JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Antimicrobial and Antifungal Evaluation of Novel *N*-Amino Benzylthiolates with Solvent-Free Synthesis

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Novel *N*-amino benzylthiolates were synthesized *via* multicomponent reaction of malononitrile, isothiocyanates and benzyl halides under conventional and solvent-free conditions. Various electron-donating and -withdrawing substitutes within both isothiocyanates and benzyl halides were used to demonstrate the efficiency of new methodology. A broad spectrum of antibacterial and antifungal activities were observed especially within benzyl halides containing electron-withdrawing aryl substituents.

Keywords: Multicomponent synthesis, Solvent-free condition, *N*-Amino benzylthiolate, Antibacterial activity, Antifungal property

INTRODUCTION

Aminothiolates are organic sulfides containing amino substituents that have been applied as starting materials in chemical reactions [1-4]. Both amino and sulphide functional groups are part of chemical structure of many biomolecules such as axitinib, ceftaroline fosamil, captodiame and ticagrelor. Axitinib is a targeted drug in the treatment of pancreatic and thyroid cancers [5] and ceftaroline fosamil is a cephalosporin antibiotic against acute skin infections [6]. Captodiame has been recommended as an antihistamine agent to prevent benzodiazepine withdrawal syndrome [7]. While ticagrelor is a preventory drug in heart attack in patients with acute coronary syndrome [8].

N-Amino alkylthiolates are useful synthons in organic synthesis. They showed significant antibacterial and antioxidant properties as well as nuclease activity toward the cleavage of genomic DNA [9]. Heterocycles such as thiazole, isothiazole and pyrazole derivatives were incorporated with them [10-13], synthesis usually includes formation of thiolate salt, followed by *S*-alkylation and final cyclization.

N-Amino thiolate salts were prepared *in situ* using reaction of active methylene compounds and alkyl or aryl isothiocyanates in the presence of bases such as KOH, K_2CO_3, NaOEt, Li_2CO_3, NaH, *n*-BuLi and NEt_3 [14-20]. A variety of organic solvents including *N*,*N*-dimethylformamide, tetrahydrofuran, toluene, ethanol and mineral oil have been applied as reaction media. These salts are alkylated with alkyl halides, α-halo carbonyl or their equivalents, oxiranes, aziridines and α-halo imines. Accordingly, *N*-amino alkylthiolates were often synthesized as intermediate or final products *via* multistep reactions [13,21-23], and multicomponent process was rarely used in their preparation [24].

To the best of our knowledge, we developed a multicomponent solvent-free procedure to synthesize several new *N*-amino benzylthiolates. No report has been published on the synthesis of these compounds under solvent-free conditions. The inhibitory potentials of all compounds were evaluated against a variety of pathogenic bacteria and fungi.

EXPERIMENTAL

Chemicals

All chemicals, solvents, antibiotics, antifungal agents and bacterial and fungal media were obtain from commercial sources (Merck, Aldrich, HiMedia) and used as received. Melting points were recorded on a Kruss type KSP1N melting point apparatus without correction. The reaction progress was monitored by aluminium TLC plates pre-coated with silica gel with fluorescent indicator F254 using CH_2Cl_2/CH_3OH (8:2, v/v). The IR spectra were recorded on KBr disks with a Bruker Tensor-27 FT-IR spectrometer. The ^1H and ^{13}C NMR spectra were acquired on a Bruker FT-NMR Ultra Shield-400 spectrometer

(400 and 100 MHz, respectively). Low-resolution mass spectra (EI, 70 eV) were measured on a Varian Mat CH-7 instrument. All CHNS/O analyses were performed by a Thermo Finnigan Flash EA microanalyzer. Initial bacterial or fungal suspensions were adjusted with a Jenway 6405 UV-Vis spectrophotometer.

General Procedure for the Synthesis of N-Amino Benzylthiolates 4a-j

Conventional method using acetonitrile as solvent. 10 mmol of each malononitrile (1) (0.66 g), isothiocyanates 2, benzylhalides 3 and potassium carbonate (1.38 g) in acetonitrile (20 ml) were heated under reflux for 3-7 h. After cooling to room temperature, 20 ml water was added to the reaction mixture and the aqueous phase was extracted twice with 15 ml of diethyl ether. The extracts were combined and washed respectively with 15 ml 5% NaOH (aq) and water. The organic phase was dried over anhydrous $MgSO_4$ and evaporated to dryness under reduced pressure. The residue was recrystallized from ethanol containing various amounts of water to afford *N*-amino benzylthiolates 4a-j. The solvent-free condition 10 mmol of each malononitrile (1) (0.66 g), isothiocyanates 2, benzylhalides 3 and triethylamine (1.01 g) were vigorously stirred at 80 °C for 2-4 h. The resulting reaction mixture was extracted and purified according to the conventional method.

2-((Benzylthio)(phenylamino)methylene)malononitrile (4a). Orange crystal; m.p.: 85-87 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.90 (s, 2H, CH_2), 7.11-7.38 (m, 10H, 2 Ar-H), 7.91 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 38.3 (CH_2), 59.1 (NC-C=C), 113.9, 114.8 (2C≡N), 124.2 (C-2",6"), 127.5 (C-4"), 128.3 (C-4'), 128.8 (C-2',6'), 128.9 (C-3',5'), 129.5 (C-3",5"), 134.3 (C1'), 137.0 (C1"), 169.3 (NC-C=C) ppm; IR (KBr) υ: 3196 (N-H), 2218 (C≡N), 1592 (C=C), 1459 (CH_2), 1096 (C-S) cm^{-1}; MS *m/z* 291 (M+, 11), 91 (100); Anal. Calcd. for $C_{17}H_{13}N_3S$: C, 70.08; H, 4.50; N, 14.42; S, 11.00. Found: C, 70.13; H, 4.52; N, 14.38; S, 10.97%.

2-(((2-Chlorobenzyl)thio)(phenylamino)methylene) malononitrile (4b). Orange crystal; m.p.: 92-94 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.06 (s, 2H, CH_2), 7.13-7.39 (m, 9H, 2Ar-H), 8.24 (br, 1H, NH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 36.5 (CH_2), 59.1 (NC-C=C), 113.9, 114.7 (2C≡N), 124.3 (C-2",6"), 127.3 (C-4"), 127.6 (C-4'), 129.6 (C-3",5"), 129.9 (C-3'), 130.0 (C-5'), 130.9 (C-6'), 132.2 (C-2'), 134.3 (C-1'), 137.0 (C-1"), 169.8 (NC-C=C) ppm; IR (KBr) υ: 3180 (N-H), 2210 (C≡N), 1617 (C=C), 1444 (CH_2), 1054 (C-S) cm^{-1}; MS *m/z* 325 (M$^+$, 9), 201 (100); Anal. Calcd. for $C_{17}H_{12}ClN_3S$: C, 62.67; H, 3.71; N, 12.90; S, 9.84. Found: C, 62.61; H, 3.69; N, 12.94; S, 9.85%.

2-(((2-Nitrobenzyl)thio)(phenylamino)methylene) malononitrile (4c). Dark brown crystal; m.p.: 195-196 °C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.59 (s, 2H, CH_2), 6.97 (t, *J* = 7.2 Hz, 1H, H-4"), 7.16-7.21 (m, 4H, H-2",3",5",6"), 7.45 (d, *J* = 7.4 Hz, 3H, H-4',5',6'), 7.96 (m, 1H, H-3'), 8.87 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 42.8 (CH_2), 60.7 (NC-C=C), 114.7, 115.6 (2 C≡N), 123.7 (C-4"), 124.4 (C-2",6"), 126.1 (C-6'), 127.3 (C-4'), 128.0 (C-3",5"), 130.9 (C-5'), 131.6 (C-3'), 137.7 (C-1"), 141.6 (C-1'), 146.9 (C-2'), 162.4 (NC-C=C) ppm; IR (KBr) υ: 3194 (N-H), 2196 (C≡N), 1625 (C=C), 1397 (CH_2), 1054 (C-S) cm^{-1}; MS *m/z* 336 (M$^+$, 4), 271 (100); Anal. Calcd. for $C_{17}H_{12}N_4O_2S$: C, 60.70; H, 3.60; N, 16.66; S, 9.53. Found: C, 60.75; H, 3.59; N, 16.63; S, 9.50%.

2-((((Perfluorophenyl)methyl)thio)(phenylamino) methylene)malononitrile (4d). Yellow crystal; m.p.: 99-101 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.19 (s, 2H, CH_2), 6.69-6.90 (m, 3H, H-3",4",5"), 7.16 (m, 2H, H-2",3"), 10.87 (br, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 22.7 (CH_2), 43.6 (NC-C=C), 112.9, 113.2 (2 C≡N), 120.4 (C-1'), 122.0 (C-2",6"), 122.9 (C-4"), 128.7 (C-3",5"), 135.3 (C-1"), 139.2 (C-2',6'), 143.0 (C-4'), 148.2 (C-3',5'), 158.9 (NC-C=C) ppm; IR (KBr) υ: 3197 (N-H), 2192 (C≡N), 1539 (C=C), 1446 (CH_2), 1128 (C-S) cm^{-1}; MS *m/z* 381 (M$^+$,

7), 168 (100); Anal. Calcd. for $C_{17}H_8F_5N_3S$: C, 53.55; H, 2.11; N, 11.02; S, 8.41. Found: C, 53.60; H, 2.10; N, 10.98; S, 8.43%.

2-(((2,4-Dinitrobenzyl)thio)(phenylamino)methylene) malononitrile (4e). Black crystal; m.p.: 125-127 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.86 (s, 2H, CH_2), 7.02 (t, J = 6.9 Hz, 1H, H-4"), 7.24-7.33 (m, 4H, H-2",3",5",6"), 7.69 (d, J = 8.8 Hz, 1H, H-6'), 8.31 (d, J = 8.8 Hz, 1H, H-5'), 8.66 (s, 1H, H-3'), 10.17 (br, 1H, NH,) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 48.6 (CH_2), 88.5 (NC-C=C), 114.5, 114.9 (2C≡N), 120.4 (C-2",6"), 121.6 (C-3'), 124.5 (C-4"), 129.5 (C-3",5"), 133.0 (C-6'), 133.4 (C-5'), 134.7 (C-1"), 143.9 (C-1'), 146.4 (C-4'), 148.4 (C-2'), 170.6 (NC-C=C) ppm; IR (KBr) υ: 3187 (N-H), 2199 (C≡N), 1627 (C=C), 1343 (CH_2), 1124 (C-S) cm^{-1}; MS m/z 381 (M^+, 11), 291 (100); Anal. Calcd. for $C_{17}H_{11}N_5O_4S$: C, 53.54; H, 2.91; N, 18.36; S, 8.41. Found: C, 53.60; H, 2.90; N, 18.32; S, 8.38%.

2-(((2,4-Dichlorobenzyl)thio)(phenylamino)methylene) malononitrile (4f). Cream crystal; m.p.: 205-206 °C (decomp.); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.43 (s, 2H, CH_2), 7.11 (d, J = 7.0 Hz, 2H, H-2",6"), 7.22 (t, J = 7.0 Hz, 1H, H-4"), 7.33-7.46 (m, 4H, H-5',6',3",5"), 7.62 (s, 1H, H-3'), 11.19 (br, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 35.0 (CH_2), 54.9 (NC-C=C), 115.5, 116.0 (2C≡N), 123.8 (C2",6"), 126.7 (C-4"), 128.1 (C-5'), 129.5 (C3",5"), 129.6 (C-3'), 133.0 (C-1'), 133.3 (C-6'), 133.9 (C-4'), 134.7 (C-2'), 139.0 (C-1"), 167.9 (NC-C=C) ppm; IR (KBr) υ: 3195 (N-H), 2215 (C≡N), 1552 (C=C), 1406 (CH_2), 1049 (C-S) cm^{-1}; MS m/z 360 (M^+, 13), 160 (100); Anal. Calcd. for $C_{17}H_{11}Cl_2N_3S$: C, 56.68; H, 3.08; N, 11.66; S, 8.90. Found: C, 56.62; H, 3.09; N, 11.64; S, 8.92%.

2-(((4-Nitrophenyl)amino)(((perfluorophenyl)methyl) thio)methylene)malononitrile (4g). Bright yellow crystal; m.p.: 98-100 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.21 (s, 2H, CH_2), 6.78 (d, J = 8.3 Hz, 2H, H-2",6"), 8.01 (d, J = 8.3 Hz, 2H, H-3",5"), 11.23 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 21.7 (CH_2), 48.7 (NC-C=C), 113.1 (C-1'), 113.8, 117.0 (2C≡N), 121.7 (C-2",6"), 124.8 (C-3",5"), 138.1 (C-4"), 139.3 (C-2',6'), 140.8 (C-1"), 143.0 (C-4'), 146.9 (C-3',5'), 160.8 (NC-C=C) ppm; IR (KBr) υ: 3416 (N-H), 2177 (C≡N), 1572 (C=C), 1400 (CH_2), 1125 (C-S) cm^{-1}; MS m/z 426 (M^+, 6), 181 (100); Anal. Calcd. for $C_{17}H_7F_5N_4O_2S$: C, 47.90; H, 1.66; N, 13.14; S, 7.52. Found: C, 47.85; H, 1.65; N, 13.17; S, 7.54%.

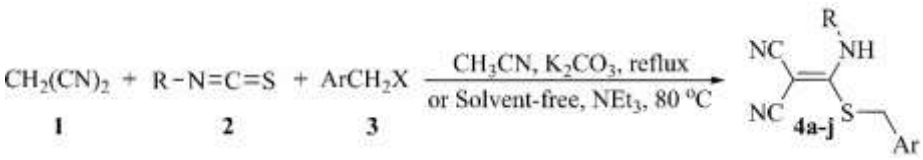
2-((((Perfluorophenyl)methyl)thio)(*p*-tolylamino) methylene)malononitrile (4h). Orange crystal; m.p.: 97-98 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.26 (s, 3H, CH_3), 4.42 (s, 2H, CH_2), 7.01 (d, J = 7.8 Hz, 2H, H-2",6"), 7.16 (d, J = 7.8 Hz, 2H, H-3",5"), 10.94 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 20.9 (CH_3), 25.2 (CH_2), 56.3 (NC-C=C), 111.4 (C-1'), 113.6, 116.4 (2C≡N), 123.7 (C-2",6"), 129.9 (C-3",5"), 135.9 (C-1"), 136.5 (C-4"), 139.4 (C-2',6"), 143.0 (C-4'), 146.9 (C-3',5'), 186.1 (NC-C=C) ppm; IR (KBr) υ: 3173 (N-H), 2210 (C≡N), 1635 (C=C), 1442 (CH_2), 1066 (C-S) cm^{-1}; MS m/z 395 (M^+, 12), 213 (100); Anal. Calcd. for $C_{18}H_{10}F_5N_3S$: C, 54.69; H, 2.55; N, 10.63; S, 8.11. Found: C, 54.73; H, 2.53; N, 10.67; S, 8.07%.

2-(((2,4-Dinitrobenzyl)thio)(ethylamino)methylene) malononitrile (4i). Black crystal; m.p.: 120-122 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.17 (t, J = 7.1 Hz, 3H, CH_3), 3.17 (q, J = 7.1 Hz, 2H, CH_2CH_3), 5.89 (s, 2H, SCH_2), 7.70 (d, J = 8.1 Hz, 1H, H-6'), 8.28 (d, J = 8.1 Hz, 1H, H-5'), 8.43 (s, 1H, NH), 8.61 (s, 1H, H-3') ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.3 (CH_3), 37.6 (CH_2CH_3), 42.0 (SCH_2), 86.5 (NC-C=C), 114.6, 115.3 (2C≡N), 121.9 (C-3'), 126.8 (C-6'), 132.3 (C-5'), 142.7 (C-1'), 145.7 (C-4'), 148.1 (C-2'), 186.1 (NC-C=C) ppm; IR (KBr) υ: 3180 (N-H), 2216 (C≡N), 1527 (C=C), 1473 (CH_2), 1058 (C-S) cm^{-1}; MS m/z 333 (M^+, 7), 239 (100); Anal. Calcd. for $C_{13}H_{11}N_5O_4S$: C, 46.84; H, 3.33; N, 21.01; S, 9.62. Found: C, 46.88; H, 3.32; N, 20.99; S, 9.65%.

2-((Ethylamino)(((perfluorophenyl)methyl)thio) methylene)malononitrile (4j). Orange crystal; m.p.: 104-106 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.24 (t, J = 7.3 Hz, 3H, CH_3), 3.40 (q, J = 7.3 Hz, 2H, CH_2CH_3), 4.55 (s, 2H, SCH_2), 7.95 (s, 1H, NH) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ: 14.4 (CH_3), 22.8 (SCH_2), 38.5 (CH_2CH_3), 59.4 (NC-C=C), 112.4 (C-1'), 115.7, 116.1 (2C≡N), 139.3 (C-2',6'), 142.8 (C-4'), 146.3 (C-3',5'), 165.8 (NC-C=C) ppm; IR (KBr) υ: 3195 (N-H), 2200 (C≡N), 1618 (C=C), 1475 (CH_2), 1045 (C-S) cm^{-1}; MS m/z 333 (M^+, 3), 123 (100); Anal. Calcd. for $C_{13}H_8F_5N_3S$: C, 46.85; H, 2.42; N, 12.61; S, 9.62. Found: C, 46.81; H, 2.41; N, 12.66; S, 9.66%.

In vitro Antimicrobial and Antifungal Activity

Gram-negative bacterial strains including *Pseudomonas*



Scheme 1. Synthesis of *N*-amino alkylthiolates 4a-j

Table 1. Multicomponent Synthesis of Aminothiolates 4a-j in Acetonitrile or under Solvent-free Condition

Products	R	Ar	X	Time (h)		Yield (%)	
				A[a]	B[b]	A	B
4a	C_6H_5	C_6H_5	Cl	7	4	80	93
4b	C_6H_5	2-Cl-C_6H_4	Cl	6	3.5	70	86
4c	C_6H_5	2-O_2N-C_6H_4	Cl	3	2	81	93
4d	C_6H_5	C_6F_5	Br	3	2.5	83	94
4e	C_6H_5	2,4-$(O_2N)_2$-C_6H_3	Cl	3	2.5	72	85
4f	C_6H_5	2,4-$(Cl)_2$-C_6H_3	Cl	3.5	2.5	75	87
4g	4-O_2N-C_6H_4	C_6F_5	Br	4	3	79	86
4h	4-H_3C-C_6H_4	C_6F_5	Br	4.5	3	78	92
4i	CH_3CH_2	2,4-$(O_2N)_2$-C_6H_3	Cl	4	3	76	92
4j	CH_3CH_2	C_6F_5	Br	5	3	74	90

[a]In the presence of acetonitrile. [b]Solvent-free conditions.

aeruginosa (PTCC 1310), *Klebsiella pneumoniae* (PTCC 1290), *Escherichia coli* (PTCC 1399), *Shigella flexneri* (PTCC 1234), *Shigella dysenteriae* (PTCC 1188), *Proteus mirabilis* (PPTC 1776), *Proteus vulgaris* (PTTC 1079), *Salmonella enterica subsp. enterica* (PTCC 1709), *Salmonella typhi* (PTCC 1609), *Enterococcus faecalis* (PTCC 1778), *Acinetobacter baumannii* (PTCC 1855), and Gram-positive bacterial strains including *Streptococcus pyogenes* (PTCC 1447), *Streptococcus agalactiae* (PTCC 1768), *Streptococcus equinus* (PTCC 1445), *Streptococcus pneumonia* (PTCC 1240), *Listeria monocytogenes* (PTCC 1297), *Staphylococcus aureus* (PTCC 1189), *Staphylococcus epidermidis* (PTCC 1435), *Bacillus cereus* (PTCC 1665), *Bacillus subtilis subsp. spizizenii* (PTCC 1023), *Bacillus thuringiensis subsp. kurstaki* (PTCC 1494), *Rhodococcus equi* (PTCC 1633) and fungal strains including *Aspergillus fumigatus* (PTCC 5009), *Candida albicans* (PTCC 5027), *Fusarium oxysporum* (PTCC 5115) were prepared from the Persian Type Culture Collection (PTCC), Karaj, Iran. The minimum inhibitory concentration (MIC), the minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC) values were determined by using broth microdilution method, according to CLSI (Clinical and Laboratory Standards Institute) guidelines M07-A9, M26-A and M-27-A2 [25,26]. The stock solutions of all derivatives and antibiotics were respectively prepared in 10% DMSO and double-distilled water at initials concentrations of 10240 and 17.6 µg ml^{-1}. All antibiogram tests were performed at least three times independently, and the results were reported as mean values.

RESULTS AND DISCUSSION

Chemistry

Novel *N*-amino alkylthiolates 4a-j were efficiently synthesized *via* multicomponent reactions of malononitrile (1), alkyl or aryl isothiocyanates 2 and benzyl halides 3 under two different conditions (Scheme 1). The higher product yields were obtained in the presence of triethylamine under solvent-free condition (Table 1). Reaction times were reduced in the absence of solvent due to packing of the reacting molecules.

Chemical structure of aminothiolates 4a-j were characterized through spectral data. *S*-Methylene groups appeared in a wide range of chemical shifts in NMR spectra due to their adjacent aryl substituents. These effects were also evident in chemical shifts of two carbons of olefinic bonds. In ^{13}C NMR, two signals were observed with the magnetically inequivalent carbons of the nitrile groups.

Evaluation of Antimicrobial and Antifungal Properties

Inhibitory properties of the synthesized aminothiolates were assessed against a variety of Gram-positive and -negative bacterial pathogens as well as some fungi (Tables 2-4). Ampicillin and fluconazole were used as positive controls in antimicrobial and antifungal susceptibility tests. In General, wider and better effects were observed with derivatives on Gram-positive strains, which is due to the impermeability of the cell well of Gram-negative bacteria. Results showed that in compounds 4a-f containing *N*-phenyl substituents, substitution of nitro groups in 2 and 4 positions on Ar rings diminished antibacterial potentials. On the other hand, we observed two chloro substituents on the ring system induced significant antibacterial effects. Introduction of electron-withdrawing nitro or electron-donating methyl groups into position 4 on R rings intensified antibacterial activities. Aminothiolate 4h containing perfluorobenzyl and *p*-tolylamino substituents could inhibit the growth of all tested bacteria except *Streptococcus equinus*. Furthermore, this compound had no effect on *Candida albicans*. Thiolates were more efficient in blocking *Candida albicans*, among which compound 4i showed the best results.

Totally different behavior was observed in experiments using compounds 4i,j containing *N*-ethyl substituents against fungi. They could not block an identical strain. In structurally similar compounds, 4d, g, h, j, antifungal effects of ethylamino thiolate 4j is only slightly more than thiolate 4d. However, antibacterial properties of thiolate 4e were more significant than those for 4i.

All derivatives were effective on Gram-negative *Acinetobacter baumannii*. Despite the fact that the most limited range of antibacterial activities were observed with *N*-amino alkylthiolate 4a, it showed inhibitory effects against this important nosocomial pathogen. In addition, compound 4a as well as derivatives 4d, f, g were the only effective agents on all three fungal strains. In series 4a-f, much better results were observed with *N*-amino alkylthiolate 4d according to the range of antifungal effects and MIC values. Changing phenyl substituents to 4-nitrophenyl improved antifungal activity, however, not much of improvement was observed when 4-tolyl group was introduced to the main cores. Broader antifungal effects were observed with alkylthiolate 4e compared to structurally similar derivatives 4a-f.

CONCLUSIONS

In conclusion, two efficient multicomponent procedures were applied to prepare novel *N*-amino alkylthiolates 4a-j. Products were synthesized within higher yield and shorter reaction time under solvent-free condition. Inhibitory activity of newly made derivatives were studied on various bacterial and fungal pathogens. The results showed that electron-donating aryl substituents in isothiocyanates and electron-withdrawing aryl substituents in benzyl halides improved range of antibacterial activities and inhibitory rates. Antifungal effects increased in the presence of electron-withdrawing groups in both isothiocyanate and benzyl halide.

ACKNOWLEDGMENTS

This work was supported by the [University of Zabol]

Table 2. Inhibitory Activities of Aminothiolates 4a-j Against Gram-negative Pathogenic Bacteria

Bacteria		Products										Antibiotic
		4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	Ampicillin
Acinetobacter baumannii	MIC[a]	64	8	256	16	1024	16	16	256	1024	1024	64
	MBC[b]	128	16	512	64	2048	64	64	512	2048	2048	128
Pseudomonas aeruginosa	MIC	-	-	-	-	-	-	-	128	128	16	1024
	MBC	-	-	-	-	-	-	-	256	256	64	2048
Klebsiella pneumonia	MIC	-	-	-	-	-	-	-	16	512	-	32
	MBC	-	-	-	-	-	-	-	64	1024	-	64
Escherichia coli	MIC	-	-	8	64	8	256	16	64	-	256	32
	MBC	-	-	16	128	16	512	64	128	-	512	64
Shigella flexneri	MIC	-	512	16	128	8	64	16	16	128	64	8
	MBC	-	1024	64	256	16	128	64	64	256	128	32
Shigella dysenteriae	MIC	-	-	16	64	8	-	-	128	-	-	256
	MBC	-	-	64	128	16	-	-	256	-	-	256
Proteus mirabilis	MIC	-	-	-	-	16	128	-	64	512	16	8
	MBC	-	-	-	-	64	256	-	128	1024	64	32
Proteus vulgaris	MIC	-	-	16	64	8	-	-	128	-	-	8
	MBC	-	-	64	128	16	-	-	256	-	-	32
Salmonella enterica	MIC	-	-	1024	512	512	1024	128	512	-	1024	8
	MBC	-	-	2048	1024	1024	2048	256	1024	-	2048	16
Salmonella typhi	MIC	-	-	8	-	8	-	-	128	1024	-	2
	MBC	-	-	16	-	16	-	-	256	2048	-	8
Enterococcus faecalis	MIC	-	-	1024	-	512	256	512	128	512	-	8
	MBC	-	-	2048	-	1024	512	1024	256	1024	-	16

No noticeable antibacterial effect at the initial concentrations. [a]Values reported as μg ml^{-1}. [b]Values reported as μg ml^{-1}.

Table 3. Inhibitory Activities of Aminothiolates 4a-j Against Gram-positive Pathogenic Bacteria

		Products										Antibiotic
Bacteria		4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	Ampicillin
Streptococcus agalactiae	MIC[a]	8	8	-	64	64	256	16	256	256	1024	8
	MBC[b]	16	16	-	128	128	512	64	512	512	2048	16
Streptococcus pyogenes	MIC	1024	-	-	-	-	128	16	16	256	256	4
	MBC	2048	-	-	-	-	256	64	64	512	512	8
Staphylococcus epidermidis	MIC	512	128	8	128	16	64	16	8	-	512	0.25
	MBC	1024	256	16	256	64	128	64	16	-	1024	2
Streptococcus pneumonia	MIC	64	512	512	-	256	64	256	64	128	-	8
	MBC	128	1024	1024	-	512	128	512	128	256	-	16
Rhodococcus equi	MIC	128	512	8	64	64	256	128	128	1024	-	8
	MBC	256	1024	16	128	128	512	256	256	2048	-	32
Staphylococcus aureus	MIC	-	16	1024	-	-	16	-	512	-	1024	8
	MBC	-	64	2048	-	-	64	-	1024	-	2048	32
Bacillus thuringiensis	MIC	-	-	8	-	64	64	64	8	16	64	8
	MBC	-	-	16	-	128	128	128	16	64	128	32
Listeria monocytogenes	MIC	-	-	8	-	16	-	256	128	-	-	8
	MBC	-	-	16	-	64	-	512	256	-	-	16
Bacillus cereus	MIC	256	128	1024	8	512	1024	1024	8	-	512	32
	MBC	512	256	2048	16	1024	2048	2048	16	-	1024	64
Streptococcus equinus	MIC	-	-	16	64	-	-	-	-	-	-	8
	MBC	-	-	64	128	-	-	-	-	-	-	32
Bacillus spizizenii	MIC	-	64	8	16	-	-	256	256	-	512	8
	MBC	-	128	16	64	-	-	512	512	-	1024	16

No noticeable antibacterial effect at the initial concentrations. [a]Values reported as µg ml^{-1}. [b]Values reported as µg ml^{-1}.

Table 4. Inhibitory Activities of Aminothiolates 4a-j Against Pathogenic Fungi

Fungi		Products										Antifungal
		4a	4b	4c	4d	4e	4f	4g	4h	4i	4j	Fluconazole
Aspergillus fumigatus	MIC[a]	16	128	-	256	-	128	64	512	256	-	32
	MFC[b]	64	256	-	512	-	256	128	1024	512	-	64
Candida albicans	MIC	128	128	64	64	16	256	16	-	8	-	256
	MFC	256	256	128	128	64	512	64	-	16	-	512
Fusarium oxysporum	MIC	256	-	16	16	16	512	64	128	-	64	128
	MFC	512	-	64	64	64	1024	128	256	-	128	256

No noticeable antibacterial effect at the initial concentrations. [a]Values reported as µg ml^{-1}. [b]Values reported as µg ml^{-1}.

under Grant [number UOZ-GR-9517-15].

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