International Journal of Research and Technology (IJRT)

International Open-Access, Peer-Reviewed, Refereed, Online Journal

ISSN (Print): 2321-7510 | ISSN (Online): 2321-7529

| An ISO 9001:2015 Certified Journal |

Rational Design and Synthesis of Diclofenac Analogues by Isosteric Replacement and Evaluation for Anti-inflammatory Activity

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Abstract

Frequently called rational drug design or, simply, rational design, drug design is the innovative process of discovering new drugs characterized by the understanding of a biological target. In this research study, we made some of the first series of Schiff base (4a 4m) and imidazo (2,1 b) 1,3,4 thiadiazole (6a d) derivatives of Diclefenac as shown in Scheme. Each of the new compounds produced by the synthesis was purified through recrystallisation or column chromatography on silica (230 400mesh). Spectral data, IR, 1H NMR, were used to determine the structures of final compounds. Acute toxicity tests of synthesized compounds did not show any toxicity irrespective of a maximum dose of 2000 mg/kg of the body weight. All the newly synthesized compounds (4a 4m and 6a 6d) were investigated to determine their anti-inflammatory activity using carrageenan induced rat paw edema method. The synthesized compounds were effective as the anti-inflammatory compound with 73.3 percent and 70.4 percent and 71.8 percent and 70.1 percent observed in records of compound 4d and 6b, and had the most activity when compared to the reference diclofenac. As against good to moderate inhibitory activity exhibited by compounds 4a, 4c, 4g and 4i. Finally, two products out of the series of synthesized derivatives compounds (4d and 6b) have shown the most promising outcomes and might also be mentioned as possible further exploitation candidates of creating the newer anti-inflammatory agents with improved efficiency and fewer safety issues, which requires further research.

***Keywords*:** Schiff base, Acute toxicity, Recrystallisation, Anti-inflammatory compound.

Introduction

Frequently called rational drug design or, simply, rational design, drug design is the innovative process of discovering new drugs characterized by the understanding of a biological target. What most often happens is that the drug is an organic small molecule and causes activation or blocking of the activity of a biomolecule (typically a protein), and the ultimate effect is a therapeutic benefit to the patient. The simplest definition of the concept of drug design could be simply the design of shape and charge opposite molecules to a biomolecular target they interact with and hence with which they will bind. Computer modelling of drugs is often though not always employed during the drug design.

There are a lot of failures during the clinical stages of drug development. Because of this, the first steps in the drug design and development process focus on picking candidate drugs whose physicochemical properties are more likely to cause fewer problems during development and lead to a drug that is approved and sold successfully.

Also, in vitro experiments that use computer methods are becoming more and more popular at the start of drug discovery to find better compounds in terms of ADME (absorption, distribution, metabolism, and excretion) and toxicological parameters.

Drug Design

- Ligand Design
- These are commonly small organic molecules, (biologics)
- Designed to activate or inhibit the enzyme activities
- Have complementary shape and charge to that of receptor sites
- Computer-aided drug design

Types of drug design

Ligand-based drug design - indirect drug design based on

•Pharmacophore

•Quantitative Structural Activity Relationship (QSAR)

Structure-based drug design – direct drug design based on

•Three-dimensional structure of biological target

Drug discovery

Phenotypic drug discovery

A common way to find new drugs is through phenotypic drug discovery, also known as forward pharmacology or classical pharmacology. The process of phenotypic screening is used on collections of synthetic small molecules, natural products, or extracts to find the material that works best as a medicine. The plan is to find out how drugs work in living things or in a lab. Then, in-vitro tests are used to find the target. It is useful and doesn't care about the target; phenotypic discovery technology creates first-pass leads to find new drug modes of action, pharmacologically active compounds and therapies.

Rationale of the study

Rational approach A systematic, step by step method in which ‘hard’ (quantitative quantitative) data obtained through observation or mathematical (statistical) analysis or modeling is used for making long term decisions.

First Generation Rational Drug Design

- In 1970s the medicinal chemists considered molecules as topological entities in 2 dimensions (2D) with associated chemical properties.
- QSAR concept became quite popular. It was implemented in computers and constituted first generation rational approach to drug design.

Second Generation Rational Drug Design

- The acceptance by medicinal chemists of molecular modeling was favoured by the fact that the QSAR was now supplemented by 3D visualization.
- The “lock and key” complementarily is actually supported by 3D model. Computer aided molecular design (CAMD) is expected to contribute to intelligent lead.

Evolutionary Drug Design

- Ancient times: Natural products with biological activities used as drugs.
- Chemical Era: Synthetic organic compounds
- Rationalizing design process: SAR & Computational Chemistry based Drugs
- Biochemical era: To elucidate biochemical pathways and macromolecular structures as target as well as drug

NSAIDs

- Although non-steroidal anti-inflammatory medicines (NSAIDs) have shown unintended gastrointestinal problems, they have proven useful in treating inflammation, discomfort, and fever. Early in the 1990s, the discovery of a second cyclooxygenase isoform (COX-2) created a novel scenario in which selective COX-2 inhibitors create a second generation of NSAIDs without the harmful side effects of gastrointestinal (GI) damage.

- There are now four selective COX-2 inhibitors available on the market, with many more having been reported since the mid-1990s (Fig.1). Celecoxib (1) was the first, followed by rofecoxib (2), etoricoxib (3), and valdecoxib (4). Second, selective COX-2 inhibitors have been investigated for a variety of potential therapeutic uses. Others, like Alzheimer's disease treatment, are undergoing clinical testing to determine their efficacy.

Aim of the present work was influenced by the following facts and hypothesis.

(1) Since the introduction of Diclofenac to the pharmaceutical market, the non-steroidal anti-inflammatory drugs have gained greater attention of medicinal chemists. NSAIDs are widely prescribed therapeutic agent for pain, fever and various inflammatory disorders.

(2) Conventional NSAIDs are non-selective inhibitors of cyclooxygenase (COX) enzymes, which catalyze the formation of prostaglandins (PGs).

(3) The above hypothesis led to the discovery many selective COX-2 inhibitors, which devoid of gastric side effects such as celecoxib, rofecoxib, etoricoxib, valdecoxib and parecoxib. These drugs were the leading drugs in the pharma market as per global pharmaceutical sales Report in 2003.

(4) Recently, some of the selective COX-2 inhibitor-based drugs are withdrawn from the market due to increased risk of cardiovascular side effects.

(5) Thus, there is a need to search for new selective COX-2 inhibitors with a mild effect on COX-1 inhibition, which could theoretically reduce the cardiovascular risk.

The two main aims of the thesis were: -

(I) To design and synthesis some Diclofenac Analogues by Isosteric Replacement.

(II) To study the anti-inflammatory effect of the newly synthesized compounds.

Methodology

All of the research chemicals used in the processes were acquired commercially from Lancaster Co. (Ward Hill, MA, USA) or Sigma Aldrich (St. Louis, Missouri, USA). The diclofenac used in this study was acquired from Elegant Drug Pvt, Ltd. in Hubballi. Sigma Aldrich Chemicals (St. Louis, Missouri, USA) is where the carrageenan was purchased. The

Bruker AVANCE II 400 (Bruker, Rheinstetten/Karlsruhe, Germany) was utilised to record the hydrogen 1 nuclear magnetic resonance (1 H NMR) using the proper solvent. The delta ppm (delta-pmm) scale is used to report these chemical changes in relation to TMS, an internal standard. The synthesised substances in the title were purified using column chromatography. Using a digital plethysmometer (Ugo Basile, Italy), an acute in vivo anti-inflammatory activity test was performed.

Results and Discussion

Preparation of methyl 2 (2 (2,6 dichlorophenylamino) phenyl) acetate) (2) The methyl ester of diclofenac was synthesized according to the older literature procedure. This was produced as a white solid that crystallized.

Synthesis Procedure

Preparation of 2 (2 (2,6 dichlorophenylamino) phenyl) acetohydrazide (3) Compound 2 (0.01 mol, 3.10 g) was reacted with hydrazine hydrate (0.02 mol, 1 g) refluxed in absolute ethanol (50 ml) at reflux conditions for 24 h (as estimated by thin layer chromatography [TLC]). Preparation of the 3 was done according to the reported literature. This mixture was condensed, cooled and emptied in ice cold water. Separation of white amorphous solid was thus carried out and filtered, dried and recrystallized using ethanol and water to give compound.

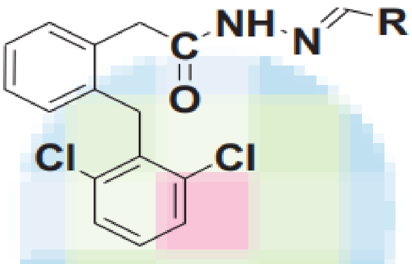
Typical synthesis of 2 (2, 6 dichlorobenzyl) phenyl) N, ethylideneaceto hydrozide derivatives (4a 4 m)6 To a constantly stirred solution of compound (3, 0.1 mol) in anhydrous toluene, the correspondingly substituted aldehyde (0.1 mol) was added and the mixture was refluxed under anhydrous conditions (4 h monitored by TLC). The mixture was then cooled back to the room temperature, filtered and the Schiff base is obtained. Absolute ethanol was used in recrystallizing the product according to the prior report. Compute and Spectral Data The physical data and spectral data of title compounds (4a 4m) are represented in Tables 1 and 2 respectively.

Table 01: Physico-chemical data of title compounds (4a-4m)

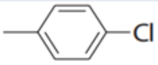
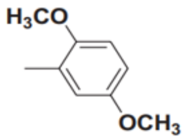
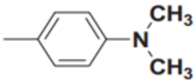
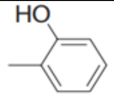
Compound	MP (C)	Yield (%)	Rf	Molecular Formula
4a	218-220	75.01	0.60	C_7H_5ClO
4b	224-226	73.23	0.52	$C_7H_4Cl_2O$
4c	236-238	68.41	0.65	$C_7H_4Cl_2O$
4d	248-250	71.62	0.48	$C_9H_{10}O_3$
4e	222-224	58.92	0.44	$C_7H_5NO_3$

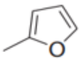
4f	234-236	64.85	0.38	$C_9H_{11}NO$
4g	240-242	70.21	0.46	$C_9H_{10}O_3$
4i	228-230	59.32	0.67	$C_{10}H_{12}O_2$
4j	238-240	72.43	0.52	C_7H_5ClO
4k	244-246	68.32	0.44	$C_7H_6O_2$
4l	230-232	74.53	0.56	$C_7H_6O_2$
4m	226-228	65.04	0.48	C_5H_6O

Table 2: Spectral data of title compounds 4a-4m



(4a-4m)

Compound	R	IR Spectra (KBr/cm)	H^1-NMR Spectra (DMSO, ppm)
4a		3337.71(N-H) 3070.43(C-H) 1657.24(C=O) 767.24(C-Cl)	11.79(s,1h-NH-CO), 11.60(s, 1H, N=CH),8,10(s,2H,ArH),8.00(s,1H, ArH), 7.41-7.37 (m,6H, ArH), 7.24-7.22(m,1H,ArH),7.08-7.06(d,1H,J=8.08Hz,ArH) 4.12(s,2H,-CH2-CO),3.70(s,1H, -NH)
4d		3334.41(N-H) 3050.43(C-H) 1357.18(C=O) 761.24(C-Cl)	10.72(s,1h-NH-CO), 11.60(s, 1H, N=CH),8,10(s,2H,ArH),8.00(s,1H, ArH), 7.45-7.27 (m,6H, ArH), 7.44-7.31(m,1H,ArH),7.18-7.21(d,1H,J=8.08Hz,ArH) 6.12(s,2H,-CH2-CO),3.50(s,1H, -NH)
4f		3332.71(N-H) 3055.43(C-H) 1621.24(C=O) 732.24(C-Cl)	11.12(s,1h-NH-CO), 11.32(s, 1H, N=CH),8,24(s,2H,ArH),8.00(s,1H, ArH), 7.35-7.37 (m,6H, ArH), 7.35-7.22(m,1H,ArH),7.08-7.08(d,1H,J=8.08Hz,ArH) 4.21(s,2H,-CH2-CO),3.21(s,1H, -NH)
4k		3337.71(N-H) 3070.43(C-H) 1657.24(C=O) 767.24(C-Cl)	11.79(s,1h-NH-CO), 11.60(s, 1H, N=CH),8,10(s,2H,ArH),8.00(s,1H, ArH), 7.41-7.37 (m,6H, ArH), 7.24-7.22(m,1H,ArH),7.08-

			7.06(d,1H,J=8.08Hz,ArH) 4.12(s,2H,-CH2-CO),3.70(s,1H, -NH)
4l		3337.71(N-H) 3070.43(C-H) 1657.24(C=O) 767.24(C-Cl)	11.79(s,1h-NH-CO), 11.60(s, 1H, N=CH),8,10(s,2H,ArH),8.00(s,1H, ArH), 7.41-7.37 (m,6H, ArH), 7.24-7.22(m,1H,ArH),7.08-7.06(d,1H,J=8.08Hz,ArH) 4.12(s,2H,-CH2-CO),3.70(s,1H, -NH)

Procedure for synthesis of 5-(2-(2,6-dichlorophenylamino

(benzyl) 1, 3, 4 -thiadiazol- 2 -amine (5) The equimolar combination of diclofenac (1, 0.051 mol), thiosemicarbazide (0.051 mol) and conc. H 2 SO 4 (0.0102 mol) were well mixed back to back considering a low temperature 10 15 o C and warmed at 90 o C and stirred continuously among 1 hour. Polyphosphoric acid(0.0408 mol) in cooled RT and water(50mL) were added. On performing refluxing, the reaction mixture was refluxed again until 7 h (using TLC method) at a temperature of 105-110 o C as reported in the previous literature. On cooling, the mixture was carefully basified over a pH range of 6.8 -7.3 by dropwise addition of NaOH solution whilst gently stirring.

Synthesis of 2,6 dichloro N (2 ((6 phenylimidazo[2,1 b] (thiadiazol 2 yl) methyl) phenyl) benzenamine derivatives (6a 6d) A mixture of equivalent aliquots of compound (5, 0.01mol) and 2, 4 or 5 substituted bromo phenyl ketone (0.01mol) in absolute ethanol(75mL) was refluxed 25 30h (At room temperature, a reaction mixture was cooled overnight. Unwanted solvent was eliminated under lowered pressure and the solid salt of hydrobromide was sieved, luxuriated using cold ethanol and dried.

Cold aqueous solution of Na2CO3 was used to neutralise the hydrobromide salts, to give the corresponding free bases (6a 6d). These compounds (6a-6d) were prepared according to the report of the previous literature.

In addition, the compounds were purified on a column chromatography using silica gel 200-400 mesh and eluted in ethyl acetate: hexane (2: 8) as mobile elaced. The functionality and the physicochemical data of title compounds (6a 6d) are illustrated in Table 3.

The filtrate was crystallized out in ethanol to obtain the crude product of 5 (2 (2,6 dichlorophenylamino) benzyl) 1, 3, 4 thiadiazol 2 amine.

Synthetic scheme*

*Reagents and conditions:

(a) Absolute methanol, conc. H2SO4 , reflux, 4 h;

(b) Hydrazine hydrate (99%), absolute ethanol, reflux, 24 h;

(c) Substituted aldehydes, anhydrous toluene, reflux, 4 h;

(d) Polyphosphoric acid, conc. H2SO4 , thiosemicarbazide, reflux, 3 h;

(e) α-bromo ketones, anhydrous ethanol, reflux, 25–30 h [Figure 3].

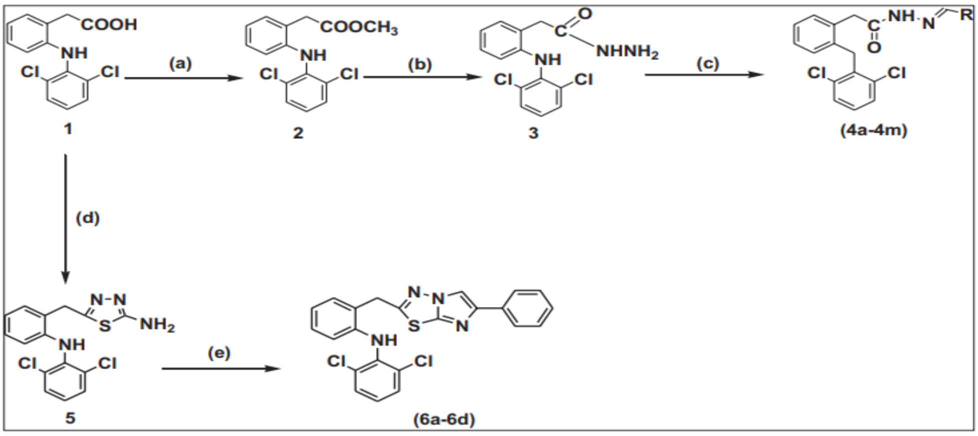
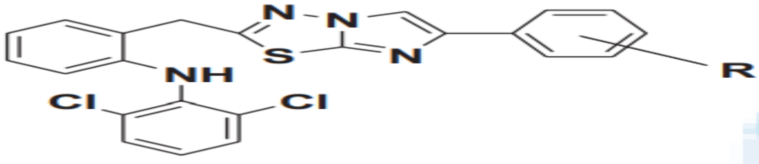


Figure 3: Synthetic scheme for Diclofenac analogs

Table 3: Physico-chemical data of title compounds (6a-6d)



(6a-6d)

Compound	**R**	**MP(C)**	**Yield (%)**	**Rf**	**Molecular formula**
6a	3,4-Dimethoxy	256-258	64.25	0.63	$C_{10}H_{12}O_3$
6b	2,5-Dimethoxy	245-247	48.32	0.58	C_8H_9NO
6c	3-Nitro	264-268	55.32	0.43	$C_{10}H_{12}O_3$
6d	3,4,5-Trimethoxy	238-240	48.21	0.58	$C_{11}H_{14}O_4$

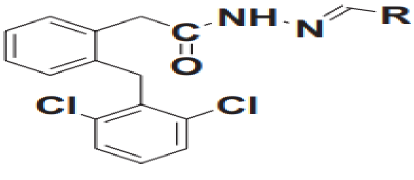
Pharmacological activities :-Animals' Acute toxicity tests and analgesic activity were done using Albino mice of both sexes weighing 20 25 g. In different pharmacological screenings, adult male albino rats with weight ranging between 150 and 230 g were utilised. Venkateshwara Enterprises, Bangalore, India, (245/CPCSEA) supplied animals that were kept single in polypropylene cages which were maintained at normal conditions of 12 h of light/dark cycle with constant temperature of 25+-20C and 35-60 relative humidity. The process of pharmacological assessment was carried out with the approval of Animal Ethical Committee of Oriental College of Pharmacy, (India). The animals were fed on a normal rat pellet diet, which was obtained from Hindustan Lever Ltd., Mumbai, India and water was allowed ad libitum.

Testing of chemical compounds: -Suspended test compounds in 0.5 percent aqueous solution of sodium carboxymethyl cellulose (sodium CMC), were orally administered to animals. The animals that were in the control group were offered the required amounts of the reference drugs and vehicle. The procedure of handling the animals involved in the experiment was the same across the animal groups.

Acute toxicity

Acute toxicity study was conducted according to the guideline of organization of economic co-operation and development revised no 423. Before the experimentation, the Institutional Animal Ethical Committee approved it. The albino mice having a weight of 25 gm to 30 gm of either sex was used as a subject of experimentation in the acute toxicity studies. The mice were starved with liberated 24 h of water and the test was conducted. On the day of the experiment, the animals were dosed with various compounds to varied groups with the increasing dose of 10, 20, 100, 200, 1000, and 2000 mg/kg bw orally. The animals were then monitored regularly (3 hours at a time) on general behavioral, neurological, autonomic profiles and subsequently 30 min after in the next 3 hours and finally 24 h or death.

Table 04: Anti-inflammatory activity data of title compounds (4a-4m) by carrageenan induced rat paw edema method



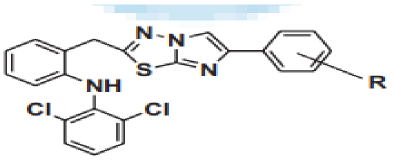
(4a-4m)

Compounds	R	Paw Volume		Percentage inhibition of oedema	
		2 h	4h	2 h	4 h

4a	4-Cholor benzaldehyde	2.49±0.12	2.06±0.04	64.3	62.4
4b	2,3-Dichloro benzaldehyde	0.88±0.03	1.05±0.31	50.1	48.3
4c	2,6-Dichloro benzaldehyde	1.25±0.05	1.64±0.05	64.1	64.2
4d	2,5-Dimethoxy benzaldehyde	0.68±0.02	0.85±0.03	74.2	70.3
4e	3-Nitro benzaldehyde	1.51±0.05	1.84±0.02	39.5	38.3
4f	4-Dimethylamino benzaldehyde	1.31±0.07	1.67±0.06	54.3	51.8
4g	3,4-Dimethoxy benzaldehyde	0.92±0.07	1.02±0.07	67.3	65.3
4h	2,5-Dimethyl-4-meyhoxy benzaldehyde	1.22±0.02	1.65±0.04	51.4	50.4
4i	3-Chloro benzaldehyde	0.85±0.02	1.50±0.13	66.7	64.3
4j	Phenyl Formate	1.22±0.01	1.65±0.03	49.5	45.3
4k	2-Hydroxy benzaldehyde	1.45±0.08	1.71±0.09	58.3	54.2
4l	Furan 2-carbaldehyde	1.19±0.04	1.65±0.02	38.9	62.5
4m	Benzaldehyde	0.86±0.08	1.12±0.14	65.3	61.3

Summarised values (mean±SEM) were derived in vivo carrageenan induced rat paw edema model by six animals per rat by using male albino rats, and significance was determined with the help of one-way ANOVA with Dunnet t test. When the P value was found, it regarded the difference in results significant.

Table 5: Anti-inflammatory activity of the title compounds (6a-6d) by carrageenan induced rat paw edema method



(6a-6d)

Compounds	**R**	**Paw Volume**		**Percentage inhibition of oedema**	
Control		**2 h**	**4h**	**2 h**	**4 h**
6a	3,4-Dimethoxy	2.49±0.13	2.06±0.04	66.43	62.4
6b	2,5-Dimethoxy	1.88±0.2	1.05±0.3	71.84	70.3
6c	3-Nitro	0.88±0.5	1.8±0.5	65.00	64.2
6d	3,4,5-trimethoxy	0.68±0.02	1.8±0.3	69.03	66.3
Diclofenac		0.86±0.09	1.12±0.14	65.5	61.4

Results and Discussion

The Following Parameters were observed:

S. No.	Parameters	Value
1	Melting Point	96–98°C
2	UV (λmax)	271.5
3	IR Spectra (KBr, νmax, cm-1)	3447 (N-H), 3082 (C-H), 1730 (C = O), 1238 (C-O-C), 783 (C-Cl).
4	NMR Spectra ($CDCl_3$, δ, ppm)	8.69 (s, 1H, N-H), 7.52–6.93 (m, 7H, Ar-H), 3.82 (s, 3H, OCH_3), 2.75 (s, 2H, CH_2)
5	Yield	71.78%.

Chemical synthesis:-New series of 2 (2 [2,6 dichlorobenzyl] phenyl) N 1 79-ethyl ideneaceto hydrazide derivatives (4a 4m) were synthesized by the general synthetically safe and very productive method as shown in general Scheme. Each of the newly synthesised compounds provided acceptable analysis based on the proposed structures that were determined based on physicochemical and spectral data, which have been compiled in Tables 1 and 2 respectively.

Diclofenac (2) was esterified in methyl ester of diclofenac. Occurring purity of the compound was determined by TLC, melting point. IR and H 1 NMR spectrographic data confirmed structure of compound 2. The typical peak of N H, C H stretching at 3447cm 1, C = O at 1729 cm 1, C O C at 1238 cm 1 and C Cl peak at 784 cm 1 were shown by IR spectra. This was indeed backed by its H1 NMR spectral data which had a 6 value of 3.72 (s, 2H, C H 2), 3.82 (s, 3H, C H 3), 6.39 (s, 1H, N H), 6.93-7.52 (m, 7H, Ar H) thereby confirming the structure. The proton of the respective synthesized compounds was supported by their chemical shifts, multiplicities, and couplings which were also used to identify the corresponding signals in the NMR spectra (1 H NMR). The synthesized compounds appeared prominent in their 1 H NMR spectra especially at 6.77 to 8.10 ppm with the aromatic protons appearing as multiples.

This methyl ester has been used in second methyl ester(2) and treated with hydrazine hydrate to involve carbohydrate for level (3). Melting point, TLC, confirmed the purity of the compound. IR and H1 NMR spectral data confirmed structure of compound 3. IR exhibited typical bands of N H frequency at 3325 cm 1, stretching frequency of C H at 3022 cm 1, C = O at 1637 cm 1, C Cl at 770cm 1. It was further buoyed by H1 NMR spectral data with values consisting of 3.64 (s, 2H, CH_2), 3.95 (s, 2H, NH_2), 6.50-7.13 (m, 7H, Ar H), 7.26 (s, 1H, N H), and 7.44 (s, 1H, CONH) which go to indicate the absence of contradiction in the structure.

When this carb hydrazide (3) was treated with various kinds of aromatic aldehydes in absolute alcohol, the title compounds (4a--4m) were obtained.

The derivation of 2 substituted 6- phenyl substituted imidazo (2,1 b) 1,3,4 thiadiazole derivatives (6 a-6 d) was done through condensation of an appropriate a bromo phenyl ketones (a d) with 5-(2-(2,6- dichlorophenylamnio) benzyl) 1,3,4 thiadiazol 2 amine (5) in refluxing ethanol in 16-20 hours and after addition of

This response is precursor-mediated through intermediate iminothiadiazole, spontaneously ring closed at room temperature below reflux to give the desired fused heterocycles in excellent yields. Purity of products and completion of the reaction was identified through chromatography analysis. Table 3 represents the physicochemical data of the compounds (6a-6d).

Synthetic Scheme

Step1:2-(2-[2,6-Dichlorobenzyl]phenyl)aceticacid

↓(a)$SOCl_2$,reflux

Intermediate A: 2-(2-[2,6-Dichlorobenzyl]phenyl)acetyl chloride

Step 2: Intermediate A+ Hydrazine hydrate ($NH_2NH_2 \cdot H_2O$)

↓ (b) EtOH, reflux

Intermediate B: 2-(2-[2,6-Dichlorobenzyl]phenyl)acetohydrazide

Step 3:Intermediate B + Substituted aldehydes (R–CHO, where R = various aromatic or alkyl groups)

↓ (c) EtOH, reflux

Final Products (4a–4m):2-(2-[2,6-Dichlorobenzyl]phenyl)-N'-ethylideneacetohydrazide derivatives

Notes:4a–4m represent derivatives formed using different substituted aldehydes in Step 3.

Conditions (a), (b), and (c) should be adjusted depending on your experimental section if you have specific temperatures, solvents, or times.

Compound 2:- Synthetic Scheme:

Preparation of Methyl Diclofenac (Compound 2)

Step: Esterification of Diclofenac

Starting Material: Diclofenac (2-[2-(2,6-dichloroanilino) phenyl] acetic acid)

Reaction:

Diclofenac (COOH) + Methanol → Methyl Diclofenac ($COOCH_3$)

|

[Conc. H_2SO_4, Reflux]

Reagents & Conditions:

- Methanol
- Catalytic concentrated H_2SO_4
- Reflux for several hours

Product: Methyl ester of diclofenac (Compound 2)

Characterization Data:

IR (cm^{-1}):

- N–H stretch: 3447
- Aromatic C–H: 3082
- Ester C=O: 1729
- C–O–C: 1238
- C–Cl: 784

1H NMR (δ, ppm):

- 3.72 (s, 2H, $–CH_2–$)
- 3.82 (s, 3H, $–OCH_3$)
- 6.39 (s, 1H, –NH)
- 6.93–7.52 (m, 7H, aromatic H)
- 6.77–8.10 (m, additional aromatic H)

Step 1: Synthesis of Carbohydrazide (3)

Compound 2 (Methyl ester) + **Hydrazine hydrate** → Reflux in ethanol → **Compound 3** (Carbohydrazide)

Characterized by IR (3325, 3022, 1637, 770 cm^{-1}) and ^1H NMR (δ 3.64, 3.95, 6.50–7.13, 7.26, 7.44)

Step 2: Formation of Schiff Bases (4a–4m)

Compound 3 +Aromaticaldehydes(various)→Reflux in absolute ethanol
→ **Compounds 4a–4m** (Schiff bases)

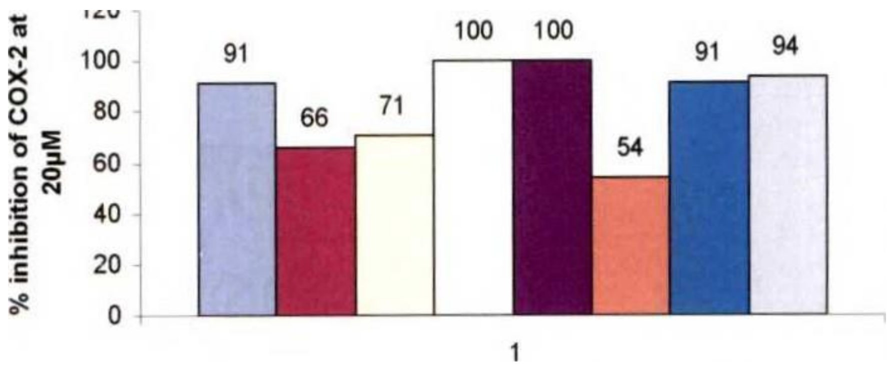
Step 3: Synthesis of Imidazo[2,1-b][1,3,4]thiadiazoles (6a–6d)

Compound5(5-(2-(2,6-dichlorophenylamino)benzyl)-1,3,4-thiadiazol-2-amine)
+α-Bromo phenyl ketones(a–d)
→Reflux in ethanol (16–20 h)
+ Add P_2O_5 → Further reflux (6–10 h)
→ Hydrobromide salts
+ Neutralization with sat. Na_2CO_3
→ **Compounds 6a–6d** (Imidazo-thiadiazole derivatives)

Reaction proceeds via iminothiadiazole intermediate followed by ring closure.

Pharmacological activity

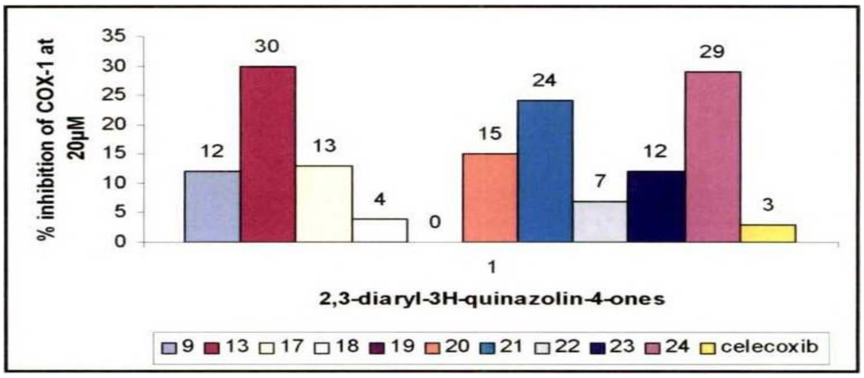
Acute toxicity Acute toxicity tests were performed in order to demonstrate how toxic the synthesized substances are and what the LD50 was. There was no toxicity (morbidity and mortality) in the synthesized compounds up to the dose level of 2000 mg/kg body weight. Therefore, the active dose of test compounds has arbitrarily been chosen in studying the acute anti inflammatory activity and that is 10 mg./kg bw.



Acute in vivo anti-inflammatory activity

In vivo anti-inflammatory experiments were carried out on compounds (4 a-4 m) and (6 a-6 d) through adopting carrageenan induced rat paw edema model by means of earlier reported Winter et al. model of nonspecific inflammation facilitated by diverse mediators.

Carrageenan has been adopted as a useful compound in investigating anti-inflammatory screening since edema of this kind is very sensitive to NSAIDs. In the second stage of inflammation, anti-inflammatory activities of test substances are picked up due to the preventive effect on the PG escalate.



This model is good at predicting the anti-inflammatory potency of the NSAIDs. This information on anti-inflammatory activity is tabulated in Table 4 and 5 and indicated moderate-to-good anti-inflammatory activity in the synthesized compounds. More specifically, compounds 4d (73.3% and 70.4%) was most active when compared to compounds 4a (64.4% and 62.1%), 4c (65.1% and 64.1%), 4g (67.2% and 65.1%), and 4i (66.7% and 64.2%) which also showed good to moderate inhibitory activity at the 2 nd and 4 th h, respectively.

Anti-inflammatory activity of these drugs was similar as that of the standard drug Diclofenac (65.5 at the 2 nd h and 61.4 at 4 th h). Among the series, the most promising activity on 2nd and the 4th h occurred in compound 6b (71.82% and 70.1) only in the case of compounds 6 (a d). These studies of the structure activity relationship of these compounds that were done over a few units of study showed that presence of electron releasing groups on the phenyl ring has tremendously increased the anti-inflammatory activity and compounds with electron withdrawing groups had moderate to poor anti-inflammatory activity.

Conclusion

In this research study, we made some of the first series of Schiff base (4a 4m) and imidazo (2,1 b) 1,3,4 thiadiazole (6a d) derivatives of Diclefenac as shown in Scheme. Each of the new compounds produced by the synthesis was purified through recrystallisation or column chromatography on silica (230 400mesh). Spectral data, IR, 1H NMR, were used to determine the structures of final compounds. Each of the newly prepared substances produced reasonable analysis of proposed structures that were validated in support of physicochemical and spectral data.Besides, the compounds are run under mass spectra to ascertain the final molecular weight (awaiting results). Finally, two products out of the series of synthesized derivatives compounds (4d and 6b) have shown the most promising outcomes and might also be mentioned as possible further exploitation candidates of creating the newer anti-inflammatory agents with improved efficiency and fewer safety issues, which requires further research.

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