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Research Paper

Ultrasound Promoted Imino Diels-Alder Reaction Of Ketimine-Isatin For The Generation Of Spiro[Indoline-3,2,-Quinoline]-2-Onesusing Peg 400 As A Green Solvent Andevaluation Of Their Anti-Microbial And Analgesic Activity

Madan Yogita[1], Gupta Ragini[2], Menghani Ekta[2]
[1]Department of Chemistry, Malaviya National Institute of Technology Jaipur-302 017, INDIA
[2]Department of Biotechnology, Mahatma Gandhi Institute of Applied Sciences, Jaipur Engineering College and Research Centre Jaipur-302 022, INDIA

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Abstract: *An easy, fast, elegantand eco-efficient protocol for the synthesis of spirotetrahydroquinoline derivatives**(4a-h)** has been explored through hetero Diels-Alder methodology by using arylamines**(1a-h)**,indole-2,3-diones**(2a/b)**and maleic anhydridecatalysed by$InCl_3$in PEG 400 as green solvent under ultrasonication. Atom economical behavior, use of green solvent PEG 400 and ultrasonicationmakes this approach anattractive pathway to achieve complex molecules.Further, the feasibility of this reactionis demonstrated in terms of short reaction time, good yield and waste minimization. Synthesized compounds **(4a-h)**were confirmed by their spectral and analytical data. The coupling of two biologically active moieties i.e.indole and tetrahydroquinoline where there is a C-3 spirooxiindole bridge in the product molecules **(4a-h)** encouraged us for evaluating their anti-microbial and analgesic activities. Some of the compounds showed promising results for future prospects.*

Keywords: Indole-2,3-diones, aza Diels-Alder reaction, tetrahydroquinoline derivatives, ultrasonication, PEG 400, Green Chemistry, Schiff's base

Introduction

Imino Diels-Alder reaction of heterodienes and numerous dienophiles has been well documented as a key step for the production of naturally occurring aromatic alkaloids [1-7], terpenes, antibiotics and several other class of *N*- containing heterocyclic natural products [8-15]. Also, it serves as an important tool for the production of biologically active spiroquinoline[16-18] and quinoline derivatives [19, 20]. These moieties are well known as an active ingredient in melanocortin receptor agonists [21], antipsychotics [22], acetylcholine esterase inhibitor which is an important target for the treatment of Alzheimer's disease [23]. This core structure is also available as a ligand for the estrogen receptors [24], protein farnesyltransferase (PTF) inhibitors (an enzyme which is necessary for the survival of pathogenic protozoa *P. falciparum*[25], cause of severe malaria).
Consequently, synthetic routes *i.e.* condensation [26], and Diels-Alder cycloadditions have been extensively developed for the production of spirodihydroquinoline derivatives. Imino Diels-Alder reaction (Povarov reaction) is an atom economical C-C and C-N bond forming reaction to produce variety of *N*-containing six membered heterocycles including tetrahydroquinolines[27].

Many catalytic systems employing Lewis and Bronsted acid catalyzed hetero Diels-Alder reactions (HDA) are enumerated in the literature [28, 29]. Kouznestov*et. al.* have reported the synthesis 3,4-dihydro-10*H*-spiro[indolin-3,20-quinolin]2-ones using the Lewis acid $BF_3.OEt_2$ as catalyst in anhydrous dichloromethane as solvent [30].

Typically, all traditional procedures suffer from low yield, cumbersome workup protocol, use of toxic reagents and volatile organic solvents. The replacement of these hazardous solvents with an environmentally benign solvent such as PEG 400 offers an advantage of being inexpensive, easily available commercially and eco-friendly solvent system with unique properties such as thermal stability, recyclable, non-toxic, safe to handle, recoverable, biologically compatible, completely non halogenated which makes it an excellent choice for sustainable Green Chemistry [31].

Various green synthetic technologies *i.e.* microwave irradiation,ultrasonication and use of catalysts have been developed for the convenience of the hetero Diels' Alder reaction (HDA)[32]. Amongst these,ultrasonication with catalysis (sonocatalysis) has

been probed as an elegant alternative and striking procedure to this cycloaddition reaction since it increases catalyst reactivity by enhanced mass-transfer and energy input [33, 34].

This research work deals with the synthesis, spectral studies, antimicrobial and analgesic activity of various 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives **(4a-h)** by reaction of aryl amines **(1a-h)**, indole-2,3-diones **(2a/b)**, and maleic anhydride in PEG-400 as a green solvent under conventional heating and ultrasonication using $InCl_3$ as catalyst. This procedure is advantageous since it has simple, effective green reaction protocol for excellent atom economy, high selectivity and less waste generation. Further it results in the junction of two biologically active moieties where there is a C-3 spiro-oxindole bridge with a heterocyclic ring in the product molecule which may enhance their biological utility [35, 36].

Material and Methods

Experimental

Melting points were determined in open glass capillaries and are reported uncorrected. The IR absorption spectra (ν_{max} in cm^{-1}) were recorded on Perkin-Elmer FT IR Spectrophotometer at Indian Institute of Technology, Bombay. ^1H NMR spectra (300 MHz) and ^{13}C NMR spectra (75 MHz)were recorded on a JEOL-AL Spectrophotometer at Indian Institute of Technology, Bombay using $CDCl_3$ as solvent and TMS was taken as internal standard. Chemical shifts are reported in parts per million (ppm, δ) downfield from TMS. ESI-MS spectra were recorded on Q-TOF MS ES^+ micro (YA-105) mass spectrophotometer.

The ESI mass spectra and CHN analyses were recorded at IIT, Bombay, India. Sonication was carried out by using Elma S 70H Elmasonic operating at 37 KHz with a power of 150 W. The purity of the compounds was assured by Thin Layer Chromatography (pre-coated silica gel 60 mesh, MERCK, as adsorbent, UV light or iodine accomplished visualization). All common reagents and solvents were used as obtained from commercial suppliers without further purification.

Antimicrobial Activity

Agar Well Diffusion Method

Agar Well Diffusion Method [37] was performed to examine antimicrobial activities of a series of 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives **(4a-h)** on selected 10 pathogens. Out of these, 6 are bacterial strains (*Klebisella, Pseudomonas, E. coli, Proteus, S. aureus, Shigella*) and 4 are fungal strains *(C. albicans, A. niger, A. flavus, T. rubrum)*. (Figure 1-4)

Concentration of the compounds (4a-h) varies from 10^{-5} mg/ml to 10^{-1} mg/ml disc of the test compounds. About 10–15 g of molten agar was spread into each sterilized petri dish by taking the usual precautions to avoid contamination. All the petri dishes were marked in a specific way. Sterile cork borer was used to make well. The agar plates were inoculated with the suspension of particular organism by spread plate technique. All the synthesized compounds (4a-h) to be tested were put on radiation sterilized disc of 6 mm diameter. After the addition of the test samples, the plates were kept in freeze for diffusion and incubated at 37 °C for 1 h. The zone of inhibition (if any) was then measured in cm for the particular compound and specific organism after 24 h (Tables-2). Discs were placed on the surface of agar plates and then inoculated at standard temperature condition for time period 10-12 hrs. Developed zone of inhibition (IZ) is measured in mm and activity index (AI) was calculated. The Minimum Inhibitory Concentration (MIC) was calculated by plotting the curve between the natural logarithm of the concentration of standard drug used against the square of the value of zone of inhibition respective to tested compounds (4a-h). Zone of Inhibition (IZ), Activity Index (AI)[38].

Analgesic Activity

Swiss albino mice (25-35 gm) of either sex were selected randomly for this experimental protocol. The animals were housed in standard rat cages (6 per cage) under standard laboratory conditions maintained at 25 ± 3 ºC in 14/10 dark/light cycle. These mice were fed with a standard laboratory chow (Aashirwaad food industries, Chandigarh) and water *ad libitum*. The animals were kept according to the guidelines of the Committee Designed for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) regulations. Experiment protocol was approved by IEC (Institutional Ethic Committee).

Radiant Heat Tail Flick Study

Analgesic activity of synthesized compounds (4a-h) was studied in rats by using radiant heat tail flick method [39]. As heat stress was applied to the tails of prescreened rats, these animals withdraw their tails within 3-6 secs. Heat stress or intensity of the light beam has experimentally been defined by using analgesiometer, indicates the drug induced changes in the sensitivity of these rats. Paracetamol at a dose level of 80 mg/kg was administrated as standard drug. All the synthesized compounds (4a-h) at the same dose level in the form of a suspension in gum acacia were introduced orally by intragastric tube to the different groups of rat. The strength of the current passing through naked nichrome wire was fixed at 5 amp. Distance of 1.5 cm was maintained between tail and heat source and heat application site on the tail is fixed within the range of 2 cm from the root of the tail.

To avoid tissue injury, cut off reaction time was fixed + 8 sec during process. Tail flick latency was studied from + 30 min after drug administration. Reaction time is defined as the time taken by rats to flick the tail. The animals were treated to the same test method after 30, 60, 120 and 180 min after the administration of the standard drug. Tail flicking method used for analgesic activity is supposed to be a spinally mediated reflex. Human pain perception in correlation to the analgesic agents is much related to the tail flick pain model [40].

Conventional method

Conventional refluxing method using PEG 400 as green solvent media

A mixture of aryl amine (1a) (1mmol) and indole-2, 3-diones (2a) (1mmol) were refluxed in PEG 400 for 1 hr at 110 ºC in a 100 ml round bottom flask. As the reaction proceeds, colour of reaction mixture darkens to red-orange indicating the formation of ketimines. The reaction was monitored *via* Thin Layer Chromatography. After the completion of the reaction, it was cooled to room temperature till red-orange needle shaped shiny crystals of Schiff's base (3a) develop which were filtered and washed well with cold pet ether (60-80 ˚C). Further, maleic anhydride (1mmol), Schiff's base (3a) and $InCl_3$ as catalyst were refluxed for 5 hrs in PEG 400. The progress of the reaction was monitored by TLC. After the completion of the reaction, product so obtained were filtered and washed well with cold pet ether to give red crystals of pure desired product 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one (4a).

Ultrasonication using PEG 400 as green solvent media

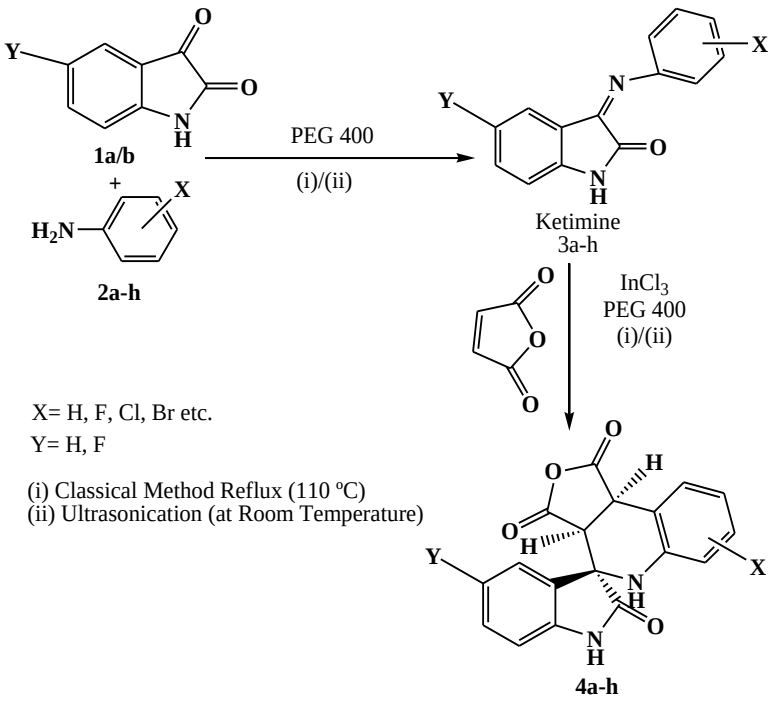
Aryl amines (1a) (1mmol) and Isatin(2a) (1mmol) were sonicated for 8 min at room temperature (25-30˚C) in PEG 400. As the reaction proceeds, colour of reaction mixture darkens to red-orange indicating the formation of ketimines. After completion of the reaction (monitored by TLC), it was cooled to room temperature till red-orange needle shaped shiny crystals of Schiff's base (3a) developed. Further, maleic anhydride (1mmol), ketimines(3a) and $InCl_3$ as catalyst were again sonicated at 80 ˚C for 45 min in PEG 400. The progress of the reaction was monitored by TLC. After the completion of the reaction, products so obtained was filtered and washed well with cold pet ether to give red crystals of pure desired product 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one (4a).

All the other 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives (4b-h) have been prepared in a similar manner. Spectral data for all the derivatives (4a-h) have been given in Table 2.

Results and Discussion

4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-ones **(4a-h)** were prepared by refluxing together equimolar amounts of iminoisatin (Schiff's base, **3a-h**) as the azadiene component and maleic anhydride as the dienophile in the presence of catalytic amount of $InCl_3$ which resulted in comparatively low yield (75-79 %). In order to improve the yield and recognizing the benefits of 'green chemistry', the same reaction was repeated by ultrasonication of the reactants (**Scheme-1**) which gave the desired products **(4a-h)** in 50 min.

In the IR spectra of compounds (**4a-h),** absorbance peak from 3449-3435 cm^{-1} is assigned to –NH stretching vibration of indole moiety. A broad absorbance peak from 3194-3167 cm^{-1} is assigned to aromatic C-H stretching vibration. The -CO stretching vibrations of anhydride moiety as well as indolic -CO appeared from 1748-1737 cm^{-1} and the absorption bands from 1618-1616 cm^{-1} have been assigned to –C=N stretching vibrations. Aliphatic stretching vibrations, initially not present in isatin Schiff's bases, appeared in the region of 2952-2941 cm^{-1}, indicating the formation of the cycloadducts**(4a-h)**.



Scheme-1:Synthesis of 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives (4a-h)

Table 1
Yield (%) and time for the synthesis of 4', 5'-dihydrofuro (4,5-a)-1,3-dione)spiro (indolin-3,2'-quinolin)-2-one derivatives (4a-h)

Compound No.	X	Y	Conventional Method		Ultrasonication	
			Time (hr)	Yield (%)	Time (min)	Yield (%)
4a	-H	H	5	77	45	88
4b	-F	H	5.5	75	41	92
4c	-Cl	H	4.5	74	42	89
4d	-Br	H	5.2	75	46	88
4e	$-NH_2$	H	4.5	76	44	89
4f	$-CH_3$	H	5	74	39	91
4g	-H	F	4.8	77	45	93
4h	-F	F	4.5	79	43	92

In the PMR spectra of compounds **(4a-h)**, -NH proton of indole moiety remained unaltered and was observed as a singlet from δ 8.10-8.13 ppm (D_2O exchangeable). A complex multiplet from δ 7.21-7.78 ppm due to the aromatic protons is observed. Appearance of new characteristic sharp singlets from δ 3.77-3.91 ppm due to $-NH_{THQ}$ andδ 4.6-4.8 ppm due to *cis*protons of THQ moiety confirmed the formation of products **(4a-h)**. Further confirmation was obtained by high resolution mass spectra (HRMS) data of compounds **(4a-h)** which displayed M+1 ion peak at m/z 321 **(4a)**, 339 **(4b)**, 355 **(4c)**, 400 **(4d)**, 336 **(4e)**, 335 **(4f)**, 339 **(4g)**, and 357 **(4h)** that agreed well with their corresponding molecular formulae.

The synthetic steps are shown in **Scheme-1**. In the mechanism, ketimine**(3a-h)** comprises azadiene system which acts as the 4π component and dienophile maleic anhydride acts as the 2π component in this [4+2] cycloaddition.

Anti microbial activity

On the basis of antimicrobial evaluation, it was observed that compound 4a, without any substitution in aryl ring of ketimine moiety shows excellent activity against *C. albicans* but was inactive against *S. flexneri*. Compounds 4b and 4c, having fluorine and chlorine group in the aryl ring of indole moiety showed enhanced activity against *S. flexneri* bacteria at higher concentration (10^{-1} mg/ml). Compound 4c also

deliberated promising activity against *A. niger, E. Coli, Klebisella and A. flavus* at all concentrations (10^{-5} mg/ml, 10^{-4} mg/ml, 10^{-3} mg/ml, 10^{-2} mg/ml and 10^{-1} mg/ml). The presence of fluorine atom in the ring of indole moiety and aniline in compound 4h resulted in significantly good activity against *C. albicans* and *A. flavus* pathogens at all concentration. Compound 4e and 4f were not very active against any of the microbes studied. (Table 3)

Analgesic activity

Radiant heat tail flick method was used to examine the analgesic activity of the synthesized compounds **(4a-h)**. In this tail flick model, the increase in latency period at different time points differed to the pretreatment value within the same drug treated. The percentage increase in latency period was dose dependent. At all the specified time intervals, the percentage of tail flick elongation time varied significantly between the 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives **(4a-h)** and standard drug paracetamol, greater for synthesized compounds **(4a-h)**. At the peak of activity, 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives **(4a-h)** showed 44.14 %, 19.20 %, 35.29 %, 56.75 %, 10.37 %, 17.91 %, 32.69 % and 88.13 % respectively while paracetamol gave 56.42 % elongation of tail flick time.

Table 4) In the model used, though the data revealed that the increase in pain tolerance or tail flick latency profiles of the synthesized 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives **4d** and **4h** were greater than that of standard drug paracetamol and time to reach peak activity was not same for 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives **(4a-h).** The percentage increase in the reaction time peaked at + 30 min for compound **4c**, +1 hr for paracetamol and compound **4h** while +2 hr for compound **4d** but thereafter the activity declined.

Table 2: Spectral data of 4', 5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives (4a-h)

Compound No.	IR (KBr) υ_{max} cm^{-1}	^1H NMR ($CDCl_3$) δ ppm	TOF Mass (ES+) m/z (M^+/M+2)
4a	3449 (-NH str.), 3194(Aro. -CH str.), 2952 (Ali. -CH str.), 1748 (-CO str.), 1616 (C=N str.), 1269 (ArC-O)	8.31 (1H, NH_{indole}), 7.21-7.78 (8H, ArH), 4.79, 4.94 (2H, *cis* -H), 3.91 (1H, $-NH_{THQ}$)	321.06
4b	3445 (-NH str.), 3196 (-Aro. C-H str.), 2950 (Ali. -CH str.), 1745 (-CO str.), 1623 (C=N str.), 1271 (ArC-O)	8.31 (1H, NH_{indole}), 6.80-7.91 (7H, ArH), 4.79, 4.94 (2H, *cis* -H), 3.91 (1H, $-NH_{THQ}$)	339.13
4c	3440 (-NH str.), 3201 (Aro. C-H str.), 2949 (Ali. -CH str.), 1744 (-CO str.), 1620 (C=N str.), 1264 (ArC-O)	8.29 (1H, NH_{indole}), 6.86-7.98 (7H, ArH), 4.82, 4.93 (2H, *cis* -H), 3.84 (1H, $-NH_{THQ}$)	355.26
4d	3429 (-NH str.), 3189 (Aro. C-H str.), 2941 (Ali. -CH str.), 1737 (-CO str.), 1612 (C=N str.), 1252 (Ar C-O)	8.10 (1H, NH_{indole}), 6.78-7.89 (7H, ArH), 4.89, 4.95 (2H, *cis* -H), 3.79 (1H, $-NH_{THQ}$)	400.06
4e	3451 (-NH str.), 3197 (Aro. C-H str.), 2950 (Ali. -CH str.), 1751 (-CO str.), 1623 (C=N str.), 1272 (ArC-O)	8.28 (1H, NH_{indole}), 6.65-7.96 (7H, ArH), 4.89, 4.93 (2H, *cis* -H), 3.89 (1H, $-NH_{THQ}$), 0.97 (s, 3H, $-CH_3$)	336.15
4f	3453 (-NH str.), 2955 (Ali. -CH str.), 1758 (-CO str.), 1629 (C=N str.), 1279 (ArC-O)	8.29 (1H, NH_{indole}), 6.71-8.03 (7H, ArH), 4.83, 4.94 (2H, *cis* -H), 3.94 (1H, $-NH_{THQ}$)	335.12
4g	3451 (-NH str.), 2952 (Aro. -CH str.), 1759 (-CO str.), 1631 (C=N str.), 1275 (ArC-O)	8.23 (1H, NH_{indole}), 6.61-7.99 (7H, ArH), 4.81, 4.92 (2H, *cis* -H), 3.89 (1H, $-NH_{THQ}$)	339.07
4h	3445 (-NH str.), 2941 (Ali. -CH str.), 1743 (-CO str.), 1623 (C=N str.), 1268 (ArC-O)	8.21 (1H, NH_{indole}), 6.67-7.94 (6H, ArH), 4.86, 4.91 (2H, *cis* -H), 3.77 (1H, $-NH_{THQ}$)	357.02

Table-3: Anti-Microbial Activity of 4',5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one Derivatives (4a-h)

Compd.	Mean value of area of inhibition in mm IZ[a] (AI)[b]																	
	4a						4b						4c					
Concentration (in ppm)→ Microbial sps. ↓	Standard	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Standard	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Standard	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Klebisella	48	34 .71	35 . 79	35 . 79	33 . 68	32 .66	48	37 . 77	36 . 75	34 .71	34 .71	31 . 64	45	33 .73	31 .68	31 .68	29 .64	29 . 64
Pseudomonas	32	12. 37	11 . 34	11 . 34	11 . 34	10 .31	32	22 . 68	21 . 65	19 .59	19 .59	18 . 56	55	31 .56	31 .56	29 .53	27 .49	28 . 51
E. coli	45	15 .33	18 . 40	17 . 37	15 . 33	15 .33	45	29 . 64	28 . 62	27 .60	27 .60	27 . 60	46	34 .74	33 .72	33 .72	31 .67	30 . 65
Proteus	50	29 .58	28 . 56	26 . 52	26 . 52	25 .50	50	36 . 72	34 . 68	33 .66	31 .62	30 . 60	48	29 .60	28 .58	27 .56	27 .56	24 . 50
S. aureus	56	35 .62	33 . 59	32 . 57	31 . 55	31 .55	56	42 . 75	41 . 73	41 .73	39 .69	38 . 68	60	47 .78	44 .73	43 . 72	43 .72	39 . 65
Shigella	55	12 .22	11 . 20	10 . 18	10 . 18	06 .11	55	24 . 43	23 . 41	22 .40	19 .35	17 . 31	50	17 .35	15 .30	15 .30	13 .26	10 . 20
C. albicans	50	38 .76	37 . 74	35 . 70	33 . 66	33 .66	50	35 . 70	35 . 70	33 .66	31 .62	31 . 62	58	42 .72	41 .71	39 .67	39 .67	38 . 65
niger	50	29 .58	28 . 56	28 . 56	26 . 52	27 .54	50	28 . 56	27 . 54	25 .50	25 .50	24 .48	52	37 .71	35 .67	35 .67	34 .65	33 . 63
A. flavus	46	21 .46	20 . 43	19 . 41	19 . 41	16 .35	46	31 . 67	29 . 63	28 .61	28 .61	26 . 56	47	29 .61	28 .59	28 . 59	27 .57	26 . 55
T.rubrum	46	16 .35	14 . 30	13 . 28	13 . 28	11 .24	46	23 . 50	22 . 48	22 .48	19 .41	18 . 39	53	24 .45	24 .45	23 . 43	21 .39	21 .39

Compounds	**Mean value of Area of inhibition in mm IZ[a] (AI)[b]**																	
	4d						**4e**						**4f**					
Concentration (in ppm)→ Microbial sps. ↓	**Standard**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**Standard**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**Standard**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
Klebisella	40	23 .57	21 . 52	20 . 50	18 .4 5	19 . 47	36	13 .3 6	12 .33	10 .27	12 . 33	11 .30	31	12 . 38	11 . 35	12 .3 8	10 . 32	11 .35
Pseudomonas	35	17 .51	17 . 43	18 . 51	15 .4 8	18 . 48	46	15 .33	13 .28	11 .24	10 . 22	08 .17	41	09 . 21	08 .1 9	07 .1 7	08 . 19	07 .17
E.coli	33	19 .57	18 . 54	18 . 54	16 .4 8	17 . 51	45	32 . 71	31 .69	31 .69	29 . 64	28 .62	30	15 . 50	15 . 50	12 .4 0	13 . 43	11 .36
Proteus	25	15 .60	13 . 52	12 . 48	12 .4 8	10 . 40	45	18 . 40	15 .33	11 .24	10 . 22	10 .22	25	13 . 52	12 . 48	12 .4 8	12 . 48	11 .44
S.aureus	28	14 .50	15 . 53	14 . 50	11 .3 9	12 . 43	55	30 . 54	28 .51	27 .49	29 . 53	28 .51	45	18 . 40	18 . 40	17 .3 7	15 . 33	15 .33
Shigella	30	18 .60	18 . 60	17 . 56	15 .5 0	16 . 53	53	32 . 60	30 .57	31 . 58	30 .5 6	32 .60	35	27 . 77	24 . 68	25 .6 3	22 . 57	20 .71
C. albicans	50	35 .70	33 . 66	32 . 64	31 .6 2	29 . 58	42	23 . 55	23 .55	22 .52	23 . 55	22 .52	31	25 . 80	24 . 77	25 .8 0	24 . 77	23 .74
A. niger	48	23 .48	21 . 44	22 . 46	20 .4 2	20 . 42	45	22 . 49	20 .44	21 .46	20 . 44	20 .44	42	-	-	-	-	-
A. flavus	48	25 .52	25 . 52	22 . 46	23 .4 8	21 . 44	45	12 .2 6	10 .22	08 .18	10 . 22	07 .15	29	12 .41	11 .37	12 .4 1	11 .37	10 .34
T.rubrum	50	27 .54	26 . 52	24 . 48	23 .4 8	23 . 46	45	18 . 40	18 .40	15 .33	15 . 33	14 .31	41	08 . 19	07 . 17	-	-	05 .12

Compounds	**Mean value of Area of inhibition in mm IZ[a] (AI)[b]**											
	4g						**4h**					
Concentration (in ppm)→ Microbial sps. ↓	**Standard**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**Standard**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**
Klebisella	46	31 .65	30 .67	28 .61	29 .63	28 .61	45	25 . 55	24 .53	22 .49	22 .49	21 .46
Pseudomonas	33	21 .63	18 .54	18 .54	16 .48	15 .45	38	21 . 55	19 .54	15 .39	12 .31	10 .26
E.coli	27	13 .48	12 .44	12 .44	09 .33	08 .30	35	21 . 60	19 . 54	18 .51	16 .46	15 .43
Proteus	22	12 .54	10 .45	10 .45	08 .36	07 .32	23	08 . 34	05 . 21	07 .30	06 .26	05 .21
S. aureus	35	17 .48	15 .43	15 .43	14 .40	13 .37	525	13 . 52	11 . 44	10 .40	08 .32	09 .36
Shigella	38	21 .55	21 .55	19 .50	18 .47	16 .42	28	18 .64	18 . 64	15 .53	13 .46	12 .43
C. albicans	47	32 .68	29 .62	26 .55	28 .59	26 .55	47	33 . 70	33 .70	31 .66	31 .66	28 .59
A. niger	33	19 .57	17 .51	16 . 48	16 .48	14 .42	45	19 . 42	20 .44	19 .42	18 .40	15 .33
A. flavus	30	13 .43	12 .40	11 .36	11 .36	09 .30	40	27 . 67	27 .67	27 .67	26 .65	25 .62
T. rubrum	30	15 .50	14 .46	12 . 40	10 .40	12 .33	50	22 . 41	20 .37	20 .37	18 .33	15 .28

[a] IZ = Inhibition area (zone) excluding diameter of disc

[b] AI (Activity Index) = Inhibition area of sample /Inhibition area of standard

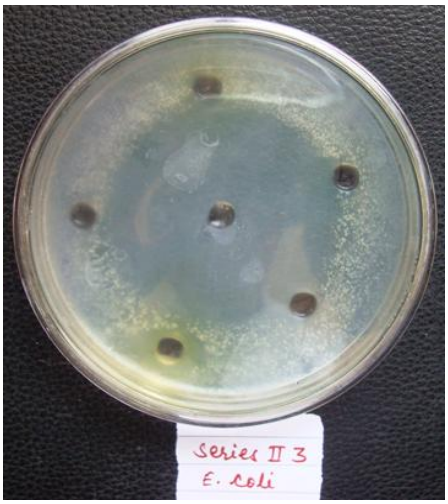


Figure 1: *E. coli* at 10^{-3} mg/ml

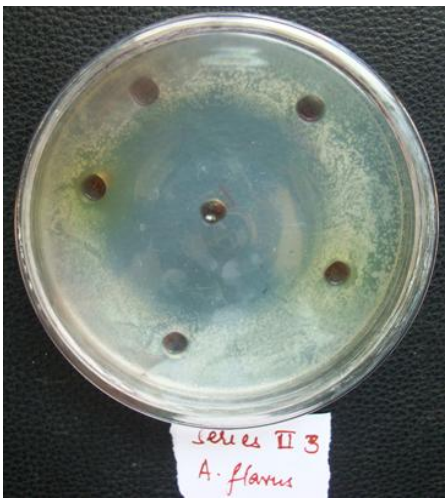


Figure 2: *A. flavus*at 10^{-3} mg/ml

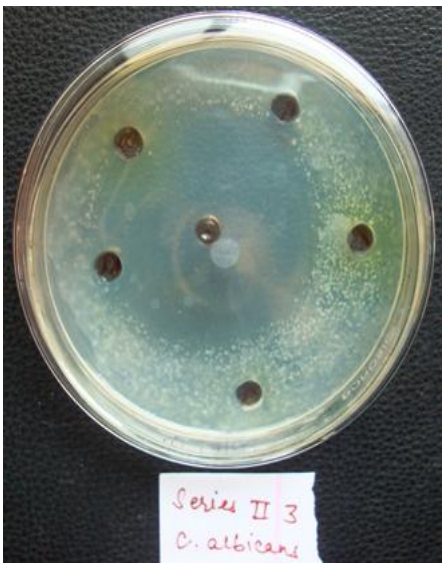


Figure 3: *C. albicans*at 10^{-4} mg/ml

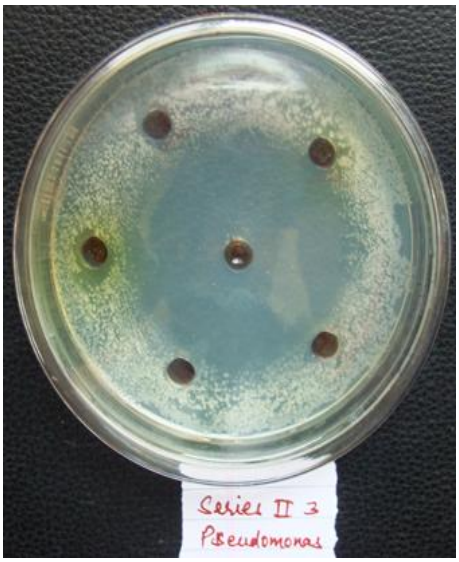


Figure 4: *Pseudomonas* at 10^{-2} mg/ml

Figures 1-4: **Zone of inhibition of Compound 4c against *E. coli, A. flavus, C. albicans*and *P. aeruginosa*at 10^{-3} mg/ml, 10^{-3} mg/ml, 10^{-4} mg/ml, and 10^{-2} mg/ml, concentration respectively**

Conclusion

We have found that ultrasonication in PEG 400 as solvent is fast, safe and eco-friendly method for synthesizing spiroquinoline derivatives. It also demonstrates the feasibility and efficiency of imino Diels Alder cycloaddition by enhancing yield in lesser reaction time. We have obtained good results for antimicrobial as well as analgesic activities in pilot study of these new synthesized compounds **(4a-h).**

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Table 4
Analgesic activity data 4', 5'-dihydrofuro(4,5-a)-1,3-dione)spiro(indolin-3,2'-quinolin)-2-one derivatives (4a-h)

Compound	Dose Interval				
	0 hr	**30 min**	**1 hr**	**2 hr**	**3 hr**
	Reaction time* in sec (% elongation)				
Control	1.45	2.01 **(37.93)**	2 **(37.93)**	2 **(37.93)**	6
Paracetamol (Standard)	1.40	1.57 **(12.14)**	2.19 **(56.42)**	2.13 **(52.14)**	2.02 **(44.28)**
4a	1.11	1.28 **(15.31)**	1.57 **(41.44)**	1.42 **(27.92)**	1.16 **(4.50)**
4b	1.25	1.32 **(5.60)**	1.44 **(19.20)**	1.22 **(NA)**	1.27 **(1.60)**
4c	0.51	0.69 **(35.29)**	0.61 **(19.60)**	0.59 **(15.68)**	0.58 **(13.72)**
4d	0.37	0.35 **(NA)**	0.45 **(21.62)**	0.58 **(56.75)**	0.53 **(43.24)**
4e	1.35	1.36 **(0.74)**	1.49 **(10.37)**	1.47 **(8.88)**	1.29 **(NA)**
4f	1.34	1.45 **(8.20)**	1.51 **(12.88)**	1.58 **(17.91)**	1.55 **(15.67)**
4g	1.04	1.02 **(NA)**	1.38 **(32.69)**	1.22 **(17.30)**	1.18 **(13.46)**
4h	1.00	0.59 **(NA)**	1.11 **(88.13)**	1.19 **(19.00)**	1.27 **(27.00)**

***Average value of reaction time in all groups has been shown in Table 4.4**

Dose level was maintained at 80 mg/kg

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