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Synthesis of Novel Methylene Bis-[1,2,4] Triazoles as Antibacterial Agents

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Abstract

A novel series of methylene-bis-[1,2,4]- triazole derivatives were synthesized by the reaction of 4-amino-5-(5-[7-(4-amino-5-sulfanyl-4H-1,2,4-triazoles-3-yl)-3-methyl benzo[b]furan5-yl]methyl benzo[b] furan-7-yl)4H-1,2,4-triazole-3-thiol and corresponding aryl isothiocyanate.All the synthesized compounds have been tesed for their antibacerial activity.

Keywords: 1,2,4 triazoles, antibacterial activity.

Introduction

Nitrogen containing heterocyclic compounds have got a broad specrum of biological and pharmacological activities. Severral research groups have contributed to the development of methods of synthesis of fused 1,2,4 triazoles. The thiazole nucleus appears frequently in the structure of various natural products and biologically active compounds, notably thiamine, penicillin, antibiotics such as micrococcin[1], troglitazone[2] and many metabolic products of fungi and primitive marine animals, including 2-(aminoalkyl)thiazole-4-carboxylic acids[3]. Numerous triazolone derivatives have shown significant pharmacological and biological activities[4] like sedative[5], anti-inflammatory[6], antibacterial[7], antifungal[8], antitubercular[9], anticancer[10], antitumor[11], analgesic and hypothermic[12], local and spinal anestetic[13], CNS stimulant[14], hypnotic[15], anti-HIV[16] and nematicidal[17].

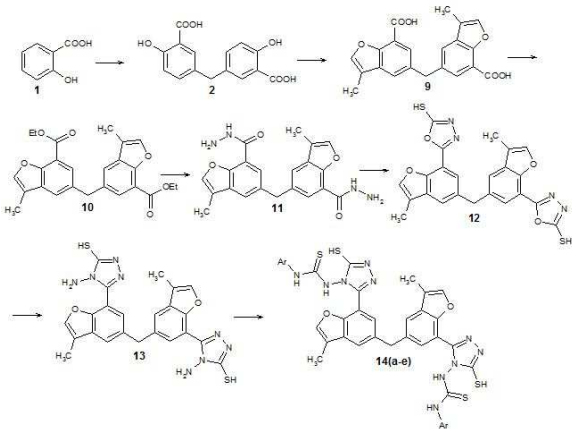
Results and Discussion

The starting material for the synthesis of target molecules is bis salicylic acid.This can be prepared by salicylic acid **1** and formaldehyde in the presence of conc.sulfuric acid for 22 h to get **2**. An intermediate for the synthesis has been prepared by the condensation of compound**2** with chloroacetone in the presence of K_2Co_3 and catalytic amount of KI at reflux for 12h followed by cyclization to yield **9**. This was treated with absolute ethyl alcohol in the presence of conc.H_2SO_4 at reflux for 3h, to get ethyl 5-[7-ethoxycarbonyl)-3-methylbenzo[b] furan-5-yl]methyl-3-methylbenzo[b] furan-7carboxylate **10** in good yield. The intemediate, 5-[7-(hydrazinocarbonyl)-3-methylbenzo[*b*]furan-5-yl]methyl-3-methylbenzo[*b*]furan-7-carbohydrazide, **11** is prepared on hydrazinolysis of compound **10** with hydrazine hydrate, in ethyl alcohol at reflux for 4 h, with 70% of yield**.**

The 5-[7-(hydrazinocarbonyl)-3-methylbenzo[*b*]furan-5-yl]methyl-3-methylbenzo [*b*]furan-7-carbohydrazide **11** was reacted with carbon disulfide in the presence of potassium hydroxide, in ethanol at reflux for 12 h, followed by acidification gave the 5-(3-methyl-5-[3-methyl-7-(5-sulfanyl-1,3,4-oxadiazol-2-yl)benzo[*b*]furan-5-yl]methyl benzo[*b*]furan-7-yl)-1,3,4-oxadiazole-2-thiol **12.** Compound **12** when reacted with hydrazine hydrate, in ethanol at reflux for 6 h, resulted the 4-amino-5-(5-[7-(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-3-methylben- zo[*b*]furan-5-yl]methyl-3-methylbenzo[*b*]furan-7-yl)-4*H*-1,2,4-triazole-3-thiol **13.**

Further, the 4-amino-5-(5-[7-(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-3-methyl benzo[*b*]furan-5-yl]methyl-3-methylbenzo[*b*]furan-7-yl)-4*H*-1,2,4-triazole-3-thiol **13** has been reacted successively with a variety of arylisothiocyanate in dry DMF at room temperature for 24 h, to get the title compounds, methylene-bis-[1,2,4]- triazoles **14(a-e).**The formation of these compounds was confirmed by their spectral and elemental analyses**.**

The IR spectrum of **11** showed absorption bands at 3270 and 1630 cm^{-1} due the NH_2 and C=O groups. The mass spectrum of compound **11** showed the peak at *m/z* 391 (M^+).In the ^1H NMR spectra of compounds **14(a-e)**, the disappearance of the signal corresponding to the -NH_2 at δ 1.37 ppm, and the appearance of the singlet signal at δ 9.85 and 10.02 ppm for NH, confirms the reaction with arylisothiocyanate involving the NH_2 group of the triazole



14;Ar=a) phenyl, b)3-fluoro phenyl, c)4-fluoro phenyl, d)4-cholo phenyl,e)4-bromophenyl,
Scheme-1

Antibacterial Activity

All the newly prepared compounds 14(a-e) were screened for their antibacterial activity against three gram-positive baceriaviz. Bacillus Subtilis Bacillus Sphaericus and Staphylococcus Aureus, and three Gram-negative bacteria viz. Pseudomonas Aeruginosa,klobsinella Aerogenes and Chromobacterium Violaceum. The minimum inhibition concentration was determined by broth dilution 41 method. The antibacterial activity of each compound was compared with the standard drugs streptomycin and penicillin. The inhibition zone data and MIC values of the compounds careened are presented in Table.

Table 4: Antibacterial activity of methylene-bis-[1, 2,4]-triazoles 14(a-e)

Comp d.	Diameter of growth inhibition zone (mm at 100 μg/mL) and Minimum inhibitiory concentration					
	B. subtilis	***B. sphaericus***	***S. aureus***	***P. aerugi nosa***	***K. aeroger es***	***C. violac eum***
14a	17 (12.5)[a]	15 (25.0)[a]	20 (12. 5)[a]	19 (12.5)[a]	16 (12.5)[a]	13 (25.0)[a]
14b	28 (6.25)	30 (3.12)	35 (3.1 2)	28 (3.12)	26 (6.25)	22 (6.25)
14c	14 (25.0)	14 (12.5)	20 (12. 5)	10 (25.0)	18 (12.5)	10 (25.0)
14d	28 (6.25)	26 (6.25)	40 (6.2 5)	20 (1.56)	28 (3.12)	22 (3.12)
14e	24 (12.5)	30 (12.5)	20 (6.2 5)	22 (25.0)	30 (25.0)	22 (25.0)
Stre pto- my cin	30 (6.25)	30 (6.25)	41 (6.2 5)	15 (6.25)	25 (1.56)	20 (3.12)
Pen icill in	25 (1.56)	28 (3.12)	40 (1.5 6)	25 (1.56)	30 (6.25)	25 (12.5)

[a]*Values in the parentheses indicate the minimum inhibitory concentration (MIC, µg/mL).*

The antibacterial screening data showed that almost all the compounds **14(a-e)** are active and showing moderate to good antibacterial activity, among the screened **14b**, **14c** and **14e** in which phenyl ring of isothiocyanate moiety bearing 3-fluoro, 4-fluoro and 4-bromo groups respectively showed high activity against all the micro-organisms employed. The activities of these compounds are almost equal to the standards. The remaining compounds showed moderate to good antibacterial activity.

Experimental Section

Synthesis of bis[3-methylbenzo[*b*]furan-7-carboxylicacid]methane (9)

To a stirred solution of **2** (5 mmol), anhydrous potassium carbonate (3 mmol) and a catalytic amount of potassium iodide in dry acetone (30 mL), was added drop wise a solution of chloroacetone (10 mmol) in dry acetone (20 mL) at reflux temperature. Reflux was continued for 12 h. The reaction mixture was concentrated to dryness and then transferred into ice water, and the solid separated was collected by filtration. The crude product was dissolved in ethanolic potassium hydroxide (10%, 100 mL) and further refluxed for 18 h. The excess ethanol was then removed by distillation *in vacuo*, the reaction mixture was poured into ice-cold aq. HCl and the solid collected by filtration, purified by column chromatography using petrolium-ether (60-80^{o}C) as eluent to give pure **9** as yellow solid, yield 82%, m.p. 182-84 ^{o}C. **IR** (KBr): ν_{max} 3400–3300, 2953, 1685, 1043 cm^{-1}. **^{1}H NMR** (DMSO-d_6 300 MHz): δ 11.4 (s, 2H, COOH), 7.60-6.85 (m, 6H, Ar-H), 3.80 (s, 2H, CH_2), 2.39 (s, 6H, CH_3). **MS**: *m/z* (%) 365 (M^++1, 10%), 106 (100%). ***Anal.* Calcd.** for $C_{21}H_{16}O_8$: C, 69.23; H, 4.43. Found: C, 69.18; H, 4.40.

Synthesis of ethyl 5-[7-(ethoxycarbonyl)-3-methylbenzo[*b*]furan-5-yl] methyl-3-methylbenzo[*b*]furan-7-carboxylate (10)

To the solution of **9** (0.01 mol) in absolute ethyl alcohol (25 mL), conc. H_2SO_4 (2 mL) was added. The mixture was refluxed for 3 h. After completion of the reaction (TLC), the mixture was poured into ice-cold water. Crude product was collected by filtration, washed with 10% $NaHCO_3$ solution, dried and recrystallized from ethyl alcohol to get pure product **10** with 74% of yield, m.p. 162-64 ^oC. **IR** (KBr): ν_{max} 2943, 1698, 1621, 1513, 1249, 1034 cm^{-1}. **^{1}H NMR** (DMSO-d_6, 300 MHz): δ 1.24 (t, 6H, CH_3), 2.40 (s, 6H, CH_3), 4.18 (s, 2H, CH_2), 4.00 (q, 4H, CH_2), 7.20 (s, 2H, ArH), 7.80 (s, 2H, ArH), 8.12 (s, 2H, ArH). **MS**: *m/z* 419 (M^+-1). ***Anal.* Calcd.** for $C_{25}H_{24}O_6$: C, 71.42; H, 5.75; N, 22.83. Found: C, 71.40; H, 5.70; N, 22.76.

Synthesis of 5-[7-(hydrazinocarbonyl)-3-methylbenzo[*b*]furan-5-yl]methyl-3-methyl benzo[*b*]furan-7-carbohydrazide (11)

A mixture of compound **10** (0.01 mol) and hydrazine hydrate (0.025 mol) in ethanol (20 mL) was refluxed for 4 h, cooled to room temperature and filtered. The crude product was recrystallized from ethanol to give the new intermediate **11** in 70% of yield, m.p. 158-60 ^oC.

IR (KBr): ν_{max} 3270, 1630, 1610, 1395, 741 cm^{-1}. **^{1}H NMR** (DMSO-d_6 300 MHz): δ 2.29 (s, 6H, CH_3), 4.25 (s, 2H, CH_2), 5.30 (s, 4H, NH_2), 7.10-7.20 (m, 6H, ArH), 7.70 (s, 2H, NH). **MS**: *m/z* 391 (M^+-1). ***Anal.* Calcd.** for $C_{21}H_{20}N_4O_4$: C, 64.28; H, 5.14; N, 14.28. Found: C, 64.22; H, 5.10; N, 14.22.

Synthesis of 5-(3-methyl-5-[3-methyl-7-(5-sulfanyl-1,3,4-oxadiazol-2-yl)benzo [*b*] furan-5-yl]methylbenzo[*b*]furan-7-yl)-1,3,4-oxadiazole-2-thiol (12)

A mixture of compound **11** (0.01 mol), potassium hydroxide (0.02 mol) and carbon disulfide (0.03 mol) in ethanol (100 mL) was heated under reflux with stirring for 12 h. The solvent was distilled *in vacuo*, the residual mass was poured over crushed ice and neutralized the alkaline solution with 10% hydrochloric acid. The precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol to get the pure compound **12** in 71% yield, m.p. 146-48 ^oC. **IR** (KBr): ν_{max} 3030, 2902, 2843, 1601, 1569, 1070 cm^{-1}. **^{1}H NMR** (DMSO-d_6 300 MHz): δ 2.38 (s, 6H, CH_3), 3.99 (s, 2H, CH_2), 7.20-7.50 (m, 4H, ArH), 7.85 (s, 2H, ArH), 11.20 (s, 1H, SH/NH). **MS**: *m/z* 475 (M^+-1). ***Anal.* Calcd.** for $C_{23}H_{16}N_4O_4S_2$: C, 57.97; H, 3.38; N, 11.76. Found: C, 57.92; H, 3.32; N, 11.75.

Synthesis of 4-amino-5-(5-[7-(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-3-methyl- benzo[*b*]furan-5-yl]methyl-3-methylbenzo[*b*]furan-7-yl)-4*H*-1,2,4-triazole-3-thiol (13)

To a warm solution of compound **12** (0.01 mol) in ethanol (50 mL), 80% hydrazine hydrate (0.03 mol) was added drop wise and the reaction mixture was heated under reflux for 6 h. The solvent was distilled off *in vacuo*, cooled and the crystals separated were filtered, washed with cold ethanol and recrystallized from chloroform to give pure compound **13**, in 68% yield, m.p. 167-69 ^oC. **IR** (KBr): ν_{max} 3343, 3068, 1663, 1030 cm^{-1}.

^{1}H NMR (DMSO-d_6 300 MHz): δ 1.37 (bs, 4H, NH_2), 2.50 (s, 6H, CH_3), 3.99 (s, 2H, CH_2), 6.90-7.50 (m, 4H, ArH), 7.90 (s, 2H, ArH), 9.70 (s, 2H, SH/NH). **MS**: *m/z* 505 (M^+ +1). ***Anal.* Calcd.** for $C_{23}H_{20}N_8O_2S_2$: C, 54.75; H, 3.99; N, 22.21. Found: C, 54.70; H, 3.90; N, 22.16.

General procedure for the synthesis of methylene-bis-[1,2,4]-triazoles 14(a-e)

To a solution of compound **13** (2 mmol) in dry DMF (15 mL), the appropriate arylisothiocyanate (4 mmol) was added and the solution was stirred at room temperature for 24 h. Water (30 mL) was then added and the mixture was stirred for 30 minutes. The separated precipitate was filtered, washed with water, dried and crystallized for ethanol to afford pure compound **14(a-e)** in 75-87% yield.

Methylene-bis-[1,2,4]-triazole-N'phenylthiourea (14a) : IR (KBr): ν_{max} 3332-3200, 3042, 1679, 1596, 1030 cm^{-1}. **^{1}H NMR** (DMSO-d_6 300 MHz): δ 4.18 (s, 2H, CH_2), 2.71 (s, 6H, CH_3), 6.90-7.00 (m, 10H, ArH), 7.30-7.40 (m, 2H, ArH), 9.80-9.85 (m, 4H, NH & ArH), 10.02 (s, 2H, NH), 12.70 (s, 2H, SH). **MS**: *m/z* 774 (M^+). ***Anal.* Calcd.** for $C_{37}H_{30}N_{10}O_2S_4$: C, 57.35; H, 3.90; N, 18.07. Found: C, 57.31; H, 3.84; N, 18.00.

Methylene-bis-[1,2,4]-triazole-N'-3-fluorophenylthiourea (14b) : IR (KBr): ν_{max} 3333-3200, 3069, 1680, 1592, 1030 cm^{-1}. **^{1}H NMR** (DMSO-d_6 300 MHz): δ 4.18 (s, 2H, CH_2), 2.71 (s, 6H, CH_3), 6.50-6.80 (m, 8H, ArH), 7.30-7.40 (m, 2H, ArH), 9.80-9.85 (m, 4H, NH & ArH), 10.02 (s, 2H, NH), 12.70 (s, 2H, SH). **MS**: *m/z* 810 (M^+). ***Anal.* Calcd.** for $C_{37}H_{28}F_2N_{10}O_2S_4$: C, 54.80; H, 3.48; N, 17.27. Found: C, 54.74; H, 3.40; N, 17.22.

Methylene-bis-[1,2,4]-triazole-N'-4-fluorophenylthiourea (14c) : IR (KBr): ν_{max} 3300-3200, 3072, 1676, 1590, 1032 cm^{-1}. **^{1}H NMR** (DMSO-d_6 300 MHz): δ 4.18 (s, 2H, CH_2), 2.71 (s, 6H, CH_3), 6.70-6.90 (m, 8H, ArH), 7.30-7.40 (m, 2H,

ArH), 9.80-9.85 (m, 4H, NH & ArH), 10.02 (s, 2H, NH), 12.70 (s, 2H, SH). **MS**: *m/z* 810 (M^+). ***Anal.* Calcd.** for $C_{37}H_{28}F_2N_{10}O_2S_4$: C, 54.80; H, 3.48; N, 17.27. Found: C, 54.76; H, 3.44; N, 17.25.

Methylene-bis-[1,2,4]-triazole-N'-4-chlorophenylthiourea (14d) : IR (KBr): ν_{max} 3300-3200, 3072, 1679, 1584, 1032, 686 cm^{-1}. **^{1}H NMR** (DMSO-d_6 300 MHz): δ 4.18 (s, 2H, CH_2), 2.71 (s, 6H, CH_3), 6.90-7.00 (m, 8H, ArH), 7.30-7.40 (m, 2H, ArH), 9.80-9.85 (m, 4H, NH & ArH), 10.02 (s, 2H, NH), 12.70 (s, 2H, SH). **MS**: *m/z* 843 (M^+). ***Anal.* Calcd.** for $C_{37}H_{28}Cl_2N_{10}O_2S_4$: C, 52.66; H, 3.34; N, 16.60. Found: C, 52.60; H, 3.30; N, 16.58.

Methylene-bis-[1,2,4]-triazole-N'-4-bromophenylthiourea (14e) : IR (KBr): ν_{max} 3300-3200, 3072, 1679, 1584, 1032, 584 cm^{-1}. **^{1}H NMR** (DMSO-d_6 300 MHz): δ 4.18 (s, 2H, CH_2), 2.71 (s, 6H, CH_3), 6.90-7.00 (m, 8H, ArH), 7.30-7.40 (m, 2H, ArH), 9.80-9.85 (m, 4H, NH & ArH), 10.02 (s, 2H, NH), 12.70 (s, 2H, SH). **MS**: *m/z* 932 (M^+). ***Anal.* Calcd.** for $C_{37}H_{28}Br_2N_{10}O_2S_4$: C, 47.65; H, 3.03; N, 15.02. Found: C, 47.61; H, 3.00; N, 15.04.

Conclusion

In conclusion, we have described the synthesis of novel methylene-bis-[1,2,4]- triazoles **14(a-e)** in good to excellent yields by the reaction of 4-amino-5-(5-[7-(4-amino-5-sulfanyl-4*H*-1,2,4-triazol-3-yl)-3-methylbenzo[*b*]furan-5-yl]methyl-3-methyl benzo[*b*] furan-7-yl)-4*H*-1,2,4-triazole-3-thiol **13** and corresponding arylisothiocyan- ate. Also evaluated their antibacterial, among the screened **14b**, **14c** and **14e** in which phenyl ring of isothiocyanate moiety bearing 3-fluoro, 4-fluoro and 4-bromo groups respectively showed high activity against all the micro-organisms employed. The activities of these compounds are almost equal to the standards. The remaining compounds showed moderate to good antibacterial activity.

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