Acta Poloniae Pharmaceutica – Drug Research, Vol. 66 No. 1 pp. 101–104, 2009 ISSN 0001-6837
Polish Pharmaceutical Society

SHORT COMMUNICATION

SYNTHESIS OF SOME NEW ISOXAZOLINE DERIVATIVES AS POSSIBLE ANTI-*CANDIDA* AGENTS

PRABODH CHANDER SHARMA[a], SUNIL V. SHARMA[b], SANDEEP JAIN[c], DALBIR SINGH[d] and BHOJRAJ SURESH[e]

[a]Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119, India
[b]School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, NR47TJ, UK
[c] Dept. of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar-125001, India
[d]Lord Shiva College of Pharmacy, Sirsa-125055, India
[e]JSS College of Pharmacy, Ootacamund-643001, India

Keywords: isoxazolines, propenamides, anti-fungal, *Candida albicans*

The isoxazolines have been reported to possess a variety of significant and diverse pharmacological activities such as antifungal (1, 2), anti-HIV (3), anti-bacterial (4), caspase inhibitory (5), antimuscarinic (6), anti-inflammatory (7), anti-cancer (8) and anti-depressant (9) activity. On the other hand, the object of antifungal drug discovery has become a subject of greater challenge due to increasing incidences of fungal drug resistance. In the past decade, number of patients diagnosed with fungal infections have increased drastically, whereas, relatively very few clinically useful drugs were discovered. As a part of our ongoing antimicrobial drug design program, quantitative structure activity relationships (QSAR) studies were performed (10). The predictive 2D and 3D QSAR studies revealed that some simple isoxazoline derivatives possessed structural and electro-chemical properties to be suitable as effective anti-*Candida* agents. Hence, in light of these findings and to check the hypothesis of QSAR studies, it was decided to synthesize certain 3-phenylamino (substituted phenyl) isoxazolines and evaluate them for anti-*Candida* activity.

EXPERIMENTAL

Chemistry

The purity of synthesized compounds was ascertained by thin layer chromatography on silica gel G in various solvent systems using iodine vapors as detecting agents. All the melting points reported were determined in open capillaries using Veego VMP-1 melting point apparatus, expressed in ^{o}C and are uncorrected. Elemental analysis was done using Carlo Erba 1106 CHN analyzer. The IR spectra of the compounds were recorded on Perkin-Elmer Infra Red-283 FTIR spectrometer in KBr phase and are expressed in cm^{-1}. ^{1}H NMR spectra recorded on Brucker 300 MHz NMR spectrometer (chemical shift in δ ppm) using TMS as internal standard.

General procedure for synthesis of N-phenyl-3-(substituted phenyl)propenamides (3a-e)

N-phenyl-3-(substituted phenyl)propenamides (**3a**-**e**) were prepared by reacting a mixture of acetanilide (**1**) (0.05 mol), 4-(dimethylamino)benzaldehyde (**2**) (0.05 mol), aqueous NaOH (10%, 5 mL) and methanol (50 mL). The reaction mixture was stirred for 10 h at room temperature using magnetic stirrer. Then, it was refluxed for further 6 h on a water bath. After completion of the reaction (monitored by TLC) an excess of solvent was removed by distillation and the resultant viscous mass was poured into ice water (100 mL) with vigorous stirring and left overnight for complete precipitation. The resultant solid product was filtered, washed with cold water, dried and recrystallized from ethanol.

Similarly, the following compounds were prepared and characterized.

* Corresponding author: phone: 094160-25460(M); e-mail: sharma_prabodh@rediffmail.com

N-Phenyl-3-phenylpropenamide (3a)

Yield 36.9%, m.p.155-157°C; IR (cm^{-1}, KBr): 3282 (NH, secondary), 2960 (CH arom./vinyl), 1650 (C=O), 1597 (C=C). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 6.58 (1H, d, *J* = 5.6 Hz, CH vinyl), 6.92 to 7.67 (10H, m, arom.), 7.72 (1H, d, *J* = 5.6 Hz, CH vinyl), 8.11 (1H, s, NH). Analysis: Calcd. for $C_{15}H_{13}NO$: C, 80.69; H, 5.87; N, 6.27%. Found: C, 80.60; H, 5.81; N, 6.11%.

N-Phenyl-3-(4-hydroxyphenyl)propenamide (3b)

Yield 39.6%, m.p. 203-204°C; IR (cm^{-1}, KBr) : 3289, 3252 (OH, NH merged), 2911 (CH arom./vinyl), 1662 (C=O), 1597 (C=C). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 5.14 (1H, bs, OH), 6.22 (1H, d, *J* = 6.2 Hz, CH vinyl), 6.82 (dd, 2H, *J* = 12.2, 1.1 Hz, arom.), 6.98 (dd, 2H, *J* = 12.2, 1.1 Hz, arom.), 7.21-7.29 (5H, m, arom.), 7.61 (1H, d, J = 6.2 Hz, CH vinyl), 7.91 (1H, s, NH); Analysis: Calcd. for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85%. Found: C, 75.32; H, 5.41;N, 5.83%.

N-Phenyl-3-(3-nitrophenyl)propenamide (3c)

Yield 41.7%, m.p. 192-195°C; IR (cm^{-1}, KBr): 3289 (NH, secondary), 2961 (CH, arom./vinyl), 1665 (C=O), 1597 (C=C), 1350 (NO_2). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 6.42 (1H, d, *J* = 6.3 Hz, CH vinyl), 7.22 to 7.65 (9H, m, arom.), 7.88 (1H, d, *J* = 6.6 Hz, CH vinyl), 7.93 (1H, s, NH). Analysis: Calcd. for $C_{15}H_{12}N_2O_3$: C, 67.16; H, 4.51; N, 10.44%. Found: C, 67.10; H, 4.49; N, 10.41%.

N-Phenyl-3-(3-methoxyphenyl)propenamide (3d)

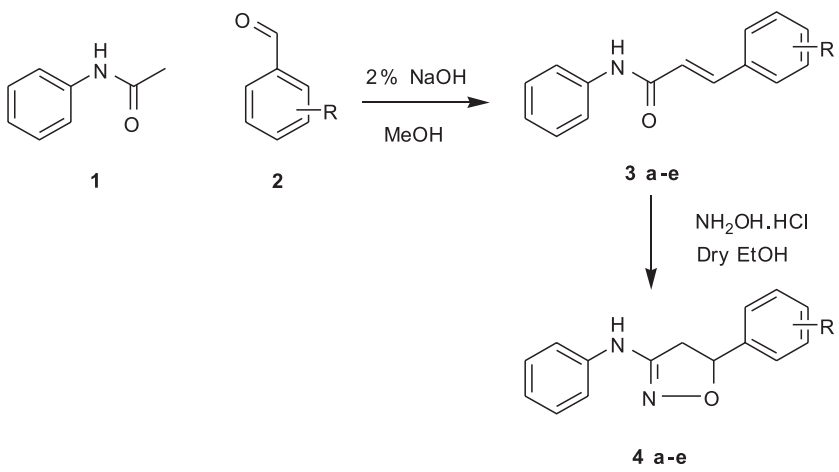
Yield 42.3%, m.p. 105-107°C; IR (cm^{-1}, KBr): 3289 (NH, secondary), 2917 (CH, arom./vinyl), 1675 (C=O), 1592 (C=C), 1499 (-OCH_3). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 3.52 (3H, s, OCH_3), 6.68 (1H, d, *J* = 6.1 Hz, CH vinyl), 6.85 to 7.35 (9H, m, arom.), 7.58 (1H, d, *J* = 6.1 Hz, CH vinyl), 7.80 (1H, s, NH); Analysis: Calcd. for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53%. Found: C, 75.81; H, 5.91; N, 5.54%.

N-Phenyl-3-[(4-dimethylamino)phenyl]propenamide (3e)

Yield 46.6%, m.p. 55-58°C; IR (cm^{-1}, KBr): 3296 (NH, secondary), 2913 (C-H, arom./vinyl), 1665 (C=O), 1597 (C=C). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 3.21 (6H, s, $N(CH_3)_2$), 6.48 (1H, d, *J* = 6.6 Hz, CH vinyl), 6.52 (dd, 2H, *J* = 12.1, 1.0 Hz, arom.), 7.08 (dd, 2H, *J* = 12.1, 1.0 Hz, arom.), 7.21-7.29 (5H, m, arom.), 7.88 (1H, d, *J* = 6.6 Hz, CH vinyl), 8.25 (1H, s, NH). Analysis: Calcd. for $C_{16}H_{15}NO$: C, 80.98; H, 6.37; N, 5.90%. Found: C, 80.93; H, 6.34; N, 5.93%.

General procedure for synthesis of 3-phenylamino-5-(substituted phenyl)isoxazolines 4(a-e)

3-Phenylamino-5-(substituted phenyl)isoxazolines (**4a-e**) were prepared by reacting a mixture of purified N-phenyl-3-(substituted phenyl)propenamides (**3a-e**) (0.01 mol), hydroxylamine hydrochloride (0.01 mol) and a solution of NaOH (0.01 mol) in dry ethanol (50 mL) by refluxing for 6 h on a water bath. After completion of the reaction,



3 and **4** - R: a = H, b = 4-OH, c = 3-NO_2, d = 3-OCH_3. e = 4-$N(CH_3)_2$

Scheme 1. Synthesis of 3-phenylamino-5-(substituted phenyl)isoxazolines (**4a-e**).

Table 1. Anti-*Candida* activity of the synthesized compounds

Compd. No.	Minimum Inhibitory concentration for various Candida species (microgram/mL)			Average MIC for each compound (microgram/mL)
	C. albicans 3102	*C. albicans*	*C. albicans* AT C C 10231	
4a	25.5	12.5	11.0	16.4
4b	12.5	12.4	13.5	12.8
4c	15.0	15.0	17.0	15.6
4d	12.0	12.0	14.0	12.7
4e	14.5	13.5	14.0	14.0
Fluconazole	10.1	12.5	1.66	8.08

an excess of the solvent was removed by distillation and the resultant mass was poured into ice water (100 mL) with vigorous stirring. It was kept in cool overnight. The resultant solid product was filtered, washed with sufficient cold water, dried and purified by recrystallization from acetone. General scheme for the synthesis is depicted in Figure 1.

3-Phenylamino-5-phenylisoxazoline (4a)

Yield 42%, m.p. 110-113°C; IR (cm^{-1}, KBr): 3296 (NH, secondary), 3135 (CH, alkyl), 2905 (CH, arom.), 1604 (C=C, arom.), 1554 (C=N), 1326 (C-O-N). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 1.78 (1H, t, *J* = 12.3 Hz, CH), 2.36 (2H, dd, *J* = 12.3, 1.2 Hz CH_2), 6.86-7.11 and 7.21-7.29 (10H, m, arom.), 9.75 (1H, s, NH); Analysis: Calcd. for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76%. Found: C, 75.63; H, 5.97; N, 11.72%.

3-Phenylamino-5-(4-hydroxyphenyl)isoxazoline (4b)

Yield 46.0%, m.p. 106-108°C; IR (cm^{-1}, KBr): 3289 (OH), 3258 (NH, secondary), 3135 (CH, alkyl), 2954 (CH, arom.) 1597 (C=C, arom.), 1560 (C=N), 1319 (C-O-N). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 1.90 (1H, t, *J* = 12.3 Hz, CH), 2.12 (2H, dd, *J* = 12.3, 1.2 Hz CH_2), 5.51 (1H, brs, OH), 6.48 (dd, 2H, *J* = 12.2, 1.2 Hz, arom.), 7.11 (dd, 2H, *J* = 12.2, 1.2 Hz, arom.), 7.28-7.36 (5H, m, arom.), 8.90 (1H, s, NH). Analysis: Calcd. for $C_{15}H_{14}N_2O_2$: C, 78.85; H, 5.55; N, 11.02%. Found: C, 78.79; H, 5.57; N, 11.05%.

3-Phenylamino-5-(3-nitrophenyl)isoxazoline (4c)

Yield 58.3%, m.p. 73-75°C; IR (cm^{-1}, KBr): 3289 (OH), 3258 (NH, secondary), 3135 (CH, alkyl), 2954 (CH, arom.) 1597 (C=C, arom.), 1560 (C=N), 1319 (C-O-N). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 1.95 (1H, t, *J* = 12.1 Hz, CH), 2.15 (2H, dd, *J* = 12.1, 1.2 Hz, CH_2), 7.11-7.59 (9H, m, arom.), 7.90 (1H, s, NH). Analysis: Calcd. for $C_{15}H_{13}N_3O_3$: C, 63.60; H, 4.63; N, 14.83%. Found: C, 63.66; H, 4.63; N, 14.89%.

3-Phenylamino-5-(3-methoxyphenyl)isoxazoline (4d)

Yield 56.4%, m.p. 116-118°C; IR (cm^{-1}, KBr) : 3258 (NH, secondary), 3060 (CH, alkyl), 2911 (CH, arom.), 1597 (C=C, arom.), 1560 (C=N), 1368 (OCH_3), 1325 (C-O-N). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 2.05 (1H, t, *J* = 11.6 Hz, CH), 2.33 (2H, d, *J* = 11.6 Hz, CH_2), 3.90 (3H, s, OCH_3), 6.96-7.11 and 7.31-7.49 (9H, m, arom.), 8.15 (1H, s, NH). Analysis: Calcd. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44%. Found: C, 71.66; H, 6.07; N, 10.37%.

3-Phenyl amino-5-(4-dimethylaminophenyl)isoxazoline (4e)

Yield 60.4%, m.p. 127-130°C; IR (cm^{-1}, KBr): 3252 (NH, secondary), 3073 (CH, alkyl), 2919 (CH, arom.), 1597 (C=C, arom.), 1559 (C=N), 1320 (C-O-N). ^{1}H NMR (300 MHz, $CDCl_3$) (δ ppm): 1.78 (1H, t, *J* = 12.1 Hz, CH), 2.18 (2H, dd, *J* = 12.1, 1.9 Hz, CH_2), 3.10 (6H, s, N-$(CH_3)_2$), 6.78 (dd, 2H, *J* = 12.1, 1.2 Hz, arom.), 7.01 (dd, 2H, *J* = 12.1, 1.2 Hz, arom.), 7.21-7.29 (5H, m, arom.), 9.75 (1H, s, NH); Analysis: Calcd. for $C_{16}H_{16}N_2O$: C, 76.16; H, 6.39; N, 10.10%. Found: C, 76.10; H, 6.33; N, 10.11%.

Antifungal screening

All the synthesized compounds were evaluated for their *in vitro* antifungal activity against *Candida albicans* by using twofold serial dilution method (11). For this purpose three different strains *C. albicans, C. albicans ATCC 10231, and C. albicans 3102* were used. Fluconazole was taken as a stan-

dard for comparison of antifungal activity. From the results (Table 1), it was observed that all the tested compounds possessed significant *in-vitro* anti-*Candida* activities. The compounds were active against all three strains of *C. albicans*. Except compound **4a**, the minimum inhibitory concentration (MIC) values of all the compounds against three different test strains were quite similar indicating their effectiveness against the wild as well as resistant types of fungi. All the compounds inhibited the growth of fungi at a concentration ranging between 11 to 25.5 μg/mL.

RESULTS AND CONCLUSION

N-Phenyl-3-(substituted phenyl)propenamides (3) were prepared by the reaction of acetanilide and appropriately substituted benzaldehyde in the presence of base. These propenamides (**3**) on refluxing with hydroxylamine hydrochloride in the presence of NaOH and dry ethanol yielded 3-phenylamino-(substituted phenyl)isoxazolines (**4**) in moderate yields. These compounds were characterized on the basis of elemental analysis and spectral data. All the synthesized compounds were evaluated for their *in vitro* antifungal activity against *Candida albicans*.

In conclusion, a series of N-phenyl-3-(substituted phenyl)propenamides has been synthesized and evaluated for antifungal activity. The compounds have exhibited moderate to significant activities.

Acknowledgments

Authors wish to thank Jagadguru Sri Shivarathri Swamiji of Suttur Mutt, Mysore for providing necessary facilities and USIC, Karnataka University, Dharwad and CDRI, Lucknow for spectral and analytical services. Quest Institute of Life Sciences, Nicholas Pyramal Ltd., Mumbai is acknowledged for anti-fungal screening.

REFERENCES

1. Kaur H., Kumar S., Renu, Manisha and. Sangal S.K: Acta Cienc. Indica Chem. 25, 49 (1999).
2. Imran M.and Khan S.A.: Indian J. Heterocyclic Chem. 13, 213 (2004).
3. Chung Y.J., Kim D.H., Choi K.Y., Kim B.H.: Korean J. Med. Chem. 5, 141 (1995).
4. Dighade S.R., Patil S.D., Chincholkar M.M., Dighade N.R.: Asian J. Chem. 15, 450 (2003).
5. Kim E.E.K., Park M.J., Park T.K., Moon K.Y.: (L.C. Chemical Ltd. South Korea) PCT Int. Appl. W00121, 600: Chem. Abstr. 134, 262851j (2001).
6. Amici M.D., Conti P., Vistoli G., Corea G., Ottolino G., Micheli C.D.: Med. Chem. Res. 10, 615 (2001).
7. Khan M.S.Y., Bawa S.: Indian J. Chem. 40, 1207 (2001).
8. Simoni D., Manfredini S., Tabrizi M.A., Bazzanini R., Guarinini M., Ferroni R., Traniello F., Nastruzzi C. G., Gambari R.: Top. Mol. Organ. Eng. 8, 119 (1991).
9. Gil J.I. A., Fernandez G.E.J., Alcazar V.M.J.: (Janssen Pharmaceutica N.V., Belgium), PCT Int. Appl. W00266, 484: Chem. Abstr**.**, 134, 201298z (2002).
10. Sharma S.V., Ashok P., Bharani P., Meyyanathan S.N., Suresh B.: Indian Drugs, 40, 88 (2003).
11. Nakahara H., Ishikawa T., Sarai Y., Kondo I.: Nature 266, 165 (1977).

Received: 27. 05. 2008