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Synthesis and antibacterial activity of 2-substituted benzothiazoles

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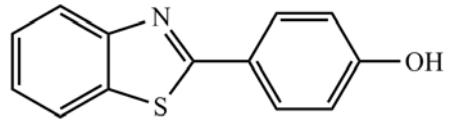
Multicomponent amidoalkylation reaction of benzothiazole, alkyl chloroformates and cyclic carbonyl compounds for the synthesis of five 2-substituted benzothiazoles is described. The conditions for chromatographic separation and isolation of the newly synthesized products by column chromatography on silica gel or neutral alumina are established. The isolated crystalline compounds are characterized spectrally and tested for antibacterial activity

Key words: *Benzothiazole, Multicomponent reaction, Antibacterial activity*

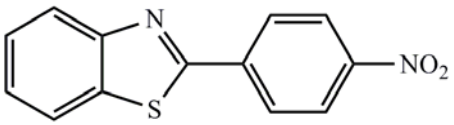
INTRODUCTION

Benzothiazole and its derivatives are important heterocyclic compounds, which are common feature of many natural products and pharmaceutical agents [1]. Benzothiazoles have attracted continuing interest because of their varied biological activities such as anticancer [2], anticonvulsant [3], antiviral [4], antitubercular [5], analgesic [6], anti-inflammatory [7], antidiabetic [8] and other activities. Moreover, 2-substituted benzothiazoles (Figure 1) exhibit activity against both Gram-positive and Gram-negative bacteria [9, 10].

Multicomponent reactions are simple and efficient method in the sustainable and diversity-oriented synthesis of heterocycles with various biological activity [11]. The ease of access to a large number of compounds, combined with high-throughput screening techniques make multicomponent reactions a very important tool in modern drug discovery [12].



4-(benzo[*d*]thiazol-2-yl)phenol

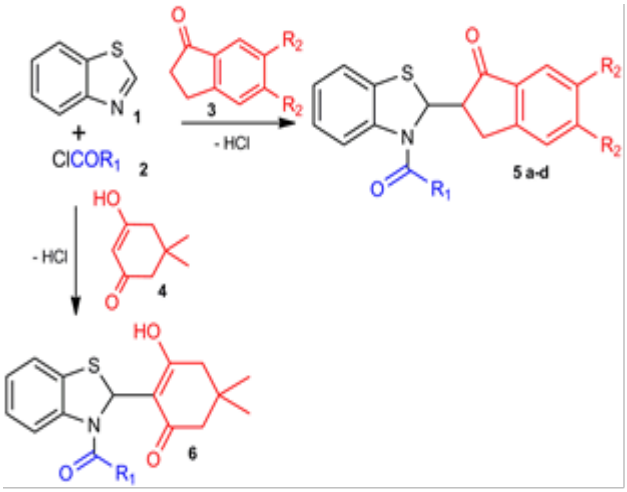


2-(4-nitrophenyl)benzo[*d*]thiazole

Figure 1. 2-substituted benzothiazoles with antimicrobial activity

This method for performing the reactions offers several advantages, such as simple procedure, short time, clean reaction conditions, simplified purification and good yields [13].

We have previously used N-acyliminium ions derived from various cyclic imines (e.g. 3,4-dihydroisoquinoline, benzimidazole, benzothiazole) and acyl chlorides as electrophilic reagents in intermolecular α-amidoalkylation reactions toward methylene active carbonyl compounds [14-19]. In continuation of our studies on the functionalization of aza-aromatic systems, we herein report a simple and catalyst free method for the direct coupling of cyclic ketones such as 1-indanone, 5,6-methylenedioxy-1-indanone and dimedone with benzothiazole, activated by alkyl chloroformates (Scheme 1).



Scheme 1. One-pot syntesis of 2-substituted benzothiazoles **5 a-d**, **6**

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EXPERIMENTAL

General information:

Commercial solvents and reagents, such as benzothiazole, alkyl chloroformates and cyclic ketones were purchased from Sigma-Aldrich and were used without further purification. Melting points were determined on a Boetius PHMKO5 hot stage apparatus and are uncorrected. IR and MS spectra were measured on Perkin Elmer 1750 Furie Transform and HRMS "Q-Exative Orbitrap" (Thermo Fisher Scientific, Waltham, MA, USA) spectrometers, respectively. ^{1}H-NMR, ^{13}C-NMR spectra were measured on Bruker Avance AV600 and DRX 300 devices in $CDCl_3$ and MeOD as solvents. Chemical shifts are given in part per million (ppm) relative to TMS and coupling constants are indicated in Hz. All the NMR spectra were taken at room temperature in $CDCl_3$ or MeOD. TLC was done on precoated 0.2 mm Merck silica gel 60 plates. Silica gel and neutral alumina were used for column chromatographic separation.

General one-pot procedure for the synthesis of 2-substituted benzothiazoles 5a-d, 6

To benzothiazole (2 mmol) dissolved in 1,2-dichloroethane (5 mL) were added alkyl chloroformate (2 mmol) and the corresponding cyclic ketone (2 mmol). The reaction mixture was stirred for 2 h at 80 °C or 24 h at room temperature (Table 1). After completion of the reaction (monitored by TLC), 30 mL $CHCl_3$ was added and the mixture was extracted successively with 50 mL 10% HCl, 50 mL 3% Na_2CO_3 and 3x20 mL water. The combined organic layers were dried (Na_2SO_4) and concentrated. After the distillation of the solvent ($CHCl_3$) the products were purified by column chromatography on silica gel or neutral alumina using mixtures of petroleum and diethyl ether as eluents.

*Ethyl-2-(1-oxo-2,3-dihydro-1H-inden-2-yl) benzo[d]thiazole-3(2H)-carboxylate (**5 a**)*

Isolated with elluents petroleum : diethyl ether 8:1, 4:1; Yield: (76 %); M.p.: 127-130 °C;

^{1}H-NMR (300 MHz, $CDCl_3$) (δ, ppm): 1.38 (t, J = 7.2, 3H, $CO_2CH_2\underline{CH_3}$), 3.00 (dd, ^{2}J = 18, ^{3}J = 5.4, 1H, Ar$\underline{CH_2}$CH), 3.10 (dd, ^{2}J = 18, ^{3}J = 7.5, 1H, Ar$\underline{CH_2}$CH), 3.54 – 3.59 (m, 1H, CH), 4.32 – 4.39 (m, 2H, $CO_2\underline{CH_2}CH_3$), 6.48 (d, J = 4.2, 1H, CH), 6.94 – 7.14 (m, 3H, Ar), 7.34 – 7.40 (m, 2H, Ar), 7.54 – 7.77 (m, 3H, Ar);

^{13}C-NMR (300 MHz, $CDCl_3$) (δ, ppm): 14.69, 27.67, 53.75, 62.77, 65.42, 117.54, 122.10, 124.11, 124.38, 125.62, 126.74, 127.71, 123.91, 154.42, 204.41;

m/z $[M+Na]^+$ calcd. 362.11, found 362.11; $[2M+Na]^+$ calcd. 701.33, found 701.33;

IR (KBr, cm^{-1}): ν(C=O) – 1703, 1724, ν(C-O) – 1268, ν(C-S-C) – 750, ν(Csp^2-H) – 2982, 3065, ν(C=C) – 1472

*Methyl-2-(1-oxo-2,3-dihydro-1H-inden-2-yl)benzo[d]thiazole-3(2H)-carboxylate (**5 b**)*

Isolated with elluents petroleum : diethyl ether 4:1, 3:1; Yield: (86 %); M.p.: 138-141 °C;

^{1}H-NMR (600 MHz, $CDCl_3$) (δ, ppm): 3.02 (dd, ^{2}J = 18, ^{3}J = 6, 1H, Ar$\underline{CH_2}$CH), 3.10 (dd, ^{2}J = 18, ^{3}J = 6, 1H, Ar$\underline{CH_2}$CH), 3.58 (br. s, 1H, CH), 3.92 (s, 3H, $CO_2\underline{CH_3}$), 6.49 (br. s, 1H, CH), 6.98 – 7.15 (m, 4H, Ar), 7.38 – 7.42 (m, 2H, Ar), 7.58 – 7.79 (m, 2H, Ar);

^{13}C-NMR (600 MHz, $CDCl_3$) (δ, ppm): 27.47, 53.42, 117.36, 121.95, 123.96, 124.34, 127.58, 135.06, 136.71, 154.30, 204.50;

m/z $[M+Na]^+$ calcd. 348.06, found 348.06, $[M-H]^-$ calcd. 324.07, found 324.07;

IR (KBr, cm^{-1}): ν(C=O) – 1698, 1722, ν(C-O) – 1271, ν(C-S-C) – 758, ν(Csp^2-H) – 2997, ν(C=C) – 1471

*Ethyl-2-(5-oxo-6,7-dihydro-5H-indeno[5,6-d][1,3]dioxol-6-yl)benzo[d]thiazole-3(2H)-carboxylate (**5 c**)*

Isolated with elluent petroleum : diethyl ether 4:1; Yield: (60 %); M.p.: 97-99 °C;

^{1}H-NMR (600 MHz, $CDCl_3$) (δ, ppm): 1.40 (t, J = 6, 3H, $CO_2CH_2\underline{CH_3}$), 2.89 (dd, ^{2}J = 18, ^{3}J = 6, 1H, Ar$\underline{CH_2}$CH), 2.97 (dd, ^{2}J = 18, ^{3}J = 6, 1H, Ar$\underline{CH_2}$CH), 3.57 (br. s, 1H, CH), 4.33 – 4.40 (m, 2H, $CO_2\underline{CH_2}CH_3$), 6.06 (d, J = 6, 2H, O$\underline{CH_2}$O), 6.47 (d, J = 6, 1H, CH), 6.77 (s, 1H, Ar), 6.97 – 7.13 (m, 4H, Ar), 7.85 (br. s, 1H, Ar);

^{13}C-NMR (600 MHz, $CDCl_3$) (δ, ppm): 14.57, 27.40, 102.35, 102.38, 105.75, 117.33, 124.18, 125.41, 131.40, 148.44, 154.61, 201.96;

m/z $[M+Na]^+$ calcd. 406.07, found 406.07;

IR (KBr, cm^{-1}): ν(C=O) – 1688, 1718, ν(C-O) – 1256, ν(C-S-C) – 754, ν(Csp^2-H) – 2980, 3056, ν(C=C) – 1468, ν(O-CH_2-O) – 2913

*Methyl-2-(5-oxo-6,7-dihydro-5H-indeno[5,6-d][1,3]dioxol-6-yl)benzo[d]thiazole-3(2H)-carboxylate (**5 d**)*

Isolated with elluent petroleum : diethyl ether 4:1; Yield: (81 %); M.p.: 92-94 °C;

^{1}H-NMR (600 MHz, $CDCl_3$) (δ, ppm): 2.79 (dd, ^{2}J = 18, ^{3}J = 6, 1H, Ar$\underline{CH_2}$CH), 2.87 (dd, ^{2}J = 18, ^{3}J = 6, 1H, Ar$\underline{CH_2}$CH), 3.47 (br. s, 1H, CH), 3.83 (s, 3H, $CO_2\underline{CH_3}$), 5.98 (d, J = 6, 2H, O$\underline{CH_2}$O), 6.36 (br. s, 1H, CH), 6.67 (s, 1H, Ar), 6.88 – 7.04 (m, 4H, Ar), 7.76 (br. s, 1H, Ar);

^{13}C-NMR (600 MHz, $CDCl_3$) (δ, ppm): 27.37, 53.42, 102.36, 105.54, 105.75, 111.20, 114.25, 117.31, 120.49, 121.91, 124.30, 125.45, 131.36, 148.45, 154.64, 201.95;

m/z $[M+Na]^+$ calcd. 392.06, found 392.06;

IR (KBr, cm^{-1}): ν(C=O) – 1694, 1719, ν(C-O) – 1258, ν (C-S-C) – 747, ν(Csp^2-H) – 2954, ν(C=C) – 1472, ν(O-CH_2-O) – 2918

*Ethyl-2-(4,4-dimethyl-2,6-dioxocyclohexyl)benzo[d]thiazole-3(2H)-carboxylate (**6**)*

Isolated with elluent petroleum:diethyl ether 4:1; Yield: (67 %); M.p.: 160-161 °C;

^{1}H-NMR (300 MHz, MeOD) (δ, ppm): 1.04 (s, 6H, 2xCH_3), 1.26 (t, J = 7.2, 3H, $CO_2CH_2\underline{CH_3}$), 2.27 (s, 4H, 2xCH_2), 4.17 (q, J = 7.2, 2H, $CO_2\underline{CH_2}CH_3$), 6.87 (s, 1H, CH), 6.88 – 6.91 (m, 1H, Ar), 6.96 – 7.03 (m, 2H, Ar), 7.72 (d, J = 7.8, 1H, Ar);

^{13}C-NMR (300 MHz, MeOD) (δ, ppm): 14.78, 28.26, 32.89, 58.99, 63.01, 116.41, 117.16, 121.71, 124.10, 125.32, 131.15, 154.37;

m/z $[M+Na]^+$ calcd. 370.11, found 370.11, $[2M+Na]^+$ calcd. 717.32, found 717.32

IR (KBr, cm^{-1}): ν(C=O) – 1708, ν(C-O) – 1247, ν(C-S-C) – 738, ν(Csp^2-H) – 2995, 3064, ν(C=C) – 1472

Antibacterial studies:

The antibacterial effect of the synthesized products against clinically isolated Gram-positive and Gram-negative bacteria – *Bacillus licheniformis ATCC 14580, Bacillus cereus ATCC 11778*, *Staphylococcus aureus ATCC 6538P* and *Escherichia coli ATCC 8739* was studied. For this purpose the method described by Marinova et al. was used [20]. The substance was dissolved in 50 % DMSO solution and added to the growing media in series of twofold decreasing concentrations between 50 – 0.012 ppm. 200 µl of each dilution were inoculated in an amount of 1×10^6 CFU (Colony-forming unit) for various bacteria and transferred in microplates. The plates were cultivated at 37 °C for 18 – 20 h. The growing media used for the cultivation of the test microorganisms was Muller-Hinton broth. The optical density of the samples was read at the end of the cultivation period with (λ = 600 nm) against inoculated media.

RESULTS AND DISCUSSION

Our primary aim was to develop an efficient multicomponent one-pot procedure for the synthesis of benzothiazole derivatives through the reaction of benzothiazole (**1**), alkyl chloroformates (**2**) and cyclic ketones (**3**, **4**) and to study their antibacterial activity. The results are shown in Table 1. The reaction conditions were optimized by varying parameters such as solvent, temperature and time. Using the optimized conditions, the reactions were carried out in dry dichloroethane for 2 h at reflux temperature (80 °C) for the reaction with 1-indanone and 24 h at room temperature for 5,6-methylenedioxy-1-indanone and dimedone, respectively. As shown in Table 1, the reaction worked well with different cyclic ketones and the desired compounds were obtained in good yields. Even though the analysis of the crude products suggests that mixtures of diastereoisomers are formed in these reactions, we only managed to isolate single diastereoisomers by preparative column chromatography (yields given in Table 1). Particularly striking example is **5b** where the TLC of the reaction mixture showed two products in nearly 1:1 ratio, but only one of these was isolated after column chromatography. Most likely this is due to epimerisation process taking place in the chromatography column. Such epimerization is plausible, considering the CH-acidity of the product and the possibility for enolisation at one of the stereogenic centers. The determination of the relative configuration of the isolated products proved difficult and was not accomplished.

The ^{1}H-NMR spectra of compounds (**5 a-d**) exhibited a distinctive set of two doublet of doublets (dd) in the range of δ = 2.79 – 3.10 ppm for diastereotopic protons from benzylic CH_2 group adjacent to the newly formed stereogenic center.

All products (Table 1) were purified by column chromatography and characterized by IR, ^{1}H-NMR, ^{13}C-NMR and ESI-MS analysis.

The synthesized benzothiazole derivatives were evaluated for antibacterial activity. The antibacterial activity of the compounds was examined via agar diffusion method with concentration of the compounds 100 µg. Effect on Gram-negative bacteria *Escherichia coli* and Gram-positive *Bacillus licheniformis*, *Bacillus cereus*, *Staphylococcus aureus* was measured.

Table 1. Synthesis of benzothiazole derivatives **(5 a-d, 6)**

Pro-duct	R_1	R_2	Reaction conditions (Time/ Temperature)	Yields %	M. p. °C
5 a	OEt	H	2 h / 80 °C	76	127–130
5 b	OMe	H	2 h / 80 °C	86	138–141
5 c	OEt	OCH_2O	24 h / r.t.	60	97–99
5 d	OMe	OCH_2O	24 h / r.t.	81	92–94
6	OEt	-	24 h / r.t.	67	160–161

Table 2. Test results for antibacterial activity of compounds **(5 a-d, 6)**

Products (5, 6)	Microorganisms (sterile zone, mm)	MIC mg/ml
5 a	*Bacillus licheniformis* - 19	0.027
	Escherichia coli - 22	0.055
5 b	Shows no activity	-
5 c	*Bacillus licheniformis* - 18	0.027
	Escherichia coli - 22	0.060
5 d	Shows no activity	-
6	*Bacillus cereus* - 10	0.031
	Staphylococcus aureus - 12	0.031

The highest activity against *Escherichia coli* and *Bacillus licheniformis* showed compounds (**5a**, **5c**) - MIC 0.027 – 0.060 mg/ml. Compound (**6**) displayed antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus* – MIC 0,031 mg/ml (Table 2).

CONCLUSIONS

An efficient method for multicomponent synthesis of 2-substituted benzothiazole derivatives is demonstrated. The presented methodology offers several advantages, such as simple procedure, clean reaction and good yields. Three of the newly synthesized compounds showed moderate activity against both Gram-positive and Gram-negative bacterial strains.

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СИНТЕЗ И АНТИБАКТЕРИАЛНА АКТИВНОСТ НА 2-ЗАМЕСТЕНИ БЕНЗОТИАЗОЛИ

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(Резюме)

Приложена е мултикомпонентна реакция на амидоалкилиране между бензотиазол, алкилхлороформиати и циклични карбонилни съединения. Успешно са синтезирани пет 2-заместени бензотиазолови производни с добри добиви (от 60 до 86 %). Намерени са условия за хроматографско разделяне и изолиране на новополучените съединения с разпределителна течно-течна колонна хроматография на силикагел или неутрален алуминиев оксид. Изолираните кристални съединения са спектрално охарактеризирани с инфрачервена, ЯМР-спектроскопия и МАС-спектрометрия.

Изследвана е антибактериалната активност на синтезираните съединения с диск-дифузионен метод срещу четири щама микроорганизми - грам-отрицателни бактерии *Escherichia coli ATCC 8739* и грам-положителни *Bacillus licheniformis ATCC 14580, Bacillus cereus ATCC 11778, Staphylococcus aureus ATCC 6538P*.

Ключови думи: *Бензотиазол, Мултикомпонентна реакция, Антибактериална активнос*