Est. 1984

ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2011, Vol. 27, No. (3):
Pg. 1101-1108

Synthesis of Bis-amide and Hydrazide containing Derivatives of Malonic Acid and Thiophenoladducts of Acidhydrazones Derived from 2-[(N-benzoyl) 2, 5-dichloroanilido] Acetohydrazide

RAJ NARAYAN SHARMA[1], K.P. SHARMA[2], S.N. DIKSHIT[2] and MANORMA SHARMA[2]

[1]Hindustan Institute of Technology Science and Management (R.G.P.V. University) (India).
[2]SMS Govnerment Model Science College, (Jiwaji University) Gwalior - 474 002 (India).
*Corresponding author: E-mail: rajnarayan1974@gmail.com

(Received: June 30, 2011; Accepted: August 02, 2011)

ABSTRACT

We have synthesized a new series of bis-amide and hydrazide-containing derivatives of malonic acid and thiophenoladducts of acidhydrazones by the reaction of 2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazide with various Carbonyl Compounds in 40 to 65% yield. Newly prepared compounds have been tested for their anti-bacterial activity against gram positive bacteria *S.albus*, *S.aureus* and gram negative bacteria *E.coli* and *Pseudomonas piosineus*. The compound (4, 5, 7, 11, 14) shown significant activities and compound (1, 6, 15, 16) have shown moderate activity. The same compounds were tested for their anti-fungal activity against *Candida albicans, Aspergillus niger and Alternaria alternata* at concentration of 30 mg/ml using Savored dextrose agar media. The compound (*2, 8, 13, 15)* shown significant activities and compound (*3, 9, 17) have* shown moderate activity against *Candida albicans and Aspergillus niger.* All the other compounds did not show significant activity against the fungi at the concentration used.

Key words: Malonicacid, Bis-amides, Acidhydrazides, Hydrazone-thiophenoladducts.

INTRODUCTION

Acidhydrazides and their condensation products possessing an azometine -NHN=CH- Proton constitute an important class of compounds for new drug development. In the past several years, numerous compounds with diverse structural features have been reported. Therefore, many researchers have synthesized these compounds as target structures and evaluated their biological activities. Hydrazides, hydrazones and their adducts have displayed diverse range of biological properties such as potential biological activities[1-6], anti-viral[7-8], anti-tuberculosis[9-10], anti-tumor[11-18], anti-fungal[19-20], anti-convulsant[21], anti-helmintic[22], anti-malerial[23], anti- Inflammatory[24], anti-cancer[25-26], anti-proliferative[27-29], anti-oxidant[30], agricultural agents[31]. Therapeutic protocols for the treatment of HIV

infection are mainly based on the combined use of reverse transcriptase, protease, and more recently, of cell fusion and entry inhibitors. Although drugs targeting reverse transcriptase and protease are in wide use and have shown effectiveness, the rapid emergence of resistant variants, often cross-resistant to the members of a given class, limits the efficacy of existing antiretroviral drugs. Therefore, it is critical to develop new agents directed against alternate sites in the viral life cycle. Moreover, many selectively chloro-substituted organic compounds show peculiar pharmacological and agrochemical properties. The work reported herein was aimed at the preparation of some new thiophenoladducts of acidhydrazones with anticipated biological activities.

MATERIAL AND METHODS

Experimental

Anhydrous solvents and all reagents were purchased from, Sigma-Aldrich, B.D.H., Excel-R, Extra pure E. Merk quality, Acros or Carlo Erba. Reactions involving air- or moisture-sensitive compounds were performed under a nitrogen atmosphere using oven-dried glassware and syringes to transfer solutions. Melting points (m.p.) were determined using an electrothermal melting point or a Köfler apparatus and are uncorrected. Infrared (IR) spectra were recorded as thin films or nujol mulls on KBr plates with a Perkin-Elmer-781 IR or 983 -Spectrophotometer and are expressed in í (cm^{-1}). Nuclear magnetic resonance spectra (^{1}H-NMR) was determined in DMSO and recorded on a Varian XL-200 (200 MHz) or a Varian VXR-300 (300 MHz). Chemical shifts (ä scale) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) used as internal standard. Splitting patterns are designated as follows: s, singlet, d, doublet, t, triplet, q, quadruplet, m, multiplet, brs, broad singlet, dd, double doublet. The assignment of exchangeable protons (-O*H* and -N*H*) was confirmed by addition of D_2O. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel, F-254 plates. For flash chromatography Merck Silica gel-60 was used as stationary phase with a particle size 0.040-0.063 mm (230-400 mesh ASTM). Elemental analyses were performed on a Perkin-Elmer-2400 spectrometer, and were within ±0.6% of the theoretical values.

Synthesis of Ethyl-2-(2, 5-dichloroanilido) ethanoate [1]

A mixture of diethylmalonate (20ml) and 2, 5-dichloroaniline (10ml) was refluxed for forty five minutes in a round bottomed flask fitted with an air condenser of such a length (14") that ethanol formed escaped and diethylmalonate flowed back into the flask. Contents were cooled, ethanol (30 ml) was added, when malon-2, 5-dichlorodianilide separated out. It was filtered under suction. The filtrate was poured on to crushed ice (Ca160g) and stirred when ethyl-2-(2, 5-dichloroanilido) ethanoate precipitated as green mass. On recrystallization from aqueous ethanol (50%), ester was obtained as white crystals. Yield: 82%, M. P.: 89^{0}C, M. W.: 276. Anal. Calculation for $C_{11}H_{11}N_1O_3Cl_2$: Found: C 47.7, H: 4.0, O: 17.2, N: 5.1, Cl: 25.4, Calcd. C: 47.8, H: 4.0, O: 17.4, N: 5.1, Cl: 25.7. *IR [KBr]* V_{max} Cm^{-1} : 1665-1660 [C=O diketone], 1290 [-O- Ester], 760-755 [2,5-disubstituted benzene], 1090 [C-Cl Stretching], 1590, 1520 , 1440 [C=C ring stretching], 3150 [N-H Stretching], 3040[C-H aromatic], 1330-1322 [C-H Stretching]. *PMR (DMSO):* δ 4.42 (2H, s, CO-CH_2-CO), 4.0 (2H, s, NH_2), 7.4-8.6 (3H, m, Ar-H), 9.2 (1H, s, CO-NH D_2O exchangeable), 10.6 [1H, s, Ar-NH D_2O exchangeable].

Synthesis of Ethyl-2-[(N-benzoyl) 2, 5-dichloroanilido] ethanoate [2]

Benzoyl chloride (8.46 gm, 0.06 mol), dioxane (6 ml), ethyl-2-(2,5-dichloroanilido) ethanoate (16.5 gm, 0.06 mol) and triethylamine (6.06 gm, 0.06 mol) were placed in a round bottomed flask carrying reflux condensor having calcium chloride guard tube. The contents were heated on a boiling water bath for two hours and kept over night when triethylamine hydrochloride separated. It was filtered under suction and the filtrate was poured on to crushed ice (Ca180 g) and stirred when ethyl-2-[(N-benzoyl) 2, 5-dichloroanilido] ethanoate separated or solid. It was filtered under suction, dried and purified by recrystallization from aqueous methanol (1:1) in white crystals. Yield = 81 %, MP = 96°C, Analytical calculation for $C_{18}H_{15}N_1O_4Cl_2$: [FW = 380] , Calculated: N 02.95 , C 45.64, H 03.38 , O 13.50 , Cl 15.00, Found : N 02.94, C 45.62 , H 03.37 , O 13.52 , Cl 15.02., *IR [KBr]* V_{max} cm^{-1}*:* 1725 [C=O

diketone], 1310 [-C-O- Ester], 765 [2,5-disubstituted benzene], 1095 [C-Cl Stretching], 1580, 1525 , 1445 [C=C Ring stretching], 3165 [N-H Stretching], 3030[C-H aromatic], 1320-1330 [C-HStretching]., *PMR (DMSO):* δ 4.45 [2H, s, CO-CH_2-CO], 4.2 [2H, s, NH_2], 7.3-8.5 [3H, m, Ar-H], 9.5 [1H, s, CO-NH D_2O exchangeable], 10.9 [1H, s, Ar-NH D_2O exchangeable].

Synthesis of 2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazide [3]

Ethyl-2-[(N-benzoyl) 2, 5-dichloroanilido] ethanoate (10.98 gm, 0.03 mol), ethanol (8 ml) and hydrazine hydrate (15 ml, 70%) were mixed together and stirred for thirty five minutes. 2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazide was filtered under suction and recrystallised from ethanol in white crystals., Yield, 79%, MP = 176°C, MW 366, Analytical calculation for $C_{16}\ H_{13}\ N_3\ O_3\ Cl_2$: Calculated: N 09.04 ,C 41.32 ,H 03.01 ,O 10.33, Cl 15.28, Found: N 09.01, C 41.30, H 03.00, O 10.31, Cl 15.27. *IR [KBr]* V_{max} cm^{-1}*:* 3165 [N-H Stretching], 3050 [C-H aromatic], 1665 [C=O diketone], 1440 [C-Cl aromatic], 1590, 1525, 1440 [C=C ring stretching]. *PMR (DMSO):* δ 4.45 (2H, s, CO-CH_2-CO), 4.2 (2H, s, NH_2), 7.2-8.6 (3H, m, Ar-H), 9.5 (1H, s, CO-NH D_2O exchangeable), 10.7 (1H, s, Ar-NH D_2O exchangeable).

Synthesis of 2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazones [4]

2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazide (0.001 mol) and (0.001 mol) of aromatic aldehyde or ketone (carbonyl compound) dissolve in absolute alcohol and added 2-drops of conc. H_2SO_4 and stirred for 25 minutes. It was filtered under suction and recrystallised from hot ethanol., Yield: 92%, M.P= 223 ^{0}C, F.W: 455, Color: White, Analytical calculation for $C_{23}H_{18}O_3N_3Cl_2$ Calculated: N 12.04, C 54.85, H 03.71, O 09.14, Cl 20.28, Found: N 11.99, C 54.83, H 03.70, O 10.31, Cl 20.25, *IR Absorption band* (cm^{-1})*:* 3155 (N–H stretching), 2965–2975 (C–H aliphatic), 1660–1665 (C=O Ketone), 785–770 (C–Cl Stretching), 765 (2, 5-disubstituted benzene), *NMR Spectra: (d DMSO*), 2.28(2 H, s, CH_2), 4.22(1 H, s, NH), 6.90–7.5 (10 H, m, ArH. Synthetic strategy has been out lined in scheme-I. Mechanism for the formation of acid hydrazones is given in chart-I.

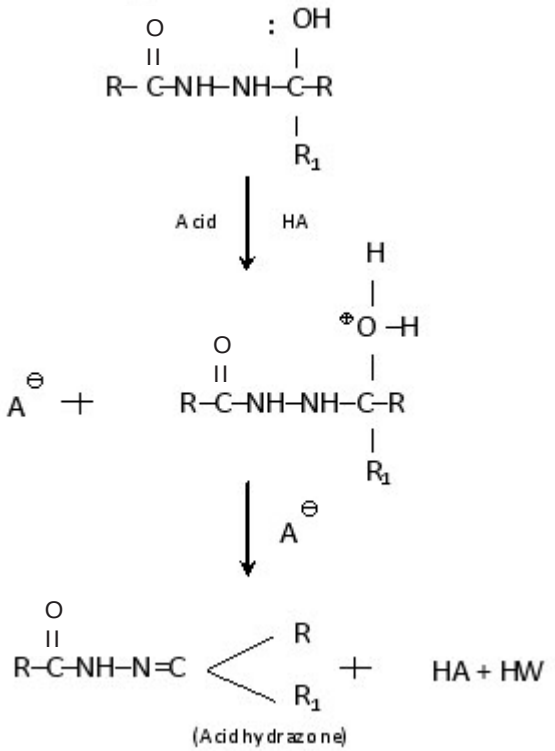


Chart 1: [Mechanism: formation of new acidhydrazones]

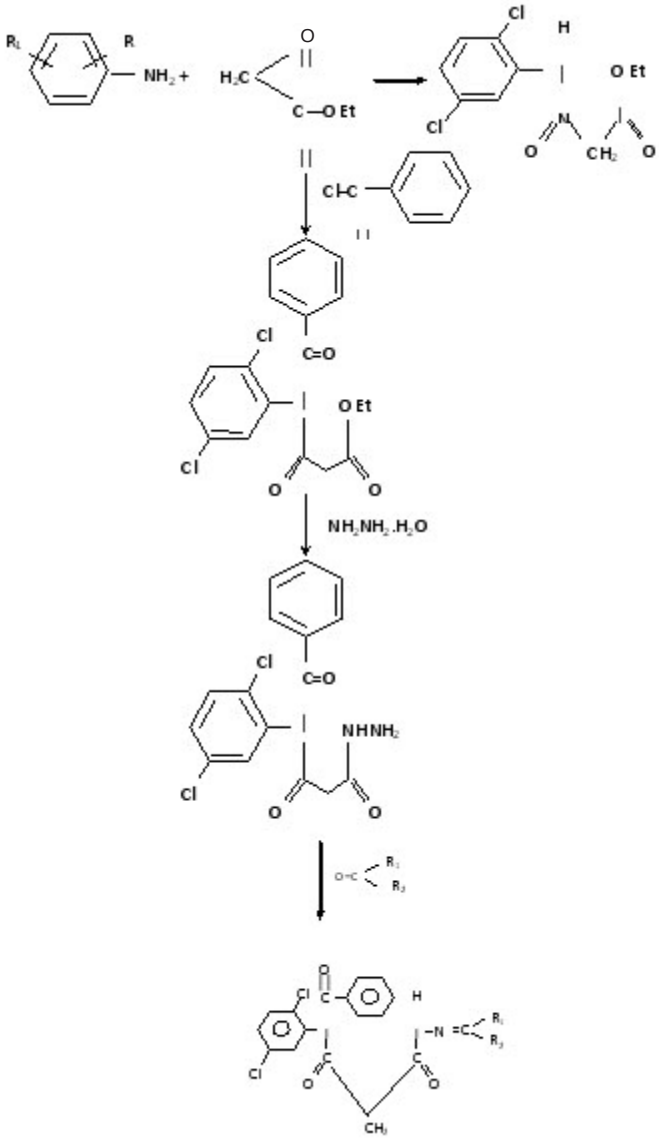
Biological evaluation

Anti-bacterial activity

Newly synthesized thiophenoladducts of acidhydrazones were screened for their anti-bacterial activity against the gram positive bacteria *S. albus, S. aureus* and gram negative bacteria *E. Coli* and *Pseudomonas piosineus* by agar plate disc diffusion method at 30 µg/mL concentration. *Ampicillin and tetracycline* were used as a reference compounds. The compound (*4, 5, 7, 11, 14) shown* significant activities and compound (*1, 6, 15, 16*) have shown moderate activity.

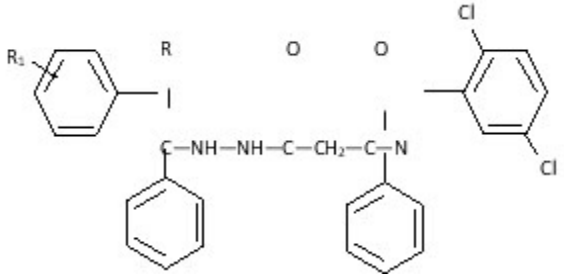
Anti-fungal activity

The same compounds were tested for their antifungal activity against *Candida albicans, Aspergillus Niger and Alternaria alternata* at concentration of 30 mg/ml using Savored dextrose agar media. The compound (*2, 8, 13, 15)* shown significant activities and compound (*3, 9, 17)* have shown moderate activity against *Candida albicans and Aspergillus niger.* All the other compounds did not show significant activity against the fungi at the concentration used.



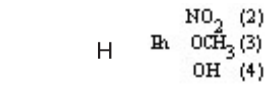
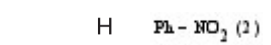
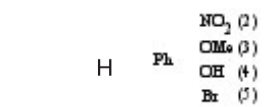
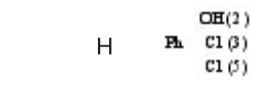
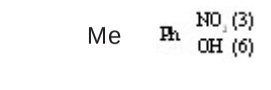
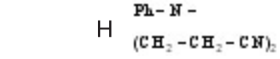
Scheme 1:

Table 1: Reaction conditions for the formation of thiophenol adducts of acidhydrazones.



(i) Quantity of acidhydrazone = 0.001 mol.
(ii) Quantity of benzene = 20 ml
(iii) Quantity of thiophenol = 0.110 g (0.001 mol)
(i) Hours of heating = 12 hours.

S. No.	Acidhydrazones	Quantity of acidhydrazone (g)	Adducts		MP (°C)	Yield (%)	Formula Weight	Molecular	Colour
			R_1	R_2					
01.	Benzaldehyde-2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazone	0.643	H	Ph	266	65	643	$C_{35}H_{30}O_3N_3Cl_2S_1$	White
02.	Vanilline-2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazone	0.690	H	Ph (OMe (3), OH (4))	242	61	690	$C_{34}H_{33}O_3N_3Cl_2S_1$	White
03.	5-chloro Salicylaldehyde-2-[(N-benzoyl) 2, 5-dichloro anilido] acetohydrazone	0.695	H	Ph (OH(2), Cl (5))	244	56	694.5	$C_{35}H_{31}O_4N_3Cl_3S_1$	White
04.	5-Bromo Salicylaldehyde-2-[(N-benzoyl) 2, 5- dichloroanilido] acetohydrazone	0.723	H	Ph (OH(2), Br (5))	235	58	723	$C_{35}H_{30}O_3N_3Cl_2BrS_1$	Silver White

05.	2-Nitro Vanilline-2-[(N-benzoyl) 2, 5-dichloroanilido]acetohydrazone	0.735	H		229	64	735	$C_{36}H_{32}O_7N_4Cl_2S_1$	Cream
06.	O-Nitrobenzaldehyde-2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazone	0.689	H		222	51	689	$C_{35}H_{30}O_5N_4Cl_2S_1$	White
07.	2-Nitro-5-Bromo Vanilline-2-[(N-benzoyl) 2, 5- dichloroanilido)] acetohydrazone	0.814	H		258	61	814	$C_{36}H_{31}O_7N_4Cl_2BrS_1$	Cream
08.	3,5-dichloro-2-hydroxy benzaldehyde-2-[(N-benzoyl) 2, 5- dichloroanilido] acetohydrazone	0.729	H		239	58	729	$C_{35}H_{29}O_4N_3Cl_4S_1$	White
09.	3-Nitro- 6-hydroxy acetophenone-2- [(N-benzoyl) 2, 5-dichloro anilido] acetohydrazone	0.719	Me		254	50	719	$C_{36}H_{32}O_6N_4Cl_2S_1$	Cream
10.	Acetone-2-[(N-benzoyl) 2, 5-di chloroanilido] acetohydrazone	0.595	Me	Me	246	64	595	$C_{31}H_{30}O_3N_3Cl_2S_1$	Cream
11.	2-Chlorobenzaldehyde-2-[(N-benzoyl) (2, 5-dichloroanilido)] acetohydrazone	0.578.5	H		257	47	578.5	$C_{35}H_{31}O_3N_3Cl_3S_1$	White
12.	4-NN-Bis-2'-cyanoethylamino benzaldehyde-2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazone	0.764	H		241	53	764	$C_{41}H_{37}O_3N_6Cl_2S_1$	Light brown
13.	2-Methyl-4-N-N-Bis-2'-cyanoethyl aminobenzaldehyde [(N-benzoyl) 2, 5-dichloroanilido] aceto hydrazone	0.779	H		262	65	779	$C_{42}H_{40}O_3N_6Cl_2S_1$	Brown

14.	2-Methoxy-4-N-N-bis-2'-cyanoethylamino benzaldehyde [(N-benzoyl) 2, 5- dichloro anilido] acetohydrazone	0.795	H		242	60	795	$C_{42}H_{40}O_4N_6Cl_2S_1$	Brown
15.	Acetophenone-2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazone	0.657	Me	Ph	231	54	657	$C_{36}H_{32}O_3N_3Cl_2S_1$	White
16.	Salicylaldehyde-2-[(N-benzoyl) 2, 5-dichloroanilido] aceto hydrazone	0.660	H	Ph – OH (2)	247	61	660	$C_{35}H_{31}O_4N_3Cl_2S_1$	White
17.	Anisicaldehyde-2-[(N-benzoyl) 2, 5-dichloroanilido] acetohydrazone	0.674	H	Ph – OCH_3 (2)	239	59	674	$C_{36}H_{33}O_4N_3Cl_2S_1$	Yellow
18.	β-Ionone-2-[(N-benzoyl) (2, 5-di chloroanilido] acetohydrazone	0.731	Me		258	40	731	$C_{41}H_{46}O_3N_3Cl_2S_1$	Buff

Solvent for crystallization - ethanol.

RESULTS AND DISCUSSION

Thiophenoladducts of various acidhydrazones have been synthesized by the reaction of 2-(2, 5-dichloroanilido) acetohydrazide with various Carbonyl compounds in 40 to 65% yield. Hydrazone-thiophenol adducts are white, brown and yellow colour solids, having high melting points. The structure of all the compounds are confirmed by IR, PMR, and Mass spectral data and are further supported by correct elemental analysis. Newly synthesized compounds have been tested for their *antibacterial activity* against gram positive bacteria *S. albus, S. aureus* and gram negative bacteria *E.Coli* and *Pseudomonas piosineus*. The compound (*4, 5, 7, 11, 14)* shown significant activities and compound (*1, 6, 15, 16*) have shown moderate activity. The same compounds were tested for their *antifungal activity* against *Candida albicans, Aspergillus niger and Alternaria alternata* at concentration of 30 mg/mL using savored dextrose agar media. The compound (*2, 8, 13, 15)* shown significant activities and compound (*3, 9, 17) have* shown moderate activity against *Candida albicans and Aspergillus Niger*. All the other compounds did not show significant activity against the fungi at the concentration used.

CONCLUSIONS

Newly synthesized compounds have been tested for their *antibacterial activity* against gram positive bacteria *S. albus, S. aureus* and gram negative bacteria *E.coli and Pseudomonas piosineus* by agar plate disc diffusion method at 30 ìg/mL concentration. *Ampicillin and tetracycline* were used as a reference compounds. The compound (*4, 5, 7, 11, 14)* shown significant activities and compound (*1, 6, 15, 16*) have shown moderate activity. The same compounds were tested for their *antifungal activity* against *Candida albicans, Aspergillus niger and Alternaria alternata* at concentration of 30 *albicans and Aspergillus niger.* All the other compounds did not show significant activity mg/mL using Savored dextrose agar media. The compound (*2, 8, 13, 15)* shown significant activities and compound (*3, 9, 17) have* shown moderate activity against *Candida* against the fungi at the concentration used.

ACKNOWLEDGEMENTS

The authors are thankful to Director, D.R.D.E. Gwalior, for spectral studies, and Director, Cancer Hospital and Research Institute, G.R. Medical College and Birla Institute of Medical Research, Gwalior, for biological activities. We are also grateful to principal SMS Government Model Science College, Gwalior, for providing research facilities.

REFERENCES

1. Rahman, V.M., Mukhtar, S., Ansari, W.H., Lemiere, G., *Eur. J. Med. Chem.* **40**: 173 - 184 (2005).
2. Dimmock, J.R., Vashishtha, S.C., Stables, J.P., *Eur. J. Med. Chem.* **35**: 241-248 (2000).
3. Yapia, R., La Mara, M.P., Massieu, G.H., *Biochem. Pharmacol.*, **16**: 1211-1218 (1967).
4. Sava, G., Perissin, L., Lassiani, L., Zabucchi, G. , *Chem. Biol. Interact.,* **53**: 37-43 (1985).
5. Ajani, O.O., Obafemi, C.A., Nwinyi, O.C., Akinpelu,D.A., *Bioorg. Med. Chem*. **18**: 214-21 (2010).
6. Bhagavan, N.V. Medical Biochemistry, Elsevier Science B.V.: Amsterdam, the Netherlands, **17**: 331-363 (2002).
7. Saulnier, M.G., Velaprthi, U., Zimmermann, K. In Progress In Heterocyclic Synthesis, Gribble, G., Ed., Elsevier Science B.V.: *Amsterdam, The Netherlands,* **16**: pp. 228-271 (2005).
8. Faroumadi, A., Kiano, Z., Soltani, F., *Farmaco* **58**: 1073-1076 (2003).
9. Holdiness, M.R., *Tubercle*, **68**: 301-309 (1987).
10. Short, E.I., *Tubercle*, **43**: 33-42 (1962).
11. Bokharev, V.V., Ghidaspov, A.A., Peresedova, E.V., *Chem. Heterocycl. Comp.*, **42**: 1096-1106 (2006).
12. Fernando, R.P., Maia, P.I., Leite, S.R., Deflon, V.M., Batista, A.A., Sato, D.N., Franzblau, S.G., Leite C.Q., *Eur. J. Med. Chem.* **45**: 1898-1905 (2010).
13. Brzozowski, Z, Czewski, F.S., *Eur. J. Med. Chem.*, **37**: 709-720 (2002).
14. Sherif, A., Rostom, F., *Bioorg. Med. Chem.*, **18**: 2767-2776 (2010).
15. Mohareb, R.M., Mohamed A.A., *Molecules,* **15**: 3602-3617 (2010).
16. Mohareb, R.M., El-Arab, E.E., El-Sharkaway, K.A., *Sci. Pharm.*, **77**: 355-365 (2009).
17. Hoda Z. Shams, Rafat M. Mohareb, Maher H. Helal ,Amira E. Mahmoud, *Molecules* , **16**: 52-73 (2011).
18. Rafat M. Mohareb, Daisy H. Fleita, Ola K. Sakka, *Molecules*, **16**: 16-27 (2011).
19. Wardakhan, W.W., Shams. H.Z., Moustafa, H.E., *Phosph. Sulf. Silicon*, **180**: 1815-1827 (2005).
20. Pinto, E., Queiroz, M.-J.R.P., Vale-Silva, L.A., Oliveira, J.F., Begouin, A., Beguin, J.-M., Kirsch, G., *Med. Chem*. **16**: 8172-8177 (2008).
21. Vicini, P., Zani, F., Cozzini, P., Doytchinova, I., *Eur. J. Med. Chem*. **37**: 553-564 (2002).
22. Isabel C.F.R., Ricardo C. C., Letícia M. E., Maria-João R.P.Q., *Bioorg. Med. Chem. Lett.*, 14, 5831-5833.
23. Melnyk, P., Leroux, V., Serghergert, C., Grellier, P. Design, *Bioorg. Med. Chem. Lett.*, **16**: 31-35 (2006).
24. Dannhardt, G., Kiefer, W., Krämer, G., Maehrlein, S., Nowe, U., Fiebich, B. , *Eur. J. Med. Chem.*, **35**: 499-510 (2000).
25. Zheng, L.W., Wu, L.L., Zhao, B.X., Dong, W.L., Miao, Y.J. , *Bioorg. Med. Chem.*, **17**: 1957-1962 (2009).
26. Xia, Y.L, Chuan-Dong, F., Zhao, B.X., Zhao, J., Shin, D.S., Miaom J.Y. , *Eur. J. Med. Chem.*, **43**: 2347-2353 (2008).
27. Brault, L., Migianu, E., Néguesque, A., Battaglia, E., Bagrel, D., Kirsch, G. , *Eur. J. Med. Chem.*, **40**: 757-763 (2005).
28. Starèeviæ, K., Karminski-Zamola, G., Piantanida, I., Ziniæ, M, SÔman, L, Kralj, M., *J. Am. Chem. Soc.*, **127**: 1074-1075 (2005).
29. Romagnoli, R., Baraldi, P.G., Carrion, M.D., Cara, C.L., Cruz-Lopez, O., Preti, D., Tolomeo, M., Grimaudo, S., Cristina, A.D., Zonta, N., Balzarini, J., Brancale, A., Sarkar, T., Hamel, E. Design, *Bioorg. Med. Chem.*, **16**: 5367-5376 (2008).
30. Ferreira, I.C.F.R., Queiroz, M.R.P., Vilas-Boas, M., Estevinho, L. M., Begouin, A., Kirsch G., *Bioorg. Med. Chem. Lett.*, **16**: 1384-1387 (2006).
31. Mamolo, M.G., Falagiani, V., Zampieri, D., Vio, L., Banfo, E., *IIFARMACO*, **56**: 587-592 (2001).