WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 15, 1313-1321. Research Article ISSN 2277–7105

DESIGN AND SYNTHESIS, CHARACTERIZATION *IN-VITRO* ANTI INFLAMMATORY ACTIVITY OF N- SUBSTITUTED 2(P-TOLYL)4-SUBSTITUTED, 1,3 DIONE INDOLE DERIVATIVES

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Article Received on
20 June 2025,

Revised on 10 July 2025,
Accepted on 31 July 2025

DOI: 10.20959/wjpr202515-37651

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ABSTRACT

The present investigation is concerned with synthesis of new substituted indole derivatives with the objective of discovering novel and potent anti-inflammatory agent. The structure of all the synthesized compounds were elucidated by spectral (IR, ^{1}HNMR ^{13}C NMR and mass spectroscopy analysis. The obtained compounds were screened for their anti-inflammatory activities at the dose of 100–500 µg/mL. The compound N-Substituted 2-(P-Tolyl)4-substituted,1,3Dione indole two showed better anti-inflammatory activities at the graded dose a concentration range of 100–500 µg/mL.

KEYWORDS: Indomethacin, Albumin Denaturation, Anti-inflammatory Activities.

INTRODUCTION

In order to manage the inflammation, pain, and fever, the most commonly used agents are NSAIDs. Existing NSAIDs aren't very good at relieving pain or fever because they block both COX enzymes, COX-1 and COX-2, which can have badeffects on the digestive system. These COX enzymes are responsible for theconversion of arachidonic acid into prostaglandin H2. COX-1 is considered responsible for the protection of gastric and intestinal cells. Conversely, COX-2 is thought to be in charge of secreting inflammatory mediators. It has been observed that drugs including indomethacin etc. as selective inhibitors of the COX-2 enzyme show significantly considerable anti-inflammatory activity. However, they are linked to elevated hepatic injury

and also results in several cardiovascular complications.[3,4] In most of the potent synthetic or natural bioactive organic compounds, heterocyclic rings is present as pharmacophores and are considered responsible for biological activity.

The compounds with heterocyclic rings are crucial in the process of chemical synthesis and also to explore their various advanced pharmaceutical applications. It is evident that a number of compounds with sulfur, nitrogen, and oxygen as heteroatoms have been demonstrated to be highly effective bioactive agents.

The proved biological response of Indole derivatives includes its role as microbicidal, fungicide, analgesic, anti-inflammatory, anti-tumor, anti-convulsant[16], anti-malarial, anti-oxidant, anti-tubercular and is presented in Fig. 1.

The computation techniques, including docking, are used to find out the best confirmations of drug-protein bindings. This is done by scoring functions, and in turn, docking is completed. This process of docking is an acceptable approach in the lead optimization. In addition to this, computer aided drug design (CADD) and structure-based drug design are done on the basis of findings of molecular docking.

In the research work presented here, some new Indole substituted Aldehyde were designed, chemically synthesised, characterised and screened for anti-inflammatory activity using Egg Alubumin. The response exhibited by these synthesised compounds exhibited anti-inflammatory effects, and the same was compared using Indomethacin as a reference standard drug.

The ten (AG-1 to AG-10) new indole-substituted Aldehyde were synthesised successfully, and the compounds were molecular docking study for anti-inflammatory activity to meet the objective of the present work.

Using the IR, ^{1}H NMR, and ^{13}C NMR spectroscopy techniques, characterization was done. All the compounds were molecularly docked with reference to the anti-inflammatory target protein. This was done to identify the mechanistic target.

MATERIAL AND METHOD

CHEMISTRY

Step 1: synthesis of 2-(P-tolyl)isoindoline-1,3-dione)

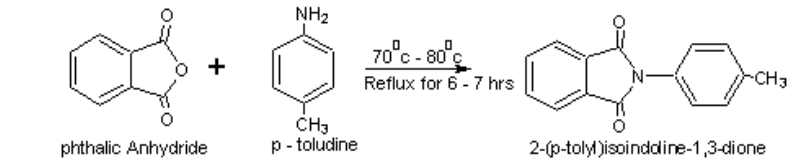
- Add 0.1M phthalic anhydride and 0.01M p-toludine in a round bottom flask containing 5ml of glacial acetic acid.
- Reflux the reaction mixture at a temperature 70-80^{o}c for 6-7hours.
- The completion of the reaction is monitored by TLCchloroform : methanol (9: 1).
- After completion of the reaction, the precipitate is cooled, recrystallized with ethanol and dried.
- The recrystallized dried product is 2-(p-tolyl)isoindoline-1,3-dione, the yield is calculated.

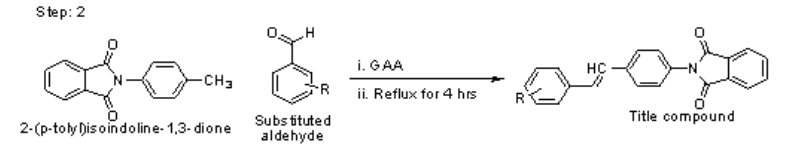
Step 2

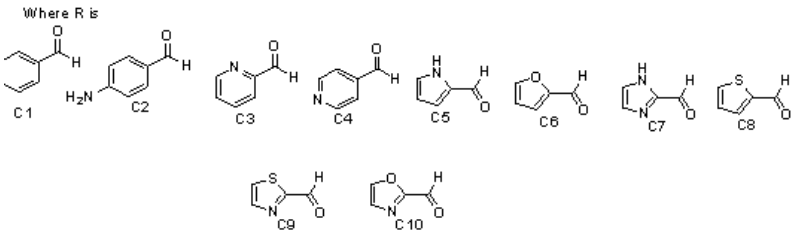
Synthesis of 2-{4-[(E)-2-substitiuted-ethenyl}phenyl}-1H-isoindole-1,3(2H)-dione

- The 2-(P-tolyl)isoindoline-1,3-dione and equimolar concentrations of various substituted aldehyde is added to the round bottom flask and add 5ml of glacial acetic acid.
- The reaction mixture was refluxed at a temperature 70-80^{o}c for 3-4 hours.
- The completion of the reaction is monitored by TLC chloroform:methanol (9 : 1).
- After completion of the reaction, the precipitate is cooled, recrystallized with ethanol and dried

Step 1.







Characterization of synthesized compounds

- **a. -2-(4-styrylphenyl)isoindoline-1,3-dione (C1)**

The 2-(p-tolyl)isoindoline-1,3-dione and equimolar concentrations of benzaldehyde is added. The completion of the reaction is monitored by TLC chloroform:methanol (9 : 1). **$C_{22}H_{15}NO_2$**; Brown colour solid; MP: 102 – 105^{O}C; Rf: 0.57; IR (KBr) cm^{-1}: 3124 (CH str alkene), 2574 (CH str aromatic), 1694 (C=O str ketone), 970 (aromatic ring), 720 (C-Clstr); ^{1}H NMR (500 MHz, DMSO-d6) δ 7.89 – 7.79 (m, 2H), 7.63 – 7.54 (m, 2H), 7.54 – 7.45 (m, 4H), 7.39 (dd, J = 7.5, 1.5 Hz, 2H), 7.31 – 7.22 (m, 3H), 7.21 – 7.09 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 167.80, 133.35, 132.62, 132.10, 131.19, 130.67, 129.72, 129.05; Mass: Actual: 325 m/z; found: 324 (M-1) m/z.

- **b. (E)-2-(4-(4-aminostyryl)phenyl)isoindoline-1,3-dione (C2)**

$C_{22}H_{16}N_2O_2$;Yellowcolour solid; MP: 110 – 113^{O}C; Rf: 0.57; IR (KBr) cm^{-1}: 2934 (CH str alkene), 2901 (CH str CH3), 2641 (CH str aromatic), 1684 (C=O str ketone), 850 (aromatic ring), 730 (C-Clstr); ^{1}H NMR (500 MHz, DMSO-d6) δ 9.92 (s, 2H), 7.89 – 7.79 (m, 2H), 7.63 – 7.53 (m, 2H), 7.48 (s, 4H), 7.18 (dd, J = 25.6, 11.3 Hz, 3H), 7.08 (d, J = 15.2 Hz, 1H), 6.57 (d, J = 7.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO) δ 167.64, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05, 23.93; Mass: Actual: 340 m/z; found: 340 m/z.

- **c. (E)-2-(4-(2-(pyridin-2-yl)vinyl)phenyl)isoindoline-1,3-dione (C3)**

$C_{21}H_{14}N_2O_2$; Brown colour solid; MP: 106 – 109^{O}C; Rf: 0.61; IR (KBr) cm^{-1}: 2971 (CH str alkene), 2815 (CH str CH_3), 2741 (CH str aromatic), 1499 (C=O str ketone), 934 (aromatic ring); ^{1}H NMR (500 MHz, DMSO-d6) δ 8.56 (dd, J = 7.5, 1.4 Hz, 1H), 7.93 – 7.83 (m, 2H), 7.70 – 7.51 (m, 4H), 7.46 – 7.32 (m, 5H), 7.32 – 7.23 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05, 54.04. Mass: Actual: 326 m/z; found: 326 (m/z).

- **d. (E)-2-(4-(2-(pyridin-4-yl)vinyl)phenyl)isoindoline-1,3-dione (C4)**

$C_{21}H_{14}N_2O_2$; Yellow colour solid; MP: 116 – 118^{O}C; Rf: 0.63; IR (KBr) cm^{-1}: 3171 (NH str amine), 2971 (CH str alkene), 2866 (CH str aromatic), 1688 (C=O str ketone), 910 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 8.56 (dd, J = 7.5, 1.4 Hz, 1H), 7.93 – 7.83 (m, 2H), 7.70 – 7.51 (m, 4H), 7.46 – 7.32 (m, 5H), 7.32 – 7.23 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05; Mass: Actual: 326 m/z; found: 326 m/z.

- **e. (E)-2-(4-(2-(1H-pyrrol-2-yl)vinyl)phenyl)isoindoline-1,3-dione (C5)**

$C_{20}H_{14}N\text{-}O_2$; Red colour solid; MP: 113 – 116^{O}C; Rf: 0.51; IR (KBr) cm^{-1}: (CH str alkene), (CH str aromatic), (C=O str ketone), (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 9.84 (s, 1H), 7.93 – 7.84 (m, 2H), 7.60 – 7.50 (m, 2H), 7.47 – 7.34 (m, 4H), 7.25 (d, J = 15.0 Hz, 1H), 7.11 (d, J = 15.0 Hz, 1H), 7.06 – 6.98 (m, 2H), 6.34 (dd, J = 7.5, 1.4 Hz, 1H), 6.14 (d, J = 7.4 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 155.36, 150.47, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05; Mass: Actual: 314 m/z; found: 314 m/z.

- **f. (E)-2-(4-(2-(furan-2-yl)vinyl)phenyl)isoindoline-1,3-dione (C6)**

$C_{20}H_{13}NO_3$; Red colour solid; MP: 121 – 123^{O}C; Rf: 0.59; IR (KBr) cm^{-1}: 3074 (CH str alkene), 2918 (CH str CH_3), 2417 (CH str aromatic), 1542 (C=O str ketone), 901 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 8.53 (d, *J* = 1.4 Hz, 1H), 7.91 – 7.81 (m, 2H), 7.63 – 7.52 (m, 4H), 7.47 (d, *J* = 7.5 Hz, 2H), 7.39 (dd, *J* = 18.3, 7.5 Hz, 3H), 7.25 (d, *J* = 15.2 Hz, 1H), 2.37 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 155.36, 150.47, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05, 56.30; Mass: Actual: 315 m/z; found: 315.

- **g. (E)-2-(4-(2-(1H-imidazol-2-yl)vinyl)phenyl)isoindoline-1,3-dione (C7)**

.$C_{19}H_{13}N_3O_2$; Orange colour solid; MP: 118 – 121^{O}C; Rf: 0.62; IR (KBr) cm^{-1}: 3050 (OH str), 2871 (CH str alkene), 2502 (CH str aromatic), 1650 (C=O str ketone), 900 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 7.63 – 7.52 (m, 2H), 8.96 – -14.00 (m, 2H), 7.44 (d, J = 7.5 Hz, 3H), 7.37 (dd, J = 15.6, 11.4 Hz, 1H), 7.78 – -14.00 (m, 1H), 7.18 (d, J = 15.0 Hz, 1H), 7.11 (d, J = 7.5 Hz, 1H), 7.03 (d, J = 7.5 Hz, 1H), 6.44 (s, 1H); ^{13}C NMR (101 MHz, DMSO) δ 167.64, 155.36, 150.47, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05; Mass: Actual: 315 m/z; found: 315 m/z.

- **h. (E)-2-(4-(2-(thiophen-2-yl)vinyl)phenyl)isoindoline-1,3-dione (C8)**

.$C_{20}H_{13}NO_2S$; Orange colour solid; MP: 114 – 117^{O}C; Rf: 0.64; IR (KBr) cm^{-1}: 3051 (OH str), 2948 (CH str alkene), 2348 (CH str aromatic), 1720 (C=O str ketone), 904 (aromatic ring), 741 (C-Br str); ^{1}H NMR (500 MHz, DMSO) δ 7.88 – 7.78 (m, 2H), 7.63 – 7.53 (m, 2H), 7.42 (dd, J = 24.7, 7.5 Hz, 4H), 7.34 – 7.25 (m, 3H), 7.19 (d, J = 15.2 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 174.26, 167.64, 155.36, 150.47, 133.35, 132.62, 132.10, 131.19, 131.19, 130.67, 129.72, 129.05, 56.30; Mass: Actual: 331 m/z; found: 331 m/z.

- **i.(E)-2-(4-(2-(thiazol-2-yl)vinyl)phenyl)isoindoline-1,3-dione (C9)**

$C_{19}H_{12}N_2O_2S$; Brown colour solid; MP: 112 – 115^{O}C; Rf: 0.65; IR (KBr) cm^{-1}:3040 (NH str amine), 2971 (CH str alkene), 2604 (CH str aromatic), 1702 (C=O str ketone), 854 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 7.88 – 7.78 (m, 2H), 7.63 – 7.53 (m, 2H), 7.48 – 7.37 (m, 7H), 7.29 (d, J = 15.2 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 170.28, 134.67, 132.94, 130.67, 128.33, 127.82, 125.48, 124.04, 121.49, 116.29; Mass: Actual: 332 m/z; found: 334 (M+2) m/z.

- **j. (E)-2-(4-(2-(oxazol-4-yl)vinyl)phenyl)isoindoline-1,3-dione (C10)**

$C_{19}H_{12}N_2O_3$; Brown colour solid; MP: 122 – 125^{O}C; Rf: 0.51; IR (KBr) cm^{-1}: 2956 (CH str alkene), 2748 (CH str aromatic), 1671 (C=O str ketone), 901 (aromatic ring); ^{1}H NMR (500 MHz, DMSO) δ 7.95 (d, J = 1.4 Hz, 1H), 7.88 – 7.78 (m, 2H), 7.76 (d, J = 1.4 Hz, 1H), 7.63 – 7.53 (m, 2H), 7.41 (dt, J = 7.5, 6.9 Hz, 5H), 7.14 (d, J = 15.2 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 134.67, 132.94, 130.67, 128.33, 127.82, 125.48, 124.04, 121.49, 116.29; Mass: Actual: 316 m/z; found: 316 m/z

CONCLUSION

The physicochemical and spectroscopic data confirmed the structural integrity of the newly synthesized compounds. The investigated molecules displayed a similar manner to protein binding to the active site of cyclooxygenase in molecular docking studies. The calculated docking energies indicated that its interaction with cyclooxygenase is favourable, but only to a limited extent. The molecular discreptor value was studied by molinsipration software and all the parameters were within a limit. All the synthesized compounds were screened for their *in vitro* anti-inflammatory activity activity. Compounds C2 and C5 are emerged to be the most active compounds against in tested enzyme and these compounds also posses a significant docking score for the studied enzyme. The study thus serves as an attempt to progress toward the discovery of novel lead molecule for the treatment of inflammation. In future the additional derivatives may be prepared and further extended in-depth investigations into *in-vivo* activity would be implemented to establish a SAR (Structural activity relationship) for rational study.

RESULTS AND DISCUSSION

Anti-inflammatory activity: At present, a study has been done with different concentrations of four synthesized compounds for anti-inflammatory activity. The anti-inflammatory study

was compared with a standard drug paracetamol. Results of in-vitro anti-inflammatory activity of the compounds were expressed as percentage values. The study was also carried out with the standard drug **indomethacin**. All the tested compounds displayed significant inhibition at a concentration range of 100–500 µg/mL. Among the tested compounds, the compound C2 which is substituted with 4-amino-benzene and the compound C5 substituted with pyrrol moiety displayed considerable anti-inflammatory activity compared to standard drug indomethacin. Primarily the inhibition of protein (albumin) denaturation was studied and was found the maximum in ethanolic solution of C2 with 98.55% at concentration of 500 µg/mL, and followed by the ethanolic solution of C5 with 97.55% at concentration of 500 µg/m

Table 1: Result for *in vitro* anti-inflammatory activity.

Percentage of albumin denaturation (%)											
Concentration (µg/ml)	**C1**	**C2**	**C3**	C4	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**Standard**
100	31.05	45.56	36.88	15.25	35.82	31.05	25.56	25.25	16.35	15.37	51.84
200	39.04	51.05	52.21	22.89	41.73	39.04	31.05	42.89	24.61	27.61	63.33
300	65.56	68.42	64.13	28.28	68.48	65.56	48.42	58.28	31.58	31.67	76.76
400	72.36	79.68	70.33	33.25	77.57	72.36	57.68	63.25	47.67	51.67	85.89
500	79.02	98.55	82.94	47.80	97.55	79.02	67.55	87.80	59.34	74.64	99.37

Molecular docking

The molecular docking studies for the designed compounds **(figure 2)** were carried out through PyRx molecular docking software to determine the free energy binding towards targeted enzymes. From the results it clearly shows that, all the compounds have promising interaction with targeted enzyme cyclooxygenase II. The interaction is mainly due to the presence of lipophilic factor of aromatic heterocyclic ring. From the docking results, compound C2(8.4 kcal/mol) shows highest binding affinity toward cyclooxygenase II enzyme compared to standard drug indomethacin Binding energy of studied compounds.

Ligand	Binding Affinity
C1	-8.3
C2	**-8.4**
C3	-8.3
C4	-8
C5	-7.8
C6	-8
C7	-7.9
C8	-7.7
C9	-7.5

C10	-7.8
Indomethacin	**-10.4**

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