Indian Journal of Heterocyclic Chemistry
Vol. 23, Oct.-Dec., 2013, pp. 149-154

SYNTHESIS, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF NOVEL 4-AMINO-SUBSTITUTED-1,2,4-TRIAZOLE-3-THIOL DERIVATIVES

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Received 30 May 2013; Accepted 10 Oct. 2013

The synthesis of a series of novel substituted-1,2,4-triazole-3-thiol derivatives were described. One of the easiest way of constructing 1,3,4-oxadiazoles is by reacting hydrazides with carbon disulfide in presence of potassium hydroxide. It is then converted to 4-amino-1,2,4-triazole (**3**) by reacting hydrazine hydrate. Target compounds 4-(4-substituted benzylideneamino)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiols (**4**a-f) were afforded by reacting 4-amino-1,2,4-triazole (**3**) with aromatic aldehydes. The selection of aromatic aldehydes is based on the electron releasing and electron withdrawing groups on aromatic ring, which would help in drawing important conclusion regarding structure activity relationship (SAR) studies during the investigation of biological activities. All synthesized compounds were confirmed by physicochemical and spectral analysis. The synthesized compounds were tested for their *in vitro* antibacterial activity against the Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Salmonella typhi* and *Escherichia coli*) bacteria. Among all the compounds synthesized, compound (**4**c) was found most active against Gram-positive bacterial strains. Also the compound (**4**e) gives a better antifungal activity against fungi (*C. albicans* and *A. niger*) than other synthesized compounds.

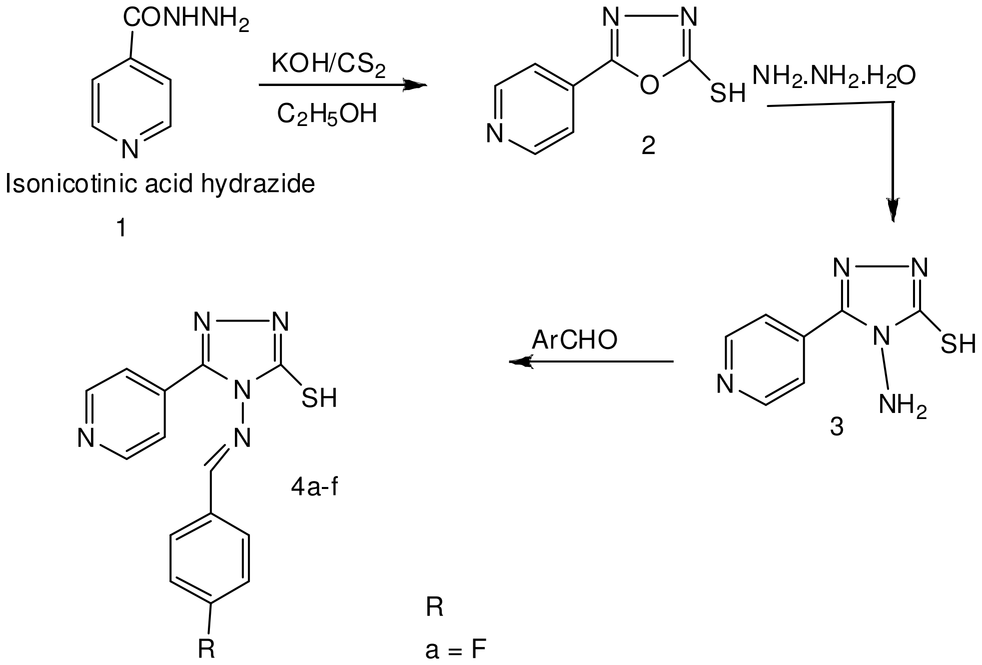
Triazole derivatives have unique position in heterocyclic chemistry due to their diverse biological activities[1]. They show antibacterial[2,3], antiviral[4], antifugal[5], antioxidant[6], anti-inflammatory[7], antituberculosis[8], antitumor[9] and antiobesity[10] activities. Many naturally occurring and synthetic compounds containing the pyridine scaffold possess interesting pharmacological properties[11-12]. Among them, amino and mercapto group together can be considered as useful intermediates in organic synthesis. By knowing the biological properties of 1,2,4-triazoles, it was planned to prepare 4-[4-(substituted-benzylidene)-amino]-5-pyridin-4-yl-4*H* [1,2,4] triazole-3-thiols where in biologically interesting pyridine moiety has been linked to triazole with an aim of obtaining enhanced biological activities.

Isonicotinic acid hydrazide was treated with carbon disulfide in presence of potassium hydroxide to afford 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-thiol[17] (**2**) which was converted to 4-amino-1,2,4-triazole (**3**) by reaction with hydrazine hydrate. Further 4-amino-1,2,4-triazole[18] (**3**) was reacted with aromatic aldehydes to afford 4-(4-substituted benzylideneamino)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiols (**4**a-f). The selection of aromatic aldehydes is based on the electron donating and electron withdrawing groups on aromatic ring, which would help in drawing important conclusion regarding structure activity relationship (SAR) studies during the investigation of biological activities.

The antibacterial activity of triazole derivatives showed a differential activity against the bacterial strains investigated.

Antimicrobial evaluation

All the compounds (**4**a-f) were screened for *in-vitro* antibacterial activity against pathogenic bacteria



R
a = F
b = Cl
c = OH
d = OMe
e = Br
f = NO_2

SCHEME-1

collected from National Chemical Laboratories, Pune. Two Gram-positive bacteria *Bacillus subtilis* National Collection of Industrial Microorganism (NCIM) No. 2063 and *Staphylococcus aureus* (NCIM No. 2079) and two Gram-negative bacteria *Salmonella typhi* (NCIM No. 2263) and *Escherichia coli* (NCIM No. 2065) were used. Antibacterial activities of novel compounds were compared using steptromycin and ampicillin as standard drugs. Similarly antifungal studies were carried out against two fungal strains *Candida albicans* (NCIM No. 3102) and *Aspergillus niger* (NCIM No. 742) using Ketoconazole as standard drug.

In-vitro antibacterial activity

For the determination of minimum inhibitory concentration (MIC) value agar dilution method was performed using Miller-Hinton agar (Hi-Media) medium. The medium was inoculated with the required amount of inoculum to obtain a suspension of microorganism which contains 10^6 colonies forming units per milliliter (cfu/mL) and applied to plates with serially diluted compounds in DMF to be tested and incubated at 37^0 overnight. The MIC value was considered to be the lowest concentration that completely inhibited growth on agar plates. MIC values were evaluated at the final range of (5-100 μg/mL) compounds.

Table-1
Physiochemical data of synthesized compounds

Compd	R	M.P. (^{0}C)	Yield (%)	R_f
4a	F	237-239	71	0.33
4b	Cl	266-270	58	0.51
4c	OH	302-305	65	0.61
4d	OMe	199-201	60	0.57
4e	Br	281-284	69	0.39
4f	NO_2	311-312	79	0.49

***In-vitro* antifungal activity**

For the determination of MIC value of the novel compounds *in-vitro* antifungal activity was tested against pathogenic fungi using agar dilution method with Saburoud's dextrose agar (Hi-Media). Suspensions of each microorganisms were prepared to contain 10^5 cfu/mL and applied to agar plates which have been serially diluted with compounds to be tested in DMF. The plates were incubated at 25^0 for 72 hr and MICs were determined. MIC values evaluated at the final concentration range of 5-100 μg/mL.

Among the all synthesized compounds, compound (**4**c) seems to be good against Gram-positive bacteria and Gram-negative bacteria may be due to the presence of electron releasing hydroxyl group at para position. Compounds (**4**a & **4**b) exhibited comparable good activity against Gram-positive and Gram-negative bacteria than other synthesized compounds due to the presence of electron withdrawing halo group at para position. Compounds (**4**e & **4**f) exhibited poor antimicrobial activity against all microbial strains. The compound (**4**e) exhibited good antifungal activity against *C. albicans* and *A. niger*. All other compounds exhibited poor antifungal activity against both the strains.

Interpretation of antimicrobial and antifungal screening data in Table-2 revealed that all the novel compounds (**4**a-f) showed moderate to good inhibition on the growth of tested micro-organisms. Among the all synthesized compounds, compound (**4**c) shows MIC value of 16 (μg/ml) for *S. aureus* and 20 (μg/ml) for *B. subtilis* against Gram-positive bacteria. Compound (**4**e) shows MIC value of 25 (μg/ml) for *E. coli* and 31 (μg/ml) for *S. typhi* against Gram-negative bacteria. Compounds (**4**a & **4**b) exhibited comparable good activity against Gram-positive and Gram-negative bacteria than other synthesized compounds. Compounds (**4**e & **4**f) exhibited poor antimicrobial activity against all microbial strains. The compound (**4**e) shows MIC value of 24 (μg/ml) against *C. albicans* and 32 (μg/ml) against *A. niger* gives a better antifungal activity than other synthesized compounds. All other compounds exhibited poor antifungal activity against both the strains.

Experimental

Melting points were determined using a VEEGO make microprocessor based melting point apparatus having silicone oil bath and are uncorrected. IR spectra (wave numbers in cm^{-1}) were recorded on a BRUKER ALPHA T FT-IR spectrophotometer using potassium

Table-2
Minimum inhibitory concentration of different compounds

Compd	MIC antibacterial activity (µg/ml)				MIC antifungal activity (µg/ml)	
	S. aureus	*B. subtilis*	*E. coli*	*S. typhi*	*C. albicans*	*A. niger*
4a	30	24	32	38	60	NA
4b	28	29	35	44	65	78
4c	16	20	22	26	42	53
4d	23	26	25	31	58	68
4e	44	52	65	58	24	32
4f	74	85	82	75	92	NA
Ampicillin	10	16	9	13	-	-
Ketoconazole	-	-	-	-	10	18

bromide discs. ^{1}H NMR spectra were recorded on BRUKER AVANCE II 400 MHz instrument in $CDCl_3$ with TMS as internal standard. Chemical shift values are mentioned in δ ppm. The progress of all reactions was monitored by TLC on 2 cm x 5 cm pre-coated silica gel 60F254 (Merck) plates of thickness of 0.25 mm. The chromatograms were visualized under UV (254 nm) and/or exposure to iodine vapours.

Synthesis of 1,2,4-triazole : General procedure (3)

A mixture of isonicotinic acid hydrazide (**1**), potassium hydroxide and carbon disulfide in ethanol was refluxed on a oil bath for 10-12 hr to obtain the compound 5-(pyridine-4-yl)-1,3,4-oxadiazole-2-thiol (**2**)[17]. Then compound (**2**) was refluxed with hydrazine hydrate (99%) in absolute ethanol for 8-9 hr on a oil bath to obtain 4-amino-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol (**3**)[18].

Synthesis of 4-(4-substituted benzylideneamino)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol (4a-f) : General procedure

Equimolar quantities of compound (**3**) (0.001 mol) and *p*-substituted benzaldehydes (0.001 mol) were dissolved in methanol. The mixture was refluxed on water bath for about 10-14 hr. Progress of the reaction was monitored by TLC. The solution was poured in ice cold water and the resulting solid was filtered, washed and recrystallized with suitable solvents viz. methanol, ethanol, acetone and benzene their physicochemical data mentioned are in Table-1.

4-(4-Fluorobenzylideneamino)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol (4a)

IR (KBr, cm^{-1}): 1604 (C=N str), 1007 (C-S str), 2710 (SH str), 1153 (C-F str); ^{1}H NMR (δ ppm): 7.79-7.86 (d, 2H, C_2 and C_6 ArH), 7.61-7.63 (d, 2H, C_3 and C_5-H), 8.23 (s, 1H, N=CH), 8.79-8.80 (d, 2H, pyridine-

C_2 and C_6-H), 7.89-7.90 (d, 2H, pyridine-C_3 and C_5-H), 2.98 (s, 1H, aromatic C-SH); Mass : m/z 299 (M^+).

4-(4-Chlorobenzylideneamino)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol (4b)

IR (KBr): 1647 (C=N str), 1027 (C-S str), 2579 (SH str), 699 (C-Cl str); ^1H NMR : 7.66-7.71 (d, 2H, C_2 and C_6 ArH), 7.45-7.56 (d, 2H, C_3 and C_5-H), 8.01 (s, 1H, N=CH), 8.81-8.88 (d, 2H, pyridine-C_2 and C_6-H), 7.79-7.85 (d, 2H, pyridine-C_3 and C_5-H), 3.11 (s, 1H, aromatic C-SH); Mass : m/z 316 (M+1).

4-(4-Hydroxybenzylideneamino)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol (4c)

IR (KBr): 1644 (C=N str), 1092 (C-S str), 2571 (SH str), 3464 (-OH); ^1H NMR : 5.12 (s, 1H, -OH), 6.82-6.93 (d, 2H, C_2 and C_6 ArH), 7.39-7.42 (d, 2H, C_3 and C_5-H), 8.14 (s, 1H, N=CH), 8.57-8.63 (d, 2H, pyridine-C_2 and C_6-H), 7.80-7.84 (d, 2H, pyridine-C_3 and C_5-H), 3.26 (s, 1H, aromatic C-SH); Mass : m/z 296 (M-1).

4-(4-Methoxybenzylideneamino)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol (4d)

IR (KBr): 1629 (C=N str), 1095 (C-S str), 2510 (SH str), 1225 (C-O-C); ^1H NMR : 3.75 (s, 3H, -OCH_3), 5.91-5.99 (d, 2H, C_2 and C_6 ArH), 5.91-5.99 (d, 2H, C_3 and C_5-H), 8.33 (s, 1H, N=CH), 8.44-8.55 (d, 2H, pyridine-C_2 and C_6-H), 7.66-7.72 (d, 2H, pyridine-C_3 and C_5-H), 3.33 (s, 1H, ar C-SH); Mass : m/z 311 (M^+).

4-(4-Bromobenzylideneamino)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol (4e)

IR (KBr): 1640 (C=N str), 1086 (C-S str), 2586 (SH str), 662 (C-Br str); ^1H NMR : 7.41-7.39 (d, 2H, C_2 and C_6 ArH), 7.02-7.16 (d, 2H, C_3 and C_5-H), 8.11 (s, 1H, N=CH), 8.69-7.73 (d, 2H, pyridine-C_2 and C_6-H), 7.90-7.92 (d, 2H, pyridine-C_3 and C_5-H), 3.23 (s, 1H, aromatic C-SH); Mass : m/z 362 (M+2).

4-(4-Nitrobenzylideneamino)-5-(pyridine-4-yl)-4*H*-1,2,4-triazole-3-thiol (4f)

IR (KBr): 1607 (C=N str), 1103 (C-S str), 2653 (SH str), 1536 (-NO_2); ^1H NMR : 9.47-9.51 (d, 2H, C_2 and C_6 ArH), 8.74-7.79 (d, 2H, C_3 and C_5-H), 8.09 (s, 1H, N=CH), 8.82-8.91 (d, 2H, pyridine-C_2 and C_6-H), 7.65-7.72 (d, 2H, pyridine-C_3 and C_5-H), 2.99 (s, 1H, aromatic C-SH); Mass : m/z 327 (M+1).

Acknowledgement

We are thankful to the director, SAIF, Punjab University, Chandigarh and Oxygen Healthcare Private Ltd. for providing spectroscopic analysis of the compounds. We would like to thanks Dr. Devanshu J. Patel, Managing Trustee Parul Trust for providing necessary infrastructure and Dr. Rajesh K.S. Principal, Parul Institute of Pharmacy, Limda, Vadodara for offering precious suggestions.

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