Research Article

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Design synthesis characterization and dual biological activity of some novel morphanthridine 6,11 dione derivatives

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ABSTRACT

In modern medicinal chemistry, molecular modeling study is effectively participating in drug discovery process to reduce and the time and cost. The present study involved in the docking of 4-alkylamide/imides substituted 5H morphanthridine 6,11-dione [5H Dibenzo(b,e) apine 6,11-dione] derivatives 1(a-d), 2(a-d), 3(a-d) and 4(a-d) with Mono Amine Oxidase A protein to find out their potency of antidepressant activity. On the basis of docking result the molecules 1a, 2a, 2b, 2c, 3a, 3b, 4a and 4c were synthesized, characterized and evaluated for antidepressant activity by Forced Swim Test. Also all the 12 molecules synthesized characterized and evaluated to their in vitro anti bacterial activity by Agar Plate Disc diffusion method.

KEYWORDS: Molecular docking, 5H Morphanthridine 6,11dione, Alkyl arylamides/imides, Antidepressant, antibacterial.

INTRODUCTION

Drug development has been one of the most prominent research areas in the pharmaceutical industry. Consequently the development of methodologies useful for the assembly of molecules containing heterocyclic templates continues to attract the attention of both the academic and industrial communities. Mood disorders such as depression are among the most prevalent forms of mental illness, affecting upto 20% of the population. Several antidepressants are currently available for clinical use however, the beneficial therapeutic effects take some weeks to appear and the effects are often accompanied by numerous side effects. Even though there are several molecular targets useful in the development of antidepressant drugs, most of current treatments for depression affect directly or indirectly the monoaminergic system [1]. According to literature majority of tricyclics and their related compounds are known for their potential biological and pharmacological properties viz antidepressant [2], anticonvulsant[3], antidiabetic[4], antimicrobial activity[5].

In the present study, the designed various 4-alkyl arylamides/imides substituted 5H dibenzo (b,e) azepine 6,11 diones have docked with Mono Amine Oxidase A (MAO-A) protein to predict the potency of the antidepressant activity before synthesizing molecues. We have synthesized 12 different inhibitor molecules by treating N-Hydroxy alkyl arylamides/imides with 2-Substituted-5H-Dibenzo (b,e) azepine-6,11 diones which is obtained by heating on equimolar mixture of Phthalic anhydride and Para substituted aniline[2] .

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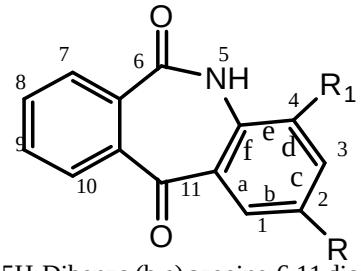


5H-Dibenzo (b,e) azepine-6,11 diones

All the synthesized molecules were characterized by IR, ^{1}HNMR and MASS, the spectral values strongly influences formation of seven membered ring and substitution in 4th position. Those molecules showed good inhibiting profile based on docking study were evaluated for antidepressant activity and antibacterial activity was carried out for the entire synthesized molecule with different bacterial strain.

DRUG DESIGN:

Prediction of Activity Spectra for Substances :(PASS)

It is a software product designed as a tool for evaluating the general biological potential of an organic drug-like molecules and it is a very easy tool for in silico screening. The structure of 12 molecules [1(a-d), 2(a-d), 3(a-d) and 4(a-d)] were drawn using12.0 and Chem Draw Ultra 8.0., and saved in Chem Sketch 2.0 document as (*.SK2) or MDL Mol files (*.mol). The saved structures are directly uploaded in the PASS prediction website (http://195.178.207.233/PASS/index.html.) and activity of the molecule was predicted by "comparing" the structure of new compound with structure of well-known biological active substrate existing in the database. This predicted that the Pa: Pi (active and inactive ratio) for antidepressant activity to most of the molecules at prediction threshold of Pa>70%.

Molecular property prediction :(OSIRIS)

The OSIRIS Property Explorer used to draw chemical structures and calculates various drug-relevant properties (CLogP, solubility, Molecular Weight, Toxicity Risk Assessment, Overall Drug-Score, Drug likeness, etc.) whenever a structure is valid. Prediction results are valued and color coded. Properties with high risks of undesired effects like mutagenicity or a poor intestinal absorption are shown in red. Whereas a green color indicates drug-conform behavior. The predicted properties are shown in table 1.

Figure 1: Docking of inhibitor molecules with MAO-A Protein

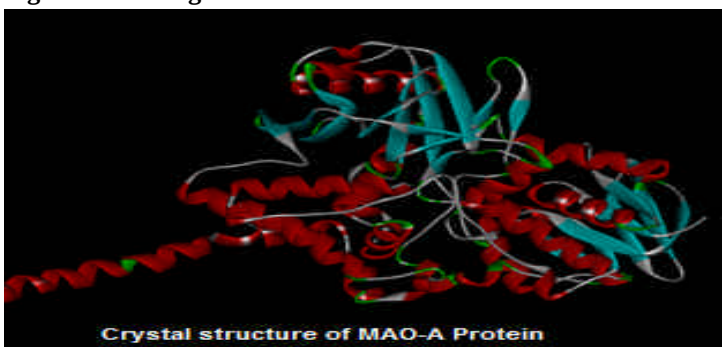


Table 1: Predcted properties by Pass And Osiris

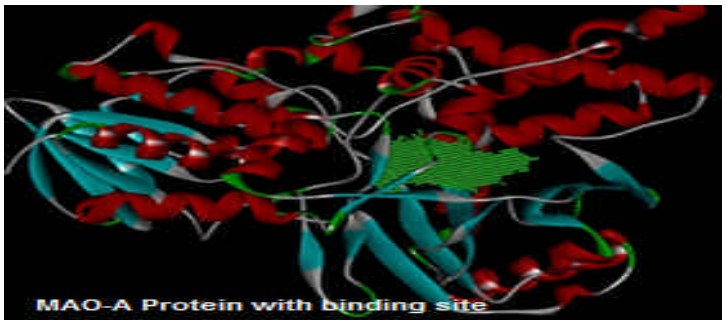
	PASS				OSIRIS						
Com	Pa	Pi	Muta	Tumero	Irrit	R.f	ClogP	Solub.	M.W	DL	DS
1a	0.51	0.04	Yellow	Green	Green	Yellow	4.12	-6.21	416	7.04	0.36
1b	0.06	0.05	Yellow	Green	Green	Yellow	5.69	-8.82	478	6.87	0.12
1c	0.43	0.06	Green	Green	Green	Green	4.31	-6.21	390	3.49	0.42
2a	0.72	0.15	Yellow	Green	Green	Yellow	3.57	-5.79	400	3.44	0.35
2b	0.38	0.02	Yellow	Green	Green	Green	5.14	-8.4	462	0.29	0.25
2c	0.67	0.12	Yellow	Green	Green	Green	3.76	-5.78	374	1.91	0.47
3a	0.57	0.20	Yellow	Green	Green	Red	3.21	-5.18	398	7.09	0.38
3b	0.20	0.04	Yellow	Green	Green	Red	4.78	-7.79	460	6.92	0.06
3c	0.42	0.15	Green	Green	Green	Green	3.4	-5.17	372	0.54	0.43
4a	0.73	0.15	Green	Green	Green	Yellow	3.4	-5.49	412	7.07	0.23
4b	0.44	0.15	Green	Green	Green	Green	4.97	-8.1	474	6.86	0.53
4c	0.57	0.11	Green	Green	Green	Green	3.59	5.49	386	0.47	0.40

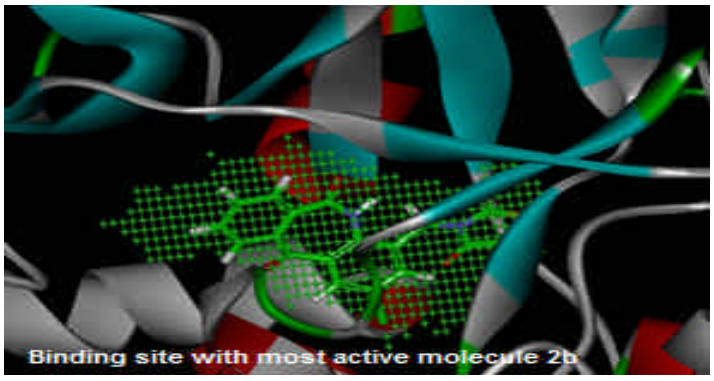
Molecular Docking :(Accelry's Discovery Studio)

Ligand-based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest. The complete docking process was carried using accelry's discovery studio software. Target protein MAO-A (Protein Code 2Z5Y) was downloaded from Protein Data bank and the structure of ligands was drawn using Chem Draw Ultra 8.0. All the possible conformation of the molecules and cavity type binding site in protein receptor was created and docked by Ligand Fit method. It offered docking score based on Force Field approximation in which two energy terms used for calculation are internal energy of the ligand and the interaction energy of the ligand with the receptor.

Dock Score (force field) = - (ligand/receptor interaction energy + ligand internal energy)

All the ligand molecules successively docked with targeted protein and the molecules with lowest dock score indicates highest activity.





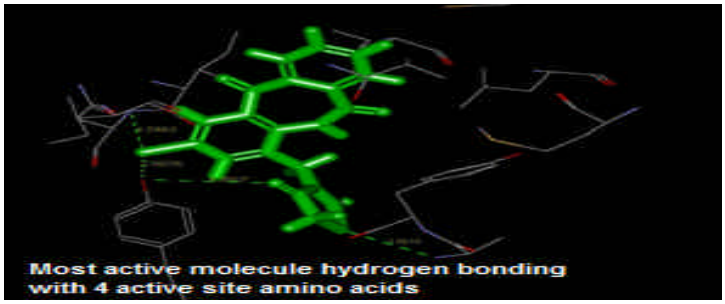


Table 2: Docking scores for inhibitor molecules on MAO-A Protein

Sl.no	PROTEIN CODE – 2Z5Y		
	Compound code	Dock score	Ligand internal energy
1	1a	52.102	6.369
2	1b	55.49	-2.965
3	1c	66.947	-2.715
4	2a	33.828	0.237
5	2b	33.828	3.22
6	2c	48.714	-2.085
7	3a	54.735	-0.051
8	3b	52.716	-0.463
9	3c	59.506	-0.015
10	4a	34.321	-2.9
11	4b	53.653	-0.34
12	4c	30.76	-3.861

MATERIALS AND METHODS

Chemistry

Melting points of all the synthesized molecules were determined by using Open capillary tube method. Pre-coated Silica gel (HF254-200 mesh) aluminium plates (E-merk) were used for determination of purity and completion of reaction, Methanol: Toluene was used as running solvent and visualized under UV chamber. IR spectra was recorded (in cm^{-1}) using KBr pellet method in Thermo Nicolet IR instrument, 1H-NMR was recorded (in δ ppm) with CDCl3 in Bruker NMR instrument and MASS spectra was recorded by Electron Impact Ionization method by JEOL GC mate.

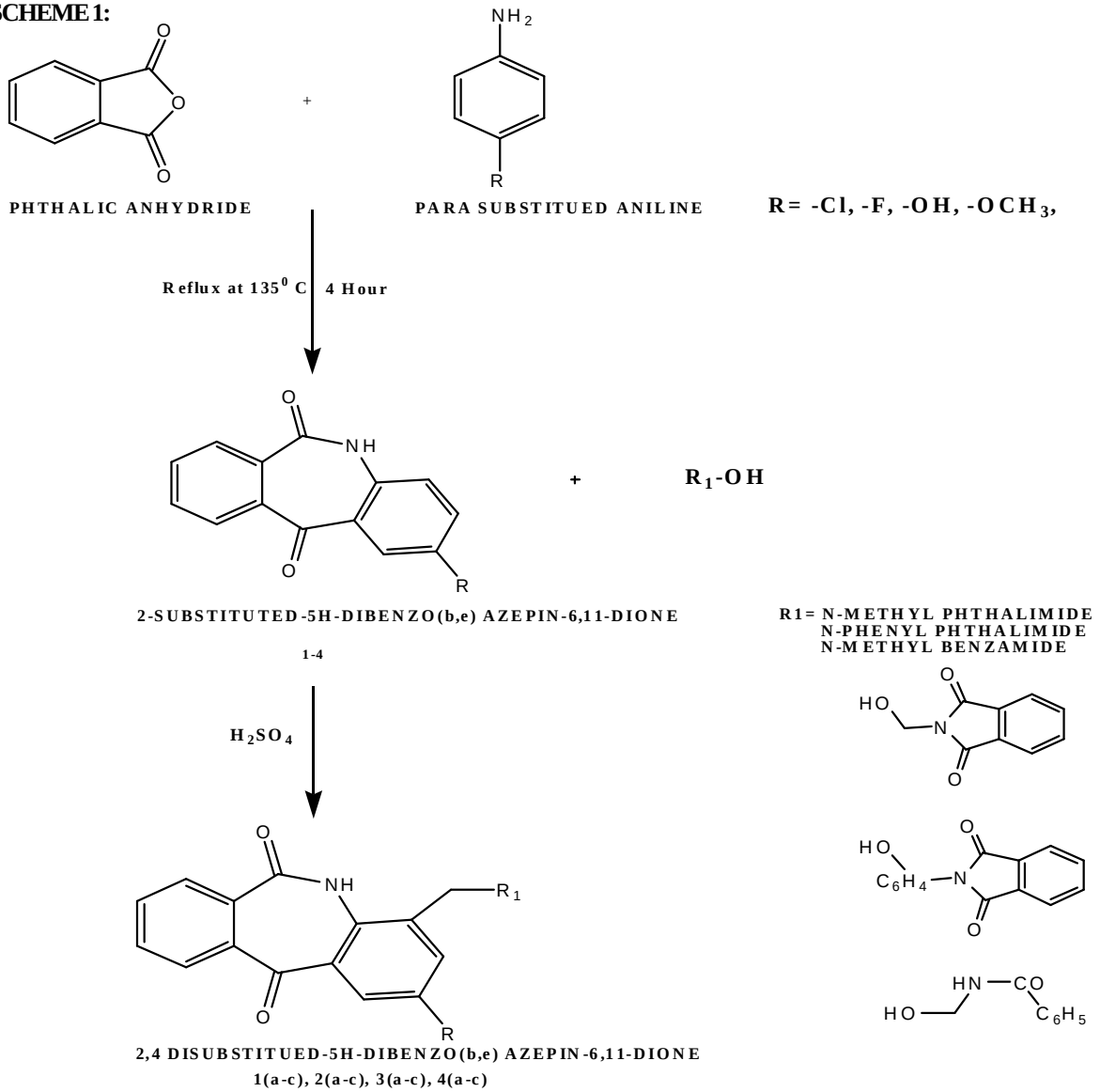
Step1: Synthesis of 2 Substituted -5H-dibenzo (b,e) azepine 6,11-dione:

A finely powdered phthalic anhydride (0.1mol) and Para substituted aniline (0.1mol) were mixed thoroughly and transferred into 500ml flat bottomed flask, if needed 5ml of ethanol was added to mix the contents well. The flask was fitted with reflux condenser, constantly stirred and heated by magnetic stir for 4 hours. Temperature is maintained at 135^0C throughout the reaction. After 4 hour the contents was filtered and washed with 0.1M hydrochloric acid initially and 10% sodium bicarbonate finally. It was allowed to dry; the solid mass obtained was recrystallized with alcohol.

Step2: Synthesis of 2, 4 substituted -5H-dibenzo (b,e) azepine 6,11-dione:

N-Hydroxy alkyl arylamides/amides (0.05mol) and 0.05 mol of the product obtained from step1 were dissolved in 50ml of Conc. Sulphuric acid carefully and cautiously by magnetic stirring. While dissolving the contents were occasionally cooling in order to prevent the decomposition of the reactants due to exothermic reaction. The resultant solution was subsequently stirred for 1 hour and kept under refrigeration overnight. It was poured on to ice cold water slowly with constant stirring, solidification occurred and the reaction was completed in half an hour. It was recrystallized with ethanol. The common procedure to synthesize the molecules are given in scheme 1.

SCHEME 1:



2-Chloro-4-methyl phthalimido-6,11-dioxo-6,11-dihydro-5H-dibenzo(b,e)azepine(1a): Yield 88.5%, M.P 159^{0}C, RF 0.672, IR: 2971.315(Ar-H), 3415(N-H), 1770.85(-C=O), 1720(C=C), 734.51(C-Cl), NMR: 8.431(s, -NH; 1H), 7.213-7.974(m, Ar-H, 8H), 4.792(-CH_2; 2H), MASS: 416.81(M^+), 381.32, 323.96, 227.25, 199.32(base beak), 107.32, 134.34.

2-chloro-4-[4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]-5*H*-dibenzo[*b,e*]azepine-6,11-dione(1b): Yield: 59.5%, M.P:210^{0}C, RF:0.689, IR: 3066.45(Ar-H), 3512.782(N-H), 1718.910(Ar C=C), 1126.214(C-N), 787.75(C-Cl), NMR: 8.478 (s, N-H; 1H), 7.264-7.971(m, Ar-H, 14H), MASS: 478.34(M^+), 442.5, 414.20, 344.32, 238.23, 210.34(base beak), 195.34, 118.25.

***N*-[(2-chloro-6,11-dioxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-4-yl)methyl]benzamide(1c):** Yield: 94.18%, M.P:184^{0}C, RF:0.710, IR: 3065.90064(Ar-H), 3494.3787(N-H amide), 1746.45(C=O Ketone), 1706.78(C=C), 787.72(C-Cl.), NMR: 7.213-7.974(m, Ar-H, 11H), 8.478(s, NH; 2H), 4.506-4.792(s, CH_2, 1H), MASS: 390.81(M^+), 355.30, 326.32, 221.76, 193.45(base beak), 178.56, 101.34.

4-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]-2-fluoro-5*H*-dibenzo[*b,e*]azepine-6,11-dione(2a): Yield: 93.78%, M.P:214^{0}C, RF:0.680, IR: 3075.1625(Ar-H), 3469.8622(N-H), 2949.03(R-H), 1768.81514(C=O), 1726.76(C=C), 1249.0032(C-F), NMR: 7.716-7.867(m, Ar-H, 10H), 7.918-7.973(s, NH; 1H), 4.738-4.979(s, CH_2, 2H, MASS: 400.35(M^+), 381.43, 323.32, 227.93, 199.45(base beak), 122.

4-[4-(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]-2-fluoro-5*H*-dibenzo[*b,e*]azepine-6,11dione(2b): Yield: 68.9%, M.P:191^{0}C, RF:0.679, IR: 3452.9591(N-H), 1789.242(C=O), 1719.226(C=C), 1230.376(C-F), NMR: 7.180-7.972(m, Ar-H; 14H), 8.451(s, NH; 1H), MASS: 462.50(M^+), 443.21, 415.34, 345.32, 239.01, 211.32(base beak), 196.45, 119.38.

***N*-[(2-fluoro-6,11-dioxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-4-yl)methyl]benzamide(2c):** Yield: 86.31%, M.P:171^{0}C, RF:0.701, IR: 3025.293(N-H), 1514.731(C=C), 1768.311(C=O), 1393.806(C-F), NMR: 8.397(s, NH; 2H), 7.180-7.972(m, Ar-H; 11H), 4.739-4.975(s, CH_2, 2H), MASS: 374.36(M^+), 355.30, 326.32, 221.76, 193.45(base beak), 178.56, 101.34.

4-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]-2-hydroxy-5*H*-dibenzo[*b,e*]azepine-6,11-dione(3a): Yield: 90.3%, M.P:189^{0}C, RF:0.721, IR: 3072.9705(Ar-H), 3348.592(N-H), 3124.1906(O-H), 1659.760(C=O), 1547.0515(C=C), NMR: 8.532 (s, NH; 1H), 7.090-7.942(m, Ar-H; 10H), 3.490-4.861(s,CH_2; 1H), MASS: 398.36(M^+), 381.32, 323.96, 227.25, 199.32(base beak), 184.34, 107.32.

4-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]-2-hydroxy-5*H*-dibenzo[*b,e*]azepine-6,11-dione(3b): Yield: 71%, M.P:204^{0}C, RF:0.656, IR: 3072.9705(Ar-H), 3348.592(N-H), 3124.9106(O-H), 1679.342(C=C), 1659.760(C=O), NMR: 8.752(s, NH; 1H), 7.090-7.942(m, Ar-H; 10H), 3.490-4.861(s, OH; 1H), MASS: 460.43(M^+), 442.98, 414.56, 344.45, 238.53, 210.70(base beak), 195.67, 118.34.

***N*-[(2-hydroxy-6,11-dioxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-4-yl)methyl]benzamide(3c):** Yield: 71%, M.P:196^{0}C, RF:0.681, IR: 3106.2179(Ar-H), 3348.592(N-H), 3124.9106(O-H), 2973.561(R-H), 1659.760(C=O), 1547.057(C=C), NMR: 7.090-7.805(m, Ar-H; 11H), 7.838-8.231(s, NH; 2H), 3.490-4.861(s, CH_2; 1H), 4.927(s, OH; 1H), MASS: 372.37(M^+), 354.29, 325.34, 220.56, 192.32(base beak), 177.23, 100.12.

4-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)methyl]-2-methoxy-5*H*-dibenzo[*b,e*]azepine-6,11-dione(4a): Yield: 78%, M.P:196^{0}C, RF:0.675, IR: 3095.2288(Ar-H), 3490.67221(N-H), 1718.91012(C=O), 1580.76752(C=C), 1126.21437(Ar-O-C3, NMR: 7.090-7.805(m, Ar-H; 13H), 8.357-7.838(s, NH; 1H), 4.861-4.927(s, CH_2; 1H), 3.490(s, CH_3; 3H), MASS: 412.36(M^+), 381.32, 323.96, 227.25, 199.32(base beak), 184.34, 107.32.

4-[(1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)phenyl]-2-methoxy-5*H*-dibenzo[*b,e*]azepine-6,11-dione(4b): Yield: 88%, M.P:213^{0}C, RF:0.68, IR: 3490.34226(Ar-H), 3490.67221(N-H), 1718.91012(C=O), 1580.76752(C=C), 1126.21437(Ar-O-C), NMR: 7.090-7.805(m, Ar-H; 14H), 7.838-8.357(s, NH, 1H), 3.490-3.927(s, CH_3, 3H), MASS: 474.46(M^+), 443.24, 421.76, 400.32, 295.3, 267.59, 252.31(base beak), 175.34.

***N*-[(2-methoxy-6,11-dioxo-6,11-dihydro-5*H*-dibenzo[*b,e*]azepin-4-yl)methyl]benzamide(4c):** Yield: 92%, M.P:190^{0}C, RF:0.599, IR: 3095.2288(Ar-H), 3490.67221(N-H), 1718.91012(C=O), 1580.76752(C=C), 1126.214(C-C, NMR: 7.213-7.822(s, Ar-H; 11H), 7.908-7.974(s, NH; 2H), 4.506-4.792(s, CH_2; 2H), 3.521(s, CH_3; 3H), MASS: 386.40(M^+), 355.40, 324.91, 221.76, 164.23(base beak), 119.02.

Pharmacology

Ethical Committee No: IAEC/XXX IV/10/CLBMCP/2011 DATED 7-12-2011

Antidepressant activity[9] :

Based on docking result 8 molecules (1a, 2a, 2b, 2c, 3a, 3b and 4a) were evaluated for antidepressant activity in 10 groups of rat by Forced Swim Test. Group-I received the vehicle (0.5 ml/100 g, i.p.) and Group-II treated with standard drug imipramine (10 mg/kg s.c.) 30 min prior to the induction of depression. Remaining groups were treated with synthesized 8 moleules (200 mg/kg p.o.) 1h prior to the induction of depression. Immobility was induced by forced swim in glass cylinder (with diameter of 18 cm and height 40 cm) filled with water at 24±10C to a depth of 15 cm. . Duration of immobility was recorded at an interval of 1h, 5 h and 24 h after drug administration. The results are shown in table 3.

Antibacterial activity: All the 12 molecules synthesized were subjected to antibacterial screening against gram positive and gram negative bacteria repectively S.aureus (ATCC 6538 P) and E.coli at 3 concentrations of 25, 50, 100 µg/disc. The Ciprofloxacin 100 µg/L was used as standard and Dimethyl formamide was employed as solvent. Agar plate disc diffusion method (Kirby-Bayer method) was employed

to perform Zone of inhibition for antibacterial activity. The results are shown in table 4.

RESULT AND DISCUSSION

In the present study two softwares PASS and OSIRIS respectively predicted the potential of antidepressant effect and molecular property of 12 molecules which are designed based on the literature survey and SAR. The MAO-A inhibition potential showed good Pa value except 1b and 2b which indicates the Phenyl Phthalimide in the 4th position may decrease the inhibiting capability due to steric hindrance. OSIRIS study revealed that all the test molecules showed Lipinski's rule of five and no or less mutagenicity, tumerogenicity, irritancy and risk factor with good drug score except 3a and 3b which are substituted –OH group in 2nd position and N-Phenyl Phthalimide in 4th position. All the test molecules are successively docked with this protein for anti-depressant activity by ligand fit method. The molecule 2a, which is substituted with fluorine and methyl Phthalimide showed low dock score i.e. 33.828 compared to other test molecules.

Table 3: Anti-depressant activity of synthesized compounds by FST

Groups	Treatment	Dose (mg/kg)	Time(hr.)	Duration of immobility Mean ±SEM	%change
II	Standard imipramine	10	1	14.33 ±0.33	80.89
			5	12.66 ±0.88	83.12
			24	10 ±0.57	86.66
III	1a	200	1	43.66 ±0.33	41.78
			5	33 ±1	56
			24	26 ±0.51	65
VI	2a	200	1	41.66 ±0.88	45.33
			5	18.66 ±0.33	75.12

Table 4: Zone of inhibition of the synthesized compounds

Comp.code	Zone of inhibition(mm)					
	Staphylococcus aureus			*Escherichia coli*		
	25	50	100	25	50	100
Control –DMF	-	-	-	-	-	-
Standard-Ciprofoxacin (100µg/L)	-	-	39	-	-	40
1a	5	17	31	2	12	25
1b	6	21	37	4	15	31
1c	3	16	27	6	11	22
2a	4	10	25	1	10	27

The lowest dock score indicates the highest anti-depressant activity, so i concluded 2a shows highest activity. The ascending order of dock score of all the molecules as follows,

2a, 2b>4c>4a>2c>1a>3b>4b>3a>1b>3c>1c

The most active molecule 2a containing 4 hydrogen bonds with 4 amino acids present in the active site compare to least active molecule 1c which is containing only 1 hydrogen bond.

12 molecules were synthesized and characterized by spectral data. Those molecules showed good inhibiting profile of MAO-A only was subjected to in vivo antidepressant activity in rats, these results are more relevant to docking result. The percentage reduction of test molecules showed mild reduction in the immobility after 1 hr. compared to the standard. After 5hr of treatment of the test molecules 2a, 2c, 4a showed very good reduction in the duration of immobility which are nearer value compared to standard. All the test molecules showed greater reduction in the immobility duration after 24 hr. The results obtained by forced swim test are given in table 3.

Most of the synthesized compounds exhibited moderate to good antimicrobial activity against the tested microorganisms S.aureus and E.coli. When compared to standard drug Ciprofloxacin 100µg/µl, the test molecules 1b, 3b, 4b and 4c (100 µg mL^{-1}) were found to exhibit good anti-bacterial activity against S.aureus and 1b, 2b, 3b, 4a, and 4b (100 µg mL^{-1}) were exhibited good anti-bacterial activity against E.coli. The compound substituted with phenyl Phthalimide, methyl Phthalimide and benzamide showing greater antibacterial activity for both organisms. The antimicrobial activity might be partly due to delocalization of the p electron along the O-C-N chain. The results obtained by agar plate disc diffusion method are given in tablem 4.
The results and discussion of my present study revealed that the alkyl arylamides/imide substituted dibenzo azepine molecules is very easy and convenient method with good yield exhibits good antidepressant and antibacterial activity with lesser side effects.

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REFERENCES

1. Thereza Christina Monteiro de Lima, Filipe Silveira Duarte , Gilliard Lach , Paulo Roberto Codeço Martins , Gilberto Alves Romeiro: Evidence for the involvement of the monoaminergic system in the antidepressant-like action of two 4-amine derivatives of 10,11-dihydro-5H-dibenzo [a,d] cycloheptane in mice evaluated in the tail suspension test. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2008; 32: 368–374.
2. Gopal Krishna Rao, Ranjit Kaur and PN Sanjay Pai. Synthesis and biological evaluation of some dibenzazepine analogs. J. Chem. Pharm. Res 2010; 2(1):489-496.
3. G Vitil, D Giannottil, M Altamural, R Riccil, G Volterraz, A Lecci, F Borsiniz, V Pestellinil: New [dibenzo(b,e)azepin-5-yl]-acetamides with anti-convulsant activity. Eur J Med Chem. 1993; 28:439-445.

4. L.P. Pathak and Rishikesh shukla: 2-nitro-6, 11-dioxo-6,11 dihydro-5h dibenzo [b,e]azepin- 4-yl-alkyl-arylamides/imides as possible hypoglycemic agents. Indian Journal of Heterocyclic Chemistry 2008 April-June; 17:373-374.
5. P. Ramalingam, S. Ganapaty, Y. Padmanaba Reddy, J. Ravindra Reddy: Synthesis and antimicrobial evaluation of dibenzo (b, e) azepin - 5, 10 - (1h)diones/ 10 – substituted dibenzo (b, e) azepines. International Journal of Pharmceutical Research and Development 2010; 1.
6. S Parasuraman: Prediction of activity spectra for substances. Journal of Pharmacology and Pharmaco therapeutics 2011; 2(1): 52-53.
7. Accelrys Software Inc., *Discovery Studio Modeling Environment, Release 3.1*, San Diego: Accelrys Software Inc., 2012.
8. A.Puratchikody, Mukesh doble and N.Ramalakshmi: Toxicity risk assessment of some novel quinoxalines. Rasayan Journal of Chemistry 2011; 4(3):636-639.
9. Patro Ganesh, CH.N.Kavitha, Malpani Amol, Monga Jeetender: Antidepressant activity of Abelmoschus esculentus alcoholic fruit extract. Int. J. Ph. Si. 2009; 1(2): 302-306.

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