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SYNTHESIS OF SOME NOVEL THIAZOLIDIN-4-ONE SUBSTITUTED 1, 2,4 - TRIAZOLES OF THEIR ANTIMICROBIAL ACTIVITY STUDIES

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ABSTRACT

Thiazolidine-4-one substituted 1, 2, 4- triazole and benzofuran heterocyclic have received considerable attention during last two decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. A literature survey indicates that benzofuran derivatives possess different pharmacological and biological activities, of which the most potent is anti-microbial activity. When one biologically active molecule is linked to another, the resultant molecule generally has increased potency. Hence two pharmacophores, i.e. benzofuran and thiazolidine-4-one substituted 1,2,4-triazole are fused to obtain highly potent, more specific and less toxic antimicrobial agent The starting product 3-methoxybenzofuran hydrazide was prepared from methylsalicylate and diethylbromomalonate and hydrazine hydrate in three steps. The hydrazide was converted into the corresponding potassium dithiocabazinate, which on cyclization with hydrazine hydrate afforded 4-amino-3-(3-methoxy benzofuran)- 5-mercapto -1, 2, 4-triazoles. Condensation of triazoles with substituted aromatic benzaldehydes gave Schiff bases, which have been cyclized by treating with mercapto acetic acid. The compounds were characterized by elemental analysis and spectral data. All the compounds showed a very good antibacterial activity and significant the compounds VIIIe and VIIIf were found to possess broad-spectrum activity, while other compounds were found to exhibit moderate activities.

Key words: Thiazolidine-4-one, benzofuran heterocyclic, substituted 1, 2, 4- triazole, antibacterial activity

INTRODUCTION

Benzofuran compounds are ubiquitous in nature, particularly among plant kingdom. Often such natural products possessing benzofuran nucleus are endowed with useful pharmacological properties. This has generated enormous interest in synthetic products containing benzofuran nucleus and has resulted in the development of benzofuran chemistry during the last several years.

Due to wide scope for synthetic investigation leading to more potent synthetic leads, voluminous synthetic work has been done. Several benzofuran compounds are reported to posses antibacterial[1-4], antifungal[2], antitumor[5-6] anti-inflammatory[7], antidepressant[8], analgesic[9] and hypoglycemic[10]activities. Substituted triazoles have been reported for antimicrobial[11-14] activity. Hence we plan to couple the benzofuran nucleus with Schiff's bases of 1, 2, 4- triazole derivatives and screen for antimicrobial activity. Thiazolidine-4-one substituted moieties have received considerable attention during last two decades as they are endowed with variety of biological activities and have wide range of therapeutic properties. When one biologically active molecule is linked to another, the resultant molecule generally has increased potency. Hence in the present study the two pharmacophores, i.e. benzofuran and

thiazolidine-4-one substituted 1, 2, 4-triazole are fused to obtain highly potent, more specific and less toxic antimicrobial agent

EXPERIMENTAL

All chemicals used were of synthetic grade. The purity of compounds was ascertained by TLC on precoated silica F254 plates (Merck, Mumbai, India) using iodine vapors and UV light as detecting agents. The melting points of the synthesized compounds were determined by open capillary method and are uncorrected. The IR spectra of synthesized compounds were recorded on a SHIMADZU FT-IR spectrophotometer in KBr. The ^{1}H-NMR were recorded in $CDCl_3$ and DMSO-d_6 using a NMR Varian-Mercury 300 MHz spectrometer and chemical shifts (δ) are given in parts per million (ppm) downfield from tetramethylsilane (TMS) as an internal standard. Physical characterization data of all the compounds are given in Table 1.

Diethylbromomalonate (I)

Diethylmalonate (160g, 1 mol) and carbon tetrachloride (150 ml) were placed in three necked flask fitted with a reflux condenser and a dropping funnel, condenser may be fixed with a cork inserted with a glass tube which may be connected to rubber tubing, the end of which may be dipped in water for absorption of hydrogen bromide. In the separating funnel 165 g (53 ml; 1.03 mol) of dry bromine was taken and added slowly, the large electric bulb was held under flask until the reaction starts. Then the rest of the bromine was added gradually at such a rate so as to keep the reaction mixture boiling gently. After the addition, the reaction mixture was refluxed until no more hydrogen bromide gas evolved (about 1 hour). The reaction mixture was cooled and washed five times with 50 ml portions of anhydrous sodium carbonate solution and solvent was removed under reduced pressure. The pure diethylbromomalonate which distilled at 132-133^{0}C under 33 mm pressure was collected (175 g).

2-Carbethoxy-3 (2H) benzofuranone (II)

A mixture of freshly distilled methylsalicylate (30.4g, 0.2mol), diethylbromomalonate (48g, 0.2mol) and anhydrous potassium carbonate (60g) in dry acetone (150ml) was heated under reflux on water bath while stirring magnetically for 12hours. The reaction mixture was filtered and potassium salts are washed with dry ether until colorless. The dry salt was suspended in water (200ml) and cooled thoroughly in ice bath. The suspension was carefully acidified with diluted hydrochloric acid and the 2-carbethoxy-3(2H) benzofuranone which separates as a colorless solid was collected by filtration. On crystallization from petroleum ether it was obtained as colorless needles. m.p 63^{0}C ; yield: 20g.

2-carbethoxy 3-methoxy benzofuran (III)

To a solution of 2-carbethoxy 3-(2H) benzofuranone (10.3g, 0.053 mol) in acetone (150 ml), anhydrous potassium carbonate (25g) and dimethylsulphate (8.1g) were added and the mixture was heated under reflux on water bath for 6 hours, cooled and filtered. Removal of solvent under reduced pressure from filtrate give a thick oil which solidified slowly. It was crystallized from petroleum ether to give 2-carbethoxy 3-methoxy benzofuran as colorless needles. m.p: 62^{0}C; yield: 9g.

3-methoxy benzofuran 2-carboxy hydrazide (IV)

To a solution of 2-carbethoxy-3-methoxy benzofuran (10.2g, 0.053mol) in ethanol (30ml), hydrazine hydrate (5ml, 99%) was added and the mixture was magnetically stirred at room temperature for 2hours. The colorless crystalline carboxyhydrazide started separating within a few minutes and was complete in about 2 hours. The reaction mixture was cooled thoroughly in ice bath and carboxyhydrazide was collected by filtration on crystallization from ethanol, it was obtained as colorless needles. M.p : 135^0 C yields: 5.2g.

3-methoxy benzofuran potassium dithiocabazinate (V)

A mixture of 3-methoxybenzofuran 2-carboxy hydrazide (2g 0.01mol), carbon disulphide (0.01mol) were taken in a round bottom flask to it alcoholic potassium hydroxide (0.15mol) was added. This reaction mixture was refluxed with stirring on a magnetic stirrer for a period of 10 hours then it is kept aside for cooling, the separated potassium salt was filtered out and washed with ether, dried and used directly for next step without further purification, Mp: 220^{0}C, yield:80%.

2-(4-amino-5-mercapto-1,2,4-triazol-3-yl) 3-methoxy benzofuran(VI)

A mixture of potassium dithiocabazinate (0.01mol), hydrazine hydrate 99% (1.5ml, 0.03mol) and water (2ml) was heated under reflux for 6 hours. Hydrogen sulphide gas evolved and homogenous solution resulted in about 5 hours. Dilution of the reaction mixture with water (50ml) and subsequent acidification with diluted hydrochloric acid gave the compound, collected by filtration, washed with water and recrystallised from ethanol; Mp. 205^0C. yield: 70%

4-(substituted benzyl amino)-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazole-3-thiol (VII)

A mixture of (0.01mol) 2-(4-amino-5-mercapto-1,2,4-triazol-3-yl) 3-methoxy benzofuran, substituted benzaldehyde (0.01mol), anhydrous sodium acetate (0.02mol) and glacial acetic acid(20ml) were taken in a round bottom flask kept under stirring and reflex for 5 hours. The reaction mixture was cooled and poured into crushed ice with vigorous stirring. The precipitated solid obtained was filtered off and recrystallized from ethanol, mp: 165^0c, yield: 80%.

3-[3-mercapto-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazol-4-yl]-2-substituted phenyl-1,3-thiazolidin-4-oneVIII (a –h)

A mixture of different substituted Schiff base (VII) (0.001 mol) in dry benzene (25 ml) was added to mercapto acetic acid (0.01 mol). The reaction mixture was refluxed for 6 hr. A solid product was obtained after cooling. The reaction was monitored by TLC plate using chloroform: benzene (1:2) used as solvent system.

VIII (a) 3-[3-mercapto-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazol-4-yl]-2-phenyl-1,3-thiazolidin-4-one (**b**) 2-(2-nitro phenyl)-3-[3-mercapto-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazol-4-yl]-1,3-thiazolidin-4-one (**c**) 2-(3-chlorophenyl)-3-[3-mercapto-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazol-4-yl]-1,3-thiazolidin-4-one (**d**) 2-(2-chlorophenyl)-3-[3-mercapto-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazol-4-yl]-1,3-thiazolidin-4-one (**e**) 2-(2-hydroxyphenyl)-3-[3-mercapto-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazol-4-yl]-1,3-thiazolidin-4-one. (**f**) 3-[3-mercapto-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazol-4-yl]-2-(2-methoxyphenyl)-1,3-thiazolidin-4-one, IR (KBr). *ν* (cm-1):3298-3071 (NH/SH str), 2950 (CH str), 1397 (C=S str), 1651 (C=N str) MS**:** m/z (%) 380 (32%) [M+.] (**g**)2-(4-bromophenyl)-3-[3-mercapto-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazol-4-yl]-1,3-thiazolidin-4-one, IR (KBr) (cm^{-1}):3258 (NH/SH str), 2927.98 (CH str), 1367 (C=S str), 1099 (C-O-C) and 1620 (C=O of thiazolidinone ring). H^1NMR (DMSO-d_6) δ ppm: 4.42 (s, 1H, CH-Ar), 11.69 (s, 1H, NH+SH), 3.08 (s, 2H, CO-CH_2-S), 2.49 (s, 3H, OCH_3), 7.19-8.30 (m, 8H, Ar-H). MS**:**m/z (%) 504.3 (10%) [M+.] (**h**)2-(4-chlorophenyl)-3-[3-mercapto-5-(3-methoxy-1-benzofuran-2-yl)-4*H*-1,2,4-triazol-4-yl]-1,3-thiazolidin-4-one, IR (KBr) (cm^{-1}):3078 (NH/SH str), 2927.93 (CH str), 1261 (C=S str), 1100 (C-O-C) and 1605 (C=O of thiazolidinone ring).H^1NMR (DMSO-d_6) δ ppm:4.39 (s, 1H, CH-Ar), 11.62 (s, 1H, NH+SH), 3.12 (s, 2H, CO-CH_2-S), 2.47 (s, 3H,OCH_3), 7.16-8.27 (m, 8H, Ar-H).MS:m/z (%) 457 (12%) [M+.]

Antimicrobial activity

Antibacterial activities were studied by subjecting the compounds to pharmacological screening by standard procedures[15].

All the compounds synthesized in the present investigation were tested for their antimicrobial activity. The antibacterial activities were tested on nutrient medium against *Bacillus pumilus, Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli,* and *Pseudomonas aeruginosa*.

Preparation of nutrient as a medium:

Media composition and procedure: The nutrient agar media was prepared by using the following ingredients. 1. Peptone 20g2. Beef extract5g 3.Sodium chloride5g 4.Agar Agar 20 g. 5. Distilled water up to 1000 ml

Weighed quantities of peptone, beef extract were dissolved in distilled water and pH was adjusted to 7.2-7.4 using pH paper. Then the specified amount of agar was added kept the beaker on hot water bath and allowed the agar to melt, it was dispensed in suitable containers and plugged them with non-adsorbent cotton they were sterilized by autoclave at 121^0C for 20 minutes.

Preparation of solutions of test compounds: Now 1mg/ml concentration of the test compounds was prepared using DMF as solvent it is considered, as stock solution from this 100µg/ml concentration is prepared using DMF as solvent and this was used for antimicrobial activity studies.

Preparation of standard antibiotic solution: Ampicillin 20µg/ml was used as a standard antibiotic for comparison and it was prepared by using sterile water.

Procedure: Sterile nutrient agar medium was cooled to 45^0C this media was inoculated with 18-24 hours old bacterial culture under aseptic conditions mixed well by gentle shaking then it was poured in to sterile petris dishes and allowed the medium to set. After setting all the seeded petris dishes were transferred to laminar flow unit and 5 cups were made by using sterile cork borer. Out of 5cups, 2cups were added with 50µl of the standard antibiotic (Ampicillin) and solvent control one in each bore, test compounds were added to the remaining 3 bores one in each bore. Then they were allowed for diffusion for 2 hours and incubated at 37^0C for 24hrs. The inhibition zone diameters were measured and the results are shown in Table 2.

Determination of MIC

Organism: (*Escherichia coli)* Minimum inhibitory concentrations of the synthesized compounds were determined by taking different concentrations of the compound in DMF. Increasing order of concentrations were added by using sterilized pipettes to different test tubes which contains sterilized broth medium inoculated with sensitive organism with respect to the particular compound. Then all the test tubes were incubated at 37^0C for 24 hours. Then after incubation period presence of growth (turbidity) was observed. Turbidity was not observed from concentration 100µg/ml this is the MIC of the compound.

Table-1: Some characterizations of the compounds

Sl. No.	Prod Code	R	Molecular Formula	Melting Point ^{0}C	Rf * Value	Yield %
1	VIIIa	Phenyl	$C_{19}H_{14}N_4O_3S_2$	250	0.68	80
2	VIIIb	2-Nitro phenyl	$C_{19}H_{13}N_5O_5S_2$	276	0.68	73
3	VIIIc	3-Chloro phenyl	$C_{19}H_{13}N_4O_3S_2Cl$	245	0.69	78
4	VIIId	2-Chloro phenyl	$C_{19}H_{13}N_4O_3S_2Cl$	250	0.69	70
5	VIIIe	2-Hydroxy phenyl	$C_{19}H_{14}N_4O_4S_2$	282	0.61	71
6	VIIIf	2-Methoxy phenyl	$C_{20}H_{14}N_4O_3S_2$	250	0.72	65
7	VIIIg	4-Bromo phenyl	$C_{19}H_{13}N_4O_3S_2Br$	240	0.71	75
8	VIIIh	4-Chloro phenyl	$C_{19}H_{13}N_4O_3S_2Cl$	248	248	68

* Chloroform, acetone, (1:1).

RESULTS AND DISSCUSION

Eleven compounds were screened for antibacterial activity studies at a concentration of 100µg/ml using DMF as a control against *Escherichia coli, S. aureus, B.pumillus, B.substilis*, and *P.aeruginosa* by cup-plate method on nutrient agar Himedia, ampicillin 20µg /ml used as standard against Gram positive and Gram negative bacteria. The data in the Table 2 indicate that VIIIe and VIIIf compounds were found to possess a board- spectrum activity. While other compounds were found to exhibited activity from the antibacterial screening, it was found that synthesized compounds showed moderate activity at the given concentration levels. Perhaps the thiazolidin-4-one substituted at 1, 2, 4-triazole moiety and 3-methoxy benzofuran moiety at 3rd position may be solely responsible for the marked bactericidal activity. The above results established the fact that thiazolidin-4-one substituted 1, 2, 4-triazoles with benzofuran moiety can be studied for further investigations in search of new antimicrobial compounds.

Table-2: Inhibition zone diameters

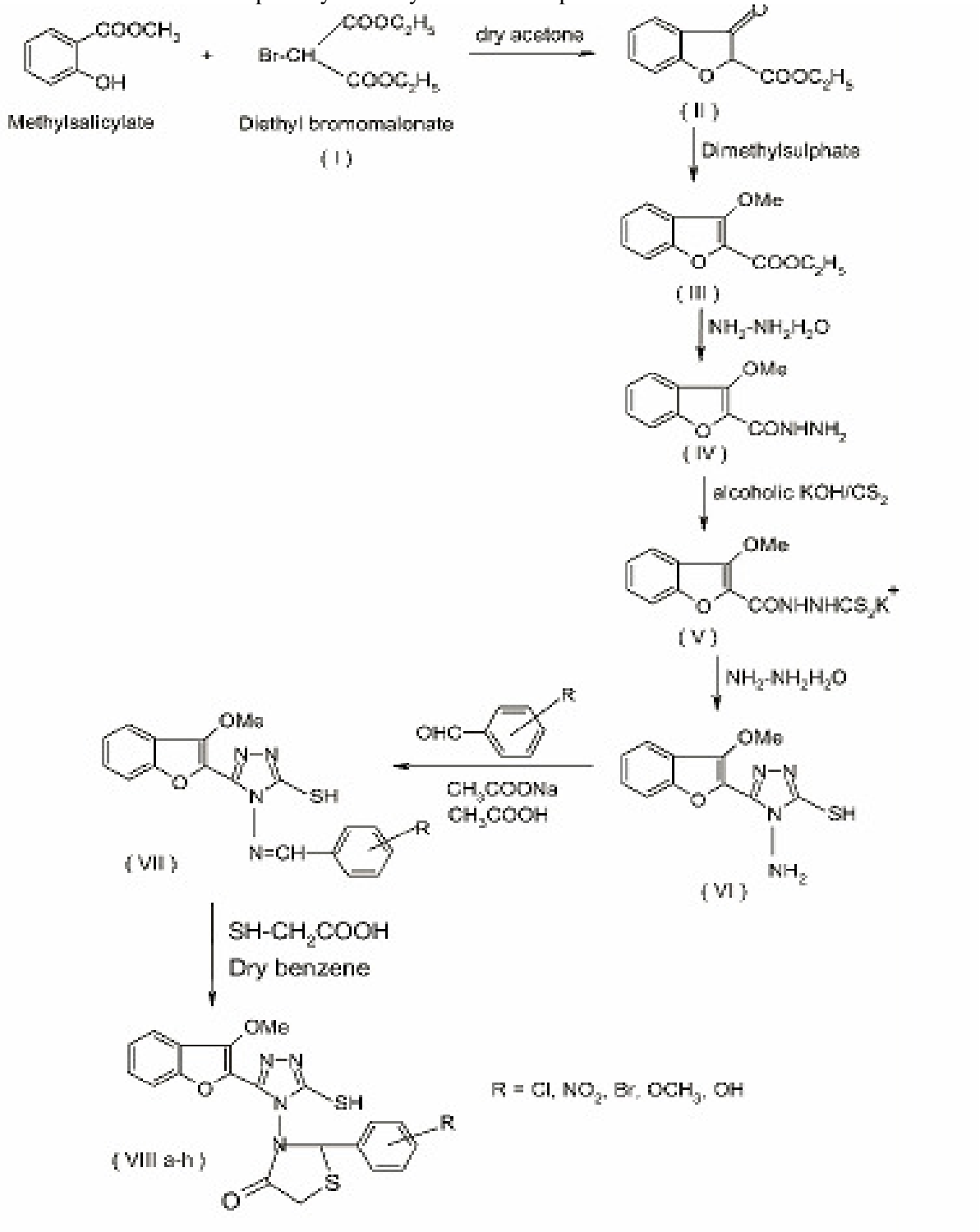
Sample Code100µg/ml	*Inhibition zone diameter in mm				
	S.aureus	*B.substilis*	*B.pumilus*	*E.coli*	*P.aeruginosa*
VII	14	12	10	10	14
VIIIa	16	12	12	12	14
VIIIb	16	16	17	15	13
VIIIc	18	16	16	16	18
VIIId	17	16	18	18	16
VIIIe	22	20	20	22.5	20
VIIIf	21	22	21	22.5	20
VIIIg	14	13	12	14	15
VIIIh	12	14	16	10	16
Ampicillin (20 µg/ml)	25	25	26	26	25
DMF	8	7	8	7	7

* Average of three independent determinations

CONCLUSION

The two moieties i.e. 3-methoxybenzofuran and thiazolidin-4-one substituted 1, 2, 4-triazole moieties independently are antibacterial agents. Here when the two moieties are fused and screened for possible antimicrobial studies, they showed a broad spectrum of antibacterial activity against G (+ve) and G (-ve) bacteria. Benzofuran and triazole molecule is responsible for antibacterial activity, but it is interesting to note that thiazolidin-4-one substituted 1, 2, 4-triazole moiety showed a broad-spectrum

antibacterial activity. The above results establish the fact that thiazolidin-4-one substituted 1, 2, 4-triazole benzofuran can be a potential source for exploitation in search of new generation of antibiotics. It may be worthwhile to explore the possibility in this area by fusing other heterocyclic moieties and increase the potency of the synthesized compounds.



Scheme-1

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