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**Research Article**

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# Design, synthesis, and biological evaluation of novel 3-(arylsulfonamido) phenyloxiindole derivatives as TNF-α and IL-6 inhibitor

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**ABSTRACT**

*A series of novel (3-sulfonamido) phenyloxindoles were prepared from (3H-indene-1,2-dione) isatin via reductive amination, reduction followed by reaction of resulting amine with different arenesulfonyl chloride and screened for their Proinflammatory cytokines inhibition (TNF-α and IL-6) on lipopolysaccharide stimulated peripheral blood mononuclear cells. The sulfonamide derivative such as compound **5d**, **5h** and **5i** exhibited promising inhibition of the release of two cytokines TNF-α and IL-6 with IC-50 values raging over 1.9-2.6 and 1.2-1.7 respectively and thus proved to be potent TNF-α and IL-6 inhibitor.*

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## INTRODUCTION

Cytokines are intercellular messengers responsible for host defense mechanisms as inflammatory, immune and hematogenic responses. Although many of them are transient, they are produced by various cells which act as urgent response mediators in cases of invasive interventions. Disruption of this biological defense mechanism and continuous excessive cytokine production contributes to pathogenesis of inflammatory diseases. One of the key pro-inflammatory cytokine, Tumor necrosis factor-α (TNF-α) is mainly produced by the activated macrophages and monocytes, which further induces the production of the several inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and granulocyte-macrophage colony-stimulating factor (GM-CSF). It is also reported that multitude of biological activities linked to pathology of autoimmune diseases such as rheumatoid arthritis (RA),[1] Crohn's disease[2], systemic lupus erythematosus [3], and multiple sclerosis [4], septic shock [5] and AIDS [6]. On the other hand, cytokine interleukin-6 (IL-6) (from the series of cytokine signaling pathway) contributes to the initiation and extension of the inflammatory process and considered as a central mediator in a range of inflammatory diseases. However, it has not yet received the desired attention in drug discovery [7]. TNF-α and IL-6 are thus pharmaceutically important molecular targets for the treatment of the above mentioned diseases.

The available biopharmaceuticals (TNF soluble receptor (EnbrelTM) and TNF antibody (RemicadeTM) are expensive, difficult to administer orally and have major side effects on prolonged clinical use. Therefore, there is an urgent need to discover small molecule agents to deal with higher levels production of TNF-α and IL-6.

Non-steroidal anti-inflammatory drugs (NSADs) are therapeutically important in the treatment of rheumatic arthritis and in various types of inflammatory conditions, but their therapeutic utility has been limited due to their frequently observed gastrointestinal side effects. Thus, there is an urgent need for new targets that are required for the design and development of novel anti-inflammatory agents as an alternative to NSAIDs [8]. (Tumor_necrosis factor alpha (TNF-α) and interleukin-6 (IL-6), the two important multifunctional proinflammatory cytokines that are involved in the pathogenesis of autoimmune, inflammatory, cardiovascular, neurodegenerative and cancer diseases through a series of cytokine signaling pathways [9, 10]. IL-6 contributes to the initiation and extension of the inflammatory process and considered as a central mediator in a range of inflammatory diseases but has not received the desired

attention in drug discovery [11]. TNF-α and IL-6 are thus pharmaceutically important molecular targets for the treatment of the above-mentioned diseases.

Sulfonamide derivatives has attracted much attention in medicinal chemistry [8, 12] due to their broad range of biological activities such as anticancer [13], antibacterial [11], hypoglycaemic [14], diuretics [15], anti-carbonic anhydrase (CA), antithyroid and anti-inflammatory [16, 17], antihypertensive [18], and anticonvulsant activity [19]. The AstraZeneca pyrazine sulphonamide[20], the Ono sulfonamide [21] and the GlaxoSmithKline indazole [22] are recently published sulfonamide CCR4 receptor antagonists (Figure 1)

Though the various sulfonamide derivatives found to exhibit the above mentioned activities, the reports on the anti-inflammatory activity of the simple sulfonamide derivatives or complex sulfonamides based hybrid scaffolds are relatively scares in the literature. In particular, the sulfonamides bearing arylureido moiety has not hitherto been tested as anti-inflammatory agents.

In recent years, we have been engaged in design, synthesis and anti-inflammatory activity and antimicrobial evaluation of novel urea and thiourea derivatives [23-27]. Herein, we disclose our another results on the design synthesis and discovery of novel sulfonamide bearing oxindole moiety as TNF-α and IL-6 inhibitors.

## EXPERIMENTAL SECTION

General techniques
All reagent used were of analytical grade (Thomas Baker, Spectrochem) $^1$HNMR spectra were recorded on Brucker Avance spectrometer (300MHz or 400MHz) using tetramethylsilane as internal standard. Chemical shifts are reported in ppm (δ) relative to the solvent peak, Mass spectra were recorded on either GCMS (focus GC with TSQ II mass analyzer and thermoelectro) with autosampler/direct injection (EI/CI) or LCMS (APCI/ESI; Buker daltanoics Micro TOFQ). HPLC purity was checked using Water Alliances or Dionex Ultima 3000 HPLC system. All purications were done by recrystallization (Methylene Dichloride / petroleum ether 9:1). Ethyl acetate and petroleum ether were used as mobile phase for TLC (Merck Kiesel 60 F254, 0.2mm thickness sheet).

**Synthesis and Analytical data of novel urea / thiourea and sulfonamide derivatives**

Synthesis of (Z)-3-((4-nitrophenyl) imino) indolin-2-one **(2)**
Procedure - To a stirred solution of 3H-indene-1, 2-dione (1equiv) and 4-nitrobenzenamine (1 equiv) in ethanol (20 mL) was added cat. PTSA (0.2 equiv) and the reaction mixture was heated under reflux in dean-stark apparatus for 3-4 h. After completion of reaction (TLC), the volatiles were removed under vacuum to get sticky solid. The residue was taken up in ethyl acetate, washed with aqueous $NaHCO_3$ and brine (NaCl) solution. After drying over anhy sodium sulphate, evaporation of the solvent followed by column chromatography, the analytical pure titled compound was obtained as pale yellow solid in 72% yield which was used as such for next step.

Synthesis of 3-(4-nitrophenylamino) indolin-2-one **(3)**
Procedure - To a stirred solution of **2** (1equiv) in methanol (20 mL) was added $NaBH_4$ (1.2 equiv) in portion at $0^0$C for 30 min. After completion of reaction (TLC), the solvent evaporated and residue was diluted with ethyl acetate, washed with saturated $NH_4Cl$ solution and water. The volatiles were removed under vacuum to get sticky material in 61% yield which was used as such for next step.

Synthesis of 3-(4-aminophenylamino) indolin-2-one **(4)**
To a stirred solution of **3** in methanol, THF, $H_2O$ (5:3:2) was added $NH_4Cl$ (3eq) and iron turning (1.5equiv) and the reaction mixture was heated at 80 $^0$C for 8 h and After completion of reaction (TLC), the reaction mixture was cooled to room temperature and then filtered through high-flow bed. The filtrate was concentrated under vacuum to get sticky. The recrystallization of above material in hexane to get free yellow solid in 52 % yield

General procedure for the synthesis of N-(4-((2-oxoindolin-3-yl) amino) aryl) benzene sulfonamides **(5a-k)**
To a soulation of **4** (1equiv.) in DCM, was added triethylamine (1.1equiv.) followed by the addition of appropriate arenesulfonyl chloride (1equiv.) and the resulting reaction mixture was stirred at 0 $^0$C for a period of 2 hours and allowed to stirred at room temperature for 6-8 hours, After completion of reaction, the reaction mixture was poured into ice cold water and extracted with Ethyl acetate (20*3), organic layer wash with aqueous $NaHCO_3$, and concentrated under vacuum to get crude compound which was purified by silica gel column chromatography (100-200#) to get off white solid in good to high yield.

4-chloro-2-fluoro-N-(4-((2-oxoindolin-3-yl) amino) phenyl) benzene sulfonamide **(5a)**
Off white solid, yield 76%, M.P.-143-145$^0$C, $^1$HNMR (DMSO, 400MHz): - 10.21 (s, 1H), 10.03 (s, 2H), 8.79 (s, 1H), 8.28 (s, 1H), 8.05-7.93 (m, 2H), 7.75-7.58 (m, 4H), 7.37-7.25 (m, 3H), 4.78 (s, 1H). MS (APCI); *m/z* 431.9 $[M+1]^+$ HPLC-99.62

2, 5-dimethoxy-N-(4-((2-oxoindolin-3-yl) amino) phenyl) benzene sulfonamide **(5b)**
Off white solid, yield 78%, M.P.-151-153$^0$C,$^1$HNMR (DMSO, 400MHz): 10.44 (s, 1H), 9.99 (s, 1H), 8.71 (s, 1H), 8.25 (s, 1H), 7.92 (m, 2H),7.58-7.53 (m, 1H), 7.34-7.33 (m, 2H), 7.26-7.24 (m, 2H), 7.17-7.09 (m,3H), 4.71 (s, 1H), 3.81 (s, 3H), 3.74 (s, 3H). *m/z* 440.0 $[M+1]^+$ HPLC-98.48%

3-methoxy-N-(4-((2-oxoindolin-3 yl) amino) phenyl) benzene sulfonamide **(5c)**
Off white solid, yield 66%, M.P.-158-161$^0$C, $^1$HNMR (DMSO, 400MHz): 10.82 (m, 2H), 8.86 (s, 1H), 8.21 (s, 1H), 7.89 (m, 2H), 7.68-7.64 (m, 3H), 7.45-7.32 (m, 3H), 7.20 (m, 3H), 4.79 (s, 1H), 3.76 (s, 3H). *m/z* 410.3 $[M+1]^+$ HPLC-98.03%

4-bromo-N-(4-((2-oxoindolin-3-yl) amino) phenyl) benzene sulfonamide **(5d)**
Off white solid, yield 66%, M.P.-166-169$^0$C, $^1$HNMR (DMSO, 400MHz): 10.90 (s, 1H), 10.61 (s, 1H), 8.85 (s, 1H), 8.22 (s, 1H), 7.91-7.74 (m, 4H), 7.64-7.36 (m, 3H), 7.31-7.20 (m, 5H), 4.34 (s, 1H). *m/z* 458.3 $[M+1]^+$ HPLC-98.33%

N-(4-((2-oxoindolin-3-yl) amino) phenyl)-3-(trifluoromethyl) benzene sulfonamide **(5e)**
Off white solid, yield 64%, M.P.-153-155$^0$C, 1HNMR (DMSO, 400MHz): 11.00 (s, 1H), 10.77 (s, 1H), 8.90 (s, 1H) 8.20 (s, 1H), 8.00-7.97 (m, 2H), 7.89-7.87 9 (m, 2H), 7.23-7.50 (m, 5H), 7.34 7.19 (m, 2H), 4.78 (s, 1H). *m/z* 447.9 $[M+1]^+$ HPLC-98.94%

4-cyano-N-(4-((2-oxoindolin-3-yl) amino) phenyl) benzene sulfonamide **(5f)**
Off white solid, yield 71%, M.P.-169-171$^0$C, $^1$HNMR (DMSO, 400MHz): 11.01 (s, 1H), 10.64 (s, 1H), 8.87 (s, 1H), 8.29-8.12 (m, 3H), 7.89-7.78 (m, 4H), 7.65-7.34 (m, 3H), 7.31-7.17 (m, 2H), 4.79 (s, 1H). *m/z* 405.1 $[M+1]^+$ HPLC-98.96%

N-(4-((2-oxoindolin-3-yl) amino) phenyl)-2(trifluoromethyl) benzene sulfonamide **(5g)**
Off white solid, yield 64%, M.P.-148-152$^0$C, $^1$HNMR (DMSO, 400MHz): 8.86 (s, 1H), 8.15 (s, 1H), 8.03-8.00 (m, 2H), 7.93-7.90 (m, 2H), 7.81-7.78 (m, 2H), 7.68-7.57 (m, 2H), 7.46-7.41 (m, 3H), 7.33-7.31 (m, 1H), 7.19 (m, 1H), 4.78 (s, 1H). *m/z* 448.1 $[M+1]^+$ HPLC-98.55%

3-fluoro-N-(4-((2-oxoindolin-3-yl) amino) phenyl) benzene sulfonamide **(5h)**
Off white solid, yield 61%, M.P.-134-136$^0$C, $^1$HNMR (DMSO, 400MHz): 10.29 (s, 1H), 10.16 (s, 1H), 10.03 (s, 1H), 8.78 (s, 1H), 8.28 (s, 1H), 8.06-7.94 (m, 3H), 7.81-7.80 (m, 1H), 7.69-7.68 (m, 2H), 7.59-7.58 (m, 2H), 7.36-7.35 (m, 1H), 7.25 (s, 1H), 4.78 (s, 1H). *m/z* 398.1 $[M+1]^+$ HPLC-98.97%

4-fluoro-N-(4-((2-oxoindolin-3-yl) amino) phenyl) benzene sulfonamide **(5i)**
Off white solid, yield 70%, M.P.-146-149$^0$C, $^1$HNMR (DMSO, 400MHz): 9.99 (s, 1H), 9.30 (s, 1H), 9.15 (s, 1H), 8.76 (s, 1H), 8.28 (s, 1H), 8.04-7.92 (m, 3H), 7.69 -7.54 (m, 5H), 7.36-7.33 (m, 1H), 7.22 (s, 1H), 4.76 (s, 1H). *m/z* 398.1 $[M+1]^+$ HPLC-98.97%

4-chloro-N-(4-((2-oxoindolin-3-yl) amino) phenyl) benzene sulfonamide **(5j)**
Off white solid, yield 70%, M.P.-154-157$^0$C, $^1$HNMR (DMSO, 400MHz): 10.01 (s, 1H), 9.87 (s, 1H), 9.79 (s, 1H), 8.77 (s, 1H), 8.28 (s, 1H), 8.03-7.93 (m, 2H), 7.71-7.58 (m, 3H), 7.37-7.24 (m, 3H), 6.94-6.91 (m, 2H), 4.75 (s, 1H). *m/z* 413.9 $[M+1]^+$ HPLC-97.43%

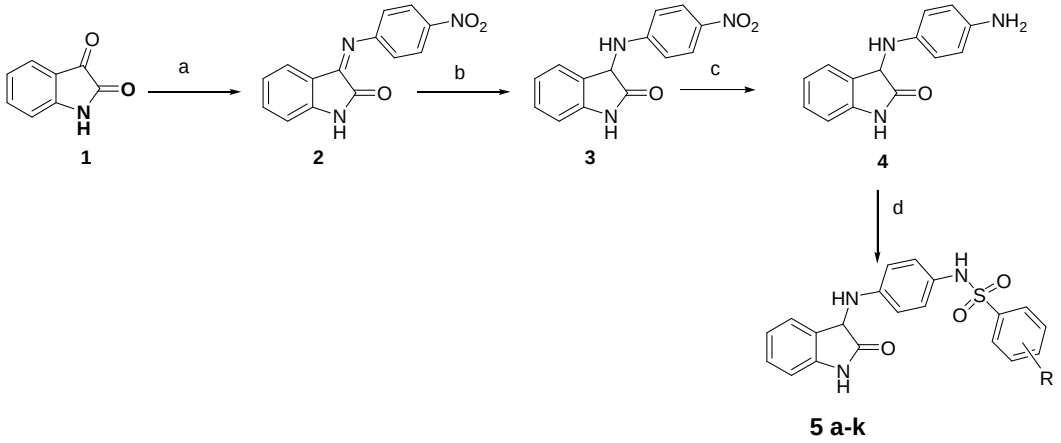
2-fluoro-N-(4-((2-oxoindolin-3-yl) amino) phenyl) benzene sulfonamide **(5k)**
Off white solid, yield 70%, M.P.-142-144$^0$C, $^1$HNMR (DMSO, 400MHz): 10.27 (s, 1H), 9.31 (s, 1H), 9.53 (s, 1H), 8.77 (s, 1H), 8.28 (s, 1H), 8.03-7.93 (m, 2H), 7.73-7.54 (m, 3H), 7.36-7.34 (m, 1H), 7.28-7.18 (m, 4H), 4.77 (s, 1H). *m/z* 398.1 $[M+1]^+$ HPLC-97.87%

## RESULTS AND DISCUSSION

### Chemistry

The synthesis of 3-(arylsulfonamido)phenyl oxindole scaffold starts with the 3H-indene-1, 2-dione (isatin) as outlined in scheme 1. The 3-(4- aminophenyl)oxindole **4** forms the key constituent for the present synthesis. The

compound **4** for was obtained by following the reaction sequence, imine formation, reductive amination and reduction from isatin in overall 75 % yield. The desired (3-arylsulfonamido) phenyloxindole derivatives were eventually obtained by the reaction of **4** with different arenesulfonyl chlorides respectively in 50-78% yield.




Reaction conditions: a) 3H-indene-1, 2-dione, 4-nitrobenzenamine PTSA, Ethanol ;
b) $NaBH_4$, MeOH, $0^0C$, 30 min; c) $NH_4Cl$, $SnCl_2$, EA; d , different arenesulfonyl chloride, THF, rt, 10-12 h.

**Biological activity**

All the synthesized oxindole-sulfonamide conjugates were screened for the inhibitory activity against the release of two cytokines, TNF-α and IL-6 from PBMCs stimulated by LPS. The biological activity data is collected in table 1. As shown in our results, among different (3-sufonamido)phenyloxiindole derivatives screened, compounds 5d, 5h and 5i exhibited promising inhibition of the release of two cytokines TNF-α and IL-6 with IC-50 values raging over 1.9-2.6 and 1.2-1.7 respectively and thus proved to be potent TNF-α and IL-6 inhibitor. Other analogues from this new sulfonamide series such as compounds **5a, 5b, 5f** and **5k** exhibited lower inhibition of the above cytokines with their IC-50 values ranging over 3.1-4.6 and 2.4-3.4 respectively and they found to be moderately potent TNF-α and IL-6 inhibitor. The other compounds such as **5c, 5e, 5**g and **5j** exhibited low or very low TNF-α and IL-6 inhibitory activity with their IC-50 values found to be greater 9.6.

We closely investigate the effect various substituent present sulfonamide moiety (structure-activity relationship, (SAR).

**Table 1. TNF-α and IL-6 inhibitory activity of oxiindolyl sulfonamide derivatives**

| Compounds | R | $IC_{50}$ (μM)TNF-α | IL-6 |
|---|---|---|---|
| 5a | 2-$CH_3$ | 4.6 | 3.4 |
| 5b | 3,5-$CF_3$ | 4.1 | 2.8 |
| 5c | 4-F | >10 | >10 |
| **5d** | 2-$CH_3$,5-F | 2.6 | **1.6** |
| 5e | 3-$CH_3$ | >10 | 9.6 |
| 5f | 4-$OCF_3$ | 3.1 | 2.4 |
| 5g | 3-CN | >10 | >10 |
| **5h** | 3-F | 2.6 | **1.7** |
| **5i** | 2-F | **1.9** | **1.2** |
| 5j | $C_6H_5$ | >10 | >10 |
| 5k | 3,5-F | 4.3 | 3.1 |
| Dexamethasone | - | 0.017 | 0.0095 |

As can be seen from biological activity data, that the fluoro substituent at 2,3 and 5- position found to be have favorable effect on the inhibition of release of TNF-α and IL-6. Table-1, entries 4, 8 and 9). However, the presence of more than one F- or trifluoromethyl ($CF_3$-) substituent found to have detrimental effect on the said activity as these substituent decreased the activity considerably (table-1, compare entry-5i with 5b and 5k). The methyl, phenyl and highly electron withdrawing CN- group at 4-position found have very little impact on the inhibition as indicated by their IC-50 value of >10.Interestingly, the presence of methyl group resulting into compounds **5a**, shown moderate inhibition of these activities. Notable Trifuoromethoxy analogue, compound 5f exhibited promising TNF-α and IL-6 inhibitory activity (table-1, entry-6). Thus the F- and trifluoro- ($CF_3$-) substituent found to be favorable for pronounced effect on the TNF-α and IL-6 inhibitory activity but more than one such a substituent found diminish the impact of these substituent on the said activity. Overall, the biological activity data suggested that the electronic nature as well as the position of the substituent had crucial impact on TNF-α and IL-6 inhibitory activity. Though the exact cause of such dramatic trend of the substituent toward said activity is not hand at this time, we speculate that the lipophilicity along the H-bond donor ability of the substituent might be the main contributing factor for the same.

## CONCLUSION

In conclusion, the structurally diversed sulfonamide derivative (oxindole-sulfonamide hybrid scaffolds) were synthesis and screened in order to search of the potent TNF-α and IL-6 inhibitor. This study leads to the discovery of compounds **5d, 5h and 5i** as a potent TNF-α and IL-6 inhibitors with reference to the standard drug dexamethason. In view of the mild and convenient approach coupled with the significant number of analogues from this novel sulfonamide series found to be potent TNF-α and IL-6 inhibitor makes the present study potentially useful in medicinal chemistry.

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