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The Evaluation Of Antifungal And Antioxidant Activity Of The Chromeno [4,3-B]Pyrrole From Baylis-Hillman Adducts Derived From Nitro Olefins Compounds

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Abstract
Most of the new compounds synthesized such as ***chromeno [4,3-b]pyrrole,*** *coumarin, oxindole annulated vicinal dispiropyrrolidines / pyrrolizidines, tricyclic quinolinopyrrolidines, tetracyclic quinolinopyrrolizidines, quinolinopyranpyrimidinediones,tetra and pentacyclic tetrahydro quinolinopyranpyrimidinediones / pyrazoles / coumarins were tested for antimicrobial activities of all human pathogens [Bacillus subtilis, Staphylococcus aureus,Escherichia coli, Vibrio cholera, Proteus mirabilis], antioxidant activities, antifungal activities and the results are encouraging.*
Keywords:- *Baylis-Hillman adducts, antifungal activity, DMSO, human fungal pathogens, Candia krusei and Candidaalbicans.*

MATERIALS AND METHODS

Antifungal activity

The antifungal activity was done by agar well diffusion technique. The human fungal pathogens *Candia krusei* and *Candidaalbicans* were maintained in Sabouraud's dextrose agar (SDA). Both the fungus were inoculated in Sabouraud's dextrose broth (SDB) and incubated at 37°C for 8h. Then the pathogens were swabbed on the sterile SDA plates using a sterile swab and wells were made using a 9 mm (diameter) sterile cork borer. The chromenopyranpyrazole compounds **(5a-k)** were added to each well at different concentrations. The commercial antibiotic Fluconazole serves as a positive control and the DMSO serves as a control. The antifungal assay plates were incubated at 28±2°C for 24 h and the zone of inhibition was observed after incubation.

Minimum inhibitory concentration: (MIC)

The minimum inhibitory concentration was done by broth dilution technique and to determine MIC, the test compound was serially diluted in Saborauds dextrose broth (SDB) as follows 1µg/mL, 500 µg/mL, 250 µg/mL to 1.9 µg/mL in the test tubes. To all the test tubes fungal suspensions at a concentration of $1x10^5$cfu/mL was added and incubated at 37°C for 24 h. The MIC was taken as the lowest concentration of test compound which inhibits the fungal growth completely. The growth inhibition of fungi (lack of turbidity) was determined visually.

Structure of the chromeno [4,3-b]pyrrole Compounds (5a-kk) Derivatives Utilized for Antibacterial and Antioxidant Activities

RESULTS:

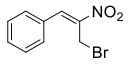
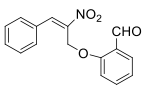
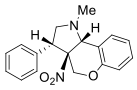
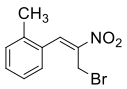
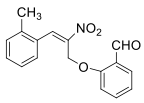
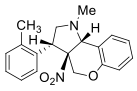
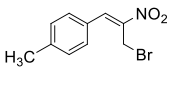
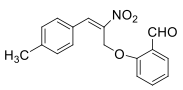
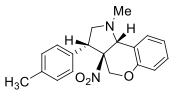
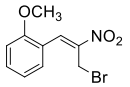
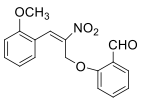
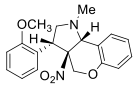
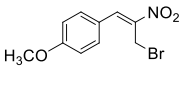
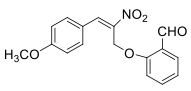
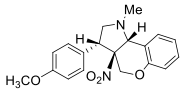
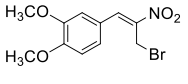
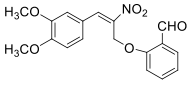
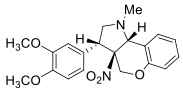
The *Candida* species are now identified as the important cause of hospital-acquired infection. Among *candida* species, the *C.albicans* is the most often associated with serious fungal infection and showing increased resistance to traditional antifungal agents (Jarvis, 1995)[1-5]. Recently other species such as *C.*

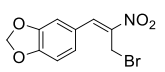
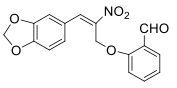
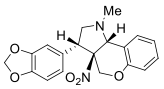
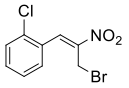
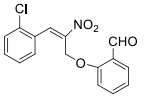
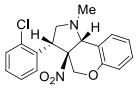
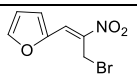
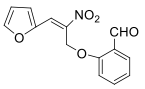
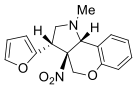
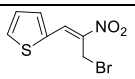
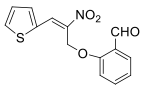
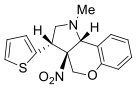
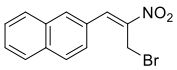
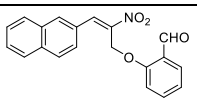
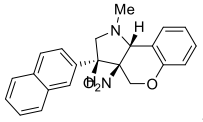
glabrata, *C. krusei* and *C.tropicalis* have also shown increases in fungal infections and antifungal resistance (Nguyen *et al*., 1996)[6-8]. The increase in drug resistance by these pathogens demonstrates the urgent for new antifungal antibiotics. In this research the **chromeno [4,3-b]pyrrole** are synthesized and screened for antifungal activity by well diffusion and broth dilution technique[9-12]. The **chromeno [4,3-b]pyrrole** shows excellent antifungal activity against the unicellular fungal pathogens *C. albicans* and *C. krusei* in the agar well diffusion assay.[13-15] The compounds **5d**, **5e** and **5i** were excellent against both the fungal pathogens showing high zone of inhibition at all concentrations followed by the compounds **5a, 5k** and **5b** with good activity. The compounds **5c** and **5g** showed moderate activity whereas the compounds **5f**, **5h** and **5j** showed weak activity when compared to other compounds. The results are tabulated in the table **5a&5b** respectively.

Table 1. Minimum inhibitory concentration of chromeno [4,3-b]pyrrole

In the broth dilution assay the compound **5i** shows very excellent activity with a MIC value of 7.81µg/mL and 3.90 µg/mL against *C albicans* and *C.krusei* respectively. The MIC for compounds **5c** and **5p** were same with a value of 7.81 µg/mL against both the fungus. The compounds **5e**, **5h** and **5j** shows good activity in broth dilution technique as similar to in the well diffusion assay. But the compounds **5g**, **5h** and **5k** shows weak activity with high MIC values 62.50 µg/mL. Thus the compound **5i** was proved to be best from these obtained results the compounds are represented as **C1-C11** in the figure 14 and 16 respectively.

Table 2. Synthesis of fused 1-methyl-3a-nitro-3-phenyl-1,2,3,3a,4,9b-hexahydro chromeno [4,3-b]pyrrole from Baylis-Hillman derivatives

Entry	Allyl bromides	Salicyaldehyde derivatives[a, b]	Yield (%)[c]	Chromenopyrrolidines[a, b]	Yield (%)[c]
1			65	 **5a**	76
2			72	 **5b**	85
3			74	 **5c**	73
4			72	 **5d**	70
5			71	 **5e**	70
6			82	 **5f**	72

7			70	 **5g**	75
8			67	 **5h**	80
9			72	 **5i**	73
10			78	 **5j**	71
11			75	 **5k**	74

[a]All reactions were carried out with 2 mmol scale of allyl bromide (**5a-k**) and 2-hydroxybenzaldehyde (2 mmol) in 10 ml of THF for 10min at room temperature. [b]All products gave satisfactory IR, ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), mass spectral data and elemental analyses.

Table2. Antifungal activity of chromeno [4,3-b]pyrrole by agar well diffusion assay:

Compounds	**Minimum inhibitory concentration (µg/mL)**	
	Candida albicans	***Candida krusei***
5a	62.50	62.50
5b	07.81	07.81
5c	15.62	07.81
5d	15.62	15.62
5e	62.50	62.50
5f	07.81	03.90
5g	62.50	62.50
5h	15.62	15.62
5i	07.81	07.81
5j	31.25	31.25
5k	31.25	15.62
Fluconazole*	**00.97**	**01.95**

*Fluconazole concentrations (1µg/mL) and 5 µL, 10 µL, 15 µL, and 25 µL were added in wells

Antioxidant Activity (DPPH radical scavenging activity)

The antioxidant activity of the sample was determined on the basis of their free radical scavenging activity and it was measured *in vitro* by using the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH). The DPPH is a stable free radical containing an odd electron in its structure and usually utilized for detection of the radical scavenging activity in chemical analysis. A solution of DPPH (0.1 mM) in methanol was prepared, and DPPH was added to test solution (pyrazoles at different concentrations (10-50 µg/mL). Incubated 30 min, after 30 min; the absorbance was measured at 517 nm using Beckman spectrophotometer. The percentage of free radical scavenging at different concentrations of pyrazoles was determined. The D-Ascorbic acid was used as a standard. The DPPH absorbs at 517 nm, and its concentration is reduced by the existence of an antioxidant. The method described by Hatano *et al.* (1988); Bhuiyan *et al.* (2009) was used as per reference, from the difference in absorbance on DPPH; the percentage of inhibition was calculated as a function of antioxidant activity. A dose response curve was plotted to determine the IC_{50} values.

The compounds were assessed for their capacity to scavenge the free radicals formation which was measured by DPPH assay. The antioxidant activities of the compounds were shown in table 10. The standard D-Ascorbic acid (Vitamin C) was used as a standard antioxidant agent and it shows activity of 91.68% of free radical scavenging at the concentration of 50 µg/mL. Compare to standard, the test compounds showed free radical scavenging activity range of 75-80% at the concentration of 50 µg/mL. The IC_{50} values for each compound was calculated and found that all the test compounds possess IC_{50} values in close ranges 27-35 µg/mL. There is no much difference between these compounds in scavenging the free radical. The best 50% of inhibition with low concentration is seen in compound **5c** with IC_{50} value of 27 µg/mL (Figure 1). The Vitamin C showed IC_{50} value at low concentration of 23 µg/mL (Figure 2; Table 3).

Compounds	Control (DMSO)	Well diffusion assay – Zone of Inhibition (dia. in mm)							
		Microbial strains							
		Candida albicans				*Candida krusei*			
		25µL	50µL	75µL	100µL	25µL	50µL	75µL	100µL
5a	–	–	–	16	27	–	–	18	20
5b	–	22	27	30	32	27	29	32	37
5c	–	17	21	27	30	26	29	31	36
5d	–	14	19	21	27	13	27	31	35
5e	–	–	–	15	32	–	–	18	33
5f	–	27	29	31	33	27	30	33	35
5g	–	–	–	13	22	–	–	15	19
5h	–	13	17	25	28	–	27	31	37
5i	–	25	27	30	32	27	31	33	35
5j	–	–	15	21	29	–	19	23	28
5k	–	–	13	22	31	–	16	28	31
Fluconazole*	–	27	31	33	35	23	27	31	38

Table 3. Antioxidant activity of test compounds measured by DPPH assay

Compound	Concentration of compound (µg/mL) and free radical scavenging (%)				
	10	20	30	40	50
5a	18.76	34.56	51.10	63.20	74.68
5c	19.10	35.54	54.56	65.00	80.50
5d	19.52	29.86	52.08	70.64	79.68
5e	18.64	30.32	52.98	60.38	74.64
5f	16.30	28.42	48.36	58.96	71.84
5g	20.01	39.76	52.12	67.13	79.42

5h	18.78	30.78	53.10	62.36	74.70
5i	16.81	35.46	49.22	61.24	74.90
5j	18.90	36.00	50.12	63.34	75.42
5k	15.20	26.54	43.90	56.48	68.22
5e	20.24	37.40	51.74	66.08	77.88
Ascorbic acid*	**26.42**	**48.24**	**62.74**	**78.12**	**91.68**

*Ascorbic acid was used as standard at 10, 20, 30, 40 & 50 µg/mL concentrations of compound

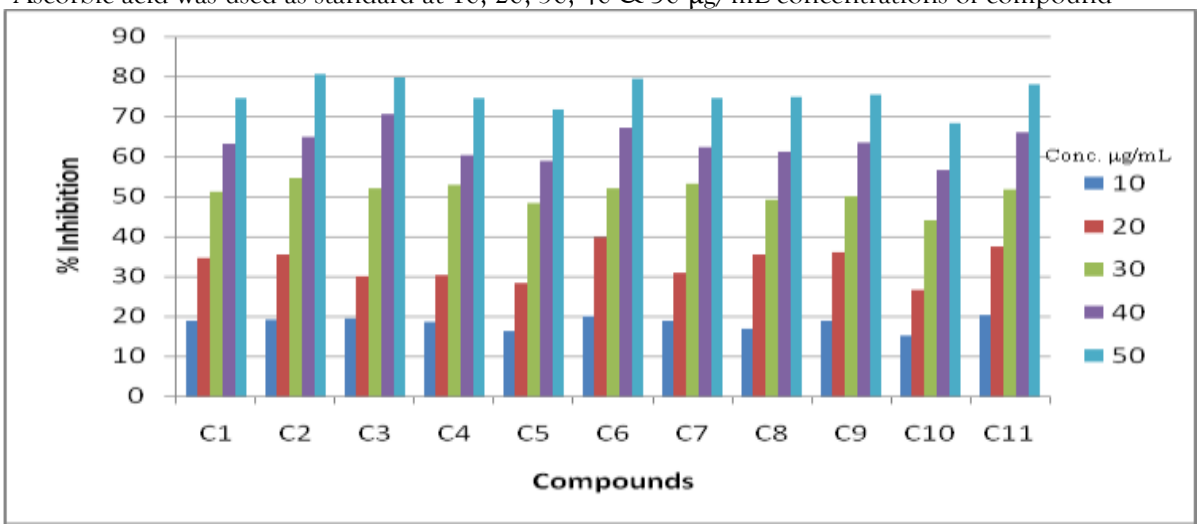


Figure 1. ***In vitro*** **DPPH radical scavenging activity of Test compounds:**

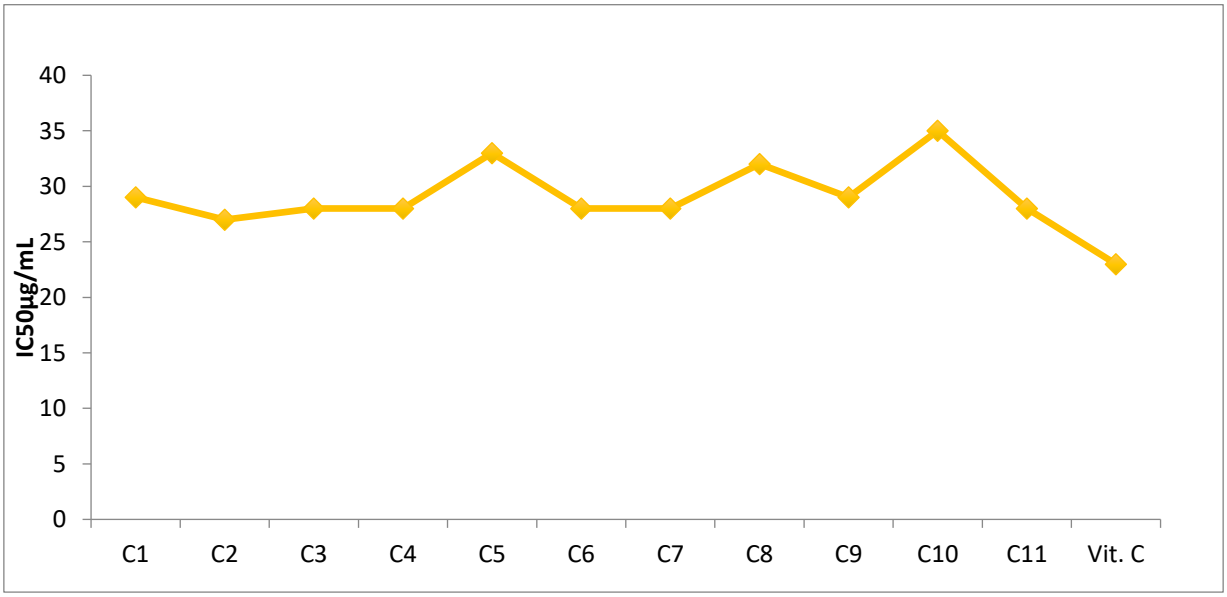


Figure 2. Free radical scavenging capacities of the compounds and Vitamin.C measured in DPPH assay

CONCLUSION

In conclusion, we have successfully developed a simple and novel protocol for the facile synthesis of complex angularly substituted tetracyclic frameworks containing a chromeno [4,3-b]pyrrole ring system *via* an intramolecular domino Knoevenagel hetero Diels-Alder reaction using Baylis-Hillman derivatives. This

reaction leads to a novel class of angularly substituted fused tetracycles which creating two new rings, three contiguous stereocentres and one tetrasubstituted carbon center in a unique fashion. Angularly substituted tetracyclic compounds were obtained in a highly stereoselective fashion with excellent yields. Some of the chromenopyranpyrazole molecules were tested for antifungal and antioxidant activities. The results are encouraging especially the compound **5i, 5e** and **5p**showed excellent antifungal activity against C. *albicans* and C. *krusei*. All the tested compunds possess IC_{50} values in close ranges 27-35 µg/ml. There is no much difference between these chromeno [4,3-b]pyrrole derivatives in scavenging the free radical. The compound **5c** showed best IC_{50} value of 27 µg/mL for antioxidant activity.

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