*Original Article*

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# SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL 3,5-DISUBSTITUTED 1,2-ISOXAZOLINYL DERIVATIVES OF NAPTHALENE-1-AMINE

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**Abstract**

Naphthalene-1-amine known as 1-amino naphthalene is an aromatic amine possesses selective serotonin reuptake inhibitor and antimicrobiological activity. New isoxazoline derivative was synthesized from α-napthylalamine to get better biological activities like anti cancer, antibiotic, antifungal, antiviral, anti-inflammatory, antidepressant, anthelmintic. The synthesized novel heterocyclic derivative i,e isoxazoline compounds or derivatives were screened for their antimicrobial and anticancer activity and compared with standard drugs.

**Keywords:** Heterocyclic derivatives, Isoxazoline, Chalcone, Antifungal, Anticancer, MTT Assay.

## Introduction

Synthesis of α -napthylamine on Acetylation reaction gives substituted aromatic ketone . The base catalyzed involves condensation of substituted aromatic ketones and substituted aldehydes to give α,β -unsaturated ketones (Chalcones), which on cyclization with hydroxylamine hydrochloride in alkaline medium gave corresponding Isoxazoline derivatives[7]. Literature review reveals that isoxazoline derivatives exhibit pharma - cological activities such as Anti-inflammatory, Antitubercular, Antibacterial, Antifungal, Antiviral, Analgesic, Antitumour, Anticoagulant, Antidepressant, Antipyschotic etc. Based on above facts it is worthwhile to prepare Isoxazolinyl derivatives of Napthalene-1-Amine[1-9] (α -Napthylamine).

**Experimental procedure synthesis**

**Synthesis of N – (naphthalene – 1- yl) acetamide**

To a solution of naphthalen -1 – amine[1] (0.01 mole) in chloroform (dry, 100ml), acetyl chloride (0.02 mole) is added drop wise at 0-5$^{o}$c with constant stirring. The reaction mixture was stirred for 2hrs by magnetic stirrer. The excess solvent was distilled off and the separated mass was poured into ice water and recrystallized from methanol.

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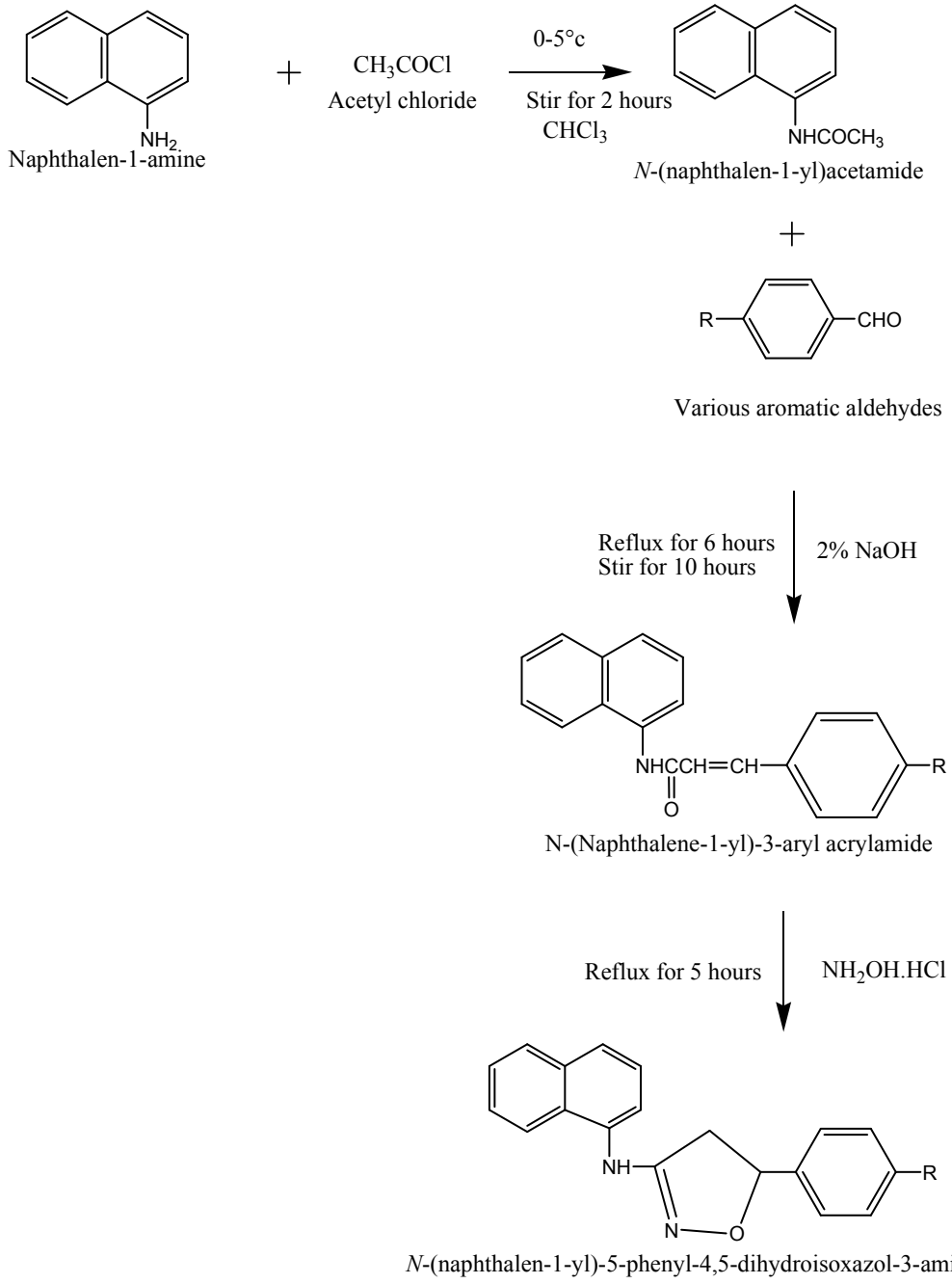
**Scheme 1:**




**Synthesis of N–(naphthalene – 1- yl) -3- aryl acrylamide derivatives ($R_1$-$R_{10}$)**

To a mixture of N- (naphthalene-1-yl) acetamide (0.01 mole) in methanol (50 ml), appropriate aromatic aldehyde[1-9]. (0.01 mole) are added in the presence of 2% NaOH solution (5ml). The reaction mixture is stirred for 10 hrs at room temperature and then refluxed for 6hrs. The excess solvent was distilled off and poured into ice water. The resulting solid thus separated, is filtered, washed with water and recrystallized from ethanol.

**Synthesis of N – (naphthalene – 1- yl) – 5- phenyl - 4, 5 - dihydroisoxazol- 3- amine derivatives ($S_1$ – $S_{10}$)**

To a mixture of N – (naphthalene -1-yl) -3- aryl acryl amide derivative (0.01mole) in absolute ethanol (50, dry), hydroxylamine hydro chloride (0.01 mole) and solid NaOH (0.4g) were added. The reaction mixture was refluxed for 5 hrs and poured in to ice water. The solid thus separated was filtered, washed with water and recrystallized from acetone. The recrystallized products purity was checked by TLC by using solvent system Ethanol, Dichloromethane (1:2) ratio.

**Experimental**

**Physicochemical Parameters**

Physical characterization of synthesized compounds was characterized. Melting points were measured in open-end capillary tube method by VEEGO digital electrically heating melting point apparatus. The purity of new compounds and reaction completion was checked by TLC by measuring $R_F$ value of products. Solubility was determined by using various organic solvents like DMSO, Chloroform and Ethanol. Physico-chemical properties are recorded in Table no. 1.

**Table No. 1: Physico-Chemical Parameters**

| Compound code | Solubility | Color | Percentage yield | Melting point | Molecular weight | Molecular formula | Rf value |
|---|---|---|---|---|---|---|---|
| $S_1$ | Ethanol, Chloroform, DMSO | Pale voilet | 85 | 90-97 | 304.34 | $C_{19}H_{16}N_2O_2$ | 0.95 |
| $S_2$ | Ethanol, Chloroform, DMSO | Reddish brown | 82 | 110-113 | 322.78 | $C_{19}H_{15}ClN_2O$ | 0.83 |
| $S_3$ | Ethanol, Chloroform, DMSO | Grey | 80 | 128-130 | 288.34 | $C_{19}H_{16}N_2O$ | 0.78 |
| $S_4$ | Ethanol, Chloroform, DMSO | Grey | 80 | 105-108 | 348.39 | $C_{21}H_{20}N_2O_3$ | 0.89 |
| $S_5$ | Ethanol, Chloroform, DMSO | Violet | 73 | 128-130 | 304.34 | $C_{19}H_{16}N_2O_2$ | 0.97 |
| $S_6$ | Ethanol, Chloroform, DMSO | Grey | 78 | 94-95 | 333.34 | $C_{19}H_{15}N_3O_3$ | 0.84 |
| $S_7$ | Ethanol, Chloroform, DMSO | Pale pink | 93 | 115-120 | 334.51 | $C_{20}H_{19}N_2O_3$ | 0.94 |
| $S_8$ | Ethanol, Chloroform, DMSO | Pink | 75 | 107-110 | 322.78 | $C_{19}H_{15}ClN_2O$ | 0.86 |
| $S_9$ | Ethanol,Chloroform, DMSO | Yellow | 83 | 117-120 | 333.34 | $C_{19}H_{15}N_3O_3$ | 0.75 |
| $S_{10}$ | Ethanol,Chloroform, DMSO | Purple | 84 | 107-110 | 348.43 | $C_{21}H_{20}N_2O_3$ | 0.93 |

**Biological Evaluation**

**Anti-bacterial activity**

Antibacterial activity of the synthesized compound was determined using Serial dilution method. Nutrient broth was used as culture media for the study. Various gram positive and gram negative bacteria were used. Standard antibiotic as Ciprofloxacin was used for comparision of activity. Nutrient broth solution was sterilized by autoclaving at 15lbs pressure for 20 min. Inoculation of bacterial strains were made in above media and incubated at 37°c for 24 hrs. Uninoculated sterile media was used as blank. Test compound was dissolved in DMSO and dilution was made to get desired concentration of 100µg/ml, 50µg/ml, 25µg/ml and 12.5µg/ml. The test tubes were visualized under bright light. The test tube with least concentration of compound showing no bacterial growth was taken as minimum inhibitory concentration (MIC) of the compound.

**Anti-fungal activity**

Asperigillus niger was used for the Serial dilution study. Sabouraud dextrose broth cultured with fungal strain was taken in series of test tubes. Test compound was dissolved in DMSO of two-fold decreasing concentrations, incubated at 37° c for 48 hrs. Uninoculated sterile media was used as blank. Ketaconazole was used as standard. The test tubes were under bright light. Test tube with least concentration of compound showing no fungal growth was taken as (MIC) of the compound.

**Evaluation of anti-cancer activity**

Microculture tetrazolium assay (MTT) was used for evaluating anticancer activity. All the synthesized compounds were evaluated for cytotoxicity using MTT assay. It is a standard colorometric assay, which measures changes in colour for the determination of viable cells. Assay is dependent on the activity of mitochondrial dehydrogenase enzymes that reduce yellow 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide(MTT) to a blue colour formazoin product. The activity of the enzyme is directly proportional to cell viability. Deoxorubicin was used as standard.

$$\text{Cell viability (\%)} = \frac{\text{Mean od}*100}{\text{Control of o.d.}}$$

**Table No. 2: Antimicrobial and Anticancer activity of synthesized compounds**

| Compound (100 µg/ml – 12.5µg/ml) | Minimum inhibitor concentration(µg/ml) | | | | $IC_{50}$ (mg/ml) |
|---|---|---|---|---|---|
| | Antibacterial activity | | | Antifungal activity | Anticancer activity |
| | E.coli | S.aureus | B.subtilis | A.niger | HEP2 Cells |
| **$S_1$** | 50 | 25 | 50 | 25 | 0.056 |
| **$S_2$** | 100 | 50 | 25 | 50 | 0,127 |
| **$S_3$** | 25 | 100 | 50 | 50 | 0.059 |
| **$S_4$** | 50 | 50 | 50 | 25 | 0.133 |
| **$S_5$** | 50 | 25 | 100 | 100 | 0.127 |
| **$S_6$** | 25 | 100 | 25 | 25 | 0.129 |
| **$S_7$** | 100 | 50 | 50 | 100 | 0.111 |
| **$S_8$** | 25 | 25 | 25 | 25 | 0.131 |
| **$S_9$** | 50 | 100 | 50 | 100 | 0.065 |
| **$S_{10}$** | 100 | 50 | 100 | 50 | 0.131 |
| **Standard** | 12.5 | 12.5 | 12.5 | 12.5 | 0.002 |

## Result and Discussion

All the synthesized compounds were characterized by TLC, Melting point and Solubility. The compounds were evaluated for their antimicrobial activity i,e antibacterial and antifungal,and anticancer activity. Minimum inhibitory concentration value of the compounds ($S_1$, $S_2$, $S_3$, $S_5$, $S_6$, $S_8$) against the bacterial, fungal strain showed moderate inhibition at 25µg/ml concentration. The bacterial screening indicated that the test compounds were found to be less active, when compared to that of standard drug Ciprofloxacin. The fungal screening indicated that the test compounds, were found to be moderately active, when compared to that of standard drug Ketaconazole. Anticancer activity evaluated by Micro culture tetrazolium assay (MTT) ($S_1$, $S_3$, $S_7$, $S_9$) In HEP2 cell lines, were found to have moderate activity compared to standard.

## Conclusion

The synthesized compounds subjected to antimicrobial and anticancer activity showed moderate activity when compared with standard drug.

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