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DESIGN, SYNTHESIS AND *IN-VITRO* STUDIES ON NOVEL CINNOLINE DERIVATIVES AS POTENTIAL ANTI-INFLAMMATORY AGENTS

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ABSTRACT

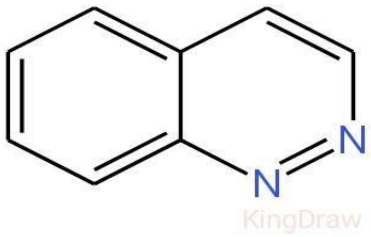
Cinnoline is a heterocyclic aromatic organic compound which is also known as 1,2 - diazanaphthalene or benzo-1,2-diazine. It's a bicyclic structure, featuring a benzene ring fused with a diazine ring (a six-membered ring with two nitrogen atoms). It is isomeric with other nitrogen-containing heterocycles like quinoline and phthalazine. Cinnoline derivatives exhibit wide-range of pharmacological activities including anti-microbial, anti-fungal, anti-malarial and anti-inflammatory properties. Pale yellow crystalline solid, Aromatic and planar in nature. The starting compound of cinnoline is Aniline and Sodium nitrite. Some of the cinnoline derivatives are 4 -phenyl cinnoline, Ethyl cinnoline, Methyl cinnoline and Cinoxacin.

INTRODUCTION

Cinnoline is a fused, aromatic, heterocyclic compound characterized by 2- nitrogen atom system within the benzene ring which means 6 -membered benzene ring is fused to another 6-membered ring containing 2 nitrogen atoms. Cinnoline is also known as “1,2-diazanaphthalene” or “benzopyridazine”. Cinnoline is isomeric to phthalazine and isosteric to quinolone or

isoquinoline. Cinnoline derivatives exhibit wide-range of pharmacological activities including anti- microbial, anti-fungal, anti-malarial and anti-inflammatory properties. Some of the mixtures in the cinnoline class are used as agrochemicals and have antithrombic and anti-warmth effects, as well as sedative and narcotic properties. Furthermore, Anti-proliferative effects of cinnoline derivatives were found in 1997. Cinnoline was first introduced by "Von Richter" in 1883. He synthesized it by cyclization of an alkyne and named the resulting heterocyclic framework as "Cinnoline". In 1957, Jacobs in his review on cinnoline and related compounds pointed out that this ring system was the least known of the condensed, bicyclic aromatic heterocycles containing two nitrogen atoms. The starting compound of cinnoline is Aniline and Sodium nitrite. Some of the cinnoline derivatives are 4-phenyl cinnoline, Ethyl cinnoline, Methyl cinnoline and Cinoxacin.

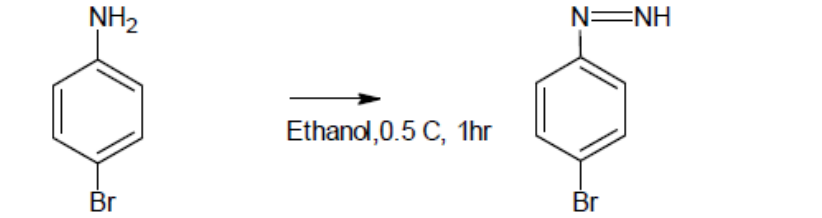
CINNOLINE



- Molecular formula : $C_8H_6N_2$
- Molecular weight : 130 gm
- Melting point : 38^oC
- Boiling point : 0.35114^o[151.4^+ 13.0^OC at 760mmHg]
- Solubility : Soluble in water, Ethanol, Methanol, DMSO, DMF etc
- Appearance : Pale yellow solid, Geranium odour.

SCHEME AND MATERIALS METHOD

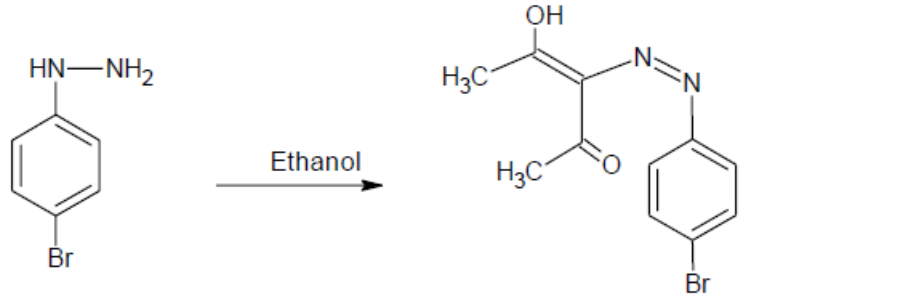
STEP-1: Synthesis of 4-bromophenyl diazene



4-bromoaniline *4-bromo phenyldiazene*

- 4-bromophenyl diazene is prepare by dissolving sodium nitrite(0.1 mole) in 26ml of water.
- Added drop wise to a solution of 4-bromoaniline (10ml of in 1N HCL) at 0^0c under stirring for about 30mins.
- Reflux for 1hr. Then orange coloured precipitate is collect by filtration and dried.

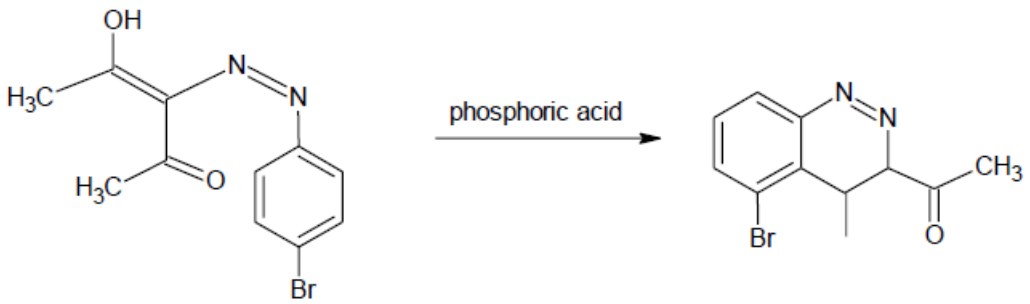
STEP-2: Synthesis of (Z)-4-hydroxy-3-{(Z)-phenyldiazenyl}pent-3-en-2-one



4-bromophenyldiazene *(Z)-4-hydroxy-3-(Z-phenyldiazenyl)pent-3-en-2-one*

- To a solution of compound (0.2mole) in ethanol, pentane-2,4-dione(0.2 mole) is added.
- The mixture is refluxed for 5hours.
- The solvent is evaporated and the solid obtained is recrystalised from petroleum ether.

STEP-3: Synthesis of 1-[4-methyl-3,4-dihydrocinnoline-3-yl]ethanone



(Z)-4-hydroxy-3-(Z-phenyldiazenyl)pent-3-en-2-one *1-(4-methyl-3,4-dihydrocinnoline-3-yl)ethanone*

- Compound 2(0.1mole) in ethanol(10ml),20ml of polyphosphoric acid is added

- The mixture is maintained under reflux for 6hours.
- After cooling, the mixture is poured on ice and the solid formed is collected by filtration, washed with cold water and recrystalised from ethanol.

CHEMICALS

Bromoaniline, Sodium nitrite,4-bromo phenyldiazene,(Z)-4-hydroxy-3-(Z-phenyl diazenyl)pent-3-en-2-one,1-(4-methyl-3,4-dihydrocinnolin-3-yl)ethanone,1-(4-methyl-3,4-dihydrocinnoline-3-yl)butan-1-one.

APPARATUS

Round bottom flask Reflex condenser Measuring cylinder Beakers
Funnel Petri plate Glass rods Water bath Weghing balance Tripod stand.

PHYSICAL CHARACTERIZATION

Molecular formula : $C_8H_6N_2$
Molecular weight : 130gm
Melting point : 38^0c
Boiling point : 0.35114^0[151.4+/-13.0^0c at 760mmHg]
Solubility : Soluble in Water, Ethanol, Methanol, DMSO, DMF etc
Apperance : Pale yellow solid, Geranium odour.

BIOLOGICAL ACTIVITY

Anti-inflammatory activity

Inflammation

- Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as injury or infection.
- It is a normal defense mechanism that aims to remove harmful stimuli, initiate tissue repair and defend against pathogens.
- Inflammation lead to chronic disease, which involves in the activation of immune cells and the release of chemical mediators.
- Signs of inflammation include,

✓ Redness (Rubor)
✓ Pain (Dolor)
✓ Heat (Calor)

- ✓ Swelling (Tumor)
- ✓ Loss of function (Functio laesa)

Anti-inflammatory

- Anti-inflammatory agent are drug or substance that reduce the inflammation in the body.
- It blocks certain substances in the body that causes inflammation such as prostaglandin and cytokines. These agents are used in a wide range of pathologic conditions including,

- ✓ Rheumatoid arthritis
- ✓ Osteoarthritis
- ✓ Migraine
- ✓ Gout

- Non-Steroidal anti-inflammatory drugs (NSAIDs) such as Aspirin, Ibuprofen, Mefenamic acid, Diclofenac are widely used anti-inflammatory agents.

Importance of Anti-Inflammatory Activity

Anti-inflammatory activity refers to the ability of a substance (natural or synthetic) to reduce inflammation, which is the body's response to injury, infection, or harmful stimuli. While inflammation is a protective mechanism, excessive or chronic inflammation can lead to many diseases. Therefore, controlling it is crucial.

1) Management of Pain and Swelling

- Inflammation often causes **pain, redness, heat, and swelling**.
- Anti-inflammatory agents relieve these symptoms, improving comfort and mobility.
- Commonly used in conditions like **arthritis**, **injuries**, and **post-surgery recovery**.

2) Treatment of Chronic Inflammatory Diseases

- Many chronic diseases involve **persistent low-level inflammation**, such as:
  - **Rheumatoid arthritis**
  - **Inflammatory bowel disease (IBD)**
  - **Psoriasis**
  - **Asthma**
- Anti-inflammatory drugs reduce tissue damage and progression of these diseases.

3) Prevention of Inflammation-Linked Disorders

- Long-term inflammation is linked to diseases like:
  - **Cardiovascular diseases**
  - **Cancer**
  - **Diabetes**
  - **Neurodegenerative disorders** (e.g., Alzheimer's)
- Anti-inflammatory strategies can potentially prevent or delay these conditions.

4) Essential in Drug Development

- Anti-inflammatory activity is a **key pharmacological property** screened during the development of new drugs.
- Many natural compounds are evaluated for this activity in **pharmacological research.**

5) Improving Quality of Life

- Reducing inflammation improves **mobility, energy levels, appetite**, and overall well-being.
- It allows patients to lead a more **functional and pain-free life**.

Types of Anti-inflammatory activity

Anti-inflammatory activity can be classified based on source of agent and phase of inflammation.

1) Phase of Inflammation

a. *Acute Anti-Inflammatory Activity*

- Targets immediate, short-term inflammation (e.g., injury, infection).
- Acts quickly to reduce swelling, redness, and pain.
- **Example**: Cold compress, NSAIDs

b. Chronic Anti-Inflammatory Activity

- Long-term control of inflammation associated with chronic diseases.
- Involves immune modulation and prevention of tissue damage.
- **Example**: Corticosteroids, DMARDs (like methotrexate).

2) Based on Source of Anti-Inflammatory Agents

a. Synthetic Agents

- Chemically designed and manufactured drugs.

- More potent but may have side effects.
- **Examples**: Naproxen, Celecoxib

b. Natural Agents

- Derived from plants, herbs, or food sources.
- Generally safer with mild effects.
- **Examples**: Curcumin (turmeric), Resveratrol (grapes), Omega-3 fatty acids

Sources of Dietary Anti-inflammatory

1) **Fruits and Vegetables:** Citrus fruits, Berries, leafy vegetables and beets**.**
2) **Nuts and Seeds:** Walnuts, Almonds, Flaxseeds and Chia seeds.
3) **Fatty fish:** Salmon, tuna and sardines.
4) **Beverages:** Green tea, Black tea and Coffee.

***IN-VITRO* Anti-inflammatory activity**

In-vitro anti-inflammatory activity refers to the testing of substances (such as plant extracts, compounds, or drugs) outside a living organism, typically in a laboratory setting using cell lines, enzymes, or biochemical assays to evaluate their potential to reduce or inhibit inflammation.

Assays for Anti-Inflammatory Activity

1. **Protein Denaturation Assay**

- **Principle**: Inflammatory conditions denature proteins; anti-inflammatory agents prevent this.
- **Common Protein Used**: Bovine Serum Albumin (BSA) or Egg Albumin
- **Result**: % Inhibition of denaturation = Anti-inflammatory effect
- **Example**: Used to screen plant extracts for aspirin-like activity

2. **Membrane Stabilization Assay (HRBC Method)**

- **Principle**: Stabilization of the red blood cell (RBC) membrane is an indicator of anti-inflammatory activity (similar to lysosomal membrane stabilization in vivo).
- **Model**: Human red blood cells exposed to hypotonic solution.
- **Result**: Less hemolysis = greater membrane stability = anti-inflammatory activity

3. **Cyclooxygenase (COX) Inhibition Assay**

- **Principle**: COX enzymes (COX-1 and COX-2) convert arachidonic acid to

prostaglandins.

- **Method**: Test compounds are incubated with COX enzymes and arachidonic acid; inhibition of prostaglandin synthesis is measured.
- **Application**: Screening for NSAID-like activity

4. Lipoxygenase (LOX) Inhibition Assay

- **Principle**: LOX enzymes produce leukotrienes, which are inflammatory mediators.
- **Method**: Test compound's ability to inhibit LOX-catalyzed reaction is observed.
- **Example**: Nordihydroguaiaretic acid (NDGA) used as positive control.

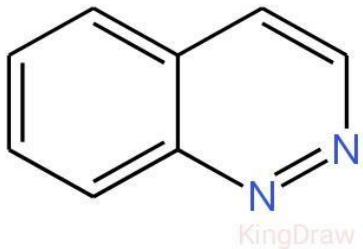
5. Cell-Based Assays Using Macrophages

- **Principle**: Macrophages stimulated with **LPS (lipopolysaccharide)** produce inflammatory markers like NO, TNF-α, IL-6.

- **Evaluation**
  - NO production (via Griess reagent)
  - ELISA for cytokines (TNF-α, IL-1β, IL-6)
  - mRNA expression (via RT-PCR)
  - NF-κB pathway activation (via western blot or reporter assay)

SPECTRAL ANALYSIS

CINNOLINE



IUPAC NAME

HNMR Interpretation

HNMR Spectral data Absorption position (in PPM)	
7.47 – 7.53	m,6H,CH
6.80 – 6.89	d,6H,CH
5.41	s,3H,CH_2
4.3	d,3H,NH

RESULTS AND DISCUSSION

Synthesis

The present study report the synthesis of cinnoline derivatives nucleophilic substitution of 4- bromoaniline with sodium nitrite was carried out stepwise at different temperature. The first step involves substitution of 4-bromo phenyl diazene and the next by polyphosphoric acid. The final cinnoline derivative in the synthesized compound 3 was replaced by benzamide. Since the report regarding this compound suggest a cinnoline possess a good bioactive moiety.

Physical Characterization

Melting points of the synthesized compound was taken in open capillary tubes and was uncorrected and were found to be in the range 112-120℃.

TLC was performed using precoated silica gel plates of 0.25mm thickness. Eluents used were Acetic acid: n-butanol: Water (9:3:2) spots were visualized in U.V. light.

At room temperature solubility of newly synthesized compounds were determined by various organic solvents and it was found that all compounds were freely soluble in Methanol, Ethanol, DMSO & DMF.

CONCLUSION

In the present study certain cinnoline derivatives were synthesized and characterized by1HNMR. The synthesized compound show characteristic absorption peaks –in 1HNMR spectra. Expected molecules in (m+) fragments were observed for the entire compounds in mass spectra.

The synthesized compound was subjected to biological evaluation. The compound were evaluated for anti-inflammatory studies revealed that the substitution of different aromatic amines to parent cinnoline nucleus show the moderate activity.

REFERENCES

1. Mathew George, Lincy Joseph & Sony S. “Synthesis and Biological screening of Novel Cinnoline derivatives for Analgesic activity”. World journal of Pharmaceutical Research, 31 August 2018; 7(16): 1704-1711.
2. Nidhi Gautham, Chourasia, O.P, “Synthesis, Antimicrobial and Insecticidal activity of some new Cinnoline derivatives”. Indian journal of Chemistry, June 2010; 49-B(06).

3. Balamurgan.K, Prashanthi Evangeline.M, and Prem kumar.P "Cinnoline Derivatives as Anti bacterial agent and Antimycobacterial agent: Synthesis, Microbial evaluation and molecular docking studies". International journal of Research in Pharmaceutical sciences, 9 October; 11[4]: 6675-6684.
4. Rosa M. Claramunt, Jose Elguero, and Ibon Alkorta, "1H-Benzo[de]Cinnolines:an interesting class of Heterocycles". A Platinum, open access journal for organic chemistry, April 5, 2022; Part i, 165-185.
5. Aswathy J., Dr. Prasobh, A. Sheejarekha, A. S. Athira, & Kavya, "A Concise review on cinnoline scaffold." World journal of Pharmaceutical Research, 10 August 2020; 9(9): 243-249.
6. S. Hurmath Unnisa, Nilofer Gayam Krishna Reddy "Synthesis ana in vitro Anti Microbial Evaluation including Anti-Malarial Activity of Pyrazole Based Novel Cinnoline Derivatives". Journal of Applied Pharmaceutical science, 27 November 2015; 5(11): 121-126.
7. Vishal Bharat Babar and Sudarshan Narayan Nagarale "Synthesis and Characterisation of some Cinnoline derivatives for its biological iterest." International Journal of Chemistry Studies, July 6, 2018; 6[4]: 3387-3395.
8. Barbar V, Dhakane.V and Chitale.V, "A Reivew on Cinnoline synthesive, its derivatives and their biological activity." International journal of Biology, Pharmacy and Allied sciences, 01 July, 2021; 10[7]: 2430-2440.
9. Vineeth Kumar Singh, Mishbahul Hassan, Vikas Saxena, Vinod Kumar Singh, Piyush Yadhav, Om Veersingh, Mukesh Kumar Bhardwaj, and Varshadeva. "A Comphrehensive review on cinnoline derivative." Journal of Pharmaceutical negative result, special issue 8, 2022; volume 13.
10. Jyoti Chaudhary, Khushbu Patel and, Dr. C N. Patel. "Synthesis and biological screening of some cinnoline derivatives". International journal universial pharmacy and biosciences, May-June 2014.
11. Pankaj Shankhadhar, Vikas Saxena, "Synthesis and Evaluation of Antihelmentic activity of some substituded Cinnolothiophen derivatives", World journal of Pharmaceutical Research,04 July, 2016; 5(8): 737-743
12. M.Prashanti Evangelin, T. Sasigold, Y.Elisha, G.Radhika, G.Krishnavamshi, and K.Aarthi, "A Concise Review on Cinnolines." International journal of Mental Healthsystems, 03April, 2020; 10(4): 879-901.
13. Mulgani. P. Evangelin, P. Premkumar and K.Balamurgan. "Design, synthesis and

molecular docking studies on novel Cinnoline derivatives as potential Anti-tuberculosis agent." International journal of Pharmaceutical sciences and research, 01 April 2020; 11(4).

14. Mulgani. P. Evangelin, P. Premkumar and K. Balamurgan. "Novice Cinnoline derivatives as Anti bacterial and Anti micobacterial agent: Synthesis, microbial Evaluation and molecular docking studies." Journal of chemical and Pharmaceutical research, 16 August 2023; 15(7): 01-11.
15. Balamurgan. K, Prashanthi Evangeline. M, and Prem kumar., "Synthesis and Anti microbial activity evaluation of 2,2,2-trifluoro-1-[6-Substituted-4-[thiophen-2-yl]Cinnolin-3-yl]ethanone Derivatives" Bulletin of Environment, Pharmacology and life sciences, April 2, 2022; [1]: 511-519.