Synthesis And Biological Evaluatuon of Pyridinederivative for Anti Microbial Activity

K. Neelaveni[1], D.Kavya Sri [2] , L.Pooja [3], Md.Rahmathullah [4]
[1,2,3,4]*CMR College of Pharmacy, Kandlakoya, Medchal, Hyderabad*

Abstarct: The present research is on synthesis of Pyridine derivative and evaluation of its antimicrobial activity. The synthesis is done by one step. In this step 1,4-dihydropyridine is synthesized and this final product has been identified by TLC and FTIR spectroscopy. The compound was tested for anti-microbial activity by using Streptomycin as standard drug against B.subtilis (gram positive bacteria) & E.coli (gram negative bacteria). The compound has significant anti-microbial activity.

Key words: 1,4-Pyridine, anti-microbial activity, B.subtilis, E.coli , Streptomycin.

INTRODUCTION

The synthesis of derivatives has been an important part of research in medicinal chemistry. Medicinal chemistry is the science that deals with the discovery and design of new therapeutic chemicals and their development into useful medicines to treat diseases. Medicinal chemistry involves

❖ Isolation of compounds from nature or synthesis of new molecules.

❖ Investigation of the relationships between the structure of natural and/or synthesized compounds and their biological activities.

❖ Elucidations of their interactions with receptors of various kinds including enzymes and DNA

❖ Determination of their absorption, transport, and distribution properties and studies of the metabolic transformations of these chemicals into other chemicals and their excretion.

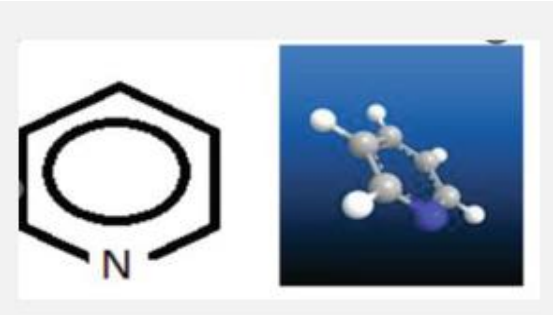
Drug discovery research is a highly creative and stimulating work environment where people are driven to succeed by personal and scientific objectives as well as with the desire to contribute to society's well-being.

Introduction to pyridine:

- Pyridine is an aromatic compound with a molecular formula (C_5H_5N)
- All the pi electrons are shared by a ring, forms one continuous circle of electrons besides the alternate double bonds shared by every atom of the circle.
- Pyridine is a unique type with nitrogen on the ring to provide a tertiary amine by undergoingreactions such as alkylation and oxidation

The pyridine has three resonance structures. Pyridine is basic in nature. In pyridine and pyrrole, lone pairs of electrons on N are different. Therefore, a H+ ion or a Lewis acid can be conveniently transferred to the lone pair of electrons on the N atom in pyridine.

Pyridine is dissolvable and is added to ethyl liquor that makes it unfit for drinking. It is changed to items such as sulfapyridine, a medication dynamic against bacterial and viral contaminations; pyribenzamine and pyrilamine, as antihistaminic drugs; and piperidine, which is utilized in elastic preparation, and as a crude substance material; and water anti-agents, bactericides, and herbicides. Compounds not using Pyridine, however, containing its ring structure incorporate niacin and pyridoxal, both Bnutrients; isoniazid, an antitubercular medication; and nicotine and a few different nitrogenous plant items. Pyridine uses in the chemical industries and enterprises as a significant crude material, used in dental consideration items for cleaning, used as a dissolvable which is appropriate for dehalogenation, Pyridine uses in pharmaceuticals, radiator fluid blends as a denaturant, Pyridine uses as a sulfonating specialist, used in colors and paints, disinfectant, a ligand in the chemical science.



Properties of pyridine :

- Molecular Formula - C_5H_5N

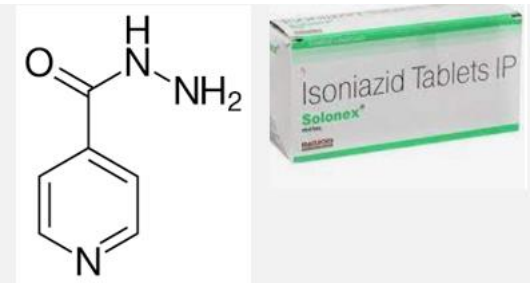
- Molar Mass - 79 .1 g/mol
- Density - 982 kg/m^3
- Boiling Point - 115 ^{0}C
- Melting point - -41.6 ^{0}C
- Nature - Basic
- State - Liquid
- Colour - Colourless
- Solubility - Alcohol, ether and other organic liquids
- pH - 8.5

Biological Activities of Pyridine:

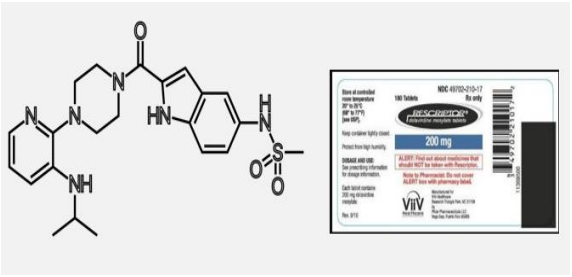
Marketed drugs of pyridine:

S.NO	CHEMICALS
1	Benzaldehyde
2	Ethyl acetoacetate
3	Ammonium acetate
4	Cerric Ammonium Sulfate (CAS)
5	Ethanol
6	Petroleum ether
7	Ethyl acetate

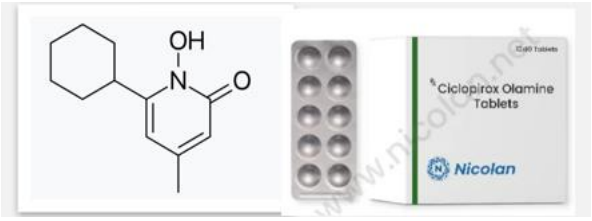
ISONIAZID: A pyridine derivative developed as tuberculosis.



DELAVIRDINE: It is a NNRTIs used for HIV/AIDS.



CICLOPIROX: It is used for anti-helminthic condition.



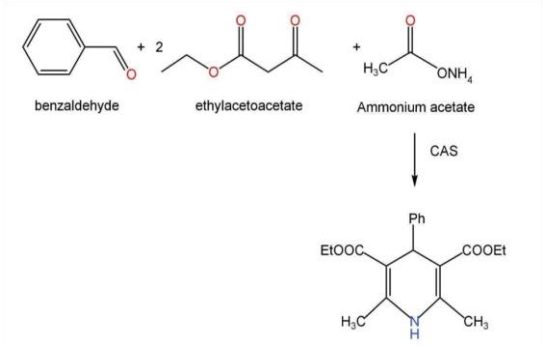
MATERIALS AND METHOD USED

All the chemicals and solvents used were of syntheticgrade from SD fine chemicals Ltd., (Mumbai, India), andAvra Chemicals (Hyderabad, India). Completion of thereactions was monitored by analytical thin layerchromatography (TLC) using E-Merck 0.25 mm silicagel plates. Visualization was accomplished with UV light(256nm) and iodine chamber. Synthesized compoundswere purified by re-crystallization process. The purity ofthe compounds was checked by a single spot in TLC andsolvent system for TLC was determined on trial and errorbasis. Melting points were determined in open capillarytubes using ANALAB melting point apparatus and wereuncorrected.

The FT-IR spectra were recorded on Schimadzu FT-IRspectrophotometer by using 1% potassium bromidediscs.

List of chemmicals used:

SCHEME



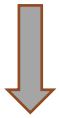
2,6-dimethyl-3,5-diethyl-4-phenyl-1,4-dihydro pyridine diacetate

SYNTHETIC PROCEDURE

Synthesis of pyridine according to the following procedure:
One pot multicomponent synthesis of dihydropyridine derivatives using the CAS as the catalyst without using any solvent. They took all reactants together and stir the reaction mixture for 1-2.5 hours at room temperature. The reaction was monitored by TLC. The product was purified and recrystallized using ethanol.

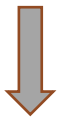
SYNTHESIS PROCEDURE:
Synthesis of 1,4-dihydropyridines :
Add benzaldehyde, ammonium acetate, ethyl acetoacetate, CAS

in a beaker

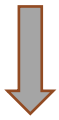


Stir the reaction mixture for 2-3hrs at room temperature

On a magnetic stirrer



Recrystallize the mixture using ethanol.



The reaction was monitored by TLC.

FIG NO.1 Synthesizing compound by using magnetic stirrer

IDENTIFICATION AND CHARACTERISATION

The identification and characterization of the prepared compound were carried out by the following procedure. 1. Melting point 2. Solubility 3. Thin layer chromatography 4. Infrared spectroscopy (I.R). Also Ultra Violet spectroscopy, Nuclear magnetic resonance are used for confirming the compounds.

1. MELTING POINT DETERMINATION: The melting points of the organic compounds were determined by open capillary tube method. Melting point is a valuable criteria of purity for an organic compound as a pure crystal is having definite and sharp melting point. The purity should not be assumed but must be established by observation of any changes in the melting point when the compounds subjected to purification by re crystallization. The synthesized compounds showed a minute change in melting point after re crystallization.

2. SOLUBILITY: The solubility of synthesized compounds were tested in various solvents. The solubility is the ability of a solid, liquid, or gaseous chemical substance to dissolve in solvent and form a solution in specific a homogenous solution.

3. THIN LAYER CHROMATOGRAPHY PROCEDURE:
Cleaned and dried glass plates were taken. Uniform slurry of silica Gel-G in water was prepared in the ratio of 1:2. The slurry was then poured into the chamber of the TLC applicator, which was fixed and thickness was set to 0.5 mm. Glass plates were moved under the applicator smoothly to get a uniform coating of slurry on the plates. The plates were dried at room temperature and then kept for activation at 110oC for 1 hr. The compound was taken in a small bored capillary tube and spotted at 2cm from the base end of the plate. Then the plate was allowed to dry at room temperature and plates were transferred to chromatographic chamber containing solvent system for development. The developed spots were detected by exposing them to iodine vapours. Then the Rf values of compounds were calculated using the formula –

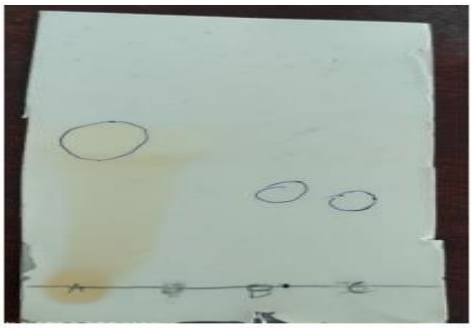


FIG NO.2 TLC

Rf value = distance moved by sample/ distance moved by solvent in a beaker

1.INFRARED SPECTROSCOPY:

Infrared spectroscopy is an important analytical technique for determining the structure of both inorganic and organic compounds. It measures the vibrations of the atoms caused by the infrared light and from these measurements it is possible to identify functional group of the compound. Infrared spectroscopy (IR spectroscopy or vibrational spectroscopy) involves the interaction of infrared radiation with matter. It covers a range of techniques, mostly based on absorption spectroscopy. As with all spectroscopic techniques, it can be used to identify and study chemical substances. Samples may be solid, liquid, or gas. The method or technique of infrared spectroscopy is conducted with an instrument called an infrared spectrometer (or spectrophotometer) to produce an infrared spectrum. An IR spectrum can be visualized in a graph of infrared light absorbance (or transmittance) on the vertical axis(y) vs. frequency or wavelength on the horizontal axis(x). Typical units of frequency used in IR spectra are reciprocal centimeters (sometimes called wave numbers), with the symbol /cm. Units of IR wavelength are commonly given in micrometers (formerly called "microns"), symbol μm, which are related to wave numbers in a reciprocal way. A common laboratory instrument that uses this technique is a Fourier transform infrared (FTIR) spectrometer.

PROCEDURE: The infrared spectrum of a sample is recorded by passing a beam of infrared light through the sample. When the frequency of the IR is the same as the vibrational frequency of a bond or collection of bonds, absorption occurs. Examination of the transmitted light reveals how much energy was absorbed at each frequency (or wavelength). This measurement can be achieved by scanning the wavelength range using a monochromator. Alternatively, the entire wavelength range is measured using a Fourier transform instrument and then a transmittance or absorbance spectrum is generated using a dedicated procedure. This technique is commonly used for analyzing samples with covalent bonds. Simple spectra are obtained from samples with few IR active bonds and high levels of purity. More complex molecular structures lead to more absorption bands and more complex spectra.

Biological Evaluation (Antimicrobial Activity)

Pyridine derivatives possess diverse variety ofpharmacological activities. Due to this pyridine haveoccupied unique place in field of medicinal chemistry. Pyridine ring system is present occasionally innature. Pyridine finds use in research as a startingmaterial for synthesis of larger, usually bioactivestructure. It is structurally similar with nucleic bases aswell as isosteres of naturally occurring cyclic nucleotidesuch as adenine and guanine that is why it probablyinteracts with biopolymers in living systems and showdiverse biological activities like antimicrobial, antiinflammatory, analgesic, antifungal, anticonvulsants,antitumor, anticancer, CNS activities, anti tubercular,anti-HIV agents anthelmintic, and other anticipatedactivities.

Principle:

The number of life threatening infections caused by multidrug resistant gram positive pathogens has reached an alarming level in hospitals and the community. The infections caused by these organisms pose a serious challenge to the specific community and the need for an effective therapy has led to search for novel antimicrobial agents. Anti-microbial drugs are effective in treatment of infection because of their selective toxicity that is they have the ability to injure or kill an invading microorganism without harming the host. It is evident from literature that Pyridine are known to be associated with broad spectrum of biological activities like antibacterial, antifungal etc.

Types of media:

Media can be classified into 3 categories on the basis of chemical constituents

l) Synthetic media

2) Complex media

3) Complex media

1) Synthetic media: Media in which all the constituents are chemically defined. They are generally used to study the specific nutritional requirements of different microbes.

2) Complex media: Media in which the media components and composition is incompletely defined. Ex: beef extract used in the nutrient media chemically complex.

3) Natural media: Substrates of natural origin that favors microbial growth are employed in this media. Ex: milk.

Media can be classified based on their functional properties:

1) Simple media: This media is used for cultivation of the most of the micro organisms. EX:- Nutrient agar.

2) Differential media: This type of media is employed to distinguish bacteria that differ in their specific property. EX: starch agar, blood agar.

3) Selective media: This type of media selectively allows the growth of particular type of microbes and prevents the growth of other microbes. EX: nutrient agar with crystal violet.
4) Selective differential media: This type of media allows only a particular type of organism to grow that can he further distinguish based on their property. EX: Macconky agar.

PREPARATION OF AGAR PLATES:
Label the Petri plates and transfer agar medium in liquid form with right hand, Turn your left hand palm side up and clamp the cotton plug between the finger, flame the mouth of the flask after removing the cotton plug. Use the hand holding cotton plug to lift the lid of Petri dish. Now pour about 20-25m of sterilized nutrient agar medium then hold the lid so that it partially covers the bottom of dish as you pour. This helps to prevent microbes and air bornedust particles from dropping into your sterilized plate and contaminating it. Immediately replace the lid. If bubbles occur on the surface of medium, quickly break them aseptically by passing Bunsen flam over the surface. Allow the plates to cool down at room temperature and store the plates. Observe all the liquid and solid media after 2 days properly sterilize medium will remain clear. The broth which was not properly sterilized would have developed turbidity use only media that has properly generalized for your work.

PREPARATION OF NUTRIENT AGAR MEDIUM:
It is an aqueous solution, obtained from beef called beef extract. Digesting protein substance Obtained by acid or enzyme hydrolysis is called peptone. Agar is used for solidifying and provides sufficient nutrients required for the growth of micro-organisms.

PRINCIPLE:
Nutrient agar is used for making plates for the growth of micro- organisms. Agar plates provide maximum surface area and it is easy to study colony characteristics.

LIMITATIONS OF AGAR:
1. Large surface area is exposed to contamination
2. Difficulty in handling
3. Media is not sterilized in the plates
4. Breaking cost of apparatus is high as Petri dishes are costly

PROCEDURE:
*Nutrient broth-
- Peptone - 10gms
- Beef extract – 10gms
- Sodium chloride – 5gms
- Water- 1000ml.

PRINCIPLE:
Follow the procedure as nutrient broth, but add 1-2percent of agar powder to nutrient broth. Weigh all the additives separately by physical balance and add all the additives in a suitable container. Dissolve with the help of stirrer and adjust the pH using sodium hydroxide, sterilize the media in autoclave at 15lb pressure at 120°c for 20 min.

Preparation of Antibiotic solution:
● Prepare different concentrations of antibiotic solution (i.e.)10 mg/ml, 20, 30, 40, solutions.
● Take 10 mg of antibiotic and dissolve in solvent and make up to 10 ml to get 1 mg/ml or 1000 µg/ml solution.
● From the above solution take 0.1, 0.2, 0.3 and 0.4 and makeup to 10ml respectively to get 10, 20, 30,40 µg/ml.

EXPERIMENTAL PROCEDURE BY CUP PLATE METHOD
● Prepare nutrient media and transfer 20 ml into boiling tube, plug and sterile them.
● After cooling inoculate each boiling tube with 0.1ml of test organism (Bacillus subtilis)
● The inoculated agar media is poured into Petri plate and solidified.
● Make holes in the solidified media at the center by using sterile borer. Add 0.1ml of prepared antibiotic solution into the holes.
● Incubate the Petri plate at 37°C for 24hrs.

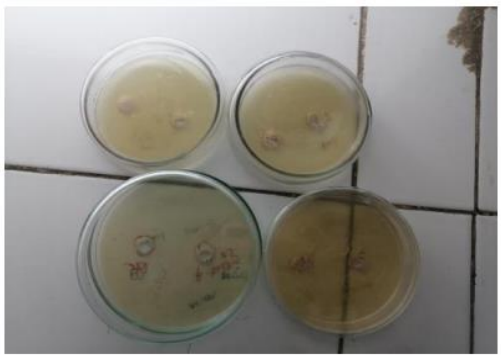


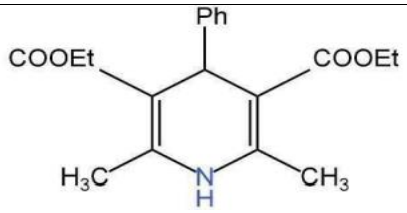
Fig no.3.Measurement of zone of inhibition

RESULTS AND DISCUSSION

Synthetic -Procedure:
The present study was aimed at synthesis of pyridine and its derivatives by a new synthetic procedure using benzaldehyde and ethyl acetoacetate as starting compounds. The resulting intermediate of these reactants were reacted with CAS in presence

of ammonium acetate resulting in generation of pyridine. The final compounds were confirmed by FT-IR studies and TLC.

Table.No1.1: IUPAC Name of Synthesized Compounds:

S.NO	STRUCTURE	IUPAC NAME OF SYNTHESISED COMPOUND
1		2,6-dimethyl-3,5-diethy-4-phenyl-1,4-dihydropyridine diacetate

TLC OF SYNTHESIZED COMPOUNDS

Rf = Distance travelled by solute/Distance travelled by solvent

Table 1.2 TLC CALACULATIONS For synthesized Pyridine compound

S.N O	COMPOUND	SOLVENT	COMPOSITION	Rf value
1	Benzaldehyde	Petroleum ether:Ethyl acetate	5:1	0.59
2	Ethyl acetoacetate	Petroleum ether:Ethyl acetate	5:1	0.51
3	Product	Petroleum ether:Ethyl acetate	5:1	0.85

SOLUBILITY:

Solubility test for synthesized Pyridine compounds are done individually.

Table 1.3 solubility

S.NO	COMPOUNDS	SOLUBLE IN
1	Benzaldehyde	Soluble in Diethyl ether,ethanol
2	Ethyl acetoacetate	Soluble in ethanol, diethyl ether.
3	Product	Soluble in Diethyl ether, ethanol DMSO

Table No 1.4 Zone of inhibition of standard { streptomycin} against B.subtillis

CONCENTRATIONS(µg/ml)	ZONE OF INHIBITION(cm)	ZONE OF INHIBITION(mm)
10	2.8	28
20	3.4	34
30	4	40
40	4.4	44

Table no.1.5 Zone of inhibition of synthesized compound against B.subtilis

CONCENTRATIONS (µg/ml)	ZONE OF INHIBITION(cm)	ZONE OF INHIBITION(mm)
10	3.2	32
20	3.6	36
30	4.4	44
40	4.8	48

Table no.1.6 Zone of inhibition of standard {streptomycin} against E.coli

CONCENTRATIONS (µg/ml)	ZONE OF INHIBITION(cm)	ZONE OF INHIBITION(mm)
10	2.6	26
20	3.4	34
30	3.8	38
40	4	40

Table no.1.7 Zone of inhibition of synthesized compound against E.coli

CONCENTRATIONS (µg/ml)	ZONE OF INHIBITION(cm)	ZONE OF INHIBITION(mm)
10	3	30
20	3.6	36
30	4	40
40	4.6	46

FT-IR GRAPH

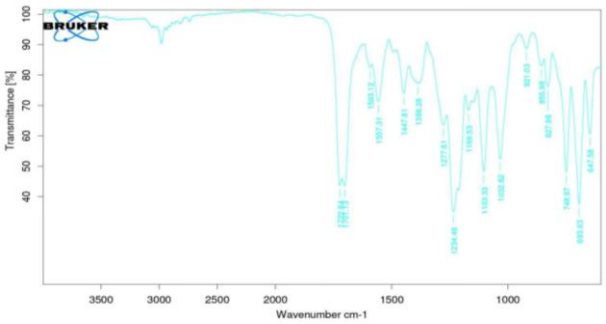


FIG N0.4 FT-IR

FT-IR SPECTRAL CHARACTERIZATION

Table no.1.8 FT-IR characterization

Standard frequency(cm^{-1})	Frequency(cm^{-1})	Type of vibration	Functional group
1700-1725	1722.64	Stretch	C=O
1700-1725	1701.13	Stretch	C=O
1550-1640	1593.12	Stretch	C=C
1550-1640	1557.31	Stretch	C=C
1400-1450	1447.81	Strong	α-CH_2
1370-1390	1386.28	Medium	CH_3
1210-1320	1277.61	Strong	C-O
1210-1320	1234.48	Strong	C-O
1000-1300	1169.53	Stretch	C-O
1000-1300	1103.33	Stretch	C-O
1000-1300	1032.83	Stretch	C-O

CONCLUSION

The present study was aimed for the synthesis of pyridine and evaluation of anti – microbial activity. Derivative of pyridine was synthesized and screened for Antimicrobial activity. The study was mainly focused on the development of a new procedure for the synthesis of pyridine. In the study following steps were performed:

1.Synthesis of pyridine derivatives were carried out by Hantzsch synthetic procedure in order to obtain desired products in acceptable yield.

2. Products formed were confirmed by TLC and characterized by FT-IR.

3. The compounds were tested for antimicrobial activity and found to have significant activity.

REFERENCE

1) Nomenclature of Organic Chemistry: IUPAC Recommendations and Preferred Names 2013 (Blue Book). Cambridge: The Royal Society of Chemistry. 2014. p. 392. doi:10.1039/9781849733069-FP001. ISBN 978-0-85404-182-4.

2) H. Andersson, F. Almqvist, R. Olsson, Org. Lett., 2007, 9, 1335-1337.

3) O. V. Larionov, D. Stephens, A. Mfuh, G. Chavez, Org. Lett., 2014, 16, 864-867.

4) X. Chen, L. Zhou, Y. Li, T. Xie, S. Zhou, J. Org. Chem., 2014, 79, 230-239.

5) J. Choi, G. Laudadio, E. Godineau, P. S. Baran, J. Am. Chem. Soc., 2021, 143, 11927-11933.

6) S. Jung, S. Shin, S. Park, S. Hong, J. Am. Chem. Soc., 2020, 142, 11370-11375.

7) I.Kim, S. Park, S. Hong, Org. Lett., 2020, 22, 8730-8734.

8) A.Cervantes-Reyes, A. C. Smith, G. M. Chinigo, D. C. Blakemore, M. Szostak, Org. Lett., 2022, 24, 1662-1667.

9) L.-Y. Xi, R.-Y. Zhang, S. Liang, S.-Y. Chen, X.-Q. Yu, Org. Lett., 2014, 16, 5269- 5271.

10) G. Asskar, M. Rivard, T. Martens, J. Org. Chem., 2020, 85, 1232-1239.

11) J. R. Colombe, S. Bernhardt, C. Stathakis, S. L. Buchwald, P. Knochel, Org. Lett., 2013, 15, 5754-5757.

12) J. Z. Deng, D. V. Paone, A. T. Ginnetti, H. Kurihara, S. D. Dreher, S. A. Weissman, S.R. Stauffer, C. S. Burgey, Org. Lett., 2009, 11, 345-347.

13) W. Miao, C. Ni, P. Xiao, R. Jia, W. Zhang, J. Hu, Org. Lett., 2021, 23, 711-715.

14) S. Sakashita, M. Takizawa, J. Sugai, H. Ito, Y. Yamamoto, Org. Lett., 2013, 15, 4308-4311.

15) T. M. Gøgsig, A. T. Lindhardt, T. Skrydstrup, Org. Lett., 2009, 11, 4886-4888.

16) Q.-L. Zahng, Q.-q. Yu, L. Ma, X. Lu, Q.-T. Fan, T.-S. Duan, Y. Zhou, F.-L. Zhang, J. Org. Chem., 2021, 86, 17244-17248.

17) H. Wei, Y. Li, K. Xiao, B. Cheng, H. Wang, L. Hu, H. Zhai, Org. Lett., 2015, 17, 5974-5977.

18) Y. Gao, R. Chen, Y. Ma, Synthesis, 2019, 51, 3875-3882.

19) H. Huang, J. Cai, L. Tang, Z. Wang, F. Li, G.-J. Deng, J. Org. Chem., 2016, 81, 1499-1505.

20) Y. Wei, N. Yoshikai, J. Am. Chem. Soc., 2013, 135, 3756-3759.

21) B.-T. Guan, Z. Hou, J. Am. Chem. Soc., 2011, 133, 18066-18089.

22) M. R. Luzung, J. S. Patel, J. Yin, J. Org. Chem., 2010, 75, 8330-8332.

23) W. Gati, M. M. Rammah, M. B. Rammah, F. Couty, G. Evano, J. Am. Chem. Soc., 2012, 134, 9078-9081.

24) T. J. Donohoe, J. A. Basutto, J. F. Bower, A. Rathi, Org. Lett., 2011, 13, 1036- 1039.

25) E. K. J. Lui, D. Hergesell, L. L. Schafer, Org. Lett., 2018, 20, 6663-6667.

26) J. P. Norman, N. G. Larson, E. D. Entz, S. R. Neufeldt, J. Org. Chem., 2022, 87, 7414-7421.

27) S. I. Scherbinina, O. V. Fedorov, V. V. Levin, V. A. Kokorekin, M. I. Struchkova, A. D.Dilman, J. Org. Chem., 2017, 82, 12967-12974.

28) J. Shen, D. Cai, C. Kuai, Y. Liu, M. Wei, G. Cheng, X. Cui, J. Org. Chem., 2015, 80, 6584-6589.

29) K. Yoshida, F. Kawagoe, K. Hayashi, S. Horiuchi, T. Imamoto, A. Yanagisawa, Org. Lett., 2009, 11, 515-518.

30) Q.-L. Zahng, Q.-q. Yu, L. Ma, X. Lu, Q.-T. Fan, T.-S. Duan, Y. Zhou, F.-L. Zhang, J. Org. Chem., 2021, 86, 17244-17248.

31) P. Lu, C. Sanchez, J. Cornella, I. Larrosa, Org. Lett., 2009, 11, 5710-5713.

32) Y.-F. Wang, S. Chiba, J. Am. Chem. Soc., 2009, 131, 12570-12572.

33) J. Wu, W. Xu, Z.-X. Yu, J. Wang, J. Am. Chem. Soc., 2015, 137, 9489-9495.

34) Y. Jiang, C.-M. Park, T.-P. Loh, Org. Lett., 2014, 16, 3432-3435.

35) P. Gao, H.-J. Chen, Z.-J. Bai, M.-N. Zhao, D. Yang, J. Wang, N. Wang, L. Du, Z.H. Guan, J. Org. Chem., 2020, 85, 7939-7951.

36) Y. Liu, W. Luo, Z. Wang, Y. Zhao, J. Zhao, X. Xu, C. Wang, P. Li, Org. Lett., 2020, 22, 9638-9643.

37) G. Chen, Z. Wang, X. Zhang, X. Fan, J. Org. Chem., 2017, 82, 11230-11237. 38) Z. Song, X.

Huang, W. Yi, W. Zhang, Org. Lett., 2016, 18, 5640-5643.
39) O. De Paolis, J. Baffoe, S. M. Landge, B. Török, Synthesis, 2008, 3423-3428. 40) P. Kumar, M. Kapur, Org. Lett., 2020, 22, 5855-5860.