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Tandem synthesis of thia-oxadiazolophanes

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ABSTRACT

Present work reports the synthesis of series of thia-oxadiazolophanes using Tandem method. Reaction of dihydrazide (oxalic acid dihydrazide, malonic acid dihydrazide, succinic acid dihydrazide, glutaric acid dihydrazide, pimelic acid dihydrazide, suberic acid dihydrazide, azelaic acid dihydrazide and sebacic acid dihydrazide) with KOH and CS_2 in alcohol resulted in the formation of K-salt of bis-[5,5'-(mercapto)-1,3,4-oxadiazole-2,2'-yl]alkane with evolution of H_2S gas. After complete removal of H_2S gas, 1,2-dibromoethane was added and the mixture was further refluxed. Structures of newly synthesized compounds were established using FTIR, ^1H NMR and elemental analysis. All the compounds are screened for their anti-inflammatory activities. IC_{50} values reveal that the newly synthesized compounds exhibit significant anti-inflammatory activities. Representative samples were studied for cytotoxicity. Results of cytotoxicity study reveal that the compounds exhibit poor cytotoxicity.

Key words: Tandem method, thia-oxadiazolophanes, anti-inflammatory, cytotoxicity.

INTRODUCTION

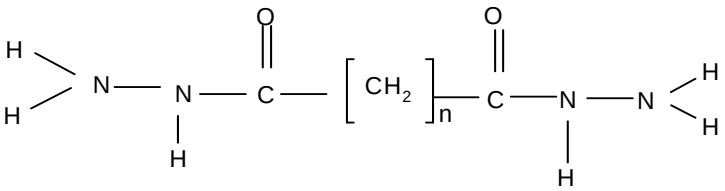
Chemistry of macrocyclic compounds has developed new dimensions through cation complexation using the legating macrocycles and their applications to biological sciences [1]. First time Izatt and co-workers recognized the special ability of sulfur containing macrocycles to form stronger complexes with transition metals which lead to the development of macrocycles having sulfur atoms [2]. Oxadiazolophanes are the macrocyclic compounds in which 1,3,4-oxadiazole unit is incorporated in macrocycle. Incorporation of heterocyclic moieties within the cavity of macrocyclic rings not only provides rigidity but also helps to participate in complexation through the soft donor atoms [3]. Among the various heterocyclic compounds that have been explored for developing pharmaceutically important molecules -1,3,4-oxadiazole derivatives have played a vital role in medicinal chemistry. Number of synthetic compounds with oxadiazole nucleus used for antibacterial [4-9], antifungal [9-13], antimicrobial [14-18], antiviral [19, 20], anti-TB [21, 22], anti-inflammatory [23-26] and analgesic activities [24]. Tandem reactions are the combination of two or more reactions whose occurrence is in the specific order, where the functionality for the second reaction has been created but the additional reagent must be added in order for the second reaction to occur. Since the intermediate formed need not be separated, Tandem reactions save on the steps, amount of the reagents, solvents and reduce the waste generated.

Present work describes the synthesis of thia-oxadiazolophanes by Tandem method. Structures of the newly synthesized compounds were established using FTIR, ^{1}HNMR and elemental analysis. Synthesized compounds were

tested for their utility as possible anti-inflammatory agents and representative samples were studied for cytotoxic activities.

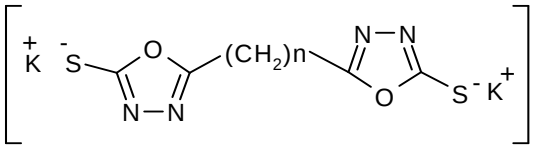
The reaction leading to the formation of the title compound is outlined in the Scheme 1

Scheme 1

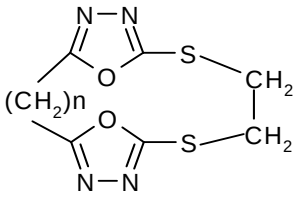


Dihydrazide (n=0,1,2,3,5,6,7,8)

CS_2
KOH/C_2H_5OH



Br CH_2 CH_2 Br
alcohol



1

n	1
0	1a
1	1b
2	1c
3	1d
5	1e
6	1f
7	1g
8	1h

Fig.1 Synthesis of thia-oxadiazolophanes

EXPERIMENTAL SECTION

Uncorrected melting points of the synthesized compounds were measured using open capillary tubes**.** FTIR spectra were recorded using BRUKER (Model 3000) FTIR spectrophotometer, ^{1}H NMR spectra were recorded using Varian, Mercury plus, 300 MHz NMR spectrophotometer. For recording NMR spectra DMSO was used as a solvent and TMS was used as an internal standard.

General procedure for synthesis of thia-oxadiazolophanes

Reaction of acid dihydrazide with KOH and CS_2 in alcohol resulted in the formation of K-salt of bis-[5,5'-(mercapto)-1,3,4-oxadiazole-2,2'-yl]alkane derivatives with evolution of H_2S gas. After complete evolution of H_2S gas, 1,2-dibromoethane was added to the reaction mixture and the mixture was further refluxed till solid product separated out.

Synthesis of thia-oxadiazolophanes1 (a-h)

To 0.01 mole carboxylic acid dihydrazide (oxalic acid dihydrazide, malonic acid dihydrazide, succinic acid dihydrazide, glutaric acid dihydrazide, pimelic acid dihydrazide, suberic acid dihydrazide, azelaic acid dihydrazide and sebacic acid dihydrazide) in alcohol, KOH (1.14 g, 0.02 mole) was added and the mixture was refluxed for 30 min. After in this hot mixture CS_2 (1.2 mL, 0.02 mole) was added slowly with constant stirring, yellow precipitate was formed which dissolves on heating. Mixture was further refluxed till H_2S gas evolves completely. After complete evolution of H_2S gas, 1,2-dibromoethane (0.9 mL,0.01 mole) was added slowly with stirring and the mixture was refluxed for 2 h. Solid product separates out. Product obtained was washed several times with warm ethanol and recrystallised with DMF: alcohol mixture having the ratio 1:10.

i) 1,2(2,5)-di(1,3,4-oxadiazola)-3,6-dithia-cyclohexaphane(1a)

Yield: 1.96 g (86%), mp182 ^oC; ir(ν cm^{-1} KBr): 1654 (C=N), 1488 (S-CH_2), 1041 (N-N), 1278 and 1153(C-O-C). ^1H nmr(δ ppm,DMSO-d_6): 3.676(dd, 4H, -S-CH_2-CH_2-S-,J= 4.75, 6.95 Hz). Anal. Cald. for $C_6H_4N_4O_2S_2$: C, 31.57; H, 1.77; N, 24.54; S, 28.10. Found: C, 31.503; H, 1.771; N, 24.440; S, 28.21.

ii) 1,3(2,5)-di(1,3,4-oxadiazola)-4,7-dithia-cycloheptaphane (1b)

Yield: 1.79 g (74%), mp charred at 210^oC; ir(ν cm^{-1}KBr): 1643 (C=N), 1498 (S-CH_2), 1038 (N-N), 1252 and 1187(C-O-C); ^1H nmr (δ ppm,DMSO-d_6): 3.403 (s, 2H, -ring-CH_2-ring-), 3.839 (s, 4H, -S-CH_2-CH_2-S-). Anal. Cald. for $C_7H_6N_4O_2S_2$: C, 34.70; H, 2.50; N, 23,12; S, 26.47. Found: C, 34.57; H, 2.506; N, 23.17; S, 26.52.

iii)1,4(2,5)-di(1,3,4-oxadiazola)-5,8-dithia-cyclooctaphane (1c)

Yield: 1.68g (66%), mp charred at 195^oC; ir(ν cm^{-1}KBr): 1661 (C=N), 1492 (S-CH_2), 1044 (N-N), 1282 and 1154(C-O-C); ^1H nmr (δ ppm, DMSO-d_6): 3.440 (s,4H, -ring-CH_2-CH_2-ring-), 3.689(s,4H, -S-CH_2-CH_2-S-). Anal. Cald. for $C_8H_8N_4O_2S_2$: C, 37.49; H, 3.14; N, 21.86; S,25.02. Found: C, 37.41; H, 3.15; N, 21.91; S, 25.07.

iv)1,5(2,5)-di(1,3,4-oxadiazola)-6,9-dithia-cyclononaphane (1d)

Yield: 2.37g (88%), mp 115^oC; ir (ν cm^{-1}KBr): 1635 (C=N), 1482 (S-CH_2), 1060 (N-N), 1259 and 1165(C-O-C); ^1H nmr(δ ppm,DMSO-d_6): 2.127(p, 2H,ring-C-CH_2-C-,J=7.1 Hz), 2.966(t,4H, -ring-CH_2-C-CH_2-ring-, J=7.1 Hz), 3.608(s,4H, -S-CH_2-CH_2-S-). Anal. Cald. for $C_9H_{10}N_4O_2S_2$: C, 39.99; H, 3.73; N, 20.72; S,23.72. Found: C, 39.85; H, 3.75; N, 20.70; S, 23.80.

v)1,6(2,5)-di(1,3,4-oxadiazola)-2,5-dithia-cycloundecaphane (1e)

Yield: 2.1g (70%), mp charred at 200^oC; ir(ν cm^{-1}KBr): 1627 (C=N), 1466 (S-CH_2), 1039 (N-N), 1242 and 1153(C-O-C); ^1H nmr (δ ppm,DMSO-d_6): 1.923(p, 2H, -ring-C-C-CH_2-,J=7.3 Hz), 2.727(p, 4H, 2x –ring-C-CH_2-,J=7.3 Hz), 3.143(t, 4H, 2x -ring-CH_2-,J=7.3 Hz), 3.755(s, 4H, -S-CH_2-CH_2-S-). Anal. Cald. for $C_{11}H_{14}N_4O_2S_2$: C, 44.28; H, 4.73; N, 18.78; S,21.50. Found: C, 44.24; H, 4.74; N, 18.80; S, 21.53.

vi)1,6(2,5)-di(1,3,4-oxadiazola)-2,5-dithia-cyclododecaphane (1f)

Yield: 2.74 g (88%), mp 102^oC; ir(ν cm^{-1}KBr): 1668 (C=N), 1486 (S-CH_2), 1009 (N-N), 1260 and 1156(C-O-C), ^1H nmr (δ ppm,DMSO-d_6): 1.348(br, 4H, 2x-ring-C-C-CH_2-) ,1.664(p, 4H, 2x -ring-C-CH_2-,J=7.2 Hz), 2.812(t, 4H, 2 x-ring-CH_2-,J=7.4 Hz), 3.602(s, 4H, -S-CH_2-CH_2-S-). Anal. Cald. for $C_{12}H_{16}N_4O_2S_2$: C, 46.13; H, 5.16; N,17.93; S,20.53. Found: C, 46.12; H, 5.15; N, 17.93; S, 20.52.

vii)1,6(2,5)-di(1,3,4-oxadiazola)-2,5-dithia-cyclotridecaphane (1g)

Yield: 2.67g (82%), mp 182^oC; ir(ν cm^{-1}KBr): 1630 (C=N), 1482 (S-CH_2), 999 (N-N), 1222 and 1146(C-O-C),^1H nmr(δppm,DMSO-d_6):1.278 (br,6H,-ring-C-C-CH_2-CH_2-CH_2-),1.662
p, 4H,2 x ring-C-CH_2-,J=7.3 Hz), 2.806(t, 4H, 2 x –ring-CH_2-, J=7.3 Hz) , 3.600(s, 4H, -S-CH_2-CH_2-S-). Anal. Cald. for $C_{13}H_{18}N_4O_2S_2$: C, 47.83; H, 5.55; N, 17.16; S, 19.64. Found: C, 47.76; H, 5.57; N, 17.19; S, 19.70.

vii)1,6(2,5)-di(1,3,4-oxadiazola)-2,5-dithia-cyclotetradecaphane (1h)

Yield: 3.0 g (90%), mp 109^oC; ir(ν cm^{-1}KBr): 1638 (C=N), 1488 (S-CH_2), 1004 (N-N), 1222 and 1156(C-O-C); ^1H nmr(δ ppm,DMSO-d_6):1.307(br,8H,2x–ring-C-C-CH_2-CH_2-,) 1.654(p, 4H, 2 x ring-C-CH_2-, J=6.8 Hz), 2.807(t, 4H, 2 x –ring-CH_2-, J= 7.3 Hz), 3.599(s, 4H, -S-CH_2-CH_2-S-). Anal. Cald. for $C_{14}H_{20}N_4O_2S_2$: C, 49.40; H, 5.92; N, 16.45; S, 18.83. Found: C, 49.29; H, 5.94; N, 16.48; S, 18.88.

BIOLOGICAL ACTIVITIES

1) Anti-inflammatory activity

Anti-inflammatory potential of the synthesized compounds were assessed using human RBC'S. Fresh human blood (5 mL) was collected and transferred to the centrifugation tubes containing sodium citrate to prevent clotting. These tubes were centrifuged at 3000 rpm for 10 min and washed three times with equal volume of normal saline.Volume of the blood was measured and reconstituted as 10 %v/v suspension with normal saline. Reaction mixture consisting 1.0 mL test sample of various concentrations in normal saline and 0.5 mL 10% HRBC suspension , 1 mL 0.2 M phosphate buffer, 1 mL hypo saline were incubated at 37 ^{0}C for 30 min and centrifuged at 3000 rpm for 30 min. Hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Dichlorofenac was used as standard. Percentage of HRBC heamolysis and the IC_{50} values were calculated and are presented in Table 1.

Table 1 Anti-inflammatory activity (IC_{50}) of the synthesized compounds

Sr.No.	Compound	IC_{50}+S.D µg/mL
1	1a	23.25 + 1.03
2	1b	19.03 +1.16
3	1c	18.01 + 1.19
4	1d	15.45+1.37
5	1e	17.69 +1.09
6	1f	23.03+ 1.45
7	1g	20.19 +1.66
8	1h	12.36 + 1.02
	Dichlorofenac	8.98 + 0.136

2) In vitro cytotoxicity study

Two representative samples were studied for short term in vitro cytotoxicity using Dalton's lymphoma ascites cells (DLA). Tumor cells aspirated from the peritoneal cavity of tumor bearing mice were washed thrice with phosphate buffered saline (PBS). Cell viability was determined by trypan blue exclusion method. Viable cell suspension (1 x10^6 cells in 0.1 mL) was added to tubes containing various concentrations of the test compounds and the volume was made to1 mL using PBS. Control tube contained only cell suspension. These assay mixtures were incubated for 3 h at 37 ^{o}C. Further cell suspension was mixed with 0.1 mL of 1% trypan blue and kept for 3 min and loaded on a haemocytometer. Dead cells take up the blue color of trypan blue while live cells do not take up dye. Number of stained and unstained cells was counted separately. Percentage cytotoxicity was calculated and the results obtained are presented in Table 2.

Table 2 Cytotoxicity of the representative compounds

Concentration µg/mL	Percentage cell death (DLA)	
	Compound 1a	*Compound 1f*
200	15	18
100	10	10
50	05	02
20	00	00
10	00	00

RESULTS AND DISCUSSION

Tashtoush et al. [27] reported the reaction of acid dihydrazides with KOH and CS_2. They reported that the dihydrazides from dimethyl glutarate, dimethyl adipate, dimethyl pimelate, dimethyl suberate, dimethyl azelate and dimethyl sebacate gives the expected bis-[5,5'-(mercapto)-1,3,4-oxadiazole-2,2'-yl] alkane derivatives. They also reported that the dihydrazides from malonate and succinate does not give the desired product, bis-[5,5'-(mercapto)-1,3,4-oxadiazole-2,2'-yl] methane and bis-[5,5'-(mercapto)-1,3,4-oxadiazole-2,2'-yl] ethane respectively. Malonic acid dihydrazide and succinic acid dihydrazide under the similar conditions undergo intramolecular cyclisation and gives the corresponding dicarboxylic acid and 2,5-dimercapto-1,3,4-thiadiazole.

Madhukar Chande and co-worker [28] reported the synthesis of bis-[5,5'-(mercapto)-1,3,4-oxadiazole-2,2'-yl] butane from adipic acid dihydrazide. Further, they have synthesized thia-oxadiazolophanes from bis-[5,5'-(mercapto)-1,3,4-oxadiazole-2,2'-yl] butane using various dibromo alkanes. This method of synthesis of oxadiazolophanes is a four step process also the yield is moderate. Further by this method for the synthesis of

oxadiazolophanes, mercapto derivative of 1,3,4-oxadiazole is required. But Tashtoush et al. [27] reported that the mercapto derivative of 1,3,4-oxadiazole from malonic acid dihydrazide and succinic acid dihydrazide cannot obtained using CS_2, KOH followed by acidification. In a present work, we report the synthesis of thia-oxadiazolophanes using Tandem method. With Tandem method, oxadiazolophanes from malonic acid dihydrazide and succinic acid dihydrazide were obtained. Also other oxadiazolophanes derived from oxalic acid dihydrazide, glutaric acid dihydrazide, pimelic acid dihydrazide, suberic acid dihydrazide, azelaic acid dihydrazide and sebacic acid dihydrazide were synthesized and gives better yields. Tandem reaction reduces the steps involved in reaction, amount of reagents and solvents used thus decreases the waste generated.

In alcoholic solution of potassium hydroxide, a potassium ion helps in template formation and it favors the regioselective S-alkylation [3]. Formation of the product is confirmed on the basis of IR and NMR data. IR spectra showed the peak between 1628 to 1668 cm^{-1} due to (C=N). Presence of peak in the range 1238 to 1281 cm^{-1} and 1148 to 1159 cm^{-1} gives the evidence for the 1,3,4-oxadiazole ring closure. Peaks in ^1H NMR spectra were consistent with the various protons. Reaction conditions and the work up procedures are mild, simple and convenient. Tandem reactions are highly desirable because they increase synthetic efficiency by decreasing the number of laboratory operations, chemicals and the solvent used. Results of anti-inflammatory activity study revealed that the synthesized compounds exhibits better anti-inflammatory activity. Representative compounds studied shows poor cytotoxic activity.

CONCLUSION

Thia-1,3,4-oxadiazolophanes have been synthesized using Tandem method and reduced the number of steps, waste generated and solvent required. Anti-inflammatory activity study revealed that the synthesized compounds possess significant anti-inflammatory activity compared with the standard. It can be concluded that this class of compounds certainly hold great promise for discovering compounds with safer pharmacological activities.

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