Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 2317–2333
Bioorganic & Medicinal Chemistry

De novo design and synthesis of HIV-1 integrase inhibitors

Mahindra T. Makhija,[a,*] Rajesh T. Kasliwal,[a] Vithal M. Kulkarni[a] and Nouri Neamati[b]

[a]*Pharmaceutical Division, Department of Chemical Technology, University of Mumbai, Matunga, Mumbai 400019, India*
[b]*School of Pharmacy, University of Southern California, 1985 Zonal Avenue, Los Angeles, CA 90089-9121, USA*

Received 1 December 2003; revised 5 February 2004; accepted 5 February 2004

Abstract—Existing AIDS therapies are out of reach for most HIV-infected people in developing countries and, where available, they are limited by their toxicity and their cost. New anti-HIV agents are needed urgently to combat emerging viral resistance and reduce the side effects associated with currently available drugs. Toward this end, LeapFrog, a de novo drug design program was used to design novel, potent, and selective inhibitors of HIV-1 integrase. The designed compounds were synthesized and tested for in vitro inhibition of HIV-1 integrase. Out of the 25 compounds that were designed, and synthesized, four molecules (compounds **23**, **26**, **43**, and **59**) showed moderate to low inhibition of HIV-1 integrase for 3′-processing and 3′-strand transfer activities. Nonetheless, these compounds possess structural features not seen in known HIV-1 integrase inhibitors and thus can serve as excellent leads for further optimization of anti-HIV-1 integrase activity.

1. Introduction

Nearly 20 years have passed since acquired immunodeficiency syndrome (AIDS) was recognized and the human immunodeficiency virus (HIV) was identified as the causative agent. Still, there is neither a cure nor a vaccine for AIDS. This pandemic has continued to exceed all expectations in the severity and scale of its impact. An estimated 36 million people worldwide are currently living with HIV, and some 20 million people have already died, giving a cumulative total number of HIV infections of 56 million.[1] Responding to HIV on a scale commensurate with the pandemic is a global imperative. This task has already begun and the important advances have been made toward suppressing the virus, bolstering the immune system, and extending and enhancing the lives of patients through combination antiretroviral therapy, which involves use of reverse transcriptase and protease inhibitors.[2,3] But challenges remain. Treatment does not suppress HIV replication in all patients, and the emergence of drug resistant virus hinders subsequent treatment. This has prompted the search for new drugs and new therapeutic strategies to control chronic viral replication.[4]

HIV-1 integrase (HIV-1 IN) is an attractive target for antivirals because it is essential for HIV replication and, unlike protease and reverse transcriptase, there are no known counterparts in the host cell.[5] Furthermore, integrase uses a single active site to accommodate two different configurations of DNA substrates, which may constrain the ability of HIV to develop drug resistance to integrase inhibitors.[6–8]

Recently, we performed a 3D QSAR on different classes of compounds using a novel alignment technique based on molecular electrostatic potentials (MEPs) to handle the tremendous diversity of this data set.[9] The results of comparative molecular field analysis (CoMFA) for this study are summarized in Table 1.

All molecular modeling techniques and CoMFA studies were performed on Silicon Graphics INDY R5000 workstation using the SYBYL 6.6 molecular modeling software from Tripos, Inc., St.Louis, MO.[10]

1.1. Molecular conformation and alignment

The compounds were built from fragments in the SYBYL database. Full geometry optimization of the constructed structures was performed using the standard Tripos force field[11] with a distance-dependent dielectric function and a 0.001 kcal/mol Å energy gradient convergence criterion. Partial atomic charges required for calculation of the electrostatic interaction were

Keywords: AIDS; HIV-1 integrase; CoMFA; LeapFrog.
* Corresponding author at present address: Western Australian Biomedical Research Institute, Curtin University of Technology, GPO Box U1987, Perth, WA 6845, Australia. Tel.: +61-8-9266-7517; fax: +61-8-9266-7485; e-mail: m.makhija@exchange.curtin.edu.au

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.02.005

Table 1. Summary of CoMFA results using MEP based alignment

Statistic	3′-Processing	3′-Strand transfer
r^2_{cv}	0.830	0.746
s_{press}	0.365	0.411
Components	5	5
r^2	0.982	0.965
SE	0.118	0.151
F value	312.01	156.68
$P(r^2 = 0)$	0.000	0.000
r^2_{bs}	0.991	0.979
r^2_{pred}	0.608	0.660

computed by a semiempirical molecular orbital method using the MOPAC 6.0 program. The charges were computed using the PM3 model Hamiltonian (keywords: 1SCF, RHF, MMOK).[12,13] Using the systematic search protocol, rotatable bonds in compounds were searched from 0° to 360° in 10° increments. The lowest energy conformation of each compound obtained using systematic search, was minimized using Tripos force field and subsequently used in the analysis. Molecules were aligned using minima and maxima of MEPs (see Ref. 9a).

1.2. CoMFA analysis

CoMFA steric and electrostatic fields were separately calculated at each lattice intersection on a regularly spaced grid of 2.0 Å units in all x, y, and z directions. The grid pattern, generated automatically by the SYBYL/CoMFA routine, extended 4.0 Å in all directions beyond the dimensions of the aligned molecules. The steric term represents the van der Waals (6–12) interactions, while the Coulombic term represents the electrostatic interactions for which a distance-dependent dielectric expression $\varepsilon = \varepsilon_0 R_{ij}$ with $\varepsilon_0 = 1.0$ was adopted. An sp^3 carbon atom with a van der Waals radius of 1.52 Å and a +1.0 charge was selected as the probe to calculate the steric and electrostatic fields. Values of the steric and electrostatic energy were truncated at 30 kcal/mol. The electrostatic contributions were ignored at lattice intersection with maximal steric interactions.[14–16]

1.3. Partial least squares (PLS) analysis

Partial least squares (PLS) fitting was applied to derive the 3D QSAR models.[17] The PLS method has been applied successfully in numerous QSAR studies aiming to rationalize those structural features affecting biological activity. PLS regression seeks a relationship between Y and X, where vector Y is the response or dependent variable and X represents the descriptor data. PLS analyses were performed following the CoMFA standard implementation in SYBYL. To check statistical significance of the models, cross-validations were done by means of the 'leave-one-out' (LOO) procedure using the enhanced version of PLS, the SAMPLS method.[18] The results from cross-validation analysis were expressed as the cross-validated r^2 value (r^2_{cv}). The cross-validated r^2 is defined as

$$r^2_{cv} = 1 - \mathrm{PRESS}/\sum (Y - Y_{mean})^2,$$

where $\mathrm{PRESS} = \sum (Y - Y_{pred})^2$.

The optimal number of components was determined by selecting the smallest s_{press} value. s_{press} is the root mean PRedictive Error Sum of Squares. It is an expected uncertainty in prediction for an individual compound based on the data available from other compounds in the set.

$$s_{press} = (\mathrm{PRESS}/(n - c - 1))^{1/2},$$

where n = number of rows and c = number of components.

Usually the smallest s_{press} value corresponds to the highest r^2_{cv} value. The optimal number of components was subsequently used to derive the final QSAR models. For all conventional analyses (no cross-validation) the 'minimum sigma' standard deviation threshold was set to 2.0 kcal/mol. The r^2_{cv}, s_{press}, r^2, and SE values were computed as defined in SYBYL. SE is the standard error of estimate. It is a measure of the target property uncertainty still unexplained after the QSAR has been derived. In Table 1, $P(r^2) = 0$ means the probability of obtaining the observed F-ratio value by chance alone, if the target and the explanatory variables themselves are truly uncorrelated. When $P(r^2) = 0$ is zero, then results are not by chance and are significant. To further assess the robustness and statistical confidence of the derived models, bootstrapping analysis (1000) runs was performed. A common test to check the consistency of the models is to scramble the biological data and repeat the model derivation process, allowing detection of possible chance correlations. After randomizing our data set, we observed very low or negative r^2_{cv} values in the PLS analyses.

1.4. Predictive r^2 value

The predictive r^2 was based only on molecules not included in the training set and is defined as:

$$r^2_{pred} = (\mathrm{SD} - \mathrm{PRESS})/\mathrm{SD},$$

where SD is the sum of the squared deviations between the biological activity of molecules in the test set and the mean biological activity of the training set molecules and PRESS is the sum of the squared deviations between predicted and actual activity values for every molecule in the test set.[19] Like r^2_{cv}, the predictive r^2 can assume a negative value reflecting a complete lack of predictive ability of the training set for the molecules included in the test set.[20]

1.5. CoMFA contour maps

The contour maps for CoMFA steric and electrostatic fields are shown in Figures 1 and 2 for 3′-processing and 3′-strand transfer activities, respectively. The green and yellow polyhedra describe regions of space around the molecules where increases in steric bulk, respectively,

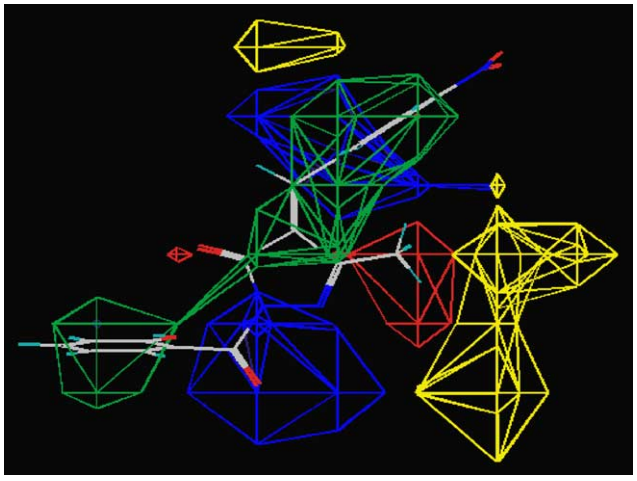


Figure 1. CoMFA steric and electrostatic fields for 3′-processing activity.

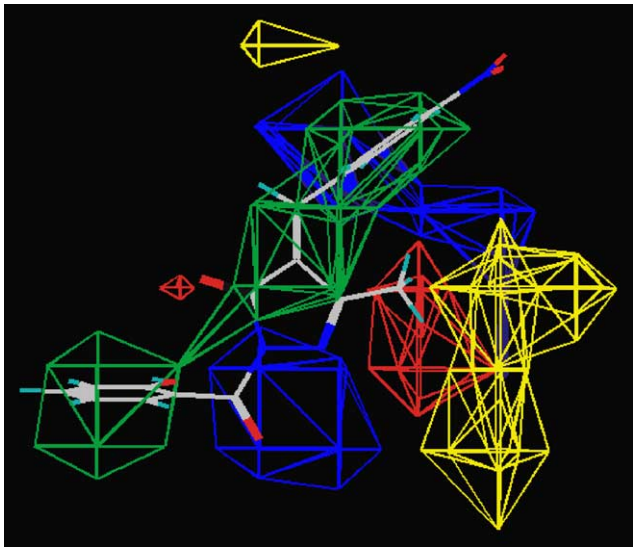


Figure 2. CoMFA steric and electrostatic fields for 3′-strand transfer activity.

enhance or diminish the inhibition of integrase. The red and blue polyhedra describe regions where a high electron density (i.e., negative charge or polarity) within the substrate structure enhances or diminishes activity, respectively. The contour maps of CoMFA thus obtained were used as pseudoreceptor model input for a de novo drug design program LeapFrog (LF) to design some novel inhibitors of HIV-1 integrase. The compounds thus designed were synthesized and tested for in vitro inhibition of 3′-processing and 3′-strand transfer activities of HIV-1 integrase.

2. LeapFrog[21]

LeapFrog (LF) is a second-generation de novo drug discovery program that performs molecular evolution or electronic screening, by repeatedly making some structural change and then either keeping or discarding the results, depending on the evolution. LF can run in three alternative modes:

OPTIMIZE: suggests improvement to existing leads;
DREAM: proposes new molecules expected to have good binding;
GUIDE: supports interactive design by performing and evaluating user modifications.

LF can start from either of two kinds of information:

Cavity: a receptor structure, usually a biopolymer;
CoMFA: a pharmacophoric model, which must be expressed as CoMFA model.

New ligand structures are evaluated mainly on their binding energy relative to their immediate precursor.

Binding energy as calculated in LF has three major components:

1. steric and electrostatic enthalpies of binding process calculated using the Tripos force field;
2. cavity desolvation energy; and
3. ligand desolvation energy.

For the purpose of this study, new ligands were generated by interactive design method in GUIDE mode using all the three components of binding energy. CoMFA contour maps were used to generate the hypothetical cavity for LF calculations.

There are two distinct stages in converting a CoMFA result into an LF cavity:

(a) a direct point-by-point mapping of the properties of a CoMFA grid to an intermediate cavity grid of the same coarse resolution,
(b) an interpolation of the intermediate cavity grid values to the closely spaced grid values actually used by LF.

The cavity thus obtained was used to generate the sitepoints. The charge of a sitepoint atom is positive, negative, or lipophilic. Its value is compared with 1.0. If the atom charge is smaller in magnitude than 1.0 the sitepoint is lipophilic; if greater than +1.0, the sitepoint seeks a negative atom; and if less than −1.0, the sitepoint seeks a positive atom in the approaching fragment.

LF's central operation is a processing loop. In each pass through the loop, a type of move is randomly selected. Depending on the move type, the fragment and probably particular atom(s) may also be randomly selected for the move to act upon. LF then tries to carry out the move. If the move succeeds, any new ligand structure(s) is evaluated using interaction with the site points. Moves normally used in LF are NEW, JOIN, COMPLEMENT, BRIDGE, CROSSOVER, FUSE, FLY, TWIST, etc.

Thus, the hypothetical cavity obtained using CoMFA maps was used along with the NEW move in LF to

generate the novel ligands, which were then built using other moves like JOIN, FUSE, COMPLEMENT, BRIDGE, and OPTIMIZE. The new ligands were checked for alternative orientations using FLY and were completely minimized using TWIST move to evaluate the binding energy for the minimum energy conformation of the ligand. The process was repeated to generate the different classes of ligands, which are described below. The ligands, which showed binding, better than the reference structure were taken up for synthesis.

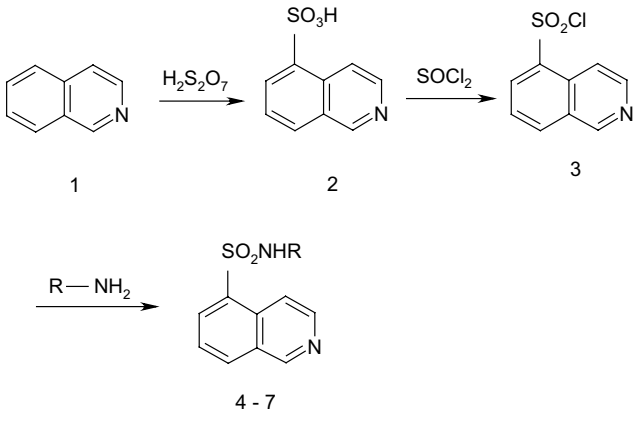
3. Chemistry

3.1. Synthesis of isoquinoline sulfonamides

The general route for the synthesis of isoquinoline sulfonamides is outlined in Scheme 1. Isoquinoline was sulfonated using 50% oleum under ice-cold conditions to get isoquinoline-5-sulfonic acid,[22–24] which was chlorinated using excess of thionyl chloride to get the sulfonyl chloride derivative.[25,26] Finally, the sulfonyl chloride was treated with appropriate amines to get the corresponding sulfonamides.

3.2. Synthesis of furoyl pyrazolones

The general route for the synthesis of furoyl pyrazolones is outlined in Scheme 2. Appropriately substituted amine was diazotized using sodium nitrite and concentrated hydrochloric acid at 0–5 °C. In case of *ortho* substituted amines, diazonium salt was reduced using stannous chloride and hydrochloric acid to the corresponding hydrazine derivative; whereas for *meta* and *para* substituted amines, sodium sulfite was used for reduction to the corresponding hydrazine derivative.[27,28] Substituted hydrazine thus obtained was reacted with ethyl acetoacetate to give substituted pyrazolone.[29,30] The pyrazolone was finally acylated using furoyl chloride to get substituted furoyl pyrazolone.



Scheme 1.

3.3. Synthesis of N-substituted indole-2,3-dione (isatins)

The general route for the synthesis of N-substituted isatins is outlined in Scheme 5.

Appropriately substituted toluenes were brominated using *N*-bromosuccinimide and carbon tetrachloride in presence of catalytic amount of benzoyl peroxide.[31–37] Substituted bromomethyl derivatives were condensed with potassium salt of isatin (1H-indole-2,3-dione), which in turn was obtained by reacting isatin with potassium carbonate, to get corresponding N-substituted indole-2,3-diones.[38,39]

3.4. Synthesis of 2-phenylmethanesulfonyl-benzothiazoles

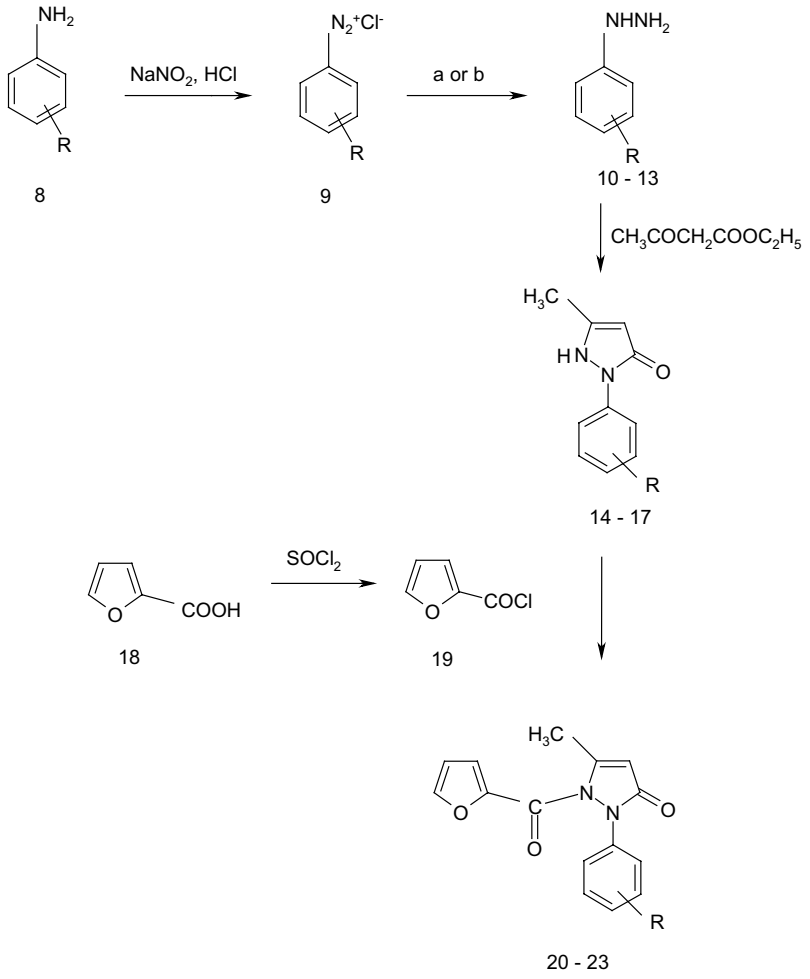
The general route for the synthesis of 2-phenylmethanesulfonyl-benzothiazoles is outlined in Scheme 6.

Appropriately substituted toluenes were brominated using *N*-bromosuccinimide and carbon tetrachloride in presence of catalytic amount of benzoyl peroxide. Substituted bromomethyl derivatives were condensed with potassium salt of benzothiazole-2-thiol, which in turn was obtained by reacting benzothiazole-2-thiol with potassium carbonate, to get the corresponding sulfides **52**–**55**.[40] These sufides were then oxidized using *meta*-chloroperbenzoic acid (MCPBA) to the corresponding sulfones **56**–**59**.

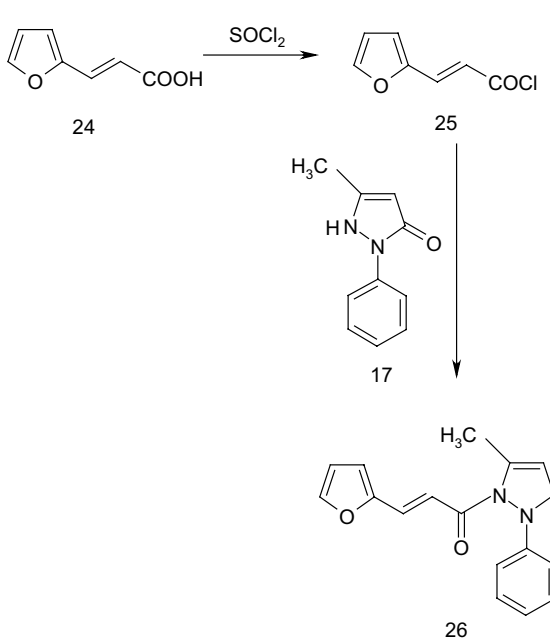
4. Results and discussion

Although several classes of inhibitors of HIV-1 IN are reported, none has reached the market. This is due to the fact that most of the reported compounds are polyhydroxylated derivatives, which are cytotoxic and nonselective. Thus, new compounds are needed, which selectively inhibit HIV-1 IN. De Novo drug design program, LeapFrog, was used to design novel inhibitors of HIV-1 integrase. The input for Leapfrog was a pharmacophoric model derived from our CoMFA study on the diverse set of HIV-1 IN inhibitors, which were aligned by a novel method based on molecular electrostatic potentials (MEPs). Novel compounds were designed using the molecular evolution process of LeapFrog and their binding energies (BE) were calculated. The compounds showing improved binding energies were selected for synthesis. These compounds were tested for in vitro inhibition of 3′-processing and 3′-strand transfer activities of HIV-1 IN. The calculated binding energies and the experimentally determined IC_{50} values are shown in Table 12. The plots of LF binding energies versus IC_{50} values for the two activities are shown in Figures 3 and 4, respectively.

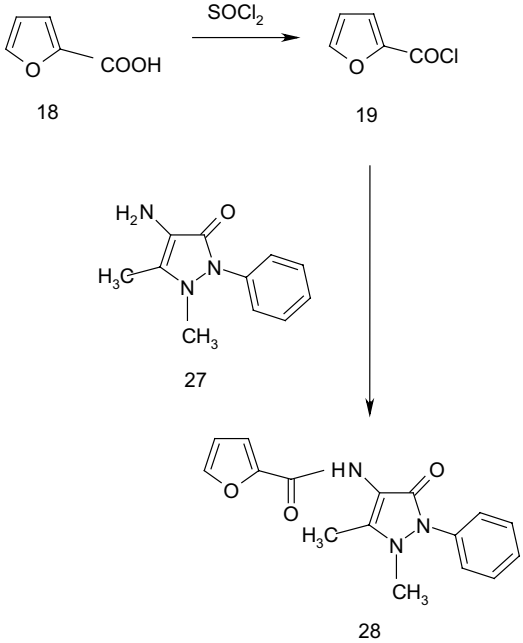
Four different series of compounds were synthesized depending on their binding energy scores:



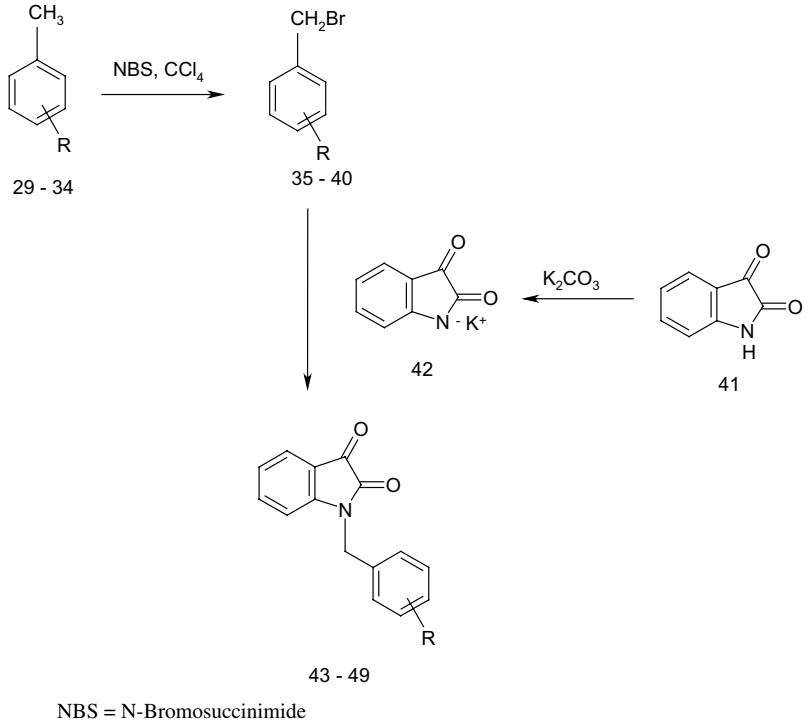
Scheme 2. Reagents and conditions: (a) $SnCl_2$, $2H_2O$ and HCl; (b) Na_2SO_3.



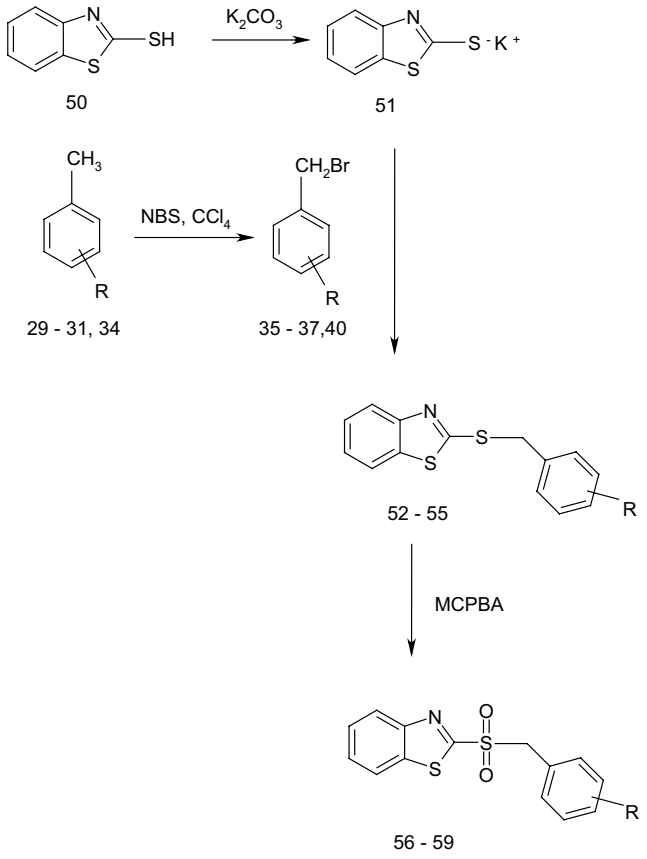
Scheme 3.



Scheme 4.



Scheme 5.



Scheme 6.

(i) *Isoquinoline-5-sulfonamides*: Compounds **4**–**7** belong to this structural class. When trifluoromethyl group was present at *ortho* position to the sulfonamide function **4**, very little activity was observed. In case of triazole derivative **5**, activity completely abolished. Moderate activity was observed for compound **6**, which has chloro substituent at the *meta* position and fluoro group at *para* position. Similarly, for *n*-propyl derivative of isoquinoline sulfonamide **7**, activity was retained. This shows that any substituent at position *ortho* to the sulfonamide moiety is detrimental to HIV-1 IN inhibitory activity. The nature of substituent hardly seems to be affecting the activity as both aryl group with free *ortho* position and alkyl group showed almost similar activities.

(ii) *Furoyl pyrazolones*: Compounds **20**–**28** belong to this structural class. In this series, Substitutions were carried out on both furoic acid as well as pyrazolone part. In case of pyrazolone part, the structural requirements for showing inhibition of HIV-1 IN activity are very rigid, that is, any substitution on phenyl ring or change in the position of furoyl group results in loss of activity. This is evident from the inactivity of compounds having 3-nitro **20**, 2-fluoro-4-bromo **21**, and 3-chloro groups **22** on the phenyl ring; and change in the position of furoyl group from ring nitrogen to side chain amino group **28**. When phenyl group in pyrazolone part was unsubstituted **23**, it showed moderate activity for both 3′-processing and 3′-strand transfer. Replacement of 2-furoyl with furan-2-ylacryloyl group **26** did not improve the activity much.

(iii) *Indole-2,3-diones*: Compounds **43**–**49** belong to this structural class. In this series, the effect of different substitutions on nitrogen of indole-2,3-dione was evaluated. The activity could directly be correlated with the electron withdrawing power of the substituent on the

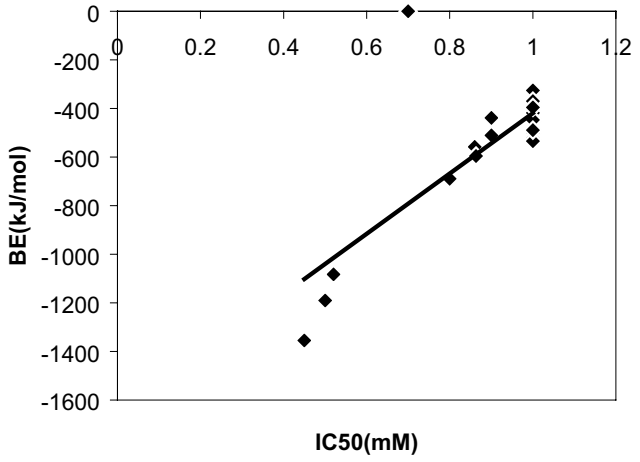


Figure 3. Plot of calculated BE versus experimentally determined IC_{50} values for 3′-processing activity.

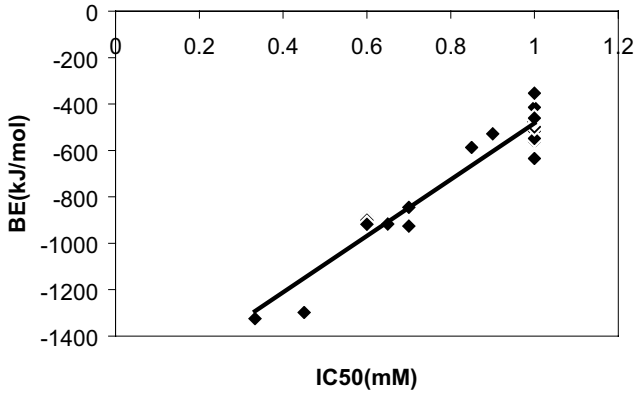


Figure 4. Plot of calculated BE versus experimentally determined IC_{50} values for 3′-strand transfer activity.

N-benzyl group. Highest activity in this series was obtained when nitro group was present at *para* position **43**. Activity somewhat decreased when the position of nitro group was changed from *para* to *ortho* **46** or when chloro group was present instead of nitro **47**. Reduced activity was also observed when nitro group was replaced with bromo **44**, or when quinolin-8-yl methyl group was substituted on the nitrogen of indole-2,3-dione **45**. Biphenyl cyano derivative **48** did not show any activity but when cyano group was hydrolyzed to carboxylic acid, some activity was observed **49**. This may be due to the involvement of COOH group in some hydrogen-bonding interaction with the enzyme, which cyano group cannot form.

(iv) *2-Phenylmethanesulfonyl-benzothiazoles*: Compounds **52**–**55** are the 2-phenylmethylthio-benzothiazoles, and **56**–**59** are their corresponding sulfones. Most of these compounds did not show any activity, with the notable exception of compound **59**. This shows that in this series, an electron withdrawing group such as cyano is required at the *ortho* position of the biphenyl ring. But, alone cyano group is not enough as the corresponding sulfide **55** is inactive. The conversion of sulfide to sulfone **59** afforded some activity consistent with the fact that most of the active inhibitors of integrase possess hydrogen bond acceptors.

5. Conclusions

Novel HIV-1 integrase inhibitors belonging to four different structural classes—isoquinoline sulfonamides, furoyl pyrazolones, indole-2,3-diones, and 2-phenylmethanesulfonyl-benzothiazoles were designed by a de novo drug design approach using LeapFrog program. The designed compounds were synthesized, and their in vitro activites were evaluated. Some compounds showed moderate inhibition of HIV-1 integrase. It was an attempt to show how a de novo method like LeapFrog predicts the activities of compounds prior to their synthesis. Since the designed compounds contain novel structural characteristics not seen in known integrase inhibitors, they can act as lead compounds for future drug design. Further modifications of these compounds can help to realize potent activity for these series of compounds.

6. Experimental

All melting points were recorded on a Oswal capillary melting point apparatus and are uncorrected. The structures of compounds were confirmed by IR, ^{1}H NMR, and mass spectroscopy. IR spectra were recorded on a Buck Scientific M-500 spectrometer using KBr pellets. ^{1}H NMR spectra were recorded on a Bruker AMX-500 MHz FT-NMR with tetramethylsilane (TMS) as the internal standard. Mass spectra were recorded on Shimadzu QP-5050 MS instrument. HPLC were recorded using JASCO PU 980 pump and UV 975 detector. All materials were purchased from commercial sources unless otherwise specified.

6.1. Synthesis of isoquinoline-5-sulfonic acid (2)

Isoquinoline (26 g, 0.201 mol) was added to 11 mL concentrated sulfuric acid with ice cooling in a 100 mL round bottom flask. With ice bath cooling and swirling 55 mL of 50% fuming sulfuric acid was added and the clear solution was allowed to stand, with occasional swirling, at room temperature for 48 h. It was then poured onto crushed ice and kept in refrigerator overnight. The long white needles of sulfonic acid were filtered and pressed dry of mother liquor with a rubber dam. The filter cake was slurried with 25 mL of water on the steam bath, cooled well in an ice bath, filtered, washed with ice water, and dried in oven at 70 °C for 4 h to get isoquinoline-5-sulfonic acid. Yield: 30 g (72.4%).

6.2. Synthesis of isoquinoline-5-sulfonyl chloride hydrochloride (3)

A mixture of isoquinoline-5-sulfonic acid, **2** (4 g, 0.019 mol), thionyl chloride (25 mL), and dimethylformamide (0.1 mL) was refluxed for 2 h, and the excess

thionyl chloride was distilled off in vacuo. The residue was suspended in dichloromethane, filtered, and washed with two portions of dichloromethane. The residue was collected and dried under vacuum to remove the solvent, yielding crude crystalline isoquinoline-5-sulfonyl chloride hydrochloride. As this compound is not stable, it was used for subsequent syntheses without further purification. Yield: 5.1 g (92.4%).

6.3. General procedure for the synthesis of compounds 4–7

To an ice cooled suspension of isoquinoline-5-sulfonyl chloride hydrochloride (5 g, 0.018 mol) in 20 mL dichloromethane, a mixture of substituted amine (0.018 mol) and pyridine (2.84 g, 0.036 mol) dissolved in 50 mL of dichloromethane was added in a dropwise manner with stirring over a period of 15 min. The reaction mixture was stirred for 1 h at 0–5 °C, and then at room temperature for 3–5 h. It was then washed twice with brine and dried over anhydrous sodium sulfate. The dried extract was evaporated to get the crude substituted isoquinoline-5-sulfonamide. The crude product was chromatographed on silica gel. The structures of compounds **4**–**7** are given in Table 2.

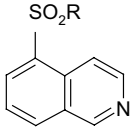
6.3.1. 5-(*N*-(2-Trifluoromethyl)phenyl)isoquinoline sulfonamide (4). The title compound was synthesized according to the general procedure using 5 g (0.018 mol) of compound **3** and 2.89 g (0.018 mol) of 2-trifluoromethyl aniline. The reaction mixture was stirred at room temperature for 4 h. The product (6.19 g) was obtained as white amorphous powder. IR (cm^{-1}): 2985.9, 2742.7, 1315, 1265.4, 1156, 1047.7; ^1H NMR ($CDCl_3$+DMSO-d_6) δ ppm: 7.18–8.62 (m, 10H), 9.32 (s, 1H); MS (*m*/*z*): 352 (M^+), 333 (M−19), 192, 176, 160, 128 (100); HPLC purity (%): 99.49.

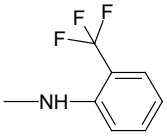
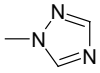
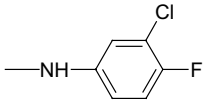
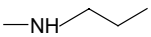
6.3.2. 5-([1,2,4]Triazole-1-sulfonyl)isoquinoline (5). The title compound was prepared using general procedure from 5 g (0.018 mol) of compound **3** and 1.242 g (0.018 mol) of 1H-[1,2,4]triazole. The reaction mixture was stirred at room temperature for 5 h. The product (4.51 g) was obtained as white crystalline powder. IR (cm^{-1}): 3128.4, 1370.4, 1322.6, 1170; ^1H NMR ($CDCl_3$+DMSO-d_6) δ ppm: 7.82 (t, 1H), 7.97 (s, 1H), 8.37 (d, 1H), 8.58 (d, 1H), 8.77 (t, 2H), 8.89 (s, 1H), 9.39 (s, 1H); MS (*m*/*z*): 260 (M^+), 196 (M−64), 192, 128 (100), 40; HPLC purity (%): 97.06.

6.3.3. 5-(*N*-(3-Chloro-4-fluoro)phenyl)isoquinoline sulfonamide (6). The title compound was synthesized following general procedure by using 5 g (0.018 mol) of compound **3** and 2.61 g (0.018 mol) of 3-chloro-4-fluoro aniline. The reaction mixture was stirred for 3.5 h. The product (6.02 g) was obtained as white needle shaped crystals. IR (cm^{-1}): 2998.2, 2730.6, 1350.5, 1320.1, 1161.9, 1131.5, 772.9, 700, 651.3; ^1H NMR ($CDCl_3$+DMSO-d_6) δ ppm: 6.75–8.73 (m, 9H), 9.38 (s, 1H); MS (*m*/*z*): 336 (M^+), 192, 128, 40 (100); HPLC purity (%): 97.60.

6.3.4. 5-(*N*-Propyl)isoquinoline sulfonamide (7). The title compound was prepared by general procedure using 5 g (0.018 mol) of compound **3** and 1.062 g (0.018 mol) of *n*-propyl amine. The reaction mixture was stirred at room temperature at 3 h. The product (4.55 g) was obtained as white amorphous powder. IR (cm^{-1}): 3095.4, 2852.2, 1368.8, 1326.3, 1131.5; ^1H NMR ($CDCl_3$+DMSO-d_6) δ ppm: 0.77 (t, 3H), 1.43 (m, 2H),

Table 2. Structures, yield, mp, and mobile phase for TLC and column chromatography of the compounds **4**–**7** synthesized according to Scheme 1



Sr. no.	R	Yield (%)	Mp (°C)	Mobile phase and R_f
4		93	182–184	Dichloromethane–methanol (1:0.05) $R_f = 0.72$
5		91.8	185–188	Dichloromethane–methanol (1:0.02) $R_f = 0.33$
6		94.6	>200	Chloroform–methanol (1:0.1) $R_f = 0.66$
7		96.2	115–120	Chloroform $R_f = 0.62$

2.91 (q, 2H), 7.68–8.68 (m, 6H), 9.36 (s, 1H); MS (m/z): 251 (M+1), 250 (M^+), 221, 192, 176, 128 (100); HPLC purity (%): 95.31.

6.4. General procedure for the synthesis of compounds 10–13

Method A: In a 250 mL, three-neck flask equipped with thermometer, stirrer, and dropping funnel were placed 5 g of substituted aniline **8** and 10 mL of water. The mixture was stirred and cooled in an ice bath while 20 mL of hydrochloric acid was added as rapidly as the temperature could be kept below 40 °C. The reaction mixture was cooled rapidly in an ice bath to 0–5 °C while a solution of sodium nitrite (equivalent mole) in 10 mL water was added through a dropping funnel slowly with stirring over a period of 10 min to get the substituted diazonium salt **9**. This diazonium salt solution was then added in a dropwise manner to a solution of sodium sulfite (5 mol equiv) in 100 mL of water in a two-neck flask equipped with the dropping funnel and reflux condenser. The reaction mixture was warmed to 60 °C for 1 h, acidified with hydrochloric acid, and heated on water bath for further 3–5 h. The reaction mixture was cooled to 0 °C and the crystals of substituted hydrazine hydrochloride were filtered. The crystals were dissolved in water and neutralized with sodium acetate solution to get the corresponding hydrazine derivatives **10–13**. These hydrazines were used in subsequent syntheses without further purification.

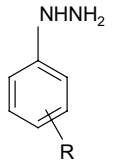
Method B: The diazonium salt was prepared in a similar way as described in method A. The diazonium salt solution was added in a dropwise manner over a period of 20 min to an ice cooled solution of stannous chloride dihydrate in 80 mL of hydrochloric acid. The reaction mixture was stirred at 0–5 °C for 3 h and then kept in a refrigerator overnight. The crystals of substituted hydrazine hydrochloride were filtered and washed twice with hydrochloric acid (25 mL each time) to remove tin. The product was dissolved in water, neutralized with sodium acetate solution as described in method A to get substituted hydrazines **10–13**. These hydrazines were used in subsequent syntheses without further purification.

The structures of compounds synthesized according to these methods (A or B) are given in Table 3.

6.4.1. 3-Nitro phenyl hydrazine (10). The title compound was synthesized according to method A using 5 g (0.036 mol) of 3-nitro aniline **8**, 2.49 g (0.036 mol) of sodium nitrite, and 22.81 g (0.181 mol) of sodium sulfite. The reaction mixture after acidification was heated for 3 h on a water bath. The product (4.68 g) was obtained as yellow amorphous powder.

6.4.2. 4-Bromo-2-fluoro-phenyl hydrazine (11). The title compound was prepared according to method B by using 5 g (0.026 mol) of 4-bromo-2-fluoro-aniline **8**, 1.81 g (0.026 mol) of sodium nitrite, and 11.87 g (0.052 mol) of stannous chloride dihydrate. The reaction mixture after acidification was heated for 3 h on a water bath. The product (4.10 g) was obtained as gray crystalline powder.

Table 3. Structures, yield, mp, and mobile phase for TLC of the compounds synthesized according to method A or B



10 - 13

Sr. no.	R	Method	Yield (%)	Mp (°C)	Mobile phase and R_f
10	3-NO_2	A	84.5	92–95	Chloroform $R_f = 0.52$
11	2-F–4-Br	B	76.2	75–78	Chloroform $R_f = 0.46$
12[27]	3-Cl	A	65.8	36–48	Chloroform $R_f = 0.31$
13[a]	H				

[a] Phenyl hydrazine was purchased from commercial source.

6.4.3. 3-Chloro phenyl hydrazine (12). The title compound was synthesized by method A using 5 g (0.039 mol) of 3-chloro aniline **8**, 2.70 g (0.039 mol) of sodium nitrite, and 24.6 g (0.195 mol) of sodium sulfite. The reaction mixture after acidification was heated for 5 h on a water bath. The product (3.67 g) was obtained as low melting solid or as brown viscous liquid.

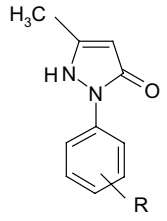
6.5. General procedure for the synthesis of compounds 14–17

To an appropriately substituted phenyl hydrazine dissolved in 20 mL of methanol, ethyl acetoacetate (equivalent mole) dissolved in 20 mL of methanol was added and the mixture was refluxed for 3–10 h. Methanol was recovered by rotary vacuum distillation. The residue was dissolved in chloroform, washed with brine, dried over anhydrous sodium sulfate, and evaporated to get crude pyrazolone derivative, which was purified by column chromatography.

The structures of the compounds synthesized according to this method are given in Table 4.

6.5.1. 5-Methyl-2-(3-nitro-phenyl)-1,2-dihydro-pyrazol-3-one (14). The title compound was synthesized according to the general procedure using 4 g (0.026 mol) of 3-nitro phenyl hydrazine **10** and 3.38 g (0.026 mol). The reaction mixture was refluxed for 4 h. The product (4.3 g) was obtained as yellow crystalline powder. IR (cm^{-1}): 3429.8, 3107.5, 1715.3, 1630.2, 1569.5, 1356.7.

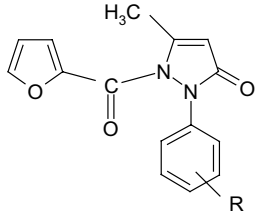
Table 4. Structures, yield, mp, and mobile phase for TLC and column chromatography of compounds **14–17**



14 - 17

Sr. no.	R	Yield (%)	Mp (°C)	Mobile phase and R_f
14[29]	3-NO_2	75.2	140–142	Chloroform $R_f = 0.52$
15	2-F–4-Br	82.1	135–138	Chloroform $R_f = 0.22$
16	3-Cl	64.7	94–98	Chloroform $R_f = 0.28$
17[30]	H	90.5	126–130	Chloroform $R_f = 0.64$

Table 5. Structures, yield, mp, and mobile phase for TLC and column chromatography of furoyl pyrazolones



20 - 23

Sr. no.	R	Yield (%)	Mp (°C)	Mobile phase and R_f
20	3-NO_2	65.8	140–142	Chloroform $R_f = 0.45$
21	2-F–4-Br	72.4	128–130	Chloroform $R_f = 0.48$
22	3-Cl	52.6	142–145	Chloroform–methanol (1:0.1) $R_f = 0.52$
23	H	78.5	154–158	Chloroform–hexane (1:1) $R_f = 0.78$

6.5.2. 2-(4-Bromo-2-fluoro-phenyl)-5-methyl-1,2-dihydro-pyrazol-3-one (15). The title compound was prepared according to the general procedure by using 3.5 g (0.017 mol) of 4-bromo-2-fluoro-phenyl hydrazine **11** and 2.21 g (0.017 mol) of ethyl acetoacetate. The reaction mixture was refluxed for 6.5 h. The product (3.79 g) was obtained as white needle shaped crystals. IR (cm^{-1}): 3113.7, 2992.1, 1721.4, 1338.4, 1235.1, 1161.9, 1119.4, 1070.7, 669.6.

6.5.3. 2-(3-Chloro-phenyl)-5-methyl-1,2-dihydro-pyrazol-3-one (16). The title compound was synthesized according to the general procedure using 3 g (0.021 mol) of 3-chloro phenyl hydrazine **12** and 2.73 g (0.021 mol) of ethyl acetoacetate. The reaction mixture was refluxed for 10 h. The product (2.84 g) was obtained as white crystalline powder. IR (cm^{-1}): 3113.7, 2979.9, 1727.4, 1624.1, 1502.5, 815.4, 748.7, 687.

6.5.4. 5-Methyl-2-phenyl-1,2-dihydro-pyrazol-3-one (17). The title compound was synthesized according to the general procedure using 4 g (0.037 mol) of phenyl hydrazine **13** and 4.81 g (0.037 mol) of ethyl acetoacetate. The reaction mixture was refluxed for 3 h. The product (5.82 g) was obtained as pale yellow amorphous powder. IR (cm^{-1}): 3332.6, 1721.4, 1605.8, 1460.

6.6. General procedure for the synthesis of compounds 20–23

To 1.5 g (0.013 mol) of furan-2-carboxylic acid **18** dissolved in 10 mL of chloroform in a two-neck 100 mL flask equipped with a dropping funnel and reflux condenser attached with a calcium chloride guard tube, 1.55 g (0.013 mol) thionyl chloride was added and it was refluxed for 1 h. The mixture was cooled to 0–5 °C and to it was added a solution of appropriately substituted pyrazolone **14–17** (0.013 mol), and 2 g (0.026 mol) pyridine in 30 mL chloroform in a dropwise manner over a period of 15 min. The reaction mixture was then stirred at room temperature for 3–6 h. It was then washed thrice with brine and dried over anhydrous sodium sulfate. The dried extract was distilled off in rotary vacuum to recover the solvent. The crude furoyl pyrazolone **20–23** was further purified by column chromatography.

The structures of compounds synthesized by this method are given in Table 5.

6.6.1. 1-(Furan-2-carbonyl)-5-methyl-2-(3-nitro-phenyl)-1,2-dihydro-pyrazol-3-one (20). The title compound was synthesized according to the general procedure by using 2.84 g (0.013 mol) of 5-methyl-2-(3-nitro-phenyl)-1,2-dihydro-pyrazol-3-one **14** and 1.5 g (0.013 mol) furan-2-carboxylic acid **18**. The reaction mixture was stirred at room temperature for 4 h. The product (2.66 g) was obtained as pale yellow amorphous powder. IR (cm^{-1}): 3177.6, 3081.9, 1778.9, 1534; ^1H NMR ($CDCl_3$) δ ppm: 2.36 (s, 3H), 6.39 (s, 1H), 6.64 (d, 1H), 7.48–8.15 (m, 5H), 8.74 (s, 1H); MS (m/z): 313 (M^+), 218, 190, 122, 95 (100); HPLC purity (%): 99.58.

6.6.2. 2-(4-Bromo-2-fluoro-phenyl)-1-(furan-2-carbonyl)-5-methyl-1,2-dihydro-pyrazol-3-one (21). The title compound was synthesized according to the general procedure by using 3.52 g (0.013 mol) of 2-(4-bromo-2-fluoro-phenyl)-5-methyl-1,2-dihydro-pyrazol-3-one **15** and 1.5 g (0.013 mol) furan-2-carboxylic acid **18**. The reaction mixture was stirred at room temperature for 4.5 h. The product (3.43 g) was obtained as white amorphous powder. IR (cm^{-1}): 3144.1, 1747.3, 1557.1, 1514.6, 1472.1, 1393, 1295.9, 1180.2, 1076.9, 760.8; ^1H NMR ($CDCl_3$) δ ppm: 2.34 (s, 3H), 6.26 (s, 1H), 6.56 (d, 1H),

7.23–7.44 (m, 4H), 7.66 (s, 1H); MS (m/z): 366 (M+2), 337 (M−28), 269, 173, 95 (100); HPLC purity (%): 99.08.

6.6.3. 2-(3-Chloro-phenyl)-1-(furan-2-carbonyl)-5-methyl-1,2-dihydro-pyrazol-3-one (22). The title compound was prepared according to the general procedure by using 2.7 g (0.013 mol) of 2-(3-chloro-phenyl)-5-methyl-1,2-dihydro-pyrazol-3-one **16** and 1.5 g (0.013 mol) furan-2-carboxylic acid **18**. The reaction mixture was stirred for 6 h at room temperature. The product (2.06 g) was obtained as white crystalline powder. IR (cm^{-1}): 2985.9, 1721.4, 1684.9, 1630.2, 1496.3, 815.4, 742.5, 681; ^1H NMR ($CDCl_3$) δ ppm: 2.40 (s, 3H), 7.04 (d, 1H), 7.24–8.16 (m, 5H), 9.04 (d, 2H); MS (m/z): 302 (M^+), 267 (M−35), 111, 90, 79, 39 (100); HPLC purity (%): 94.87.

6.6.4. 1-(Furan-2-carbonyl)-5-methyl-2-phenyl-1,2-dihydro-pyrazol-3-one (23). The title compound was synthesized according to the general procedure by using 2.26 g (0.013 mol) of 5-methyl-2-phenyl-1,2-dihydro-pyrazol-3-one **17** and 1.5 g (0.013 mol) furan-2-carboxylic acid **18**. The reaction mixture was stirred for 3 h at room temperature. The product (2.73 g) was obtained as white amorphous powder. IR (cm^{-1}): 3119.8, 2913, 1747.9, 1754.7, 1599.9, 1539.1, 1502.5; ^1H NMR ($CDCl_3$) δ ppm: 2.30 (s, 3H), 6.58 (d, 1H), 7.24–7.70 (m, 8H); MS (m/z): 268 (M^+), 173, 95 (100), 77; HPLC purity (%): 99.66.

6.6.5. 1-(3-Furan-2-yl-acryloyl)-5-methyl-2-phenyl-1,2-dihydro-pyrazol-3-one (26). The title compound was synthesized as outlined in Scheme 3.

To 1.79 g (0.013 mol) of 3-furan-2-yl-acrylic acid **24** dissolved in 10 mL of chloroform in a two-neck 100 mL flask equipped with a dropping funnel and reflux condenser attached with a calcium chloride guard tube, 1.55 g (0.013 mol) thionyl chloride was added and it was refluxed for 2 h. The mixture was cooled to 0–5 °C and to it was added a solution of 2.26 g (0.013 mol) of 5-methyl-2-phenyl-1,2-dihydro-pyrazol-3-one **17**, and 2 g (0.026 mol) pyridine in 30 mL chloroform in a dropwise manner over a period of 20 min. The reaction mixture was then stirred at room temperature for 2.5 h. It was then washed thrice with brine and dried over anhydrous sodium sulfate. The dried extract was distilled off in rotary vacuum to recover the solvent. The crude product **26** was further purified by column chromatography. The yield, melting point, and mobile phase for compound **26** are shown in Table 6. IR (cm^{-1}): 3113.7, 2985.9, 1721.4, 1684.9, 1630.2; ^1H NMR ($CDCl_3$) δ ppm: 2.34 (s, 3H), 6.18 (s, 1H), 6.40 (d, 1H), 6.51 (s, 1H), 6.70 (d, 1H), 7.29–7.58 (m, 7H); MS (m/z): 294 (M^+), 173, 121 (100), 77; HPLC purity (%): 97.47.

6.6.6. *N*-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-furan-2-carboxamide (28). The title compound was synthesized as outlined in Scheme 4.

Table 6. The yield, mp, and mobile phase for chromatography of compound **26**

Sr. no.	Yield (%)	Mp (°C)	Mobile phase and R_f
26	76.4 (2.91 g)	84–86	Chloroform–methanol (2:0.1) $R_f = 0.86$

To 1.5 g (0.013 mol) of furan-2-carboxylic acid **18** dissolved in 10 mL of chloroform in a two-neck 100 mL flask equipped with a dropping funnel and reflux condenser attached with a calcium chloride guard tube, 1.55 g (0.013 mol) thionyl chloride was added and it was refluxed for 1 h. The mixture was cooled to 0–5 °C and to it was added a solution of 2.63 g (0.013 mol) 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-pyrazol-3-one **27** and 2 g (0.026 mol) pyridine in 30 mL chloroform in a dropwise manner over a period of 15 min. The reaction mixture was then stirred at room temperature for 2 h. It was then washed thrice with brine and dried over anhydrous sodium sulfate. The dried extract was distilled off in rotary vacuum to recover the solvent. The crude product **28** was further purified by column chromatography. The yield, melting point, and mobile phase for compound **28** are shown in Table 7. IR (cm^{-1}): 3217, 3137.9, 1654.5, 1643.9, 1587.5, 1490.4; ^1H NMR ($CDCl_3$) δ ppm: 2.3 (s, 3H), 3.1 (s, 3H), 6.48 (d, 1H), 7.15–7.47 (m, 7H), 8.20 (s, 1H); MS (m/z): 297 (M^+), 187, 95 (100), 77; HPLC purity (%): 99.24.

6.7. General procedure for the synthesis of compounds 35–40

Appropriately substituted toluene **29**–**34** was dissolved in 50 mL of dry carbon tetrachloride. To this solution was added an equivalent mole of *N*-bromosuccinimide (NBS) and 0.05 g (0.0002 mol) of benzoyl peroxide and the mixture was refluxed for 4–12 h. After cooling, the succinimide was removed by filtration and the filtrate was concentrated under vacuum. The crude bromomethyl derivatives were used in subsequent syntheses without further purification. The structures of the compounds synthesized according to this method are given in Table 8.

6.7.1. 4-Nitro-benzylbromide (35). The title compound was synthesized according to the general procedure using 3 g (0.021 mol) of 4-nitro-toluene **29**, 3.73 g (0.021 mol) of NBS, and 0.05 g (0.0002 mol) of benzoylperoxide. The reaction mixture was refluxed for 4 h. The product (3.57 g) was obtained as lachrymatory yellow needles.

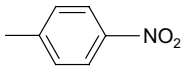
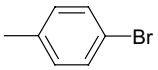
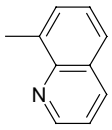
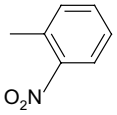
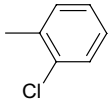
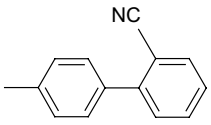
Table 7. The yield, mp, and mobile phase for chromatography of compound **28**

Sr. no.	Yield (%)	Mp (°C)	Mobile phase and R_f
28	78.12	136–138	Chloroform–methanol (1:0.1) $R_f = 0.57$

Table 8. Structures, yield, mp, and mobile phase for TLC and of compounds **35–40**

R–CH_2Br

35–40

Sr. no.	R	Yield (%)	Mp (°C)	Mobile phase and R_f
35		75.7	94–96	Chloroform $R_f = 0.82$
36[32]		70.2	64–66	Hexane $R_f = 0.78$
37		73.6	80–84	Chloroform $R_f = 0.84$
38[32]		40.3	42–44	Chloroform $R_f = 0.9$
39		53.7	Yellow oil	Chloroform $R_f = 0.91$
40[37]		78.5		Chloroform $R_f = 0.79$

6.7.2. 4-Bromo-benzylbromide (36). The title compound was synthesized according to the general procedure using 3 g (0.017 mol) of 4-bromo-toluene **30**, 3.02 g (0.017 mol) of NBS, and 0.05 g (0.0002 mol) of benzoylperoxide. The reaction mixture was refluxed for 4 h. The product (3.07 g) was obtained as lachrymatory brown needles.

6.7.3. 8-Bromomethyl-quinoline (37). The title compound was synthesized according to the general procedure using 3 g (0.02 mol) of 8-methyl-quinoline **31**, 3.55gm (0.02 mol) of NBS, and 0.05 g (0.0002 mol) of benzoylperoxide. The reaction mixture was refluxed for 6 h. The product (3.42 g) was obtained as lachrymatory pale brown crystals.

6.7.4. 2-Nitro-benzylbromide (38). The title compound was synthesized according to the general procedure using 3 g (0.021 mol) of 2-nitro-toluene **32**, 3.73 g (0.021 mol) of NBS, and 0.05 g (0.0002 mol) of benzoylperoxide. The reaction mixture was refluxed for 12 h. The product (1.90 g) was obtained as lachrymatory orange oil that crystallized upon keeping in refrigerator for 2 h.

6.7.5. 2-Chloro-benzylbromide (39). The title compound was synthesized according to the general procedure using 3 g of 2-chloro-toluene **33**, 4.21 g (0.023 mol) of NBS, and 0.05 g (0.0002 mol) of benzoylperoxide. The reaction mixture was refluxed for 10 h. The product (2.61 g) was obtained as lachrymatory yellow oil that crystallized upon keeping in refrigerator for 2 h.

6.7.6. 4′-Bromomethyl-biphenyl-2-carbonitrile (40). The title compound was synthesized according to the general procedure using 3 g (0.015 mol) of 4′-methyl-biphenyl-2-carbonitrile **34**, 2.66 g (0.015 mol) of NBS, and 0.05 g (0.0002 mol) of benzoylperoxide. The reaction mixture was refluxed for 4 h. The product (3.31 g) was obtained as white amorphous powder.

6.8. General procedure for the synthesis of compounds 43–49

To 1 g (0.006 mol) of 1H-indole-2,3-dione (isatin) **41** dissolved in 30 mL acetonitrile was added 1.65 g (0.012 mol) of potassium carbonate and 0.1 g (0.0006 mol) potassium iodide and the mixture was stirred at room temperature for 30 min. To this mixture was then added 0.006 mol of appropriately substituted bromomethyl derivative **35–40** dissolved in 20 mL acetonitrile, in a dropwise manner over a period of 15 min and the mixture was stirred at room temperature for 2–4 h. The product was purified by column chromatogra-

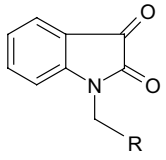
phy. The structures of compounds synthesized by this method are given in Table 9.

6.8.1. 1-(4-Nitro-benzyl)indole-2,3-dione (43). The title compound was synthesized according to the general procedure using 1 g (0.006 mol) of 1H-indole-2,3-dione and 1.29 g (0.006 mol) of 4-nitro-benzylbromide **35**. The reaction mixture was stirred at room temperature for 2 h. The product (1.6 g) was obtained as orange red amorphous powder. IR (cm^{-1}): 2979.9, 2876.6, 1733.6, 1697, 1557.1, 1514.6, 1368.8, 1308; ^1H NMR ($CDCl_3$) δ ppm: 5.1 (s, 2H), 6.72 (d, 1H), 7.15 (t, 1H), 7.53 (t, 3H), 7.67 (d, 1H), 8.23 (d, 2H); MS (m/z): 282 (M^+), 146, 90 (100), 78; HPLC purity (%): 99.92.

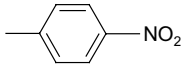
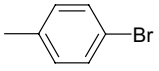
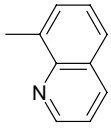
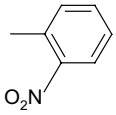
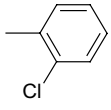
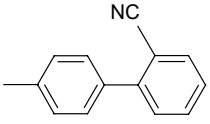
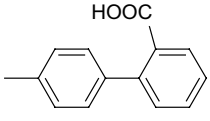
6.8.2. 1-(4-Bromo-benzyl)indole-2,3-dione (44). The title compound was synthesized according to the general procedure using 1 g (0.006 mol) of 1H-indole-2,3-dione and 1.5 g (0.006 mol) of 4-bromo-benzylbromide **36**. The reaction mixture was stirred at room temperature for 2 h. The product (1.69 g) was obtained as orange red crystalline powder. IR (cm^{-1}): 3052.9, 1727.4, 1599.9, 1465.9, 754.6; ^1H NMR ($CDCl_3$) δ ppm: 4.9 (s, 2H), 6.75 (d, 1H), 7.1 (t, 1H), 7.22 (d, 2H), 7.5 (m, 3H), 7.65 (d, 1H); MS (m/z): 316 (M^+), 315 (M−1), 236 (M−80), 169, 146 (100), 90; HPLC purity (%): 99.33.

6.8.3. 1-(Quinolin-8-ylmethyl)indole-2,3-dione (45). The title compound was synthesized according to the general procedure using 1 g (0.006 mol) of 1H-indole-2,3-dione and 1.33 g (0.006 mol) of 8-bromomethyl-quinoline **37**. The reaction mixture was stirred at room temperature for 3.5 h. The product (1.50 g) was obtained as dark red crystals. IR (cm^{-1}): 3046.7, 1733.6, 1612, 1472.1; ^1H NMR ($CDCl_3$) δ ppm: 5.7 (s, 2H), 7.0–7.8 (m, 8H), 8.2 (d, 1H), 9.0 (d, 1H); MS (m/z): 288 (M^+), 155 (100), 142, 129, 90; HPLC purity (%): 94.87.

Table 9. Structures, yield, mp, and mobile phase for TLC and column chromatography of compounds **43–49**



43 - 49

Sr. no.	R	Yield (%)	Mp (°C)	Mobile phase and R_f
43[38]		83.6	190–192	Chloroform $R_f = 0.48$
44		79.2	178–180	Chloroform $R_f = 0.8$
45		77.3	130–135	Chloroform $R_f = 0.36$
46		72.8	185–187	Chloroform $R_f = 0.66$
47		70.4	158–160	Chloroform $R_f = 0.82$
48		87.4	200–202	Chloroform $R_f = 0.66$
49[39]		67.9	195–197	Chloroform–methanol (1:0.1) $R_f = 0.63$

6.8.4. 1-(2-Nitro-benzyl)indole-2,3-dione (46). The title compound was synthesized according to the general procedure using 1 g (0.006 mol) of 1H-indole-2,3-dione and 1.29 g (0.006 mol) of 2-nitro-benzylbromide **38**. The reaction mixture was stirred at room temperature for 4 h. The product (1.39 g) was obtained as orange red crystalline powder. IR (cm^{-1}): 3077.1, 1733.6, 1612, 1520.8, 1453.8, 1344.3; ^1H NMR ($CDCl_3$) δ ppm: 5.4 (s, 2H), 6.7 (d, 1H), 7.16–7.71 (m, 6H), 8.2 (d, 1H); MS (m/z): 282 (M^+), 236 (M−46), 146 (100), 135, 90; HPLC purity (%): 99.15.

6.8.5. 1-(2-Chloro-benzyl)indole-2,3-dione (47). The title compound was synthesized according to the general procedure using 1 g (0.006 mol) of 1H-indole-2,3-dione and 1.23 g (0.006 mol) of 2-chloro-benzylbromide **39**. The reaction mixture was stirred at room temperature for 4 h. The product (1.29 g) was obtained as orange crystalline powder. IR (cm^{-1}): 3046.7, 1745.7, 1612, 1472.1, 760.8; ^1H NMR ($CDCl_3$) δ ppm: 5.1 (s, 2H), 6.77 (d, 1H), 7.1–7.67 (m, 7H); MS (m/z): 271 (M^+), 236 (M−35, 100), 146, 125, 90; HPLC purity (%): 99.33.

6.8.6. 4′-(2,3-Dioxo-2,3-dihydro-indol-1-ylmethyl)-biphenyl-2-carbonitrile (48). The title compound was synthesized according to the general procedure using 1 g (0.006 mol) of 1H-indole-2,3-dione and 1.63 g (0.006 mol) of 4′-bromomethyl-biphenyl-2-carbonitrile **40**. The reaction mixture was stirred at room temperature for 2 h. The product (2 g) was obtained as orange amorphous powder. IR (cm^{-1}): 3052.9, 2225.9, 1745.7, 1612, 1472.1; ^1H NMR ($CDCl_3$) δ ppm: 5.0 (s, 2H), 6.84 (d, 1H), 7.1–7.67 (m, 10H), 7.77 (d, 1H); MS (m/z): 338 (M^+), 192, 177, 146 (100), 90, 77; HPLC purity (%): 99.25.

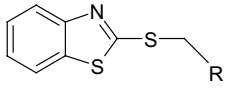
6.8.7. 4′-(2,3-Dioxo-2,3-dihydro-indol-1-ylmethyl)-biphenyl-2-carboxylic acid (49). To a mixture of 0.5 g (0.0014 mol) of 4′-(2,3-dioxo-2,3-dihydro-indol-1-yl-methyl)-biphenyl-2-carbonitrile **48** and 25 mL water was added 0.24 g (0.004 mol) of potassium hydroxide and the mixture was heated at 100–110 °C for 5 h. After cooling in an ice bath, it was acidified with dilute hydrochloric acid (10%) to get the crude product **49**. The product (0.358 g) was purified by column chromatography. IR (cm^{-1}): 3375.1, 3162.3, 2919.1, 1739.5, 1642.4, 1599.9, 1465.9; ^1H NMR ($CDCl_3$+DMSO-d_6) δ ppm: 5.0 (s, 2H), 6.84 (d, 1H), 7.1–7.66 (m, 10H), 7.73 (d, 1H); MS (m/z): 356 (M^+), 339 (M−17), 311 (M−45), 210, 146 (100), 90, 77; HPLC purity (%): 98.92.

6.9. General procedure for the synthesis of compounds 52–55

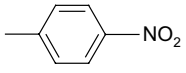
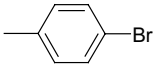
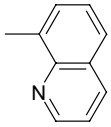
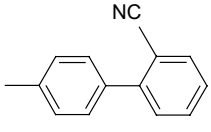
To 1 g (0.0059 mol) of benzothiazole-2-thiol dissolved in 30 mL acetonitrile was added 1.52 g (0.011 mol) of potassium carbonate and 0.1 g potassium iodide and the mixture was stirred at room temperature for 15 min. To this mixture was then 0.0059 mol of appropriately substituted bromomethyl derivative **35**–**37**, **40** dissolved in 20 mL acetonitrile, in a dropwise manner over a period of 20 min and the mixture was stirred at room temperature for 1–2 h. The product was purified by column chromatography. The structures of compounds synthesized by this method are given in Table 10.

6.9.1. 2-(4-Nitro-benzylthio)-benzothiazole (52). The title compound was prepared according to the general procedure using 1 g (0.0059 mol) of benzothiazole-2-thiol and 1.27 g (0.0059 mol) of 4-nitro-benzylbromide **35**.

Table 10. Structures, yield, mp, and mobile phase for TLC and column chromatography of compounds **52**–**55**



52 - 55

Sr. no.	R	Yield (%)	Mp (°C)	Mobile phase and R_f
52[40]		92.4	72–76	Chloroform $R_f = 0.88$
53		88.3	74–78	Chloroform $R_f = 0.9$
54		83.6	68–70	Chloroform $R_f = 0.82$
55		94.7	120–125	Chloroform $R_f = 0.85$

The reaction mixture was stirred at room temperature for 1 h. The product (1.67 g) was obtained as pale yellow amorphous powder. IR (cm^{-1}): 3065, 1593.7, 1514.6, 1457.5, 1344.3; ^1H NMR ($CDCl_3$) δ ppm: 4.67 (s, 2H), 7.20–8.23 (m, 8H); MS (m/z): 302 (M^+, 100), 256 (M−46), 223 (M−79), 166, 135, 78; HPLC purity (%): 99.92.

6.9.2. 2-(4-Bromo-benzylthio)-benzothiazole (53). The title compound was prepared according to the general procedure using 1 g (0.0059 mol) of benzothiazole-2-thiol and 1.47 g (0.0059 mol) of 4-bromo-benzylbromide **36**. The reaction mixture was stirred at room temperature for 1 h. The product (1.77 g) was obtained as pale yellow crystalline powder. IR (cm^{-1}): 3052.9, 1453.8, 1423.4, 748.7; ^1H NMR ($CDCl_3$) δ ppm: 4.55 (s, 2H), 7.29–7.45 (m, 6H), 7.75 (d, 1H), 7.90 (d, 1H); MS (m/z): 337 (M+1), 336 (M^+), 255 (M−81), 169 (100), 167; HPLC purity (%): 96.22.

6.9.3. 2-(Quinolin-8-ylmethylthio)-benzothiazole (54). The title compound was prepared according to the general procedure using 1 g (0.0059 mol) of benzothiazole-2-thiol and 1.309 g (0.0059 mol) of 8-bromomethyl-quinoline **37**. The reaction mixture was stirred at room temperature for 2 h. The product (1.53 g) was obtained as pale yellow amorphous powder. IR (cm^{-1}): 2925.1, 1490.4, 1460; ^1H NMR ($CDCl_3$) δ ppm: 5.29 (s, 2H), 7.26–8.25 (m, 9H) 9.0 (d, 1H); MS (m/z): 308 (M^+), 275 (M−33), 243 (M−65), 141 (100); HPLC purity (%): 93.27.

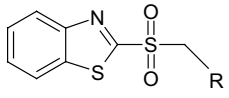
6.9.4. 4′-(Benzothiazol-2-ylthiomethyl)-biphenyl-2-carbonitrile (55). The title compound was prepared according to the general procedure using 1 g (0.0059 mol) of benzothiazole-2-thiol and 1.60 g (0.0059 mol) of 4′-bromomethyl-biphenyl-2-carbonitrile **40**. The reaction mixture was stirred at room temperature for 1 h. The product (2.02 g) was obtained as white amorphous powder. IR (cm^{-1}): 3028.6, 2225.9, 1453.8, 1423.4; ^1H NMR ($CDCl_3$) δ ppm: 4.67 (s, 2H), 7.29–7.92 (m, 12H); MS (m/z): 358 (M^+), 325 (M−33), 192 (100), 166; HPLC purity (%): 99.25.

6.10. General procedure for the synthesis of compounds 56–59

To 1 g of sulfide **52–55** dissolved in 25 mL of dichloromethane and maintained at 0–5 °C was added a solution of equivalent 3 mol of *m*-chloroperbenzoic acid dissolved in 15 mL of dichloromethane in a dropwise manner over a period of 10 min and the reaction was first stirred at 0–5 °C for 1 h and then at room temperature for 10–15 h. The reaction mixture was washed with a 25 mL saturated solution of sodium bisulfite, 25 mL saturated solution of sodium carbonate, and then twice with brine. It was dried over anhydrous sodium sulfate and solvent was recovered under vacuum. The crude sulfone **56–59** was further purified by column chromatography. The structures of compounds synthesized by this method are given in Table 11.

6.10.1. 2-(4-Nitro-phenylmethanesulfonyl)-benzothiazole (56). The title compound was synthesized according to the general procedure by using 1 g (0.0033 mol) of 2-(4-nitro-benzylthio)-benzothiazole **52** and 1.70 g (0.0099 mol) of MCPBA. The reaction mixture was stirred at room temperature for 12 h. The product

Table 11. Structures, yield, mp, and mobile phase for TLC and column chromatography of compounds **56–59**



56 - 59

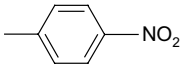
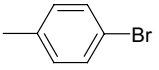
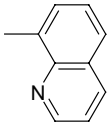
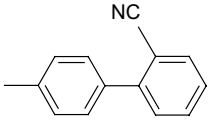
Sr. no.	R	Yield (%)	Mp (°C)	Mobile phase and R_f
56		65.4	175–178	Chloroform $R_f = 0.48$
57		61.8	160–164	Chloroform–hexane (1:1) $R_f = 0.53$
58		53.5	138–140	Chloroform $R_f = 0.63$
59		62	152–154	Chloroform–hexane (1:1) $R_f = 0.3$

Table 12. Calculated LeapFrog binding energies (BE) versus experimentally determined IC_{50} values

Compd	3′-Processing		3′-Strand transfer	
	BE (kJ/mol)	IC_{50} (mM)	BE (kJ/mol)	IC_{50} (mM)
4	−500.2	1	−561.3	1
5	−523.6	>1	−449.2	>1
6	−558.1	0.86 ± 0.12	−587.5	0.85 ± 0.13
7	−595.3	0.863; 0.98	−528.6	0.9; 1
20	−450.8	>1	−515.9	>1
21	−378.5	>1	−453.8	>1
22	−418.8	>1	−548.1	>1
23	−1354.8	0.45 ± 0.11	−1298.3	0.45 ± 0.06
26	−1082.4	0.52 ± 0.26	−917.2	0.65 ± 0.08
28	−523.7	>1	−624.6	>1
43	−1190.7	0.500	−1324.6	0.333
44	−438.2	0.900	−886.4	0.600
45	−449.7	1.000	−845.3	0.700
46	−693.2	0.800	−897.3	0.600
47	−689.3	0.800	−900.8	0.600
48	−484.3	>1	−498.2	>1
49	−510.5	0.900	−918.6	0.600
52	−534.6	>1	−634.7	>1
53	−445.3	>1	−473.5	>1
54	−325.6	>1	−467.9	>1
55	−446.9	>1	−414.7	>1
56	−370.3	>1	−473.5	>1
57	−489.2	>1	−353.1	>1
58	−395.7	>1	−461.0	>1
59	−934.8	0.700	−925.4	0.700

(0.722 g) was obtained as pale yellow amorphous powder. IR (cm^{-1}): 2967.7, 1520.8, 1465.9, 1344.3, 1149.8; ^1H NMR ($CDCl_3$) δ ppm: 4.87 (s, 2H), 7.49 (d, 2H), 7.60–7.70 (m, 2H), 7.97 (d, 1H), 8.15 (d, 2H), 8.25 (d, 1H); MS (m/z): 334 (M^+), 270 (M−64), 269 (M−65, 100), 167, 136; HPLC purity (%): 94.87.

6.10.2. 2-(4-Bromo-phenylmethanesulfonyl)-benzothiazole (57). The title compound was synthesized according to the general procedure by using 1 g (0.0029 mol) of 2-(4-bromo-benzylthio)-benzothiazole **53** and 1.50 g (0.0087 mol) of MCPBA. The reaction mixture was stirred at room temperature for 10 h. The product (0.676 g) was obtained as white amorphous powder. IR (cm^{-1}): 2973.8, 1478.3, 1332.2, 1137.7, 639.2; ^1H NMR ($CDCl_3$) δ ppm: 4.71 (s, 2H), 7.14 (d, 2H), 7.41 (d, 2H), 7.58–7.68 (m, 2H), 7.96 (d, 1H), 8.24 (d, 1H); MS (m/z): 369 (M+1), 368 (M^+), 304 (M−64), 198, 169 (100); HPLC purity (%): 91.33.

6.10.3. 2-(Quinolin-8-ylmethanesulfonyl)-benzothiazole (58). The title compound was synthesized according to the general procedure by using 1 g (0.0032 mol) of 2-(quinolin-8-ylmethylthio)-benzothiazole **54** and 1.65 g (0.0096 mol) of MCPBA. The reaction mixture was stirred at room temperature for 15 h. The product (0.59 g) was obtained as white amorphous powder. IR (cm^{-1}): 2919.1, 1460, 1320.1, 1149.8; ^1H NMR ($CDCl_3$) δ ppm: 5.59 (s, 2H), 7.19–8.13 (m, 9H), 8.47 (d, 1H); MS (m/z): 390 (M^+), 325 (M−65), 192 (100), 165; HPLC purity (%): 93.61.

6.10.4. 4′-(Benzothiazole-2-sulfonylmethyl)-biphenyl-2-carbonitrile (59). The title compound was synthesized according to the general procedure by using 1 g (0.0027 mol) of 4′-(benzothiazol-2-ylthiomethyl)-biphenyl-2-carbonitrile **55** and 1.4 g (0.0081 mol) of MCPBA. The reaction mixture was stirred at room temperature for 10 h. The product (0.675 g) was obtained as white amorphous powder. IR (cm^{-1}): 2973.8, 2225.9, 1465.9, 1320.1; ^1H NMR ($CDCl_3$) δ ppm: 4.82 (s, 2H), 7.39 (d, 2H), 7.43–7.67 (m, 7H), 7.73 (d, 1H), 7.96 (d, 1H), 8.27 (d, 1H); MS (m/z): 341 (M+1), 276 (M−64, 100), 198, 142; HPLC purity (%): 99.15.

7. Anti-HIV-1 integrase activity testing[41–47]

The HIV-1 integrase inhibitory activity of these compounds was evaluated in vitro as follows:

IN Assay. IN was preincubated at a final concentration of 200 nM with the inhibitor in reaction buffer [50 mM NaCl, 1 mM HEPES, pH 7.5, 50 μM EDTA, 50 μM dithiothreitol, 10% glycerol (w/v), 7.5 mM $MnCl_2$, 0.1 mg/mL bovine serum albumin, 10 mM 2-mercaptoethanol, 10% dimethyl sulfoxide, and 25 mM 3-(*N*-morpholino) propanesulfonic acid (MOPS), pH 7.2] at 30 °C for 30 min. Then, 20 nM of the 5′-end 32P-labeled linear oligonucleotide substrate was added, and incubation was continued for an additional 1 h. Reactions were quenched by the addition of 8 μL of loading dye (98% deionized formamide, 10 mM EDTA, 0.025% xylene cyanol, 0.025% bromophenol blue). An aliquot (5 μL) was electrophoresed on a denaturing 20% polyacrylamide gel (0.09 M Tris–borate, pH 8.3, 2 mM EDTA, 20% acrylamide, 8 M urea). Gels were dried, exposed in a Molecular Dynamics PhosphorImager cassette, and analyzed using a Molecular Dynamics PhosphorImager (Sunnyvale, CA). Percent inhibition was calculated using the following equation:

$$\%I = 100 \times [1 - (D - C)/(N - C)],$$

where C, N, and D are the fractions of 21-mer substrate converted to 19-mer (3′-processing product) or 3′-strand transfer products for DNA alone, DNA plus IN, and IN plus drug, respectively. IC_{50} values were determined by plotting the drug concentration versus percent inhibition and determining the concentration that produced 50% inhibition.

Acknowledgements

The recording of mass spectra by IPCA laboratories, Mumbai, and NMR spectra by Tata Institute of Fundamental Research, Mumbai is gratefully acknowledged.

References and notes

1. Piot, P.; Bartos, M.; Ghys, P. D.; Walker, N.; Schwartlander, B. *Nature* **2001**, *410*, 968.
2. Hariprasad, V.; Talele, T. T.; Kulkarni, V. M. *Pharm. Pharmacol. Commun.* **1998**, *4*, 365.
3. De Clercq, E. *J. Med. Chem.* **1995**, *38*, 2491.
4. Richman, D. D. *Nature* **2001**, *410*, 995.
5. Goldgur, Y.; Craigie, R.; Cohen, H. G.; Fujiwara, T.; Yoshinaga, T.; Fujishita, T.; Sugimoto, H.; Endo, T.; Murai, H.; Davies, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13040.
6. Sakai, H.; Kawamura, M.; Sakuragi, J.; Sakuragi, S.; Shibata, R.; Ishimoto, A.; Ono, H.; Ueda, S.; Adachi, A. *J. Virol.* **1993**, *7*, 1169.
7. Taddeo, B.; Haseltine, W. A.; Farnet, C. M. *J. Virol.* **1994**, *68*, 8401.
8. Engelman, A.; Englund, G.; Orenstein, J. M.; Martin, M. A.; Craigie, R. *J. Virol.* **1995**, *69*, 2729.
9. (a) Makhija, M. T.; Kulkarni, V. M. *J. Comput.-Aided Mol. Des.* **2001**, *15*, 961; (b) Makhija, M. T.; Kulkarni, V. M. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 1569; (c) Makhija, M. T.; Kulkarni, V. M. *Bioorg. Med. Chem.* **2002**, *10*, 1483; (d) Makhija, M. T.; Kulkarni, V. M. *J. Comput.-Aided Mol. Des.* **2002**, *16*, 181.
10. SYBYL Molecular Modeling System, version 6.6; Tripos, Inc., St. Louis, MO, 63144-2913.
11. Clark, M.; Cramer, R. D.; Van Opdenbosh, N. J. *J. Comput. Chem.* **1989**, *10*, 982.
12. MOPAC 6.0 is available from Quantum Chemistry Program Exchange, Indiana University.
13. Stewart, J. J. P. *J. Comput.-Aided Mol. Des.* **1990**, *4*, 1.
14. Cramer, R. D.; Patterson, D. E.; Bunce, J. D. *J. Am. Chem. Soc.* **1988**, *110*, 5959.
15. Kulkarni, S. S.; Kulkarni, V. M. *J. Med. Chem.* **1999**, *42*, 373.
16. Gokhale, V. M.; Kulkarni, V. M. *J. Med. Chem.* **1999**, *42*, 5348.
17. Wold, S.; Albano, C.; Dunn, W. J.; Edlund, U.; Esbensen, K.; Geladi, P.; Hellberg, S.; Johansson, E.; Lindberg, W.; Sjostrom, M.; Kowalski, B. *CHEMOMETRICS: Mathematics and Statistics in Chemistry*; D. Reidel: Dordrecht, 1984.
18. Bush, B. L.; Nachbar, R. B. *J. Comput.-Aided Mol. Des.* **1993**, *7*, 587.
19. Waller, C. L.; Oprea, T. L.; Giolliti, A.; Marshall, G. R. *J. Med. Chem.* **1993**, *36*, 4152.
20. Cramer, R. D.; Bunce, J. D.; Patterson, D. E. *Quant. Struct. Act. Relat.* **1988**, *7*, 18.
21. SYBYL 'Ligand-Based Design Manual' version 6.6; Tripos, Inc., St. Louis, MO.
22. Georgian, V.; Harrisson, R. J.; Skaletzky, L. L. *J. Org. Chem.* **1962**, *27*, 4571.
23. Koelsch, C. F.; Albertson, N. F. *J. Am. Chem. Soc.* **1953**, *75*, 2095.
24. Robinson, R. A. *J. Am. Chem. Soc.* **1947**, *69*, 1939.
25. Morikawa, A.; Sone, T.; Asano, T. *J. Med. Chem.* **1989**, *32*, 42.
26. Morikawa, A.; Sone, T.; Asano, T. *J. Med. Chem.* **1989**, *32*, 46.
27. Bullock, W. M.; Hand, J. J. *J. Am. Chem. Soc.* **1956**, *78*, 5854.
28. March, J. *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*; 4th ed.; Wiley Interscience: NY, 1992; pp 1220–1221.
29. Duffy, K. J.; Darcy, M. G.; Delorme, E.; Dillon, S. B.; Eppley, D. F.; Erickson-Miller, C.; Giampa, L.; Hopson, C. B.; Huang, Y.; Keenan, R. M.; Lamb, P.; Leong, L.; Liu, N.; Miller, S. G.; Price, A. T.; Rosen, J.; Shah, R.; Shaw, T. N.; Smith, H.; Stark, K. C.; Tian, S.; Tyree, C.; Wiggall, K. J.; Zhang, L.; Luengo, J. I. *J. Med. Chem.* **2001**, *44*, 3730.
30. Chmutova, G. A.; Kataeva, O. N.; Ahlbrecht, H.; Kurbangalieva, A. R.; Movchan, A. I.; Lenstra, A. T. H.; Geise, H. J.; Litvinov, I. A. *J. Mol. Struct.* **2001**, *570*, 215.
31. Day, J. C.; Lindstrom, M. J.; Skell, P. S. *J. Am. Chem. Soc.* **1974**, *96*, 5616.
32. Weizmann, M.; Patai, S. *J. Am. Chem. Soc.* **1946**, *68*, 150.
33. Brian, P. R.; Wilson, C. *J. Chem. Soc., Chem. Commun.* **1978**, *17*, 752.
34. Skell, P. S.; Day, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 1951.
35. Daub, H. G.; Castle, R. N. *J. Org. Chem.* **1954**, *19*, 1571.
36. Baldwin, J. E.; Cha, J. K.; Kruse, L. I. *Tetrahedron* **1985**, *41*, 5241.
37. Carini, D. J.; Duncia, J. V.; Aldrich, P. E.; Chiu, A. T.; Johnson, A. L.; Pierce, M. E.; Price, W. A.; Santella, J. B.; Wells, G. J. *J. Med. Chem.* **1991**, *34*, 2525.
38. Tacconi, G.; Gamba, A.; Marinone, F.; Desimoni, G. *Tetrahedron* **1971**, *27*, 561.
39. Cramer, R. D.; Poss, M. A.; Hermsmeier, M. A.; Caulfield, T. J.; Kowala, M. C.; Valentine, M. T. *J. Med. Chem.* **1999**, *42*, 3919.
40. Kočí, J.; Klimešová, V.; Waisser, K.; Kaustová, J.; Dahse, H.; Möllmann, U. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3275.
41. Mazumder, A.; Neamati, N.; Sunder, S.; Owen, J.; Pommier, Y. *Retroviral Integrase: A Novel Target in Antiviral Development; Basic In Vitro Assays with the Purified Enzyme*; The Humana: Totowa, NJ, 1999; pp 327–338.
42. Fesen, M. R.; Kohn, K. W.; Leteurte, F.; Pommier, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 2399.
43. Mazumder, A.; Engelman, A.; Craigie, R.; Fesen, M.; Pommier, Y. *Nucl. Acid Res.* **1994**, *22*, 1037.
44. Mazumder, A.; Gupta, M.; Pommier, Y. *Nucl. Acid Res.* **1994**, *22*, 4441.
45. Mazumder, A.; Pommier, Y. *Nucl. Acid Res.* **1995**, *23*, 2865.
46. Neamati, N.; Hong, H.; Mazumder, A.; Wang, S.; Sunder, S.; Milne, G. W. A.; Proksa, B.; Pommier, Y. *J. Med. Chem.* **1997**, *40*, 942.
47. Craigie, R.; Mizuuchi, K.; Bushman, F. D.; Engelman, S. A. *Nucl. Acid Res.* **1991**, *19*, 2729.