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Design And Microwave Assisted Synthesis Of Dual Inhibitors

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ABSTRACT

N-[5-(4–Amino-5-mercapto-4*H*-[1,2,4]triazol-3-yl)-4-methyl-1,3-thiazol-2-yl]benzamide **(1)** on condensation with chloroacetic acid, α-haloketone and benzoin furnished [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine derivatives **(2)**, **(3)** and **(4)** respectively, while condensation with 2,3-dichloroquino xaline, carbon disulphide, aromatic carboxylic acid and aromatic carbox aldehydes yielded the cyclic products, [1,2,4] triazolo[3,4-*b*][1,3,4] thiadiazole derivatives **(5)**, **(6)**, **(7)**, **(8)** respectively. The compounds have been characterized on the basis of elemental analysis and spectral data. The antibacterial and antiinflammatory activities of the compounds have also been evaluated.

KEYWORDS

Triazole;
Thiazole;
Anti-inflammatory;
Antibacterial.

INTRODUCTION

Identification of novel compounds which treat effectively both infectious and inflammatory states devoid of side effects associated, remains a major challenge in biomedical research. Since large number of functional derivatives of thiazole and triazole were reported to exhibit interesting anti-inflammatory and antimicrobial properties[1-10], we have established a program to discover agents that have a dual effect, as anti-inflammatory–antimicrobial agents.

Microwave assisted reactions[11] using dry media[12] have attracted much interest because of the simplicity in operation, greater selectivity and rapid synthesis of variety of heterocyclic compounds[13]. Keeping this in view, it appeared worthwhile to develop rapid syntheses of title compounds under solvent free conditions using MWI. The synthesis entails the union of two biologically active nuclei, viz. triazole and thiadiazole and also triazoles and thiadiazine. Earlier thiadiazoles and thiadiazines were synthesized in 6-7hr[14], while on solid support under microwave, the reaction was completed within 40-80 sec. with improved yield. Thus it was thought

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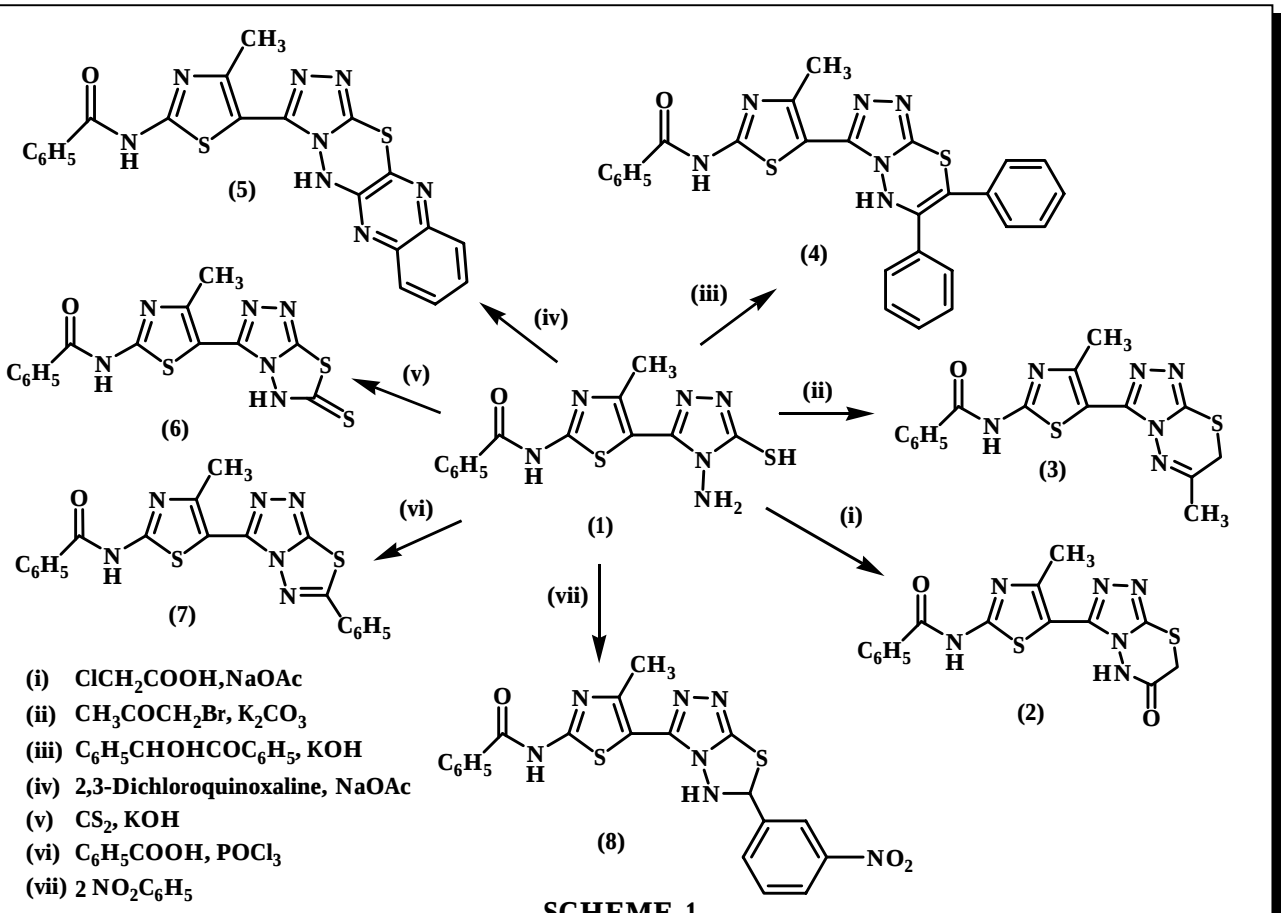

SCHEME 1

worthwhile to carry out synthesis by MORE method and perform pharmacological study of newly synthesized compounds. (SCHEME 1). The structures (1)-(8) have been established on the basis of their ^{1}H NMR, IR and elemental analysis.

EXPERIMENTAL

The melting points were recorded on electrothermal apparatus and are uncorrected. IR spectra were recorded in KBr on a Perkin Elmer-983; ^{1}H NMR spectra on a Bruker Avance 300 MHz instrument using $CDCl_3$ as solvent (chemical shifts in δ ppm) using TMS as internal standard; mass spectra on a Finning LCQ mass spectrometer. Microwave irradiation was carried out in Padmini Essentia oven, Model Brownie at 2450 MHz. Elemental analysis were performed on a Heracus CHN-Rapid analyser. The purity of the compounds was checked on silica gel coated Al plates (Merck).

N-[5-(4–Amino-5-mercapto-4H-[1,2,4]triazol-3-yl)-4-methyl-1,3-thiazol-2-yl]benzamide (1)

It was synthesized by reported method[15-19]. Yield 89%; m.p. 210-212°C; IR (KBr) ν cm^{-1} 1540 (C-N stretching), 1630 (C=N), 1670 (C=O); ^{1}H NMR (TMS) δ ppm: 2.12 (s, 2H, NH_2), 2.39 (s, 3H, CH_3), 5.92 (s, 1H, NH), 7.44 (t, 2H, ArH, *J*= 12.7 MHz), 7.60 (t, 1H, ArH), 7.87 (d, 2H, ArH, *J*= 15.9 MHz); Anal., Calcd. For $C_{13}H_{12}N_6OS_2$ (332): C, 46.97, H, 3.63, N, 25.28 Found: C, 46.91, H, 3.69, N, 25.22%.

N-[4-methyl-5-(5-oxo(4H,6H-1,2,4-triazolo[3,4-b]1,3,4-thiadiazaperhydroin-3-yl)-1,3-thiazol-2-yl] benzamide (2)

A solution of I (0.01mole), chloroacetic acid (0.01 mole) and freshly prepared fused sodium acetate (0.01 mole) was prepared. Acidic alumina (Aluminium oxide, acidic, Brockmann I, ~150 mesh, 58Å CAMAG 506-C-I, Surface area 155m^2/g. pH=6.0) was added to the above solution at room tempera-

ture. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath[19] and irradiated for 60 sec at 145^{0}C. The mixture was cooled and then product was extracted with dry methanol and poured onto crushed ice. The solid thus separated was filtered, washed thoroughly with water and recrystallized from aq. ethanol. Yield 93%; m.p. 232-235^{o}C; IR (KBr) ν cm^{-1} 1547 (C-N stretching), 1612 (C=N), 1645 (C=O); ^{1}H NMR (TMS) δ ppm: 2.31 (s, 3H, CH_3), 3.82 (s, 2H, CH_2), 5.87 (s, 2H, NH), 7.40 (t, 2H, ArH, *J*= 12.1 MHz), 7.67 (t, 1H, ArH), 7.72 (d, 2H, ArH, *J*= 15.7 MHz); Anal., Calcd. For $C_{15}H_{12}N_6O_2S_2$ (372): C, 48.37, H, 3.24, N, 22.56 Found: C, 48.91, H, 3.69, N, 22.22 %.

N-[4-Methyl-5(5-methyl(6H-1,2,4-triazolo[3,4-b]1,3,4-thiadiazin-3-yl)-1,3-thiazol-2-yl]-benzamide (3)

Solution of (1) (0.01mole) and p-bromophenacyl bromide (0.01 mole) was added to acidic alumina at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 80 sec at 168^{0}C. The mixture was cooled and then product was extracted with dry methanol and neutralized with aq.potassium carbonate. The solid thus separated was filtered, washed thoroughly with water and recrystallized from ethanol. Yield 94%; m.p. 229-231^{o}C; IR (KBr) ν cm^{-1} 835 (1,4-disubstituted benzene ring), 1530 (C-N stretching), 1622 (C=N), 3030 (aromatic C-H stretching); ^{1}H NMR (TMS) δ ppm: 1.1 (s, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.14 (s, 2H, CH_2), 5.93 (s, 1H, NH), 7.37 (t, 2H, ArH, *J*= 12.4 MHz), 7.61 (t, 1H, ArH), 7.76 (d, 2H, ArH, *J*= 14.9 MHz); Anal., Calcd. For $C_{13}H_{12}N_6OS_2$ (332): C, 46.97, H, 3.63, N, 25.28 Found: C, 46.91, H, 3.69, N, 25.22 %.

N-[5-(5,6-diphenyl(4H-1,2,4-triazolo[3,4-b]1,3,4-thiadiazin-3-yl))-4-methyl-1,3-thiazol-2-yl]-benzamide (4)

A solution of **(1)** (0.01mole), benzoin (0.01 mole) and 2N KOH solution was prepared. Acidic alumina was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40 sec at 135^{0}C. The mixture was cooled and then product was extracted with acetone and was evaporated to dryness. The solid thus separated was washed thoroughly with water and recrystallized from ethanol. Yield 84%; m.p. 242-245^{o}C; IR (KBr) ν cm^{-1} 715, 755 (monosubstituted benzene ring), 1600, 1620 (C=C), 1665 (C=N), 3040 (aromatic C-H stretching), 3410 (N-H stretching; ^{1}H NMR (TMS) δ ppm: 2.31 (s, 3H, CH_3), 5.73 (s, 2H, NH), 7.14 - 7.98 (m, 15H, ArH); Anal., Calcd. For $C_{27}H_{20}N_6OS_2$ (508): C, 63.75, H, 3.96, N, 16.52 Found: C, 63.12, H, 3.57, N, 16.32.

N-(5-(4H-quinoxalino[2,3-e]1,2,4-triazolo[3,4-b]1,3,4-thiadiazin-3-yl)-4-methyl-1,3-thiazol-2-yl)benzamide (5)

Solution of **(1)** (0.01mole), 2, 3-dichloro quinoxaline (0.01 mole) and fused sodium acetate (0.02 mole) was added to acidic alumina at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 35 sec at 180^{0}C. The mixture was cooled and then product was extracted with dry methanol, concentrated and cooled. The solid thus separated was filtered, washed thoroughly with water and recrystallized from ethanol. Yield 84%; m.p. 242-245^{o}C; IR (KBr) ν cm^{-1} 740 (1,4-disubstituted benzene ring), 1520 (C-N stretching), 1610 (C=C), 1660 (C=N), 3050 (aromatic C-H stretching); ^{1}H NMR (TMS) δ ppm: 2.24 (s, 3H, CH_3), 5.54 (s, 2H, NH), 7.47 - 7.90 (m, 9H, ArH); Anal., Calcd. For $C_{21}H_{14}N_8OS_2$ (508): C, 55.00, H, 3.07, N, 24.43 Found: C, 54.76, H, 3.31, N, 24.72.

N-[4-methyl-5-(5-thioxo(4H-1,2,4-triazolo[3,4-b]1,3,4-thiadiazolidin-3-yl)-1,3-thiazol-2-yl] benzamide (6)

Carbon disulphide (0.015 mole) was added dropwise with constant stirring to the solution of **(1)** (0.01mole) in methanolic KOH solution. Acidic alumina was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40 sec at 180^{0}C. The mixture was cooled and then product was extracted with dry methanol, which was then poured onto ice and acidified with dil. HCl. The solid thus separated was fil-

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tered and recrystallized from aq. ethanol. Yield 87%; m.p. 221-224°C; IR (KBr) ν cm^{-1} 1130 (CS), 1520 (C-N stretching), 1600 (C=C), 1665 (C=N), 3050 (aromatic C-H stretching); ^1H NMR (TMS) δ ppm: 2.33 (s, 3H, CH_3), 5.95 (s, 2H, NH), 7.31 (t, 2H, ArH, *J*= 12.1 MHz), 7.47 (t, 1H, ArH), 7.75 (d, 2H, ArH, *J*= 15.3 MHz); Anal., Calcd. For $C_{14}H_{10}N_6OS_3$ (354): C, 44.90, H, 2.69, N, 22.44 Found: C, 44.67, H, 2.34, N, 22.23.

N-[4-methyl-5-(5-Phenyl-(1,2,4-triazolo[3,4-b] 1,3,4-thiadiazolin-3-yl))-1,3-thiazol-2-yl] benzamide (7)

A solution of **(1)** (0.01mole) and p-toluic acid (0.01 mole) in $POCl_3$ (5mL) was prepared. Acidic alumina was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40 sec at 180°C. The mixture was cooled and then poured onto ice and neutralized with aq. potassium carbonate solution. The solid thus separated was filtered and recrystallized from hexane. Yield 85 %; m.p. 217-221°C; IR (KBr) ν cm^{-1} 830 (1,4-disubstituted benzene ring), 1520 (C-N stretching), 1600 (C=C), 1620 (C=N), 3060 (aromatic C-H stretching); ^1H NMR (TMS) δ ppm: 2.24 (s, 3H, CH_3), 5.78 (s, 1H, NH), 7.21 –7.94 (m, 10H, ArH); Anal., Calcd. For $C_{20}H_{14}N_6OS_2$ (418): C, 57.40, H, 3.37, N, 20.08 Found: C, 57.17, H, 3.56, N, 20.35.

N-{4-methyl-5-[5-(3-nitrophenyl)(4H,5H-1,2,4-triazolo[3,4-b]1,3,4-thiadiazolidin-3-yl)]-1,3-thiazol-2-yl}benzamide (8)

A solution of **(1)** (0.01mole) and m-nitrobenzal dehyde (0.01 mole) was prepared. Acidic alumina was added to the above solution at room temperature. The reaction mixture was mixed, adsorbed, dried and kept inside the alumina-bath and irradiated for 40 sec at 180°C. The mixture was cooled and then product was extracted with dry toluene, concentrated and cooled. The solid thus separated was filtered and recrystallized from ethanol. Yield 85 %; m.p. 217-221°C; IR (KBr) ν cm^{-1} 1350, 1540 (NO_2), 1520 (C-N stretching), 1600 (C=C), 1620 (C=N), 3070 (aromatic C-H stretching); ^1H NMR (TMS) δ ppm: 2.37 (s, 3H, CH_3), 4.72 (d, 1H, CH), 5.58 (s, 1H, NH), 5.73 (d, 1H, NH), 7.43 –7.87 (m, 9H, ArH); Anal., Calcd. For $C_{20}H_{15}N_7O_3S_2$ (465): C, 51.60, H, 3.24, N, 21.06 Found: C, 51.36, H, 3.53, N, 21.41.

(Temperature for all reactions were measured by stirring the reaction mixture with thermometer and then immediately observing the temperature.)

RESULTS AND DISCUSSION

Antibacterial activity

Antibacterial activity of compounds was tested using microbroth dilution method[20-22].

Tested microorganism strains were; *Escherichia coli* (ATCC 10798), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 7700). Chloramphenicol was used as control drug. The observed data on the antibacterial activity of the compounds and control drugs were given in TABLE 1.

TABLE 1: MIC values of the compounds as μg ml^{-1}

Compd.	*E. coli*	*S. aureus*	*P. aeruginosa*
1	100	100	200
2	100	400	400
3	100	400	400
4	400	400	400
5	100	100	200
6	200	200	200
7	200	400	400
8	400	200	400
Chloramphenicol	50	12.5	200

Anti-inflammatory activity

Acute inflammation was produced by injecting 0.1 ml of (1%) carrageenan into plantar surface of rat hind paw[23]. The test compounds 1-8 (100 mg/kg orally) and phenylbutazone (100 mg/kg orally) as reference agent were administered 60 min before carrageenan injection. The paw volume was measured at 0, 1, 2, 3 and 4 h, using a thread to determine the diameter of edema formation size. The difference in diameter between the left and right hind paws was taken as a measure of edema. The results of the anti-inflammatory effect of the synthesized compounds on carrageenan-induced edema in rat's right hind paws are presented in TABLE 2. There was a gradual increase in edema paw volume of rats

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TABLE 2: Anti-inflammatory activity of methanolic extract of *Cassia auriculata* root and phenylbutazone (100 mg/kg) on carrageenan induced rat paw oedema in the right hind-limb paw of rats

Treatment	Dose (mg/kg)	Time (h)					Average oedema Formation
		0	1	2	3	4	
Control		-	0.41 ± 0.11	0.73 ± 0.02	0.79 ± 0.10	0.93 ± 0.20	0.71 ± 0.10
Compound -1	100	-	0.30 ± 0.11	0.32 ± 0.11*	0.23 ± 0.11*	0.16 ± 0.14*	0.24 ± 0.01*
Compound -2	100	-	0.31 ± 0.11	0.33 ± 0.11*	0.22 ± 0.11*	0.15 ± 0.14*	0.22 ± 0.01*
Compound -3	100	-	0.33 ± 0.11	0.31 ± 0.11*	0.22 ± 0.11*	0.14 ± 0.14*	0.22 ± 0.01*
Compound -4	100	-	0.33 ± 0.11	0.31 ± 0.11*	0.22 ± 0.11*	0.17 ± 0.14*	0.21 ± 0.01*
Compound -5	100	-	0.32 ± 0.11	0.29 ± 0.11*	0.20 ± 0.11*	0.14 ± 0.14*	0.21 ± 0.01*
Compound -6	100	-	0.32 ± 0.11	0.31 ± 0.11*	0.25 ± 0.11*	0.15 ± 0.14*	0.23 ± 0.01*
Compound -7	100	-	0.28 ± 0.11	0.30 ± 0.11*	0.22 ± 0.11*	0.17 ± 0.14*	0.25 ± 0.01*
Compound -8	100	-	0.30 ± 0.11	0.35 ± 0.11*	0.21 ± 0.11*	0.15 ± 0.14*	0.22 ± 0.01*
Phenylbutazone	100	-	0.21 ± 0.10	0.23 ± 0.02*	0.23 ± 0.01*	0.18 ± 0.05*	0.22 ± 0.03*

Values are mean ± S.E.M. (n = 6), *P < 0.05 of the difference between the left and the right hind paws

in the control [carrageenan treated]. However, in the test groups, the compounds showed a significant reduction in the edema paw volume. As indicated in TABLE 2, a dose-related inhibition of hind paws edema between 2 and 4 h was observed.

Animals: Swiss albino mice of both sex weighing between (20-25 g) and Albino Wistar rats of either sex (180-200 g) were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet supplied by Hindustan lever Ltd. Kolkata, India, and water *ad libitum*. The study was duly approved by IAEC.

Grouping of animal for anti-inflammatory activity (n=6)

1. Control (Vehicle treated)
2. Compound-1
3. Compound-2
4. Compound-3
5. Compound-4
6. Compound-5
7. Compound-6
8. Compound-7
9. Compound-8
10. Phenylbutazone (100 mg/kg)

Acute toxicity study

Acute toxicity study was performed in rats divided into different groups of 6 each. After an over night fast, the test drug was administered orally in graded doses [100-500 mg/kg]. They were observed continuously for the first 2 h for toxic symptoms and up to 24 h for mortality[24].

Statistical analysis

The results and data obtained in this study were evaluated using the one-way analysis of variance (ANOVA) test between two mean groups; control and test groups, followed by Student's *t*-test. Significant levels were at $P < 0.05$

CONCLUSION

From the result, it is suggested that anti-edematogenic effects of the compounds **(1-8)** on carrageenan-induced edema may be related to inhibition of inflammation mediator formation; however, it is necessary to identify the exact mechanism.

Based on the results of antibacterial study it can be concluded that compounds **(1-8)** has potential antibacterial activity. Thus it can be stated that, the chemical moieties discussed here, may undergo lead optimization and by applying 3D QSAR a better dual inhibitor can be designed.

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