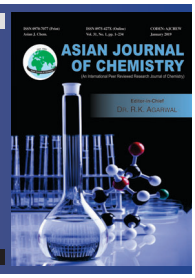
Asian Journal of Chemistry; Vol. 31, No. 4 (2019), 760-762

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.21165



Synthesis, Characterization and Biological Evaluation of Isoxazoles Derived from 3-Aminoacetophenone

T. Raj Kumar[1,*], S.V. Suresh Kumar[2] and N. Srinivasan[3]

[1]Department of Pharmaceutical Chemistry, Creative Educational Society's College of Pharmacy, Kurnool- 518218, India
[2]Department of Pharmacognosy, Creative Educational Society's College of Pharmacy, Kurnool-518218, India
[3]Department of Pharmacognosy, Annamalai University, Annamalainagar-608002, India

*Corresponding author: E-mail: rajkutty1983@gmail.com

Received: 30 July 2018; *Accepted*: 15 October 2018; *Published online*: 27 February 2019; AJC-19281

Isoxazoles were synthesized by condensing different aldehydes with 3-aminoacetophenone coupled to N-methyl piperazines. All the compounds were screened for anticancer activity against human breast cancer cell lines- MCF-7 and MDA-MB-468. The antimycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate alamar blue assay (MABA). The structures of newly synthesized compounds established on the basis of their m.p., TLC, FTIR, mass and ^{1}H NMR data. The results of antimycobacterial and anticancer studies shows that some of it posses mild to moderate activity against standard. These new synthesized compounds were identified and characterized, demonstrated antitubercular, *in vivo* efficacy in tumor models like MCF-7 and MDA-MB-468.

Keywords: Isoxazoles, 3-Aminoacetophenone, MCF-7, MDA-MB-468.

INTRODUCTION

Oxygen-containing heterocyclic building blocks are of great importance in medicinal chemistry and posses antibacterial, antiviral, antifungal, anti-inflammatory, antitubercular, anticancer, insecticidal activities [1-3]. Hence they are used extensively as useful synthons in organic synthesis. In this respect, we envisioned and implemented the synthesis of several novel molecules containing the fused isoxazole skeleton and evaluated their inhibition on cancer cells and tubuerculosis.

In an effort to continually develop potent antitubercular and anticancer agents, a novel series of isoxazoles with piperazine nucleus were synthesized and characterized. These derivatives were tested using estrogen receptor positive breast cancer cell lines MCF-7 and MDA-MB-468.

EXPERIMENTAL

3-Aminoacetophenone, N-methyl piperazine and other aldehydes derivatives purchased from the SD Fine Chemicals Ltd., Mumbai and were used without further purification. Melting points were determined on a Fisher-Johns Melting Point Apparatus and were uncorrected. Infrared spectra were obtained using KBr Pellet technique on a Bruker IR spectrometer. ^{1}H NMR spectra was obtained on Bruker AV III 500 MHz spectrometer & Mass spectra obtained from Thermo Scientific MS instrument at the IIT Madras, Chennai. Reaction progress was checked by TLC in a solvent vapour-saturated chamber on glass plates coated with Silica Gel GF_{254} followed by visualization under UV light (254 nm). The solvent system used for thin layer chromatography was *n*-hexane:ethyl acetate (7:3).

Diazotization and coupling of 3-aminoacetophenone with N-methyl piperazine: 0.01 mol of 3-Aminoacetophenone in 3 mL of conc. HCl & 6 mL water and 1.35 g of sodium nitrite in 6 mL of water were prepared separately and both the solutions were placed in ice bath. To this sodium nitrite solution added dropwise with continuous stirring and diazotized. After the completion of diazotization, N-methyl piperazine added to the reaction mixture with continuous stirring at the same temperature *i.e.*, 0-5 °C and a yellow coloured precipitate is obtained by adding sodium bicarbonate. The product was filtered, washed with ice-cold water and dried.

Synthesis of chalcones: 0.005 mol of Diazotized and coupled product reacted with equimolar amount of aldehydes in a round bottomed flask containing 50 mL of ethanol. 1 mL

of 40 % KOH added and stirred continuously on magnetic stirrer for 2 h. Completion of the reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was poured into crushed ice and neutralized with dilute HCl. Then the solid is filtered, washed with cold water, dried and recrystallized from ethanol [4,5].

Synthesis of isoxazoles: 0.002 mol of chalcone derivatives reacted with equimolar hydroxylamine hydrochloride and sodium acetate in 25 mL ethanol and refluxed for 6 h. The mixture was concentrated and poured into crushed ice and stirred well to obtain the precipitate [6-10]. The precipitate obtained was filtered, washed and recrystallized. TLC solvent system used was *n*-hexane:ethyl acetate (7:3) (**Scheme-I**).

Anti-TB activity using Alamar blue dye [11]**:** The anti mycobacterial activity of compounds were assessed against *M. tuberculosis* using microplate Alamar blue assay (MABA) BACTEC radiometric method and the activity expressed as the minimum inhibitory concentration (MIC) in µg/mL. *M. tuberculosis* (H37 RV strain): ATCC No. 27294 was used for the activity. The standard values are pyrazinamide 3.125 µg/mL, streptomycin 6.25 µg/mL and ciprofloxacin 3.125 µg/mL.

Anticancer activity by MTT assay method [12]**:** Cell lines of MCF-7 and MDA-MB-468 were procured from NCCS, Pune. MTT assay was performed based on the procedure given by Dolly and Griffiths [12]. The cell lines were maintained in 96 wells microtiter plate containing MEM media supplemented with 10 % heat inactivated fetal calf serum (FCS), containing 5 % of the mixture of gentamycin (10 µg), penicillin (100 units/mL) and streptomycin (100 µg/mL) in the presence of 5 % CO_2 at 37 °C for 48-72 h.

Cytotoxicity assay: *in vitro* Growth inhibition effect of test compound was assessed by conversion of MTT into "Formazan blue" by living cells and determined spectrophotometrically. The supernatant from the plate was removed and fresh MEM solution added, treated with different concentrations of compound appropriately diluted with DMSO.

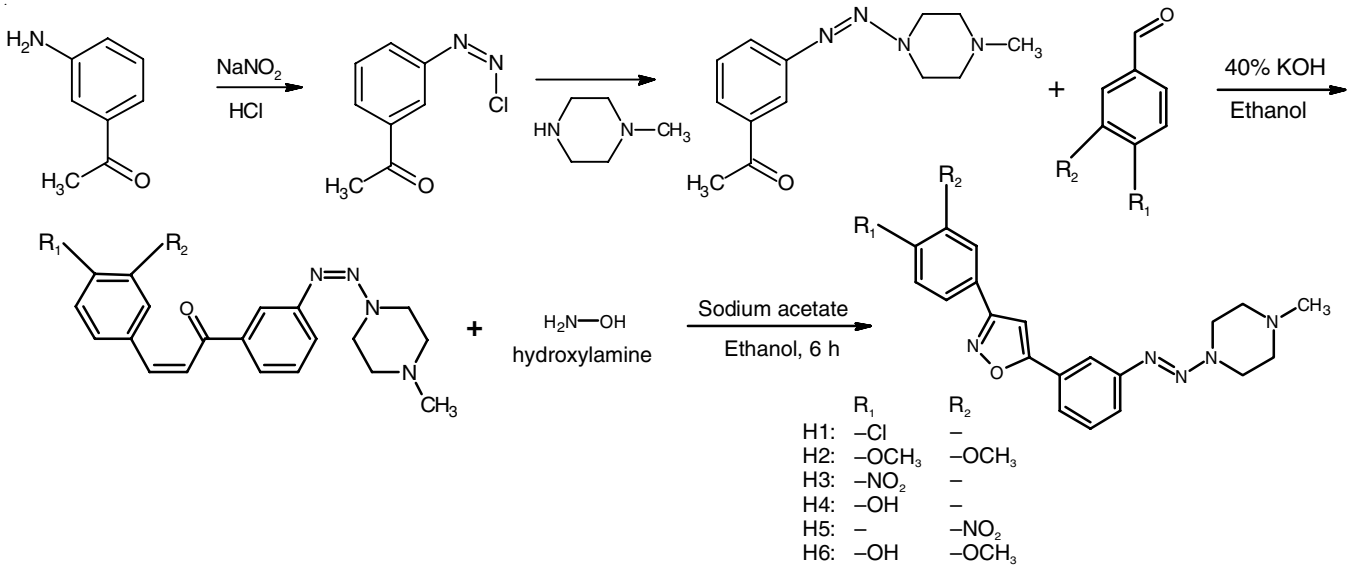
Control group contains only DMSO. 10, 20, 25, 30 and 50 µL of the stock solution (10 mg/mL prepared in DMSO) were added to respective wells containing 100 µL of the medium to make the final concentration of 10, 20, 25, 30 and 50 µg/mL. After 48 h of incubation at 37 °C in a humidified atmosphere of 5 % CO_2, stock solution of MTT was added to each well (20 µL, 5 mg per mL in sterile PBS) for further 4 h incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazan blue" were solubilized by adding DMSO (100 µL) and optical density was measured at wavelength of 570 nm. The results represent the mean of five readings. The concentration at which the OD of treated cells was reduced by 50 % with respect to the untreated control.

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD at control}} \times 100$$

1-[(*E*)-{3-[3-(4-Chlorophenyl)-1,2-oxazol-5-yl]phenyl}-diazenyl]-4-methylpiperazine (H1): Yellow crystals; yield: 60 %; m.p.: 180 °C; m.w. 410.50. TLC 0.66; IR (KBr, ν_{max}, cm^{-1}): 3417.58 (N-H amine *str.*), 2924.01-2853.36 (alkyl C-H *str.*), 1518.75 (aromatic C=C bend.); ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 8.026-8.219 (1H, d, CH-Ar); δ 6.532-6.922 (8H, m, Ar-H); δ 3.702-3.793 (3H, s, CH_3), δ (2H, d, CH_2 of isoxazole); Mass: *m/z* 383.12 $[M+H]^+$. Elemental analysis of $C_{22}H_{25}N_5OCl$ calcd. (found) %: C, 63.50 (63.48); H, 5.00 (5.08); Cl, 9.01 (9.34); N, 18.00 (18.10).

1-[(*E*)-{3-[3-(3,4-Dimethoxyphenyl)-1,2-oxazol-5-yl]phenyl}diazenyl]-4-methylpiperazine (H2): Dark brown crystals; yield: 58 %; m.p.: 185 °C; m.w. 407.47. TLC 0.74; IR (KBr, ν_{max}, cm^{-1}): 3461.41 (N-H amine *str.*), 2923.41 (carboxylic acid O-H *str.*), 2852.9 (alkyl C-H *str.*); ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 7.963-7.987 (1H; d; CH-Ar); δ 6.836-6.922 (8H; m, Ar-H); δ 3.664-3.711 (3H, s, CH_3); δ 6.533-6.540 d (1H, -CH=CH); Mass: *m/z* 408.10 $[M-H]^+$. Elemental analysis of $C_{22}H_{25}N_5O_3$ calcd. (found) %: C, 65.49 (65.51); H, 6.08 (6.10); N, 17.12 (17.10).

1-Methyl-4-[(*E*)-{3-[3-(4-nitrophenyl)-1,2-oxazol-5-yl]phenyl}diazenyl]piperazine (H3): Brownish crystals; yield: 62 %; m.p.: 170 °C; m.w. 392.41. TLC 0.54; IR (KBr, ν_{max}, cm^{-1}): 3417.38 (N-H amine *str.*), 2923.38, 2853.15 (alkyl C-H *str.*), 1590.53 (aromatic C=C bend.); ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 7.907-8.108 (1H; d; CH-Ar); δ 6.904-6.965 (8H;



Scheme-I

m, Ar-H); δ 3.659-3.781 (3H, s, CH_3); δ 6.552 d(1H, -CH=CH); δ 5.113 (1H, d, CH of isoxazole); Mass: *m/z* 394.32 (M^+). Elemental analysis of $C_{20}H_{20}N_6O_3$ calcd. (found) %: C, 61.19 (61.10); H, 5.08 (5.10); N, 22.12 (22.02).

4-(5-{3-[(*E*)-(4-Methylpiperazin-1-yl)diazenyl]phenyl}-1,2-oxazol-3-yl)phenol (H4): Brownish crystals; yield: 66 %; m.p.: 17-60 °C; m.w. 363.41. TLC 0.69; IR (KBr, ν_{max}, cm^{-1}): 3347.18 (N-H amine *str.*), 2923.59, 2852.67 (alkyl C-H *str.*), 1706.38 (aldehyde C=O *str.*); ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 7.907-8.108 (1H; d; CH-Ar); δ 6.912-6.916 (8H; m, Ar-H); δ 3.669 (3H, s, CH_3); δ 6.445 d (1H, -CH=CH); δ 9.45 (s, 1H, OH); δ 5.04 (1H, d, CH of isoxazole); Mass: *m/z* 365.23 (M^+). Elemental analysis of $C_{20}H_{21}N_5O_2$ calcd. (found) %: C, 66.29 (66.15); H, 6.12 (6.10); N, 19.35 (19.32).

1-Methyl-4-[(*E*)-{3-[3-(3-nitrophenyl)-1,2-oxazol-5-yl]-phenyl}diazenyl]piperazine (H5): Brownish crystals; yield: 58 %; m.p.: 175 °C; m.w. 392.41. TLC 0.55; IR (KBr, ν_{max}, cm^{-1}): 3676.23 (amide N-H *str.*), 3435.24 (N-H amine *str.*), 2923.69, 2853.40 (alkyl C-H *str.*), 1525.74 (aromatic C=C bend.); ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 7.941-8.007 (1H; d; CH-Ar); δ 7.439 (8H; m, Ar-H); δ 3.693-3.733 (3H, s, CH_3); δ 6.890-6.894 d (1H, -CH=CH); δ 5.764 (1H, d, CH of isoxazole); Mass: *m/z* 394.26 (M^+). Elemental analysis of $C_{20}H_{20}N_6O_3$ calcd. (found) %: C, 61.56 (61.42); H, 5.17 (5.20); N, 22.23 (22.22).

1-[(*E*)-{3-[3-(3-Methoxy-4-hydroxy phenyl)-1,2-oxazol-5-yl]phenyl}diazenyl]-4-methylpiperazine (H6): Brownish crystals; yield: 68 %; m.p.: 180 °C; m.w. 393.44. TLC 0.71; IR (KBr, ν_{max}, cm^{-1}): 3427.62 (N-H amine *str.*), 2924.28, 2853.52 (alkyl C-H *str.*), 1508.72 (aromatic C=C bend.); ^{1}H NMR ($CDCl_3$) in δ (ppm): δ 7.920-7.940 (1H; d; CH-Ar); δ 7.285-7.562 (8H; m, Ar-H); δ 3.693-3.733 (3H, s, CH_3); δ 6.678-6.708 d(1H, -CH=CH); δ 5.802 (1H, d, CH of isoxazole); δ 9.58 (s, 1H, OH); Mass: *m/z* 395.38 (M^+). Elemental analysis of $C_{21}H_{23}N_5O_3$ calcd. (found) %: C, 64.44 (64.42); H, 6.17 (6.20); N, 18.30 (18.22).

RESULTS AND DISCUSSION

The novel isoxazoles have been synthesized from the chalcone intermediates. The synthesis of the isoxazole is a three step method. The yields of the synthesized compounds were found to be significant. The synthesized derivatives were undergone physicochemical characterization. The structures of the synthesized compounds was confirmed by IR, mass and ^{1}H NMR. The results obtained from this study confirmed that the product has formed.

The IR spectrum, showed a strong absorption at 1656 cm^{-1}, which is a characteristic band for the carbonyl group of the chalcones. Absorption around 1400 cm^{-1} was assigned to the N=N *str*. The other C=C, C-H, Ar-H stretching absorptions were noticed in accordance with the structure of synthesized compounds. ^{1}H NMR has shown signal around at δ 5 accounting for isoxazole nucleus. Signal for the aromatic protons were present in between δ 8 and 7. Thus, all the protons were accounted for the respective structures. Mass spectra were also in accordance with the proposed structures.

These isoxazole derivatives have biological activities like antibacterial and anti-inflammatory may be a pave for synthesis and characterization of some new derivatives. All the synthesized compounds were tested for antitubercular activity, the compounds were assessed against *M. tuberculosis* using microplate Alamar blue assay (MABA). All the compounds have mild active against *M. tuberculosis* at 100 µg/mL. It also have moderate to good anticancer activity (Table-1). The α,β-unsaturared part of chalcone along with isoxazole seems to be essential for the activity. But substitution on the phenyl ring decides the extent of potency of the compounds. Compounds with substituent's like chloro, nitro and hydroxyl group at *para* position and compound with 3-methoxy substituent's (H_1, H_2, H_3, H_4 and H_6) were cytotoxic against MCF-7 cell lines at IC_{50} 25-30 µg. *para* Chloro, nitro substituted derivatives (H_1, H_3, H_5) were cytotoxic against MDA-MB-468 cell lines at IC_{50} 30-35 µg.

TABLE-1
IC_{50} (µg) OF SYNTHESIZED COMPOUNDS AGAINST MCF-7 AND MDA-MB-468

Sample code	IC_{50} (µg)	
	MCF	MDA-MB-468
H1	25-30	30-35
H2	25-30	30
H3	25-30	30
H4	25-30	30-35
H5	20	30
H6	25-30	30

REFERENCES

1. J. Kumar, G. Chawla, M. Akhtar, K. Sahu, V. Rathore and S. Sahu, *Arabian J. Chem.*, **10**, 141 (2017); https://doi.org/10.1016/j.arabjc.2013.04.027.
2. K.A. Kumar and P. Jayaroopa, *Int. J. Pharm. Chem. Biol. Sci.*, **3**, 294 (2013).
3. C.B. Patil, S.K. Mahajan and S.A. Katti, *J. Pharm. Sci. Res.*, **1**, 11 (2009).
4. M.A. Rahman, *Chem. Sci. J.*, **2011**, 29 (2011).
5. H. Suwito, Jumina, Mustofa, A.N. Kristanti and N.N.T. Puspaningsih, *J. Chem. Pharm. Res.*, **6**, 1076 (2014).
6. G. Thirunarayanan, *Org. Chem. Ind. J.*, **12**, 1 (2016).
7. K.C. Gautam and D.P. Singh, *Chem. Sci. Trans.*, **2**, 992 (2013); https://doi.org/10.7598/cst2013.478.
8. R Kalirajan, S. Jubie and B. Gowramma, *Peertechz J. Med. Chem. Res.*, **1**, 1 (2015).
9. L.S.S. Reddy, M. Bhagavanraju and C. Sridhar, *Inventi Impact: Med. Chem.*, **4**, 133 (2015).
10. L.S.S. Reddy, M. Bhagavanraju and C. Sridhar, *J. Chem. Pharm. Res.*, **7**, 211 (2015).
11. M.C.S. Lourenço, M,V.N. de Souza, A.C. Pinheiro, M. de L. Ferreira, R.S.B. Gonçalves, T.C.M. Nogueira and M.A. Peralta, *ARKIVOC*, 181 (2007); https://doi.org/10.3998/ark.5550190.0008.f18.
12. A. Dolly and J.B. Griffiths, Cell and Tissue Culture for Medical Research, John Wiley & Sons, New York, pp. 62-65 (2000).