

MICROWAVE ASSISTED SYNTHESIS OF BIOLOGICALLY ACTIVE 4-HYDROXY-N'-(PHENYL CARBONYL)-2H-1,2-BENZOTHAZINE-3-CARBOHYDRAZIDE 1,1-DIOXIDE DERIVATIVES

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ABSTRACT

Microwave assisted synthesis of a series of 4-hydroxy-N'-(phenylcarbonyl)-2H-1,2-benzothiazine-3-carbohydrazide 1,1-dioxides from sodium saccharin is reported. Sodium o-benzosulfimide was N-alkylated with ethyl chloroacetate followed by base catalyzed ring expansion to six membered 1,2-benzothiazine through Gabriel-Coleman type rearrangement yielding ethyl 4-hydroxy-2H-1,2-benzothiazine-3-carboxylate 1,1-dioxide. It was later condensed with a series of benzohydrazides synthesized from various carboxylic acids to get the title compounds. All the synthesized compounds were subjected to preliminary evaluation for their biological activity against series of Gram positive, Gram negative bacteria and fungi. Some of the compounds showed marked activity against the selected microorganisms.

Key Words: Microwaves; Benzohydrazide; Carbohydrazides; 1,2-Benzothiazine; Anti-bacterial; Anti-fungal.

INTRODUCTION

There is a need for development of clean chemical processes including synthetic procedures, catalysis and reaction conditions to reduce the environmental impact of chemical & pharmaceutical industry. To achieve this, innovative and unconventional synthetic procedures using water as solvent¹ under normal² and super heated conditions³, in supercritical fluids⁴, ionic liquids⁵ and micro-emulsions⁶ are being used now a days. Besides these, ultrasound⁷ and microwaves⁸ assisted reactions both in solvent as well as in solventless conditions are becoming popular due to their shorter reaction times and greater yields.

Hydrazides and related compounds are known as useful building blocks for the synthesis of various heterocyclic rings⁹. A large number of carbohydrazides and their derivatives have been reported in literature focusing their biological activities which include anti-fungal, anti-viral & bacteriostatic¹¹, anti-parasite¹², anti-tuberculous¹³ and insecticidal¹⁴ activities. A large number of carbohydrazides and related compounds which are usually synthesized by hydrazinolysis of carboxylic acid esters are found to possess versatile activities.^{15,16}

1,2-Benzothiazine 1,1-dioxide nucleus, firstly synthesized by Braun et al¹⁷ is familiar for its various derivatives like Sudoxicam®, Ampiroxicam®, Meloxicam® and Lornoxicam®. These are known as oxicams and are being used worldwide as non-steroidal anti-inflammatory drugs (NSAIDs)¹⁸ [Figure 1]. Benzothiazine derivatives are also familiar for their other biological activities e.g., 3,4-dihydro-1,2-benzothiazine-3-carboxylate 1,1-dioxide α -ketomide and P(2)–P(3) peptide mimetic aldehyde compounds are reported as potent calpain I inhibitors¹⁹ while 1,2-benzothiazin-3-yl-quinazolin-4(3H)-ones possess anti-bacterial properties²⁰. They have also been found as anti-oxidants²¹⁻²⁴ and for the treatment of rheumatoid arthritis, ankylosing spondylitis, osteoarthritis and other inflammatory rheumatic and non-rheumatic processes²⁵.

Keeping in view the potential biological activities of 1,2-benzothiazine-1,1-dioxides and carbohydrazides, it was perceived that if both the heterocyclic moieties are synergized in a single nucleus, the newly obtained compounds may possess significant biological activities of individual compounds. In this quest, novel 4-hydroxy-N'-(phenylcarbonyl)-2H-1,2-benzothiazine-3-carbohydrazide 1,1-dioxides derivatives (**8a-l**) are synthesized by microwave assisted reactions of various benzohydrazides (**7a-l**) and ethyl 4-hydroxy-2H-1,2-benzothiazine-3-carboxylate 1,1-dioxide (**4**) along with their preliminary assay for anti-bacterial and anti-fungal activities [Scheme 1].

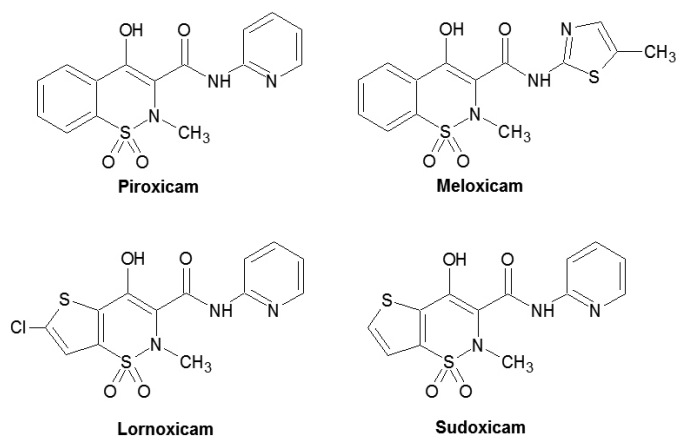


Figure 1: Structures of few commercially available oxicam drugs

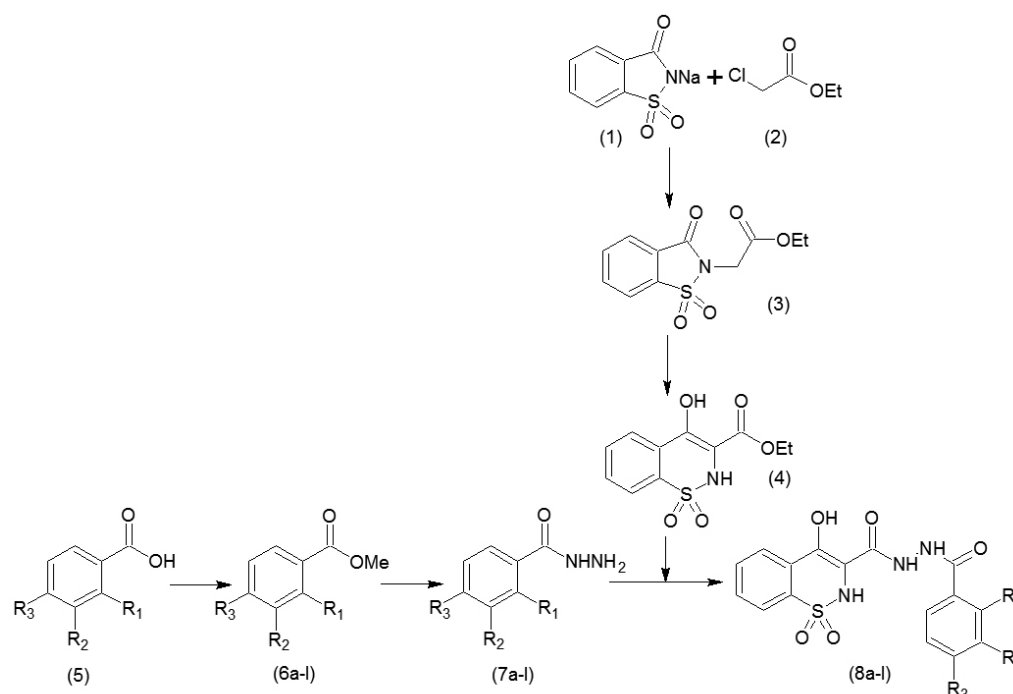
EXPERIMENTAL

Chemistry:

All the chemicals were purchased from E. Merck, BDH or Fluka and used without purification. However, solvents were purified through distillation. ¹H-NMR spectra were recorded on a Bruker DPX-400 instrument at 400 MHz. Chemical shifts are reported in ppm referenced to the residual solvent signal. FT-IR spectra were recorded on a Bruker Tensor 27 spectrometer. Mass spectra were recorded on Agilent 5973 N instrument using EI mode. Melting points were recorded on a Mettler Toledo FP 62 melting point apparatus and are uncorrected. Microwave assisted reactions were carried out in a household Microwave oven (Orient eNNe781JF) equipped with inverter technology (generating fixed frequency throughout the required time) for realistic control of the microwaves operating at multiples of 100 Watts up to 1000 Watts generating 2450 MHz frequency. The apparatus was modified for laboratory applications, equipped with magnetic stirrer and an external reflux condenser.

Synthesis of ethyl (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3H)-yl)-acetate (**3**)

A mixture of ethylchloroacetate (1.19 g, 9.71 mmol), sodium saccharin (1.50 g, 7.31 mmol) and dimethylformamide (10 ml) was taken in a round bottom flask. The mixture was heated at water bath for 4 hours. The reaction mixture was cooled to room temperature and poured into ice cold water (60 ml) to get white solid, which was filtered and washed with water. The solid was dried to get white crystalline solid mp 105-106 °C (Lit. mp 104-106 °C).¹⁷ IR (KBr): 1752, 1743, 1340, 1181 cm⁻¹. ¹H-NMR (CDCl₃), δ : 1.29 (t, J = 7.2 Hz,



Scheme 1: Schematic synthesis of 4-hydroxy-*N'*-(phenylcarbonyl)-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxides from carboxylic acids and sodium saccharin

3H), 4.30 (q, $J = 7.2$ Hz, 2H), 4.41 (s, 2H), 7.74 (dd, $J = 5.6, 3.2$ Hz, 2H), 7.80 (dd, $J = 5.6, 3.2$ Hz, 2H). MS m/z : 269 [M^+], 224 [$M^+ - OC_2H_5$]. Anal. Calcd for $C_{11}H_{11}NO_5$: C, 49.06; H, 4.12; N, 5.20; Found: C, 49.08; H, 4.11; N, 5.19. HRMS: Calcd. 269.0358, Found 269.0360.

Synthesis of ethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide (4)

Sodium metal (2.7 g, 115 mmol) was added to dry ethanol (60 ml) in small portions. On the complete dissolution of sodium, the solution was concentrated in vacuum for the synthesis of sodium ethoxide suspension. To it ester (4) (10.0g, 37.17 mmol) and absolute ethanol (30 ml) were added and sonicated for 4 minutes in ultrasonic bath at room temperature. Hydrochloric acid (430 ml) was added to the reaction mixture to precipitate the product which was filtered and washed with water.

White crystalline solid; mp 138–139 °C (Lit. mp 136–139 °C).¹⁷ IR (KBr): 3180, 1667, 1338, 1179 cm^{-1} . 1H -NMR ($CDCl_3$), δ : 1.33 (t, $J = 7.2$ Hz, 3H), 4.25 (q, $J = 7.2$ Hz, 2H), 6.42 (br s, NH), 7.74 (dd, $J = 5.6, 3.2$ Hz, 2H), 7.80 (dd, $J = 5.6, 3.2$ Hz, 2H), 12.38 (s, 1H). MS m/z : 269 [M^+], 268 [$M^+ - H$], 224 [$M^+ - OC_2H_5$]. HRMS: Calcd: 269.0358, Found: 269.0359.

General procedure for the synthesis of alkyl benzoates compounds (6a-l)

A mixture of substituted benzoic acid in methanol (50ml) and concentrated sulphuric acid (2–3 drops) was refluxed in microwave oven till the completion of reaction as indicated by TLC. After completion of reaction; excess methanol was distilled off and the content were poured in ice cold aqueous sodium bicarbonate (4%). The product obtained was washed with ice cold water and dried in oven. Experimental details are given in Table 1.

General procedure for the synthesis of substituted benzohydrazides (7a-l)

A mixture of substituted alkyl benzoate (6a-l) (0.001 moles) and hydrazine hydrate (0.001 moles) in ethanol (50ml) was refluxed in a microwave oven till the completion of reaction as indicated by TLC. After the completion of reaction; excess ethanol was distilled off and acidified with hydrochloric acid (5%). The product obtained was washed with ice cold water and dried in oven. Experimental details are given in Table 1.

General procedure for the synthesis of derivatives of 4-hydroxy-*N'*-(phenylcarbonyl)-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxides (8a-l)

A mixture of substituted benzohydrazide [7a-l] (0.001 moles) and ethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide (4) (0.001 moles; 0.269g) in ethanol (50ml) was refluxed in a microwave oven till the completion

of reaction. After the completion of reaction, excess ethanol was distilled off and acidified with HCl (5%). The product obtained was washed with ice cold water and dried in oven.

4-Hydroxy-*N'*-(2-hydroxyphenyl)carbonyl]-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8a)

Pale yellow powder; mp: 116 °C. IR (KBr) Cm^{-1} : 3749, 3653, 3562, 2365, 1643, 1348, 1177, 1069. 1H NMR ($DMSO-d_6$) (400 MHz) δ : 3.43 (1H, s, SO_2NH), 6.90–6.97 (4H, m, ArH), 7.75–7.89 (4H, m, ArH), 8.18 (1H, s, -OH), 9.72 (1H, s, -NH), 9.94 (1H, s, -NH), 13.55 (1H, s, -OH). MS m/z 375.0 [M^+]

4-Hydroxy-*N'*-(3-hydroxyphenyl)carbonyl]-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8b)

Dirty yellow powder; mp: 150 °C. IR (KBr) Cm^{-1} : 3760, 3657, 3568, 2366, 1642, 1349, 1180, 1070. 1H NMR ($DMSO-d_6$) (400 MHz) δ : 3.31 (1H, s, SO_2NH), 6.96 (1H, d, $J = 7.6$ Hz, ArH), 7.12 (1H, d, $J = 8.0$ Hz, ArH), 7.25–7.42 (4H, m, ArH), 7.77 (1H, s, ArH), 7.87 (1H, d, $J = 7.2$ Hz, ArH), 8.19 (1H, s, -OH), 9.72 (1H, s, -NH), 9.94 (1H, s, -NH), 13.55 (1H, s, -OH). MS m/z 375.0 [M^+]

4-Hydroxy-*N'*-(4-hydroxyphenyl)carbonyl]-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8c)

Dirty yellow powder; mp: 185 °C. IR (KBr) Cm^{-1} : 3753, 3655, 3568, 2365, 1648, 1345, 1189, 1078. 1H NMR ($DMSO-d_6$) (400 MHz) δ : 3.36 (1H, s, SO_2NH), 6.92 (2H, d, $J = 8.0$ Hz, ArH), 7.36 (2H, d, $J = 8.4$ Hz, ArH), 7.97–8.05 (4H, m, ArH), 8.20 (1H, s, -OH), 9.78 (1H, s, -NH), 9.99 (1H, s, -NH), 13.57 (1H, s, -OH). MS m/z 375.0 [M^+]

4-Hydroxy-*N'*-(2-methoxyphenyl)carbonyl]-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8d)

Brownish yellow powder; mp: 112 °C. IR (KBr) Cm^{-1} : 3751, 3654, 3564, 2364, 1642, 1349, 1178, 1069. 1H NMR ($DMSO-d_6$) (400 MHz) δ : 3.49 (1H, s, SO_2NH), 3.90 (3H, s, -OCH₃), 6.89–6.95 (4H, m, ArH), 7.77–7.90 (4H, m, ArH), 10.18 (1H, s, -NH), 10.31 (1H, s, -NH), 13.60 (1H, s, -OH). MS m/z 389.3 [M^+]

4-Hydroxy-*N'*-(3-methoxyphenyl)carbonyl]-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8e)

Yellow powder; mp: 152 °C. IR (KBr) Cm^{-1} : 3754, 3653, 3564, 2364, 1645, 1352, 1182, 1071. 1H NMR ($DMSO-d_6$) (400 MHz) δ : 3.37 (1H, s, SO_2NH), 3.93 (3H, s, -OCH₃), 6.95 (1H, d, $J = 7.6$ Hz, ArH), 7.16 (1H, d, $J = 8.0$ Hz, ArH), 7.26–7.45 (4H, m, ArH), 7.75 (1H, s, ArH), 7.88 (1H, d, $J = 7.2$ Hz, ArH), 9.79 (1H, s, -NH), 9.96 (1H, s, -NH), 13.61 (1H, s, -OH). MS m/z 389.3 [M^+]

4-Hydroxy-*N'*-(4-methoxyphenyl)carbonyl]-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8f)

Brownish yellow powder; mp: 119 °C. IR (KBr) Cm^{-1} : 3751, 3654, 3564,

2364, 1642, 1349, 1178, 1069. ¹H NMR (DMSO-*d*₆)(400MHz) δ: 3.39(1H, s, SO₂NH), 3.91(3H, s, -OCH₃), 6.96(2H, d, *J*=8.0 Hz, ArH), 7.29(2H, d, *J*=8.4 Hz, ArH), 7.90-8.01(4H, m, ArH), 10.19(1H, s, -NH), 10.32(1H, s, -NH), 13.56(1H, s, -OH). MS *m/z* 389.3 [M⁺].

***N*'-[(2-Bromophenyl)carbonyl]-4-hydroxy-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8g)**

Light yellow powder; mp: 116°C. IR (KBr) *Cm*⁻¹: 3751, 3654, 3564, 2364, 1642, 1349, 1178, 1069. ¹H NMR (DMSO-*d*₆)(400MHz) δ: 3.49(1H, s, SO₂NH), 6.90-6.95(4H, m, ArH), 7.78-7.89(4H, m, ArH), 10.17(1H, s, -NH), 10.30(1H, s, -NH), 13.61(1H, s, -OH). MS *m/z* 438[M⁺]

***N*'-[(3-Bromophenyl)carbonyl]-4-hydroxy-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8h)**

Light brown powder; mp: 216°C. IR (KBr) *Cm*⁻¹: 3754, 3656, 3563, 2365, 1643, 1348, 1180, 1072. ¹H NMR (DMSO-*d*₆)(400MHz) δ: 3.47(1H, s, SO₂NH), 6.95(1H, d, *J*=7.6 Hz, ArH), 7.19(1H, d, *J*=8.0 Hz, ArH), 7.25-7.44(4H, m, ArH), 7.78(1H, s, ArH), 7.89(1H, d, *J*=7.2 Hz, ArH), 10.19(1H, s, -NH), 10.27(1H, s, -NH), 13.59(1H, s, -OH). MS *m/z* 438[M⁺]

***N*'-[(4-Bromophenyl)carbonyl]-4-hydroxy-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8i)**

Light brownish powder; mp: 260°C. IR (KBr) *Cm*⁻¹: 3751, 3654, 3564, 2364, 1642, 1349, 1178, 1069. ¹H NMR (DMSO-*d*₆)(400MHz) δ: 3.39(1H, s, SO₂NH), 6.96(2H, d, *J*=8.0 Hz, ArH), 7.30(2H, d, *J*=8.4 Hz, ArH), 7.92-8.02(4H, m, ArH), 10.19(1H, s, -NH), 10.34(1H, s, -NH), 13.60(1H, s, -OH). MS *m/z*: 438[M⁺], 440[M⁺+2].

4-Hydroxy-*N*'-[(2-nitrophenyl)carbonyl]-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8j)

Pale yellow powder; mp: 155°C. IR (KBr) *Cm*⁻¹: 3754, 3651, 3563, 2363, 1644, 1347, 1182, 1071. ¹H NMR (DMSO-*d*₆)(400MHz) δ: 3.50(1H, s, SO₂NH), 6.90-6.96(4H, m, ArH), 7.80-7.91(4H, m, ArH), 10.16(1H, s, -NH), 10.29(1H, s, -NH), 13.62(1H, s, -OH). MS *m/z* 403.9 [M⁺]

4-Hydroxy-*N*'-[(3-nitrophenyl)carbonyl]-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8k)

Yellow powder; mp: 230°C. IR (KBr) *Cm*⁻¹: 3751, 3654, 3564, 2364, 1642, 1349, 1178, 1069. ¹H NMR (DMSO-*d*₆)(400MHz) δ: 3.31(1H, s, SO₂NH), 6.98(1H, d, *J*=7.6 Hz, ArH), 7.14(1H, d, *J*=8.0 Hz, ArH), 7.23-7.41(4H, m, ArH), 7.79(1H, s, ArH), 7.88(1H, d, *J*=7.2 Hz, ArH), 9.77(1H, s, -NH), 9.98(1H, s, -NH), 13.59(1H, s, -OH). MS *m/z* 403.9 [M⁺]

4-Hydroxy-*N*'-[(4-nitrophenyl)carbonyl]-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxide (8l)

Pale yellow powder; mp: 155°C. IR (KBr) *Cm*⁻¹: 3754, 3651, 3563, 2363, 1644, 1347, 1182, 1071. ¹H NMR (DMSO-*d*₆)(400MHz) δ: 3.49(1H, s, SO₂NH), 6.87-6.97(4H, m, ArH), 7.67-7.82(4H, m, ArH), 10.17(1H, s, -NH), 10.33(1H, s, -NH), 13.62(1H, s, -OH). MS *m/z* 403.9 [M⁺]

ANTI-MICROBIAL ACTIVITIES:

Anti-Bacterial Activity: The anti-bacterial assay was carried out by microdilution method^{26,27} against human pathogenic bacteria. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0 x 10⁵ colony forming unit (CFU)/ml. The inocula were prepared daily and stored at +4°C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum. The minimum inhibitory concentrations (MICs) were determined using 96-well microtitre plates. Compounds under investigation were dissolved in broth LB medium (100 µl) with bacterial inoculum (1.0 x 10⁴ cfu per well) to achieve the desired concentrations (1mg/ml). All the microplates were incubated for 24 hours at 48°C. The optical density of each well was measured at a wavelength of 655 nm and compared with a blank. Streptomycin was used as a positive control (1 mg/ml DMSO).

Anti-fungal Activity: Anti-fungal screening of the newly synthesized compounds was carried out by micro dilution technique²⁶ against *Aspergillus flavus*, *Trichoderma reesei*, *Drechslera australiensis*, *Trichoderma viride*, *Alternaria alternata* and *Macrophomina phaseolina*. The micromycetes were maintained on malt agar and the cultures were stored at 4°C and sub-cultured once a month²⁸. Fungal spores were washed from the surface of agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v) and the spore suspension was adjusted with sterile saline to a concentration of approximately 1.0 x 10⁵ in a final volume of 100 µl per well. Minimum inhibitory concentration (MIC) determinations were performed by a serial dilution technique using 96-well microtiter plates. The compounds investigated were dissolved in DMSO (1 mg/ml) and were added in broth malt medium with inoculum. The microplates were incubated for 72 hours at 28°C. The lowest concentrations without visible growth (at the binocular microscope) were defined as MICs.

RESULTS AND DISCUSSION

During the course of the work, using the green innovative technology ethyl (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)- acetate (**3**) was synthesized by the reaction of sodium saccharin (**1**) and ethyl chloroacetate (**2**) followed by its ring expansion to six membered benzothiazine through Gabriel-Coleman type rearrangement yielding ethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide (**4**). The formation of ethyl (1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)- acetate (**3**) was carried out on water bath and the formation of ethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide (**4**) was carried out using ultrasonic waves.

Synthesis of alkyl benzoates (**6a-l**), substituted benzohydrazides (**7a-l**) and 4-hydroxy-*N*'-(phenylcarbonyl)-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxides compounds (**8a-l**) were synthesized as per scheme 1 through environment friendly fashion by the use of microwaves. Alkyl benzoate derivatives (**6a-l**), were synthesized by acid catalysis of respective benzoic acid under microwaves in excellent yields (92 - 97%; Table 1) which were converted to the corresponding benzohydrazide derivatives (**7a-l**) by microwave induced hydrazinolyses in very good to excellent yields (81-96%). These benzohydrazides were then reacted with ethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide (**4**) to get a series of 4-hydroxy-*N*'-(phenylcarbonyl)-2*H*-1,2-benzothiazine-3-carbohydrazide 1,1-dioxides (**8a-l**). These reactions were carried out under the influence of microwaves and were completed in good yields with in shorter reaction times (Table 1). All of the newly synthesized compounds were characterized through spectroscopic techniques and their data was found in accordance to the proposed structures.

Anti-bacterial activity

All the newly synthesized carbohydrazide derivatives (dissolved in dimethylformamide) were subjected to anti-bacterial screening against three gram positive bacteria (*Bacillus cereus*, *Bacillus subtilis* and *Bacillus thuringiensis*) and three gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*) by determining their minimum inhibitory concentrations (MICs) using agar dilution technique²⁹ and streptomycin as a reference drug. The choice of streptomycin as a clinical standard was made due to the fact that at lower concentrations, it only inhibits growth of the bacteria through induction of prokaryotic ribosomes to misread mRNA. It also prevents initiation of protein synthesis and leads to death of microbial cells. Also, in humans, they have structurally different ribosomes from bacteria, thereby allowing the selectivity of this antibiotic for bacteria²⁷.

The Minimum inhibitory concentrations of the synthesized compounds against selected bacteria are presented in Table 2. Results of anti-bacterial activity of compounds show that the minimum inhibitory concentration (MIC) of compounds varies in the range of 6.0 - 67.0 x 10⁻² µmol/ml. It was found that the title compounds were found more active against gram positive bacteria than gram negative bacteria. The most sensitive bacterial species on these compounds is *Bacillus subtilis*, while *Pseudomonas aeruginosa* is the most resistant species. An insight to the structure-activity relationship gives an idea that activity generally increases with the incorporation of hydroxyl and methoxy groups at the benzene ring of the title compounds. Hydroxy derivatives (**8a-8c**) were found the most active with MIC values comparable to the standard and this may be attributed to the presence of lone pairs on oxygen atoms of hydroxyl groups perhaps by blocking the active sites through hydrogen bonding. Compounds having methoxy groups are found weaker than the hydroxyl analogues probably due to steric hindrance of methyl group. Compounds possessing nitro groups (**8j-8l**) were found least active against both the gram positive and gram negative strains.

Table 1. Data of the synthesized compounds (6a- 8l).

Entry	Compound	R ₁	R ₂	R ₃	M.P/ B.P	Reaction time	Yield
	6a	OH	H	H	224°C*	17 minutes	94%
	6b	H	OH	H	70°C	19 minutes	93%
	6c	H	H	OH	126°C	13 minutes	96%
	6d	OCH ₃	H	H	247°C*	18 minutes	94%
	6e	H	OCH ₃	H	238°C*	20 minutes	95%
	6f	H	H	OCH ₃	50°C	15 minutes	96%
	6g	Br	H	H	241°C*	15 minutes	95%
	6h	H	-Br	H	140°C*	18 minutes	94%
	6i	H	H	Br	78°C	12 minutes	96%
	6j	NO ₂	H	H	275°C*	19 minutes	92%
	6k	H	NO ₂	H	78°C	14 minutes	97%
	6l	H	H	NO ₂	96°C	16 minutes	95%
	7a	OH	H	H	148°C	10 minutes	85%
	7b	H	OH	H	88°C	15 minutes	81%
	7c	H	H	OH	266°C dec.	12 minutes	93%
	7d	OCH ₃	H	H	78°C	13 minutes	87%
	7e	H	OCH ₃	H	92°C	16 minutes	86%
	7f	H	H	OCH ₃	137°C	11 minutes	96%
	7g	Br	H	H	152°C	14 minutes	89%
	7h	H	Br	H	155°C	15 minutes	83%
	7i	H	H	Br	168°C	14 minutes	91%
	7j	NO ₂	H	H	120°C	18 minutes	88%
	7k	H	NO ₂	H	152°C	12 minutes	95%
	7l	H	H	NO ₂	215°C	15 minutes	91%
	8a	OH	H	H	116°C	21minutes	81%
	8b	H	OH	H	150°C	23 minutes	79%
	8c	H	H	OH	185°C	17 minutes	84%
	8d	OCH ₃	H	H	112°C	15 minutes	81%
	8e	H	OCH ₃	H	152°C	18 minutes	77%
	8f	H	H	OCH ₃	119°C	12 minutes	85%
	8g	Br	H	H	116°C	20 minutes	75%
	8h	H	Br	H	216°C	25 minutes	73%
	8i	H	H	Br	260°C	15 minutes	80%
	8j	NO ₂	H	H	155°C	22 minutes	82%
	8k	H	NO ₂	H	230°C	14 minutes	86%
	8l	H	H	NO ₂	155°C	20 minutes	74%

* = Boiling point

Dec. = Decomposition

Table 2. Anti-bacterial activity of compounds (8a-l) tested by micro dilution method (MIC $\mu\text{mol} \times 10^{-2}$).

Entry	Compound	Susceptible Bacteria					
		<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>	<i>B. cereus</i>	<i>B. subtilis</i>	<i>B. thuringiensis</i>
1	8a	25	26	22	9	8	22
2	8b	21	23	21	11	6	20
3	8c	30	28	24	14	9	23
4	8d	39	35	38	17	14	20
5	8e	37	31	33	16	18	19
6	8f	40	46	42	19	15	21
7	8g	60	56	55	22	22	25
8	8h	66	59	51	18	24	23
9	8i	61	67	49	20	20	27
10	8j	58	44	48	20	17	18
11	8k	59	49	42	20	19	20
12	8l	63	51	52	23	24	18
13	Control	0	0	0	0	0	0
14	Streptomycin	16.5	16.5	16.5	4.3	4.2	4.4

Anti-fungal activities

All the newly synthesized compounds **8a–8l** (dissolved in dimethylsulfoxide) along with ethyl 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide (**4**), bifonazole (reference drug) and control **0** were subjected to in-vitro anti-fungal screening by determining the minimum inhibitory concentration (MICs) through micro dilution technique²⁹ against a panel of six strains of fungi i.e., *Aspergillus flavus*, *Trichoderma reesei*, *Drechslera australiensis*, *Trichoderma viride*, *Alternaria alternata* and *Macrophomina phaseolina*.

Results of anti-fungal activity of compounds show that the minimum inhibitory concentration (MIC) of compounds varies in the range of 68.1 – 124.0 x 10⁻² µmol/ml [Table 3]. Compounds **8j**, **8k** and **8l** showed the best activity against all the fungi, with **8i** exhibiting the highest antifungal potential (MIC 68.1 x 10⁻² µmol/ml, while compounds **8g**, **8h** and **8i** were found moderately active. Rest of the compounds were found almost inactive (with MIC >100 x 10⁻² µmol/ml).

Table 3. Anti-fungal activity of compounds (8a-l) tested by micro dilution method (MIC µmol x 10⁻²).

Entry	Compound	Susceptible Fungi					
		<i>Aspergillus flavus</i>	<i>Trichoderma reesei</i>	<i>Drechslera australiensis</i>	<i>Trichoderma viride</i>	<i>Alternaria alternata</i>	<i>Macrophomina phaseolina</i>
1	8a	>100	98.0	>100	97.3	98.0	96.5
2	8b	>100	96.5	>100	94.2	>100	95.2
3	8c	>100	99.6	>100	97.9	>100	>100
4	8d	>100	81.3	>100	98.8	>100	>100
5	8e	>100	80.6	92.8	97.6	>100	>100
6	8f	>100	79.9	89.9	95.1	93.3	96.2
7	8g	>100	77.4	99.7	75.6	81.5	87.4
8	8h	>100	74.9	98.8	74.4	78.9	85.6
9	8i	>100	72.7	95.4	71.6	77.3	83.9
10	8j	>100	72.5	92.0	71.9	80.1	82.6
11	8k	>100	70.1	90.9	69.6	77.8	81.2
12	8l	>100	68.4	87.0	68.1	74.8	78.3
13	0	0	0	0	0	0	0
14	4	>100	>100	>100	>100	>100	>100
15	Bifonazole	48.0	60.2	57.5	64.0	58.0	58.0

Results showed moderate to significant activity of almost all the compounds against *Trichoderma reesei*, *Drechslera australiensis*, *Trichoderma viride*, *Alternaria alternata* and *Macrophomina phaseolina* while these were found inactive against *Aspergillus flavus*; all of the compounds were either found inactive or with MIC greater than 100. Majority of the compounds showed good activity against *Trichoderma viride* and *Trichoderma reesei* while *Aspergillus flavus* is the most resistant species. An insight to the structure–activity relationship gives an idea that activity generally increases with substitution groups having electron withdrawing nature.

CONCLUSION

The present study revealed that the compounds obtained by synergism of 4-hydroxy-2*H*-1,2-benzothiazine-3-carboxylate 1,1-dioxide moiety with different carbonylhydrazides in one frame were found to possess anti-bacterial & anti-fungal activities and could be useful as a template for future development through modification or derivatization to design more potent biologically active compounds. The new skeleton may also possess other biological activities of the parent ring systems, if tested.

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