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Synthesis, Characterization And Biological Evaluation Of Novel Amides Containing Spiro [Chromeno[4,3-D]Thiazole-4,1'-Cyclohexan]-2-Amine Derivatives

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ABSTRACT

A series of novel N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)alkyl/aryl amide derivatives were synthesized for evaluation of their antimicrobial activity. The newly synthesized compounds were characterized by spectroscopic studies such as IR, ^{1}H NMR and LC-Mass analysis. All the synthesized compounds were screened for their in vitro antimicrobial activity. Some of the compounds showed good biological activity.

Keywords: Antimicrobial activity, spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane, spectroscopic studies, potent antimicrobial derivatives.

INTRODUCTION

Heterocyclic compounds are one of the main groups of organic compounds possessing wide range of applications in various areas of science and high technologies. Many heterocyclic compounds are natural compounds that can also be formed by biosynthesis. From the important group of heterocyclic compounds a growing interest is given to the thiazole derivatives, especially after the identification thiazole ring in the structures of some active compounds and alkaloids (thiazole in vitamin B1 and carboxylase, thiazolidine in penicillin, etc.). It is known about forty alkaloids bearing thiazole ring: antibiotic coumermycine and acidomycine, anthelmintic micothiazole, macrocyclic alkaloids tantazole, sisomycine, etc. Reduced thiazoles serve in the study of polypeptides and proteins and occur as structural units in compounds of biological significance, for example; firefly luciferins, antibiotics bacitracin A and thiostrepton. Equally some derivatives of the 2-aminothiazoles are used as fungicides, pesticides, and bactericides; other possesses mitodepressive and mitostatic properties, and a large range of 2-amino (and hydrazino) 5-nitrothiazoles (nitridazole) are devoid of schistosomicidal activity. Fused thiazoleamine continues to attract considerable attention because of their great particular usefulness primarily, due to a very wide spectrum of biological activities. Thiazole core unit were found to show interesting biological activities such as anti-anoxic (AA) [1], allosteric enhancer of adenosine A1 receptors [2], mycobacterium tuberculosis methionine amino peptidases [3], anti-helicobacter pylori (H-pylori) agent [4] and adenosine A2B receptor

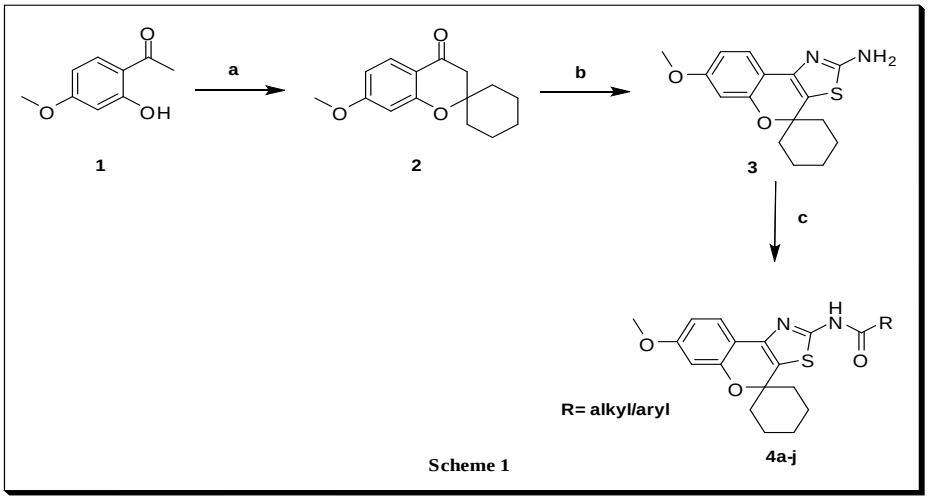
antagonist [5] etc. In the view of biological importance of 2-aminothiazole derivatives we aimed the synthesis of a series of novel N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)alkyl/aryl amide.

MATERIALS AND METHODS

All chemicals were purchased from commercial suppliers. Pyrrolidine and THF were purified by distilling from sodium spheres through a Vigereaux column. The progress of reaction was monitored by Analytical TLC in EtOAc-Hexane solvent system on precoated plates (silica gel 60, F254) and visualized with UV light. Column chromatography was performed with silica gel 60(60-120 mesh). NMR spectra (^{1}H at 400 MH_z) were recorded using $CDCl_3$ or DMSO-d^6 as a solvent. Infrared spectra were determined on a Shimadzu FT-IR. The specifications of the LC/MS are as follows: electrospray (+) ionization, mass range 100-1500 Da, 20-V cone voltage, and Xterra MS C_{18} column (2.1 mm x 50 mm x 3.5 µm). Melting points were determined using Lab India V10 Thermovar apparatus and were uncorrected.

Reagents:(**a**) cyclohexanone, pyrrolidine, methanol, reflux; (**b**) thiourea, iodine, 120 ºC; (**c**) R-CO-Cl, Sodium hydride (60% dispersion in mineral oil), anhydrous THF, RT.

Synthesis of 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one(2). 1-(4-Methoxy-2-hydroxypheny1) ethanone (10.0g, 60.2mmol) and cyclohexanone (5.9g, 60.2mmol) were dissolved in anhydrous methanol (100 mL). Pyrrolidine (4.3g, 60.2mmol) was added and the reaction mixture was allowed to reflux at 80 ºC overnight under N_2. The mixture was then concentrated and water (100 mL) and EtOAc (200 mL) were added. The layers were formed and the aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexanes, 1:9) to yield 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **2** (8.6g, 58% yield) as a colorless solid. M.P.: 73.5-74.1 °C; IR (KBr cm^{-1}): 1620, 1597, 1507, 1466; MS: m/z 247 $(M+H)^+$.



Scheme 1

Synthesis of 7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine(3). Thiourea (3.71g, 48.7mmol) and iodine (3.4g, 13.4mmol) were added in 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **2** (3.0g, 12.2mmol) . The reaction mixture was heated at 100ºC for 3 h. The residue was washed with EtOAc (3 x 25 mL), dissolved in water (50 mL) and heated for 30 min. and cooled. The brown solid was filtered, dried, and recrystallized from 9:1 EtOH–H_2O and dried under vacuum to afford the hydroidide salt of **3**. The salt was taken in dichloromethane (100 mL) and upon a quick free-basing by washing with 5% NaOH and evaporating the dichloromethane layer to yield 7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-

cyclohexan]-2-amine **3** (2.95g, 80% yield) as a brown solid. M.P.: 247.5-248.9 °C; IR (KBr cm^{-1}): 3320, 1510, 1460, 1045; MS: m/z 303 $(M+H)^+$.

General Procedure for the Preparation of Substituted-N-(7-methoxyspiro [chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide (4a-j) To a stirred solution of 7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine **3** (0.3g, 0.99mmol) in dry THF (3 mL) sodium hydride (60% dispersion in mineral oil) (0.06g, 2.5mmol)was added under nitrogen atmosphere at 0 ºC followed by the addition of substituted benzoyl chloride (1.5 mol), and the mixture was stirred at room temperature for overnight. The mixture was then concentrated and water (25 mL) and EtOAc (50 mL) were added. The layers were formed and the aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated by rotary evaporation. The crude material was purified by column chromatography (EtOAc/Hexanes, 3:7) to yield Substituted-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide**4a-j**. All the compounds were found to be brown solid. Characteristic physical data of all the final compounds are represented in table 1.

Table 1: Characteristics physical data of amide derivatives **4a-j**.

Sr. No.	Compound Code	R	M.P. (°C)	Yield (%)
1	4a	2,5-difluoro phenyl	142-143	66
2	4b	2-trifluoromethyl phenyl	156-157	62
3	4c	4-n-butoxy phenyl	138-139	69
4	4d	4-ethyl phenyl	159-160	68
5	4e	4-n-pentyl phenyl	117-118	65
6	4f	2,4,5-trifluoro phenyl	175-176	58
7	4g	Isobutyryl	165-166	52
8	4h	4-morpholino phenyl	99-100	61
9	4i	4-trifluoromethyl phenyl	123-124	68
10	4j	Phenyl	135-136	71

All compounds are either crystalline or amorphous solid.

2,5-difluoro-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide(4a) ^{1}H NMR (400 MHz, DMSO-d^6): δ =1.37-1.80(8H, m), 2.09(2H, m), 3.77(3H, s), 6.606(2H, d), 7.444(1H, d), 7.468(1H, d), 7.503(1H, d), 7.622(1H, s), 12.95(1H, s) ppm.; IR (KBr cm^{-1}): 3431, 2925, 1669, 1541, 1499, 1150; MS: m/z 443 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)-2-(trifluoromethyl) benzamide (4b) ^{1}H NMR (400 MHz, DMSO-d^6): δ =1.31-1.79(8H, m), 2.11(2H, m), 3.75(3H, s), 6.611(2H, d), 7.440(1H, d), 7.474(1H, t), 7.497(1H, t), 7.763(1H, d), 7.801(1H, d), 12.98(1H, s)ppm. IR (KBr cm^{-1}): 3435, 2941, 1665, 1531, 1485, 1147; MS: m/z 475 $(M+H)^+$.

4-butoxy-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide(4c) ^{1}H NMR (400 MHz, $CDCl_3$): δ =0.995-1.044(5H, m), 1.542(2H, m), 1.650-1.876(8H, m), 2.241(2H, m), 3.864(3H, s), 4.077(2H, t), 6.613-6.669(1H, 3sd), 6.955(2H, d), 7.040(1H, d), 8.087(2H, d), 8.208(1H, d), 12.98(1H, s) ppm. IR (KBr cm^{-1}): 3429, 2927, 1673, 1554, 1470, 1133; MS: m/z 479 $(M+H)^+$.

4-ethyl-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide(4d) ^{1}H NMR (400 MHz, $CDCl_3$): δ =1.288(3H, t), 1.354-1.929(8H, m), 2.242(2H, m), 2.749 (2H, q), 3.837(3H, s), 6.534-6.588(1H, d), 6.583(1H, s), 7.347(2H, d), 7.551(1H, d), 7.951(2H, d), 10.8 (1H, broad)ppm.IR (KBr cm^{-1}): 3441, 2958, 1677, 1538, 1491, 1168; MS: m/z 436 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)-4-pentylbenzamide(4e) ^{1}H NMR (400 MHz, $CDCl_3$): δ =0.903-0.948(3H, t), 1.272-1.378(6H, m), 1.660-1.901(8H, m), 2.246(2H, m), 2.709(2H, t), 3.861(3H, s), 6.612-6.660(1H, 35d), 7.309(2H, d), 7.374(1H, d), 7.691(1H, d), 8.079-8.169(2H, dd), 12.98(1H, s) ppm. IR (KBr cm^{-1}): 3435, 2928, 1655, 1545, 1479, 1146; MS: m/z 477 $(M+H)^+$.

2,4,5-trifluoro-N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)benzamide(4f)^{1}H NMR (400 MHz, DMSO-d^6): δ =1.274-1.439(2H, m), 1,644-1.922(6H, m), 2.246(2H, m), 3.850(3H, s), 6.598(1H, s), 6.616-6.622(1H, d), 7.034-7.163(1H, m), 7.597(1H, d), 7.857-8.002(1H, m), 12.96(1H, s) ppm. IR (KBr cm^{-1}): 3437, 2935, 1653, 1577, 1490, 1153; MS: m/z 461 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)isobutyramide(4g) ^{1}H NMR (400 MHz, DMSO-d^6): δ =1.282(6H, d), 1.327-1.902(8H, m), 2.206(2H, m), 2.628(1H, m), 3.837(3H, s), 6.560-6.565(1H, d), 6.583(1H, s), 7.542(1H, d), 9.109(1H, broad)ppm. IR (KBr cm^{-1}): 3432, 2941, 1679, 1535, 1495, 1158; MS: m/z 373 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)morpholine-4-carboxamide (4h) ^{1}H NMR (400 MHz, DMSO-d^6): δ =1.322-1.941(8H, m), 2.216(2H, m), 3.321(4H, t), 3.753(4H, t), 3.864(3H, s), 6.660-6.675(2H, d), 7.341(2H, d), 7.541(1H, s), 7.959(2H, d),11.98(1H, s)ppm.IR (KBr cm^{-1}): 3438, 2932, 1679, 1543, 1482, 1148; MS: m/z 492 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl)-4-(trifluoromethyl) benzamide (4i)^{1}H NMR (400 MHz, DMSO-d^6): δ =1.364-1.923(8H, m), 2.236(2H, m), 3.877(3H, s), 6.539(1H, d), 6.581(1H, s), 7.537(2H, d), 7.553(1H, d), 7.951(2H, d), 10.8 (1H, broad) ppm. IR (KBr cm^{-1}): 3447, 2912, 1650, 1561, 1478, 1163; MS: m/z 475 $(M+H)^+$.

N-(7-methoxyspiro[chromeno[4,3-d]thiazole-4,1'-cyclohexane]-2-yl) benzamide (4j)^{1}H NMR (400 MHz, DMSO-d^6): δ =1.351-1.919(8H, m), 2.232(2H, m), 3.869(3H, s), 6.539(1H, d), 6.578(1H, s), 7.551(1H, d), 7.769(5H, m), 11.92 (1H, broad) ppm. IR (KBr cm^{-1}): 3422, 2936, 1644, 1556, 1483, 1147; MS: m/z 407 $(M+H)^+$.

RESULTS AND DISCUSSION

Chemistry: As delineated in synthetic **Scheme 1**, 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **2** were prepared by Chandrasekhar's enamine-mediated crossed Aldol condensation method [6-9]. This efficient one-pot transformation employs freshly distilled catalytic pyrrolidine to condense reagent 2-hydroxy 4-methoxy acetophenone **1** with cyclohexanone; subsequent Michael addition of the phenoxy moiety to the newly generated enone delivers chromanone **2** in good yields. The formation of 7-methoxyspiro[chroman-2,1'-cyclohexan]-4-one **2** was evident from the IR and Mass spectrometry. Condensation of **2** with thiourea in the presence of iodine at 120 ºC led to formation of 7-methoxyspiro [chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine **3** [2,5,10-11]. The target compounds**4a-j** was obtained with variable yield by reacting primary amine **3** with various aryl or alkyl acid chlorides. In this preparation various bases like potassium carbonate, cesium carbonate, DBU, and 60% sodium hydride in mineral oil in the final step were used. Among them 60% sodium hydride in mineral oil with anhydrous THF shows best result. Based on the data given, the reaction appears to be compatible with both aryl and alkyl substitutions. All the reactions have provided the products in 52-71% yields. The compounds were purified by column chromatography using 30% ethyl acetate in hexanes as the eluent.

Biological activities

Antibacterial and antifungal activities: The newly synthesized derivatives were evaluated for their in vitro antibacterial activity against gram positive staphylococcus aureus and streptococcus pyogenes, gram negative Escherichia coli and pseudomonas aeruginosa, and antifungal activity against candida albicans and aspergillusniger by micro broth dilution method [12-14]. The standard strains used for screening antibacterial and antifungal activities were procured from institute of microbial technology (IMTECH), Chandigarh, India. The MIC values are given in Table-2. The standard drugs used for antibacterial activity were ampicillin and ciprofloxacin and nystatin for antifungal activity. Mueller Hinton Broth was used as neutriant medium for bacteria and Sabouraud Dextrose Broth for fungal to grow. Inoculums size for test strain was adjusted to 10^8 CFU mL^{-1} by comparing the turbidity. The serial dilutions were prepared in primary and secondary screening. The target compounds and standard drugs were dissolved in DMSO-water at a concentration of 2.0 mg mL^{-1}. In primary screening, 500 µg mL^{-1}, 250 µg mL^{-1}, and 125 µg mL^{-1} concentrations of the synthesized drugs were taken. Data were not taken for the initial solution because of the high DMSO concentration (10%). The actively synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. In secondary screening, the drugs found active in primary screening were similarly diluted to obtain 100 µg mL^{-1}, 50 µg mL^{-1}, and 25 µg mL^{-1}, 12.5 µg mL^{-1}, and 6.25 µg mL^{-1} concentrations. The inoculated wells were incubated overnight at 37ºC in a humid atmosphere overnight. The highest dilution showing at least 99% inhibition zone is taken as MIC. The MIC values revealed that some of the newly synthesized compounds showed moderate to good inhibition. Compounds 4b, 4f and4iexhibited good activities against all the four bacterial strains. The MIC values of antifungal activity revealed that compound 4a, 4f exhibited good activity against C. albicans and A. niger fungal strain. Rest of all compounds did not exhibit comparable activity against both the fungal strains.

Table 2: Antibacterial and antifungal activity of amide derivatives **4a-j.**

	Antibacterial MIC (µg mL^{-1})				Antifungal MIC (µg mL^{-1})		
Compounds	E. coli MTCC 443	P. aeruginosa MTCC 1688	S. aureus MTCC 96	S. pyogenes MTCC 442	C. albicans MTCC 227	A. niger MTCC 282	A. clavatus MTCC 1323
Ampicillin	100	100	250	100	-	-	-
Ciprofloxacin	25	25	50	50	-	-	-
Nystatin	-	-	-	-	100	100	100
4a	62.5	62.5	100	125	100	500	500
4b	125	125	200	250	500	500	1000
4c	250	250	250	500	1000	500	500
4d	200	200	250	500	1000	1000	1000
4e	250	125	250	500	1000	500	1000
4f	62.5	62.5	62.5	100	200	100	500
4g	500	250	250	500	1000	1000	500
4h	125	100	100	200	500	1000	1000
4i	100	62.5	125	200	1000	1000	500
4j	200	200	125	250	500	500	1000

APPLICATIONS

In the present study the derivatives which we have synthesized were screened for their antimicrobial activity, which are promising as active pharmacophore. Further studies are undergoing to explore the scope of the various biological activities.

CONCLUSIONS

An efficient method for preparing Spiro[chromeno[4,3-d]thiazole-4,1'-cyclohexan]-2-amine Derivatives was described and the structure of synthesized compounds was determine by IR, ^{1}H NMR, and LC-Mass spectroscopic analysis and evaluated for their in vitro antimicrobial activity by broth dilution method.

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