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Synthesis of novel isoindolinone derivatives containing azetidinones and thiazololidinone and their anti-inflammatory evaluation

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ABSTRACT

A series of new leads towards potent anti-inflammatory agents, azetidinone congeners:1,3-dihydro-2-[2'-(3''-chloro-2''-oxo-4''-substitutedaryl-1''-azetidinyl)-1',3'-thiazol-4'yl]-isoindol-1-ones (9-13) and thiazololidinone congeners: 1,3-dihydro-2-[2'-(2''-substitutedaryl-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-ones derivatives (14 -18) have been synthesized from Schiff bases: 1,3-dihydro-2-(2'-substitutedarylimino-1',3'-thaizol-4'-yl)-isoindol-1-ones (4-8). All synthesized compounds were evaluated for their toxicity profile, anti-inflammatory activity and ulcerogenic liability and the results were possessed positive activity anti-inflammatory activity. SAR studies have shown that compound 1,3-dihydro-2-[2'-(2''-(p-chlorophenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-ones (15) displayed better anti-inflammatory activity.

Keywords: Isoindolinone, Azetidinones, Thiazolidinones, Anti-Inflammatory, Toxicity and Ulcerogenic activity.

1. INTRODUCTION

In the past three decades, it has also become evident that a much larger variety of diseases have telltale cellular and molecular evidence for inflammation. These include chronic arterial and venous disease [1-2], myocardial ischemia [3-4], acute cerebral stroke and alzheimer's chronic disease [5-6], arterial hypertension [7], cancer [8] and various kinds of rheumatoid arthritis [9]. So, the discovery of novel anti-inflammatory drugs has attracted a lot of interests. The problems in treating these diseases particularly in chronic setting are that these are multifactorial, involve several changes in the system affected by the diseases, have several proteins involved in the metabolic cascade and are multigenic. Moreover, regularly prescribed drugs viz. NSAIDs and corticosteroids exhibited serious side effects. So, there is not a single drug available, which can be termed as ideal in terms of risk-benefit ratio. Hence, there is still critical need for new anti-inflammatory agents. Thus, as a part of our program of synthesizing new chemical entities possessing anti-inflammatory profile, our interests have been focused on isoindolin-1-one derivatives. A literature survey has revealed that congeners of isoindolin-1-one [10-14], thiazole [15-18], thiazolidinones [19-21] and azetidinones [22-25] are associated with promising anti-inflammatory activity. So, in the present study, isoindolin-1-one derivatives have been synthesized by incorporating thiazole followed by thiazolidinone or azetidinone in one frame. This may lead to compounds with interesting anti-inflammatory profile.

2. EXPERIMENTAL

All the reagents and solvents were generally received from commercial supplier *viz*. Spectochem, Rankem, RFCL, Merck, Qualigens and Sigma-aldrich. All melting points are in centigrade scale, uncorrected and taken on digital melting point apparatus. All organic solvents were dried and distilled before use petroleum ether used had a boiling range 60-80℃. All ethereal or ethyl acetate extracts were dried over anhydrous sodium sulphate. Solvents were generally removed under reduced pressure. R_f values were recorded for TLC that was carried out on glass plates coated with 0.2 mm layer of silica gel-G and also on Merck plates. The compounds on the TLC plate were visualized in UV light (254 and 366 nm) or developed in iodine atmosphere and also located by spraying various reagents. Spraying agents used for TLC were 5% aqueous sulphuric acid and 1% ethanolic ferric chloride. Infra-red (IR) spectra were recorded on Shimadzu model 435 spectrophotometer using KBr pallets and only major absorption bands are quoted in cm^{-1}. Proton nuclear magnetic resonance (^1H NMR) spectra

were recorded on JEOL (400MHz) spectrometers with reference to tetramethyl silane as the internal standard. The chemical shifts are expressed in δ values and coupling constant (*J*) in Hertz. The abbreviation s, brs, d, t and m indicate the signals as singlet, broad singlet, doublet, triplet and multiplet, respectively. The Mass spectra were recorded on a Varian MAT 311A instrument by using electron ionization (EI) at 70ev and TOF MS on LCT micromass and only the major peaks are quoted. Apparatus was dried in electrical oven at 120℃, flushed with nitrogen prior to use. The carbon, hydrogen and nitrogen analysis were performed on Carlo Erba-1108 (Carlo Erba, Milan, Italy), and the results were found with in + 0.4% of the theoretical values. All spectral analysis was analyzed in Department of Chemistry, University of Delhi.

2.1. General procedure for the synthesis of 1,3-dihydro-2-(acetyl)-isoindol-1-one (2)

To the solution of 1,3-dihydro-isoindol-1-one (**1**) (30g ,225mmol) in dry benzene (150 ml), acetyl chloride (19.43g, 248mmol) was added drop wise with constant stirring at temperature of 0-5 ^{o}C. Further, this mixture was stirred for 4 hours and then kept overnight. The excess of solvent was removed by distillation and remnant was poured onto crushed ice. The solid mass obtained was crystallized with ethanol to yield compound **2**. Yield: 24.8g (62.3%)**.** MP: 160-164 ^{o}C. IR[cm^{-1} ,KBr]: 3073(C-H aromatic),2941(C-H aliphatic),1717 (C=O), 1615 (C=C of aromatic ring). ^{1}H-NMR[400 MHz, DMSO-D_6-ppm]: δ 8.09-7.50 (m, 4H, Ar-H) 7.26 (s, 2H, CH_2 of isoindolin-1-one) 3.34 (s, 3H, -$COCH_3$). MS: $[M]^+$ at m/z 175. Anal Cacld for $C_{10}H_9NO_2$: C, 68.56 H, 5.18 N, 8.00. Found: C, 68.71 H, 5.02 N, 8.16.

2.2. General procedure for the synthesis of 1,3-dihydro-2-(2'-amino-1',3'-thiazol-4'-yl)-isoindol-1-one (3)

1,3-Dihydro-2-(acetyl)-isoindol-1-one (**2**), (22g, 126mmol), acetophenone (16.65g, 139mmol), catalytic amount of iodine and thiourea (19.12g, 251mmol) was well crushed in crucible. The mixture was taken in 250 ml round bottom flask and heated at 110℃ for 24 hours. A reaction mixture was cooled to room temperature and diluted with 100 mL of water and extracted with ether to remove unreacted iodine and acetophenone. Excess of ether was distilled off. Residue was dissolved in boiling water and filtered off the hot solution. It was allowed to stand for 30 minutes. Make the reaction mixture alkaline (upto pH 8-9) using ammonium hydroxide solution. The solid obtained was filtered and washed successively with water (2 X 150 ml). The separated solid was crystallized by aqueous ethanol (1:1). Yield: 15.5g (53.37%)**.** MP: 145-148℃. IR [cm^{-1},KBr]: 3062(N-H), 2924(C-H aromatic), 2852(C-H aliphatic), 1685(C=O), 1649(C=C of aromatic ring),1612(C=N). ^{1}H-NMR[400 MHz, DMSO-D_6-ppm]: δ 7.50-6.80 (m, 4H, Ar-H) 5.86 (s, 1H, CH of thiazole ring) 3.35 (s, 2H, CH_2 of isoindolin-1-one) 2.49 (s, 2H, NH_2, exchangeable with D_2O). MS: $[M]^+$ at m/z 231. Anal Cacld for $C_{11}H_9N_3OS$: C, 57.13 H, 3.92 N, 18.17. Found: C, 57.30 H, 3.78 N, 18.30.

2.3. General procedure for the synthesis of 1,3-dihydro-2-(2'-substitutedarylimino-1',3'-thaizol-4'-yl)-isoindol-1-ones (4-8)

A solution of compound **3** (12.9mmol) and proper aromatic aldehyde (14.3mmol) in absolute ethanol (30 ml) in presence of few drops of glacial acetic acid was refluxed for 8-12 hours. Progress and completion of the reaction was followed by TLC. After completion of reaction, excess of solvent was distilled off, cooled and then poured onto crushed ice and filtered. The solid thus separated was recrystalized form the appropriate solvent to give compounds **4-8**. By employing above procedure, compounds **4**, **5**, **6**, **7** and **8** were synthesized starting form benzaldehyde, *p*-chlorobenzaldehyde, *p*-methoxybenzaldehyde, *p*-hydroxybenzaldehyde and *p*-aminodimethyl benzaldehyde, respectively. The physical, analytical and spectral data of these compounds are given below:

2.4. General procedure for the synthesis of 1,3-dihydro-2-(2'-(phenyl)-imino-1',3'-thaizol-4'-yl)-isoindol-1-one (4)

A solution of compound **3** (3g,12.9mmol) and benzaldehyde (1.52g, 14.3mmol) in absolute ethanol (30 ml) in presence of few drops of glacial acetic acid was refluxed for 8-12 hours. Progress and completion of the reaction was followed by TLC. After completion of reaction, excess of solvent was distilled off, cooled and then poured onto crushed ice and filtered. The solid thus separated was recrystallized form the ethanol solvent to give compound **4**. Yield: 2.7g (65.22%) MP: 129- 131 ^{o}C. IR[cm^{-1} ,KBr]: 3078 (C-H aromatic), 2939 (C-H alipahatic), 1734 (C=O), 1615 (C=N), 1495 C=C of aromatic ring), 1220 (C-N), 1108 (C-S-C). ^{1}H-NMR[400 MHz, DMSO-D_6-ppm]: δ 8.77-7.35 (m, 9H, Ar-H) 7.33(s, 1H, CH of thiazole ring) 7.24 (s, 1H, CH-Ar) 2.60 (s, 2H, CH_2 of isoindolin-1-one). MS: $[M]^+$ at m/z 319. Anal Cacld for $C_{18}H_{13}N_3OS$: C, 67.71 H, 4.07 N, 13.16. Found: C, 67.76 H, 4.15 N, 13.20.

2.5. General procedure for the synthesis of 1,3-dihydro-2-(2'-(p-chlorophenyl)-imino-1',3'-thaizol-4'-yl)-isoindol-1-one (5)

A solution of compound **3** (3.0g , 12.9mmol) and *p*-chlorobenzaldehyde (2.0g, 14.3mmol) in absolute ethanol (30 ml) in presence of few drops of glacial acetic acid was refluxed for 8-12 hours. Progress and completion of the reaction was followed by TLC. After completion of reaction, excess of solvent was distilled off, cooled and then poured onto crushed ice and filtered. The solid thus separated was recrystallized form methanol solvent to give compound **5**. Yield: 2.5g (54.47%). MP: 109-113 ^{o}C. IR[cm^{-1},KBr]: 2994(C-H aromatic), 2934(C-H aliphatic), 1685(C=O), 1648(C=C of aromatic ring), 1618(C-N), 1226(C-N). ^{1}H-NMR[400 MHz, DMSO-D_6-ppm]: δ 7.26-6.93 (m, 8H, Ar-H) 6.92 (s, 1H, CH of thiazole ring) 6.80 (s, 1H, CH-Ar) 3.45 (s, 2H, CH_2 of isoindolin-1-one). MS: $[M]^+$ at m/z 353.5 and [M+2] at m/z 355.5. Anal Cacld for $C_{18}H_{12}N_3OSCl$: C, 61.10 H, 3.39 N, 11.88. Found: C, 61.18 H, 3.45 N, 11.70.

2.6. General procedure for the synthesis of 1,3-dihydro-2-(2'-(p-methoxyphenyl)-imino-1',3'-thaizol-4'-yl)-isoindol-1-one (6)

A solution of compound **3** (3.0g,12.9mmol) and *p*-methoxybenzaldehyde (1.95g, 14.3mmol) in absolute ethanol (30 ml) in presence of few drops of glacial acetic acid was refluxed for 8-12 hours. Progress and completion of the reaction was followed by TLC. After completion of reaction, excess of solvent was distilled off, cooled and then poured onto crushed ice and filtered. The solid thus separated was recrystallized by ethanol solvent to give compound **6**. Yield: 2.65g(58.5%). MP: 133- 135 ^{o}C. IR[cm^{-1},KBr]: 3060(C-H aromatic), 2868(C-H aliphatic),1692(C=O),1647 (C=C of aromatic ring), 1561(C=N),1192(C-N). ^{1}H-NMR[400 MHz, DMSO-D_6-ppm]: δ 7.87-7.27 (m, 8H, Ar-H) 6.80 (s, 1H, CH of thiazole ring) 6.78(s, 1H, CH-Ar) 3.30 (s, 3H, OCH_3) 2.49(s, 2H, CH_2 of isoindolin-1-one).

MS: $[M]^+$ at m/z 349. Anal Cacld for $C_{19}H_{15}N_3O_2S$: C, 65.32 H, 4.30 N, 12.03. Found: C, 65.24 H, 4.36 N, 11.95.

2.7. General procedure for the synthesis of 1,3-dihydro-2-(2'-(p-hydroxyphenyl)-imino-1',3'-thaizol-4'-yl)-isoindol-1-one (7)

A solution of compound **3** (3.0g,12.9mmol) and *p*-hydroxybenzaldehde (1.74g, 14.3mmol) in absolute ethanol (30 ml) in presence of few drops of glacial acetic acid was refluxed for 8-12 hours. Progress and completion of the reaction was followed by TLC. After completion of reaction, excess of solvent was distilled off, cooled and then poured onto crushed ice and filtered. The solid thus separated was recrystallized by using methanol solvent to give compound **7**. Yield: 2.60g (59.78%). MP: 123-126 ^{o}C. IR[cm^{-1} , KBr]: 3394 (O-H), 2989 (C-H aromatic), 2907 (C-H aliphatic), 1705 (C=O), 1623 (C=C), 1606 (C=N), 1217 (C-N). ^{1}H-NMR[400 MHz, DMSO-D_6-ppm]: δ 8.41 (s, 1H, OH, exchangeable with D_2O) 8.19-6.98 (m, 8H, Ar-H) 6.96 (s, 1H, CH of thiazole ring) 6.87 (s, 1H, CH-Ar) 3.33 (s, 2H, CH_2 of isoindolin-1-one). MS: $[M]^+$ at m/z 335. Anal Cacld for $C_{18}H_{13}N_3O_2S$: C, 64.48 H, 3.88 N, 12.54. Found: C, 64.57 H, 3.93 N, 12.65.

2.8. General procedure for the synthesis of 1,3-dihydro-2-(2'-(p-aminodimethylphenyl)-imino-1',3'-thaizol-4'-yl)-isoindol-1-one (8)

A solution of compound **3** (3.0g, 12.9mmol) and *p*-aminodimethylbenzaldehyde (2.13g, 14.3mmol) in absolute ethanol (30 ml) in presence of few drops of glacial acetic acid was refluxed for 8-12 hours. Progress and completion of the reaction was followed by TLC. After completion of reaction, excess of solvent was distilled off, cooled and then poured onto crushed ice and filtered. The solid thus separated was recrystallized acetic acid solvent to give compound **8**. Yield: 2.35g (49.89%), MP: 150-153 ^{o}C. IR[cm^{-1} ,KBr]: 3089 (C-H aromatic), 2931 (C-H aliphatic), 1694 (C=O), 1615(C=C), 1567(C=C of aromatic ring), 1251 (C-N). ^{1}H-NMR[400 MHz, $CDCl_3$-ppm] : δ 7.77-7.24 (m, 8H, Ar-H) 6.99 (s, 1H, CH of thiazole ring) 6.98(s, 1H, CH-Ar) 3.65(s, 2H, CH_2 of isoindolin-1-one) 2.66 (s, 6H, -$N(CH_3)_2$). MS: $[M]^+$ at m/z 362. Anal Cacld for $C_{20}H_{18}N_4OS$: C, 66.30 H, 4.97 N, 15.47. Found: C, 66.43 H, 5.10 N, 15.40.

2.9. General procedure for the synthesis of 1,3-dihydro-2-[2'-(3''-chloro-2''-oxo-4''-substituted aryl-1''-azetidinyl)-1',3'-thiazol-4'yl]-isoindol-1-ones (9-13)

To a solution of compound **4-8**, 1g each in dry dioxane chloroacetlyl chloride and triethyl amine were added with constant stirring at temperature of 0-5 ^{o}C. This reaction mixture was, then, reluxed for 6-8 hours, the progress and completion of reaction was checked by TLC. After completion of the reaction, excess of solvent was removed by distillation, residue was poured on crushed ice and then filtered. The separated product was recrystallized form proper solvent to furnish compounds **9-13**. By following above method, compounds **9**, **10, 11**, **12** and **13** were obtained from compounds **4**, **5**, **6**, **7** and **8** respectively. Their physical, analytical and spectral data are given below:

2.10. General procedure for the synthesis of 1,3-dihydro-2-[2'-(3''-chloro-2''-oxo-4''-(phenyl)-1''-azetidinyl)-1',3'-thiazol-4'yl]-isoindol-1-one (9)

To a solution of compound **4** (1g, 3.13 mmol) in dry dioxane (10ml), chloroacetlyl

chloride (0.39g, 3.44mmol) and triethyl amine (0.48ml, 3.44mmol) were added with constant stirring at temperature of 0-5 ^{o}C. This reaction mixture was, then, refluxed for 6-8 hours, the progress and completion of reaction was checked by TLC. After completion of the reaction, excess of solvent was removed by distillation, residue was poured on crushed ice and then filtered. The separated product was recrystallized by using DMF solvent to furnish compound **9**. Yield: 0.65g (52.42%). MP: 142-145 ^{o}C. IR[cm^{-1} ,KBr]: 3079 (C-H aromatic), 2976 (C-H aliphatic),1705 (C=O), 1606 (C=C), 1564 (C=C of aromatic ring) , 1216 (C-N). ^{1}H-NMR[400 MHz, $CDCl_3$-ppm] : δ 7.26-6.43 (m, 9H, Ar-H) 6.41 (s, 1H, CH of thiazole ring) 3.42 (d, 1H, CH-Ar) 3.09 (d, 1H, CH-Cl) 2.69 (s, 2H, CH_2 of isoindolin-1-one). MS: $[M]^+$ at m/z 395.5 and [M+2] at m/z 397.5. Anal Cacld for $C_{20}H_{14}N_3O_2SCl$: C, 60.68 H, 3.53 N, 10.61. Found: C, 60.75 H, 3.68 N, 10.53.

2.11. General procedure for the synthesis of 1,3-dihydro-2-[2'-(3''-chloro-2''-oxo-4''-(p-chloro phenyl)-1''-azetidinyl)-1',3'-thiazol-4'yl]-isoindol-1-one (10)

To a solution of compound **5** (1g, 2.83 mmol) in dry dioxane (10ml), chloroacetlyl chloride (0.35g , 3.11mmol) and triethyl amine (0.43ml,3.11mmol) were added with constant stirring at temperature of 0-5 ℃. This reaction mixture was, then, reluxed for 6-8 hours, the progress and completion of reaction was checked by TLC. After completion of the reaction, excess of solvent was removed by distillation, residue was poured on crushed ice and then filtered. The separated product was recrystallized by using benzene solvent to furnish compound **10**. Yield: 0.62g(50.82%). MP: 120-124 ℃. IR[cm^{-1},KBr]: 3075 (C-H aromatic), 2915 (C-H aliphatic),1702 (C=O), 1627(C=C), 1606(C=N), 1211 (C-N). ^{1}H-NMR[400 MHz, $CDCl_3$-ppm] : δ 7.84-7.02 (m, 8H, Ar-H) 6.74 (s, 1H, CH of thiazole ring) 1.63 (d, 1H, CH-Ar) 1.60 (d, 1H, CH-Cl) 1.36 (s, 2H, CH_2 of isoindolin-1-one). MS: $[M]^+$ at m/z 430. Anal Cacld for $C_{20}H_{13}N_3O_2SCl_2$: C, 55.81 H, 3.02 N, 9.77. Found: C, 55.68 H, 2.90 N, 9.69.

2.12. General procedure for the synthesis of 1,3-dihydro-2-[2'-(3''-chloro-2''-oxo-4''-(p-methoxy phenyl)-1''-azetidinyl)-1',3'-thiazol-4'yl]-isoindol-1-one (11)

To a solution of compound **6** (1g, 2.86 mmol) in dry dioxane (10ml), chloroacetlyl chloride (0.36g, 3.15mmol) and triethyl amine (0.44ml, 3.15mmol) were added with constant stirring at temperature of 0-5℃. This reaction mixture was, then, refluxed for 6-8 hours, the progress and completion of reaction was checked by TLC. After completion of the reaction, excess of solvent was removed by distillation, residue was poured on crushed ice and then filtered. The separated product was recrystallized by using diethyl ether solvent to furnish compound **11**. Yield: 0.60g (49.18%). MP: 144-147^{o}C. IR[cm^{-1} ,KBr]: 3026 (C-H aromatic),2925(C-H aliphatic), 1727 (C=O), 1602 (C=C), 1508 (C=C) , 1276 (C-N). ^{1}H-NMR[400 MHz, $CDCl_3$-ppm]: δ 8.58-7.14 (m, 8H, Ar-H) 6.97 (s, 1H, CH of thiazole ring) 3.83 (s, .3H, OCH_3) 3.80 (s, 1H, CH of thiazole ring) 3.33 (d, 1H, CH-Ar) 2.55 (d, 1H, CH-Cl). MS: $[M]^+$ at m/z 425.5 and [M+2] at m/z 427.5. Anal Cacld for $C_{21}H_{16}N_3O_3SCl$: C, 59.22 H, 3.76 N, 9.87. Found: C, 59.37 H, 3.81 N, 9.92.

2.13. General procedure for the synthesis of 1,3-dihydro-2-[2'-(3''-chloro-2''-oxo-4''-(p-hydroxy phenyl)-1''-azetidinyl)-1',3'-thiazol-4'yl]-isoindol-1-one (12)

To a solution of compound **7** (1g, 2.98 mmol) in dry dioxane (10ml), chloroacetlyl chloride (0.37g ,3.28mmol) and triethyl amine (0.46ml, 3.28mmol) were added with constant stirring at temperature of 0-5 ^{o}C. This reaction mixture was, then, reluxed for 6-8 hours, the progress and completion of reaction was checked by TLC. After completion of the reaction, excess of solvent was removed by distillation, residue was poured on crushed ice and then filtered. The separted product was recrystallized by using dioxane solvent to furnish compound **12**. Yield: 0.67g(54.47%). MP: 136-139 ^{o}C. IR[cm^{-1} ,KBr]: 3327 (O-H), 2937 (C-H aromatic), 2840 (C-H aliphatic), 1717 (C=O), 1617 (C=C aromatic), 1508 (C=N), 1198 (C-N). ^{1}H-NMR[400 MHz, $CDCl_3$-ppm] : δ 13.07 (s, 1H, OH, exchangeable with D_2O) 9.94-7.01 (m, 8H, Ar-H) 6.92 (s, 1H, CH of thiazole ring) 3,87 (d, 1H, CH-Ar) 3.41 (s, 2H, CH_2 of isoindolin-1-one) 2.55 (d, 1H, CH-Cl). MS: $[M]^+$ at m/z 411.5. Anal Cacld for $C_{20}H_{14}N_3O_3SCl$: C, 58.61 H, 3.43 N, 10.20. Found: C, 58.69 H, 3.38 N, 10.13.

2.14. General procedure for the synthesis of 1,3-dihydro-2-[2'-(3''-chloro-2''-oxo-4''-(p-aminodimethyl phenyl)-1''-azetidinyl)-1',3'-thiazol-4'yl]-isoindol-1-one (13)

To a solution of compound **8** (1g, 2.76 mmol) in dry dioxane (10ml), chloroacetlyl chloride (0.34g, 3.03mmol) and triethyl amine (0.42ml, 3.03mmol) were added with constant stirring at temperature of 0-5 ^{o}C. This reaction mixture was, then, reluxed for 6-8 hours, the progress and completion of reaction was checked by TLC. After completion of the reaction, excess of solvent was removed by distillation, residue was poured on crushed ice and then filtered. The separated product was recrystallized by using DMF solvent to furnish compound **13**. Yield: 0.58g (47.93%). MP: 167-170 ^{o}C. IR[cm^{-1}, KBr]: 3026 (C-

H aromatic), 2951 (C-H aliphatic), 1729 (C=O), 1608 (C=C aromatic), 1515 (C=N), 1215 (C-N). ^{1}H-NMR[400 MHz, DMSO-D_6-ppm]: δ 7.78-7.25 (m, 8H, Ar-H) 6.70 (s, 1H, CH of thiazole ring) 3.35 (s, 6H, -N(CH_3)$_2$) 2.49 (s, 2H, CH_2 of isoindolin-1-one) 2.30 (d, 1H, CH-Ar) 2.00 (d, 1H, CH-Cl). MS: $[M]^+$ at m/z 438.5 and [M+2] at m/z 440.5. Anal Cacld for $C_{22}H_{19}N_4O_2SCl$: C, 60.20 H, 4.33 N, 12.77. Found: C, 60.25 H, 4.20 N, 12.85.

2.15. General procedure for the synthesis of 1,3-dihydro-2-[2'-(2''-substitutedaryl-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-ones (14-18)

A solution of compounds **4-8** (1g each), thioglycolic acid and anhydrous $ZnCl_2$ in DMF (10 ml) was refluxed for 8-11 hours, then concentrated, cooled and poured into ice-water and then filtered. The product obtained was purified by recystallization from appropriate solvent to get compounds **14-18**, and the homogeneity of these was checked by TLC. By using above method, compounds **14, 15, 16, 17** and **18** were procured form compounds **4**, **5**, **6**, **7** and **8** respectively. The physical, analytical and spectral data of compounds **14-18** are furnished below:

2.16. General procedure for the synthesis of 1,3-dihydro-2-[2'-(2''-(phenyl)4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-one (14)

A solution of compound **4** (1g, 3.13mmol), thioglycolic acid (0.32g, 3.44mmol) and anhydrous $ZnCl_2$ (0.51g, 3.76mmol) in DMF (10 ml) was refluxed for 8-11 hours. The progress of the reaction was checked by TLC, then concentrated, cooled and poured into ice-water and then filtered. The product obtained was purified by recystallization by using benzene solvent to get compound **14**. Yield: 0.52g(42.3%). MP: 152-156 °C. IR[cm^{-1} ,KBr]: 3067 (C-H aromatic), 2957(C-H aliphatic), 1685 (C=O), 1640 (C=C aromatic), 1560 (C=N) , 1212 (C-N). ^{1}H-NMR[400 MHz, $CDCl_3$-ppm]: δ 7.37-7.07 (m, 9H, Ar-H) 6.78 (s, 1H, CH of thiazole ring) 2.58 (s, 1H, CH attached with S and N) 1.86 (s, 2H, CH_2 of isoindolin-1-one), 1.71 (s, 2H, CH_2 attached with >C=O). MS: $[M]^+$ at m/z 393. Anal Cacld for $C_{20}H_{15}N_3O_2S_2$: C, 61.07 H, 3.82 N, 10.69. Found: C, 61.15 H, 3.70 N, 10.60.

2.17. General procedure for the synthesis of 1,3-dihydro-2-[2'-(2''-(p-chlorophenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-ones (15)

A solution of compound **5** (1g, 2.83mmol), thioglycolic acid (0.29g, 3.11mmol) and anhydrous $ZnCl_2$ (0.46g, 3.39mmol) DMF (10 ml) was refluxed for 8-11 hours. The progress of the reaction was checked by TLC, then concentrated, cooled and poured into ice-water and then filtered. The product obtained was purified by recystallization by using acetone solvent to get compound **15**. Yield: 0.55g (45.45%). MP: 132-135 °C. IR[cm^{-1} ,KBr]: 2925 (C-H aliphatic), 1741 (C=O), 1602 (C=C aromatic), 1552 (C=N), 1219 (C-N). ^{1}H-NMR[400 MHz, $CDCl_3$-ppm]: δ 7.34-7.07 (m, 8H, Ar-H) 6.77 (s, 1H, CH of thiazole ring) 2.47(s, 1H, CH attached with S and N) 1.78 (s, 2H, CH_2 of isoindolin-1-one), 1.52 (s, 2H, CH_2 attached with >C=O). MS: $[M]^+$ at m/z 427.5 and [M+2] at m/z 429.5. Anal Cacld for $C_{20}H_{14}N_3O_2S_2Cl$: C, 56.14 H, 3.27 N, 9.82. Found: C, 56.25 H, 3.35 N, 9.90.

2.18. General procedure for the synthesis of 1,3-dihydro-2-[2'-(2''-(p-methoxyphenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-one (16)

A solution of compound **6** (1g, 2.86mmol), thioglycolic acid (0.29g, 3.15mmol) and anhydrous $ZnCl_2$ (0.47g, 3.43mmol) in DMF (10 ml) was refluxed for 8-11 hours. The progress of the reaction was checked by TLC, then concentrated, cooled and poured into ice-water and then filtered. The product obtained was purified by recystallization by using benzene solvent to get compound **16**. Yield: 0.59g (48.76%). MP: 156-160 ^{o}C. IR[cm^{-1} ,KBr]: 2930 (C-H aliphatic), 1734 (C=O), 1654 (C=C aromatic), 1615 (C=N), 1206 (C-N). ^{1}H-NMR[400 MHz, DMSO-D_6-ppm]: δ 7.92-7.10 (m, 8H, Ar-H) 7.08 (s, 1H, CH of thiazole ring) 3.20 (s, 3H, OCH_3) 2.96(s, 1H, CH attached with S and N) 2.45 (s, 2H, CH_2 of isoindolin-1-one) 2.28 (s, 2H, CH_2 attached with >C=O). MS: [M]+ at m/z 422. Anal Cacld for $C_{21}H_{17}N_3O_3S_2$: C, 59.56 H, 4.05 N, 9.95. Found: C, 59.55 H, 4.0 N, 9.80.

2.19. General procedure for the synthesis of 1,3-dihydro-2-[2'-(2''-(p-hydroxyphenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-one (17)

A solution of compound **7** (1g, 2.98mmol), thioglycolic acid (0.30g, 3.28mmol) and anhydrous $ZnCl_2$ (0.49g, 3.58mmol) in DMF (10 ml) was refluxed for 8-11 hours. The progress of the reaction was checked by TLC, then concentrated, cooled and poured into ice-water and then filtered. The product obtained was purified by recystallization by using DMF solvent to get compound **17**. Yield: 0.62g (50.82%). MP: 149-152 ^{o}C. IR[cm^{-1} , KBr]: 3449 (O-H), 3083 (C-H aromatic), 1720 (C=O), 1617 (C=C aromatic), 1460 (C=N), 1210 (C-N). ^{1}H-NMR[400 MHz, $CDCl_3$-ppm]: δ 10.39 (s, 1H, OH, exchangeable with D_2O), 8.56-7.52(m, 8H, Ar-H) 7.26 (s, 1H, CH of thiazole ring) 3.50 (s, 2H, CH_2 of isoindolin-1-one) 2.75 (s, 2H,

CH_2 attached with >C=O) 2.18 (s, 1H, CH attached with S and N). MS: [M]+ at m/z 409. Anal Cacld for $C_{20}H_{15}N_3O_3S_2$: C, 58.68 H, 3.67 N, 10.27. Found: C, 58.75 H, 3.55 N, 10.35.

2.20. General procedure for the synthesis of 1,3-dihydro-2-[2'-(2''-(p-aminodimethylphenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-ones (18)

A solution of compound **8** (1g, 2.76mmol), thioglycolic acid (0.28g, 3.03mmol) and anhydrous $ZnCl_2$ (0.45g, 3.31mmol) in DMF (10 ml) was refluxed for 8-11 hours. The progress of the reaction was checked by TLC, then concentrated, cooled and poured into ice-water and then filtered. The product obtained was purified by recystallization by using DMF solvent to get compound **18**. Yield: 0.55g (45.83%). MP: 161-164 ^oC. IR[cm^{-1},KBr]: 3073 (C-H aromatic), 2928 (C-H aliphatic), 1717 (C=O), 1615 (C=C aromatic), 1508 (C=N), 1208 (C-N). ^{1}H-NMR[400 MHz, DMSO-D_6-ppm]: δ 7.99-6.94 (m, 8H, Ar-H) 6.82 (s, 1H, CH of thiazole ring) 3.87 (s, 6H, -$N(CH_3)_2$) 3.29 (s, 2H, CH_2 of isoindolin-1-one), 2.58 (s, 2H, CH_2 attached with >C=O) 2.58 (s, 1H, CH attached with S and N). MS: $[M]^+$ at m/z 436.5. Anal Cacld for $C_{22}H_{20}N_4O_2S_2$: C, 60.53 H, 4.62 N, 12.83. Found: C, 60.09 H, 4.18 N, 12.21.

2.21. Pharmacology

All the compounds **4**-**18** were evaluated for their anti-inflammatory and acute toxicity. The experiments were performed on albino rats of Charles Foster strain of either sex, excluding pregnant females, of 70 to 90 days weighing 68-115g. Acute toxicity of numerous compounds was tested in albino mice (22–28 g). (Rats and mice were purchased from LABO-AIDS, Meerut, U.P., India). Food (chaw pallet) and water were given to the animals *ad libitum*. The test compounds were dissolved in propylene glycol. Indomethacin (from commercial source) was used as reference drugs. The biological activities were carried out in Late Dr. Ashok Kumar's (ex supervisor) lab, Medicinal Chemistry Division, Department of Pharmacology, Lala Lajpat Rai Memorial Medical College, Meerut (U.P.), India and the protocol was approved by the ethical committee of Lala Lajpat Rai Memorial Medical College, Meerut (U.P.), India.

2.22. Acute toxicity study

The test compounds were investigated for acute toxicity profile (LD_{50}) in albino mice, according to the method of Smith Q. E. [26]. The test compounds were given orally at different dose levels in separate groups of animals. After 24 hours of drug administration, percent mortality in each group was observed. LD_{50} was calculated from the data obtained.

2.23. Anti-inflammatory activity

This activity was done according to the given procedure by Winter *et al.,* [27]. Indomethacin was used as reference drugs. The rats were divided into three groups (control, drug treated and standard drug) of six animals each. A freshly prepared suspension of carrageenan (1% in 0.9% saline), 0.05 mL was injected under planter aponeurosis of right hind paw of each rat. One group was kept as control and treated with propylene glycol. Test compounds and standard drugs were administered to the animals of drug treated and standard drug group, respectively, 1 h before to carageenan injection. The paw volume of each rat was measured before 1 and 3 h after carrageenan treatment with the help of Plethysmometer. The percent anti-inflammatory activity was calculated according to the formula given below.

Percentage of inhibition of oedema = $(1 - V_t / V_c) \times 100$

Where, V_t and V_c are the mean increase in paw volume of rats of treated group and the control group, respectively. Results obtained were statistically analyzed.

2.24. Ulcerogenic Activity

The ulcerogenic activity was done on albino rats according to the procedure of Verma *et al*., [28]. The rats were fasted for 24 hours prior to the administration of the test compounds. Water was given to the animals *ad libitum*. The most promising compounds **15**, **16** and reference drugs were given intraperitoneally, and then these animals were sacrificed 8 hours after drug treatment. The stomach, duodenum and jejunum were removed and examined with a hand lens for any evidence of the following: (a) shedding of epithelium, (b) petechial or frank hemorrhage, (c) erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic liability.

2.25. Cyclooxygenase activity

This test was carried out *in vitro* on the microsmal fraction of mucosal preparations of rabbit distal colon in order to search out the possible mechanism of action of the test compounds by Calderano *et al.,*[29]. Colonic mucosa (=2-3g), stripped as previously described, was minced and homogenized (Potter homogenizer) in 3 volumes of Tris buffer, 0.1 M, pH 8.0. The homogenate was centrifuged for 30 min at 10 000 g. The resulting supernatent was centrifuged for 1 hour at 100 000 g. The precipitate was suspended

in Tris buffer, 0.1M, pH 8.0, and re-centrifuged for 1 hour at 100 000 g. The microsomal pellet was used immediately for an enzyme assay cyclooxygenase activity was assayed by measuring the rate of conversion of arachidonic acid to PGE_2. Microsomal fractions (50 µ L) were incubated with test agents for 5 min at 37°C in 30 mL Tris HCl, pH 8.0 containing 2 mM reduced glutathione, 5mM L-tryptophan, and 1µM hematin. The substrate, 20 µM arachidonic acid with tracer amount of [1- ^{14}C] arachidonic acid [=20 000 cpm] was then added and the reaction proceeded for 3 min at 37 °C. The reaction was stopped by addition of 0.2 mL of ethyl ether/methanol /citric acid 0.2 M (30:4:1), which was pre-cooled at -25 °C. PGE_2 was extracted twice into the same mixture. The solvent was evaporated under a N_2 stream and radilabelled arachidonic acid was separated from radio-labeled PGE_2 by RP-HPLC. HPLC analysis was performed on a Hitachi spectrophotometer (Hitachi High Technologies America, San Jose, CA, USA) equipped with a flow cell. The sample was injected on an ultra sphere column (Beckman Coulter Ltd., High Wycombe, Buckinghamshire, UK) ODS 5 mm, 4.6 mm X 25 cm with 2 mmol unlabelled PGE_2 as an internal standard, PG chromatographic profile was obtained by isocratic elution with 150 mM H_3PO_4 in water, pH 3.5, containing 30% acetonitrile a flow rate of 1mL min^{-1} monitoring the UV absorption at 214 nm. Radioactivity that coeluted with authentic PGE_2 was quantified by liquid scintillation spectrometry. Test samples were compared to parried control incubations. The percentage of inhibition was calculated as follows:

[(cpm control – cpm test / (cpm control)) X 100]

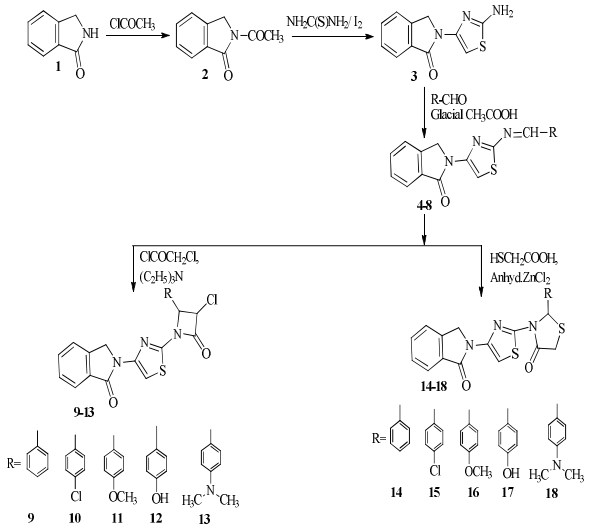
3. RESULTS AND DISCUSSION

3.1. Chemistry

Reactions leading to the formation of proposed compounds of present series are displayed in Scheme 1. Acetylation of 1,3-Dihydro-isoindol-1-one (**1**) occurred to give 1,3-Dihydro-2-(acetyl)-isoindol-1-one (**2**). This compound on reaction with thiourea and iodine has yield the 1,3-Dihydro-2-(2'-amino-1',3'-thiazol-4'-yl)-isoindol-1-one (**3**). Furhter, compound **3** was condensed with numerous aromatic aldehydes to furnish Schiff bases: 1,3-Dihydro-2-(2'-substitutedarylimino-1',3'-thaizol-4'-yl)-isoindol-1-ones (**4-8**). From these compounds **4-8**, azetidinone congeners: 1,3-Dihydro-2-[2'-(3''-chloro-2''-oxo-4''-substitutedaryl-1''-azetidinyl)-1',3'-thiazol-4'yl]-isoindol-1-ones (**9-13**) have been procured on reaction with triehtyl amine and chloracetyl chloride. Compound **9** is confirmed by IR peak at 1705 cm^{-1} (C=O) and 1216 cm^{-1} (C-N). The main evidence of compound **9** formation came from its ^{1}H-NMR spectrum at δ 6.41 (s, 1H, CH of thiazole ring), 3.42 (d, 1H, CH-Ar) and 3.09 (d, 1H, CH-Cl). Its mass spectrum displayed a molecular ion peak (M^+) at m/z 395.5 corresponding to its molecular formula $C_{20}H_{14}N_3O_2SCl$ was confirmed that compound **9** was 1,3-dihydro-2-[2'-(3''-chloro-2''-oxo-4''-(phenyl)-1''-azetidinyl)-1',3'-thiazol-4'yl]-isoindol-1-one.

Similarly other structural assignments of the compounds **10-13** is confirmed by spectral and elemental analysis.

Finally, thiazololidinone derivatives: 1,3-Dihydro-2-[2'-(2''-substitutedaryl-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-ones (**14-18**) have also been synthesized form compounds **4-8** and thioglycoic acid and anhydrous $ZnCl_2$. Compound **14** is confirmed by confirmed by IR peak at 1685 cm^{-1} (C=O) and 1560 cm^{-1} (C=N). The main evidence of compound **14** formation came from its ^{1}H-NMR spectrum at δ 6.78 (s, 1H, CH of thiazole ring), 2.58 (s, 1H, CH attached with S and N), 1.86 (s, 2H, CH_2 of isoindolin-1-one) and 1.71 (s, 2H, CH_2 attached with >C=O). Its mass spectrum displayed a molecular ion peak (M^+) at m/z 393 corresponding to its molecular formula $C_{20}H_{15}N_3O_2S_2$ was confirmed that compound **14** was 1,3-dihydro-2-[2'-(2''-(phenyl)-4''-oxo-1'',3''-thiazolidin-3''-yl)-1',3'-thiazol-4'yl]-isoindol-1-ones.



Scheme - 1: Synthesis of isoindolin-1-one derivatives.

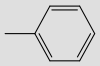
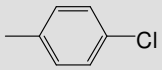
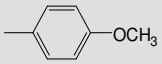
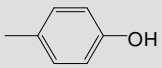
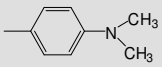
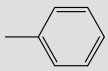
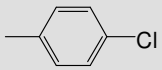
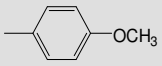
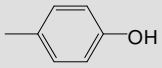
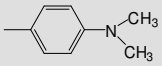
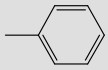
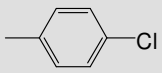
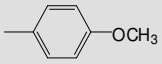
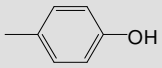
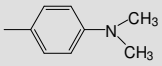
Similarly other structural assignments of the novel compounds **15-18** is confirmed by spectral and elemental analysis.

3.2. Biological Activity

3.2.1. Acute toxicity study

The test compounds **4-18** were investigated *in vivo* for acute toxicity profile (LD_{50}) in albino mice [**26**]. All the compounds of the present series have shown LD_{50} > 800 mg kg^{-1} p.o., except compounds **4**, **7** and **8** exhibiting LD_{50} >600, >600 and >400 mg kg^{-1} p.o., respectively (Table 1).

Table -1: Pharmacological results of compounds (4-18)

Compound	R	Acute toxicity	Anti-inflammatory activity		Ulcerogenic activity	Cyclooxygenase assay
		LD_{50} (mg Kg^{-1} p.o.)	Dose (mg/kg^{-1} p.o.)	% inhibition of oedema	UD_{50} (mg kg^{-1} i.p.)	(% inhibition 10µM)
Control [d]		-	1 mL	0.0	-	-
4		>600	50	34.50 [a]	-	-
5		>800	50	49.30 [b]	-	-
6		>800	50	44.00 [b]	-	-
7		>600	50	40.00 [b]	--	-
8		>400	50	36.72 [a]	-	-
9		>800	50	38.30 [b]	-	-
10		>800	50	57.21 [a]	-	-
11		>800	50	54.10 [a]	-	-
12		>800	50	47.22 [a]	-	-
13		>800	50	40.05 [b]	-	-
14		>800	50	41.25 [b]	-	-
15		>800	25 50 100	44.73 [a] 70.26 [c] 88.45 [b]	130.0	80.0
16		>800	25 50 100	40.10 [b] 61.56 [c] 80.23 [a]	120.0	75.0
17		>800	50	52.50 [a]	-	-
18		>800	50	45.43 [a]	-	-
Indomethacin		-	25 50 100	35.5 [b] 56.31 [c] 78.0 [b]	90	86

3.2.2. Anti-inflammatory activity

Random screening of compounds **4-18** and reference drug, indomethacin, was performed at a dose of 50 mg kg^{-1} p.o. [27]. All the tested compounds have displayed statistically significant anti-inflammatory activity (Table 1). Two compounds **15** and **16** were found to possess more potent anti-inflammatory activity as

compared to indomethacin, while compound **10** showed comparable anti-inflammatory activity with standard drug (Table-1). By considering the potentiality of compounds **15** and **16**, these compounds along with indomethacin were subjected to screening at two more doses of 25 and 100 mg kg^{-1} p.o. for anti-inflammatory activity. Interestingly, compounds **15** and **16** yielded improved anti-inflammatory profile than indomethacin. However, compound **15** exhibited maximal anti-inflammatory activity of the present study (Table-1).

3.2.3. Ulcerogenic activity

Two most active compounds **15**, **16** and standard drug indomethacin were screened for their ulcerogenic liability [28]. The UD_{50}'s of compounds **15**, **16** and reference drug were 130.0, 120.0 and 90.0 mg kg^{-1} i.p., respectively. Hence, compound **15** possessed less ulcerogenic liability (Table-1).

3.2.4. Cyclooxygenase assay

The most promising compounds **15**, **16** and reference drug idomethacin underwent cyclooxygenase assay to determine their most likely mechanism of action [29]. Results given in table-1 suggested that these two compounds inhibited rat's paw oedema by inhibiting the prostaglandins' (PG) synthesis by inhibiting the cyclooxygnease (COX) enzyme.

4. Discussion

Fifteen compounds **4-18** were evaluated *in vivo* in order to determine their toxicity and anti-inflammatory profile, and the results are given in Table-1. The structural feature of compounds **4-8**, **9-13** and **14-18** is presence of the Schiff base, azetidinone and thiazolidinone moieties, respectively, at 2-postion of thiazolylisoindolin-1-one moiety. The LD_{50} data of all compounds were very high suggesting good safety margin except compounds **4**, **7** and **8** (Table-1). All fifteen compounds showed statistically significant anti-inflammatory activity (Table-1).

All the five Schiff bases **4 - 8** exhibited mild anti-inflammatory activity (34.50%. 49.30%, 44.00%, 40.00%, and 36.72%, respectively). Cyclization of compounds **4 - 8** into their corresponding azetidinone **9 - 13** and thiazolidinone **14 -18** congeners enhanced the anti-inflammatory activity (38.30%, 57.21%, 54.10%, 47.22%, 40.05% and 41.25%, 70.26%, 61.56%, 52.50%, 45.43%, respectively). Hence, these results indicated that compounds **14-18** containing thiazolidineone moiety yielded maximal anti-inflammatory activity.

It is significant to note form the observations that the compounds **4**, **9** and **14** having phenyl group as a substitution showed least percentage of inhibition of oedema (34.50%, 38.30% and 41.25%, respectively), whereas substitution with *p*-chlorophenyl group as seen in compounds **5**, **10** and **15** displayed most significant anti-inflammatory activity (49.30%, 57.21% and 70.26%, respectively). *p*-Methoxyphenyl group substitution in compounds **6**, **11** and **16** exhibited significant anti-inflammatory activity. Substitution *p*-hydroxyphenyl **7**, **12** and **17** and *p*-aminodimethylphenyl **8**, **13** and **18** yielded less but still adequate anti-inflammatory activity.

Two compounds **15** and **16** were found to exhibit better anti-inflammatory profile as compared standard drug indomethacin. These compounds along with standard drug were also tested at two more doses of 25 and 50 mg kg^{-1} p.o., these two compounds yielded maximal activity than standard drug at tested doses. These two compounds along with standard drug were also checked for their ulcerogenic liability. These compounds were found to be less ulcerogenic as compared to standard drug. However, compound **15** were found to be the best one in terms of anti-inflammatory and ulcerogenic profile.

5. CONCLUSION

Out of eleven compound examined, compound **15** and **16** were found to be the most promising compounds of the present study. LD_{50} of these compounds was very high suggesting good safety margin and also these compounds possessing less ulcerogenic liability as compared to standard drug: indomethacin. However, compound **15** yielded outstanding biological results as compared compound **16**.

At the end, following conclusion can be drawn:

- Presence of *p*-chlorophenyl group as a substituent may elicit a remarkable increase in anti-inflammatory activity.
- *p*-methoxyphenyl substitution showed significant activity.
- Presence of electronegative atom may play an important role in the modulation of activity.

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