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SYNTHESIS AND ANALGESIC ACTIVITY OF 4-(2H-PYRIDO[3,2-b] (1,4) OXAZIN-4(3H)-YL SULFONYL) ANILINO- STEARIC ACID

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ABSTRACT

Morpholine is an organic chemical compound. The heterocyclic features consists of both amine and ether functional groups. Because of the amine function group morpholine acts as a base. Morpholine is a back bone is essential in different pharmacologically active synthetic compounds. Present work summarizes the synthesis of morpholine nucleus and its derivatives. The morpholine and its compounds where synthesized in multistep reaction with more efficient process. Starting compound is available commercially. Starting compound used in this reaction is 2-aminopyridin-3-ol. Morpholine nucleus shows a broad spectrum of pharmaceuticals applications. The chemical structures of the synthesized were confirmed by means of NMR, UV and MASS spectral data. High yield and purity of the derivatives indicates lack of side reaction. In recent years various new method were developed for the synthesis of morpholine analogues. The synthesized compounds and its derivatives were then examined for antibacterial, antifungal, analgesic and anti inflammatory activities.

KEYWORDS: Morpholine, Pharmaceutical applications, Analgesic activity.

INTRODUCTION

Morpholine is an organic chemical compound having the chemical formula $O(CH_2CH_2)_2NH$. This heterocycle features both amine and ether functional groups. Because of the amine, morpholine is a base; its conjugate acid is called morpholinium. For example, treating morpholine with hydrochloric acid makes the salt morpholinium chloride. The naming of morpholine is attributed to Ludwig Knorr, who incorrectly believed it to be part of the structure of morphine.[5]

Morpholines is an open source framework that reduces the time and efforts necessary to build and change Hadoop ETL stream processing applications that extract, transform and load data into Apache Solar, HBase, HDFS, Enterprise Data Warehouses, or Analytic Online Dashboards. Want to build or facilitate ETL jobs without programming and without substantial MapReduce effort Get the job done with a minimum amount of fuss and support costs Here is how to get started.

A morpholine is a rich configuration file that makes it easy to define a transformation chain that consumes any kind of data from any kind of data source, processes the data and loads the results into a Hadoop component. It replaces Java programming with simple configuration steps, and correspondingly reduces the cost and integration effort associated with developing and maintaining custom ETL projects.

Morpholines is a library, embeddable in any Java codebase. A morpholine is an in-memory container of transformation commands. Commands are plugins to a morpholine that perform tasks such as loading, parsing, transforming, or otherwise processing a single record. A record is an in-memory data structure of name-value pairs with optional blob attachments or POJO attachments. The framework is extensible and integrates existing functionality and thirdparty systems in a straightforward manner.

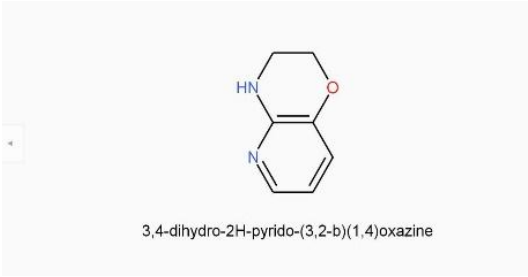
The morpholine commands were developed as part of Cloudera Search. s power ETL data flows from Flume and MapReduce and HBase and Spark into Apache Solar. Flume and Spark cover the real time case, whereas MapReduce covers the batch processing case. Since the launch of Cloudera Search morpholine development graduated into the Kite Software Development Kit (Kite SDK) in order to make the technology accessible to a wider range of users and products, beyond Search. The Kite SDK is a set of libraries, tools, examples, and documentation focused on making it easier to build systems on top of the Hadoop ecosystem.

The Kite SDK is hosted on GitHub and encourages involvement by the community. For example, morpholines could be embedded into Crunch, HBase, Impala, Pig, Hive, or Sqoop. Let us know where you want to take it!

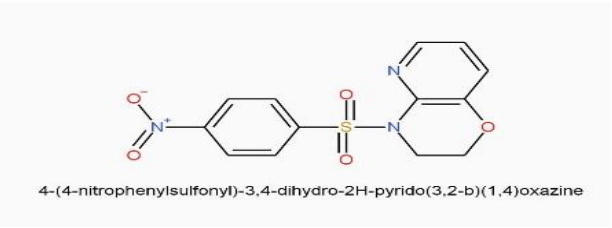
MATERIALS AND METHODS

Scheme of work

Step 1: Synthesis of 2H-pyrido [3,2] [1,4] oxazin-3(4H)-one.

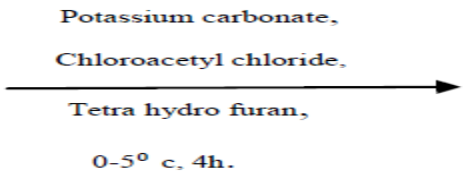


3,4-dihydro-2H-pyrido-(3,2-b)(1,4)oxazine

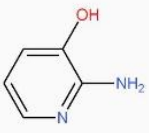


4-(4-nitrophenylsulfonyl)-3,4-dihydro-2H-pyrido(3,2-b)(1,4)oxazine

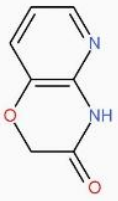
The chloroacetyl chloride (0.1mol) is added drop-wise to the solution of potassium carbonate (0.1 mol) and 2-amino -3-hydroxy pyridine -3-ol (0.1 mol) in THF (250 ml) at 0^0c. The resulting suspension was stirred at room temperature for 1hr. then the reaction mixture heated to reflux and maintained for 4hr. after completion of reaction, the reaction was cooled to room temperature and the inorganic solids were removed by filtration washed with THF (25 ml). filtered and washed with water (25 ml).



Step 2: synthesis of 3,4-dihydro-2H-pyrido[3,2-b] [1,4] oxazine.



2-aminopyridine-3-ol



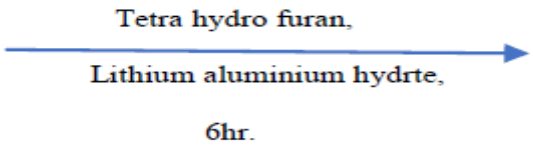
2H-pyrido(3,2-b)(1,4)oxazin-3(4H)One

A mixture of THF 25 ml and compound-1 (0.1 mole) in 30 ml of LiAlH4 was heated with occasional stirring at 80^0c for 6 hr.

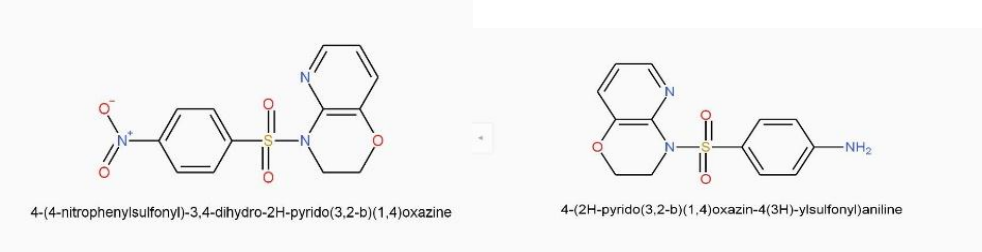
At the end of this period, the mixture was cooled and poured into ice cold water.

The separated solid was filtered.

The crude product obtained above was recrystallised from methanol -DMF solution to obtain pure compound -2.



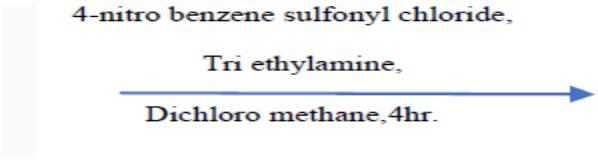
Step 3: synthesis of 4(4-nitrophenylsulfonyl)-3,4-dihydro-2H-pyrido[3,2-b] [1,4] oxazine.



A mixture of compound -2 (0.1 mole), 4-nitro benzene sulphonyl chloride (0.1 mole), triethylamine 10ml and dichloromethane (0.1 mole) in a round bottomed flask was heated with occasional stirring for 4 hrs.

At the end of this period the mixture was poured into ice cold water.

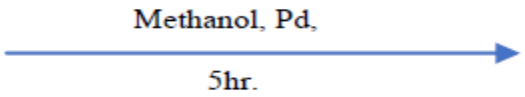
The separated solid was filtered and dried to obtain compound -3, which are recrystallised from the methanol to obtain ccompound-3.



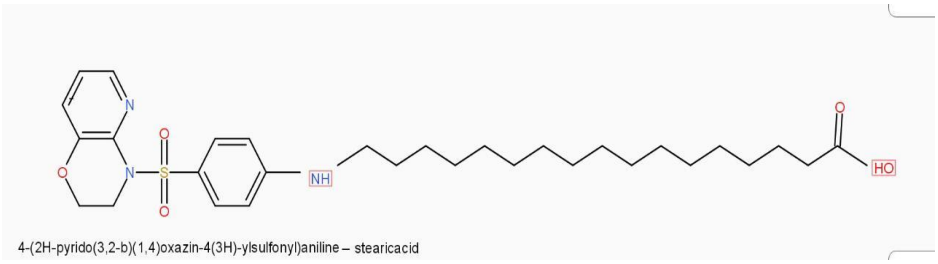
Step 4: synthesis of 4-(2H-pyrido(3,2-b) (1,4) oxazin-4(3H)-sulfonyl) aniline.

A mixture of compound-3 (0.1 mole) and phenyl hydrazine (0.1 mole), 50ml of acetic acid and ethanol (30 ml) was refluxed for 5hrs.

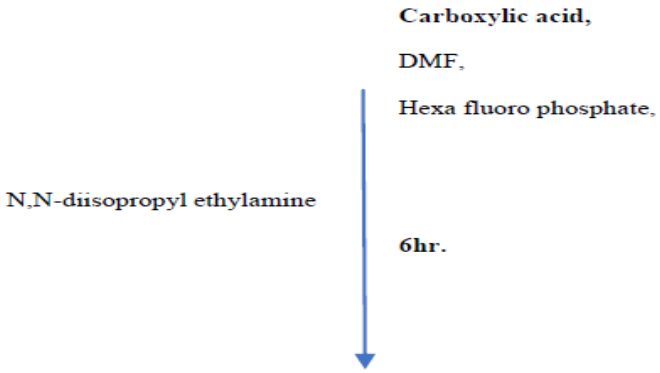
At the end of this period the mixture was cooled and poured into ice cold water.



Step 5: synthesis of 4-(2H-pyrido(3,2-b) (1,4) oxazin-4(3H)-yl sulfonyl) anilino- stearic acid



The corresponding carboxylic acid (1 mole) was dissolved in DMF (30ml). followed by compound-4 (1mol), hexafluoro phosphate (0.1 mole) and N,N-diisopropyl ethylamine (0.1mole). then the mixture was continued to stir for 6hr room temperature. After completion of reaction, the reaction mass poured into cold water and the suspension was stirred for 2hr at room temperature.



Chemicals

2-aminopyridine-3-ol, potassium carbonate, chloro acetyl chloride, tetra hydro furan, lithium aluminium hydride, 4- nitro benzene sulfonyl chloride, tri ethylamine, dichloro methane, palladium, methanol, carboxylic acid, DMF, hexafluoro phosphate, N, N-diisopropyl ethylamine, stearic acid.

Physical characterization

- ✓ Molecular formula: $C_{31}H_{48}N_3SO_5$
- ✓ Molecular weight: 574 g/mole
- ✓ Soluble in Methanol, Ethanol, DMSO and DMF
- ✓ Melting point: 121-130℃
- ✓ Melting point were determined using Veego Digital melting point apparatus.
- ✓ The purity of synthesis compound was monitored on TLC.

Biological screening

- ✓ **Analgesic activity**
- ✓ **Materials and Methods**
- ✓ **Acute toxicity**
- ✓ The acute toxicity study was carried out as per OECD-425 Guidelines. Mortality in each group within 24 hr was recorded. The animals were observed for a further 14 days for any signs for delayed toxicity. The compound has good margin of safety and did not show the lethal effects on the animals up to the doses of 500 mg/kg. Hence LD50 of triazine derivative considered as 500mg/kg, studies were carried out with 1/10 of the LD50 dose is 50mg/kg.

- ✓ **Evaluation of analgesic activity**
- ✓ **Tail immersion method**
- ✓ Swiss albino mice were screened by exposure to the thermal stimulus. The mice showing positive response were divided in to four groups of six animals each. The animals of Group I, II, III and IV were received DMSO (1ml/kg/p.o.), indomethacin (10 mg/kg/p.o.) and triazine derivative i.e. (50 mg/kg) respectively. After half an hour of treatment, the tail of mice was dipped in warm water kept constant at 55 ± 1^{o} C upto 2cm from the tip of the tail. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cut of time being 60 sec. The observations were made at 0 min, 30 min, 60 min, 120 min, and 180 min.[9]

- ✓ **Acetic acid induced writhing test**
- ✓ The triazine a derivative was evaluated for its analgesic activity by acetic acid induced writhing model. Swiss albino mice were divided in to four groups of six animals each. First group was served as a negative control received DMSO (1ml/kg). Second group served as positive control received indomethacin (10 mg/kg). While the third and fourth

groups were administered orally with triazine derivative. Half an hour after the administration of above drugs 0.6% v/v acetic acid (10ml/kg) i.p was given to all animals and observed for 15minutes. The number of abdominal constriction (writhing) and stretching with a jerk of the hind limb was counted for 15 minutes after administering acetic acid.[10]

- ✓ % Protection = 1- (Experimental/control) x 100

- ✓ **Statistical analysis**
- ✓ One way analysis of variance (ANOVA) by Dunnett's method was employed using Graphpad instat 3.0 software for statistical analysis of the data. A probability value of < 0.01 was considered statistically significant. Values in the text and tables are represented as Mean ± SEM.

Spectral analysis

IUPAC NAME: 4-(2H-pyrido(3,2-b) (1,4) oxazin-4(3H)-yl sulfonyl) anilino stearic acid

^{1}HNMR Interpretation:

1HNMR Spectral data Absorption position (in PPM)	
7.48 - 7.81	**m, 6H,**
6.16 – 6.36	d, 6H, CH
5.38	s, 2H, CH2
4.52	d, 2H, NH

RESULTS AND DISCUSSION

Synthesis: The present study report the synthesis of Morpholine derivatives electrophilic substitution of 2-amino-pyridin-3-ol in THF & chloro acetyl chloride was carried out stepwise at different temperature by stearic acid. The final Morpholine derivative was synthesized. Compound 4 was replaced by stearic acid. Since the report regarding this compound suggest a Morpholine possesses 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 121-130˚C.

TLC was performed using precoated silica gel plates of 0.25mm thickness. Eluents used were Acetic acid: n-butanol: Water (10:3:1) 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 and DMF.

In-Vitro Anti-Inflammatory Activity

The synthesized compounds are to be used for this study. They are to be made into doses of 1000 µg/ml with DMSO (5.0 %) solution. Diclofenac sodium is taken as standard. The reaction mixture (4.5 ml) consist of 2 ml of hypotonic saline (0.36 % sodium chloride), 1 ml of 0.15 M phosphate buffer (Ph 7.4) ,1 ml of the test solution (1000 µg/ml) in normal saline and 0.5 ml of HRBC suspension in normal saline. For control test, 1 ml isotonic saline is to be used instead of test solution while product control lacked RBC. The mixture is then incubated at 56˚C for 30 minutes, then to be cooled under running tap water and centrifuged at 3000 rpm for 20 minutes. The absorbances of the supernatants are read at 560 nm. Percent membrane stabilization activity is calculated as follows.

$$\% \text{ stabilization} = \frac{\text{OD of test control} - \text{OD of test sample}}{\text{OD of test control}} \times 100$$

S. No	Compound code	Percentage Stabilization
1	Morpholine derivative	70
2	STD (Diclofenac)	86.4

CONCLUSION

In the present study certain Morpholine 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 Morpholine nucleus show the moderate activity.

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