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Synthesis, Characterization and Antimicrobial Evaluation of New 3-(Alkyl/Arylamino)benzo[d]isothiazole 1,1-Derivatives

DHANRAJ P. KAMBLE[1], ANIL G. SHANKARWAR[1], YOGESH D MANE[2], RADHAKRISHNA M. TIGOTE[3], YUVARAJ P. SARNIKAR[4] and BALAJI R. MADJE[5]*

[1]Saraswati Bhuwan College of Science, Aurangabad, Dist. Aurangabad, M.S, India.
[2]BSS Arts, Science & Commerce College, Makni, Dist-Osmanabad, M.S, India.
[3]Dr. Babasaheb Ambedkar Marathwada University Subcampus, Osmanabad, M.S, India.
[4]Dayanand Science College, Latur, Dist. Latur, M.S, India.
[5]Vasantrao Naik College, Aurangabad, Dist. Aurangabad, M.S, India.
*Corresponding author E-mail: drmadjebr@gmail.com

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ABSTRACT

The saccharine nucleus has long been recognized as a significant component in medicine. A series of pseudo-saccharine amines derivatives (7a-j) were synthesized and examined for their antibacterial activity. After testing all compounds, 7b, 7f, 7g, 7i and 7j were found most effective against *Escherichia coli, Streptococcus aureus* and *Bacillus subtilis* strains. The MIC of the compound was found from 4.6 to 16.1 µM. Further, compound 7f and 7i exhibited excellent activity against *E.coli* and *Bacillus subtilis* with MIC value 4.6 and 4.7 µM respectively. The compound 7b and 7i was found active against all the three bacteria. The zone inhibition was observed at 10 µM against *Escherichia coli, Staphylococcus aureus* and *Bacillus subtilis* at 0.9, 1.8, 3.9 respectively for 7b and 1.0, 1.8 and 2.0 cm respectively for 7i.

Keywords: Saccharine, Pseudo saccharine and Pseudo saccharine amine.

INTRODUCTION

The saccharine (1,2-Benzisothiazole-3-one 1,1-dioxide) is the main core of several compound shows biological potency.[1-3] Different kind of $5HT_{1a}$ antagonists[4], human leukocyte elastase (HLE) inhibitors[5], analgetics[6], human mast cell tryptase inhibitors[7], $\alpha_{1\text{-}a}$ adrenergic receptor antagonists[8] and aldehyde dehydrogenase inhibitors have identified the saccharine nucleus as the main molecular ingredient.[9] Bioactive compounds harbouring Saccharine nucleus are known as inhibitors of serine proteasees[10], cathepsin G proteinase3,[11] α_{1a} and α_{1c} adrenergic receptor atagonists,[12] human mast cell tryptase inhibitors,[13] analgesics,[14] 5-HT_{1a} receptors,[15] anti-anxiety and antibacterial.[16] The analogues of benzo[d]isothiazole were demonstrates the central nervous system and mycobacterium activity.[17-18] The Benzo[d]isothiazole 1,1-dioxide derivatives are known as dual functional inhibitors of 5-lipoxygenase and microsomal prostaglandin E2 synthase-1.[19]

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Hence, considerable observation have been made to synthesize 1,2-Benzisothiazol-3-one 1,1-dioxide derivatives, specifically in those having N-basic side chain so as add to their pharmacological value. By considering the biological importance of compounds harbouring saccharine moiety, we decided to synthesize 3-(alkyl/arylamino) benzo[d]isothazole 1,1-dioxide analogues. This work reports synthesis, characterization and antibacterial evaluation of 3-(alkyl/arylamino)benzo[d]isothiazole 1,1-dooxide analogues.

MATERIAL AND METHODS

From the commercial sources the reagents and solvent were purchased and it has been used without further purification. The melting points were taken in open capillary tubes and are uncorrected. During the reaction, the synthesis of compounds was examined using TLC on 0.5 mm thick silica-gel plates, and the location of spots was checked using iodine and UV-light. All of the chemicals were purified utilizing suitable organic solvents and a recrystallization/silica gel (100-200 mesh) gravity column. The compound's mass spectra were determined using the Shimadzu GC-MS-QP-2010 model and the direct inlet probe technique. ^{1}H NMR, ^{13}C NMR was recorded in $CDCl_3$ and DMSO-d6 solution on a Bruker Ac 200 0r 400 MHz spectrometer.

EXPERIMENTAL

The Psuedo-saccharinchloride (6) were synthesized by taking 18 g of saccharin with 56.25 mL of 1,4 dioxane, 56.25 mL of thionyl chloride and 4 mL of DMF in round bottomed flask and refluxing it for 24 h at 100°C. After completion of 24 h reflux add 18 ml of $SOCl_2$ and 4 mL of DMF and continue to reflux for another 12 hours. The formed crude product was recrystallized from toluene to afford yellow-colored fine crystals of Psuedo-saccharin chloride.

Procedure for the synthesis of Pseudo-saccharine amines analogues (7a-j)

Take 500 mg (1eq) of saccharine chloride and alkyl or aryl amine (1eq) was dissolved in 1,4-dioxan and refluxed the content for 2h at 100°C. After completion of the reaction add water to quench the reaction to get a solid product, which then recrystallized by using toluene. The formation of the product was validated by ^{1}H NMR, ^{13}C NMR and ES-MS.

3-((2-hydroxyethyl)amino)benzo[d]isothiazole 1,1-dioxide (7a)

It is whitish solid crystal, yield 75%, ^{1}H NMR (200 MHz, DMSO-d6) δH: 3.6 (m, 4H), 3.1 (s, 1H),7.8 (d,2H), 7.9 (d, 1H),8.1 (d, 1H), 9.5 (s,1H); ^{13}C NMR (50 MHz, DMSO0d6) δc: 45, 47, 53, 58, 121, 122, 132, 142, 159; MS (ESI) m/e = 227 (M+1).

3-((4-chlorophenyl)amino)benzo[d]isothiazole 1,1-dioxide (7b)

It is whitish solid crystal, yield 65%, ^{1}H NMR (200 MHz, DMSO-d6) δH: 7.5 (d, 2H), 7.6 (d, 2H), 8.0 (d, 1H), 8.2 (d, 2H), 8.4 (d,2H), 10.7 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d6) δc: 126,128,132,133, 134, 138, 139, 141, 154, 161; MS (ESI) m/e = 293 (M+1).

3-(o-tolylamino)benzo[d]isothiazole 1,1-dioxide (7c)

Off white yellow crystal, yield 62%, ^{1}HNMR (200 MHz, DMSO-d6) δH: 2.2 (s,3H), 7.3 (d, 1H), 7.4 (d, 2H), 7.8 (d, 1H), 7.9 (d, 2H), 8.0 (d, 1H), 8.4 (d1H) 10.8 (s,1H); ^{13}CNMR (50 MHz, DMSO-d6) δC: 22, 26, 126, 128, 131, 132, 133, 136, 138, 139, 140, 147, 163; MS (ESI) m/e = 273 (M+1).

3-(pyridin-2-ylamino)benzo[d]isothiazole 1,1-dioxide (7d)

Off brown crystal, yield 60%, ^{1}H NMR (200 MHz, DMSO-d6) δH: 5.6 (d, 1H), 7.4 (d,2H), 7.5 (d, 2H), 7.8 (d, 2H), 8.4 (d, 2H), 9.9 (s, 1H); ^{13}C NMR (50 MHz, DMSO-d6) δC: 60, 121, 124, 127, 129, 133, 134, 135, 136, 142, 159, 171; MS (ESI) m/e = 259 (M+1).

2-((1,1-dioxidobenzo[d]isothiazol-3-yl)amino)-3-(1H-indol-3-yl)propanoic acid (7e)

Off yellow crystal, yield 65%, ^{1}H NMR (200 MHz, DMSO-d6) δH: 2.5 (s,1H), 3.6 (s,1H), 5.6 (d, 2H), 7.4 (d, 2H), 7.5 (s, 1H), 7.7 (d, 2H), 8.0 (d, 2H), 8.5 (d, 2H), 9.9 (s,1H), 13.5 (s,1H); ^{13}C NMR (50 MHz, DMSO-d6) δC: 60, 79, 124, 127, 128, 129, 133, 134, 135, 142, 159, 171; MS (ESI) m/e = 367 (M+1).

Biological evaluation

All the Pseudo-saccharine amine derivatives were check for in vitro antimicrobial activity against *Escherichia coli, Bacillus subtilis* and *Streptococcus aureus* using Disc diffusion method and time dose dependent growth inhibition assay. The results of antimicrobial activity of tested compounds (7a-j), using tetracycline as reference standard, are shown in Table1, 2 and Figure 1.

General protocol for antibacterial activity

Growth of pathogenic microorganisms

Clinical isolates were growth in Luria bertini medium (pH 6.8) for 24 h for activation of cultures. The colony forming units (CFUs) were calculated from the broth. The 100 uL ($100x10^2$ CFUs/mL) of the medium were inoculated into fresh Luria bertini broth (5 mL) and kept for 16 h to 18 h for log phase culture. The log phase culture was used for the antimicrobial assay.

Preparation of compounds

The compounds stock solution was prepared in DMSO and diluted further for antimicrobial action.

Antimicrobial effect of compounds on pathogenic microorganism by using Disc diffusion method

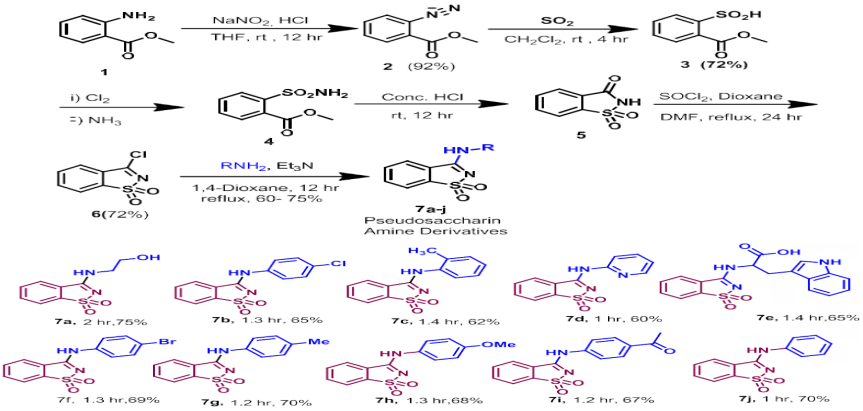
For the determination of antimicrobial activities of every compound, the Disc diffusion method was used. We use a multidrug resistant strain of *Escherichia coli, Staphylococcus aureus*, and *Bacillus subtilis* culture for this. Several bacterial species were cultured on nutrient agar media. Microorganisms in broth media were used to make inoculum suspensions (100 CFU per uL), nutrient broth inoculated with bacteria species was incubated for 24 h at 37°C. The sterile filter paper disc of 4mm in diameter were impregnated with 10 µL, 50 µL and 100 µL (stock concentration 10 ug/mL) of each compound. For drying purpose the desk were kept for 1 h at room temperature in a sterile airflow laminar chamber. After that it placed in the center of fresh nutrient agar plates which earlier seeded with 100 µL of inoculum suspension of each bacteria. The culture was kept incubation purpose either at 37°C for 24–48 hours. The every experiment was repeated three times. The antibiotics were used as positive control and use tetracycline as reference antibacterial standard. The antimicrobial activities were checked by measuring the zone inhibition diameters (Millimetres) surrounding in each disk.

Time and dose dependent effect of compounds on the growth of the pathogenic microorganisms

The culture of MDR strain of *Escherichia coli, Streptococcus aureus* and *Bacillus subtilis* were inoculated separately into LB medium and incubated at 37°C for 16-18 hours. After 16 to 18 h the cultured tubes were exposed to the compounds at concentration of 10, 25 and 50 µg/mL. The optical density was recorded at 660 nm after fixed interval of time. The time-dependent development of microorganisms was investigated using Graph pad prism 7.

RESULTS AND DISCUSSION

The following procedure was used for the synthesis of pseudo saccharine amine derivatives **(7a-j)** in the Scheme 1. The starting material 2-(methoxycarbonyl)benzenesulfinic acid **(3)** has been prepared by diazotization of commercially available 2-amonomethylbenzoate **(1)**. Cholorination of acid **(3)** followed by ammonia reaction yields methyl 2-sulfamoylbezoate **(4)**, which when cyclized with Conc. HCl yields benzo[d]isothiazol-3-(2H)-one 1,1-dioxide **(5)**. The compound **(5)** on chlorination with thionylchloride gives 3-chlorobenzo[d]isithiazole 1,1-dioxide **(6)**. Compound **(6)** was treated with different substituted or non-substituted aryl or aliphatic amines in presence of triethylamine as a base in 1,4 dioxane affords the target pseudo saccharine amines derivatives **(7a-j)** in very good yield. After the formation of derivatives **(7a-j)** was justify by ^{1}H NMR, ^{13}C NMR and MS analysis.



Scheme 1. Synthesis of Pseudo-saccharine amine derivatives (7a-j)

All the newly synthesized compounds were screened for *In vitro* antimicrobial activity, against *Escherichia coli, Streptococcus aureus* and *Bacillus subtilis* using Disc diffusion method. Time and dose dependent growth inhibition assay with the result of antimicrobial activity of tested compounds **(7a-j)**, using tetracycline as reference standard, are shown in Table 1, 2 and Figure 1

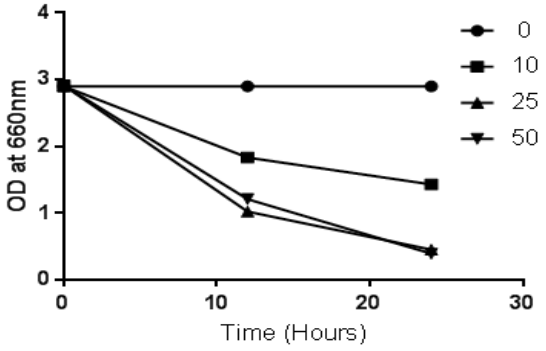
Table 1: Antimicrobial effect of compounds (7a-j) on pathogenic microorganism by using Disc diffusion method

Compound	Organism	Zone of inhibition (cm) 10 µL	50 µL	100 µL
7a	*E. coli*	0	1.5	2.5
	S. aureus	0	0	0
	Bacillus	0	0	0
7b	*E. coli*	0.9	no growth	no growth
	S. aureus	1.8	no growth	no growth
	Bacillus	3.4	no growth	no growth
7c	*E. coli*	0	1.3	2.5
	S. aureus	0	0	1.7
	Bacillus	0	0	0
7d	*E. coli*	0	2	2.5
	S. aureus	0	0	0
	Bacillus	0	1.5	2
7e	*E. coli*	0	0	0
	S. aureus	0	0	2
	Bacillus	0	0	0
7f	*E. coli*	0	1	2.2
	S. aureus	0	0	0
	Bacillus	2.3	no growth	no growth
7g	*E. coli*	2.5	no growth	no growth
	S. aureus	2.9	no growth	no growth
	Bacillus	no growth	no growth	no growth
7h	*E. coli*	0	0	0
	S. aureus	0	1.5	2.5
	Bacillus	0	1.8	2.7
7i	*E. coli*	1	no growth	no growth
	S. aureus	1.8	no growth	no growth
	Bacillus	2	no growth	no growth
7j	*E. coli*	0	0	0
	S. aureus	0	0	2
	Bacillus	1	2	3.3

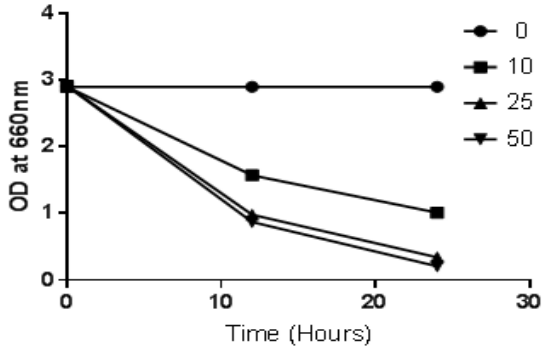
Table 2: Time and dose dependent growth inhibition assay compounds (7a-j) on pathogenic microorganism

Compound	MIC (μM) *E. coli*	*S. aureus*	*Bacillus subtilis*
7a	14.5	Inactive	Inactive
7b	6.8	8.23	7.49
7c	12.5	10.84	Inactive
7d	8.3	Inactive	9.25
7e	Inactive	Inactive	Inactive
7f	4.6	9.1	Inactive
7g	7.25	6.8	Inactive
7h	Inactive	16.1	12.6
7i	5.93	8.6	4.7
7j	Inactive	Inactive	7.01

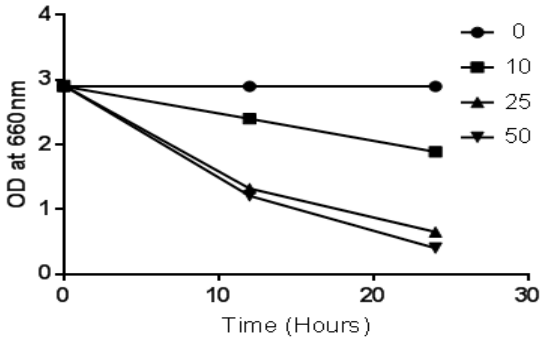
The stock concentration of the compound was 10 µg/mL



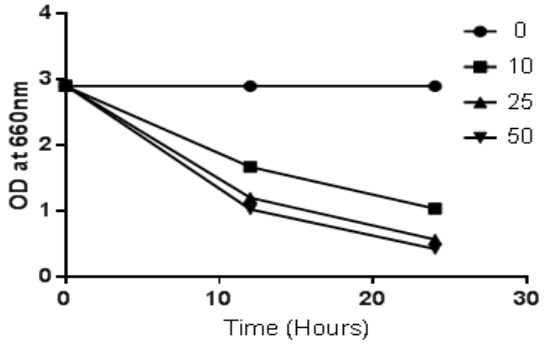
Compound 7a (*E. coli*)



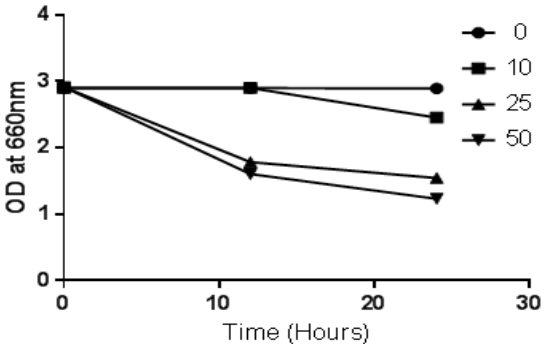
Compound 7a (*E. coli*)



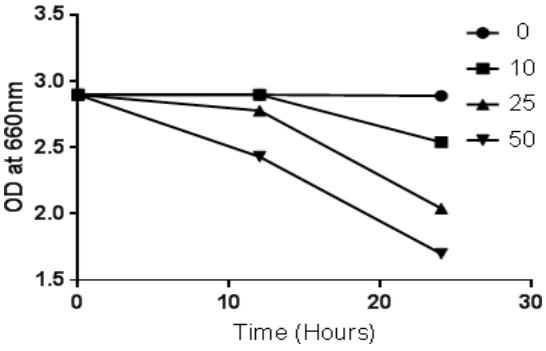
Compound 7b (*S. aureus*)



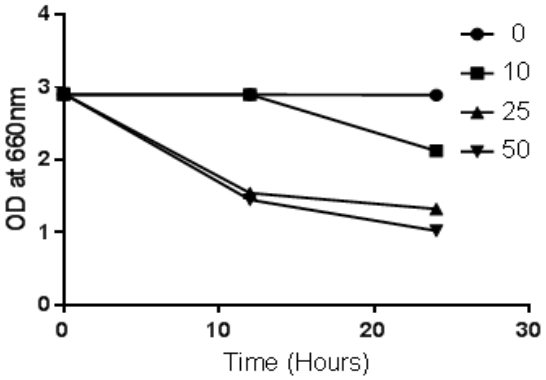
Compound 7b (*Bacillus subtilis*)



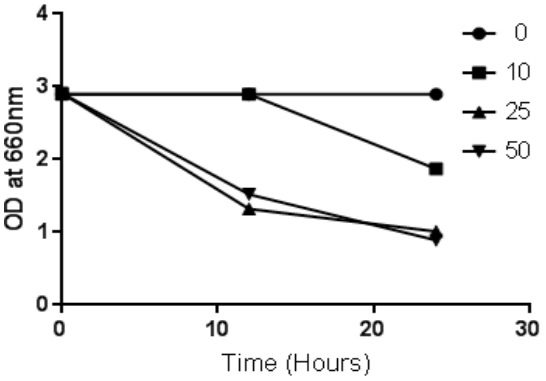
Compound 7c (*E.coli*)



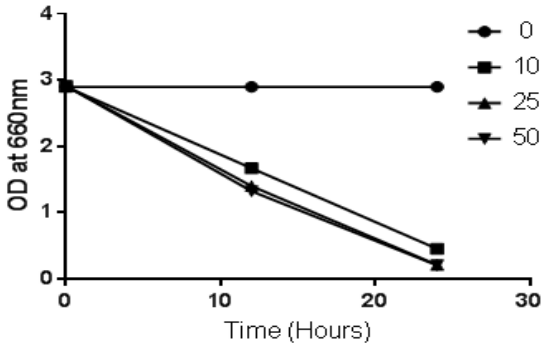
Compound 7c (*S. aureus*)



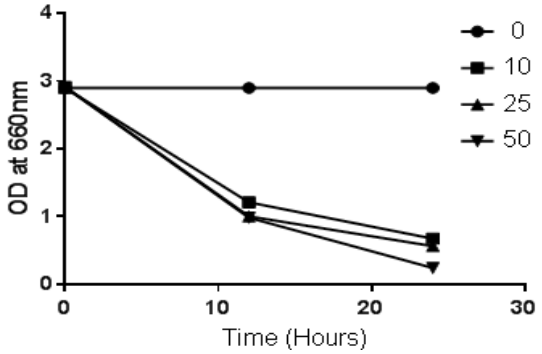
Compound 7d (*E. coli*)



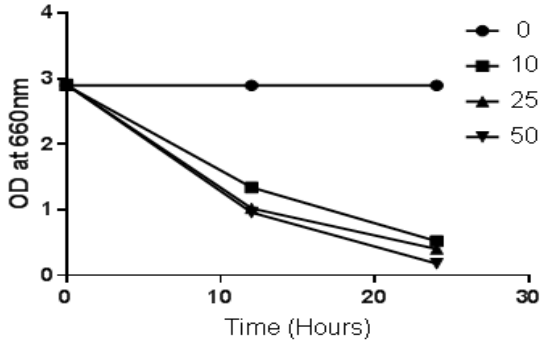
Compound 7d (*Bacillus subtilis*)



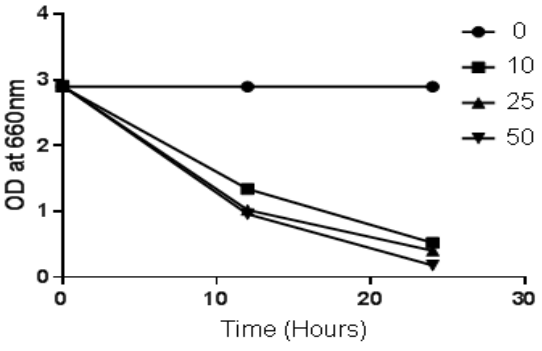
Compound 7f (*E.coli*)



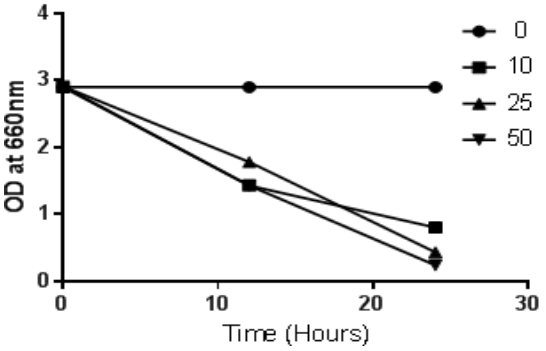
Compound 7f (*S. aureus*)



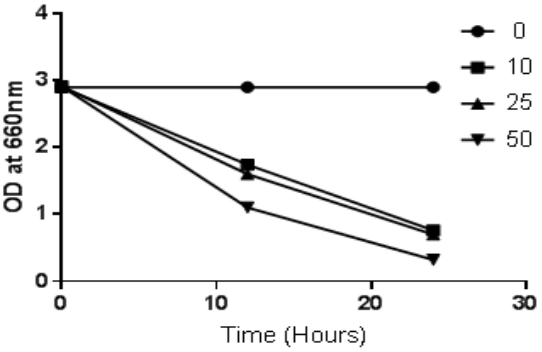
Compound 7g (*E.coli*)



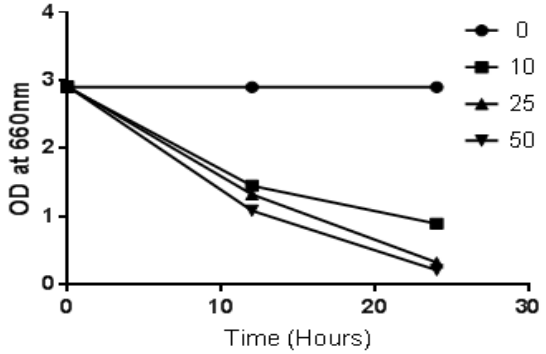
Compound 7g (*S. aureus*)



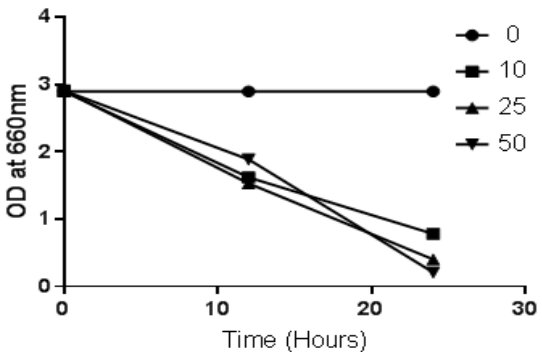
Compound 7h (*S. aureus*)



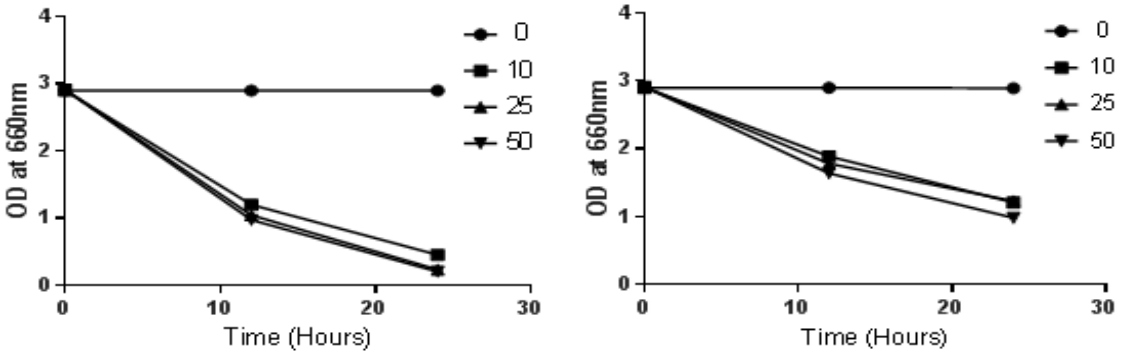
Compound 7h (*Bacillus subtilis*)



Compound 7i (*E.coli*)



Compound 7i (*S. aureus*)



Compound 7i (Bacillus subtilis) **Compound 7j (Bacillus subtilis)**

Fig. 1. Time and concentration dependent effect of compounds (7a-j) on microbes

Compound **7a** was found active against the pathogenic *E. coli* with promising zone of inhibition 1.5 and 2.5 cm at 50 µL and 100 µL respectively and not found active against *Bacillus subtilis* and *S. aureus* at any concentration. The compound **7b** was found very active against all the three bacteria. The zone of inhibition was observed at 10 µL against *E. coli, S. aureus* and *Bacillus subtilis* 0.9, 1.8 and 3.4 cm, respectively. The compound **7c** was found active against the pathogenic *E.coli* at concentration 50 µL and 100 µL with the zone of inhibition was found 1.3 and 2.5 cm respectively. The Compound 3 was also found active against *S. aureus* at 100 µL with zone of inhibition 1.7 cm. The compound **7d** was found active against *E. coli* at concentration 50 µL and 100 µL with zone of inhibition 2 and 2.5 cm respectively. The zone of inhibition was recorded at 50 µL and 100 µL concentrations against *Bacillus subtilis* were 1.5 and 2 cm respectively. The compound **7e** was found not active against any *E.coli, Bacillus subtilis* and *S. aureus* at concentration 10 µL, 50 µL and 100 µL. Compound **7f** was found active against the *E. coli* at concentration 50 and 100 µL with zone of inhibition 1.0 and 2.2 cm respectively. It was found active with *Bacillus subtilis* at concentration of 10 µL with zone inhibition at 2.3 cm and shows no growth with *S. aureus*. Compound **7g** was found active against the pathogenic *E. coli* at concentraion[10] subtilis at zone inhibition at 2.5 cm and it also found active for *S. aureus* with promising zone of inhibition 2.9 cm at 10 µL and no growth was observed at higher concentration. The compound **7h** was found active against *S. aureus* and *Bacillus subtilis* with zone of inhibition 1.5 and 1.8 cm at 50 µL concentrations respectively. The compound **7i** was found active against all the three bacteria. The zone of inhibition was observed at 10 µL against *E. coli, S. aureus* and *Bacillus subtilis* at 1.0, 1.8 and 2.0 cm respectively. The compound **7j** was found active against *Bacillus subtilis* 10 µL, 50 µL and 100 µL concentrations with zone of inhibition, 1, 2 and 3.3 cm respectively. All three bacteria were shown to be susceptible to the compounds **7b** and **7i**. At 10µM, zone inhibition against *Escherichia coli, Staphylococcus aureus*, and *Bacillus subtilis* was seen at 0.9, 1.8, and 3.9 cm for **7b** and 1.0, 1.8, and 2.0 cm for **7i**, respectively.

CONCLUSION

In summary, the eco-friendly and catalyst free protocol was developed and applied for the synthesis of saccharin derivatives. Saccharin derivatives showed significant inhibition of microbial growth. These analogues are chemically tractable and hence provide ample opportunities for further modification to obtain potent anti-microbial agents. The isolated yield of the saccharin derivatives is excellent, so gram scale synthesis is possible.

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Conflict of interest

Authors declare that they have no conflict of interest.

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