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"EXPLORING THE ANTIMICROBIAL EFFICACY OF 2-AMINO BENZAMIDE DERIVATIVES AGAINST PATHOGENIC MICRO-ORGANISUMS"

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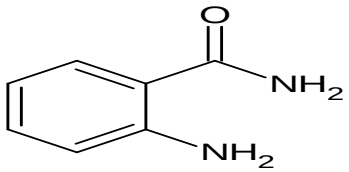
ABSTRACT

A series of new 2-aminobenzamide derivatives (2a-2i) has been synthesized in good to excellent yields by adopting conventional methodologies starting from 2-amino salicylic acid and characterized on the basis of their analytical and spectral. Selected compounds of this series were then tested antimicrobial activity against different strains of gram positive and gram-negative bacteria and fungal strain by using cup plate method. among this series of compounds **2f** shows broad spectrum antibacterial activity. Whereas, the tested compounds **2d** and **2e** and exhibited moderate activity. and compound **2c** shows good activity against gram negative bacteria. For antifungal activity compounds **2g** shows potent activity. whereas the test compounds **2f** and **2i** exhibited moderate activity,

KEYWORDS: 2-Amino Benzamide, Antibacterial activity, Antifungal activity. Cup plate method.

INTRODUCTION

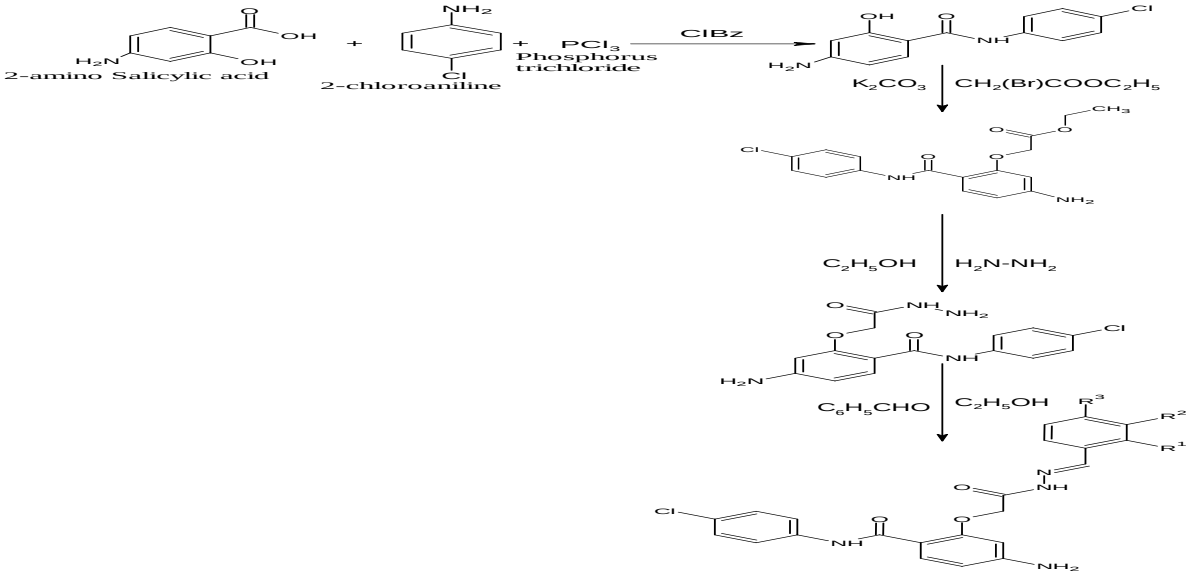
Benzamide is an aromatic amide that consists of benzene bearing a single carboxamido substituent. Benzamide used in medicinal as well as in synthetic industry.[1] Amides are found in various natural products in organic chemistry. Benzamide are a significant class of amide compounds. These compounds have been widely used in medical, industrial, biological and potential drug industries.: Juvenile hyperactivity.[2] The treatment of Cancer.[3], Hypercholesterolemia.[4] Antitumour.[5] Anti-microbial.[6] Antibacterial.[7] Anti-fungal.[8] Anti-HSV.[9] Antioxidant.[10] Analgesic.[11] and Anti-inflammatory.[12] Amide derivatives also show Anti-platelet activity.[13] Recently, amide compounds have been used in drug discovery. Other fields in which amide compounds are broadly used include industrial sectors such as plastic, rubber industry, paper industry, and agriculture. 2-Aminobenzamide is chemically reactive due to its amine and amide groups. which are prone to reactions such as reductive amination, forming heterocyclic rings like quinazolinones when reacted with aldehydes and ketones. Its amide functional group can undergo amidation reactions, and its amine group can act as a ligand in forming complexes with metal ions. 2-Amino Benzamide is also an inhibitor of histone deacetylases (HDACs), demonstrating biological reactivity.[14] 2-aminobenzamide is an amphoteric compound with both acidic and basic properties due to its amide (-$CONH_2$) and amine (-NH2) groups. Therefore, it has two different ionizations constants. one for the acidic ionization and one for the basic ionization.



MATERIALS AND METHODS

Chemistry (Scheme)

Synthesis of 2-Amino Benzamide derivatives (2a-2i) from 2-Amino Salicylic acid by Conventional method.



$\mathbf{R^1}$=NO_2.
$\mathbf{R^2}$= NO_2.
$\mathbf{R^3}$=OCH_3. CH_3. Cl. $N(CH_3)_2$. $N(CH_3)_2$-CH=CH. NO_2.

General procedure for scheme

Step-1; 4-Amino-(4-Chlorophenyl)-2-Hydroxybenzamide

To a mixture of 2-amino salicylic acid, 2-chloroaniline and phosphorus trichloride 1:1:0.3, chlorobenzene was added in RBF. The mixture was stirred and heated to reflux for 5 hrs. The completion of reaction was monitored by TLC. After being cooled at room temperature, the obtained, crude product was filtered off, washed successively with 10% sodium carbonate solution. The solid product was then filtered off, dried and recrystallized using dimethylformamide as a solvent.

Step-2; Ethyl {2-[(4-Chlorophenyl) Carbamoyl] Phenoxy} Acetate

Ethyl/Methyl esters were added dropwise to a mixture of appropriate salicylamide and anhydrous K_2CO_3 , refluxed in 2-butanone, ethyl Bromo-ester. The Optimum molar ratio was amide: ester: K_2CO_3 = 1:1:1. The mixture was stirred and heated to reflux for 5 hrs. cooling at room temperature, after filtration and evaporation of solvent, esters were obtained in crystalline form and were recrystallized using ethanol as a solvent.

Step-3:4-Amino-*N*-(4-Chlorophenyl)-2-(2-Hydrazinyl-2-Oxoethoxy) Benzamide

A mixture of ethyl ester and hydrazine hydrate 1:1 was refluxed in ethanol for 3 hrs. The reaction mixture was cooled, it is filtered then the precipitate is recrystallized using dimethylformamide, as a solvent.

Step-4;5-Amino-2-{2-[(2*E*)-2-Benzylidenehydrazin-1-yl]-2-Oxoethoxy}-*N*-(4-Chlorophenyl) Benzamide

Hydrazones - were added to a solution of hydrazide in ethanol, the appropriate various aromatic aldehyde was (1:1). The reaction mixture was refluxed for 5 hrs. The solid obtained after cooling was filtered off, and recrystallized using ethanol as a solvent.

BIOLOGICAL ACTIVITY

In-vitro ANTIBACTERIAL ACTIVITY

The anti-bacterial activity was carried out by cup-plate method.[15] Zone of inhibition of the synthesized compounds were measured and compared with the Zone of inhibition of the standard reference drug. The standard reference drug used in the research work was Ciprofloxacin.[16] Antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis. Escherichia coli, Pseudomonas aeruginosa, Shigella.* Nutrient agar media was prepared by dissolving Peptone (10g), Beef extract (1.5g) Agar (15g) Sodium chloride (5g) Distilled water(1000ml).20 ml of agar media was poured on previously sterilized Petri plates and the media was allowed to solidify in room temperature. After solidification 0.2 ml of bacterial culture was speeded with the help of spreader. Then bored with the help of borer and the synthesized compounds were added to the bore at 50,100,150µg/ml concentration. The standard was also placed in all the plates for the comparison along with negative (DMSO) control was also added. The concentration of the standard was 2 µg/ml ciprofloxacin was taken as a standard for comparison of the activity of the newly synthesized compounds against various bacterial organisms. The plates were incubated at 37°C

for 18-24 hours in the incubator and then the zone of inhibition has been measured for each concentration and reported.[17,18]

***In-vitro* ANTIFUNGAL ACTIVITY**

2-amino benzamide derivatives (2a-2i) were screened for their antifungal activity against Aspergillus Niger and Candida albicans by Cup plate method. Potato dextrose agar media was prepared by dissolving potato extract (20 g), D-glucose (2 g) and agar (2 g) in distilled water (100 ml).20 ml of agar media was poured on previously sterilized Petri plates and the media was allowed to solidify in room temperature. After solidification 0.2 ml of fungal culture was speeded with the help of spreader. Then bored with the help of borer and the synthesized compounds were added to the bore at 50,100,150µg/ml concentration. The standard was also placed in all the plates for the comparison along with negative (DMSO) control was also added. The concentration of the standard was 2 µg/ml fluconazole[19] was taken as a standard for comparison of the activity of the newly synthesized compounds against various fungal organisms. The plates were incubated at 37°C for 18-24 hours in the incubator and then the zone of inhibition has been measured for each concentration and reported.[20]

Table 1: Antibacterial activity of 2-amino benzamide derivatives(2a-2i) against gram positive bacteria.

SI. NO	Name of the compound	Zone of inhibition (in mm)								
		Staphylococcus aureus			Bacillus substiles			Staphylococcus epidermidis		
		50µg	100µg	150µg	50µg	100µg	150µg	50µg	100µg	150µg
01	2a	2	3	5	-	-	-	-	-	-
02	2b	3	4	5	-	-	-	-	-	-
03	2c	-	-	-	-	-	-	3	4	8
04	2d	2	6	8	3	7	8	4	7	8
05	2e	6	8	9	-	-	-	3	4	8
06	2f	4	6	8	5	8	10	4	9	11
07	2g	2	3	5	-	-	-	2	4	6
08	2h	4	5	6	1	2	4	3	4	5
09	2i	2	4	6	-	-	-	-	-	-
10	STD (2µg)	10			10			11		

Table 2: Antibacterial activity of 2-amino benzamide derivatives(2a-2i) against gram negative bacteria.

SI. N	Name of the compound	Zone of inhibition (in mm)								
		Escherichia coli			Pseudomonas aeruginosa			Shigella		
		50µg	100µg	150µg	50µg	100µg	150µg	50µg	100µg	150µg
01	2a	2	3	4	3	4	6	-	-	-
02	2b	-	-	-	2	3	4	-	-	-
03	2c	3	6	8	4	6	8	5	6	9
04	2d	3	5	7	-	-	-	6	7	8
05	2e	4	5	7	-	-	-	5	6	7
06	2f	3	6	9	3	7	10	3	8	11
07	2g	2	4	5	2	4	6	-	-	-
08	2h	1	3	5	4	5	6	-	-	-
09	2i	3	4	5	4	6	8	3	5	4
10	STD (2µg)	10			10			10		

Table 3: Antifungal activity of 2-amino benzamide derivatives(2a-2i) against *Candida albicans and Aspergillus Niger*.

SI. N	Name of the compound	Zone of inhibition (in mm)					
		Candida albicans			Aspergillus Niger		
		50µg	100µg	150µg	50µg	100µg	150µg
01	2a	2	4	5	3	4	5
02	2b	3	4	7	4	5	7
03	2c	2	4	5	2	4	5
04	2d	4	6	7	2	6	8
05	2e	3	4	6	3	5	7
06	2f	4	6	8	2	4	8

07	2g	5	8	11	6	8	10
08	2h	3	5	6	2	4	5
09	2i	3	6	8	4	6	9
10	STD (2µg)	10			10		

RESULTS AND DISCUSSION

Antimicrobial susceptibility testing serves various purposes such as drug discovery, epidemiology, and prediction of therapeutic outcomes. The widespread use of antimicrobial substances has significantly reduced microbial contamination and helped treat various diseases. So, we made an attempt to synthesize 2-amino benzamide derivatives to possess better antimicrobial property.

The antibacterial activity of the synthesized series of 2-amino benzamide derivatives (2a-2i) was studied by cup plate method. The standard drug used was ciprofloxacin. antibacterial activity among the test compounds is presented in table. All the test compounds **[2a-2i]** showed a varied degree of antibacterial activity with broad spectrum of activity against the Gram positive (*Staphylococcus aureus, Bacillus subtilis, Staphylococcus epidermidis,*) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Shigella*) bacterial strains employed. However, among this series of compounds **2f** shows broad spectrum activity. Whereas the test compounds **2d** and **2e** and exhibited good activity against gram positive bacteria. And compound **2c** shows good activity against gram negative bacteria.

The anti-fungal activity was carried out by cup-plate method. Here responses of microorganisms to the synthesized compounds were measured and compared with the response of the standard reference drug. The standard reference drug used in the research work was fluconazole. The antifungal activity was carriedout aginest *Candida albicans and Aspergillus Niger*. However, among this series of compounds **2g** shows potent activity. whereas the test compounds **2f** and **2i** exhibited moderate activity.

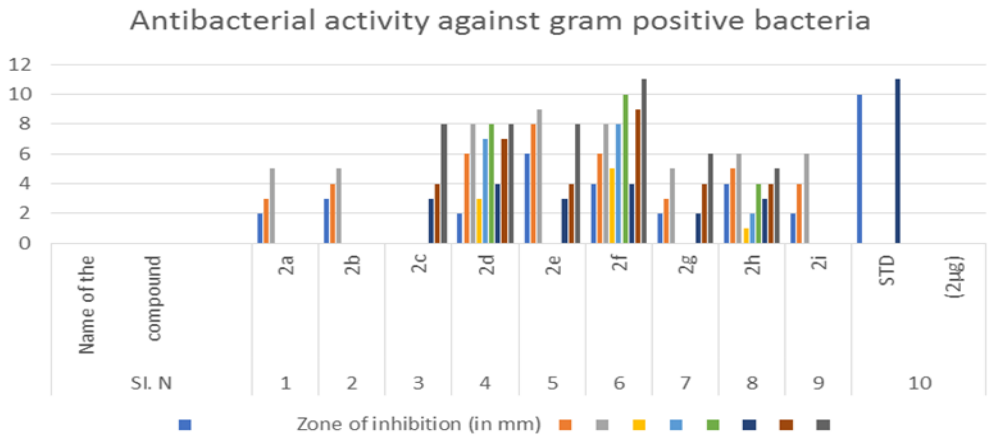


Fig.1: Graphical representation of antibacterial activity of Novel 2-amino Benzamide derivatives (2a-2i) – Zone of Inhibition(mm)for gram positive bacteria.

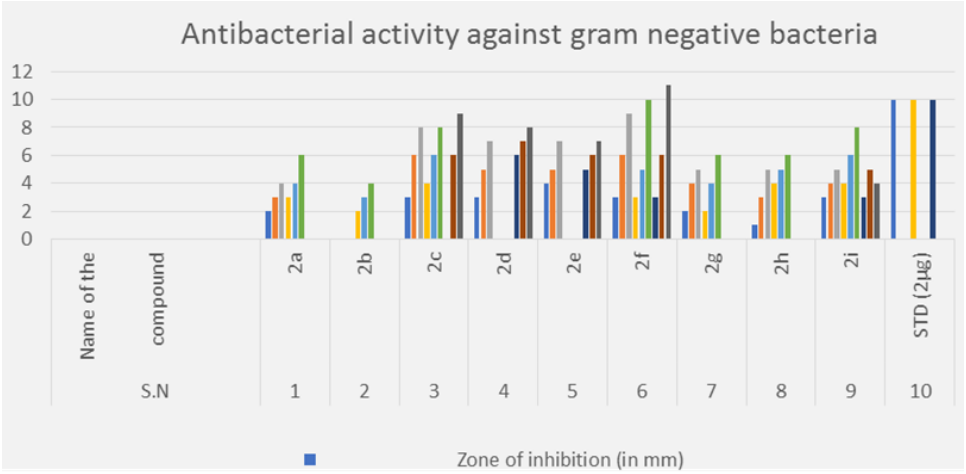


Fig.2: Graphical representation of antibacterial activity of Novel 2-amino Benzamide derivatives (2a-2i) – Zone of Inhibition(mm) for gram negative bacteria.

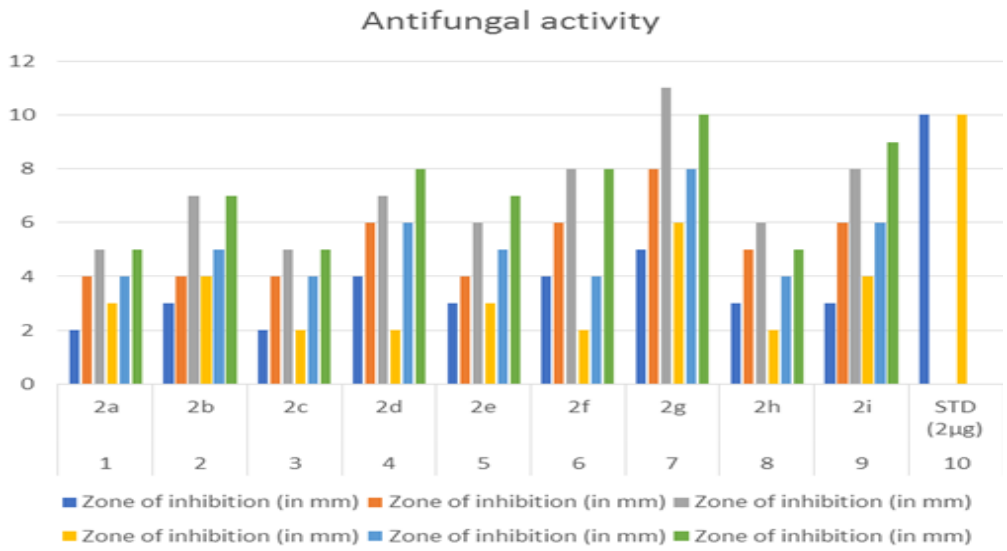


Fig.3: Graphical representation of antifungal activity of Novel 2-amino Benzamide derivatives (2a-2i) – Zone of Inhibition(mm).

CONCLUSION

The synthesized compounds were evaluated for their *in-vitro* Antibacterial, Antifungal activity Among the 9 synthesized derivatives, compound **2f** shows a broad-spectrum activity at the concentration of 150µg/ml, due to the presence of double bond. Meanwhile **2d** shows good activity because it contains chloro which is potent antibacterial and the aromaticity due to the sum of inductive and mesomeric effect it shows good antibacterial activity. against gram positive bacteria and compound **2e** shows activity agonist Staphylococcus *aureus*, *Staphylococcus epidermidis*, due the presence of methyl substitution which is electron releasing in nature. The remaining compound **2c** shows moderate activity against Escherichia coli, Pseudomonas aeruginosa, Shigella,

The antifungal activity was carriedout aginest *Candida albicans and Aspergillus Niger*. However, among this series of compounds **2g** shows potent activity. Whereas the test compounds **2f** and **2i** exhibited moderate activity.

In summary, structural modifications significantly influenced antimicrobial activity. Electron-withdrawing groups (such as nitro and chloro) enhanced antibacterial and antifungal potency, while electron-donating groups (such as methyl) selectively improved activity against Gram-negative bacteria. These findings suggest that benzaldehyde derivatives are promising scaffolds for the development of new antimicrobial and antifungal agents.

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