ORIGINAL RESEARCH PAPER

Chemistry

GREEN SYNTHESIS OF BENT SHAPED MESOGENIC THIADIAZOLES AND ITS PERFORMANCE EVALUATION OF BIOLOGICAL PROPERTIES AND CYTOTOXIC ACTIVITIES

KEY WORDS: Thiadiazoles, bent shaped, green synthesis, biological activity, Liquid Crystal

Prabha Modi* Assistant Professor, Parul University *Corresponding Author

Priyanka Sindhwa M. Sc. Student, Parul University

Vishal Modi Owner, Innovestra Solution

ABSTRACT

While 80% of commercial medications are based on heterocyclic moieties, many synthesis processes lack sustainability, highlighting the need for environmentally friendly techniques. N-heterocyclic scaffolds play a crucial role in drug design, with an increasing number of new analogues demonstrating significant physiological and pharmacological value. To meet current demands for effective and sustainable methods, traditional synthetic protocols need modification. Recently, various techniques have been developed to promote the environmentally friendly production of important N-heterocyclic compounds. In this context, Rod shaped 4-substitutedbenzoic acid-N'-(4'-n-alkoxybenzoyl) hydrazide (series I) have been synthesized by the Schotten-Baumann reaction of 4-Subsitutedbenzhydrazide with 4-n-alkoxybenzoyl chloride using triethyl amine as basic media in dry dichloromethane, as a solvent. The series I compounds have been cyclized to bent shaped mesogenic 2-(4'-Substituedphenyl)-5-(4''-n-alkoxyphenyl)-1, 3, 4-thiadiazoles (series II) using lawesson's reagent under microwave condition. The synthesized compounds are characterized by combination of elemental analysis and standard spectroscopic methods. In series II, all the synthesized compounds mesogenic properties. The biological properties and cytotoxic activities of mesogenic compounds in the present work are compared with each other to evaluate the effect on properties.

INTRODUCTION

A significant class of organic compounds, which is characterized by ring structures having at least one atom other than carbon, commonly may be nitrogen (N), Oxygen (O) or Sulphur (S) known as a heteroatom, is known as heterocyclic compounds. These heterocyclic compounds are having various biological properties and synthetic materials, including nucleic acids, vitamins, and pharmaceuticals. Synthesis of Three, Four, Five, Six, and Seven member heterocyclic compounds are very well reported. Among these azole family having a structural formula containing two carbon atoms, two nitrogen atoms, and one sulfur atom named as thiadiazoles five-membered heterocyclic compounds are well reported. Thiadiazole ring is aromatic due to the presence of double bonds and a lone pair of electrons from sulfur, contributing to its stability and reactivity. The most common isomer is 1,3,4-thiadiazole, which has gained significant attention in pharmaceutical applications due to its diverse biological activities. 1, 3, 4-oxadiazole derivatives are reported by several methods (18) compare to 1, 3, 4-thiadiazoles. The synthesis of 1, 3, 4-thiadiazole ring is reported by ring formation using by sulphuration of the N, N'-diacylhydrazines using reagents such as P4S10 and Lawesson's reagent.

The synthesis of thiadiazoles has gained attention due to their potential in liquid crystal technology and biology. Their cytotoxic effects vary with structure and substituents, with certain compounds inducing apoptosis in cancer cell lines, making them candidates for cancer therapies. Mesogenic thiadiazoles show promise for developing therapeutic agents with diverse activities. Their dual role as medicinal compounds and technological materials highlights their significance in chemistry and pharmacology. Further research is needed to clarify their mechanisms and optimize clinical efficacy. Thiadiazole synthesis involves reactions that exploit their electron-deficient nature, allowing for nucleophilic substitution and the creation of derivatives with enhanced biological activity. Compounds with mesogenic properties exhibit both solid and liquid characteristics, akin to the phospholipid structure of microbial cell walls, allowing for easy penetration. Thiadiazoles with bent shapes were traditionally synthesized, but this work presents a green synthesis method for these compounds to compare their biological properties and cytotoxic activities against the conventional method.

Experiment

MATERIAL AND METHODS

The required starting materials such as 4-methylbenzoic acid, 4-nitrobenzoic acid, 4-hydroxybenzoic acid, 4-chlorobenzoic acid. lawesson's reagent, potassium hydroxide, thionyl chloride, hydrazine hydrate (99%), 1-bromoalkane, sulphuric acid, methanol, dichloromethane, triethyl amine, hydrochloric acid, ethyl acetate, diethyl ether, hexane, silica gel (100-200 mesh) etc. were procured from Aldrich Company and used without any further purification. All the solvents were purified and dried by standard method. Analytical TLC was conducted on Merck aluminium plates with 0.2 mm of silica gel 60 F-254. Microwave synthesis was carried out by using Samsung GW71B domestic equipment

General Procedure

Methyl-4-susbstitutedbenzoate (A) and 4-substitutedbenzhydrazide (B) were synthesized by reported method. 4-n-Alkoxybenzoic acid (C) and 4-n-alkoxybenzoyl chloride (D) were synthesized by modified method of Dave and Vora.

General methods for synthesis of (4-Substituedbenzoic acid-N'-(4'-n-alkoxybenzoyl) hydrazide (series I):

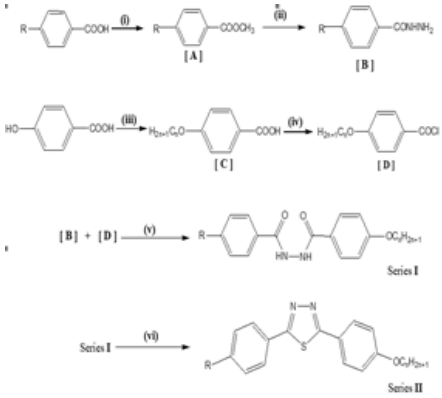
4-Substitutedbenzoic acid-N'-(4'-n-alkoxybenzoyl) hydrazide (series I) was synthesized by condensing equimolar quantity of 4-substitutedbenzhydrazide (B) and 4-n-alkoxybenzoyl chloride (D) and triethyl amine, in dry dichloromethane with stirring. The reaction mixture was poured in cold aqueous (1:1) HCl. The solid material obtained was filtered off and was chromatographed on silica gel (100-200 mesh) using mixture of ethyl acetate and hexane (20:80) as eluent. Removal of solvent from the eluted afforded a solid material, which was crystallized repeatedly from alcohol. The reaction products were obtained as off-white solids in 80-85 % yield. Initially the purity of all these compounds was checked by thin layer chromatography (Merck kieselgel 60F254 pre-coated plates). 1H NMR, FTIR and Elemental analysis, for the n-decyloxy and n-dodecyloxy derivatives as representative members of series are carried out and found satisfactory.

General Microwave synthesis method for synthesis of 2-(4'-

Substituedphenyl)-5-(4''-n-alkoxyphenyl)-1, 3, 4-thiadiazole (series II)

2-(4'-Substitutedphenyl)-5-(4''-n-alkoxyphenyl)-1, 3, 4-thiadiazole (series II) was synthesized by reaction mixture of equimolar of series I compound and lawensson's reagent was kept under microwave for 50 seconds to 75 seconds at 760 W. The mixture of reaction was cooled to ambient temperature followed by pouring in to sufficient quantity of ice. Crude solid was observed which was filtered off. It was further purified by column chromatography on silica gel (100-200 mesh) using mixture of polar and non-polar mixture of solvent i.e. by ethyl acetate and hexane (20:80) as eluent. Solvent was removed by distillation on rota-vapor and observed a solid material, which was further recrystallized using alcohol. The reaction products were obtained as off-white solids in 85-95 % yield.

n = 1 to 8, 10, 12, 14 and 16 (series I and II) R = -CH3, -NO2, -Cl
Reagents and Conditions: (i) CH3OH, H2SO4, Reflux (ii) Hydrazine hydrate, Ethanol, Reflux (iii) R-Br, Alcoholic KOH, Reflux (iv) SOCl2 (v) Triethyl amine, Methylene dichloride, Stirring (vi) Lawesson's reagent, Microwave at 760 W



Scheme 1: Synthetic route to series I-II compounds

Detection Method

By thin layer chromatography (Merck kieselgel 60F254 pre-coated plates) purity of all synthesized compounds were checked. Spectral and Elemental analysis data for the n-decyloxy, n-dodecyloxy, n-tetradecyloxy and n-hexadecyloxy derivatives as representative members, which found satisfactory.

Biological properties and cytotoxic activities: Antibacterial and antifungal activity (Barry, 1976)

The synthesized compounds were tested in vitro for their antibacterial activities and antifungal activities against Escherichia coli; Staphylococcus aureus and Aspergillus oryzae; Aspergillus niger by cup-plate agar diffusion method, which are pathogenic in human beings. The procedure was followed as per reported method. The synthesized were dissolved in DMF having 1000 ppm concentration and added 0.1 mL of tested. It was diffused at 4 C. The plate was incubated overnight at 37 C after 30 minute of diffiusion. The zone of inhibition was observed and reported in table.

Cytotoxicity Test: Brine shrimp lethality bioassay (BSLT) was performed as per reported.

All synthesized compound were added in vial at room temperature for 24 hours under the light and were counted. Test was performed along with control standard at different concentration varies from 1 – 5000 µg / mL. Replicate results were generated to get accurate results.

RESULTS AND DISCUSSION

The synthetic route of 2-(4'-Substituedphenyl)-5-(4''-n-alkoxyphenyl)-1,3,4-thiadiazole is outlined in Scheme 1. The synthesis of 2-(4'-Substituedphenyl)-5-(4''-n-alkoxyphenyl)-1,3,4-thiadiazole is reported by conventional method. In present work, we report green synthesis of bent shaped mesogenic 2-(4'-Substituedphenyl)-5-(4''-n-alkoxyphenyl)-1,3,4-thiadiazole by microwave method to do comparison over conventional method, it is reported in table 2. Initially, purity of synthesized compounds were checked by TLC. Final characterization was done by spectroscopic method i.e. 1H NMR, FTIR technique. It is found satisfactory and identical as conventional method. Mesogenic properties was checked on optical polarizing microscope. Mesogenic behavior of synthesized compounds are reported in table 3. The mesophase length and thermal stability of 2-(4'-Chlorophenyl)-5-(4''-n-alkoxyphenyl)-1,3,4-thiadiazole are more, as compared to 2-(4'-Nitrophenyl)-5-(4''-n-alkoxyphenyl)-1,3,4-thiadiazole and 2-(4'-Methylphenyl)-5-(4''-n-alkoxyphenyl)-1,3,4-thiadiazole, which satisfactory found in reported value. All the synthesized compounds are evaluated its microbial properties. We observed that –Cl and –NO2 substituted thiadiazole having moderate antibacterial activities than –CH3 substituted thiadiazole. In other word, we found that compounds having smectic mesophase having moderate antibacterial activities.

Bioassay of toxic substances are tested by Brine Shrimp Lethality Test. All the synthesized compounds were tested for cytotoxic activity by the BSLT bioassay method. Synthesized compounds showed a dose dependent cytotoxic activity at different concentrations, which is reported in table 4. The degree of lethality is directly proportional to the concentration of the synthesized compounds. Podophyllotoxin was used as a standard drug for BSLT assay method.

Table 1: Comparison of Microwave over Conventional Synthesis Route for Series II

Compounds n =	Conventional Method		Microwave Method	
	Reaction time* (Hours)	Yield (%)	Reaction time* (Seconds)	Yield (%)
Series -CH_3				
1	8.5	60.0	60	88
2	8.5	61.0	70	85
3	8.5	58.0	65	90
4	8.0	61.0	65	88
5	8.0	64.0	65	90
6	8.0	65.0	75	94
7	8.0	55.0	70	88
8	8.0	58.0	70	85
10	8.0	70.0	65	90
12	8.0	64.0	60	85
14	8.0	67.0	70	90
16	8.0	66.0	65	90
Series -NO_2				
6	8.0	75.0	50	90
7	8.0	72.0	55	95
8	7.5	70.0	50	90
10	7.0	78.0	60	94
12	7.0	75.0	55	93
14	7.0	70.0	55	95
16	7.0	74.0	55	95
Series -Cl				
5	8.0	77.0	60	85
6	8.5	75.0	65	88
7	8.0	76.0	55	90
8	7.5	77.0	65	88
10	8.0	79.0	65	92
12	7.5	80.0	65	90
14	7.5	76.0	55	92
16	7.5	75.0	50	92

*Completion of reaction inially monitored by TLC.

Table 2: Transition temperature (°C) of the Series II compounds

Compounds n =	Crystal		Smectic A		Nematic		Isotropic (Liquid)
Series - CH_3							
1	●	145	-	-	●	222	●
2	●	118	-	-	●	218	●
3	●	120	-	-	●	210	●
4	●	121	-	-	●	208	●
5	●	118	-	-	●	204	●
6	●	120	-	-	●	192	●
7	●	112	-	-	●	186	●
8	●	110	-	-	●	187	●
10	●	91	-	-	●	172	●
12	●	85	-	-	●	155	●
14	●	95		138	●	154	●
16	●	90		134	●	150	●
Series - NO_2							
6	●	170	-	-	-	-	●
7	●	178	-	-	-	-	●
8	●	200	(	185)	-	-	●
10	●	177		218	-	-	●
12	●	155		230	-	-	●
14	●	150		239	-	-	●
16	●	165		248	-	-	●
Series - Cl							
5	●	151	-	-	-	-	●
6	●	128		251	-	-	●
7	●	135		244	-	-	●
8	●	114		241	-	-	●
10	●	113		239	-	-	●
12	●	110		238	-	-	●
14	●	120		230	-	-	●
16	●	128		225	-	-	●

Table 3: Microbial Properties of the Series II compounds

Compounds n =	Anti bacterial Blank 12 mm		Anti fungal Blank 10 mm	
	E. coli	S. aureus	A. niger	A. oryzae
Series -CH_3				
1	12.50	12.25	11.75	11.00
2	12.75	12.50	11.25	10.50
3	12.50	12.50	11.75	11.00
4	12.50	12.25	11.00	10.75
5	12.75	12.50	11.25	10.50
6	12.50	12.25	11.75	11.00
7	12.75	12.50	11.25	10.50
8	12.75	12.50	11.25	10.50
10	12.50	12.25	11.75	11.00
12	12.75	12.25	11.25	10.50
14	13.00	12.50	11.75	11.00
16	13.00	12.50	11.75	11.00
Series -NO_2				
6	13.25	13.00	11.75	11.25
7	13.50	13.25	11.50	11.00
8	13.25	13.00	11.50	11.00
10	13.00	13.25	11.75	11.25
12	13.25	13.00	11.50	11.00
14	13.50	13.25	11.75	11.25
16	13.50	13.00	11.50	11.00
Series -Cl				
5	14.00	13.25	11.00	10.75
6	14.25	13.50	11.25	10.25
7	14.00	13.75	11.25	10.75
8	13.75	13.50	11.50	10.50
10	14.00	13.25	11.25	11.00
12	13.50	13.25	11.50	10.50
14	13.75	13.75	11.25	10.25
16	14.00	13.25	11.50	10.75

Furacin (as a standard): E. coli: 14.75; S. aureus: 14.75; A. niger: 12.00; A. oryzae: 12.00 mm.

Grieseofulvin (as a standard): E. coli: 12.00; S. aureus: 12.00; A. niger: 10.00; A. oryzae: 11.75 mm

Table 3: Cytotoxic activity of data for synthesized Series II

Compounds n =	Solubility in DMSO	Compound n =	Solubility in DMSO	Compounds n =	Solubility in DMSO
	Ed_{50} µg/mL		ED_{50} µg/mL		ED_{50} µg/mL
Series -CH_3		Series -Cl		Series -NO_2	
1	35.20	5	37.55	6	38.25
2	37.50	6	39.76	7	40.00
3	39.32	7	38.28	8	39.22
4	40.25	8	39.65	10	41.25
5	32.12	10	39.53	12	42.52
6	38.24	12	40.15	14	41.55
7	39.23	14	39.43	16	40.33
8	38.25	16	41.25		
10	38.60	Podophyllotoxin	3.88		
12	39.22				
14	41.25				
16	42.52				

CONCLUSION

In present work, we reported green synthesis of bent shaped mesogenic 2-(4'-Substituedphenyl)-5-(4''-n-alkoxyphenyl)-1,3,4-thiadiazole by microwave method and compare over conventional method. We conclude that compounds are synthesized by microwave having better yield, lesser by product, consume less time and no solvent was used to complete the reactions. We had evaluated for microbial properties and cytotoxic activity. We conclude that compounds having smetic mesophase having moderate microbial activities than other mesophase. The cell wall of organisms are made of phospholipids and it is lyotropic mesophase which has similar pattern as smetic mesophase. The degree of toxicity is directly proportional to the concentration of the synthesized compounds

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