Synthesis and antimicrobial activity of new triazolopyridinyl phenothiazines

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Abstract

A variety of *N* (2-methyl-7-aryl-8-cyano-[1,2,4] triazolo [1,5-*a*] pyridin-5-yl) phenothiazines **6a – d** were synthesized by using chalcones of *N* – acetylphenothiazine. And the structures of these compounds were confirmed by IR, NMR (^{1}H & ^{13}C) & Mass spectral analysis. The newly synthesized compounds were also evaluated for antimicrobial activity against variety of bacterial strains and some of these compounds have shown significant antibacterial and antifungal activities.

Keywords: Chalcones, phenothiazines, triazolopyridinyl phenothiazines, IR, NMR (^{1}H & ^{13}C), Mass spectral analysis, Active MnO_2, antibacterial and antifungal activities

Introduction

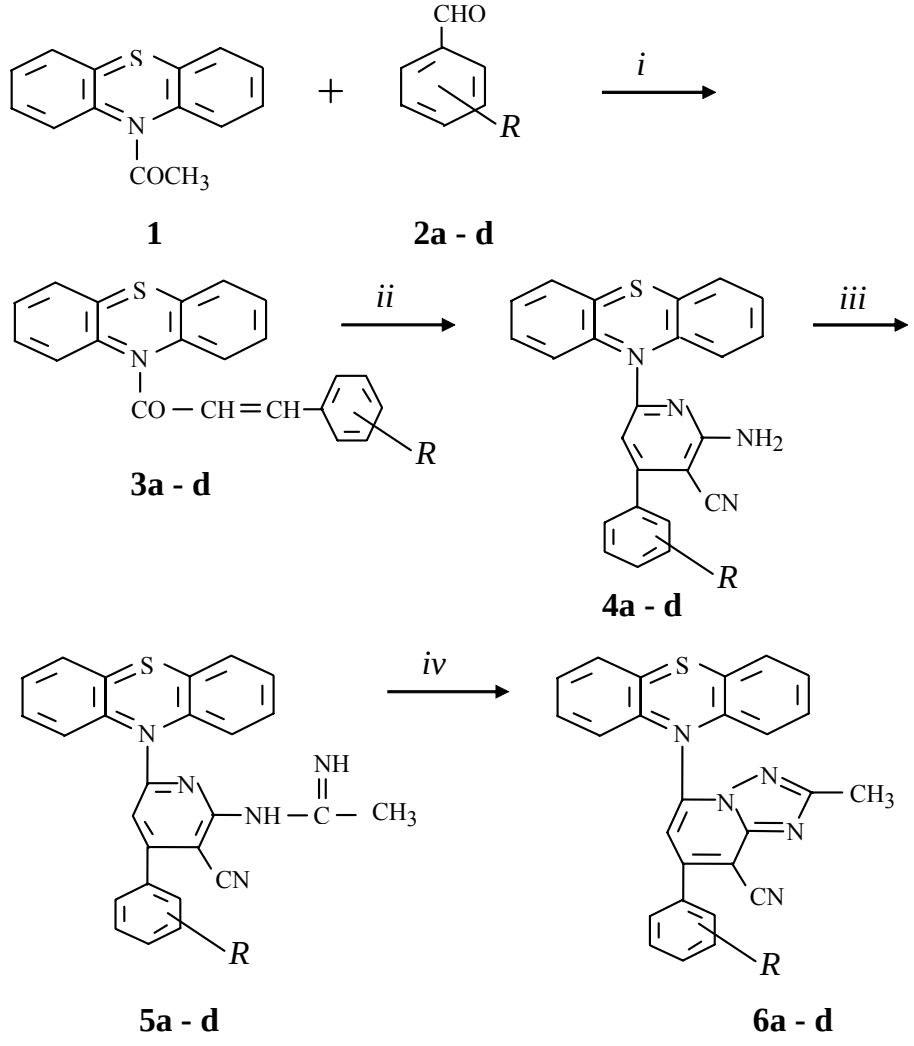
Among a wide variety of heterocycles that have been explored for developing pharmaceutically important molecules such as cyanopyridines[1] and triazolopyridines[2] have played an important role in medicinal chemistry. They are reported to possess a broad spectrum of biological activity such as potential cardiovascular agents[2(c)] antiviral,[3] CNS depressant,[4] bactericidal,[5] ulcer inhibitors[6] etc. Furthermore researchers have also revealed that Phenothiazine derivatives constitute an important class of compounds possessing diverse type of biological properties including antiviral,[7] antiparasitic,[8] antiparkinsonian,[9] anticonvulsant,[10] antihistaminic[11] as well as anthelmintic[12] properties.

These observations led the authors to undertake the synthesis of some *N* (2-methyl-7-aryl-8-cyano[1,2,4]triazolo[1,5-a]pyridin-5-yl)phenothiazine **6a – d** and evaluate their antimicrobial activities.

Results and Discussion

Chemistry

Chalcones **3a-d** of *N*-Acetylphenothiazine were obtained from *N*-acetylphenothiazine **1** using various aldehydes **2a-d**. Chalcones **3a-d** on treatment with malonitrile in the presence of ammonium acetate gave **4a-d**. Compounds **4a-d** when treated with acetonitrile in the presence of $AlCl_3$ afforded **5a–d**. Compounds **5a–d** on oxidation with active MnO_2 furnished **6a–d** (Scheme.1). The structures of the above compounds were in agreement with spectral and analytical data.



Scheme 1. R = H, 4-OCH_3, 4-$N(CH_3)_2$, 4-OH-3-OCH_3.
Reagents and reaction conditions. (i) NaOH / MeOH ; (ii) $CH_2(CN)_2$, CH_3COONH_4 ; (iii) acetonitrile, $AlCl_3$; (iv) MnO_2.

Antimicrobial activity

The minimum inhibition concentration (MIC) was determined using the streak plate and cup plate method by measuring the zone of inhibition according to a standard procedure.[13] All the newly synthesized compounds were screened in vitro for their antimicrobial activity against a variety of bacterial strains such as *Staphylococcus aureus, Salmonella paratyphi, Escherichia coli, Shigella flexneri, Pseudomonas auregenosa, Bacillus subtilis, and fungi such as Cerevesae vitae, Candida albicans, Aspergillus niger* (Table.1). The MIC of the compounds was defined, as the lowest concentration at which there was 80% inhibition of growth compared with the growth for a drug free control[14]. Standard inhibition of zone size for Ciprofloxacin, Cloxacillin and for Gentamycin[14] is (++++) at $\leq$ 50 μgm/mL against all microbes.

Table 1. Antimicrobial activities of the compounds **4a – d**, **5a – d**, and **6a – d**

Compd	Antibacterial activity						Antifungal activity		
	S.a	*S. p*	*E. c*	*S. f*	*P. a*	*B. s*	*C. a*	*A. n*	*C. v*
4a	++	++	++	++	+	++	++	++	++
4b	++	++	++	++	++	+++	-	-	-
4c	++++	++	+++	++	+	-	++	+	+
4d	++	++	++	++	+	++	+	++	++
5a	++++	++	+++	+	++	++	++	-	-
5b	+++	++	+++	++	++	++	-	++	++
5c	++	+	++	+	+++	++	++	+	+
5d	++	++	+++	+	++	++	-	-	-
6a	++++	++	+++	+	+	++	++	+	+
6b	++++	++	++	+	+++	++	+	+	+
6c	++	++	+++	++	++	++	++	-	+
6d	++	+	+++	+++	+	++	+	++	++

50 μgm/mL = ++++, 100 μgm/mL = +++, 150 μgm/mL = ++, 200 μgm/mL = + ,

Not active upto 200 μgm/mL = –

Ciprofloxacin, Cloxacillin & Gentamycin[14] is (++++) at $\leq$ 50 μgm/mL

S. a = Staphylococcus aureus, S. p = Salmonella paratyphi, E. c = Escherichia coli,
S. f = Shigella flexneri, P. a = Pseudomonas auregenosa, B. s = Bacillus subtilis,
C. v = Cerevesae vitae, C. a = Candida albicans, A. n = Aspergillus niger

Conclusions

A new series of antimicrobial agents was designed and by visualizing the antimicrobial data it could be observed that compounds of the series showed activity ranging from 50 μgm/mL to 200 μgm/mL. The standard drugs used for comparision were Ciprofloxacin, Cloxacillin and Gentamycin. By visualizing the antimicrobial data it could be observed that some of the compounds possess significant activity.

Experimental Section

General Procedures. All the melting points were determined on a Cintex melting point apparatus and are uncorrected. The IR – spectra (ν_{max} cm^{-1}) were recorded on Perkin – Elmer 783 Spectrophotometer and NMR (^1H and ^{13}C) spectra in TFA (Chemical shifts in δ ppm) on Bruker AMX500 MHz using TMS as an internal standard, and mass spectra on Jeol D-300 spectrometer. Elemental analysis was performed on Carlo Erba – 1108 analyzer.

***N*-Acetylphenothiazine (1).** [15] To the solution of 10H–phenothiazine(0.01 mole) in dry benzene(50mL), acetyl chloride (0.01 mole) was added drop wise at 0 – 5ºC. the reaction mixture was stirred for 3 – 4 hr. at room temperature. After being stirred, the reaction mixture was kept overnight. The resulting mixture was distilled off and poured onto ice. The solid thus obtained was recrystallized from ethanol/ water to afford **1**. yield 76%, m.p. 197 – 98ºC (reported[15] m.p. 198ºC); IR (KBr) cm^{-1}: 1650 (CO), 1450 (C – N), 1580 (C = C of aromatic ring); ^1H NMR ($CDCl_3 – d_6$**)** : δ 7.00 –7.48 (m, 8h, Ar – H), 2.60 (s, 3H, $COCH_3$); MS : m/z (%) : 241(64%)M^+, 266(77), 198 (100),166 (66), 153 (34); Anal. Calcd. for $C_{14}H_{11}NOS$: C, 69.71; H, 4.56; N, 5.81. Found: C, 69.77; N, 4.59; N, 5.79%.

***N*-Benzylideneacetylphenothiazine (3a).** [16] To the solution of *N*-acetylphenothiazine **1** (0.01 mole) in absolute methanol(50mL), benzaldehyde **2a** (0.01 mole) was added on the presence of 2% NaOH(2mL) and refluxed for 10 – 12 hr. After refluxing, the reaction mixture was concentrated to half of its volume and poured onto ice, extracted with benzene and solvent was removed to get residue, which was washed several times with water and finally recrystallized from ethanol to give **3a**: yield 72%, m.p. 168ºC; IR (KBr) cm^{-1}: 1650 (CO), 1620 (CH = CH), 1575 (C = C aromatic ring) cm-1; ^1H NMR ($CDCl_3 – d_6$) : δ 8.61 (d,1H, = CH – Ar), 7.00 – 7.67 (m, 12H, Ar – H), 6.71 (d, 1H, COCH =); MS : m/z (%) : 329 (88.5)M^+, 266 (72), 198 (100), 166 (63), 153 (31),103 (65), 90 (77), 77 (72).

Similarly, other chalcone derivatives **3b–d** were synthesized by condensing *N* – Acetylphenothiazine with various aldehydes **2b–d** according to method reported in the literature.[16]

General procedure for (4a–d) *N*-(2-Amino-4-phenyl-3-cyanopyridin-6-yl)phenothiazine[17] 4a
A mixture of chalcones **3a – d** (0.01 mole), malonitrile (0.01 mole, 0.66 g) and ammonium acetate (0.08 mole, 6.16 g) dissolved in ethanol(20mL) was refluxed on a water bath for 6 hr. It was then decomposed in crushed ice, neutralized with dil. HCl and recrystallized from methanol to give product (**4a)**. m.p. 197ºC; ^{1}H NMR (DMSO – d_6): δ 8.41 (s, 2H, NH_2, D_2O exchangeable), 7.00 – 8.00 (m, 13H, Ar – H). ^{13}C NMR : 118.6 (- CN), 120 – 140 (Ar – C), 159.9 (C_5, >C = N), 178.2 (C_2, N – C – N); MS : m/z (%) : 392 (72) M^+ ,350 (3.4), 301 (32.6), 286 (33.4), 198 (100), 166 (65), 153 (35), 108 (30), 77 (33.7); Anal. Calcd. for $C_{24}H_{16}N_4S$: C, 73.46; H, 4.08; N, 14.28. Found: C, 73.51; H, 4.09; N, 14.26%.
***N*-(2-Amino-4-(4-methoxyphenyl)-3-cyanopyridin-6-yl)phenothiazine (4b).** M.p. 213ºC; ^{1}H NMR (DMSO – d_6) : δ 3.72 (s, 3H,OCH_3), , 8.19 (s, 2H, NH_2, D_2O exchangeable), 7.00 – 8.10 (m, 12H, Ar – H). ^{13}C NMR **:** 55.6 (OCH_3), 114.6 (- CN), 125 – 140 (Ar – C), 157.9 (C_5, >C = N), 179.1 (C_2, N – C – N); MS : m/z (%) : 422 (62) M^+, 394 (5.5), 391 (31.6), 345 (77.0), 331 (12.7), 199 (95), 165 (35), 153 (30), 78 (32.7); Anal. Calcd. for $C_{25}H_{18}ON_4S$: C, 71.09; H, 4.26; O, 3.79; N, 13.27. Found: C, 71.01; H, 4.29;N, 3.77; N, 13.29%.
***N*-(2-Amino-4-(4-*N*,*N*-dimethylaminophenyl)-3-cyanopyridin-6-yl)phenothiazine (4c).** M.p. 237 ºC; ^{1}H NMR (DMSO – d_6): δ 3.11 [s, 6H, $N(CH_3)_2$], 8.49 (s, 2H, NH_2, D_2O exchangeable), 7.00 – 8.10 (m, 12H, Ar – H). ^{13}C NMR : 40.3 [$N(CH_3)_2$], 59.6 (OCH_3), 115.9 (- CN), 130 – 140 (Ar – C), 152.9 (C_5, >C = N), 178.9 (C_2, N – C – N); MS : m/z (%) : 435 (72) M^+ ,420 (7.4), 407 (42.1), 390 (33.4), 358 (47.7), 343 (65), 199 (100), 166 (30),153 (42.6), 108 (19.7), 78 (33.5); Anal. Calcd. for $C_{26}H_{21}N_5S$: C, 71.72; H, 4.85; N, 16.09. Found: C, 71.77; H, 4.89; N, 16.07%.
***N*-(2-Amino-4-(3-hydroxy-4-methoxyphenyl)-3-cyanopyridin-6-yl)phenothiazine (4d).** M.p. 254 ºC; ^{1}H NMR (DMSO – d_6): δ 3.77 (s, 3H,OCH_3); 8.46 (s, 2H, NH_2, D_2O exchangeable), 7.00 – 8.00 (m, 11H, Ar – H), 10.77 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR : 59.6 (OCH_3), 111.6 (- CN), 120 – 150 (Ar – C), 160.9 (C_5, >C = N), 162.0 (C– OH), 179.9 (C_2, N – C – N); MS : m/z (%) : 438 (72) M^+ ,421 (4.4), 410 (22.6), 407 (23.4), 361 (45.6), 346 (65.7), 198 (100), 166 (75), 154 (45), 108 (50), 77 (31.7); Anal. Calcd. for $C_{25}H_{18}O_2N_4S$: C, 68.49; H, 4.10; O, 7.30; N, 12.78. Found: C, 68.41; H, 4.11; O, 7.28; N, 12.79%.

General procedure for (5a – d) *N*-(2-Acetamidino-4-phenyl-3-cyanopyridin-6-yl)phenothiazine 5a
A mixture **3 a – d** (0.01 mole), powdered anhydrous $AlCl_3$ and acetonitrile(1.0mL) was heated on an oil – bath at 150 – 160 ºC for 8 hr. The contents were cooled and decomposed in ice – cold HCl. The product obtained was filtered, washed with water and recrystallized from ethanol to give **5a**. m.p. 207 ºC; ^{1}H NMR (DMSO – d_6): δ 2.51 (s, 3H, CH_3); 4.48 (s, 1H, NH, D_2O exchangeable), 7.10 – 8.20 (m, 13H, Ar – H). ^{13}C NMR: 34.9 (CH_3), 115.9 (- CN), 130 – 140 (Ar – C), 151.1 (C_6, >C = N), 175.7 (C_2, N – C = N), 180.0 (C_2', N – C = N): MS : m/z (%) : 433 (32) M^+ , 418 (54), 405 (23), 376 (15.3), 356 (27), 331 (29.2), 263 (57.7), 199 (99), 167 (62), 153 (59.2), 108 (34), 78 (6.8); Anal. Calcd. for $C_{26}H_{19}N_5S$: C, 72.05; H, 4.38; N, 16.16. Found: C, 72.01; H, 4.33; N, 16.19%.

***N*-(2-Acetamidino-4-(4-methoxyphenyl)-3-cyanopyridin-6-yl)phenothiazine(5b).** M.p. 187 ºC; ^{1}H NMR (DMSO – d_6): δ 2.57 (s, 3H, CH_3); 3.85 (s, 3H, OCH_3), 4.46 (s, 1H, NH, D_2O exchangeable), 7.10 – 8.10 (m, 12H, Ar – H). ^{13}C NMR : 33.9 (CH_3), 58.9 (OCH_3), 115.2 (- CN), 130 – 140 (Ar – C), 152.5 (C_6, >C = N), 174.9 (C_2, N – C = N), 181.0 (C_2', N – C = N): MS : m/z (%) : 463 (35) M^+ , 448 (51), 432 (47.7), 406 (25), 386 (25.2), 361 (26), 293 (39.1), 199 (100), 165 (72), 152 (57.1), 166 (62), 76 (7.8); Anal. Calcd. for $C_{27}H_{21}ON_5S$: C, 69.97; H, 4.53; O, 3.45; N, 15.11. Found: C, 69.92; H, 4.52; O, 3.42; N, 15.19%.

***N*-(2-Acetamidino-4-(4-*N*,*N*-dimethylaminophenyl)-3-cyanopyridin-6-yl)phenothiazine(5c).** M.p. 218ºC; ^{1}H NMR (DMSO – d_6) : δ 2.46 [s, 6H, $N(CH_3)_2$], 2.59 (s, 3H, CH_3); 3.85 (s, 3H, OCH_3), 4.44 (s, 1H, NH, D_2O exchangeable), 7.10 – 8.20 (m, 12H, Ar – H). ^{13}C NMR: 34.2 (CH_3), 49.3 [(N– (CH_3)$_2$], 114.9 (- CN), 130 – 140 (Ar – C), 154.1(C_6, >C = N), 171.7 (C_2, N – C = N), 182.1 (C_2', N – C = N): MS : m/z (%) : 477 (52) M^+ , 462 (54), 446 (19.7), 420 (21), 400 (13.5), 375 (10.1), 306 (26), 198 (99), 166 (65), 153(69.2), 106 (31), 76(6.9).

***N*(2-Acetamidino-4-(3-hydroxy-4-methoxyphenyl)-3-cyanopyridin-6-yl)phenothiazine (5d).** M.p. 198ºC; ^{1}H NMR (DMSO – d_6): δ 3.61 (dd, 1H, OH), 3.82 (s, 3H, OCH_3), 2.57 (s, 3H, CH_3); 3.85 (s, 3H, OCH_3), 4.49 (s, 1H, NH, D_2O exchangeable), 7.10 – 8.10 (m, 11H, Ar – H). ^{13}C NMR : 35.1 (CH_3), 58.7 (OCH_3), 115.7 (- CN), 159.1 (C– OH), 130 – 140 (Ar – C), 152.3 (C_6, >C = N),171.9(C_2,N–C=N), 181.9 (C_2', N – C = N); MS: m/z (%) : 479 (41) M^+ ,464 (64), 448 (29.2), 422 (24), 402 (14.2), 309 (37), 198 (99.2), 166 (62.7), 152 (59.7), 108 (34), 76 (6.9); Anal. Calcd. for $C_{28}H_{25}N_6S$: C, 70.44; H, 5.24; N, 17.61. Found: C, 70.42; H, 5.21; N, 17.67%.

Active MnO_2. A solution of Manganese (II) sulphate tetrahydrate ($MnSO_4.4H_2O$) 223 g, 1 mole) in 300 mL of water and 240 mL (2.5 moles) of 40% aqueous NaOH solution was added simultaneously during 1 hr to a hot stirred solution of 190 g of $KmnO_4$ in 1200 mL of water. The stirring was continued for further 1 hr and the brown precipitate of MnO_2 was filtered and washed with water until the washings were colourless. The precipitate was dried in oven at 100 – 120ºC and grinded to fine powder.

General procedure for (6a – d) *N*-(2-Methyl-7-phenyl-8-cyano-[1,2,4]triazolo[1,5-a]pyridin-5-yl)phenothiazine 6a.

A mixture of **5a** (0.01 mole) and active MnO_2 (4.0 g) was taken in benzene (50mL). The contents were refluxed for 11 hr. and filtered hot. Solvent was removed to give the product. It was filtered, washed and recrystallized from alcohol to give product. **6a**. m.p. 231ºC; ^{1}H NMR (DMSO – d_6): δ 2.55 (s, 3H, CH_3), 7.00 – 8.00 (m, 13H, Ar – H). ^{13}**C** NMR: 32.2 (CH_3), 115.4 (- CN), 125 – 140 (Ar – C), 150.7 (C_8, >C = N), 174.4 (N – C = N), 182 (C_3, N – C = N); MS : m/z (%) : 431 (20) M^+, 372 (3.5), 340 (3.5), 312 (15.2), 302 (16.5), 199 (99.1), 166 (31.2), 152 (32.2), 106 (23.5), 77 (32.6); Anal. Calcd. for $C_{26}H_{17}N_5S$: C, 72.38; H, 3.94; N, 16.24. Found: C, 72.39; H, 3.95; N, 16.27%.

***N*-(2-Methyl-7-(4-methoxyphenyl)-8-cyano-[1,2,4]triazolo[1,5-a]pyridin-5-yl)phenothiazine (6b).** M.p. 224ºC; ^{1}H NMR (DMSO – d_6): δ 2.51 (s, 3H, CH_3), 3.35 (s, 3H, OCH_3), 7.00 – 8.00 (m, 12H, Ar – H). ^{13}C NMR: 32.5 (CH_3), 114.7 (- CN), 125 – 140 (Ar – C), 149.1 (C_8, >C = N),

154.8 (C_4', C – OCH_3), 175.2 (N – C = N), 184 (C_3, N – C = N); MS : m/z (%): 461 (27) M^+, 444 (15.2), 430 (16.5), 402 (4.5), 370 (4.8), 332 (8.5), 199 (100), 163 (31.2), 153 (25.9), 106 (31.7), 77 (30.6); Anal. Calcd. for $C_{27}H_{19}ON_5S$: C, 70.28; H, 4.12; O, 3.47; N, 15.18. Found: C, 70.29; H, 4.15; O, 3.45; N, 15.19%.

***N*-(2-Methyl-7-(4-*N*,*N*-dimethylaminophenyl)-8-cyano-[1,2,4]trazolo[1,5-a]pyridin-5-yl) phenothiazine (6c).** M.p. 213ºC; ^{1}H NMR (DMSO – d_6) : δ 2.46 [s, 6H, $N(CH_3)_2$], 2.59 (s, 3H, CH_3), 7.00 – 8.00 (m, 11H, Ar – H). ^{13}C NMR: 32.7 (CH_3), 114.9 (- CN), 125 – 140 (Ar – C), 150.1 (C_8, >C = N), 175.1 (N – C = N), 185 (C_3, N – C = N) ; MS : m/z (%) : 474 (29) M^+, 457 (25.1), 443 (26.1), 383 (3.9), 355 (3.6), 345 (4.6), 199 (99.9), 164 (29.2), 152 (32.2), 106 (31.8), 78 (31.6); Anal. Calcd. for $C_{28}H_{22}N_6S$: C, 70.88; H, 4.64; N, 17.72. Found: C, 70.87; H, 4.68; N, 17.77%.

***N*-(2-Methyl-7-(3-hydroxy-4-methoxyphenyl)-8-cyano-[1,2,4]trazolo[1,5-a]pyridin-5-yl) phenothiazine (6d).** M.p. 221ºC; ^{1}H NMR (DMSO – d_6) : δ 2.57 (s, 3H, CH_3), 3.39 (s, 3H, OCH_3), 7.00 – 8.00 (m, 11H, Ar – H), 11.09 (s, 1H, OH, D_2O exchangeable); ^{13}C NMR: 32.9 (CH_3), 115.1 (- CN), 125 – 140 (Ar – C), 149.9 (C_8, >C = N), 151.8 (C_4', C – OCH_3), 175.7 (N – C = N), 182 (C_3, N – C = N); MS : m/z (%) : 477 (30) M^+, 418 (3.8), 358 (3.1), 386 (5.7), 348 (16.9), 460 (26.9), 446 (27.9), 199 (100), 162 (31.9), 152 (25.9), 106 (33.7), 77 (32.9); Anal. Calcd. for $C_{27}H_{19}O_2N_5S$: C, 67.92; H, 3.98; O, 6.70; N, 14.67. Found: C, 67.95; H, 3.92; O, 6.71; N, 14.77%.

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