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Synthesis, spectral characterization and antifungal activities of 2,6-dimethyl-7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5]decane

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Abstract

The reaction between t(3)-methyl-r(2),c(6)-diphenylpiperidin-4-one and Propane-1,2-diol yielded a diastereomeric product of 2,6-dimethyl-7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5]decane. The structure of the newly synthesized compound was analysed by using spectroscopic methods such as IR, one-dimensional NMR (^1H and ^{13}C) and two-dimensional NMR (NOESY). The spectral data revealed that the compound adopt chair conformation with equatorial orientation of all the substituents. The newly synthesized compound was tested for their antifungal activity against *Penicillium notatum*, *Candida albicans*, *Mucor sp*, *Aspergillus nige and*, *Fusarium solani* using micro dilution method. The compound 2,6-dimethyl-7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5] decane displayed inhibitory activity with MIC value of 15.625 µg ml^{-1} in controlling the growth of *M. indicus*, *A.niger* and, *F. solani* and 31.25 µg ml^{-1} for *P.notatum C. albicans*.

Keywords: Antifungal, Dioxolanes, NOESY, Spiro heterocylic

INTRODUCTION

Spiro heterocyclic compounds are considered the most important and essential derivatives in the field of organic chemistry. Many outstanding chemists devoted a lot of efforts which paved the way for the synthesis of new spiro heterocyclic derivatives as well as for studying and understanding their valuable applications (Teng et al., 2012; Subba Reddy et al., 2012; Han et al., 2012; Sakhuja et al., 2011). The skeletal ring of piperidine nucleus can also be often found in the molecular framework of many synthetic and natural medicaments (Daly and Cordell, 1998). The 1,3-dioxolanes are the important intermediates and end-products in the pharmaceutical, fragrance and polymer industries (Greene and Wuts,1991; Kociensky and George,1994; Gemal and Luche, 1979). Recently, spiro compounds receive more attention due to their conformational features and their structural implications on biological systems (Wang et al., 2010). Many spiro compounds possess very promising biological activities such as anticancer (Young-Won et al., 2008; Wen Liang et al., 2007), antibacterial (Manivannan et al., 2012), anticonvulsant (Krzysztof et al., 2008) anti-tuberculosis (Jolanta et al., 2006), anti-Alzheimer's (Chande et al., 2005), anti-dermatitis (Hans et al., 2006), antioxidants (Elanchezhian et al., 2014a, b) and antimicrobial (Nakao et al., 2008; Pawar et al., 2009; Thadhaney et al., 2010). In addition to their medicinal uses, some spiro-compounds have other uses in the agricultural and industrial fields For example, they are used as antifungal agents (Umamaheswari et al., 2010), pesticides (Hejiao et al., 2006), laser dyes (Lindell et al., 22001) and electroluminescent devices (Kreuder et al., 1999).

Consequently, many synthetic methodologies have been developed for constructing these spirocycles, most of which were based on cyclo addition or condensation reactions (Yong et al., 2007)..Therefore the present investigation was carried out to establish a simple synthetic method for the efficient preparation of spiro hetero cycles containing piperidine ring and dioxolane. The compound 2,6-dimethyl-7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5]decane was synthesized by condensation of t(3)-methyl-r(2),c(6)-diphenylpiperidin-4-one with Propane-1,2-diol. Further the synthesized compound was characterized using IR, MS, 1D and 2D NMR spectroscopy.

EXPERIMENTAL

MATERIAL AND METHODS

Column chromatography and TLC were carried out to monitor the course of the reaction and purity of the product. The melting points were recorded in open capillaries and are uncorrected. IR spectra of compound were recorded in KBr disc on AVATAR-330 FT-IR spectrophotometer (Thermo Nicolet) and noteworthy absorption levels (reciprocal centimeters) alone are listed. ^1H and 2D NMR spectra were recorded BRUKER AMX 400 MHz spectrophotometer using $CDCl_3$ as solvent and TMS as internal standard. ^{13}C NMR spectra were recorded at 100.6 MHz on BRUKER AMX 400 MHz spectrometer in $CDCl_3$.

GENERAL PROCEDURE

PREPARATION OF COMPOUND

The compound t(3)-methyl-r(2),c(6)-diphenyl piperidin-4-one (1) was prepared Baliah *et al*., (Noller and Balliah, 1948), and the compound 2,6-dimethyl-7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5]decane was synthesized as described by Chao Gao *et al*., (Gao et

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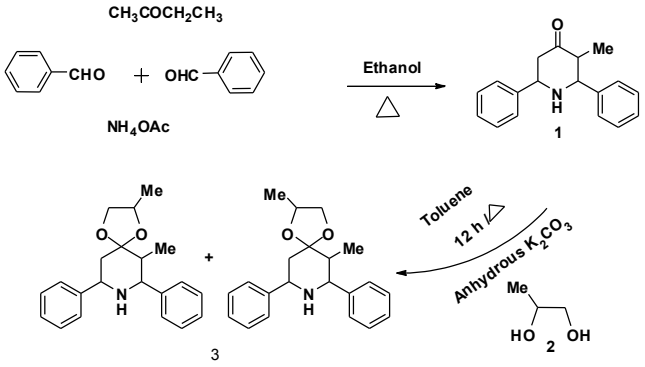
al., 2013), Propane-1,2-diol (11 mM) , t(3)-methyl-r(2),c(6)-diphenylpiperidin-4-one (5 mM) and p-toluene sulphonic acid (0.05 mM) catalyst in 25 mL of toluene, The reaction flask was fitted with a Dean stark water separator, which was charged with anhydrous K_2CO_3 and the solution was gently refluxed for 12 h. It was then cooled to room temperature and evaporated under reduced pressure. To the crude residue 20 mL dichloromethane was added and the solution was washed with saturated sodium bicarbonate (30 mL). The organic layer was then washed twice with water and dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give the crude product. Then it was purified using silica gel column chromatography to get colourless solid. Purity of the products was checked by TLC . The melting point is found to be at 226-228 °C (yield 90%).

ANTI-FUNGAL ACTIVITY

A microdilution method as described by Eloff (1998) and modiûed for fungi by Masoko et al. (2007) was used to determine the antifungal activity of the extracts against *Penicillium notatum, Candida albicans, Mucor sp, Aspergillus niger and Fusarium solani*. An overnight fungal culture was prepared in Yeast Malt (YM) broth. Four hundred microlitres of the overnight culture were added to 4 ml of sterile saline and absorbance was read at 530 nm. The absorbance was adjusted with sterile saline to match that of a 0.5 M McFarland standard solution. From this standardized fungal stock, a 1:1000 dilution with sterile YM broth was prepared to a ûnal inoculum of approximately 106 CFU/ml. The compound was resuspended in absolute ethanol to known concentrations. One hundred microlitres of the compound was serially diluted to two-fold with sterile distilled water in a 96-well microtitre plate for each of the five fungal strains. A similar two-fold serial dilution of Amphoterecin B (Sigma Aldrich) (0.1mg/ml) was used as a positive control against each fungus. One hundred microlitres of each fungal culture were added to each well. Water and 70% ethanol were included as negative and solvent controls respectively. The plates were covered with paraûlm and incubated at 37°C for 24 h. Fungal growth was indicated by adding 50µl of 0.2 mg/ml *p*-iodonitrotetrazolium chloride (INT) (Sigma–Aldrich) with further incubation at 37 °C for 24 h. The wells remained clear where there was inhibition of fungal growth. MIC values were recorded as the lowest concentrations that inhibited the fungal growth after 48 h.

RESULTS AND DISCUSSION

After some preliminary experimentation, maximum yield of 90% of the product was achieved by refluxing for 12h in toluene medium with PTSA catalyst (Scheme 1).



Scheme-1

The compound is a stable solid, its structure is fully supported by IR, Mass, ^1H ,^{13}C and 2D NOESY NMR spectroscopy.

IR Spectral analysis

The carbonyl group stretching of compound (1) at 1713cm^{-1} disappeared in compound (3). The IR spectral data of compound 2,6-dimethyl-7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5]decane are given in Table-1

Table 1: IR stretching frequencies (cm^{-1}) of the compound

Absorption band	Assignment (cm^{-1})
NH	3446
Aromatic CH	3040
CH_3 in aliphatic	2970, 1459
Benzene ring stretching	1599, 1497
C-C-N bend	1224
C-O-C bend	513
C-N-C bend	439

Mass spectral analysis

ESI Mass m/z calculated for $C_{21}H_{25}NO_2$ ($[M+H]^+$), found to be 324.20. It agrees with formula mass.

^{1}H NMR spectral analysis

The signals were assigned based on their positions and multiplicities. Chemical shift and coupling constant values suggest that the compound have chair conformation. The ^{1}H NMR spectral data are given in Table-2.

^{13}C NMR spectral analysis

The ^{13}C NMR spectral data confirm the Carbon skeleton of the compound. The ipso carbon or spiro carbon is confirmed by the signal at 95.08 and 107.86 ppm. ^{13}C NMR spectral data are given in Table-3

2D NMR spectral data

The NOESY spectrum and their spectral data are given in Fig-1 and Table - 4.

Table 2: ^{1}H NMR chemical shift values (ppm) of the compound

S.No	Proton assignment	Chemical shift	Multiplicity	Coupling constant (Hz)
1	$6CH_3$	0.360-0.495	Multiplet	4.8
2	$2CH_3$	1.208 - 1.25	Doublet	16.8
3	10Ha,e	1.740-1.803	Doublet	25.2
4	6Ha	2.521 - 2.74	Multiplet	12, 13.6, 14.8, 12.
5	9Ha	3.198-3.337	Multiplet	8, 6.8
6	$0CH_2$	3.949-4.153	Multiplet	
7	7H	4.254	Broad singlet	
8	OCH	4.545	Broad singlet	
9	Phenyl protons	7.073-7.561	Multiplet	
10	NH proton	1.634	Broad singlet	

Table 3: ^{13}C NMR chemical shift values (ppm) of the compound

S.No	Carbon assignment	Chemical shift
1	$6CH_3$	9.66
2	$2CH_3$	18.81,18.88
3	C-10	40.42, 40.68
4	C-6	42.62,42.97
5	C-9	60.38,60.60
6	C-7	65.75, 65.89
7	OCH2	72.13
8	OCH	72.24
9	C-5 ipso	95.08, 107.86
10	Phenyl	126.05 -129.15
11	phenyl ipso	139.58, 142.83

Fig-1 NOESY Spectrum

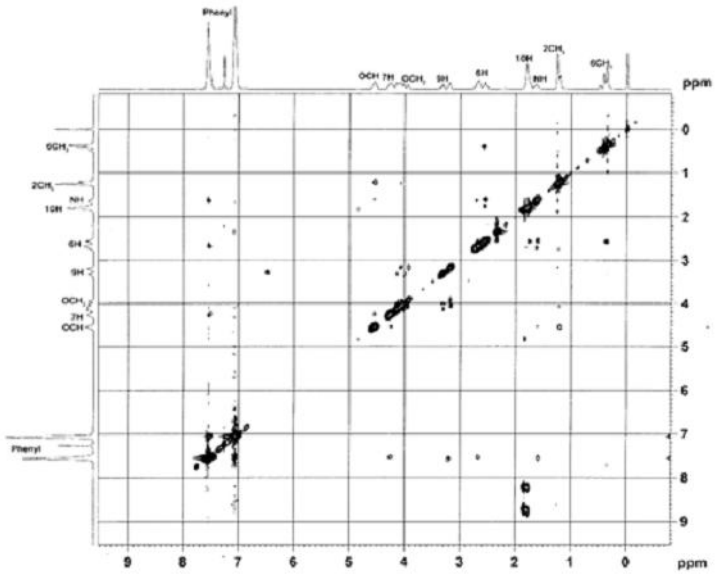


Table-4 NOESY spectral correlation of the compound

Proton/ Proton	**ppm**	**$6CH_3$ 0.36**	**$2CH_3$ 1.208**	**10Ha,e 1.74**	**6H 2.521**	**9H 3.198**	**OCH2 3.949**	**7H 4.254**	**OCH 4.545**	**Phenyl 7.073**	**NH 1.634**
$6CH_3$	**0.36**				Bonded						
$2CH_3$	**1.208**						Bonded		Bonded		
10Ha,e	**1.74**				Bonded						
6H	**2.521**	Bonded		Bonded						Bonded	
9H	**3.198**						Bonded			Bonded	
OCH2	**3.949**		Bonded			Bonded				-	
7H	**4.254**								Bonded	Bonded	
OCH	**4.545**		Bonded					Bonded			
Phenyl	**7.073**				Bonded	Bonded		Bonded			Bonded
NH	**1.634**							-		Bonded	-

Table-5 Minimum inhibitory concentrations of 2,6-dimethyl-7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5]decane (ug/ml)

	P.notatum	*C.albicans*	*Mucor sp*	*A. niger*	*F.solani*
2,6-dimethyl-7,9-diphenyl-1, 4-dioxa-8-azaspiro[4.5]decane	31.25	31.25	15.625	15.625	15.625
Amphoterecin B	7.81	7.81	3.91	3.91	3.91

3.5.2 NOESY Spectral analysis

NOESY spectral correlations are given in Table-4**.** The multiplet 6CH_3 protons at 0.360 ppm nOe with 6H.The 2CH_3 protons at 1.208 ppm strong nOe with OCH_2 and OCH .The NH proton at 1.634 nOe with phenyl protons. The 10Ha,e at 1.740 ppm nOe with 6H proton. The 9H proton at 3.198 ppm nOe with OCH_2 and phenyl protons. The phenyl proton nOe with 6H, 9H, 7H and NH. The most probable structure is given in Fig-2.

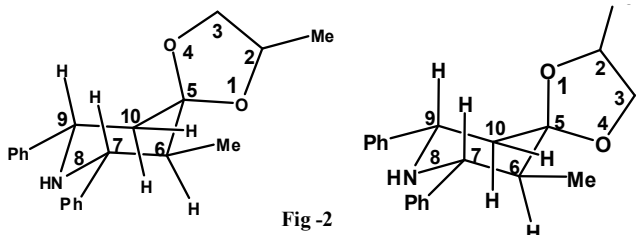


Fig -2

3.6 Antifungal activity of compound

The ethanolic extract of synthesized spiro heterocyclic compound was active against all the fungal pathogens tested namely, *Penicillium notatum*, *Candida albicans*, *Mucor sp*, *Aspergillus niger and Fusarium solani*. Minimum Inhibitory Concentrations (MIC) of the ethanol extracts of 2,6-dimethyl-7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5]decane against the five pathogens are depicted in Table -5. The maximum inhibitory activity with MIC value of 15.625 µg ml^{-1} in controlling the growth of *Mucor sp, A. niger* and, *F. solani* and 31.25 µg ml^{-1} for *P. notatum C. albicans* was recorded. The positive control Amphoterecin B was inhibitory to all the tested pathogen at MIC value of 3.291 µg ml^{-1} against *M. indicus A. niger* and, *F. solani and 7.81* µg ml^{-1} against *P. notatum and C. albicans*.

CONCLUSION

2,6-dimethyl-7,9-diphenyl-1,4-dioxa-8-azaspiro[4.5] decane was synthesized by condensation of t(3)-methyl-r(2),c(6)-diphenylpiperidin-4-one with Propane-1,2-diol. The spectral data revealed that the compound exhibits chair conformation with all the substituent's in equatorial orientations. The compound was inhibitory to the tested pathogens with MIC value of 15.625 µg ml^{-1} for *M. indicus A. niger* and, *F. solani* and 31.25 µg ml^{-1} for *P. notatum and C. albicans*.

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