Pure Appl. Chem., Vol. 83, No. 9, pp. 1643–1650, 2011.
doi:10.1351/PAC-CON-11-02-11

Changing paradigm for drug development: A case study of natural products*

Atta-ur-Rahman[1,‡], M. Iqbal Choudhary[1,2], and Naik Tameen Khan[1]

[1]H. E. J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan; [2]Department of Chemistry, School of Sciences, King Saud University, Riyadh-11451, Saudi Arabia

Abstract: Natural products have been a rich source of lead identification for drug discovery and development. Over the last two decades, the impetus for natural product-based lead discovery in pharmaceutical industry has declined, resulting in a productivity and innovation crisis. Most of the focus is now on high-throughput screening of synthetic libraries of compounds against defined biological targets; however, discovery of new molecular entities (NMEs) is still declining in this postgenomic era. With the availability of modern purification, characterization, and yield optimization techniques, and with an established intellectual property rights (IPR) regime, it is now possible to circumvent the problems once associated with natural product-based drug discovery. Our work demonstrates that natural products can lead to cost-effective identification of lead molecules against a defined molecular target. During our recent studies, novel classes of urease and α-glucosidase inhibitors were initially obtained from medicinal plants, and based on these novel structural leads, synthetic libraries of structural analogues were synthesized for structure–activity relationship (SAR) studies. These synthetic analogues have shown potent inhibitory activities against the target enzymes, and their mechanisms of action were studied by kinetic, computational, and NMR-based methods.

Keywords: biscoumarins; α-glucosidase inhibitors; natural products; urease inhibitors.

INTRODUCTION

Modern drug development is an expensive and lengthy process which requires enormous investments and focused efforts of a large interdisciplinary team of scientists involving years of work and screening of thousands of compounds. This level of investment and human resource is only available with large multinational conglomerates (MNCs) [1]. Unfortunately, this situation has diminished the role of academic institutions and pharmaceutical R & D of developing nations in drug development. Ironically, the decision of developing a drug by MNCs is largely on the basis of commercial reasons rather than human needs. As a result, a large number of diseases affecting the lives of poor populations of the south remain untreated [2]. This situation demands a major rethinking and change by pharmaceutical scientists who wish to serve humanity through the skills they possess. This change in attitude and approach must involve a number of measures such as the effective use of indigenous knowledge and resources, cost-effective preclinical and clinical studies, and rapid and free-of-cost regulatory approval, etc. [3].

*Paper based on a presentation made at the 11th Eurasia Conference on Chemical Sciences, The Dead Sea, Jordan, 6–10 October 2010. Other presentations are published in this issue, pp. 1643–1799.
‡Corresponding author: Fax: +92-21-34819018-9; E-mail: hej@cyber.net.pk

Natural products and their traditional uses combined with modern scientific advancement can play a very important role in lead identification for drug development for the poor masses by researchers of the developing world. A mission-based approach is required to increase access to much needed drugs at affordable prices in the drug development process, parallel to MIT's $100 laptop, or TATA's under $2,000 Nano car.

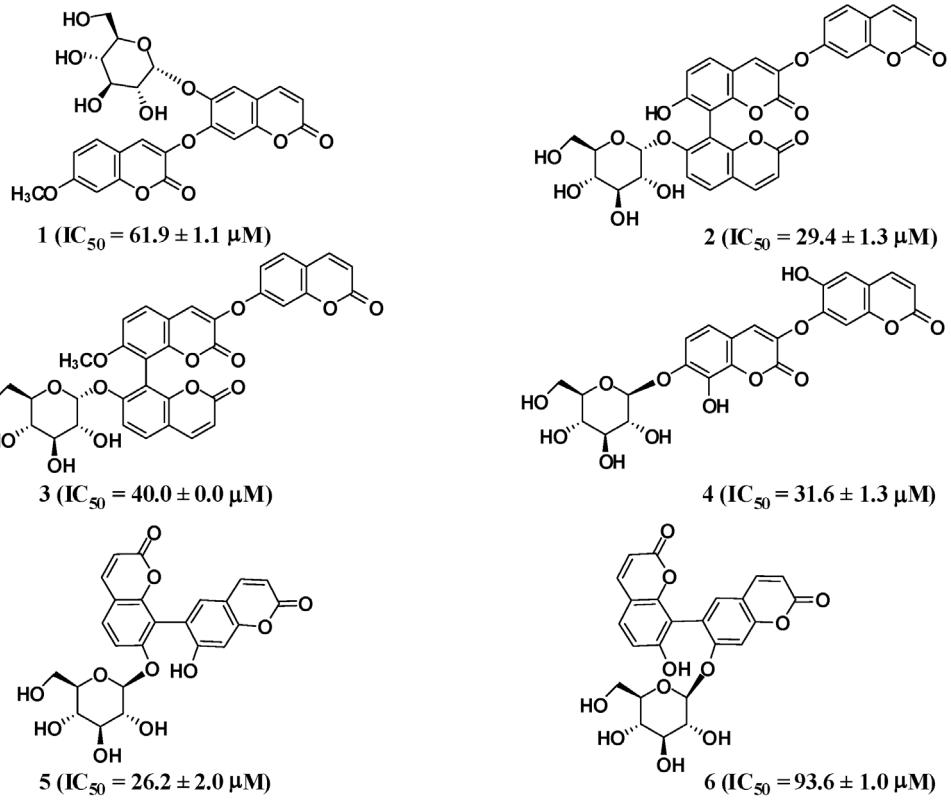
The primary focus of our studies has been to identify new lead molecules by using appropriate conventional and mechanism-based biological screenings on natural products. Some of the results of recent studies are reviewed here.

NATURAL ENZYME INHIBITORS

Enzymes play a vital role in regulating physiological activities. Several enzymes are found to be involved in the development of various diseases. Inhibition of such enzymes in vivo can be an effective strategy to treat diseases. We have discovered several novel classes of natural products, capable of inhibiting the activity of clinically important enzymes, such as urease and α-glucosidase in vitro.

Urease inhibitors

Urease has been an important virulence factor in the pathogenic conditions associated with human and animal health. This enzyme is known to be a major source of gastric and peptic ulcers, induced by *Helicobacter pylori*. Urease of *Proteus mirabilis* is also directly involved in urolithiasis as well as pyelonephritis, hepatic coma, and urinary catheter encrustation [4]. Thus, urease enzyme has been an



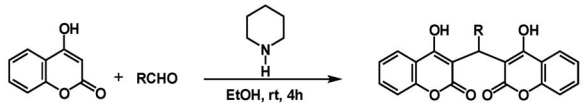
Scheme 1 Structures of natural coumarins isolated from *Daphne oleoids*.

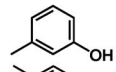
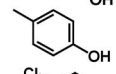
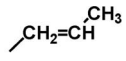
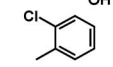
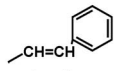
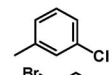
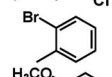
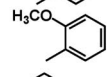
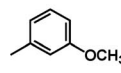
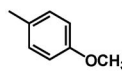
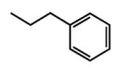
important target of our research, which has led to the discovery of several new classes of inhibitors. During the systematic search of new urease inhibitors, we initially discovered that *O*- and *C*-linked biscoumarins **1–6**, isolated from *Daphne oleoides*, have shown strong inhibitory activities (Scheme 1) [5].

However, the *C*-linked biscoumarins **2** and **5** with IC_{50} values 29.4 and 26.2 μM, respectively, were found to be the most active. A closer inspection of the structures of the natural coumarins showed that in all these compounds phenolic substituents were present, which resulted in a pronounced affinity for urease as compared to those compounds having methoxy substituents, except for compound **3**.

Based on this initial observation, we decided to synthesize a library of *C*-linked biscoumarins and compared their structure–activity relationship (SAR) with those of natural coumarins [6]. The least hindered biscoumarin **7** (R = H, IC_{50} = 15.0 μM) was found to be the most active member of the series (Table 1).

Table 1 Synthesis and urease (Jack bean) inhibitory activities of biscoumarins **7–27**.



Comp.	R	(IC_{50} ± SEM[a]) μM	Comp.	R	(IC_{50} ± SEM[a]) μM
7	-H	15.0 ± 0.0	**18**	$-(CH_2)_4CH_3$	86.3 ± 0.0
8	$-CH_3$	61.3 ± 0.2	**19**		71.9 ± 0.0
9	$-(CH_2)_2CH_3$	66.4 ± 0.0	**20**		73.7 ± 0.1
10		42.9 ± 0.0	**21**		84.5 ± 0.0
11		59.0 ± 0.0	**22**		72.9 ± 0.0
12		35.0 ± 0.0	**23**		73.8 ± 0.0
13		65.0 ± 0.0	**24**		81.8 ± 0.0
14		46.0 ± 0.0	**25**		59.8 ± 0.0
15		48.4 ± 0.0	**26**		55.3 ± 0.0
16		52.1 ± 0.0	**27**		67.3 ± 0.0
17	$-(CH_2)_3CH_3$	84.7 ± 0.0	Std.[b]	-	21.0 ± 0.1

[a]Standard error of the mean.
[b]Standard: thiourea.

The biscoumarins **1–27** inhibited the urease enzyme in a concentration-dependent fashion with K_i values ranging between 12.2–78.0 μM and 15.0–5.0 μM for the natural compounds **1–6** and synthetic compounds **7–27**, respectively. Lineweaver–Burk, Dixon plots and their secondary replots indicated that the compounds **1–6**, **8**, **10**, **14**, and **15** caused a competitive inhibition of urease because in these cases V_{max} remained unchanged, whereas K_m increased. Compounds **7**, **9**, **11–13**, and **16–27** displayed an uncompetitive inhibition because in these cases there was a decrease in both V_{max} and K_m values.

Modern NMR experiments, such as saturation-transfer difference (STD) and transfer nuclear Overhauser enhancement (TrNOE), and computational techniques like comparative molecular field analysis (CoMFA) and similarity indices analysis (CoMSIA) were employed on compounds **7–27** in order to identify the structural features in biscoumarins responsible for high affinity toward urease (Table 1).

The STD and TrNOE experiments clearly indicated that the aromatic protons experienced a saturation transfer from the protein (urease), which suggested the proximal contacts of phenyl rings of biscoumarins with the active site residues of the protein. This helped us to identify epitopes in ligand molecules. Moreover, CoMFA and CoMSIA analysis suggested that the electron-rich moieties (phenyl ring, lactone, and hydroxyl) interacted favorably with electropositive Ni ions in the active site of urease. CoMSIA analysis and docking studies of the various factors responsible for high affinity of the various biscoumarins is shown in Figs. 1–3.

The most potent urease inhibitor **7** was subjected to molecular docking simulation studies in order to understand its mechanism of inhibition. Docking studies indicated that the complex of compound **7** with urease is stabilized mainly through chelation of phenyl ring, which is symmetrically placed between the positively charged Ni ions in the active site and thus hinder the entry of substrate. The demonstration of Ni-phenyl interactions using a competitive inhibitor, where the spectrally observed *Kd* is identical to the *Ki*, provides strong support for a mechanism where urea also binds to the Ni metallocenter. Hydrogen bondings of the ligand **7** with the Arg 339 (3.15 Å) and His 323 (3.09 Å) provide further stability. Hydrophobic contacts with the certain residues (His 275, Gly 280, Ala 366, Met 367, Ala 170, His 137, Asp 363, His 139, and His 222) also contribute to potent competitive inhibition by compound **7**. All other ligands, which inhibited the urease (*Bacillus pasteurii*) competitively, placed themselves in the active site and their phenyl rings were symmetrically placed between the two positively charged Ni ions. However, their hydrogen bonding and hydrophobic interactions were found to be different.

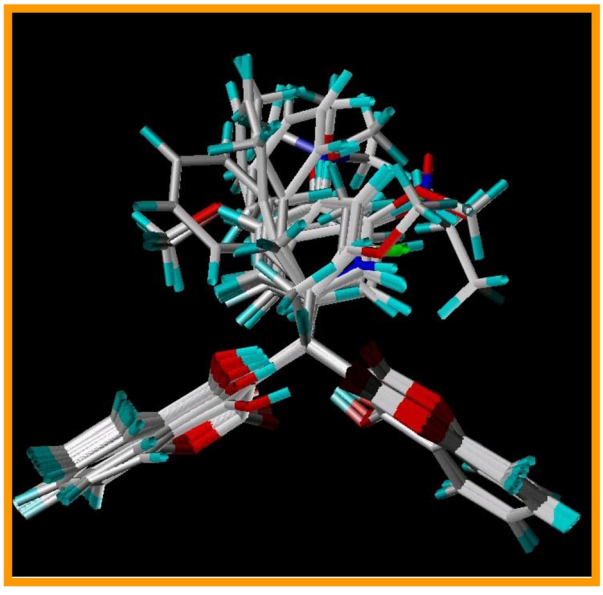


Fig. 1 Plot of all the twenty-one biscoumarins **7–27** used for 3D-QSAR CoMSIA analysis.

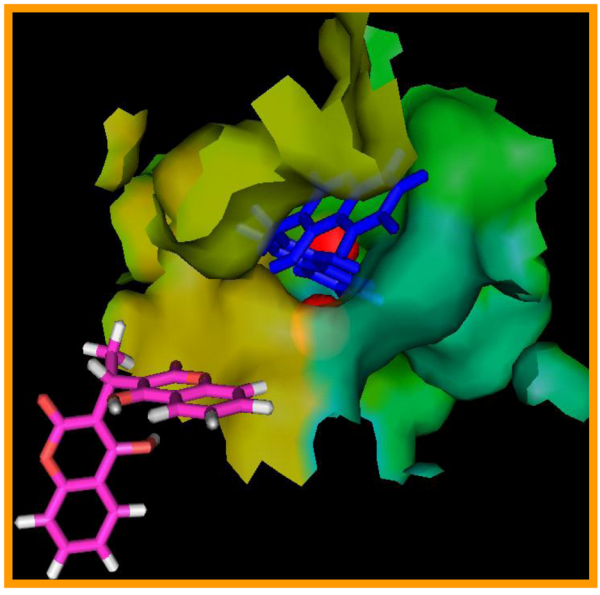


Fig. 2 The interactions of compounds **7** (blue) and **8** (red) with urease (*Bacillus pasteurii*). Ligand **7** is penetrated inside the active site and interacted with nickel metalo center of urease, where as ligand **8** was unable to reach into the narrow pocket.

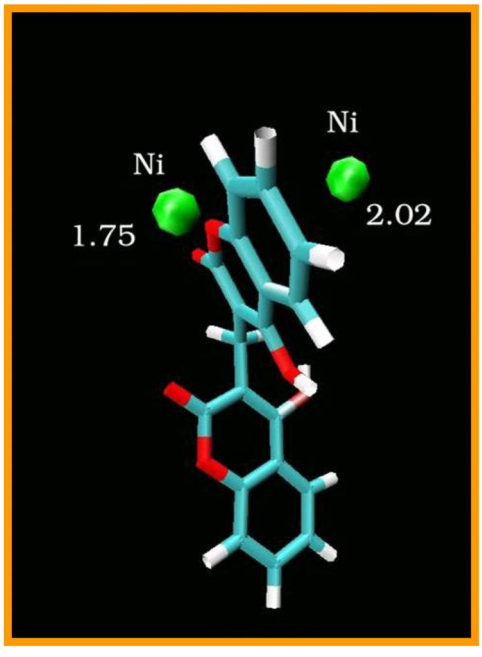


Fig. 3 A view showing that the benzene ring of ligand **7** is sandwiched between the two nickel ions in the active site of urease (*Bacillus pasteurii*).

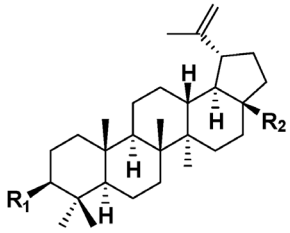
α-Glucosidase inhibitors

α-Glucosidase is an enzyme that catalyzes the conversion of disaccharides into monosaccharides, which can then be easily absorbed. The discovery of effective α-glucosidase inhibitors is considered to be an important therapeutic intervention in the management of type II diabetes. Moreover, α-glucosidase inhibitors are also used as antiobesity agents, fungistatic, antiviral, antifeedant, immuno-modulators, and inhibitors of tumor metastasis [7]. In continuation of our research for potent α-glucosidase inhibitors from antidiabetic plants, we have discovered several new classes of α-glucosidase inhibitors. Through a systematic compound library screening and bioassay-directed phytochemical studies on medicinal plants, we have discovered several new classes of α-glucosidase inhibitors, as shown in Table 2. All the tested compounds were far more active than the clinically used drugs (deoxynojirimycin and acarbose), used as standards in the study, except for the triterpenes **29**, **35**, and **36**, which were less active than deoxynojirimycin, but more active than acarbose. Compound **28**, isolated from *Diospyros mespiliformis*, was several folds more active than both the standards. Compounds **35** and **36** were obtained from the biotransformation of **34** with *Fusarium lini*. The introduction of additional hydroxyl functionalities in compound **34** decreased the activity. Similarly, the introduction of acetyl functionality in **28**, **32**, and **37** to afford **31**, **33** (also occurs naturally), and **38**, respectively, also decreased the α-glucosidase inhibitory potential. This helped in the identification of structural features responsible for α-glucosidase inhibitory activity (Scheme 2).

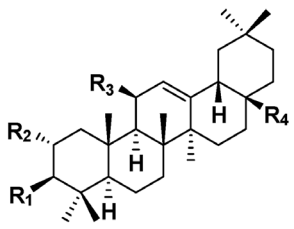
Table 2 α-Glucosidase inhibitory activities of compounds **28–47**.

Compound	(IC_{50} ± SEM[a]) μM	Source	Ref.
28	2.0 ± 0.0	*Diospyros mespiliformis*	8
29	460.0 ± 0.0	*D. mespiliformis*	8
30	62.4 ± 0.0	*D. mespiliformis*	8
31	66.8 ± 0.0	Acetylation of **28**	8
32	10.5 ± 0.5	*Millettia conraui*	9
33	22.2 ± 0.1	*Tabernaemontana dichotoma*	10
34	12.8 ± 0.0	*Musanga cecropioides*	11
35	444.0 ± 8.0	*Biotransformation*	11
36	666.0 ± 20.0	*Biotransformation*	11
37	51.9 ± 1.1	*Cichorium intybus*	12
38	10.0 ± 0.6	Acetylation of **37**	12
39	6.3 ± 0.2	*Harungana madagascariensis*	13
40	12.0 ± 0.3	*H. madagascariensis*	13
41	21.9 ± 1.2	*H. madagascariensis*	13
42	6.0 ± 0.1	*H. madagascariensis*	13
43	192.3 ± 0.0	*H. madagascariensis*	13
44	84.3 ± 3.0	*Myrtus communis*	7
45	46.6 ± 0.0	*M. communis*	7
46	35.4 ± 1.15	*M. communis*	7
47	39.9 ± 1.0	*M. communis*	7
Acarbose (**48**)[b]	780.0 ± 0.0	–	–
Deoxynojirimycin (**49**)[b]	425.6 ± 8.1	–	–

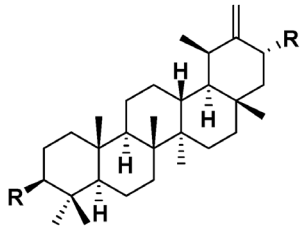
[a]Standard error of the mean.
[b]Standard used.



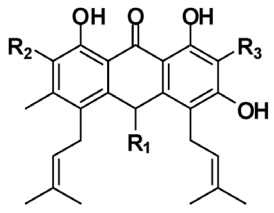
28. $R_1 = OH, R_2 = CH_3$
29. $R_1 = OH, R_2 = CH_2OH$
30. $R_1 = O, R_2 = CH_3$
31. $R_1 = CH_3COO, R_2 = CH_3$

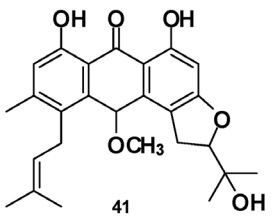


32. $R_1 = OH, R_2 = R_3 = H, R_4 = CH_3$
33. $R_1 = CH_3COO, R_2 = R_3 = H, R_4 = CH_3$
34. $R_1 = OH, R_2 = R_3 = H, R_4 = COOH$
35. $R_1 = R_2 = OH, R_3 = H, R_4 = COOH$
36. $R_1 = R_2 = R_3 = OH, R_4 = COOH$

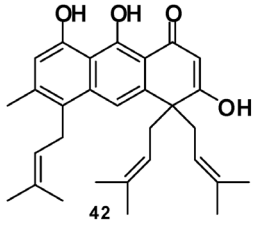


37. R = OH
38. $R = CH_3COO$



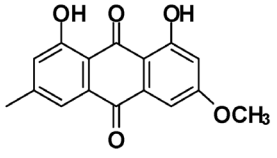


41

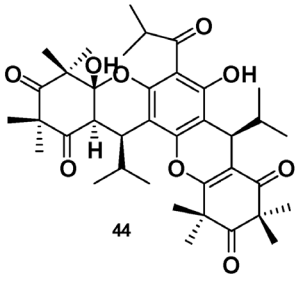


42

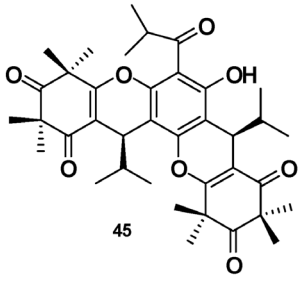
39. $R_1 = OH, R_2 = H, R_3 = prenyl$
40. $R_1 = R_3 = H, R_2 = prenyl$



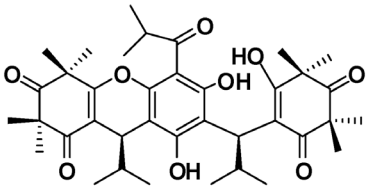
43



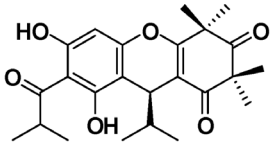
44



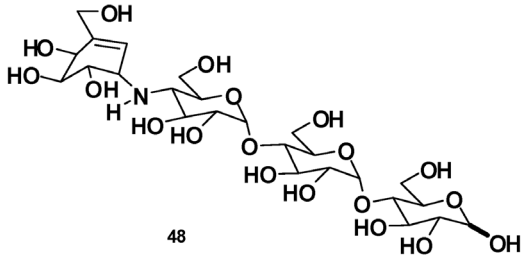
45



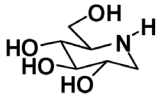
46



47



48



49

Scheme 2 Structures of natural α-glucosidase inhibitors.

CONCLUSIONS

In conclusion, the work presented herein suggests the advantage of natural products over combinatorial synthesis in chemical-diversity-based drug discovery process. Several new classes of enzyme inhibitors were discovered during this systematic study through an approach based on ethnobotanic information, bioassay-directed phytochemical investigations, medicinal chemistry, and modern computational and spectroscopic methods. This has also led to the identification of several exciting templates for future drug development.

ACKNOWLEDGMENTS

The research work presented in this manuscript was mainly carried out in collaboration with Prof. Dr. Abdul Malik, Prof. Dr. Khalid Muhammad Khan, Dr. Zaheer Ul-Haq, Prof. Dr. Mirihanage Don Jayantha Wijayabandara, and Dr. Farzana Shaheen, by a group of Ph.D. students and co-workers, Drs. Shamsun Nahar Khan, Muhammad Arif Lodhi, Sajjad Ali, Manzoor Ahmad, Nasim Hasan Rama, El Bushra E. El Nur, and Alembert T. Tchinda.

REFERENCES

1. G. M. Cragg, P. G. Grothaus, D. J. Newman. *Chem. Rev.* **109**, 3012 (2009).
2. P. J. Hotez, A. Fenwick, L. Savioli, D. H. Molyneux. *Lancet* **373**, 1570 (2009).
3. A. L. Harvey. *Curr. Opin. Chem. Biol.* **11**, 480 (2007).
4. I. Ahmad, I. Fatima, N. Afza, A. Malik, M. A. Lodhi, M. I. Choudhary. *J. Enzym. Inhib. Med. Chem.* **23**, 918 (2008).
5. M. Ayaz, M. A. Lodhi, M. Riaz, Azhar-ul-Haq, A. Malik, M. I. Choudhary. *J. Enzym. Inhib. Med. Chem.* **21**, 527 (2006).
6. K. M. Khan, S. Iqbal, M. A. Lodhi, G. M. Maharvi, Zia-Ullah, M. I. Choudhary, Atta-ur-Rahman, S. Parveen. *Bioorg. Med. Chem.* **12**, 1963 (2004).
7. F. Shaheen, M. Ahmad, S. N. Khan, S. S. Hussain, S. Anjum, B. Tashkhodjaev, K. Turgunov, M. N. Sultankhodzhaev, M. I. Choudhary, Atta-ur-Rahman. *Eur. J. Org. Chem.* 2371 (2006).
8. I. E. Mohamed, El B. E. El Nur, M. I. Choudhary, S. N. Khan. *Rec. Nat. Prod.* **3**, 198 (2009).
9. A. T. Tchinda, S. N. Khan, V. Fuendjiep, F. Ngandeu, A. N. Ngane, M. I. Choudhary. *Chem. Pharm. Bull.* **55**, 1402 (2007).
10. M. D. J. Wijayabandara, S. N. Khan, M. I. Choudhary. U.S. Patent 0103201, Filed 26 October 2006, Issued 1 May 2008.
11. M. I. Choudhary, I. Batool, S. N. Khan, N. Sultana, S. A. A. Shah, Atta-ur-Rahman. *Nat. Prod. Res.* **22**, 489 (2008).
12. Atta-ur-Rahman, S. Zareen, M. I. Choudhary, M. N. Akhtar, S. N. Khan. *J. Nat. Prod.* **71**, 910 (2008).
13. S. F. Kouam, S. N. Khan, K. Krohn, B. T. Ngadjui, D. G. W. F. Kapche, D. B. Yapna, S. Zareen, A. M. Y. Moustafa, M. I. Choudhary. *J. Nat. Prod.* **69**, 229 (2006).

