Full Paper

# Design and Synthesis of New 1,3-Benzdiazinan-4-one Derivatives as Selective Cyclooxygenase (COX-2) Inhibitors

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A new group of regioisomeric 2,3-diaryl-1,3-benzdiazinan-4-ones, possessing a methyl sulfonyl pharmacophore, were synthesized and their biological activities were tested for cyclooxygenase-2 (COX-2) inhibitory activity. *In vitro* COX-1/COX-2 inhibition studies identified 3-(*p*-fluorophenyl)-2-(4-methylsulfonylphenyl)-1,3-benzdiazinane-4-one (**2b**) as a potent and highly selective ($IC_{50}$ = 0.07 μM; selectivity index = 572.8) COX-2 inhibitor.

**Keywords:** 1,3-Benzdiazinan-4-ones / COX-2 inhibition / Docking studies / SAR

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## Introduction

The cyclooxygenase (COX) enzymes catalyze a key step in the conversion of arachidonate to PGH2, the immediate substrate for a series of cell specific prostaglandin and thromboxane synthases. At least two COX isoforms have been identified thus far: the constitutively expressed COX-1 and COX-2, which is inducible under pathologic conditions and of which increased concentrations have been observed in inflamed and tumorous tissues [1, 2]. In addition to COX-1 and COX-2, a third isoform of COX enzyme (COX-3) has been also identified. It is considered to play a key role in the biosynthesis of prostanoids known to be important mediators in pain and fever [3]. It is known that selective COX-2 inhibitors can provide anti-inflammatory agents devoid of the undesirable gastro-intestinal side effects [4]. In addition to the role of COX-2 in inflammatory disorders such as rheumatoid arthritis and osteoarthritis, it is also implicated in cancer and angiogenesis. In this regard, several epidemiologic studies have reported that inhibitors of COX-2 enzyme reduce the risk of several cancer types such as colon, breast, lung and prostate cancers [5–8] and neurodegenerative diseases such as Parkinson [9] and Alzheimer's [10] disease. Selective COX-2 inhibitors frequently belong to a class of tricycles that possess vicinal diaryl moieties attached to a central ring scaffold in conjunction with a COX-2 pharmacophore such as a *para*-$SO_2NH_2$, a *para*-$SO_2Me$, substituent on one of the phenyl rings [11]. Celecoxib and rofecoxib are two typical selective COX-2 inhibitors in this class (COXIBs). However, the market withdrawal of some COXIBs such as rofecoxib due to their increasing the risk of heart attack and cardiovascular side effects [12] encourages the researchers to explore new selective COX-2 inhibitors to evaluate their effects and may improve the safety profiles. For this reason novel scaffolds with high selectivity for COX-2 inhibition need to be found and evaluated for their biological activities. In this regard, we recently reported several investigations describing the design, synthesis, and COX inhibitory activities of new compounds possessing a bicyclic 1,3-benzthiazinan **(A)** [13] or quinoline **(B)** [14, 15] structural template (Fig. 1). Our results showed that both 1,3-benzthiazinan and quinoline rings are very suitable scaffolds for COX-2 inhibitory activity and some of these synthesized compounds exhibited high potency and selectivity on COX-2 inhibition even more than the reference drug celecoxib. As part of our research program, aimed at discovering new selective COX-2 inhibitors, we focused our attention on the synthesis, COX inhibitory acitivity and some molecular modeling studies of

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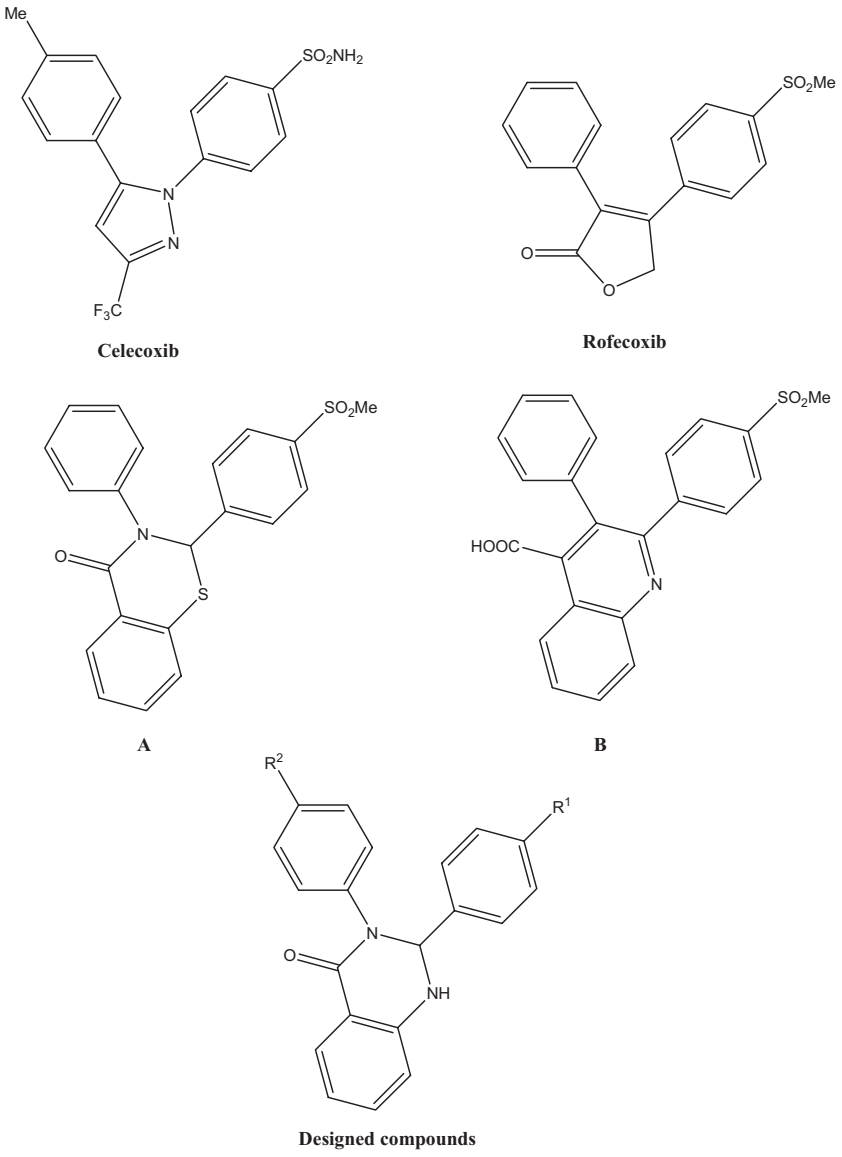



**Figure 1.** Some representative examples of COXIBs (celecoxib and rofecoxib), 1,3-benzthiazinane-4-one (**A**), quinoline-4-caboxylic acid (**B**) lead compounds and our 1,3-benzdiazinan-4-one scaffolds.

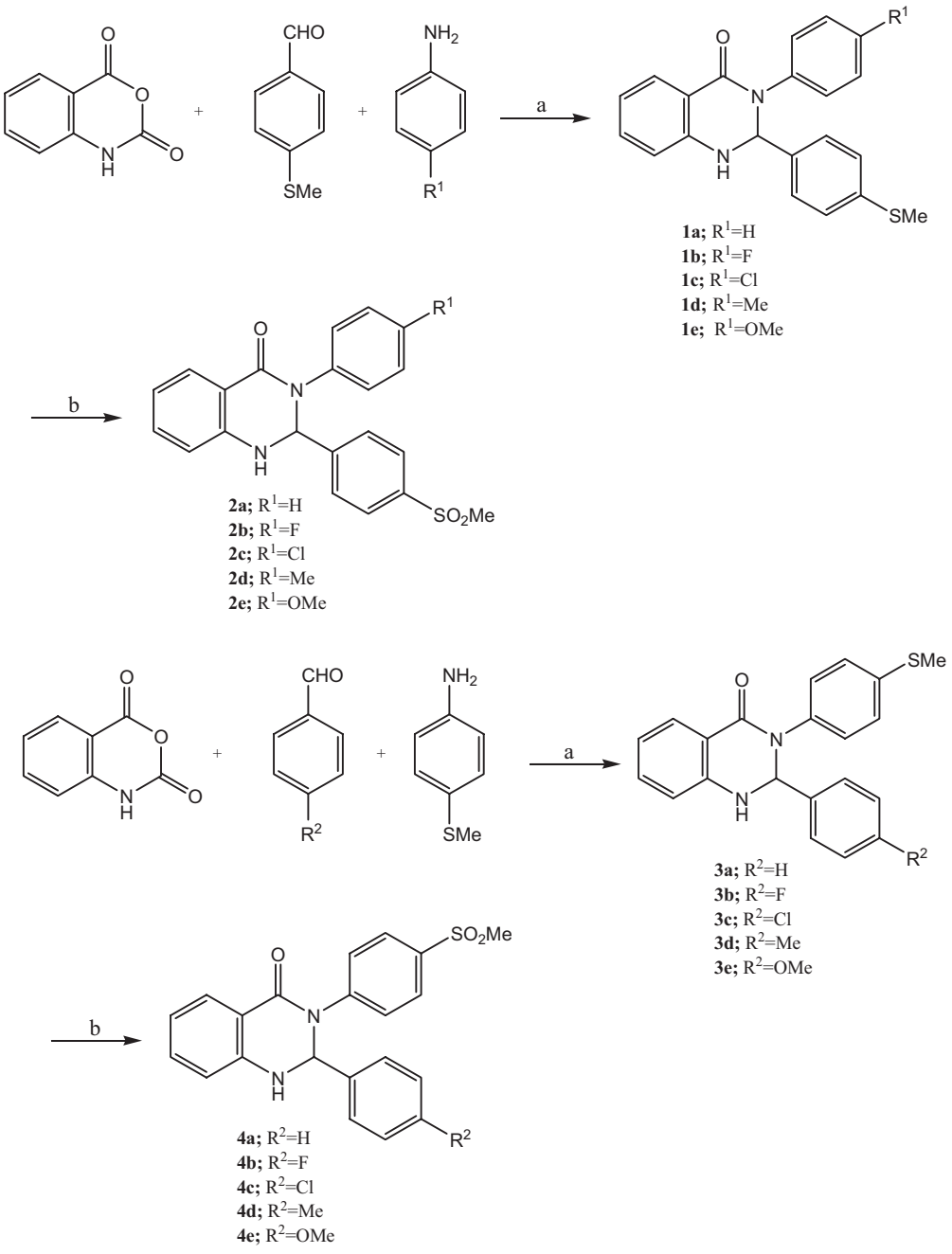
a new group of regioisomeric 2,3-diaryl-1,3-benzdiazinan-4-ones. The rationale for the design of these compounds was based on hybrid structure of 2,3-diaryl-1,3-benzthiazinan-4-ones and 2,3-diaryl-quinolines in our previously reported COX-2 inhibitors.

## Results and discussion

### Chemistry

The two sets of 1,3-benzdiazinan-4-one regioisomers in which the 4-methylsulfonylphenyl substituent is attached to C-2 (**2a–e**) or to N-3 (**4a–e**), were synthesized via the route outlined in Scheme 1. Accordingly, a one-pot three-component cyclocondensation of a mixture of isatoic anhydride, aldehyde and primary amine in ethanol using alum ($KAl(SO_4)_2.12H_2O$) as catalyst was used to prepare 1,3-bendiazinan-4-ones (**1** or **3**; yield: 14–30%) [16]. Presumably, the reaction proceeds through condensation of isatoic anhydride with primary amine followed by decarboxylation to yield the corresponding 2-aminobenzamide. Then, condensation of the aromatic aldehyde with the amino group of 2-aminobenzamide gives the imine intermediate which undergoes cyclization to afford the 1,3-bendiazinan-4-one product [16]. The low yield of the product may be explained by the possible formation of byproducts such as the reaction between the amine and aldehye or low activity of starting materials such as the aldehyde having electron donating group. Oxidation of cyclic intermediate using 30% $H_2O_2$ in




**Scheme 1.** Reagents and conditions: (a) Alum, EtOH, reflux, 1 h; (b) $H_2O_2$ 30%, $WO_3$, 25°C, 4–5 h.

hydromethanol in the presence of a trace amount of $WO_3$ gave the target products (**2** or **4,** yield: 22–36%) [17].

## Biological evaluation

A group of 1,3-benzdiazinan-4-one derivatives having a $MeSO_2$ group at the *para*-position of the C-2 phenyl ring containing a variety of substituents (H, F, Cl, Me, OMe) at the *para*-position of the N-3 phenyl ring (**2a–e**), and the corresponding regioisomers (**4a–e**), were synthesized to investigate the effect of these substituents on COX-2 selectivity and potency. The ability of the 1,3-benzdiazinan-4-ones to inhibit the COX-1 and COX-2 isozymes was determined using chemiluminescent enzyme assays (see enzyme inhibition data in Table 1) according to our previously reported method [18]. In vitro COX-1/COX-2 inhibition studies showed that all compounds were selective inhibitors of the COX-2 isozyme with

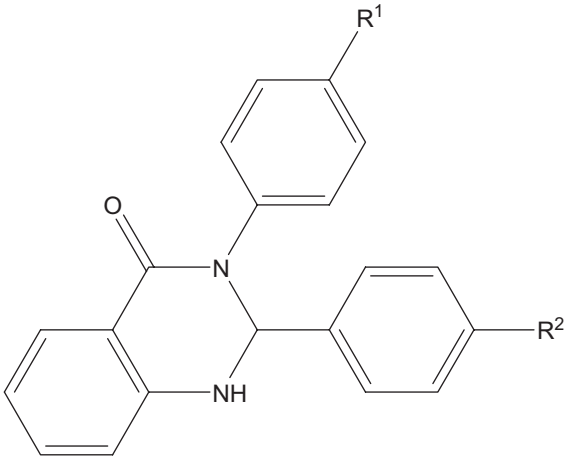
 

$IC_{50}$ values in the highly potent 0.07 to 0.22 μM range, and COX-2 selectivity indexes (S.I.) in the 95.0 to 572.8 range. SAR data ($IC_{50}$ μM values) acquired by determination of the *in vitro* ability of the title compounds to inhibit the COX-1 and COX-2 isozymes showed that the position of the COX-2 $SO_2Me$ pharmacophore and the nature of the *para*-substituents on the C-2 or N-3 phenyl ring were either individual, or collective, determinants of COX-2 inhibitory potency and selectivity. *In vitro* COX-1/COX-2 inhibition studies showed that compounds having a $MeSO_2$ group at the *para*-position of the C-2 phenyl ring (**2a–e**) were more selective COX-2 inhibitors compared to their corresponding regioisomers (**4a–e**). These data showed that the nature and size of substituent attached to C-2 or N-3 of the 1,3-benzdiazinan-4-one ring influenced both selectivity and potency for COX-2 inhibitory activity. Our results indicated that the introduction of suitable substituents such as F (**2b**) and OMe (**2e**) at the *para*-position of N-3 phenyl ring increased both selectivity and potency for COX-2 inhibitory activity. The similar results were obtained for their corresponding regioisomers (**4b** and **4e**). However, compounds having Cl substituent (**2c** and **4c**) showed less selectivity and potency for COX-2 isozyme. According to these results, 3-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one **2b** was the most potent ($IC_{50}$ = 0.07 μM), and selective (S.I. = 572.8), COX-2 inhibitor among the synthesized compounds. It was more selective than celecoxib ($IC_{50}$ = 0.06 μM; S.I. = 405) in terms of COX-2 inhibitory activity but showed less potency. These data suggest that the compound **2b** should inhibit the biosynthesis of prostaglandins via the cyclooxgenase pathway at sites of inflammation and be devoid of ulcerogenicity due to the absence of COX-1 inhibition.

## Molecular modeling studies

The orientation of the highly potent and selective COX-2 inhibitor, 3-(4-fluorophenyl)-2-(4-methylsulfonylphenyl)-1,3-

**Table 1.** In vitro COX-1 and COX-2 enzyme inhibition assay data **3–9**.




| Compoud | $R^1$ | $R^2$ | $IC_{50}$ (μM)[a] | | COX-2 S.I.[b] |
|---|---|---|---|---|---|
| | | | COX-1 | COX-2 | |
| 2a | H | $SO_2Me$ | 99.8 | 0.35 | 285.1 |
| 2b | F | $SO_2Me$ | 40.1 | 0.07 | 572.8 |
| 2c | Cl | $SO_2Me$ | 17.7 | 0.17 | 104.1 |
| 2d | Me | $SO_2Me$ | 37.2 | 0.10 | 372.0 |
| 2e | OMe | $SO_2Me$ | 33.4 | 0.07 | 477.1 |
| 4a | $SO_2Me$ | H | 24.1 | 0.12 | 200.8 |
| 4b | $SO_2Me$ | F | 31.8 | 0.09 | 353.3 |
| 4c | $SO_2Me$ | Cl | 20.9 | 0.22 | 95.0 |
| 4d | $SO_2Me$ | Me | 24.1 | 0.11 | 219.1 |
| 4e | $SO_2Me$ | OMe | 29.7 | 0.11 | 270.0 |
| Celecoxib | | | 24.30 | 0.06 | 405 |

[a] Values are means of two determinations acquired using an ovine COX-1/COX-2 assay kit and the deviation from the mean is <10% of the mean value.
[b] *In vitro* COX-2 selectivity.

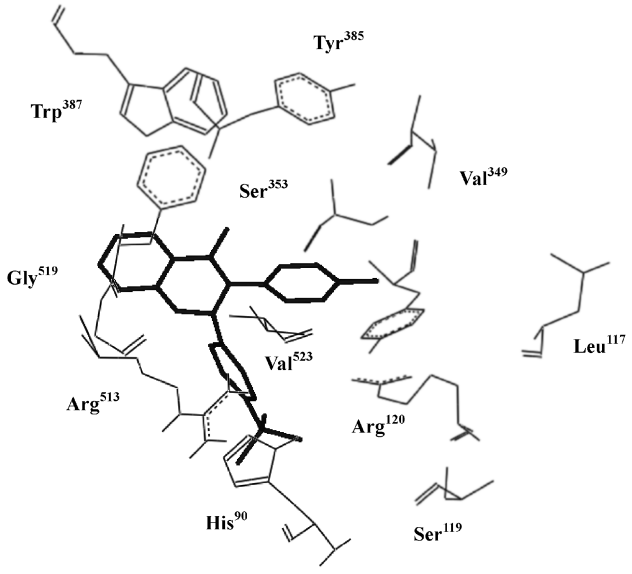



**Figure 2.** Compound **2b** 3-(*p*-fluorophenyl)-2-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one docked in the active site of murine COX-2 isozyme..

benzdiazinan-4-one **2b** in the COX-2 active site was examined by a docking experiment (Fig. 2) [19, 20]. This molecular modeling shows that it binds in the primary binding site such that the C-2 *para*-$SO_2$Me substituent inserts into the secondary pocket present in the COX-2 isozyme. One of the *O*-atoms of *p*-$SO_2$Me forms a hydrogen bonding interaction with amino group of $Arg^{513}$ (distance = 2.9 Å) whereas the other *O*-atom is close to N*H* of $His^{90}$ amino acid (distance = 3.4 Å). Also, the N*H* group of benzdiazinan central ring is very close to carbonyl group of $Gly^{519}$ (distance = 2 Å) and can form hydrogen binding. In addition, the C=*O* of benzdiazinan-4-one ring is almost close to (distance = 5.4 Å) N*H* of $Tyr^{385}$. It was interesting to note that the *para*-fluoro substituent of N-3 phenyl ring was forming a hydrogen bond with hydroxyl group (O*H*) of $Tyr^{355}$ (distance = 4.8 Å). These observations together with experimental results provide a good explanation for the potent and selective inhibitory activity of **2b**.

## Conclusions

The results of this investigation indicate that (i) the 1,3-benzdiazinan-4-one moiety is a suitable scaffold (template) to design COX-2 inhibitors, (ii) in this class of compounds COX-1/-2 inhibition is sensitive to the position of the COX-2 $SO_2$Me pharmacophore and the nature of the C-2 or N-3 phenyl substituents, and (iii) 3-(*p*-fluorophenyl)-2-(4-methylsulfonylphenyl)-1,3-benzdiazinane-4-one (**2b**) is not only a potent, but also a highly selective, COX-2 inhibitor.

## Experimental

### General

All chemicals and solvents used in this study were purchased from Merck AG and Aldrich Chemical. Melting points were determined with a Thomas–Hoover capillary apparatus. Infrared spectra were acquired using a Perkin Elmer Model 1420 spectrometer. A Bruker FT-500 MHz instrument (Bruker Biosciences, USA) was used to acquire $^1$HNMR spectra with TMS as internal standard. Chloroform-D and DMSO-$D_6$ were used as solvents. Coupling constant (*J*) values are estimated in Hertz (Hz) and spin multiples are given as s (singlet), d (double), t (triplet), q (quartet), m (multiplet), and br (broad). The mass spectral measurements were performed on an 6410 Agilent LCMS triple quadrupole mass spectrometer (LCMS) with an electrospray ionization (ESI) interface. Microanalyses, determined for C and H, were within ± 0.4% of theoretical values.

### *General procedure for preparation of 2,3-diaryl-1,3-benzdiazinan-4-one (**1a–e** and **3a–e**)*

Alum (0.2 g) was added to a mixture of isatoic anhydride (1 mmol), aldehyde (1 mmol) and primary amine (1.1 mmol) in ethanol. The mixture was heated at reflux for 1 h. After completion of the reaction, the solid 1,3-bendiazinan (**1** or **3**) was separated, washed with water and recrystallized from ethanol (yields: 14–30%). The physical and spectral data for **1a–e** and **3a–e** are listed below.

### *2-(4-Methylthiophenyl)-3-phenyl-1,3-benzdiazinan-4-one (**1a**)*

Yield, 20%; white crystalline powder, mp 202–203°C; IR (KBr): $\nu$ $cm^{-1}$ 3307 (NH), 1660 (C=O); LCMS (ESI): 347.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.48 (s, 3H, SMe), 6.09 (s, 1H, CH), 6.67 (d, 1H, benzdiazinan $H_8$, $J$ = 7.9 Hz), 6.94 (m, 1H, benzdiazinan $H_6$), 7.16 (d, 2H, 4-methylthiophenyl $H_3$ and $H_5$, $J$ = 8.3 Hz), 7.22 (m, 1H, benzdiazinan $H_7$), 7.23 (d, 2H, 4-methylthiophenyl $H_2$ & $H_6$, $J$ = 8.3 Hz), 7.30–7.36 (m, 5H, phenyl), 8.06 (d, 1H, benzdiazinan $H_5$, $J$ = 7.7 Hz); Anal. Calcd. for $C_{21}H_{18}N_2OS$: C, 72.80; H, 5.24; N, 8.09. Found: C, 72.99; H, 5.62; N, 8.31.

### *3-(4-Fluorophenyl)-2-(4-methylthiophenyl)-1,3-benzdiazinan-4-one (**1b**)*

Yield, 22%; white crystalline powder, mp 242–243°C; IR (KBr): $\nu$ $cm^{-1}$ 3312 (NH), 1642 (C=O); LCMS (ESI): 365.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.49 (s, 3H, SMe), 6.07 (s, 1H, CH), 6.69 (d, 1H, benzdiazinan $H_8$, $J$ = 7.9 Hz), 6.96 (m, 1H, benzdiazinan $H_6$), 7.02 (t, 2H, 4-fluorophenyl $H_3$ & $H_5$, $J$ = 8.3 Hz), 7.15 (d, 2H, 4-methylthiophenyl $H_3$ & $H_5$, $J$ = 8.3 Hz), 7.18 (d, 4-methylthiophenyl $H_2$ & $H_6$, $J$ = 8.3 Hz), 7.28 (m, 1H, benzdiazinan $H_7$), 7.42 (d, 2H, fluorophenyl $H_2$ & $H_6$, $J$ = 8.7 Hz), 8.05 (d, 1H, benzdiazinan $H_5$, $J$ = 7.7 Hz); Anal. Calcd. for $C_{21}H_{17}FN_2OS$: C, 69.21; H, 4.70; N, 7.69. Found: C, 69.39; H, 4.42; N, 7.88.

### *3-(4-Chlorophenyl)-2-(4-methylthiophenyl)-1,3-benzdiazinan-4-one (**1c**)*

Yield, 20%; white crystalline powder, mp 230–232°C; IR (KBr): $\nu$ $cm^{-1}$ 3314 (NH), 1640 (C=O); LCMS (ESI): 381.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.34 (s, 3H, SMe), 5.96 (s, 1H, CH), 6.03 (s, 1H, NH),

6.61 (d, 1H, benzdiazinan $H_8$, $J = 8.0$ Hz), 6.72 (m, 1H, benzdiazinan $H_6$), 7.02 (d, 2H, 4-chlorophenyl $H_2$ & $H_6$, $J = 8.3$ Hz), 7.08 (d, 2H, 4-methylthiophenyl $H_3$ & $H_5$, $J = 8.3$ Hz), 7.15 (d, 4-methyl thiophenyl $H_2$ and $H_6$, $J = 8.3$ Hz), 7.19 (d, 2H, 4-chlorophenyl $H_3$ and $H_5$, $J = 8.7$ Hz), 7.21 (m, 1H, benzdiazinan $H_7$), 7.83 (d, 1H, benzdiazinan $H_5$, $J = 7.7$ Hz); Anal. Calcd. for $C_{21}H_{17}ClN_2OS$: C, 66.22; H, 4.50; N, 7.35. Found: C, 66.49; H, 4.22; N, 7.68.

### *3-(4-Methylphenyl)-2-(4-methylthiophenyl)-1,3-benzdiazinan-4-one (**1d**)*

Yield, 20%; white crystalline powder, mp 217–218°C; IR (KBr): $\nu$ $cm^{-1}$ 3298 (NH), 1633 (C=O); LCMS (ESI): 361.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.27 (s, 3H, Me), 2.41 (s, 3H, SMe), 6.19 (s, 1H, CH), 6.72 (d, 2H, 4-methylphenyl $H_3$ & $H_5$, $J = 7.8$ Hz), 7.14–7.16 (m, 3H, 4-methylphenyl $H_2$ and $H_6$ & benzdiazinan $H_8$), 7.18 (d, 2H, 4-methylthiophenyl $H_3$ & $H_5$, $J = 8.4$ Hz), 7.25 (m, 1H, benzdiazinan $H_6$), 7.30 (d, 4-methylthiophenyl $H_2$ & $H_6$, $J = 8.4$ Hz), 7.55 (m, 1H, benzdiazinan $H_7$), 7.70 (d, 1H, benzdiazinan $H_5$, $J = 7.7$ Hz); Anal. Calcd. for $C_{22}H_{20}N_2OS$: C, 73.30; H, 5.59; N, 7.77. Found: C, 72.99; H, 5.32; N, 7.90.

### *3-(4-Methoxyphenyl)-2-(4-methylthiophenyl)-1,3-benzdiazinan-4-one (**1e**)*

Yield, 30%; white crystalline powder, mp 248–250°C; IR (KBr): $\nu$ $cm^{-1}$ 3295 (NH), 1632 (C=O); LCMS (ESI): 377.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.36 (s, 3H, SMe), 3.72 (s, 3H, OMe), 6.16 (s, 1H, CH), 6.74 (d, 2H, 4-methoxyphenyl $H_3$ & $H_5$, $J = 7.9$ Hz), 6.88 (d, 2H, 4-methylthiophenyl $H_3$ & $H_5$, $J = 8.4$ Hz), 7.17 (d, 2H, 4-methyoxyphenyl $H_2$ & $H_6$, $J = 7.9$ Hz), 7.25 (m, 1H, benzdiazinan $H_8$), 7.25 (m, 1H, benzdiazinan $H_6$), 7.29 (d, 4-methylthiophenyl $H_2$ & $H_6$, $J = 8.4$ Hz), 7.50 (m, 1H, benzdiazinan $H_7$), 7.71 (d, 1H, benzdiazinan $H_5$, $J = 7.7$ Hz); Anal. Calcd. for $C_{22}H_{20}N_2O_2S$: C, 70.19; H, 5.35; N, 7.44. Found: C, 70.39; H, 5.21; N, 7.53.

### *3-(4-Methylthiophenyl)-2-phenyl-1,3-benzdiazinan-4-one (**3a**)*

Yield, 24%; white crystalline powder, mp 193.5–194.5°C; IR (KBr): $\nu$ $cm^{-1}$ 3299 (NH), 1638 (C=O); LCMS (ESI): 347.9 $(M+1)^+$, 369.8 $(M+23)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.47 (s, 3H, SMe), 4.78 (s, 1H, NH), 6.12 (s, 1H, CH), 6.67 (d, 1H, benzdiazinan $H_8$, $J = 8$ Hz), 6.93 (m, 1H, benzdiazinan $H_6$), 7.14 (d, 2H, methylthiophenyl $H_3$ & $H_5$, $J = 8.5$ Hz), 7.2 (d, 2H, methylthiophenyl $H_2$ & $H_6$, $J = 8.5$ Hz), 7.31–7.40 (m, 5H, phenyl $H_2$ & $H_6$ & 1H, benzdiazinan $H_7$), 8.06 (d, 1H, benzdiazinan $H_5$, $J = 7.7$ Hz); Anal. Calcd. for $C_{21}H_{18}N_2OS$: C, 72.80; H, 5.24; N, 8.09. Found: C, 73.03; H, 5.42; N, 8.38.

### *2-(4-Fluorophenyl)-3-(4-methylthiophenyl)-1,3-benzdiazinan-4-one (**3b**)*

Yield, 14%; white crystalline powder, mp 191–192°C; IR (KBr): $\nu$ $cm^{-1}$ 3296 (NH), 1638 (C=O); LCMS (ESI): 365.8 $(M+1)^+$, 387.7 $(M+23)^+$; $^1$HNMR ($CDCl_3$) δ 2.47 (s, 3H, SMe), 6.12 (s, 1H, CH), 6.69 (d, 1H, benzdiazinan $H_8$, $J = 8$ Hz), 6.95 (m, 1H, benzdiazinan $H_6$), 6.99 (m, 2H, 4-fluorophenyl $H_2$ & $H_6$), 7.1 (d, 2H, methylthiophenyl $H_3$ & $H_5$, $J = 8.6$ Hz), 7.2 (d, 2H, methylthiophenyl $H_2$ & $H_6$, $J = 8.6$ Hz), 7.35–7.39 (m, 3H, 4-fluorophenyl $H_3$ & $H_5$ & 1H, benzdiazinan $H_5$); Anal. Calcd. for $C_{21}H_{17}FN_2OS$: C, 69.21; H, 4.70; N, 7.69. Found: C, 69.43; H, 4.99; N, 7.96.

### *2-(4-Chlorophenyl)-3-(4-methylthiophenyl)-1,3-benzdiazinan-4-one (**3c**)*

Yield, 15%; white crystalline powder, mp 238.5–240°C; IR (KBr): $\nu$ $cm^{-1}$ 3328 (NH), 1645 (C=O); LCMS (ESI): 403.6 $(M+23)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.49 (s, 3H, SMe), 6.11 (s,1H, CH), 6.69 (d, 1H, benzdiazinan $H_8$, $J = 7.9$ Hz), 6.97 (m, 1H, benzdiazinan $H_6$), 7.13 (d, 2H, methylthiophenyl $H_3$ & $H_5$, $J = 8.6$Hz), 7.21 (d, 2H, methylthiophenyl $H_2$ & $H_6$, $J = 8.6$ Hz), 7.29 (d, 2H, 4-chlorophenyl $H_2$ & $H_6$, $J = 8.2$ Hz), 7.32 (d, 2H, 4-chlorophenyl $H_3$ & $H_5$, $J = 8.2$ Hz), 7.36 (m, 1H, benzdiazinan $H_7$), 8.06 (d, 1H, benzdiazinan $H_5$, $J = 7.8$ Hz); Anal. Calcd. for $C_{21}H_{17}ClN_2OS$: C, 66.22; H, 4.50; N, 7.35. Found: C, 65.95; H, 4.75; N, 7.61.

### *2-(4-Methylphenyl)-3-(4-methylthiophenyl)-1,3-benzdiazinan-4-one (**3d**)*

Yield, 20%; white crystalline powder, mp 217–218°C; IR (KBr): $\nu$ $cm^{-1}$ 3298 (NH), 1633 (C=O); LCMS (ESI): 361.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.27 (s, 3H, Me), 2.41 (s, 3H, SMe), 6.19 (s, 1H, CH), 6.72 (d, 2H, 4-methylphenyl $H_3$ & $H_5$, $J = 7.8$ Hz), 7.14–7.16 (m, 3H, 4-methylphenyl $H_2$ and $H_6$ & benzdiazinan $H_8$), 7.18 (d, 2H, 4-methylthiophenyl $H_3$ & $H_5$, $J = 8.4$ Hz), 7.25 (m, 1H, benzdiazinan $H_6$), 7.30 (d, 4-methylthiophenyl $H_2$ & $H_6$, $J = 8.4$ Hz), 7.55 (m, 1H, benzdiazinan $H_7$), 7.70 (d, 1H, benzdiazinan $H_5$, $J = 7.7$ Hz); Anal. Calcd. for $C_{22}H_{20}N_2OS$: C, 73.30; H, 5.59; N, 7.77. Found: C, 73.59; H, 5.88; N, 7.60.

### *2-(4-Methoxyphenyl)-3-(4-methylthiophenyl)-1,3-benzdiazinan-4-one (**3e**)*

Yield, 15%; white crystalline powder, mp 199–201°C; IR (KBr): $\nu$ $cm^{-1}$ 3307 (NH), 1638 (C=O); LCMS (ESI): 377.4 $(M+1)^+$, 399.3 $(M^++23)^+$; $^1$HNMR (DMSO-$d_6$) δ ppm 2.46 (s, 3H, SMe), 3.68 (s, 3H, OMe), 6.19 (d, 1H, CH, $J = 2.1$ Hz), 6.58 (m, 1H, benzdiazinan $H_8$), 6.71 (m, 1H, benzdiazinan $H_6$), 6.74 (d, 2H, 4-methoxyphenyl $H_3$ & $H_5$, $J = 7.9$ Hz), 6.85 (d, 2H, methylthiophenyl $H_3$ & $H_5$, $J = 8.2$ Hz), 7.24 (d, 2H, 4-methyloxyphenyl $H_2$ & $H_6$, $J = 7.9$ Hz), 7.29 (d, 2H, methylthiophenyl $H_2$ & $H_6$, $J = 8.2$ Hz), 7.51 (d, 1H, NH, $J = 2.1$ Hz) 7.59 (m, 1H, benzdiazinan $H_7$), 7.71 (d, 1H, benzdiazinan $H_5$, $J = 7.7$ Hz); Anal. Calcd. for $C_{22}H_{20}N_2O_2S$: C, 70.19; H, 5.35; N, 7.44. Found: C, 70.41; H, 5.66; N, 7.71.

### *General procedure for preparation of aryl-4-methylsulfonylphenyl-1,3-benzdiazinan-4-one (**2a–e** and **4a–e**)*

0.5 g of **1** or **3** was dissolved in 10 mL methanol and 5 mL 30% hydrogen peroxide solution and 100 mg $WO_3$ as catalyst were added. The mixture was stirred at room temperature for 5–6 hours, after evaporation of methanol, the mixture was extracted with ethyl acetate and dried with anhydrous sodium sulfate and then evaporated, the product was recrystallized in ethanol (yields: 22–36%). The physical and spectral data for **2a–e** and **4a–e** are listed below.

### *2-(4-Methylsulfonylphenyl)-3-phenyl-1,3-benzdiazinan-4-one (**2a**)*

Yield, 30%; yellow crystalline powder, mp 175–176°C; IR (KBr): $\nu$ $cm^{-1}$ 3298 (NH), 1645 (C=O), 1320, 1165 ($SO_2$); LCMS (ESI): 379.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 3.03 (s, 3H, $SO_2CH_3$),

6.24 (s, 1H, CH), 6.73 (d, 1H, benzdiazinan $H_8$, $J = 8.4$ Hz), 6.98 (t, 1H, benzdiazinan $H_6$, $J = 7.5$ Hz), 7.25 (d, 2H, phenyl $H_3$ & $H_5$ $J = 7.9$ Hz), 7.28 (m, 1H, benzdiazinan $H_7$), 7.35–7.40 (m, 3H, phenyl $H_3$-$H_5$), 7.62 (d, 2H, 4-methylsulfonylphenyl $H_2$ & $H_6$, $J = 8.0$ Hz), 7.89 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J = 8.0$ Hz), 8.07 (d, 1H, benzdiazinan $H_5$, $J = 8.0$ Hz); Anal. Calcd. for $C_{21}H_{18}N_2O_3S$: C, 66.65; H, 4.79; N, 7.40. Found: C, 66.34; H, 4.62; N, 7.29.

### *3-(4-Fluorophenyl)-2-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one (**2b**)*

Yield, 35%; cream crystalline powder, mp 215–217°C; IR (KBr): $\nu$ $cm^{-1}$ 3400 (NH), 1610 (C=O), 1300, 1150 ($SO_2$); LCMS (ESI): 397.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 3.04 (s, 3H, $SO_2CH_3$), 4.9 (s, 1H, NH), 6.20 (s, 1H, CH), 6.72 (d, 1H, benzdiazinan $H_8$, $J = 8.0$ Hz), 6.98 (t, 1H, benzdiazinan $H_6$, $J = 7.5$ Hz), 7.04 (t, 2H, 4-fluorophenyl $H_3$ & $H_5$, $J = 8.4$ Hz), 7.21 (dd, 2H, 4-fluorophenyl $H_2$ & $H_6$), 7.40 (m, 1H, benzdiazinan $H_7$), 7.61 (d, 2H, 4-methylsulfonylphenyl $H_2$ & $H_6$, $J = 8.2$ Hz), 7.89 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J = 8.2$ Hz), 8.07 (d, 1H, benzdiazinan $H_5$, $J = 6.7$ Hz); Anal. Calcd. for $C_{21}H_{17}FN_2O_3S$: C, 63.62; H, 4.32; N, 7.07. Found: C, 63.48; H, 4.12; N, 7.22.

### *3-(4-Chlorophenyl)-2-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one (**2c**)*

Yield, 31%; cream crystalline powder, mp 191–193°C; IR (KBr): $\nu$ $cm^{-1}$ 3315 (NH), 1648 (C=O), 1322, 1159 ($SO_2$); LCMS (ESI): 413.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 3.20 (s, 3H, $SO_2CH_3$), 6.39 (s, 1H, CH), 6.72 (d, 1H, benzdiazinan $H_8$, $J = 8.1$ Hz), 6.78 (t, 1H, benzdiazinan $H_6$, $J = 8.3$ Hz), 7.12 (d, 2H, 4-chlorophenyl $H_2$ & $H_6$, $J = 8.1$ Hz), 7.25 (d, 2H, 4-chlorophenyl $H_3$ & $H_5$, $J = 8.1$ Hz), 7.30 (m, 1H, benzdiazinan $H_7$), 7.56 (d, 2H, 4-methylsulfonylphenyl $H_2$ & $H_6$, $J = 8.7$ Hz), 7.80 (d, 1H, benzdiazinan $H_5$, $J = 6.7$ Hz), 7.87 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J = 8.7$ Hz); Anal. Calcd. for $C_{21}H_{17}ClN_2O_3S$: C, 61.09; H, 4.15; N, 6.78. Found: C, 59.87; H, 4.36; N, 6.88.

### *3-(4-Methylphenyl)-2-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one (**2d**)*

Yield, 31%; cream crystalline powder, mp 181–182°C; IR (KBr): $\nu$ $cm^{-1}$ 3312 (NH), 1632 (C=O), 1315, 1151 ($SO_2$); LCMS (ESI): 393.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.33 (s, 3H, $CH_3$), 3.04 (s, 3H, $SO_2CH_3$), 6.38 (s, 1H, CH), 6.69 (d, 1H, benzdiazinan $H_8$, $J = 8.1$ Hz), 6.95 (t, 1H, benzdiazinan $H_6$, $J = 7.5$ Hz), 7.12 (d, 2H, 4-methylphenyl $H_3$ & $H_5$, $J = 8.4$ Hz), 7.16 (d, 2H, 4-methylphenyl $H_2$ & $H_6$, $J = 8.4$ Hz), 7.36 (m, 1H, benzdiazinan $H_7$), 7.59 (d, 2H, 4-methylsulfonylphenyl $H_2$ & $H_6$, $J = 8.3$ Hz), 7.87 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J = 8.7$ Hz), 8.04 (d, 1H, benzdiazinan $H_5$, $J = 6.6$ Hz); Anal. Calcd. for $C_{22}H_{20}N_2O_3S$: C, 67.33; H, 5.14; N, 7.14. Found: C, 67.30; H, 5.26; N, 7.22.

### *3-(4-Methoxyphenyl)-2-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one (**2e**)*

Yield, 30%; cream crystalline powder, mp 218–220°C; IR (KBr): $\nu$ $cm^{-1}$ 3298 (NH), 1628 (C=O), 1320, 1152 ($SO_2$); LCMS (ESI): 409.1 $(M+1)^+$; $^1$HNMR ($CDCl_3$) δ ppm 3.05 (s, 3H, $SO_2CH_3$), 3.66 (s, 3H, $OCH_3$), 6.18 (s, 1H, CH), 6.86 (d, 2H, 4-methoxyphenyl $H_3$ & $H_5$, $J = 8.9$ Hz), 6.99 (d, 1H, benzdiazinan $H_8$, $J = 8.1$ Hz), 7.15 (d, 2H, 4-methoxyphenyl $H_2$ & $H_6$, $J = 8.9$ Hz), 7.36 (t, 1H, benzdiazinan $H_6$, $J = 7.3$ Hz), 7.57 (m, 1H, benzdiazinan $H_7$), 7.60 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J = 8.3$ Hz), 7.89 (d, 2H, 4-methylsulfonylphenyl $H_2$ & $H_6$, $J = 8.7$ Hz), 8.07 (d, 1H, benzdiazinan $H_5$, $J = 6.1$ Hz); Anal. Calcd. for $C_{22}H_{20}N_2O_4S$: C, 64.59; H, 4.93; N, 6.86. Found: C, 64.77; H, 4.74; N, 6.95.

### *3-(4-Methylsulfonylphenyl)-2-phenyl-1,3-benzdiazinan-4-one (**4a**)*

Yield, 34%; yellow crystalline powder, mp 208–209°C; IR (KBr): $\nu$ $cm^{-1}$ 3327 (NH), 1647 (C=O), 1326, 1162 ($SO_2$); LCMS (ESI): 401.7 $(M+23)^+$; $^1$HNMR ($CDCl_3$) δ ppm 3.21 (s, 3H, $SO_2CH_3$), 6.26 (s, 1H, CH), 6.37 (m, 1H, benzdiazinan $H_6$), 6.80 (d, 1H, benzdiazinan $H_8$, $J = 8.1$ Hz), 7.29–7.32 (m, 3H, phenyl $H_3$–$H_5$), 7.35 (m, 1H, benzdiazinan $H_7$), 7.39 (d, 2H, phenyl $H_2$ & $H_6$ $J = 7.6$ Hz), 7.58 (d, 2H, 4-methylsulfonylphenyl $H_2$ & $H_6$, $J = 8.7$ Hz), 7.83 (d, 1H, benzdiazinan $H_5$, $J = 7.8$ Hz), 7.87 (d, 1H, NH, $J = 2.9$ Hz), 7 .90 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J = 8.7$ Hz); Anal. Calcd. for $C_{21}H_{18}N_2O_3S$: C, 66.65; H, 4.79; N, 7.40. Found: C, 66.29; H, 4.51; N, 7.32.

### *2-(4-Fluorophenyl)-3-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one (**4b**)*

Yield, 27%; yellow crystalline powder, mp 170–171°C; IR (KBr): $\nu$ $cm^{-1}$ 3369 (NH), 1666 (C=O), 1298, 1140 ($SO_2$); LCMS (ESI): 419.3 $(M+23)^+$; $^1$HNMR ($CDCl_3$) δ ppm 3.21 (s, 3H, $SO_2CH_3$), 6.48 (s, 1H, CH), 6.73 (m, 1H, benzdiazinan $H_6$), 6.8 (d, 1H, benzdiazinan $H_8$, $J = 8.1$ Hz), 7.16 (m, 2H, 4-fluorophenyl $H_3$ & $H_5$), 7.31 (m, 1H, benzdiazinan $H_7$), 7.42 (dd, 2H, 4-fluorophenyl $H_2$ & $H_6$), 7.56 (d, 2H, 4-methylsulfonylphenyl $H_2$ & $H_6$, $J = 8.7$Hz), 7.74 (d, 1H, benzdiazinan $H_5$, $J = 7.8$ Hz), 7.84 (d, 1H, NH, $J = 2.9$ Hz), 7.89 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J = 8.7$ Hz); Anal. Calcd. for $C_{21}H_{17}FN_2O_3S$: C, 63.62; H, 4.32; N, 7.07. Found: C, 63.29; H, 4.22; N, 7.12.

### *2-(4-Chlorophenyl)-3-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one (**4c**)*

Yield, 22%; yellow crystalline powder, mp 220–221°C; IR (KBr): $\nu$ $cm^{-1}$ 3338 (NH), 1650 (C=O), 1294, 1147 ($SO_2$); LCMS (ESI): 413.1 $(M+1)^+$, 435.1 $(M+23)^+$; $^1$HNMR ($CDCl_3$) δ ppm 3.21 (s, 3H, $SO_2Me$), 6.48 (d, 1H, CH, J = 3.1 Hz), 6.74 (m, 1H, benzdiazinan $H_6$), 6.8 (d, 1H, benzdiazinan $H_8$, $J = 8.3$ Hz), 7.31 (m, 1H, benzdiazinan $H_7$), 7.4 (m, 4H, 4-chlorophenyl $H_2$–$H_6$), 7.57 (d, 2H, 4-methyl sulfonylphenyl $H_2$ & $H_6$, $J = 8.7$ Hz), 7.74 (d, 1H, benzdiazinan $H_5$, $J = 7.8$ Hz), 7.88 (d, 1H, NH, $J = 3.1$ Hz), 7.9 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J = 8.7$ Hz); Anal. Calcd. for $C_{21}H_{17}ClN_2O_3S$: C, 61.09; H, 4.15; N, 6.78. Found: C, 61.29; H, 4.29; N, 6.84.

### *2-(4-Methylphenyl)-3-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one (**4d**)*

Yield, 24%; yellow crystalline powder, mp 177–179°C; IR (KBr): $\nu$ $cm^{-1}$ 3038 (NH), 1670 (C=O), 1326, 1158 ($SO_2$); LCMS (ESI): 415.6 $(M+23)^+$; $^1$HNMR ($CDCl_3$) δ ppm 2.22 (s, 3H, Me), 3.2 (s, 3H, $SO_2Me$), 6.39 (d, 1H, CH, $J = 2.9$ Hz), 6.72 (m, 1H, benzdiazinan $H_6$), 6.78 (d, 1H, benzdiazinan $H_8$, $J = 8.1$ Hz), 7.12 (d, 2H, 4-methylphenyl $H_3$ & $H_5$, $J = 8.0$ Hz), 7.26 (d, 2H, 4-methylphenyl $H_2$ & $H_6$, $J = 8.0$ Hz), 7.99 (m, 1H, benzdiazinan $H_7$), 7.56 (d, 2H, 4-methylsulfonylphenyl $H_2$ & $H_6$, $J = 8.7$ Hz), 7.73 (d, 1H, benzdiazinan $H_5$, $J = 7.7$ Hz), 7.8 (d, 1H, NH, $J = 2.9$ Hz), 7.88 (d, 2H, 4-methylsulfonyl phenyl $H_3$ & $H_5$,

$J$ = 8.7 Hz); Anal. Calcd. for $C_{22}H_{20}N_2O_3S$: C, 67.33; H, 5.14; N, 7.14. Found: C, 67.19; H, 5.31; N, 7.18.

### *2-(4-Methoxyphenyl)-3-(4-methylsulfonylphenyl)-1,3-benzdiazinan-4-one (**4e**)*

Yield, 29%; yellow crystalline powder, mp 175–176°C; IR (KBr): $\nu$ $cm^{-1}$ 3313 (NH), 1632 (C=O), 1323, 1152 ($SO_2$); LCMS (ESI): 409.6 $(M+1)^+$, 431.5 $(M+23)^+$; $^1$HNMR ($CDCl_3$) δ ppm 3.2 (s, 3H, $SO_2Me$), 3.68 (s, 3H, OMe), 6.38 (d, 1H, CH, $J$ = 2.7 Hz), 6.72 (m, 1H, benzdiazinan $H_6$), 6.78 (d, 1H, benzdiazinan $H_8$, $J$ = 8.1 Hz), 6.87 (d, 2H, 4-methoxyphenyl $H_3$ & $H_5$, $J$ = 8.7 Hz) 7.29 (d, 2H, 4-methoxyphenyl $H_2$ & $H_6$, $J$ = 8.6 Hz), 7.3 (m, 1H, benzdiazinan $H_7$), 7.55 (d, 2H, 4-methylsulfonyl phenyl $H_2$ & $H_6$, $J$ = 8.7 Hz), 7.73 (d, 1H, benzdiazinan $H_5$, $J$ = 7.7 Hz), 7.76 (d, 1H, NH, $J$ = 2.7 Hz), 7.88 (d, 2H, 4-methylsulfonylphenyl $H_3$ & $H_5$, $J$ = 8.7 Hz); Anal. Calcd. for $C_{22}H_{20}N_2O_4S$: C, 64.59; H, 4.93; N, 6.86. Found: C, 64.39; H, 4.71; N, 6.88.

### *In vitro cyclooxygenase (COX) inhibition assays*

The assay was performed using an enzyme chemiluminescent kit (Cayman Chemical, MI, USA) according to our previously reported method [17]. The Cayman chemical chemiluminescent COX (ovine) inhibitor screening assay utilizes the heme-catalyzed hydroperoxidase activity of ovine cyclooxygenases to generate luminescence in the presence of a cyclic naphthalene hydrazide and the substrate arachidonic acid. Arachidonate-induced luminescence was shown to be an index of real-time catalytic activity and demonstrated the turnover inactivation of the enzyme. Inhibition of COX activity, measured by luminescence, by a variety of selective and nonselective inhibitors showed potencies similar to those observed with other *in vitro* and whole cell methods.

### *Molecular modeling (docking) studies*

Docking studies were performed using Autodock software Version 3.0. The coordinates of the X-ray crystal structure of the selective COX-2 inhibitor SC-558 bound to the murine COX-2 enzyme was obtained from the RCSB Protein Data Bank (1cx2) and hydrogens were added. The ligand molecules were constructed using the Builder module and were energy minimized for 1000 iterations reaching a convergence of 0.01 kcal/mol Å. The energy minimized ligands were superimposed on SC-558 in the PDB file 1cx2 after which SC-558 was deleted. The purpose of docking is to search for favorable binding configuration between the small flexible ligands and the rigid protein. Protein residues with atoms greater than 7.5 Å from the docking box were removed for efficiency. These docked structures were very similar to the minimized structures obtained initially. The quality of the docked structures was evaluated by measuring the intermolcular energy of the ligand-enzyme assembly [19, 20].

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