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Research Article

MICROWAVE-ASSISTED EFFICIENT SYNTHESIS AND ANTIFUNGAL EVALUATION OF QUINOXALINE DERIVATIVES

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ABSTRACT

Microwave assisted organic reaction enhancement (MORE) has emerged as a new 'lead' in organic synthesis. The technique offers simple, clean, fast, efficient economic and environment friendly method for the synthesis of a large number of organic molecules. During our studies, the conventional synthesis of N-(phenyl/alkyl/dialkyl/amines)-2-(3-oxo-1,2,3,4-tetrahydroquinoxaline-2-yl) acetamide derivatives required time (12-16 h) and the yield were often poor (36.9-48.6%). Hence application of microwave technique for synthesis of the title compounds with an objective to reduce reaction time and increase yield was explored, Using microwave irradiation, all the reaction could be completed in very short duration (4-7 min) with considerable increase in the yields (53.2-80.8%).All the synthesized compounds were evaluated for their anti-fungal activity against *Tricophytonrubrum*, *Epidermophytonfloccosum* and *Malassazia furfur.*The minimum inhibitory concentration was found to be ranging between 13-19 μg/ml.

KEY WORDS: MORE, Microwave Chemistry, Quinoxaline, Antifungal Activity.

INTRODUCTION

In the recent years, microwave assisted organic reactions have emerged as a new toolin organic synthesis. Important advantages of this technique include highly accelerated rate of the reaction, reduction in reaction time with an improvement in the yield and quality of product. Moreover, the technique is considered as an important approach towards 'green chemistry' because of its eco-friendly nature. Conventional methods of organic synthesis usually need longer heating time, elaborate and tedious apparatus set up, which result in higher cost of process and the excessive use of solvents/ reagents leads to environmental pollution.

The microwave technique has been applied to qualitative/ extraction of natural products. The experiments like determination of saponification value, degradation of atropine, estimationof loss on drying could be performed within minutes with the help of microwave-assisted technique and are being used for routine practical classes (Sharma, et al., 2002).

Incidences of fungal infections have been increased dramatically in the last few decades. The infection in hair, nails and the outer layer of epidermis are very common in India (Rastogi, et al., 2010) This type of infection is collectively known as Dermatophytosis and caused by dermatophytes (superficial fungi) (Jawetz, et al., 1997: Watkins, et al., 2003). Intact dry skin is an effective barrier against many diseases but aforementioned incidences of infections develop with fungal infection (Peleczar, et al.,1993). The increased intensity of these life threatening fungal infections and the development of resistance to the currently used antifungal agents warrant the search for novel, alternative chemical moieties.

During our synthetic studies, it was observed that the synthesis of N-(phenyl/alkyl/dialkyl/amines)-2-(3-oxo-1, 2, 3, 4-tetrahydroquinoxaline-2-yl) acetamide required a reaction time of 12-16 h while the yields were always poor (<50%). Therefore, it was felt worthwhile to study these reactions under microwave irradiation with the aim of decreasing the reaction time and increasing the yield.

The new microwave procedures were developed by considering two important parameters: minimum reaction time and maximum yield of the pure product. This was achieved by carrying out each reaction in two major ways. Firstly, optimization of the microwave power (intensity) was performed by conducting the reactions at different microwave powers/ intensities (160, 350 & 500W) setting for a fixed time of five minutes. The microwave intensity giving the maximum yield was selected for optimizing the reaction time (**Table-1**). Each time, the product was isolated; the yield and quality of the product was compared with the one obtained by conventional method. Finally, by using the optimized microwave intensity and time, each reaction was repeated at least three times and the products were compared with the conventional products by studying their melting point, mixed melting point, TLC, Co-TLC and IR spectra.

TABLE 1: Optimization of Microwave Intensity And Reaction Time For The Synthesis Of N-(Phenyl/Alkyl/Dialkyl/Amines)-2-(3-Oxo-1,2,3,4-Tetrahydroquinoxaline-2-Yl) Acetamide (4a-F)

Compd.no	Microwave Intensity(W)	Yield* %	Optimum Intensity (W)	Reaction time(min)	Yield** %
4a	160	24.6		4	47.9
	350	45.8		5	70.7
	500	70.6	500	6	61.5
4b	160	40.5		4	76.4
	350	69.8		5	65.0
	500	43.8	350	6	56.9
4c	160	56.9		4	46.7
	350	52.8		5	75.8
	500	78.5	500	6	80.5
4d	160	56.4		4	65.8
	350	80.8		5	76.0
	500	53.8	350	6	74.6
4e	160	77.9		4	67.9
	350	66.9		5	65.4
	500	62.5	160	6	56.7
4f	160	45.7		4	44.6
	350	65.8		5	56.8
	500	61.8	350	6	65.9

*Average of three separate reaction after microwave irradiation for 5 minute

**Average of three readings

MATERIALS AND METHODS

The study was conducted randomly by buying the drugs for patients. The survey form designed and questionnaires were comprised, open/closed and yes/no questions were asked randomly from selected 90pharmacists/chemist. The study was conducted among the pharmacists in Moradabad who cooperated well during this study. The study was conducted in the following step. Step-1 to buy the drugs from 90 pharmacists for acidity without prescription. Step-2 the questionnaire was prepared and asked from the pharmacists whether pharmacist dispensed the drugs. Whether the patients was referred to the physician.heather

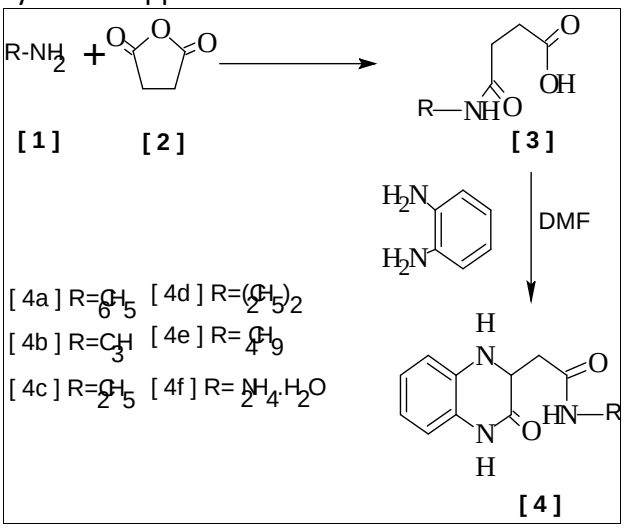
they can ask about the severity/history of the disease. Step-3 the drugs were categorized and the results were assessed and interpreted. After collecting the data the necessary interpretation was done and the outcome of the study was assessed.

RESULT

All the chemicals used were obtained from S.D. Fine Chem. Ltd., Mumbai and E-Merck Ltd., Mumbai while the reagents and solvents were of analytical grade. Heating was done in a microwave oven (LG-Healthcare System, MG-605 AP, and 900 W). The melting points were carried out in open capillary tube and were uncorrected. Thin layer chromatography was performed using silica gel coated on a glass plate. IR spectra in KBr disc and the absorption bands are expressed in cm^{-1} were recorded on a Shimadzu 8201 PC FTIR spectrophotometer. ^{1}H NMR spectra were recorded in $CDCl_3$+acetone-d_6 on a FX 90Q FTNMR spectrometer using TMS as an internal standard (chemical shifts in δ, ppm). The ^{13}C-NMR spectrums of synthesized derivatives were recorded on varian-VXR-300S at 125 MHz; Mass spectra of synthesized derivatives were recorded at MAT-120.

The reactions were monitored by thin layer chromatography on silica gel G coated glass plates using benzene: ethyl acetate (7:3) solvent system. The purity of synthesized compounds was ascertained by TLC using iodine vapours as detecting agents.

N-(phenyl/alkyl/dialkyl/amines)-2-(3-oxo-1, 2, 3, 4-tetrahydroquinoxaline-2-yl) acetamide**(4a-f)** were prepared by the reaction of o-phenylinediamine with 3-(phenyl, methyl, ethyl, diethyl, isobutyl, hydrazino) carbamoyl acrylic acid **(3a-f)** in DMF solvent. The 3-(phenyl, methyl, ethyl, diethyl, isobutyl, hydrazino) carbamoyl acrylic acid **(3a-f)** were prepared by the reaction of maleic anhydride with aniline, methyl amine, ethylamine, diethyl amine, isobutylamine and hydrazine hydrate in the presence of diethyl ether solvent. The synthetic approach outlined in **Scheme 1**



Scheme 1: scheme showing the synthesis of N-(phenyl/alkyl/dialkyl/amines)-2-(3-oxo-1,2,3,4-tetrahydroquinoxaline-2-yl) acetamide (4a-f)

Conventional method:

3-(phenyl/methyl/ethyl/diethyl, isobutyl/hydrazino) carbamoyl acrylic acid (3a-f)

To the solution of maleic anhydride (9.8gm; 0.1mole) in diethyl ether (30 ml), the solution of substituted amine (0.1mole) in diethyl ether (30 ml) was added. The reaction mixture was stirred at room temperature for 5-7 minute. The precipitate was filtered, washed with ether (2×60 ml) and then methanol to get the compound **3a-f**.

N-(phenyl/alkyl/dialkyl/amines)-2-(3-oxo-1,2,3,4-tetrahydroquinoxaline-2-yl) acetamide (4a-f)

To the solution of **3a-f** (0.078 mole) in DMF (25ml) was added; solution of o-phenylenediamine (8.43gm, 0.078 mole) in DMF (25ml) was added. The resulting mixture was refluxed for 7 hrs. The solution was cooled and poured into crushed ice and set aside for an overnight. The solid that are separated out and washed by ethanol to get the compounds **4a-f**

Microwave method:

N-(phenyl/alkyl/dialkyl/amines)-2-(3-oxo-1,2,3,4-tetrahydroquinoxaline-2-yl) acetamide (4a-f)

To the solution of **3a-f** (0.078 mole) in DMF (10ml) was added; solution of o-phenylenediamine (8.43gm, 0.078 mole) in DMF (10ml) was added and the reaction mixture was placed in a conical flask, covered with a glass funnel. A Petri-dish containing few ice pieces was kept on the funnel to prevent excess evaporation of the solvent. The reaction mixture was irradiated with microwaves at different microwave intensities for different duration by following the pulse heating approach (irradiation in 30 seconds increments). A beaker containing water was also kept in the oven to serve as a 'heat sink'. To monitor the progress of reaction, a TLC was run after every one minute of microwave irradiation using benzene: ethyl acetate (7:3) solvent system. After completion of reaction, the work-up was done in a manner similar to the conventional procedure.

The authenticity of product was confirmed by physical (melting point, mixed melting point, Co-TLC), chemical and spectral analysis by comparison with samples obtained in conventional manner. All the six compounds were prepared in yields that were appreciably more than the conventional methods **(Table 2)**

TABLE 2: Comparative Reaction Time and Percentage Yield of Synthesized Compounds (4a-F) By Conventional & Microwave Method

Compd no.	Reaction time		Yield (%)	
	Conventional(h)	MW(min)	Conventional	MW
4a	11	5	39.0	70.7
4b	15	4	44.0	76.4
4c	14	6	43.5	80.5
4d	16	6	36.7	74.6
4e	15	4	42.6	67.9
4f	15	6	41.5	65.9

Characterstics of 4a :

m.p. 201-203^{0}C, IR (KBr)(cm^{-1}) 3294.79 (N-H Str. in 2^0 amide), 3054.69 (C-H Str. In aromatic ring), 1643.05 (C=0 Str. in 2^0 amide), 1335.46 (C-N vibr. aromatic 2^0 amine), 748.25(C-H bend in aromatic, subst.4-adjacent H-atom) **^{1}H NMR** δ (ppm)(300 MHz $CDCl_3$): 7.08 – 7.71(m, 5H, aryl-H group), 6.1 – 6.7(m, 4H, aryl-H group), 2.72 – 2.87(broad t, 1H, N-**CH**-CH_2 group), 2.40 – 2.50(d.2H, CH-**CH_2** group).**^{13}C-NMR** (125MHz, $CDCl_3$) δ (ppm) 119.6, 118.0, 117.1, 114.3(CH-aryl), 134.6, 128.6(C-aryl), 150.7(C-methylene), 62.2(CH-aliphatic), 82.7 (CH_2-ethylene), 33.9(CH_2-aliphatic), 173.7(C-amide), 138.5, 121.6, 129, 124.4, 129.0 (CH-phenyl). **MS (EI. 70eV) m/z (%):** 281.12(100%), 282.12 (17.6%).

Characterstics of 4b:

m.p. 180-182^{0}C, IR (KBr)(cm^{-1}) 3383.50(N-H Str. in 2^0 amide), 1657.52(C=0 Str. in 2^0 amide), 1544.70(N-H bend. in 2^0 amide), 1316.18 (C-N vibr. aromatic 2^0 amine), 746.32 (C-H bend in aromatic, subst.4-adjacent H-atom) ^{1}H NMR δ (300 MHz $CDCl_3$): 7.49 – 7.74 (m, 2H, aryl-H group), 7.1 – 7.3 (m, 2H, aryl-H group), 3.2 – 3.5 (d, 3H, NH-**CH_3** group), 2.7 – 2.8 (m,1H, CH-**CH_2** group), 2.5(d,2H, CH –**CH_2** group),**^{13}C-NMR** (125MHz, $CDCl_3$) δ (ppm) 119.6, 118.0, 117.1, 114.3(CH-aryl), 134.6, 128.6(C-aryl), 150.7(C-methylene),62.7(CH-aliphatic), 82.7 (CH_2 ethylene), 34.0 (CH_2-aliphatic)173.7(C-amide), 26.3(CH_3). **MS (EI. 70eV) m/z (%):** 219.10(100%), 220.10 (13.1%), 221.1(1.1%)

Characterstics of 4c:

m.p. 165-167^{0}C, IR (KBr)(cm^{-1}) 2974.66 (N-H Str. in 2^0 amide), 2938.02 (C-H Str. in alkane), 1692.23 (C=0 Str. in 2^0 amide), 1549.25 (N-H bend. in 2^0 amide), 1376.93(C-H bend in alkane), 1348.00 (C-N vibr. aromatic 2^0 amine),

745.35 (C-H bend in aromatic, subst.4-adjacent H-atom) **^{1}H NMR** δ (300 MHz $CDCl_3$): 9.8(d,1H,**NH**-CH group), 7.3 – 7.6 (m, 2H, aryl-H group), 6.9 – 7.2 (m, 2H, aryl-H group), 2.9 (m, 2H, **CH_2**CO group), 2.7 – 2.8 (m,1H, CH-**CH_2** group), 1.2 (t,3H, CH_2-**CH_3** group).**^{13}C-NMR** (125MHz, $CDCl_3$) δ (ppm) 119.6, 118.0, 117.1, 114.3(CH-aryl), 134.6, 128.6(C-aryl), 150.7(C-methylene), 62.2(CH-aliphatic), 82.7 (CH_2-ethylene), 34.3(CH_2-aliphatic), 173.4(C-amide), 15.1(CH_3), 34.2(CH_2-aliphatic). **MS (EI. 70eV) m/z (%):** 233.12(100%), 234.12 (13.2%), 234.11(1.1%)

Characterstics of 4d:

m.p. 110-112^{0}C, IR (KBr)(cm^{-1}) 1664.27 (C=0 Str. in 3^0 amide), 1376.96(C-H bend, gem. diethyl), 1305.57 (C-N vibr. aromatic 2^0 amine), 744.39 (C-H bend in aromatic, subst.4-adjacent H-atom) **^{1}H NMR** δ (300 MHz $CDCl_3$): 7.1 – 7.9 (m, 2H, aryl-H group), 6.1 – 6.9 (m, 2H, aryl-H group), 2.8 – 2.9 (m, 4H, **CH_2**CH_3 group), 2.7 – 2.8 (t,1H, **CH**-CH_2 group), 0.8 – 1.2 (m,6H, CH_2**CH_3** group). **^{13}C-NMR** (125MHz, $CDCl_3$) δ (ppm) 119.6, 118.0, 117.1, 114.3(CH-aryl), 134.6, 128.6(C-aryl), 150.7(C-methylene), 62.5 (CH-aliphatic), 82.7 (CH_2-ethylene), 32.1(CH_2-ethylene), 32.1(CH_2-aliphatic), 171.8(C-amide), 12.9(CH_3-aliphatic), 41(CH_2), **MS (EI. 70eV) m/z (%):** 262.16(100%), 263.16 (15.4%), 263.15(1.1%)

Characterstics of 4e:

m.p. 80-82^{0}C, IR (KBr)(cm^{-1}) 3339.14(N-H Str. in 2^0 amide) , 2958.27 (C-H Str. in alkane), 1706.69 (C=0 Str. in 2^0 amide), 1500.35 (N-H bend. in 2^0 amide), 1352.82 (C-H Str. gem.dimethyl),1321.00 (C-N vibr. aromatic 2^0 amine), 744.39 (C-H bend in aromatic, subst.4-adjacent H-atom) **^{1}H NMR**δ (300 MHz $CDCl_3$): 7.1 – 7.7 (m, 2H, aryl-H group), 6.6 – 6.7 (m, 2H, aryl-H group), 3.1 – 3.85 (m, 2H, **NH-CH_2** group), 2.7 – 2.8 (broad t,1H, **CH**-CH_2 group), 2.5 (d,2H, HC-**CH_2** group), 0.76 – 0.89 (d,6H, CH-**$(CH_3)_2$** group), **^{13}C-NMR** (125MHz, $CDCl_3$) δ (ppm) 119.6, 118.0, 117.1, 114.3(CH-aryl), 134.6, 128.6(C-aryl), 150.7(C-methylene), 62.2 (CH-aliphatic), 82.7 (CH_2-ethylene), 34.3(CH_2-aliphatic)173.4(C-amide), 13.8(CH_3), 19.9, 32.3, 40.1(CH_2-aliphatic), **MS (EI. 70eV) m/z (%):** 261.15(100%), 262.15 (15.4%), 262.14(1.1%)

Characterstics of 4f:

m.p. 207-209^{0}C, IR (KBr)(cm^{-1}) 3172.33 (N-H Str. in 2^0 amide), 1661.37 (C=0 Str. in 2^0 amide), 1626.66 (N-H bend. in 1^0 amide), 1321.00 (C-N vibr. aromatic 2^0 amine), 742.43 (C-H bend in aromatic, subst.4-adjacent H-atom), **^{1}H NMR**δ (300 MHz $CDCl_3$): 7.0 – 7.6 (m, 2H, aryl-H group), 6.6 – 6.9 (m, 2H, aryl-H group), 2.7 – 2.8 (t, 1H, **=CH-** group), 2.5 – 2.55 (d,2H, HC-**CH_2**-C group), **^{13}C-NMR** (125MHz, $CDCl_3$) δ (ppm) 119.6, 118.0, 117.1, 114.3(CH-aryl), 134.6, 128.6(C-aryl), 150.7(C-methylene), 62.5(CH-aliphatic), 82.7 (CH_2-ethylene),168.2(C-amide), 36.0, 82.7(CH_2). **MS (EI. 70eV) m/z (%):** 220.10(100%), 221.10 (11.0%), 222.10(1.1%).

ANTIFUNGAL ACTIVITY

All the synthesized compounds were evaluated for their *in vitro* antifungal activity against fungi as *Tricophytonrubrum*,*Epidermophytonfloccosum* and *Malassazia furfur* by using two fold serial dilution method. 0.2 ml of 1 mg/ml stock solution (using DMSO) of the test substance was added to 1.8 ml of seeded broth, which was previously standardized to produce a concentration of 100 μg/ml of the test substance.1 ml from 100 μg/ml was added to 1 ml of seeded broth (50 μg/ml). Likewise, the test compounds were serially diluted in two-fold manner to obtained different concentrations (100, 50, 25, 12.5, 6.25 and 3.125 μg/ml)

Then it was incubated at 27˚C for 48 h. the MIC was found out by visual comparison method (presence of turbidity due to growth). In experimental terms, the MIC is the concentration of the drug present in the last clear tube, i.e. in the tube having the lowest drug concentration in which growth is not

observed (Cappuccino, et al., 1996: Greenwood, et al., 1997).

Apart from this, one positive control (without test substance), one negative control (only broth) and one solvent control were kept. All the tests were done in triplicate. To determine the exact MIC of each compound, the essay was repeated by taking several concentrations showing growth and lowest concentration without it. Fluconazole was used as a standard drug for comparison of antifungal activity of synthesized compounds listed in **Table-3**.

TABLE 3: Antifungal Activity of Synthesized Compounds by MIC Method

Compd no.	Fungal strain			MIC average («g/ml)
	Tricophyton rubrum	*Epidermophyton floccosum*	*Malassazia furfur*	
4a	14.00	-	13.80	13.90
4b	14.50	-	14.50	14.50
4c	14.50	14.00	14.00	14.20
4d	19.00	17.50	-	18.20
4e	15.50	-	15.50	15.50
4f	15.00	17.00	-	16.30
Fluconazole	20.00	19.00	18.75	19.50

RESULTS AND DISCUSSION

A new microwave procedure for the rapid and efficient synthesis of N-(phenyl/alkyl/dialkyl/amines)-2-(3-oxo-1,2,3,4-tetrahydroquinoxaline-2-yl) acetamide has been developed. The microwave heating effectively reduced the reaction time from 12-16 h to a few minutes (4-7 min). By using microwave irradiation for heating, all the six compounds were prepared in yields that were appreciably more than the conventional methods.

Highest yield improvement of about 91% was observed for compound **4c**, when compared with conventional method. The quality of the products formed was found to be better showing less number of impurities on TLC when compared to the conventional products. The physical, chemical and spectral (IR, ^{1}HNMR, ^{13}CNMR & Mass) properties of the microwave reaction products were found to be the same when compared with the conventional products prepared simultaneously. The experiment could be conducted in much shorter duration by new microwave methods. From the result, it was observed that all the test compounds possessed significant antifungal activity. The compounds were active against all the three strain of fungi. 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 type of fungi. All the compounds inhibited growth of the fungi at a concentration ranging between 12.5-19μg/ml.

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REFERENCES

1. Sharma, S.V., Sharma, G.V.S. and Suresh, B. (2002). MORE Chemistry: An Eco-friendly Synthetic Technique. Indian J. Pharm. Sci., 64, 337.
2. Rastogi, S. and Rastogi, H. (2010). An efficient synthesis of some substituted piperidin-4-one thio-semicarbazone

derivatives as potential anticonvulsant under microwave irradiation. Indian J. Chem., 49(B), 547-553.
3. Rastogi, S. and Pathak, D. (2008). Microwave-assisted efficient synthesis and antimicrobial evaluation of some N-Aryl-2-(3-oxo-1, 4-benzothiazin-2-yl) acetamide derivatives. J. Pharmaceutical Res., 34, 234-238.
4. Jawetz, E. Melnick, J. and Adelberg, E.A. (1997). Review of Medicinal Microbiology. 7, 244.
5. Watkins, W.S. and Renau, J.E. (2003). Antifungal agents, Burger's medicinal chemistry & drug discovery. 6th ed. John-Wiley & Sons inc; 5, 881-918.
6. Peleczar, M.J. Chan, E.C.S. and Krieg, N.R. (1993). Microbiology, 5th eds. Tata Mcgraw Hill Publishing Company Ltd. New Delhi. 511.
7. Cappuccino, J.G. and Sherman, N. (1996). Microbiology- A Laboratory Manual 6. 199.
8. Greenwood, D. (1997). Antimicrobial agent, Medical Microbiology. 15th edi., ChurchilLingstone. 196-195.

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