Asian Journal of Chemistry; Vol. 24, No. 10 (2012), 4361-4364

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Structure Activity Relationship Studies of Some Potent Antifungal Flavones, 4-Thioflavones and 4-Iminoflavones

AURANGZEB HASAN[1,*], E.U. MUGHAL[2] and A. SADIQ[2]

[1]Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur-50603, Malaysia
[2]Department of Chemistry, Bielefeld University, Universit¨atsstraße 25, Bielefeld 33615, Germany

*Corresponding author: Fax: +603 79674193; Tel: +603 79675165; E-mail: flavonoids@hotmail.com

(*Received*: 11 July 2011; *Accepted*: 2 May 2012) AJC-11388

A series of flavones, carrying halogens, methoxy and nitro groups at various positions were synthesized by the Baker-Venkataraman rearrangement and were subsequently converted to 4-thioflavones and 4-iminoflavones. The synthesized compounds were evaluated for their *in vitro* antifungal activity against *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata*. All synthesized compounds showed significant activity against *T. longusus*, *A. flavus*, *M. canis* and *F. Solani* but inactive against *C. albicans* and *C. glabrata*, respectively. 2-phenyl-4*H*-1-benzopyran-4-thiones were relatively more active than flavones and 4-iminoflavones. However, some compounds were even more active than standard miconazol and amphotericin B drugs.

Key Words: Flavones, 4-Thioflavones, 4-Iminoflavones, Synthesis, Antifungal activity.

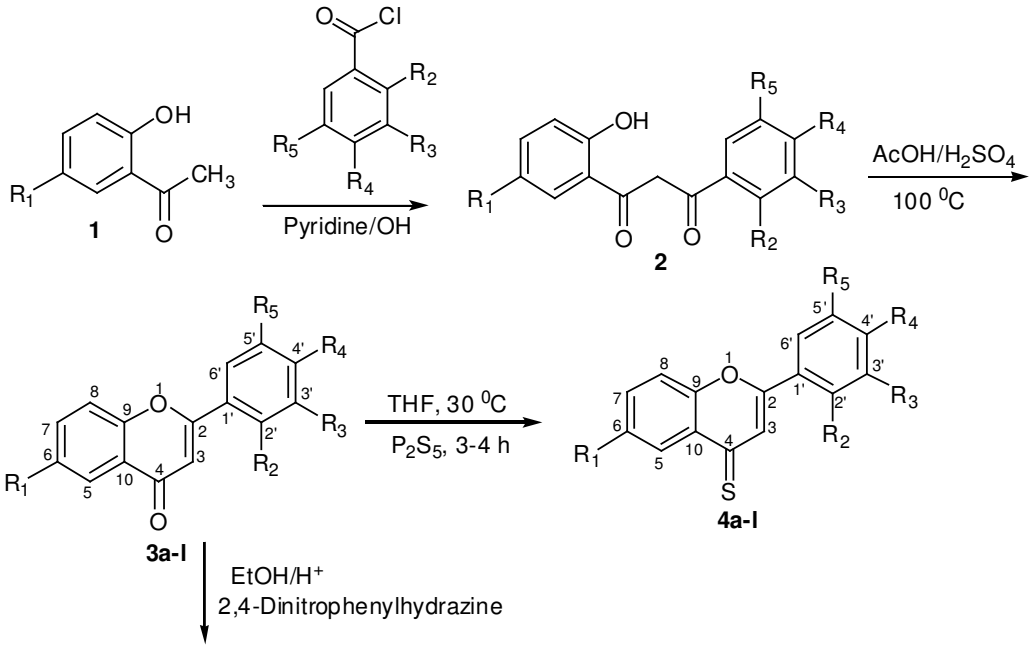
INTRODUCTION

Flavones (2-phenyl-4*H*-1-benzopyran-4-ones) are well known natural products of the plant kindom[1], some of which have been found to possess important bioactivities[2,3]. Large numbers of flavones are known to exhibit several activities like anticancer[4], antiviral[5], antitumor[7], antifungal[7], antioxidant[8], antiinflammatory[9], antibacterial[10]. 4-Thioflavones (2-phenyl-4*H*-1-benzopyran-4-thiones) are synthetic thioanalogue of the flavones. Comparatively less work has been carried out on these sulphur containing flavones. But thioanaloges of flavones and isoflavones have received considerable attention due to their photochemistry[11] and their usefulness as precursors for the synthesis of a variety of organic compounds[12,13]. Similarly, 4-iminoflavones (2-phenyl-4*H*-1-benzopyran-4-imines) are synthetic imines prepared from the direct reaction of flavones with primary amino compounds. Iminoflavone have been found to show antimicrobial and antimalarial activity[14]. It is well known that natural flavonoids are mostly hydroxylated, methoxylated, prenylated and glycosylated both in ring A and ring B and are mainly responsible for the reported biological activities. On the other hand halogenated, sulphur and nitro compounds are also biologically active but in nature no flavonoids have been found having these as substituents. The present work was therefore devoted to the synthesis of variably substituted flavones, 4-thioflavones and 4-iminoflavones either on ring A, B or both and to study the structure activity relationship (SAR) of these modified flavonoids.

Flavones (4*H*-1-benzopyran-4-one) (**3a-l**) were synthesized by the well known Baker-Venkataraman[15] rearrangement (**Scheme-I**). Whereas 5-substituted 2- hydroxyacetophenone (**1**) was converted to 1,3-diketone (**2**) by treating with benzoyl chloride in the presence of KOH and pyridine followed by reaction with H_2SO_4 and glacial acetic acid. Different flavones (**3a-l**), were converted to 4-thioflavone-4*H*-1-benzopyran-4-thione) (**4a-l**) by the reaction with phosphorus pentasulfide, solid sodium hydrogen carbonate and dry THF. Finally different 4-iminoflavones (**5a-l**)[16] were obtained by treating flavones (**3a-l**) with 2,4-dinitrophenyl hydrazine in the presence of EtOH/H_2SO_4 and the mixture was kept for 24 h[17,18].

EXPERIMENTAL

The synthesized compounds were tested by agar tube dilution method[19] for their *in vitro* fungicidal activity. Miconazol and amphoterine B (200 µg/mL) were used as positive control. All experiments were done in three replicates. Six fungal strains: *Trichophyton longifusus*, *Candida albicans*, *Aspergillus flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glabrata*. were used. 24 h old culture containing *ca.* 10^4-10^6 colony forming unit (CFU) was spread on the surface of Muller Hinton agar (MHA) plates. Wells were created in the medium with the help of a sterile metallic borer. Test samples of different



	R_1	R_2	R_3	R_4	R_5
3a, 4a, 5a	H	H	$-OCH_3$	H	H
3b, 4b, 5b	H	H	H	$-CH_3$	H
3c, 4c, 5c	H	H	H	$-NO_2$	H
3d, 4d, 5d	H	H	H	$-OCH_3$	H
3e, 4e, 5e	H	$-NO_2$	H	H	H
3f, 4f, 5f	H	H	$-NO_2$	H	H
3g, 4g, 5g	H	H	H	F	H
3h, 4h, 5h	H	H	$-OCH_3$	$-OCH_3$	H
3i, 4i, 5i	F	H	H	H	H
3j, 4j, 5j	F	I	H	H	H
3k, 4k, 5k	Cl	I	H	H	H
3l, 4l, 5l	Br	I	H	H	H

Scheme-I: Synthesis of flavones (**3a-l**), 4-thioflavones (**4a-l**) and 4-iminoflavones (**5a-l**)

concentrations were added in their respective wells. Experimental plates were incubated at 27 ºC for 7 days and zone of inhibition were measured and compared with standard drugs.

RESULTS AND DISCUSSION

Results were reported as linear growth inhibition (LG1) against some human pathogens (Table-1).

Considering the varied structure-activity relationships of different series of compounds, it cannot be inferred that the biological behaviour of a drug is determined by the influence of a single parameter or variable. Furthermore, in most cases, the presence or introduction of various functional groups in a compound does not allow to accurately explain the kind and intensity of its biological activity. However, taking necessary precautions, the information in Table-1 may allow us to make some general remarks on the structure and antifungal activity of the analyzed flavones (**3a-l**), 4-thioflavones (**4a-l**) and 4-iminoflavones (**5a-l**).

From Table-1, it is clear that antifungal activity of flavones increases on replacing oxygen atom with sulphur (4-thioflavones) while in case of 4-iminoflavones (replacing oxygen atom with nitrogen atoms at 4-position) the activity decreases or increases depending upon the tested fungi.

Furthermore, it was noticed that when flavones, 4-thioflavones and 4-iminoflavones have methoxy group in the B-ring at position 3', the linear percentage inhibition against *T. longifusus* and *A. flavus* was 65 and 35 (**3a**), 75 and 40 (**4a**) and 20 and 0 (**5a**), respectively as compared to the standard drugs miconazol which showed 70 and 0, whereas there was no inhibition against *T. longifusus* and *A. flavus* by the second standard drug amphotericin B. However, when a methoxy group was present at 4'-position in ring-B, the activity increased accordingly in flavones, 4-iminoflavones and 4-thioflavones. The same pattern of activity is found with the replacement of -OMe group in ring-B with $-NO_2$ group but the percentage of inhibition decreased relatively. Similarly compounds **3h**, **4h** and **5h** which have methoxy groups in B-ring at positions 3' and 4' showed strong antifungal activity against *T. longifusus*. Thus increase in activity is probably due to the resonance effect of -OMe group that is more prominent at 4'-position than 3'-position, whereas in case of a nitro group, it operates in the opposite direction.

TABLE-1
PERCENTAGE INHIBITION OF TESTED FUNGI BY FLAVONES (**3a-l**), 4-THIOFLAVONES (**4a-l**) AND 4-IMINOFLAVONES (**5a-l**) RELATED TO STANDARD DRUGS MICONAZOL AND AMPHOTERICIN B

Compound	*T. longifusus*	*C. albicans*	*A. flavus*	*M. canis*	*F. solani*	*C. glabrata*
3a	65	0	35	0	20	0
4a	75	0	40	0	0	0
5a	20	0	0	0	0	0
3b	45	0	30	0	0	0
4b	60	0	35	0	0	0
5b	50	0	0	0	0	0
3c	65	0	48	20	0	0
4c	70	0	55	0	0	0
5c	0	0	0	0	0	0
3d	0	0	50	55	0	0
4d	80	0	60	30	50	0
5d	50	0	35	40	0	0
3e	60	0	45	20	0	0
4e	68	0	0	35	0	0
5e	0	0	40	42	0	0
3f	50	0	40	0	0	0
4f	55	0	30	0	10	0
5f	40	0	0	0	0	0
3g	70	0	50	30	0	0
4g	80	0	50	35	15	0
5g	60	0	40	25	20	0
3h	70	0	45	40	40	0
4h	75	0	45	50	50	0
5h	55	0	0	0	0	0
3i	55	0	55	0	40	0
4i	60	0	60	0	50	0
5i	70	0	50	40	30	0
3j	60	0	40	50	70	0
4j	0	0	0	85	50	0
5j	80	0	60	70	60	0
3k	70	0	35	20	20	0
4k	0	0	0	0	70	0
5k	80	0	60	70	60	0
3l	55	0	55	50	70	0
4l	55	0	0	0	60	0
5l	80	0	0	0	0	0
Miconazol	70	110.8	0	98.4	73.25	110.8
Amphotericin B	0	0	20	0	0	0

Moreover, when a methoxy group was present at both positions *i.e.*, 3' and 4', then both groups mutually enhanced the activity against *T. longifusus*, *A. flavum*, *M. canis* and *F. solani*. Linear percentage inhibition against these four fungi, in this case (**3h**), was 70, 45, 40 and 40, respectively, whereas in case of **4h** percentage inhibition was enhanced to 75, 45, 50 and 50, respectively. The 4-imino analogue, **5h** exhibited lower percentage inhibition, 55 for *T. longifusus* and 0 for remaining three fungal strains. In the presence of electron-withdrawing groups like $-NO_2$ at 2'- and 5' positions in ring-B (compounds **3e**, **4e** and **5e**), the percentage inhibition decreased further.

Flavones, 4-thioflavones and 4-iminoflavones having F atom at position 4' in ring-B showed significant percentage inhibition against *T. longifusus* and *A. flavum*, but were inactive against *C. albicans* and *C. glabrata*. This increase in activity is probably due to the presence of fluorine at 4'-position which is the most electronegative atom. However, compounds (**3b**, **4b** and **5b**) with a methyl group as a substituent on B-ring were found to be only active against *T. longifusus* and *A. flavum*.

The results show that the electron-donating and electronegative groups are responsible for the antifungal activity of flavones, 4-thioflavones and 4-iminoflavones provided these groups are present at 4'-position in ring-B rather than any other position. Additionally, increase in inhibition of thio-analogues is probably due to the larger size of the sulphur atom. Antifungal analysis of flavones (**3a-l**) revealed that all flavones except **3d** show good or moderate activity against *T. longifusus*, *A. flavus*, *M. canis*, *F. solani* but were inactive against *C. albicans* and *C. glabrata*, whereas their respective sulphur and nitrogen analogues exhibited significant activity against *T. longifusus*, *A. flavus*, however, they were also found to be inactive against *C. albicans* and *C. glabrata*.

A comparison of the antifungal activities of the three series of compounds (Table-1) show that the flavone **3l** having a bromine atom at 6-position in ring-A exhibit percentage inhibition of 55, 55 and 70 against *T. longifusus*, *A. flavus* and

F. solani and its respective thio- and imino- analogues exhibit 55, 0, 60 and 80, 0, 0, respectively, against the same fungal species. However, when the bromine atom in ring-A is replaced by chlorine (**3k**) and fluorine (**3j**), percentage inhibition is changed to 70 and 60 against *T. longifusus*, respectively. The same results are found in case of their respective thio-[**4k** (0 %), **4j** (0 %)] and imino-[**5k** (80 %), **5j** (80 %)] analogues, whereas standard drug miconazol shows 70 % inhibition. None of the compounds have inhibitory effect against *C. albicans* and *C. glabrata*. However, all compounds showed significant activity against *T. longifusus* and *A. flavus* and moderate activity against *M. canis* and *F. solani*.

It was also noticed that all 2-phenyl-4*H*-1-benzopyran-4-thiones (**4a-l**) were relatively more active than flavones and 4-iminoflavones. This enhanced activity is due to replacement of oxygen atom with sulphur at 4-position of flavones while same extent of activity is reduced in case of 4-iminoflavones where 2,4-dinitrophenylhydrazyl group is placed instead of oxygen atom at 4-position of flavone. Less activity was noted against *M. canis* and *F. solani*, the most potent compounds against *T. longifusus* were **4a**, **4d**, **4g**, **4h**, **5k**, **5l** with % inhibition of 75, 80, 80, 75, 80, 80, respectively while standard drug miconazole has 70 % inhibitory effect. These compounds exhibit even greater activity than the standard drugs miconazole and amphotericin.B and can easily replace these as antifungal drugs.

Conclusion

This research finding demonstrates that 4-thioflavones show greater antifungal activity than flavones and 4-iminoflavones. Furthermore, the electron donating groups such as -OCH_3 present at 4-position make these compounds more active towards the fungi under investigation. Similarly, compounds having fluorine group at 6-position also results in enhanced activity. Almost all the synthesized compounds have shown potent antifungal activity against *A. flavus* as compared to standard drug amphotericin B. Similarly compounds **4a**, **4d**, **4g**, **4h**, **5k**, **5l** exhibited stronger activity against *T. longifusus* as compared to standard drug miconazol. Interestingly, compounds of the series 4-iminoflavones did not show any activity against *A. flavus*, *M. canis*, *F. solani*, *C. albicans* and *C. glabrata*but compounds of the same series *i.e.*, **5i**, **5j** and **5k** exhibited significant antifungal activities against *T. longifusus*, *A. flavus*, *M. canis* and *F. solani*.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Dr. Khalid Khan, HEJ Research Institute of Chemistry, International Centre for Chemical Sciences, Karachi, for his cooperation in spectroscopic analysis.

REFERENCES

1. T.A. Geissman, The Chemistry of Flavonoid Compounds, Pergamon Press, Oxford (1962).
2. K. Rohn, Die Flavonoide; Eine Übersicht über ihre Physiologie, Pharmakodynamik und Therapeutisch Verwendung, Cantor Verlog, Aulendorf (1967).
3. M. Gabor, The Pharmacology of Benzopyran Derivatives and Related Compounds, Akademici Kido, Budapest (1988).
4. A.R. Ibrahim and Y.J. Abdul-Haji, *J. Steroid Biochem. Mol. Biol*., **37**, 257 (1990).
5. B.L. Deng J.A. Lepoivre and G. Lemiere, *Eur. J. Org. Chem*., 2683 (1999).
6. G. Kaur, M. Stetler-Stevenson, S. Sebers, P. Worland, H. Sedlacek, C. Myers, J. Czeck, R. Naik and E. Sausville, *J. Natl. Cancer Inst*., **84**, 1736 (1992).
7. M. Cotoras, C. Garica, C. Lagos, C. Folch and L. Mendoza, *J. Chil. Chem. Soc.*, **46**, 433 (2001).
8. P. Montana, N. Pappano, N. Debattista, V. Avila, A. Posadaz, S.G. Bertolotti and N.A. Garcia, *Can. J. Chem*., **81**, 909 (2003).
9. M.S.Y. Khan and S.M. Hasan, *Indian J. Chem.*, **42B**, 1970 (2003).
10. R.B. Palkar and H.E. Master, *Indian J. Chem.*, **39B**, 141 (2000).
11. P. de Mayo, L.K. Sydnes and G. Wenska, *J. Org. Chem*., **45**, 1549 (1980).
12. P. Reynaud, Y. El Hamad, C. Davrinche, T.X.E. Nguyen, G. Tran and A. Rinjard, *J. Heterocycl. Chem*., **29**, 991 (1992).
13. G.F. Meijs, E. Rizzardo, T.P.T. Le and Y.C. Chen, *Makromol. Chem.*, **193**, 369 (1992).
14. A.W. Raut, A.G. Doshi and P.B. Raghuwanshi, *Orient. J. Chem*., **14**, 337 (1998).
15. W. Baker, *J. Chem. Soc*., 1381 (1933).
16. E.U. Mughal, M. Ayaz, Z. Hussain, A. Hasan, A. Sadiq, M. Riaz, A. Malik, S. Hussain and M.I. Choudhary, *Bioorg. Med. Chem*., **14**, 4704 (2006).
17. E.U. Mughal, A. Hasan. R. Lubna, *Heterocycl. Commun*., **11**, 445 (2005).
18. R. Mozingo and H. Adkins, *J. Am. Chem. Soc*., **60**, 669 (1938).
19. M.I. Choudhary, Z. Dur-e-Shahwar, A. Parveen, I.A. Jabbar and Atta-ur-Rahman, *Phytochemistry*, **40**, 1243 (1995).