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ARTICLE

Synthesis of Novel Oxazin Analogs of Thio-4-azaspiro[4.5]decan-3-one for their Antimicrobial Activity

Raj Bahadur Singh[1], Krishna Srivastava[1,✉], Ram Prakash Tiwari[1] and Jyoti Srivastava[2]

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ABSTRACT

The present work reports the synthesis of 4-(1-(substituted phenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)-1-thia-4-azaspiro-[4.5]-decan-3-one **IV(a-h)** by 4-(((substituted phenyl)(2-hydroxy-naphthalen-1-yl) ethyl)amino)-1-thia-4-azaspiro[4.5]decan-3-one with formaldehyde in acetonitrile, containing a spiro ring obtained from the reaction of cyclohexylidene hydrazine and thioglycollic acid in DMF (cyclohexanone reacts with hydrazine hydrate in pyridine). The structures of the synthesized compounds have been established on the basis of elemental analysis, UV-vis absorption spectroscopy, IR, ^{1}H NMR and mass spectral studies. The *in vitro* antimicrobial screening of all novel compounds was done against *S. aureus, E. coli*, *P. aeruginosa* and *B. subtilis*. The activity of compounds **IVb**, **IVc**, **IVe** and **IVf** compounds showed moderate to good activity against the tested microbes.

KEYWORDS

Thioglycolic acid, β-Naphthol, Aromatic aldehyde, Hydrazine hydrates, Antimicrobial activity, Cyclohexone.

INTRODUCTION

A large number of heterocyclic compounds are synthesized in the laboratory or are isolated from the natural resources everyday signifying their immense biological and industrial importance and utility [1-5]. The heterocyclic compounds, primarily consisting of nitrogen, oxygen ring system display a broad range of biological and immunological applications such as antimicrobial, antioxidant, anti-inflammatory, anti-HIV, anticonvulsant, agent and play a dynamic role in the pharmaceutical and drug industry apart from their other diverse utilizations [6-12].

The enormous mainstream difficulties, confronting the people, in maintaining their well-being, presently are cancer and contagious microbial diseases [13]. As cancer remains one of the most common reason for the death of people all over the world, bacterial infections also presents a severe menace to human lives, owing to its incipient resistant ability to prevailing antimicrobial drugs. The identification of new specific anticancer agents with tissue selective activity for use in chemotherapy, therefore, is an area of immense importance in drug research [14]. Therefore, there is a vigorous necessity

Author affiliations:

[1]Faculty of Chemical Sciences, Shri Ramswaroop Memorial University, Lucknow-225003, India
[2]Department of Environmental Science, Babasaheb Bhim Rao Ambedkar University, Lucknow-226025, India

✉To whom correspondence to be addressed:

E-mail: rintu_srivastava@rediffmail.com

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for the development of new anticancer compounds with improved efficacy and reduced side effects as well as antimicrobial molecules with effective activity and lesser resistance against broad array of microorganisms.

EXPERIMENTAL

The melting points were examined in open capillaries on Jindal melting point apparatus and are uncorrected. Routine assessment of the purity of the compounds was determined by thin layer chromatography (TLC) using silica gel G (Merck). The ^{1}H & ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO on Bruker NMR spectrophotometer at 300 MHz. Tetramethylsilane (TMS) was taken as the internal standard and chemical shift value (δ) are given in part per million (ppm). The following instruments were employed: Jasco FTIR-470 spectrophotometer (KBr) with diffuse reflectance method; MS-JEOL SX102 Mass spectroscopy by using Argon/Xenon (6 kV, 10 mA) as the FAB gas and *m*-nitro benzyl alcohol (NBA) as the matrix. The UV analysis of the sample were carried out on double beam UV spectrophotometer.

Synthesis of cyclohexylidenehydrazine (I): A cyclohexanone (0.02 mol) and hydrazine hydrate (0.02 mol) in pyridine (30 mL) was heated under reflux on a sand-bath for 6 h. The reaction was monitored by thin-layer chromatography (TLC) with the methanol-chloroform. After completion, the reaction mixture was poured into crushed ice and acidified with dil. HCl. The cyclohexylidenehydrazine precipitates out as solid. Then, it was filtered and crystallized from ethanol. Yield: 88%; m.p.: 130-131 ºC; Anal. calcd. for $C_6H_{12}N_2$ (m.w. = 112.17) calcd. (found) %: C, 64.24 (64.19), N, 24.97 (24.91). IR (KBr, ν_{max}, cm^{-1}): 1632 (C=N), 2342 (–N–N–).

Synthesis of 4-amino-1-thio-4-azaspiro[4.5]decan-3-one (II): Cyclohexylidenehydrazine (**I**) (0.02 mol) of cyclohexylidenehydrazine and thioglycollic acid (0.01 mol) containing in trace $ZnCl_2$ (0.1 g) in DMF was heated under reflux for 4 h. It was poured into crushed ice and stirred vigorously. Solidification occurred after 15 min, which was filtered off and washed with cold water. Recrystallization from ethanol gave pure sample. Yield: 77%; m.p.: 142-143 ºC; Anal. calcd. (found) % for $C_8H_{14}N_2OS$ (m.w. = 186.27): C, 51.58 (51.46); N, 15.02 (14.97). IR (KBr, ν_{max}, cm^{-1}): 1757 (C=O), 1629 (CH=CH), 3142 (OH).

Synthesis of 4-(((substituted phenyl)(2-hydroxynaphthalen-1-yl)methyl)amino)-1-thia-4-azaspiro[4.5]decan-3-one (IIIa-h): Compound **II** (0.02 mol), aromatic aldehyde (0.02 mol) and β-naphthol (0.02 mol) in DMF (25 mL) was refluxed with stirring at 110-120 ºC for 4 h. After completion of the reaction as indicated by TLC, the reaction was cooled to room temperature. The reaction mixture poured over ice solution. The solid obtained was filtered, washed with ethanol (3 × 10 mL) thrice. The resulting product was recrystallized in ethanol to obtain the product.

4-(((4-Chlorophenyl)(2-hydroxynaphthalen-1-yl)-methyl)amino)-1-thia-4-azaspiro[4.5]decan-3-one (IIIa): Yield: 60%; m.p.: 178-179 ºC; Anal. calcd. (found) % for $C_{25}H_{25}ClN_2O_2S$ (m.w. = 453): C, 66.28 (66.21); N, 6.18 (6.12); IR (KBr, ν_{max}, cm^{-1}): 1664 (C=O), 1066 (C-S-C), 3110 (arom. C-H str.), 1595 (C=C skeletal), 855 (C-N), 844 (N-N), 580 (C-Cl), 1430 (cyclohexane, CH_2); ^{1}H NMR ($CDCl_3$) (δ ppm): 6.90-7.82 (m, 10H, Ar-H), 3.21 (s, 2H, O=CCH_2-S), 1.2-1.9 (m, 10H, cyclohexane ring), 4.71 (s, 1H, Ar-OH), 8.92 (1H, -N-NH-C-), 3.89 (d, 1H, NCH-R); Mass: M^+ 452, 418, 268, 186, 118, 84.

4-(((4-Bromophenyl)(2-hydroxynaphthalen-1-yl)-methyl)amino)-1-thia-4-azaspiro[4.5]decan-3-one (IIIb): Yield: 71%, m.p.: 166-167 ºC; Anal. calcd. for $C_{25}H_{25}BrN_2O_2S$ (m.w. = 497.45): C, 60.36 (60.29); N, 5.63 (5.58); IR (KBr, ν_{max}, cm^{-1}): 1678 (C=O), 3585 (Ar-OH), 1067 (C-S-C), 3115 (arom. C-H *str.*), 1591 (C=C skeletal), 852 (N-N), 865 (C-N), 638 (C-Br); ^{1}H NMR ($CDCl_3$) (δ ppm): 6.71-7.77 (m, 10H, Ar-H), 3.31 (s, 2H, O=CCH_2-S), 1.2-2.1 (m, 10H, cyclohexane ring), 4.53 (s, 1H, Ar-OH), 8.86 (1H, -N-NH-C-), 3.79 (d, 1H, NCH-R); Mass: M^+ 498, 418, 312, 186, 118, 84.

4-((2-Hydroxynaphthalen-1-yl)((3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)amino)methyl)benzaldehyde (IIIc): Yield: 59%, m.p.: 148-149 ºC; Anal. calcd. (found) % for $C_{26}H_{26}N_2O_3S$ (m.w. = 446.56): C, 69.93 (69.85); N, 6.27 (6.23); IR (KBr, ν_{max}, cm^{-1}): 1648 (C=O), 3566 (Ar-OH), 1041 (C-S-C), 3012 (arom. C-H str.), 1582 (C=C skeletal), 874 (N-N), 892 (C-N); ^{1}H NMR ($CDCl_3$) (δ ppm): 8.96 (1H, -N-NH-C-), 6.81-7.96 (m, 11H, Ar-H)), 3.18 (s, 2H, O=CCH_2-S), 1.2-2.9 (m, 10H, cyclohexane ring), 4.52 (s, 1H, Ar-OH), 3.62 (d, 1H, NCH-R); 446, 418, 234, 186, 118, 84.

4-(((2-Hydroxynaphthalen-1-yl)(4-hydroxyphenyl)-methyl)amino)-1-thia-4-azaspiro[4.5]decan-3-one (IIId): Yield: 66%, m.p.:190-191 ºC; Anal. calcd. (found) % for $C_{25}H_{26}N_2O_3S$ (m.w. = 434.55): C, 69.10 (69.08); N, 6.45 (6.38); IR (KBr, ν_{max}, cm^{-1}): 1654 (C=O), 3566 (Ar-OH), 1037 (C-S-C), 3044 (arom. C-H *str.*), 1562 (C=C skeletal), 863 (N-N), 878 (C-N); ^{1}H NMR ($CDCl_3$) (δ ppm): 6.78-7.87 (m, 10H, Ar-H), 3.12 (s, 2H, O=CCH_2-S), 1.2-2.6 (m, 10H, cyclohexane ring), 4.61 (s, 1H, Ar-OH), 3.52 (d, 1H, NCH-R), 8.92 (1H, -N-NH-C-); Mass: M^+ 434, 418, 250, 186, 118, 84.

4-(((2-Hydroxynaphthalen-1-yl)(2-hydroxyphenyl)-methyl)amino)-1-thia-4-azaspiro[4.5]decan-3-one (IIIe): Yield: 64%, m.p.: 175-176 ºC; Anal. calcd. (found)% for $C_{25}H_{26}N_2O_3S$ (m.w. = 434.55): C, 69.10 (69.05), N, 6.45 (6.41); IR (KBr, ν_{max}, cm^{-1}): 1677 (C=O), 3640 (Ar-OH), 1071 (C-S-C), 3082 (arom. C-H str.), 1590 (C=C skeletal), 870 (N-N), 885 (C-N); ^{1}H NMR ($CDCl_3$) (δ ppm): 4.78 (s, 1H, Ar-OH), 6.81-7.90 (m, 10H, Ar-H), 4.74 (brs, 1H, s, replaceable-OH), 3.35 (s, 2H, O=CCH_2-S), 1.2-2.6 (m, 10H, cyclohexane ring), 4.61 (s, 1H, Ar-OH), 8.91 (1H, -N-NH-C-), 3.81 (d, 1H, NCH-R); Mass: M^+ 434, 418, 250, 186, 118, 84.

4-(((2-Hydroxynaphthalen-1-yl)(2-nitrophenyl)-methyl)amino)-1-thia-4-azaspiro[4.5]decan-3-one (IIIf): Yield: 68%, m.p.: 131-132 ºC; Anal. calcd. (found) % for $C_{25}H_{25}N_3O_4$ (m.w. = 463.55): C, 64.78 (64.73), N, 9.06 (9.01); IR (KBr, ν_{max}, cm^{-1}): 1644 (C=O), 3590 (Ar-OH), 1075 (C-S-C), 3090 (arom. C-H *str.*), 1585 (C=C skeletal), 855 (N-N), 874 (C-N),1522 (N=O *str*. asym), 1338 (N=O *str.*, sym); ^{1}H NMR ($CDCl_3$) (δ ppm): 6.81-7.90 (m, 10H, Ar-H), 3.11 (s, 1H, -N-CHS-R), 3.26 (s, 2H, O=CCH_2-S), 8.82 (1H, -N-NH-C-), 3.81 (d, 1H, NCH-R), 1.1-2.8 (m, 10H, cyclohexane ring), 4.75 (s, 1H, Ar-OH); Mass: M^+ 463, 418, 279, 186, 118, 84.

4-(((3-Hydroxy-4-methoxyphenyl)(2-hydroxynaphthalen-1-yl)methyl)amino)-1-thia-4-azaspiro[4.5]decan-3-one (IIIg): Yield: 57%, m.p.: 182-183 ºC; Anal. calcd. (found) % for $C_{26}H_{28}N_2O_4S$ (m.w. = 464.58): C, 67.22 (67.17); N, 6.03 (6.01); IR (KBr, ν_{max}, cm^{-1}): 1670 (C=O), 3620 (Ar-OH), 1052 (C-S-C), 3095 (arom. C-H *str.*), 1580 (C=C skeletal), 890 (N-N), 870 (C-N *str*.), 3436 (3-OH, 3-hydroxyphenyl), 1685 (OCH_3, *p*-methoxyphenyl); ^{1}H NMR (δ in ppm) ($CDCl_3$): 6.85-7.90 (m, 09H, Ar-H), 4.80 (brs, 1H, s, replaceable-OH), 3.15 (s, -N-CHS-R 1H), 3.44 (s, 2H, O=CCH_2-S), 1.3-2.7 (m, 10H, cyclohexane ring), 8.97 (1H, -N-NH-C-), 3.95 (d, 1H, NCH-R), 2.68 (s, 3H, Ar-OCH_3); Mass: M^+ 464, 418, 280, 186, 118, 84.

4-(((4-Hydroxy-3-methoxyphenyl)(2-hydroxynaphthalen-1-yl)methyl)amino)-1-thia-4-azaspiro[4.5]decan-3-one (IIIh): Yield: 70%, m.p.: 189-190 ºC; Anal. calcd. (found) % for $C_{26}H_{28}N_2O_4S$ (m.w. = 464.58): C, 67.22 (67.16); N, 6.03 (5.96); IR (KBr, ν_{max}, cm^{-1}): 1680 (C=O), 3645 (Ar-OH), 1062 (C-S-C), 3085 (arom. C-H *str.*), 1590 (C=C skeletal), 875 (N-N), 895 (C-N *str*.), 3445 (3-OH, 3-hydroxyphenyl), 1675 (OCH_3, *p*-methoxyphenyl); ^{1}H NMR ($CDCl_3$) (δ ppm): 6.88-7.92 (m, 9H, Ar-H), 4.68 (brs, 1H, s, replaceable-OH), 3.34 (s, -N-CHS-R 1H), 3.42 (s, 2H, O=CCH_2-S), 2.88 (s, 3H, N=C-CH_3), 3.86 (d, 1H, NCH-R), 2.85 (s, 3H, Ar-OCH_3), 1.1-2.6 (m, 10H, cyclohexane ring), 8.95 (-N-NH-C-); Mass: M^+ 464, 418, 280, 186, 118, 84.

Synthesis of 4-(1-(substitutedphenyl)-1*H*-naphtho [1,2-*e*][1,3]oxazin-2(3*H*)-yl)-1-thia-4-azaspiro[4.5]decan-3-one (IVa-h): The target compounds were synthesized by taking (0.02 mol) compound **III(a-i)** and formedehyde (37%, 0.02 mol) were taken in acetonitrile (15 mL). The reaction was refluxed while stirring for 4 h. After the completion of the reaction (monitored by TLC), the solvent was removed and 30 mL water was added. The content was extracted with ethyl acetate and solvent distilled out under reduce pressure (**Scheme-I**). The residue obtained was purified by recystallization from ethanol to produce good yield.

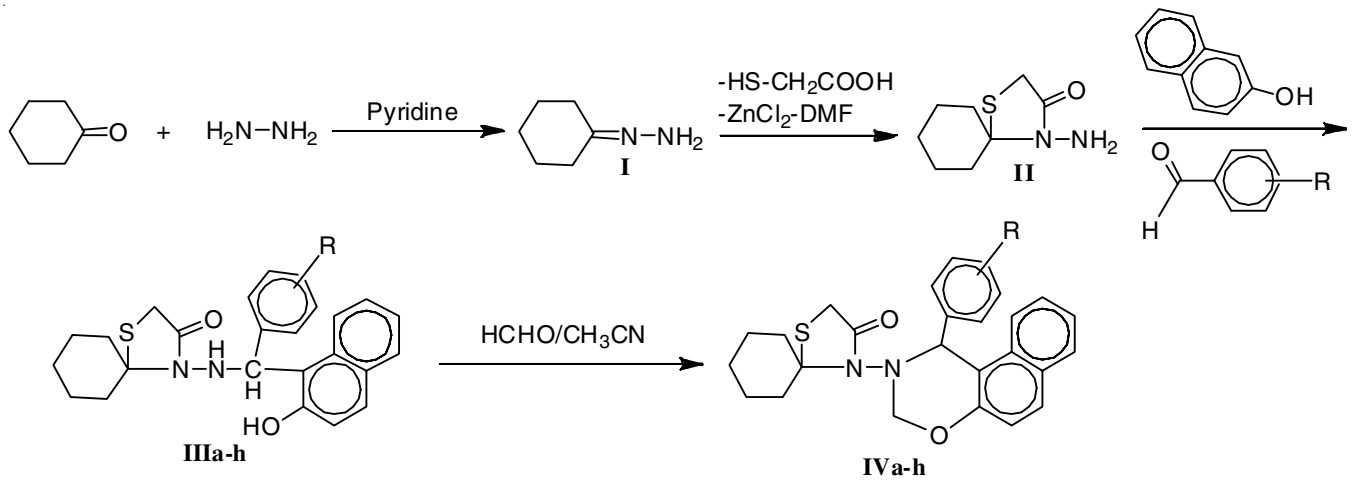
4-(1-(4-Chlorophenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)-1-thia-4-azaspiro[4.5]decan-3-one (IVa): Yield: 68%, m.p.: 133-134 ºC; Anal. calcd. (found) % for $C_{26}H_{25}ClN_2O_2S$ (m.w. = 465.01): C, 67.16 (67.11); N, 6.02 (5.95); IR (KBr, ν_{max}, cm^{-1}): 1678 (C=O), 1088 (C-S-C), 3086 (arom. C-H *str.*), 1570 (C=C skeletal), 870 (C-N), 850 (N-N.), 578 (C-Cl), 1117 (C-O-C), 1439 (cyclohexane, CH_2); ^{1}H NMR ($CDCl_3$) (δ ppm): 6.70-8.78 (m, 10H, Ar-H), 3.36 (s, 2H, O=CCH_2-S), 3.95 (s, 2H, C-O-CH_2-N), 1.3-1.8 (m, 10H, cyclohexane ring). Mass: M^+ 464, 430, 295, 261, 171, 84.

4-(1-(4-Bromophenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)-1-thia-4-azaspiro[4.5]decan-3-one (IVb): Yield: 71%. m.p.: 116-117 ºC; Anal. calcd. (found) % for $C_{26}H_{25}BrN_2O_2S$ (m.w. = 409.46): C, 61.30 (61.22); N, 5.50 (5.47); IR (KBr, ν_{max}, cm^{-1}): 1132 (C-O-C), 1437 (cyclohexane, CH_2), 1683 (C=O), 1076 (C-S-C), 3078 (arom. C-H *str.*), 1573 (C=C skeletal), 880 (C-N), 864 (N-N), 560 (C-Br); ^{1}H NMR ($CDCl_3$) (δ ppm): 6.80-8.74 (m, 10H, Ar-H), 1.3-1.8 (m, 10H, cyclohexane ring), 3.32 (s, 2H, O=CCH_2-S), 3.98 (s, 2H, C-O-CH_2-N), Mass: M^+ 510, 430, 339, 261, 171, 84.

4-(2-(3-Oxo-1-thia-4-azaspiro[4.5]decan-4-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazin-1-yl)benzaldehyde (IVc): Yield: 61%. m.p.: 111-112 ºC; Anal. calcd. (found) % for $C_{27}H_{26}N_2O_3S$ (m.w. = 458.57): C, 70.71 (70.68); N, 6.11 (6.06); IR (KBr, ν_{max}, cm^{-1}): 1672 (C=O), 1068 (C-S-C), 3090 (arom. C-H *str.*), 1572 (C=C skeletal), 855 (N-N), 871 (C-N *str*.), 1134 (C-O-C), 1452 (cyclohexane, CH_2); ^{1}H NMR ($CDCl_3$) (δ ppm): 6.68-7.81 (m, 11H, Ar-H), 3.36 (s, 2H, O=CCH_2-S), 3.95 (s, 2H, C-O-CH_2-N), 1.3-1.7 (m, 10H, cyclohexane ring); Mass: M^+ 458, 430, 261, 171, 84.

4-(1-(4-Hydroxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]-oxazin-2(3*H*)-yl)-1-thia-4-azaspiro[4.5]decan-3-one (IVd): Yield: 65%. m.p.: 139-140 ºC; Anal. calcd. (found) % for $C_{26}H_{26}N_2O_3S$ (m.w. = 446.56): C, 69.93 (69.93); N, 6.27 (6.21); IR (KBr, ν_{max}, cm^{-1}): 1128 (C-O-C), 1447 (cyclohexane, CH_2), 1658 (C=O), 3635 (Ar-OH), 1085 (C-S-C), 3098 (arom. C-H *str*.), 1574 (C=C skeletal), 1631 (N-N), 1136 (C-N *str.*); ^{1}H NMR ($CDCl_3$) (δ ppm): 4.65 (s, 1H, Ar-OH), 6.71-8.88 (m, 10H, Ar-H), 4.71 (brs, 1H, s, replaceable-OH), 3.46 (s, 2H, O=CCH_2-S), 3.88 (s, 2H, C-O-CH_2-N), 1.2-1.9 (m, 10H, cyclohexane ring); Mass: M^+ 446, 430, 277, 261, 171, 84.

4-(1-(2-Hydroxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]-oxazin-2(3*H*)-yl)-1-thia-4-azaspiro[4.5]decan-3-one (IVe): Yield: 58%. m.p.: 169-170 ºC; Anal. calcd. (found) % for $C_{26}H_{26}N_2O_3S$ (m.w. = 446.56): C, 69.93 (69.91); N 6.27 (6.20); IR (KBr, ν_{max}, cm^{-1}): 1681.93 (C=O), 3211.48 (Ar-OH), 1064.71 (C-S-C), 3008.95 (arom. C-H *str*.), 1599-1391 (C=C skeletal), 844.82 (N-N), 862.18 (C-N *str.*), 1120.64 (C-O-C), 1444.68



Scheme-I

(cyclohexane, CH_2); ^1H NMR ($CDCl_3$) (δ ppm): 4.68 (s, 1H, Ar-OH), 6.68-8.58 (m, 10H, Ar-H), 4.66 (brs, 1H, s, replaceable-OH), 3.5 (s, 2H, O=CCH_2-S), 4.6 (s, 2H, C-O-CH_2-N), 1.2-1.7 (m, 10H, cyclohexane ring); Mass: M^+ 446, 430, 277, 261, 171, 84.

4-(1-(2-Nitrophenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)-1-thia-4-azaspiro[4.5]decan-3-one (IVf): Yield: 64%. m.p.: 188-189 ºC; Anal. calcd. (found) % for $C_{26}H_{25}N_3O_4S$ (m.w. = 475.56): C, 65.67 (65.60); N, 8.84 (8.77); IR (KBr, ν_{max}, cm^{-1}): 1681.81 (C=O), 1064.47 (C-S-C), 3008.70 (arom. C-H *str*.), 1390.52-1598.94 (C=C skeletal), 844.61 (N-N), 860.11 (C-N *str*.), 1120.21 (C-O-C), 1448.38 (cyclohexane, CH_2); ^1H NMR ($CDCl_3$) (δ ppm): 6.74-7.81 (m, 10H, Ar-H), 3.42 (s, -N-CHS-R 1H), 3.53 (s, 2H, O=CCH_2-S), 3.19 (s, 3H, N=C-CH_3), 3.48 (dd, 1H, CH_2), 3.88 (d, 1H, NCH-R); Mass: M^+ 475, 430, 306, 261, 171, 84.

4-(1-(4-Hydroxy-3-methoxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)-1-thia-4-azaspiro[4.5]decan-3-one (IVg): Yield: 66%. m.p.: 155-155 ºC; Anal. calcd. (found) % for $C_{27}H_{28}N_2O_4S$ (m.w. = 476.59): C, 68.04 (68.10); N, 5.88 (5.86); IR (KBr, ν_{max}, cm^{-1}): 1688 (C=O), 1066.40 (C-S-C), 3016 (arom. C-H *str*.), 1392 (C=C skeletal), 852 (N-N), 872 (C-N *str*.), 1135 (C-O-C), 1466 (cyclohexane, CH_2), 3388 (3-OH, 3-hydroxyphenyl), 672 (OCH_3, *p*-methoxyphenyl); ^1H NMR ($CDCl_3$) (δ ppm): 6.74-7.82 (m, 09H, Ar-H), 3.18 (s, -N-CHS-R 1H), 3.56 (s, 2H, O=CCH_2-S), 2.65 (s, 3H, N=C-CH_3), 3.33 (dd, 1H, CH_2), 3.66 (d, 1H, NCH-R), 2.87 (s, 3H, Ar-OCH_3), 4.62 (brs, 1H, s, replaceable-OH), Mass: M^+ 476, 430, 307, 261, 171, 84.

4-(1-(3-Hydroxy-4-methoxyphenyl)-1*H*-naphtho[1,2-*e*][1,3]oxazin-2(3*H*)-yl)-1-thia-4-azaspiro[4.5]decan-3-one (IVh): Yield: 56%. m.p.: 119-120 ºC; Anal. calcd. (found) % for $C_{27}H_{28}N_2O_4S$ (m.w. = 476.59): C, 68.04 (68.11); N, 5.88 (5.82); IR (KBr, ν_{max}, cm^{-1}): 1682.26 (C=O), 1070 (C-S-C), 3037 (arom. C-H *str*.), 1387 (C=C skeletal), 863 (N-N), 864 (C-N str.), 1144 (C-O-C), 1466 (cyclohexane, CH_2), 3377 (3-OH, 3-hydroxyphenyl), 681 (OCH_3, *p*-methoxyphenyl); ^1H NMR ($CDCl_3$) (δ ppm): 6.65-7.79 (m, 09H, Ar-H), 3.22 (s, -N-CHS-R 1H), 3.48 (s, 2H, O=CCH_2-S), 2.71 (s, 3H, N=C-CH_3), 3.42 (dd, 1H, CH_2), 3.58 (d, 1H, NCH-R), 2.91 (s, 3H, Ar-OCH_3), 4.71 (brs, 1H, s, replaceable-OH), Mass: M^+ 476, 430, 307, 261, 171, 84.

***in vitro* Antimicrobial activity:** The synthesized compounds were evaluated for their toxicity towards some Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*) using well-known antimicrobial test. The test microbial species were grown in various identical sets of LB media containing variable concentrations of the compound (**IVa-h**). Growth was estimated, by recording the optical density of the solution at 600 nm, after 24 h of all the test microbial species. The optical density (OD) *versus* compound concentration growth curve was plotted for each set to get the minimum inhibitory concentration (MIC) values of the tested compounds against the pathogenic Gram-positive and Gram-negative bacteria. In a conical tube containing 10 mL of LB media, *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa,* were incubated for 8 h at 37 ºC and their growth were measured by recording the optical density of the solution at 600 nm.The bacterial suspension (100 µL) was added into each conical tube containing the test compound with final concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, 1.95, 0.98, 0.49 and 0.244 µg mL^{-1} in 10 mL of LB media, respectively. The parallel control experiment was done by adding the same 100 µL of the bacterial suspension to conical tube containing 10 mL of LB media alone. The growth of bacteria for each conical tube was measured by recording the optical density of the solution at 600 nm after 24 h time. The optical density *versus* compound concentration was plotted together. The drop in optical density at respective antibacterial compound concentration was taken as the MIC of that compound.

RESULTS AND DISCUSSION

Antibacterial activity: In Gram-positive bacterial strains, compounds **IVb** and **IVf** showed exceptional activity (12.5-50 µg/mL) against *S. aureus* and *B. subtilis* where as in Gram-negative bacterial strains, compounds **IVc** (12.5-50 µg/mL) was extremly potent followed by **IVe** which showed very good activity (12.5 µg/mL) against *E. coli* compared with ampicillin. All other compounds show moderate activity or less activity against the test bacterial strains (Table-1).

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TABLE-1
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED (**IVa-h**) COMPOUNDS AGAINST GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Compd. No.	R	Gram-positive		Gram-negative	
		S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
IVa	*p*-Chloro benzaldehyde	50	50	>100	>100
IVb	*p*-Bromo benzaldehyde	25	12.5	50	50
IVc	Benzaldehyde	>100	>100	12.5	25
IVd	*p*-Hydroxy benzaldehyde	100	50	50	50
IVe	*o*-Hydroxy benzaldehyde	>100	50	>100	12.5
IVf	*o*-Nitro benzaldehyde	25	12.5	100	>100
IVg	3-OH,4-OCH_3-Benzaldehyde	100	25	50	100
IVh	4-OH,3-OCH_3-Benzaldehyde	100		50	50

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