IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
December 2021 Vol.:23, Issue:1

Synthesis, In Silico Studies and Pharmacological Evaluation of Amide Derivatives of Sulphosalicylic Acid

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Sheena Mathew*, Jijo James, Nair, Reshma Sanal, Tintu Johnson

Nazareth College of Pharmacy, Othera, Thiruvalla, Kerala, India

Submitted: 25 November 2021
Accepted: 30 November 2021
Published: 30 December 2021

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: sulphosalicylic acid, *in silico*, Lipinski's Rule of Five, PASS, IR spectroscopy, antimicrobial activity

ABSTRACT

The process of establishing a new pharmaceutical is exceedingly complex and involves the talents of people from a variety of disciplines, including chemistry, biochemistry, molecular biology, physiology, pharmacology, pharmaceutics and medicine. This work was aimed to synthesize a novel series of amido derivatives of sulphosalicylic acid and to conduct there *in silico* studies and pharmacological evaluation. The present work involved the preliminary *in silico* screening of various analogues using suitable software. Various derivatives of 5-sulphosalicylic acid-like diphenyl amines derivative, 2,4 dinitro phenyl derivative, and p-nitro aniline derivative were prepared and characterized using IR spectroscopy. The compounds were then evaluated for their antimicrobial activity by the cup plate agar diffusion method.

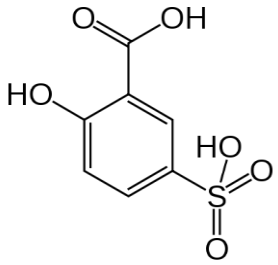
INTRODUCTION

Drug discovery is mostly portrayed as a linear, consecutive process that starts with target and leads the discovery, followed by lead optimization and pre-clinical *in vitro* and *in vivo* studies to determine if such compounds satisfy several pre-set criteria for initiating clinical development. *In-silico* approach attempts to identify and quantify the physicochemical properties of a drug and to see whether any of these properties affects drugs biological activity. Using the software ACD Labs, Chemsketch, LogP, molar volume, molar refractivity, parachor, polarizability, surface tension were determined. Drug likeness and violation of the "Lipinski rule of 5" are carried out using Molinspiration software.

Sulpho salicylic acid is the lead molecule here which is a salicylic acid derivative having the IUPAC name2-hydroxy-5-sulphobenzoic acid.[1]

Table 1

Molecular formula	$C_7H_6O_6S$
Molar mass	218.185 g/mol



The nitrogen and sulfur-containing compounds plays important role in medicine for the treatment of various kinds of bacterial and fungal diseases caused by different pathogenic bacterial and fungal strains. The amides of 5-sulphosalicylic acid were tested for antibacterial activity against E coli. The biological activity of these entire compounds generally occurs due to the presence of nitrogen and sulphur contents in the molecule, the presence of hydrogen bonding in 5-sulfosalicylic acid, presence of polar OH groups which increases the water solubility of the compound. These compounds generally react with the bacterial cell wall and damage it and kill the bacteria.[5]

MATERIALS AND METHODS

Softwares such as Molinspiration, ACD Labs Chemsketch 12.0 and PASS were used for the in silico screening of the molecules.

All the chemicals and reagents used were of analytical or synthetic grades.

Reagents used are Sulphosalicylic acid, Diphenylamine,2,4 dinitrophenyl hydrazine, Paranitroaniline, Ethanol (99.9%).

The synthesized compounds were purified by using by recrystallisation using ethanol as well as Thin Layer Chromatography using the solvent system Choloroform: Ethanol (9:1), which featured as a single spot using Iodine chamber and concordance in R_f value.

The synthesized compounds were characterized by Melting point and Vibrational spectra (IR) spectra.

The melting points were determined using the open capillary tube method on the melting point apparatus and were presented uncorrected.

IR Spectra of the synthesised analogues were recorded using the KBr pellets FTIR instrument.

IN-SILICO MOLECULAR STUDIES:

Analysis of Lipinski's Rule of Five

Lipinski's Rule of Five describes molecular properties vital for a drug's pharmacokinetics in the human body, including its absorption, distribution, metabolism and excretion (ADME).

The 'Rule of Five' is so named due to the features being multiples of five. Molecules that violate more than one of these rules may encounter problems with bioavailability. The descriptors are calculated using software like molinspiration and ACD Chemsketch 12.

In general, an orally active drug exhibits the following: Molecular weight not greater than 500, Not more than 5 hydrogen bond donors (OH and NH groups), Not more than 10 hydrogen bond acceptors (notably O and N), Calculated octanol/water partition (C Log P) not greater than 5 and Number of rotatable bonds not more than 5.[2]

Prediction of Activity Spectra for Substances (Pass)

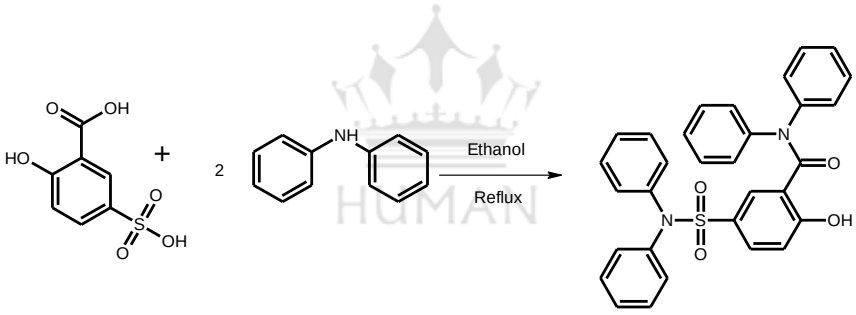
The approach used in PASS is based on the suggestion that Activity = f (structure). Thus, by comparing the structure of a new compound with structures of well- known biologically active substance it is possible to estimate if a new compound may have a particular effect.

The result of prediction is presented as the list of activities with appropriate Pa and Pi, sorted in descending order of the difference (Pa –Pi) > 0. Pa and Pi are the estimates of probability

for the compound to be active or inactive respectively for each type of activity from the biological activity spectrum. Their values vary from 0.000 to 1.000. If Pa > 0.7 the compound is very likely to reveal this activity in experiments, but in this case the chance of being the analogue of the known pharmaceutical agents for this compound is also high. If 0.5 < Pa < 0.7, the compound is likely to reveal its activity in experiments, but this probability is less, and the compound is not so similar to the known pharmaceutical agents.If Pa< 0.5, the compound is unlikely to reveal its activity in experiments, but if the presence of this activity is confirmed in the compound, it might be a new chemical entity.[3]

METHOD OF SYNTHESIS OF DIPHENYLAMINE DERIVATIVE[A]

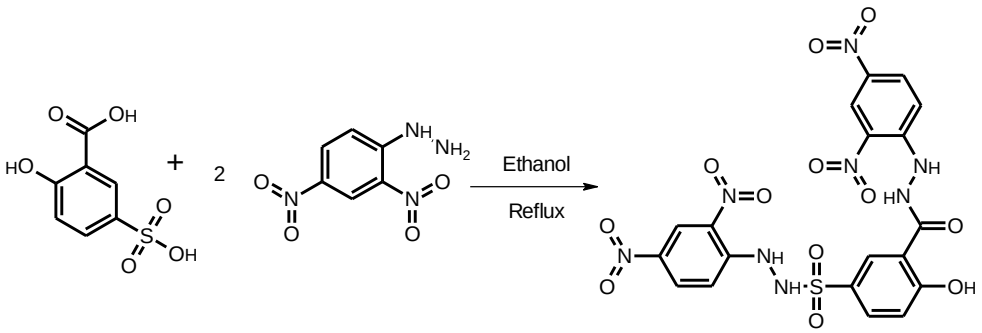
An ethanolic solution of 5-sulphosalicylic acid was prepared by taking 2.18g of sulphosalicylic acid and 3.38g of diphenylamine and 15ml of ethanol and it was then refluxed for 6-7 hours at room temperature followed by evaporation and concentration in a vacuum to give a crystalline solid product which was recrystallized in ethanol to the give respective amide of 5-sulphosalicylic acid.



N-(diphenyl)-5-(diphenyl) sulphamoyl-2-hydroxy benzamide

METHOD OF SYNTHESIS OF 2,4-DINITROPHENYL HYDRAZINE DERIVATIVE[B]

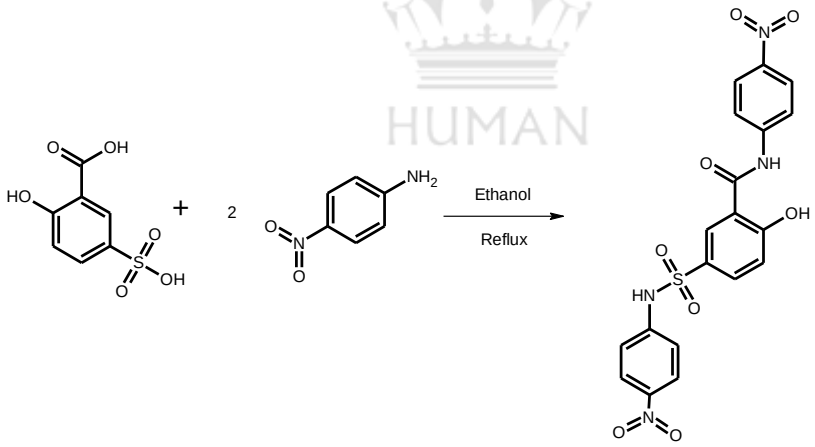
An ethanolic solution of 5-sulphosalicylic acid was prepared by taking2.18g of sulphosalicylic acid and 3.96g of 2,4-dinitro phenylhydrazine in 15ml ethanol and it was then refluxed for 6-7hours at room temperature followed by evaporation and concentration in a vacuum to give a crystalline solid product. This was recrystallized in ethanol to give the respective amide of 5-sulphosalicylic acid.



N-(2,4-dinitrophenylhydrazine)-5-(2,4-dinitrophenylhydrazine)sulphamoyl-2-hydroxy benzamide

METHOD OF SYNTHESIS OF PARA NITRO ANILINE DERIVATIVE[C]

An ethanolic solution was prepared by taking 2.18g of sulphosalicylic acid and 2.76g of p-nitro aniline and 15ml of ethanol and it was then refluxed for 6-7hours at room temperature followed by evaporation and concentration in a vacuum to give a crystalline solid product. This was then recrystallized in ethanol to give the respective amide of 5-sulphosalicylic acid.[7,8]



2-hydroxy-*N*-(4-nitrophenyl)-5-[(4-nitrophenyl)sulfamoyl]benzamide

The antimicrobial activity of synthesized compounds was determined by cup plate agar diffusion method. The organism selected for antimicrobial activity was *Escherichia coli*.

RESULTS AND DISCUSSION

IN-SILICO MOLECULAR STUDIES:

Analysis of Lipinski's Rule of Five

Analysis of Lipinski rule of five for selected novel Sulphosalicylic acid analogues was conducted. Compound B shows two violations against Lipinski's rule of five.

Table 2: Analysis of Lipinski rule of five for selected novel Sulphosalicylic acid analogues

Compound	Log P	Molecular Weight	No. of Hydrogen Bond donors	No. of Hydrogen Bond acceptors	No. of Rotatable Bonds	Number of violations
A	4.942	520.61	1	6	7	1
B	3.269	578.432	5	20	11	2
C	2.928	458.40	7	8	5	1

Prediction of Activity Spectra for Substances (Pass)

Table 3: Prediction of biological activity of selected Sulphosalicylic acid analogues

Compound	Effect	Pa	Pi
A	Antidiabetic	0.714	0.005
	Bone diseases treatment	0.651	0.005
	Calcium channel N-type blocker	0.595	0.004
	Antiobesity	0.599	0.011
	Analgesic	0.516	0.031
	Antibacterial activity	0.313	0.025
B	Antitubercular	0.571	0.006
	Antimycobacterial	0.468	0.023
	Antiprotozoal	0.316	0.051
	Antidiabetic	0.272	0.056
	Diuretic	0.251	0.048
	Antibacterial activity	0.353	0.056
C	Antiprotozoal	0.727	0.003
	Sickle-cell anemia treatment	0.678	0.002
	Antituberculosic	0.595	0.005
	Antipyretic	0.530	0.010
	Antiseptic	0.517	0.011
	Antimycobacterial	0.519	0.015
	Antianginal	0.485	0.048
	Antibacterial activity	0.267	0.070

Synthetic Methodology

Synthesis of derivatives of 5-sulphosalicylic acid was carried out using ethanol, diphenylamine,2,4-dinitro phenyl hydrazine and p-nitroaniline. The prepared derivatives were recrystallised from ethanol. Melting point and TLC determination [chloroform: ethanol(90:10)] were performed to ascertain the purity as well as the completion of the reaction.

Table 4

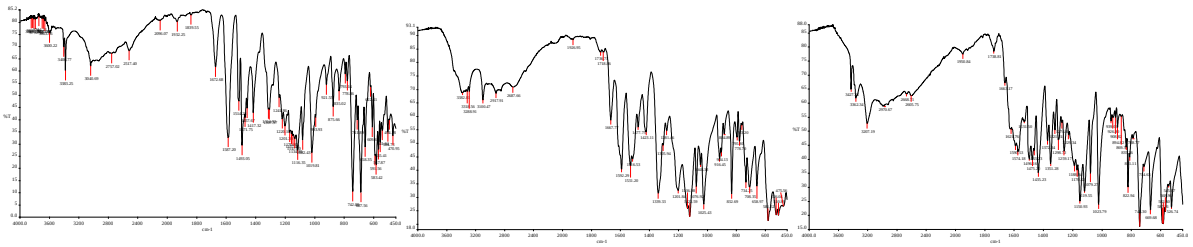
Compound	Log P	Molecular Weight	No. of Hydrogen Bond donors	No. of Hydrogen Bond acceptors	No. of Rotable Bonds	Number of violations
A	4.942	520.61	1	6	7	1
B	3.269	578.432	5	20	11	2
C	2.928	458.40	7	8	5	1

Table 5

SLNO.	Compounds	(%)w/w	Meltingpoint(°C)	Rf value
1.	**A (**Diphenylamine derivative)	87%	135	0.94
2.	**B (**2,4 Dinitro phenyl hydrazine)	70%	140	0.95
3.	**C** (p-nitroaniline derivative)	68%	145	0.92

IR SPECTRAL ANALYSIS:[9]

A **B** **C**

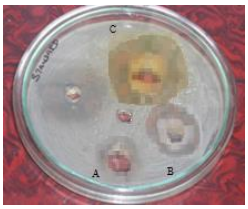


ANTIMICROBIAL ACTIVITY

The prepared derivatives of 5-sulphosalicylic acid were screened for their antimicrobial activity using cup plate agar diffusion method against *Escherichia coli*.

Table 6: Antimicrobial Activity

Sl no	Compounds	Zone of inhibition(mm) Escherichia coli
1	A	15
2	B	18
3	C	21
4	Standard	22
5	Control	Nil



Standard - Gentamicin

DISCUSSION

IN-SILICO MOLECULAR STUDIES:

Analysis of Lipinski's Rule of Five

The compounds A and C may have good oral activity as they follow Lipinski's Rule of Five. Compound B may not be orally active as it shows two violations against Lipinski's Rule.

Prediction of Activity Spectra for Substances (Pass)

The compounds are predicted to show various activities. Further studies on the compounds can be done according to the predictions.

Antimicrobial Activity

Compound C shows comparable activity to that of the standard gentamicin.

CONCLUSION

The present work involved the preliminary *in silico* screening of various analogues using suitable software. Analysis of Lipinski Rule of Five was conducted for the proposed derivatives of 5-sulphosalicylic acid. The biological activities of the derivatives were predicted using the software PASS (Prediction of Activity Spectra for Substances). Various derivatives of 5-sulphosalicylic acid-like diphenyl amines derivative,2,4 dinitro phenyl derivative, and p-nitro aniline derivative were prepared and characterized using IR spectroscopy. The compounds were then evaluated for their antimicrobial activity by the cup plate agar diffusion method. All synthesized compounds were found to possess considerable antimicrobial activity. Among the derivatives compound, C showed maximum activity against *E.coli*.

ACKNOWLEDGEMENT

The authors are thankful to the Principal, and all other faculties of Nazareth College of Pharmacy and also the faculties of the Department of Chemical Sciences, M G University, Kottayam.

REFERENCES

1. en.wikipedia.org/wiki/5-sulphosalicylic acid.
2. Available from en.wikipedia.org/wiki/lipinski's rule of five.
3. Available from URL:http://www.ibnh.msk.su/PASS
4. Available fromhttp://en.wikipedia.org/wiki/Antibacterial
5. Barry, A.L., The Antimicrobial Susceptibility test, Principle and practices, BiolAbstr, 1976,64,180,257-263.
6. Arora. D.R, Textbook of Microbiology, 2,645-647.
7. Alfa XeniaLupea ,MirabelaPadure, Synthesis and characterisation of some N-ubstituted Amides of Salicylic acid, Proceedings for Natural Sciences, 2003; 104 : 5-10
8. EvgenijaDjurendić et al, Synthesis and biological evaluation of some new 2-oxazoline and salicylic acid derivatives, ARKIVOC 2011; (ii) 83-102
9. Robert.m.Silverstein, Spectrometric Identification of Organic Compounds, 2005, 71-143.