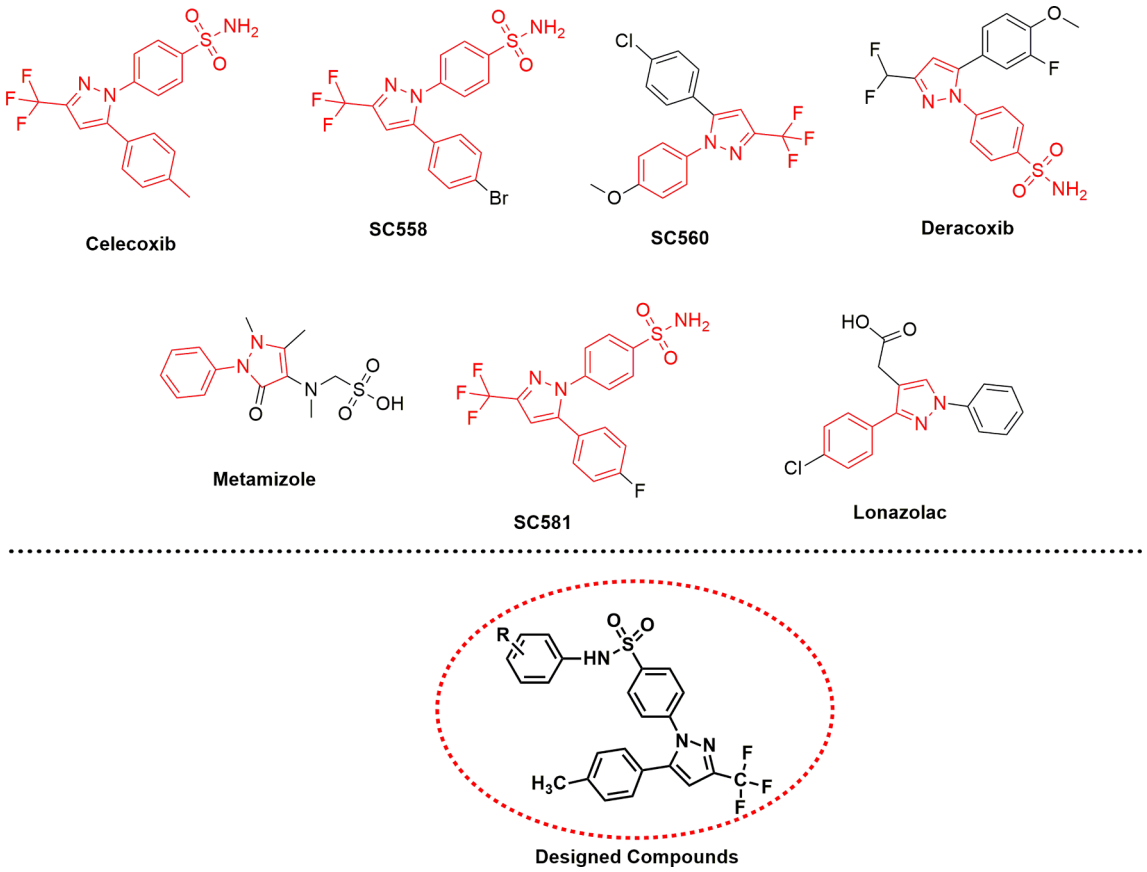

Fig. 1. Some examples of pyrazole based NSAID and design of target compounds.

Table 1
. Inhibitory activity of compound **7 (a-m)** against COX-1 and COX-2 isoenzyme.

Compound	R_1	IC_{50} (in μM)		Selectivity Index (SI)[a]
		COX-1	COX-2	
7a	H	42.16	28.45	1.48
7b	4-CH_3	23.35	15.71	1.48
7c	2-CH_3	20.30	18.25	1.11
7d	4-OCH_3	22.34	19.74	1.13
7e	2-OCH_3	22.20	20.91	1.06
7f	4-NO_2	22.32	1.23	18.14
7g	2-NO_2	26.43	2.14	12.35
7h	4-Cl	32.05	3.35	9.56
7i	2-Cl	36.40	5.62	6.47
7j	4-F	17.22	0.78	22.07
7k	2-F	20.61	0.97	21.24
7l	2-CF_3	27.13	1.14	4.03
7m	4-CF_3	11.51	0.04	287.75
Celecoxib		13.37	0.09	148.55

[a] SI: (COX-1 IC_{50}/COX-2 IC_{50}).

2.3. In-vivo pharmacological activity

The *in-vitro* activity of compound against COX isoenzyme disclosed compound **7m** as highly potent inhibitor of COX-2. Therefore, it is worthwhile to assess the pharmacological activity of compound **7m** against experimentally induced SCI in rats.

2.3.1. Effect of compound *7m* on the motor function of SCI rats

The loss of motor function is a characteristic hallmark of the SCI [35]. In many experimental studies, the affected motor function was assessed by Basso-Beattie-Bresnahan (BBB) scores and is widely accepted [36–38]. Therefore, in the present study, the effect of compound **7m** was assessed on Adult male Sprague-Dawley rats *via* BBB scores on 1, 4, 7, 10 and 14 day post-SCI. The results are presented in Fig. 2. The sham group showed no alteration in motor function, whereas, in surgery group the motor function was altered significantly as compared to sham. The **7m** treated rats showed dose-dependent improvement of motor function near to normal as compared to SCI group.

2.3.2. Effect of compound *7m* on neurons in SCI rats

The loss of neuronal normal architecture is well-evidenced in numerous clinical and non-clinical studies reporting SCI [39]. Therefore, in the next part, we investigate the effect of compound **7m** on the histopathology of neurons *via* H and E and Nissl staining. As evidence by Fig. 3, the Sham treated rats showed normal neuronal architecture, which was later found deteriorated in SCI treated group due to the presence of edema, necrosis and enhanced infiltration. On the other hand, **7m** treated rats showed reversal of these features near to normal in concentration-dependent manner as compared to SCI group. The result suggests that compound **7m** showed protective effect against SCI might be due to restoration of integrity of neurons lost after SCI.

2.3.3. Effect of compound *7m* on oxidative stress in SCI rats

The production of reactive oxygen species (ROS) due to impaired oxidative defense mechanism leads to the development of oxidative stress. This oxidative stress showed to be very detrimental to the neurons after SCI due to cyto-toxic effects and enhanced lipid-peroxidation. The central nervous system consists of large amount of lipids, thus it became very labile to the damage caused by oxidative stress [40]. Therefore, in the next part of the study we examined the effect **7m** on the endogenous biomarkers of the oxidative stress and the results are presented in Fig. 4. It was found that, compound **7m** decreases the MDA level and enhances the activity of SOD and GSH level as compared to SCI-treated group. It has been suggested that, compound **7m**

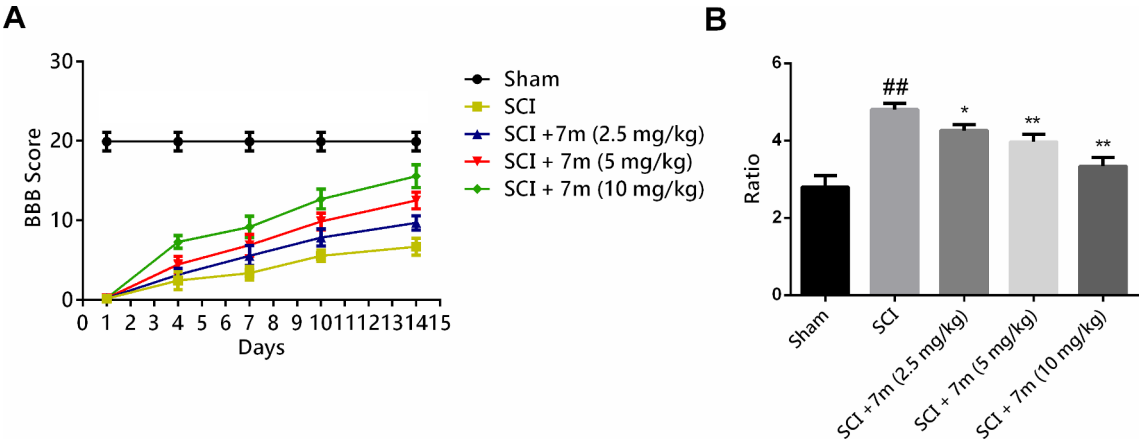

Fig. 2. Effect of compound **7m** on motor function as determined by (A) BBB Score and (B) ratio of wet/dry spinal cord. Values represent the mean ± SD and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post hoc* test.

significantly reduces the burden of oxidative stress caused by SCI, which might be accountable for its neuro-protective effect against SCI.

2.3.4. *Effect of compound **7m** on inflammation SCI rats*

Inflammation was markedly reported in patients affected by SCI followed by oxidative stress which leads to the progression of disease [41]. Therefore, the effect of compound **7m** was investigated on various pro-inflammatory cytokines and interleukins and results are presented in Fig. 5. The level of TNF-α, IL-1β and IL-6 were found to be enhanced in the injured spinal cord of the rats. Whereas, in **7m** treated rats, the level of these tested cytokines were considerably reduced in dose-dependent manner with maximum in 10 mg/kg treated group. Thus, it

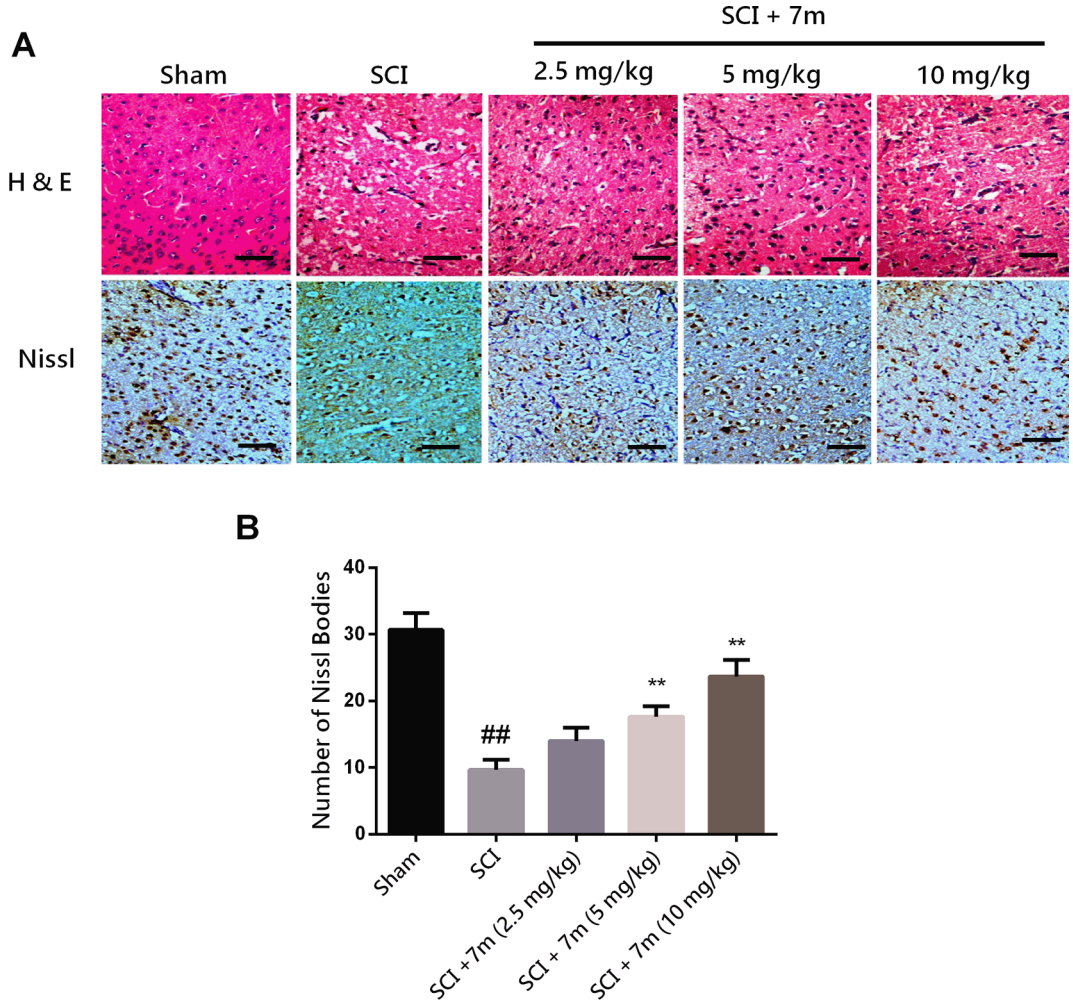

Fig. 3. Effect of compound **7m** on the neurons after SCI. (A) H & E and (B) Nissl staining. Values represent the mean ± SD and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post hoc* test.

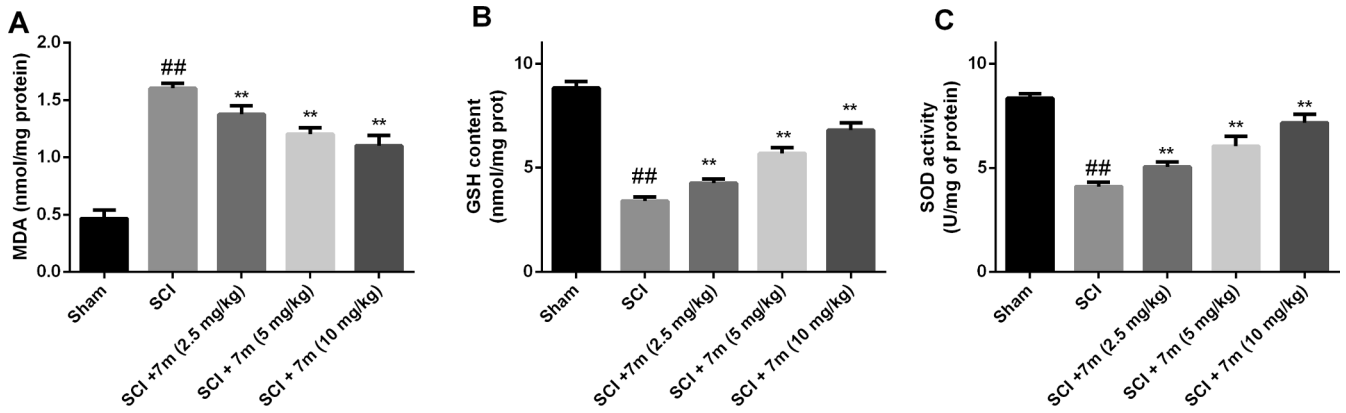

Fig. 4. . Effect of compound **7m** on the oxidative stress of spinal cord of rats after SCI induction (A) MDA, (B) GSH and (C) SOD. Values represent the mean ± SD and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post hoc* test.

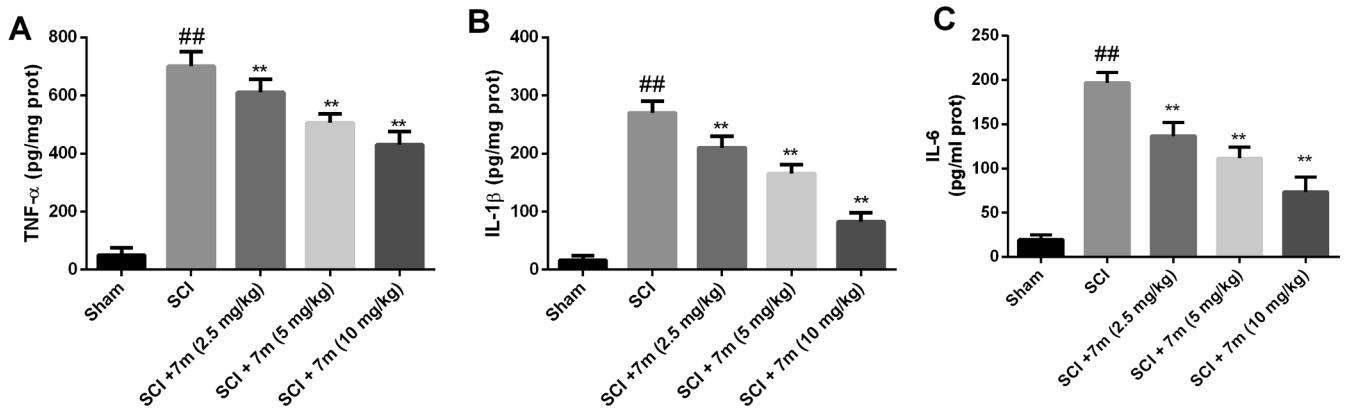

Fig. 5. . Effect of compound **7m** on the proinflammtory cytokines as determined by ELISA analysis (A) TNF-α, (B) IL-1β and (C) IL-6. Values represent the mean ± SD and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post hoc* test.

could be suggested that compound **7m** causes attenuation for inflammation which might be suggested as its plausible mechanism underlying neuro-protective effect against SCI.

2.3.5. Effect of compound *7m* on apoptosis in SCI rats

Apoptosis promoted by inflammation is the critical pathway which is found to be significantly altered during SCI. It is believed to play key a role in the pathogenesis of acute and chronic SCI *via* promoting necrosis of neurons and oligodendrocytes which ultimately reduces the function of neurons [42]. Therefore, it is suggested that, limiting the inflammation and oxidative stress in SCI delay the apoptosis and increases the chances of neuronal regeneration. Encouraged by the excellent anti-inflammatory and antioxidant activity of compound **7m**, it is worthwhile to determine its effects on apoptotic biomarkers. Various studies showed that mitochondrial genes have momentous impact on the apoptotic pathway, such as the Bcl-2 and Bax which is considered as anti-apoptotic and pro-apoptotic gene, respectively. The delicate balance between these two genes governs apoptosis, and any imbalance causes defect in apoptotic pathway. In western blot analysis (Fig. 6), the level of Bcl2 was found to be decreased together with increased Bax expression in SCI group as compared to control. Whereas upon treatment with compound **7m**, the level of these mitochondrial genes was restored near to normal. Concerning the role of COX-2 in the SCI and excellent *in-vitro* inhibitory activity of compound **7m** against COX-2, the next part of the study was aimed to assess whether **7m** could able to inhibit COX-2 *in-vivo* or not. As shown in Fig. 7, compound **7m** causes does-dependent decrease in expression of iNOS and COX-2 after SCI as determined by western blot analysis.

3. Material and methods

3.1. Chemistry

3.1.1. Synthesis of target molecules

The chemicals used in the current work were obtained from Sigma Aldrich (USA). ^{1}H NMR spectra were recorded in *d6*-DMSO on a Bruker Avance-400 NMR spectrometer with TMS as the internal reference. ^{13}C NMR spectra were recorded on a Bruker Avance-100 NMR spectrometer in *d6*-DMSO on the same spectrometers with TMS as the internal reference. The multiplicity of a signal is indicated as: s – singlet, d – doublet, t – triplet, q – quartet, m – multiplet, br – broad, dd – doublet of doublets, *etc*. Coupling constants (*J*) are quoted in Hz and reported to the nearest 0.1 Hz. Infrared spectra were recorded as a neat thin film on a Perkin-Elmer Spectrum One FT-IR spectrometer using Universal ATR sampling accessories. Letters in the parentheses refer to the relative absorbency compared to the most intense peak: w – weak, < 40%; m – medium, 40–75%; s – strong, greater than 75%. Melting points were obtained using an OptiMelt automated melting point system. MS spectra were recorded on an Agilent 1100 LC/MS.

Synthesis of compound **2**, **4** and **5** was performed with earlier reported procedure given elsewhere, and identities of the compounds were ascertained with the help of melting point, mass and elemental analysis [43].

3.2. General synthesis of compounds *7 (a-m)*

Compound **5** (12 mmol) was added over a period of time of 15 min

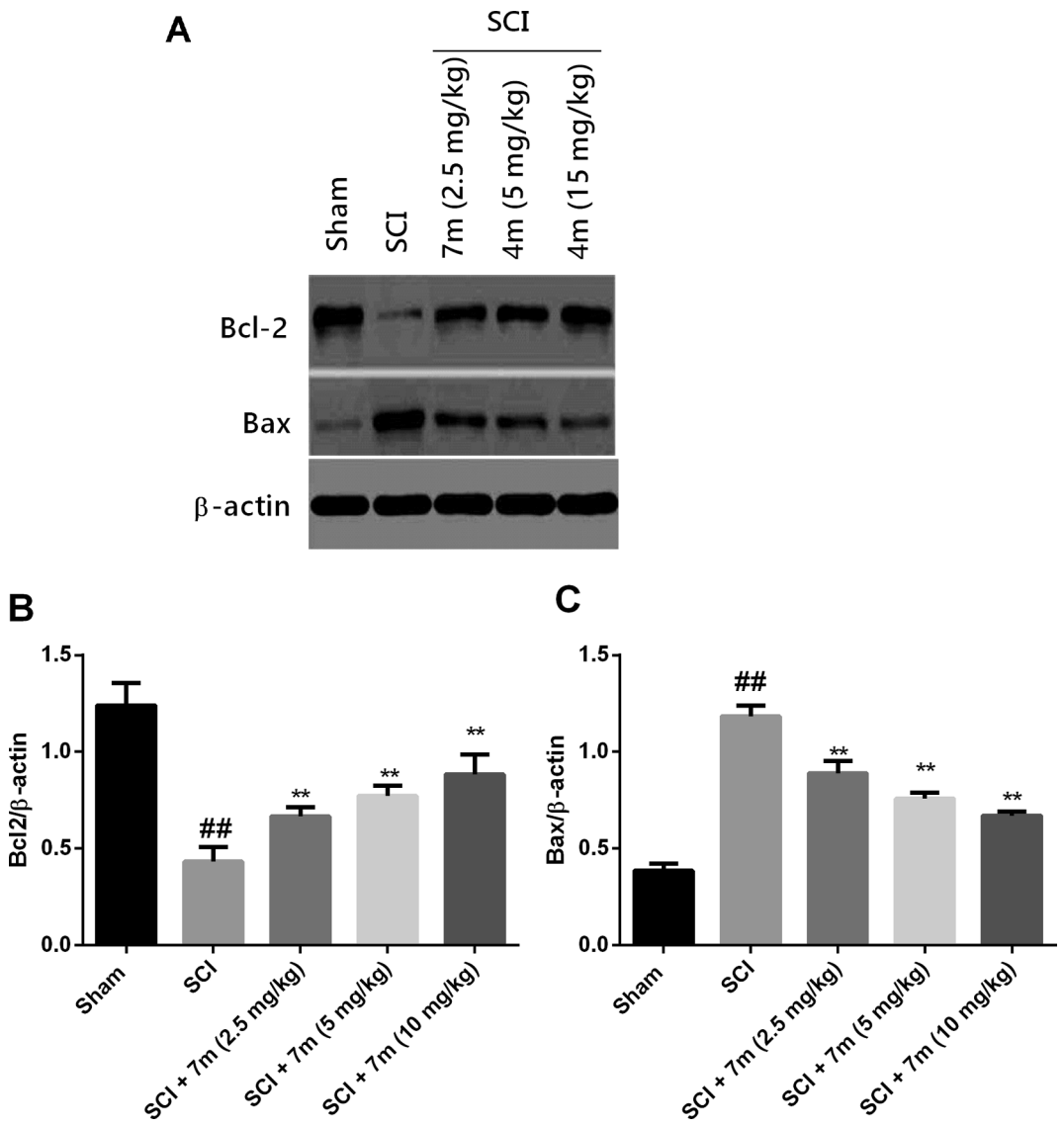

Fig. 6. . Effect of **7m** on the (a) expression of Bcl-2 and Bax in spinal cord of SCI rats *via* western blot analysis; and representative quantitative analysis of (b) Bcl2 and (c) Bax Values represent the mean ± SD and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post hoc* test.

to an aqueous mixture of corresponding anilines **6 (a-m)** (10 mmol) and Na_2CO_3 (12 mmol) in water (50 mL) and temperature was maintained at 0 °C. The resulting mixture was then continuously stirred for 4–6 h at room temperature and then acidified with 10% HCl at 0 °C. The target product **7 (a-m)** was obtained as precipitate after filtration, washed with water, dried, and then recrystallized.

*3.2.1. N-phenyl-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl) benzenesulfonamide (**7a**)*

Yield: 79%; M.p: 193–194 °C; MW: 457.47 ; R_f: 0.72; FTIR (ν_{max}; cm^{-1} KBr): 3249 (N—H stretching), 3083 (C—H $_{broad}$), 2959 (alkyl C—H stretching), 1681 (C═N $_{aromatic}$), 1646 (C═C), 1328 (aromatic CF_3 stretching), 1154 (SO_2 stretching), 1079 (C—N stretching), 954 (S—N stretching), 814 (C—S stretching), 735; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.86 (d, 2H, J = 1.91 Hz, Ar—H), 7.80 (d, 2H, J = 1.86 Hz, Ar—H), 7.59 (d, 2H, J = 1.43 Hz, Ar—H), 7.18 (d, 2H, J = 1.63 Hz, Ar—H), 7.15 (d, 2H, J = 1.46 Hz, Ar—H), 6.82 (t, 1H, J = 1.26 Hz, Ar—H), 6.80 (d, 2H, J = 1.35 Hz, Ar—H), 6.51 (s, 1H, pyrazole-H), 3.98 (s, 1H, NH—SO_2—), 2.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.6, 143.5, 142.9, 137.2, 131.7, 130.1, 129.5, 129.2, 128.9, 127.9, 125.7, 123.1, 122.4, 119.4, 117.3, 107.5, 21.3; Mass: 458.53 $(M+H)^+$; Elemental analysis for $C_{23}H_{18}F_3N_3O_2S$: Calculated: C, 60.39; H, 3.97; N, 9.19. Found: C, 60.42; H, 3.95; N, 9.21.

*3.2.2. N-(p-tolyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl) benzenesulfonamide (**7b**)*

Yield: 82%; M.p: 205–206 °C; MW: 471.50 ; R_f: 0.76; FTIR (ν_{max}; cm^{-1} KBr): 3251 (N—H stretching), 3087 (C—H $_{broad}$), 2952 (alkyl C—H stretching), 1687 (C═N $_{aromatic}$), 1649 (C═C), 1329 (aromatic CF_3 stretching), 1152 (SO_2 stretching), 1075 (C—N stretching), 955 (S—N stretching), 816 (C—S stretching), 731; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.85 (d, 2H, J = 1.90 Hz, Ar—H), 7.81 (d, 2H, J = 1.84 Hz, Ar—H), 7.58 (d, 2H, J = 1.42 Hz, Ar—H), 7.16 (d, 2H, J = 1.45 Hz, Ar—H), 7.02 (d, 2H, J = 1.29 Hz, Ar—H), 6.96 (d, 2H, J = 1.32 Hz, Ar—H), 6.51 (s, 1H, pyrazole-H), 3.97 (s, 1H, NH-SO_2-), 2.34 (s, 3H, CH_3), 2.32 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.5, 143.6, 142.9, 137.3, 134.7, 131.8, 131.2, 130.1, 129.8, 129.5, 127.9, 125.8, 123.1, 119.5, 117.3, 107.5, 21.3; Mass: 472.59 $(M+H)^+$; Elemental analysis for $C_{24}H_{20}F_3N_3O_2S$: Calculated: C, 61.14; H, 4.28; N, 8.91. Found: C, 61.18; H, 4.28; N, 8.94.

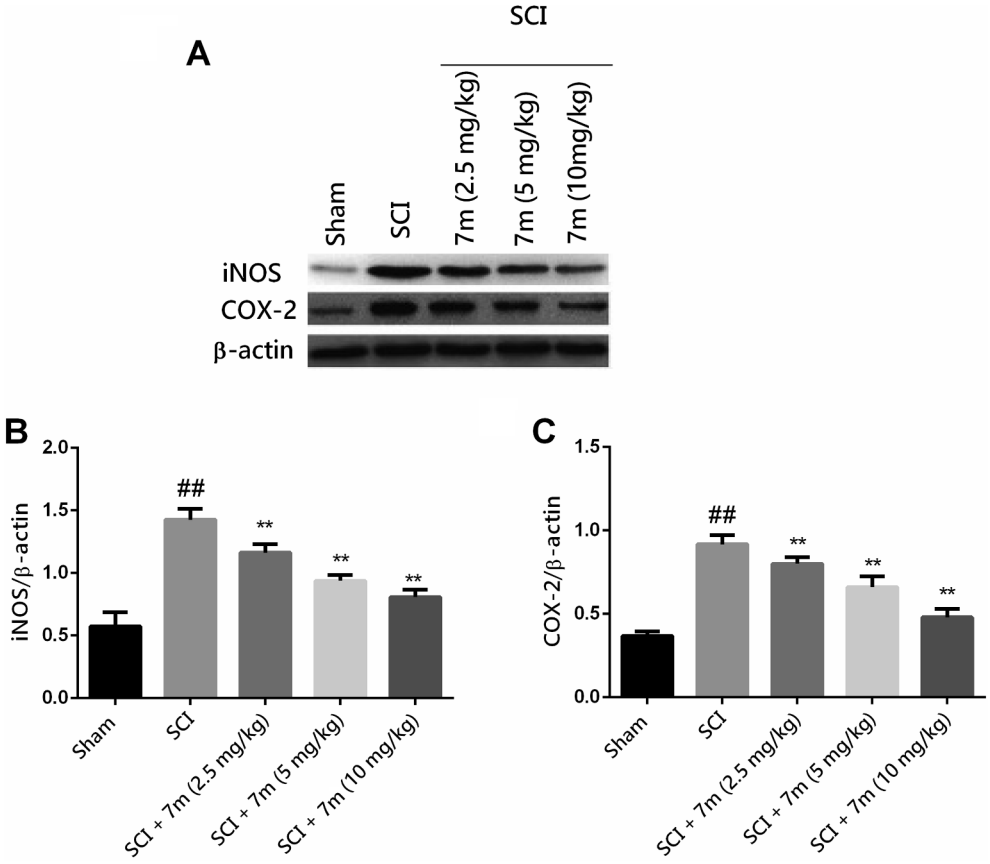

Fig. 7. . Effect of compound **7m** on COX-2 and iNOS expression in spinal cord of SCI rats *via* western blot analysis; and representative quantitative analysis of (b) iNOS and (c) COX-2. Values represent the mean ± SD and are representative of three independent experiments. $^{\#\#}P < 0.05$ *vs* sham; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* SCI, one-way analysis of variance followed by a Tukey's *post hoc* test.

*3.2.3. N-(o-tolyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl) benzenesulfonamide (**7c**)*

Yield: 78%; M.p: 212–213 °C; MW: 471.50 ; R_f: 0.82; FTIR (ν_{max}; cm^{-1} KBr): 3256 (N—H stretching), 3085 (C—H $_{broad}$), 2954 (alkyl C—H stretching), 1689 (C═N $_{aromatic}$), 1648 (C═C), 1326 (aromatic CF_3 stretching), 1153 (SO_2), 1078 (C—N stretching), 956 (S—N stretching), 815 (C—S stretching), 728; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.86 (d, 2H, J = 1.92 Hz, Ar—H), 7.79 (d, 2H, J = 1.82 Hz, Ar—H), 7.59 (d, 2H, J = 1.41 Hz, Ar—H), 7.15 (d, 2H, J = 1.47 Hz, Ar—H), 7.08–6.71 (m, 4H, CHx4, Ar—H), 6.52 (s, 1H, pyrazole-H), 3.97 (s, 1H, NH—SO_2—), 2.35 (s, 3H, CH_3), 2.13 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.5, 143.4, 142.9, 137.2, 136.1, 133.8, 131.7, 131.4, 130.2, 129.5, 127.9, 126.5, 125.7, 124.7, 123.9, 123.1, 117.4, 107.5, 21.3, 17.3; Mass: 472.54 $(M+H)^+$; Elemental analysis for $C_{24}H_{20}F_3N_3O_2S$: Calculated: C, 61.14; H, 4.28; N, 8.91. Found: C, 61.15; H, 4.26; N, 8.89.

*3.2.4. N-(4-methoxyphenyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide (**7d**)*

Yield: 73%; M.p: 217–218 °C; MW: 487.50 ; R_f: 0.85; FTIR (ν_{max}; cm^{-1} KBr): 3258 (N—H stretching), 3083 (C—H $_{broad}$), 2955 (alkyl C—H stretching), 2821 (OCH_3 stretching), 1686 (C═N $_{aromatic}$), 1642 (C═C), 1325 (aromatic CF_3 stretching), 1152 (SO_2 stretching), 1073 (C—N stretching), 956 (S—N stretching), 813 (C—S stretching), 709; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.84 (d, 2H, J = 1.94 Hz, Ar—H), 7.81 (d, 2H, J = 1.84 Hz, Ar—H), 7.57 (d, 2H, J = 1.43 Hz, Ar—H), 7.16 (d, 2H, J = 1.4 Hz, Ar—H), 6.98 (d, 2H, J = 1.52 Hz, Ar—H), 6.80 (d, 2H, J = 2.25 Hz, Ar—H), 6.51 (s, 1H, pyrazole-H), 3.98 (s, 1H, NH—SO_2—), 3.82 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.5, 153.2, 143.5, 142.9, 137.2, 131.8, 130.1, 130.0, 129.5, 127.9, 125.7, 124.5, 123.1, 117.4, 115.1, 107.5, 55.8, 21.3; Mass: 488.52 $(M+H)^+$; Elemental analysis for $C_{24}H_{20}F_3N_3O_3S$: Calculated: C, 59.13; H, 4.14; N, 8.62. Found: C, 59.12; H, 4.18; N, 8.65.

*3.2.5. N-(2-methoxyphenyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide (**7e**)*

Yield: 71%; M.p: 215–216 °C; MW: 487.50 ; R_f: 0.81; FTIR (ν_{max}; cm^{-1} KBr): 3254 (N—H stretching), 3085 (C—H $_{broad}$), 2952 (alkyl C—H stretching), 2821 (OCH_3 stretching), 1687 (C═N $_{aromatic}$), 1645 (C═C), 1323 (aromatic CF_3 stretching), 1156 (SO_2 stretching), 1078 (C—N stretching), 955 (S—N stretching), 816 (C—S stretching), 704; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.86 (d, 2H, J = 1.92 Hz, Ar—H), 7.82 (d, 2H, J = 1.83 Hz, Ar—H), 7.58 (d, 2H, J = 1.45 Hz, Ar—H), 7.14 (d, 2H, J = 1.3 Hz, Ar—H), 7.36–6.82 (m, 4H, CHx4, Ar—H), 6.52 (s, 1H, pyrazole-H), 3.99 (s, 1H, NH—SO_2—), 3.83 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.6, 147.4, 143.5, 142.9, 137.2, 131.7, 130.1, 129.5,128.4, 127.9, 127.1, 125.7, 123.2, 122.6, 122.3, 117.3, 113.4, 107.5, 55.8, 21.3 ; Mass: 488.56 $(M+H)^+$; Elemental analysis for $C_{24}H_{20}F_3N_3O_3S$: Calculated: C, 59.13; H, 4.14; N, 8.62. Found: C, 59.14; H, 4.15; N, 8.64.

*3.2.6. N-(4-nitrophenyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide (**7f**)*

Yield: 84%; M.p: 228–229 °C; MW: 502.47 ; R_f: 0.86; FTIR (ν_{max}; cm^{-1} KBr): 3259 (N—H stretching), 3084 (C—H $_{broad}$), 2954 (alkyl C—H stretching), 1685 (C═N $_{aromatic}$), 1643 (C═C), 1534 (NO_2 stretching), 1327 (aromatic CF_3 stretching), 1158 (SO_2 stretching), 1077 (C—N stretching), 957 (S—N stretching), 816 (C—S stretching), 702; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.85 (d, 2H, J = 1.93 Hz, Ar—H), 7.81 (d, 2H, J = 1.84 Hz, Ar—H), 7.57 (d, 2H, J = 1.44 Hz, Ar—H), 7.16 (d, 2H, J = 1.5 Hz, Ar—H), 7.92 (d, 2H, J = 1.85 Hz, Ar—H), 7.06 (d, 2H, J = 2.24 Hz, Ar—H), 6.51 (s, 1H, pyrazole-H), 3.97 (s, 1H, NH—SO_2—), 2.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ

ppm:153.6, 143.8, 143.5, 142.9, 137.8, 137.2, 131.7, 130.1, 129.5, 127.8, 125.7, 124.7, 123.1, 119.1, 117.3, 107.5, 21.3; Mass: 502.49 (M + H)$^+$; Elemental analysis for $C_{23}H_{17}F_3N_4O_4S$: Calculated: C, 54.98; H, 3.41; N, 11.15. Found: C, 54.93; H, 3.45; N, 11.13.

3.2.7. N-(2-nitrophenyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide (7g)

Yield: 78%; M.p: 223–224 °C; MW: 502.47 ; R_f: 0.82; FTIR (ν_{max}; cm^{-1} KBr): 3257 (N—H stretching), 3089 (C—H $_{broad}$), 2956 (alkyl C—H stretching), 1687 (C=N $_{aromatic}$), 1645 (C=C), 1537 (NO_2 stretching), 1329 (aromatic CF_3 stretching), 1156 (SO_2 stretching), 1076 (C—N stretching), 953 (S—N stretching), 817 (C—S stretching), 712; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.86 (d, 2H, J = 1.92 Hz, Ar—H), 7.83 (d, 2H, J = 1.85 Hz, Ar—H), 7.59 (d, 2H, J = 1.46 Hz, Ar—H), 7.14 (d, 2H, J = 1.4 Hz, Ar—H), 8.09–7.06 (m, 4H, CHx4,Ar—H), 6.52 (s, 1H, pyrazole-H), 3.98 (s, 1H, NH—SO_2—), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.7, 143.5, 142.9, 137.2, 137.1, 135.6, 134.1, 131.7, 130.1, 129.5, 127.8, 125.9, 125.7, 123.1, 119.8, 119.6, 117.3, 107.5, 21.3; Mass: 502.52 (M + H)$^+$; Elemental analysis for $C_{23}H_{17}F_3N_4O_4S$: Calculated: C, 54.98; H, 3.41; N, 11.15. Found: C, 54.97; H, 3.40; N, 11.17.

3.2.8. N-(4-chlorophenyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide (7h)

Yield: 85%; M.p: 231–232 °C; MW: 491.91 ; R_f: 0.80; FTIR (ν_{max}; cm^{-1} KBr): 3254 (N—H stretching), 3083 (C—H $_{broad}$), 2957 (alkyl C—H stretching), 1689 (C=N $_{aromatic}$), 1642 (C=C), 1327 (aromatic CF_3 stretching), 1159 (SO_2 stretching), 1078 (C—N stretching), 957 (S—N stretching), 812 (C—S stretching), 792 (C—Cl stretching), 701; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.87 (d, 2H, J = 1.92 Hz, Ar—H), 7.79 (d, 2H, J = 1.87 Hz, Ar—H), 7.56 (d, 2H, J = 1.42 Hz, Ar—H), 7.14 (d, 2H, J = 1.4 Hz, Ar—H), 7.32 (d, 2H, J = 1.51 Hz, Ar—H), 7.12 (d, 2H, J = 1.84 Hz, Ar—H), 6.53 (s, 1H, pyrazole-H), 3.99 (s, 1H, NH—SO_2—), 2.33 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.6, 143.5, 142.8, 137.3, 135.8, 131.7, 130.1, 129.7, 129.5, 127.8, 127.6, 125.8, 123.1, 122.1, 117.4, 107.4, 21.4; Mass: 492.94 (M + H)$^+$; Elemental analysis for $C_{23}H_{17}ClF_3N_3O_2S$: Calculated: C, 56.16; H, 3.48; N, 11.59. Found: C, 56.18; H, 3.52; N, 11.57.

3.2.9. N-(2-chlorophenyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide (7i)

Yield: 81%; M.p: 228–229 °C; MW: 491.91; R_f: 0.79; FTIR (ν_{max}; cm^{-1} KBr): 3257 (N—H stretching), 3085 (C—H $_{broad}$), 2959 (alkyl C—H stretching), 1684 (C=N $_{aromatic}$), 1645 (C=C), 1329 (aromatic CF_3 stretching), 1157 (SO_2 stretching), 1079 (C—N stretching), 953 (S—N stretching), 815 (C—S stretching), 789 (C—Cl stretching), 693; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.85 (d, 2H, J = 1.94 Hz, Ar—H), 7.82 (d, 2H, J = 1.82 Hz, Ar—H), 7.60 (d, 2H, J = 1.41 Hz, Ar—H), 7.16 (d, 2H, J = 1.5 Hz, Ar—H), 7.38–6.74 (m, 4H, CHx4,Ar—H), 6.53 (s, 1H, pyrazole-H), 3.97 (s, 1H, NH-SO_2-), 2.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.7, 143.5, 142.9, 137.2, 131.8, 130.7, 130.1, 129.5, 127.9, 127.7, 127.6, 125.8, 125.4, 125.2, 123.1, 122.6, 117.3, 107.5, 21.3; Mass: 492.90 (M + H)$^+$; Elemental analysis for $C_{23}H_{17}ClF_3N_3O_2S$: Calculated: C, 56.16; H, 3.48; N, 11.59. Found: C, 56.14; H, 3.51; N, 11.61.

3.2.10. N-(4-fluorophenyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide (7j)

Yield: 75%; M.p: 223–224 °C; MW: 475.46; R_f: 0.87; FTIR (ν_{max}; cm^{-1} KBr): 3259 (N—H stretching), 3087 (C—H $_{broad}$), 2954 (alkyl C—H stretching), 1686 (C=N $_{aromatic}$), 1643 (C=C), 1328 (aromatic CF_3 stretching), 1159 (SO_2 stretching), 1152 (C-F stretching), 1074 (C—N stretching), 952 (S—N stretching), 817 (C—S stretching), 698; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.89 (d, 2H, J = 1.96 Hz, Ar—H), 7.82 (d, 2H, J = 1.81 Hz, Ar—H), 7.58 (d, 2H, J = 1.49 Hz, Ar—H), 7.17 (d, 2H, J = 1.5 Hz, Ar—H), 6.98 (d, 2H, J = 1.43 Hz, Ar—H), 6.78 (d, 2H, J = 1.87 Hz, Ar—H), 6.51 (s, 1H, pyrazole-H), 3.96 (s, 1H, NH—SO_2—), 2.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:157.3, 153.7, 143.4, 142.9, 137.2, 133.4, 131.7, 130.1, 129.5, 127.9, 125.7, 123.1, 117.9, 117.3, 116.3, 107.5, 21.3; Mass: 476.48 (M + H)$^+$; Elemental analysis for $C_{23}H_{17}F_4N_3O_2S$: Calculated: C, 58.10; H, 3.60; N, 8.84. Found: C, 58.12; H, 3.63; N, 8.85.

3.2.11. N-(2-fluorophenyl)-4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)benzenesulfonamide (7k)

Yield: 70%; M.p: 220–221 °C; MW: 475.46; R_f: 0.83; FTIR (ν_{max}; cm^{-1} KBr): 3258 (N—H stretching), 3089 (C—H $_{broad}$), 2956 (alkyl C—H stretching), 1687 (C=N $_{aromatic}$), 1645 (C=C), 1327 (aromatic CF_3 stretching), 1158 (SO_2 stretching), 1154 (C-F stretching), 1075 (C—N stretching), 952 (S—N stretching), 811 (C—S stretching),696; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.87 (d, 2H, J = 1.98 Hz, Ar—H), 7.78 (d, 2H, J = 1.89 Hz, Ar—H), 7.62 (d, 2H, J = 1.46 Hz, Ar—H), 7.14 (d, 2H, J = 1.4 Hz, Ar—H), 6.94–6.63 (m, 4H, CHx4,Ar—H), 6.54 (s, 1H, pyrazole-H), 3.98 (s, 1H, NH—SO_2—), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:157.5, 153.6, 143.5, 142.9, 137.3, 133.7, 131.7, 130.1, 129.5, 127.9, 125.7, 125.1, 123.4, 123.1, 117.9, 117.4, 116.3, 107.5, 21.3; Mass: 476.52 (M + H)$^+$; Elemental analysis for $C_{23}H_{17}F_4N_3O_2S$: Calculated: C, 58.10; H, 3.60; N, 8.84. Found: C, 58.14; H, 3.59; N, 8.83.

3.2.12. 4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)-N-(2-(trifluoromethyl)phenyl)benzenesulfonamide (7l)

Yield: 77%; M.p: 245–246 °C; MW: 525.47; R_f: 0.88; FTIR (ν_{max}; cm^{-1} KBr): 3256 (N—H stretching), 3086 (C—H $_{broad}$), 2959 (alkyl C—H stretching), 1689 (C=N $_{aromatic}$), 1643 (C=C), 1329 (aromatic CF_3 stretching), 1157 (SO_2 stretching), 1152 (C-F stretching), 1078 (C—N stretching), 956 (S—N stretching), 814 (C—S stretching), 698; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.88 (d, 2H, J = 1.94 Hz, Ar—H), 7.83 (d, 2H, J = 1.84 Hz, Ar—H), 7.57 (d, 2H, J = 1.42 Hz, Ar—H), 7.16 (d, 2H, J = 1.5 Hz, Ar—H), 7.46–6.73 (m, 4H, CHx4,Ar—H), 6.52 (s, 1H, pyrazole-H), 3.97 (s, 1H, NH—SO_2—), 2.35 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.6, 143.6, 142.8, 137.1, 132.8, 132.2, 131.8, 130.1, 129.5, 127.3, 127.8, 125.7, 125.2, 125.1, 123.1, 119.1, 117.3, 115.1 107.5, 21.3; Mass: 526.49 (M + H)$^+$; Elemental analysis for $C_{24}H_{17}F_6N_3O_2S$: Calculated: C, 54.86; H, 3.26; N, 8.00. Found: C, 54.89; H, 3.24; N, 8.02.

3.2.13. 4-(5-(p-tolyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl)-N-(4-(trifluoromethyl)phenyl)benzenesulfonamide (7m)

Yield: 79%; M.p: 241–242 °C; MW: 525.47; R_f: 0.85; FTIR (ν_{max}; cm^{-1} KBr): 3259 (N—H stretching), 3087 (C—H $_{broad}$), 2958 (alkyl C—H stretching), 1687 (C=N $_{aromatic}$), 1645 (C=C), 1327 (aromatic CF_3 stretching), 1159 (SO_2 stretching), 1151 (C—F stretching), 1075 (C—N stretching), 956 (S—N stretching), 817 (C—S stretching), 696; ^{1}H NMR (400 MHz, DMSO-d_6, TMS) δ ppm: 7.87 (d, 2H, J = 1.94 Hz, Ar-H), 7.81 (d, 2H, J = 1.79 Hz, Ar—H), 7.60 (d, 2H, J = 1.45 Hz, Ar—H), 7.15 (d, 2H, J = 1.4 Hz, Ar—H), 7.35 (d, 2H, J = 1.82 Hz, Ar—H), 7.31 (d, 2H, J = 1.28 Hz, Ar—H), 6.52 (s, 1H, pyrazole-H), 3.98 (s, 1H, NH—SO_2—), 2.34 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO) δ ppm:153.7, 143.5, 142.8, 141.2, 137.2, 131.8, 130.1, 129.6, 127.8, 126.5, 125.9, 125.7, 124.1, 123.1, 119.8, 117.3, 107.5, 21.3; Mass: 526.53 (M + H)$^+$; Elemental analysis for $C_{24}H_{17}F_6N_3O_2S$: Calculated: C, 54.86; H, 3.26; N, 8.00. Found: C, 54.85; H, 3.28; N, 8.01.

3.3. In-vitro COX inhibitory activity

The inhibitory activity against COX-1/2 of entire target compounds was measured using colorimetric COX (ovine) Inhibitor Screening Assay Kit (catalog number 560131, Cayman Chemical, MI, USA) as per the manufacturer's instruction. This method allows to estimate the peroxidase activity of COX by colorimetric monitoring of presence of the oxidized form of *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) at

590 nm. The test is based on the oxidation of TMPD during the reduction of PGG2 (prostaglandin G2) to PGH2, which is reflected by a change in color, measured spectrophotometrically (Victor2 microspectrophotometer, PerkinElmer Waltham, MA, USA). The assay uses Tris-HCl buffer

(0.1 M assay buffer, pH 8.0), a solution of heme in dimethylsulfoxide (DMSO), enzymes (COX-1, COX-2), arachidonic acid (100 μM), KOH (0.1 M) and a solution of TMPD. The assay mixture contains: 150 μL of assay buffer, 10 μL of heme, and 10 μL of COX-1 or COX-2. To the wells for determination of 100% enzyme activity (each COX sample was assayed in triplicate) were added 10 μL of the substances used as solvents. To the other wells were added 10 μL of tested inhibitors at 10 μM. To all wells were added 20 μL of TMPD. The reaction was initiated by addition of arachidonic acid. The effect of tested inhibitors on COX-1 and COX-2 enzyme activity was measured by assaying the rate of TMPD oxidation within 2 min in a spectrophotometer at 590 nm. We determined the activity factor at 2 min of incubation with the tested compounds in comparison to the initial activity of the enzyme. This enabled the calculation of IC_{50} values (concentrations at which 50% inhibition of enzyme activity occurred).

3.4. In vivo pharmacology

3.4.1. Animals

The Sprague-Dawley rats (male adult, 230–260 gm) rats after procuring from animal house and were kept in polypropylene cages with *ad libitum* supply of food and water at controlled humidity and temperature with alternate, dark and light cycle.

3.4.2. Establishment of spinal cord injury (SCI)

The SCI injury was induced in the rats as per Allens method with modifications [32]. Initially, a 2 cm midline incision was made on skin targeting the eighth thoracic segment (T8) along the vertebrae. The laminectomy was performed by removing vertebral lamina of T8. The SCI injury was induced dropping 10 g rod from the distance of 5 cm on the spinal cord of rats and rats were put on rest for 3 min. The sham treated rats observe only laminectomy. The surgical site was sutured together, and intramuscular injection of penicillin (200,000 U) was administered for three consecutive days after surgery. Compound **7m** after suspending in 5% CMC was administered once a day for 14 days to rats in graded dose of 2.5 mg/kg; 5 mg/kg; and 10 mg/kg *via i.p.* immediately after surgery.

3.4.3. Evaluation of motor function

The effect of compound **7m** on rats motor function was determined using Basso, Beattie and Bresnahan (BBB) locomotor rating scale between 0 and 21 at the day 1, 4, 7, 10 and 14 day post-SCI. The effect was evaluated by person who was blinded to the treatment group, and the mean of the three measurements was recorded [44].

3.4.4. Histological examinations

The segments of spinal cord in the vicinity of the lesion epicenter were collected and 5 μm-thick sections was obtained after embedded in paraffin for transverse sectioning. Sections were stained with hematoxylin and eosin together with cresyl violet for HE staining and Nissl staining, respectively.

3.4.5. Biochemical determination

The spinal cord homogenates were centrifuged at 4 °C and resulting supernatant was used for the measurement of MDA and GSH levels and SOD activity via commercially available kits according to the given protocols.

3.4.6. Enzyme-linked immunosorbent assay (ELISA)

The determination of TNF-α (tumor necrosis factor-α), IL (interleukin)-1β and IL-6 were performed using commercially available ELISA kits as per manufacturer's instructions.

3.4.7. Terminal dexynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining

The spinal cord sections were stained using a TUNEL apoptosis detection kit (Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China) according to the manufacturer's instruction.

3.4.8. Western blot assay

10% SDS-PAGE was carried out to separate the proteins and transferred on PVDF membranes (Millipore, Bedford, MA, USA). The membrane were incubated primary antibodies (1:1000; CST or Abcam, USA) after blocking for overnight. The corresponding secondary antibodies (1:2000; Abcam, USA) were further incubated for 2 h. The proteins bands were observed using enhanced chemiluminescence substrate (Millipore, Billerica, MA, USA) and photographed with Image Quant LAS 4000 (GE, USA) and was analyzed by Image J software (NIH, Bethesda, MD, USA).

3.5. Statistical analysis

All data are recorded as mean ± SD of three independent experiments. Data were statistically analyzed by one-way analysis followed by the by a Tukey's *post hoc* test using statistical software GraphPad Prism 5.0 (California, USA). The P value < 0.05 was considered as statistically significant.

4. Conclusion

Our study has shown the development of novel celecoxib derivatives *via* facile synthetic route as COX-2 inhibitor. Among the synthesized derivatives, compound **7m**, a highly potent COX-2 inhibitor showed protective action against SCI *via* attenuation of COX-2, oxidative stress, apoptosis and inflammation in Male Sprague-Dawley rats.

Ethics approval and consent to participate

The animal use protocol listed below has been reviewed and approved by the Animal Ethical and Welfare Committee of Beijing Jishuitan Hospital, Beijing, China.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104044.

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