***Current Pharma Research***
ISSN-2230-7842
***CODEN-CPRUE6***
www.jcpronline.in/

***Research Article***

# Synthesis and antimicrobial evaluation of 2-amino-6-[(5-pyridin-4-yl-1, 2, 4-triazole-4(H)-phenyl-3-ylthio) methyl]-4-arylnicotinonitriles.

**Niranjan S. Mahajan*[1], Shashikant C. Dhawale[2]**

[1]Department of Pharmaceutical Chemistry, Adarsh College of Pharmacy, Vita, Tal-Khanapur, Sangali-415311, Maharashtra, India.

[2]Department of Pharmacology, SRTM University, Nanded-431606, Maharashtra, India.

Received 11 May 2019; received in revised form 01 June 2019; accepted 02 June 2019

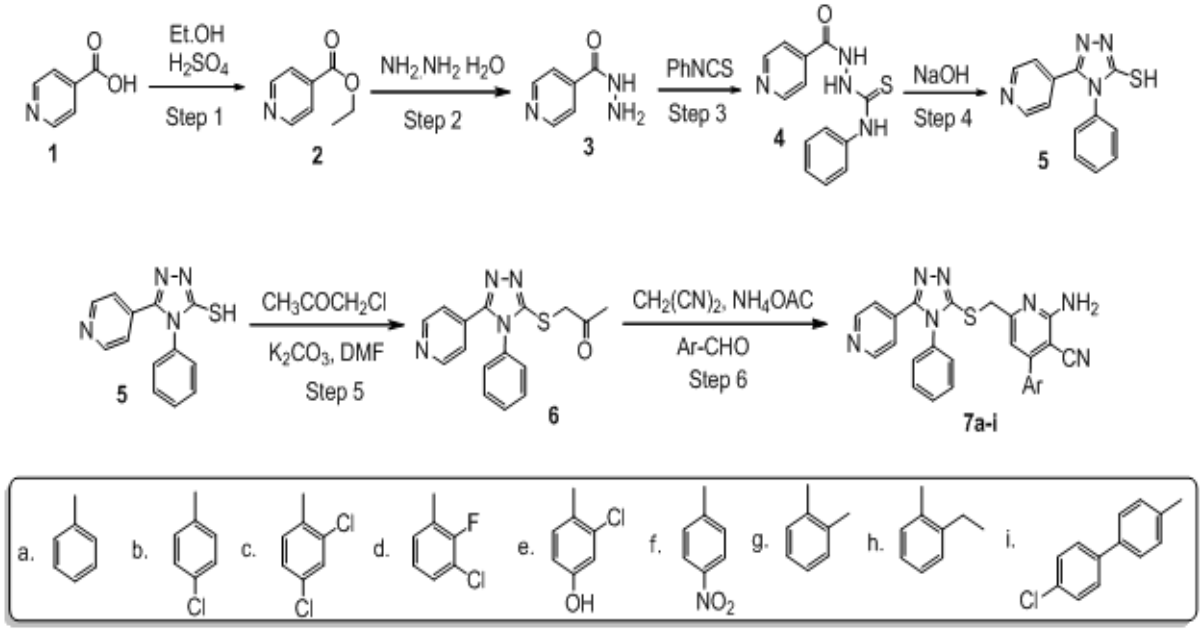
***Corresponding author E-mail address:** *nsmahajan17@gmail.com*

## ABSTRACT
Novel biheterocycle compounds 2-amino-6-[(5-pyridin-4-yl-1, 2, 4-triazole-4(*H*)-phenyl-3-ylthio) methyl]-4-arylnicotinonitriles were designed, synthesized and proved effective against both bacteria and fungi. These novel compounds were prepared using one-pot synthesis from malononitrile, aromatic aldehyde, acetone derivative and ammonium acetate using conventional method reported in the literature. This procedure has the advantage of short route, good yields, convenient workup and being environmentally friendly. These new compounds were obtained in satisfactory yields and characterized successfully using IR, $^1$HNMR, Mass spectral results. These newly prepared compounds were then screened for both antibacterial and anti-fungal activities. Out of which compound 7c found to exhibit comparable antibacterial activity with standard drug Ciprofloxacin, whereas all the compounds 7a-i have shown poor antifungal activity compared to standard drug fluconazole.

## KEYWORDS
1, 2, 4- Triazole**,** pyridine, antibacterial, antifungal.

## 1. INTRODUCTION

1, 2, 4-Triazole derivatives represent a novel emerging major chemical group as antimicrobial agent [1]. Various substitutions have been successfully tried at the 3 &/or 5 positions of the 1, 2, 4-triazole ring, to design potential antimicrobial agents to overcome resistance problems [2]. These compounds have drawn great attention to medicinal chemists since two decades due to its readily binding property with a variety of enzymes and receptors in biological system *via* diverse non-covalent interactions such as coordination bonds, hydrogen bonds, ion-dipole, cation-π, π - π stacking, hydrophobic effect, van der Waals force and so on, because of this, these derivatives displaying broad spectrum of biological activities, exhibit low toxicity and good pharmacokinetic and pharmacodynamic profiles [1-2]. Moreover, 1,2,4-triazole can function as attractive linker units which could connect two pharmacophore to give an innovative bifunctional drug, and thus have become increasingly useful and important in constructing bioactive and functional molecules [3]. Literature survey revealed that, 1, 2, 4-triazole derivatives exhibit wide range of biological activities including antibacterial, antifungal, antitumor, anti-inflammatory, anti-tubercular, hypoglycemic, antidepressant, anticonvulsant, anticancer, anti-malarial, antiviral, anti-proliferative, analgesic and anti-migraine [4]. A wide variety of antifungal agents such as fluconazole, voriconazole, ketoconazole, itraconazole, posaconazole etc of this class of compounds have been successfully launched in the market [5-9]. In addition to this, many natural occurring and synthetic compounds containing the pyridine scaffold possess interesting pharmacological properties [10]. These observations initiated a program of clubbing these two heterocycles 1, 2, 4-triazoles and pyridine in search of novel chemical entities with enhanced biological and pharmacological spectrum [11-13].




**Scheme 1.** Synthetic pattern of 2-amino-6-[(5-pyridine-4-yl-1, 2, 4-triazole-4(H)-phenyl-3-ylthio) methyl]-4-substitutedphenylnicotinonitriles.

## 2. MATERIALS AND METHODS

*2.1. Experimental*

*2.1.1. Synthesis of 2-amino-6-[(5-pyridine-4-yl-1, 2, 4-triazole-4(H)-phenyl-3-ylthio-) methyl-]-4-substituted phenylnicotinonitriles.*

*2.1.1.1. Preparation of ethyl isonicotinate 2*

Isonicotinic acid (1) (140 g, 1.14 mol) was suspended in 1000 ml of absolute ethanol and cooled to 0 °C. Dry hydrogen chloride was bubbled in until the solution saturated. Then with the gas still being passed in, it was refluxed until the all solid dissolved. The excess ethanol was removed under diminished pressure, the solid dissolved in sufficient aq. medium, cooled and treated with an excess of saturated sodium carbonate solution, filtered and extracted with ether. On distillation, a clear; colorless ester (2) was obtained. Yield 58%, b.p. 103-106 $^0$C (Lit. 105-108 °C) [14].

***TLC;*** $R_f$ 0.47 (ethylacetate: cyclohexane, 4:1).

*2.1.1.2. Preparation of Isonicotinic acid hydrazide (INH) 3*

Ethyl isonicotinate (**2**) (0.1 mol) was dissolved in 30 ml of ethanol; to this, drop wise hydrazine hydrate (0.1 mol) was added with stirring. The resulting mixture was refluxed for 6 h. then excess of ethanol was distilled off and the contents were allowed to cool. The crystals formed (3) were filtered off and thoroughly washed with water dried and recrystallized from ethanol. Yield 58%, m.p. 169-170 $^0$C.

**TLC**; $R_f$ 0.54 (ethyl acetate: cyclohexane, 4:1)

2.1.2. *Synthesis of 1-isonicotinoyl-4-phenylthiosemicarbazide/2-isonicotinoyl-N-phenylhydrazine carbothioamide 4*

A mixture of isonicotinic acid hydrazide (3) (10 mmol) and phenylisothiocyanate (15 mmol) was refluxed in ethanol for 4 h. The solution was cooled and a white solid appeared. This was filtered and recrystallized from ethanol to afford the desired product. Yield 90%, m.p. 119 °C (Lit. 120 °C) [15].

***TLC;*** $R_f$ 0.32 (ethylacetate: cyclohexane, 4:1)

*2.1.3. Synthesis of 4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole-3-thiol 5*

A solution of 10 mmolcarbothioamide (4) and sufficient 2N NaOH was refluxed for 3 h. After this the resulting reaction solution was cooled to room temperature and acidified to pH 3-4 with 37% hydrochloric acid solution. The resulting solution was then filtered; pptobtaind was washed with water and recrystallized from ethanol/water (1:1) to get the desired compound (5). Yield 83%, m.p. 199 °C (Lit. 195-200 °C) [16].

***TLC;*** $R_f$ 0.28 (ethylacetate: cyclohexane, 4:1)

*2.1.4. Synthesis of 1-(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole-3-ylthio)acetone 6*

A mixture of (5) (0.01 mol), 0.011 mol chloroacetone, and 2 g of potassium carbonate in 20 ml dimethylformamide was stirred for 2 h, at 90 °C, in an oil-bath. Then cooled the reaction mixture

afterwards poured into water (60 ml). This solution was filtered. The precipitated product obtained was washed thoroughly with cold water and recrystallized from ethanol to afford the desired product. Yield 80%, m.p. 179 °C [17].
**TLC**; $R_f$ 0.23 (ethylacetate: cyclohexane, 4:1)

*2.1.5. Synthesis of 2-amino-6-[(5-pyridin-4-yl-1,2,4-triazole-4(H)-phenyl-3-ylthio)methyl]-4-substitutedphenylnicotinonitriles 7a-i*

A mixture of acetone derivative (6) (10 mmol), malononitril (10 mmol), aromatic aldehyde (10 mmol), and ammonium acetate (10 mmol) in ethanol (50 ml) was refluxed for 10 h. After completion of reaction, the resulting reaction mixture was cooled and poured on to crushed ice. The product separated was filtered and recrystallized from ethanol [17].

*2.2. Antimicrobial Activity of compounds 7a-i.*
The synthesized compounds were tested for their *in vitro* antibacterial activity against the Gram-positive organisms *S. aureus, B. subtilis* and the Gram-negative organisms *P. aeruginosa, E. coli,* and fungi, *C. albicans* and *A. niger*. The primary screening was carried out by Broth dilution method using nutrient broth medium. The minimum bactericidal concentration (MBC) against the same microorganisms used in the preliminary screening was carried out using Broth dilution susceptility method. Ciprofloxacin and Fluconazole were used as control drugs. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the compounds were recorded as the lowest concentration of each chemical compounds in the tubes with no turbidity (i.e. no growth) of inoculated bacteria/fungi.

## 3. RESULTS AND DISCUSSION

*3.1. Chemistry*
Commercially available isonicotinic acid (1) was esterified to ethyl isonicotinate (2) which on treatment with hydrazine hydrate provided isonicotinic acid hydrazide (3). This transformation was done as per literature and obtained compounds matched reported melting point (2: B.P. 103-106°C& 3: M.P. 169-170°C) and IR (3: 3302, 3112, 1678, 1545 $cm^{-1}$) values. The hydrazine compound (3) was converted to 1-isonicotinoyl-4-phenylthiosemicarbazide (4) by Phenyl isothiocyanate. Mass and IR values (272; 3370, 3292, 1716, 1308 $cm^{-1}$) indicated successful transformation. The cyclization of compound (4) in the presence of sodium hydroxide, gave away 4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole-3-thiol (5). Mass spectrum (254) and thione-thioltautomeric form in IR was also observed (2682 (SH), 1320, 1628, and 1558 $cm^{-1}$). Chemo-transformation of (5) with chloroacetone in presence of potassium carbonate in dimethylformaamide resulted in the formation of 1-(4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazole-3-ylthio)acetone (6). Absence of SH signal and appearance of additional signals for originating from $CH_3$ (4.50) and $CH_2$ (2.78) concluded conversion of (5) to (6). This molecule (6) when treated with malononitrile and aromatic aldehyde in presence of ammonium acetate in ethyl alcohol was converted to title compound (7). In the 1H NMR spectrums of compound (7) signals owing to $CH_3$ group remain absent; instead, new signals derived from $NH_2$ appeared. The IR spectra of compounds (7a-i) showed multiple bands in the 3478-3272 $cm^{-1}$ region due to NH

stretching vibrations of the amino group, bands around 1600cm$^{-1}$ characteristic of NH bending vibrations and C≡N around 2210 cm$^{-1}$. The 1H NMR spectra of compound (7) also displayed additional signals owing to the aromatic ring derived from aldehyde moiety in the aromatic region. The physicochemical data of the synthesized compounds is tabulated in Table 1, while spectral data is tabulated in Table 2.

**Table 1.** Physico-chemical data of compounds 7a-i

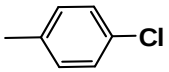
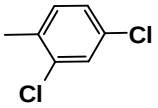
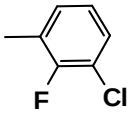
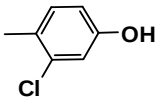
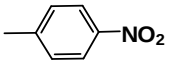
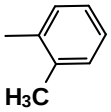
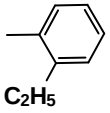
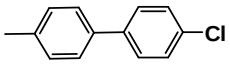
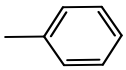
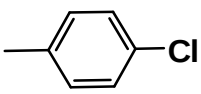
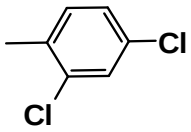
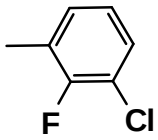
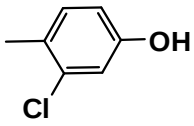
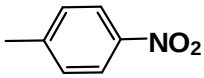
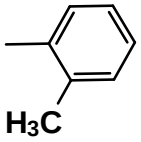
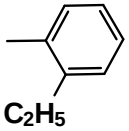
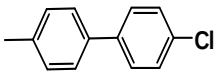
| Comp. No | Ar | MF(MW) | M.P. °C | %Yield (MW) |
|---|---|---|---|---|
| **7a** | $C_6H_5$ | $C_{26}H_{19}N_7S$ (461.55) | 182 | 62.30 |
| **7b** |  | $C_{26}H_{18}ClN_7S$ (496) | 172 | 60.30 |
| **7c** |  | $C_{26}H_{17}Cl_2N_7S$ (529.10) | 176 | 62.22 |
| **7d** |  | $C_{26}H_{17}ClFN_7S$ (514.00) | 185 | 66.30 |
| **7e** |  | $C_{26}H_{18}ClN_7OS$ (512.00) | 146 | 56.74 |
| **7f** |  | $C_{26}H_{18}N_8O_2S$ (506.50) | 168 | 66.28 |
| **7g** |  | $C_{27}H_{21}N_7S$ (475.60) | 154 | 62.32 |
| **7h** |  | $C_{28}H_{23}N_7S$ (489.60) | 165 | 56.74 |
| **7i** |  | $C_{32}H_{22}ClN_7S$ (572.10) | 141 | 60.28 |

**Table 2.** Spectral data of compounds 7a-i

| No. | Ar | IR (KBr)cm$^{-1}$ | $^1$H NMR ($CDCl_3$) | Mass *m/z* |
|---|---|---|---|---|
| **7a** |  | 3350 ($NH_2$), 3170 (Ar-H), 2208 (C≡N), 1602(C=N) | 7.12 (s,1H,5-H of pyridine), 7.20-7.37 (m,10H,2$C_6H_5$), 7.60 (d,2H, CH-C-CH), 8.60 (d, 2H, CH-N-CH) 4.48 (s,2H,$CH_2$), 4.10 (br,s,2H,$NH_2$) | $(M+2)^+$ 463.1 |
| **7b** |  | 3350, 3272 ($NH_2$), 3161 (Ar-H), 2208 (C≡N), 1585 | 7.18 (s,1H,5-H of pyridine), 7.25-7.48 (m,9H, $C_6H_5$, $C_6H_4Cl$), 7.88 (d,2H, CH-C-CH), 8.60 (d, 2H, CH-N-CH), 4.38 | $(M+2)^+$ 498.1 |

| | | | | |
|---|---|---|---|---|
| | | (C=N) | (s,2H,$CH_2$), 4.06 (br,s,2H,$NH_2$) | |
| **7c** |  | 3462,3361 ($NH_2$), 3078 (OH), 2212 (C≡N), 1566 (C=N) | 7.18 (s,1H,5-H of pyridine), 7.35-7.70 (m,8H, $C_6H_5$, $C_6H_3Cl_2$), 7.58 (d,2H, CH-C-CH), 8.60 (d, 2H, CH-N-CH), 4.42 (s,2H,$CH_2$), 4.02 (br,s,2H,$NH_2$) | $(M+2)^+$ 531.1 |
| **7d** |  | 3478, 3366 ($NH_2$), 3178 (Ar-H), 2210 (C≡N), 1615 (C=N) | 7.38-7.59(m,9H,$C_6H_5$, $C_6H_3FCl$ and 5-H of pyridine), 7.65 (d,2H, CH-C-CH), 8.60 (d, 2H, CH-N-CH), 4.44 (s,2H,$CH_2$), 3.98 (br,s,2H,$NH_2$), 4.35 (s,2H,$CH_2$) | $(M+2)^+$ 515.9 |
| **7e** |  | 3458 (O-H), 3356 ($NH_2$), 3147 (Ar-H), 2210 (C≡N), 1618 (C=N) | 6.68-7.29(m,10H,$C_6H_5$, $C_6H_4OH$ and 5-H of pyridine), 7.60 (d,2H, CH-C-CH), 8.65 (d, 2H, CH-N-CH), 4.48 (s,2H,$CH_2$), 4.0 (br,s,2H,$NH_2$), 4.98 (s, H, OH) 4.42 (s,2H,$CH_2$) | $(M+2)^+$ 514.1 |
| **7f** |  | 3458,3365 ($NH_2$), 2218 (C≡N), 1555 (C=N)1345 ($NO_2$) | 7.14 (s,1H,5-H of pyridine), 7.33-8.41 (m,9H,$C_6H_5$ and $C_6H_4NO_2$ ), 7.55 (d,2H, CH-C-CH), 8.59 (d, 2H, CH-N-CH) 4.48 (s,2H,$CH_2$), 3.98 (br,s,2H,$NH_2$) | $(M+1)^+$ 507.2 |
| **7g** |  | 3380 ($NH_2$), 3098 (Ar-H), 2195 (C≡N), 1610(C=N) | 2.28 (3H, $CH_3$), 7.20-7.58 (m,9H,$C_6H_5$, $C_6H_4$-$C_2H_5$)$_2$ and 5-H of pyridine), 7.63 (d,2H, CH-C-CH), 8.62 (d, 2H, CH-N-CH), 4.22 (s,2H,$CH_2$), 4.06 (br,s,2H,$NH_2$) | $(M+1)^+$ 476.2 |
| **7h** |  | 3358 ($NH_2$), 3145 (Ar-H), 2235 (C≡N), 1560 (C=N) | 1.25 (3H, $CH_3$), 2.58 (2H, $CH_2$), 7.17-7.63 (m,10H,$C_6H_5$, $C_6H_5$-$C_2H_5$)$_2$ and 5-H of pyridine), 7.64 (d,2H, CH-C-CH), 8.61 (d, 2H, CH-N-CH), 4.32 (s,2H,$CH_2$), 4.04 (br,s,2H,$NH_2$) | $(M+1)^+$ 490 |
| **7i** |  | 3422,3388 ($NH_2$), 3099 (Ar-H), 2218 (C≡N), 1574 (C=N) | 7.25-7.58(m,14H,$C_6H_5$, $C_{12}H_8Cl$ and 5-H of pyridine), 7.63 (d,2H, CH-C-CH), 8.66 (d, 2H, CH-N-CH), 4.45 (s,2H,$CH_2$), 4.08 (br,s,2H,$NH_2$), 4.36 (s,2H,$CH_2$) | $(M+2)^+$ 574.1 |

*3.2. Antimicrobial Activity of compounds 7a-i.*

In this series of new compounds, 7a (MIC; 62.5µg/ml), 7b (MIC; 31.25µg/ml) exhibited poor antibacterial activity compared to standard drug Ciprofloxacin (MIC; <1 µg/ml).Compound 7c (MIC; 8 µg/ml) showed good inhibition against gram-negative organisms; *E. coli* and *P. aeruginosa* and also against gram-positive microbes; *B. subtilis* (MIC; 8 µg/ml) but not against *S. aureus* (MIC; 16 µg/ml). The other compounds 7d, 7f, 7h-i were found to exhibit poor activities against *S. aureus*, *E. coli*, *P. aeruginosa* and *B. subtilis*(MIC; 16-62.5 µg/ml), while compound 7e showed moderate activity (MIC; 16 µg/ml). Amongst all the newly synthesized compounds, 7c showed promising activity. Antifungal activity results of all these newly synthesized compounds 7a-i (MIC; 62.5-125 µg/ml) exhibited poor activity against fungal

strains; *A. niger* and *C. albicans* compared to fluconazole (MIC;0.25 µg/ml).Data of antimicrobial activities of compounds 7a-I is presented in Table 3.

**Table 3.** Data of antimicrobial activities of compounds 7a-i.

| Comp | MIC values (µg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Antibacterial activity | | | | Antifungal activity | |
| | Gram-positive | | Gram-negative | | | |
| | S. aureus | B. subtilis * | E. coli | P. aeruginosa | A. niger | C. albicans |
| **7a** | 62.5 | 62.5 | 62.5 | 62.5 | 125 | 125 |
| **7b** | 31.25 | 16 | 16 | 16 | 125 | 62.5 |
| **7c** | 16 | 8 | 8 | 8 | 31.25 | 31.25 |
| **7d** | 31.25 | 31.25 | 16 | 16 | 62.5 | 62.5 |
| **7e** | 16 | 16 | 16 | 8 | 31.25 | 62.5 |
| **7f** | 62.5 | 62.5 | 31.25 | 31.25 | 62.5 | 62.5 |
| **7g** | 62.5 | 31.25 | 31.25 | 31.25 | 125 | 125 |
| **7h** | 62.5 | 31.25 | 31.25 | 31.25 | 125 | 125 |
| **7i** | 31.25 | 31.25 | 31.25 | 16 | 62.5 | 62.5 |
| **Cip**[b] | <5 | <1 | <1 | <5 | - | - |
| **Fluc**[c] | - | - | - | - | 0.25 | 0.25 |

## 4. CONCLUSION

In the present series total 9 novel analogues of 1, 2, 4-triazole clubbed with pyridine were synthesized and assessed for their antibacterial and anti-fungal activities. The activity data indicates that compound 7c emerged as good antibacterial amongst the series, whereas none of the compound from the series 7a-i was shown good antifungal activity compared to standard fluconazole.

## 5. ACKNOWLEDGEMENT

Authors are thankful to Govt. College of Pharmacy, Karad and Adarsh College of Pharmacy, Vita for providing the necessary facilities to carry out the above mentioned research work.

## 6. REFERENCES

1. J.L. Mi, J. Wu, C.H. Zhou. (2008) *West China J. Pharm. Sci.*23, 84–86.
2. J.L. Mi, C.H. Zhou,X. Bai. (2007) *Chin. J. Antibiot.* 32, 587–593.
3. Y. Wang, C.H. Zhou. (2011) *Sci. Sin.Chemi.*41, 1429–1456.
4. K. Shalini, N. Kumar,S. Drabhu, P.K. Sharma. (2011) *Beil. J. Chem.* 7, 668-677.
5. G. Kofla, M. Ruhnke. (2005) *Expert Opinion on Pharmacotherapy.* 6, 1215-1229.

**6.** E. Rodriguez-Fernandez, J.L. Manzano, J.J. Benito, R. Hermosa, E. Monte, J.J. Criado. (2005) *J. Inorg. Biochem.* 99, 1558-1572.

**7.** H.A. Torres, R.Y. Hachem, R.F. Chernaly, D.P. Kantoyiannis, I. I. Raad. (2005) *Lancet Infect. Dis.* 5, 775-785.

**8.** C.A. Kauffman. (2006) *Curr. Opin. Microbiol.* 9, 483-488.

**9.** G. Aperis, E. Mylonakis. (2006) Expert Opinion Invest. Drugs. 15, 579-602.

**10.** T. Murata, M. Shimada, S Sakakibara, T. Yoshino, H. Kadono et. al. (2003) *Bioorg Med Chem Lett,* 13, 913.

**11.** Birsen Tozkkoparam, Esra Kupeli, Erdem Yesilada, Mevlut Ertan. (2007) Bioorg Med Chem, 15, 1808.

**12.** Zafer AsimKaplancikh, Gulhan Turan-Zitouni, Ahmet Ozdemir, Gilbert Revial. (2007) *Eur J Med Chem,* 42,268.

**13.** Guniz Kuckguzel S, Ilkey Kuckguzel , Esara Tatar, Sevim Rollas, Firkrettin Sahin, Medine Gulluce, Erik De Clercg, Levent Kabasakal. (2007) *Eur J Med Chem,* 42 ,893.

**14.** S. Kushner, R.I. Cassell, J. Morton, J.H. Williams. (1951) *J. Org. chem.* 15, 1283-1288.

**15.** Organic Synthesis, Collective Volume V, Editor, Henry Baumgarten, John Wiley and Sons Inc., New York (1973) 1070-1072.

**16.** S.R. Pattan, A.M. Manikrao, M.H. Maste, S. Talath. (2002) *Indian Drugs.* 39, 265-269.

**17.** S.A. Khanum, S. Shashikanth, S. Umesha, R. Kavitha. (2005) *Eur. J. Med. Chem.* 40, 1156-1162.