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Research Article

SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF 6(3,5-SUBSTITUTED-2-HYDROXY PHENYL) 1,2,4-TRIAZINE DERIVATIVES AS ANTIMICROBIAL AGENTS.

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ABSTRACT

6-(2-Amino-3,5-substituted phenyl)-1,2,4-triazines were prepared by refluxing semicarbazones or thiosemicarbazones in presence of basic medium. 6(3,5-Substituted-2-hydroxyphenyl)1,2,4-triazine derivatives were prepared from 6-(2-amino-3,5-substituted phenyl)-1,2,4-triazine derivatives by sodium nitrite and sulfuric acid. The structures of all the titled compounds were confirmed by FT-IR and H1-NMR spectral data. The newly synthesized compounds were evaluated in-vitro for their antimicrobial activity against four bacterial strains (Bacillus pumulis, Escherichia coli, Klebsiella pneumoniae and Staphylococcus aureus) and two fungal strains (Aspergillus niger and Candida albicans) by using agar well diffusion method. Some of these compounds showed good antimicrobial activity. The in vitro anticancer evaluation of selected compounds was also carried out by NCI 60 Cell screen at a single high dose (10–5 M) on various panel/cell lines. Compound 5e revealed significant antibacterial activity against S. aureus. Compound 5g showed moderate antifungal activity against Candida albicans. Compounds 5b, 5e and 5g were found to be the most active on UO-31, renal cancer cell line with 41.96, 42.57 and 40.18 growth percentage inhibition, respectively. Compound 5i was moderately active on UO-31, renal cancer cell line with 39.15 growth percentage inhibition.

KEY WORDS: (3,5-Substituted-2-hydroxy phenyl)1,2,4-triazines, antimicrobial activity, anticancer activity.

INTRODUCTION

Microbes including bacteria and fungi cause severe life threatening infections such as pneumonia and surgical wound infections. Development and spread of multi-drug resistant strains of bacteria and fungi pose a public health hazard worldwide (Shanmugam, et al., 2013) Although several potent antibiotics usable in the therapy are available, research on new substances possessing an antimicrobial activity is still of considerable interest owing to the continuous increase in bacterial resistance. 1,2,4-Triazines are a class of nitrogen containing heterocyclic compounds. Various 1,2,4-triazine derivatives are well known to possess an array of physiological activities, such as antimalarial (Gupta, et al 2010), and antiviral (Rusinov, et al., 2012) agents. Certain compounds containing a 1,2,4-triazine nucleus have been reported to possess antifungal (Ibrahim, et al, 2008), antimicrobial (Rauf, et al, 2007, Pandey, et al, 2009, Dabholkar, 2010, Ali, et al, 2010, Farshoi,

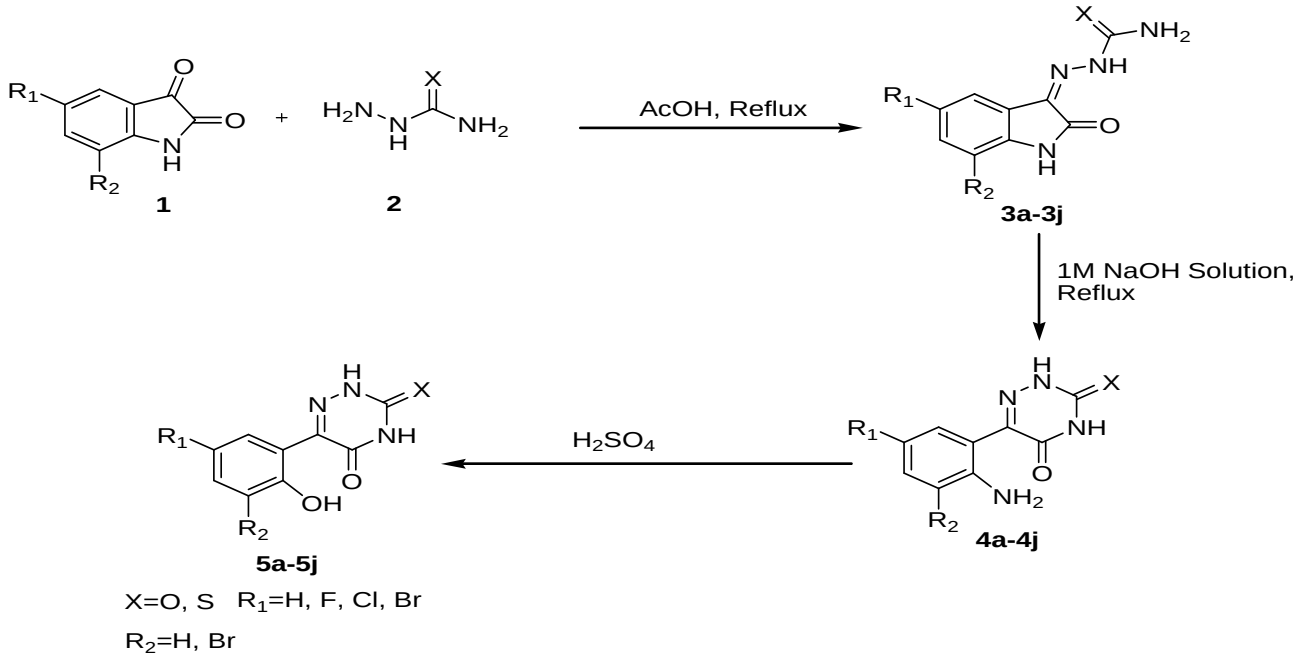
et al, 2011), antitumor (Sztanke, et al, 2007, Nassar, et al, 2013, El-Gendy, 2003) properties. Prompted by the varied biological activities of 1,2,4-triazine compounds, we envisioned our approach toward the synthesis of novel 6(3,5-substituted-2-hydroxy phenyl) 1,2,4-triazine derivatives would possess high anticancer and antimicrobial activities.

MATERIALS AND METHODS

a. Chemistry

The reaction sequence leading to the formation of the titled compounds, viz**.** 6(3,5-substituted-2-hydroxy phenyl)1,2,4-triazines (**5a-j**) is shown in Scheme **1.** 6(3,5-Substituted-2-hydroxyphenyl)1,2,4-triazines (**5a-j**) were synthesized from 6-(2-amino-3,5-substituted phenyl)-1,2,4-triazine derivatives with sodium nitrite and sulfuric acid. 6-(2-Amino-3,5-substituted phenyl)-1,2,4-triazine derivatives (**4a-l**) were prepared by refluxing semicarbazones or thiosemicarbazones in basic medium. Semicarbazones were obtained by the reaction of substituted isatin with semicarbazide hydrochloride. Thiosemicabazone derivatives were synthesized by refluxing substituted isatin with thiosemicarbazide. Thin layer chromatography (TLC) was run throughout the reactions to optimize the reactions for purity and completion. The structural assignments to new synthesized titled compounds were based on their elemental analysis and spectral (FT-IR and ^{1}H-NMR) data. All the details of synthesized compounds were already published in Kumar, R., et al. 2015.

Scheme 1 for synthesis of compounds 5a-5j.



b. Pharmacology:

I. Antimicrobial activity: Antibacterial and antifungal activities of synthesized compounds (**5a-5j**) were evaluated by using agar well diffusion method against selective strains i.e. gram-positive bacteria strains like *Staphylococcus aureus* (MTCC-737), *Bacillus pumilus* (MTCC-1607) and gram-negative bacteria strains like *Klebsiella pneumonia* (MTCC-109), *Escherichia coli* (MTCC-1687) and fungal strains like *Aspergillus niger* (MTCC-282) and *Candida albicans* (MTCC-183). Nutrient agar medium was autoclaved at 15 lbs pressure (121°C) for 15 minutes and then was cooled upto 50-54°C at room temperature after removing it from autoclave. Agar medium was transferred into sterile petri plate's upto a uniform depth of 4 mm in laminar air flow. Once medium gets solidified, 1 mL of standardized organism suspension (10^5 CFU/mL) (0.5 McFarland Nephelometery Standards) culture

was inoculated in medium. Sterile cotton swab was used for distribution of organisms on surface of nutrient agar medium and plates were
undisturbed for 30 minutes. Agar wells (diameter = 7 mm) were made by sterilized cork borer. Each well was filled with test compound stock solution (concentration = 1 mg/mL). DMSO was used as a control. Plates were incubated for 72 h at 28° C for fungi and 24 h at 30° C for bacteria. Then, diameter of inhibition zone (mm) was measured. Fluconazole and Ciprofloxacin were used as standard drugs in antifungal and antibacterial activities, respectively. (Barry, 1976, Indian Pharmacopoeia. Vol. II, Published by the controller of publications, Delhi, 1996).

II. Anticancer activity: The data of the all synthesized compounds was submitted to NCI, Betehesda, MD, USA for anticancer activity. As per the protocol of NCI, only six compounds of the series were selected and granted **NSC Code** D-768149 for screening of antiproliferative activity at a single high dose (10^{-5} M) in full NCI 60 cell panel representing leukemia, melanoma and cancers of lung, colon, brain, breast, ovary, kidney and prostate. The one-dose data was reported as a mean graph of the percent growth of treated cells. The number reported for the one-dose assay is growth relative to the no-drug control, and relative to the time zero number of cells. The anticancer screening was carried out as per the NCI US protocol reported elsewhere (Turner, 1964, Monks et al., 1991, Boyd and Paull, 1995, Shoemaker, 2006). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition is calculated as:

[(Ti-Tz)/(C-Tz)] x 100 for concentrations for which Ti>/=Tz

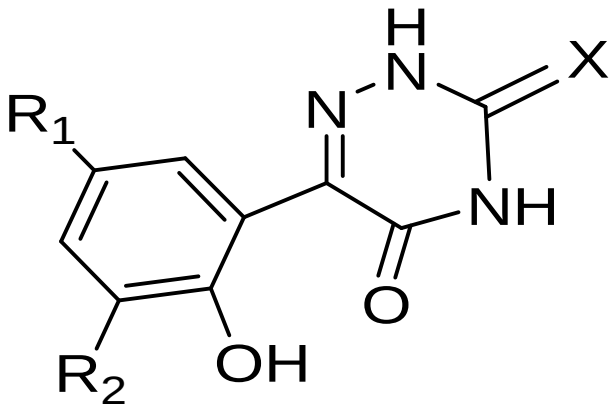
[(Ti-Tz)/Tz] x 100 for concentrations for which Ti<Tz.

RESULT

a. Antimicrobial activity: The newly synthesized titled compounds (5a-j) were screened in vitro for their antibacterial activity against gram positive bacteria i.e Bacillus pumulis (MTCC-1607), Staphylococcus aureus (MTCC-737) and gram-negative bacteria i.e Escherichia coli (MTCC-1687), Klebsiella pneumoniae (MTCC-109) and also antifungal activity against Candida albicans (MTCC-183) and Aspergillus niger (MTCC-282) by using agar well diffusion method. The synthesized compounds were dissolved in DMSO to get 1 mg/mL solution. The diameter of inhibition zone was measured as an indicator for the activity of the compounds (Table 2). Ciprofloxacin and fluconazole were used as standard drugs in antibacterial and antifungal activities, respectively. DMSO showed no inhibition zones. All the tested compounds showed variable activities toward different strains in comparison to the standard ciprofloxacin and flucanazole which revealed that these compounds are biologically active due to the presence of different functional groups (i.e. H, F, Cl, Br, O and S). The results for antibacterial activities depicted in Table 1 revealed that all the tested compounds reveal lower to good antibacterial activity. Compound 5a having R1=H, R2=H and X=O groups exhibited moderate antibacterial activity against B. pumulus with 24 mm zone of inhibition. Compounds 5c, 5d, 5h and 5i revealed moderate antibacterial activity against all bacteria i.e. E. coli, K. pneumoniae, B. pumulus and S. aureus strains. Compound 5b containing R1=H, R2=H and X=S groups revealed moderate antibacterial activity against E. coli and S. aureus with 18 and 22 mm zones of inhibition, respectively. Compound 5g having R1=Br, R2=H and X=O groups was found to be moderate active against E. coli with 20 mm zone of inhibition. This compound also showed lower activity against B. pumulus, K.

pneumoniae and S. aureus strains. Compound 5j containing R1=Br, R2=Br and X=S groups showed moderate antibacterial activity against E. coli, K. pneumoniae and S. aureus with 24, 16 and 20 mm zones of inhibition, respectively. This compound was lower active against B. pumulus bacteria stain. Compound 5f having R1=Cl, R2=H and X=S groups exhibited moderate antibacterial activity against E. coli, S. aureus and B. pumulus with 22, 24 and 20 mm zones of inhibition, respectively. Among all the tested compounds Compound 5e containing R1=Cl, R2=H and X=O groups was the most active compound against S. aureus with 31 mm zone of inhibition. This compound also showed moderate activity against E. coli, K. pneumoniae and B. pumulus bacterial stains with 20, 18 and 22 mm zones of inhibition, respectively. Antibacterial activity of all the synthesized compounds (5a-j) was found to be less active than that of Ciprofloxacin (Standard Drug).

Table No. 1: **Molinspiration calculations**:



Code No.	R_1	R_2	X	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
5a	H	H	O	-0.84	-0.60	-0.80	-1.08	-1.28	-0.67
5b	H	H	S	-0.97	-0.74	-1.14	-1.30	-1.41	-0.76
5c	F	H	O	-0.69	-0.55	-0.63	-0.86	-1.15	-0.60
5d	F	H	S	-0.81	-0.68	-0.94	-1.07	-1.27	-0.69
5e	Cl	H	O	-0.75	-0.57	-0.73	-0.96	-1.24	-0.63
5f	Cl	H	S	-0.87	-0.70	-1.05	-1.17	-1.35	-0.72
5g	Br	H	O	-0.92	-0.65	-0.79	-1.15	-1.36	-0.69
5h	Br	H	S	-1.04	-0.78	-1.11	-1.36	-1.48	-0.78
5i	Br	Br	O	-0.90	-0.69	-0.67	-1.07	-1.10	-0.60
5j	Br	Br	S	-1.02	-0.81	-0.97	-1.27	-1.21	-0.68
Ciprofloxacin				0.12	-0.04	-0.07	-0.19	-0.21	0.28
Fluconazole				0.04	0.01	-0.09	-0.23	-0.09	0.03
Chloroamphenicol				-0.22	-0.28	-0.38	-0.41	-0.21	-0.00
Penicillin G				-0.56	-0.41	-0.74	-0.45	---	---

Table No. 2: *In vitro* antimicrobial activity of compounds (5a-5j) against selected strains (Zone of inhibition in mm).

Code No.	R_1	R_2	X	Zone of Inhibition in mm					
				E. coli	***K. pneumoniae***	***B. pumilus***	***S. aureus***	***C. albicans***	***A. niger***
5a	H	H	O	12	18	16	24	18	14
5b	H	H	S	18	14	22	12	14	22
5c	F	H	O	16	18	12	16	12	15
5d	F	H	S	17	19	17	19	14	15
5e	Cl	H	O	20	18	**31**	22	22	16
5f	Cl	H	S	22	12	24	20	12	20
5g	Br	H	O	20	14	12	14	26	12
5h	Br	H	S	19	17	19	17	17	16
5i	Br	Br	O	21	18	16	15	15	18
5j	Br	Br	S	24	16	20	12	18	16
Control				00	00	00	00	00	00
Ciprofloxacin				46	47	45	40	---	---
Fluconazole				---	---	---	---	40	32

Positive control (Standard) = Ciprofloxacin, Negative control =DMSO, --- = Not performed
Lower active = inhibition zone <15 mm, Moderately active = inhibition zone 16–30 mm and High active = inhibition zone > 31 mm.

The in vitro antifungal activity results (Table 3) revealed that the synthesized compounds show lower to moderate antifungal activity. Compounds 5c and 5d exhibited lower antifungal activity against Aspergillus niger and Candida albicans. Compounds 5h, 5i and 5j revealed moderate antifungal activity against both Aspergillus niger and Candida albicans. Compound 5a having R1=H, R2=H, X=O showed moderate antifungal activity against Candida albicans with 18 mm zone of inhibition and lower activity against Aspergillus niger. Compounds 5b and 5f displayed moderate activity against Aspergillus niger with 22 and 20 mm zones of inhibition, respectively. Compound 5e containing R1=Cl, R2=H, X=O showed moderate antifungal activity against Candida albicans and Aspergillus niger with 22 and 16 mm zones of inhibition, respectively. Compound 5g having R1=Br, R2=H and X=O groups was the most active compound against Candida albicans with 26 mm zone of inhibition. This compound also showed lower activity against Aspergillus niger. Antifungal activity of all synthesized compounds (5a-j) was found to be less active than that of fluconazole (Standard Drug). It is fairly ambiguous from the virtual studies, that compounds (5a-5j) show probable mode of antibacterial action like Penicillin (Table 1).

Table No. 3. *In-vitro* percentage growth inhibition of cancer cell line by titled compounds

	60 cell lines assay in 1 dose 10^{-5}M conc.					
Comp. Code	**NSC Code**	**Mean Growth %**	**Range of growth %**	**Most Cancer Cell lines**	**Growth % of the most sensitive cell line**	**Growth Percentage Inhibition**
	D-768153	101.60	35.72-117.98	Non-small cell lung cancer (NCI-H322M)	84.10	15.90
5a				SNB-75 (CNS cancer)	84.20	15.80
	D-768152	100.17	63.35-128.46	UO-31 (Renal cancer)		
				SNB-75 (CNS	65.11	41.96
5b				cancer)	83.00	17.00
	D-768154	102.04	39.36-119.63	UO-31 (Renal cancer)		
				CAKI-1(Renal	80.27	19.73
5c				cancer)	87.02	12.98
	D-768157	98.29	68.62-126.05	UO-31 (Renal cancer)		
				SNB-75 (CNS	57.43	42.57
5e				cancer)	77.53	22.47
	D-768155	98.87	73.65-133.47	UO-31 (Renal cancer)		
				SF-295 (CNS	59.82	40.18
5g				cancer)	80.77	19.23
	D-768156	99.34	73.31-134.16	UO-31 (Renal cancer)		
				MOLT-4	60.85	39.15
5i				(Leukemia)	79.08	20.92

b. Anticancer activity: The screened compounds showed low to moderate activity in the in vitro screen on all tested cancer cell lines. Screening results (Mean growth %, range of growth %, most cancer cell lines, growth % of the most sensitive cell line and percentage growth inhibition) of the selected compounds were given in table 3. The tested compounds were found to be the most active against CVS and renal cancer cell lines among all tested cell lines. In general, in vitro anticancer activity of the titled compounds was higher against renal cell lines as compared to CVS cancer cell lines. Compound 5e having chloro and oxo groups was most active compound of the all screened compounds that showed 42.57 and 22.47 growth percent inhibition (GPI) against UO-31, renal cancer and SNB-75, CNS cancer cell lines, respectively. Compound 5b with thio group displayed 41.96 and 17.00 GPI against UO-31, renal cancer and SNB-75, CNS cancer cell lines respectively. Compound 5g having bromo and oxo groups also revealed significant activity against UO-31, Renal cancer (40.18 GPI) and SF-295, CNS cancer (19.23 GPI) cell lines. The compounds 5i and 5e (bromo) showing moderate activity were highly active on UO-31, renal cancer cell line with GPI of 39.15 and 38.62,

respectively. While compounds 5a and 6c showed less activity with an average growth percent inhibition of 12.98115.90.

CONCLUSION

Antimicrobial data of present research work showed that some analogues have shown moderate to good activity. One compound showed good antibacterial activity against B. pumilus so these compounds can be further modified to develop potent antibacterial agents. Hence these compounds may be considered as clinical candidates for development of future novel antibacterial agents. Chemical modifications as well as synthesis of some new 1,2,4-triazine analogues have been tried to find out novel anticancer compounds for treatment of deadly diseases like cancer. 1,2,4-Triazine analogs, showing encouraging anticancer activity, can be used to develop future generation of compound with high affinity and specificity.

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REFERENCES

1. Shanmugam M., Narayanan K., Chidambaranathan V., Kabilan S. (2013). Synthesis, spectral characterization and antimicrobial studies of novel s-triazine derivatives. Spectrochim. Acta Part A: Molecular and Biomolecular Spectroscopy. 105, 383-390.
2. Gupta L., Sunduru N., Verma A., Srivastava S., Gupta S., Goyal N, Chauhan P. M.S. (2010). Synthesis and biological evaluation of new [1,2,4]triazino[5,6-b]indol-3-ylthio-1,3,5-triazines and [1,2,4]triazino[5,6-b]indol-3-ylthio-pyrimidines against Leishmania donovani. Eur. J. Med. Chem., 45, 2359-2365.
3. Rusinov V.L., Egorvor I.N., Chupakhin O.N., Belanov E.F., Bormotov N.I., Serova O.A. (2012). Synthesis and antiviral activity of 1,2,4-triazine derivatives. Pharm. Chem. J., 45(11), 655-659.
4. Magdy A.I., Reda M.A.R., Ali M.A.H., Salah S.I., Hassan A.A. (2008). Synthesis and antifungal activity of novel polyheterocyclic compounds containing fused 1,2,4-triazine moiety. Arkivoc, 16, 202-215.
5. Rauf A., Sharma S., Gangal S. (2007). Microwave assisted efficient one-pot synthesis of 3,5,6-trisubstituted-1,2,4-triazines from fatty acid hydrazides under solvent-free conditions and their antimicrobial activity. Arkivoc, (16), 137-147.
6. Pandey S.K., Singh A., Singh A., Nizauddin (2009). Antimicrobial studies of some novel quinazolinones fused with [1,2,4]-triazole, [1,2,4]-triazine and [1,2,4,5]-tetrazine rings. Eur. J. Med. Chem., 44, 1188-1197.
7. Dabholkar V.V., Tripathi D.R. (2010). Efficient synthesis of some novel spiro heterocycles containing triazine nucleus and their microbiological activity. Indian J. Chem., 49B, 593-597.
8. Ali, T.E.S. and Ibrahim, M.A. (2010). Synthesis and antimicrobial activity of chromone-linked 2-pyridone fused with 1,2,4-triazoles, 1,2,4-triazines and 1,2,4-triazepines ring systems. J. Braz. Chem. Soc., 21(6), 1007-1016.

9. Farshoi N.F., Banday M.R., Ahmad A., Khan A.U., Rauf A. (2011). Facile one-pot synthesis of novel 3-substituted-1, 6-dihydro-1,2,4-triazin-5-(2H)-ones from fatty acid hydrazides and their in-vitro antimicrobial activity, Indian J. Chem., 50B, 605-610.
10. Sztanke K., Pasternak K., Rzymowska J., Sztanke M., Kandefer-Szerszen M., Dybala I., Koziol A.E. (2007). Identification of antitumor activity of novel derivatives of 8-aryl-2,6,7,8-tetrahydroimidazo[2,1-c][1,2,4]triazine-3,4-dione and 8-aryl-4-imino-2,3,7,8-tetrahydroimidazo-[2,1-c][1,2,4]triazin-3(6H)-one. Bioorg. Med. Chem., 15, 2837-2849.
11. Nassar, I.F. (2013). Synthesis and antitumor activity of new substituted mercapto-1,2,4-triazine derivatives, their thioglycosides, and acyclic thioglycoside analogs. J. Heterocyclic Chem., 50, 129-134.
12. El-Gendy Z., Morsy J.M., Allimony H.A., Abdel-Monem W.R., Abdel-Rahman R.M. (2003). Synthesis of heterobicyclic nitrogen systems bearing a 1,2,4-triazine moiety as anticancer drugs: Part IV. Phosphorus, Sulfur, and Silicon, 178, 2055–2071.
13. Kumar, R., Singh A., Roy R.K. (2015). Synthesis, characterization and pharmacological evaluation of some new 6-(3,5-substituted-2-bromo/hydroxy phenyl)-1,2,4-triazine derivatives as anticonvulsant agents. Int. J. Pharm. Sci. Res., 6(8), 1000-1011.
14. Barry, A.L. (1976). The antimicrobial susceptibility test, principles and practices, Illus Lea and Febiger, Philadeliphia, PA, USA, 180.
15. Indian pharmacopoeia. (1996). Vol. II, Published by the controller of publications, Delhi, A-100-124.
16. Turner, R.A., (1964). Screening methods in pharmacology. Academic Press, New York, London, 165-172.
17. Monks, A., Scudiero D., Skehan P., Shoemaker R., Paull K., Vistica D., Hose C., Langley J., Cronise P., Vaigro-Wolff A. (1991). Feasibility of a high-flux anticancer drug screen utilizing a diverse panel of human tumor cell lines in culture. J. Natl. Cancer Inst., 83(11), 757-766.
18. Boyd, M.R. and Paull, K.D. (1995). Some practical considerations and applications of the National Cancer Institute in vitro anticancer drug discovery screen. Drug Dev. Res., 34(2), 91-109.
19. Shoemaker, R.H. (2006). The NCI 60 human tumour cell line anticancer drug screen. Nat. Rev. Cancer, 6(10), 813-823.